Master's thesis

The effects of propolis screen on the amounts of *Varroa destructor* mites and honey production in honeybee hives

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European honeybee (*Apis mellifera*) is an eusocial insect species that is as an important pollinator in agriculture, but their numbers are declining at an alarming rate. Perhaps one of the most significant known factors for the decline of honeybees is the ectoparasitic mite *Varroa destructor* originating from Asian honeybee *Apis cerana*. Unlike *A. cerana*, *A. mellifera* has almost no tolerance towards this threat and the *V. destructor* can destroy an entire *A. mellifera* colony it has infested by physically weakening the colony and by spreading severe pathogens inside the hive. Fortunately, honeybees have ways to counter these threats, one of which is the usage of propolis. Propolis is a substance honeybees produce from different materials from different plants, and its composition differs based on the materials it is produced of and therefore based on the geographical location of the hive. Propolis has significant antimicrobial properties and has been observed to affect certain parasite species and honeybees use it to fill unnecessary spaces inside the hive. Beekeepers can collect propolis for commercial purposes using specifically made propolis screens that induce propolis production behaviour in honeybees by creating unnecessary draft inside the hive. The purpose of this study was to determine whether a propolis screen affects the severity of *V. destructor* infestation in a honeybee hive. An experiment was conducted using a total of 10 *A. mellifera* hives; 5 target hives with propolis screens and 5 control hives without propolis screens. All the hives were naturally infested with *V. destructor*. The mite counts of each hive were recorded and compared. Additionally, the honey amounts of each hive were recorded and the interaction between the honey production and the propolis screen as well as the mite counts were explored. No statistical difference was found in the total number of mites during the summer between the hive groups. No significant differences were found between the honey production and the propolis screen, but a positive correlation was found between the number of mites and the honey production. Based on this experiment, the propolis screens did not affect the *V. destructor* counts or the honey production of honeybee hives during 11 weeks.

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Puro Simo S.	Propolislevyn vaikutus *Varroa destructor*-punkkien määriin ja hunajatuotantoon tarhamehiläispesissä
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Hakusanat: *Apis mellifera*, tarhamehiläinen, propolis, *Varroa destructor*

Tarhamehiläinen (*Apis mellifera*) on aitososiaalinen hyönteislaji, joka on erittäin tärkeä pölyttäjä maataloudessa, mutta lajin yksilömäärät vähenevät maailmanlaajuisesti hälyttävää vauhtia. Yksi merkittävimmistä tunnetuista mehiläiskatoon vaikuttavista tekijöistä ovat intianmehiläisestä (*Apis cerana*) peräisin olevat *Varroa destructor* -punkit. Toisin kuin intianmehiläisillä, tarhamehiläisillä ei ole luontaista vastustuskykyä punkkeja vastaan, vaan varroapunkit voivat tuhota koko yhdyskunnan heikentämällä sitä fyysisesti ja levittämällä vakavia taudinaiheuttajia pesissä. Tarhamehiläisillä on kuitenkin keinoja torjua punkkeja ja yksi näistä keinoista on propoliksen eli kittivahan tuottaminen ja sen käyttö pesissä. Propolis on useista eri yhdisteistä koostuva aine, jota mehiläiset valmistavat eri luonnonmateriaaleista, kuten kasveista kerätyistä eritteistä ja hartseista ja sen koostumus vaihtelee pesän maantieteellisen sijainnin ja siten käytettyjen materiaalien mukaan. Propoliksella tiedetään olevan merkittäviä antimikrobisia ominaisuuksia ja sen on myös havaittu torjuvan joitakin loislajeja. Mehiläiset käyttävät propolista pesän sisällä olevien ylimääräisten aukkojen täyttämiseen. Mehiläistarhaajat voivat kerätä propolista kaupallisiin tarkoituksiin propolislevyjen avulla, jotka luovat tarpeetonta vetoa mehiläispesässä, mikä saa mehiläiset valmistamaan lisää propolista aukkojen tukkimista varten. Tässä gradututkimuksessa selvitettiin, vaikuttavatko propoliksen keräämiseen käytetyt levyt varroapunkkien määriin tai hunajatuotantoon sekä vaikuttaako varroapunkkien määrä hunajatuotantoon mehiläispesissä. Kokeessa käytettiin kymmentä tarhamehiläispesää, joista puoleen oli laitettu propolislevyt ja puolet toimivat kontrollipesinä ilman levyä. Kaikki pesät olivat saaneet luontaisesti varroatartunnan. Punkkien määrissä ei havaittu tilastollisesti merkitsevää eroa koe- ja kontrollipesien välillä kesän aikana. Propolislevyjen ja hunajatuotannon välillä ei myöskään havaittu eroa, mutta punkkien määrän ja hunajatuotannon välillä havaittiin positiivinen korrelaatio. Tämän kokeen perusteella propolislevyt eivät vaikuta varroapunkkien määriin eivätkä hunajatuotantoon mehiläispesissä 11 viikon aikana.

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1 INTRODUCTION

European honeybee (*Apis mellifera*) (hereafter referred to as honeybee) is among the most studied insect species, and it plays an important role in human agriculture (Florio 2012). Honeybees are both found in the wild and managed by beekeepers and at least 28 subspecies are known to exist (Ruttner 1988). Honeybees are important pollinators for a wide array of different plant species and honey they produce is a food source for animals, such as bears, and the bees themselves are prey for many bird, spider and insect species (Florio 2012). Honeybees can also be considered as one of the most important and lucrative insect species utilised by humans. Bee farming and honey production employs millions of people worldwide with millions of registered beehives and the market value of honey totals up to almost 7,2 billion EUR worldwide (Shahbandeh 2021).

1.1 Honeybees and humans

Humans and honeybees have a long history together, the earliest findings of honey 'hunting' and beekeeping dating back thousands of years (Crane 1999). Beekeeping can thus be considered as one of the oldest professions and it still remains today as a global multi-billion-dollar industry (Shahbandeh 2021) that provides crucial services to agriculture (Florio 2012, Litchwark 2013). It has been estimated that 84% of the European Union's crops are at least partly dependent on insect pollination (Williams 1994) with the honeybees most likely taking the largest responsibility in the pollination services as they are highly efficient pollinators and the easiest to manage (Delaplane & Mayer 2000, Potts *et al.* 2010). Honeybees are considered to be the most efficient pollinators in agriculture for a number of reasons, for example they have highly specialised body parts to collect the pollen efficiently (Usha & Padam 2015). Moreover, honeybee hives are relatively easy to maintain, they can adapt to a wide array of different environments very quickly and can give significant benefits to the pollinated crops compared to those pollinated by other means (Usha & Padam 2015). The crops that are honeybee-pollinated have been reported to have faster growth, enhanced resistances to diseases, increased seed size and count, and higher production rate of nectar among numerous other benefits (Usha & Padam 2015). Pollination services are not the only benefit that beekeeping can produce. Most commonly known product harvested from beekeeping is honey, but many other products can be produced from beekeeping as well, such as beeswax (candles and cosmetics), royal jelly (cosmetics and protein source), bee pollen (protein source), bee venom (medicine) and propolis (medicine and cosmetics) (Florio 2012, Usha & Padam 2015).

1.2 The biology and the social structure of honeybees

Honeybees are a haplodiploid species, and a colony consists of both haploid (one set of chromosomes) males and diploid (two sets of chromosomes) females (Menzel 2012). Honeybee colonies have three distinct castes that have different roles in the colony: The drones (a few hundred per colony), the workers (thousands per colony), and the queen(s) (1-2 per colony) (Mortensen *et al.* 2015). Drones are haploid males, and their only task is to mate with the queen (Florio 2012). They are usually larger than the workers, possess no sting and have no contribution to the colony other than mating with a queen and are even fed by the workers (Florio 2012). The workers and the queens are diploid females. The worker's tasks include the overall defence, maintenance and growth of the colony while the queen's only task is to mate with drones and to lay eggs (Mortensen *et al.* 2015). The queens are initially identical to workers and whether a female pupa will become a queen or a worker is determined by the food that is fed to it; if it is fed with bee pollen it will become a worker and if it is fed with royal jelly, it will become a queen (Usha & Padam 2015, Mortensen *et al.* 2021). The queens possess a sting which is merely used to fight with potential other queens for the right to 'rule' (Mortensen *et al.* 2015). The queens are larger than drones or workers and live by far the longest, up to several years, while the drones live only two to three weeks and the workers one to two months or even four to nine months depending on the month they are born (those born during the winter live longer than those born during the other seasons) (Mortensen *et al.* 2015).

Honeybees are often used as an example of a highly social insect species. Sociality in insects has been defined by Mortensen, Smith & Ellis in 2015 using three distinct characteristics: 1. reproductive division of labour: only certain castes in the colony are allowed to or even able to reproduce and the other castes are responsible for colony growth, maintenance and defence 2. cooperative brood care: individuals take care for the offspring of other individuals from the colony 3. overlapping generations: offspring contribute to the workload in the colony while at least one of their parents is still alive and contributing to the colony. Honeybees possess all three of the mentioned characteristics and can thus be classified as eusocial insect species (Mortensen *et al*. 2015).

1.3 The impact of *Varroa destructor* on the decline of honeybees

Both wild and managed bee species are declining at an alarming rate worldwide. Between 2006 and 2015, 25% fewer bee species were reported than before the 1990s (Zattara & Aizen 2021) and recent observations reveal that also the number of honeybees in Central America and Europe is declining (National Research Council 2006, Potts *et al.* 2010). Humans are affected globally by this rather abrupt drop since honeybees have been demonstrated to directly affect both global food security and human welfare (Litchwark 2013). Although many factors contribute to this decline (such as pesticide usage and the increased intensity of agriculture), perhaps one the most damaging factor is *Varroa destructor* mite (formerly known as *Varroa jacobsoni*) (Wilfert *et al.* 2016, Koziy *et al.* 2019). *Varroa destructor* is an ectoparasitic mite that mainly feeds on the honeybee fat tissue weakening the host directly while simultaneously spreading severe pathogens (Ramsey *et al.* 2019). *Varroa destructor* has been an important topic for research for decades and its contribution to the honeybee decline was first recorded in the Philippines in 1957 (Delfinado 1963) and the biology of this mite species has been well documented.

Varroa destructor's life cycle consists of two phases: reproductive phase and the phoretic, or the dispersal phase (Koziy *et al.* 2019). During the reproductive phase, the mite enters the honeybee brood cell before its capped (closing of the open cell with a wax 'cap'), feeds on the pupa and reproduces safely inside the capped brood cell. The mite exits the brood cell with its offspring when the fully developed honeybee adult emerges (Koziy *et al.* 2019). In the phoretic phase, mites function as ectoparasites on honeybees, spreading throughout the colony (Koziy *et al*. 2019).

Varroa destructor originates from Asian honeybee *Apis cerana* and has spread via global queen trade to other continents and to *A. mellifera* during the 20^{th} century (Navajas 2010). *Apis cerana* has a long evolutionary history with the mite that has led to a stable host-parasite relationship between these two species (Locke 2016). Two primary strategies are employed by *A. cerana* to maintain a mite population below a level that is viable for the colony: first, the mites are limited to infecting drone brood cells by the expression of *Varroa* sensitive hygienic behaviour (VSH) (Harris 2007). When a mite is discovered in a worker pupa, both the pupa and the mites are removed (Boot *et al.* 1999). Second, the workers responsible for grooming behaviour catch and kill mites that are in the phoretic phase of their life cycle (Peng *et al.* 1987).

Several pathogens use *V. destructor* as a vector to spread around honeybee colonies and many of them cause severe diseases (Ramsey *et al.* 2019). One of the most damaging diseases that the *V. destructor* spread around honeybee colonies is caused by the deformed-wing virus (DWV) (Martin *et al.* 2013). DWV is a single-stranded RNA virus that causes wing deformity, bloated abdomens, colouring and shortened lifespans in honeybees when infected at larval or pupal stage of the honeybees' life cycle (Benaets *et al.* 2017). If a honeybee individual is infected at the adult stage of its life cycle, the symptoms are less obvious, causing typically no visible morphological alterations, but instead causing physical weakness and the impairment of the hosts associative learning abilities and memory formation (Iqbal & Mueller 2007, Benaets *et al.* 2017). Widespread DWV infection in a colony can even lead to a sudden collapse of the entire colony (Benaets *et al.* 2017). DWV has affected honeybees prior to the *V. destructor* invasion, but the extreme increase in the mite populations worldwide has caused an alarming spread of the virus in honeybee colonies (Ryabov *et al.* 2019).

Apis mellifera does not possess the same adapted defensive capabilities towards *V. destructor* as their relative *A. cerana* does but even if it is vulnerable to *V. destructor*, *A. mellifera* is not completely defenceless against this foreign pest. Honeybees are genetically highly adaptable and are able to swiftly adjust to new threats (Oddie *et al.* 2021). Their genetic recombination processes are targeted toward quick behavioural modifications in worker bees, which suggests a very flexible adaptive strategy according to a thorough analysis of their adaptive potential (Oddie *et al.* 2021).

Honeybees exhibit a variety of adapted behavioural traits related to social immunity, enabling responses to threats like *V. destructor* (Oddie *et al.* 2021). Similar to *A. cerana*, *A. mellifera* expresses hygienic behaviour, albeit with less specialization in countering *V. destructor* compared to *A. cerana*. Perhaps the most important part of the hygienic behaviour present in *A. mellifera* for countering *V. destructor* is the cell recapping (Oddie *et al.* 2021). In this behaviour, the workers uncap, inspect and then recap the targeted brood cell without removing the pupae. If the cell is infected, the pupa along with the mites are removed from the cell. Most of the mites usually escape during this process instead of being killed, but this behaviour has nevertheless been documented to disturb the mites since it interrupts their reproduction cycle (Oddie *et al.* 2021).

1.3.1 Current methods in apiculture to protect honeybees from *V. destructor*

Varroa destructor poses a significant threat to the apiculture worldwide (Popova *et al.* 2016, Traynor *et al.* 2020) and a number of both chemical and practical methods are used to counter the mites and the pathogens they spread (Haber *et al.* 2019). Beekeepers tend to prefer certain characteristics in the chemicals they use for this purpose, such as price, applicability and chemical properties, such as lipophilicity (do not contaminate the honey, commonly synthetic chemicals), hydrophilicity and volatility (do not accumulate in wax, commonly natural chemicals) (Haber *et al.* 2019). Synthetic chemicals used for this purpose include the organophosphate coumaphos, the pyrethroid fluvalinate and the formamidine amitraz (Rosenkranz *et al.* 2010), and natural chemicals include organic acids such as hop oil, formic acid and oxalic acid, and thymol (Haber *et al.* 2019). Both the synthetic and natural chemicals help keep the *V. destructor* populations down to a more sustainable level for the honeybee colonies, but each have their own disadvantages (Johnson *et al.* 2009, Johnson *et al.* 2010, Rosenkranz *et al.* 2010, Traynor *et al.* 2016). Synthetic chemicals tend to accumulate in the hive structures and may compromise the health of the colony (Traynor *et al.* 2016) and recent studies have shown that *V. destructor* have become increasingly resistant to the chemicals (Johnson *et al.* 2010). Natural chemicals are less likely to accumulate in the hive structures, but their efficacy is more dependent on local conditions (colony conditions, environmental factors) (Haber *et al.* 2019). Practical methods are used to slow down the mite population growth by removing the drone broods, using screened bottom boards and splitting the hives (Sammataro *et al.* 2000, Haber *et al.* 2019). However, these methods are not commonly favoured by beekeepers as they require a considerable amount of work while their effects are often insignificant (Sammataro *et al.* 2000, Haber *et al.* 2019).

Potts *et al.* (2010) found a steady reduction in the number of beekeepers throughout Europe, which is most likely related to the *V. destructor*. Since *V. destructor* has a major financial impact on beekeeping, its significance in the industry cannot be overstated. *Varroa destructor* directly affects a honeybee hive's health (Wilfert *et al.* 2016, Koziy *et al.* 2019), which directly impacts the hive's ability to produce honey (Neupane *et al.* 2012), therefore influencing the beekeeper's earnings. Therefore, it is reasonable to argue that the introduction of *V. destructor* to the honeybees has reduced the profitability and, consequently, appeal of beekeeping as a career. Undoubtedly, the decrease in beekeepers has contributed to the decline in managed honeybees; therefore, studies into novel approaches to assist beekeepers in combating *V. destructor* may also mitigate the decline of managed honeybees.

1.4 Propolis

Propolis, or commonly known as 'bee glue' is a substance produced by honeybees from materials collected from different plants (such as resins, conifer secretions, gums) and it has a long history of human utilization, with documented medicinal use dating back to ancient civilizations such as the Greeks, Romans, and Egyptians (Kuropatnicki *et al.* 2013).Today, humans continue to harvest propolis from honeybee hives for its versatile applications in biomedicine as well as using it in a multitude of products, such as in cosmetics, dentistry and hygiene products, cakes, candies and antiseptic mixtures (Anjum *et al.* 2019).

The biological properties of propolis vary based on its chemical composition, which depends on the material(s) it is produced from and therefore on the geographical zone of the hive and on the season it is produced on (Anjum *et al.* 2019, Pusceddu *et al.* 2021). Propolis is composed of hundreds of different compounds, of which more than 300 have been identified, including flavonoids, benzoic acids, waxes, amino acids, aromatic acids, essential oils, sugars, different vitamins, esters, minerals and enzymes (Anjum *et al.* 2019).

Honeybees have a wide range of ways to utilise propolis. For example, they can use it as a cement to coat the walls of the hive, to seal unnecessary spaces inside the hive, for decreasing size of the entrances during cold seasons and to embalm bodies of a dead intruders that could not be moved outside the hive (Pusceddu *et al.* 2021). Honeybees can use propolis also to protect themselves against different kinds of threats, such as pathogens, and it has been documented to possess antimicrobial properties (Pusceddu *et al.* 2021). Most importantly, recent studies show that honeybees could also use propolis to defend themselves against ectoparasites, such as *V. destructor* (Pusceddu *et al.* 2021). An increase in the number of resin foragers (adult honeybees tasked with the collection of resins) has been reportedly found in hives that have a *V. destructor* infestation, and the proportion of these foragers seems to correlate with the severity of the infestation (Pusceddu *et al.* 2019). Propolis has also been reported to increase *V. destructor* mite mortality and to decrease mite reproduction rate when applied to brood cells before oviposition (Pusceddu *et al.* 2021).

Propolis is an important produce in apiculture and beekeepers use specifically made propolis screens to collect propolis from beehives. The propolis screens work by creating 'unnecessary' space within the hive, which then causes the honeybees to fill the cavities with propolis, just as they would do in a natural hive. The purpose of the propolis screens is not only to induce propolis material foraging behaviour in honeybee workers, but also to have them produce the propolis to a more easily collectable location and form.

It is crucial to research ways to protect *A. mellifera* and its subspecies against their major enemy, *V. destructor*, to provide more information on how to slow down the global decline of honeybees. Here, I conducted a study, in which propolis screens were applied to naturally *V. destructor* infested honeybee hives and the number of mites per hive were counted weekly to test if propolis screens have a negative effect on the total mite amounts of the hives. The honey amounts were also recorded so that the interactions between honey production and mite amounts, and between propolis screen and honey production could be explored for determining other possible effects that the propolis screens and *V. destructor* could produce.

2 MATERIALS AND METHODS

2.1 The experimental setup

I conducted the experiment during the summer of 2022 using naturally *V. destructor* infested honeybee hives rented from a local beekeeper in Jyväskylä. The hives were located about 20 km west to the University of Jyväskylä in Central-Finland at a field surrounded by forest patches. The area near the hives contained scattered patches of trees and bushes, varying flowering herbaceous plants (fireweeds, dandelions) and the hives were surrounded by an electric fence for deterring off threats such as bears (Figure 1.).

Figure 1. Honeybee hives used in the experiment. Photo by Simo Puro.

A total of 10 hives were used, of which five were target hives and five were control hives. All the hives were of same size and material (Styrofoam). Target hives were inserted with commercial use propolis screens (SKU: Anel-PropolisScreen) (Figure 2.). The purpose of the propolis screens was to induce propolis material foraging behaviour in honeybee workers to naturally create a difference of propolis amounts between the target and the control hives. Both the target and the control hives were also inserted with mite traps (Figure 3.) which collect dead mites and ones that have fallen from their hosts. Since the intensity of mite fall (the number of found dead mites) is known to be closely correlated with the size of the mite population in a colony, it can be used as a proxy for overall mite infestation at moderate infestation levels (Delaplane *et al*. 2013, Drescher *et al.* 2017). The mite traps were placed under each hive at the start of the experiment and exchanged once a week during the course of 11 weeks. First traps were placed on 6.6.2022 and last traps were collected on 22.8.2022, the same traps were reused after examination and cleaning.

Figure 2. Opened hive no. 2. A propolis screen is indicated with a red arrow. Photo by Simo Puro.

Figure 3. Cleaned mite trap. Photo by Simo Puro.

After collection, each trap was brought to the Jyväskylä University and saved in a freezer room (-20 °C) for later inspection. Each trap was thoroughly examined using a tiny paintbrush, and mites were counted, collected and recorded. Besides the mites, the traps also contained relatively large amounts of small debris fallen from the hive structures, larvae, small insects (such as ants), spiders and dead honeybees or parts of them. Finally, the mite traps were carefully cleaned. Additionally, the amount of produced honey during the summer was recorded by the beekeeper after the honey collection in September 2022.

2.2 Statistical analysis

For determining whether the mite counts differed significantlybetween the control and the target groups, the data were analysed using an analysis of variance (ANOVA) for repeated measures design with SPSS (29.0.1.0 (171) IBM Statistics). A t-test was used to determine whether the propolis screen influenced the total amount of honey produced. The mite counts were compared with the total amount of honey produced per honeybee hive using a two-way t-test and a correlation test to determine whether the mite counts affected the honey production. The t-tests and the correlation test were conducted using RStudio (2023.09.01, Build 494).

3 RESULTS

3.1 *Varroa destructor* counts

Weekly *V. destructor* counts in each honeybee hive were recorded (Figure 4.) to determine whether there were differences between the mite counts of the hives. The statistical analysis showed that there were no significant differences in the total mite counts or in the weekly accumulation of mites between the control and the target hives (Table 1. and Figure 5. and Figure 6.). The interaction between the *V. destructor* counts and time, as well as between the treatment and time, were not significant (Table 1.)

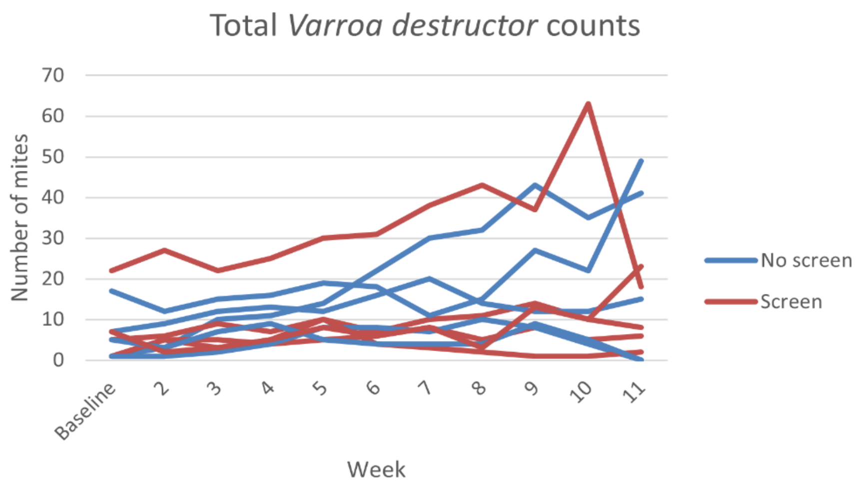

Figure 4. Weekly *Varroa destructor* counts per honeybee hive, starting at 6.6.2022. Baseline measure was taken before inserting propolis screens into the hives. Hives without a propolis screen (No screen) are shown with blue and hives with a propolis screen (Screen) with red colour.

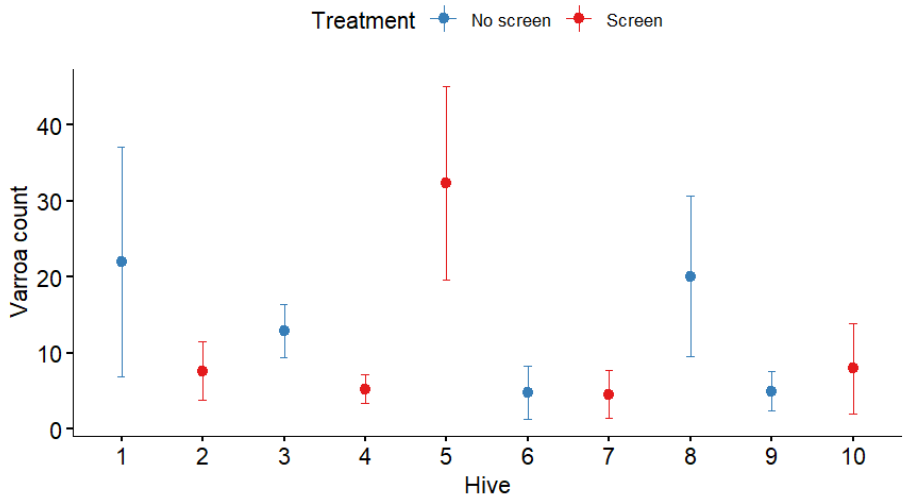

Figure 5. The averages and the standard deviations of the total *Varroa destructor* counts per honeybee hive. The data points represent the average mite counts for each honeybee hive, while the whiskers indicate the standard deviations in the mite counts. The honeybee hives without a propolis screen are shown with blue (No screen) and the hives with a screen with red colour (Screen).

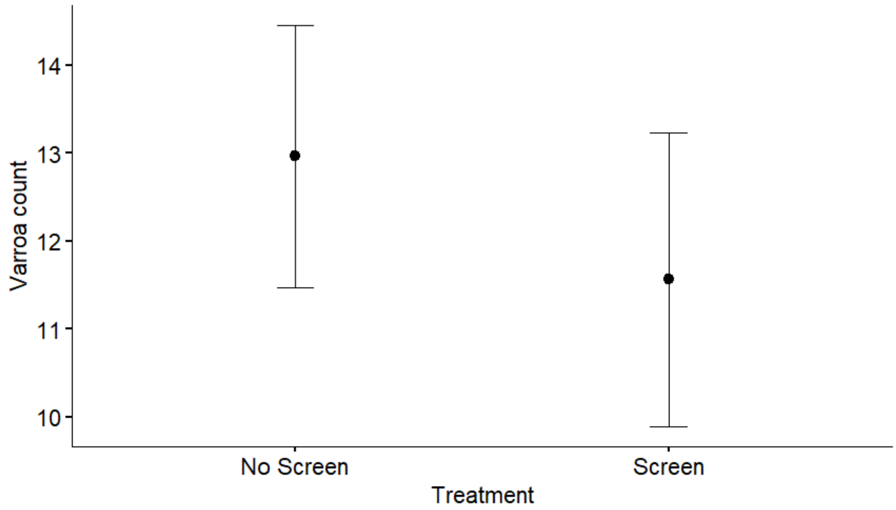

Figure 6. The means and the standard errors of the total *Varroa destructor* counts per treatment group. 'No Screen' group consisted of 5 honeybee hives without a propolis screen, and the 'Screen' group consisted of 5 honeybee hives with a propolis screen. The data points represent the mean total mite counts per treatment group, and the whiskers denote the standard errors of the mean mite counts.

Table 1. Summary of results of the analysis of variance for the repeated measures design. *Varroa destructor* counts were compared between the two treatment groups (honeybee hives with and without a propolis screen). "Propolis Screen" shows the effect that propolis screen had on the *V. destructor* counts, "Time" gives the effect of time on the *V. destructor* counts and "Interaction" gives the interaction between the propolis screen and time. None of the p-values are significant.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
Propolis Screen	53,9	1	53,9	,048	,834
Time	1381	4,061	340	2,607	,053
Interaction	330	4,061	81,5	,625	,651

3.2 Honey production

Honey was collected from each hive at the end of summer 2022 by the beekeeper and the amounts were recorded (Appendix 1.). The honey amounts were compared with the mite counts of each hive to determine if the number of *V. destructor* mites and the treatment (propolis screen) affected the honey production of the honeybee hives. The correlation analysis revealed a positive correlation ($r = 0{,}42$) between the quantity of honey and the total mite count, with a statistically significant difference detected through the two-way t-test ($p = 0{,}02$). No statistically significant difference was detected ($p = 0{,}832$) between the means of total honey produced during the summer by the two treatment groups (hives with and without a propolis screen) (Figure 7.).

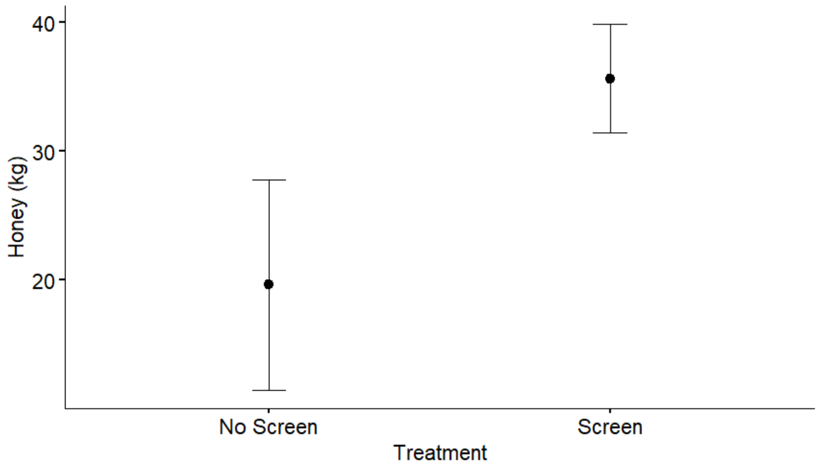

Figure 7. The quantities of honey (in kg) in the two treatment groups. 'No Screen' group consisted of 5 honeybee hives left without a propolis screen and the 'Screen' group of 5 honeybee hives with a screen. The plot displays the means and the standard errors of the honey measurements for the two treatment groups.

4 DISCUSSION

The number of honeybees worldwide has been decreasing at an alarming rate (Bauer & Wing 2010, Potts *et al.* 2010) as a consequence of several factors such as the increasing intensity of agriculture and pesticide use, but perhaps the most damaging factor has been the spread of *Varroa destructor* mite (Wilfert *et al.* 2016, Koziy *et al.* 2019). The negative impact of *V. destructor* on the health of honeybees worldwide has been significant (Popova *et al.* 2016, Traynor *et al.* 2020) and more and better ways to lessen the negative effects caused by *V. destructor* are needed. This subject has been of great interest to researchers for the past 20 years and a great number of studies have been conducted to find a viable solution to the problem. A study by Pusceddu *et al.* (2021) proposed that propolis, the antimicrobial substance produced by the honeybees themselves, could affect the mortality rates of *V. destructor* inside the honeybee hives. Here, we tested if propolis, in its natural form, could affect the *V. destructor* counts of the hives. We used propolis nets to induce propolis material foraging behaviour in honeybees to create a natural difference in propolis amounts between the experimental hives and recorded the weekly mite amounts of each hive for 11 weeks, from the early June to the end of August. Additionally, we also recorded the total amount of honey produced by each hive to test whether the propolis or the amount of *V. destructor* inside the honeybee hives affects honey amounts.

4.1 Effects of propolis screen on *Varroa destructor* infestation

Our results show that the propolis screens did not affect the *V. destructor* counts in the studied *Apis mellifera* hives during the course of 11 weeks. This could result from multiple factors. Mites could not have had enough physical contact with propolis, since the propolis screens were applied to the top of each target hive, between the topmost hive compartment and the hive lid. The location and the distribution of propolis in the hives matters if only direct physical contact with propolis has a strong enough effect on *V. destructor* to increase its mortality rates. It might be that physical contact is required, as a similar study by Drescher *et al.* (2017) showed that raw propolis volatiles do not affect the mortality rates of *V. destructor*. The same study also manipulated propolis intakes of experimental honeybee hives and found no correlation between the amounts of propolis and the number of *V. destructor* in the honeybee hives (Drescher *et al.* 2017), aligning with the results of our study, but contradicting the results of the study by Pusceddu *et al.* (2021). The different results of the studies could be explained by the fact that the final composition of propolis greatly varies based on the local conditions of the honeybee hive producing it, which significantly affects its biological properties (Anjum *et al.* 2019, Pusceddu *et al.* 2021).

The local flora near a honeybee colony has a significant role in the final composition of the propolis the colony produces, since honeybee workers choose the appropriate source plants based on what is available (Bankova 2005). Therefore, the location of the hive affects the composition and properties of the produced propolis. A few honeybee colonies in France have been reported to possess a significant increase in resistance towards *V. destructor* (Fries *et al.* 2006, Le Conte *et al.* 2007, Locke *et al.* 2012). It was found that the studied *V. destructor* resistant hives produced propolis that had a higher relative concentration of certain compounds (different caffeic acids and caffeic acid pentenyl esters) compared to the propolis extracted from hives that were not resistant to *V. destructor* (both hive groups were located in Avignon, France) (Popova *et al.* 2014). The observed increase in the concentration of specific compounds in propolis produced by *V. destructor* resistant hives implies a potential influence of propolis composition on its efficacy against *V. destructor* infestation. The chemical composition of the propolis in this study's experimental hives was not analysed, and it could be that the propolis did not contain strong enough concentrations of specific compounds that could affect the *V. destructor*. However, even if the propolis of the experimental hives did not have an effect on the *V. destructor* amounts, it still could have affected the strength and/or health of the honeybee hives in a different way, such as reducing the amounts of pathogens in the hives. As known, the effects of a *V. destructor* infestation on a honeybee hive are affected by number of factors, such as the local climate conditions which further affects which viruses are present in the area (Giacobino *et al.* 2016). It could be that the local area in which the *V. destructor* resistant honeybee hives were located did not have large enough numbers of severe pathogens (such as DWV) that the mites would spread in the hives, which would then make the effects of the infestation less harmful. While the viral loads in the honeybee hives in this study were not analysed, it is worth considering the potential impact of propolis screens, given the significant antiviral properties of propolis (Drescher *et al.* 2017). If propolis screens are indeed capable of reducing viral loads in honeybee hives, they could improve overall hive health. Healthier honeybee colonies are better equipped to defend themselves against threats like *V. destructor*, potentially magnifying the positive effects of propolis screens over time, but further research is required to determine the long-term effects of propolis screens on hive health.

4.2 Honeybee adaptations to *Varroa destructor*

Honeybees possess a tremendous capability for adapting to local threats (Oddie *et al.* 2021) and one of the most significant methods they use in the defence against microbes, parasites and viruses is the usage of propolis (Pusceddu *et al.* 2021). It was shown by Pusceddu *et al.* (2021) that some honeybee colonies use propolis as a natural pesticide against *V. destructor*. Since *V. destructor* has just spread to honeybees during the 20th century (Matheson 1996) it could be that the honeybees are only beginning to adapt to using propolis against the mite. There have been reports of honeybee colonies that have seemingly adapted to *V. destructor* around the world. Several studies have found that the africanized honeybees (*Apis mellifera adansoni)* in Southern and Northern America have developed a strong tolerance towards *V. destructor* and have been able to coexist with the mite even when left untreated (Boecking & Ritter 1993, Rosenkranz 1999). More recently, a study was conducted by Fries *et al.* (2006) in Gotland, Sweden to determine if honeybees could adapt to *V. destructor* in Nordic climate. They found that 5 out of 150 honeybee colonies survived and were able to coexist with *V. destructor* after 6 years of infestation. Four years later, Locke & Fries (2011) conducted a subsequent study and found that the colonies had become resistant towards *V. destructor* by suppressing the reproduction of the mite. Therefore, it is possible for honeybees to adapt to *V. destructor* when left completely untreated. The propolis produced by the hives of the aforementioned studies was not analysed, but since the mechanisms behind the honeybee adaptation to *V. destructor* is not fully understood, it could be that the propolis has played a role in the adaptation. Our results cannot affirm that propolis has a negative effect on a *V. destructor* infestation, but the possibility that the honeybees could produce propolis of different properties in another year still exist, as it was shown by Peixoto *et al.* (2021) that the same honeybee hive may produce propolis of different composition on a different year. So, it could be that the propolis screens used in our study could produce a different effect when used for a longer period of time than just 11 weeks. Therefore, it cannot be yet concluded whether the propolis screens could impact the *V. destructor* amounts in honeybee hives or not. The possibility of honeybees producing propolis of different composition in response to *V. destructor* is highly interesting but requires further research.

4.3 Effects of *Varroa destructor* and propolis on honeybee hive's health

The strength of a honeybee hive can be measured using the number of worker brood cells in the hive and it has a strong effect on the total honey production of the hive (Neupane *et al.* 2012). Therefore, the total amount of honey produced by a honeybee hive gives information about its condition. The amount of produced honey per hive was measured in this study at the end of the experiment to determine if the propolis screens had an effect on the total amount of honey produced during the summer. Our results showed that the honeybee hives without a propolis screen (control hives) produced only 19,62 kg of honey on average, while the hives with a propolis screen (target hives) produced 35,64 kg of honey on average, but no significant difference between the honey amounts of the hive groups was found in the statistical analysis. Since the control and the target groups each consisted of only five hives with only one measurement point for the amount of honey per hive, each hive obviously causes a significant effect on the average of total honey produced per group. In particular, two control hives had a high impact on the average honey produced by the whole group: the hive no. 6, which produced no honey at all, and the hive no. 9, which only produced 5,1 kg of honey. It could be just a coincidence that the two weakest hives were both control hives, since propolis nets did not affect the amounts of *V. destructor* found in the hives. However, considering that all the target hives produced at least 25 kg of honey, it could also be a sign that the propolis nets could impact the strength and/or health of the hive in another way.

The overall health of a honeybee hive affects the total honey produced by the hive (Neupane *et al.* 2012), so the intensity of the *V. destructor* infestation will have an effect on the total amount of honey produced. We found a statistically significant difference and a positive correlation between the number of mites and the total honey produced by the honeybee hives. However, this result does not indicate a direct relationship between the number of mites and the total honey produced by the hives, nor does a positive correlation between the honey production and the mite counts necessarily mean that more mites cause higher honey production or vice versa. There could be several other factors that influence both the honey production and the mite count independently. For example, both the honey production and the mite counts may be influenced by factors such as the health of the honeybee hive, environmental conditions during the summer and management practices.

Keeping honeybee hives in good strength and health is crucial in beekeeping, as it has a direct effect on the total honey produced by the hives and therefore affects beekeepers economically. *Varroa destructor* and the pathogens that the mite spreads have thus far been countered mostly using synthetic and natural chemicals. This has a number of disadvantages such as the impact of the chemicals on the surrounding environment as well as on the honeybees themselves, and the chemicals have been shown to accumulate on the hive structures (such as wax) most likely producing a negative effect on the health of the hive (Traynor *et al.* 2016). In addition, recent studies show that *V. destructor* has become increasingly resistant towards all the used chemicals (Johnson *et al.* 2010) and hence better ways to control it are crucially needed. While our study did not demonstrate direct benefits of propolis screens for honeybee hives, I still recommend their use by beekeepers. Propolis screens are cost-effective, easy to use, and do not significantly increase workload. Moreover, they offer additional revenue through propolis extraction and sale. Additionally, there is potential for the long-term use of propolis screens to improve hive health by reducing viral loads (Drescher *et al.* 2017). Further research is needed to determine whether propolis screens could help beekeepers in combating *V. destructor* and associated diseases.

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APPENDIX 1. TOTAL HONEY PRODUCED PER HIVE