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AN ANALYSIS OF THE HISTORY AND CURRENT TREATMENT TRENDS OF THE PARASITIC MITE VARROA DESTRUCTOR

(ACARI: VARROIDAE) IN MAINE BEEKEEPING

by

Patrick Hurley

A Thesis Submitted in Partial Fulfillment of the Requirements for a Degree with Honors (Ecology and Environmental Sciences)

The Honors College

The University of Maine

May 2020

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ABSTRACT

Varroa mites, Varroa destructor (Acari: Varroidae), are a parasitic mite of honey bee colonies worldwide. Varroa mites feed on both adult honey bees and developing brood, easily spread between colonies, and can kill European honey bee colonies within just a few years. Beekeepers must apply mite treatments to maintain healthy colonies. This thesis is an overview of the currently available mite treatments in the United States and how they relate to Maine Beekeeping. There are three main research components of this thesis. The first is the analysis of two surveys that Maine beekeepers completed in 2019. The second is a research project testing the efficacy of a new approach to two commonly use mite treatments with the largest commercial beekeeper in Maine. The third is the generation of mite treatment resources based on the previous two components and subsequent presentation to beekeepers across Maine. Numerous mite treatment information sources already exist, but the amount of information can often be difficult for beekeepers unfamiliar with treating. Most Maine beekeepers are small-scale and provided feedback that helped make these outputs applicable to a wider range of beekeeper demographics. Beekeeping is an important part of Maine's economy and lifestyle, and varroa mite treatment is an essential part of beekeeping. This thesis is a collection of literature, stakeholder-engaged research, and personal anecdotes that is intended to further the field of varroa mite IPM and provide useful resources for beekeepers in Maine and elsewhere to consult when approaching difficult mite treatment decisions.

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I would like to thank the numerous members of the UMaine Honors College Sustainable Food System Research Collaborative for providing my summer 2019 employment. I want to especially thank Sara Velardi for informing me of the Sweet Spot Fellowship and introducing me to the Maine beekeeping scene during the Honors tutorial she taught, *Reflections on Cultural Aspects of Maine Agriculture*, and for serving as a mentor to the other fellows and myself during the 2019 research season. Sara also came

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up with the whole idea for the bee club outreach in a proposal that was unfortunately never funded but nonetheless provided inspiration for much of this research, so thank you Sara! Special thanks also to Jessica Leahy for providing the employment opportunity for me to continue working with survey data during the academic year.

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INTRODUCTION

This thesis is the result of a three-pronged research project on beekeeping in the state of Maine and specifically on varroa mite treatments. The project began in the spring of 2019 in the Honors Tutorial, *Reflections on Cultural Aspects of Maine Agriculture*. During this small tutorial focused on Maine agriculture, beekeeping, and maple syrup production, I was introduced to relevant research topics in the fields of bees and maple, to the community of Maine beekeepers, and to my summer 2019 employment opportunity as a student research fellow on the project Finding the Sweet Spot: Scale Challenges and Opportunities for Beekeeping and Maple Syrup Production in Maine (Sweet Spot) under the University of Maine Honors College Sustainable Food System Research Collaborative (SFSRC). I had previously taken a beekeeping course during my sophomore year through which I ended up with a hive of my own, and I was ecstatic for the opportunity to do research on honey bees. As a summer research fellow, I participated in group research projects on maple and honey while working an independent varroa mite treatment research project.

The first prong of my research project involved working with the largest commercial beekeeper in Maine who was also a member of the Sweet Spot Project stakeholder advisory board. This opportunity allowed me to get hands on experience with a few varroa mite treatments while researching the various treatments available, not to mention the results ended up being a major part of this thesis. The importance of stakeholder feedback was apparent from the beginning of this project. Stakeholder are often not considered research experts, however stakeholder-engaged research has proven to produce more relevant and usable results than traditional research methods (Hall et al.,

2012; Jansujwicz et al., 2013; Oscarson & Calhoun, 2007). There is often a disconnect between researchers and relevant stakeholders, and by working side-by-side with one of the biggest stakeholders in the Maine beekeeping community, we were able to minimize this disconnect.

During the Sweet Spot Fellowship, we also sent out a survey to licensed honey producers in the state. The second prong of my research is the analysis of data from that survey and from another yearly survey that the Maine State Apiarist sends to beekeepers. This survey data analysis was a key component to my research, as it allowed me to get a better idea of the Maine beekeeping industry and greatly influenced the next steps in the research process.

After working so closely with one very knowledgeable Maine beekeeper, I was eager to engage with more. The third prong of my research involved generating outputs that I felt were relevant to Maine beekeepers based on my work in the summer of 2019 and the survey results. I then set up meetings with Maine bee clubs and local chapters of the Maine State Beekeepers Association (MSBA) to present my research and outputs for feedback.

Guided by input from Maine beekeepers, this research project has evolved immensely since my original idea. Through phone calls, presentations, and hands-on work with bees; in about a year, I have gone from knowing very little about varroa mites to creating this thesis with its various mite treatment information and decision-making recommendations. I hope that those reading this document learn something new about varroa mites or honey bees, and understand more about the tremendous Maine beekeeping industry and community.

CHAPTER 1

VARROA DESTRUCTOR

The Homeland of Varroa

Bees and Mites

Apis Cerana. There are nine recognized species of *Apis* honey bees on Earth, four of which nine are predominantly used for honey production (Raffiudin & Crozier, 2007). Three of those four—*Apis dorsata*, the giant honey bee; *Apis florea*, the dwarf honey bee; and *Apis cerana*, the eastern or Asian honey bee—are native to Asia (De Jong, De Andrea Roma, et al., 1982). *Apis mellifera*, the European honey bee, is the most commonly recognized species and has been transported worldwide for honey and pollination. *Apis mellifera* has a native range that includes Europe and Africa, but as it is more productive and easily kept than the other species, humans have brought these honey bees to nearly every corner of the earth (De Jong, De Andrea Roma, et al., 1982; Queensland Department of Agriculture and Fisheries, 2013). As a result of this global transport, European honey bees have come in contact with their Asian relatives, and with the novel pests and diseases they carry.

As is the case with honey bees, there are also multiple species of mites on the planet. *Varroa jacobsoni* has been a known pest of Asian honey bees since the early 1900s (De Jong, De Andrea Roma, et al., 1982). These mites have co-evolved with Asian honey bees for hundreds of thousands of years, and over time, *Apis cerana* populations have developed natural defenses to the mites to prevent total colony loss (Spivak, 1996).

These defenses include grooming of both adult bees and brood to remove mites (Spivak, 1996). This evolutionary race has allowed both the mites and honey bees to persist in their native habitat. These mites feed on bodily fluids of adult bees and reproduce within the colony's brood cells (Huang, 2013). There are many other pests, including brood, phoretic, and flower mites that are native to both Europe and Asia (De Jong, Morse, & Eickwort, 1982). *Varroa jacobsoni*, however, has been seen as the major threat to European honey bees for most of the last century (Anderson & Trueman, 2000).

An Unwelcome Guest

Spread by Humans. As stated previously, *Varroa jacobsoni* has been a longstanding pest of Asian honey bees. It wasn't until the 1950s and 60s that these mites were first detected in European honey bee colonies that were brought to the Philippines and Russia (De Jong, De Andrea Roma, et al., 1982). Interactions such as robbing between colonies of *Apis cerana* and *Apis mellifera* and beekeeper manipulations, such as brood transfers between different colonies, are suspected to have transported the mite from one species to another (De Jong, De Andrea Roma, et al., 1982). From there, varroa mites continued to spread throughout Asia, Africa, and Europe, eventually infesting honey bees in most of eastern Europe by the 1980s. Since European honey bees did not co-evolve with these mites, they do not have innate defenses and colonies that become infested will collapse within a few years. The continuous transport of European honey bees around the globe for honey production and pollination has caused varroa mites to become established everywhere European honey bees are kept, except for Australia (Queensland Department of Agriculture and Fisheries, 2013).

<u>Varroa Travels to the United States</u>. Varroa mites were first detected in honey bee colonies in the United States in 1986-1987 (Rosenkranz et al., 2010; M. Scott, personal communication, April 3, 2020). In the first 20 years or so after its introduction, the number of managed honey bee colonies dropped 26% in the United States (vanEngelsdorp & Meixner, 2010). This is thought to be due to smaller beekeepers leaving the industry due to increased stress of varroa management. During the same time period, however, the remaining, larger-scale beekeepers in the U.S. increased the number of colonies they managed by 66% (vanEngelsdorp & Meixner, 2010). Today, varroa mites are seen as the most serious threat to honey bees in the United States and globally (Milbrath, 2016).

Varroa mites were first detected in 1986 in Maine by Maine State Apiarist at the time, Tony Jadczak. He found varroa mites and tracheal mites while inspecting honey bee colonies brought into downeast Maine for blueberry pollination (M. Scott, personal communication, April 3, 2020). At the time, the only way to detect the presence of mites in a hive was to identify them during a visual hive inspection.

<u>A New Varroa...destructor</u>. In 2000, Anderson and Trueman's research changed the field of science on varroa mites. Their paper mapped the genotypic, phenotypic, and reproductive variation of *Varroa jabsconi* in *Apis cerana* colonies across Asia (Anderson & Trueman, 2000). Anderson and Trueman identified nine different haplotypes of *Varroa jabsconi*. They reclassified six of these nine as *Varroa destructor* due to differences in the adult female body shape and reproductive isolation from *Varroa jabsconi*. Furthermore, they found two haplotypes to be particularly detrimental to *Apis mellifera* colonies that were more widespread than the others, both of which were *Varroa destructor* (Anderson & Trueman, 2000).

The Life of Varroa

Appearance

Varroa mites are arachnids and therefore have 4 pairs of legs. They are only about a millimeter in length and lack the ability to see and hear but they are covered with tiny hairs that allow them to sense their environment. They have piercing-sucking mouthparts and tiny suction cups on their feet that allow them to grip onto bees and pierce their exoskeleton. Additionally, they are dark reddish-brown, oval-shaped, and almost flat to blend in seamlessly on the exoskeleton of bees (Bayer Bee Care, 2019).

Feeding

All species of varroa mite in the family Varroidae are a parasite solely of honey bee colonies (Rosenkranz et al., 2010). Adult female *V. destructor* survive by attaching themselves to adult honey bees. Prior to 2018, it was thought that *V. destructor* feeds on the hemolymph, or a bee's circulatory fluid. It is now understood that they feed primarily on the fat body tissue of adult honey bees (Ramsey et al., 2019). Honey bee fat body tissue is responsible for immune function in honey bees and provides more sustenance for adult mites than hemolymph alone. By feeding on the organs that honey bees rely on to be healthy, *V. destructor* are able to quickly spread pathogens, deteriorate colonies, and disperse to surrounding colonies as collapsing colonies become defenseless and desperate (David Thomas Peck & Seeley, 2019; Ramsey et al., 2019).

Life History

Dispersal. The life cycle of *Varroa destructor* consists of two stages. The first, the dispersal stage, involves adult female mites attaching to adult honey bees and feeding on their fat body tissues and hemolymph (Huang, 2013; Ramsey et al., 2019; Rosenkranz et al., 2010; Traynor et al., 2020). In this stage, all the mites are female, have already mated, and are gathering nutrients to lay eggs. The dispersal stage usually lasts between five and eleven days, with exceptions occurring in broodless periods such as winter months (Huang, 2013). During this stage, mites can and often do switch hosts (David Thomas Peck & Seeley, 2019; Rosenkranz et al., 2010). Once they are ready to reproduce, adult mites crawl into a cell with a developing larva just before it is about to be capped (Huang, 2013). This is usually seven to eight days after an egg is laid (Huang, 2013). Mites bury themselves under the food placed in a cell for the developing larva, remaining hidden until the cell is capped (Huang, 2013; Rosenkranz et al., 2010).

Reproductive. About three days after a cell is capped, an adult female *V*. *destructor* lays her first egg. This egg is a male mite and roughly each day after that, a female egg will be laid. Like honey bees, varroa mites have a haplodiploid sexdetermination mechanism (Huang, 2013). Once the eggs hatch, the adult female creates a feeding site in the bee pupa at which the mites feed on the hemolymph and fat body tissue of the developing bee. It takes six days for a daughter mite to mature, at which point she will mate with the male mite in the cell and wait until the bee finishes developing to emerge with it and find a host. Male mites and female mites that don't develop in time will die and remain in the cell upon the adult bee emerging.

The length of time between a honey bee egg being laid and an adult bee emerging is about 21 days for a worker bee and about 24 days for a drone. The length of time a varroa mite has to reproduce within a cell is about 11-12 days for a worker bee cell and 14-15 days for a drone bee cell. A detailed drawing of the varroa mite and honey bee life cycle can be seen below in **Figure 1**. Typically, 1-2 mature female mites will emerge from an infested worker bee cell and 2-3 mature female mites will emerge from a drone bee cell. This two-staged life cycle and exponential population increase can lead to infestation levels higher than the bees or beekeepers can handle.

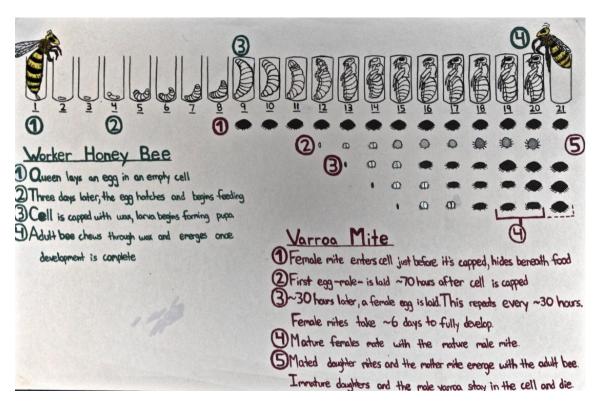


Figure 1. Visual timeline of the development of a worker honey bee and the

corresponding reproduction of a varroa mite.

Spread

Disease. Since mites can feed on multiple hosts in their dispersal stage and also feed on both adults and developing larvae, they are vectors and amplifiers for honey bee diseases (Huang, 2013; Ramsey et al., 2019; vanEngelsdorp & Meixner, 2010; Yang et al., 2005). Some of these diseases include deformed wing virus (DWV), which inhibits a honey bee's ability to fly properly, and acute bee paralysis virus (ABPV), which impairs orientation and brain development in adult bees, ultimately leading to death. Typically, when either of these diseases become apparently visible in a colony, infestation levels are beyond levels of reasonable control. Furthermore, the way in which mites feed weakens bees' immune systems, they are more susceptible to any viruses that enter their system (Ramsey et al., 2019; Yang et al., 2005).

Dispersal. Varroa mites can travel not only between bees in the same colony, but also between bees of nearby colonies (Peck & Seeley, 2019). Mites have occasionally been observed on flowers, allowing them to attach to visiting bees (David T. Peck et al., 2016), but more often spread between colonies via robbing or the 'mite bomb' effect (Peck & Seeley, 2019). The 'mite bomb' effect is often the term used when a colony with a high mite infestation dies and the remaining bees disperse to adjacent hives, bringing mites with them. Until recently, this was thought to be the major cause of mite dispersal between nearby colonies. Peck and Seeley suggested that mite dispersal occurs more via worker bees robbing nearby collapsing colonies and subsequently picking up mites in the process (2019). While the method of mite transport is important, the fact that they bring with them viruses and the ability to destroy colonies is far more so.

CHAPTER 2

VARROA MITE CONTROL

Monitoring for Mites

Varroa mites are found in nearly every honey bee colony in the world and must be managed by beekeepers if they are to maintain healthy colonies. Today, mites are considered permanent residents of honey bee hives and mite monitoring and management is considered an essential component of keeping honey bees. Below are a few ways in which beekeepers can monitor varroa mite infestation levels.

Sticky Board

The first method of mite monitoring is the sticky board method, which involves placing a typically white board just above the bottom board of a hive, or below a hive with a screened bottom board (Bayer Bee Care, 2019; Aleš Gregorc et al., 2016). The board is coated with a sticky substance that traps any falling hive debris, including mites, and is typically left in for one to three days. Since mites must emerge from cells after maturing to find a new host, they can often fall from the combs to the bottom of the hive. A sticky board allows beekeepers to visually count and estimate the infestation levels, but they are not very accurate and are more often used to test efficacy of mite treatments. <u>Mite Roll</u>

The second, and more popular, method for monitoring for varroa mites is the mite roll. This method involves scooping a half cup of bees (approx. 300 bees) into a jar or

container and shaking them around in a substance that removes mites from the bodies of adult bees. Several different substances can be used depending on the intentions of the beekeeper.

Sugar. Powdered sugar is an option that dislodges mites from bees but doesn't kill the bees in the process, allowing them to return to the hive slightly frazzled and covered in sugar. This option is not completely effective at removing mites but it is inexpensive and causes minimal harm to the bees. Bees are shaken in sugar and the sugar is then poured out of the jar through a screen that keeps bees in but allows mites to pass through for a count

<u>Alcohol, etc</u>. Other options that are more effective at dislodging mites but kill the bees in the process include isopropyl alcohol, the most common method, and soapy water. After shaking the bees in the jar or container with the solution, the main lid of the jar is replaced with a screen small enough to keep the bees in but allow mites to pass through. This allows for a final count of the number of mites per half cup of bees, which is roughly 300 bees (Lund & Skyrm, 2019).

Infestation Levels

Monitoring for mites is how beekeepers become aware of infestation levels and decide whether they need to treat. Infestation levels can be difficult to calculate from sticky boards, which are more often used to judge efficacy of mite treatments. Infestation levels can easily be calculated from mite rolls since the number of mites and an estimate of the number of bees in a sample is known. An estimate of the infestation level of the colony is found by dividing the number of mites counted in a half-cup roll by three. Since

there are roughly 300 bees in ¹/₂ cup, dividing the number found in the role by three gives an estimate of the number of mites per 100 bees (Lund & Skyrm, 2019). According to Jennifer Lund, the Maine State Apiarist, infestation levels at or above 3% warrant treatment and infestation levels above 5% are considered on their way to collapsing. Due to the exponential growth rate of the varroa mite population, frequent monitoring can help beekeepers make crucial decisions about managing mites before colony collapse.

Mite Treatments

There are three main categories of miticides currently on the market. They are synthetic chemicals, organic acids, and natural or essential oils (Rosenkranz et al., 2010). Other methods of treatment include mechanical manipulations of colonies by beekeepers.

Synthetic Chemicals

Synthetic chemical acaricides were among the first products used to combat varroa mites in beekeeping. Some of the more popular of these acaricides include Checkmite+® (A.I. coumaphos), Apistan® (A.I. tau-fluvalinate), and Apivar® (A.I. amitraz) (Honey Bee Health Coalition, 2018; Lund & Skyrm, 2019; Rosenkranz et al., 2010). These products have persisted due to their simple application requirements and their ability to target mites with minimal effects on honey bees. Most of these products are applied via an impregnated plastic strip that is placed directly into the brood chamber of hives. Today, however, mite populations resistant to all three of these chemicals have been documented especially those that have received repeated exposure of the same chemical (Maggi et al., 2010; Pettis, 2004; Trouiller, 1998)

Organic Acids

Three different organic acids are currently used as varroa mite treatments. The term organic here refers to carbon-based substances that are naturally found on the planet and does not imply or adhere to 'organic certification' standards. These organic acid treatments are valued for their efficacy and also because they generally do not accumulate in beeswax or honey and no resistance has been reported thus far (Maggi et al., 2010; Rosenkranz et al., 2010).

<u>Formic Acid</u>. Formic acid is currently sold as Mite-Away-Quick-Strips[®] and Formic Pro[®]. Both are applied via a gel pad that is placed directly on the brood chamber and releases vapors that kill mites. Formic acid is currently the only miticide registered for use in beehives that targets mites beneath capped cells and those attached to adult bees. Furthermore, it is safe to use when producing honey since there are no significant residual effects on the honey crop (Honey Bee Health Coalition, 2018; Rosenkranz et al., 2010).

<u>Oxalic Acid</u>. Oxalic acid is applied either as a vapor via a sublimator (vaporizer) or in a sugar solution applied directly to adult bees. Oxalic acid only targets mites in the dispersal phase and is often used during a time when colonies are broodless such as early spring or late fall. Oxalic acid kills mites upon contact (Honey Bee Health Coalition, 2018; Maggi et al., 2010; Rosenkranz et al., 2010).

<u>Hops Beta Acid</u>. The most recent organic acid mite treatment to enter the market is hops beta acid, applied in the form of Hopguard $II(I)^{\text{®}}$. It consists of cardboard strips that are impregnated with potassium salts of hops beta acids and placed directly in the brood chamber of a hive. Hopguard $II(I)^{\text{®}}$ kills mites it comes into contact with and is

spread throughout the colony via active removal of the strips by worker bees (Honey Bee Health Coalition, 2018).

Essential Oils

Thymol and menthol are the two main essential oils used in mite treatment products. Apiguard[®] is a mite treatment that contains just thymol while Api Life Var[®] contains thymol, menthol, and eucalyptus oil. Both are applied via a gel strip, are most effective in broodless periods, and are not approved for use during honey production (Honey Bee Health Coalition, 2018).

Hive Manipulations

Numerous other options exist for beekeepers wishing to be more proactive in their mite treatments or limit their use of chemicals to combat mites.

<u>Screened Bottom</u>. Screened bottom boards allow falling mites to drop through the screen and out of the hive once they emerge from a cell to look for a host. It is nearly impossible for them to crawl back into the hive, eliminating their chance of finding a host and reproducing. Screened bottom boards have questionable efficacy and are not often used in colder climates where honey bee thermal regulation is more difficult.

<u>Brood Cycle Disruption</u>. Another option is to create broodless conditions in hives to prevent mites from reproducing. This is usually done either by caging the queen or splitting hives to simulate a swarm scenario. Swarming disrupts the colony brood cycle and is a natural reproductive process during which the colony makes a new queen and the old queen leaves with half of the workers to start a new colony with half of the mite load.

This usually happens when colonies are overcrowded. Once the old queen leaves, it usually takes a few weeks for the new queen to emerge, orient herself, mate, and begin laying eggs. During most of this period, the colony is broodless. By monitoring colonies, beekeepers can proactively split colonies to create a broodless period which temporarily prevents mites from reproducing and provides a perfect opportunity for a mite treatment (Lund & Skyrm, 2019).

Drone Brood Removal. Varroa mites can only reproduce in the drone brood cells of their native host, *Apis* Cerana, due to hygienic removal from worker brood (Spivak, 1996). In *Apis* mellifera, however, mites can reproduce in both drone and worker brood cells, but prefer drone brood since drone development time is longer, giving female mites more time to produce more offspring (Spivak, 1996). Beekeepers can purchase or construct frames which promote the production of drone comb. They can then remove that frame later and scrape it off once drone cells are capped, mites included. While this does remove resources from the colony, drones do not contribute to brood or honey production, so the overall loss to the colony is minimal.

CHAPTER 3

MAINE BEEKEEPING

A Sweet, Fruitful History

The Beekeepers of Maine

Bee-ginnings. Beekeeping has a rich history in the state of Maine. Mainers have kept bees for hundreds of years, and over this time beekeeping has evolved tremendously. Documentation of beekeeping practices in Maine exists dating back to the late 19th century. The Langstroth Hive, the conventional hive setup that is used by most beekeepers today, was invented in 1852 (**Figure 2**). Lizzie Cotton, a beekeeper from West Gorham, ME, claimed in publications throughout the 1880s that her "Controllable Hive" design was far superior to the Langstroth (Cotton, 1887). Not only have beekeeping equipment and practices changed, but bees are also much more available today. For example, in 1887, Lizzie Cotton offered the equivalent to a package of bees and her complete Controllable Hive setup for \$28, or \$762 today (Cotton, 1887). Current prices for packaged bees are \$100-\$180 depending on origin and a complete hive setup can be purchased for \$50-\$150. Today, both packages and hive equipment can be purchased and shipped overnight from nearly anywhere in the country.

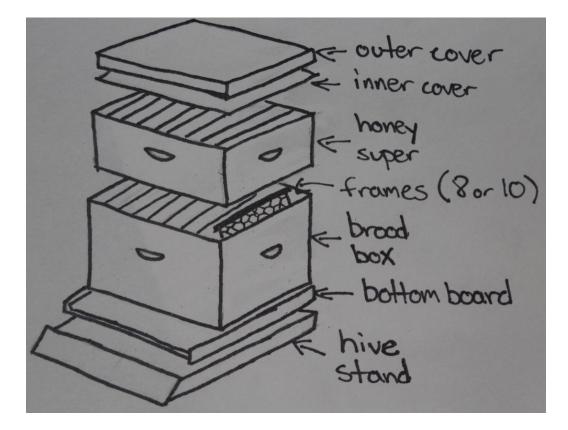


Figure 2. Labeled components of the Langstroth Hive Setup, the conventional hive setup used by most beekeepers today that was first invented during the 1800s.

Dr. Charles Dirks published the first University of Maine Cooperative Extension services bulletin on Maine beekeeping in 1936 (M. Scott, personal communication, April 3, 2020). Around the same time, the largest commercial beekeeping operation in Maine, R.B. Swan and Sons, began. Interest in beekeeping and pollination continued to grow in Maine, and the University of Maine started conducting pollination research projects on lowbush blueberries, apples, and squash during the 1950s and 60s.

Apples and Lowbush Blueberries

Two of the state's historically largest fruit industries, apples and lowbush blueberries are reliant on insect pollination for fruit set. Beekeeping plays a critical role in these industries today. Little documentation on beekeeping in the state exists before the 20th century, however it is well-documented that Mainers have grown apples, primarily on family farms, for much of the state's history (Bunker, 2008). This history is evident when walking through the woods and fields of Maine, much of which still has cultivated apple trees from the orchards that used to exist there.

Today, Maine is known for its lowbush blueberry industry. Wild lowbush blueberries have been a part of Maine's landscape since de-glaciation around 10,000 years ago (Yarborough, 2018). During the late 1900s, growers figured out how to maximize their blueberry production not only via land management, but also by bringing in honey bees (UMaine Cooperative Extension, n.d.; Yarborough, 2018). In 1965, about 500 out-of-state honey bee colonies were brought in for blueberry pollination. Just twenty years later, that number had increased to 25,000 (UMaine Cooperative Extension, n.d.). By 2000, that number was up to over 60,000 hives. Since then, that number has fluctuated in response to blueberry grower demand, but honey bees remain an essential part of Maine blueberry production.

Maine State Beekeepers Association (MSBA)

Bees and Maine. The Maine State Legislature adopted the honey bee as the official state insect in 1975 (*Title 1, §214: State Insect*, 1975). The Maine Association of Beekeepers was created during the late 1940s and consisted of a number of chapters

across the state that received support from the Swan family (M. Scott, personal communication, April 3, 2020). Since it was not state recognized, this group remained more of a social club for beekeepers (M. Scott, personal communication, April 3, 2020). As the pollination and beekeeping industries grew in Maine, so grew the demand for research in pollination and honey bee diseases, and for Maine Department of Agriculture regulation of honey bees brought in for commercial pollination.

<u>Organization</u>. On July 22, 1976, Bill Nolet, Bill Rich, and Matt Scott organized a meeting at the Androscoggin County Extension Service Office with the intention of creating a new beekeeping organization recognized by the state legislature (M. Scott, personal communication, April 3, 2020). Twenty-one people attended the meeting and signed paperwork later submitted to the Secretary of State to recognize the Maine State Beekeepers Association as nonprofit corporation under Maine law (M. Scott, personal communication, April 3, 2020). The first official annual meeting of the MSBA took place on January 13, 1977 at the Augusta Civic Center. Later that year, the MSBA was granted 501(c)(3) status as a nonprofit by the IRS, which allowed for the creation of chapters in each state county via the MSBA bylaws. The first ever newsletter of the MSBA can be found below in **Appendix A**.

Legislation. In 1983, the MSBA created the legislation to establish the position of the Maine State Apiarist to inspect commercial hives brought into the state for diseases and parasites. Prior to that point, there was no full-time bee inspector employed by the Maine Department of Agriculture. In 1983, Tony Jadczak was the first person to be appointed to the full-time position of Maine State Apiarist. He held this position, inspecting millions of commercial hives and assisting local beekeepers, until 2016 when

the current State Apiarist, Jennifer Lund, was hired. Since then, her job responsibilities have included inspection of bees brought in for pollination; inspection, outreach, and education services for Maine beekeepers of all size; and monitoring hive losses and other beekeeping statistics via the annual Maine Honey Bee Survey.

Maine Beekeeper Surveys

This section will summarize findings from two surveys conducted during 2019 that reflect the common practices and interests of Maine beekeepers. These surveys provide valuable information not only on important statistics such as honey production and colony loss, but also on the demographics and decision-making process of Maine beekeepers. The first survey is the Maine Honey Bee Survey Maine beekeepers fill out electronically each year. The Maine State Apiarist and Bee Inspector created this survey to record data on Maine beekeeping. This data set came from the April 2018-April 2019 beekeeping season. The second survey is a paper survey sent out by the University of Maine Honors College Sustainable Food System Research Collaborative as a part of the USDA-NIFA funded research project, Finding the Sweet Spot: Scale Challenges and Opportunities for Beekeeping and Maple Syrup Production in Maine (USDA-NIFA Award #2017-69006-26573). This project focused on production and marketing challenges for small and medium-scale maple syrup and honey producers in the state of Maine. The project research team sent the survey to registered honey producers in the state. Information from these surveys were used to develop the later portions of this project and ultimately generate outputs.

<u>Methods</u>

<u>Maine Honey Bee Survey</u>. The Maine State Apiarist conducts a yearly Maine Honey Bee Survey to keep track of colony losses and other beekeeping statistics in the state. Each year, a link to the survey is posted on the Maine Department of Agriculture, Conservation, and Forestry Apiary Program website. This survey asks questions about Maine beekeeping operations. These questions include but are not limited to location, size of operation, reasons for keeping bees, experience, winter preparations, colony losses, mite monitoring/treatments, and types of equipment used. Most of the data utilized in this project pertains to mite monitoring, treatment choices, and other mite management practices.

Sweet Spot Survey. Faculty and student researchers from the University of Maine and College of the Atlantic collaborated with stakeholders in the Maine beekeeping industry to produce the 2019 Maine Honey Bee Survey (Sweet Spot Survey). The State of Maine provided us with a list of 204 registered honey producers and their contact information. This list included only beekeepers who are licensed to sell honey in the state of Maine, while the Maine Honey Bee Survey was filled out by beekeepers who register their hives with the Maine State Apiarist, but aren't necessarily licensed to sell honey. Subject recruitment for this survey followed Dillman's Tailored Design Methods for surveys (Dillman et al., 2014). We recruited respondents by mail. The first mailing announced the mail survey and questionnaire, described the study, and invited participants to participate. Five days later, we set the second mailing, which included a cover letter describing the survey, an informed consent statement, the questionnaire, and a self-addressed and stamped reply envelope. We sent out a thank you and reminder

postcard 14 days after the survey was sent out to help remind participants to complete the survey. About one week after we sent the reminder postcard, we contacted non-responsive producers via phone call, if phone numbers were available, to encourage them to respond and answer any questions they had about the survey. Multiple contact modes has proven to increase the likelihood of participation by sample members and specifically, a contact by telephone has shown to be effective in improving response rates for mail surveys (Brick et al., 2012; Dillman et al., 2014). Finally, 14 days after the reminder, we mailed a second round of the cover letter, survey, and reply envelope to those individuals who had still not responded to the first mailing. We kept records of returned surveys and post cards throughout this process.

We removed 3 names initially due to incomplete or insufficient address, and sent pre-survey postcards informing of the survey details to the remaining 201 producers. Nineteen postcards were returned-to-sender, leaving us with 182 potential participants to which paper surveys were sent. Fifteen of those producers informed us that they no longer produce honey, therefore declining participation. Our effective sample size then became 167. We received 87 completed surveys for a response rate of 52.1%. The 54question survey included but was not limited to questions on location, beekeeping operation, scale decisions within operations, collaboration between beekeepers, mite treatments, and labor concerns. The data used for this project pertains to mite treatments and various factors that influence beekeepers' mite treatment decisions.

Results: Maine Beekeepers Today

Operation Size. Most of the beekeepers in Maine have small-scale beekeeping operations. 360 beekeepers responded to the online Maine Honey Bee Survey posted on the Maine Department of Agriculture Website. Most (97%) of respondents were hobbyist or backyard beekeepers. The term hobbyist beekeeper refers to beekeepers with 30 or fewer hives and is a scale descriptor, not a judgement on producers' dedication to beekeeping. There were 9 sideliner beekeepers, keeping 30-300 hives, and 2 commercial beekeeper respondents, keeping more than 300 hives. Of the 87 respondents to the Maine Honey Producer Survey (Sweet Spot Survey), 76 were hobbyist or backyard beekeepers, 8 were sideliner beekeepers, and 3 were commercial beekeepers. Forty-six percent of those respondents said they plan to increase the number of colonies they manage by between 1 and 500 hives, however the majority intend to add just a few colonies to their operation, keeping them in the hobbyist beekeeper category.

<u>Why Keep Bees</u>? When asked in the Maine Honey Bee Survey why they keep bees, most respondents chose enjoyment/hobby (89%), honey/pollen/propolis/wax production for personal use (67%), and to help the bee population (65%). These results align with the size distribution of Maine beekeepers as hobbyists or backyard beekeepers typically don't profit from their beekeeping. Only 25% of respondents keep bees to sell honey or other hive products and very few respondents sell commercial beekeeping products such as pollination services, Queens, packages, or nucs. **Figure 3** shows the full distribution of responses to this question.

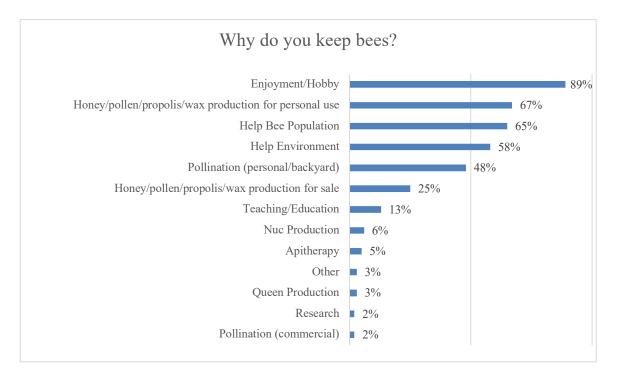


Figure 3. Distribution of responses to the question *Why do you keep bees?* from the 2019 Maine Honey Bee Survey (n=360).

Economic Dependency. Most Maine beekeepers do not depend on their beekeeping operations as a primary source of income. Most (60%) of respondents to the Sweet Spot Survey reported that their economic livelihoods are not at all dependent on their beekeeping and honey enterprise. Just 5% of respondents were completely or considerably dependent. Most (69%) of respondents reported having occupations outside of their beekeeping operation. For the remaining 31%, comments and results from the economic dependency and age questions suggest that many of these respondents are retired and continue to keep bees as a hobby or for supplementary income.

<u>Age & Experience</u>. Respondents' experience keeping bees ranges from as few as 1 year to well over 50 years. Most respondents to the Sweet Spot Survey (66%) have received a college degree or higher, while 11% have a technical or vocational degree. **Figure 4** shows the age distribution of respondents to the Sweet Spot Survey. Most Maine beekeepers are over the age of 55, but results from the 2019 Maine Honey Bee Survey and my personal experiences with bee clubs suggest that beekeeping is slowly growing as a hobby or side practice for younger Mainers.

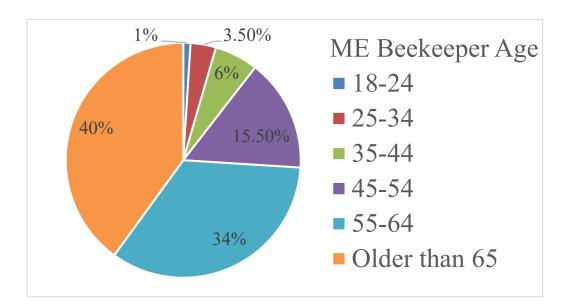


Figure 4. Age distribution of respondents to the Sweet Spot Survey (n=87).

Respondents answered questions about their varroa mite treatment choices, and how their operation size and values relate to their treatment choices. Since most of the beekeepers in Maine are small-scale, this information is important to consider because beekeeping practices and values as well as varroa mite treatment choices can be vastly different between commercial beekeepers, whose livelihood is often tied to their operations, and hobbyist beekeepers, who usually value the best interest of the bees and do not wish to be profitable.

Results: Varroa Mites

<u>Monitoring</u>. The 2019 Maine Honey Bee Survey asked whether producers monitor for mites and if so, which method they use. Of the 360 respondents, the most popular monitoring methods were sticky board (32%) and alcohol wash (31%). Ten percent of respondents reported not monitoring at all. (**Table 1**).

 Table 1. Mite monitoring methods by respondents to the Maine Honey Bee Survey

 (n=360)

Monitoring Method	Maine Honey Bee Survey
Sticky Board	32%
Mite Roll- Alcohol Wash	31%
Mite Roll- Powdered	19%
Sugar	
Drone Brood Survey	6%
Mite Roll- Ether	1%
Visual Inspection of Bees	1%
None	10%

<u>Treatments</u>. Formic acid, in the form of Mite-Away-Quick-Strips[®] (MAQS) and Formic Pro[®] strips, and oxalic acid vaporization were the two most commonly used mite treatments. The majority of respondents also reported using some sort of mechanical control, such as screened bottom boards or drone brood removal, in addition to regular mite treatments. **Table 2** below shows the treatments that respondents used from both surveys. While most beekeepers use just one or two treatments, as the number of hives kept by respondents to the Sweet Spot Survey increased, so did the total number of different mite treatments used.

Table 2. Respondents' Treatment Choices, 2019 Maine Honey Bee Survey and the 2019Maine Honey Producer Survey (Sweet Spot Survey).

<u>Treatment</u>	<u>Maine Honey Bee</u> <u>Survey</u> (n=360)	Sweet Spot Survey (n=87)
Apiguard®	13%	13%
Api Life Var®	4%	3%
Apistan®	<1%	3%
Apivar®	6%	20%
Checkmite+®	<1%	2%
Formic Pro®	21%	31%
Mite-Away-Quick-Strips®	23%	33%
Hopguard II®	11%	9%
Oxalic Acid Dribble	3%	2%
Oxalic Acid Vaporization	42%	44%
Powdered Sugar	3%	3%
Mechanical Control (Drone Brood Trapping, Screened Bottom Board, Brood Cycle Disruption)	32%	47%
None	11%	1%

<u>Treatment Decisions</u>. The Sweet Spot Survey asked respondents to score eight different factors regarding their mite treatment decision-making process. Each factor pertained to a reason for choosing or not choosing mite treatments. The majority of respondents considered effectiveness on mites, impact on bee health, impact on the quality of the honey product, and ease of application to be important. **Figure 5** below shows the percentages of respondents that find each factor either important or very important, broken up by the category of beekeeper size.

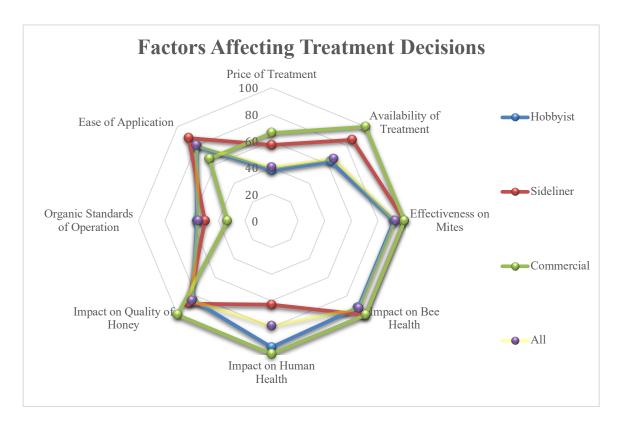


Figure 5. Radar graph showing percentages of respondents (Sweet Spot Survey, n=87) who consider each factor important or very important when making mite treatment decisions. (Appendix B-Q30)

<u>Varroa Information Source</u>. In the Sweet Spot Survey, we also asked where beekeepers get their information on varroa mite treatments. We provided five options to select, such as the MSBA or State Apiarist, and gave respondents an option to write in answers. Respondents could select as many options as they needed to, and the most common information sources were fellow beekeepers, the MSBA, and the State Apiarist. **Figure 6** below shows the complete question results.

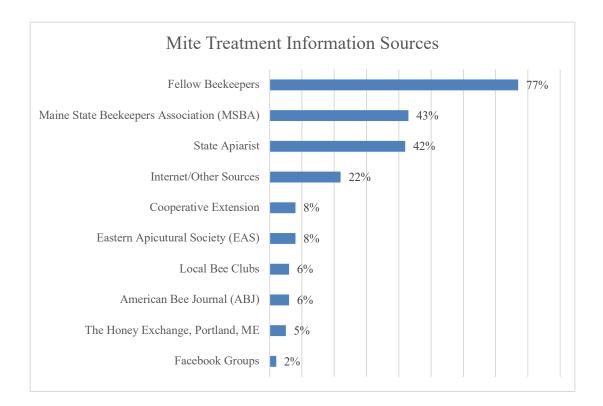


Figure 6. Respondents (Sweet Spot Survey, n=87) sources for information on varroa mite treatments.

Discussion.

<u>Maine Beekeepers</u>. Beekeeping is ever-evolving and is gaining popularity in Maine and around the world as the concern for honey bee health and pollination services continues to increase. Survey results described above confirm the passion for beekeeping in Maine made evident by conversations with new and experienced beekeepers (M. Scott, personal communication, April 3, 2020). Most of the beekeepers in Maine are retired or have jobs outside of their beekeeping enterprise. The majority have small-scale operations and keep bees for personal reasons. Unlike the few commercial beekeepers, they are not economically dependent on honey production or pollination. As a result, the personal values of most Maine beekeepers play a larger role in their honey production or mite treatment decision-making process than does the opportunity for economic gain. These differences in demographics and economic dependency between the majority of Maine beekeepers and the few that provide essential, large-scale pollination services was an important influence on this research and its outputs.

In Chapter 4, I discuss my experience working with a large-scale beekeeper in Maine. The survey results described above show that the majority of beekeepers in Maine are not on the same scale as Lincoln. Lincoln's experience with mite treatments and his knowledgeable decision-making process was extremely valuable to this research. The hobbyists and sideliners that make up a large portion of the Maine beekeeping community, however, are likely to vary in their approaches to mite treatments and other beekeeping practices, as their primary motivation is not their economic livelihood. In Chapter 5, I combine my results from working with Lincoln with the survey results to generate outputs relevant to Maine beekeeping. I then ground truth those outputs with Maine bee clubs to gather feedback from a wider range of beekeepers whose decisionmaking processes and reasons for keeping bees vary.

Mite Treatments. Regardless of beekeeper size or experience, mite treatments are essential, however survey results show that not all Maine beekeepers monitor and/or treat for mites. Survey results provide valuable information on the mite treatments Maine beekeepers use and their values pertaining to mite treatment decisions. Furthermore, it is clear from both surveys and from my experience with Lincoln and other stakeholders that varroa mite treatment resources and practices vary greatly throughout the state. Formic and oxalic acid are the two most commonly used mite treatments in the state of Maine, yet application methods, reported efficacy, and reported adverse effects often vary between beekeepers, especially those of different scales. This variation in values and the decision-making process became an essential component to my research, especially in Chapter 5. There are multiple factors to consider when approaching varroa mite treatments, such as timing, beekeeper values pertaining to health effects, available labor and equipment, etc. A useful mite treatment resource must consider all of these factors. While there are several treatment resources available to beekeepers, they often contain an overwhelming amount of information and conflicting recommendations. My ultimate goal from this research was to create a resource that would consider all of the necessary factors that go into mite treatment decision and provide guidance for varroa mite treatment choices based on individual beekeepers' scale, concerns, and motivations.

CHAPTER 4

MITES, CAMERA, ACTION: BIOPHYSICAL RESEARCH WITH FORMIC AND OXALIC ACID

Literature Review

Formic Acid

Formic acid (FA) is not only one of the most commonly used varroa mite treatments in Maine, but it is also an effective and unique chemical that is used to combat mites across the world. FA is naturally found in numerous fruits and vegetables, in the poison glands of ants and bees that use it as a defensive chemical, and even in honey (Cheremisinoff & Rosenfield, 2010). When sold as a miticide, it is a fumigant that irritates and kills mites it comes into contact with and is currently the only registered miticide able to penetrate cell cappings to reach mites in their reproductive stage (Calderón et al., 2000; Ostermann & Currie, 2004). Other treatments target only mites in the dispersal phase, or those attached to adult honeybees (Cheremisinoff & Rosenfield, 2010; Rosenkranz et al., 2010; Traynor et al., 2020). FA is safe to use in the presence of honey, as it is naturally present in honey and doesn't significantly accumulate in honey or beeswax during treatments (Honey Bee Health Coalition, 2018; Rosenkranz et al., 2010). It has proven to be very effective during periods of brood production, however, there is potential for FA to cause brood and queen mortality, which negatively affects colony growth and honey production (Pietropaoli & Formato, 2018). During FA treatments, open and closed brood can be killed due to lack of oxygen in the hive (Ostermann & Currie, 2004)

FA is typically applied in the form of strips or a pad composed of FA and a binding agent, such as gel, to ensure extended release over time. For this experiment, we used MAQS[®]. One full treatment of MAQS[®] consists of two strips applied to a hive for a 7-day duration while some beekeepers use a half treatment consisting of one strip at a time for 21 days each (Honey Bee Health Coalition, 2018). Once the gel pad is removed from its packaging, FA fumes are released. The rate of release of these fumes is influenced by temperature. Guidelines for applying FA include a temperature window roughly between 50°F and 90°F depending on the brand of treatment used (Honey Bee Health Coalition, 2018; NOD Apiary Products, n.d.). Below 50°F, fumes are not released in high enough concentrations to be effective on mites and above 85°F, fumes are released too rapidly, making ventilation much harder on the colony. Honey bees regulate the temperature within their hives by fanning. When FA strips are placed in a hive, they will begin ventilating in an attempt to remove the fumes, which circulates the fumes around the hive to ultimately reach more mites. If temperatures are above the threshold, fumes may be too strong for the bees to adequately ventilate and some bee mortality may occur. Queens are subject to mortality or supersedure during FA treatments as well. Very little literature exists on the interaction between formic acid and a honey bee queen's pheromone, yet we observed multiple queens either superseded or killed during FA treatments during my research, despite following all temperature guidelines.

Oxalic Acid

Oxalic acid (OA) is an organic acid that is also found naturally in foods such as rhubarb and spinach, and has been commercialized as a varroa mite treatment. OA is toxic to varroa mites it contacts, yet the exact mode of action is unknown. OA is very effective at treating mites in the dispersal phase, but is unable to reach mites in the brood cells, which can be up to 80% of the mite population within a hive (Honey Bee Health Coalition, 2018; Huang, 2013). For this reason, the majority of OA treatments involve multiple treatments to target mites emerging at different stages of the brood cycle. OA has proven to be most effective in broodless periods, such as in the fall or early spring, or in conjunction with other practices that reduce the amount of brood, such as queen caging or brood removal (Gregorc & Planinc, 2001; Hatjina & Haristos, 2005). Currently, OA is not registered to be used in the presence of honey supers, although some studies hint that the residual effects on honey are minimal or comparable to formic acid (Bogdanov et al., 2002).

OA is typically applied in the form of vapor or as part of a sugar solution that is dribbled directly on bees between frames or sprayed in a mist. Spraying OA solutions directly on brood has proven to have negative effects on development, in some cases for up to four months after application (Gregorc et al., 2004; Higes et al., 1999). Trickling is regarded as a safer method as it is not sprayed directly on the brood, but rather on adult bees in between frames, however as the solution is mixed with sugar water, adult bees ingest more of the OA and can die they consume a lethal amount (Hatjina & Haristos, 2005). Sublimating, or vaporizing the OA is the most popular and most effective method for OA mite treatments. This method ensures that the OA crystals spread through the entire hive. It is quick to apply, can be done in hot or cold temperatures, and does not require the hive to be taken apart (Honey Bee Health Coalition, 2018).

Applying OA via vaporizer is a relatively new method, as OA was only approved to be used on honey bee colonies in 2015 by the EPA. Numerous vaporizers have entered the market since then to make applying OA in the form of airborne crystals to honey bee colonies an easy task. Applying OA via vapor is regarded as safer for the bees than trickling, as it exposes more mites to the OA crystals without the bees consuming excessive amounts of OA. Common issues with these vaporizers include the potential to harm the applicator, uncertainty in application consistency, and price. PPE such as a respirator, long sleeves, and gloves, is required when applying oxalic acid vapor. A number of beekeepers have also created their own vaporizers to avoid paying for costly, brand-name ones. The official EPA pesticide label for OA lists an application rate of 35 g OA per 1 liter of 1:1 sugar water for the dribble/spray method, and 1 gram per brood box chamber for vaporization (EPA, n.d.). When vaporizing, entrances should be reduced and plugged to prevent bees and the OA from leaving the hive during treatment.

Stakeholder Engagement

Sweet Spot Project

During the summer of 2019, I was employed as a student research fellow through the University of Maine Honors College Sustainable Food Systems Research Collaborative on the Sweet Spot Project. Goals of this project included connecting university researchers with stakeholders in the maple and honey industries in Maine and facilitating research projects to help small- and medium-scale producers with production

and marketing challenges. Students were tasked with individual research projects while participating in industry stakeholder engagement activities with the rest of the research team throughout the summer.

Research conducted with frequent stakeholder involvement has the potential to not only produce more applicable results, but also help those results resonate with people outside of the scientific community (Hall et al., 2012; Jansujwicz et al., 2013; Oscarson & Calhoun, 2007). For this reason, stakeholder involvement and feedback was a top priority of this project and stakeholder feedback dramatically improved my research methods. I was interested in conducting research on varroa mite treatments, yet I had limited experience with and knowledge of mite treatments prior to my fellowship. Collaboration with Maine beekeepers through stakeholder advisory board meetings held by the Sweet Spot Project provided direction for the rest of the research project. Specifically, a mentorship through the Sweet Spot Project with Lincoln Sennett of Swan's Honey allowed me to pursue relevant research topics on mite treatments with a very experienced Maine beekeeper.

Swan's Honey

<u>History</u>. R.B. Swan and Son has been producing honey and providing pollination services in the state of Maine since the 1940s. It was founded in 1946 by Reginald Swan and his son Harold Swan, who was an activist in the Maine beekeeping community until his passing in 2018. Since its creation, Swan's Honey has become a well-known name in Maine and elsewhere and is found on Hannaford grocery shelves across the state. In 2002, the Swan label was purchased by the Sennett family and relocated from its original

home in Brewer, ME to Albion, ME, where it still thrives. Lincoln Sennett, the head beekeeper and owner of Swan's today, is an active member in the Maine beekeeping community and a member of the Sweet Spot Project stakeholder advisory group. I had the opportunity to work with Lincoln, who runs over 3000 hives that he moves between Maine and Georgia each year for honey production and pollination services for several fruit crops.

The Research Project. Lincoln has many years of experience treating honey bees for mites using FA and OA in various ways. Going into the summer field season, I began researching mite treatments that don't involve miticides, such as colony manipulations or powdered sugar dusting. While I was reading a lot about these sorts of mite treatments, I had no real sense of what worked because I had only treated for mites once, and the professor of my beekeeping class did most of the work. This sort of knowledge gap often exists between the academic and industry worlds, but conversing with on-the-ground experts, or Maine beekeepers in this case, helps to eliminate that gap. Additionally, there is a knowledge gap between the communities of hobbyist and commercial beekeepers, however organizations like bee clubs that advocate for educational programs are a way to bridge that gap. Upon talking with stakeholders by email and in-person, I soon realized that these treatments were ineffective and unrealistic, especially for larger beekeepers. Lincoln's experience using FA and OA led him to develop several research questions about new treatment methods. Some of those questions included efficacy of new OA vaporizers, such as the ProVap 110, and the efficacy of a combined FA and OA treatment. It was from his experience treating mites that the entire basis for this research project came from. Lincoln also felt that there was a disconnect in the beekeeping

community between companies manufacturing and 'testing' mite treatments, commercial beekeepers experiencing their efficacy, and small-scale beekeepers who don't have the opportunity to test every available treatment, but rather make their treatment decisions based on the available resources. Since these resources are often guidelines and efficacy reports published by mite treatment manufacturing companies, they may not have the interests of individual beekeepers in mind. Furthermore, if there is a product to sell, companies will always opt to promote pros rather than cons.

For the two most popular mite treatments in Maine, formic and oxalic acid (see Chapter 3), numerous information sources and efficacy reports exist, yet they are often inconsistent. For example, NOD Apiary Products, the manufacturer of MAQS®, states on the label that MAQS® may cause queen supersedure or death, yet they fail to mention that basically all open brood, and some capped brood, is killed and removed during treatment (NOD Apiary Products, n.d.). Lincoln had also identified issues, such as inconsistent testing and efficacy reports, with several of the oxalic acid vaporizers that have entered the market in recent years. Through stakeholder meetings and communication via email and phone, Lincoln and I developed and tested a new treatment timeline for FA and OA during summer honey and brood production. We developed this timeline based on Lincoln's experience using the two treatments and tested it during the summer of 2019 on research hives that he generously provided.

Biophysical Research

Treatment Timeline

Research Questions. When used according to their labels, FA and OA are effective varroa mite treatments. There are currently no studies that test the combined use of FA and OA as mite control during a period of brood production in honeybee colonies. During his use of FA in the form of Mite Away Quick Strips[®] (MAQS), Lincoln observed that during roughly the first three days of a full treatment, queens stop laying eggs and most uncapped brood is removed. He suspected this to be due to more vapor released during the first three days or that it takes several days for bees to properly ventilate the fumes and resume brood production. These reasons could also account for queen mortality, which has been reported numerous times with FA use and which we also observed a handful of times (NOD Apiary Products, n.d.). The treatment strategy that Lincoln came up with involved using those negative effects of FA to our advantage by following up with an OA treatment during the broodless period created by the FA treatment.

<u>Treatment Approach</u>. A detailed drawing of the treatment timeline can be seen below in **Figure 7**. It is based on the life cycle of both the honey bee and the varroa mite and involves utilizing the 'broodless' period created during FA use to target more mites with a follow-up OA treatment. Mites typically enter cells about a day before they are to be capped. They then develop within the cells and emerge with the young bee about 10 days later. Timing of emergence is dependent on several factors, but generally is 21 days after the egg is laid for workers, 10-12 days after the capping of the cell. Our theory was that treating with FA, therefore delaying brood production for about 3 days, and

following up with an OA on day 10-12 after the FA treatment would target more mites than either of the treatments alone. FA targets phoretic mites as well as those in the reproductive stage beneath cell cappings (Calderón et al., 2000). Any mites that survive the FA treatment either beneath cell cappings or on adult bees will be in the dispersal phase 10-12 days after the start of the FA; 2-3 days broodless + 8-9 days after egg laying before cells are capped. The delay of egg-laying, and therefore mite reproduction, leaves a window of opportunity during which all mites will be phoretic and therefore susceptible to an OA treatment.

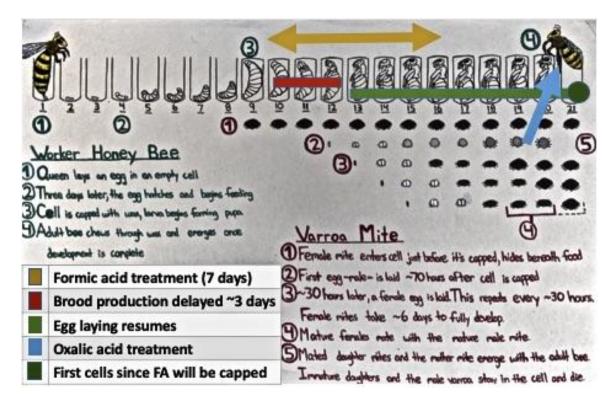


Figure 7. FA/OA treatment timeline in line with reproductive cycle of mites and worker bees, explaining each treatment step and corresponding development stage.

Research Methods

Location and Initial Inspections. Lincoln set up two bee yards in Monticello, ME for us to test the new approach to mite treatment with FA and OA described above. We started with 72 colonies split between the two yards, Yard A in an old air strip and Yard B in an old orchard field. Each hive was first labeled with a number. Relative strength of each colony was assessed by counting the number of frames of bees and number of frames of capped brood during an initial hive inspection (Delaplane et al., 2013; Aleš Gregorc & Planinc, 2001). Presence of eggs and larvae, swarm or supersedure cells, and visible viruses were also recorded. Queens of each hive were visually located and returned to the brood box.

During initial inspections, we took mite roll samples from each colony to determine initial infestation rates. ¹/₄ cup of bees (~150 bees) was shaken from a brood frame not containing the queen into a Tupperware container, then transferred into a jar with 70% isopropyl alcohol (Lund & Skyrm, 2019). Due to the number of mite rolls being done throughout the experiment, ¹/₄ cup was used instead of the typical ¹/₂ cup to regularly monitor for mites.

<u>Treatment Groups</u>. Queenless or deadout colonies were removed from the yards. The remaining colonies were then split into three different treatment groups based on findings during inspections. If the hive had swarmed or superseded recently and no capped brood was present, they were placed into the oxalic only group (OA). These hives were treated with OA only, which is most effective in the absence of brood (Aleš Gregorc et al., 2017). Colonies from both Yard A and Yard B were placed into the OA group, as it was based on hive condition not location. All queenright (with a healthy and laying

queen) colonies with capped brood in Yard A were placed in the formic plus oxalic group (FA/OA). All queenright colonies in Yard B were placed in the formic only group (FA). We intended to use the FA only group as a baseline to compare with the combined formic plus oxalic treatment.

Treatments. Any colony that received formic acid treatment was left with two honey supers on and all entrances open during treatment to allow for adequate ventilation, in compliance with label instructions. Most hives used in the study consisted of a single deep brood box, with the exception of a few hives in Yard B which had two deep brood boxes. Honey supers were removed from any colonies receiving oxalic acid treatment to comply with label instructions and food safety regulations (EPA, n.d.; Honey Bee Health Coalition, 2018). Lower entrances to hives receiving oxalic acid treatment were also reduced and top entrances were plugged. For colonies receiving the formic plus oxalic treatment in Yard A, honey supers were removed and entrances were reduced before applying oxalic acid.

The formic acid treatments consisted of two MAQS[®] pads placed in between the brood box and first honey super of each hive, staggered a couple inches apart (**Figure 8**). MAQS[®] contain 46.7% formic acid in a gel strip that allows the acid to vaporize in the hive. We used one full dose, two pads, of MAQS[®] and left them in for 7 days (Honey Bee Health Coalition, 2018; Lund & Skyrm, 2019).



Figure 8. Formic acid (MAQS[®]) treatment on a brood box.

Oxalic acid treatments were applied with a ProVap 110 vaporizer by Oxavap. Single deep hives with reduced and plugged entrances were treated with approximately 1 gram of oxalic acid dihydrate. ¹/₄ teaspoon, or roughly one gram of oxalic acid dihydrate was used for each deep brood box (**Figure 9**). Oxalic acid was loaded into the ProVap which was immediately inserted into the hive entrance (**Figure 10**). A piece of burlap was used to attempt to cover the rest of the entrance and prevent bees or oxalic acid from escaping. OA application was done at dawn or dusk to ensure that most of the field bees were in the hive receiving treatment.



Figure 9. Bag of oxalic acid and measuring/loading equipment.

<u>PPE</u>. Proper personal protective equipment (PPE) was worn for all treatments with FA and OA. This included gloves and long sleeves for OA, and welding gloves, a respirator, and long sleeves for OA (**Figure 10**).



Figure 10. PPE (respirator, gloves) for OA treatments (both). ProVap 110 being inserted into a reduced hive entrance (right).

Monitoring Efficacy. Mite rolls were done prior to any treatment, after each treatment, and again after a full brood cycle. Exact treatment application and mite roll dates can be seen below in **Table 3.** Mite rolls were used to calculate the infestation levels of each colony. A typical mite roll consists of ½ cup or about 300 bees. The number produced by the roll is then divided by 3 to determine the percent infestation of the colony. For our experiment, we used ¼ cup of bees to minimize the effect of repeated rolls on colonies, and divided the number of mites found in each roll by 1.5. Equipment used for the mite roll can be seen in **Figure 11** below.

Yard	Group	Treatment	Application Date	Mite Roll	Date
	FA/OA	Formic Acid	7/14/2019	Initial	7/11/2019
		Oxalic Acid	7/24/2019 6 am	Post FA	7/23/2019
А				Final	8/30/2019
				Initial	7/11/2019
	OA	Oxalic Acid	7/14/2019 8 pm	Post OA	7/19/2019
				Final	8/30/2019
	FA	Formic Acid	7/22/2019	Initial	7/14/2019
В				Final	8/30/2019
				Initial	7/14/19
	OA	Oxalic Acid	7/16/2019 11 pm	Post OA	7/19/19
				Final	8/30/2019

Table 3. Application and Mite Roll dates during summer 2019 research.



Figure 11. Mite roll equipment (alcohol, Tupperware, ¹/₄ cup scoop, mason jar with screened lid, fine mesh strainer)

Results and Analysis

Sample Sizes. Lincoln provided a total of 72 colonies for the experiment, 40 in Yard A and 32 in Yard B. During initial inspections, we found seven total colonies that were dead or queenless that were removed from the study. A total of 18 colonies in both yards contained no capped brood and were placed in the OA only group. Twenty-five colonies in Yard A were queenright with capped brood and were placed in the FA/OA group. Twenty colonies in Yard B were queenright with capped brood and were placed in the FA only group. Of the colonies that received formic acid treatments in both yards, 11% lost their queens during treatment. Two colonies in Yard B were given the wrong treatment and were also left out of the study. Another three colonies in the FA/OA group lost their queens after both treatments in Yard A and were removed. Results were based on analysis of 15 colonies in the OA only group, 19 colonies in the FA group, and 21 colonies in the FA/OA group.

<u>OA Only</u>. Of the 15 colonies in the OA only treatment group, 13 colonies were at or above treatment threshold of 3%, and the remaining two were at 2% infestation prior to treatment. After a single application of OA and a full brood cycle, just three of the fifteen colonies were still above treatment threshold, all in Yard A. The average infestation level after one treatment of OA was 3.17% in Yard A, 0.76% in Yard B, and 2.04% in both yards combined. **Tables 4a** and **4b** below shows the infestation levels of the OA only group.

Yard	Hive	Treatment	% Infestation Before	% Infestation After
А	1	OA	4.67	1.33
А	2	OA	4	2
А	3	OA	12	2.67
А	23	OA	2	3.33
А	26	OA	6.67	7.33
А	37	OA	4.67	1.33
А	39	OA	2	6
А	40	OA	3.33	1.33
		Average:	4.92	3.17

Table 4a. Infestation levels before and after treatments for the OA only group in Yard A.

Table 4b. Infestation levels before and after treatments for the OA only group in Yard B

Yard	Hive	Treatment	% Infestation	% Infestation
			Before	After
В	21	OA	8.67	0.67
В	23	OA	6	0.67
В	24	OA	9.33	1.33
В	25	OA	4	0.67
В	26	OA	11.33	0.67
В	27	OA	4	0.67
В	28	OA	8	0.67
		Average:	7.33	0.76
	Combined (A&B)	0	6.04	2.04

FA Only. Five days after the start of the MAQS[®] treatment in Yard A, formic acid's effects were visible in the yard in the form of brown or dead vegetation around the hives and in some cases piles of dead bees and larvae by hive entrances (Figure 12). Despite temperatures being below the recommended threshold in the MAQS® treatment guidelines, a near complete loss of open brood was noted in almost every colony. Very few eggs and uncapped larvae survived the FA treatment. We found that most queens

resumed egg-laying three to five days after the start of treatments. These results were consistent with the FA treatment in Yard B as well.



Figure 12. Dead grass and bees in the bee yard after formic acid treatments

Of the nineteen colonies in the FA only treatment, twelve of them were at or above the treatment threshold of 3% infestation prior to treatment. After receiving a full dose of MAQS[®] and going through a full brood cycle, none of the colonies in Yard B were above the treatment threshold of 3%. Six colonies had an infestation level of zero. Average infestation in this group prior to treatment was 4.91% and dropped down to 0.95% after treatment. **Table 5** shows infestation levels before and after treatment in the FA only group.

Yard	Hive	Treatment	% Infestation Before	% Infestation After
В	1	FA	4	0
В	2	FA	10.67	2.67
В	4	FA	3.33	0.67
В	5	FA	2.67	1.33
В	6	FA	4	0.67
В	7	FA	7.33	1.33
В	9	FA	3.33	1.33
В	12	FA	1.33	1.33
В	13	FA	6	2.67
В	14	FA	8.67	2
В	15	FA	4	2
В	16	FA	2.67	0
В	17	FA	0.67	0
В	18	FA	0	0
В	19	FA	2	0
В	20	FA	3.33	0.67
В	22	FA	13.33	0.67
В	29	FA	14.67	0
В	30	FA	1.33	0.67
		Average:	4.91	0.95

Table 5: Infestation levels of FA only group before and after treatment with MAQS and a full brood cycle.

<u>FA Plus OA</u>. Of the 21 colonies in FA/OA treatment in Yard A, nine were at or above treatment threshold of 3% infestation prior to treatment. An additional five colonies were just below treatment threshold.

After the initial formic acid treatment, five colonies were still at or above treatment threshold and four colonies were just below treatment threshold at 2% infestation.

After an oxalic acid treatment on day 10 and a full brood cycle, just two colonies were still at or above treatment threshold and four colonies were just below. By the end of our experiment with both treatments, the average infestation in the study colonies was 1.30%, down from 1.84% after the FA treatment and 3.87% from prior to any treatment.**Table 6** below shows the infestation levels before and after each treatment in the FA/OA group.

			% Infestation	% Infestation	% Infestation
Yard	Hive	Treatment	Before	After FA	After FA/OA
А	4	FA/OA	2	0.67	2
А	5	FA/OA	1.33	0	1.33
А	6	FA/OA	0.67	2	1.33
А	9	FA/OA	4	4.67	0.67
А	10	FA/OA	5.33	3.33	0
А	11	FA/OA	1.33	0	0.67
А	12	FA/OA	1.33	1.33	0.67
А	13	FA/OA	0	0.67	0.67
А	14	FA/OA	4.67	0	2
А	15	FA/OA	1.33	2	0
А	16	FA/OA	4	0	0
А	18	FA/OA	12	1.33	0
А	21	FA/OA	2	4	2.67
А	25	FA/OA	7.33	4.67	3.33
А	27	FA/OA	18.67	6.67	2.67
А	30	FA/OA	3.33	1.33	0
А	31	FA/OA	2	0.67	1.33
А	32	FA/OA	1.33	0.67	0
А	33	FA/OA	2.67	2	3.33
А	36	FA/OA	4	2	2
А	38	FA/OA	2	0.67	2.67
		Average:	3.87	1.84	1.30

Table 6: Infestation levels of FA/OA group before and after each treatment.

<u>Statistical Analyses</u>. I first ran a two-sample t test on the initial infestation levels in the two yards to determine if I could group them together. Infestation levels were not significantly different between the two yards. Post treatment infestation levels were also not significantly different between the FA and FA/OA groups. Post treatment infestation levels were, however, significantly different between the OA only groups in the two yards. Significant results from a linear regression test also indicate that the FA/OA treatment may be more effective at bringing infestation levels below treatment threshold when infestation levels are higher to begin with than just a FA treatment. Results from statistical analyses are summarized in **Table 7** and **Figures 13, 14, and 15** below.

Yard	Treatment	Condition	Test	Results
A vs. B	All (with outliers)	Initial % Infestation	2 sample t test	t=-1.3014 df=52.51 p=0.1988
A vs. B	All (without outliers)	Initial % Infestation	2 sample t test	t=-2.9915 df=35.302 p=0.005037
A vs. B	FA/OA vs. FA	Post treatment % infestation	2 sample t test	t=1.0829 df=37.122 p=0.2858
A vs. B	OA only	Post treatment % infestation	2 sample t test	t=2.9293 df=7.1875 p=0.0214
A	FA/OA	Post Treatment, Initial Infestation vs. % Change in Infestation	Linear regression	RSE: 4.017 (df=19) R2=0.1781 Adjusted R2: 0.1349 F= 4.118 (DF=1, 19) p=0.05667*
Yard	Treatment	Condition	Test	Results
В	FA	Post Treatment, Initial Infestation vs. % Change in Infestation	Linear regression	RSE= 3.927 (df=17) R2:0.1724 Adjusted R2: 0.1237 F=3.54 (df=1, 17) p=0.7714

Table 7. Statistical tests and results from summer 2019 research.

Initial Percent Infestation in Both Yards (with outliers)

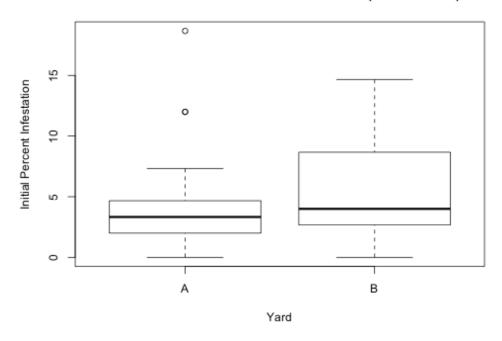
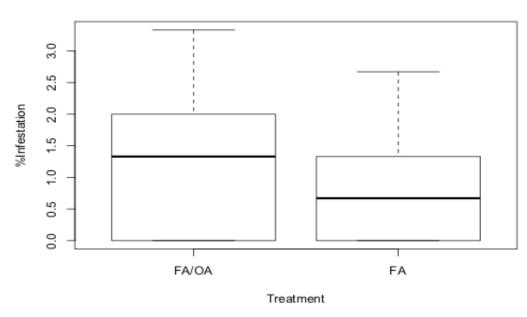


Figure 13. Box plot showing initial infestation in both yards, including outliers.



Post-treatment Infestation Levels

Figure 14. Box plot showing post-treatment infestation levels for FA/OA and FA only groups.

Post-treatment Infestation Levels: OA Only Treatment

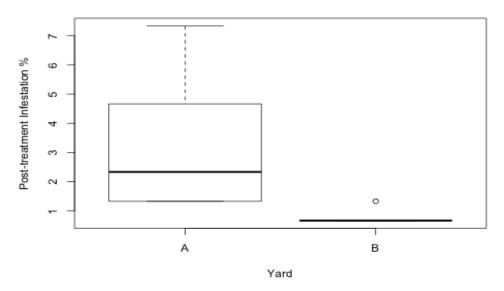


Figure 15. Box plot showing post-treatment infestation levels for the OA only treatment group in both yards.

Discussion

The most valuable recommendation from my biophysical research results is that beekeepers base mite treatments and timing on hive conditions. This is made evident from the results of the OA only group. OA has proven to be effective in the absence of capped brood. Infestation levels of all colonies dropped to below treatment threshold (3%), in Yard B with an OA only treatment on the colonies with no capped brood present. These results are consistent with the findings of other studies that report success with oxalic acid during a broodless period (Gregorc et al., 2017; Moro & Mutinelli, 2019). This was with just a single application of OA during a summer honey flow. Had we treated these colonies with FA instead, the recently mated or virgin queens would have been subjected to the harsh fumes and the colony would have to deal with

ventilation while working to resume brood production. The OA only group in Yard A also shows that it is important to be consistent in application timing to prevent reinfestation, but more importantly to treat according to hive conditions. If an additional treatment of OA had been done on these colonies 15 days after the start of the FA treatment, we likely would have observed lower final infestation levels. If a stricter treatment and monitoring schedule had also been followed, overall efficacy in Yard A may have increased, however scheduling and weather conflicts did not allow. Many of the efficacy reports of OA are from studies done during broodless periods (Gregorc et al., 2016, 2017; Moro & Mutinelli, 2019). OA is much less effective if brood is present, however linking treatments with colony conditions can improve treatment success overall.

FA was effective at killing mites, but we did observe numerous negative effects from the result of its use. For this reason, it is important to use caution when treating with FA, especially in weaker colonies or those with virgin or old queens. Since FA causes most uncapped and even some capped brood to be removed, colonies treated with FA lose a generation of bees. This loss can be a major setback, especially for weaker colonies. If an established queen is killed by FA, the ability of her colony to re-queen itself rests on the chance that one of her eggs survives the FA treatment. If a virgin queen is killed by FA before she is given the opportunity to mate and begin laying, that colony will be unable to generate another queen without the presence of eggs. It is important to check colony health and queen vitality before and after treating with FA.

The combined FA and OA treatment approach was effective at killing mites and likely even more effective in bringing colonies with very high infestation levels below

the treatment threshold of 3% infestation. This treatment is best used when temperatures are below 80°F and can be done during a summer honey flow as long as honey supers are either removed or blocked during the OA treatment. We treated with OA ten days after the start of the FA treatment. This was a novel approach to the two treatments, Previous studies have reported adverse effects on brood production by formic acid (Ostermann & Currie, 2004; Underwood & Currie, 2003) and highly successful oxalic acid treatments of the absence of brood (Aleš Gregorc et al., 2017; Moro & Mutinelli, 2019). This study, however, used the temporary 'broodless' period caused by formic acid treatment and the effectiveness of oxalic acid in the absence of brood to increase the potential efficacy of both treatments combined. Lincoln and I both agreed that an OA treatment eleven or twelve days after would likely be more effective. This would allow for more mites to emerge from capped brood cells, if they survive the FA treatment. An additional OA treatment fifteen days after the start of the FA treatment would likely be even more effective as it would target mites reproducing in drone cells that would emerge three days later than those in worker cells.

Lincoln's expertise in mite treatments and in beekeeping led to a unique approach to formic and oxalic acid applications. Our methods, results, and new treatment timeline can be adjusted and improved in future studies. Our success in bringing down infestation levels to below the treatment threshold of 3% in all but one treatment group, and our novel approach to applying formic and oxalic acid treatments opens a promising opportunity for researchers to explore further.

CHAPTER 5

OUTPUT GENERATION AND BEE CLUB FEEDBACK

Phase 3: Further Stakeholder Involvement

Stakeholder Feedback

Importance. Research is critical to scientific, technological, and cultural advancement. Research also has potential value to community stakeholders when it is designed with the collaboration and livelihoods of stakeholders in mind when generating outputs. Stakeholder-engaged research has the potential to generate outputs that are accessible outside of the scientific world. Much of the disconnect between the scientific community and the general public is that it is often difficult to communicate scientific results in a way that resonates with people, let alone in a form that people can successfully use. There has been growing interest in "knowledge to action research" in which research is conducted with goals in mind that incorporate the interests of all stakeholders involved (Hall et al., 2012; Jansujwicz et al., 2013). This sort of approach has the potential to not only further scientific research, but also generate outputs from research that meet relevant stakeholder needs (Hall et al., 2012; Jansujwicz et al., 2013; Oscarson & Calhoun, 2007). Stakeholder involvement has been critical in developing my research outputs described in this chapter.

A large part of my research has involved gathering information from literature and other sources on current mite treatments. While I describe briefly the groups of mite treatments (Chapter 2), the second half of my research has focused on relevant outputs to

the Maine beekeeping community. Phases 1 and 2 of this project consisted of stakeholder-guided research on popular varroa mite treatments. The knowledge gap in the application methods and efficacy of these treatments, and their popularity with Maine beekeepers was evident anecdotally and confirmed through stakeholder engagement and survey results. Information from Phases 1 and 2 helped identify further research questions that were implemented in Phase 3. Through analysis of survey data, meetings with stakeholders, and results from my research with Lincoln, it became clear that there were differences in Maine beekeepers' values in their beekeeping operations. These values ultimately influence mite treatment choices. Phase 3, as described below, involves generating relevant outputs for Maine beekeepers, returning to stakeholders for feedback, and adjusting those outputs to meet the values and concerns made evident during the feedback process.

<u>Collaborative Feedback</u>. While the biophysical research for this project was conducted with stakeholders in mind, survey results indicate that most Maine beekeepers are small-scale, unlike the stakeholder I worked most closely with. Since they are not economically dependent or may not have the experience that commercial beekeepers do, the majority of Maine beekeepers likely do not have the same mite treatment decisionmaking process that Lincoln does. For this reason, I wanted to expand the diversity of beekeepers providing me with feedback. The first part of Phase 3 involved generating several outputs useful to the beekeeping community based on my research experience with Lincoln and results from the two 2019 beekeeping surveys.

When I initially began researching the available varroa mite treatments during my Sweet Spot Fellowship, I created a table of treatments as a resource for myself during my

research. My project evolved as I engaged with more stakeholders and furthered my research, and this treatment table turned into a critical decision-making output. Upon engaging with stakeholders prior to and during the Sweet Spot stakeholder advisory board meeting, I learned more about the current Maine beekeepers and their most popular mite treatments.

This engagement led to the development of a research project with Lincoln Sennett using FA and OA, two very popular mite treatments. The FA/OA treatment guideline, which I present first, is a result of this collaborative research project. After the biophysical research portion, I began to look at survey data from Maine beekeepers and analyze the results of questions about mite treatments. This led to several research questions and ultimately to the development of these three outputs.

Using my expanded table of registered mite treatments (Appendix D), and beekeeper survey data focused on mite treatments, I have built a framework for an interactive varroa mite treatment decision-making tool. This research goal grew based on the knowledge gap in application and effects of the available mite treatments made evident by Maine beekeepers through both personal conversations and formal surveys. The decision-making tool includes pros and cons of each treatment based on literature review and personal anecdotes, and highlights common concerns from both survey results and bee club meetings. The draft presented in Appendix C is meant to be a dynamic framework, based on currently available data and beekeeper concerns, that will incorporate new information and concerns as they arise.

<u>Bee Clubs</u>. There are currently sixteen local chapters, or bee clubs, of the Maine State Beekeepers Association located throughout the state. These chapters hold monthly

meetings to bring beekeepers together, organize outreach and education events, and to act as a bank of information on all things related to beekeeping. Some bee clubs are more active than others, but they all have the same goal of promoting the wellbeing of honey bees and their keepers in the state of Maine.

I presented drafts of the three outputs below to bee clubs across the state for feedback. Some of the feedback that was consistent between bee clubs was the unrealistic nature of organic standards, the enormous variability in oxalic acid application methods, and the overwhelming amount of information available on mite treatments. At each meeting, there were a diversity of beekeepers, which provided me the opportunity to learn how size, values, and numerous other factors impact their mite treatment decisions. This gave me an idea of how to steer the outputs towards better supporting the entire Maine beekeeping community. After each meeting, I updated the drafts using the concerns, interests, and knowledge of Maine beekeepers as guides. This chapter contains the three main outputs that resulted from this collaboration with Maine bee clubs.

FA/OA Treatment Guideline

Results from the summer 2019 research project with Lincoln Sennett may offer guidance for future mite treatment research. Even if beekeepers are not willing to use the methods described below and in Chapter 4, our approach to the treatment in which we based application dates on the life cycles of both honey bees and varroa mites can be applied elsewhere and can be valuable to varroa management in a broader sense.

Formic and Oxalic acid are the two most popular mite treatments for Maine beekeepers. Many Maine beekeepers are familiar with application techniques and best

practices for the two acids, however there is still an overwhelming amount of information, often conflicting, available on the internet and other sources for application tips and tricks. Here (and in Chapter 4) I have attempted to provide a clear, concise description, based on my personal experience and review of literature, of the best practices for and any adverse effects that may result from using FA and OA to treat for mites during a summer honey flow. A timeline of suggested FA and OA application dates in line with the honey bee and varroa mite life cycle can be seen below in **Figure 16**.

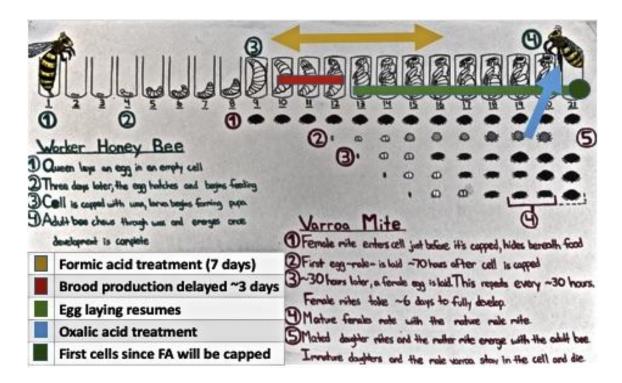


Figure 16. FA/OA treatment timeline in line with reproductive cycle of mites and worker bees, explaining each treatment step and corresponding development stage.

Decision-Making Tool

Numerous tools exist on the internet and in publication to assist beekeepers in making their mite treatment decisions. A few examples of such tools are the Honey Bee

Health Coalition Varroa Guide and the Massachusetts Department of Agricultural Research Varroa Mite Brochure. Both resources list descriptions of all the available treatment and considerations and best practices when using them. I have used these resources throughout my research and have attempted to generate a decision-making resource of sorts that will not only reinforce these considerations and best practices, but also add a new element to the mite treatment decision-making process that helps define beekeeping operation values standards and how they pertain to mite treatment choices.

I used the results from the survey data (Chapter 3) as a starting point for my decision-making tool. The end goal was to make a framework similar to a dichotomous key, in which beekeepers would answer a series of yes or no questions that would eventually prompt them to a list of suggested treatments based on their hive conditions, available equipment, willingness to use certain types of treatments, etc. I began with a few questions on the mite treatment decision-making to which respondents varied in their responses. An example of the early stages of this output can be seen below in **Figure 17**. The questions continued to evolve throughout the research project and eventually turned into the final product which can be found in **Appendix C.** It is intended to help beekeepers choose treatments relevant to their hive conditions and scale decisions and provides my own sustainability ranking of the treatments along with best practices and considerations.

1.	Are you concerned with maintaining organic standards with your mite t	reatment?
	a. Yes	go to 2
	b. No	go to 13
2.	(from 1a.) Is there capped brood in your hive?	
	a. Yes	go to 3
	b. No	go to 4
3.	(from 2a.) Is your hive strong (8+ frames of bees) and queen healthy an	d laying?
	a. Yes	go to 5
	b. No	go to 4
4.	(from 2b.) Do you have access to PPE (gloves, goggles, respirator)?	
	a. Yes	go to 6
	b. No	go to 9

Figure 17. Sample of questions from the first draft of the mite treatment decision-making tool.

Mite Treatment Information Table

The final table with information on varroa mite treatments and their cost, application method, and other . can be found in **Appendix D**. This table is based on a number of a similar fashion, but includes additional information, however most of the information was gathered from these sources and other literature (Honey Bee Health Coalition, 2018; Lund & Skyrm, 2019). The paragraphs that follow are intended to provide a concise overview of the pros, cons, and special considerations of each treatment. Feedback from beekeepers and additional literature review throughout my research has helped improve the treatment table and make it more relevant for beekeepers.

<u>Apiguard.</u>[®] Apiguard[®] (A.I. thymol) is a mite treatment derived from the thyme plant that is sold either as a gel in a large bucket or individual trays. When gel is placed into the hive, it releases thymol fumes that come into contact with and kill mites. One treatment of Apiguard[®] consists of 50 grams of gel per brood box, placed directly on top

of the brood frames. Hygienic behavior by the bees to remove the gel also disperses the treatment throughout the hive. Apiguard is not approved to be used in the presence of honey supers and beekeepers must wait 48 hours after starting Apiguard[®] treatments to re-enter their hives. Treatments can last for 4-6 weeks depending on the rate of removal of the gel. Apiguard[®] is most effective in the spring or fall when temperatures are between 60°F and 105°F and colonies are not producing honey for human consumption. The higher the temperature, the faster the thymol fumes will be released.

<u>Api Life Var.</u>[®] Api Life Var[®] (A.I. thymol, menthol, eucalyptus oil) is sold as a tablet that releases fumes that come in contact with and kill mites. A full treatment of Api Life Var[®] consists of three 11 gram tablets that are replaced every 7-10 days. Tablets are broken into pieces and placed into the corners of each hive, just above the brood box. Entrances should be reduced when using Api Life Var[®] to keep the fumes in the hive. Since it is fumigant, Api Life Var[®] has an application temperature range of 64°F-95°F to ensure treatment is released but not in such high concentrations that it harms the bees. Like Apiguard[®], Api Life Var[®] is not approved to be used in the presence of honey supers and works best during the spring and fall when less sealed brood is present. When treating with Api Life Var[®], beekeepers cannot have surplus honey supers on hives and must wait 30 days after the end of the treatment before extracting honey.

<u>Apistan.</u>[®] Apistan[®] (A.I. tau-fluvalinate) comes in the form of a plastic strip impregnated with miticide. Tau-fluvalinate is a pyrethroid, a group formulated to be similar to natural pyrethrins found in the flowers of the plant genus pyrethrum and used in many insecticides. One treatment of Apistan[®] consists of two strips per brood box, spaced evenly apart. Strips are left in for six or seven weeks, and no more than eight

weeks. Apistan[®] is most effective when colonies have less brood and when temperatures are above 50°F. It is not approved to be used during honey production, beekeepers can super hives immediately after removing the strips. Widespread mite resistance to Apistan[®] has been reported due its continuous use (Maggi et al., 2010; Trouiller, 1998). For this reason, it is advised that beekeepers either check for resistance before the treatment or check for effectiveness after treating with Apistan[®]. Furthermore, fluvalinate has been documented to accumulate in wax and can lead to development issues if levels become too high (Maggi et al., 2010). It can also negatively affect queen and drone reproductive health.

<u>Apivar.</u>[®] Apivar[®] (A.I. amitraz) is a type of formamidine insecticide that kills mites on contact. It is applied via a slow-release polymer strip impregnated with amitraz that is placed directly in the brood chamber. Two strips per brood chamber are used, spaced evenly apart, and are left in for 6-8 weeks. Bees come into contact with the strip and distribute the chemical throughout the colony. Apivar[®] is most effective during spring and fall when colonies have less brood and is not approved for use during honey production. Beekeepers must wait two weeks after removing Amitraz before supering hives. Apivar[®] was one of the first miticides to enter the market and is very effective at killing mites, but mite resistance to amitraz has also been reported numerous times (Maggi et al., 2010). For this reason, Apivar[®] is not to be used more than twice per year and should be alternated with other mite treatments. Amitraz is also known to accumulate in wax, which can further contribute to resistance issues.

<u>Checkmite+.</u>[®] Checkmite+[®] (A.I coumaphos) is a type of organophosphate. One treatment of Checkmite+[®] consists of two plastic strips, impregnated with coumaphos,

per brood box, spaced evenly apart. Strips are left in for a 6 week treatment period, during which bees come into contact with the coumaphos and spread it throughout the colony. Checkmite+[®] is not approved to be used during honey production, but hives can be supered immediately after removing strips, however it is most effective during the spring and fall when colonies have less brood. Resistance has been widely reported in mite populations to coumaphos (Pettis, 2004). It should not be used more than twice per year and should be alternated with other mite treatments. Coumaphos can also accumulate in wax which allows for the development of resistance even after treatment (Pettis, 2004). Furthermore, it can have negative effects on queen and drone reproductive health and should not be used in queen-rearing colonies (Pettis, 2004).

Formic Acid. Formic acid is the only miticide that targets varroa mites in their reproductive stage beneath capped brood cells. There are currently two approved formic acid mite treatments, Formic Pro[®] and Mite Away Quick Strips[®] (MAQS). MAQS[®] contain 46.7% formic acid in a saccharide gel strip. Strips are placed directly above the brood box. Two treatment options exist for MAQS[®]. The first is a full dose or two strips left in the hive for seven days. The second option is to use one strip at a time for 14 days and then replace it with a fresh strip for another seven days. Formic Pro[®] consists of 42.25% formic acid in a similar saccharide gel strip. Two treatment options also exist for Formic Pro[®]. Option one is two strips left in for fourteen days while option two is one strip for ten days followed by a fresh strip for another ten days. Both Formic Pro[®] and MAQS[®] are safe to use while producing honey, however MAQS[®] should only be used when temperatures are between 50°F and 85°F and Formic Pro should only be used when

brood production and queen health even when temperature guidelines are followed (source, summer). It is not uncommon for egg laying to be delayed or queens to be superseded during formic acid treatments. MAQS[®] has been on the market for a few years now while Formic Pro[®] is a newer product. From my research, I presume that Formic Pro[®] has a lower concentration of formic acid and releases vapor slower than MAQS[®] in an effort to alleviate some of the queen and brood loss that beekeepers have experienced during formic acid treatments.

<u>Hopguard II(I).</u> [®] Hopguard II[®] contains 16% potassium salt of hops beta acids in a cardboard strip that is placed directly in the brood chamber. Two strips per brood box are used and left in for four weeks. Hygienic behavior of the bees moves the sticky substances on the strips throughout the colony. Hopguard II[®] can be used up to three times per year and is safe to use when producing honey, however it is most effective when colonies are broodless or have very little brood, as it is unable to penetrate wax cappings. Hopguard II[®] is also a relatively new mite treatment, emerging in the past few years, and no resistance has been reported to it thus far. Some bee mortality has been observed when treating with Hopguard II[®], but only because the sticky nature of the treatment traps some adult bees and suffocates the brood it covers. A new formulation, Hopguard III[®] has recently entered the market, but is not yet approved for use in Maine

Oxalic Acid. Oxalic acid was approved by the EPA as a miticide to be used in honey bee colonies in 2015. There are two main methods by which oxalic acid can be applied, vaporization and via dribbling sugar syrup on bees. Vaporization is much more popular, however it can be much more expensive. The oxalic acid dihydrate itself is very cheap, but vaporizers can range from under \$100 to over \$400. One treatment of oxalic

acid consists of one gram per brood boxed applied via vaporization. Entrances should be plugged before vaporizing oxalic acid and it should be done at a time when most of the colony is in the hive. Since oxalic acid does not penetrate wax cappings, many beekeepers will treat via multiple applications of oxalic acid vapor, spaced 5-7 days apart to cover an entire brood cycle. Oxalic acid vaporization is not approved for use during honey production, however numerous studies suggest that residues in honey may not be significantly higher after treatment (Bogdanov et al., 2002; Papežíková et al., 2017). Oxalic acid via the dribble method, however, is approved to be used during honey production only in Europe. The dribble method involves 35 grams of oxalic acid being mixed into 1 liter of 1:1 sugar water and then trickled directly onto the bees in the brood box, 5 mL per space between frames. This same solution can also be sprayed onto package bees, ensuring they've been fed first. Oxalic acid is most effective when colonies are broodless or have very little brood. While oxalic acid has the potential to negatively impact brood and colony health, the main issue in regards to oxalic acid as a mite treatment is application inconsistency. The EPA pesticide label approves one gram of oxalic acid to be applied at a time per brood box. Since many beekeepers don't measure at all, the true amount of oxalic acid being applied to hives is often unknown. A new method for oxalic acid application, shop towels, is also an area of study today. While it is not yet approved for use, some studies suggest positive results from using shop towels soaked with an oxalic acid solution as a mite treatment (Oliver, 2011, 2013).

Collaborative Feedback (cont.)

Funding and Outreach. I received funding as an academic year student fellow from the University of Maine Center for Undergraduate Research (CUGR) to travel to bee clubs, present my research, and gather feedback for the third portion of this thesis. In January of 2020, I reached out to six bee clubs that were within driving distance of my apartment in Orono. Five of those had not yet booked speakers for February or March and offered me the opportunity to present my research at their monthly meetings. During February and March, I presented my research in-person to the Northern Penobscot Beekeepers, the Knox-Lincoln County Beekeepers, the Penobscot County Beekeepers Association, and the Somerset County Beekeepers. I had scheduled an additional inperson presentation to the Oxford Hills Honey Bee Club in late March, however it was cancelled due to COVID-19 facility closures and took place later via Zoom in April, albeit less interactively. During these presentations, I went through my research process and results from the summer of 2019 and introduced data from the Maine beekeeping surveys while requesting feedback the entire time and allowing for open dialogue. I then presented briefly the drafts of my outputs and again asked for feedback. Next, I will highlight a few of the most common themes discussed during my presentations with bee clubs and how each influenced the direction of my final outputs.

<u>FA/OA Treatment.</u> Beekeepers at all five clubs I visited, as well as some of those present at my presentation at the Maine State Beekeepers Association's annual meeting in October of 2019, expressed interest in the combined FA and OA treatment approach. Testing two treatments combined during honey production in line with the mite and honey bee life cycle makes for an interesting study, however not a very realistic treatment

strategy for most Maine beekeepers. For a commercial beekeeper with thousands of hives and high mite loads, it makes sense, but for an average Maine beekeeper with four hives in their backyard, it doesn't. A beekeeper may think a vaporizer is too expensive, or may not have the time or strength to manipulate honey supers and entrances on colonies that require treatment. While we experienced some success with this treatment approach and many Maine beekeepers expressed interest in it, it is unrealistic for a small-scale beekeeper to carry out such an elaborate mite treatment plan with proper monitoring.

Organic Standards. I chose to start the first draft of my decision-making tool with a question on organic standards based on the results from the Sweet Spot Survey. When asked how important organic standards were in making their mite treatment choices, nearly half of respondents said organic standards were important while the other half either were not concerned with organic standards or felt they were unimportant when making mite treatment choices. Organic standards, however, do not exist for honey produced in the United States. The USDA does not have a certification program or requirements for organic honey, yet organic honey is often found on the shelves of U.S. supermarkets. There are two main reasons for this. The first is that other countries have organic standards for producing organic honey that require certain feed, treatment, and extraction processes by beekeepers. They also require a certain radius of foraging habitat around the apiary to be free of agricultural, garden, and household chemicals and human or agricultural waste. Honey produced according to these standards in other countries can be sold as organic in the United States. The second main reason organic honey can be found in U.S. supermarkets is third party certification of honey produced according to the National Organic Standards Board organic livestock standards. These standards also

require a chemical-free forage area and certain beekeeping and honey extraction processes.

So, what do Maine beekeepers consider to be organic standards in beekeeping? Roughly 46% of survey respondents consider them important when choosing mite treatments, yet organic standards in beekeeping are somewhat of a grey area in terms of regulation in the United States. The general consensus on organic beekeeping, based on conversations with nearly 100 Maine beekeepers, is that the process of organic certification in the United States is unattainable. Many beekeepers may try to practice chemical-free beekeeping or organic gardening to maintain the integrity of their honey, but the reality is that honey bees fly for miles, and often come into contact with a variety of different substances during those foraging flights. Even if bees are kept on a 500-acre farm, the right of way 2 miles down the road is likely treated with herbicides on a regular basis. For this reason, organic certification is not plausible for many beekeepers in urban, suburban, or even agricultural settings. Since most beekeepers in Maine are small-scale, even organic honey sold at a premium price would likely not pay for the investment to become certified organic which can cost from hundreds to thousands of dollars. Furthermore, since there are few government regulations on organic honey, small producers could likely get away with labeling their honey as organic without consequence, as long as their operation remains small. Almost all of the beekeepers I spoke with at bee clubs were not willing to pay for organic certification at this time.

<u>Sustainable Practices?</u> I asked all five bee clubs their opinions on organic beekeeping standards, however I did not include it as the introductory question to my decision-making tool after the first two meetings. It became clear that the word organic

carries too much weight with it and organic honey standards cannot be realistically regulated or achieved in the majority of Maine beekeeping. I then began to ask bee club members what other standards or best practices they try to adhere to, if not organic. This connection between scale decisions and standards of beekeeping operations eventually came to be a focus point of my decision-making tool. While U.S. organic honey is unrealistic, several mite treatments are approved for use in organic apiaries elsewhere in the world. But at what point does an 'organic' beekeeper using formic acid to combat mites begin to question whether or not a chemical that can kill any plants within 6 inches of a hive and potentially the queen inside of it should really be considered organic? The organic acid mite treatments are only labeled organic because they are not made of synthetic chemicals and are naturally found in the environment. The true integrity of organic standards would favor practices that minimize harm and maximize sustainability for the bees, beekeepers, and consumers.

Ask Ten Beekeepers. After numerous conversations about mites, treatments, organic honey, non-organic honey, and who has packages available this season, I learned that beekeeping is much more than just a hobby or form of extra income to a lot of Maine beekeepers. Most devote an enormous amount of time to their yellow and black ladies and see beekeeping as a major part of their lives. As a result, there is not one set of rules or standards that any one of them applies to his or her beekeeping operation. Beekeeping is unique and so are the folks who do it. Each beekeeper has their own set of values that they adhere to. These values are often flexible and adapt with experience. It is important to replicate this flexibility in all aspects of beekeeping, but especially in mite treatments. There will always be a better treatment method, depending on the views of the beekeeper.

That is why it's important to consider beekeeper values in addition to colony status when approaching mite treatments because there is never one correct answer. As the old saying goes, ask ten beekeepers, get thirteen answers.

Integrated Mite Control (IMC)

Monitoring

Monitoring for mites is the first step to efficient mite control. Whether it be via alcohol wash, sticky board, or drone brood surveys, for beekeepers to combat mites and maintain healthy colonies they must stay on top of monitoring. More experienced beekeepers often choose to treat on a schedule based on successful treatments in the past. Monitoring is especially important for newer beekeepers because it allows the chance to learn about the mite and honey bee population cycle.

Life Cycle Approach

Regardless of the mite treatment used, it is important to have a treatment strategy. The easiest and, in my opinion, the best strategy is to treat in accordance with the mite and honey bee reproductive cycles. Each treatment on the market has pros and cons that can be best utilized if treatments are done with the colony life stage in mind. For example, OA treatments are most effective when colonies have little or no capped brood. If a colony is observed to have no capped brood, a timely OA treatment can easily knock mite infestation levels to below treatment threshold.

Hive and Environmental Conditions

Noting the stage colonies and therefore varroa mites are at in their population cycles is a part of observing hive conditions, but it is not the only aspect of colony status that beekeepers should make note of. It is also important to observe colony strength, queen health, and any other signs of poor health that might impact the effectiveness of mite treatments. Regardless of the reported harm that each treatment causes to the bees, the bees are still being exposed to pesticides or manipulation during treatment. This is inevitable, and it is in the best interest of beekeepers to ensure that hives are ready for treatment on an individual basis. It is also in the best interest of beekeepers to monitor the weather when treating for mites. Most miticides have temperature guidelines that can cause adverse effects on the colony if not followed correctly.

Beekeeping Standards of Operation

As mentioned above, standards of operation must be considered in every aspect of beekeeping, including mite treatments. Beginner beekeepers may wish to use natural mite treatment methods to minimize harm to the bees, but often lack experience to effectively treat. For this reason, I have attempted to make a tool that incorporates the three aspects of beekeeping described above, but also whichever standards individual beekeepers wish to maintain in their honey production.

IMC- Interactive Decision-Making Tool

Appendix C shows the final (current) decision-making tool. It is modeled after a few industry sustainability recommendation websites such as seafoodwatch.org from

Monterey Bay Aquarium. This website allows consumers to choose a category of fish and see multiple options that rank from most to least sustainable based on their research and recommendations. I have created a framework similar in the decision-making tool while basing recommendations on things such as hive and environmental conditions, colony growth stage, and infestation levels. As mentioned previously, this tool is supposed to replicate the diversity of beekeepers and mite treatment options and is subject to future change as both continue to evolve.

In Conclusion

Mites and Treatments

Varroa mites and honey bees have a long, complex history that is still working itself out today. Most beekeepers recognize varroa mites as a permanent resident of honey bee hives that must be dealt with regularly to prevent colony losses. As the parasite-host interaction of varroa mites and honey bees continues to evolve, so will the research on honey bees, varroa mites, and mite treatments. In the words of longtime Maine beekeeper Matt Scott, "If the bees are doing fine, the mites are doing fine."

From wanting to initially do research on treating mites with powdered sugar to holding my own in conversations with veteran beekeepers, I feel I have come a long way in regards to varroa mite treatments. Based on my research, I can conclude that so has the field of varroa mite treatments as a whole. It truly is an ongoing arms race between mites and bees, and successful beekeepers are those that stay on top of mite management. Information on mite treatments and the treatments themselves must also constantly be adapting. This brings me again to the importance of stakeholder involvement, which should play a major part in the creation of any mite treatment information source.

Maine Beekeeping

Beekeeping has been and will continue to be an important part of Maine agriculture, industry, and culture for some time. Most beekeepers in Maine are older and keep only a few hives, however my experience with beekeepers suggests that it is growing as a hobby or even profession in more young people. Through conversing with, working with, and sometimes just hanging out with Maine beekeepers, I have developed this thesis with its outputs to act as a mite treatment information source to articulate the relevant needs of beekeepers in Maine and elsewhere.

This thesis would not have been possible without the continuous support I received from the Maine beekeeping community. Stakeholder engagement has been the most valuable asset to my research, and I learned a tremendous amount of beekeeping information from the friendly Maine beekeeping community. While beekeeping is considered to be a somewhat solitary hobby, passion, or profession, it is through the continuous collaboration of beekeepers that beekeeping continues to evolve and mites continue to be combatted. I know now more than ever how useful and impactful this collaboration can be.

The beekeepers of Maine are extremely passionate about their hobby, interest, side gig, or job of beekeeping, whatever it may be to them. They are an immense resource that constantly exchanges knowledge on everything from mite treatments to honey recipes. It was a great pleasure to have the opportunity to learn from them and get

a sense of their passion for honey bees. In my four years in Orono, I have learned there are two major staples in Maine—Mainahs and blueberries. As long as there is one of those two things, there will also be bees.

IMC Moving Forward

With more time and funding for this research, I would have likely turned the IMC decision-making approach into an online platform. As mentioned previously, the recommendations made in the decision-making tool are subject to change with future mite treatments and research.

REFERENCES

- Anderson, D. L., & Trueman, J. W. H. (2000). Varroa jacobsoni (Acari: Varroidae) is more than one species. 25.
- Bayer Bee Care. (2019). The Varroa Mite. Bayer AG.
- Bogdanov, S., Charrière, J. D., Imdorf, A., Kilchenmann, V., & Fluri, P. (2002). Determination of residues in honey after treatments with formic and oxalic acid under field conditions. https://doi.org/10.1051/apido:2002029
- Brick, J. M., Andrews, W. R., Brick, P. D., King, H., Mathiowetz, N. A., & Stokes, L. (2012). Methods for Improving Response Rates in Two-Phase Mail Surveys.
 Survey Practice, 5(3), 1–6. https://doi.org/10.29115/SP-2012-0016
- Bunker, J. (2008). Keynote: The Apples of Maine.

https://www.mofga.org/Publications/The-Maine-Organic-Farmer-

Gardener/Winter-2008-2009/Bunker-Keynote

Calderón, R. A., Ortiz, R. A., Arce, H. G., van Veen, J. W., & Quan, J. (2000).
Effectiveness of Formic Acid on Varroa Mortality in Capped Brood Cells of Africanized Honey Bees. *Journal of Apicultural Research*, *39*(3–4), 177–179. https://doi.org/10.1080/00218839.2000.11101039

- Cheremisinoff, N. P., & Rosenfield, P. E. (2010). *Formic Acid—An overview* | *ScienceDirect Topics*. https://www.sciencedirect.com/topics/earth-and-planetarysciences/formic-acid
- Cotton, L. E. (1887). *The Controllable Bee Hive and New System of Bee Management:* 1887 Annual Circular. 24.

De Jong, D., De Andrea Roma, D., & Gonçalves, L. S. (1982). A COMPARATIVE ANALYSIS OF SHAKING SOLUTIONS FOR THE DETECTION OF VARROA JACOBSONI ON ADULT HONEYBEES. *Apidologie*, 13(3), 297– 306. https://doi.org/10.1051/apido:19820308

De Jong, D., Morse, R. A., & Eickwort, G. C. (1982). Mite Pests of Honey Bees. Annual Review of Entomology, 27(1), 229–252. https://doi.org/10.1146/annurev.en.27.010182.001305

Delaplane, K. S., van der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of Apis mellifera colonies. *Journal of Apicultural Research*, 52(1). https://doi.org/10.3896/IBRA/1.52.1.03

Dillman, D. A., Smyth, J. D., & Christian, L. M. (2014). *Internet, Phone, Mail, and Mixed-Mode Surveys: The Tailored Design Method*. John Wiley & Sons.

EPA. (n.d.). *EPA Oxalic Acid Dihydrate MSDS*. Retrieved June 26, 2019, from https://www3.epa.gov/pesticides/chem_search/ppls/091266-00001-20151013.pdf

Gregorc, Ale?, Pogacnik, A., & Bowen, I. D. (2004). Cell death in honeybee (Apis mellifera) larvae treated with oxalic or formic acid. Apidologie, 35(5), 453–460. https://doi.org/10.1051/apido:2004037

Gregorc, Aleš, Adamczyk, J., Kapun, S., & Planinc, I. (2016). Integrated varroa control in honey bee (*Apis mellifera carnica*) colonies with or without brood. *Journal of Apicultural Research*, 55(3), 253–258.

https://doi.org/10.1080/00218839.2016.1222700

Gregorc, Aleš, Alburaki, M., Werle, C., Knight, P. R., & Adamczyk, J. (2017). Brood removal or queen caging combined with oxalic acid treatment to control varroa mites (Varroa destructor) in honey bee colonies (Apis mellifera). *Apidologie*, *48*(6), 821–832. https://doi.org/10.1007/s13592-017-0526-2

- Gregorc, Aleš, & Planinc, I. (2001). Acaricidal effect of oxalic acid in honeybee (apis mellifera) colonies. *Apidologie*, 32(4), 333–340. https://doi.org/10.1051/apido:2001133
- Hall, D., Silka, L., & Lindenfeld, L. (2012). Advancing Science and Improving Quality of Place: Linking Knowledge with Action in Maine's Sustainability Solutions Initiative. 9.
- Hatjina, F., & Haristos, L. (2005). Indirect effects of oxalic acid administered by trickling method on honey bee brood. *Journal of Apicultural Research*, 44(4), 172–174. https://doi.org/10.1080/00218839.2005.11101174
- Higes, M., Meana, A., Suárez, M., & Llorente, J. (1999). Negative long-term effects on bee colonies treated with oxalic acid against Varroa jacobsoni Oud. *Apidologie*, 30(4), 289–292.

Honey Bee Health Coalition. (2018). Honey Bee Health Coalition Varroa Guide.

Huang, Z. (2013). Varroa Mite Reproductive Biology. Extension, 19.

- Jansujwicz, J. S., Calhoun, A. J. K., & Lilieholm, R. J. (2013). The Maine Vernal Pool Mapping and Assessment Program: Engaging Municipal Officials and Private Landowners in Community-Based Citizen Science. *Environmental Management*, 52(6), 1369–1385. https://doi.org/10.1007/s00267-013-0168-8
- Lund, J., & Skyrm, K. (2019). Varroa Mite IPM Program for New England Honey Beekeepers. 60.

- Maggi, M. D., Ruffinengo, S. R., Negri, P., & Eguaras, M. J. (2010). Resistance phenomena to amitraz from populations of the ectoparasitic mite Varroa destructor of Argentina. *Parasitology Research*, 107(5), 1189-. Academic OneFile.
- Milbrath, M. (2016). *KEEPING HONEY BEE COLONIES SAFE FROM THE VARROA MITE*. 12.
- Moro, A., & Mutinelli, F. (2019). Field evaluation of *Maqs* [®] and *Api-Bioxal* [®] for late summer control of *Varroa* mite infestation in Northeastern Italy. *Journal of Apicultural Research*, 58(1), 53–61.

https://doi.org/10.1080/00218839.2018.1494921

- NOD Apiary Products. (n.d.). *MAQS*® *Label*. Retrieved September 7, 2019, from http://nodglobal.com/wp-content/uploads/2016/03/US-M-PL-003.pdf
- Oliver, R. (2011, February 21). *The Learning Curve Part 3: The Natural Miticides*. Scientific Beekeeping. http://scientificbeekeeping.com/the-learning-curve-part-3-the-natural-miticides/
- Oliver, R. (2013, August 22). *Mite Management Update 2013*. Scientific Beekeeping. http://scientificbeekeeping.com/mite-management-update-2013/
- Oscarson, D. B., & Calhoun, A. J. K. (2007). Developing vernal pool conservation plans at the local level using citizen-scientists. *Wetlands*, 27(1), 80–95. https://doi.org/10.1672/0277-5212(2007)27[80:DVPCPA]2.0.CO;2
- Ostermann, D. J., & Currie, R. W. (2004). Effect of Formic Acid Formulations on Honey Bee (Hymenoptera: Apidae) Colonies and Influence of Colony and Ambient

Conditions on Formic Acid Concentration in the Hive. *Journal of Economic Entomology*, *97*(5), 1500–1508. https://doi.org/10.1603/0022-0493-97.5.1500

- Papežíková, I., Palíková, M., Kremserová, S., Zachová, A., Peterová, H., Babák, V., & Navrátil, S. (2017). Effect of oxalic acid on the mite Varroa destructor and its host the honey bee Apis mellifera. *Journal of Apicultural Research*, 56(4), 400–408. https://doi.org/10.1080/00218839.2017.1327937
- Peck, David T., Smith, M. L., & Seeley, T. D. (2016). Varroa destructor Mites Can Nimbly Climb from Flowers onto Foraging Honey Bees. *PLoS ONE*, 11(12), e0167798. Gale In Context: Opposing Viewpoints.
- Peck, David Thomas, & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and robbing in Varroa destructor transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, *14*(6), e0218392. Opposing Viewpoints in Context.
- Pettis, J. S. (2004). A scientific note on *Varroa destructor* resistance to coumaphos in the United States. *Apidologie*, *35*(1), 91–92. https://doi.org/10.1051/apido:2003060

Pietropaoli, M., & Formato, G. (2018). Liquid formic acid 60% to control varroa mites (*Varroa destructor*) in honey bee colonies (*Apis mellifera*): Protocol evaluation. *Journal of Apicultural Research*, 57(2), 300–307.

https://doi.org/10.1080/00218839.2017.1376767

Queensland Department of Agriculture and Fisheries. (2013). Varroa mites. https://www.daf.qld.gov.au/business-priorities/biosecurity/animal-biosecuritywelfare/animal-health-pests-diseases/beekeeping-in-queensland/diseases-andpests/asian-honey-bees/varroa-mites

- Raffiudin, R., & Crozier, R. H. (2007). Phylogenetic analysis of honey bee behavioral evolution. *Molecular Phylogenetics and Evolution*, 43(2), 543–552. https://doi.org/10.1016/j.ympev.2006.10.013
- Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., Lim, D., Joklik, J., Cicero, J. M., Ellis, J. D., Hawthorne, D., & vanEngelsdorp, D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences*, *116*(5), 1792–1801. https://doi.org/10.1073/pnas.1818371116
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of Varroa destructor. *Journal of Invertebrate Pathology*, *103*, S96–S119. https://doi.org/10.1016/j.jip.2009.07.016
- Scott, M. (2020, April 3). *ME Beekeeping History* [Phone call].
- Spivak, M. (1996). Honey bee hygienic behavior and defense against Varroa jacobsoni. *Apidologie*, 27(4), 245–260. https://doi.org/10.1051/apido:19960407
- *Title 1, §214: State insect.* (1975).

https://legislature.maine.gov/statutes/1/title1sec214.html

- Traynor, K., Mondet, F., Miranda, J. de, Techer, M., Kowallik, V., Oddie, M., Chantawannakul, P., & McAfee, A. (2020). Varroa destructor: A Complex Parasite, Crippling Honeybees Worldwide. https://doi.org/10.20944/preprints202002.0374.v1
- Trouiller, J. (1998). Monitoring Varroa jacobsoni resistance to pyrethroids in western Europe. *Apidologie*, *29*(6), 537–546.

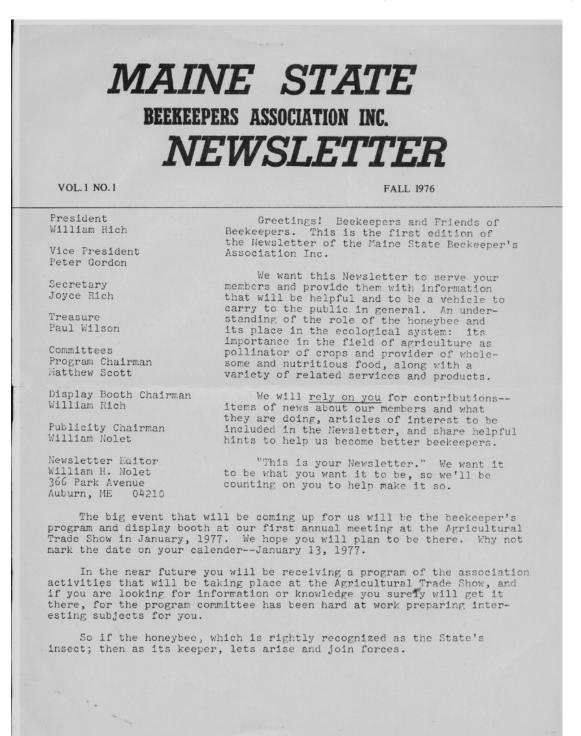
- UMaine Cooperative Extension. (n.d.). Fact Sheet 629-Honey Bees and Blueberry
 Pollination. *Cooperative Extension: Maine Wild Blueberries*. Retrieved April 19, 2020, from https://extension.umaine.edu/blueberries/factsheets/bees/629-honey-bees-and-blueberry-pollination/
- Underwood, R. M., & Currie, R. W. (2003). The effects of temperature and dose of formic acid on treatment efficacy against Varroa destructor (Acari: Varroidae), a parasite of Apis mellifera (Hymenoptera: Apidae). 12.
- vanEngelsdorp, D., & Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology*, 103, S80–S95. https://doi.org/10.1016/j.jip.2009.06.011
- Yang, X., Cox-Foster, D. L., & Berenbaum, M. R. (2005). Impact of an Ectoparasite on the Immunity and Pathology of an Invertebrate: Evidence for Host Immunosuppression and Viral Amplification. *Proceedings of the National Academy of Sciences of the United States of America*, 102(21), 7470–7475.
 JSTOR.

Yarborough, D. (2018). History of Maine's Wild Blueberry Industry. 8.

APPENDICES

APPENDIX A

FIRST NEWSLETTER PUBLISHED BY THE MSBA (FROM MATTHEW SCOTT)



APPENDIX B

2019 MAINE HONEY PRODUCER (SWEET SPOT) SURVEY,

RELEVANT QUESTIONS

2019 Maine Honey **Producer Survey**



o by Greg A. Ha





About this survey

About this survey This survey is part of a study administered by researchers at the University of Maine and College of the Atlantic. A version of this survey is being distributed to all registered honey producers in Maine. This survey will help us understand more information about honey production and beekeeping in the state of Maine and help improve beekeeping outreach and education. If you decide to complete this survey it will take approximately 30 minutes. All data from this survey will be kept confidential, and no personal names or personal information will ever be released. Your privacy is our top priority. Thank you for helping us support the continued viability and sustainability of honey production and pollination in the state of Maine!

Distance to potential 1 out-yards (e.g. driving distance/time)		2	3	4	5
Section E. Varroa mite	treatment	8			
33. What mite treatment(s) did yo that apply.	u apply betwe	een August 2	2018-August 201	9. Please check all	
□ None (thymol)	🗆 Apigu	ard (thymol) [] Api Life Var	
Apistan (fluvalinate)	🗆 Apiva	r (amitraz)	C	Brood cycle	
CheckMite+ (coumaphos) (formic acid)		e brood trap	ping(removal) [Formic Pro	
Mite-Away Quick Strips (forr vaporization	nic) 🗌 Oxali	c acid dribbl	e D	Oxalic acid	
Powered sugar dusting	□ Scree	ened bottom	board		

34. How important were the following factors in choosing the mite treatment(s) that you did? *Please circle one number for each row.*

Other *Please list:*

	Very unimportant	Unimportant	Neutral	Important	Very important
Price of the treatment	1	2	3	4	5
Availability of the treatment	1	2	3	4	5
Effectiveness on mites	1	2	3	4	5
Health impacts on the bees	1	2	3	4	5
Impact on the quality of honey product	1	2	3	4	5
Ease of application	1	2	3	4	5

35. Based on your mite treatment(s), have you noticed any of the following adverse effects? *Please check all that apply.*

No adverse effects	Queen health (reduced egg laying)	Adult
mortality		
Reduced brood area	Removal of capped brood	
□ Other Please list:		

36. How effective would you rate your treatments against mites between August 2018-	17
August 2019. Please check one.	

□ Not at all effective	Somewhat effective	Neutral	Somewhat effective	
Very effective				

Section G. Honey Producer Demographics

48. What is your age? Please check one.

□ 18-25	□ 25-34	□ 35-44
□ 45-54	□ 55-64	□ Older than 65

19
49. What level of formal education have you completed? <i>Please check one.</i> □ High school, no degree earned □ High school diploma or GED □ College, no degree earned
College, degree earned College, degree earned school, no degree
Advanced degree (e.g., MS, PhD, JD)
50. What is your gender? <i>Please list:</i>
51. How many years have you been beekeeping and producing honey? <i>Please list:</i>
52. How many years have you been selling your honey/beekeeping products? <i>Please list:</i>
53. Do you have an occupation outside of beekeeping and honey production? <i>Please check one.</i>
□ Yes □ No
 54. How dependent is your economic livelihood on the success of your beekeeping and honey enterprise? <i>Please check one.</i> Completely dependent Considerably dependent Neutral Slightly dependent Not at all dependent
 55. Are you currently a member of the Maine State Beekeepers Association (MSBA)? Please check one. □ Yes □ No
56. What concerns do you have about pollination and honey production in Maine? <i>Please</i> respond in the space below.

APPENDIX C

FINAL (CURRENT) DRAFT OF THE IMC DECISION-MAKING TOOL

Part 1: Decision-Making Tool

1.	Are yo	ou treating while producing honey?		
	a.	Yes		go to 2
	b.	No		go to 8
2.	(from	1a.) Is there capped brood in your hive?		
	a.	Yes		go to 3
	b.	No		go to 7
3.	(from	2a.) Is your hive strong (8+ frames of bees) a	nd queen healt	hy/laying?
	a.	Yes		go to 4
	b.	No		Hopguard II®
4.	(from	3a.) Is the temperature outside between 50°F	and 85°F?	
		Yes		go to 5
	b.	No		go to 7
5.	(from	4a.) Is colony infestation level above 5%?		
		Yes		go to 6
		No	Formic Acid,	Hopguard II®
6.		5a.) Do you have access to a vaporizer?		
		Yes	Formic Acid -	1
		No	Formic Acid,	Hopguard II®
7.	•	2b.) Do you have access to a vaporizer?		
		Yes		OA Vapor
_		No		Hopguard II®
8.		1b.) Do you have access to a vaporizer?		
		Yes		OA Vapor
_		No		go to 9
9.	•	8b.) Do you plan to put honey supers on in th	ne next 30-40 d	-
		Yes		go to 10
		No		go to 11
10.	(from	9a.) Is your hive strong (8+ frames of bees) a	nd queen healt	
	a.	Yes		Formic Acid
		No		Hopguard II®
11.	•	9b.) Are you opposed to using synthetic mitie	eides or have y	ou used
	•	tic miticides in the past three years?		. 10
		Yes		go to 12
		No		go to 13
	c.			

12. (from 11a.) Do you plan to put on honey supers in the next 60 days?

- a. Yes Apiguard®, Hopguard II®
- b. No Api Life Var®
- 13. (from 11b.) Do you use this colony for queen production?
 - a. Yes Apistan®, Apivar®
 - b. No Apistan®, Apivar®, Checkmite+®

*OA Vapor is not yet approved to be applied to hives with honey supers on, however evidence suggests that it does not pose any serious risk. Measures should be taken, either by removing honey supers or placing a barrier between them and the brood box(es) if using OA vapor during honey production.

Part 2: Mite Treatment Sustainability Rankings

I have rated each available treatment on my own personal scale(1-5) based on literature review and personal anecdotes. There are three categories that contribute to the overall score, based off the considerations listed below:

Efficacy	<u>Health</u>	Ease of App
-efficacy on mites	-impact on colony health	-labor and time
-resistance	-risk to beekeeper health	-equipment/considerations

Scores highlighted in green are the best choices, in my opinion, based on the reasons listed below each treatment. Category scores highlighted in green (4.2-5.0) indicate how well a treatment meets the relevant components of that category.

Scores highlighted in yellow may be good choices, but have extenuating treatment circumstances or other factors that should be considered when using these treatments. Category scores highlighted in yellow (3.0-4.19) indicates a treatment adequately, but not superiorly meets the relevant components of that category.

Scores highlighted in red are poor mite treatment choices, in my opinion. The conditions listed below each category indicate why each overall or individual score highlighted in red failed to meet the relevant components of that category. Scores ranked as red were 2.99 or lower.

Hopguard II® - 4.67



Efficacy-

Hopguard II® does not reach mites within capped brood cells and for this reason is usually most effective in the spring or fall when colonies have little or no brood, or after a brood cycle disruption.

Health-

Hopguard II® contains potassium salts of hops beta acids that kill mites but are not harmful to bees or beekeepers. The sticky substance on the strips can be a skin irritant, but is easy to avoid. The same substance can also suffocate brood directly beneath the strips and trap bees that get caught in it, however overall colony losses are minimal. Application is also minimally invasive to the colony. Hopguard II® can be used while producing honey

Ease of Application-

The application process for Hopguard II® is very easy. One treatment consists of two cardboard strips per brood box placed in between brood frames. Hopguard II® can be used while producing honey and treatments only last about two to four weeks, after which most of the strips have usually been removed by the bees' hygienic behavior. If Hopguard II® is being used during honey production, application will involve temporary removal of honey supers, which can be an additional labor concern, so that strips can be placed in the brood box. Hopguard II® is also relatively low in cost compared to other name brand mite treatments.

Formic Acid – 4.23

	<u>Efficacy</u>	<u>Health</u>	Ease of App	<u>Overall</u>
Formic Acid	5	3.5	5	4.5

Efficacy-

Formic acid is the only miticide currently available on the market that targets both phoretic varroa mites and those reproducing inside capped brood cells.

Health-

Formic acid can have negative effects on queens, brood, and adult bees. Capped and uncapped brood can suffocate if formic vapors block the available oxygen. Queens usually stop laying eggs and all uncapped brood is usually removed during the first three days of treatment with formic acid. Queen and adult bee deaths may also occur, especially in colonies with older queens. Formic acid can also be harmful to beekeepers, but minimal PPE in the form of eyewear, long sleeves, and gloves is required, most of which beekeepers wear anyway.

Ease of Application.

Formic acid pads are very easy to apply. Honey supers must be removed, which may be a labor concern, but pads are placed directly on top of the brood box and left alone for the duration of the treatment. Minimal PPE is required and treatments last as little as one to as many as three weeks.



Formic Acid Plus Oxalic Acid Vapor - 4.33

Efficacy-

Both formic and oxalic acid are effective mite treatments alone, but combining them has the potential to increase efficacy even more. By treating with oxalic acid 10-12 days after formic pads are applied, it gives the opportunity to target mites that may have survived the initial formic treatment before they have the opportunity to crawl back into cells about to be capped. Treating as such in line with the reproductive cycle of varroa mites has the potential to target the majority of both phoretic and reproductive mites (see Figure 16 above).

Health-

Formic acid can have adverse effects on honey bee colonies, especially on weaker colonies and at higher temperatures. For this reason, it is important to monitor colony strength and queen health both before and after treatment. Oxalic acid has the potential to harm adult bees and brood, but adverse effects with oxalic acid are less frequent and less severe.

Beekeepers must wear a respirator, long sleeves, and gloves when applying oxalic acid via vapor because it is toxic to humans. Long sleeves and gloves should also be worn when applying formic acid pads and direct contact with the pads should be avoided. Beekeepers should also avoid entering hives during formic acid treatments, and during the first few days especially, due to irritating and potentially harmful fumes. <u>Ease of Application-</u>

Formic acid pads are very easy to apply, but they do require honey supers to be temporarily removed to access the brood box. Applying oxalic acid via vaporization is a quick and easy process, however preparation can be time-consuming and expensive. Vaporizers can cost hundreds of dollars and must be loaded with a specific dose per hive for proper treatment. Oxalic acid is also not approved to be applied during honey production, so supers must either be removed or separated from brood boxes via a barrier to comply with food safety guidelines during this treatment. Formic acid, on the other hand, is approved to be used during honey production. Since many OA treatment strategies require consecutive applications over multiple weeks, this approach can save time and reach similar, if not better, efficacy.

Oxalic Acid Vapor - 4.27



Efficacy-

Oxalic acid is very effective at killing phoretic mites, but it does not target mites in their reproductive stage within capped brood cells. For this reason, multiple applications over a three- or four-week period are typically done with oxalic acid vapor to target mites emerging throughout a brood cycle. <u>Health-</u> Oxalic acid vapor has minimal negative effects on adult bees. Brood and adult bees exposed to too much vapor can die, but the colony is usually minimally affected. Oxalic acid vaporization does require the use of proper PPE, however, as vapor can be toxic to humans. With proper PPE, there are little to no negative health affects for beekeepers applying oxalic acid vapor.

Ease of Application-

Since a vaporizer must be purchased or built, applications can be time consuming or expensive. Oxalic acid dihydrate is very inexpensive, however, and beekeepers with numerous hives might find it cost effective to purchase a vaporizer. PPE also must be purchased before applying OA vapor. The application process itself, however, is very simple and minimally invasive. Vaporizers are usually inserted into hive entrances or a separate hole that doesn't require the hive to be taken apart, and treatments can take just seconds per hive depending on the vaporizer. Hive entrances must also be reduced or plugged during treatments.

Apiguard® - 4.23

	<u>Efficacy</u>	<u>Health</u>	Ease of App	<u>Overall</u>
Apiguard ®	4	4	4.7	4.23

Efficacy-

Apiguard[®] can be up to 80-90% effective, but it does not penetrate capped brood and this efficacy is over a treatment period of about 40 days. It is most effective when colonies have little or no brood present.

Health-

Thymol fumes from Apiguard® can be harmful to humans, so beekeepers must wait at least 48 hours after application before entering hives again. These fumes can also be toxic to adult bees and suffocate developing brood, especially at higher temperatures. Ease of Application-

Apiguard® is extremely ease to apply. The gel or gel tray is placed on top of the brood box and no hive manipulation is required since it can't be applied during honey production. A treatment length of about 40 days however prevents beekeepers from treating too close to honey season.

Api Life Var® - 4.17



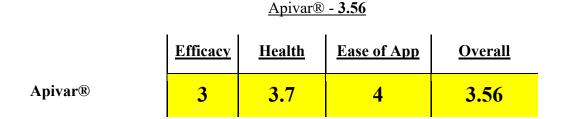
Efficacy-

Api Life Var® releases thymol, menthol, and eucalyptus oil fumes that kill phoretic mites on contact, but do not penetrate wax cappings. It is most effective during the fall or early spring when colonies have little to no brood. Health-

Api Life Var® can be toxic to humans if contacted, swallowed, or if fumes are inhaled, however risk during treatment is minimal, especially when proper PPE such as gloves and long sleeves, is worn. Api Life Var® also has the potential to cause brood and adult bee mortality, but only becomes a real concern as temperatures reach the maximum allowed treatment temp. of 94°F.

Ease of App-

Api Life Var® is very easy to apply. Tablets are broken into pieces and placed towards the perimeter of the hive, directly above the brood box. Tablets are usually replaced in intervals, which can cause labor to add up, however no honey supers will have to be removed as it is not approved for use during honey production. Furthermore, supers can't be put on hives until thirty days after the treatment ends, which can last for up to thirty days itself.



Efficacy-

Apivar® was one of the first miticides to enter the market once varroa mites became an issue. It has been effective at treating mites since then, however numerous cases of resistance have been reported. For this reason, it is important for beekeepers who use Apivar® to rotate mite treatments, treat with Apivar® no more than twice per year and to not use Apivar® for more than two consecutive years. Apivar® also does not penetrate wax cappings and is most effective when colonies have little or no brood. <u>Health-</u>

Apivar® is harmless to humans if applied properly and has minimal effects on colony or individual bee health on an individual treatment basis, but repeated doses can lead to accumulation in beeswax. This can cause development issues and potentially create a safety risk for beeswax products.

Ease of Application-

Apivar® is very easy to apply. Two plastic strips are placed directly over frames in the brood chamber and left alone for the duration of the treatment, which cannot be done during honey production. One Apivar® treatment lasts 40-50 days.

Apistan[®] - 3.0

	<u>Efficacy</u>	<u>Health</u>	Ease of App	<u>Overall</u>
Apistan®	3	2	4	3.0

Efficacy-

Apistan® is also a synthetic miticide that has been effective for years, but numerous cases of resistant mites have been reported since its introduction. Apistan® treatments should be alternated with other treatments and done no more than twice per year. It is most effective during the fall or early spring when colonies are as close to broodless as possible.

<u>Health-</u>

Apistan® can significantly accumulate in wax and can lead to lethal doses for adult bees and brood if combs are exposed too to the chemical too frequently. Tau-fluvalinate is harmful to humans, but if applied properly, there is minimal risk. <u>Ease of App-</u>

One treatment of Apistan[®] consists of two plastic strips per brood box, placed directly over brood frames. It is very easy to apply, cannot be used with honey supers, and must be left in for 40-50 days.

Checkmite+® - 2.67



Efficacy-

Checkmite+® is unable to penetrate wax cappings and targets only phoretic mites. It is most effective during the fall or early spring when colonies have little or no brood present. Like the other synthetic miticides, numerous cases of resistance to coumaphos have been reported. Checkmite+® should not be used more than twice per season and should be alternated with other mite treatments.

<u>Health-</u>

Checkmite+® can accumulate in wax and propolis over time and lead to brood and adult bee death if repeated exposure causes lethal levels to occur. It is also known to have adverse effects on queen health and for that reason should not be used in queenrearing colonies or colonies with weaker queens.

Ease of Application-

Checkmite+® is very easy to apply. One application consists of two plastic strips placed directly in the brood chamber. It cannot be used during honey production and treatments last 40-50 days, so beekeepers must plan accordingly around honey season.

Ingredient Amitraz Coumaphos (org Fluvalinate Formic Acid (Oxalic Acid (A carricide Synthetic chemical (amidine) Synthetic chemical Organic a cid Organic a cid	Product Name Apivar [®] Apistan [®] Formic Pro Formic Pro Birips Strips [®] (MAQS) Oxalic acid Dihydrate	Application Method(s) Plastic strip Plastic strip Gel pad Gel pad vaporizer dribbe	Treatment Length 6-10 weeks 42.45 days 45 days 14-20 days 7-21 days 7-21 days varies varies	Homey Safe? No No Yes Yes	Mite Resistan Yes Yes No No	Product Name Apivar® Check- mite+® Apistan® Formic Pro Formic Pro Mite-Away Quick Strips ®(MAQS) ®(MAQS) OA- Vaporizer OA- Dribble	Cost per Treatment 57.20 (Da.dant) 58 86.80 (Da.dant) 55.70 (Da.dant) 55.70 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.20 (Da.dant) 55.70 (Da.dant) 55.70 (Da.dant) 55.70 (Da.dant) 55.70 (Da.dant) 56.80 (Da.dant) 55.70 (D	Notes Wait 14 days after removal to put on supers Don't use in queen- rearing colonies Some toxicity to queen and workers and workers De lays egg laying for3 days, may cause and workers to a queen death, or removal of capped throod. Penetrates wax cappings* Most effective when the atment period
		_1	Shop towel	varies			O.A. Shop Towel	~\$0.2.7 ²	Only experimental, not yet approved for mite control
Hops Beta (Organic acid	Hopguard 11 ⁸	Cardboard Strips	14-30 days	Yes	No	Hopguar d 11 ²	\$4.17 (MannLako)	Brood removed behind strip, most effective when broodless
Thymol	Essential oil	Apiguard®	Gel or Gel Trap	4-6 weeks	No	No	Apiguard®	\$7 (Du dant)	Fumes can be harmful in high temps (>100° F)
		Api Life Var®	Tablet	21-30 days	No	No	Api Life Var [®]	\$7.50 (MannLake)	Wait 30 days after tablet removal to super

APPENDIX D: MITE TREATMENT INFORMATION TABLE

price per treatment not including cost of vaporizer (range from <\$100.5500)
 price per ½ shop towel. Directions from http://scientificbeekeeping.com/oxalic-shop-towel-updates/
 also contains menthol and eucalyptus oil *only treatment that penetrates cappings

APPENDIX E

IRB APPLICATION AND LETTER OF APPROVAL FOR STAKEHOLDER-

INVOLVED RESEARCH DURING THE SWEET SPOT SUMMER RESEARCH

FELLOWSHIP

Part 1: Application and Proposal

Proposal

Sara Velardi

IRB Application for Finding the Sweet Spot: Survey of Scale Challenges and Opportunities for Maple Syrup Production and Beekeeping in Maine

Summary of Proposal:

Due to increased industrialization and consolidation of the farming sector in the United States, larger farms are becoming more prevalent across the farming landscape, achieving economies of scale with fewer input costs and greater outputs (USDA-ERS 2018). While traditional economics argues that larger farmers are more efficient than small- to medium-sized farms (Paul et al. 2004; Hoppe et al. 2010; MacDonald et al. 2013), small- and medium-sized farms provide a multitude of economic, social, and nutritional benefits including restoring natural resources and supporting rural communities (USDA-NIFA 2018; Marsden et al. 2002).

The state of Maine is comprised predominantly of small- and medium-sized farms and with the oldest population in the U.S., farming has been viewed as a solution to prevent the mass exodus of young people from the state (Beal and Jemison 2011). Specifically, there has been renewed interest in the maple and beekeeping industries in Maine as both have experienced significant growth within the last ten years. Maine's maple syrup production doubled between 2010-2016 and with the state's abundant resource base of sugar (Acer saccharum) and red (Acer rubrum) maple, there is significant potential for further growth and expansion (USDA 2015). Beekeeping in Maine has also become more popular, similar to trends throughout the U.S., due to increasing awareness of the threats facing honey bees (Apid mellifera), native bees, and other crop pollinators as well as greater emphasis on producing and consuming local food (e.g. the Maine Food Strategy, Food Solutions New England) (Goulson et al. 2015; Potts et al. 2010). In Maine, the number of beekeepers have increased from 379 to 909 in the last decade and the number of colonies have increased by almost 4,000. As each of these industries are becoming vital sources of Maine's growing economy and farm base, and capable of expansion and growth, it is important to understand how producers are making scale management decisions in terms of increasing size and scope of their business operations. By evaluating current business characteristics of these industries including technology use, services offered, types of products, marketing choices and size as well as producers' intentions in terms of scale decisions, and the challenges and opportunities they face in making such decisions, we can gain an understanding of how these industries are currently operating, expectations for growth and expansion in the future, and ways in which to facilitate and support producers' scale management decisions.

In addition, we intend to assess forms of cooperation among producers and beekeepers in Maine. Formal and informal cooperation among farmers have served as a helpful resource for small-scale and/or beginning farmers with initial high input costs or land and labor requirements where farmers can access shared equipment, land, and labor help (Swindal et al. 2010; Hassanshahi et al. 2008). Specifically, Lucas et al. (2019) evaluated "local inter-farm cooperation" among farmers in France that they defined as "the formal and informal collaborative practices between farmers to share, manage, and/or exchange equipment, labor and/or material resources" (147) to understand the transformation to agroecological practices among farmers in France. We adopt a similar definition as Lucas et al. (2019) in our exploration into the different types of cooperation among producers within each distinctive industry and assess producer motivations for participating in cooperation and interest in potential participation.

Methods

This study will encompass a mail survey distributed to registered maple syrup producers and registered beekeepers in the state of Maine (Appendix A). Basic demographic information will be gathered for each individual including gender, age, occupation, years' experience in their respective industry as well as background business information (size of operation, number of employees, amount of production, years established). Questions on the survey will pertain to (1) their perspective on the current scale and intended future changes of their management practices (2) their current modes of cooperation and interest in other potential forms of cooperation, (3) their access to land, landowner relationships, and challenges with land access, and (4) their labor practices and needs.

References:

- Beal, A., and J. Jemison. 2011. Resource, environment and energy considerations for Maine food security in 2050 and beyond." *Maine Policy Review* 20(1): 172 -182.
- Dillman, D.A., J.D. Smyth, and L.M. Christian. 2014. Internet, Phone, Mail, and Mixed-Mode Surveys: The Tailored Design Method. Hoboken, NJ: John Wiley & Sons, Inc.
- Goulson, D., E. Nicholls, C. Botias, and E. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1-16.
- Hassanshahi, H., H. Irvani, K. Kalantari, and A. Rezaei. 2008. Analysis of capital assests of natural resources management in the Agricultural Production Cooperatives (APCs) in Fars Province, Iran. American-Eurasian Journal of Sustainable Agriculture, 150.
- Hoppe, R.A., J.M. MacDonald, and P. Korb. 2010. Small farms in the United States persistence under pressure. USDA Economic Research Service. Economic Information Bulletin Number 63.
- Lucas, V., P. Gasselin, and J.D. van Der Ploeg. 2019. Local inter-farm cooperation: a hidden potential for the agroecological transition in northern agricultures. *Agroecology and Sustainable Food Systems* 43(2): 145-179.
- MacDonald, J.M., P. Korb, and R.A. Hoppe. 2013. Farm size and the organization of U.S. crop farming. USDA Economic Research Service. Economic Research Report Number 152.
- Marsden, T., J. Banks, and G. Bristow. 2002. The social management of rural nature: understanding agrarian-based rural development. *Environment and Planning A* 34: 809-825.
- Paul, C.M., R. Nehring, D. Banker, and A. Somwaru. 2004. Scale economies and efficiency in U.S. agriculture: are traditional farms history? *Journal of Productivity Analysis* 22: 185-205.
- Potts, S.G., S.P.M. Roberts, R. Dean, G. Marris, M. Brown, R. Jones, P. Neumann, and J. Settele. 2010. Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49(1): 15-22.
- Swindal, M.G., G.W. Gillespie, and R.J. Welsh. 2010. Community digester operations and dairy farmer perspectives. Agriculture and Human Values 27: 461-474.
- USDA (United States Department of Agriculture). 2015. Forests of Maine, 2015. Accessed May 31, 2018. https://www.fs.fed.us/nrs/pubs/ru/ru_fs86.pdf.
- USDA-ERS (United States Department of Agriculture Economic Research Service). 2018. Farming and farm income. USDA. Accessed May 22, 2018. <u>https://www.ers.usda.gov/data-products/ag-and-food-statistics-charting-the-essentials/farming-and-farm-income/</u>.

USDA-NIFA (United States Department of Agriculture – National Institute of Food and Agriculture). 2018. Small and family farms. USDA. Accessed May 22, 2018. https://nifa.usda.gov/topic/small-and-family-farms.

Personnel:

Sara Velardi, Postdoctoral Research Associate in the School of Forest Resources is the principal investigator conducting this study. She has six years' experience conducting research with human subjects. Jessica Leahy, Professor in the School of Forest resources is the coprincipal investigator supervising the project and has 15 years' experience conducting research with human subjects. All personnel have completed the online human subjects training and passed.

Participant recruitment:

There will be two sets of participants for this study: (1) maple syrup producers and (2) beekeepers in Maine. Maple syrup producers and honey producers will be selected from the publicly available 2019 Maine Department of Agriculture, Conservation, and Forestry (MDACF) registered maple syrup and honey producer lists. Based on Dillman et al. (2014) recommended sampling method and accounting for non-response rate we will survey the 540 registered maple producers and 200 honey producers from each of the producer lists.

Subject recruitment will follow Dillman's Tailored Design Methods for surveys (Dillman et al. 2014). Respondents will be recruited through mailing. The first mailing will announce the mail survey and questionnaire, describe the study, and invite participants to participate (Appendix B). Five days later, the second mailing will be sent out which will include a cover letter describing the survey (Appendix C), an informed consent statement (Appendix D), the questionnaire (Appendix A), and a self-addressed and stamped reply envelope. A thank you and reminder postcard (Appendix E) will be mailed 14 days after the survey has been sent out to help remind participants to complete the survey. About one week after the reminder postcard is sent out, if a phone number is available for those who have not responded yet, the researcher will call the contact number for the participant listed to let them know that a questionnaire was sent to them previously, encourage them to respond, and answer any questions that individuals may have about the survey (Appendix F). They can also let the researcher know specifically if they will need another survey if the previous survey was thrown out. If they indicate that they would not like to participate, it will be marked on the recruitment document so a second mailing is not sent to them. Finally, 14 days after the reminder has been sent to participants, a second round of the cover letter (Appendix G), survey, and reply envelope will be mailed to those individuals who had not responded to the first mailing.

Informed consent: Mail survey respondents will have the choice to participate or not. An informed consent statement will be mailed to them in the survey packet (Appendix D).

Confidentiality: A unique identifier, a number, will be included on the mail survey (Appendix A) to allow for tracking of response and non-response. Only members of the research team (Velardi and Leahy) will have access to identifier information used to recruit respondents. The master list of names and codes will be stored in a locked office when not in use. The data from the paper survey will be entered in an electronic file. The electronic data will be kept on a password protected computer, and paper data, such as they physical surveys and the paper key

will be kept in a locked office. The paper key linking the code to participants' names will be destroyed within two years, June 2021 after data analysis is complete, and the physical surveys and electronic data will be destroyed within five years, June 2024. All reports and papers summarizing mail survey results will only report general findings and will not include identifiable information to ensure confidentiality of participants.

Risks to Participants: There are minimal risks to participants, such as cost of individual time and inconvenience. Some business information may be considered sensitive to some interviewees. We will assure participants they can skip any question they prefer not to answer with no penalty. Any identification information will be excluded from reports or presentations.

Benefits: There will be no direct benefit for participation but intended outcomes include outreach materials for maple syrup producers and beekeepers to help inform their scale management decisions such as a decision tree as well as possible collaborative or cooperative models for producers to emulate. Results from this study may lead to a greater understanding of factors guiding decision-making, cooperation strategies among producers/beekeepers and collaborative potential with landowners within the maple syrup and beekeeping industries in Maine.

Compensation: There will be no compensation.

Pre-Survey Notification Letter

Date

<<Address Block>>

Dear << Participant Name>>

I am writing to ask for your help with a University of Maine School of Forest Resources study on beekeepers' [maple producers'] decision-making, challenges, and needs in Maine. Your name and address were obtained from the state's honey and/or hive [maple syrup] registration records. In the next week you will be receiving a request to participate in our research project by filling out a survey that ask questions about your bee and/or honey [maple] business decisions, participation in informal and formal cooperation, your experience with varroa mite treatments, and concerns related to labor.

We want to make participating in our study easy and pleasant for you. We are writing in advance because we know many people like to know ahead of time that they will be asked to take time to fill out a survey. The success of our research depends on the generous help of people like you. It is our hope that it will take 30 minutes to fill out our survey. We will provide a <u>postage-paid</u> envelope for you to use to return the survey. We hope you enjoy filling out the survey and the opportunity to share your thoughts and opinions about your experience as a maple producer [beekeeper] in the state of Maine.

Best wishes,

Sara Velardi, Ph.D. Research Associate 249 Nutting Hall School of Forest Resources University of Maine Orono, ME 04469 203-583-0181 sara.velardi@maine.edu Jessica Leahy, Ph.D. Professor 241 Nutting Hall School of Forest Resources University of Maine Orono, ME 04469 207-581-2834 jessica.leahy@maine.edu

Survey Cover Letter

Date

<<Address Block>>

Dear << Participant Name>>

Recently, you may have received a mailed invitation from me about completing a survey about your experience, needs and challenges as a [maple syrup producer] beekeeper in Maine. Your name was selected from all registered honey producers and/or hive owners [maple syrup producers] in Maine to participate in the survey group. Honey production and beekeeping [maple syrup production] provide vital contributions to the state's economy and rural community development. As the industry continues to grow in Maine and across the U.S. it is important to understand the current state of the industry in Maine, including the needs and challenges addressed by beekeepers [maple syrup producers] themselves.

I am writing to you today to ask for your assistance in helping us understand more about the beekeeping [maple syrup] industry in Maine and in particular related to how you are making business decisions, your participation in formal or informal cooperation amongst beekeepers [producers], your varroa mite treatments, and your needs or concerns related to labor. By completing this questionnaire, you will contribute to understanding some of these components of the beekeeping [maple industry] in Maine.

Please have the primary business owner who makes the majority of operation decisions (over 18 years old) complete the survey. Please return the questionnaire to the University of Maine using the addressed and postage paid envelope enclosed with the survey. Your responses are voluntary and will be confidential. If you have any questions about the survey, please contact Sara Velardi by email at <u>sara.velardi@maine.edu</u> or by phone at 203-583-0181.

By taking time to participate in the survey you will be contributing greatly to the understanding of the maple syrup [beekeeping] industry in Maine as well as challenges and needs. We hope you enjoy completing the questionnaire and we look forward to receiving your responses.

Thank you, Sara Velardi Research Associate University of Maine

Informed Consent Form

You are invited to participate in a research project being conducted by Sara Velardi, a postdoctoral research associate in the School of Forest Resources at the University of Maine. This project is overseen by Dr. Jessica Leahy, a professor in the School of Forest Resources. The purpose of the research is to understand maple syrup producers' and beekeepers' scale management decision-making, needs and challenges in the state of Maine. You must be at least 18 years of age to participate.

What Will You Be Asked to Do?

If you decide to participate, you will be asked to take a confidential survey. It should take you about 30 minutes to complete.

Risks

Aside from your time and inconvenience, there is minimal risk of identification of survey respondents.

Benefits

While there is no direct benefit to you for participating in this survey, the results will provide the state of Maine and maple producer and beekeeping associations with valuable information to help inform decisions that impact maple syrup production and beekeeping. This is an opportunity to hear from a large sample of the maple producers and beekeeping communities to help understand needs and challenges to help support the continued sustainability and vitality of these industries in the state.

Confidentiality

This study is confidential. Please do not write your name on the survey. Paper surveys will be kept in a locked drawer in Nutting Hall of the University of Maine. Answers to the survey will be entered onto a computer and stored electronically. All electronic data will be kept on a password-protected computer. All confidential data, including paper surveys will be destroyed after five years (by July 2024). The key linking participant's registration numbers to responses will be destroyed in June 2021.

Voluntary

Participation is voluntary. If you choose to take part in this study, you may skip questions or stop at any time. Submission of the survey implies consent to participate.

Contact Information

If you have any questions about this study, please contact me at <u>sara.velardi@maine.edu</u>. If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, 207-581-2657 (or e-mail at umric@maine.edu).

Thank You/Reminder Post-card

Dear Registered Maine Beekeeper/Hive Owner [Maple Syrup Producer],

Last week, we mailed a survey asking for your help with a study on honey [maple syrup] production and beekeeping, along with and your experience as a beekeeper [maple syrup producer]in Maine. If you or someone in your household has already completed the questionnaire, please accept our sincere thanks. If not, please have the primary business owner who makes the majority of operation decisions (over 18 years old) complete and return the questionnaire as soon as possible. We are especially grateful for your help with this study. If you do not have a questionnaire, or if you have any questions, please contact Sara Velardi by email at sara.velardi@maine.edu or by phone at 203-583-0181.

Thank you for your time and assistance, Sara Velardi Research Associate University of Maine

Telephone Reminder Script

Hello [Participant name],

My name is Sara Velardi and I am from the University of Maine. A couple of weeks ago you should have received a survey request in the mail to share your experience has a beekeeper [maple syrup producer] in the state of Maine. Your name and phone number were obtained from the registered honey producers and/or hive owners [maple syrup producers] in Maine to participate in the survey group. the best of our knowledge we have not received your responses yet. We would greatly appreciate your input and response to help us understand more about the needs and challenges for beekeepers [maple syrup producers] in Maine.

Can I answer any questions that you have about the survey and would you like us to send you another paper copy?

Thank you very much!

Second Notification Cover Letter

Date

<<Address Block>>

Dear << Participant Name>>

Three weeks ago, we sent you a survey about your experience as a beekeeper [maple syrup producer] in the state of Maine. This survey is part of a study being conducted by Dr. Sara Velardi and Dr. Jessica Leahy with the University of Maine's School of Forest Resources. To the best of our knowledge, we have not received your response. Honey production and beekeeping [maple syrup production] provide vital contributions to the state's economy and rural community development. As the industry continues to grow in Maine and across the U.S. it is important to understand the current state of the industry in Maine, including the needs and challenges addressed by beekeepers [maple syrup producers] themselves.

We write to you again today because of the importance of your response to the study being conducted. Only by hearing from a wide range of beekeepers [maple syrup producers] can we fully understand the current state of the industry along with needs and challenges. We hope that the primary decision-maker for your beekeeping [maple syrup] operation can fill out the questionnaire soon.

For your convenience we have sent another enclosed questionnaire with a postage-paid, addressed envelope to return to the University of Maine. Your answers will never be associated with your name or mailing address in any way. If you have any questions about this survey please contact Sara Velardi by email at sara.velardi@maine.edu or by phone at 203-583-0181.

Thank you,

Sara Velardi Research Associate University of Maine

Part 2: IRB Letter of Approval

1

APPLICATION FOR APPROVAL OF RESEARCH WITH HUMAN SUBJECTS Protection of Human Subjects Review Board, 400 Corbett Hall

PRINC CO-IN CO-IN FACUI (Requ TITLE	nside gray areas) IPAL INVESTIGATOR: VESTIGATOR: VESTIGATOR: .TY SPONSOR: ired if PI is a student): OF PROJECT:	Jessica Leahy Finding the Sweet S	EMAII EMAII Spot: Scale Challenges					
Maple Syrup Production in Maine: Interviews								
	TDATE: NG AGENCY (if any):	7/16/18 PI USDA	DEPARTMENT:	School of Forest Resources				
STATU	JS OF PI: FACULTY/ST	AFF/GRADUATE/U	NDERGRADUATE	Postdoctoral Faculty (F,S,G,U)				
1.	If PI is a student, is this	research to be perfor	med:					
	for an honors th for a doctoral d other (specify)	nesis/senior thesis/cap issertation?	ostone?	for a master's thesis? for a course project?				
2.	Does this application modify a previously approved project? N (Y/N). If yes, please give assigned number (if known) of previously approved project:							
3.	Is an expedited review requested? Y (Y/N).							
	Submitting the application indicates the principal investigator's agreement to abide by the responsibilities outlined in <u>Section I.E. of the Policies and Procedures for the Protection of Human Subjects</u> .							
Faculty Sponsors are responsible for oversight of research conducted by their students. The Faculty Sponsor ensures that he/she has read the application and that the conduct of such research will be in accordance with the University of Maine's Policies and Procedures for the Protection of Human Subjects of Research. REMINDER : if the principal investigator is an undergraduate student, the Faculty Sponsor MUST submit the application to the IRB.								
Email this cover page and complete application to UMRIC@maine.edu								
FOR IF		**************************************		**************************************				
	Judged Exempt; category 2 Modifications required? Yes Accepted (date) 7/10/2018 Approved as submitted. Date of next review: by Degree of Risk: Approved pending modifications. Date of next review: by Degree of Risk: Modifications accepted (date): Not approved (see attached statement) Judged not research with human subjects							
	FINAL APPROVAL TO) BEGIN	7/10/2018 Date	01/2017				

AUTHOR'S BIOGRAPHY

Patrick Hurley, the youngest of five kids (and five more step-kids), was born in Pineville, North Carolina on October 6, 1998. He has lived in Medford Lakes, New Jersey since the age of four and graduated from Shawnee High School in Medford, NJ in 2016. At UMaine, he majored in ecology and environmental sciences with a concentration in aquatic ecosystem ecology. During his sophomore year, he added a sustainable food systems minor to his degree. His hobbies outside of coursework include fishing, music, hiking, and beekeeping, of course.

Upon graduation, Patrick will begin working for a local beekeeper near his NJ home and to continue to grow his own apiary.