

THE EFFECT OF NASONOV BASED DISPENSERS ON HONEY BEE BEHAVIOR AND
ON POLLINATION IN BLUEBERRY, APPLE, AND CHERRY

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ABSTRACT

THE EFFECT OF NASONOV BASED DISPENSERS ON HONEY BEE BEHAVIOR AND ON POLLINATION IN BLUEBERRY, APPLE, AND CHERRY

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Honey bee attractants are not novel, but the time is right to continue to investigate their importance in the growing fruit industry. Due to pollinator declines, potential honey bee attractants have been used for the purpose of improving and increasing pollination services by bees. Polynate™ is a potential product manufactured for this purpose that contains a synthetic mixture similar to that of the Nasonov pheromone in honey bees. In this thesis, two main objectives are investigated: 1) to determine the ability of Nasonov based dispensers to increase fruit set and visitation in blueberries, apples and cherries and 2) to quantify behavioral changes associated with dispenser application. The first objective was accomplished by assessing the differences in forager visitation rates and fruit yield in crops treated and untreated with Nasonov based dispensers. The second objective was accomplished by directly observing the responses of honey bees in a hoop house when exposed to Nasonov based dispensers on artificial feeders. Dispensers did not increase fruit set or forager activity in blueberries, apples or cherries. A half rate of Polynate also did not cause a difference between treated and untreated areas. Given a choice between two identical feeders, one treated with a dispenser and one not, the honey bees had no visible preference.

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I dedicate this thesis to my entire family, especially to my fiancé Jason. Thank you all for your continuous support throughout my entire career especially during my years as a graduate student. Your patience and listening ears were greatly appreciated.

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TABLE OF CONTENTS

LIST OF TABLES	viii
LIST OF FIGURES	x
CHAPTER 1 Honey Bees, Pollination and the History of Attractants	1
The Importance of Pollination	1
Measures of Pollination and Bee Efficiency	3
Honey Bees and Pollination	4
Biology	4
Colony Health	5
Role as Pollinators	6
Honey Bee Visual and Olfactory Cues	9
Scent Taste and Sight	9
Pheromones and Attractants	10
The Nasonov Gland	10
The Components of Nasonov Pheromone	11
The Functions of Nasonov Pheromone	12
Queen Mandibular Pheromone	13
Pollination Enhancement	14
Nectar Guides	14
Scent Guides	15
Project Aims and Objectives	19
CHAPTER 2 Dispensing Components of the Nasonov Gland to Enhance Bee Visitation and Pollination in Blueberry, Apple and Cherry	21
Introduction	21
Materials and Methods	25
Blueberry	26
Apple	29
Cherry	30
Statistical Analysis	31
Results	31
Blueberry	31
Apple	35
Cherry	36
Discussion	38
CHAPTER 3 The Response of Honey Bees Reared in a Hoop House to Polynate and SPLAT	43
Introduction	43
Materials and Methods	46
Nuclear Colony (Nuc) Rearing	46

Artificial Feeders and Sucrose Solutions	47
Experimental Design and Protocols.....	48
Statistical Analysis	50
Results.....	51
Foraging Intensity	52
Feeding Duration	52
Discussion	53
CHAPTER 4 The Use of Polynate and SPLAT in Agriculture.....	56
APPENDICES	60
APPENDIX 1	61
APPENDIX 2.....	62
BIBLIOGRAPHY	66

LIST OF TABLES

Table 1. The mean number of blueberry fruit per flower \pm SEM at three distances from honey bee hives in untreated and Polynate treated fields during 2012.....	32
Table 2. The mean number of blueberry fruit per flower \pm SEM in untreated and half or full Polynate treated fields during 2013	32
Table 3. The mean count of honey bees, native bees and other pollinators per minute \pm SEM at three distances from honey bee hives in untreated and Polynate treated fields during 2012	33
Table 4. The mean count of honey bees, bumble bees, other bees and flies in blueberry fields per minute \pm SEM during 2013.....	33
Table 5. The mean mass (g) \pm SEM of blueberries in untreated and full or half Polynate treated fields during 2012 and 2013	34
Table 6. The mean mature seed count \pm SEM of blueberries in untreated and Polynate treated fields during 2012	34
Table 7. The mean number of apple fruit per flower \pm SEM in untreated and Polynate treated orchards during 2013	35
Table 8. The mean count of honey bees, native bees and other pollinators per minute \pm SEM at three distances from honey bee hives in untreated and Polynate or SPLAT treated orchards during 2012	35
Table 9. The mean count of honey bees, bumble bees, other bees and flies per minute \pm SEM in untreated or Polynate treated orchards during 2013	36
Table 10. The mean number of cherry fruit per flower \pm SEM at three distances from honey bee hives in untreated and Polynate treated orchards during 2012	37
Table 11. The mean number of cherry fruit per flower \pm SEM in untreated and Polynate or SPLAT treated orchards during 2013	37
Table 12. The mean count of honey bees, native bees and other pollinators per minute \pm SEM at three distances from honey bee hives in untreated or Polynate treated orchards during 2012	38
Table 13. The mean count of honey bees, bumble bees, other bees and flies per minute \pm SEM in untreated and Polynate or SPLAT treated orchards during 2013	38

Table 14. The mean count of honey bee foragers per minute and the mean time spent feeding \pm SEM for each treatment.	52
Table 15. The organization of field sites, treatment plots and sampling in 2012	62
Table 16. The organization of field sites, treatment plots and sampling in 2013	62

LIST OF FIGURES

Figure 1. A diagram of the dorsal side of a honey bees abdomen showing the Nasonov gland (McIndoo 1914).....	11
Figure 2. Example of plot and sampling location arrangement within each block for field experiments in 2012.....	63
Figure 3. Example of plot and sampling location arrangement within each block for field experiments in 2013.....	63
Figure 4. A single SPLAT dollop applied in cherry	64
Figure 5. A single Polynate dispenser applied in blueberry	64
Figure 6. The hoop house used in my experiments without the end partitions (pictured in figure 8).....	65
Figure 7. An experimental feeder during a trial.....	65
Figure 8. An experimenter videotaping one experimental feeder and the set-up of the nuc and stock feeder.....	65

CHAPTER 1

Honey Bees, Pollination and the History of Attractants

The Importance of Pollination

Most of our food comes from cereals or grains that rely only on gravity or wind to disperse pollen. However, about one third of our food comes from plants that need bee pollination to produce fruit (Free 1970; McGregor 1976). This includes foods like dairy and meat, which are reliant on plant products that are pollinated by bees. It was estimated that honey bees could bring as much as 1.6-8.3 billion dollars to agriculture in the United States (Southwick and Southwick Jr. 1992). While it is true that mankind could probably survive without bee pollination, many luxury foods that we take for granted such as ice cream or watermelon, would no longer be available to even the richest of people if bees did not pollinate plants. Around 130 crops grown in the United States require pollination by bees. Thus, much of the specialty crop agricultural economy in the United States is dependent on bees (McGregor 1976; Delaplane and Mayer 2000). Some time ago, it was estimated that the bee pollination in the United States was worth approximately 9 billion dollars. Honey bees specifically were estimated to provide 5.7 billion dollars and non-managed bees approximately 1.6 billion dollars in pollination services (Robinson, Nowogrodzki, and Morse 1989). In 2000, honey bees brought in \$14.6 billion through the increase of yield and produce quality of crops (Morse and Calderone 2000). It is reasonable to assume that these numbers would be higher now in 2014.

Crops need varying levels of pollination due to factors such as self-fertility, parthenocarpy, and plant diversity. Some crops and varieties are self-infertile, meaning that the ovum cannot be fertilized by pollen from the same plant. In these cases, it is essential for a bee to visit multiple flowers to facilitate pollen transfer. Parthenocarpy occurs when an ovule is not fertilized and a seedless fruit is produced. Growers are encouraged to select cultivars that exhibit

parthenocarpic traits so that their reliance on pollination is less (Allsopp, de Lange, and Veldtman 2008). Pollination requirements also differ among crops. For example, apples have a king bloom, one blossom that produces a fruit that is bigger and better quality than the others. It is more important for this king bloom to be pollinated than the other blossoms. Blueberries have flowers that are oriented downward which makes it difficult for some pollinators to collect pollen from them. The ovules of cherries start to deteriorate before the flowers open. Thus, fertilization must occur shortly after bloom for fruit to set (Delaplane and Mayer 2000).

It is important to note that bees can pollinate certain crops more efficiently than others. A bee's ability to pollinate depends on factors such as flower shape, and the orientation of the stamen and stigma (Delaplane and Mayer 2000). There are many species of bee that provide pollination services. However, not all of these bees are at sufficient numbers to sustain agriculture. For example, bumble bees are extremely efficient pollinators, but are harder to manage commercially than honey bees. Unfortunately, the protocols for managing other species of bees are, in most cases, proprietary or not readily accessible. Although honey bees may not be the most efficient pollinators, they are by far the easiest to manage on a commercial scale (Delaplane and Mayer 2000).

Of crops that are produced globally, 13 are dependent on pollination for production while 57 are highly to moderately dependent and 28 are only slightly or not dependent (Klein et al. 2007). Pollination of apple, cherry, and blueberry are the primary focus of this thesis. Out of these crops, apple is most dependent on bee pollination to produce fruit, followed by cherry then blueberry (Robinson, Nowogrodzki, and Morse 1989; Southwick and Southwick Jr. 1992; Williams 1994).

Measures of Pollination and Bee Efficiency

Since better pollination results in higher fruit quality and quantity, it is important to determine if we can produce a method to measure pollination and bee efficiency. It can be challenging to quantify the efficiency of bees in regards to pollination because pollinators have particular behaviors that can affect pollen transfer. For example, the total amount of time a bee spends foraging, the number of flowers visited per unit time, and the efficiency of pollen deposition all affect pollination. It is generally assumed that the longer a bee spends visiting a flower; the more time she spends collecting pollen. This could result in an increase in the amount of pollen being transferred from flower to flower as well which may result in increased fruit set. However, it may be detrimental to the level of pollination achieved if different flower species are visited during a single foraging trip.

It is even harder to quantify the amount of pollination that a crop is receiving. One way is to look at the number of seeds and the quality of fruit. Bee pollination significantly improves the quality of fruit in apple and blueberries (Dulta and Verma 1987; MacKenzie 1997). When a flower is pollinated, its seeds are fertilized; quite simply, the more seeds the more pollination that occurred. The number of seeds influences fruit quality. For example, a fruit that develops from a flower that has been poorly pollinated is usually misshapen and undersized (Delaplane and Mayer 2000). Another way is to count the number of blossoms during bloom versus the number of fruit that develop from those blossoms to determine fruit set. In crops without parthenocarpy, the greater the level of fruit set, the more pollination occurred. In other crops, the size of the fruit is proportional to the level of pollen deposited.

Honey Bees and Pollination

Biology

The European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is not native to the United States. As the name suggests, it is native to Europe as well as parts of Africa and the Middle East. Honey bees were brought to the United States (and many other countries) by European settlers in the 1700's where they flourished for years due to their high capacity to adapt (Delaplane and Mayer 2000; vanEngelsdorp and Meixner 2010). Honey bees are perhaps one of the most studied social insects in the world. There is no question about the numerous services that they provide us, from pollination of crops to production of wax and honey. They are more easily managed than other species of bee for our purposes because of their hive structure.

A honey bee hive consists of several castes, the queen, the drones and the workers. The majority of honey bees that we see are the workers, since they are responsible for collecting resources as well as maintaining the hive through various tasks. The queen is the only reproductive female, though in dire need the workers also have the ability to develop mature sexual organs and lay eggs. The drones are the only males in the hive and have one purpose; to mate with queens from other hives.

This distribution of responsibilities makes the worker bees the most important caste from a pollination standpoint. While the queen regulates much of the activity of the hive through pheromones, the workers alone are responsible for the decisions that they make outside of the hive while foraging for resources. There are other factors within the hive that have an influence on foraging behavior such as quantity of pollen and nectar, presence of brood and egg laying (Jay 1986). However, since on-site visual and chemical cues are only available for the foragers to interpret, they are the most influential on foraging based decisions. These cues include nectar

quality, distance traveled and floral patterns (Dyer 2002). Honey bees use memories of landmarks and the orientation of the sun to locate food sources. The information about food source location is relayed in the hive by means of the waggle dance (von Frisch 1993), but the actual cues are only experienced by individual workers.

Colony Health

Declines in honey bee numbers have not only been occurring in the United States, but worldwide (Ghazoul 2005; Aizen et al. 2008). Overwintering losses on average in the United States were 30.6% from 2012-2013 which is consistent with previous years (Steinhauer et al. 2014). Due to the recent decline in honey bees, there has been a greater effort to preserve them and the pollination services that they provide. These declines are not specific to honey bees, but other bee species as well. Declines of other pollinators however, are intermittent and dependent on species and location (Ghazoul 2005). In some regions, such as south west China, wild bee loss is so severe that apple and pear crops must now be hand pollinated (Partap and Ya 2012). Even though the bee losses in the United States have yet to reach a level that seriously effects crop yield, there is evidence to suggest that this will soon occur because of an increase in the use crops that are dependent on bee pollination (Ghazoul 2005; Klein et al. 2007; Aizen et al. 2008).

When managing honey bee colonies for crop pollination, it is important to maintain colony health. To accomplish this, honey bees need a constant supply of nectar and pollen during the spring, summer and fall seasons in addition to treatment for diseases. However, crops do not bloom all year long; most of them only have a short period of time in which they bloom. Placing a hive in the middle of one crop would not be sufficient to support the colony throughout their active season. This means that either multiple varieties or crops with overlapping bloom

periods must be available to the colonies, or the colonies must be moved to other locations with blooming flowers once the initial crop's bloom is over. Currently, beekeepers are paid to do just that; ship their bees across the country to aid in pollination. Inevitably, whenever a colony is moved, even at night, it will suffer the loss of many foragers (Delaplane and Mayer 2000). In 2000, more than 2 million out of 2.9 million colonies in the United States were shipped across the country for the pollination of various crops as well as for harvesting honey and wax (Morse and Calderone 2000). At this rate it would not be a surprise if there were a correlation between how much a hive has been moved throughout the summer, and its likelihood to survive to the next season (Stokstad 2007).

Over the years honey bees have been plagued by pests such as *Varroa destructor*, tracheal mites, Nosema, and now a disease with an unknown cause called Colony Collapse Disorder or CCD (Tew and Ferree 1998; Delaplane and Mayer 2000). Many blame this issue on pesticides, the arrival of new pests, poor nutrition, and even a small gene pool. However, the cause of CCD is most likely not due to any one problem but the synergistic effects of all that plagues honey bees (vanEngelsdorp et al. 2009).

Role as Pollinators

Honey bees are generalist foragers, meaning that they will forage on a wide range of flowers if they are an adequate source of pollen and nectar. This makes them very versatile because they can pollinate a variety of crops. However, this also means that they tend to pollinate other non-target flowers instead of crop flowers. von Frisch (1966) noted that once an individual honey bee has found a type of flower with good resources, she will consistently re-visit the same type of flower over others. His reasoning was that because honey bees must learn

how to locate the nectar in a particular flower, it is much easier for them to continue to visit the same type of flower instead of learning to collect from another (von Frisch 1966).

Undoubtedly, honey bees that are foraging for pollen are better crop pollinators than bees that are foraging for nectar because nectar collectors have a habit of bypassing the anthers and stigma of flowers to get to the nectar. Without the bee-to-anther contact, pollen is not transferred from plant to plant as the bee forages. Even if the bee is covered with pollen, they must also make contact with flower stigmas for pollination to occur (Jay 1986).

Distance is also a huge factor for honey bee foragers. There has been a debate on the precise distances that honey bees will travel to forage but the general consensus is that honey bees prefer to forage closer to their hive than travel longer distances. However, honey bees have been found to prefer foraging more than three to four miles (Beekman and Ratnieks 2000).

Even though honey bees provide excellent pollination services to a large variety of crops, their generalist nature can cause them to be less efficient than other bees. For example, Orchard mason bees, *Osmia lignaria* Say (Hymenoptera: Megachilidae), are more efficient in pollinating apple blossoms because of their tendency to land directly on the stigma and anthers of the flowers instead of the petals like honey bees. Bumble bees, *Bombus* spp. (Hymenoptera: Apidae), may also be more efficient pollinators of apple and blueberry (Delaplane and Mayer 2000; Javorek, Mackenzie, and Vander Kloet 2002). Honey bees will sometimes collect nectar without brushing against the sexual parts of the flower; this results in pollen neither being carried away nor deposited. Bumble bees, in contrast, almost always make contact with the stigma and anthers; therefore a higher percentage of flowers that are visited are also pollinated (Delaplane and Mayer 2000). Blueberry flowers are more efficiently pollinated by bees like bumble bees that sonicate flowers through buzzing (Javorek, Mackenzie, and Vander Kloet 2002). The

vibrations from their buzzing shakes the blueberry pollen loose which will fall onto the visiting bee (Delaplane and Mayer 2000). However, bumble bees tend to avoid foraging on less rewarding flowers (Townsend-Mehler, Dyer, and Maida 2011), and unfortunately, most crop flowers are neither as rewarding or attractive as other flowers in the surrounding area (Free 1968b). This suggests that bumble bees may be more attracted to the ground cover flowers than honey bees.

It is no secret that native bees are more efficient pollinators for specific crops than honey bees. This however, does not mean that honey bees are not capable of adequately pollinating crops. In fact, crop yield would decrease about 30-50% without the aid of honey bees in crops such as almond, apple, and cherry (Southwick and Southwick Jr. 1992). In addition, managing native bee colonies at the scale required by our agricultural needs may not be feasible because they are difficult to manage. Conversely, the art of honey bee keeping has been practiced for centuries and is not part of some trade secret like some rearing practices of native bees. The ability to move honey bee colonies long distances makes them especially versatile as individual hives can be moved among crops as bloom occurs. It would be more beneficial to use native bees such as bumble bees, orchard mason bees, soil-nesting bees and leafcutting bees, as a supplement when honey bee numbers are low or when they are inefficient at pollinating some varieties (Delaplane and Mayer 2000). Since it is unrealistic to completely rely on other pollinators and it is becoming harder for growers to purchase additional honey bee hives, it would be advantageous to discover a way to increase the efficiency of honey bees.

Honey Bee Visual and Olfactory Cues

Scent Taste and Sight

The senses of sight and smell are extremely important to foragers and honey bees in general (von Frisch 1950; Winston 1987). Foragers need both senses to locate pollen and nectar resources. Sight is most important during long-range exploration. However, honey bees can see no more than four colors and can't make out the exact shape of flowers which makes their sense of smell paramount during close range exploration (von Frisch 1966; Winston 1987). Bees trained to a scented box of a specific color containing food tended to investigate other boxes of the same color, but only entered boxes that emitted the scent; color or not (von Frisch 1971). This seems to suggest that honey bees use sight to find the general location of food, but use scent to find the more exact location.

Training bees based on sent cues has been well documented. Artificial feeders that have an attractive sent associated with them are visited with a higher frequency than sources that are not (von Frisch 1966). One explanation is that the scent makes it easier for the honey bee to pinpoint the exact location of the food source.

Contrary to what one may believe, honey bees actually have a poor sense of taste. A 2% sugar solution, which would taste sweet to humans, is no more attractive to a honey bee than a solution of pure water. This doesn't mean that taste is not important in terms of pollination. On the contrary, it prevents bees from wasting time visiting less-rewarding sugar sources (von Frisch 1966).

For a flower to be the most attractive, it must have strong visual, olfactory, and somatosensory cues. This is because foragers take cues from all of these senses to ensure that they are foraging on the most rewarding of flowers.

Pheromones and Attractants

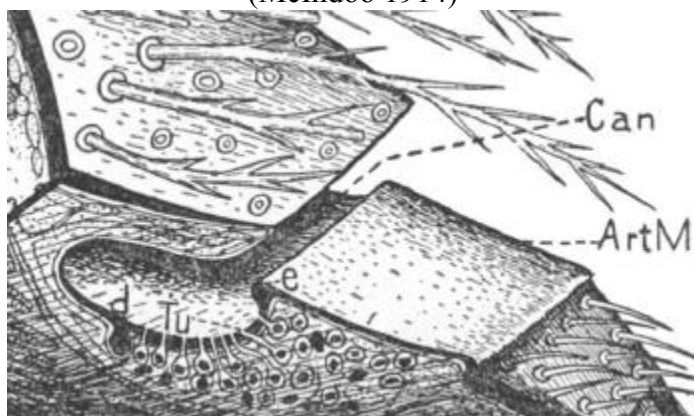
Because honey bees are social insects, they require a complex means of communicating with each other. Pheromones are chemicals released by an individual as a signal to a member of that individual's same species (Nordlund and Lewis 1976). Attractants can be pheromones, but not always. Attractants are materials that draw an organism to it either for the organism's benefit or demise. In the case of a bee, attractants include numerous types of scents from flowers, nectar scents, pheromones, and many others. Attractants may or may not have an effect on the target organism, but pheromones always elicit either a positive or negative response (Schmidt 1999).

Honey bees use many types of pheromones such as Queen Mandibular Pheromone (QMP), Nasonov pheromone, mating pheromones, brood pheromones alarm pheromones and trail pheromones to name a few (Free 1987; Pankiw 2004). All of these chemicals are emitted from one bee, or even sometimes the whole hive, to communicate with another. Nasonov pheromone and QMP have been studied most extensively for the purpose of increasing pollination services in crops.

The Nasonov Gland

The Nasonov or scent gland is located under a honey bees sixth intertergal membrane; in-between tergite six and seven (McIndoo 1914; Snodgrass 1984). A honey bee releases Nasonov pheromone when it lifts up its abdomen and exposes a part of the Nasonov gland (Wells et al. 1993). She may also fan her wings so that the pheromone is dispersed. The rest of the gland that is not exposed is invaginated, forming an internal canal (McIndoo 1914). To the naked eye, the gland appears to be a small white strip just above the tip of the abdomen.

Figure 1. A diagram of the dorsal side of a honey bees abdomen showing the Nasonov gland (McIndoo 1914)



Generally, honey bees will only release Nasonov pheromone when they are collecting water and not nectar or pollen. This is because nectar and pollen have their own natural scents to attract bees and water is usually void of any distinct scent (Free and Williams 1970). However, since honey bee dances are not precise and recruits are not able to follow exact directions (von Frisch 1967), pheromone may be used to draw them in. Honey bees that are visiting a food source that is already marked with Nasonov pheromone will not expose their Nasonov glands to discharge extra pheromone (Free 1968a). To do so would be a waste of energy. However, honey bees do expose their Nasonov glands when Nasonov pheromone is present at their hive entrance (Ferguson and Free 1981).

The Components of Nasonov Pheromone

The components of the honey bee Nasonov pheromone are geraniol (100 parts), nerolic acid (75 parts), geranic acid (12 parts) E-citral (1 part), Z-citral (1 part), E,E-farnesol (50 parts) and nerol (1 part) (Boch and Shearer 1962; Boch and Shearer 1964; Shearer and Boch 1966; Pickett et al. 1980; Free et al. 1984). E,E-farnesol is a sesquiterpenoid while the other six

components are monoterpenes. Honey bees will expose their Nasonov glands at their hive entrance when (E)-citral, geraniol, nerolic acid and geranic acid are individually applied to the front of the hive. However, (E, E) – farnesol and nerol did not elicit the same response, and actually decreased the honey bees response to the previous mentioned chemicals (Free, Pickett, and Ferguson 1983).

The Functions of Nasonov Pheromone

Nasonov pheromone is thought to be the most significant pheromone that bees use for orientation and organization during swarming (Morse and Boch 1971; Jay 1986). It is also thought to be the main pheromone that honey bees use during clustering, and may help a disorientated queen-less swarm re-locate their queen (Morse and Boch 1971; Mautz, Boch, and Morse 1972).

Wild honeybee swarms found artificial nest sites set up by scientists more often when accompanied by synthetic Nasonov pheromone when compared to nesting sites without the pheromone (Schmidt 1994). Again in 1999, Schmidt and colleagues tested the hypothesis that Nasonov pheromone was indeed a pheromone that is used in part to orient honey bees seeking nest sites and not is just a simple odor. They found that swarms were almost five times more likely to choose a nest site including a synthetic Nasonov mixture than a nest site that had none. They also tested four other odors, linalool, skatole, clove oil and wax moth pheromone, all of which attracted far fewer swarms than the nest sites with Nasonov pheromone. They concluded that Nasonov is indeed a pheromone that is essential to the nest-seeking behavior (Schmidt 1999).

von Frisch (1923) conducted a study which rejected his hypothesis that the Nasonov pheromone is attractive to honey bees during foraging. In 1947, he conducted a similar study and found the exact opposite, so he changed his view and maintained that the Nasonov pheromone was still important in foraging (Wenner and Wells 1990; Wells et al. 1993). In an additional experiment in 1993, Wells and colleagues were unable to conclude that foraging bees are attracted to Nasonov pheromone or its components. However, they also stated that it is useful to train bees to respond to different motivators (Wells et al. 1993).

There have obviously been some mixed results and opinions regarding the usefulness of the Nasonov pheromone, its components, and synthetic reproductions in attracting honey bees. Therefore, more research should be done to clarify their effects.

Queen Mandibular Pheromone

QMP is also a honey bee pheromone that appears to have many functions. It is not the only pheromone that the queen possesses, but it is by far the most important (Strauss et al. 2008). Unlike the Nasonov pheromone, QMP mainly functions in the hive level and honey bees rarely encounter it outside of the hive. It stimulates young workers to perform retinue behaviors such as feeding and grooming the queen. It also directs workers in colony maintenance and facilitated comb building (Beggs et al. 2007; Ledoux et al. 2001). Other functions of QMP include decreasing the chance of another queen being reared, delaying the age of first foraging, suppressing juvenile hormone, and inhibiting the development of worker ovaries (Pankiw et al. 1998; Hoover et al. 2003; Strauss et al. 2008).

QMP has been hypothesized to be useful to attract honey bee foragers to crops because it is used to orientate workers to the queen during swarming. However, while testing the

attractiveness of nesting sites to honey bees, researchers demonstrated that the presence of Nasonov pheromone increased site attractiveness more than the presence of QMP. However, a combination of Nasonov and QMP attracted the greatest number of swarms (Schmidt, Slessor, and Winston 1993). The queen scent alone may not be enough to move a swarm and the combined scents of queens and workers are very attractive to a queen-less swarm (Morse and Boch 1971).

Pollination Enhancement:

Due to the recent declines in pollinators and the concern that many crops are no longer being adequately pollinated, there has been an increase in conservation efforts (Ghazoul 2005) which have also led to the development of many products geared to increase pollination in crops. However, this phenomenon is not new; pollinator declines in the past (vanEngelsdorp and Meixner 2010) also spurred an increase in interest in pollination enhancement.

Nectar Guides

Nectar guides are honey bee attractants based on sugar content and smell to a lesser extent. As mentioned before, honey bees can only recognize sugar solutions that are relatively strong. This means that they are only attracted to the most rewarding resources (von Frisch 1966). Numerous studies have concluded that spraying sugar water on flowers does not increase pollination (Free 1965; Jay 1986). While they may increase pollinator activity, they decrease the number of bees actually pollinating flowers. This is because bees are more likely to collect the sugar syrup on the leaves rather than the nectar from flowers (Free 1965; Mayer and Johansen 1982).

Beelure® and Beeline® are two products that have been used in the past as nectar guides. Beelure is a colored corn syrup containing strawberry flavoring. Before being sprayed onto target crops, it is diluted with water. It was found to be ineffective aiding pollination in apple (Rajotte and Fell 1982). Later in 1984, Tew and Ferree found that Beelure increased the number of foragers in apple, though not significantly. Instead of visiting flowers, however, bees were often observed collecting syrup from the tree leaves. This explains why scientists did not also observe a higher fruit yield (Tew and Ferree 1984).

Beeline is a wettable powder containing lactose, fat, proteins, sucrose, pollens and vitamins. It was intended to be a food supplement for bees as well as a nectar guide. In red clover, an increased number of honey bee foragers and a higher yield were observed in untreated plots compared to plots that were treated with Beeline. However, the researchers observed a greater number of foragers in the untreated plots even before Beeline was applied. It was therefore determined that Beeline had no effect on foragers or yield in red clover instead of having a negative effect (Burgett and Fisher 1979). Beeline also did not increase pollinator activity in apple or pears. This is consistent with further research (Mayer and Johansen 1982). Beeline also had no effect on pollination through number of visits or yield in either cucumber or watermelon (Schultheis et al. 1994).

Scent Guides

Scent guides are attractive to honey bees solely based on smell. Placing bouquets of flowers in orchards will increase pollinator activity but decrease pollination (Waller 1970). Again, this is because the bees are more interested in visiting the flowers in the bouquets and collecting their pollen, than the pollen of the target crop. Scent guides that do not include a

pheromone component and have been studied include anise oil, lemon grass extract, and Bee-Here®.

Sugar added to anise oil did not increase pollinator activity in apples or pears (Mayer and Johansen 1982). The extract from lemon grass when diluted with water was found to be attractive to honey bees in sweet citrus (Malerbo-Souza, Nogueira-Couto, and Couto 2004). Bee-Here when diluted with water and not sugar syrup was found to attract honey bees to sweet orange orchards (Malerbo-Souza, Nogueira-Couto, and Couto 2004).

Other scent guides include Pollenaid®, Pollenaid-D®, Bee-Scent®, Fruit Boost® and Polynate™. Pollenaid is part Nasonov pheromone components plus some sugars and attractive oil (Dag 2011). Pollenaid-D is a source of nutrients consisting of iron, nitrogen and gluconic acid. It did not increase pollinator activity in apples or pears (Mayer and Johansen 1982). Bee-Scent was found to have varying percents increase of both honey bee foraging and fruit set in apple cherry and pear (Mayer, Lunden, and Britt 1989). It appeared to increase foraging seed content and fruit yield in watermelon (Elmstrom and Maynard 1991). However, a few years later, it was found to have no effect on number of visits or yield in either cucumber or watermelon (Schultheis et al. 1994). It also was reported to increase honey bee foraging on apple, but not fruit set (Tew and Ferree 1998).

The use of QMP and its components for the purpose of increasing pollination has had mixed results. Spraying QMP in blueberry and cranberry did not increase the number of flower visits, but foragers did spend a longer time in direct contact with flowers (Higo, Winston, and Slessor 1995). Fruit Boost®, which is composed of synthetic QMP components, did not increase pollinator frequency or fruit set in pear or sweet cherry. However, a 7% increase in pear weight was observed that led to a \$400 per hectare increase (Naumann et al. 1994). Fruit Boost also did

not increase honey bee visitation or fruit set in watermelon (Ellis and Delaplane 2009). Honey bees do not normally encounter queens outside of the hive (except in swarms). Thus, products containing QMP are most likely not going to attract foragers to crops.

Polynate, the main subject of this thesis, is a Nasonov based pollination enhancement product. It is hand-applied prior to bloom at a rate of 500-100 dispensers per acre. It has shown promise in the past to increase the pollination services honey bees provide (Gut and Isaacs, 2011 unpublished data). The Polynate dispenser is loaded with Z-citral (neral), E-citral (geranial), nerol, geraniol, geranic acid and anethole (whereas Nasonov pheromone contains Z-citral, E-citral, nerol, geraniol, nerolic acid, geranic acid and E,E-farnesol), components that have been documented as attractants for honey bees and other insects. Below, I review the relative attractiveness of each of the components individually to honey bees.

Geraniol in Japanese beetle traps, when combined with anethole and eugenol, is attractive to bumble bees (Hamilton, Schwartz, and Townshend 1970). Similar results were found again with a trap containing only a geraniol lure (Ladd, Beroza, and McGovern 1974). However, traps containing a geraniol lure did not catch significantly more honey bees than un-baited traps (Ladd, Beroza, and McGovern 1974; Allsopp and Cherry 1991). Geraniol also did not increase pollinator activity in apples or pears (Mayer and Johansen 1982). However, when ten focal bees were given a choice between feeding on sucrose solutions marked with several scents (anise, bay, citral, geraniol and nerol) some bees did prefer the geraniol marked solution (Wells et al. 1993).

Traps containing a citral lure caught a similar number of honey bees as un-baited traps (Allsopp and Cherry 1991). When ten focal bees were given a choice between feeding on sucrose solutions marked with several scents (anise, bay, citral, geraniol and nerol), no bees

visited the citral marked solution (Wells et al. 1993). However, citral was found to be attractive to honey bees in sweet orange when diluted with water (Malerbo-Souza, Nogueira-Couto, and Couto 2004). (E)-citral has specifically been shown to elicit Nasonov gland exposure in honey bees. The other isomer (Z)-citral also seems to be important in eliciting honey bees to expose their Nasonov glands at the hive entrance. There is evidence that these two isomers have the same level of attractiveness to honey bees (Free, Pickett, and Ferguson 1983).

When ten focal bees were given a choice between feeding on sucrose solutions marked with several scents (anise, bay, citral, geraniol and nerol) no bees significantly preferred the nerol marked solution. However, the feeder did receive some bee visits (Wells et al. 1993).

Geranic acid has been shown to elicit Nasonov gland exposure in honey bees (Ferguson and Free 1981; Free, Pickett, and Ferguson 1983; Free et al. 1984).

Anethole, a chemical used in Japanese beetle traps, was found to be attractive to honey bees. Anethole is not a honey bee pheromone but is thought to act as a kairomone to them since it is found in anise oil (van Praagh and von der Ohe 1983). When studying a Japanese beetle trap, scientists observed many honey bees and bumble bees were also being caught (Hamilton, Schwartz, and Townshend 1970). Traps containing an anethole lure caught significantly more honey bees and bumble bees than un-baited traps (Ladd, Beroza, and McGovern 1974; Allsopp and Cherry 1991).

There is evidence that combinations of these potential attractants may work better to increase pollination than just one attractant alone. However, the presence of a scent will not stimulate bees to forage and recruit without the presence of a reward. For example, citral, geraniol and anise were found only to be attractive to bees (alone and in various combinations with each other) when accompanied by a sucrose solution. Geraniol was found to be more

attractive than citral only when combined with sucrose. Without sucrose, citral was the more potent attractant (Waller 1970). Artificial hives with lures containing a mixture of neral, geraniol, nerolic acid and geranic acid were effective in catching Africanized honey bee swarms (Schmidt and Thoenes 1987).

Project Aims and Objectives

Because honey bees are so beneficial to agriculture, it is important to maintain and enhance the pollination services that they provide. This is becoming harder as the years pass due to their declining health and the increased demand for pollination. Development of a pollination attractant, such as Polynate, could provide improved pollination or more efficient pollination of hard-to pollinate cultivars.

The many products that have been developed and tested to date have generated some success, but results have been variable. Even when increased crop quality and yield have been obtained, the mechanism by which it is achieved is still unknown. Furthermore, any positive effects have been short-lived, lasting for only a day or two. In Michigan, sprayable products would be expected to wash off readily during spring rainfall. As early as 1986, it was proposed that the use of long-release dispensers, which protect the chemical from rain and degradation on leaf surfaces, would be beneficial for field deployment (Jay 1986).

Polynate is a reservoir-type device that contains the same chemicals that honey bees use to recruit each other. This plastic dispenser cannot be washed off and protects the components from degradation. However, it is possible to impregnate the same chemical composition into a wax paste (SPLAT: Specialized Pheromone and Lure Application Technology). The overall aim of this project is to test if Polynate and SPLAT can be applied in blueberry, apple or cherry by

growers to increase pollination, yield and profit. The specific objectives are: 1) to determine the attractiveness of Polynate and SPLAT and their chemical components to honey bees and 2) to determine whether deployment of Polynate and SPLAT dispensers loaded with bee pheromone and other attractants increases bee visitation and pollination of highbush blueberry, apple, and cherry.

CHAPTER 2

Dispensing Components of the Nasonov Gland to Enhance Bee Visitation and Pollination in Blueberry, Apple and Cherry

Introduction

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), provide pollination services that are extremely important to agriculture. In North America, apples and cherries are primarily pollinated by honey bees. Blueberries have other major pollinators, but honey bees are still important in producing a higher yield of berries. Due to the recent decline in honey bee populations because of colony collapse disorder, it is becoming difficult for beekeepers to supply enough bees to pollinate these fruit crops, as well as the many other pollination-dependent crops in the United States (McGregor 1976; Delaplane and Mayer 2000). This has resulted in it becoming harder to rent hives, as well as more costly. On average, renting a honeybee hive cost about \$70.85 in 1999 in the Pacific North West (Burgett 2011). The California Beekeeper's Association estimates that the average cost of renting honey bee hives for almond pollination in 2014 will be as much as \$170 per hive ("CSBA Pollination Survey Results" 2013). It is estimated that over 2 million honey bee colonies are required for crop pollination each year (Morse and Calderone 2000). A possible option for decreasing the demand and therefore the cost of renting hives would be to maximize the pollination services that each hive provides.

Some varieties of apple have the ability to produce fruit without pollination, but the quality and quantity of fruit produced is low. About six to seven of the ten ovules must be fertilized to produce a well shaped fruit (Brault and de Oliveira 1995). Fertilization is more likely to happen when cross pollination by pollinators occurs. Cross pollination can also

increase the amount of calcium in apples, which maintains apple quality in storage (Volz, Tustin, and Ferguson 1996).

Highbush blueberries are able to produce a relatively constant number of blueberries through self-fertilization and parthenocarpy (El-Agamy, Sherman, and Lyrene 1979; MacKenzie 1997). However, as in apples, cross-pollination increases the quality and quantity of the fruit as well as increasing the rate of fruit maturation through the development of a higher number of mature seeds. When adequately pollinated, a blueberry can have as many as 65 mature seeds. A blueberry flower must be pollinated within three days of opening otherwise fruit will not set (Delaplane and Mayer 2000).

Sour cherry, in contrast to sweet cherry varieties, can self-fertilize their flowers whereas most sweet cherries need cross-pollination to produce fruit. In both types of cherry, it is very important that pollination occurs quickly, because they only have one ovary (thus two ovules) that starts to decay even before the flower opens (Delaplane and Mayer 2000). This can pose a problem since cherries bloom in the early spring, when it may still be too cold for some pollinators to forage.

To meet the pollination needs of these crops, many growers buy or rent honey bee hives in the spring to ensure adequate pollination. Even though there are other types of pollinators, honey bees are the most versatile because they are generalist feeders, meaning that they visit all types of flowers to acquire food. The foraging behaviors of honey bees also facilitate a high rate of cross pollination. They prefer to visit consecutive trees or bushes in a row instead of flying through rows (Delaplane and Mayer 2000). They also tend to visit multiple flowers on the same plant before moving down the row to another (Lyrene 1989). This means that not only are honey

bees in contact with a generous amount of pollen from each plant, but they are also spreading the pollen throughout the crop.

The extent that honey bees are successful at pollinating is determined by the flowers' characteristics. For example, honey bees are less effective at pollinating the Delicious apple variety due to their tendency to collect nectar from the flowers without brushing against the pollen covered anthers (Delaplane and Mayer 2000). The shape and downward orientation of the blueberry flower makes it difficult for honey bees to pollinate them. The pollen containing anthers are located further back within the flower which decreases the chance that a honey bee will brush against them while trying to extract nectar. It would be beneficial to find a way to increase the attractiveness of crops to honey bees so that they are more persistent at flowers that are more difficult to pollinate.

PolynatTM is a product manufactured by BioGlobal in Australia that is intended to increase honey bee visitation and thus, pollination (Figure 5). It contains a synthetic mixture of the compounds found in the Nasonov gland in honey bees. In the wild, this pheromone is used by foraging honey bees to attract more foragers to food sources with high rewards or water. It is also used during the swarming process, to keep the swarm together (Free 1987). Other honey bee attractants have been tested in the past with uncertain success (Burgett and Fisher 1979; Mayer and Johansen 1982; Rajotte and Fell 1982; Tew and Ferree 1984; Mayer, Lunden, and Britt 1989; Elmstrom and Maynard 1991; Naumann et al. 1994; Schultheis et al. 1994; Tew and Ferree 1998; Malerbo-Souza, Nogueira-Couto, and Couto 2004; Ellis and Delaplane 2009). A major advantage of Polynat over these previous products is that it is applied manually rather than sprayed on the crop. Thus, the release rate is controlled and it is not susceptible to wash off by rain.

Polynate has shown promise in preliminary trials as a pollination enhancer. Polynate was found to increase fruit set as much as 10-15% in blueberries, apples and cherries (Gut and Isaacs, 2011 unpublished data). When applied to a target crop, Polynate may cause honey bees to become more attracted to its flowers. It is expected that Polynate will cause honey bees to visit crop flowers more frequently and for a shorter period of time (thus visiting a higher number of flowers throughout time) causing an increase in pollen flow throughout the crop. It has been shown that visitation rates increase in flower patches with a higher reward (Southwick, Loper, and Sadwick 1981). This change in bee behavior could be measured as an increase in fruit production which would increase revenue. However, there is some concern that Polynate would repel other native foragers that are also important in pollination since it only contains components of honey bee pheromone and not pheromones of other important pollinators.

The objectives for this study were 1) to determine the potential of Nasonov based dispensers to increase the number of honey bee foragers in blueberries, apples and cherries; 2) to determine if Nasonov based dispensers have a repellent effect on other non-honey bee foragers; 3) to determine the potential of Nasonov based dispensers to increase fruit set in blueberries, apples, and cherries; 4) to determine if Nasonov based dispensers are equally effective at various distances from honey bee hives; 5) to determine the most effective rate of application for Polynate in blueberries; and 6) to compare the effectiveness of SPLAT or Specialized Pheromone and Lure Application Technology (a wax paste impregnated with the same chemical composition of Polynate in figure 4) to Polynate.

Materials and Methods

Experiments were conducted in four blueberry fields located in Grand Junction MI (Van Buren County), four apple orchards on “The Ridge” in Grand Rapids MI (Kent Co.), and two cherry orchards located in the southwest near Hartford MI (Van Buren Co.) and two in Traverse City MI (Leelanau Co.). The efficacy of Polynate dispensers or SPLAT was determined by comparing fruit set and pollinator counts between plots of treated and untreated blueberry, apple and cherry. The experimental design was a randomized complete block with fields or orchards at four sites per crop. Each site was split into pairs of plots with one randomly assigned a potential attractant and the other designated as the untreated control. Experimental blocks were selected that had a consistent elevation throughout the block and treatments at each site were positioned such that pheromone would not be carried by prevailing winds from the treated plots to the untreated plots. Honey bee hives were supplied by the growers and positioned centrally with respect to the two treated plots. The aim was to place honey bee hives equidistant from the two treatments. The total number of hives differed between crop and field site due to adhering to the grower’s usual practices in renting hives. However, there were approximately 4-8 two-high hives in apple and cherry for each field site. In blueberry, there was an average of 50 two-high and 54 three-high per field site. However, there was as many as 44 to 188 hives (both two and three-high hives) in one field site. Colony health was not assessed to determine the exact number of foragers. There was a possibility that wild honey bees or other managed bees from different hives visited the field sites. However, since it was impossible to distinguish bees from the test hives or other sources, it was assumed that each plot had the potential to be visited equally in the absence of treatment.

Polynate (Figure 5) or SPLAT (Figure 4) dispensers were applied 7-14 days prior to bloom at the recommended rate of 1,000 units/ha. Polynate dispensers were applied at 1.5-2.0 m height in the canopy by twisting them onto branches to ensure that they would remain in place throughout the duration of bloom. SPLAT was applied with a caulking gun to deposit quarter sized dollops (approximately 1 g) on the trunks of the trees at the same height and potency.

Within the treated and untreated plots, bee activity and fruit set were assessed to determine treatment effects on pollination. The position and number of sampling locations differed from 2012 and 2013 and between crops. Details on bee activity and fruit set or quality measurements are provided below for each crop, as well as summarized in Table 15 and 16. In general, there were three sampling locations in each crop in 2012: near honey bee hives, in the middle of the plot and far from the hives (Figure 2). Due to a limited supply of Polynate in 2013, only a 0.8 ha section in the middle of each block was treated, thus sampling of bee activity and pollination were restricted to a single location in the center of the treated or untreated plots (Figure 3).

Blueberry

In 2012, the experiment was conducted in four 1.6 ha Jersey variety blueberry fields. At each site, a 0.81 ha section was treated with Polynate at the rate of 1000 dispensers/ha by placing a dispenser on every third bush. The remaining 0.81 ha section was left untreated. Bee activity, pollination and fruit set were measured in three sampling locations: near the honey bee hives, in the middle of the plot and far from the hives (Figure 2). The near sampling locations were located in the first row along the perimeter where the hives were placed, about 10-20 m from the

hives, the intermediate locations were approximately 30 bushes into the plot or 30-45 m from the hives and the far locations were approximately 60 bushes or 60-90 m from the hives.

Timed observations were used to determine the number of honey bees, native bees and other pollinators visiting blueberry flowers in treated and untreated plots. One minute counts were conducted on each of 10 bushes randomly selected in the near, intermediate and far sampling locations. During each one-minute count, the observer would circle around the bush, or count pollinators on one side of two bushes. Observers counted the number of honey bees, native bees and other potential pollinators that made contact with the flowers. Pollinator observations were only conducted during peak bloom and on days with optimal foraging conditions (sunny days at approximately 20°C with wind blowing no more than 16 kmh). Observations were conducted in the afternoon, during peak foraging times when honey bees and other pollinators were visibly active (12:00 to 4:00). Observations were only conducted once during bloom on the same day at a given site to minimize differences between treatments in foraging due to weather conditions.

The impact of treatment on fruit set, berry size, and blueberry weight (an indication of the number of mature seeds) was determined by comparing open pollinated fruit clusters versus clusters that were enclosed in a fine mesh bag to prevent pollinators from accessing the flowers. The bags consisted of a sheer fabric and were held in place with an elastic band around the opening. Prior to bloom (mid-May) two flower clusters on each of four bushes (in the same row) at each of the three sampling locations per plot were selected for sampling and marked with flagging tape. The number of buds on each cluster (at least ten buds per cluster) were counted and subsequently one cluster on each bush was bagged and the other left unbagged. After bloom, when berries started to develop (June), bags were removed and the number of berries

formed on each cluster was counted. A final assessment was made when blueberries were ripe (July) by removing the clusters and bringing them back to the laboratory to determine berry size. Approximately ten berries from each cluster were weighed and dissected to determine the number of seeds per fruit (two sites for seed counts and three sites for weights).

The experimental set-up was modified in 2013 due to a limited supply of Polynate dispensers. The experiment was conducted in four 6.1 ha Jersey variety blueberry fields. Each field was divided into three experimental units that were randomly assigned one of three treatments: 1) Polynate deployed at 1,000 units/ha, 2) half of the Polynate dispenser deployed at the rate of 1,000 units/ha, and 3) an untreated control. The Polynate dispenser is a twin-tube design making it easy to split the dispenser in half for the low rate treatment (Figure 5). Only a 0.4 ha section in the center of each block was treated, thus sampling of bee activity and pollination were restricted to a single location in the center of the treated or untreated plots (Figure 3). The impact of treatment on fruit set, berry size, and blueberry weight and bee activity were assessed as described above. However after the fruit were counted, the bags were replaced to prevent berry loss due to animals or pickers. Fruit counts as previously described were taken from 16 bushes in a 40 m² area located centrally in each 0.4 ha experimental area. To determine the berry size all the ripe berries from each cluster were weighed, but seed counts were not taken. Pollinator counts as described above were taken from 20 random bushes located in the 0.4 ha experimental area. The number of honey bees, bumble bees, other bees and flies were counted per minute.

Apple

In 2012, experiments were conducted in three 2.4 ha apple orchards. A total of three treatment plots were used in each orchard. Polynate and SPLAT were applied to 0.81 ha sections at a rate of 1000 dispensers/ ha. The remaining 0.81 ha was left untreated. Apple varieties varied across sites and included Jonagold, Golden Delicious, Gala, Honey Crisp, Empire, Paula Red, Portland and Ida Red. However, varieties were the same in treated and untreated plots at each of the three sites. Due to a severe early season frost, accurate fruit counts were unobtainable at harvest in 2012. Pollinator activity was assessed using timed observations as described for blueberries. Sampling was conducted on 12 trees in each of three sampling locations in treated and untreated plots. The near sampling locations were located in the closest row to the honey bee hives, the intermediate locations were approximately 15 trees or 30-40 m from the hives and the far locations were approximately 30 trees or 60-80 m from the hives.

As with blueberries, the experimental set-up was modified in 2013 due to the limited supply of dispensers. The experiment was conducted in three 4 ha apple orchards. Varieties were the same as in 2012. Each orchard was divided into two experimental plots and a 0.6 ha area within the center of one plot was treated with Polynate at 1000 dispensers/ha and a 0.6 ha area within the center of the other plot was delineated with flagging tape and left untreated. Fruit set was assessed by counting flower buds on 25 trees in a 250-300 m² area located centrally in each 0.6 ha experimental plot. Prior to bloom (May), a pair of branches on each tree that had at least 30 fruit buds were identified, marked with flagging tape and the total number of buds counted. Four to five weeks later, when fruit were 10-15 mm in circumference, the total number of fruit present on the same branches was counted. The percent of flowers that produce fruit is expected to be anywhere from 0.2% to 25.9% (Stephenson 1981). Timed observations as

described previously were used to determine pollinator activity in treated and untreated plots. One-minute counts were conducted on each of 24 trees randomly selected in the near, intermediate and far sampling locations.

Cherry

In 2012, experiments were conducted in three 1.6 ha ‘Montmorency’ cherry orchards. At each site, Polynate was applied to 0.81 ha section at a rate of 1000 dispensers/ ha. The remaining 0.81 ha was left untreated. Pollinator activity was assessed using timed observations as described previously. Sampling was conducted on 20 trees in each of three sampling locations. The near sampling locations were located in the closest row to the honey bee hives, the intermediate locations were approximately 15 trees or 30-45m from the hives and the far locations were approximately 30 trees or 60-90m from the hives. Fruit set was assessed by counting fruit buds on 10 trees located in the middle of each 0.81 ha plot. Prior to bloom (May), a suite of four branches on each tree that had at least 100 fruit buds were identified, marked with flagging tape and the total number of buds counted. Four to five weeks later when set fruit were present, the total number of fruit present on the same branches was counted.

The experimental set-up was modified in 2013 due to the limited supply of dispensers. The experiment was conducted in three 4 ha ‘Montmorency’ cherry orchards. Each orchard was divided into two experimental units and a 0.6 ha area within the center of one plot was treated with Polynate at 1000 dispensers/ha and a 0.6 ha area within the center of the other plot were delineated with flagging tape and left untreated. Fruit set was assessed as in 2012 by counting the number of set fruit from a know number of fruit buds. The expected percentage of flowers that produce fruit is 23.5% to 50.1% (Stephenson 1981). Timed observations as described

previously were used to determine pollinator activity in treated and untreated plots. One-minute counts were conducted on each of 24 trees randomly selected in the near, intermediate and far sampling locations.

Statistical Analysis

All data were analyzed using a randomized complete block design in SAS 9.3 (SAS Institute Inc. 2013). A one-way Analysis of Variance (ANOVA) was performed using PROC MIXED and treatment as the independent variable. This was used to determine the effect of treatment on pollinator abundance and fruit yield (i.e. dependent variables). Pollinator abundance and fruit set data were transformed using a square root transformation to normalize the data. Mean separations and comparisons were calculated using Tukey's Honest Significant Difference test (HSD) at a 5% confidence level (SAS Institute Inc. 2013).

Results

Blueberry

Fruit set or the number of fruit produced was significantly lower for the bagged than the un-bagged clusters in 2012. However, there was no significant difference between Polynate treated or untreated plots for either unbagged or bagged clusters ($F = 0.84$; $df = 1,1$; $p = 0.53$). The untreated plots tended to have a slightly higher fruit set than the treated plots but there was no significant difference in fruit set between the three sampling locations ($F = 0.42$; $df = 2,6$; $p = 0.67$) (Table 1).

Table 1. The mean number of blueberry fruit per flower \pm SEM at three distances from honey bee hives in untreated and Polynate treated fields during 2012

Sampling Location	Bag	Untreated	Polynate
Near	Yes	0.17 ± 0.05	0.24 ± 0.09
	No	0.76 ± 0.07	0.73 ± 0.18
Intermediate	Yes	0.10 ± 0.02	0.15 ± 0.11
	No	0.84 ± 0.03	0.80 ± 0.04
Far	Yes	0.24 ± 0.04	0.14 ± 0.05
	No	0.76 ± 0.02	0.70 ± 0.19

In 2013, fruit set was significantly lower for clusters that were bagged compared to those not bagged. There was not a significant difference in fruit set between Polynate or untreated plots ($F = 2.75$; $df = 2,2$; $p = 0.27$), but the blocks treated with a half rate had numerically fewer fruit per flower compared to bagged and unbagged exposures (Table 2).

Table 2. The mean number of blueberry fruit per flower \pm SEM in untreated and half or full Polynate treated fields during 2013

Bag	Untreated	Full Polynate	Half Polynate
Yes	0.33 ± 0.09	0.32 ± 0.09	0.22 ± 0.10
No	0.84 ± 0.02	0.86 ± 0.02	0.74 ± 0.04

The number of pollinators observed per minute visiting blueberry flowers in 2012 was not significantly different between the Polynate treated or untreated blocks ($F = 0.94$; $df = 1,57$; $p = 0.34$). However, there was a slight trend for higher counts in the treated blocks. There also was not a significant difference in pollinator activity between the three sampling locations ($F = 0.90$; $df = 2,6$; $p = 0.45$). Honey bees were the most abundant pollinator in both treatments. The numbers of bumble bees and other bees were extremely low (Table 3).

Table 3. The mean count of honey bees, native bees and other pollinators per minute \pm SEM at three distances from honey bee hives in untreated and Polynate treated blueberry fields during 2012

Sampling Location	Untreated	Polynate
Near		
Honey bees	3.60 \pm 0.45	3.25 \pm 0.46
Native bees	0.05 \pm 0.03	0.05 \pm 0.03
Other Pollinators	0.10 \pm 0.08	0.03 \pm 0.03
Intermediate		
Honey bees	3.75 \pm 0.36	3.48 \pm 0.45
Native bees	0.03 \pm 0.03	0.08 \pm 0.04
Other Pollinators	0.13 \pm 0.09	0.03 \pm 0.03
Far		
Honey bees	3.73 \pm 0.32	3.33 \pm 0.43
Native bees	0.13 \pm 0.05	0.15 \pm 0.05
Other Pollinators	0.10 \pm 0.05	0.05 \pm 0.03

There was not a significant difference between treatments for pollinator counts per minute in 2013 ($F = 0.11$; $df = 2,39$; $p = 0.89$) although, plots treated with a full or half rate of Polynate had numerically higher numbers of flower visitors. Honey bees again were the most abundant pollinator observed. Counts of other pollinators were very low making it difficult to measure any differences between the treatments (Table 4).

Table 4. The mean count of honey bees, bumble bees, other bees and flies in blueberry fields per minute \pm SEM during 2013

Organism	Untreated	Full Polynate	Half Polynate
Honey bees	2.78 \pm 0.77	3.20 \pm 0.99	3.43 \pm 0.69
Bumble bees	0.04 \pm 0.02	0.03 \pm 0.01	0.00 \pm 0.00
Other bees	0.03 \pm 0.03	0.05 \pm 0.03	0.01 \pm 0.01
Flies	0.20 \pm 0.06	0.28 \pm 0.11	0.26 \pm 0.11

There was not a significant difference between treatments in berry mass for 2012 ($F = 3.52$; $df = 1,1$; $p = 0.31$) and 2013 ($F = 0.09$; $df = 2,2$; $p = 0.92$). Polynate treated plots had slightly heavier blueberries in 2012, but slightly lighter blueberries in 2013 (Table 5). There was not a significant difference between sampling locations in 2012 ($F = 0.98$; $df = 2,2$; $p = 0.51$). There also was not a significant difference in berry mass between bagged and unbagged clusters in either year, although the unbagged berries were always heavier.

Table 5. The mean mass (g) \pm SEM of blueberries in untreated and full or half Polynate treated fields during 2012 and 2013

Year	Sampling location	Bag	Untreated	Full Polynate	Half Polynate
2012	Near	Yes	0.43	0.84 ± 0.15	-
		No	1.01 ± 0.05	1.12 ± 0.13	-
	Intermediate	Yes	0.79 ± 0.20	0.93 ± 0.05	-
		No	1.03 ± 0.06	1.22 ± 0.04	-
	Far	Yes	0.57 ± 0.15	0.62 ± 0.06	-
		No	1.04 ± 0.28	1.14 ± 0.26	-
2013	NA	Yes	0.83 ± 0.10	0.73 ± 0.08	0.49
		No	1.27 ± 0.11	1.05 ± 0.11	0.80 ± 0.21

There was not a significant difference in fertilized seed counts between Polynate or untreated plots in 2012 ($F = 0.10$; $df = 1,1$; $p = 0.80$). There also was not a significant difference in seeds between locations ($F = 0.05$; $df = 2,2$; $p = 0.95$) or between bagged and unbagged clusters (Table 6).

Table 6. The mean mature seed count \pm SEM of blueberries in untreated and Polynate treated fields during 2012

Sampling location	Bag	Untreated	Polynate
Near	Yes	19.00	13.17 ± 9.77
	No	44.20 ± 7.10	33.98 ± 2.73
Intermediate	Yes	15.50 ± 1.50	23.00 ± 7.50
	No	37.41 ± 4.71	40.98 ± 2.16

Table 6. (cont'd)

Far	Yes	13.30 ± 0.70	25.63 ± 11.63
	No	31.70 ± 0.00	40.55 ± 3.45

Apple

Due to freezing temperatures in the spring, we were not able to collect reliable data for apple fruit set in 2012. There was not a significant difference in fruit set between treatments ($F = 2.86$; $df = 1,1$; $p = 0.34$) in 2013. However, the untreated plots had a slightly higher numerical fruit set than the Polynate treated plots (Table 7).

Table 7. The mean number of apple fruit per flower ± SEM in untreated and Polynate treated orchards during 2013

Treatment	Untreated	Polynate
Fruit/Flower	0.19 ± 0.04	0.16 ± 0.02

There was not a significant difference between the Polynate, SPLAT or untreated plots in the number of honey bees, other bees or flies visiting apple flowers in 2012 ($F = 0.08$; $df = 2, 68$; $p = 0.92$) per minute. There was also not a significant effect of distance from the hive on pollinator activity ($F = 0.69$; $df = 2,4$; $p = 0.55$) (Table 8).

Table 8. The mean count of honey bees, native bees and other pollinators per minute ± SEM at three distances from honey bee hives in untreated and Polynate or SPLAT treated orchards during 2012

Sampling Location	Untreated	Polynate	SPLAT
Near			
Honey bees	1.94 ± 0.54	1.86 ± 0.43	1.61 ± 0.59
Native bees	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03
Other Pollinators	0.00 ± 0.00	0.06 ± 0.03	0.06 ± 0.06

Table 8. (cont'd)

Intermediate			
Honey bees	1.44 ± 0.56	0.97 ± 0.34	1.19 ± 0.56
Native bees	0.06 ± 0.06	0.03 ± 0.03	0.00 ± 0.00
Other Pollinators	0.06 ± 0.06	0.00 ± 0.00	0.03 ± 0.03
Far			
Honey bees	1.44 ± 0.48	1.12 ± 0.70	2.64 ± 0.77
Native bees	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
Other Pollinators	0.06 ± 0.06	0.12 ± 0.03	0.03 ± 0.03

Pollinator counts per minute in Polynated and untreated plots were not significantly different in 2013 ($F = 1.13$; $df = 1,17$; $p = 0.30$) either. However, the Polynated treated plots consistently had a higher number of pollinators. Honey bees comprised the majority of flower visitors in 2012 and 2013; the number of native bees and bumble bees visiting flowers were very low (Table 9).

Table 9. The mean count of honey bees, bumble bees, other bees and flies per minute ± SEM in untreated or Polynated treated orchards during 2013

Organism	Untreated	Polynated
Honey bees	8.40 ± 0.38	10.51 ± 1.13
Bumble bees	0.00 ± 0.00	1.67 ± 0.07
Other bees	0.40 ± 0.33	0.35 ± 0.27
Flies	0.03 ± 0.01	0.04 ± 0.02

Cherry

There were no significant differences in fruit set between treatments in 2012 ($F = 0.74$; $df = 1,5$; $p = 0.43$). The untreated plots had a higher numerical fruit set in the near and far locations, but the opposite was true for the intermediate location. Distance was also not a significant predictor of the number of fruit per flower ($F = 0.98$; $df = 2,2$; $p = 0.51$) (Table 10).

Table 10. The mean number of cherry fruit per flower \pm SEM at three distances from honey bee hives in untreated and Polynate treated orchards during 2012

Sampling Location	Untreated	Polynate
Near	0.16 ± 0.02	0.08 ± 0.04
Intermediate	0.11 ± 0.04	0.14 ± 0.02
Far	0.09 ± 0.00	0.08 ± 0.05

Overall, treatment was not a significant factor affecting the fruit per flower ratio in 2013 ($F = 7.82$; $df = 2,3$; $p = 0.06$). The plots treated with SPLAT had a numerically higher number of fruit per flower than the Polynate treatment and the untreated plots. However, since SPLAT was only tested in a single plot the difference in fruit set could be associated with plot differences rather than the treatment (Table 11).

Table 11. The mean number of cherry fruit per flower \pm SEM in untreated and Polynate or SPLAT treated orchards during 2013

Treatment	Untreated	Polynate	SPLAT
Fruit/Flower	0.16 ± 0.03	0.12 ± 0.02	0.29

There was not a significant difference between treatments in the number of pollinators observed per minute in 2012 ($F = 1.01$; $df = 1,16$; $p = 0.33$). However, untreated plots tended to numerically have more flower visitors than treated plots. Honey bees were the most frequently counted pollinator. There was a numerical trend of more native bees in untreated plots compared to treated plots. Distance was not found to be a significant factor in predicting pollinator counts ($F = 5.53$; $df = 2,2$; $p = 0.15$) (Table 12). A greater number of pollinators were observed in the more distant plots compared to the near plots.

Table 12. The mean count of honey bees, native bees and other pollinators per minute \pm SEM at three distances from honey bee hives in untreated or Polynate treated orchards during 2012

Sampling Location	Untreated	Polynate
Near		
Honey bees	5.20 \pm 0.40	4.53 \pm 1.28
Native bees	0.43 \pm 0.28	0.30 \pm 0.25
Intermediate		
Honey bees	7.68 \pm 1.48	6.58 \pm 1.63
Native bees	1.03 \pm 0.23	0.00 \pm 0.00
Far		
Honey bees	7.88 \pm 0.33	8.18 \pm 0.38
Native bees	1.20 \pm 0.75	0.65 \pm 0.05

There was not a significant difference in pollinator counts per minute between treatments in 2013 ($F = 1.12$; $df = 2,26$; $p = 0.34$). However, plots treated with SPLAT had numerically higher number of honey bees. Honey bees were the most frequent pollinator, followed by other bees (Table 13).

Table 13. The mean count of honey bees, bumble bees, other bees and flies per minute \pm SEM in untreated and Polynate or SPLAT treated orchards during 2013

Organism	Untreated	Polynate	SPLAT
Honey bees	1.18 \pm 0.27	1.57 \pm 0.57	1.83
Bumble bees	0.13 \pm 0.06	0.03 \pm 0.02	0.13
Other bees	0.45 \pm 0.13	0.44 \pm 0.12	0.54
Flies	0.18 \pm 0.06	0.25 \pm 0.17	0.08

Discussion

Overall there was a lack of consistency in measured differences in pollinator activity and fruit set in plots treated with Polynate or SPLAT and plots left untreated. There were no significant differences between treatments in either fruit set or pollinator counts. Although there

were some trends, they were not consistent between crops or years. Therefore, Polynate and SPLAT did not have a significant effect on pollinators or fruit crop pollination. The addition of a Nasonov based dispenser also did not influence the activity of other flower visitors. We did not expect to see an increase in activity as the compounds present in Polynate or SPLAT are only known to influence honey bee behavior. Although the number of other pollinators was generally low, we did not observe any adverse affects on activity of treating with Polynate or SPLAT. Additional studies in which one or more species of native bees are released in plots are needed to confirm our findings.

Surprisingly, there were no differences in pollinator counts between the near, intermediate and far sampling locations. We hypothesized that there would be less honey bee activity in the sampling locations further from the hives compared to close to the hives leading to a greater positive effect of Nasonov based dispenser treatment at these locations. Honey bees have been documented to fly as far as 9.5 km from their hive (Beekman and Ratnieks 2000). Thus it is probable that the furthest sampling locations were not far enough away from the hives to result in major differences in foraging activity. Indeed, overall we observed similar numbers of honey bees in the untreated plots across the three sampling locations. It is also a possibility that honey bees from other hives on the farm visited the test plots. The relative positions of the near, intermediate and far sampling locations for other hives could have been different compared to the experimental hives.

During bloom in commercial apple, blueberry, and cherry, farms used in these experiments had a substantial number of honey bees and other potential pollinators visiting flowers. It may be very difficult to attain measurable improvements in pollination under these circumstances. It is possible that a pollination threshold is reached where flowers receive the

maximum amount of pollination possible. This would mean that no matter what is done to increase honey bee activity there would not be a measurable increase in fruit set and size. The maximum number of fruit produced is clearly limited to the number of flowers during bloom and the amount of natural pollination tends to surpass the amount needed to set fruit (Stephenson 1981). Also, it is possible that too much pollen on a stigma can actually cause pollination failure in some plants (Wilcock and Neiland 2002).

In these trials where high pollinator activity trends were observed, they generally did not produce significant positive effects on fruit set. This also occurred when queen mandibular pheromone sprays were applied in cranberry and blueberry (Currie, Winston, and Slessor 1992a) and when Bee-Scent was applied in apples (Tew, Ferree 1998). Future studies with Nasonov based dispensers or other potential pollination enhancements should focus on the potential for increasing fruit set in orchards that are known to be difficult to pollinate and achieve high fruit set. Only when pollinator activity does not meet a pollination threshold, can pheromone attractants become economically feasible (Currie Winston, and Slessor 1992a; Currie, Winston, and Slessor 1992b). However, it has been suggested that Nasonov pheromone applications may not aid in honey bee attraction if flowers provide low reward (Free 1987) which may be the case for some crops that are harder to pollinate. Studies could be conducted in orchards where few or no honey bee hives are placed near the orchard during bloom.

Polynate and SPLAT were applied at a rate of 1000/ha, primarily based on the recommended application rate for similar devices containing sex pheromone and used to disrupt mating of the targeted species (Gut et al 2004). It is possible that this application rate is either too high or too low to achieve the desired result of pollination enhancement. Nothing is known about how the chemicals loaded into Polynate or SPLAT disperse and how large or small the

resulting plume is. At a high application or release rate, this could result in plumes spreading into the untreated areas adjacent to treated plots. Under this circumstance, honey bees would be equally attracted to the treated or untreated blocks.

Moreover, the threshold at which honey bees respond or fail to respond to the compounds present in Polynate or SPLAT is unknown. The high application or release rate of Polynate and SPLAT might have overloaded the sensory system of the honey bees. Alternatively, the plumes emanating from individual dispensers may have been too small. Perhaps the plume of a dispenser did not extend past the tree that it was applied and honey bees only sensed its presence locally rather than from further distances. Honey bee sensitivity to odors is well known and studied (von Frisch 1950; Winston 1987). We could readily perceive the odor emanating from Polynate when it was applied, suggesting that high quantities of the volatiles were present. It is reasonable to assume that foraging honey bees could detect the odors as well (von Frisch 1950; Kaissling 1971). This suggests that the rates applied were too high rather than too low. There was no significance difference in the effect of Polynate applied at a full or half rate. Much lower release rates may be needed to have the desired effects on honey bee foragers.

The effect that Polynate or SPLAT might have on pollinators may be more subtle than the hypothesized increase in numbers of individuals visiting flowers. Our sampling methods were designed to maximize measuring any increase in the numbers of flower visitors. Polynate or SPLAT may have the more subtle effect on the time an individual pollinator spends foraging in the treated area. Honey bees are known to spend more time feeding at artificial feeders with a high reward (von Frisch 1950). In the field, this behavior could increase the amount of pollen carried from the flower to the bee, but might decrease the actual number of flowers visited. In reality, honey bees tend to spend less time feeding on individual flowers in attractive patches to

maximize energy efficiency. The less time a bee spends on one flower, the more flowers she can visit in one foraging trip (Schmid-Hempel, Kacelnik, and Houston 1985; Delaplane and Mayer 2000). Thus, flower visitation rates and total time spent are increased in flower patches with higher rewards (Southwick, Loper, and Sadwick 1981). This could cause an increase in pollen transfer from flower to flower, but this might not cause an increase in fruit production, especially if a flower's pollination needs are already met. Since we only counted the number of visits (defined as every time a pollinator made contact with a flower) and not the number of flowers visited or the total time each bee spent at a flower, we are unable to discern any effect of Polynate or SPLAT on these behaviors. Changes in the number of flowers visited or time spent at a flower may not greatly change the number of fruit produced per flower (fruit set) but they may increase the quality of fruit, which was not measured in apples or cherries.

Our results demonstrate the complexities of pollination in crops. Due to recent declines, pollinators and pollination are receiving increased attention from the scientific community and the general public. This had led to increased effort to enhance honey bee habitat or efficiency to improve pollination (Potts et al. 2010). Perhaps lost in the intense effort to address the pollination problem is the recognition of the complexity of the process by which honey bees and other insects pollinate crops. Honey bee workers perform pollination services until they die, and it may not be possible to entice them to work any harder simply by improving the attractiveness of crops through pheromones. There are many factors that go into a forager's choices and behaviors. Due to these complexities, developing a product that will increase pollination services may be a difficult task. However, the less than desirable results obtained with Polynate and SPLAT should not discourage us from continuing to pursue attractant-based approaches to enhancing pollination.

CHAPTER 3

The Response of Honey Bees Reared in a Hoop House to Polynate and SPLAT

Introduction

Honey bee behavior has been heavily investigated throughout the years, starting with the famous work of Karl von Frisch (von Frisch 1950, 1966, 1967). It is clear that the factors affecting honey bee behavior are very complex and that they use many cues to make choices. One of the most significant social cues honey bees use are chemicals called pheromones. There are many different types of pheromones such as alarm pheromones, brood pheromones, queen mandibular pheromone and Nasonov pheromone that honey bees use to communicate with each other within the hive and outside (Free 1987; Pankiw 2004). Pheromones are used to convey information from one honey bee to the next and can cause changes in the behaviors of individual bees for the benefit of the hive.

Honey bees do not start to react to Nasonov pheromone until they are about 28 days old; when they start to forage (Winston 1987). Nasonov pheromone is thought to be used for orientation and organization during swarming (Morse and Boch 1971; Jay 1986) and is also important in nest-seeking behavior (Schmidt 1999) and marking the hive entrance (Winston 1987). Honey bees often expose their Nasonov glands and fan their wings after some type of disturbance of the hive such as when a beekeeper moves it (Free 1987). There also is evidence that Nasonov pheromone is used to make rewarding food resources more attractive. More specifically, Nasonov pheromone may increase recruitment of honey bees by the forager that encounters it. However, there is mixed support for this theory (Wenner and Wells 1990; Wells et al. 1993).

It is common for scientists to attempt to manipulate honey bee behavior using pheromones because of the way the bees naturally use them. One of the more recent examples of

this is to use pheromones to attract honey bees to target crops in the hope of increasing fruit quantity and quality. It has been suggested many times that it could be beneficial to apply synthetic Nasonov pheromone to crops in need of further pollination (Free 1968; Free 1987). A specific pheromone laden dispenser (Polynate™, BioGlobal, Australia) that contains a synthetic version of Nasonov pheromone was applied to blueberries, apples, and cherries and the impacts on fruit quantity, quality or pollinator activity was quite variable, with no significant increase in pollination levels (Chapter 2). However, it was unclear whether Polynate or SPLAT had any effect on the behavior of honey bees specifically. Thus, it is also important to investigate if these Nasonov based dispensers are attractive to honey bees in a controlled setting and if it has any subtle effects that were not measured previously. In the field, the number of honey bees that visited the experimental blocks could not be controlled. Feed time, flower visit duration or specific behaviors of individual bees were also not measured. In this controlled study, behaviors were observed directly instead of only quantifying potential outcomes of changed behaviors (such as increased fruit set).

Honey bees make foraging decisions based on energy profitability and their foraging background. For example, a resource with a higher quality of food (higher nectar quality) at a closer distance is thought to be more rewarding, thus more attractive to honey bees (Shafir 2011). It is possible to measure how attractive a food source is to honey bees by using the number of visits it receives. When a honey bee returns from a foraging flight, she will relay information about the location of desirable food sources through the waggle dance to other foragers in the hive. Thus, if a food source is attractive, the number of recruited foragers visiting that food source will increase exponentially depending on the quality of the food source and forager availability (von Frisch 1967; Núñez 1970; Dyer 2002). Interestingly, once a honey bee

exposes her Nasonov gland, other honey bees are prone to do so as well (Winston 1987). This behavior could be used to draw additional foragers to a rewarding food source.

While foraging on flowers which present a consistent reward, honey bees don't completely fill their crops because flying back to the hive with a full crop takes more energy (Schmid-Hempel, Kacelnik, and Houston 1985). This crop filling behavior also occurs because individual flowers do not contain enough nectar to completely fill a honey bee crop. However, artificial feeders have plenty of sucrose solution. Indeed, von Frish observed that honey bees completely fill their crops at artificial feeders with a high sucrose concentration but do not at a feeder with a low sucrose concentration (von Frisch 1950). Since it is likely that completely filling a crop takes more time than partially filling, this could mean that honey bees feed for a longer period of time on artificial feeders that they find attractive. Thus, the attractiveness of an artificial feeder treated with Nasonov based dispensers could be measured by comparing the time each bee spends feeding at it with another untreated feeder.

Two pollination enhancement products, Polynate and SPLAT, have shown promise in preliminary field studies as a tool for increasing fruit set and foragers in blueberries, apples, and cherries (Gut and Isaacs, 2011 unpublished data). However, more recent field studies did not yield the same positive results (Chapter 2). Due to inconsistencies in field studies, a series of controlled studies were undertaken to determine more directly how Polynate and SPLAT might increase the attraction of a food source to foraging honey bees. The specific objectives were to

- 1) determine if Polynate or SPLAT increase the number of honey bee visits to an artificial feeder
- 2) determine if Polynate or SPLAT have an effect on the time that honey bees spend feeding in one foraging trip
- 3) to observe the general behavior of honey bees around a pollination

enhancement dispenser and 4) determine the attractiveness of different concentrations of Polynate to foraging honey bees.

Materials and Methods

All experiments were conducted in an outdoor hoop house located on the Southern part of the Michigan State University (MSU) campus and described in Townsend-Mehler et al. (2010). The hoop house is 35 m long, 5.6 m wide and 2.3 m high and is covered with 30% greenhouse shade cloth (Pictured in Figure 6, without end partitions). Only about a 27 m area on one end was used to avoid interfering with experiments being conducted at the other end of the facility. Previous studies conducted in this particular hoop house concluded that the 30% light reduction this shade cloth produces has no effect on the behavior of the bees being studied (Townsend-Mehler et al. 2010).

Nuclear Colony (Nuc) Rearing

Confining a honey bee hive in a small area such as the hoop house on MSU's campus presented some complications. Once a honey bee leaves its hive for the first time, she does something called an orientation flight. This is so that she will know what her hive looks like as well as its location. She will often expose her Nasonov gland at the hive entrance afterwards (Hazelhoff 1941; Free 1987). After the orientation flight, she will start to forage and develop an impression of the outside world. This means that once foragers in a colony have their first flights, the colony can no longer be confined within a small area. If a honey bee hive is placed within the confines of a hoop house the bees will become disorientated and form swarms in the corners, constantly trying to find ways to escape confinement. Therefore, conducting studies in the hoop house required the use of a hive that was set up inside the house before the bees had

their first orientation flights. In other words, the honey bees must have their first flight inside of the hoop house and perceived the confined space as their entire foraging area.

To establish a small nuclear colony (a four frame hive) inside of the hoop house, four frames of honey bee brood were taken from a hive maintained at MSU in mid-June and reared in an incubator kept at 35°C until young bees emerged. Newly emerged bees were brushed into the nuclear hive located within the hoop house with a handheld bee-brush. A queen was removed from a colony maintained at MSU and put into a queen cage stopped with candy along with several worker bees. The queen cage consisted of a small hollowed out wooden block about the size of a thumb with screen for one side. The queen cage was installed into the two-frame hive, screen side facing outward (so that worker bees could feed her) and left there until the worker bees chewed through the candy stopper. The honey bees were provided with pollen (sold commercially and slightly ground up) and gave foraging bees daily access to a sucrose solution artificial feeder. All other food sources were eliminated.

Artificial Feeders and Sucrose Solutions

Artificial sucrose feeders were used to control the quality and consistency of food that the honey bees received. Feeders consisted of a baby food jar upended on a clear plastic platform and filled with a sucrose solution (Figure 7). Because honey bees are generalists, it is easy to encourage them to forage on artificial feeders if the reward is enough. In fact honey bees tend to expose their Nasonov glands near artificial feeders more than near flowers (Free and Williams 1972).

It also was important to provide bees with a sugar source that simulates natural nectar sources. Honey bees prefer sucrose solutions of about 0.88-1.5 M (30-50% concentration).

Lower or higher concentrations of sucrose may cause aversion to the sucrose solution. However, honey bees are less discriminatory if there are more foragers (Waller 1972) or if the number of food sources in the area is low (von Frisch 1950; Seeley 1989). When testing the attractiveness of Polynate or SPLAT it was important to keep a balance between providing bees with an extremely attractive feeder vs. one that caused aversion. If the feeder was too rewarding, then the attractiveness of the treatment would be irrelevant because the feeder would attract more foragers regardless of the presence of Polynate or SPLAT. However, if the opposite was true, then the honey bees may have learned to associate the Polynate or SPLAT odor with a negative stimulus.

For the first trial, a 1.5 M sucrose solution was used for the two experimental feeders at either end of the hoop house (this first trial was thrown out in the end due to a camera malfunction which led to one side not being filmed). The sugar concentration was decreased to 1 M and 0.5 M for subsequent experiments. Sucrose solutions of lower sugar content were not used because there is evidence that this would have caused an aversion to the feeders (Waller 1972).

Experimental Design and Protocols

Experiments were conducted between the 20th of August and 23rd of September at approximately 12:00pm in 2013. For all experiments there were a total of three feeders in the hoop house (Figure 7). One of the feeders, defined as the stock feeder contained a lower sucrose concentration (0.25 M) and was placed directly in front of the hive. The other two feeders, defined as the experimental feeders, contained a higher sucrose concentration of 1.0 M or 0.5 M (relative to the activity of the honey bees). The two experimental feeders were placed on

opposite sides of the hoop house (north and south), equidistant from the hive. Each platform had a crisscross pattern of colored tape that mimicked natural flower patterns so that the bees could easily see them. The effect of a Polynate or SPLAT dispenser on bee behavior was evaluated by placing a dispenser on the same platform as one of the feeders while leaving the other feeder untreated. Experiments also were conducted with blank dispensers (containing no chemicals; trial 7) or no dispensers at all (trials 8 and 22) at both feeders. These trials served as additional controls but were not analyzed statistically due to low replication.

Before the start of each trial, the stock feeder was set up and any bee that visited it was tagged. Colored and numbered tags, slightly larger than the size of a pinhead were glued with wood glue to the back of 50 bee's thoraxes using a toothpick. Additional bees were tagged (in increments of ten) as the original tagged bees died or lost their tags. At the start of each experiment, 0.25 M stock feeder was placed in front of the hive and bees (tagged and non-tagged) were allowed to visit it. Once at least five untagged bees and one tagged bee were visiting the feeder sucrose solution was switched to a higher concentration and allowed the unsettled foragers to settle and start feeding again. The stand and the feeder were moved to one side of the hoop house (north or south) where an observer counted and recorded the number of untagged bees and the identity of any tagged bees. The experimental feeder containing a 0.5 M sucrose solution was replaced with another 0.5 M experimental feeder on another stand back in front of the hive soon after. Once it was again visited with foragers, it was transported to the other side of the hoop house where another observer was waiting. One side was randomly chosen to be treated with a Nasonov based dispenser, which was placed on the stand by the observer once it reached its side. The other side contained the experimental feeder only. The following pollination enhancement treatments were tested: 1) Full rate of Polynate, 2) a half rate

of Polynate in which the twin-tube was split in half, 3) a quarter rate of Polynate in which one side of the twin-tube was cut in half and 4) a 1 g SPLAT dollop. There was not a significant amount of time between the placing of each feeder on opposite sides (not more than 1-3 minutes). After both sides received a feeder, the 0.25M stock feeder was placed back in front of the hive and left it for the bees even after the completion of the trial. This was an alternative method to train bees to visit the experimental feeders and was done to avoid the possibility that honey bees wouldn't find the feeders throughout the duration of the trials.

For 30-45 minutes, each side was videotaped by an observer (Figure 8) using an Aiptek Handycam camera filming 29 frames/second. The trial was stopped when either of the sucrose feeders ran out, when the memory on the cameras was full or if honey bee activity was so high, that the number of visits was too difficult to count. The observer vocalized the feeding behavior of the tagged bees; specifically when they started and stopped feeding (defined by the duration of time between the protraction and retraction of the proboscis (Townsend-Mehler, Dyer, and Maida 2011) and when they arrived and left the feeder. After each trial, each video was watched and the total number of visits each feeder received was counted (foraging intensity). The total feeding time in seconds was also counted for each tagged bee. If no tagged bees visited the feeder throughout the duration of filming, several bees whose activity was easily seen throughout their entire visit were randomly chosen and the time they spent feeding was recorded.

Statistical Analysis

All data were analyzed using SAS 9.3 (SAS Institute Inc. 2013). A one-way Analysis of Variance (ANOVA) was performed using PROC MIXED and treatment as the independent variable. This was used to determine the effect of treatment on foraging intensity and feeding

time (i.e. dependent variables). The foraging intensity data were transformed using arcsin transformation to normalize the data. Mean separations and comparisons were calculated using Tukey's Honest Significant Difference test (HSD) at a 5% confidence level (SAS Institute Inc. 2013).

Results

The honey bees reared in the hoop house fed at the stock and experimental feeders and the colony thrived. The hive was strong throughout the summer without having to add supplementary newly emerged bees to it. The queen laid eggs continuously throughout the summer, but very little brood was raised. The bees were able to maintain an adequate supply of honey and pollen with the amount of sugar water that was supplied to them through the experiments.

Forager activity was high in the hoop house in general. However, very few bees foraged before the continuous stock feeder in front of the hive was re-filled with 0.25 M sucrose solution prior to the onset of the experiment. Once the stock feeder was refilled, honey bees were visiting it and other bees were foraging within minutes. After only a few experiments, the bees seemed to remember the location of the experimental feeders. Once the stock feeder was refilled, many foragers immediately went to the locations of the two experimental feeders in anticipation of sucrose being placed there. During the experiment, when the experimental feeders were available, the 0.25 M stock feeder was relatively abandoned. Honey bees resumed feeding at the stock feeder once the other experimental feeders were removed. Forager activity was lower on days after the experiment had been delayed; like after a spell of bad weather or the weekend for example.

Foraging Intensity

There were no significant differences between the full Polynate and untreated treatments ($F = 3.57$; $df = 4,3$; $p = 0.62$) the half Polynate and untreated treatments ($F = 0.26$; $df = 4,3$; $p = 1.00$) the quarter Polynate and untreated treatments ($F = 0.03$; $df = 4,3$; $p = 1.00$) and the SPLAT and untreated treatments ($F = 3.31$; $df = 4,3$; $p = 0.65$) in the number of visits/min to the feeders as shown in table 14. There also were no differences between trials or between the north and south feeders. The number of foragers trained to each feeder for each trial did not have an effect on the number of forager visits per minute.

Feeding Duration

There were no significant differences between the full Polynate and untreated treatments ($F = 1.59$; $df = 4,3$; $p = 0.87$) the half Polynate and untreated treatments ($F = 0.55$; $df = 4,3$; $p = 0.99$) the quarter Polynate and untreated treatments ($F = 0.21$; $df = 4,3$; $p = 1.00$) and the SPLAT and untreated treatments ($F = 0.20$; $df = 4,3$; $p = 1.00$) in the length of time spent feeding per visit. The location of the feeder (north or south) also did not predict the amount of time a honey bee spent feeding (Table 14). The foraging intensity count per minute was not significant in predicting the feeding duration. The time honey bees spent feeding was affected by the trial (day that the trial was conducted).

Table 14. The mean count of honey bee foragers per minute and the mean time spent feeding \pm SEM for each treatment.

Treatment	Forager Intensity (visits/min)	Feeding Duration (s)
Untreated	24.01 ± 2.21	57.17 ± 1.85
Full Rate	29.60 ± 7.78	52.10 ± 3.28
Half Rate	20.36 ± 3.48	55.12 ± 2.92

Table 14 (cont'd)		
Quarter Rate	26.38 ± 4.41	48.25 ± 1.31
SPLAT	17.69 ± 2.50	62.68 ± 5.97

Discussion

None of the Nasonov based dispenser treatments increased the number of honey bee visits to an artificial feeder or influenced the time each visitor spends feeding. This included both the Polynate and SPLAT formulations, as well as reduced rates of the Polynate dispenser. It was expected that a lower treatment rate would be more effective, but there was no significant variation among the rates tested. The number of visits per minute did not affect the time a bee spent feeding. It was observed that during times of high activity, a bee was often pushed out of the way by a newcomer and thus spent less time feeding.

Honey bees are more likely to expose their Nasonov glands to mark water or an artificial feeder than to mark flowers (Free and Williams 1970; Free and Williams 1972). This means that even if Polynate was observed to increase the attractiveness of on one feeder over the other, the same observation may not be made in the field. In one study, honey bees did scent-mark flowers that they had visited, but this decreased the chance of another honey bee visiting the same flower. Perhaps this behavior warned other bees that the flower had already been visited and been depleted of nectar (Giurfa and Núñez 1992). Although the particular ‘scent’ the honey bees used to mark the flowers was unclear, they may have been referring to Nasonov pheromone which could mean that it has a repellent nature instead of an attractant one in the field. This could help explain why Polynate did not increase the attractiveness of an artificial feeder. However, no difference between the Polynate treated feeder and the control feeder was observed.

It is thought that honey bees can change the concentration of the Nasonov components during emission, which could in turn alter the reactions to other bees to it (Winston 1987). This

seems to be plausible since an earlier study found that honey bees did not show aversion to flowers that had been recently visited (Ribbands 1949). Since the concentration of the Polynate dispenser components was fixed for a particular treatment (full, half or quarter rate), it could be a reasonable explanation as to why honey bees did not respond to the dispenser as they do to their own emissions.

It is also possible that activity in the hoop house was too high to determine a difference between the two feeders. Because there were no other food sources besides the three feeders it is likely that honey bees foraged on them equally simply because there was no other option for them to forage on. When there is a food shortage, honey bees tend to be less discriminatory against food sources, visiting both high and low rewarding flowers (von Frisch 1950; Seeley 1989). They simply visit what is there because there is nothing else for them to do. In fact, the number of foragers is known to alter the attractiveness of a food source (Waller 1972). It would be interesting to see what would happen if additional food sources were provided or if the whole apparatus was moved outside of the hoop house. This way, there would still be some control over the bees, but the set up would be more natural to them so behaviors would more closely mimic what happens in the field.

It would also be beneficial to replicate this study without training the bees to feeders before each trial. If Polynate works by attracting foragers, it would make sense to allow the foragers to find the artificial feeders on their own. It may take a longer period of time for the bees to find the feeders if they are able to find them at all. In fact, honey bees tend to have a hard time finding a feeder that is unscented (Tautz and Sandeman 2003; Riley et al. 2005). Thus, in the absence of training, there may be a more noticeable difference in foraging intensity

between treatments. Since honey bees are not trained to a food source in nature, not training them would more closely replicate what occurs in field conditions.

Through this research, it has been determined that Nasonov based dispensers do not impact the foraging of honey bees. In fact, these results support my earlier conclusion that Nasonov based dispensers do not change honey bee behavior such that an increase in fruit set or forager activity in the field is increased (Chapter two). Polynate is not the only product that has failed to increase yield in a crop (Chapter one). The biology of honey bees is extremely complex and they use pheromones differently than many other insect species. Instead of using one pheromone to elicit one response, they use numerous pheromones in different combinations to change minute behaviors in the hive. For example, footprint pheromones are thought to increase the attractiveness of Nasonov pheromone (Winston 1987). These results combined with the knowledge of honey bee biology leads me to conclude that simply putting one pheromone containing device in front of honey bees and expecting them to respond in a specific way is not practical. Perhaps a combination of pollination attractants and other pollination enhancing practices will be able to produce the results that growers desire.

CHAPTER 4

The Use of Polynate and SPLAT in Agriculture

Throughout this thesis, the effects of potential products for increasing honey bee attraction to fruit crops were evaluated and determined to be minimal. Polynate™ and SPLAT had no significant effect on fruit set or pollinator activity in blueberries, apples or cherries. They also did not increase the number of honey bee visits to an artificial feeder nor alter the amount of time each forager spent feeding. It is possible that Polynate or SPLAT have effects that were not measured, but studies provided little evidence that they could produce an outcome that is economically beneficial for growers. Despite these discouraging results, there is still a possibility that another product or attractants produce positive results. However, it may also be best to pursue other pollination enhancement options.

Thus far, pollination products such as Beelure®, Beeline®, Bee-Here®, Pollenaid®, Pollenaid-D®, Bee-Scent®, Fruit Boost® have been largely unsuccessful in increasing fruit set and pollinator counts in numerous different crops (Burgett and Fisher 1979; Mayer and Johansen 1982; Rajotte and Fell 1982; Tew and Ferree 1984; Mayer, Lunden, and Britt 1989; Elmstrom and Maynard 1991; Naumann et al. 1994; Schultheis et al. 1994; Higo, Winston, and Slessor 1995; Tew and Ferree 1998; Malerbo-Souza, Nogueira-Couto, and Couto 2004; Ellis and Delaplane 2009). Additionally, there is evidence to suggest that the addition of volatile compounds can't make a completely unattractive flower, such as one that has a poor amount of nectar for example, attractive. Unfortunately, crops that growers are most interested in applying attractant pollination enhancement chemicals are ones that have flowers that are considered to be unattractive to bees. These attractants may increase visitation but an increase in pollinator activity does not directly translate to an increase in pollination or fruit set (Currie et al. 1992;

Delaplane and Mayer 2000). Even if a bee's pollination efficiency is improved, an increase in crop yield may not occur because of potential pollination thresholds (Currie et al. 1992). Even if an increase in crop yield is observed, the number of additional fruit may not be high enough to increase revenue to a level that would at minimum pay for the application of the attractant.

Polynate and other potential pollination attractants should still be studied however, especially if they alter pollinator behavior in subtle ways. Since honey bees are so sensitive to their surroundings, it is possible that Nasonov based dispensers cause behavioral responses that I was unable to measure in the field or in the controlled study. The same could be true for other potential attractants as well. However if the behavioral responses are not measureable, and do not have the potential to increase yield and revenue, it would be hard to justify private industry pursuing them or to procure funds for research (Currie et al. 1992).

Despite the current findings, there are still avenues of research to be pursued for Polynate and other potential pollination enhancement devices. Some studies on scent guides have stressed the importance of placing the test scent in the hive as well as in the field (von Frisch 1950). This way, foragers will experience the scent of Polynate before they forage thus encouraging them to seek the same scent in the field. It would be interesting to see if one could cause Polynate to be attractive simply by placing a couple of dispensers in hives as well as in the field or next to an artificial feeder. Although this would not guarantee an increase in pollination, it might increase the forager frequency within the treated areas.

Also, the hoop house study could be replicated providing honey bees with a variety of natural food sources instead of artificial feeders. It would also be interesting to see how honey bees would react to the same experimental design, but outside of the hoop house. The results may also differ without training the bees to feeders before each trial. In nature, not only do

honey bees have to choose between numerous different resources, but they have to find them on their own. Additionally, it was impossible to determine if Nasonov based dispensers had any adverse effects on other essential pollinators in the field; it would be interesting to perform another similar hoop house study with colonies of native bees.

In the first chapter of this thesis, I contrasted the activity of honey bees to that of native bees. While native bees may be more efficient pollinators, they may not be abundant and are also harder and more expensive to manage than honey bees. Since honey bees are easier to manage, they also are the most cost effective organism to use during bloom to increase pollination. It is still possible to manage some native bee species, even if it is more difficult (Delaplane and Mayer 2000). Native bee habitat conservation is also important in preserving pollination services. It may be more advantageous to pursue this route instead of developing more honey bee attractants, or it is entirely possible that the combination of pollination attractants and native bee conservation would result in the increase of pollination services.

Due to the recent bee declines all over the world, a solution to the corresponding decrease in crop pollination has been sought. One solution is applying possible pollination attractants like Polynate in crops. However, the intricacies of honey bee biology are often overlooked. Worker bees provide pollination services in the most energy efficient ways possible until they die. It is unlikely that chemical attractants will entice pollinators to work harder simply by making target crops more attractive. Pollination attractants may work partially but it is impractical to think that one pheromone based product will solve the pollination problem. There are many decision factors such as energy profitability and foraging background that go into honey bee choices and behaviors (Shafir 2011) and their pheromones are not used for any one purpose, but many

(Winston 1987). Due to these complexities, pollination attractants may be useful, but will not likely contribute substantially to solving the pollination crisis as bee numbers decline.

APPENDICES

APPENDIX 1
RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2014-06

Author and Title of thesis:

Author: Julie Adams

Title: The Effect of Nasonov Based Dispensers on Honey Bee Behavior and on Pollination in Blueberry, Apple, and Cherry

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

<u>Family</u>	<u>Genus-Species</u>	<u>Life Stage</u>	<u>Quantity</u>	<u>Preservation</u>
Apidae	<i>Apis mellifera</i>	adult	10	pinned

APPENDIX 2
ADDITIONAL TABLES AND FIGURES

Table 15. The organization of field sites, treatment plots and sampling in 2012

Crop	Field sites		Plots		N Sampling locations/plot	Pollinator activity		Fruit set		
	N	Area (ha)	N	Area (ha)		N sites	N trees	N sites	Dimensions	Limbs
Blueberry	4	1.6	2	0.81	3	4	10	4	4X1	2
Apple	3	2.4	3	0.81	3	3	12	Na	Na	Na
Cherry	2	1.6	2	0.81	3	2	20	2	5X2	4

Table 16. The organization of field sites, treatment plots and sampling in 2013

Crop	Field sites		Plots		N Sampling locations/plot	Pollinator activity		Fruit set		
	N	Area (ha)	N	Area (ha)		N sites	N trees	N sites	Dimensions	Limbs
Blueberry	4	6.1	3	0.4	1	4	20	4	4X4	2
Apple	3	4	2	0.6	1	3	24	2	5X5	2
Cherry	5	4	2	0.6	1	5	24	5	5X5	2

Figure 2. An example of plot and sampling location arrangement within each block for field experiments in 2012. Dark grey: treated plot, light grey: untreated plots, white box: sampling locations, black box: honey bee hives.

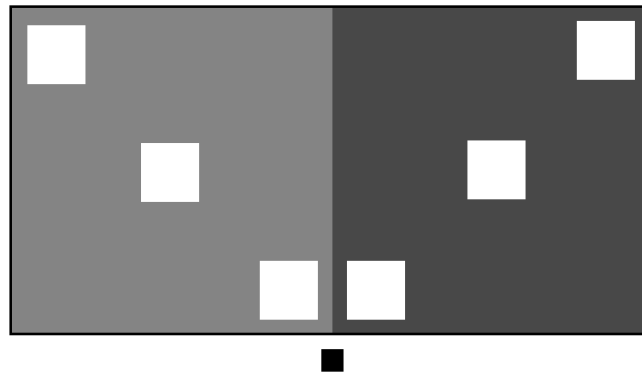


Figure 3. An example of plot and sampling location arrangement within each block for field experiments in 2013. Dark grey: treated, light grey: untreated plots, white box: sampling locations, dotted white: unused area, black box: honey bee hives.

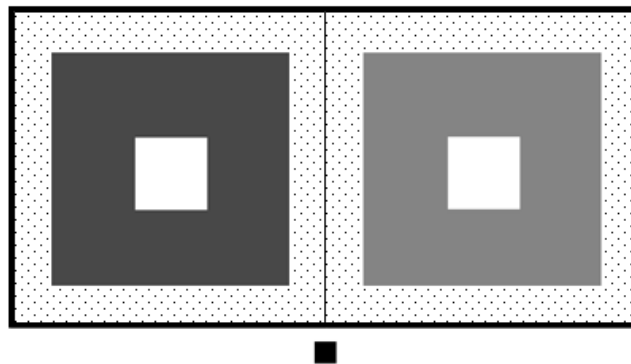


Figure 4. A single SPLAT dollop applied in cherry



Figure 5. A single Polynate dispenser applied in blueberry



Figure 6. The hoop house used in my experiments without the end partitions (pictured in figure 8)



Figure 7. An experimental feeder during a trial



Figure 8. An experimenter videotaping one experimental feeder and the set-up of the hive and stock feeder



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