Evaluating the effects of novel application methods of oxalic acid on *Varroa destructor* and *Apis mellifera* in the Southeastern United States

by

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Abstract

Varroa destructor is a devastating ectoparasitic mite of *Apis mellifera* honey bees. To manage it, beekeepers employ Integrated Pest Management, including the strategic use of chemicals like oxalic acid. Here, I performed colony-level field studies to examine the effects of continuous and multi-day release methods of oxalic acid application that target *V. destructor* as it emerged from brood cells alongside young *A. mellifera*. I observed that the tested continuous release method employing shop towels and cellulose boards had no effect on *V. destructor* or *A. mellifera* colonies. However, I observed that the tested multi-day application of oxalic acid via sublimation had a negative effect on *V. destructor* but no effect on *A. mellifera* colonies. Because of the need to identify diverse *V. destructor* management options for beekeepers, more work is needed to fine-tune the practical use of oxalic acid in *A. mellifera* colonies rearing brood.

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Chapter 1: Literature Review

Statement of purpose

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman) (Acari: Varroidae) is a major threat to beekeeping globally, causing widespread mortality of managed *Apis mellifera* L. (Hymenoptera: Apidae) honey bee colonies (Koleoglu et al. 2017). Oxalic acid is a natural varroacide with considerable promise; however, its current use is limited by colony and environmental conditions. Investigating novel ways for beekeepers to use oxalic acid would promote effective Integrated Pest Management against this devastating parasite.

1.1 Honey bees (*Apis mellifera)*

Angiosperms, or flowering plants, depend on the movement of pollen from the anther, the male part of the flower, to the stigma, the female part of the flower, for sexual reproduction (Yamaji and Ohsawa 2016). Often, the plant is unable to do this on its own, and instead relies upon an animal to complete this process, known as pollination (Mitchell et al. 2009). Pollination by insects is among the most important ecosystem services provided by animals (Sandhu et al. 2016). One of the most important animal pollinators is the Western honey bee, *Apis mellifera,* which is services both agricultural and natural systems (vanEngelsdorp and Meixner 2010; Hung et al. 2018). In the United States, pollination by *A. mellifera* is estimated to be worth $15 billion (Ricigliano et al. 2019), and globally 87 of the most important food crops depend upon them and other bees for pollination (Klein et al. 2007). Many examples include economically important crops such as almonds, blueberries, and cucurbits (Klein et al. 2007). Although not all flowering plants rely on insect pollinators, they do benefit from increased pollen movement from insect

pollinators. One great example is increased cotton yields between 27-31% in the presence of *A. mellifera* (Stein et al. 2017).

A. mellifera is regarded as one of the most efficient pollinators is due to its biology and sociality, which has historically allowed for its management in large numbers (Degrandi-Hoffman et al. 2019). As a result, *A. mellifera* can be found outside of its native Eurasian range, inhabiting every continent permanently occupied by humans (Weber 2012). Furthermore, innovation in management over the last several hundred years has allowed it to be relatively easily transported to areas of crop production in high volumes during brief periods when pollination is required (Sáez et al. 2020).

Apis mellifera is unique among insect pollinators, as they are eusocial. This highly advanced social structure in animals is characterized by overlapping generations, cooperative brood care, and reproductive division of labor in females (Wilson and Hölldobler 2005). Because of their eusociality, the entire colony is frequently regarded as a superorganism (Page et al. 2016) composed of a single queen (the reproductive female), many thousands of workers (the non-reproductive females), and a few thousand drones (the reproductive males). Drones are only present during certain times, however, such as during the spring and summer, whereas the queen and workers are present during all times of the year (Rangel and Fisher 2019).

The queen is arguably the most important individual within the *A. mellifera* colony. The queen has the shortest development time of all other bee types within the colony, which is 16 days (Sammataro and Avitabile 2011). Also unique to queens are the cells in which they develop, which are oriented vertically compared to horizontal workers and drone cells (Pirk 2018). Additionally, a developing queen is fed large amounts of royal jelly, a protein and nutrient-rich food produced by the workers that determines whether the female larva will become

a queen or worker (Merzendorfer 2011, Sagili et al. 2018). The queen is the sole reproductive force within the colony, laying up to 2,000 eggs per day during the active season (Leimar et al. 2012).

The queen, unlike workers and drones, is the only perennial member of the colony, living several years, though they are commonly replaced annually by beekeepers (Sammataro and Avitabile 2011). Upon cell-emergence, the newly emerged virgin queen will orient herself with her new colony. After orientation, she will then leave the hive and commonly mate with up to 12-14 drones at drone congregation areas, a form of lek mating behavior (Richard et al. 2007, Beaurepaire et al. 2014, Heidinger et al. 2014). This polyandrous mating behavior increases genetic diversity of future generations of the colony which subsequently improves resistance or tolerance to disease (Ostroverkhova et al. 2016, Ding et al. 2017). The reproductive performance of queens is heavily dependent on the quantity and quality of sperm obtained during mating. This in turn also relies on the quality of the drones she had mated with previously (Al-Sarhan et al. 2019). Mating quality and proper care of queens by the workers enhance the survival and acceptance of the queen by the workers of the colony. If the queen begins to fail, or lays fewer eggs or more drone brood, the workers may then replace her (Sammataro and Avitabile 2011).

Workers are nonreproductive females, and as their name implies perform housekeeping duties of the *A. mellifera* colony. Workers, similar to queens, are fed royal jelly, but are switched to beebread, a mixture of nectar and pollen, after three days of feeding (Sammataro and Avitabile 2011). Workers have a development time of 21 days, which is intermediate compared to queens and drones. Workers are the most numerous individuals of the colony, and are present at all times of the year although they can be physiologically different depending on the season. For example, winter *A. mellifera* workers are produced towards the end of the active season and

exhibit low titers of Juvenile Hormone and have larger fat bodies that ultimately allows them to live up to 6 months. During the winter months, the workers participate in thermoregulation and care for the queen, and if present, brood (Mattila et al. 2001, Behrends and Scheiner 2010). This is in stark contrast to *A. mellifera* workers during the active season, which live up to 42 days, and have lower levels of proteins and lipids (Sammataro and Avitabile 2011, Kunc et al. 2019).

Workers also exhibit temporal polyethism (Beshers et al. 2001). Juvenile hormone, secreted by the corpora allata, regulates the physiological processes associated with division of labor and age polyethism in *A. mellifera* workers (Robinson 1987, Beshers et al. 2001, Johnson 2010). When workers first emerge, their duties are restricted to the brood nest where they clean brood cells and feed the developing larvae. Workers then transition to tasks outside of the the brood nest, such as building comb and processing nectar and pollen. Finally, once they are around 3-weeks old, they then transition into foragers – the life stage which perform pollinations via gathering food from floral resources (Page et al. 2006, Johnson 2010, Sagili et al. 2011). Workers are also responsible for feeding the queen, and if present, any drones in the colony (Johnson 2010). Workers are also the caste that makes honey, a highly prized food substance throughout the world. Honey is made from nectar, which is gathered from floral resources. The nectar then undergoes chemical transformation, breaking down sugars. Water is then evaporated from the nectar by the workers and eventually capped with wax (Sammataro and Avitabile 2011, Eyer, Neumann, et al. 2016).

Workers may also be the most infamous individual type of *A. mellifera*, as it is capable of stinging in self-defense. Although queens possess stingers, they are not barbed like stingers of workers (Sammataro and Avitabile 2011, Wu et al. 2014). Workers that defend the colony are older, and have transitioned to foragers near the end of their lives (Nouvian et al. 2016). The

venom possessed by *A. mellifera* is not unlike other venomous animals, and is composed of protens, alkaloids and peptides (Danneels et al. 2015).

Drones, or male honey bees, have the longest development time, which lasts 24 days. They are easily distinguishable from workers and queens, as they have very large eyes and rounded abdomens (Sammataro and Avitabile 2011). Drones are only present during the active season,when they seek to mate with virgin queens from other colonies at drone congregation areas (Langowska and Zduniak 2020).There, hundreds of drones gather high above the ground. Sensing the virgin queens' sex pheromone, they will then follow, and mate with her (Butler et al. 1962, Bastin et al. 2017). Upon mating, the drone then dies. The population dynamics of drones fluctuate throughout the year, and is believed to be connected to the population of workers present within the colony (Rowland and McLellan 1987). Although drones were thought to not perform any work in the hive, it has been shown they do contribute to some thermoregulation of brood during times of colony stress (Harrison 1987).

The *Apis mellifera* colony, made up of thousands of individuals that work together, acts as a single organism. Worker *A. mellifera* work together to raise brood, regulate internal conditions, such as temperature and humidity, within the hive, defend the hive, and gather food for all members (Sammataro and Avitabile 2011, Straub et al. 2015, Page et al. 2016). However, a minimum number of workers are needed to carry out normal colony conditions and behaviors. Stressors, both biotic and abiotic, threathen the life of *A. mellifera* colonies when enough workers have succumbed to these stressors (Straub et al. 2015).

Many factors contribute to the health and quality of drone sperm. Drones are born with all the sperm they will use in their lifetime, though viability and quantity released has been shown to decrease over time (Rousseau et al. 2015). Other factors that contribute to sperm quality include

the in-hive environment, such as brood-nest temperature (Stürup et al. 2013). Commonly used insecticides, such as the neonicotinoids, and acaricides, such as fluvalinate, also influence the health of sperm (Ciereszko et al. 2017, Rangel and Fisher 2019).

1.2 *Apis mellifera* colony loss

Despite the widespread distribution and commercial management of *Apis mellifera*, beekeepers have experienced increased colony loss across the globe in recent years (Kulhanek et al. 2017, vanEngelsdorp et al. 2017, Bruckner et al. 2020, Gray et al. 2020, Oberreiter and Brodschneider 2020, Stahlmann-Brown et al. 2020). Many groups, such as the Bee Informed Partnership in the United States and COLOSS in Europe, have conducted colony loss surveys via information from participating beekeepers in order to understand the spatial and temporal dynamics of colony loss. In the years 2019-2020, it was reported that U.S. beekeepers lost 43.7% of their colonies (Bruckner et al. 2020), which is about double what beekeepers deem to be normal (Kulhanek et al. 2017). Reasons for colony mortality include both abiotic and biotic factors, acting singly or in combination (vanEngelsdorp and Meixner 2010, Havard et al. 2020).

Abiotic factors that have led to *A. mellifera* colony loss include environmental conditions, pesticides, and beekeeper management (Kulhanek et al. 2017). Because workers regulate the brood temperature, and keep the colony at a constant temperature during the winter, high and low temperatures in the extremes can be detrimental to *A. mellifera* colonies (Southwick and Heldmaier 1987, Petz et al. 2004). Extreme high and low temperatures prohibit foraging workers from collecting pollen and nectar (Park et al. 2015, Li et al. 2018). Pesticides, especially insecticides, are often discussed as a reason for colony loss, and can elicit lethal and sub-lethal effects, such as oxidative stress (Chakrabarti et al. 2015). Furthermore, because some pesticides

are systemic in plants, these chemicals can leave residues in nectar and pollen, contaminating the stored food in colonies (Mullin et al. 2010). It has been observed that migratory beekeepers, or those that transport their colonies to different agricultural areas for pollination often experience high colony loss (Simone-Finstrom et al. 2016).

Biotic stressors to *Apis mellifera* include floral resources, pests, and parasites. Though *A. mellifera* are extremely important and used extensively in agricultural systems, monocropping, or only one type of plant available, reduces floral diversity, restricting *A. mellifera*'s intake of appropriate nutrients (Rands and Whitney 2010). Furthermore, the landscape, being natural, agricultural or suburban, has an effect on nutritional intake of *A. mellifera*, due to differences in floral diversity (Smart et al. 2019). This can result in increased susceptibility of colonies to parasites (Branchiccela et al. 2019). Pests, such as the small hive beetle (*Aethina tumida* Murray (Coleoptera: Nitidulidae)) cause damage to wax and can make hive products, such as honey, unsellable for the beekeeper (Stief et al. 2020). Finally, a plethora of endemic and introduced parasites play an important role in *A. mellifera* health. Among the most damaging s the introduced ectoparasitic mite *Varroa destructor*, which feeds on the fat body of *A. mellifera* and can cause significant disease (Koleoglu et al. 2017b).

1.3 Varroa mite (*Varroa destructor*)

Varroa destructor, an introduced parasite to *A. mellifera*, was originally a parasite of the Eastern honey bee *Apis cerana* (Beaurepaire et al. 2015). The first report of this host switch occurred in 1957 in Japan (Sakai and Okada 1973). *V. destructor* arrived in Europe in the early 1970s, and in America in the 1980s, plaguing beekeeping industries since its introduction (Griffiths and Bowman 1981). Unlike *A. cerana*, *A. mellifera* did not evolved alongside *V.*

destructor; therefore, *A. mellifera* lacks the defense strategies that *A. cerana* developed to keep infestations at a non-damaging level. These defense stragies include grooming behavior, drone brood trapping, and hygienic behavior (Peng et al. 1987).

The lifecycle of *V. destructor* is entirely dependent on the lifecycle of its honey bee host. For *A. mellifera* and other honey bee species, there are two distinct life stages of *V. destructor* – the dispersal stage, when mites travel and feed on adult *A. mellifera*, and the reproductive stage, when mites reproduce and develop alonside *A. mellifera* late stage larvae and pupae within capped brood cells (Mondet et al. 2018, Roth et al. 2020, Traynor et al. 2020). *Varroa destructor* is most often introduced to colonies via robbing of collapsing colonies and drifting of *A. mellifera* workers (Peck and Seeley 2019). The reproductive stage begins when a parasitized adult worker comes in contact with a brood cell containing a 5^{th} instar larvae. The mite then leaves her adult host and enters the cell. From there, she buries herself under the brood food and waits for cell-capping. Once the cell is capped, the foundress mite begins to feed on the developing *A. mellifera* brood by creating a small slit in the cuticle with her gnathosoma (Ramsey et al. 2019). Approximately seventy hours later, the foundress lays a single haploid egg on the wall of the brood cell, which develops into a male, followed by diploid eggs that become daughter mites (Rosenkranz et al. 2010, Piou et al. 2016). On average, up to 1.5 and 2.5 daughters are produce in worker and drone cells, respectively (Traynor et al. 2020). Because drones take longer to develop and nurses tend to drone cells more than worker cells (Boot et al. 1995, Rosenkranz et al. 2010). Once the *A. mellifera* worker or drone pupates, they emerge alongside fully developed female *V. destructor* mites (Rosenkranz et al. 2010, Roth et al. 2020, Traynor et al. 2020). During the dispersal stage, adult female *V. destructor* mites adhere themselves to *A. mellifera* adults and feed on the underside of their host's abdomen on the fat

body (Li et al. 2019, Ramsey et al. 2019). Nurse *A. mellifera* are the preferred life stage of workers, as they have larger fat bodies (Xie et al. 2016, Ramsey et al. 2019), and stay near the brood nest. This stage of life of *V. destructor* lasts up to 10 days when brood are present, but up to 6 months when brood are absent during winter (Piou et al. 2016).

Feeding by *V. destructor* elicits both sub-lethal and lethal effects on *A. mellifera* (Koleoglu et al. 2017b). The fat body, the organ which *V. destructor* feeds upon, is among the most important organs in insects, functioning in storage and regulation of metabolites and immunity (Raikhel et al. 1997, Arrese and Soulages 2010, Ramsey et al. 2019). Feeding by *V. destructor* can result in reduced longevity, weight, and size of individuals, vectoring of viruses, anatomical deformities, and immunosuppression (Bowen-Walker and Gunn 2001, Annoscia et al. 2012, 2019, Dainat et al. 2012, Noël et al. 2020). Furthermore, drones parasitized by *V. destructor* during development are less capable of reaching drone congregation areas, and have lower sperm production and weight (Duay et al. 2002, 2003). These viruses act synergistically with *V. destructor*, threatening and causing widespread losses to *A. mellifera* colonies (Francis et al. 2013). In addition to the direct negative effects of *V. destructor*, feeding can also result in susceptibility to other pests and pathogens and insecticides (Blanken et al. 2015, Rinkevich et al. 2017, Noël et al. 2020).

At high infestations, *V. destructor* severely weakens colonies, ultimately leading to colony death. Because the life cycle of *V. destructor* is highly correlated with that of *A. mellifera*, *V. destructor* infestations can quickly reach damaging levels (Noël et al. 2020). Without intervention, severe infestations can negatively affect individuals so that the colony is unable to fend the infection, eventually resulting in colony death (Straub et al. 2015, Morawetz et al. 2019, Thoms et al. 2019).

1.4 *Varroa destructor* control

At high infestations without successful treatment, *V. destructor* severely weakens *A. mellifera* colonies, and will eventually cause death (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017). Because of the threats posed by *V. destructor*, a successfully implemented management strategy employing Integrated Pest Management (IPM) is the best opportunity for a beekeeper prevent colony death due to this devastating parasite (Delaplane et al. 2005). Setting an action threshold, or a point at which pest populations reach a level in which control is needed, is necessary for a successful IPM Program (US EPA 2015c). Currently, an infestation of 3% *V. destructor* per 100 *A. mellifera* is the standard action threshold for *V. destructor* treatment (Caron et al. 2018). Prevention strategies, such as cultural and mechanical practices, are the next steps of an IPM program. Cultural practices include the use tolerant or resistant stocks, some of which can detect and remove *V. destructor* from within brood cells, or groom *V. destructor* from themselves or conspecifics (van Alphen and Fernhout 2020). Mechanical means by beekeepers include drone brood removal, whereby a beekeeper removes an entire frame of parasitized drone brood, and making colonies artificially broodless to limit *V. destructor* reproduction (Gregorc et al. 2017). When all other treatment methods have not successfully maintained infestations below the action threshold, chemical treatments are often used.

Currently synthetic and organic (i.e., plant derived compounds) are available for use for beekeepers against *V. destructor*; however, many of the former compounds have recently demonstrated development of resistance by *V. destructor* because of improper use (Hillesheim et al. 1996, Elzen et al. 2000, Thompson et al. 2002, Sammataro, Untalan, et al. 2005, Rinkevich 2020). Furthermore, synthetic treatments can have long-lasting negative effects on *A. mellifera*, possibly because residues remain in bee products like bee bread, honey, and wax (Wallner 1999,

Mullin et al. 2010). For example, one such active ingredient, tau-Fluvalinate, can negatively impact *A. mellifera* learning and survival (Frost et al. 2013). Both synthetic and natural treatments are also limited to use, as some cannot be used when honey supers are present, or are restricted by temperature (US EPA 2016, Caron et al. 2018).

Due to the limitations of effective control using synthetic varroacides, beekeepers have begun to rely on natural compounds for control. Compounds such as oxalic acid have considerable promise due to high efficacy, natural content in nectar, and low probability of resistance development in *V. destructor* (Bogdanov et al. 2002, Rademacher and Harz 2006a, Toufailia et al. 2015, Adjlane et al. 2016). Oxalic acid, though used extensively throughout Europe and Canada, was registered for apicultural use in the United States in 2015 (US EPA 2015). Current registered application methods using oxalic acid include trickling, which is the deposition of liquid formulation of oxalic acid dissolved in a sucrose solution onto the colony, and sublimation, which is the application of gaseous oxalic acid to a colony using a heating device (Rademacher and Harz 2006a, US EPA 2015a). Although natural treatments such as oxalic acid are highly effective, their use is limited by weather and internal conditions within the colony. For example, the acaricidal properties of oxalic acid do not penetrate the wax capped brood cells where *A. mellifera* and *V. destructor* reside (Adjlane et al. 2016). Because of this, treatment using oxalic acid is limited to broodless periods, such as during the winter (Higes et al. 1999). Oxalic acid has very few to no negative effects on *A. mellifera* (Rademacher et al. 2017a). Due to its high acaricidal effects and low negative effects on *A. mellifera*, oxalic acid continues to be an important tool in IPM among beekeepers. However, more research is required to understand and develop effective uses of oxalic acid during the active brood rearing season of

A. mellifera when the majority of *V. destructor* reside within the protective confines of capped brood cells.

1.5 Oxalic acid

Oxalic acid is a naturally occurring plant compound, found in commonly consumed foods by humans and other animals such as spinach, rhubarb, beets, and honey (Moosbeckhofer et al. 2003, Morrison and Savage 2003). An organic compound with the molecular formula of C2H2O4, it commonly occurs in its crystalline as a dihydrate ($C_2H_6O_6$) in its solid form. It has a molecular weight of 90.03 g/mol and is a white, odorless solid, and is an end product of ascorbic acid (Vitamin C) oxidation (Knight et al. 2016, National Center for Biotechnology Information 2020). Oxalic acid is often sold as wood bleach, as it removes stains but does not affect the natural coloration of wood (Herstedt and Herstedt 2017). Oxalic acid has been used extensively in beekeeping in both Europe and Canada to control the *V. destructor* mite, but was not approved for use in the United States until 2015 (Gregorc and Planinc 2001, Rademacher and Harz 2006a, US EPA 2015b). It is believed that oxalic acid damages the mouthparts and cuticular damage of *V. destructor* (Toufailia et al. 2015, Papežíková et al. 2017). Although it has been used for many years, *V. destructor* (Anderson and Trueman) have not developed resistance, as it did to synthetic compounds that also had widespread use. Possible reasons for its low probability of resistance development include rapid degradation of oxalic acid within the colony, its contact toxicity, and its limited use by beekeepers (Imdorf et al. 1999, Maggi et al. 2016). Because of its acaricidal effects and low probability of resistance development, oxalic acid is a promising tool for a successful Integrated Pest Management for *V. destructor*.

Thesis objectives

The overall objective of my thesis research was to investigate novel application methods for oxalic acid against the *Apis mellifera* honey bee parasite *Varroa destructor*. I did this by performing colony-level studies that investigated the continuous release of a liquid oxalic acid solution from shop-towels and cellulose boards, and by investigating intermittent release of oxalic acid gas via sublimation.

References

Adjlane, N., E.-O. Tarek, and N. Haddad. **2016**. Evaluation of oxalic acid treatments against the mite *Varroa destructor* and secondary effects on honey bees *Apis mellifera*. J Arthropod Borne Dis. 10: 501–509.

Allsopp, M. H., W. J. de Lange, and R. Veldtman. **2008**. Valuing insect pollination services with cost of replacement. PLOS ONE. 3: e3128.

van Alphen, J. J. M., and B. J. Fernhout. **2020**. Natural selection, selective breeding, and the evolution of resistance of honeybees (*Apis mellifera*) against Varroa. Zool Lett. 6: 6.

Al-Sarhan, R., N. Adgaba, Y. Tadesse, Y. Alattal, A. Al-Abbadi, A. Single, and A. Al-Ghamdi. **2019**. Reproductive biology and morphology of *Apis mellifera jemenitica* (Apidae) queens and drones. Saudi Journal of Biological Sciences. 26: 1581–1586.

Anderson, D. L., and J. W. Trueman. **2000**. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol. 24: 165–189.

Annoscia, D., S. P. Brown, G. Di Prisco, E. De Paoli, S. Del Fabbro, D. Frizzera, V. Zanni, D. A. Galbraith, E. Caprio, C. M. Grozinger, F. Pennacchio, and F. Nazzi. **2019**. Haemolymph removal by *Varroa* mite destabilizes the dynamical interaction between immune effectors and virus in bees, as predicted by Volterra's model. Proc R Soc B. 286: 20190331.

Arrese, E. L., and J. L. Soulages. **2010**. Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol. 55: 207–225.

Aslan, C. E., C. T. Liang, B. Galindo, H. Kimberly, and W. Topete. **2016**. The role of honey bees as pollinators in natural areas. Nat. Areas. J. 36: 478–488.

Bastin, F., H. Cholé, G. Lafon, and J.-C. Sandoz. **2017**. Virgin queen attraction toward males in honey bees. Sci Rep. 7: 6293.

Beaurepaire, A. L., T. A. Truong, A. C. Fajardo, T. Q. Dinh, C. Cervancia, and R. F. A. Moritz. **2015**. Host specificity in the honeybee parasitic mite, *Varroa* spp. in *Apis mellifera* and *Apis cerana*. PLOS ONE. 10: e0135103.

Behrends, A., and R. Scheiner. **2010**. Learning at old age: a study on winter bees. Front Behav Neurosci. 4.

Benaets, K., A. Van Geystelen, D. Cardoen, L. De Smet, D. C. de Graaf, L. Schoofs, M. H. D. Larmuseau, L. E. Brettell, S. J. Martin, and T. Wenseleers. **2017**. Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. Proceedings of the Royal Society B: Biological Sciences. 284: 20162149.

Birkhofer, K., E. Diehl, J. Andersson, J. Ekroos, A. Früh-Müller, F. Machnikowski, V. L. Mader, L. Nilsson, K. Sasaki, M. Rundlöf, V. Wolters, and H. G. Smith. **2015**. Ecosystem services—current challenges and opportunities for ecological research. Front. Ecol. Evol. 2.

Blanken, L. J., F. van Langevelde, and C. van Dooremalen. **2015**. Interaction between Varroa destructor and imidacloprid reduces flight capacity of honeybees. Proceedings of the Royal Society B: Biological Sciences. 282: 20151738.

Boot, W. J., M. van Baalen, and M. W. Sabelis. **1995**. Why do Varroa mites invade worker brood cells of the honey bee despite lower reproductive success? Behav Ecol Sociobiol. 36: 283–289.

Botías, C., R. Martín-Hernández, L. Barrios, A. Meana, and M. Higes. **2013**. *Nosema* spp. infection and its negative effects on honey bees (*Apis mellifera iberiensis*) at the colony level. Vet Res. 44: 25.

Bowen-Walker, P. L., and A. Gunn. **2001**. The effect of the ectoparasitic mite, *Varroa destructor* on adult worker honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels. Entomologia Experimentalis et Applicata. 101: 207–217.

Branchiccela, B., L. Castelli, M. Corona, S. Díaz-Cetti, C. Invernizzi, G. Martínez de la Escalera, Y. Mendoza, E. Santos, C. Silva, P. Zunino, and K. Antúnez. **2019**. Impact of nutritional stress on the honeybee colony health. Sci Rep. 9: 10156

Bruckner, S., N. Steinhauer, J. Engelsma, A. M. Fauvel, K. Kulhanek, E. Malcolm, A. Meredith, M. Milbrath, E. L. Niño, J. Rangel, K. Rennich, D. Reynolds, R. Sagili, J. Tsuruda, D. vanEngelsdorp, S. D. Aurell, M. Wilson, and G. R. Williams. **2020**. 2019-2020 honey bee colony losses in the United States: Preliminary results. (https://beeinformed.org/wp-content/uploads/2020/06/BIP_2019_2020_Losses_Abstract.pdf).

Butler, C. G., R. K. Callow, and N. C. Johnston. **1962**. The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honeybee pheromone. Proceedings of the Royal Society of London. Series B, Biological Sciences. 155: 417–432.

Caron, D. M., M. Dykes, D. Epstein, G. Hansen, M. Nasr, D. Downey, K. Rennich, D. Rogers, R. M. Sears, and T. Steeger. **2018**. Tools for varroa management: a guide to effective varroa sampling & control. (https://honeybeehealthcoalition.org/wp-content/uploads/2018/06/HBHC-Guide_Varroa_Interactive_7thEdition_June2018.pdf).

Chakrabarti, P., S. Rana, S. Sarkar, B. Smith, and P. Basu. **2015**. Pesticide-induced oxidative stress in laboratory and field populations of native honey bees along intensive agricultural landscapes in two Eastern Indian states. Apidologie. 46: 107–129.

Ciereszko, A., J. Wilde, G. J. Dietrich, M. Siuda, B. Bąk, S. Judycka, and H. Karol. **2017**. Sperm parameters of honeybee drones exposed to imidacloprid. Apidologie. 48: 211–222.

Dainat, B., J. D. Evans, Y. P. Chen, L. Gauthier, and P. Neumann. **2012**. Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. Appl Environ Microbiol. 78: 981–987.

Danneels, E. L., M. Van Vaerenbergh, G. Debyser, B. Devreese, and D. C. de Graaf. **2015**. Honeybee venom proteome profile of queens and winter bees as determined by a mass spectrometric approach. Toxins (Basel). 7: 4468–4483.

Degrandi-Hoffman, G., H. Graham, F. Ahumada, M. Smart, and N. Ziolkowski. **2019**. The economics of honey bee (Hymenoptera: Apidae) management and overwintering strategies for colonies used to pollinate almonds. J Econ Entomol. 112: 2524–2533.

Delaplane, K. S., J. A. Berry, J. A. Skinner, J. P. Parkman, and W. M. Hood. **2005**. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. J Apic Res. 44: 157–162.

Ding, G., H. Xu, B. P. Oldroyd, and R. S. Gloag. **2017**. Extreme polyandry aids the establishment of invasive populations of a social insect. Heredity (Edinb). 119: 381–387.

Duay, P., D. De Jong, and W. Engels. **2002**. Decreased flight performance and sperm production in drones of the honey bee (*Apis mellifera*) slightly infested by *Varroa destructor* mites during pupal development. Genet Mol Res. 1: 227–232.

Elzen, P. J., J. R. Baxter, M. Spivak, and W. T. Wilson. **2000**. Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. Apidologie. 31: 437–441.

Eyer, M., P. Neumann, and V. Dietemann. **2016**. A Look into the cell: honey storage in honey bees, *Apis mellifera*. PLOS ONE. 11: e0161059.

Fisher, A., and J. Rangel. **2018**. Exposure to pesticides during development negatively affects honey bee (*Apis mellifera*) drone sperm viability. PLoS One. 13: e0208630.

Francis, R. M., S. L. Nielsen, and P. Kryger. **2013**. Varroa-virus interaction in collapsing honey bee colonies. PLOS ONE. 8: e57540.

Frost, E. H., D. Shutler, and N. K. Hillier. **2013**. Effects of fluvalinate on honey bee learning, memory, responsiveness to sucrose, and survival. J Exp Biol. 216: 2931–2938.

Gallai, N., J.-M. Salles, J. Settele, and B. Vaissière. **2009**. Economic valuation of the vulnerability of world agriculture confronted to pollinator decline. Ecol Econ. 68: 810–821.

Gray, A., N. Adjlane, A. Arab, A. Ballis, V. Brusbardis, J.-D. Charrière, R. Chlebo, M. F. Coffey, B. Cornelissen, C. A. da Costa, B. Dahle, J. Danihlík, M. M. Dražić, G. Evans, M. Fedoriak, I. Forsythe, A. Gajda, D. C. de Graaf, A. Gregorc, I. Ilieva, J. Johannesen, L. Kauko, P. Kristiansen, M. Martikkala, R. Martín-Hernández, C. A. Medina-Flores, F. Mutinelli, S. Patalano, A. Raudmets, G. S. Martin, V. Soroker, J. Stevanovic, A. Uzunov, F. Vejsnaes, A. Williams, M. Zammit-Mangion, and R. Brodschneider. **2020**. Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018–2019, and the effects of a new queen on the risk of colony winter loss. J of Apic Res. 59: 744–751.

Gregorc, A., M. Alburaki, C. Werle, P. R. Knight, and J. Adamczyk. **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: 821–832.

Griffiths, D. A., and C. E. Bowman. **1981**. World distribution of the mite *Varroa Jacobsoni*, a parasite of honeybees. Bee World. 62: 154–163.

Guzmán-Novoa, E., L. Eccles, Y. Calvete, J. Mcgowan, P. G. Kelly, and A. Correa-Benítez. **2010**. *Varroa destructor* is the main culprit for the death and reduced populations of

overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie. 41: 443–450.

Harrison, J. M. 1987. Roles of individual honeybee workers and drones in colonial thermogenesis. J Exp Biol. 129: 53–61.

Havard, T., M. Laurent, and M.-P. Chauzat. **2020**. Impact of stressors on honey bees (*Apis mellifera*; Hymenoptera: Apidae): Some guidance for research emerge from a meta-analysis. Diversity. 12: 7.

He, C., K. Zhang, X. Hou, D. Han, and S. Wang. **2019**. Foraging behavior and pollination efficiency of *Apis mellifera* L. on the oil tree Peony 'Feng Dan' (*Paeonia ostii* T. Hong et J.X. Zhang). Insects. 10.

Heidinger, I. M. M., M. D. Meixner, S. Berg, and R. Büchler. **2014**. Observation of the mating behavior of honey bee (*Apis mellifera* L.) queens using radio-frequency identification (RFID): Factors influencing the duration and frequency of nuptial flights. Insects. 5: 513–527.

Herstedt, L., and M. Herstedt. **2017**. Chemical bleaching of wood: an investigation into the bleaching of mahogany, walnut, rosewood, padauk, and purpleheart. Stud Conserv. 62: 162–172.

Higes, M., A. Meana, M. Suárez, and J. Llorente. **1999**. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. Apidologie. 30: 289–292.

Hillesheim, E., W. Ritter, and D. Bassand. **1996**. First data on resistance mechanisms of *Varroa jacobsoni* (OUD.) against tau-fluvalinate. Exp Appl Acarol. 20: 283–296.

Hung, K.-L. J., J. M. Kingston, M. Albrecht, D. A. Holway, and J. R. Kohn. **2018**. The worldwide importance of honey bees as pollinators in natural habitats. Proc R Soc B. 285: 20172140.

Iqbal, J., and U. Mueller. **2007**. Virus infection causes specific learning deficits in honeybee foragers. Proceedings of the Royal Society B: Biological Sciences. 274: 1517–1521.

Johnson, B. R. 2010. Division of labor in honeybees: form, function, and proximate mechanisms. Behav Ecol Sociobiol. 64: 305–316.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. **2007**. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences. 274: 303–313.

Knight, J., K. Madduma-Liyanage, J. A. Mobley, D. G. Assimos, and R. P. Holmes. **2016**. Ascorbic acid intake and oxalate synthesis. Urolithiasis. 44: 289–297.

Koleoglu, G., P. H. Goodwin, M. Reyes-Quintana, M. M. Hamiduzzaman, and E. Guzman-Novoa. **2017**. Effect of *Varroa destructor*, wounding and varroa homogenate on gene expression in brood and adult honey bees. PLOS ONE. 12: e0169669.

Kulhanek, K., N. Steinhauer, K. Rennich, D. M. Caron, R. R. Sagili, J. S. Pettis, J. D. Ellis, M. E. Wilson, J. T. Wilkes, D. R. Tarpy, R. Rose, K. Lee, J. Rangel, and D. vanEngelsdorp. **2017**. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. JApic Res. 56: 328–340.

Langowska, A., and P. Zduniak. **2020**. No direct contact needed for drones to shorten workers lifespan in honey bee. J Apic Res. 59: 88–94.

Leimar, O., K. Hartfelder, M. D. Laubichler, and R. E. Page. **2012**. Development and evolution of caste dimorphism in honeybees – a modeling approach. Ecol Evol. 2: 3098–3109.

Li, A. Y., S. C. Cook, D. E. Sonenshine, F. Posada-Florez, N. I. I. Noble, J. Mowery, C. J. Gulbronson, and G. R. Bauchan. **2019**. Insights into the feeding behaviors and biomechanics

of *Varroa destructor* mites on honey bee pupae using electropenetrography and histology. J Insect Physiol. 119: 103950.

Li, G., H. Zhao, Z. Liu, H. Wang, B. Xu, and X. Guo. **2018**. The wisdom of honeybee defenses against environmental stresses. Front Microbiol. 9: 722.

Martin, S. J., A. C. Highfield, L. Brettell, E. M. Villalobos, G. E. Budge, M. Powell, S. Nikaido, and D. C. Schroeder. **2012**. Global honey bee viral landscape altered by a parasitic mite. Science. 336: 1304–1306.

Mattila, H. R., J. L. Harris, and G. W. Otis. **2001**. Timing of production of winter bees in honey bee (*Apis mellifera*) colonies. Insectes soc. 48: 88–93.

Merzendorfer, H. 2011. Active component in honeybees' royal jelly discovered. J Exp Biol. 214: iv–iv.

Mitchell, R. J., R. E. Irwin, R. J. Flanagan, and J. D. Karron. **2009**. Ecology and evolution of plant–pollinator interactions. Ann Bot. 103: 1355–1363.

Mondet, F., A. Rau, C. Klopp, M. Rohmer, D. Severac, Y. Le Conte, and C. Alaux. **2018**. Transcriptome profiling of the honeybee parasite *Varroa destructor* provides new biological insights into the mite adult life cycle. BMC Genomics. 19: 322.

Moosbeckhofer, R., H. Pechhacker, H. Unterweger, F. Bandion, and A. Heinrich-Lenz. **2003**. Investigations on the oxalic acid content of honey from oxalic acid treated and untreated bee colonies. Eur Food Res Technol. 217: 49–52.

Morawetz, L., H. Köglberger, A. Griesbacher, I. Derakhshifar, K. Crailsheim, R. Brodschneider, and R. Moosbeckhofer. **2019**. Health status of honey bee colonies (*Apis mellifera*) and disease-related risk factors for colony losses in Austria. PLoS One. 14: e0219293.

Morrison, S. C., and G. P. Savage. **2003**. Oxalates, pp. 4282–4287. *In* Caballero, B. (ed.), Encyclopedia of Food Sciences and Nutrition (Second Edition). Academic Press, Oxford.

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp, and J. S. Pettis. **2010**. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLOS ONE. 5: e9754.

National Center for Biotechnology Information. **2020**. PubChem compound summary for CID 971, oxalic acid. (https://pubchem.ncbi.nlm.nih.gov/compound/971).

Navajas, M. J. 2010. Tracking the colonisation history of the invasive species *Varroa destructor*, pp. 375–378. *In* Sabelis, M.W., Bruin, J. (eds.), Trends in Acarology. Springer Netherlands, Dordrecht.

Noël, A., Y. Le Conte, and F. Mondet. **2020**. *Varroa destructor*: how does it harm *Apis mellifera* honey bees and what can be done about it? Emerg Top Life Sci. 4: 45–57.

Nouvian, M., J. Reinhard, and M. Giurfa. **2016**. The defensive response of the honeybee *Apis mellifera*. Journal of Experimental Biology. 219: 3505–3517.

Oberreiter, H., and R. Brodschneider. **2020**. Austrian COLOSS survey of honey bee colony winter losses 2018/19 and analysis of hive management practices. Diversity. 12: 99.

Ostroverkhova, N. V., O. L. Konusova, A. N. Kucher, and T. N. Kireeva. **2016**. Investigation of polyandry in honey bees (*Apis mellifera*) using microsatellites. Entmol. Rev. 96: 389–394.

Page, P., Z. Lin, N. Buawangpong, H. Zheng, F. Hu, P. Neumann, P. Chantawannakul, and V. Dietemann. **2016**. Social apoptosis in honey bee superorganisms. Sci Rep. 6: 27210.

Page, R. E., R. Scheiner, J. Erber, and G. V. Amdam. **2006**. The development and evolution of division of labor and foraging specialization in a social insect (*Apis mellifera* L.). Curr Top Dev Biol. 74: 253–286.

Papežíková, I., M. Palíková, S. Kremserová, A. Zachová, H. Peterová, V. Babák, and S. Navrátil. **2017**. Effect of oxalic acid on the mite *Varroa destructor* and its host the honey bee *Apis mellifera*. Journal of Apicultural Research. 56: 400–408.

Park, D., J. W. Jung, B.-S. Choi, M. Jayakodi, J. Lee, J. Lim, Y. Yu, Y.-S. Choi, M.-L. Lee, Y. Park, I.-Y. Choi, T.-J. Yang, O. R. Edwards, G. Nah, and H. W. Kwon. **2015**. Uncovering the novel characteristics of Asian honey bee, *Apis cerana*, by whole genome sequencing. BMC Genomics. 16: 1.

Peck, D. T., and T. D. Seeley. **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: e0218392.

Peng, Y.-S., Y. Fang, S. Xu, and L. Ge. **1987**. The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. J Invertebr Pathol. 49: 54–60.

Petz, M., A. Stabentheiner, and K. Crailsheim. **2004**. Respiration of individual honeybee larvae in relation to age and ambient temperature. J Comp Physiol B. 174: 511–518.

Pirk, C. W. W. 2018. Honeybee evolution: royal jelly proteins help queen larvae to stay on top. Curr Biol. 28: R350–R351.

Rademacher, E., and M. Harz. **2006**. Oxalic acid for the control of varroosis in honey bee colonies – a review. Apidologie. 37: 98–120.

Rademacher, E., M. Harz, and S. Schneider. **2017**. Effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae). Insects. 8: 84.

Raikhel, A. S., K. W. Deitsch, and T. W. Sappington. **1997**. Culture and analysis of the insect fat body, pp. 507–522. *In* Crampton, J.M., Beard, C.B., Louis, C. (eds.), The Molecular Biology of Insect Disease Vectors: A Methods Manual. Springer Netherlands, Dordrecht.

Ramsey, M.-T., M. Bencsik, M. I. Newton, M. Reyes, M. Pioz, D. Crauser, N. S. Delso, and Y. Le Conte. **2020**. The prediction of swarming in honeybee colonies using vibrational spectra. Sci Rep. 10: 9798.

Ramsey, S. D., R. Ochoa, G. R. Bauchan, C. J. Gulbronson, J. D. Mowery, A. J. Cohen, D. W. Lim, J. P. Joklik, J. M. Cicero, J. D. Ellis, D. J. Hawthorne, and D. vanEngelsdorp. **2019**. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *In* Proceedings of the National Academy of Sciences of the United States of America. 116: 1792-1801.

Rands, S. A., and H. M. Whitney. **2010**. Effects of pollinator density-dependent preferences on field margin visitations in the midst of agricultural monocultures: a modelling approach. Ecol Modell. 221: 1310–1316.

Rangel, J., and A. Fisher. **2019**. Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review. Apidologie. 50: 759–778.

Richard, F.-J., D. R. Tarpy, and C. M. Grozinger. **2007**. Effects of insemination quantity on honey bee queen physiology. PLoS One. 2: e980.

Ricigliano, V. A., B. M. Mott, P. W. Maes, A. S. Floyd, W. Fitz, D. C. Copeland, W. G. Meikle, and K. E. Anderson. **2019**. Honey bee colony performance and health are enhanced by apiary proximity to US Conservation Reserve Program (CRP) lands. Sci Rep. 9: 4894.

Rinkevich, F. D. 2020. Detection of amitraz resistance and reduced treatment efficacy in the varroa mite, *Varroa destructor*, within commercial beekeeping operations. PLOS ONE. 15: e0227264.

Rinkevich, F. D., R. G. Danka, and K. B. Healy. **2017**. Influence of varroa mite (*Varroa destructor*) management practices on insecticide sensitivity in the honey bee (*Apis mellifera*). Insects. 8: 9.

Robinson, G. E. 1987. Regulation of honey bee age polyethism by juvenile hormone. Behav Ecol Sociobiol. 20: 329–338.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. **2010**. Biology and control of *Varroa destructor*. J Invertebr Pathol. 103: S96–S119.

Roth, M. A., J. M. Wilson, K. R. Tignor, and A. D. Gross. **2020**. Biology and Management of *Varroa destructor* (Mesostigmata: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) Colonies. J Integr Pest Manag. 11: 1.

Rousseau, A., V. Fournier, and P. Giovenazzo. **2015**. *Apis mellifera* (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of breeding. Can Entomol. 147: 1–10.

Rowland, C. M., and A. R. McLellan. **1987**. Seasonal changes of drone numbers in a colony of the honeybee, *Apis mellifera*. Ecol Modell. 37: 155–166.

Rubanov, A., K. A. Russell, J. A. Rothman, J. C. Nieh, and Q. S. McFrederick. **2019**. Intensity of *Nosema ceranae* infection is associated with specific honey bee gut bacteria and weakly associated with gut microbiome structure. Sci Rep. 9: 3820.

Ryabov, E. V., A. K. Childers, Y. Chen, S. Madella, A. Nessa, D. vanEngelsdorp, and J. D. Evans. **2017**. Recent spread of *Varroa destructor* virus-1, a honey bee pathogen, in the United States. Sci Rep. 7: 17447.

Sáez, A., M. A. Aizen, S. Medici, M. Viel, E. Villalobos, and P. Negri. **2020**. Bees increase crop yield in an alleged pollinator-independent almond variety. Sci Rep. 10: 3177.

Sagili, R. R., B. N. Metz, H. M. Lucas, P. Chakrabarti, and C. R. Breece. **2018**. Honey bees consider larval nutritional status rather than genetic relatedness when selecting larvae for emergency queen rearing. Sci Rep. 8: 7679.

Sagili, R. R., T. Pankiw, and B. N. Metz. **2011**. Division of labor associated with brood rearing in the honey bee: how does it translate to colony fitness? PLoS One. 6: e16785.

Sammataro, D., and A. Avitabile. **2011**. The beekeeper's handbook, 4th ed. Cornell University Press, Ithaca, New York.

Sammataro, D., P. Untalan, F. Guerrero, and J. Finley. **2005**. The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. Int J Acarol. 31: 67–74.

Sandhu, H., B. Waterhouse, S. Boyer, and S. Wratten. **2016**. Scarcity of ecosystem services: an experimental manipulation of declining pollination rates and its economic consequences for agriculture. PeerJ. 4: e2099.

Simone-Finstrom, M., H. Li-Byarlay, M. H. Huang, M. K. Strand, O. Rueppell, and D. R. Tarpy. **2016**. Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. Sci Rep. 6: 32023.

Smart, M. D., C. R. V. Otto, and J. G. Lundgren. **2019**. Nutritional status of honey bee (*Apis mellifera* L.) workers across an agricultural land-use gradient. Sci Rep. 9: 16252.

Southwick, E., and G. Heldmaier. **1987**. Temperature control in honey bee colonies. AIBS. 37: 395–399.

Spleen, A. M., E. J. Lengerich, K. Rennich, D. M. Caron, R. Rose, J. S. Pettis, M. Henson, J. T. Wilkes, M. Wilson, J. Stitzinger, K. Lee, M. Andree, R. Snyder, and D. vanEngelsdorp. **2012**. A national survey of managed honey bee 2011–12 winter colony losses in the United States: results from the Bee Informed Partnership: J Apic Res: Vol 52, No 2. (https://www.tandfonline.com/doi/abs/10.3896/IBRA.1.52.2.07).

Stahlmann-Brown, P., T. Robertson, and O. Borowik. **2020**. Report on the 2019 New Zealand Colony Loss Survey. Biosecurity New Zealand. 1-74. https://www.mpi.govt.nz/dmsdocument/40349/direct

Stein, K., D. Coulibaly, K. Stenchly, D. Goetze, S. Porembski, A. Lindner, S. Konaté, and E. K. Linsenmair. **2017**. Bee pollination increases yield quantity and quality of cash crops in Burkina Faso, West Africa. Sci Rep. 7: 17691.

Stief, K., B. Cornelissen, J. D. Ellis, and M. O. Schäfer. **2020**. Controlling small hive beetles, *Aethina tumida*, in western honey bee (*Apis mellifera*) colonies by trapping wandering beetle larvae. J Apic Res. 59: 539–545.

Straub, L., G. R. Williams, J. Pettis, I. Fries, and P. Neumann. **2015**. Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. Curr Opin Insect Sci, Neuroscience * Special Section: Insect conservation. 12: 109–112.

Stürup, M., B. Baer-Imhoof, D. R. Nash, J. J. Boomsma, and B. Baer. **2013**. When every sperm counts: factors affecting male fertility in the honeybee *Apis mellifera*. Behav Ecol. 24: 1192–1198.

Thompson, H. M., M. A. Brown, R. F. Ball, and M. H. Bew. **2002**. First report of *Varroa destructor* resistance to pyrethroids in the UK. Apidologie. 33: 357–366.

Thoms, C. A., K. C. Nelson, A. Kubas, N. Steinhauer, M. E. Wilson, and D. vanEngelsdorp. **2019**. Beekeeper stewardship, colony loss, and *Varroa destructor* management. Ambio. 48: 1209–1218.

Toufailia, H. A., L. Scandian, and F. L. W. Ratnieks. **2015**. Towards integrated control of varroa: 2)comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts. J Apic Res. 54: 108–120.

Traynor, K. S., F. Mondet, J. R. de Miranda, M. Techer, V. Kowallik, M. A. Y. Oddie, P. Chantawannakul, and A. McAfee. **2020**. *Varroa destructor*: A complex parasite, crippling honey bees worldwide. Trends Parasitol. 36: 592–606.

US EPA. **2015a**. Regulations.gov - Supporting & related material document. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2015-0043-0119).

US EPA, O. 2015b. Integrated pest management (IPM) Principles. US EPA. (https://www.epa.gov/safepestcontrol/integrated-pest-management-ipm-principles).

US EPA, O. 2016. EPA-registered pesticide products approved for use against varroa mites in bee hives. US EPA. (https://www.epa.gov/pollinator-protection/epa-registered-pesticide-products-approved-use-against-varroa-mites-bee-hives).

vanEngelsdorp, D., and M. D. Meixner. **2010**. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol. 103: S80–S95.

vanEngelsdorp, D., K. S. Traynor, M. Andree, E. M. Lichtenberg, Y. Chen, C. Saegerman, and D. L. Cox-Foster. **2017**. Colony Collapse Disorder (CCD) and bee age impact honey bee pathophysiology. PLOS ONE. 12: e0179535.

Wallner, K. 1999. Varroacides and their residues in bee products. Apidologie. 30: 235–248.

Weber, E. 2012. *Apis mellifera*: the domestication and spread of European honey bees for agriculture in North America. 4: 20-23.

Wilson, E. O., and B. Hölldobler. **2005**. Eusociality: origin and consequences. PNAS. 102: 13367–13371.

Wu, J., S. Yan, J. Zhao, and Y. Ye. **2014**. Barbs facilitate the helical penetration of honeybee (*Apis mellifera ligustica*) stingers. PLoS One. 9: e103823

Xie, X., Z. Y. Huang, and Z. Zeng. **2016**. Why do *Varroa* mites prefer nurse bees? Sci Rep. 6: 28228.

Chapter 2

Effects of multi-day continuous release oxalic acid on *Varroa destructor* mites and *Apis mellifera* honey bees

2.1 Abstract

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman) is among the most detrimental biological stressors to the Western honey bee, *Apis mellifera* L.. Parasitism can result in multiple negative effects, including virus transmission, deformities, and reduced longevity. The organic compound oxalic acid is often used by beekeepers to manage *V. destructor* due to its acaricidal properties. Unfortunately, existing application methods do not allow oxalic acid to pass through the wax cappings of honey bee brood cells, where *V. destructor* reproduces. Due to this limitation, there is a need to develop a novel multi-day continuous release technique using oxalic acid that targets *V. destructor* as it emerges from *A. mellifera* brood cells. To investigate this, we established 54 double-deep brood chambered colonies; 19 received cloth towels containing 6g oxalic acid, 5 ml glycerin and 6.5 ml water, 16 received cardboard strips containing 17 g oxalic acid and 33.33 ml glycerin, and 19 were untreated controls. Treatments were applied to colonies for 42 days, or two worker brood cycles. During the experiment, colonies were assessed twice for *V. destructor* abundance, and once for *A. mellifera* adult workers and capped brood, as well as mass. We found no effect of treatment on *V. destructor* abundance, *A. mellifera* worker adults and brood cells, and colony mass. This study showed that under our experimental design that the tested multi-day continuous release oxalic acid treatments had no effect on *V. destructor* or *A. mellifera*.

2.2 Introduction

Pollinators are crucial for successful crop production in global agricultural systems. Of the roughly 300 agricultural crops, more than 80% benefit from insect pollination (Allsopp et al. 2008). Among the most commonly used commercial insect pollinators is the Western honey bee, *Apis mellifera* L. (vanEngelsdorp and Meixner 2010). *Apis mellifera,* a generalist pollinator, feeds on a variety of flowering plants, both managed and unmanaged, that benefit or rely on insects for pollination (Allsopp et al. 2008, Aizen et al. 2009). Part of the widespread use of *A. mellifera* for global agriculture is due to its biology and sociality. *Apis mellifera* live in colonies composed of thousands of workers, the caste which pollinates flowers. Colonies can be easily transported to areas of crop production, such as in the early spring for almond pollination in California (Hung et al. 2018, Sáez et al. 2020). However, the global decline in insect pollinators, especially *A. mellifera*, has led to concerns about agricultural production and food security.

Recently, it has been highlighted that the American beekeeping industry has been threatened by increased colony mortality (vanEngelsdorp et al. 2017). In years 2019-2020, United States beekeepers reported an annual loss of 43.7%, with a 22.2% winter loss (Bruckner et al. 2020). The reasons for the increase in colony loss can be attributed to both abiotic and biotic stressors (Havard et al. 2020). Many stressors affect the health of *A. mellifera,* including insecticides, pests and diseases, and nutrition, among other stressors (Rosenkranz et al. 2010, Zhang and Nieh 2015, Pettis et al. 2016, Branchiccela et al. 2019, Burnham 2019, Havard et al. 2020).

Among the most detrimental biological stressors associated with colony mortality is the introduced obligate ectoparasitic mite, *Varroa destructor* (Anderson and Trueman) (Rosenkranz et al. 2010). Historically a parasite of the Eastern honey bee, *Apis cerana* (Fabricius), the

lifecycle of *V. destructor* can be categorized into two major phases – the dispersal phase, which travels and feeds on adult *A. mellifera*, and the reproductive phase, which reproduces and develops within *A. mellifera* brood cells (Rosenkranz et al. 2010, Roth et al. 2020). The reproductive stage begins when a female *V. destructor* mite enters an *A. mellifera* brood cell containing a 5th instar larvae. Once the brood cell is capped, the foundress mite begins feeding on the developing *A. mellifera*. Seventy hours later, the foundress lays a single haploid egg that develops into a male, followed by repeated deposition of diploid eggs that develop into females (Rosenkranz et al. 2010, Piou et al. 2016). On average, up to 1.5 and 2.5 daughters are produced in *A. mellifera* worker and drone brood cells, respectively (Sammataro and Avitabile 2011, Traynor et al. 2020); the difference in number of offspring produced is because drone development time is longer than that of workers (Sammataro and Avitabile 2011). The female *V. destructor* remains in the dispersal stage for up to 10 days, until they once again come in contact with a cell containing a 5th instar *A. mellifera* larva (Piou et al. 2016). During the dispersal stage, the adult female *V. destructor* feeds on the fat body of nurse *A. mellifera*, which is the preferred life stage of worker *A. mellifera*, as nurses have larger fat bodies (Xie et al. 2016, Ramsey et al. 2019). Damage to the fat body includes decreased lipid synthesis, impaired metabolic function and decreased longevity (van Dooremalen et al. 2013, Ramsey et al. 2019).

Varroa destructor elicits both lethal and sub-lethal effects on *A. mellifera* (Koleoglu et al. 2017b). *V. destructor* feeds on the fat body (Ramsey et al. 2019), an important organ of the insect involved in storage and regulation of metabolites and immunity, of *A. mellifera* (Raikhel et al. 1997, Arrese and Soulages 2010). Additionally, feeding can result in virus transmission, reduced immune response, shortened lifespan, anatomical deformities, among other negative effects to *A. mellifera* (Yang and Cox-Foster 2007, Conte et al. 2010, Rosenkranz et al. 2010,

Annoscia et al. 2012). At high infestations, *V. destructor* severely weakens the colony, ultimately leading to its death if left unmanaged by the beekeeper (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017).

To mitigate the negative effects of *V. destructor*, beekeepers rely on Integrated Pest Management strategies to keep infestation at a non-damaging level. (Delaplane et al. 2005). Integrated Pest Management begins with prevention strategies such as cultural practices like using tolerant or resistant stock, and mechanical means like drone brood removal or making colonies broodless to limit *V. destructor* reproduction (Gregorc et al. 2017, Panziera et al. 2017, Roth et al. 2020). Chemical treatment is most often used when all other control strategies have not successfully maintained infestation below the treatment threshold, which is currently set at 3 percent infestation of adult *A. mellifera* (Caron et al. 2018). Currently, both synthetic and natural compounds are available to beekeepers for *V. destructor* control (US EPA 2016). Overuse of synthetic varroacides without changing modes-of-action has led to resistance in *V. destructor* populations, as is the case with many pests in important agricultural crops (Thompson et al. 2002, Sammataro, Olafson, et al. 2005, Silva et al. 2012, Gregorc et al. 2018a, Rinkevich 2020). It has also led to chemical residues detected in bee products (Mullin et al. 2010). Due to the limited availability of efficacious varroacides, as well as product use limitations with respect to colony condition and weather (US EPA 2016, Underwood and López-Uribe 2019), there is a need to identify new compounds and control techniques that can be used to treat *V. destructor* effectively under a variety of conditions (Caron et al. 2018).

Organic acids, such as oxalic acid, have become increasingly popular among beekeepers due to their high efficacy and low probability of resistance development in *V. destructor* (Maggi et al. 2015, Adjlane et al. 2016). The low probability of resistance development is thought to be

due to its rapid degradation within the colony, limited use by beekeepers, and that it does not affect neurons (Imdorf et al. 1999, Maggi et al. 2016). Oxalic acid is most often applied via trickling, sublimation, or spraying (Rademacher and Harz 2006a). Although oxalic acid has shown considerable promise for managing *V. destructor* infestations, its effectiveness is dependent on weather and conditions within the hive. For example, its acaricidal effects do not extend to within the wax capped brood cells where *V. destructor* reproduce and develop alongside *A. mellifera* (Adjlane et al. 2016). Therefore, treatment with oxalic acid is most often implemented in winter, when there is little to no *A. mellifera* brood within the colony (Higes et al. 1999). Due to this limitation, there is an interest to develop novel delivery systems for oxalic acid to effectively control *V. destructor* during the brood-rearing season.

Recently, Maggi et al. (2015) developed a multi-day continuous release technique using cellulose strips containing oxalic acid, glycerin, and water draped over *A. mellifera* brood frames for 42 days, or two *A. mellifera* worker brood cycles, to gain control of *V. destructor* as they emerged from *A. mellifera* brood cells and entered the dispersal phase. Oliver (2017) experimented with different ratios of oxalic acid, glycerin, and water using different matrices, like cloth shop towel and cellulose boards placed between the hive brood boxes for 42 days in an effort to identify a product with high efficacy that could be easily applied by commercial beekeepers managing thousands of *A. mellifera* colonies. Subsequent work found that using a ratio of equal parts oxalic acid, glycerin and water for oxalic acid shop towels led to ease of application and effective *V. destructor* control.

Because promise of these treatments has been shown by Maggi et al. (2015) and Oliver (2017) in Argentina and California, and because environmental conditions are known to influence effectiveness of chemical treatments against *V. destructor*, the specific objective of this

study was to determine the effects of these continuous release applications on *V. destructor* abundance and *A. mellifera* colony strength in the Southeast United States during the brood rearing season of *A. mellifera*. Based on the results of Maggi et al. (2015) and Oliver (2017), a negative effect of treatment on *V. destructor* abundance, and no effect on *A. mellifera* colony strength, was predicted.

2.3 Methods

2.3.1 Experimental design

The experiment was performed in Lakemont, Georgia from August to September 2017 using *Apis mellifera* honey bee colonies maintained in double-deep Langstroth hive equipment. Prior to the study, colonies were preliminarily screened for queen status, worker adult and capped brood numbers, mass, and *Varroa destructor* abundance to identify those uniform in strength and health (Delaplane et al. 2013, Dietemann et al. 2013). Ultimately, 54 colonies were allocated to one of three experimental groups – 16 received an oxalic acid cellulose board treatment (hereafter OA Cardboard), 19 received an oxalic acid shop towel treatment (hereafter OA Shop Towels), and 19 received no treatment (hereafter Control). Within the apiary, colonies were arranged ~3m apart in a non-uniform row with entrances randomly directed Northeast, East, or Southeast to minimize drifting of workers that could have adhering *V. destructor* (Bordier et al. 2017). Prior to treatment application, colonies were again assessed for *V. destructor*. Colonies were exposed to their respective treatment group applications for 42 days, which corresponds to two worker brood cycles (Sammataro and Avitabile 2011) and to similar *V. destructor* control products marketed in other countries (Maggi et al. 2015, Rodríguez Dehaibes et al. 2020).

2.3.2 Treatment Groups

OA Shop Towels were made one day prior to application using a modified protocol based on Oliver (2017) and Maggi et al. (2015). In brief, 715 ml distilled water (Nice!, Deerfield, IL) was slowly brought to 83 °C, then added to a 400 ml beaker containing 660 g oxalic acid dihydrate (Brushy Mountain Bee Farm, Moravian Falls, NC), which was further heated to 325 °C using a hot plate (VWR, Radnor, PA) until the oxalic acid dihydrate was fully dissolved (Fig. 1). These temperatures were monitored using a calibrated thermometer (VWR, Radnor, PA). Next, 550 ml vegetable glycerin (Froggy's Fog, Columbia, TN), previously warmed in a microwave (Sunbeam, Boca Raton, FL) for 1 min, was added and homogenized by stirring. The solution was allowed to cool, then poured onto a 13.95 x 13.2 cm 55-sheet-roll of blue shop towels (Scott, Neenah, WI) that was previously cut in half transversely and placed in a metal cake pan (Mainstays, Bentonville, AR) (Fig. 2). The fully soaked towel roll, containing 55 sheets cut in half, was left in a ventilated room overnight to allow evaporation of excess moisture. Similar to the OA Shop Towels, OA Cardboard were cut into 11.43 x 25.4 cm sections and soaked in a solution containing 2400 g of oxalic acid and 4800 ml of glycerin per cellulose board; they were also allowed to evaporate overnight (Fig. 3). This solution was enough to make 60 cellulose boards.

The following day, three 14 x 27 cm OA Shop Towels, each containing ~6 g oxalic acid dihydrate, 5 ml vegetable glycerin, and 6.5 ml distilled water were placed across the top bars of the bottom brood box of each colony allocated to the OA Shop Towels treatment group (n=19). Similarly, three 11.43 x 25.4 cm cardboard strips, each containing 17 g oxalic acid dihydrate and 33.33 ml vegetable glycerin, were placed across the top bars of the bottom brood box of each colony allocated to the OA Cardboard treatment group (n=16). OA Shop Towels and OA

Cardboard were spaced ~2.5 cm apart to allow *A. mellifera* workers to move between brood boxes (Fig. 4). In summary, colonies from both the OA Shop Towels and OA Cardboard treatment groups were each exposed to 18 and 51 g oxalic acid dihydrate, respectively. These amounts are similar to the amounts used in the studies conducted by Maggi et al. (2015) and Oliver (2017).

Fig. 1. Mixing of oxalic acid dihydrate in distilled water for the OA Shop Towel treatment. For OA Shop Towels, 660 g of oxalic acid dihydrate was added to 715 ml hot distilled water until fully dissolved. 550 ml of vegetable glycerin was then added to the oxalic acid/distilled water mixture, homogenized, and then poured over the half-roll of shop towels and allowed to evaporate excess moisture overnight in a ventilated room.

Fig. 2: For the OA Shop Towel treatment, each 14 x 27 cm half-sheet containing 6 g of oxalic acid dihydrate, 5 ml of vegetable glycerin, and 6.5 ml of distilled water. OA Shop Towels were placed on the top bars of the lower brood box's frames, with the upper brood box placed on top, and were left in the colony for 42 days.

Fig. 3. For the OA Cardboard treatment, each 11.43 x 25.4 cellulose board containing 17 g of oxalic acid dihydrate and 33.33 ml of vegetable glycerin. Similar to OA Shop Towels, three OA Cardboard strips were placed on the top bars of the lower brood box's frames, with the upper brood box placed on top, and were left in the colony for 42 days. In total, each colony received ~ 51 g of oxalic acid and 100 ml of vegetable glycerin.

Fig. 4. Application of shop towels onto the top bars of the bottom brood box in an *Apis mellifera* honey bee colony belonging to the OA Shop Towel treatment group. Each colony received ~ 18 g of oxalic acid, 15 ml of vegetable glycerin, and 19.5 ml of distilled water . OA Shop Towels were left in the colony for 42 days.

2.3.3 Colony strength

Each colony was visually assessed on Day 21 for the presence of an egg-laying queen and rough estimates of frames containing *A. mellifera* worker adults and capped brood. This was to ensure that potentially collapsing colonies could be removed from the trial to in order to prevent their influence on other colonies (Peck and Seeley 2019). Furthermore, colony strength was quantified twice during the experiment according to Delaplane et al. (2013) – immediately prior to experimental treatment application at Day 0 and then again at Day 42 days post application. During evaluations on Days 0 and 42, colony mass was quantified using a tripod crane scale (KLAU, China), whereas total percent coverage of deep Langstroth frames was estimated for worker adults and capped brood. Total number of adult workers and developing worker brood was estimated by assuming that 1215 adults and 3.8 brood cells per cm^2 occupy a deep Langstroth frame (Delaplane et al. 2013). Total percent coverage by adult workers was divided by 100 and multiplied by 1215 to determine the total number of individuals inside the colony. Similarly, the total percent coverage by capped brood was divided by 100 and then multiplied by 880 and 3.8 to find the total number of capped brood cells inside the colony. For colony mass, contributions from woodenware, including frames with new foundation, was removed from the overall measurement to identify mass of drawn comb, its contents such as brood or stored food, and adult workers. Colony death, defined by when colonies were hopelessly queenless or when less than 2 deep frames of adult workers were present, was recorded at each inspection.

2.3.4 *Varroa destructor* abundance

Varroa destructor was quantified every 21 days in each colony by collecting ~300 adult workers from a frame containing open worker brood (Dietemann et al. 2013). Individuals were

shaken into a 40 x 31.8x15.2 cm holding container (Sterilite, Townsend, MA), collected using a ½ cup scoop (Measurex, Cupertino, CA), and placed in a 1-gallon Ziploc freezer bag (Ziploc, Racine, WI). Samples were subsequently frozen for 24 hours, then 100 bees were weighed, along with the total weight of the sample, to estimate total number of workers collected. Adult workers were then washed with 70% isopropyl alcohol using a Burrell Wrist-Action Shaker (Pittsburgh, PA) for 35 minutes (Bee Informed Partnership 2017). The alcohol, including its contents, was then passed through a fine-mesh strainer (Oneida, Lincolnshire, IL) so that mites could be counted. *V. destructor* abundance was determined by dividing the number of mites washed by the total weight of the sample, then multiplied by the weight of 100 bees.

2.3.5 Statistical analyses

All data manipulation and analyses were performed in RStudio (Version 1.1.463). We removed any colonies from the analysis that did not have complete data for the 42 day experimental period. For each variable (*V. destructor* abundance, worker and capped brood, and colony mass) we used two analyses. For the first analysis, we used a generalized linear mixed modelling (GLMMs) for analyzing data with multiple timepoints (repeated measures) to account for nested structure. For the GLMMs, we used the 'afex' package (Singmann et al. 2020), which interfaces with the 'lme4' package (Bates et al. 2015). For the second analysis, we used the mixed 'mixed' function with likelihood-ratio tests to perform type-III anovas to confirm significance. We used Poisson-distributed error structures for *V. destructor* abundance data after rounding upwards to the nearest whole *V. destructor* (ceiling transformation), and Gaussian-distributed error structures for worker adults and capped brood, and colony mass. The 'emmeans' package was used to generate compact letter displays for plotting of data (Lenth,

2020). This package generates compact letter displays of all pairwise comparisons of estimated marginal means. When a p-value exceeds 0.05, the means will have at least one letter in common, denoting similarities between data.

We also analyzed *V. destructor* by calculating a change between Days 0 and 42 – effectively a 'delta (Δ)' value. For the effect of treatment on these single-value per colony data (delta variables), we used type-II anovas ('car' package (Fox and Weisberg, 2019)) assuming a Gaussian-distribution. In all cases, for both mixed-models and anovas, response variable distributions were graphically inspected as histograms and tested using a Shapiro-Wilk test for normality, although emphasis was principally placed on inspection of residuals vs fitted values. QQ-Plots were used to check analytical and model assumptions, with the exception of the Poisson-distributed *V. destructor* abundance response variable using mixed modelling.

For *V. destructor* abundance, we also calculated a Henderson-Tilton's efficacy measure of each treatment by comparing each treatment block to the control block; for this we used the mean values of all colonies in a block for the 'plot' value (Gama 2015). This efficacy measure accounts for a non-uniform population, and for infestations of individuals.

2.4 Results

2.4.1 Colony Strength

There was no significant difference among treatment groups concerning the number of *A. mellifera* worker adults ($p=0.25$), or between sampling period (Days 0 and 42) ($p=0.46$) (Fig. 5). Although there was no significant difference among treatment groups on the number of *A. mellifera* worker capped brood cells ($p=0.95$), there was a significant difference between sampling period (Days 0 and 42) ($p<0.001$) (Fig. 6). Furthermore, there was no significant difference among treatment groups concerning colony mass ($p=0.15$), but there was a significant difference between sampling period (Days 0 and 42) ($p<0.001$) (Fig. 7).

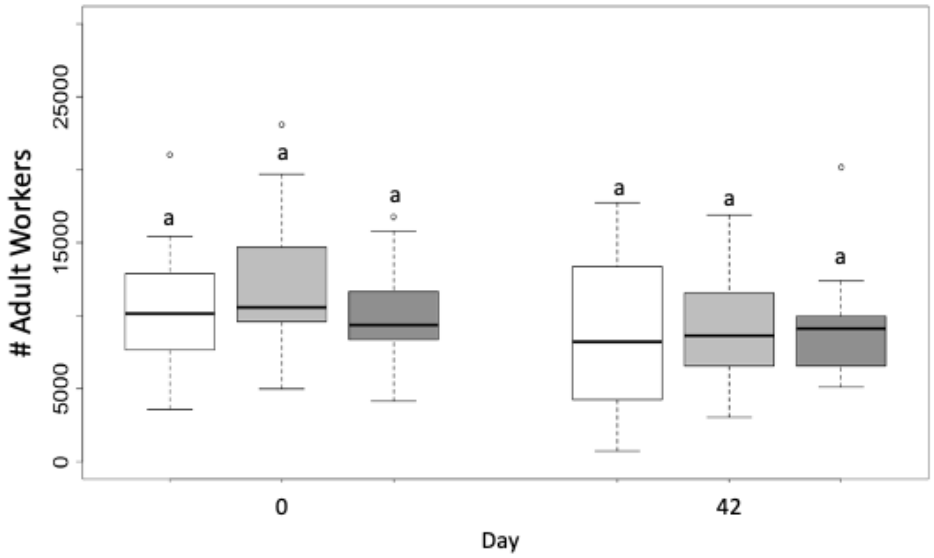

Fig. 5: *Apis mellifera* worker adult populations at each sampling period (Day 0 and Day 42) among treatment groups - Control (n=19) (white), OA Cardboard (n=16) (light grey), and OA Shop Towel (n=19) (dark grey). There was no statistical difference among treatment groups ($p=0.25$) or sampling period ($p=0.46$). Different letters above each plot indicate significant differences ($p<0.05$). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

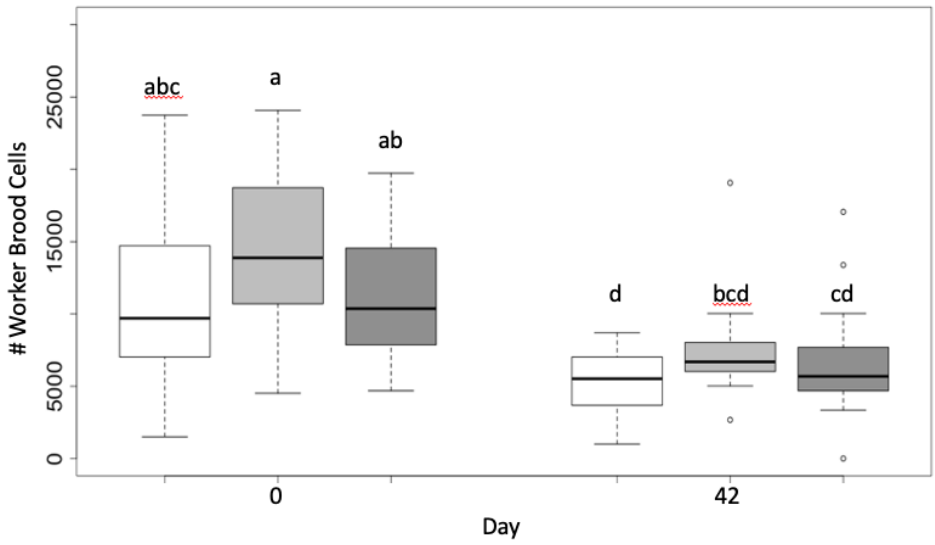

Fig. 6: *Apis mellifera* worker capped brood populations at each sampling period (Day 0 and Day 42) among treatment groups Control (n=19) (white), OA Cardboard (n=16) (light grey), and OA Shop Towel (n=19) (dark grey). There was no significant effect among treatment groups ($p=0.95$); however, there was a significant effect between sampling days ($p<0.001$). Different letters above each plot indicate significant differences ($p<0.05$). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

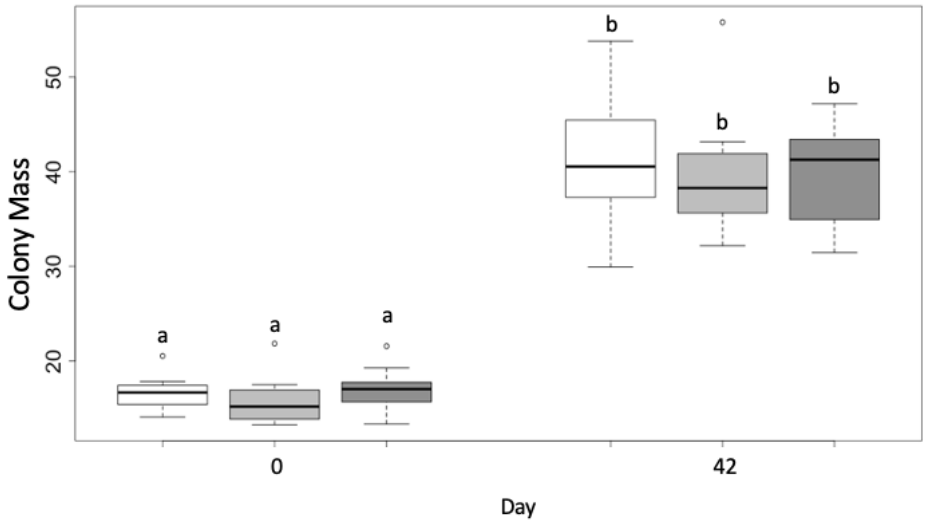

Fig. 7: Colony mass (kg) at each sampling period (Day 0 and Day 42) among treatment groups Control (n=19) (white), OA Cardboard (n=16) (light grey), and OA Shop Towels (n=19) (dark grey). There was no significant difference among treatment groups (p=0.15); however, there was a significant difference between sampling period (p<0.001). Different letters above each plot indicate significant differences (p<0.05). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

2.4.2 *Varroa destructor* abundance

There was no significant difference of *V. destructor* abundance among treatment groups (p=0.23); however, there was a significant difference among sampling period (Days 0, 21, and 42) (p=0.044) (Fig. 8). Compared to Control, efficacy for OA Cardboard and OA Shop Towel was 61% and 49%, respectively. We found evidence for significantly increasing *V. destructor* abundance with time across the 42-day experiment (p = 0.04, positive coefficient), but no effect

of treatment on this rate of increase (p=0.23). Furthermore, we found no significant effect of treatment on change in *V. destructor* abundance between Day 0 and 42 (p=0.071) (Fig. 9).

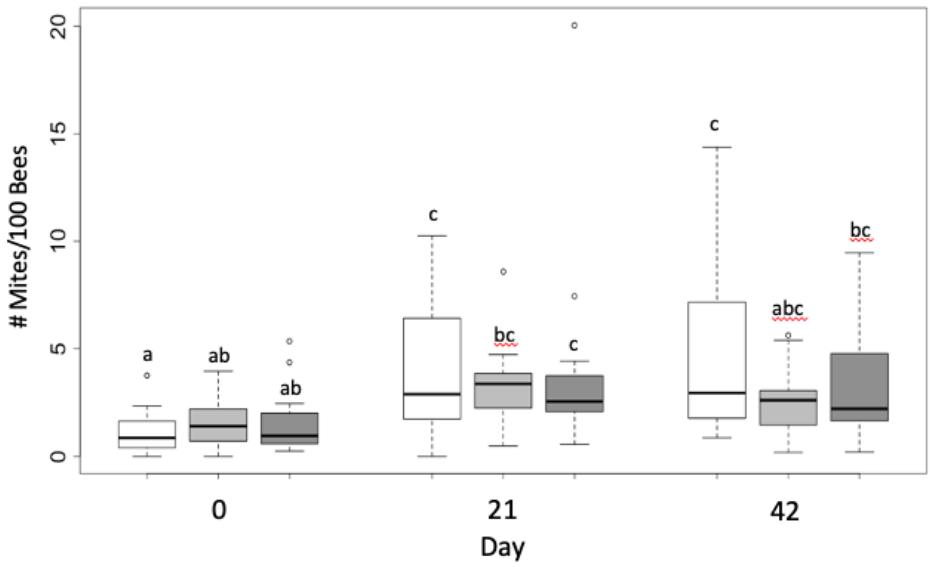

Fig. 8: *Varroa destructor* abundance at each sampling period (Day 0, Day 21, and Day 42) among treatment groups Control (n=19) (white), OA Cardboard (n=16) (light grey), and OA Shop Towel (n=19) (dark grey). Different letters above each plot indicate significant differences. We found no significant difference among treatment groups (p=0.23); however, there was a significant difference among sampling periods (p=0.044). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

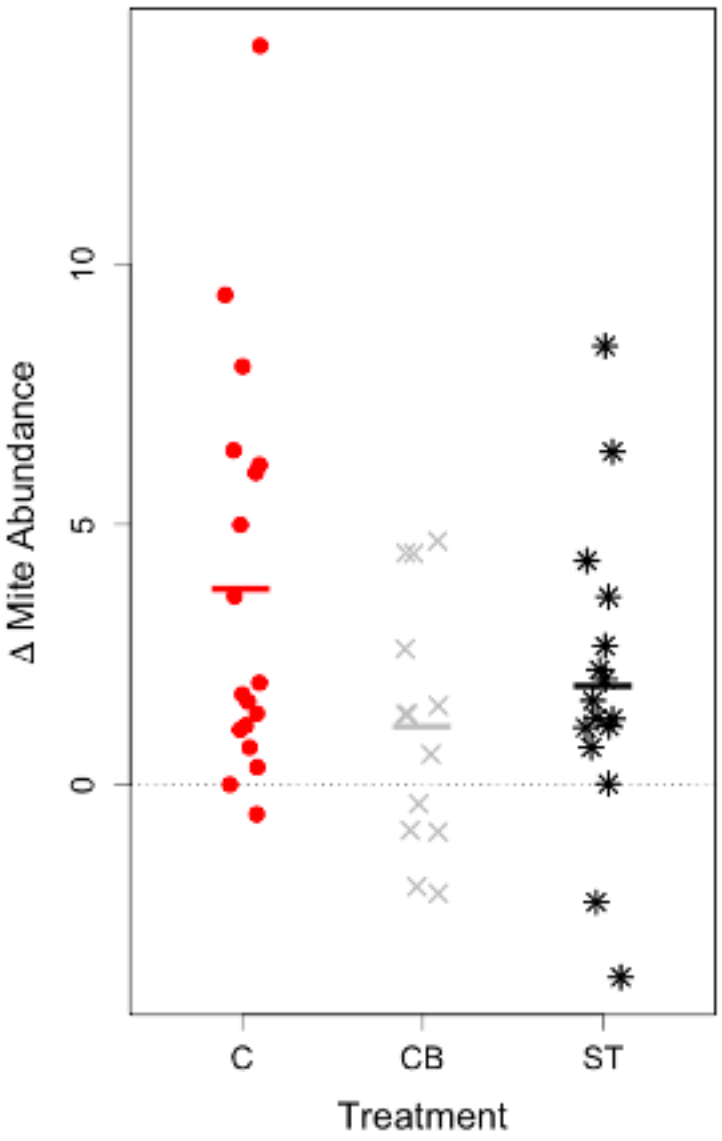

Fig. 9: The effect of treatment on the change of the number of *Varra destructor* mites for each *Apis mellifera* colony modeled over the 42-day experimental period. Control (n=19) is abbreviated as “C”, OA Cardboard (n=16) as “CB”, and OA Shop Towel (n=19) as “ST”. There was no significant difference for the change in *V. destructor* abundance (p=0.071).

2.5 Discussion

Oxalic acid is believed to be a promising tool of an Integrated Pest Management program for beekeepers to limit the negative effects of *Varroa destructor* on *Apis mellifera* colonies (Toomemaa et al. 2010, Gregorc and Sampson 2019). In this study, we found that multi-day continuous release oxalic acid shop towels or cellulose boards did not significantly reduce *V. destructor* in *A. mellifera* colonies. This is in contrast to Maggi et al. (2015) and Oliver (2017). However, similar to Maggi et al. (2015) and Oliver (2017, 2018), we observed that the multi-day continuous release treatments did not have any negative effects on *A. mellifera* worker adults and capped brood, or colony mass.

Although many compounds are available to beekeepers for control of *V. destructor,* colony loss due to this devastating parasite is still increasing (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017). Due to instances of acaricide resistance to synthetic chemicals in *V. destructor* populations, natural acaricides have become frequently used among beekeepers because there has been no evidence of resistance of *V. destructor* to these compounds (Sammataro, Olafson, et al. 2005, Rademacher and Harz 2006a, Gregorc et al. 2018b, Rinkevich 2020). Our study suggests that currently communicated methods of multi-day continuous release of oxalic acid using shop towel or cellulose matrices placed between *A. mellifera* brood chambers does not effectively control *V. destructor*, at least under our experimental conditions.

As a natural compound, oxalic acid has become increasingly popular among beekeepers. It is commonly used during the fall or winter when brood is absent by trickling, spraying or sublimation (Rademacher and Harz 2006a). Due to the limitation of oxalic acid to only effect *V. destructor* present on adult worker *A. mellifera*, research is ongoing to establish a continuous release formulation that will kill *V. destructor* emerging from *A. mellifera* brood cells over

sustained periods of time, especially during major brood-rearing periods. Previous studies using cellulose strips containing oxalic acid and glycerin (Maggi et al. 2015), and shop towels containing oxalic acid, water, and glycerin (Oliver 2017), found significant control of *V. destructor* during brood-rearing periods under their experimental conditions.

We found no difference among treatment groups for *V. destructor* abundance. Our results differed from both Maggi et al. (2015) and Oliver (2017), who found a significant difference between treatment groups for *V. destructor* quantity. It is worth noting that Maggi et al. (2015) used less oxalic acid and vegetable glycerin for their oxalic acid treatments. The possible impact of differing environmental conditions (i.e., humidity) may have led to different results than Maggi et al. (2015) and Oliver (2017). It is possible that the high environmental humidity caused colonies to remove more moisture from the air, thus causing the cellulose boards and shop towels to lose moisture making the oxalic acid less available for *A. mellifera* to pick up and come in contact with *V. destructor* (Human et al. 2006, Brasil et al. 2013). We did find that colonies that received both oxalic acid continuous release treatments had a diminished range of *V. destructor* abundance when compared to controls. Therefore, treatment using shop towels and cellulose boards may be better for use as a maintenance strategy for keeping *V. destructor* infestation at a non-damaging level within a colony, rather than a control strategy to severely reduce high levels of *V. destructor* infestation.

It is interesting to note that Maggi et al. (2015) used cellulose matrices cut into 45 cm x 3 cm x 1.5 mm strips that were draped over frames 2, 4, 6 and 8 of the bottom brood box, directly next to emerging *V. destructor* and *A. mellifera*. The cellulose matrices each held 10 g of oxalic acid and 20 ml of glycerin. In our study, both cardboard strips and shop towels were placed over the tops of frames in the bottom brood box in an attempt to reduce potential

application time by beekeepers. Our cellulose boards were cut into 11.43 cm x 25.4 cm strips and each held 17 g of oxalic acid and 33.33 ml of vegetable glycerin. This difference in delivery method may have led to a difference in results for *V. destructor* abundance, although each cardboard strip in our experiment contained more oxalic acid. Oliver's (2017) treatment using a 1:1:1 ratio of 18 g oxalic acid and 18 ml each of glycerin and water impregnated in shop towels per colony had considerable efficacy. It is also interesting that Oliver (2017) study used the same amount of oxalic acid, though we used different amounts of vegetable glycerin and water. Current registered varroacides delivering active ingredients like amitraz and formic acid are placed between brood frames, thus more directly exposing nurse and emerging *A. mellifera* which are more likely to carry *V. destructor* (Rosenkranz et al. 2010). However, some currently registered varroacides, such as thymol, are placed on top of the frames of the upper brood box. Evaluating how product placement of registered treatments influences efficacy is certainly warranted.

In this study, we did not find that multi-day, continuous release of oxalic acid via shop towels or cellulose boards had a negative effect on *A. mellifera* worker adults. These results were similar to both Maggi et al. (2015) and Oliver (2017), in which neither study found any negative effects. It has been documented that *A. mellifera* are able to tolerate normal oxalic acid treatments (trickling, spraying, sublimation), although an oral application of oxalic acid has caused high mortality to *A. mellifera* in a laboratory setting (Rademacher et al. 2017b). Additionally, the spraying method of oxalic acid treatment can have more adverse effects on *A. mellifera* worker adults colonies than the trickling or sublimation methods of application (Toufailia et al. 2015). This could be caused by increased acidity to the cuticle or organs of the *A. mellifera* worker adults and brood (Higes et al. 1999, Toomemaa et al. 2010, Terpin et al.

2019). Currently, there are no data on the effects of a long-term continuous release oxalic acid formulation on *A. mellifera* mortality. Based on this work and others, it appears to be relatively safe to *A. mellifera*.

The effects of long-term continuous oxalic acid treatments on *A. mellifera* worker capped brood were also examined. We did not observe that the continuous release oxalic acid treatments had a negative effect on *A. mellifera* worker capped brood when compared to control colonies. These results are also similar to Maggi et al. (2015) and Oliver (2017), in which neither studies found any negative effects. There was a decline in worker capped brood in all treatment groups, which is congruent with season, as this study was performed in autumn when colonies reduce worker population due to environmental cues in preparation for winter (Mattila et al. 2001). It is interesting that brood was not negatively affected by the increased acidity in the colony from the oxalic acid treatments, since liquid oxalic acid treatments like trickling and spraying can result in brood death (Higes et al. 1999, Gregorc et al. 2004, Rademacher et al. 2017b). Any potential long-term effects of the oxalic acid shop towels and cardboard were not determined in this study, so we cannot confirm that our 42 day continuous release oxalic acid treatments had any impacts on *A. mellifera* brood during the winter.

The effects of continuous release oxalic acid via shop towels and cardboard on colony mass was also examined. We did not observe any negative effects of either oxalic acid treatment on colony mass. These results are similar to Oliver (2017), in which there were no observed negative effects of oxalic acid shop towels on colony mass. Colony mass is mostly comprised of carbohydrate storage – nectar, honey, and sugar water – but there are some contributions to mass from *A. mellifera* adults and brood, wax, and pollen. Carbohydrates and protein, obtained via flower nectar and pollen, respectively, are especially important for winter bee production and

colony survival (Kunc et al. 2019). Additionally, nutritional stress can have adverse effects on *A. mellifera* colonies fending infection from viruses and other diseases such as *Nosema* (Branchiccela et al. 2019, Castelli et al. 2020). This highlights the importance of nutrition availability within *A. mellifera* colonies during the wintering period (Branchiccela et al. 2019).

This study elucidates the potential of developing a multi-day continuous release formulation using oxalic acid to treat for *V. destructor* over an extended period of time that would potentially allow effective *V. destructor* control as foundress mites emerge from *A. mellifera* brood cells alongside her son and daughters. Although the two multi-day continuous released oxalic acid application methods studied here did not significantly control *V. destructor* under our experimental conditions, other authors have found promising results using similar delivery mechanisms (Maggi et al. 2015, Oliver 2017). Because there were no observable effects on colony strength from the oxalic acid treatments, a multi-day continuous release formulation appears safe to *A. mellifera*. As stated by Maggi et al. (2015), a continuous release treatment using oxalic acid may offer an effective alternative technique of applying oxalic acid to *A. mellifera* colonies possessing worker brood. This is the first study using a continuous release oxalic acid technique in the southeastern United States, which differs in climate from the western United States and South America where previous studies have been done. To establish a continuous release technique, different ratios of oxalic acid, glycerin, and water should be examined to find effective control in a laboratory setting, prior to moving to field trials. Future studies should investigate different delivery mechanisms and formulations that can be used in diverse climates across seasons in order to effectively control *V. destructor*, while maintaining safety to *A. mellifera* worker adults and capped brood and beekeeper applicators.

References

Adjlane, N., E.-O. Tarek, and N. Haddad. **2016**. Evaluation of oxalic acid treatments against the mite *Varroa destructor* and secondary effects on honey bees *Apis mellifera*. J Arthropod Borne Dis. 10: 501–509.

Aizen, M. A., L. A. Garibaldi, S. A. Cunningham, and A. M. Klein. **2009**. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Ann Bot. 103: 1579–1588.

Allsopp, M. H., W. J. de Lange, and R. Veldtman. **2008**. Valuing insect pollination services with cost of replacement. PLOS ONE. 3: e3128.

Annoscia, D., F. Del Piccolo, and F. Nazzi. **2012**. How does the mite *Varroa destructor* kill the honeybee *Apis mellifera*? Alteration of cuticular hydrocarbons and water loss in infested honeybees. J Insect Physiol. 58: 1548–1555.

Arrese, E. L., and J. L. Soulages. **2010**. Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol. 55: 207–225.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bee Informed Partnership. 2017. The Bee Informed Partnership honey bee tech-transfer team protocol manual. 7.0

Branchiccela, B., L. Castelli, M. Corona, S. Díaz-Cetti, C. Invernizzi, G. Martínez de la Escalera, Y. Mendoza, E. Santos, C. Silva, P. Zunino, and K. Antúnez. **2019**. Impact of nutritional stress on the honeybee colony health. Sci Rep. 9: 10156.

Brasil, D. de F., M. de O. Guimarães, J. A. D. Barbosa Filho, and B. M. Freitas. **2013**. Internal ambience of bee colonies submitted to strengthening management by adding broods. Engenharia Agrícola. 33: 902–909.

Bruckner, S., N. Steinhauer, J. Engelsma, A. M. Fauvel, K. Kulhanek, E. Malcolm, A. Meredith, M. Milbrath, E. L. Niño, J. Rangel, K. Rennich, D. Reynolds, R. Sagili, J. Tsuruda, D. vanEngelsdorp, S. D. Aurell, M. Wilson, and G. R. Williams. **2020**. 2019-2020 honey bee colony losses in the United States: preliminary results. (https://beeinformed.org/wp-content/uploads/2020/06/BIP_2019_2020_Losses_Abstract.pdf).

Burnham, A. J. 2019. Scientific advances in controlling *Nosema ceranae* (*Microsporidia*) infections in honey bees (*Apis mellifera*). Front Vet Sci. 6: 79.

Caron, D. M., M. Dykes, D. Epstein, G. Hansen, M. Nasr, D. Downey, K. Rennich, D. Rogers, R. M. Sears, and T. Steeger. **2018**. Tools for varroa management: a guide to effective varroa sampling & control. (https://honeybeehealthcoalition.org/wp-content/uploads/2018/06/HBHC-Guide_Varroa_Interactive_7thEdition_June2018.pdf).

Conte, Y. L., M. Ellis, and W. Ritter. **2010**. Varroa mites and honey bee health: Can varroa explain part of the colony losses? Apidologie. 41: 353–363.

Delaplane, K. S., J. A. Berry, J. A. Skinner, J. P. Parkman, and W. M. Hood. **2005**. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. J Apic Res. 44: 157–162.

Dietemann, V., J. Pflugfelder, D. Anderson, J.-D. Charrière, N. Chejanovsky, B. Dainat, J. de Miranda, K. Delaplane, F.-X. Dillier, S. Fuch, P. Gallmann, L. Gauthier, A. Imdorf, N. Koeniger, J. Kralj, W. Meikle, J. Pettis, P. Rosenkranz, D. Sammataro, D. Smith, O.

Yañez, and P. Neumann. **2012**. *Varroa destructor*: research avenues towards sustainable control. J Apic Res. 51: 125–132.

Fox, J. and S. Weisberg. 2019. An {R} companion to applied regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Gama, J. 2015. Ecotoxicology: methods for ecotoxicology. R package version 1.0.1. https://CRAN.R-project.org/package=ecotoxicology

Gracia, M. J., C. Moreno, M. Ferrer, A. Sanz, M. Á. Peribáñez, and R. Estrada. **2017**. Field efficacy of acaricides against *Varroa destructor*. PLoS One. 12: e0171633.

Gregorc, A., and B. Sampson. **2019**. Diagnosis of varroa mite (*Varroa destructor*) and sustainable control in honey bee (*Apis mellifera*) colonies - A review. Diversity. 11: 243.

Gregorc, A., M. Alburaki, B. Sampson, P. R. Knight, and J. Adamczyk. **2018b**. Toxicity of selected acaricides to honey bees (*Apis mellifera*) and varroa (*Varroa destructor* Anderson and Trueman) and their use in controlling varroa within honey bee colonies. Insects. 9: 55.

Gregorc, A., M. Alburaki, C. Werle, P. R. Knight, and J. Adamczyk. **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: 821–832.

Gregorc, A., A. Pogacnik, and I. D. Bowen. **2004**. Cell death in honeybee (*Apis mellifera*) larvae treated with oxalic or formic acid. Apidologie. 35: 453–460.

Guzmán-Novoa, E., L. Eccles, Y. Calvete, J. Mcgowan, P. G. Kelly, and A. Correa-Benítez. **2010**. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie. 41: 443–450.

Haber, A. I., N. A. Steinhauer, and D. vanEngelsdorp. **2019**. Use of chemical and nonchemical methods for the control of *Varroa destructor* (Acari: Varroidae) and associated winter colony losses in U.S. beekeeping operations. J Econ Entomol. 112: 1509-1525.

Havard, T., M. Laurent, and M.-P. Chauzat. **2020**. Impact of stressors on honey bees (*Apis mellifera*; Hymenoptera: Apidae): Some guidance for research emerge from a meta-analysis. Diversity. 12: 7.

Higes, M., A. Meana, M. Suárez, and J. Llorente. **1999**. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. Apidologie. 30: 289–292.

Human, H., S. W. Nicolson, and V. Dietemann. **2006**. Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest? Naturwissenschaften. 93: 397–401.

Hung, Keng-Lou James, Kingston, Jennifer M., Albrecht, Matthias, Holway, David A., and Kohn, Joshua R. 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proc R Soc B. 285: 20172140.

Koleoglu, G., P. H. Goodwin, M. Reyes-Quintana, M. M. Hamiduzzaman, and E. Guzman-Novoa. **2017**. Effect of *Varroa destructor*, wounding and varroa homogenate on gene expression in brood and adult honey bees. PLOS ONE. 12: e0169669.

Kulhanek, K., N. Steinhauer, K. Rennich, D. M. Caron, R. R. Sagili, J. S. Pettis, J. D. Ellis, M. E. Wilson, J. T. Wilkes, D. R. Tarpy, R. Rose, K. Lee, J. Rangel, and D. vanEngelsdorp. **2017**. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apic Res. 56: 328–340.

Kunc, M., P. Dobeš, J. Hurychová, L. Vojtek, S. B. Poiani, J. Danihlík, J. Havlík, D. Titěra, and P. Hyršl. **2019**. The year of the honey bee (*Apis mellifera* L.) with respect to its physiology and immunity: A search for biochemical markers of longevity. Insects. 10: 244.

Lenth, R. 2020. emmeans: Estimated marginal means, aka least-squares means. R package version 1.5.1. https://CRAN.R-project.org/package=emmeans

Maggi, M., E. Tourn, P. Negri, N. Szawarski, A. Marconi, L. Gallez, S. Medici, S. Ruffinengo, C. Brasesco, L. Feudis, S. Quintana, D. Sammataro, and M. Eguaras. **2015**. A new formulation of oxalic acid for *Varroa destructor* control applied in *Apis mellifera* colonies in the presence of brood. Apidologie. 47: 596-605.

Martel, A.-C., S. Zeggane, C. Aurières, P. Drajnudel, J.-P. Faucon, and M. Aubert. **2007**. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol®50. Apidologie. 38: 534–544.

Mattila, H. R., J. L. Harris, and G. W. Otis. **2001**. Timing of production of winter bees in honey bee (*Apis mellifera*) colonies. Insectes soc. 48: 88–93.

de Mattos, I. M., A. E. E. Soares, and D. R. Tarpy. **2017**. Effects of synthetic acaricides on honey bee grooming behavior against the parasitic *Varroa destructor* mite. Apidologie. 48: 483–494.

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp, and J. S. Pettis. **2010**. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLOS ONE. 5: e9754.

Oliver, R. 2017. Extended release oxalic acid progress report. American Bee Journal. https://americanbeejournal.com/extended-release-oxalic-acid-progress-report/

Oliver, R. 2018. Extended-release oxalic acid progress report #4. Scientific Beekeeping. (http://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-4/).

Panziera, D., F. van Langevelde, and T. Blacquière. **2017**. Varroa sensitive hygiene contributes to naturally selected varroa resistance in honey bees. J Apic Res. 56: 635–642.

Pettis, J. S., N. Rice, K. Joselow, D. vanEngelsdorp, and V. Chaimanee. **2016**. Colony failure linked to low sperm viability in honey bee (*Apis mellifera*) queens and an exploration of potential causative factors. PLoS One. 11: e0147220

Piou, V., J. Tabart, V. Urrutia, J.-L. Hemptinne, and A. Vétillard. **2016**. Impact of the phoretic phase on reproduction and damage caused by *Varroa destructor* (Anderson and Trueman) to its host, the European honey bee (*Apis mellifera* L.). PLoS One. 11: e0153482.

Rademacher, E., and M. Harz. **2006**. Oxalic acid for the control of varroosis in honey bee colonies – a review. Apidologie. 37: 98–120.

Rademacher, E., M. Harz, and S. Schneider. **2017**. Effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae). Insects. 8: 84.

Raikhel, A. S., K. W. Deitsch, and T. W. Sappington. **1997**. Culture and analysis of the insect fat body, pp. 507–522. *In* Crampton, J.M., Beard, C.B., Louis, C. (eds.), The Molecular Biology of Insect Disease Vectors: A Methods Manual. Springer Netherlands, Dordrecht.

Ramsey, S. D., R. Ochoa, G. R. Bauchan, C. J. Gulbronson, J. D. Mowery, A. J. Cohen, D. W. Lim, J. P. Joklik, J. M. Cicero, J. D. Ellis, D. J. Hawthorne, and D. vanEngelsdorp. **2019**. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *In* PNAS. 116: 5.

Rinkevich, F. D. 2020. Detection of amitraz resistance and reduced treatment efficacy in the varroa mite, *Varroa destructor*, within commercial beekeeping operations. PLOS ONE. 15: e0227264.

Rodríguez Dehaibes, S. R., F. R. Meroi Arcerito, E. Chávez-Hernández, G. Luna-Olivares, J. Marcangeli, M. Eguaras, and M. Maggi. **2020**. Control of *Varroa destructor* development

in Africanized *Apis mellifera* honeybees using Aluen Cap (oxalic acid formulation). Int J Acarol. 1–4.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. **2010**. Biology and control of *Varroa destructor*. J Invertebr Pathol. 103: S96–S119.

Roth, M. A., J. M. Wilson, K. R. Tignor, and A. D. Gross. **2020**. Biology and management of *Varroa destructor* (Mesostigmata: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies. J Integr Pest Manag. 11: 1.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Sáez, A., M. A. Aizen, S. Medici, M. Viel, E. Villalobos, and P. Negri. **2020**. Bees increase crop yield in an alleged pollinator-independent almond variety. Sci Rep. 10: 3177.

Sammataro, D., P. Untalan, F. Guerrero, and J. Finley. **2005**. The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. Int J Acarol. 31: 67–74.

Silva, A. X., G. Jander, H. Samaniego, J. S. Ramsey, and C. C. Figueroa. **2012**. Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) I: a transcriptomic survey. PLOS ONE. 7: e36366.

Singmann, H., B. Bolker, J. Westfall, F. Aust and M. S. Ben-Shachar. (2020). afex: Analysis of factorial experiments. R package version 0.27-2. https://CRAN.R-project.org/package=afex

Terpin, B., D. Perkins, S. Richter, J. K. Leavey, T. W. Snell, and J. A. Pierson. **2019**. A scientific note on the effect of oxalic acid on honey bee larvae. Apidologie. 50: 363–368.

Thompson, H. M., M. A. Brown, R. F. Ball, and M. H. Bew. **2002**. First report of *Varroa destructor* resistance to pyrethroids in the UK. Apidologie. 33: 357–366.

Toomemaa, K., A.-J. Martin, and I. H. Williams. **2010**. The effect of different concentrations of oxalic acid in aqueous and sucrose solution on varroa mites and honey bees. Apidologie. 41: 643–653.

Toufailia, H. A., L. Scandian, and F. L. W. Ratnieks. **2015**. Towards integrated control of varroa: 2)comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts. J Apic Res. 54: 108–120.

Traynor, K. S., F. Mondet, J. R. de Miranda, M. Techer, V. Kowallik, M. A. Y. Oddie, P. Chantawannakul, and A. McAfee. **2020**. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends Parasitol. 36: 592–606.

Underwood, R. M., and M. M. López-Uribe. **2019**. Methods to control varroa mites: an integrated pest management approach. Penn State Extension. (https://extension.psu.edu/methods-to-control-varroa-mites-an-integrated-pest-management-approach).

US EPA, O. 2016. EPA-registered pesticide products approved for use against varroa mites in bee hives. US EPA. (https://www.epa.gov/pollinator-protection/epa-registered-pesticide-products-approved-use-against-varroa-mites-bee-hives).

van Dooremalen, C., E. Stam, L. Gerritsen, B. Cornelissen, J. van der Steen, F. van Langevelde, and T. Blacquière. **2013**. Interactive effect of reduced pollen availability and *Varroa destructor* infestation limits growth and protein content of young honey bees. J Insect Physiol. 59: 487–493.

vanEngelsdorp, D., and M. D. Meixner. **2010**. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol. 103: S80–S95.

vanEngelsdorp, D., K. S. Traynor, M. Andree, E. M. Lichtenberg, Y. Chen, C. Saegerman, and D. L. Cox-Foster. **2017**. Colony Collapse Disorder (CCD) and bee age impact honey bee pathophysiology. PLOS ONE. 12: e0179535.

Yang, X., and D. L. Cox-Foster. **2005**. Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. PNAS. 102: 7470–7475.

Zhang, E., and J. C. Nieh. **2015**. The neonicotinoid imidacloprid impairs honey bee aversive learning of simulated predation. J Exp Biol. 218: 3199–3205.

Chapter 3

Effects of intermittent release of oxalic acid on *Varroa destructor* mites and *Apis mellifera* honey bees

3.1Abstract

Varroa destructor (Anderson and Trueman), a mite that parasitizes the Western honey bee, *Apis mellifera* L., is a devastating biotic stressor that commonly causes colony mortality. To combat the negative effects of *V. destructor*, beekeepers frequently rely on oxalic acid, a plant derived compound. Oxalic acid is usually applied in one of three ways − in liquid form via trickling or spraying, or in a gaseous form via sublimation − when colonies are broodless, with no developing individuals, as the acaricidal effects of oxalic acid do not penetrate the wax cappings of brood cells. To investigate the effects of oxalic acid sublimation on *V. destructor* abundance and *A. mellifera* colony strength during the brood-rearing season, we established 30 double-deep brood chambered colonies − 15 received 1 g oxalic acid per brood box via sublimation seven times at 5 day intervals over a 30 day period; 15 were untreated controls. Colonies were assessed twice for *V. destructor* abundance, *A. mellifera* adult workers and brood cells, and stored carbohydrates. Oxalic acid sublimation reduced *V. destructor* abundance, but did not affect any *A. mellifera* colony strength measure. These results suggest that under certain circumstances, oxalic acid sublimation may be a useful tool for *V. destructor* management in brood-rearing *A. mellifera* colonies.

3.2 Introduction

The Western honey bee, *Apis mellifera* L., is among the most important insect pollinator to agricultural and natural systems (Allsopp et al. 2008, vanEngelsdorp and Meixner 2010). They are used extensively throughout the world for pollination, as they can be easily transported, are generalist pollinators, and live in colonies consisting of thousands of workers (Aizen et al. 2009, Sáez et al. 2020). Despite its importance and widespread use, there is consensus that a global decline in *A. mellifera* health is occurring in many regions of the world. For *A. mellifera* in the United States and abroad, this decline manifests as increased colony losses (Kulhanek et al. 2017, vanEngelsdorp et al. 2017, Bruckner et al. 2020, Gray et al. 2020, Oberreiter and Brodschneider 2020, Stahlmann-Brown et al. 2020). Abiotic and biotic stressors are believed to play a major role in these losses. Abiotic stressors include mismanagement of *A. mellifera* colonies, pesticides, and weather, whereas, biotic stressors include pests, parasites, and forage availability (Zhang and Nieh 2015, Havard et al. 2020, Roth et al. 2020).

The most damaging biotic stressor associated with *A. mellifera* colony loss is *Varroa destructor* (Anderson and Trueman), an introduced obligate ectoparasitic mite (Rosenkranz et al. 2010). *Varroa destructor* was historically a parasite of the Eastern honey bee, *Apis cerana* (Fabricius), but experienced a host switch in the early 20th century to *A. mellifera* (Beaurepaire et al. 2015). The lifecycle of *V. destructor* is closely tied to that of its honey bee host, and can be categorized into two distinct phases – the dispersal stage, when a mite travels and feeds on adult *A. mellifera,* and the reproductive phase, when a mite reproduces and develops within sealed *A. mellifera* brood cells (Rosenkranz et al. 2010, Roth et al. 2020). The reproductive stage begins when a female *V. destructor* mite enters an *A. mellifera* brood cell containing a 5th instar larvae. Once the *A. mellifera* brood cell is capped, the foundress mite then lays one haploid egg that

develops into a male, followed by repeated deposition of diploid eggs which develop into females alongside a developing *A. mellifera* (Rosenkranz et al. 2010, Piou et al. 2016). Once the fully developed, the *A. mellifera* individual will emerge from its brood cell as an adult alongside the fully developed female *V. destructor* mites. Approximately 1.5-2.5 daughter mites are produced in *A. mellifera* worker and drone brood cells, respectively (Sammataro and Avitabile 2011, Lin et al. 2018, Traynor et al. 2020). During the dispersal stage, the adult female *V. destructor* feeds on the fat body of nurse *A. mellifera,* which is the preferred worker life stage as nurses have large fat bodies (Xie et al. 2016, Ramsey et al. 2019). The dispersal stage can last up to 10 days in the presence of brood (Roth et al. 2020), ending when a mature female *V. destructor* enters an *A. mellifera* brood cell, marking the start of the reproductive phase.

Varroa destructor elicits both lethal and sub-lethal effects on *A.* mellifera (Koleoglu et al. 2017b). By feeding on the fat body (Ramsey et al. 2019), an important organ involved in storage and regulation of metabolites and immunity, as well as other structures of *A. mellifera* (Raikhel et al. 1997, Arrese and Soulages 2010), reduced longevity, vectoring of viruses, and immunosuppression can occur (Williams et al. 2009, Straub et al. 2019). At high infestations, *V. destructor* severely weakens the *A. mellifera* colony, ultimately leading to its death if left unmanaged by the beekeeper (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017).

To mitigate the negative effects of *V. destructor*, beekeepers rely on Integrated Pest Management (IPM) to keep an infestation at a non-damaging level (Delaplane et al. 2005, Roth et al. 2020). Prevention strategies are the first step to a successful IPM program for *V. destructor*, and include cultural practices such as using tolerant or resistant stocks of *A. mellifera*, and mechanical means like drone brood removal or making colonies broodless to limit reproduction of *V. destructor* (Gregorc et al. 2017, Panziera et al. 2017, Jack et al. 2020, Roth et al. 2020).

Chemical intervention is most often used when all other control strategies have not successfully maintained infestation below the treatment threshold, which is currently set at three *V. destructor* mites per 100 *A. mellifera* workers (Caron et al. 2018). There are currently both synthetic and natural compounds available to beekeepers for *V. destructor* control (US EPA 2016). Overuse of synthetic acaricides especially with minimal rotation among different active ingredients, has led to resistance in *V. destructor* populations (Thompson et al. 2002, Sammataro, Olafson, et al. 2005, Gregorc et al. 2018a, Rinkevich 2020). Furthermore, synthetic pesticides, including acaricides, have been found to be present in wax, wax comb, and stored food in colonies (Mullin et al. 2010). As a result, beekeepers have begun to embrace organic compounds for successful treatment of *V. destructor*.

Organic acids, such as oxalic acid, are naturally derived plant extracts that have become increasingly popular among beekeepers due to their high efficacy and low probability of resistance development (Adjlane et al. 2016, Maggi et al. 2016). The low probability of resistance development is thought to be due to its rapid degradation within the colony, limited use by beekeepers, and that it does not affect neurons (Imdorf et al. 1999, Maggi et al. 2016). Oxalic acid, though used previously throughout Canada and Europe, was only registered for apicultural use in the United States in 2015 (US EPA 2015a). Labelled use includes two application methods – trickling, which is the deposition of a liquid application of oxalic acid dissolved in sucrose solution onto the colony, and sublimation, which is the application of gaseous oxalic acid to a colony using a heating device (Rademacher and Harz 2006, US EPA 2015). Although oxalic acid has shown considerable promise for managing *V. destructor* infestations, its effectiveness is dependent on weather and in-hive conditions. For example, its acaricidal effects do not extend to within the wax capped brood cells of *A. mellifera*, where *V.*

destructor reproduce and develop (Adjlane et al. 2016). Therefore, treatment using oxalic acid is most often implemented in winter, when there is little to no *A. mellifera* brood within the colony (Higes et al. 1999).

Sublimation of oxalic acid can be highly effective against *V. destructor* and relatively safe to *A. mellifera* workers and brood (Rademacher and Harz 2006, Rademacher et al. 2017). Sublimation is performed by inserting a heating device into a hive, the wood structure housing an *A. mellifera* colony, that subsequently releases oxalic acid vapors. This method of oxalic acid treatment has several practical advantageous, including the need for limited manipulation of hives and colonies, which reduces exposure to the environment (Toufailia et al. 2015). However, treatment of oxalic acid via sublimation is time consuming, as colonies have to be sealed for the duration of treatment, which may be up to several minutes. Beekeepers are permitted to use oxalic acid sublimation when *A. mellifera* brood are present; however, specific treatment regimens are left to the manufacturers of heating devices (US EPA 2015) . The current user manual of a very common heating device is four times every five days (OxaVap 2020); the previous recommendation was three times every seven days. This application rate is designed to target *V. destructor* mites during their typical 5-10 day long dispersal stage when brood are present (Piou et al. 2016), thus providing effective control by repeatedly targeting *V. destructor* as cohorts emerge from brood cells alongside their *A. mellifera* hosts.

Most studies examining the effects of oxalic acid on *V. destructor* have been conducted during broodless periods, and therefore observe significant control of *V. destructor*. However, Jack et al. (2020) and Gregorc et al. (2017) performed studies using oxalic acid during the active season. Both focused on the effects of oxalic acid, and oxalic acid in combination with artificial brood-breaks, on *V. destructor*. Gregorc et al. (2017) used the liquid trickle method and found

that oxalic acid applied 4 times every 8 days had a negative effect on *V. destructor*. Jack et al. (2020) used the sublimation method applied 3 times every 8 days, and found that it had no effect on *V. destructor*. However, neither of these studies used the current recommended application rate of 4 times every 5 days.

Oxalic acid can be effective against *V. destructor*, but little effort has investigated its potential for use during the active brood rearing season. Therefore, the objective of this study was to determine the effects of intermittent release of oxalic acid via sublimation on *V. destructor* abundance and *A. mellifera* colony strength in the Southeastern United States during the brood-rearing season. Based on preliminary data that revealed that five day application intervals were successful at reducing *V. destructor* without affecting *A. mellifera* colonies, coupled with the promising use of repeated liquid applications of oxalic acid for *V. destructor* control during brood rearing (Gregorc et al. 2017), we predicted a negative effect of treatment on *V. destructor* abundance and no effect on *A. mellifera* colony strength.

3.3 Methods

3.3.1 Experimental design

The experiment was performed at a research apiary in Auburn, AL, USA from August to September 2020 using *Apis mellifera* honey bee colonies maintained in double-deep Langstroth hive equipment. Prior to the study, colonies were preliminarily screened for queen status, worker adult and capped brood numbers, number of stored carbohydrate cells, and *Varroa destructor* mite abundance to identify those uniform in strength and health (Delaplane et al. 2013, Dietemann et al. 2013). All colonies had naturally occurring *V. destructor* infestations with a upper limit of 10 *V. destructor* mites per 100 *A. mellifera* adult workers. Ultimately, 30 colonies

were allocated to one of two treatment groups – 15 were exposed to sublimated oxalic acid that was administered seven times every 5 days (hereafter OA Sublimation), and 15 were not exposed (hereafter Control). Within the apiary, colonies were arranged ~3m apart in a non-uniform row with entrances randomly directed Northeast, East or Southeast to minimize drifting of workers that could have adhering *V. destructor* (Bordier et al. 2017). Colonies were exposed to their respective treatment group applications for 30 days, which fully encompasses both ~21 day worker and ~24 day drone brood development times (Sammataro and Avitabile, 2011), as other registered *V. destructor* control products (Maggi et al. 2015, Rodríguez Dehaibes et al. 2020). All colonies were fed *ad libitum* with 2:1 white granulated sugar water solution (w:w) during the experiment because of the dearth of seasonal forage.

3.3.2 Oxalic acid sublimation

Oxalic acid was applied to colonies (n=15) via sublimation according to the label of the registered product and the user manual of the application equipment (US EPA 2015, OxaVap 2020). Sublimation occurred before dawn to ensure *A. mellifera* would be inside the hive and not outside performing activities like guarding or foraging. Hives were in good condition, with limited space between equipment components. Entrances and screened bottom boards were sealed with duct tape (Duck Brand, Avon, OH) and corrugated plastic boards (Mann Lake, Hackensack, MN), respectively, to prevent gas leaking from the hives. A ProVap 110 Sideliner/Commercial Vaporizer (OxaVap, Manning, SC) was used to sublimate 1 g solid oxalic acid dihydrate (Brushy Mountain Bee Farm, Moravian Falls, NC) per deep brood box. The device was heated to 230°C, and 1 g of solid oxalic acid was placed into the Teflon cap. Keeping the device inverted so that oxalic acid would not fall into the bowl and sublimate into

the environment, the stem was inserted into a pre-drilled hole in the bottom deep brood box and the Teflon cap was placed on the bowl of the vaporizer. The heating device was then turned right-side-up so that the solid oxalic acid fell into the heated bowl and was heated into its gaseous state (Fig. 10). Oxalic acid vapor was allowed to permeate throughout the colony for roughly 30 seconds, or until the vaporizer returned to a temperature of 230°C, as recommended by the product user manual. These steps were repeated on all colonies of the OA Sublimation treatment group. After 10 minutes of being sealed, the duct tape and corrugated plastic boards were removed from all experimental colonies belonging to both treatment groups (OxaVap 2020).

Fig. 10: Placement of a ProVap 110 Sideliner/Commercial Vaporizer (OxaVap, Manning, SC) in the bottom brood box of a hive housing an *Apis mellifera* honey bee colony. Colonies receiving oxalic acid sublimation were sealed at their entrances and screened bottoms prior to treatment to prevent oxalic acid vapors from exiting the hive for 10 minutes per colony. For photographic purposes, this image was taken during daylight. Actual treatment application occurred prior to sunrise.

3.3.3 *Varroa destructor* abundance

Varroa destructor abundance was quantified on Days 0, 21, and 42 for each colony by collecting ~300 adult workers from a frame containing open worker brood (Dietemann et al. 2013). Individuals were shaken into a 40 x 31.8 x 15.2 cm holding container (Sterilite, Townsend, MA), collected using a ½ cup scoop (Measurex, Cupertino, CA), and placed in the basket of a Varroa EasyCheck (Mann Lake, Hackensack, MN) containing 70% isopropyl alcohol. The sample of adult workers with adherent *V. destructor* was shaken vigorously for 1 minute to dislodge *V. destructor* from the worker adults, as per the product user manual. The deceased worker adults were discarded from the basket, and the alcohol, including its contents of the Varroa EasyCheck, were then passed through a fine-mesh strainer (Oneida, Lincolnshire, IL) so that mites could be counted. *V. destructor* abundance was determined by dividing the total number of mites by 300, and then multiplied by 100 to determine the number of mites per 100 *A. mellifera* worker adults (Veto-Pharma 2018).

3.3.4 Colony strength

Each colony was visually assessed on Days 13 and 35 for the presence of an egg-laying queen and to estimate of number of frames containing *A. mellifera* worker adults and capped brood, and stored carbohydrates (nectar or sugar syrup). These experimental days were chosen as they were halfway points between colony strength assessments. This was to ensure that potentially collapsing colonies could be removed from the trial to prevent their influence on other colonies (Peck and Seeley 2019). Furthermore, colony strength was quantified three times during the experiment according to Delaplane et al. (2013) – immediately prior to the first treatment application on Day 0, and then again on Days 21 and 42. During evaluations on Days

0, 21 and 42, percent coverage of deep Langstroth frames was estimated for worker adults and capped brood, and stored carbohydrates. Total number of adult workers and developing brood and stored carbohydrate cells were estimated by assuming 1215 adults and 3.8 cells per cm^2 occupy a deep Langstroth frame (Delaplane et al. 2013). Total percent coverage by adult workers was divided by 100 and multiplied by 1215 to determine the total number of individuals inside the colony. Similarly, the total number of percent coverage by capped brood and stored carbohydrate was divided by 100 and then multiplied by 880 and 3.8 to determine the total number of capped brood and stored carbohydrate inside the colony.

3.3.5 Statistical analysis

All data manipulation and analyses were performed in RStudio (Version 1.1.463). Colonies that did not have complete data for the 42-day experimental period were removed from the analysis. For each variable (*V. destructor* abundance, *A. mellifera* worker adults and capped brood, and stored carbohydrate), two analyses were performed. For the first analysis, we used generalized linear mixed models (GLMMs) to analyze data with multiple timepoints (repeated measures) to account for nested structure. For the GLMMs, we used the 'afex' package (Singmann et al. 2020), which interfaces with the 'lme4' package (Bates et al. 2015). For the second analysis, we used the 'mixed' function with likelihood-ratio tests to perform type-III anovas to confirm significance. We used Poisson-distributed error structures for *V. destructor* abundance data after rounding upwards to the nearest whole *V. destructor* (ceiling transformation), and Gaussian-distributed error structures for worker adults and capped brood, and stored carbohydrates. The 'emmeans' package was used to generate compact letter displays for plotting of data (Lenth, 2020). This package generates compact letter displays of all pairwise

comparisons of estimated marginal means. When a p-value exceeds 0.05, the means will have at least one letter in common, denoting similarities between data.

We also analyzed *V. destructor* by calculating change between Days 0 and 42 – effectively a 'delta (Δ)' value of the change in each colony during that specific time period. For the effect of treatment on these-single value per colony data (delta-variables), we used type-II anovas ('car' package, (Fox and Weisberg, 2019)) assuming a Gaussian-distribution. For both mixed-models and anovas, response variable distributions were graphically inspected as histograms and tested using a Shapiro-Wilk test for normality, although emphasis was principally placed on inspection of residuals vs fitted values. QQ-Plots were used to check analytical and model assumptions, with the exception of the Poisson-distributed *V. destructor* abundance response variable using mixed-modelling. For *V. destructor* abundance, a Henderson-Tilton's efficacy measure was calculated for each treatment at days 21 and 42 by comparing mean values of each treatment block to controls (Gama 2015). This efficacy measure accounts for a non-uniform population, and for infestations of individuals.

3.4 Results

3.4.1 *Varroa destructor* abundance

There was a significant difference in *V. destructor* abundance between treatment groups ($p<0.001$) and sampling periods (Days 0, 21, 42) ($p=0.011$) (Fig. 11). Compared to untreated control($n=15$), efficacy for oxalic acid sublimation ($n=15$) was 45.15% after 21 days and 70.99% after 42 days. We found evidence for significantly increasing *V. destructor* abundance with time during the experiment ($p=0.011$), and an effect of treatment on this rate of increase ($p<0.001$). Furthermore, we found a significant effect of treatment on *V. destructor* abundance by days 21 ($p=0.02$) and 42 ($p=0.020$) (Fig. 12).

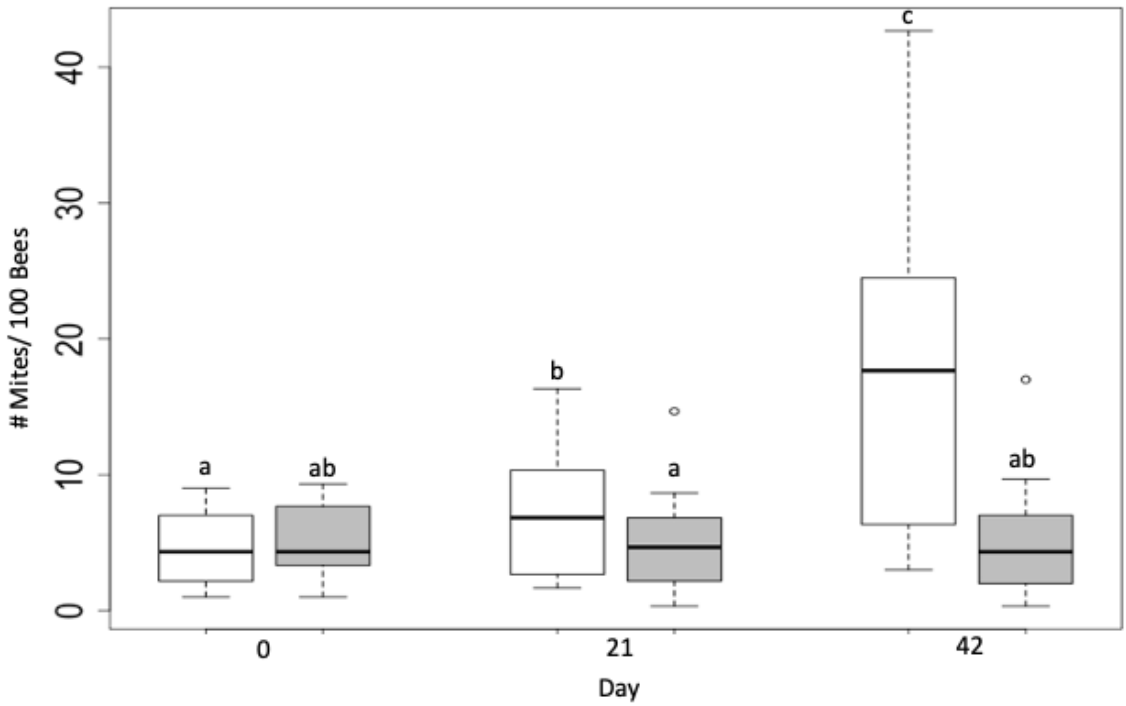

Fig. 11: *Varroa destructor* abundance at each sampling day (Day 0, 21, 42) for Control (n=15) (white) and Oxalic Acid (n=15) Sublimation (gray) treatment groups. Different letters above each plot indicate significant difference ($p<0.05$). A significant difference was observed between treatment groups ($p<0.001$) and sampling days ($p=0.011$). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

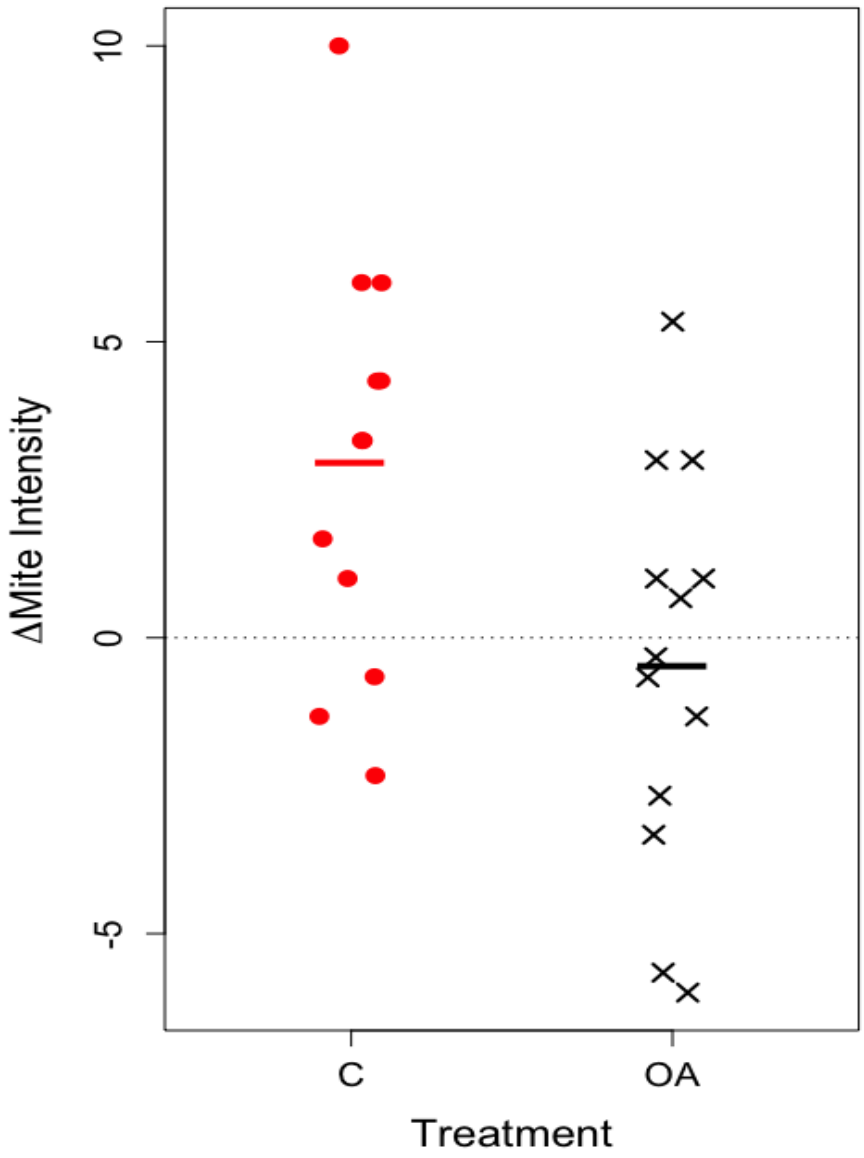

Fig. 12: The effect of treatment on the change in number of *Varroa destructor* mites for each *Apis mellifera* colony modeled over the 42-day experimental period. Control (n=15) is abbreviated as "C" and Oxalic Acid Sublimation (n=15) as "OA". There was a significant difference for the change in *V. destructor* abundance (p=0.020). The circles and crosses represent change in *V. destructor* abundance, and the bars demonstrate the mean.

3.4.2 Colony strength

There was no significant difference between treatment groups in the number of *A. mellifera* worker adults (p=0.95); however, there was a significant difference between sampling periods (p=0.012) (Fig. 13). Although there was no significant difference between treatment groups on the number of *A. mellifera* worker capped brood cells (p=0.57), there was a significant difference between sampling periods (p=0.003) (Fig. 14). Furthermore, for stored carbohydrate cells there was no significant difference between treatment groups (p=0.13) or between sampling periods (p=0.19) (Fig. 15).

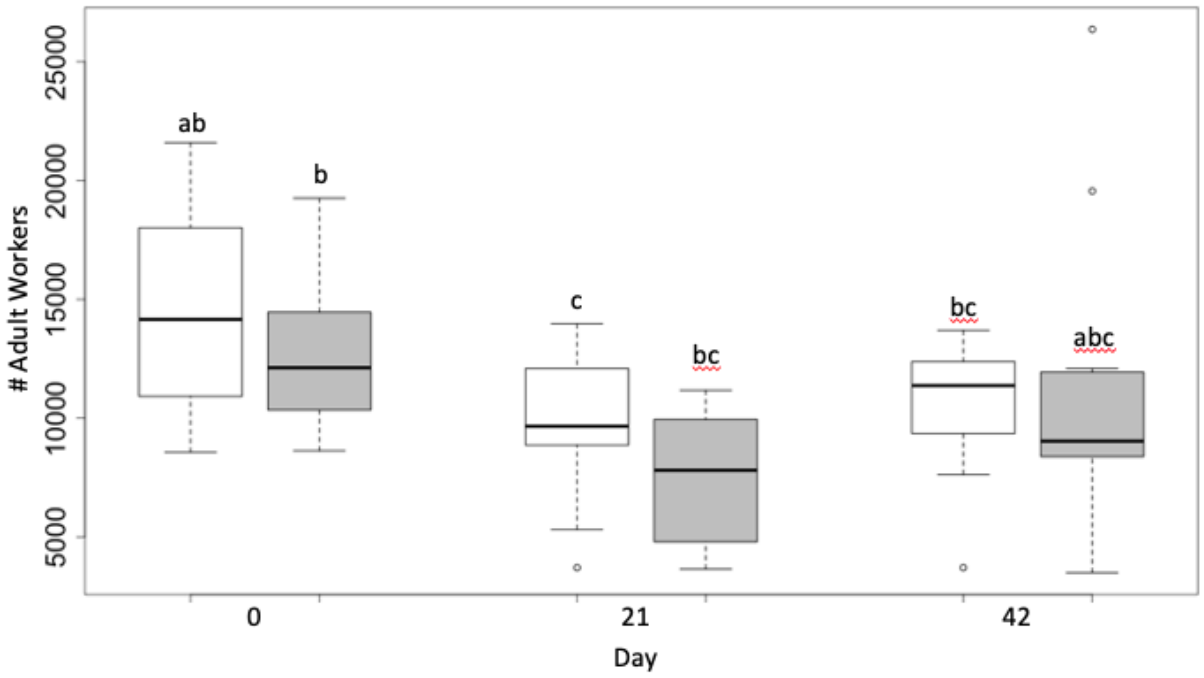

Fig. 13: *Apis mellifera* worker adult populations at each sampling day (Day 0, 21 and 42) between treatment groups Control (n=15) (white) and Oxalic Acid Sublimation (n=15) (gray). Different letters above each plot indicate significant differences ($p<0.05$). There was no significant difference between treatment ($p=0.947$); however, there was a significant difference between sampling day ($p=0.012$). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

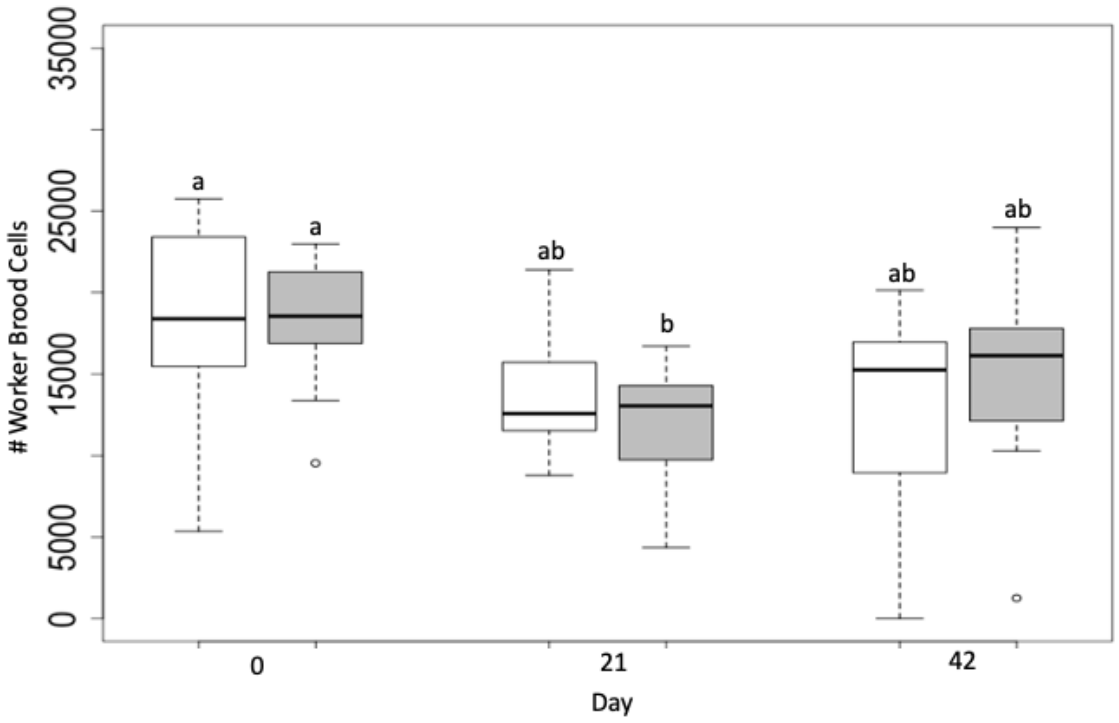

Fig. 14: *Apis mellifera* worker capped brood populations at each sampling day (Day 0, 21 and 42) between treatment groups Control (n=15) (white) and Oxalic Acid Sublimation (n=15) (gray). Different letters above each plot indicate significant differences. There was no significant difference between treatment (p=0.57); however, there was a significant difference between sampling day (p=0.003). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

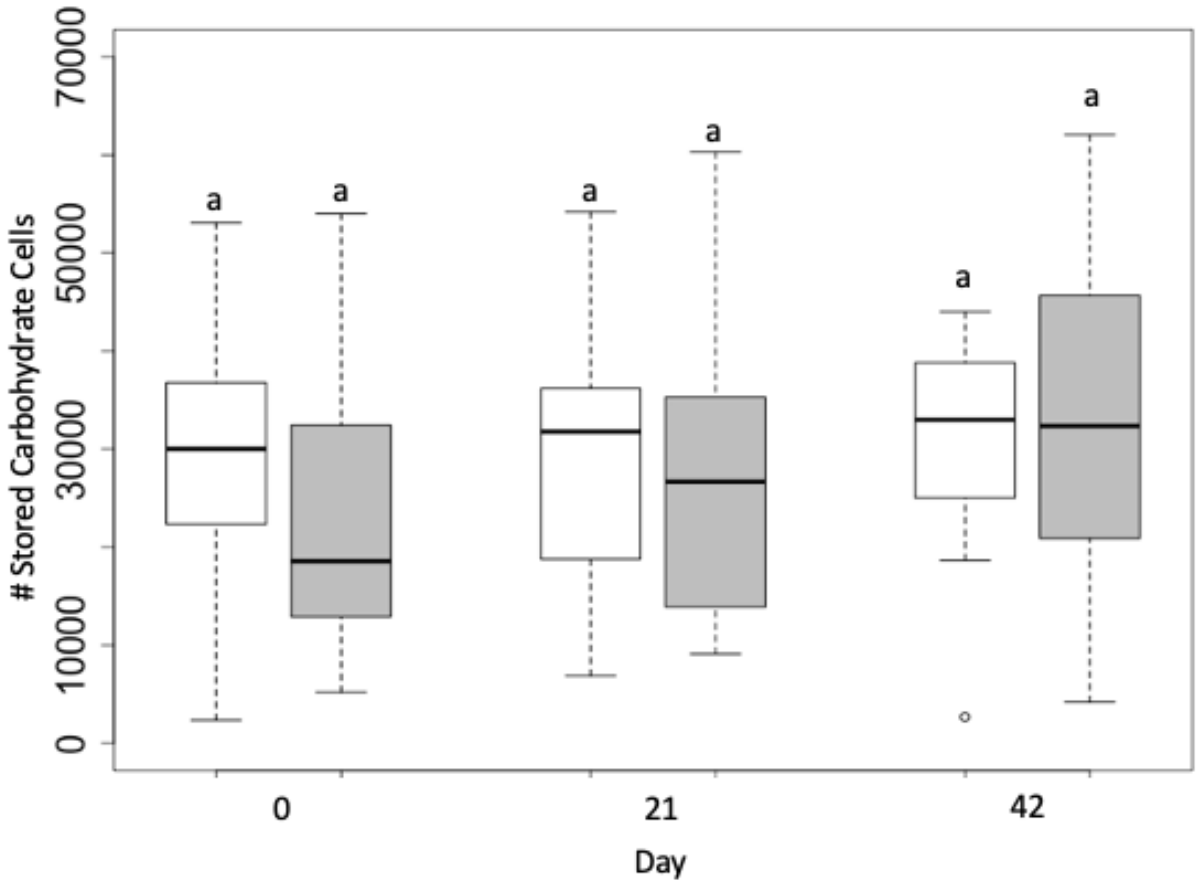

Fig. 15: Stored carbohydrate cells at each sampling day (Day 0, 21 and 42) between treatment groups Control (n=15) (white) and Oxalic Acid Sublimation (n=15) (gray). Different letters indicate significant differences ($p<0.05$). We found no significant difference between treatment groups ($p=0.13$) or sampling day ($p=0.19$). The boxplots show the inter-quartile range (box), the median (black line within the box), data range (horizonal black lines from box), and outliers (black circles).

3.5 Discussion

Varroa destructor is a detrimental ectoparasite to *Apis mellifera* honey bees, causing both lethal and sub-lethal effects and widespread colony mortality (Koleoglu et al. 2017b, Kulhanek et al. 2017, Oberreiter and Brodschneider 2020, Stahlmann-Brown et al. 2020). Oxalic acid is a chemical treatment option showing incredible promise for *V. destructor* management, with little to no adverse effects on *A. mellifera* (Rademacher and Harz 2006, Rademacher et al. 2017). Here we found that intermittent release of oxalic acid via sublimation every five days had a significant negative effect on *V. destructor* after seven applications. Furthermore, we observed that oxalic acid sublimation did not have an effect on *A. mellifera* worker adults and capped brood, or stored carbohydrates.

Treatment of oxalic acid is most commonly implemented in late autumn or winter, or when colonies have limited brood (Rademacher and Harz 2006). The US EPA (2015) currently permits two types of application of oxalic acid to *A. mellifera* colonies − via sublimation and liquid sugar solution. The primary reason why treatment of oxalic acid is highly effective when *A. mellifera* brood is absent is because its acaricidal properties cannot penetrate the wax cappings of the brood cells that also contain reproducing and developing *V. destructor* (Rosenkranz et al. 2010, Toufailia et al. 2015). Previous studies have shown that treatment during broodless periods result in up to 99% efficacy; however, during the brood rearing season efficacy of oxalic acid is more variable, often resulting in less effectiveness, sometimes in the range of 29-45% (Gregorc and Planinc 2001, Rademacher and Harz 2006, Rosenkranz et al. 2010, Gregorc and Planinc 2012, Toufailia et al. 2015). Until now, only anecdotal evidence suggested that the recommended four applications of sublimated oxalic acid every 5 days was effective at *V. destructor* control (OxaVap 2020). Furthermore, conflicting results between studies examining the effects of oxalic

acid applied multiple times by either sublimation or liquid trickling cast doubt into its effectiveness during the active brood season (Gregorc et al. 2017, Jack et al. 2020).

We found a significant difference between treatment groups for *V. destructor* abundance, both at days 21 and 42. This result supports current recommendations by one of the most common oxalic acid sublimation heating devices on the market. However, it appears that additional applications, beyond the recommended four applications every five days, resulted in improved efficacy. Given the resources required to perform even four applications of oxalic acid via sublimation, efficacy of ~45% halfway through our experiment is unlikely to convince beekeepers that it is an ideal management option. Our results correspond to Gregorc et al. (2017), but not Jack et al. (2020), who also performed oxalic acid management studies during *A. mellifera* brood rearing. These conflicting results are difficult to explain, as Gregorc et al. (2017) applied oxalic acid four times via trickling every 7-10 days, whereas Jack et al. (2020) applied oxalic acid via sublimation, like our study, but as 3 applications spaced 7 days apart. It could be that the additional treatments and extended application period by our study and Gregorc et al. (2017) was important for *V. destructor* control, particularly by encompassing the entire *A. mellifera* drone development period (Rangel and Fisher 2019). Other studies examining the effects of oxalic acid sublimation on *V. destructor* during broodless periods often documented high (>93%) efficacy (Rademacher and Harz 2006, Toufailia et al. 2015, Papežíková et al. 2016). It is also interesting to note that Toufailia et al. (2015) found that the most effective dose of oxalic acid via sublimation was more than 4 times greater than the current label rate (4.5 g oxalic acid). This dose resulted in a 98.2% reduction in *V. destructor* infestation, and no negative effects on *A. mellifera*, thus suggesting that increasing the legal dose of oxalic acid may provide

beekeepers with improved results. Investigations on how increased doses might influence both *V. destructor* and *A. mellifera* during brood rearing are certainly warranted.

Oxalic acid is regarded as a relatively safe treatment to *A.* mellifera, although physiological damage from liquid application has been documented (Rademacher et al. 2017). We did not observe any negative effect of our oxalic acid sublimation treatment on *A. mellifera* worker adults, even for our extended 42-day period. Although both Papežíková et al. (2017) and Toufailia et al. (2015) found that *A. mellifera* workers treated with sublimated oxalic acid did not experience increased mortality, both studies observed an effect on *A. mellifera* when oxalic acid was applied via trickling. Reasons for this could include increased cell death from ingestion of the liquid oxalic acid dissolved in sugar syrup, a common method for feeding carbohydrates to *A. mellifera* colonies. Rademacher and Harz (2006) reported that sublimation of 0.5-5 g oxalic acid did not result in significant *A. mellifera* mortality in an extensive review of the existing literature. Indeed, sublimation is regarded as a superior oxalic acid treatment during winter compared to the trickle method, as the latter method can kill workers that cannot be immediately replaced, and also requires colonies to be exposed to the environment during treatment.

We did not observe any negative effects of oxalic acid sublimation on *A. mellifera* worker capped brood. Our results were similar to Jack et al. (2020), who found treatment groups that received oxalic acid via sublimation had similar amounts of brood as controls. In contrast, Toufailia et al. (2015) found that oxalic acid sublimation resulted in increased brood, possibly because sublimation is less harmful to workers and the queen. In a study conducted by Higes et al. (1999), no immediate effects to brood were observed during the treatment period. However, their long-term study found that colonies treated with oxalic acid had significantly less brood. They applied oxalic acid via liquid spray, which is not registered as an application method in the

United States and could cause increased acidity to the cuticle and organs as a result of less homogenous liquid formulation (Higes et al. 1999, Toomemma et al. 2010, Rademacher et al. 2017, Terpin et al. 2019. No studies conducted thus far have examined the long-term effects of oxalic acid sublimation on *A. mellifera* brood, but this is certainly warranted if this method is to be more frequently employed in early fall, when colonies in many regions rely on brood production to create long-lived winter workers that must subsequently survive several months in a broodless colony (Amdam et al. 2004, Bagheri and Mirzaie 2019).

We did not observe any negative effects of treatment on the number of stored carbohydrate cells, which colonies heavily rely on for a source of energy during floral deaths in summer and winter (Eyer, Greco, et al. 2016). Our results were similar to Jack et al. (2020), who also did not find an effect. It can therefore be inferred that oxalic acid via sublimation does not interfere with *A. mellifera* worker ability to gather nectar. It is also worth noting that colonies used in our study were fed *ad libitum*, as at the time they were experiencing a nectar dearth in the field. Because there have been no long-term studies on the effects of oxalic acid sublimation on *A. mellifera*, we cannot confirm how colonies respond over time. Future studies should investigate this, as well as possible long-term effects of oxalic acid sublimation on traits important to colony performance, such as foraging or homing behavior.

In this study, we found that the current label rate of 1 g oxalic acid per deep brood box of *A. mellifera* honey bees (US EPA 2015) was sufficient for *V. destructor* control, though there was not a large reduction in *V. destructor* abundance. Most studies evaluating the acaricidal effects of oxalic acid have investigated its effects during broodless periods (Toomeemaa et al. 2010, Toufailia et al. 2015, Adjlane et al. 2016 Papežíková et al. 2016); however, there is an urgent need to identify *V. destructor* management practices during the brood rearing season

because of current issues related to resistance of mites to chemical controls and efficacy of treatments depending on colony and environmental conditions. It is worth noting that repeated applications of oxalic acid via sublimation are likely not practical for commercial beekeepers, as this treatment is time consuming and labor intensive. Therefore, it is likely to be more adopted by small-scale beekeepers managing fewer colonies. Though the *V. destructor* in our treated colonies were not totally diminished, there was a relative negative effect of treatment on *V. destructor* abundance 21 and 42 days post initial treatment. Because there are no known instances of resistance or residues of oxalic acid, a label revision permitting the use of oxalic acid via sublimation during major brood-rearing periods, or when honey supers are on, will likely allow small-scale beekeepers to treat their colonies for *V. destructor* using this organic approach, and when other treatments are not allowed or are not effective (Bogdanov et al. 2002).

References

Adjlane, N., E.-O. Tarek, and N. Haddad. **2016**. Evaluation of oxalic acid treatments against the mite *Varroa destructor* and secondary effects on honey bees *Apis mellifera*. J Arthropod Borne Dis. 10: 501–509.

Aizen, M. A., L. A. Garibaldi, S. A. Cunningham, and A. M. Klein. **2009**. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Ann Bot. 103: 1579–1588.

Allsopp, M. H., W. J. de Lange, and R. Veldtman. **2008**. Valuing insect pollination services with cost of replacement. PLOS ONE. 3: e3128.

Amdam, G. V., K. Hartfelder, K. Norberg, A. Hagen, and S. W. Omholt. **2004**. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering? J Econ Entomol. 97: 741–747.

Arrese, E. L., and J. L. Soulages. **2010**. Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol. 55: 207–225.

Bagheri, S., and M. Mirzaie. **2019**. A mathematical model of honey bee colony dynamics to predict the effect of pollen on colony failure. PLOS ONE. 14: e0225632.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Beaurepaire, A. L., T. A. Truong, A. C. Fajardo, T. Q. Dinh, C. Cervancia, and R. F. A. Moritz. **2015**. Host specificity in the honeybee parasitic mite, *Varroa* spp. in *Apis mellifera* and *Apis cerana*. PLOS ONE. 10: e0135103.

Bogdanov, S., J.-D. Charrière, A. IMDORF, V. KILCHENMANN, and P. Fluri. **2002**. Determination of residues in honey after treatments with formic and oxalic acid under field conditions. Apidologie. 33: 399–409.

Bordier, C., M. Pioz, D. Crauser, Y. Le Conte, and C. Alaux. **2017**. Should I stay or should I go: honeybee drifting behaviour as a function of parasitism. Apidologie. 48: 286–297.

Bruckner, S., N. Steinhauer, J. Engelsma, A. M. Fauvel, K. Kulhanek, E. Malcolm, A. Meredith, M. Milbrath, E. L. Niño, J. Rangel, K. Rennich, D. Reynolds, R. Sagili, J. Tsuruda, D. vanEngelsdorp, S. D. Aurell, M. Wilson, and G. R. Williams. **2020**. 2019-2020 honey bee colony losses in the United States: preliminary results. (https://beeinformed.org/wp-content/uploads/2020/06/BIP_2019_2020_Losses_Abstract.pdf).

Burnham, A. J. 2019. Scientific advances in controlling *Nosema ceranae* (*Microsporidia*) infections in honey bees (*Apis mellifera*). Front Vet Sci. 6: 79.

Caron, D. M., M. Dykes, D. Epstein, G. Hansen, M. Nasr, D. Downey, K. Rennich, D. Rogers, R. M. Sears, and T. Steeger. **2018**. Tools for varroa management: a guide to effective varroa sampling & control. (https://honeybeehealthcoalition.org/wp-content/uploads/2018/06/HBHC-Guide_Varroa_Interactive_7thEdition_June2018.pdf).

Charrière, J.-D., and A. Imdorf. **2002**. Oxalic acid treatment by trickling against *Varroa destructor*: recommendations for use in central Europe and under temperate climate conditions. Bee World. 83: 51–60.

Delaplane, K. S., J. A. Berry, J. A. Skinner, J. P. Parkman, and W. M. Hood. **2005**. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. J Apic Res. 44: 157–162.

Delaplane, K. S., J. van der Steen, and E. Guzman-Novoa. **2013**. Standard methods for estimating strength parameters of *Apis mellifera* colonies. J. Apic. Res. 52: 1–12.

Dietemann, V., F. Nazzi, S. J. Martin, D. L. Anderson, B. Locke, K. S. Delaplane, Q. Wauquiez, C. Tannahill, E. Frey, B. Ziegelmann, P. Rosenkranz, and J. D. Ellis. **2013**. Standard methods for varroa research. J. Apic. Res. 52: 1–54.

Eyer, M., M. K. Greco, J. Lang, P. Neumann, and V. Dietemann. **2016**. No spatial patterns for early nectar storage in honey bee colonies. Insect. Soc. 63: 51–59.

Fox, J. and S. Weisberg. 2019. An {R} companion to applied regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Gray, A., N. Adjlane, A. Arab, A. Ballis, V. Brusbardis, J.-D. Charrière, R. Chlebo, M. F. Coffey, B. Cornelissen, C. A. da Costa, B. Dahle, J. Danihlík, M. M. Dražić, G. Evans, M. Fedoriak, I. Forsythe, A. Gajda, D. C. de Graaf, A. Gregorc, I. Ilieva, J. Johannesen, L. Kauko, P. Kristiansen, M. Martikkala, R. Martín-Hernández, C. A. Medina-Flores, F. Mutinelli, S. Patalano, A. Raudmets, G. S. Martin, V. Soroker, J. Stevanovic, A. Uzunov, F. Vejsnaes, A. Williams, M. Zammit-Mangion, and R. Brodschneider. **2020**. Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018–2019, and the effects of a new queen on the risk of colony winter loss. J Apic Res. 59: 744–751.

Gregorc, A., M. Alburaki, B. Sampson, P. R. Knight, and J. Adamczyk. **2018**. Toxicity of selected acaricides to honey bees (*Apis mellifera*) and varroa (*Varroa destructor* Anderson and Trueman) and their use in controlling varroa within honey bee colonies. Insects. 9: 55.

Gregorc, A., M. Alburaki, C. Werle, P. R. Knight, and J. Adamczyk. **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: 821–832.

Gregorc, A., and I. Planinc. **2001**. Acaricidal effect of oxalic acid in honeybee (*Apis mellifera*) colonies. Apidologie. 32: 333–340.

Gregorc, A., and I. Planinc. **2012**. Use of thymol formulations, amitraz, and oxalic acid for the control of the varroa mite in honey bee (*Apis mellifera carnica*) colonies. J Apic Sci. 56: 61–69.

Gregorc, A., and B. Sampson. **2019**. Diagnosis of varroa mite (*Varroa destructor*) and sustainable control in honey bee (*Apis mellifera*) colonies - A review. Diversity. 11: 243.

Guzmán-Novoa, E., L. Eccles, Y. Calvete, J. Mcgowan, P. G. Kelly, and A. Correa-Benítez. **2010**. Varroa destructor is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie. 41: 443–450.

Havard, T., M. Laurent, and M.-P. Chauzat. **2020**. Impact of stressors on honey bees (*Apis mellifera*; Hymenoptera: Apidae): some guidance for research emerge from a meta-analysis. Diveristy. 12: 7.

Higes, M., A. Meana, M. Suárez, and J. Llorente. **1999**. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. Apidologie. 30: 289–292.

Jack, C. J., E. van Santen, and J. D. Ellis. **2020**. Evaluating the efficacy of oxalic acid vaporization and brood interruption in controlling the honey bee pest *Varroa destructor* (Acari: Varroidae). J Econ Entomol. 13: 582-588.

Koleoglu, G., P. H. Goodwin, M. Reyes-Quintana, M. M. Hamiduzzaman, and E. Guzman-Novoa. **2017**. Effect of *Varroa destructor*, wounding and varroa homogenate on gene expression in brood and adult honey bees. PLOS ONE. 12: e0169669.

Kulhanek, K., N. Steinhauer, K. Rennich, D. M. Caron, R. R. Sagili, J. S. Pettis, J. D. Ellis, M. E. Wilson, J. T. Wilkes, D. R. Tarpy, R. Rose, K. Lee, J. Rangel, and D. vanEngelsdorp. **2017**. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apic Res. 56: 328–340.

Lenth, R. 2020. emmeans: Estimated marginal means, aka least-squares means. R package version 1.5.1. https://CRAN.R-project.org/package=emmeans

Lin, Z., Y. Qin, P. Page, S. Wang, L. Li, Z. Wen, F. Hu, P. Neumann, H. Zheng, and V. Dietemann. **2018**. Reproduction of parasitic mites *Varroa destructor* in original and new honeybee hosts. Ecol Evol. 8: 2135–2145.

Maggi, M., N. Damiani, C. Brasesco, N. Szawarski, G. Mitton, F. Mariani, D. Sammataro, S. Quintana, and M. Eguaras. **2016**. The susceptibility of *Varroa destructor* against oxalic acid: A study case. Bull Insectology. 70.

Maggi, M., E. Tourn, P. Negri, N. Szawarski, A. Marconi, L. Gallez, S. Medici, S. Ruffinengo, C. Brasesco, L. Feudis, S. Quintana, D. Sammataro, and M. Eguaras. **2015**. A new formulation of oxalic acid for *Varroa destructor* control applied in *Apis mellifera* colonies in the presence of brood. Apidologie. 47: 39-44.

Oberreiter, H., and R. Brodschneider. **2020**. Austrian COLOSS survey of honey bee colony winter losses 2018/19 and analysis of hive management practices. Diversity. 12: 99.

Panziera, D., F. van Langevelde, and T. Blacquière. **2017**. Varroa sensitive hygiene contributes to naturally selected varroa resistance in honey bees. J Apic Res. 56: 635–642.

Papežíková, I., M. Palíková, S. Kremserová, A. Zachová, H. Peterová, V. Babák, and S. Navrátil. **2017**. Effect of oxalic acid on the mite *Varroa destructor* and its host the honey bee *Apis mellifera*. J Apic Res. 56: 400–408.

Papežíková, I., M. Palíková, S. Navrátil, R. Heumannová, and M. Fronc. **2016**. The effect of oxalic acid applied by sublimation on honey bee colony fitness: a comparison with amitraz. Acta Veterinaria Brno. 85: 255–260.

Peck, D. T., and T. D. Seeley. **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: e0218392.

Piou, V., J. Tabart, V. Urrutia, J.-L. Hemptinne, and A. Vétillard. **2016**. Impact of the phoretic phase on reproduction and damage caused by *Varroa destructor* (Anderson and Trueman) to its host, the European honey bee (*Apis mellifera* L.). PLoS One. 11: e0153482.

Rademacher, E., and M. Harz. **2006**. Oxalic acid for the control of varroosis in honey bee colonies – a review. Apidologie. 37: 98–120.

Rademacher, E., M. Harz, and S. Schneider. **2017**. Effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae). Insects. 8: 84.

Raikhel, A. S., K. W. Deitsch, and T. W. Sappington. **1997**. Culture and analysis of the insect fat body, pp. 507–522. *In* Crampton, J.M., Beard, C.B., Louis, C. (eds.), The Molecular Biology of Insect Disease Vectors: A Methods Manual. Springer Netherlands, Dordrecht.

Ramsey, S. D., R. Ochoa, G. R. Bauchan, C. J. Gulbronson, J. D. Mowery, A. J. Cohen, D. W. Lim, J. P. Joklik, J. M. Cicero, J. D. Ellis, D. J. Hawthorne, and D. vanEngelsdorp. **2019**. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *In* PNAS. 116: 5.

Rangel, J., and A. Fisher. **2019**. Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review. Apidologie. 50: 759–778.

Rinkevich, F. D. 2020. Detection of amitraz resistance and reduced treatment efficacy in the varroa mite, *Varroa destructor*, within commercial beekeeping operations. PLOS ONE. 15: e0227264.

Rodríguez Dehaibes, S. R., F. R. Meroi Arcerito, E. Chávez-Hernández, G. Luna-Olivares, J. Marcangeli, M. Eguaras, and M. Maggi. **2020**. Control of *Varroa destructor* development in Africanized *Apis mellifera* honeybees using Aluen Cap (oxalic acid formulation). Int J Acarol. 1–4.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. **2010**. Biology and control of *Varroa destructor*. J Invertebr Pathol. 103: S96–S119.

Roth, M. A., J. M. Wilson, K. R. Tignor, and A. D. Gross. **2020**. Biology and management of *Varroa destructor* (Mesostigmata: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies. J Integr Pest Manag. 11: 1.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Sáez, A., M. A. Aizen, S. Medici, M. Viel, E. Villalobos, and P. Negri. **2020**. Bees increase crop yield in an alleged pollinator-independent almond variety. Sci Rep. 10: 3177.

Sammataro, D., P. Untalan, F. Guerrero, and J. Finley. **2005**. The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. Int J Acarol. 31: 67–74.

Sammataro, D., and A. Avitabile. **2011**. The beekeeper's handbook, 4th ed. Cornell University Press, Ithaca, New York.

Singmann, H., B. Bolker, J. Westfall, F. Aust and M. S. Ben-Shachar. (2020). Afex: analysis of factorial experiments. R package version 0.27-2. https://CRAN.R-project.org/package=afex

Stahlmann-Brown, P., T. Robertson, and O. Borowik. **2020**. Report on the 2019 New Zealand colony loss survey. Biosecurity New Zealand. 1-74. https://www.mpi.govt.nz/dmsdocument/40349/direct

Straub, L., G. R. Williams, B. Vidondo, K. Khongphinitbunjong, G. Retschnig, A. Schneeberger, P. Chantawannakul, V. Dietemann, and P. Neumann. **2019**. Neonicotinoids and ectoparasitic mites synergistically impact honeybees. Sci Rep. 9: 8159.

Terpin, B., D. Perkins, S. Richter, J. K. Leavey, T. W. Snell, and J. A. Pierson. **2019**. A scientific note on the effect of oxalic acid on honey bee larvae. Apidologie. 50: 363–368.

Thompson, H. M., M. A. Brown, R. F. Ball, and M. H. Bew. **2002**. First report of *Varroa destructor* resistance to pyrethroids in the UK. Apidologie. 33: 357–366.

Toomemaa, K., A.-J. Martin, and I. H. Williams. **2010**. The effect of different concentrations of oxalic acid in aqueous and sucrose solution on Varroa mites and honey bees. Apidologie. 41: 643–653.

Toufailia, H. A., L. Scandian, and F. L. W. Ratnieks. **2015**. Towards integrated control of varroa: 2)comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts. J Apic Res. 54: 108–120.

Traynor, K. S., F. Mondet, J. R. de Miranda, M. Techer, V. Kowallik, M. A. Y. Oddie, P. Chantawannakul, and A. McAfee. **2020**. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends Parasitol. 36: 592–606.

US EPA. **2015**. US EPA, Pesticide product label, oxalic acid dihydrate, 10/13/2015. (https://www3.epa.gov/pesticides/chem_search/ppls/091266-00001-20151013.pdf).

US EPA, O. 2016. EPA-registered pesticide products approved for use against varroa mites in bee hives. US EPA. (https://www.epa.gov/pollinator-protection/epa-registered-pesticide-products-approved-use-against-varroa-mites-bee-hives).

vanEngelsdorp, D., and M. D. Meixner. **2010**. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol. 103: S80–S95.

vanEngelsdorp, D., K. S. Traynor, M. Andree, E. M. Lichtenberg, Y. Chen, C. Saegerman, and D. L. Cox-Foster. **2017**. Colony Collapse Disorder (CCD) and bee age impact honey bee pathophysiology. PLOS ONE. 12: e0179535.

Williams, G. R., R. E. L. Rogers, A. L. Kalkstein, B. A. Taylor, D. Shutler, and N. Ostiguy. **2009**. Deformed wing virus in western honey bees (*Apis mellifera*) from Atlantic Canada and the first description of an overtly-infected emerging queen. J of Invertebr Pathol. 101: 77–79.

Xie, X., Z. Y. Huang, and Z. Zeng. **2016**. Why do varroa mites prefer nurse bees? Sci Rep. 6: 28228.

Zhang, E., and J. C. Nieh. **2015**. The neonicotinoid imidacloprid impairs honey bee aversive learning of simulated predation. J Exp Biol. 218: 3199–3205.

Chapter 4

Thesis conclusions

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman) is considered to be among the most important of biotic stressors to *Apis mellifera* L. honey bees, despite significant efforts to better understand its biology and mitigate its effects over several decades (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017). In the United States, oxalic acid has emerged as a promising chemical treatment component for Integrated Pest Management programs seeking to control *V. destructor*. Current approved treatment options for it include relatively short-lasting aqueous or gas applications. Multi-day continuous release methods for oxalic acid have received attention recently because they target *V. destructor* as it emerges from *A. mellifera* brood cells alongside its host. My thesis work re-affirmed the limitations of using oxalic acid during the active *A. mellifera* brood rearing season, and highlighted the importance of the need to consider the biology of both the pest – *V. destructor* – and the beneficial – *A. mellifera* – species. I found that liquid oxalic acid applied via cloth shop towels and cellulose boards did not significantly reduce *V. destructor* abundance in *A. mellifera* colonies, which was contrast to the findings of Oliver (2017, 2018) and Maggi et al. (2015). Additionally, I found that sublimation of oxalic acid had a negative effect on *V. destructor*, although efficacy data suggest that repeated application for about three weeks was only moderately effective. This aligns with previous work that investigated repeated application using a liquid oxalic acid formulation (Gregorc et al. 2017). Extending treatment application an additional three times resulted in a stronger negative effect on *V. destructor*, and improved efficacy, but is likely impractical for large-scale beekeepers because of the resources required. Given the urgent need for effective *V. destructor* control options for *A. mellifera* colonies during the active brood rearing season, more research

into novel active ingredients or novel application methods of currently registered active ingredients is warranted. This would promote further use of Integrated Pest Management programs in apiculture, which is severely limited due to a lack of available products and low adoption of monitoring techniques. Although oxalic acid is effective, other control strategies considering the biology of both *V. destructor* and *A. mellifera* should be considered for an effective control strategy. Using an Integrated Pest Management program that focuses on the biology of both species, with employment of chemical methods when needed, will limit the devastating effects of *V. destructor* on *A. mellifera*.

References

Gregorc, A., M. Alburaki, C. Werle, P. R. Knight, and J. Adamczyk. **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: 821–832.

Guzmán-Novoa, E., L. Eccles, Y. Calvete, J. Mcgowan, P. G. Kelly, and A. Correa-Benítez. **2010**. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie. 41: 443–450.

Kulhanek, K., N. Steinhauer, K. Rennich, D. M. Caron, R. R. Sagili, J. S. Pettis, J. D. Ellis, M. E. Wilson, J. T. Wilkes, D. R. Tarpy, R. Rose, K. Lee, J. Rangel, and D. vanEngelsdorp. **2017**. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apic Res. 56: 328–340.

Maggi, M., E. Tourn, P. Negri, N. Szawarski, A. Marconi, L. Gallez, S. Medici, S. Ruffinengo, C. Brasesco, L. Feudis, S. Quintana, D. Sammataro, and M. Eguaras. **2015**. A new formulation of oxalic acid for *Varroa destructor* control applied in *Apis mellifera* colonies in the presence of brood. Apidologie. 47: 596-605.

Oliver, R. 2017. Extended release oxalic acid progress report. American Bee Journal. https://americanbeejournal.com/extended-release-oxalic-acid-progress-report/

Oliver, R. 2018. Extended-release oxalic acid progress report #4. Scientific Beekeeping. (http://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-4/).