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PROPOSED GUIDELINES FOR A SUSTAINABLE BEEKEEPING THAT IMPROVES ENVIRONMENTAL QUALITY

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ABSTRACT

Le api e il miele hanno svolto un ruolo culturalmente e economicamente rilevante per l'uomo fin dai tempi antichi. Numerosi sono gli studi che negli anni hanno avuto l'obiettivo di comprendere la vita di questo insetto, nel tentativo di migliorarne sia la qualità della sua vita, sia la produzione di miele, che la qualità di questa produzione. È tuttavia noto come il delicato sistema su cui si basa la vita all'interno di un alveare, sia soggetto a numerosi stress sia di natura abiotica che biotica. La presente tesi affronta alcuni dei principali problemi che attualmente affliggono il mondo dell'apicoltura e degli apicoltori, sia da parte del produttore che del consumatore. Per quanto riguarda le avversità che causano la morìa di api, gli studi si sono concentrati sulla lotta alla varroa. Sono stati effettuati studi che hanno riguardato alcuni prodotti a base di acidi beta e alfa di luppolo, nel tentativo di verificare se questi acidi, in soluzione, potessero contribuire alla lotta alla varroa riducendo il numero di parassiti all'interno degli alveari. Le modalità di somministrazione dei trattamenti sono state diverse rispetto agli altri lavori che si trovano in letteratura, spruzzando direttamente il trattamento sui telaini. I risultati hanno mostrato come la soluzione di beta acidi di luppolo al 2% abbia avuto un buon effetto acaricida, al pari del controllo, effettuato con acido ossalico. Allo stesso tempo, le soluzioni di alfa acidi testate a percentuali dello 0,3% e 0.6% non hanno portato ad una riduzione del carico di varroa prevista, anche sulla base della caduta dell'acaro avuta con il controllo a base di acido ossalico. Parlando delle attività effettuate in ambito di qualità della produzione di miele e sicurezza per il consumatore, vista la necessità di garantire prodotti sicuri ed esenti da dichiarazioni mendaci riguardo alla loro origine, il presente lavoro ha voluto studiare le relazioni esistenti tra il profilo elementare, i rapporti isotopici, alcuni parametri chimico-fisici e l'origine di uno dei più pregiati e rappresentativi mieli dell'apicoltura mediterranea: il miele di agrumi *(Citrus spp.).* A tal fine, sono stati caratterizzati tramite parametri chimico fisici, profilo elementare e rapporti isotopici di 81 campioni di miele proveniente da Italia, Grecia ed Egitto. Il principale obiettivo è stato quello di validare l'uso di queste analisi negli studi di tracciabilità geografica del miele. I risultati dell'anali statistica multivariata (PCA), sebbene abbiano mostrato una parziale discriminazione dell'origine geografica dei mieli di agrumi, hanno alcuni elementi e parametri con un potenziale discriminatorio notevole (pH, Pb, Mg, Fe, Cd, Rb, δ15 N) consentendo di distinguere le provenienze dei campioni analizzati.

Bees and honey have played a culturally and economically relevant role for humans since ancient times. Numerous studies over the years have aimed to understand the life of this insect in an attempt to improve both its quality of life and honey production. However, it is well known how the delicate system on which life within a hive is based is subject to numerous stresses of both abiotic and biotic nature. This thesis addresses some of the major problems currently plaguing the world of beekeeping and beekeepers, both on the part of the producer and the consumer. Regarding the adversities that cause bee death, studies have focused on varroa control. Studies were carried out involving some beta and alpha hop acid products in an attempt to test whether these acids, in solution, could contribute to varroa control by reducing the number of pests within the hives. The mode of treatment administration was different from other work found in the literature, spraying the treatment directly on the frames. The results showed that the 2% hops beta acid solution had a good acaricidal effect, on par with the control, made with oxalic acid. At the same time, the alpha acid solutions tested at rates of 0.3 percent and 0.6 percent did not lead to a reduction in the expected varroa load, also based on the mite drop had with the oxalic acid-based control. Speaking of the activities carried out in the field of honey production quality and consumer safety, given the need to ensure safe products free from misrepresentation regarding their origin, the present work aimed to study the relationships existing between the elemental profile, isotope ratios, some chemical and physical parameters and the origin of one of the most valuable and representative honeys of Mediterranean beekeeping: citrus honey (Citrus spp.). To this end, 81 honey samples from Italy, Greece and Egypt were characterized through chemical-physical parameters, elemental profile and isotope ratios. The main objective was to validate the use of these analyses in honey geographic traceability studies. The results of multivariate statistical analysis (PCA), although they showed a partial discrimination of the geographic origin of citrus honeys, have some elements and parameters with remarkable discriminatory potential (pH, Pb, Mg, Fe, Cd, Rb, δ15 N) allowing to distinguish the origins of the analysed samples.

Keywords

Honey, Bee, Varroa, IPM, Traceability

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1 INTRODUCTION

1.1 THE HONEY BEES

Honeybees are pollinating insects that belong to the order *Hymenoptera* and the family *Apidae*, which includes various species of social bees. Among these species are honeybees, specifically the species *Apis mellifera* (Linnaeus, 1758), although some authors and taxonomists use the name *Apis mellifica* (Carlo Linnaeus, Fauna Suecica, 1761)(M. L. Winston 1991).

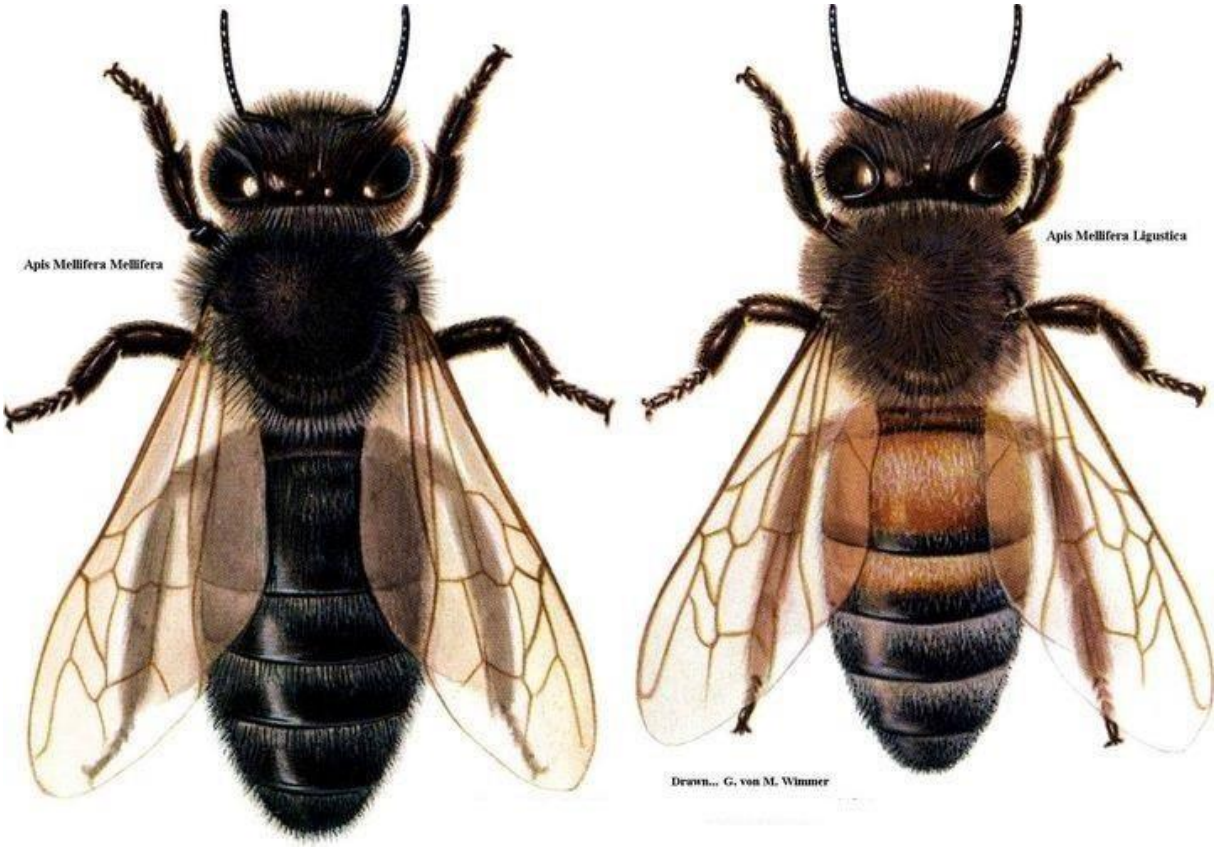

Figure 1. Differences between *Apis Mellifera Mellifera* and *Apis Mellifera Ligustica* (G. Von Wimmer)

A. mellifera lives in colonies that typically consist of an average of 60,000-80,000 individuals. These colonies generally include a fertile queen bee, a few hundred fertile males known as drones, and thousands of worker bees (M. L. Winston 1991).

The life cycle of bees consists of four stages: egg, larvae, pupae, and adult. The development of a worker bee takes approximately 21 days, while it takes 15 days for a queen and 24 days for a drone (Oliver, 2010).

The mating process of the queen with drones follows a specific pattern. The virgin queen flies to a congregation area where drones from different colonies gather, even if they are located several kilometers apart. Around fifteen drones deposit approximately 90 million spermatozoa in the queen's oviducts (polyadry). Out of this amount, around seven million spermatozoa are stored in

a specialized sac in her body called the *spermatheca* (Zagnoli 2013). The sperm will be released from the *spermatheca* only when the eggs are fertilized (Heimpel and De Boer 2008).

Figure 2. A closed queen cage (author's photo). Figure 3. A queen outside the hive (author's photo).

The bee egg is a sort of white stick 1.5 mm long, attached to the bottom of the cell where the queen bee lays it. After three days of incubation, a small larva is born, which is fed by the nurse bees. After five days, the larvae stop feeding, they isolate themselves in a cocoon and they have woven themselves while from the outside the workers seal the cell with a wax operculum; Metamorphosis begins, transforming the larva into a prenymph and subsequently into a nymph. Eventually, it takes on the appearance of an adult insect. The timing of this process varies depending on the individual's role as an adult. Drones and worker bees have an adult lifespan of about 6-8 weeks during peak activity, except during winter periods when worker bees can survive for up to 6-8 months (David Aston 2021; Heimpel and De Boer 2008).

Immediately after emerging, young worker bees begin their assigned tasks, starting with cleaning the cells in which they were born. Since they have underdeveloped reproductive organs, their tasks differ based on their age. During the first three weeks of life, they remain inside the hive and engage in activities such as cell cleaning, brood feeding, honeycomb construction, food storage, and colony defense. In the subsequent period, worker bees gather essential elements for sustaining the entire colony, including nectar, pollen, propolis, and water (Döke, Frazier, and Grozinger 2015).

In Europe, various subspecies of A. mellifera are recognized. Among them, the most commonly farmed ones are *A. mellifera ligustica* in Italy, *A. m. mellifera* (Linnaeus, 1758) in central and northern Europe, *A. m. carnica* (Polmann, 1869) and *A. m. caucasica* (Gorbatschew, 1916) in central-eastern Europe, *A. m. sicula* (Montagnano, 1911) on the mentioned island, and *A. m. iberiensis* (Goetze, 1964) in Spain and Portugal (Spinola, 1806).

Honeybees require specific nutrients in their diet to support their growth and development. Proper nutrition plays a crucial role in colony growth and immune responses (Alaux et al. 2011; Rosenkranz, Aumeier, and Ziegelmann 2010). Among the main nutrients needed by bees we find:

- Carbohydrates: Bees derive carbohydrates as a source of energy primarily from nectar, which is transformed into glucose with the help of certain enzymes. Pollen also serves as a significant source of carbohydrates (Huang 2010).
- Proteins, in particular the essential amino acids, deriving from the collection of pollen, of which it is composed of about 20% (Thakur and Nanda 2020), then transformed into bee bread which the young bees consume as soon as they are born (Brodschneider and Crailsheim 2010; Janmaat and Winston 2000; Somerville 2005). Without sufficient pollen, brood growth may be impaired and bees may have a shorter life span (Brunner, Schmid-Hempel, and Barribeau 2014).
- Lipids, important for the development, nutrition and reproduction of bees, whose needs are met by the consumption of pollen, which has an average lipid concentration between 5% and 8% (Manning 2001; Svoboda et al. 1980).
- Vitamins, especially the B and C complexes, which bees always obtain from pollen. However, since the vitamins are not stable, their value can be reduced in pollen stored for periods longer than 12 months (Sahinler, Gül, and Şahin 2005; Sakla and El-shafeiy 2022).
- Minerals, including potassium, phosphorus (predominant), calcium, and iron and zinc, present in both pollen and nectar in smaller quantities. Among the various minerals we find iron, useful both for maintaining iron homeostasis and for orientation outside the hive (Beinert, Kennedy, and Stout 1996; Kuterbach and Walcott 1986; Valverde et al. 2023).

1.1.1 THE HIVE'S PRODUCTS

Apis mellifera collects various substances to ensure the survival of the colony. This includes nectar, which adult bees transform into honey and store inside beeswax cells. Propolis, another substance collected by bees, acts as a natural adhesive and is used to seal cracks and maintain hive temperature and hygiene. Pollen is also collected and provides essential nutrients such as protein, amino acids, fatty acids, vitamins, and minerals. Additionally, bees collect water to regulate temperature and humidity within the hive(Somerville 2005)

Among the products of bees that are consumed directly by humans, the main one is definitely honey. According to Directive 2001/110/EC

"Honey is defined as the natural sweet substance that bees (Apis Mellifera. L) produce from the nectar of plants or secretions from living parts of plants or from substances secreted by sucking insects on living parts of plants, which they forage, process, combine with their own specific substances, deposit, dehydrate, store and allow to ripen in the combs of the hive".

So, honey is a complex product that is challenging to define. Its chemical composition, botanical origin, and extraction methods are among the factors that determine its identity. Honey can be considered to have a dual nature, both animal and plant. Bees play a vital role in the processing of raw materials, while the plant origin of these materials is also significant (Contessi 2016).

The primary plant-based raw materials used in honey production are nectar and honeydew. Nectar is a sugary secretion produced by specialized glands called "*nectaries*" located at the base of flowers. Honeydew, on the other hand, is a sugary substance found in plant parts affected by certain parasitic insects belonging to the order *Rhynchus homoptera* (such as Aphids, *Psyllidae*, *Coccidae*). These insects extract sap from plants and excrete excess substances in the form of droppings, which include water, sugars, enzymes, mineral salts, and organic acids. Once the foraging bees collect nectar or other sugary liquids, they deposit them into the honeycomb cells, initiating the transformation into honey by adding enzymes like diastase, invertase, glucose oxidase, and others. Based on botanical origin, honey can be classified into three types: unifloral honeys, derived mainly from a single plant species; multifloral honeys, where no predominant origin can be determined; and honeydew honeys, primarily derived from the collection of honeydew by bees (Ballabio et al. 2018; Everstine, Spink, and Kennedy 2013; Oddo et al. 1995; Oddo L. P., Piana L. 2007)

1.2 THE BEES FOR THE PLANET

Honey bees are important for many ecological and economic reasons. In fact, a large part of agricultural crops requires zoophilic or entomophilous pollination (Vaudo et al. 2015). Bees are part, together with other Hymenoptera, Lepidoptera and Coleoptera, of that category of insects

defined as "pollinators", e.g., insects which, through their biological activity, allow the sexual reproduction of plants with entomophilous pollination. There are in fact about 300,000 plant species that require an entomophilous type of pollination.

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services è un'organizzazione intergovernativa creata per migliorare l'interfaccia tra scienza e politica su questioni di biodiversità e servizi ecosistemici (IPBES 2019; ISPRA 2021).

Among the above mentioned 300,000 plant species relying on bee pollination, three-quarters of the main crops that are essential for human sustenance depend on pollinators like bees. Without them, our food diets would be severely limited, lacking the variety of essential nutrients such as vitamins and minerals that are necessary for the body (ISPRA 2020).

Bees are the dominant taxonomic group among pollinators. It is worth noting that of the 107 most important crops for human nutrition globally, over 90% rely on bee visits. The loss of these pollinators is therefore of great significance and could lead to a 5-8% reduction in the quantity of food produced worldwide (Ponisio et al. 2015).

Insect pollination is a process of utmost importance in terrestrial environments, as it provides vital ecosystem services for human well-being. A decrease in insect pollination could potentially limit yields by about 40% (ISPRA 2020; Khalifa et al. 2021).

1.3 THE PROBLEMS OF THE APICULTURE

1.3.1 THE COLONY LOSS

Bees are insects that face different risks, both biotic and abiotic. The presence of bees is decreasing in different parts of the planet, and among the most common causes are intensive agriculture, monocultures, the excessive use of chemical products such as pesticides, and the rise in temperatures due to climate change. These factors have an impact on both crops and bee nutrition (Desneux, Decourtye, and Delpuech 2007; Paleolog et al. 2023).

However, there are also other significant factors to consider, such as mites, viruses, and bacteria. *Varroa destructor*, *Paenibacillus larvae* (the cause of American foulbrood), *Melissococcus plutonius* (the cause of European foulbrood), *Aethina tumida*, *Nosema ceranae*, and *Nosema apis* are among the agents that pose a significant threat to bees (Krupke et al. 2012; Neumann and Carreck 2010; S.G. Potts et al. 2010; Staveley P.J. 2014).

Analyzing the risk factors for bees, however, is not a simple task. Pathological and non-pathological factors can interact with each other, creating effects that are not only additive but also synergistic or multiplicative (Bortolotti et al. 2009; Gray et al. 2020).

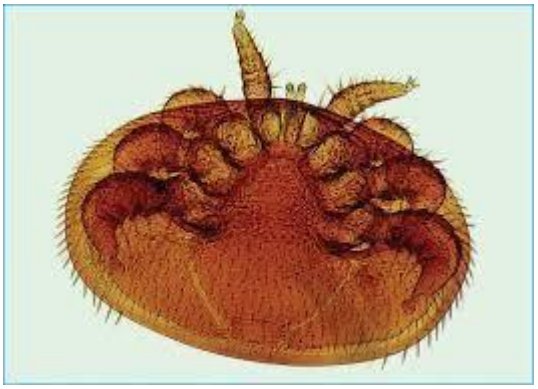

Figure 3. *Varroa destructor* apacame.org.br.

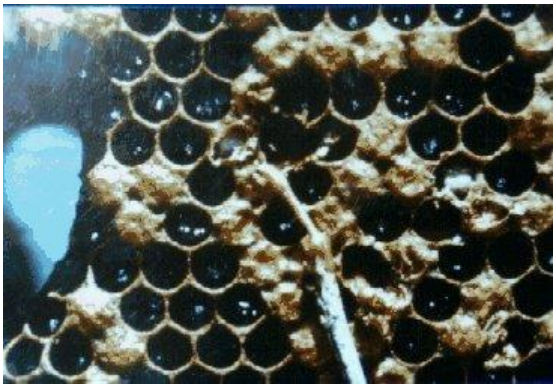

Figure 4. American bee pest (apicolturaonline.it).

Figura 5. European pest (www.izslt.it)

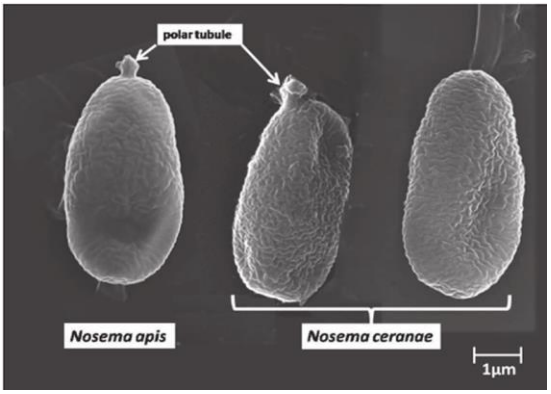

Figure 6. *Nosema apis* and *N. ceranae* spores (Ptaszyńska et al. 2012).

Stress phenomena, with the consequent effects on colony loss, can also be divided into different periods of the year (Bortolotti et al. 2009): January-February, when beekeeping activity resumes, as a result of orphanhood and/or insufficient stocks. March-April, in particular in correspondence with the spring sowing of some herbaceous crops and treatments on orchards. And finally in autumn, in the period in which the high infestation by the *V. destructor* mite is associated with the spread of particularly lethal viruses, which can compromise the correct development of the pre-winter bees.

1.3.2 VARROA DESTRUCTOR: MORPHOLOGY, LIFE CYCLE AND BEHAVIOR

Currently, one of the biggest problems facing beekeeping is *V. destructor*, the parasitic mite that has spread from its native host, the Asian bee (*A. cerana*) to the commercially used European bee (*A. mellifera*), used for pollination and honey production worldwide. This parasitic mite has a big impact on colony decline and bee production loss (Traynor et al. 2020; Vilarem, Piou, and Vogelweith 2021). From a taxonomic point of view, the Varroa mite belongs to the Varroidae family, belonging to the class of arachnids (Arachnida) and to the subclass of mites (Acari). The

genus includes several species of obligate ectoparasites, of which the most relevant and widespread are:

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- *V. jacobsoni Oudemans*, natural ectoparasite of the asiatic bee (*A. cerana*), discovered by Oudemans in 1904 in Java, which later became a parasite of *A. mellifera* in different parts of the world.
- *V. destructor*, considered a separate species from jacobsoni in 2000 by Anderson and Trueman (who define the two species, *V. destructor* and *V. jacobsoni*, not interbreeding and therefore quite distinct from each other), of similar constitution but whose adult females are significantly larger (over 1 mm diameter)(Anderson and Trueman 2000b).

As regards the morphological characteristics of the mite (relevant for the purposes of parasitization), the buccal apparatus of the Varroa includes two chelicerae, which the parasite uses to cut the cuticle of the adult bee, larvae and pupae, thus having access to the nutrients it feeds on (the opening formed is called the "feeding site"). Another function of the chelicerae is to allow the Varroa to anchor on the adult bee, thanks to small spurs that grip the cuticle (called pedipalps).

Varroa destructor is closely related to its host and does not have a free life stage. The life cycle of Varroa is divided into a phoretic phase on adult bees and a reproductive phase inside sealed brood cells, preferably male but also female, with an annexed oviposition phase.

During the reproductive phase the parasite feeds on the bee larvae inside the brood cell protected by the operculum and also exploits this condition to reproduce and raise the offspring up to the adult stage the penetration of the mite in a cell is strongly regulated by time, a cell is infested a few hours (about 24 hours) before capping.

After the operculature, the oviposition phase begins, where the female Varroa lays haploid male eggs, the born males (after reaching the mature stage) will reach the nutrition site and mate with the female mite, making her able to lay eggs female diploids (which once adults will then be able to re-infest other brood cells), mating is a very delicate process as a failure in fertilization can make the female mite irreversibly sterile (due to regression of the genital system). After the reproductive phase, the female Varroa parasitize the adults by clinging to the flickering bee (phoretic phase)(Reams and Rangel 2022).

V. destructor mites are highly sexually dimorphic with many morphological adaptations to their host. A characteristic that unites the two sexes is the division of the body into two well-defined

parts: the *idiosoma* and the *gnatosoma*. Females have a flattened, ellipsoidal *idiosoma* with a width greater than the length; the legs of the female are short and show specialized structures, called *apothelia*, for adhesion to the host (A. De Ruijter, 1987).

As regards the body of the male, this is pear-shaped and shows only a weak sclerotization which is present above all in the legs and in the dorsal shield; at all developmental stages, males are smaller than females (Rosenkranz, Aumeier, and Ziegelmann 2010). The males and nymphal stages of the mite are short-lived and can only be found within sealed brood cells (Kuenen and Calderone 1997).

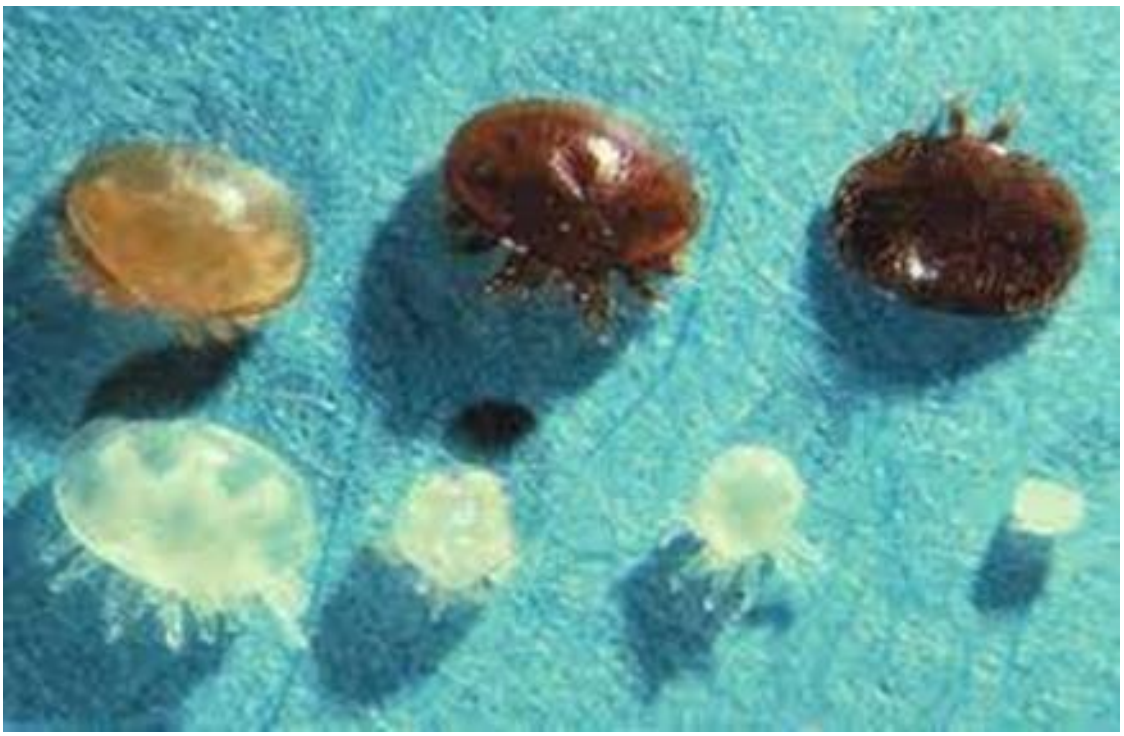


Figure 7. Different maturation states of *Varroa destructor* (VETO-PHARMA 2020)

Through adult bees, Varroa females are transported to brood cells (Fig. 8) for their reproduction or disseminated to foraging and swarming bees (Kuenen and Calderone 1997). In the phoretic phase, the mites hide under the sternites of the bee, which transports them from the preimaginal stages of the host into the capped brood cells (De D'Aubeterre et al. 1999).

The parasite harms host bees by consuming the fat body, an integral tissue for proper immune function, pesticide detoxification, overwinter survival, and many other processes essential for healthy bees. In general, the effects of Varroa parasitosis generate a negative influence on the immune response of the bee, thus making it more vulnerable to microbial agents, determining the onset of viruses, and inducing the replication of viruses already present in the bee (Johnson et al. 2010; Ramsey et al. 2019; Shen et al. 2005; Yves, Marion, and Wolfgang 2010).

Figure 8. Schematization of the reproductive cycle of varroa within the bee's brood (www.izslt.it/apicoltura/stadi/varroa). Figure 9. A bee larva with varroa mite (author's photo)

1.3.3 VARROA SPREAD

Varroa has a great ability to spread over the territory, using the bees themselves as a vector, which guarantees the mite's penetration into a new hive. To start an infestation, even just one female mite is enough, which will then lay the eggs of the males. There are four main ways of propagating Varroa:

• Looting: The hive brought to collapse by Varroa itself, therefore weakened, will then be subject to looting by nearby bee colonies, allowing the latter to come into contact with a large number of Varroa mites (Norberto Milani 1999).

• Drift: this mainly affects bee colonies kept in very close hives. Foraging bees returning from a foraging flight or drones can enter a hive other than their own, and if they carry Varroa in the phoretic state, they can increase its diffusion within and between apiaries (Goodwin and Van Eaton 2001).

• Swarming: Swarms can settle in an area different from their origin, bringing the phoretic Varroa mites with them (Paleolog et al. 2023).

• Normal beekeeping operations can also facilitate the spread of Varroa. For example, the transfer of honeycombs with sealed brood from a stronger colony to a weaker one can introduce Varroa mites, depending on the degree of infestation in the honeycomb (Wilde et al. 2005).

The origin of the Varroa infestation in European bees was due to the introduction of *A. mellifera* colonies into the range of *A. cerana* in East Asia, particularly in Japan and Korea (Crane, E., Graham 1985; Oldroyd 1999). From there, the mite spread from Asia to South America and then to North America (De Jong, Morse, and Eickwort 2003). Simultaneously, the parasite began to expand towards the European continent (Rosenkranz, Aumeier, and Ziegelmann 2010). In Italy, the spread started with outbreaks identified on the border of Trieste in 1980, and the first official case of infestation on the peninsula was recorded in June 1981 in Staranzano (Gorizia). Despite the implementation of containment and prevention measures, such as acaricidal treatments, disinfection, and trade restrictions with the destruction of potentially infected material, the mite's spread was nearly uncontrolled in all Italian regions by 1984 (Commission of the European Communities 1988; Frilli 1983).

1.3.4 VARROA DAMAGES

Varroa destructor is considered the main cause of winter hive mortality in temperate climate countries (Rosenkranz, Aumeier, and Ziegelmann 2010), and the collapse of heavily infested colonies during the active season, known as “colony collapse disorder” This syndrome presents

a complex of symptoms at both the individual bee and colony levels, including a numerical reduction in population, malformed adult bees with signs of paralysis, mosaic brood, dead larvae, and viral infections carried by Varroa. These stressors significantly reduce bee survival, leading to rapid depopulation of hives (Sabahi et al. 2020; Vesco 2013).

In general, Varroa parasitism has a negative influence on the bee's immune response, making it more vulnerable to microbial agents and leading to the onset of viruses and replication of existing viruses within the bee (Ramsey et al. 2019; Shen et al. 2005). Specifically, the damage caused by *V. destructor* can be categorized as direct and indirect.

Among the direct damages, the main one is the depletion of nutrients from the host. Varroa is a relatively large parasite compared to the bee, and although its nutritional requirements are not high in relation to the host, it exploits up to 25% of the larva's reserves due to an inefficient metabolic mechanism with high nutritional demands (Garedew, Schmolz, and Lamprecht 2004). A worker bee emerging from a parasitized larva weighs 3% to 25% less than a bee emerging from a non-parasitized larva (De Jong, Morse, and Eickwort 2003). Nutrient withdrawal also alters the chemical composition of the cuticle of parasitized bees, making it inadequate for retaining body water, which partially explains the reduced longevity of these bees (Annoscia, Del Piccolo, and Nazzi 2012). Additionally, the mite produces substances with proteolytic action to obtain a high content of free amino acids, inhibiting protein synthesis in the pupa and altering its development (Vesco 2013). The parasite also harms host bees by consuming the fat body, an integral tissue for proper immune function, pesticide detoxification, overwinter survival, and many other processes essential in healthy bees. In general, the effects of Varroa parasitosis generate a negative influence on the immune response of the bee, thus making it more vulnerable to microbial agents, determining the onset of virosis and inducing the replication of the virus already present in the bee (Ramsey et al. 2019; Shen et al. 2005).

Indirect damages include weakening. Parasitized bees show lower quantities of vitellogenin, an important protein that serves as a reserve for royal jelly production, immune regulation, worker task regulation, and longevity (Quigley, Amdam, and Harwood 2019). Vitellogenin deficiency can lead to winter colony losses in the presence of heavy Varroa infestations (Dainat et al. 2012; Dietemann et al. 2012). Infested foragers also exhibit reduced learning abilities in recognizing sugary sources and during orientation flights (Dietemann et al. 2012; Kralj et al. 2007).

Immunosuppression is another consequence of Varroa parasitism. The mite's saliva contains substances that damage the bee's hemocytes and reduce their aggregation capacity, which is crucial for wound healing and pathogen phagocytosis (Richards, Jones, and Bowman 2011). Furthermore, parasitism inhibits the synthesis of phenoloxidase (Shen et al. 2005; Yang and

Cox-Foster 2007), an enzyme on which the melanization reaction is based, which is an indispensable process in insects for protection from infections(Kanost and Gorman 2008). In infested bees, the production of glucose oxidase is also inhibited (Yang and Cox-Foster 2007), an enzyme normally synthesized in the hypopharyngeal glands and secreted in royal jelly which preserves the meal of the larvae from contamination (Alaux et al. 2011). Thus, reducing the effectiveness of some mechanisms of social immunity of the hive (Yang and Cox-Foster 2007). Varroa, as already mentioned, also acts as a vector for viruses. Many bee viruses are present at low levels in hives, but the presence of Varroa can convert silent infections into symptomatic infections (Joachim R de Miranda and Genersch 2010), as the mite can alter the balance between the host and the viruses. The most prominent example is the transmission of "deformed wing virus" (DWV). Without Varroa, DWV primarily employs vertical transmission, where infected queens and drones transmit the virus to their offspring (J R de Miranda and Fries 2008). Furthermore, DWV can be transmitted from adults to offspring via dietary nutrition (Bowen-Walker, Martin, and Gunn 1999; Y P Chen et al. 2004; Yue et al. 2007; Yue and Genersch 2005). All this does not generate an evident symptomatology either in the bees or at the colony level (Fries and Camazine 2001; J R de Miranda and Fries 2008; Joachim R de Miranda and Genersch 2010). The transmission of the virus mediated by the Varroa vector instead generates a serious pathological picture in bees, the most evident symptoms of which are wing deformations, swollen and shortened abdomen, alteration of coloration and various physiological processes (Möckel, Gisder, and Genersch 2011). Therefore, strains of viruses are selected which, instead of allowing the bee good conditions to favor its transmission, maximize virulence(Yan Ping Chen and Siede 2007; Iqbal and Mueller 2007).

According to some authors (Dainat et al. 2012; Genersch and Aubert 2010), the winter collapse of the colonies is given by the combined action of DWV and Varroa also assisted by abiotic stress factors such as the seasonal drop in temperatures (Carreck, Ball, and Martin 2010; Martin, Ball, and Carreck 2010; Prisco et al. 2011).

In addition to the deformed wing virus (DWV), there are other viruses for which the ability to exploit Varroa for replication has been demonstrated, such as: *Varroa Destructor* Virus-1 (VDV-1), Kakugo virus (KV) (Ongus et al. 2004) and acute paralysis virus (ABPV) (Genersch et al. 2006; Sumpter and Martin 2004).

Figure 10-11. Symptom of deformed wing on bee, on the right, and a dead worker bee with symptoms of deformed wing virus on the left (www.agronotizie.imagelinenetwork.com/zootecnia).

1.3.5 VARROA CONTROL METHODS

The fight against *Varroa destructor* aims to contain the infestation rate by reducing the quantity of mites in the colony and, consequently, the viral load. Various studies argue that total eradication of Varroa is extremely difficult, if not impossible, especially in vast areas (Koeniger and Fuchs 1989).

There are numerous types of acaricidal treatments available to beekeepers. Practical implementation of acaricidal treatment requires consideration of various factors, as the control strategy strongly depends on local climatic conditions and beekeeping production guidelines. It is essential to consider the need to reach certain temperatures, which are essential for the effectiveness of various acaricidal treatments that will be analysed later. Other factors to consider include exploiting the brood block or applying the biological control criterion. It is also important to determine the level of Varroa infestation in colonies to establish an adequate control strategy. It should be noted that approximately 95% of phoretic Varroa tend to position themselves on the ventral part of bees. Therefore, evaluating infestation visually (Varroa positioned on the back of bees) can lead to erroneous conclusions (Ramsey et al. 2019). More reliable methods, such as counting natural fall or conducting the icing sugar test or inspection of capped brood, can be adopted (Goodwin and Van Eaton 2001). Additionally, taking advantage of sensitive periods for Varroa can help reduce the infestation rate. Pre-winter treatment is particularly important because only bees that have not been parasitized during larval development have the possibility of overwintering. Treating before the birth of winter bees ensures sufficient support for their survival until the following spring (Quigley, Amdam, and Harwood 2019).

Currently, there are numerous control methods used to combat Varroa mites, including chemical treatments with synthetic or natural active ingredients, as well as alternative biomechanical techniques. The main acaricidal treatments can be divided into two groups: acaricides of natural origin, which include formulations based on oxalic acid, formic acid, and thymol, and synthetic acaricides, which are distinguished from natural acaricides based on their chemical class and related neurotoxic mechanism of action. Synthetic acaricides include products based on Fluvalinate, Flumethrin, and Amitraz. Various acaricidal treatments and beekeeping techniques are illustrated below. The effectiveness of Varroa control methods is usually assessed by quantifying and comparing the infestation rate before and after treatment using monitoring methods such as natural shedding analysis. Follow-up treatments, involving the use of highly effective acaricides after the initial treatment, are often conducted to evaluate the remaining Varroa population.

CONTROL METHODS USING SYNTHETIC ACARICIDES

Synthetic acaricides are distinguished from acaricides of natural origin based on their chemical class and the related neurotoxic mechanism of action. It is important to emphasize that these acaricides must not leave any treatment residues in honey intended for human consumption beyond the maximum permissible limits for health. In some cases, being lipophilic substances, certain active ingredients can easily be absorbed by the wax (Bogdanov, Kilchenmann, and Imdorf 1998; Wallner 1999), and in the long term determine accumulation phenomena inside the hive (Johnson, Pollock, and Berenbaum 2009; Wallner 1999). The main active ingredients used are (Johnson et al. 2013): Amitraz, That is an acaricidal active ingredient that has been used for decades in beekeeping with great efficacy in reducing the infestation but a marked onset of resistance phenomena even after consecutive treatments (Chuda-Mickiewicz et al. 2007; Faucon et al. 2007; Reza et al. 2008; Semkiw, Skubida, and Pohorecka 2013). However, some metabolites may remain in honey and especially wax (Jiménez et al. 2005; Korta et al. 2003).

Figure 12. The placement of a few strips of an Amitraz-based product. (https://www.blog-veto-pharma.com/. 01/2022)

Fluvalinate is a neurotoxic active ingredient that blocks nerve transmission. In various tests carried out, an excellent efficacy on Varroa mortality was highlighted (Ferrer-Dufol, Martinez-Viñuales, and Sanchez-Acedo 1991; Salima and Haddad 2015). Although even in this case, more than in the case of amitraz, the mite is able to develop resistance (Ansari et al. 2018; Norberto Milani 1999). At the level of residues, being the lipophilic fluvalinate, it can contaminate the wax (Bonzini et al. 2011; Johnson, Pollock, and Berenbaum 2009). Furthermore, high doses of this active ingredient can cause some problems in terms of queen size reduction, locomotor deficits and mnemonic learning (Frost, Shutler, and Hillier 2013; Teeters et al. 2012).

Coumaphos is a non-volatile fat-soluble phosphorothioate with ectoparasiticidal properties. Coumaphos, too, kills through the inactivation of acetylcholinesterase, thereby interfering with nerve signaling and function. However, even in this case populations of coumaphos-resistant mites were found as early as 2001 (Elzen and Westervelt 2002; Jack and Ellis 2021a; Johnson et al. 2010).

Flumethrin is an active ingredient belonging to the pyrethroid group. The active ingredient has shown great efficacy (>80%) (Blacquière, Altreuther, and Krieger 2017), however it shows strong resistance even after only a few applications (Ansari et al. 2018; Salima and Haddad

2015). In terms of toxicity, it has been demonstrated that oral administration to bees is highly toxic (Oruc et al. 2012). Residual values found in several studies did not show high values of this acaricide on both wax and honey (Jamal et al. 2020).

Cymiazole hydrochloride, an iminophenyl thiazolidine derivative, widely used in veterinary medicine for the control of mites, and also of Varroa. Cimiazole hydrochloride residues can be detected not only in honey bees (Stanimirovic Z et al.,2003), but also in beeswax and other bee products. Considering that cimiazole hydrochloride residues can be ingested by consumers of apiculture products, this active ingredient has a limited use (Boi et al. 2016; Korta et al. 2003).

METHODS OF CONTROL WITH NATURAL ACARICIDES

Naturally derived acaricides include formulations with a low risk of residue accumulation after several treatments in honey or beeswax (Bogdanov, Kilchenmann, and Imdorf 1998; Bogdanov and Martin 2002) and drug resistance (Norberto Milani 1999). Among these we find organic acids. Usually this methods are influenced in a decisive way by the climate, by the harvest, beekeeping activity and the development of Varroa infestation (Imdorf et al. 2003).

Among the organic acids, it is possible to underline the most used: Oxalic acid (dihydrate), an active ingredient which is not able to target Varroa in the brood but acts on Varroa phoretic (Ales Gregorc and Planinc 2001; Imdorf and Berna 1999; Nanetti et al. 2003). The administration of oxalic acid in beehives can take place by dripping, spraying or sublimation (Al Toufailia, Scandian, and Ratnieks 2015). Therefore, its use is limited to the period of brood blockage or in combination with another slow-release acarid product or with an effect on the sealed brood. Oxalic acid can exhibit a very high efficacy of over 90%, which can vary markedly with sugar, as it is an adjuvant factor (Bogdanov et al. 2002; Coffey and Breen 2016; Nanetti et al. 2003). Oxalic acid in excessive doses can lead to important toxic effects on the larvae exposed to the treatment (Aliano and Ellis 2009; Ebert et al. 2007). At the same time, it has some limits of effectiveness deriving from environmental temperatures (Llorente and Suárez 1999; Rademacher and Harz 2006), it has also been shown that some oxalotrophic actinobacteria in association with VM can cause the degradation of oxalic acid with consequent loss of toxicity for the mite itself (Maddaloni and Pascual 2015). Furthermore, by acting on the phoretic Varroa, the period of use is limited to the brood block period(Ales Gregorc and Planinc 2001).

Figure 13. The treatment of a hive with dripped oxalic acid (https://unaapi.it/sanita-dell-alveare/varroatosi/varroa-acido-ossalico. 12/2014)

Formic acid, another active ingredient which has the characteristic of penetrating the wax, killing the Varroa found in the sealed brood (Fries and Camazine 2001). Being a corrosive substance, it is able to release toxic vapours to the respiratory tract, therefore care must be taken in its use. formic acid has good efficacy in breaking down Varroa (Bellucci et al. 2019; Pietropaoli 2013) although it can be negatively influenced by temperatures (Pietropaoli and Formato 2019a). Some studies have underlined a toxicity for bees which led to a reduction in brood or a higher mortality compared to bees treated with oxalic acid (Giovenazzo and Dubreuil 2011; Pietropaoli and Formato 2019b).

Lactic acid, that it is also an organic acid that has good efficacy on Varroa mortality (Girisgin and Aydin 2010; Kraus and Berg 1994). As for oxalic acid, it is administered by spray and acts on phoretic Varroa. However, the application of sprayed lactic acid is an expensive technique; therefore, it is valid only for beekeepers with a small number of colonies. lactic acid can have the effect of acidifying honey intended for human consumption, it is therefore necessary to refrain from dealing with this product during the laying of the supers (Bogdanov, Kilchenmann, and Imdorf 1998; Imdorf et al. 2003).

Citric acid, a tricarboxylic organic acid which has a certain acaricidal efficacy, albeit lower than oxalic acid, thus requiring a greater quantity of active ingredient (N. Milani 2001). In general, citric acid as the sole component for the acaricidal treatment does not show great results, as well as having a high variability (N. Milani 2001; Vanlalhmangaiha et al. 2022). On the contrary,

using the active principle with oxalic acid has been obtained a high mortality of Varroa (Coffey and Breen 2016; Howis and Nowakowski 2009).

Thymol, a monoterpenic phenol with good acaricidal efficacy on arthropods and Varroa (Gashout 2009; Sabahi et al. 2020). Being a volatile substance, its administration takes place by evaporation. It is usually used both as a mitigating treatment against phoretic Varroa, and as a real and proper treatment also extended to the Varroa that emerges from the brood over time (not penetrating the wax). The effectiveness of this active ingredient can vary greatly according to the external temperature which influences its effectiveness (especially at low temperatures (Coffey and Breen 2016; Leza, Llado, and Miranda-Chueca 2015; Lodesani and Costa 2008; Al Naggar et al. 2016), in the presence of brood (Giacomelli et al. 2016; A. Gregorc and Planinc 2012).

Hop acids, a group of acids that come from the resin of the hop plant (Humulus lupulus L.). Two main compounds capable of having acaricidal efficacy have been extracted from hop acids: beta acids (BA/HBA) and alpha acids (AA), these differ from each other in their acaricidal efficacy. There are currently no hop acid products marketed in Italy. HBAs are the most studied ones, which have demonstrated a good acaricidal potential (70-80%) although with variable results. HBAs are well tolerated by bees. (DeGrandi-Hoffman et al. 2012, 2014; Rademacher, Harz, and Schneider 2015; Vandervalk, Nasr, and Dosdall 2014)

ALTERNATIVE CONTROL METHODS

The alternative control methods are types of acaricidal techniques capable of controlling the infestation rate but, unlike the chemical ones (which kill the mite through an active ingredient), the former exploit different systems for the removal of the mite: such as mechanical removal (icing sugar, removal of the male brood), heat (hyperthermia) or they favor the fall of the mite, both natural and after a treatment (Spazio Mussi, anti-Varroa grid), or they still hinder the reproduction of the mite (size reduction of cells, selection of resistant/tolerant genotypes). All of these methods are aimed more at containing the increase of varroa infestations in colonies. The main methods used include: the removal of the male brood, a varroa control technique which aims at the mechanical removal of the mite inside the brood, given the propensity of the mite to infest the male brood more (Fuchs 1990). The brood is removed using supports such as trap frames or tripartite frames (Fig. 13 and 14), which particular conformation stimulates the bees to build natural honeycombs with male cells. The trap frame is not a reliable means of varroa monitoring since there is no correlation between the rate of varroa infestation in male broods and mite infestation in the whole colony, therefore it cannot be used as the only means of control against varroa (Bogdanov, Kilchenmann, and Imdorf 1998; Wantuch and Tarpy 2009).

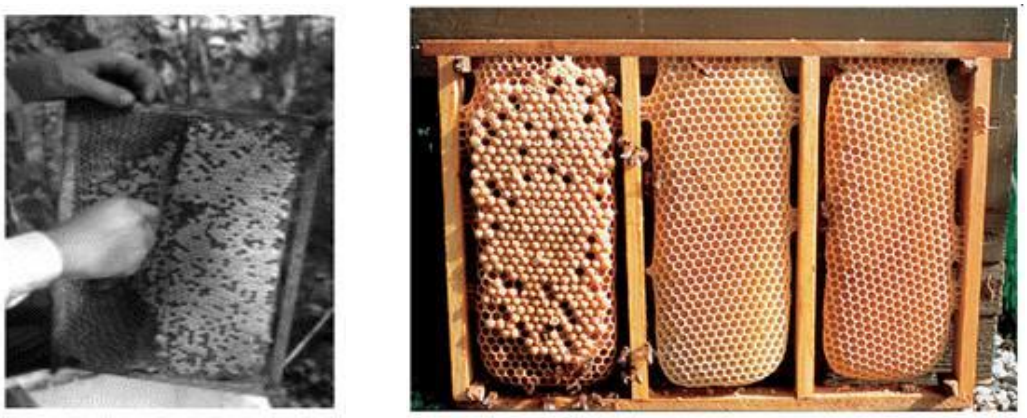


Figure 14-15. On the left Trap frame for male brood removal (Imdorf *et al*., 2003); on the right, tripartite frame, where the three frame spaces can be seen, two spaces can, if necessary, be closed to facilitate the bees in building the honeycomb in the space left open. (Jensen *et al*., 2016).

The use of powdered sugar, as a powdery substance, that is a non-chemical technique to combat varroa infestation (Macedo, Wu, and Ellis 2002; Stevanovic et al. 2012). Icing sugar is a weak acaricidal product whose effectiveness can only increase by frequently (weekly) repeating the treatment. It is a treatment that has no negative effects on bees (Aliano and Ellis 2009; Stevanovic et al. 2012).

The anti-varroa grid (Fig. 15), actually a component of the most commons beehives, the size of the holes in the grid allows the passage of the varroas, and therefore, following a fall of these from the bees (after a treatment or due to a natural fall), through the grid, the varroas can be removed from the hive (Harbo and Harris 2004). On the contrary, in the absence of an anti-varroa grid, the mites would accumulate on the bottom of the hive and could easily go back to the brood. The anti-varroa grid is useful for increasing the effectiveness of conventional treatments (Harbo and Harris 2004; Poonia, Gulati, and Sharma 2009).

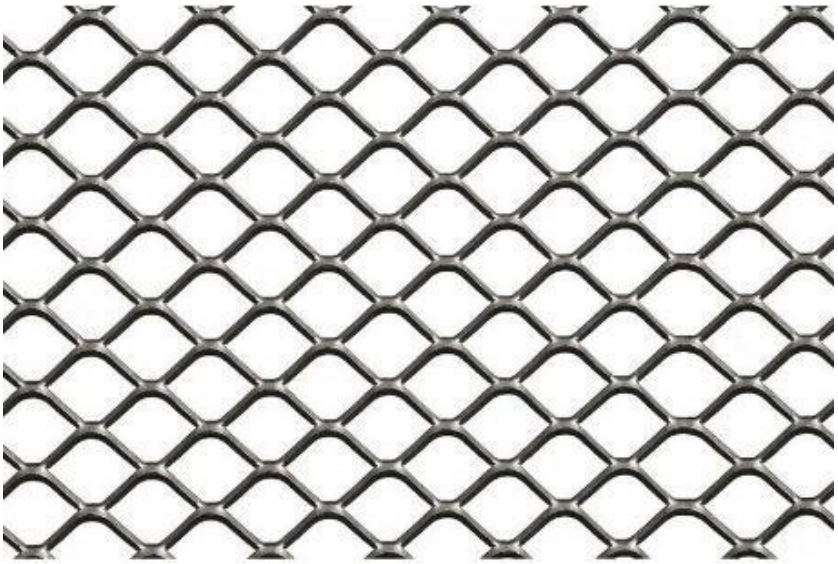


Figure 16. Mesh of an anti-varroa grid (the width of the holes is 4 mm) (www.quartiitaly.it).

The Spazio Mussi®, that is a larger space between the combs than in the standard Dadant-Blatt hives. Instead of the 37.5 mm standardized for hives, the Spazio Mussi® has a size of 45 mm; this space would allow to facilitate the hygienic behavior of the bees (or grooming), although its effectiveness is not significantly different from standard spaces, this type of space leads to a greater fall of Varroa (Manino and Patetta 2004)

Iron salts can stimulate bees to exhibit hygienic behaviors such as cleaning or removing parasitized larvae. Among the various iron salt solutions, ferrous fumarate has proven to be particularly suitable because, when administered to bees in a sugar solution, it has good acaricidal efficacy and is easily obtainable (Van Hoorn, M. Ter Horst 2014)(Van Hoorn and Ter Horst, 2014). In fact, in the tests conducted by Danieli and Ter Horst, the administration of ferrous fumarate in a sugar solution resulted in a higher mite drop compared to the control groups, leading to increased winter survival of the colonies (Van Hoorn and Ter Horst, 2014).

1.4 BEEKEEPING, PRODUCTION AND ITS PROBLEMS

1.4.1 HISTORICAL BACKGROUND ON APICOLTURE

The first evidence of beekeeping activities would date back to the period following the ice age, around 7000 BC, these are rock paintings found in the Turkish province of Çatalhöyük. interpreted as depictions of honeycombs (Crane, E., Graham 1985); the rock drawing, found in 1924 in the 'spider cave' of Valencia, would appear to be from the same period, depicting a person suspended on lianas, intent on collecting honey while bees buzz around her (Barbattini and Fugazza 2006). Other testimonies, particularly important for their antiquity and rarity

(Crane, E., Graham 1985), are those found in Egypt, in which, compared to other cultures, the representation of a good is determined not only economic but also of religious and medical value . In particular, in addition to the bas-relief of the temple of Niuserra, in Abu Gurab, and the decorations in the tomb of Pabasa, in El-Asasif, and in the tomb of Rekhmire, in Luxor, beehives made with clay pipes have been found which testify to the use of a particular 'mobile' breeding technique that follows the progress of the blooms (Barbattini and Fugazza 2006; Martano, M., Maiolino, P., Power 2016; Pinzauti, M. 1998). As a religious aspect, always in Egypt honey was considered a precious food of divine origin. An Egyptian custom wanted honey or whole wax honeycombs to be placed inside the sarcophagi (Bormetti 2014).

Figure 17. A detail of Pasaba tomb in Egypt (author’s photo)

The same importance was attributed in Greece, where, according to what Aristotle reported, honey would have been a good that 'falls from the sky' transported (but not produced) by bees (Giuliani 2017). It was Aristotele himself who handed down the interest in beekeeping with a description in the 'Storia degli Animali' of the social organization, morphology, biological and zootechnical aspects of bees (Burkert 2003; Martano, M., Maiolino, P., Power 2016). The breeding system, in this case, provided for a movement of the swarms, in order to guarantee the presence of a greater climatic and vegetal variety. The same attention in the choice of bee production areas was applied by the Romans. To the food use as a sweetener, as an ingredient in confectionery production in Imperial Rome (Calzoni, G. L., Rondinini 2012; Pinzauti, M. 1998), is added the belief that there was a curative effect for sick people, and, although there was no knowledge of its antibacterial properties, it was used as an ointment against bruises and wounds and for the preparation of purifying drinks (Bormetti 2014; Delaplane 1999).

With the fall of the Roman Empire, the honey trade - which at the time extended from North Africa to the Middle East and the Adriatic area - died out, and the beekeeping practiced by the wealthy owners was replaced by the convent beekeeping for the production of precious honey and beeswax that was used for worship. With the discovery of the New World, the purely sweetening function given by honey gradually took a back seat due to the spread of cane cultivation and later of sugar beet. In the 19th century, the beekeeping sector therefore found new impetus, even if traditional-type beekeeping with the use of rusticated ashlars remained until 1851, when L.L Lagstroth discovered the "honeycombs with mobile frames" (Fig. 16) and was no longer necessary to destroy the honeycombs to extract the products. In the 70s the scientific foundations were laid to implement the work of characterization of unifloral honeys with the first melissopalynological analyses (Louveaux, Maurizio, and Vorwohl 1978; Von Der Ohe et al. 2004).

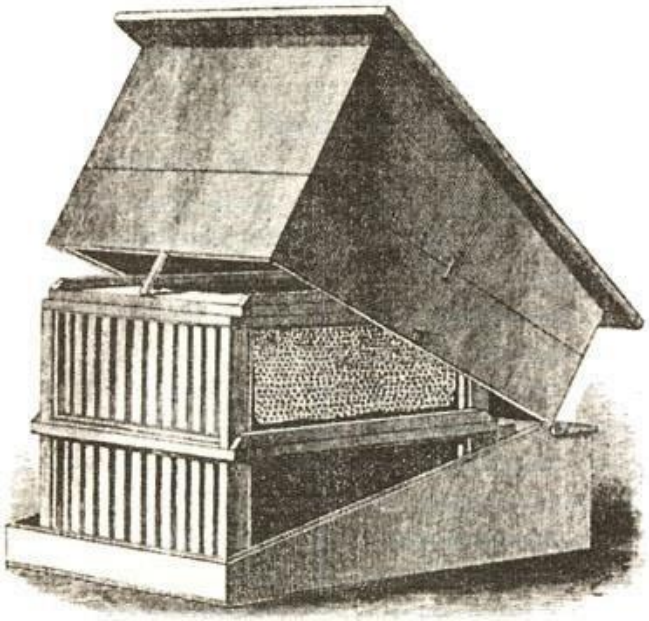


Figure 18. Lagstroth's moving frame hive, based on bee-space discovery (www.unaapi.it).

1.4.2 HONEY PRODUCTION

World honey production stands at 1.8 million tons per year (FAO STAT). Half of the world's honey is produced on the Asian continent. This is followed by the European continent (28%), the Americas (18%), Africa (11%) and, to a lesser extent, Oceania (1%). Six countries are the holders of about 50 percent of the world's production (China, Turkey, Argentina, Iran, Ukraine and the USA). The EU is the world's second largest producer, with Spain taking the lead (EU COMMISSION 2023; Honey source 2023).(Fig. 1.4).

Even among exporters, it is the Asian continent in the major exporter, with China playing the predominant role. Different discourse, however, if we go to see who are importers, whereby the United States, with 30 percent of the imported volumes on a global scale, is the main importer in the world, followed by Germany and Japan.

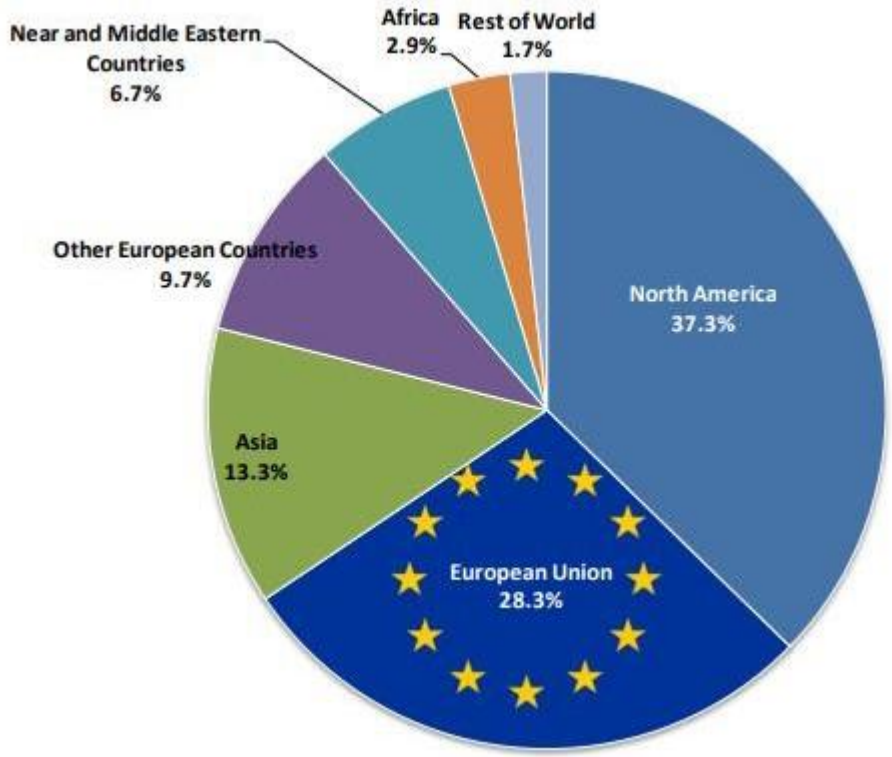


Fig 19. 2021 world's honey importers (UN Comtrade)

At the European level, in 2018, beekeeping in EU countries produced 280,000 t of honey with a self-sufficiency rate of 60 percent; this leads to the need to import considerable quantities of honey to make up for the considerable domestic demand (ISMEA 2019).

Although these data referring to individual continents are useful for gaining a global view of the honey market, in the specific case of the Mediterranean, it is important to consider that the countries bordering it share such a productive identity that it is commonly accepted to speak of the Mediterranean basin as a single productive entity. This is justified by the common biogeographical and production characteristics of the countries that are part of it, which determine the production of honeys conventionally recognized as "typically Mediterranean." (ISMEA 2019).

As for the specific case of the countries of the Mediterranean basin, honey production has been estimated at about 250,800 t (FAO-STAT). Among the main producers there are (Fig. 1.5) Turkey (114,113 t), Spain (36,394 t), Greece (21,400 t), France (17,489 t), Italy (9500 t), Croatia (8,727 t), Morocco (7,430 t) and Algeria (7,327 t). Italy, according to the latest ISMEA report, is the fourth largest European country in terms of number of hives (1.38 million) after Spain (3 million hives), Romania and Poland (2 and 1.7 million hives, respectively).

Although honey production in the Mediterranean reaches quite considerable values, this is not enough to meet the growing domestic demand. According to the latest Eurostat data, honey imports to EU countries in 2019 were 162,000 tons. The bulk of imports came mainly from the Asian market (40 percent), Ukraine (27 percent) and Argentina (13 percent).
The significant imports of honey within the Mediterranean Basin countries, create several problems at the economic level for domestic producers. In fact, over the past decade, European producers have been faced with ever-increasing imports, especially from China, diu a product sold at very low prices compared to the price that local producers can set. Creating a competitiveness problem. (Copa and Cogeca statement to ANSA, Feb. 14, 2020).

At the same time, "foreign" products are often cheaper than local products but offer fewer hygienic-sanitary guarantees for the final consumer (M. Carmen, Escuredo, and Fernández-González 2011). These products satisfy a market segment that is not particularly attentive to quality, but their presence in large-scale distribution, even in the form of various of different origins, in any case reduces the marketing margins of the domestic product (Milko Sinacori 2014).
Another worrying aspect concerns the phenomenon of counterfeiting. According to the Food Fraud Database of the United States Pharmacopeial Convention, honey is the third most counterfeited food in the world after milk and olive oil with losses of over 600 million dollars annually.
It should also be considered that, although the monetary value moved by the beekeeping sector, in terms of GDP, is not among the most important, in reality, behind every beehive product, there is the conservation of the pollinating insect "par excellence". Pollination activity has by now become, especially for areas with intensive agriculture, the most important aspect of bee breeding which completely replaces the pollinating insects which have largely disappeared in degraded production areas. The value of pollination, for these reasons, has been estimated at 153 billion euros, equal to 9.5% of the GDP deriving from food production. Hence the need to support the beekeeping sector of the Mediterranean.
The need to "defend" the typicality of local productions and promote the economic sustainability of the sector has meant that in recent years forms of cooperation and aggregation have emerged among Mediterranean beekeepers, with the aim of protecting and enhancing local productions by promoting more rational and competitive beekeeping. Even the EU, according to the latest report from the Commission to the European Parliament and the Council on the implementation of apiculture programs - highlights how the sector needs constant support, given the importance of bees for the environment and agriculture. Among the objectives of the European financial

measures made available are: technical assistance to beekeepers and beekeepers' organizations, support measures for apiculture product analysis laboratories, the restocking of the beekeeping stock and the improvement of product quality for their greater valorisation.

Several producers' associations are active in the promotion of local honey production. Among the many FED-API-MED (Mediterranean Beekeepers Federation) associates various cooperatives and beekeeping associations of many countries of the Mediterranean basin (Italy, Morocco, Tunisia, Algeria, Lebanon, Albania, France, Turkey, Palestine, Iraq, Jordan and Egypt) and promoted the "Charter of Mediterranean honeys": a "document of historical significance, which, aligning them with European standards, defines and frames the honeys of the Mediterranean from a qualitative point of view".

In today's global market, it is obvious that the competition of the domestic product must be based mainly on higher quality standards, largely determined by climatic factors, the professionalism of the operators and the industriousness of the bees (Milko Sinacori 2014). Furthermore, if one looks at Italy, it is also possible to focus on the floristic-vegetational variety and on the diffusion of nomadic techniques, which make it possible to hold the record for the number of unfloral honeys (Oddo L. P., Piana L. 2007), which have always been considered higher value than the more common millefiori. In fact, Italy holds the world record for honey varieties: it has over 50 (ISMEA 2019).

1.5 COUNTERFEITING AND FOOD SAFETY

1.5.1 FOOD FRAUDS

Food fraud is a set of actions that do not comply with current legislation, perpetrated in the production and marketing of food products. It is usual to distinguish fraud into two large groups: commercial fraud (foodstuffs different from those declared in terms of origin, provenance, quality or quantity) and health fraud (food dangerous to public health because it is poisoned, adulterated or counterfeited). As far as commercial fraud is concerned, these can be voluntary and involuntary and can be classified according to the following types:

• adulteration: modification of the natural composition of the food by subtracting useful elements or by adding lower quality materials;

• counterfeiting: partial or total replacement of substances different in quality and quantity from those that normally contribute to forming it;

• sophistication: addition of foreign substances to its composition with the aim of improving its appearance or covering its defects;

• alteration: modification of the chemical-physical and/or organoleptic characteristics due to spontaneous degenerative processes.

Honey for human consumption, according to article 4 of Legislative Decree 179/2004, must not undergo the addition of any food ingredient or other product that is not honey. Furthermore, honey must not contain organic and inorganic substances extraneous to its composition and must not have an anomalous taste or smell, have started a fermentation process and have been heated in such a way as to destroy or inactivate its natural enzymes.

A very common fraud in honey concerns the addition of exogenous sugars. This can happen directly or through bee feeding. The substances used are mainly sucrose and sugary syrups produced from corn starch or other cereals by enzymatic hydrolysis which transforms the sugars so as to obtain a high fructose content (Soares et al. 2017; Tosun 2013).

In the specific case of honey, adulteration commonly sees the addition of sucrose and sugary syrups directly into the honey. Syrups have different compositions according to the processes they have undergone and their intended use: some are very similar to honey (HFC = high fructose corn syrup), others differ in the high glucose or dextrin content. In other cases, aromatic substances are added to emphasize certain aromas rather than caramel to make it more similar to honeydew honey (Contessi 2016).

When the product is subjected to actions that result in a new product, using substances other than those that should be present in the original product, a counterfeit occurs. The most frequent counterfeits in honey concern, in the case of false geographical indications, both the country of origin (mandatory level) and the territorial origin (optional level). According to the latest report carried out by the ICQRF, 5,200 kg of honey that did not comply with the regulations was seized in Italy in 2019.

Another counterfeiting activity of honey is heating treatments. According to Codex Alimentarius standards, honey must not be subjected to any term treatment to the extent that it causes alterations in its essential composition and qualitative characteristics (Directive 2001/110/EC; FAO 2001). One of the reasons why honey is heated is to increase its fluidity during processing. Moreover, since consumers erroneously associate crystallised honey as being of inferior quality, some producers perform a heat treatment in order to dissolve the crystals and eliminate air bubbles so as to delay the start of crystallisation for at least 12 months.

Honey owes its special characteristics to the presence of multiple 'minor compounds' - thermolabile and unstable over time - that derive directly from flowers and bees and that confer special properties such as aroma, taste and biological properties. Legislation recognises this prerogative and sets maximum values of hydroxymethylfurfural (HMF) and a minimum value of diastasis index as parameters for the qualitative integrity of honey.

1.5.2 FOOD SAFETY AND TOXIC CONTAMINATION

RESIDUES AND CONTAMINANTS IN HONEY

The chemical composition of honey, more than any other food, is closely influenced by the production environment, where by environment all the factors that compose it must be considered, including possible sources of pollution. The peculiar behavioral and biological characteristics of bees make honey particularly exposed to the presence of polluting substances (Contessi 2016), so much so that there are many authors who consider the bee as a valid bioindicator of the state of pollution of a given territory (Quigley, Amdam, and Harwood 2019; Skorbiłowicz, Skorbiłowicz, and Cieśluk 2018). In fact, each beehive has thousands of foragers which are renewed cyclically, and which take numerous substances such as nectar, pollen, water, honeydew and propolis from the surrounding area, including distances of up to 3 km from the hive. Furthermore, it intercepts and carries the particles in atmospheric suspension during the flight and then return to the hive (Contessi 2016). Polluting substances from the environment such as pesticides, heavy metals, radionuclides, polycyclic aromatic hydrocarbons or dioxins represent a serious risk to the health of consumers and their monitoring is extremely necessary especially if we consider that honey is a product used in human nutrition.

For "organic" products, compliance with the indications present in the relative production specification is added to the parameters of the law (Soares et al. 2017). Furthermore, Regulation 1829/2003 states that there is an obligation on the part of the producer to declare the presence of GMOs on the label if the maximum permitted threshold of 0.9% is exceeded. Furthermore, according to Reg. 834/2007, with reference to organic production, GMOs are incompatible with the concept of organic production, which is why they should be totally absent. However, in the case of honey, since bees also travel long distances in their search for raw materials, the possibility that pollen from GMO plants is transported is very frequent. The greatest risk occurs above all in those honey-producing countries where the cultivation of GMO plants is authorized, such as Spain, France, Portugal and Romania in the European Union (Soares et al. 2017). Furthermore, if we consider that the European honey market satisfies just 40% of the needs and that most of the imports come from countries with large extensions of GMOs (Latin America and Asian countries), it is indicative to say that before marketing, honey produced in these countries should ideally be tested.

PESTICIDE RESIDUES

The European Union (EU) has established, in order to protect human and animal health, maximum residue levels (MRL) of plant protection products in the composition of all foods intended for human and animal consumption. The possibility that pesticide residues are found in foods is linked to their solubility and their biodegradability. The most relevant consequences that pesticide residues can cause in the consumer are those related to chronic manifestations, often connected to the ability of these substances to accumulate and give rise to a sum of effects. For honey, the reference legislation is represented by Reg. (EC) 396/2005 concerning the maximum limits of pesticide residues in or on food and feed products of vegetable and animal origin. The legislation establishes for all the active ingredients that make up plant protection products that the maximum limit of 0.01mg/kg (10ppb) (Engwa et al. 2019) while specifically for honey, Directive 96/23/EC establishes specific limits for pyrethroids (50 ppb), organochlorines (5 ppb) and organophosphorus (10 ppb). The EU has set up a special database that is continuously updated where the maximum permitted levels of pesticide residues are present for each authorized active ingredient.

HEAVY METALS

"Heavy metals" can be defined as metallic elements that have a specific density higher than 5 g/cm3 and a higher atomic weight which is generally higher than 10. These are represented by Antimony, Tellurium, Bismuth, Tin, Selenium, Thallium, Gold, Arsenic, Gallium, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Mercury, Manganese, Nickel, Platinum, Silver, Uranium, Vanadium and Zinc. Some metals, such as iron (Fe), zinc (Zn), chromium (Cr), copper (Cu), cobalt (Co), molybdenum (Mo) and manganese (Mn), perform important biological activities but, if they exceed safety, they can show toxic effects (Codex Alimentarius). Others, however, such as Arsenic (As) Lead (Pb), the methyl forms of Mercury (Hg) and Cadmium (Cd), even in small concentrations can have deleterious effects on human health. A phenomenon that most heavy metals have in common is their ability to accumulate in the body. In the case of lead, it is transported by red blood cells to the liver and kidneys to then be redistributed to the teeth, bones and hair, in the form of phosphate salt (Engwa et al. 2019). Cadmium initially binds to blood cells and albumin and, by binding to metallothionein’s, accumulates in kidney and liver tissue. Arsenic accumulates in the heart, lung, liver, kidney, muscle and neuronal tissues, as well as in the skin, hair and nails.

The major problem of these elements is given by their lasting resistance in the environment being almost indestructible by nature. For this reason, their dispersion causes a large

accumulation in environmental substrates which leads to the presence of high concentrations of metalloids especially in plant products, since they draw sustenance from all three environmental matrices (soil, air, water). The concentration of heavy metals in the specific case of honey therefore depends on the botanical and geographical origin and on the sources of pollution that occur in the place of production (agro-chemical substances, industrial foundries, vehicular traffic, petrol- and chemical industries, etc..) or due to incorrect production practices. (Pinzauti, M. 1998) reported a higher concentration of lead in honey from a permanent apiary located near a highway. Weather conditions can also affect the heavy metal content. High temperatures and dry climates, in particular, favor the increase of pollutants in the hives due to the absence of "leaching" of heavy metals from the flowers (Conti et al. 2007).

For honey there is no legislation that specifies the content of heavy metals in honey except for Regulation (EU) 2015/1005 where only for lead it sets the limit at 0.10 mg/kg of honey.

1.5.3 HONEY TRACEABILITY

The globalisation of markets, the diversification of food products and the increased awareness assumed by consumers with regard to food safety have led to a growing attention in public opinion towards the origin of food products (Carmen Gigliotti 2007). In fact, issues such as 'traceability', 'quality control', 'typical product' or 'organic product' are particularly topical, requirements that are sought not only to certify the quality or distinctiveness of a product but also to protect public health. Transparency regarding origin has therefore become a fundamental component of food quality and safety, and thus a valid element of competitiveness on the national and international market. In the case of honey, in particular, this is even more evident due to the highly competitive market due to the availability of a wide range of honey varieties with very individual quality characteristics that require their authentication. The possibility of tracing the origin of honey is a key aspect in protecting consumers and producers from fraudulent practices. The market value of honey is in fact linked, especially for monofloral honeys, to its botanical, geographical and process origin, which are the main determinants of its quality characteristics. Although uncommon, the frauds most commonly perpetrated in honey are: misrepresentation of the origin on the label, adulteration (addition of foreign sugars, flavourings or other substances), mixing of the more expensive and valued monofloral honeys with cheaper mixed-flower honeys.

Monofloral, PDO and PGI honeys are among the most vulnerable to fraudulent practices due to their higher commercial value. During the marketing phase, it is also important to implement an information system that allows the consumer to recognise and evaluate the product, preventing deception and fraud on the denomination (Leida, Della Valle, and Piana n.d.; Persano Oddo and

Piro 2004). No less important are the health and hygiene aspects established at European level and by Codex standards. In this regard, it is useful to classify honey traceability according to the control objective: traceability of production and/or process; traceability of origin, both geographical and botanical.

The purpose of process traceability is to identify any adulteration and/or transformation processes that do not comply with the regulations in force. It therefore investigates the various stages of honey production and the extraction and transformation processes, which represent a critical point in guaranteeing a healthy finished product free from fraudulent practices. While traceability of origin is aimed at discriminating honeys according to their geographical and botanical origin. The botanical and geographical origin of honey is decisive in defining its qualitative and product characteristics. Monofloral honeys, compared to multifloral honeys, are more appreciated by consumers because of their particular organoleptic, biological and chemical-physical properties. For this reason, the former is among the most susceptible to fraud, also because their commercial value is generally higher than their counterparts. Honey traceability can be distinguished into botanical and geographical. Botanical traceability refers to the possibility of tracing the botanical species that have contributed to the composition of a certain type of honey with the aim of protecting producers and consumers from misrepresentation. Geographical traceability, on the other hand, aims to establish the production area of a given type of honey. The production area is a not insignificant aspect, especially if one considers that the declarations of origin on the label are an important aspect both from a food safety and a purely commercial point of view. In fact, there are certain production areas that are more favoured by consumers as synonymous with quality and safety. Tracing the geographical origin, therefore, is a traceability aspect of priority importance. The production area also contributes to determining the quality characteristics of honey. In this respect, it is not possible to establish absolute and immutable quality rankings on the basis of geographical origin, but it is certainly clear that there are relatively constant differences, recognisable both at organoleptic level and in terms of composition, which make products of different geographical origins not equivalent to one another. The production area certainly influences the botanical origin but there are other factors linked to the territory that can influence honey, such as the type of soil, production techniques and different human activities that can have negative effects on the healthiness of the product (Persano and Lucia n.d.; Persano Oddo and Bogdanov 2004). To the objective and analytically verifiable differences must be added, as already mentioned, those of image that make consumers prefer one product to another even without knowing their objective characteristics. Today, in fact, the concept of quality is associated with the typicality of the product; where 'typicality' means a physical place to which is linked a production tradition

established over time and referring to a specific territorial context. In this regard, the 'quality marks' (PDO, PGI and TSG) of food products in the EU were created with the precise aim of protecting the identity of these productions. For honey, some typical examples concern the Spanish PDO 'Miel de Granada', the Greek fir honey 'Menalon' or in Italy the PDO 'Miel Varesino', 'Honey from Lunigiana', 'Honey from the Belluno Dolomites' or the Amalfi Coast lemon honey. In addition, the regulatory framework on honey provides for two levels of geographical denomination, one voluntary and the other compulsory, to which is linked the problem of establishing its veracity.

1.5.4 METHODOLOGIES USED TO VERIFY THE QUALITY AND TRACEABILITY OF HONEY

For the determination of the botanical and geographical origins of honey, different methods have been developed, applied and evaluated over the last decades. It must be underlined that between botanical and geographical origins there is often a strong relationship; some nectareous plant species are strictly related to a specific geographic area (e.g., the Mediterranean basin), making rather easy the identification of the geographical origin of the honey looking at its botanical composition, or at the most to reduce the number of possible origins (Jovetić et al. 2017a)(Trifković et al. 2017).

Composition, physical and organoleptic properties of honey depend mainly on the types of flowers or plant/insect secretions gathered by the honey bees. In principle, different types of honey having different chemical profiles should be rather easy to distinguish, and to identify. However, in some circumstances, the chemical profiles of different monofloral honeys are similar, making the discrimination work difficult (M.S. Rodríguez-Flores et al. 2019). To identify the origin (botanical and/or geographic) of the honey, is an issue of the traceability which main goal is tracing the history of a food product from the source (i.e., the hive) to the consumer.

in recent years the study of the traceability of honey is evolving in the search for increasingly reliable, accurate, economic and fast methods suitable for standardization (Laube et al. 2010; Soares et al. 2015; Valentini, Miquel, and Taberlet 2010). Below are described briefly the main methods used to trace honey.

Melissopalynological analysis

Melissopalynology, one of the most affirmed methods for honey identification, is based on the analysis of the pollen grain naturally occurring in honey. A microscopic analysis if performed identifying all the component of the so-called pollen spectrum, which can be compared with

already known pollen spectra (Anklam 1998). The strength of this method is that it unequivocally provides some important information about the nectar source, giving evidence of the botanical origin of a honey sample (Corvucci et al. 2015; Von Der Ohe et al. 2004). In addition, this method is a valid choice for the geographical fingerprint of the honeys in case of areas with a site-specific melliferous flora (Anklam, 1998). Through the melissopalynological analysis, honey can be classified as monofloral if the characteristic pollen (i.e., the pollen granules referable to a specific botanical species) exceeds a specific threshold, usually expressed as percentage of the total pollen amount (Louveaux, Maurizio, and Vorwohl 1978).

Though a well-recognized method for honey typing, melissopalynology brings with it also some disadvantages, such as (Molan 1998; Von Der Ohe et al. 2004): 1) the considerable amount of experience asked to the analyst, 2) the seasonal variability of the amount of pollen grains, 3) the lack of a sufficient amount of pollen grains due to filtration processes, or 4) pollens can be added fraudulently or added involuntarily by the bees themselves, that is, pollen of flowers that the bee has not visited directly, but which can accidentally ends up on its body. In this respect, the risk of air contamination where the honey is managed must also be considered (Corvucci et al. 2015).

Physical-chemical parameters

Physio-chemical profiling of honey mainly concerns sugars' content, acidity, humidity, conductivity, pH, colour, enzymatic activity and some specific chemical markers. Each physical-chemical trait has a different discriminatory value for each group of floral honeys. In general, the minimum/maximum values are used as diagnostic tools rather than the average values (Devillers et al. 2004; Kadar et al. 2011; Kavanagh et al. 2019).

Bioactive compounds content

Honey is a natural antioxidant food, with many beneficial properties (Aljadi and Kamaruddin 2004; Chua et al. 2013). The different geographical and botanical origin of honey leads to variable content of phenolic compounds and different antioxidant properties (Dzugan et al. 2018). Overall, the botanical origin of the honey has the greatest influence on its antioxidant activity, while processing, handling and storage affect honey antioxidant activity only to a minor degree (Attali et al. 2007; Beretta et al. 2005; Bertoncelj et al. 2007).

Mineral profile

Normally the mineral content in honey is between 0.02% and 1%. Potassium is the one most represented. Different honeys are characterized by different mineral profiles, which, together with other types of analysis (e.g., physicochemical) can potentially be used to discriminate them botanically and/or geographically (Bergamo et al. 2018; Jovetić et al. 2017a; Maria Rizelio et al. 2012).

Chemical-physical properties evaluable through spectroscopy/spectrometry

The advantage of using some spectroscopic techniques on honey samples lies in their low invasiveness and their suitability to work with non-manipulated samples (Minaei et al. 2017). A promising approach in this field is the VIS-NIR hyperspectral imaging, a technique that involves both spectroscopy and the use of digital imaging (Minaei et al. 2017; Schaepman 2007).

The rapid evaporative ionization mass spectrometry analysis (REIMS) is a form of ambient mass spectrometry, which was initially used for in situ real-time analysis of tissue samples for medical and surgical purposes (Balog et al. 2010; Wang et al. 2019). REIMS has more recently been used in food analysis applications, also for food safety purposes (Connolly, Wallace, and Stead 2016; Wang et al. 2019).

Another possibility to get reliable origin information of honey samples lays in their stable isotopic abundance measurable through the Isotope Ratio Mass Spectroscopy (IRMS). The main application of this techniques was for the identification of honey adulteration by exogenous sugar sources (Magdas et al. 2021; Zhou et al. 2018).

Omics approaches

In the last decade, some studies have been carried out on the omics sciences, with particular attention to the metabolomic and genomic approaches. As for the metabolomic methods, these all concern the analyse of the metabolites, targeted and untargeted, in a quantitative or qualitative way (Cajka et al. 2016; Cevallos-Cevallos et al. 2009; Cubero-Leon, Peñalver, and Maquet 2014; Koulis et al. 2021), and their usefulness has been proven in food traceability, and against fraud as well. Among these techniques, there is the analysis of the amino acid profile of honey. Infact also the variation of the amino acid profile could be used to determine the geographical and botanical source of a honey (Anklam 1998).

The Nuclear Magnetic Resonance Spectroscopy (NMR) can be used for metabolomic purposes allowing the identification of chemical compounds occurring in food even simultaneously (Consonni and Cagliani 2019; Siddiqui et al. 2017). As for the applicability of NMR to investigate the geographical origin of honey, its botanical origin, and possible adulterations, there are currently different types of use (Schievano et al. 2019).

Melissopalynological analysis is currently the most used method to investigate the geographical and botanical origin of honey, and it is standardized according to specific protocols such as the

ISO method N. TC34/SC19. However, over the last ten years, other analysis methods have been integrated because bibliographic research has shown that a single parameter is not sufficient to trace the origin of honey. As a result, a series of methods are increasingly used in combination, the most reliable of which combine data from different parameters (multicomponent analysis), especially with current statistical data evaluation techniques. The new frontier concerns methodologies aimed at reducing analysis times, experience and personnel, automating processes and obtaining more accurate results. Genomics and other "omics" sciences seem to have interesting prospects, but preliminary digitization and cataloging of plants are necessary. At the moment, these methodologies seem to be limited and dependent on other verification methods, including melissopalynology, losing the advantages of speed, accuracy, simplicity and cost. In the future, for economic, time and cost savings, analyses will be needed that deal with the control of honey adulteration, its origin, authenticity and typicality. Finally, the standardization of the main analysis methods would lead to data homogeneity, more effective product traceability, and simpler data interpretation (Danieli and Lazzari 2022).

2 THESIS STRUCTURE

This paper addresses some of the major problems currently affecting the world of beekeeping, both from the perspective of the producer and the consumer. On the producer's side, the study focuses on the development of alternative methods of integrated pest management. On the other hand, the analysis of the traceability of the geographical origin of monofloral honeys has been a central focus of the research. This study is derived from work conducted as part of the European project PRIMA PLANT B. The objective of this project is to develop an innovative and sustainable agricultural system that combines two important Mediterranean crops, such as citrus and aromatic/medicinal plants (AMPs), with high-value livestock, such as honeybees (Apis mellifera L.). This system aims to ensure high yields in terms of food production and ecosystem services like pollination and biological control.

Specifically, this thesis work is based on two work packages:
1. WP2 - Development of biological control tools and integrated pest management (IPM) for the control of pests in citrus, AMPs, and honeybee colonies.
2. WP5 - Assessment of pesticide residues in citrus production, determination of quality, safety, and traceability specifications in honey production in the PLANT-B breeding system.

Regarding WP2, the study focuses on the proper management of varroa parasitosis through the development of an IPM strategy that incorporates different strategies and interventions based on treatment types. The objective of this study was to apply an IPM approach to control varroa mites, using Mussi® space hives and organic acids, specifically hop beta acids and hop alpha acids (case studies 1-2). The efficacy of these hop acids on varroa mortality and bee survival has not been extensively studied. Laboratory tests were conducted to determine the most effective concentration, followed by field tests comparing Mussi® space hives and regular space hives to assess any additional effects on varroa drop.

In the case of WP5, the aim of the study was to investigate the geographical origin discrimination of citrus honey (case study 3). Citrus honey, produced from the nectars of various Citrus species, is highly popular in the Mediterranean region. It exhibits a nearly colourless to straw yellow colour in its liquid state, while in the crystalline state, it appears white, sometimes pearly, or light beige. Citrus honey's aroma is characterized by the presence of caffeine. Monofloral citrus honey typically contains a relatively low percentage of citrus pollen, usually not exceeding 10% of the total pollen. To establish an effective methodology for geographic discrimination of these honey samples, the study combined the analysis of the mineral profile of

Citrus spp. honey from three Mediterranean countries (Italy, Greece, and Egypt) and three different harvest seasons (2020-2021-2022) with the analysis of physicochemical parameters and carbon and nitrogen isotope ratios. The data obtained were evaluated based on European guidelines for honey quality (moisture, pH, electrical conductivity, free acidity, and colour) and food safety standards (lead concentration < 0.1 mg/kg). For other data, particularly potentially toxic elements for which no specific European directives indicate limits (such as As, Cd, Cr, and Hg), a comparison was made with existing literature on the elemental profile of citrus honey produced in the Mediterranean basin. The objective of this study was also to assess whether the combination of these analysis methods can effectively discriminate the geographical origin of the honey samples, identifying which elements or correlations provide the best discrimination.

The results obtained from three different studies will be presented: the first focuses on the efficacy of hop beta acids in a controlled environment and in the field, in combination with Mussi® space hives; the second examines the efficacy of alpha hop acids, previously tested in the laboratory and then in the field; and the third presents the findings of the analysis conducted on citrus honey samples from countries in the Mediterranean basin.

3. IPM METHODS BASED ON HOP ACIDS AGAINST THE VARROA *DESTRUCTOR*

The western honey bee (*Apis mellifera* L.)(HB) plays an important role both economically and culturally in many countries of the world, contributing to the agricultural sector not merely under a productive point of view, but also through the pollination of many agricultural crops (Bartlett 2022; Berry et al. 2021; Manzano Sánchez et al. 2021). However, there are several threats that hamper the productivity of the beekeeping sector and the health of the HB colonies causing their decline, a phenomenon that has increased word-wide in recent decades (Paudel et al. 2015; Simon G Potts et al. 2010; Shumkova, Balkanska, and Hristov 2021). Among various pests, the ectoparasitic mite *Varroa destructor* (Anderson and Trueman 2000a) is the major parasite of the HBs, which has been reported causing yearly dramatic losses of bee colonies (Annoscia, Del Piccolo, and Nazzi 2012). In fact, it has been shown that varroosis can cause the collapse of bee colonies. It is also estimated that in Europe, varroa mite (VM) infestation can lead to an increase in winter mortality of bee colonies of 15% (Brodschneider et al. 2018; Gray et al. 2020; Reams, Rangel, and Lopez-Uribe 2022). The VM parasitosis is also related to changes in strain diversity within the Deformed Wing Virus that are likely related to increase the viral load of HB colonies (Annoscia, Del Piccolo, and Nazzi 2012; Martin et al. 2012). At present, the control of VM is one of the most difficult challenges for beekeepers (Allabergenova, Turganbayeva, and Nurseitova 2021; Bahreini et al. 2020; Pietropaoli and Formato 2019a; Ward, Coffey, and Kavanagh 2022). This is partly due to the limited use that can be made of veterinary drugs such as acrinathrin, flumethrin, fluvalinate, amitraz, coumaphos, cymizole, but also to the ability of the parasite to develop anti-acaricidal resistance (Ivana Smodiš Škerl et al. 2021; Rademacher, Harz, and Schneider 2015). At the same time, other factors that can alter the effectiveness of acaricidal treatments, such as seasonality, active ingredients used, and health status of the bee colonies must be taken into account (Giacobino et al. 2015). This dramatic situation highlights the need for a different and integrated approach for the management of VM that includes an increasingly usage of organic acids (formic acid, oxalic acid, lactic acid) and essential oils (Bahreini et al. 2020) which, overall, pose low risks of residue accumulation and mite-resistance (Bogdanov et al. 2002; Norberto Milani 1999). However, such natural substances have some limits of applicability such as the treatment period or the variable efficacy (Lodesani and Costa 2008; Maddaloni and Pascual 2015; Pietropaoli and Formato 2019a).

In particular, oxalic acid appears to be one of the most widespread and effective substances used by beekeepers, but at the same time it has some limits of effectiveness (Llorente and Suárez 1999; Rademacher and Harz 2006). In addition, it has been shown that some oxalotrophic

actinobacteria in association with VM can cause the degradation of oxalic acid with consequent loss of toxicity to the mite itself (Maddaloni and Pascual 2015). Among the natural compounds that are being studied, few researches (DeGrandi-Hoffman et al. 2012, 2014; Moškrič et al. 2018; Rademacher, Harz, and Schneider 2015; Vandervalk, Nasr, and Dosdall 2014) have focused their work on the efficacy of beta and alfa acids from hop (*Humulus lupulus* L.), which are weak organic acids repelling some parasites such as the hop aphid (*Phorodon humuli* Schrank) (Jones et al. 2003), and are not toxic to humans (DeGrandi-Hoffman et al. 2012). Hop acids are widely used in the food sector, with a main role in the production of beer (Almaguer et al. 2014; Karabín et al. 2016). In recent years there has been an great attention on alternative methods for the containment of VM infestations of HBs. These methods are conceived to enhance the VM fall (Manino and Patetta 2004; Poonia, Gulati, and Sharma 2009), to mechanically remove the VM (icing sugar, male brood removal) (Macedo, Wu, and Ellis 2002; Wantuch and Tarpy 2009), or hinder the reproduction of the mite (reduced the cell size, selection of resistant/tolerant genotypes) (Piccirillo and De Jong 2003; Rinderer et al. 2010). There is today wide agreement on the fact that the best strategy to be implemented for the mitigation of parasitic VMs should be based on a management program based on multiple tactics and activities, known as Integrated Pest Management (IPM)(Underwood and Lopez-Uribe 2019). IPM is an ecological and sustainable approach to pest managements, which involves a program of interventions over the course of a season/year (Jack and Ellis 2021b; Rice, Winston, and Higo 2004). These interventions can be carried out using organic acids or chemical compounds, in combination with alternative mechanical methods (Calderone 2005).

In this context, the aim of the present studies was to apply an IPM approach to the fight against VM consisting of the use of wider-spaced hives and hop beta acids and hop alfa acids applied topically in the bees, tested first in the laboratory with bees taken from hives with a high VM infestation and then in the field. The field tests were aimed at assessing whether this approach actually had an impact on VM mortality and was tolerable by the bees in terms of hive survival.

3.1 HOP BETA ACIDS TRIALS (CASE STUDY 1)

Material and methods

The efficacy of hop beta acids was preliminarily tested in the laboratory at different concentrations and solubilized in ethanol or acetic acid. Once all the solutions had been tested, moving the activities to the field, the concentration that had yielded the maximum in terms of VM and tolerable for HB was tested. The field test was foreseen on Mussi® space and regular space hives, in such a way as to be able to evaluate whether the type of hive, with the same treatment, could have an additional effect on the fall of varroa.

Chemical and reagents

For laboratory and field treatments, solutions based on Beta Rich Hop Extract 40% (with 20% Propylene Glycol and 1,5% hop oils) (Hopsteiner, Germany) (HBA) dissolved in 96% ethanol (EtOH) (Carlo Erba Reagents, Italy) or acetic acid (Carlo Erba Reagents, Italy) were used. Oxalic acid dihydrate 99.5% (OA)(Carlo Erba reagents, Italy) was used as a control. As needed, both the HBA and OA solutions were dissolved in 50:50 (w/w) sugar syrup (SS50) with 0.4% citric acid (99.8% purity) (Carlo Erba reagents, Italy) to promote the sucrose inversion.

Laboratory trials

Three consecutive trials under controlled conditions (34 ± 1 C°; 72 ± 1% RH) were carried out. The caged bees were placed inside a Carbolite incubator (Carbolite Gero Ltd, UK) and temperature and humidity were monitored with a TESTO 175H (Testo SE & Co., Germany) datalogger. The HB used were collected from the Plant-B apiary located at the Experimental Teaching Farm “N. Lupori” (Tuscia University, Viterbo, Italy) (42.4271454°E, 12.0796127°N, 302.2 m EASL) from two different hives, which had an average varroa drop value/24h of 88 VM in the week prior to the start of activities. Emerging HBs (0-24h old) were used. An *A. m. ligustica* Spin. queen was caged in a queen-excluder frame cage (Fig. 1) and left to lay inside her hive. About four days before the emergence of the first young workers, the brood comb was moved to the incubator inside a net cage (Fig. 1) provided with SS50 and tap water *ad libitum*, so to avoid the starvation of the bees. Once the comb began uncap, every 24 h the bees were individually captured with a battery vacuum cleaner (Fig. 1), and caged in 75 cm^2 flasks for cell culture (Corning Incorporated, USA) in which six 4.5 mm holes were drilled for positioning syringes for feeding and for gas exchange.

Figure 1 The materials used for collecting bees for laboratory tests. On the left, the queen-excluder frame cage, in the center the vacuum cleaner for collecting young bees, on the right the net cage equipped with 1 mL syringes for sugar syrup and water.

Caged bees (35 ± 1 per cage, three cage per treatment) were submitted to an experimental scheme including: 6% OA (w/w) in water as a positive control, tap water as negative control, five treatments with HBA at concentrations of 0.125, 0.25, 0.5, 1, and 2% (w/w) in acetic acid (ACOH). A second set of HBA treatments at the same concentrations, but dissolved in EtOH, was set up. The hoice to dissolve HBA in EtOH or in ACOH was dictated by the difficulty of dissolving the HBA in water or in SS50. Through preliminary solubilisation tests, EtOH and ACOH were found suitable to dissolve the HBA that, in its original state was similar to a very thick molasses. Aliquots of 2.0 g of HBA were weighted in 25 mL centrifuge tubes; then 16.0 mL of ethanol or acetic acid and 2 mm diameter borosilicate glass beads (SIGMA ALDRICH, Germany) were added before vortexing the mixture at room temperature until complete dissolution (approximately 15-25 minutes vortexing time). Finally, the HBA/EtOH or HBA/ACOH mixtures were diluted in SS50 to the desired concentrations to be tested. After 24 h of acclimation, a volume of 56 µL of treatment/control per flask (1.6 µL/bee) was spread on the bees using a 100 µL pipette (Sartorius AG, Germany). Water and SS50 consumption, varroa mite death (DVM) and HB death (DHB) were recorded daily. Each test lasted 240 hours. At the end of each test, the caged bees were frozen and subsequently removed from the cages to be weighed.

Apiary trials

The two-years study was carried out from September to October 2021 and 2022 in the Plant-B apiary. The apiary consisted of twenty Italian-Dadant hives. Ten hives were equipped with Mussi-type spacers® (45 mm of distance between the centers of the adjacent honeycombs) (WS), and ten equipped with regular spacers (37.5 mm spaced) (RS). In order to use hives as much homogeneous as possible as far as colony strength and stores, the number of hives used for the field test was 16 in 2021 and 14 in 2022, respectively. One HBA treatment per year was scheduled, at the end of September. Half of the hives in both WS or RS groups were used as a control (6% OA in SS50). Based on the tests carried out in the laboratory, it was chosen to test in operative conditions the 2% HBA/EtOH solution diluted in SS50. Unlike DeGrandi-Hoffman et al. (2012), the acaricidal solutions of HBA and OA were applied by spraying treatment or control solutions on the bees at a rate of 5 mL/comb.

Before, during, and after the treatment, hive inspections were performed and data on the number of bee sixths, capped brood and sores (honey, bee bread) were recorded (Imdorf and Berna 1999). In addition, the presence and state of the queen (marked/non marked, other observations), eggs and larvae in the brood cells were noticed. In-hive mortality (IHM) was assessed by using the underbasket traps (Porrini 2007) which were checked for dead bees every 7 days for a total of six weeks. The dead bees and bee parts were collected, counted, weighed, and then frozen for further evaluations. The VM fall was carried out through 35 × 50 cm adhesive paper sheets placed just beneath the anti-VM screen of each hive. The sheets were changed every 48 hours for the first two weeks. In the following fou weeks they were placed once a week for 48 hours. Mature and immature VM specimen were manually counted.

Data processing and statistical analysis

At the end of the test, the survival rate was calculated, expressed as a percentage based on the number of bees still living on the tenth day compared to the initial one.

The statistical analysis was performed by the software STATISTICA 10 (StatSoft, Hamburg, Germany). A General Linear Model were set up as follow: cumulate dead bees (%), consumption of SS50 and water (ml/day), cumulate VM fall (%), for laboratory trials, and in hive mortality, VM fall, bee sixths as depending variables, treatment and hive type as categorial factor and experimental days as co-variable.

RESULTS

LABORATORY TRIALS

GLM analysis (Tab.1) showed that the phase effect, between before and after treatment was statistically significant ($p<0.05$) with respect to the varroa mortality parameter. While the treatment effect was significant ($p<0.05$) with respect to the parameter SS50 consumption. The phase × treatment interactions showed no significant effects. From the three laboratory tests, it was seen that the greatest impact on VM mortality (DVM%) at 120 hs was the EtOH/HBA_2% (57 ± 37%) (Fig. 3), very similar to the performance that the OA-based positive control (OAdiidr_6%/W) had (60 ± 38%). Turkey's test showed that all treatments administered had a statistically significant ($p<0.001$) greater effect than the water-based negative control, which had the absolute lowest percentage of dead varroa (21 ± 23%). In contrast, bee mortality (DHB%) (Fig. 2) was lower in the ACOH/W negative control (45 ± 34) and in the ACOH/HBA_0.125% (46 ± 38%) and EtOH/HBA_1% (48 ± 37%) treatments. In contrast, the treatments that had the greatest impact on bee mortality were the OAdiidr_6%/W oxalic acid control (65 ± 39%), and the ACOH/HBA_1% (67 ± 40%) and EtOH/HBA_0.25% (67 ± 39%) treatments. Talking about the consumption of SS50 (Fig. 4) during the test, the bees recorded an average consumption of 101.11 ± 86.20 µl during the 120 hs of the test. The bees treated with EtOH/HBA_2% recorded the lowest consumption (31.20 ± 18.00 µl), along with the ACOH/HBA_2% treatments (33.24 ± 18.10 µl) and the water-based negative control (40.40 ± 23.10 µl) while the EtOH/HBA_0.125% treatment recorded the highest consumption (192.10 ± 109.30 µl). As for water consumption (Fig. 5), the average consumption was 24.50 ± 21.10 µl. Again, bees treated with EtOH/HBA_2% had the lowest consumption (13.30 ± 10.00 µl), along with the ACOH/HBA_2% treatments (15.01 ± 10.10 µl). Also, the water-based negative control (40.40 ± 23.10 µl) and OAdiidr_6%/W-based positive control recorded low bee water consumption (17.01 ± 11.20 µl and 17.20 ± 11.10 µl, respectively). At the same time, the **bees that consumed**

the most water during the trial were those treated with the ACOH/W-based negative control (43.40 ± 94.84 µl).

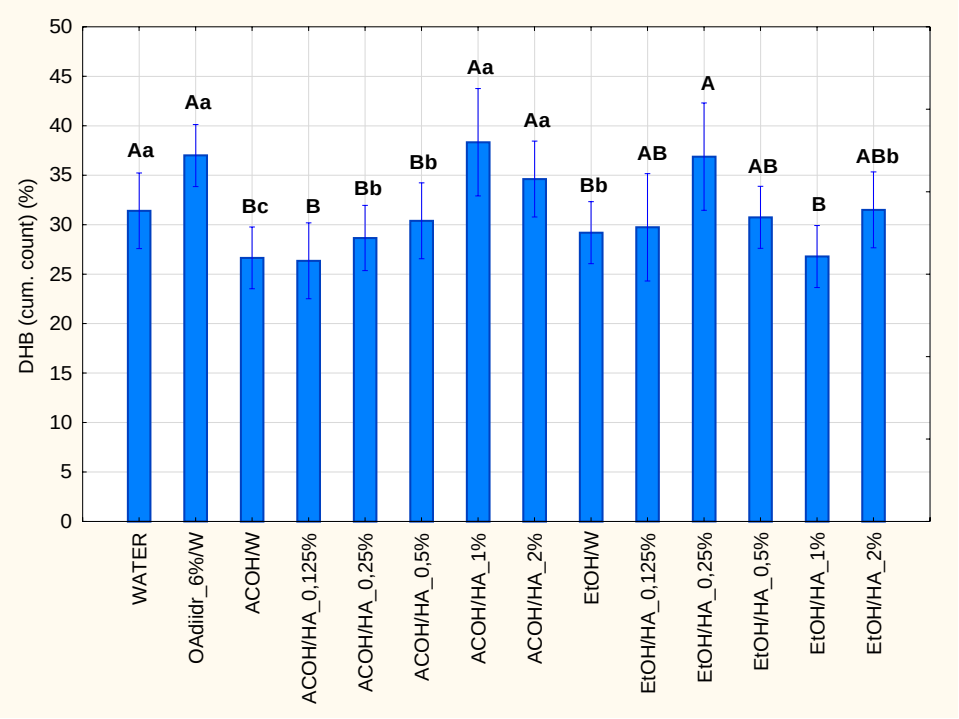
From these evidences, it could be preliminary concluded that the treatments that have had the best results, that is highest VM mortality with the lowest impact on the bees, were the EtOH/HBA_2%.This solution was then chosen to be tested in the apiary.

Table 1 Effect of phase[1], the treatment on the bees in the laboratory trials

	PHASE	TREATMENT	PHASE × TREATMENT
DHB (cum. count) (%)	n.s	n.s	n.s
DVM (cum. count) (%)	<0.05	n.s	n.s
Cons. SS50 (cum. vol.) (µl)	n.s	<0.05	n.s
Cons. water (cum. vol.) (µl)	n.s	n.s	n.s

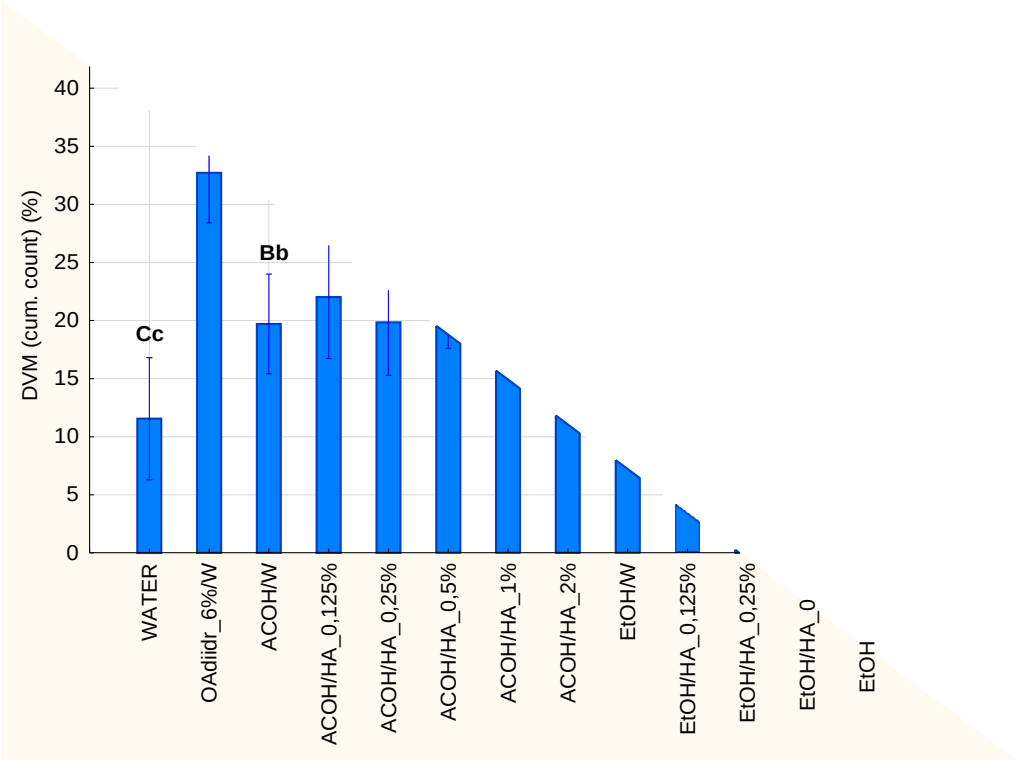
[1]*PRE and POST treatments;*

Figure 2 the effect of the different treatments on the bee's mortality.



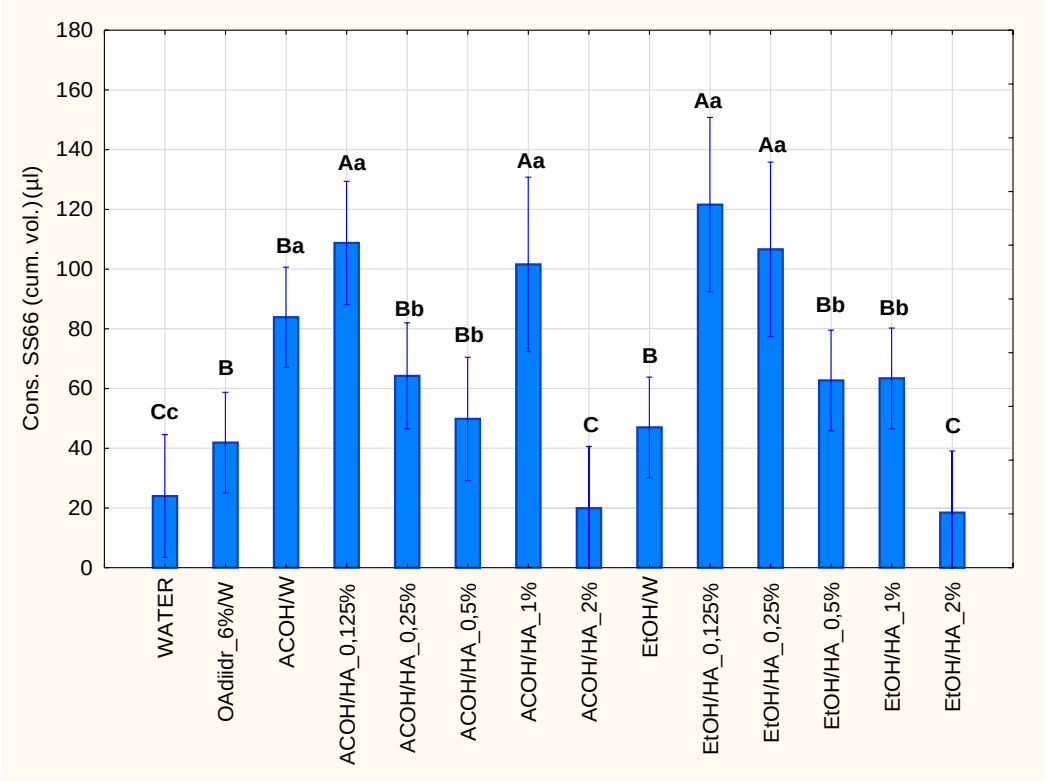
Different letters affixed above the bars denote mean values significantly different at p<0.001; [abc]—p<0.05

Figure 3 Effect of the different treatments on the varroa mortality.



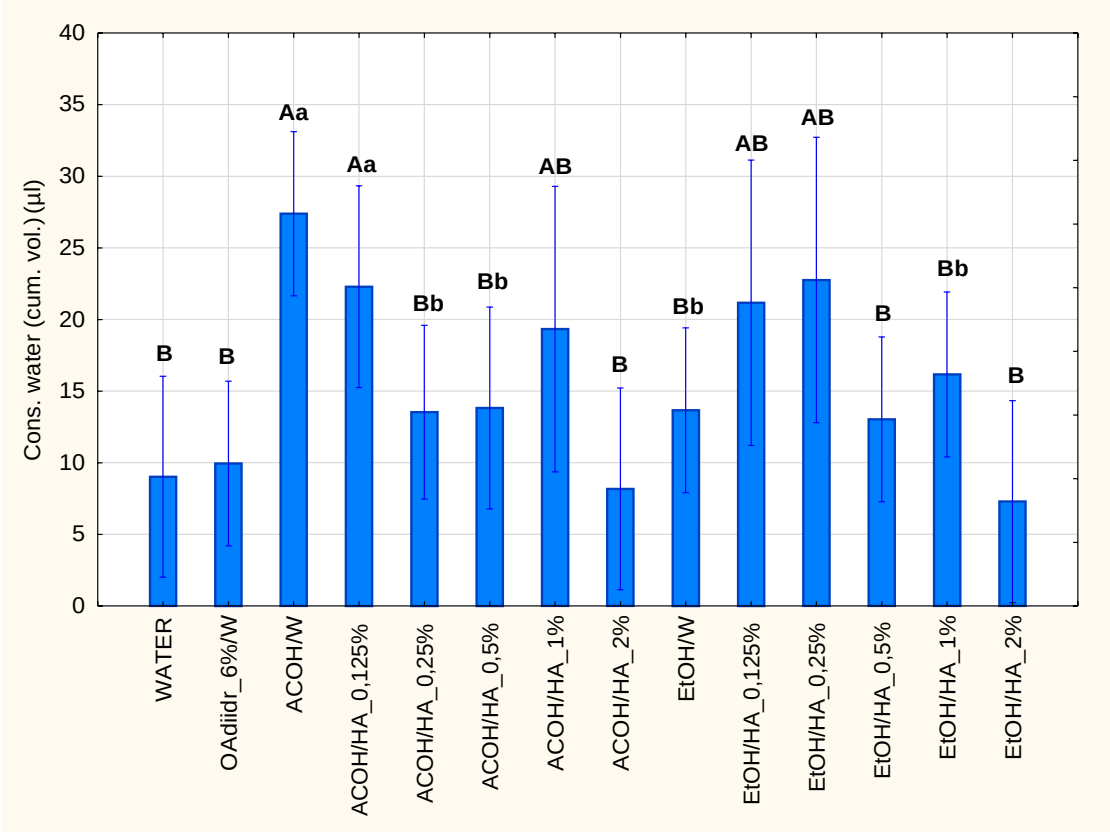
Different letters affixed above the bars denote mean values significantly different at p<0.001; [abc]—p<0.05

Figure 4 the effect of the different treatments on the SS50 consumption.



Different letters affixed above the bars denote mean values significantly different at p<0.001; [abc]—p<0.05

Figure 5 the effect of the different treatments on the water consumption



Different letters affixed above the bars denote mean values significantly different at p<0.001; [abc]—p<0.05

APIARY TRIALS

Hive inspections

Regarding the analysis on the hive inspections conducted before and after the 2021 field treatment, the hive type effect was significant ($p<0.05$) for the stores sixth parameter (Fig 1 supplementary materials). The interactions hive type × experimental phase and experimental phase × treatment effects, although there was a reduction in stocks after treatment, this did not appear to be statistically significant. Regarding bee sixth, again, although there was a reduction in the number of bee sexes present in the hives after treatment, but this was not found to be statistically significant in any case.

Speaking instead of data collected from inspections conducted before and after the 2022 treatment, the experimental phase ($p<0.001$) was significant for the parameter regarding the number of bee sixths (Fig. 2 supplementary materials). Indeed, it is possible to show that the number of bees present in the hives decreased after the treatments, both HBA-based and OA-based. In the case of stores sixths, on the other hand, the reduction in the number of sixths in this case is not statistically significant.

Combining the data from the two years of treatments (2021-2022) (Tab. 2). The experimental phase effect and experimental phase x treatment interaction were both found to be significant ($p<0.001$ and $p<0.05$ respectively) for the variable bee sixths. registering a reduction in the number of bee sixths after the two treatments in the two years of the experiment combined (Fig. 5).

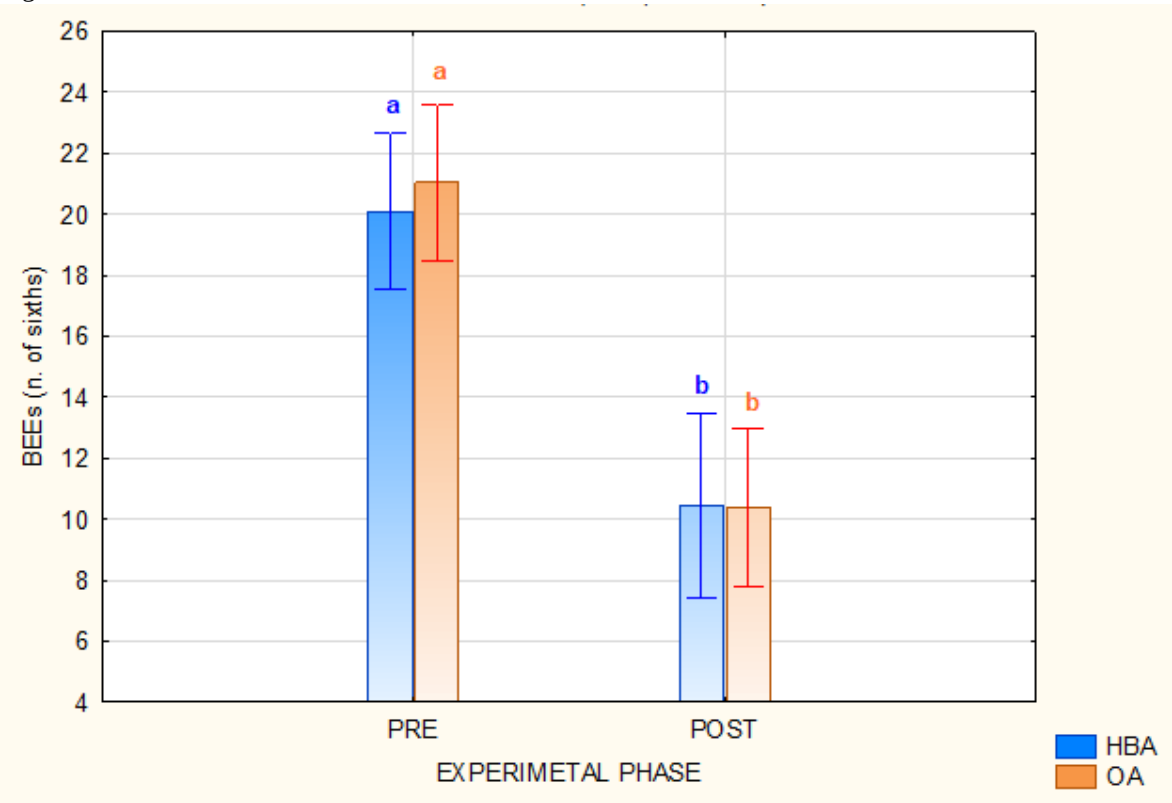
Overall, in no case the treatment effect was statistically significant, indicating a similarity in the impact the two treatments had on the different colonies.

Table *2* Effect of phase[1], treatment and hive type[2] on the hives inspections in 2021-2022 treatments.

2021-2022 INSPECTIONS	TRT	EXP. PHASE	HIVE TYPE	EXP. PHASE*TRT	HIVE TYPE*TRT
BEEs (n. of sixths)	n.s.	<0.001	n.s.	<0.05	n.s.
STORES (n. of sixths)	n.s.	n.s.	n.s.	n.s.	n.s.

[1]PRE and POST treatments,[2]Mussi space and Italian Dadant standard space

Figure 6 the effect of the EXPERIMENTAL PHASE × TREATMENT interaction on the bee sixths.



Different letters affixed above the bars denote mean values significantly different at p<0.05;

In hive bees' mortality

Regarding the in-hive mortality, statistical analysis of treatments carried out in 2021 (Tab. 1 supplementary materials) showed that the control n. effect was significant ($p<0.001$) for the variable dead bees. The interaction treatment × control n. was also significant ($P<0.001$) for the same parameter (Fig. 3 supplementary materials). In fact, it can be seen that bee mortality was stable and did not change from before treatment until the fifth control made with under baskets. From the sixth control (five controls after treatment), there is an increase in mortality in the OA-treated hives, a statistically significant increase at Turkey test ($p<0.001$) compared to the HBA-treated hives. In contrast, at the last control, while mortality in OA-treated hives remained stable, there was a greater increase in mortality in HBA-treated hives than in OA-treated hives ($p<0.001$ by Turkey test). The HIVE TYPE effect did not show statistically significant differences.

Speaking of the statistical analysis performed on the 2022 treatments (Tab. 2 supplementary materials), the control n. effect was significant ($p<0.001$) for the dead bees. In contrast to 2021, after treatment before the third control, bee mortality had an increasing trend, particularly in the fourth and fifth controls. Bee mortality was found to be increasing especially in OA-treated hives with a statistically significant difference at Turkey test ($p<0.05$) with HBA-treated hives. Again, the hive type effect was not significant.

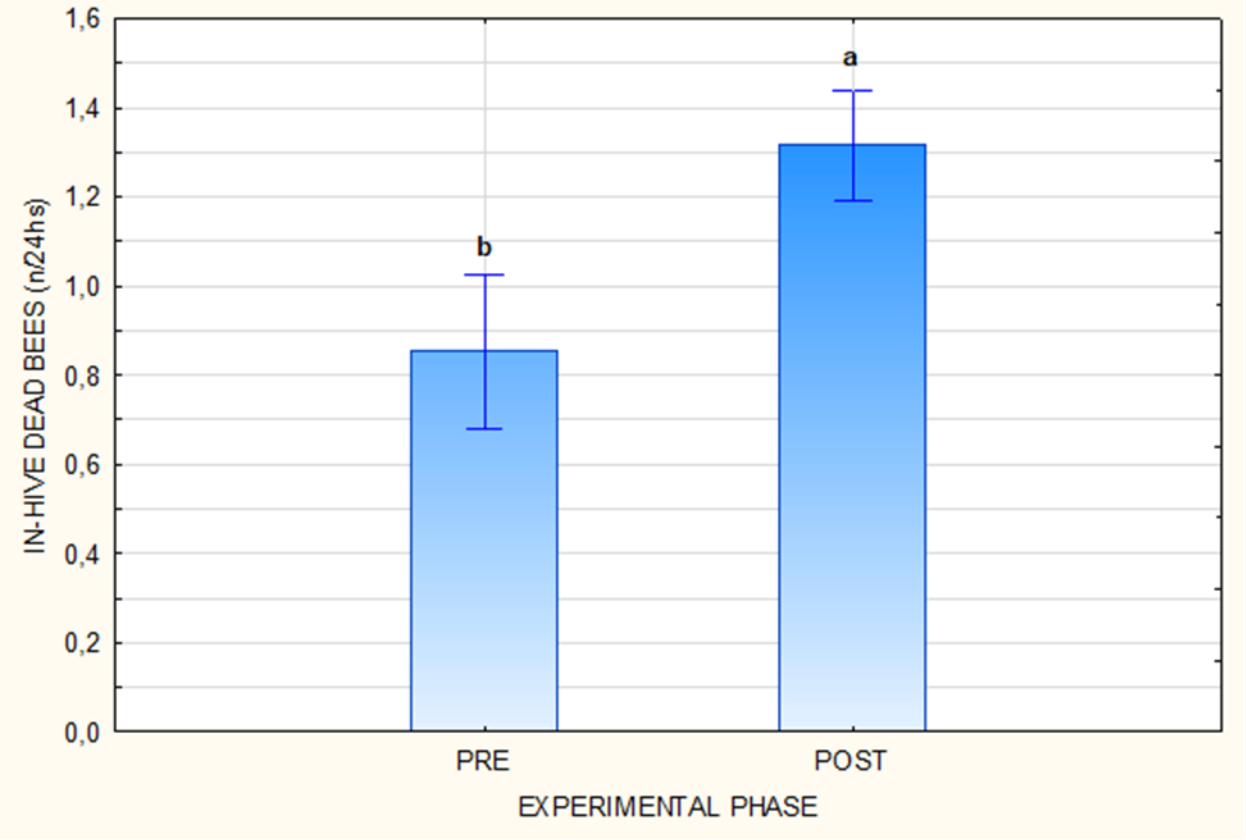
Finally, talking about the overall statistical analysis performed on the two years of treatments (Tab. 3), the experimental phase effect (before and after treatment) was significant ($p<0.05$) on bee mortality (Fig. 6). All other effects, including HIVE TYPE again were not significant.

Table 3 Effect of experimental phase[1], treatment and hive type[2] on the in-hive mortality during 2021-2022 treatments.

2021-2022 IN HIVE MORTALITY	TRT	EXP. PHASE	HIVE TYPE	EXP. PHASE*TRT	HIVE TYPE*TRT
IN-HIVE DEAD BEES (n/24hs)	n.s.	<0.05	n.s.	n.s.	n.s.

[1]PRE and POST treatments,[2]Mussi space and Italian Dadant standard space

Figure 7 the effect of the EXPERIMENTAL PHASE on the in-hive mortality during the 2021-2022 treatment.



Different letters affixed above the bars denote mean values significantly different at p<0.05

Varroa fall

Speaking of mature and immature VM fall in the 2021 treatments (Tab. 4), the control n. effect was statistically significant ($p<0.001$) for both parameters analysed (VM FALL and IMMATURE VM FALL). Since, the control n. × treatment effects was not significant, it was evident that the two treatments had the same outcome.

On the other hand, the hive type effect was significant ($p<0.05$) as far as the VM fall parameter was concerned (Fig. 4 of supplementary materials), Dadant space hives recounted a higher varroa fall. The hive type × treatment effects was also significant ($p<0.001$) on the VM FALL, with VM fall being greater in D-space hives treated with HBA. In contrast, there were no significant differences in immature varroa fall in the different type of hives.

Regarding the 2022 treatments (Tab. 5), the control n. effect was statistically significant ($p<0.05$) for the VM FALL. As the control n. × treatment effect was not significant like the treatment effect significant, it was evident that the two treatments had the same outcome. On the other hand, the HIVE TYPE effect was significant ($p<0.001$) with regard to the VM FALL parameter,

and the IMMATURE VM FALL paramenter ($p<0.05$) the MUSSI-spaced hives found higher VM and immature VM FALL.

Table 4 Effect of control n., treatment and hive type[1] on the mature and immature VM fall during 2021 treatments**.**

VM FALL 2021	TRT	CONTROL N.	HIVE TYPE	CONTROL N.*TRT	HIVE TYPE*TRT
VM FALL (n/24hs)	n.s.	<0.001	<0.05	n.s.	<0.001
IMMATURE VM FALL (n/24h)	n.s.	<0.001	n.s.	n.s.	n.s.

[1]Mussi space and Italian Dadant standard space

Regarding the 2022 treatments (Tab. 5), the control n. effect was statistically significant ($p<0.05$) for the VM FALL. As the control n. × treatment effect was not significant like the treatment effect significant, it was evident that the two treatments had the same outcome. On the other hand, the HIVE TYPE effect was significant ($p<0.001$) with regard to the VM FALL parameter, and the IMMATURE VM FALL paramenter ($p<0.05$) the MUSSI-spaced hives found higher VM and immature VM FALL.

Table 5 Effect of control n., treatment and hive type[1] on the mature and immature VM fall during 2022 treatments

beta 2022 control	TRT	CONTROL N.	HIVE TYPE	CONTROL N.*TRT	HIVE TYPE*TRT
VM FALL (n/24hs)	n.s.	<0.05	<0.001	n.s.	n.s.
IMMATURE VM FALL (n/24h)	n.s.	n.s.	<0.05	n.s.	n.s.

[1]Mussi space and Italian Dadant standard space

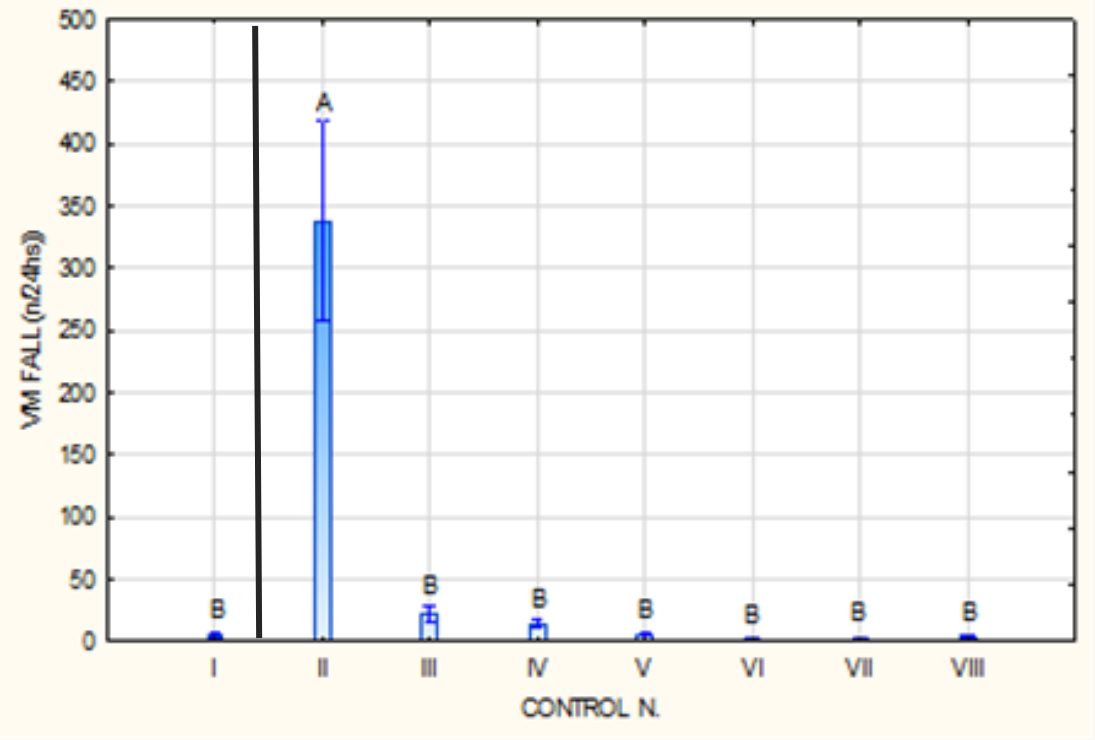
Finally, talking about the 2021-2022 data (Tab. 6), the control effect was significant ($p<0.001$) for both parameters analysed (Fig. 7 and 8) . The hive type x control effect was also significant ($p<0.05$) for the VM FALL parameter. No statistically significant difference was found between the two types of treatments (HBA and OA)

Table 6 Effect of control n., treatment and hive type[1] on the mature and immature VM fall during 2022 treatments

beta 2021-2022 control	TRT	CONTROL N.	HIVE TYPE	CONTROL N.*TRT	HIVE TYPE*TRT
VM FALL (n/24hs)	n.s.	<0.001	<0.05	n.s.	n.s.
IMMATURE VM FALL (n/24h)	n.s.	<0.001	n.s.	n.s.	n.s.

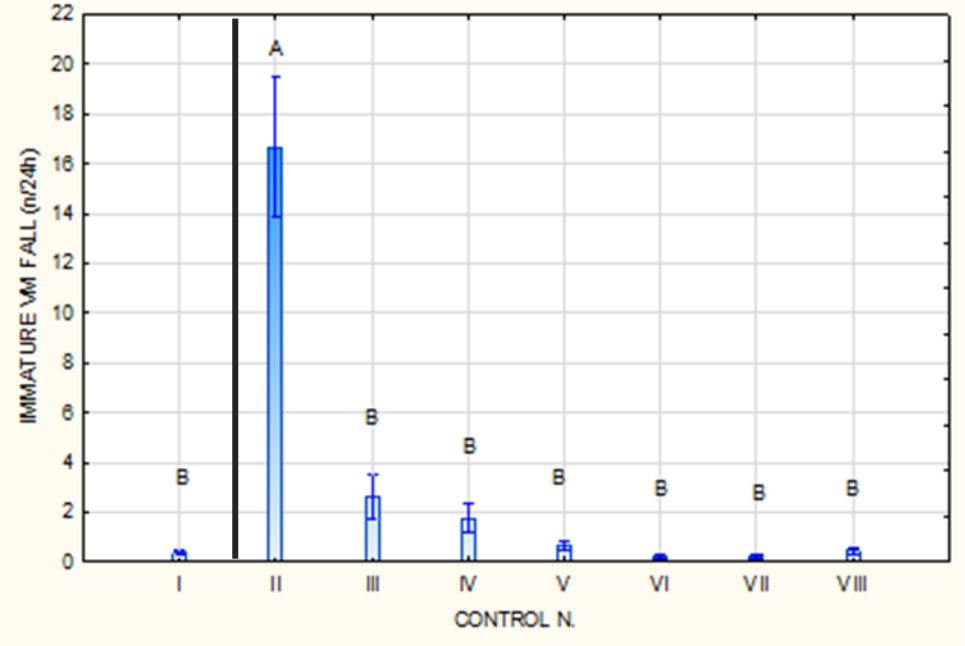
[1]*Mussi space and Italian Dadant standard space*

Figure 8. The effect of the control number[1] on the mature VM fall during the 2021-2022 treatments.



Different letters affixed above the bars denote mean values significantly different at p<0.001;[1] the black bar in the graph shows the time of treatment.

Figure 9 The effect of the control number[1] on the immature VM fall during the 2021-2022 treatment.



Different letters affixed above the bars denote mean values significantly different at p<0.001;[1] the black bar in the graph shows the time of treatment.

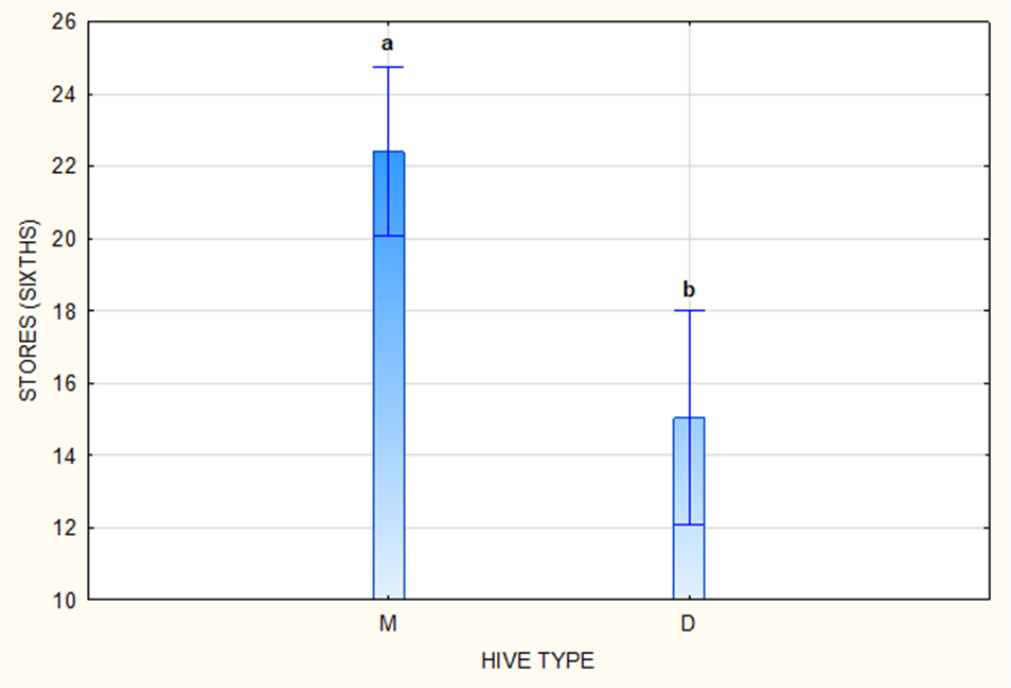
Conclusions

This study represents step toward the implementation of an integrated pest management (IPM) program to combat *Varroa destructor*, a major threat to honey bee colonies. Our method, based on the use of higher spacing hives (MUSSI SPACE) and topical application of hop beta acids, has shown potential both in the laboratory and in the field.

Field tests were conducted differently from the studies listed above by directly spraying the treatment on the combs. Results showed that the proposed approach had a significant impact on varroa mortality, making it a promising strategy for containing infestations of this pest. In addition, the approach was tolerated by the bees in terms of hive survival, suggesting that the use of hop beta acids can be implemented in practice without affecting bee health or productivity. In fact, HBAs were found to be a viable alternative to the use of oxalic acid, as no statistically significant differences were found between the two treatments in either year of operation. However, statistical analysis also showed that the use of Mussi space did not make an overall statistically significant improvement in varroa fall or bee survival.

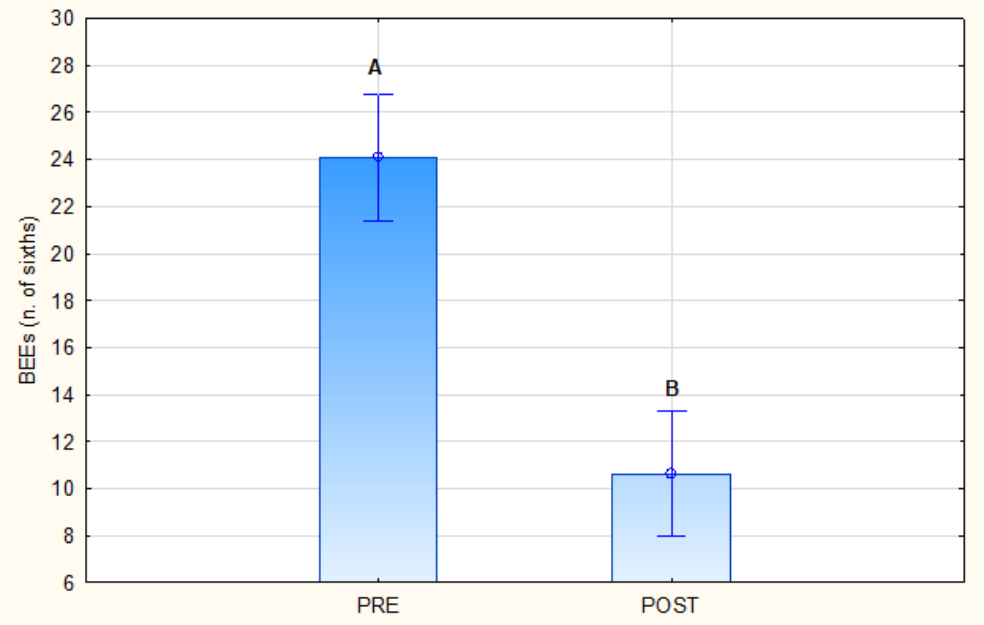
Supplementary materials

Figure 1 the effect of the different Hive type space on the bee stores in 2021 treatment.



Different letters affixed above the bars denote mean values significantly different at p<0.05; M--MUSSI space, D--Italian Dadant standard space.

Figure 2 the effect of the experimental phase on the bee sixths in the 2022 treatment.



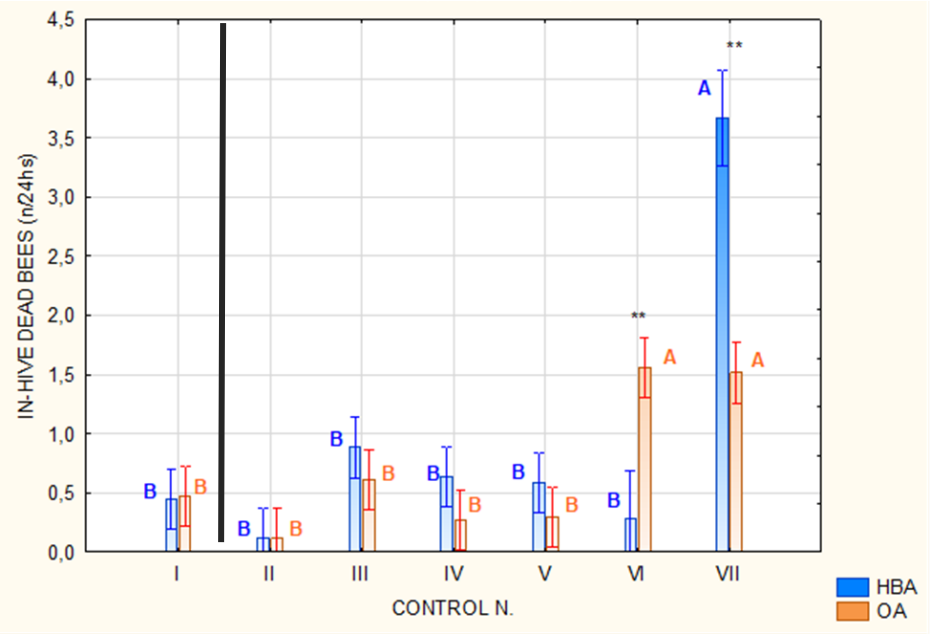
Different letters affixed above the bars denote mean values significantly different at p<0.001;

Table 1 Effect of control n., treatment and hive type[1] on the in-hive mortality during 2021 treatment.

2021 treatment	TRT	CONTROL N.	HIVE TYPE	CONTROL N.*TRT	HIVE TYPE*TRT
IN-HIVE DEAD BEES (n/24hs)	n.s.	<0.001	n.s.	<0.001	n.s.

[1]*Mussi space and Italian Dadant standard space*

Figure 3 the effect of the CONTROL NUMBER[1] × TREATMENT on the in-hive mortality during the 2021 treatment.



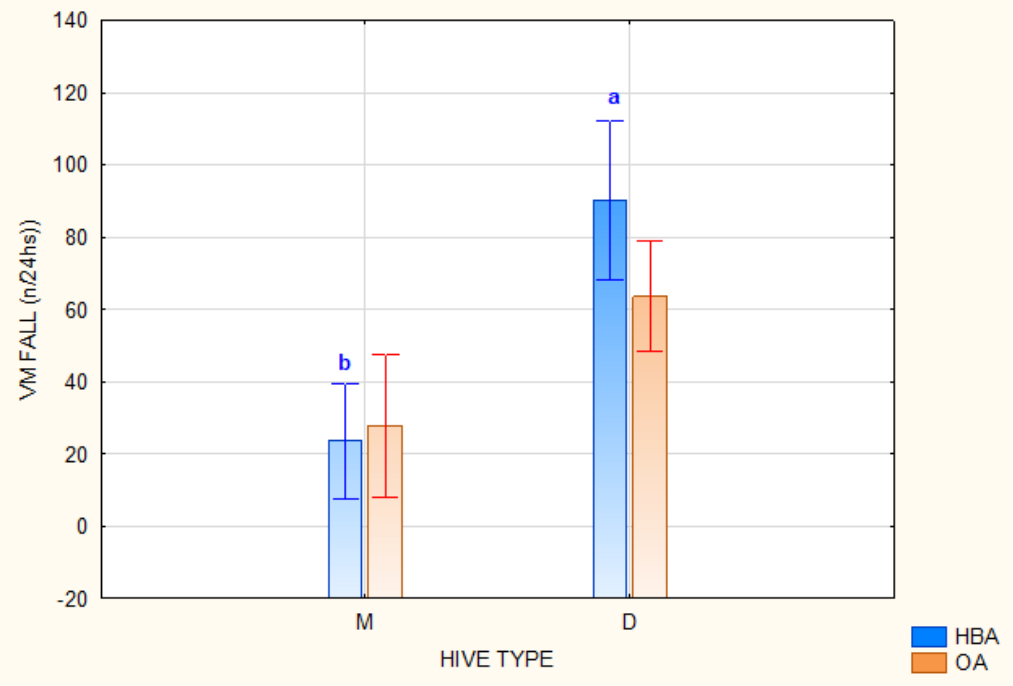
*Different letters affixed above the bars denote mean values significantly different at p<0.001; **—p<0.001;[2] the black bar in the graph stands for the time of treatment.*

Table 2 Effect of control n., treatment and hive type[1] on the in-hive mortality during 2022 treatments.

2022 IN HIVE MORTALITY	TRT	CONTROL N.	HIVE TYPE	CONTROL N.*TRT	HIVE TYPE*TRT
IN-HIVE DEAD BEES (n/24hs)	n.s.	<0.001	n.s.	n.s.	n.s.

[1]*Mussi space and Italian Dadant standard space*

Figure 4 The effect hive type × treatment on the mature VM fall during the 2021 treatment.



Different letters affixed above the bars denote mean values significantly different at p<0.05;M--MUSSI space, D--Italian Dadant standard space

3.2 HOP ALFA ACIDS TRIALS (CASE STUDY 2)

Material and methods

The efficacy of hop alfa acids was preliminarily tested in the laboratory at different concentrations. Once all the solutions had been tested, moving the activities to the field, the concentration that had yielded the maximum in terms of VM and tolerable for HB was tested. The field test was foreseen on Mussi® space and regular space hives, in such a way as to be able to evaluate whether the type of hive, with the same treatment, could have an additional effect on the fall of varroa.

Chemical and reagents

For all the treatments, solutions based on Hexa-Tetra-Blend Hop Alpha Acids (Hopsteiner, Germany). Oxalic acid dihydrate 99.5% pure (OA) (Carlo Erba reagents, Italy) was instead used as a control. To dilute the various solutions, a water-sugar solution in a 50:50 (w/w) ratio with 0.4% citric acid 99.8% pure (Carlo Erba reagents, Italy) was used (SS50).

Laboratory trials

In four consecutive trials under controlled conditions (34±1 C°; 72.0±0.6 % RH). The caged bees were placed inside a PID SYSTEM thermostat (MPM Instruments s.r.l., Bernareggio, Italy) inside the laboratories of the DAFNE department of the University of Tuscia, and temperature and humidity were monitored with a TESTO 175H (Testo SE & Co., Germany) datalogger.

The HB used were collected from the Plant-B apiary located at the Experimental Teaching Farm “N. Lupori” (Tuscia University, Viterbo, Italy) (42.4271454°E, 12.0796127°N, 302.2 m EASL). We focused on using young (1-3 weeks) bees. The method described by (Pickard and Kither 1983) was used to separate the adult bees from the young ones. After the division, the bees were collected using a self-produced tool (Fig.2), composed of a collector with which the bees were taken, and a narrow exit that allowed the bees to exit one by one allowing the operator to count the bees collected more accurately.

The bees (32.2±2.2 per cage) were taken from three bee hives with different VM infestation levels (from 23 up to 412 VM/48 hs) in the week prior to the star of the activity, and caged in 75

cm^2 flasks for cell culture (Corning Incorporated, USA) in which six 4.5 mm holes were drilled for positioning syringes for feeding (based on SS50), water and for gas exchange (Fig. 1).

After collection, and waiting 24 hours of acclimatation, young bees were submitted to an experimental scheme including: 6% oxalic acid (OAdiidr) in a 50:50 sugar solution (SS50) as the positive control; SS50 the negative control, and the treatments namely HA at 0.1%, 0.3%, 0.6%, 1%, 2% in SS50. A mean volume of 1.6 µl of treatment/control per bee was spread on the bees using a 100 µL pipette (Sartorius AG, Germany). Four replicates for each treatment/trial were set up.

Fig. 1 and 2. On the right, caged bees immediately before the trial. On the left, the self-produced tool for the bee catching.

The bees were regularly checked at 6, 24, and every 24 hs until 120 hs after treatment. During the checks, the consumption of water and sugar solution (SS50), the possible presence of dead bees (DHB) and dead varroas (DVM) were verified, and everything was noted. Four repetitions of this experiment were carried out. At the end of each test, the bees were frozen and subsequently removed from the cages to be weighed.

Apiary trial

The study was conducted from September to October 2020 in the Plant-B apiary. The apiary consisted of twenty Italian-Dadant hives. Ten hives were equipped with Mussi-type spacers® (45 mm of distance between the centers of the adjacent honeycombs) (WS), and ten equipped with regular spacers (37.5 mm spaced) (RS).

Two treatment per hive were scheduled, at the end of September. Half of the hives in both WS or RS groups were used as a control (6% OA in SS50). Based on the tests carried out in the laboratory, it was chosen to test in operative conditions the 0.3% HA solution diluted in SS50 for

the first treatment, and the 0.6% HA for the second treatment, after a week. Unlike DeGrandi-Hoffman et al. (2012), the acaricidal solutions of HA and OA were applied by spraying treatment or control solutions on the bees at a rate of 5 mL/comb.

Hives controls

Before, during, and after the treatment, hive inspections were performed and data on the number of bee sixths, capped brood and sores (honey, bee bread) were recorded (Imdorf and Berna 1999). In addition, the presence and state of the queen (marked/non marked, other observations), eggs and larvae in the brood cells were noticed. In-hive mortality (IHM) was assessed by using the underbasket traps (Porrini 2007) which were checked for dead bees every 7 days for a total of six weeks. The dead bees and bee parts were collected, counted, weighed, and then frozen for further evaluations. The VM fall was carried out through 35 × 50 cm adhesive paper sheets placed just beneath the anti-VM screen of each hive. The sheets were changed every 48 hours for the first two weeks. In the following fou weeks they were placed once a week for 48 hours. Mature and immature VM specimen were manually counted.

Data processing and statistical analysis

At the end of the test, the survival rate was calculated, expressed as a percentage based on the number of bees still living on the tenth day compared to the initial one.

In order to ensure that the parameters which values were percentages could have a normal distribution for the statistical analysis, the arcsine transformation as described by Zar et al. (2014) was applied for the following variables: cumulate death bees (%), cumulate VM fall (%). The statistical analysis was performed by the software STATISTICA 10 (StatSoft, Hamburg, Germany).

A General Linear Model were set up as follow: cumulate dead bees (%), consumption of SS50 and water (ml/day), cumulate VM fall (%), for laboratory trials, and in hive mortality, VM fall, bee sixths as depending variables, treatment and hive type as categorial factor and experimental days as co-variable.

Results and discussion

Laboratory trials

GLM analysis (Tab. 1) showed that the PHASE effect, between before and after treatment and statistically significant ($p<0.001$) with respect to the parameter's bee mortality, varroa mortality, water consumption and SS50.

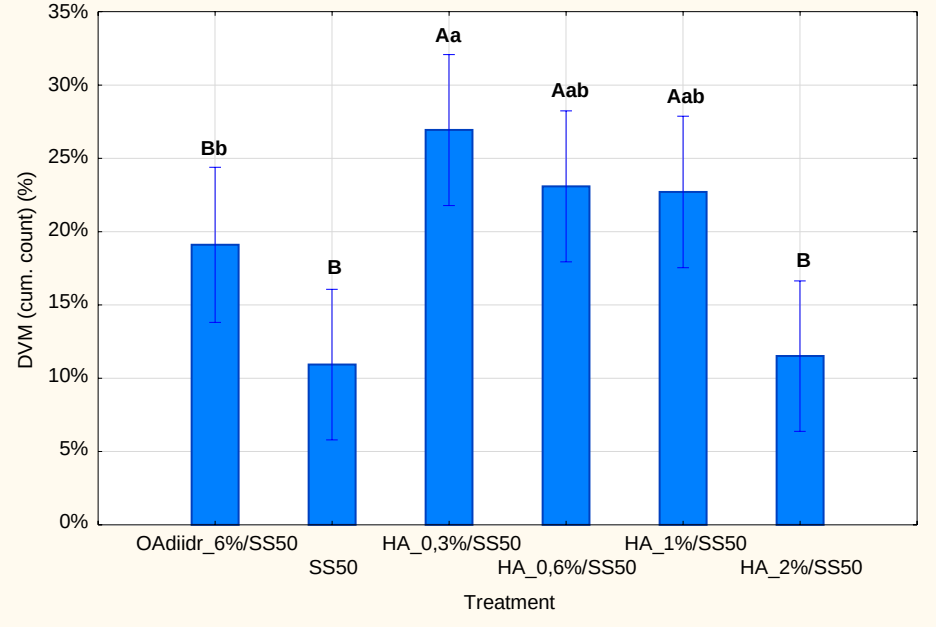
Table 2 Effect of phase[1], the treatment on the bees in the laboratory trials

Laboratory trial	TREATMENT	PHASE	TREATMENT*PHASE
DHB (cum. count) (%)	0,180220	0,000000	0,208649
DVM (cum. count) (%)	0,151989	0,000000	0,644122
Cons. SS66 (cum. vol.) (µl)	0,827066	0,000000	0,993800
Cons. water (cum. vol.) (µl)	0,962361	0,000000	0,917172

[1]PRE and POST treatments;

From the three laboratory tests, it was evident that the greatest impact in terms of VM mortality at 120 hs was the 0.3% solution in SS50 (38 ± 47%) (Fig.2). HA at 1% and 0.6% were also found to cause somewhat high VM mortality rates, respectively 34 ± 39% and 29 ± 37% ($p<0.001$) with respect to the positive control of OA (25 ± 37%), with a statistically significant difference ($p<0.001$) evidenced by Turkey's test.

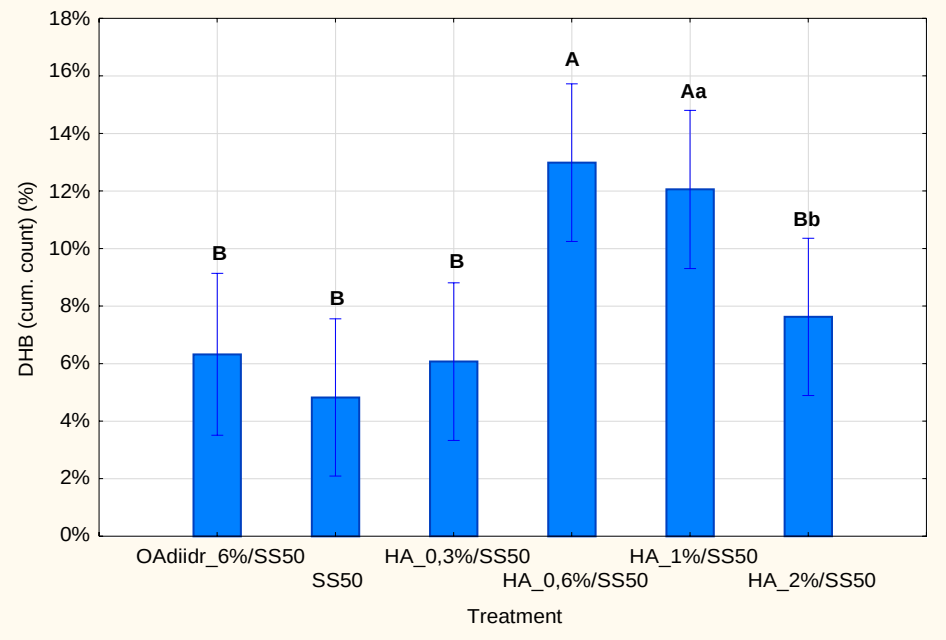
Figure 2 the effect of the different treatments on the VM mortality



Different letters affixed above the bars denote mean values significantly different at $p<0.001$; [ab]— $p<0.05$

In contrast, bee mortality was lower in the HA_0.03% treatment (9 ± 16%) similar to the OA positive control (10 ± 19%) and SS50 negative control (8 ± 10%). THE highest mortality, on the other hand, was recorded in the HA_0.6% (21 ± 27%) and HA_1% (19 ± 26%) treatments with a statistically significant difference at Turkey's test ($p<0.001$) compared with the positive and negative controls and the HA_0.3% treatment (Fig. 3).

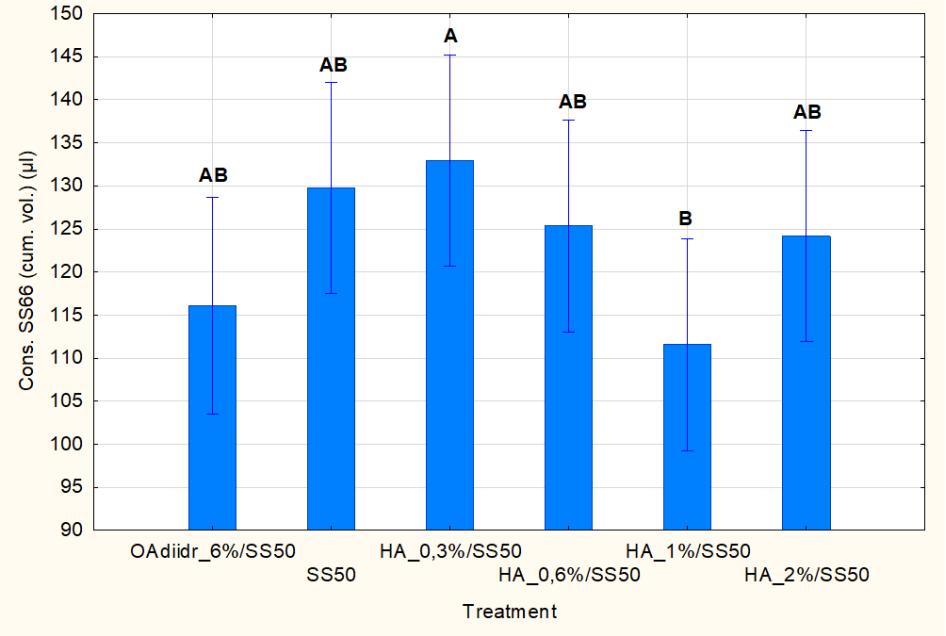
Figure 3 the effect of the different treatments on the bee's mortality



Different letters affixed above the bars denote mean values significantly different at $p<0.001$; ^{ab}— $p<0.05$

Speaking of SS50 consumption during the test (Fig. 4), the bees recorded an average consumption of 178 ± 106 µl during the 120 hs of the test. Bees treated with HA_1 % recorded the lowest consumption (162 ± 100 µl), while the HA_0.3% treatment recorded the highest consumption (189 ± 109 µl).

Figure 4 the effect of the different treatments on the nutritive consumption of the bees



Different letters affixed above the bars denote mean values significantly different at p<0.001;

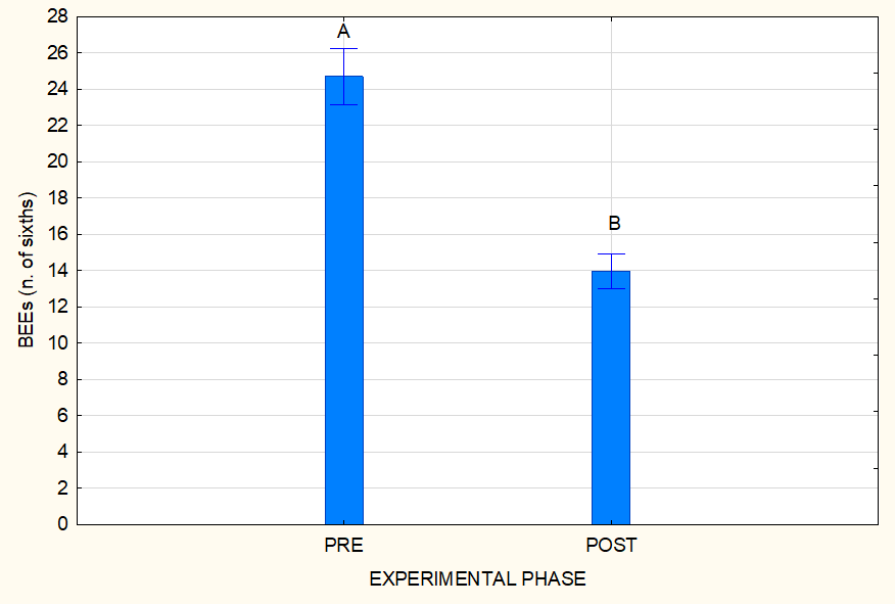
Turkey's test showed no statistically significant differences in water consumption between treatments (Fig. 1 supplementary materials). The average consumption was 34 ± 19 µl. From these evidences, it can be preliminary concluded that the treatments that have had the best results, that is highest VM mortality with the lowest impact on the bees, were the 0.3%. This solution was then chosen to be tested in the apiary. At a later stage, the 0.6% HA solution was chosen to carry out a second treatment

Apiary trials

Hive inspections

Statistical analysis showed that there was a decrease in the number of bees present (Fig. 4) in the hives after the treatments were carried out ($p<0.001$). No significant differences were shown between the two treatments used. The interaction between treatment and hive type was significant ($p<0.05$) for the number of bee sixths (Fig. 2 of supplementary materials). Speaking of the stocking sixths, statistical analysis showed no significant differences, which means that there was no decrease in the stocks present in the treated hives.

Figure 5 the effect of the experimental phase on the bee sixth

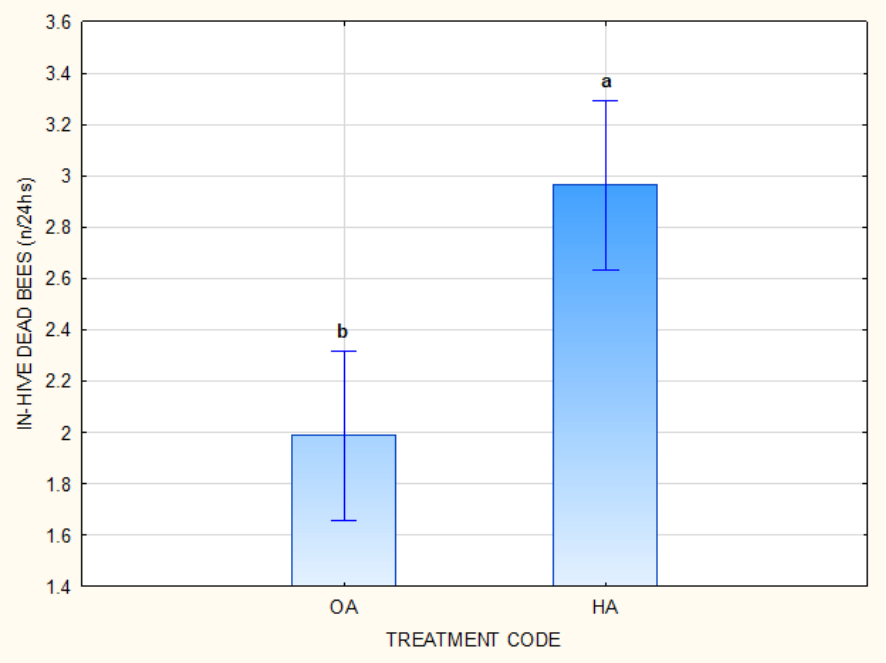


Different letters affixed above the bars denote mean values significantly different at p<0.001

In hive mortality

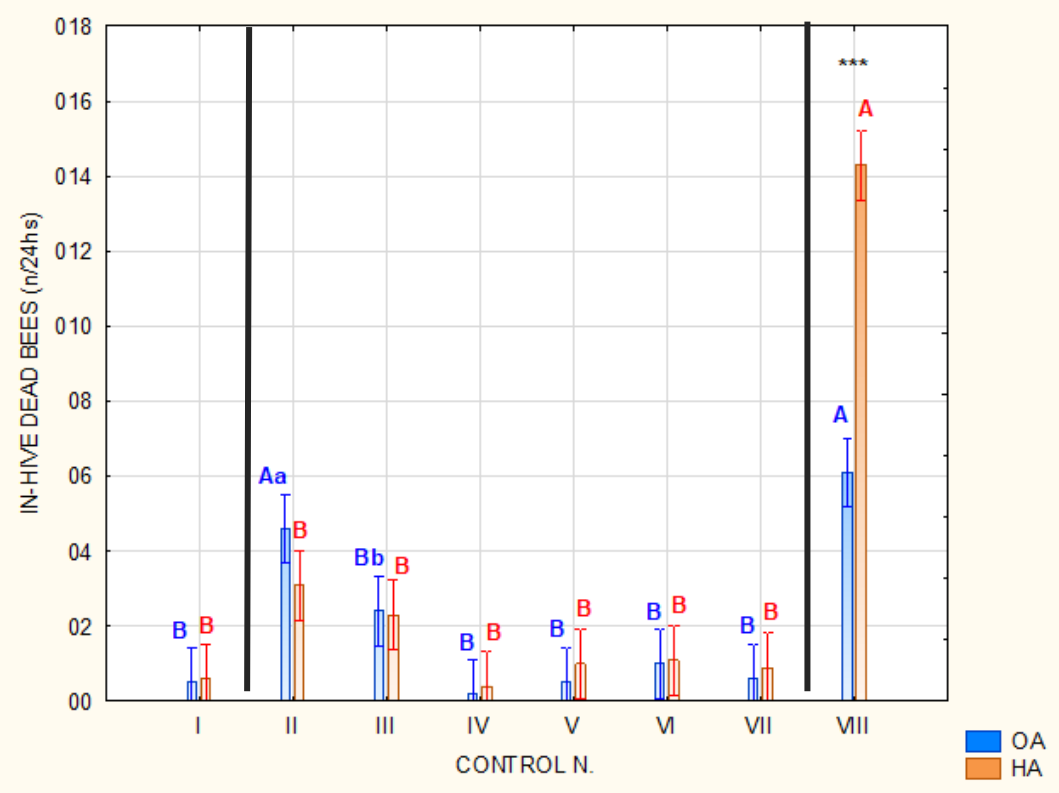
Regarding hive mortality, the treatment effect was statistically significant ($p<0.05$), with hives treated with hop acids having the highest mortality (Fig. 5). The controls effect was significant ($p<0.001$) on bee mortality (Fig. 3 of supplementary materials). The interaction between the two effects (Fig. 6) treatment and controls was also significant ($p<0.001$). indeed, it is evident that mortality in treated hives, especially after the second treatment, was found to increase. At the same time, HA-treated hives recorded the highest mortality only in the second treatment, while in the first treatment it was the OA-treated hives that recorded the highest mortality.

Figure 6. The effect of the different treatments on the in-hive mortality



Different letters affixed above the bars denote mean values significantly different at p<0.05

Figure 7. The effect of the control number × treatment on the in-hive mortality.



Different letters affixed above the bars denote mean values significantly different at p<0.001, [ab]—p<0.05; the black bar in the graph stands for the time of treatments.

Varroa fall

With regard to VM fall (Tab.2), the treatment effect was significant ($p<0.05$), with hives treated with oxalic acid showing greater VM fall (Fig. 7). The CONTROL × TREATMENT interaction was significant ($p<0.001$) for both mature and immature (Fig..of supplementary material) varroa fall. In fact, in both cases VM fall was higher in the first oxalic acid treatment than in the HA 0.3% treatment. In the second treatment, the fall was about the same and not statistically significant for both treatments. It should be noted how in both cases, in the case of both immature and mature, the varroa fall after HA treatments, although it did not peak as much as it did in the oxalic acid-treated hives, was more constant in the post-treatment controls.

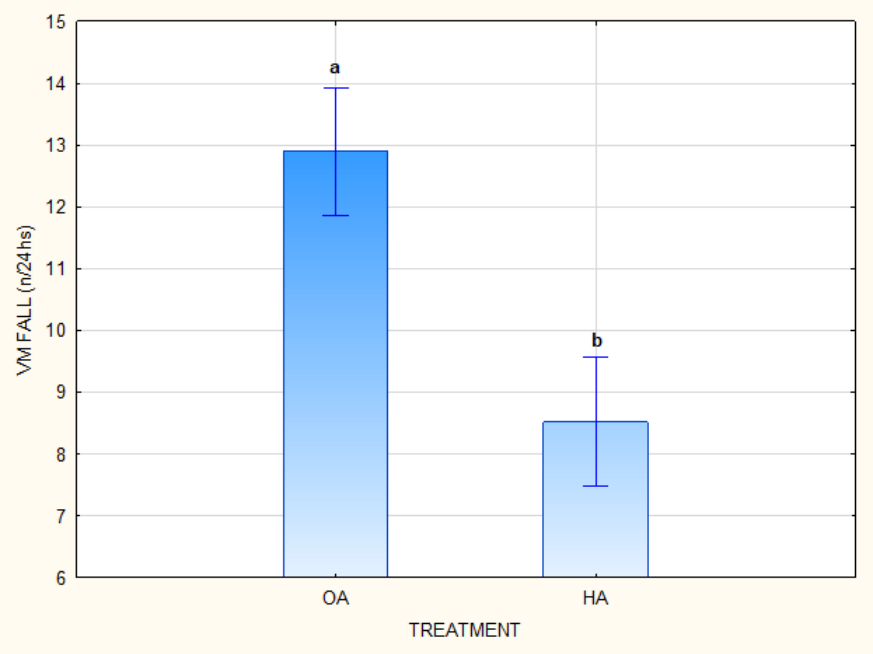
On the other hand, with regard to the interaction HIVE × TREATMENT (Fig. 9), this effect was statistically significant ($p<0.001$) for both parameters analysed, particularly with regard to the fall of mature VM, this fell more ($p<0.001$) in the MUSSI hives treated with OA than in the same type of hives treated with HA ($p<0.001$). Similarly, immature varroa (fig… of supplementary material) also fell more in OA-treated MUSSI hives, but in this case the fall in DADANT hives was greater in HA-treated hives, although the finding was not statistically significant.

Table 2 Effect of control number, treatment and hive type[1] on the mature and immature varroa fall

VARROA FALL	TRT	HIVE TYPE	CONTROL N.	HIVE TYPE×TRT	CONTROL N.×TRT
VM FALL (n/24hs)	<0.05	n.s.	<0.001	<0.001	<0.001
IMMATURE VM FALL (n/24h)	n.s.	n.s.	<0.001	<0.001	<0.001

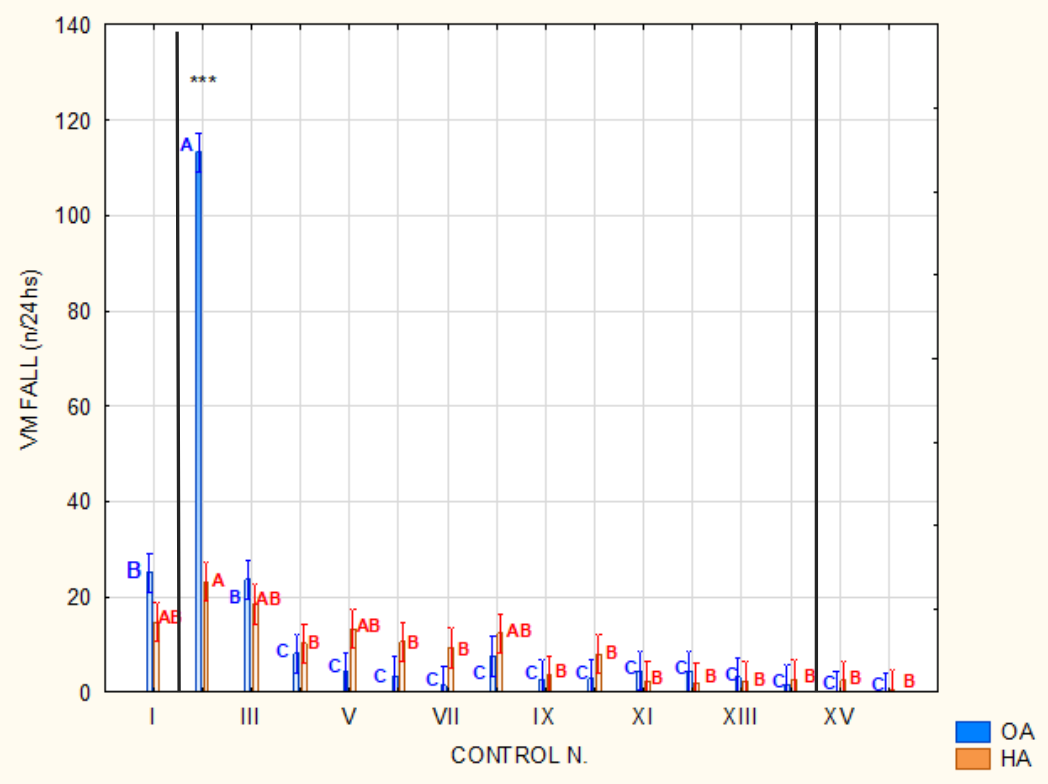
[1]*Mussi space and Italian Dadant standard space*

Figure 7. The effect of different treatments on the varroa mortality



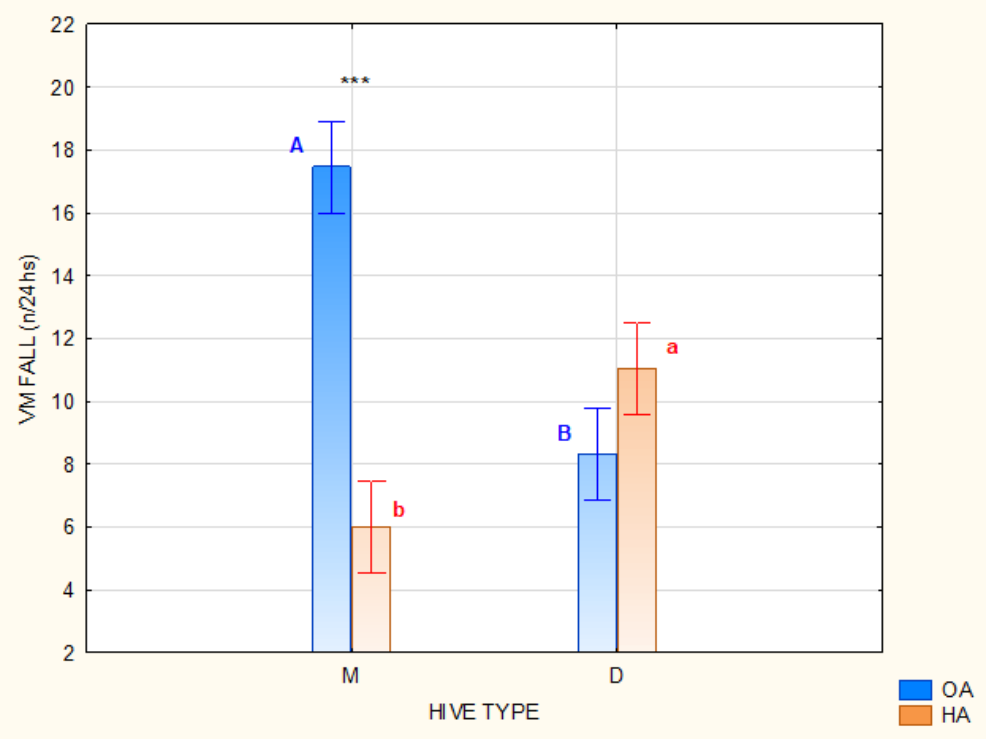
Different letters affixed above the bars denote mean values significantly different at p<0.05,

Figure 8. The effect of different treatments on the varroa mortality



Different letters affixed above the bars denote mean values significantly different at p<0.001, the black bars in the graph stands for the time of treatments.

Figure 9. The effect of different hive type on the varroa mortality



Different letters affixed above the bars denote mean values significantly different at p<0.001, [ab]—p<0.05.

Conclusions

The paper presents a study on the implementation of an integrated pest management (IPM) program to combat the *Varroa destructor*. The method involved the use of hives with greater spacing (MUSSI SPACE) and the topical application of hop alpha acids.

Field tests were conducted differently from the studies previously reported in the literature, by directly spraying the treatment on the combs. The results showed that the proposed approach had a significant impact on varroa mortality, however the impact of the alpha acid treatment was lower than that of oxalic acid, both on mature and immature varroa. The HA-based treatment also had a statistically significant impact on the mortality of bees in the hive, a mortality rate higher than that suffered by hives treated with OA. The statistical analysis also showed that the use of MUSSI space did not result in an overall improvement in varroa drop, but only on hives treated with oxalic acid. Looking forward, it will be interesting to test hop alpha acids at higher concentrations, previously testing their tolerability by bees.

Supplementary materials

Figure 1 The effect of the different treatments on the bee's water consumption.

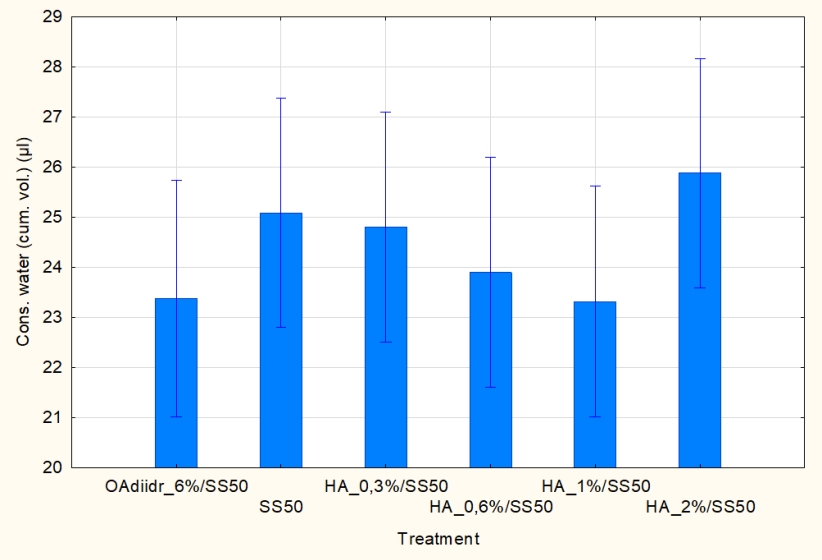
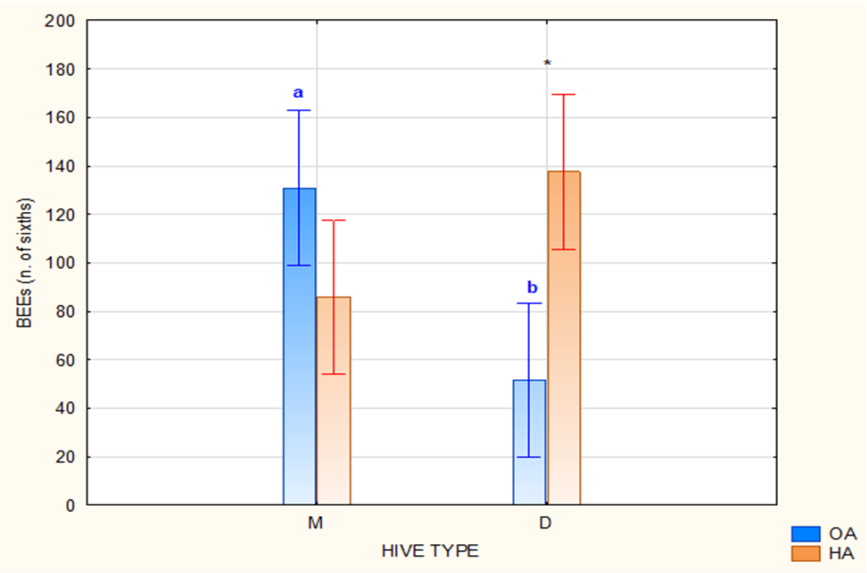
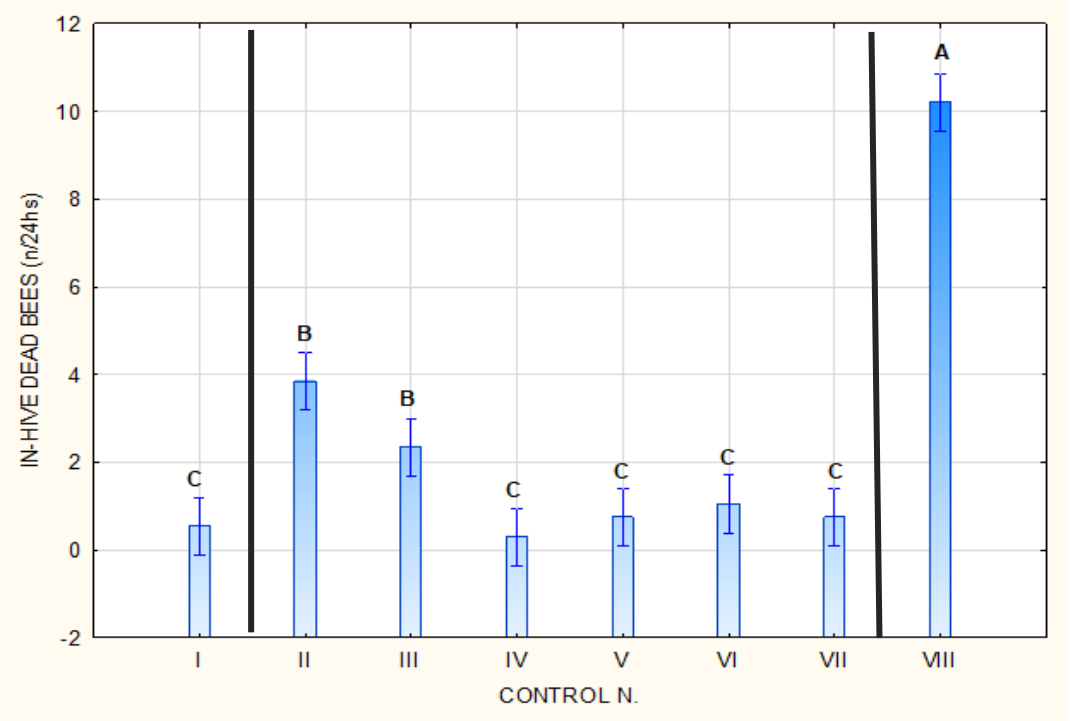


Figure 2. The effect of the different Hive type space on the bee sixth



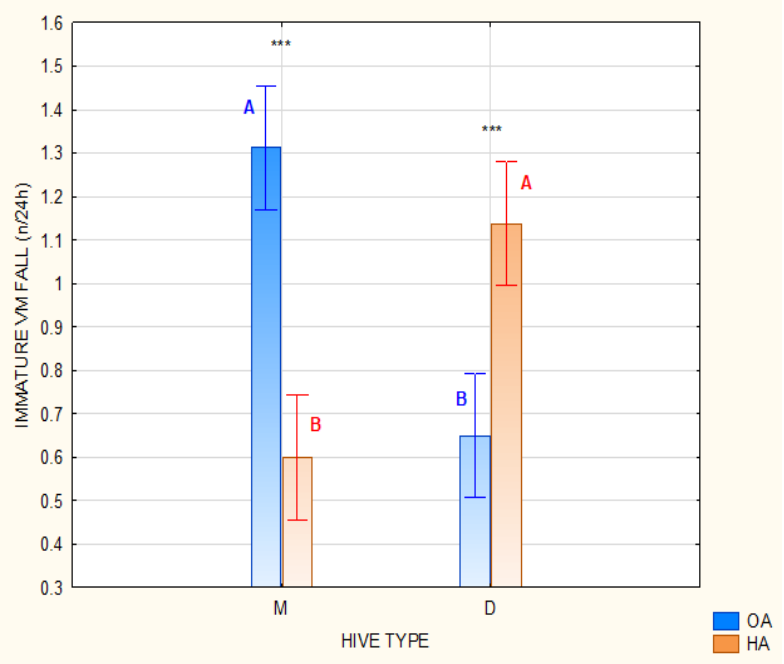
*Different letters affixed above the bars denote mean values significantly different at p<0.05; *--- p<0.05*

Figure 3. The effect of the control number on the in-hive mortality



Different letters affixed above the bars denote mean values significantly different at p<0.001; the black bar in the graph stands for the time of treatments.

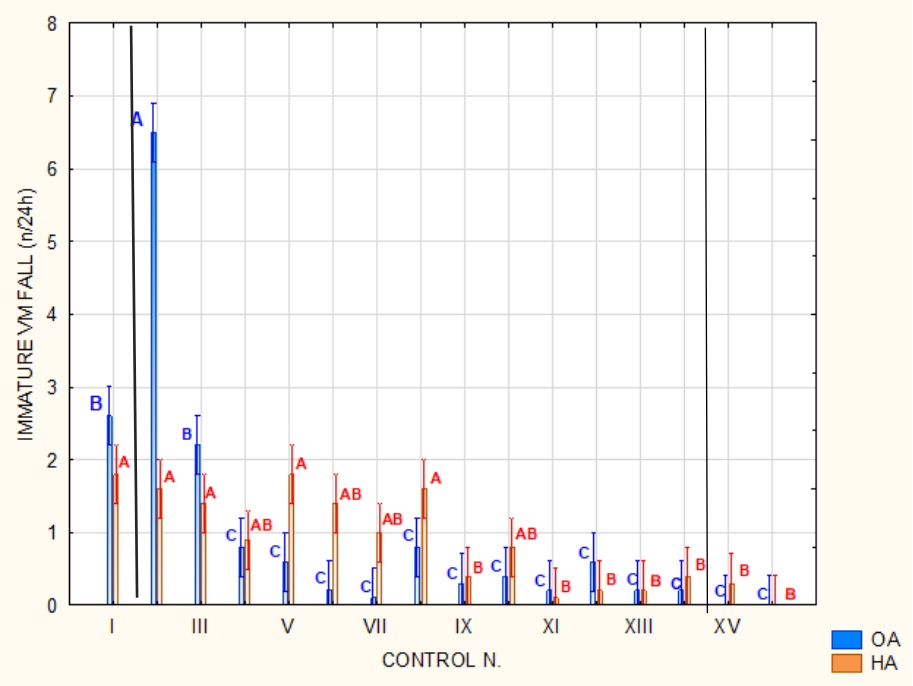
Figure 4. The effect of different treatmens × hive type on the immature varroa mortality



Different letters affixed above the bars denote mean values significantly

*different at p<0.001,**—p<0.001*

Figure 5. The effect of different treatments × control n. on the immature varroa mortality



Different letters affixed above the bars denote mean values significantly different at p<0.001, the black bars in the graph stands for the time of treatments.

References

Allabergenova, A., G. Turganbayeva, and M. Nurseitova. 2021. Control of Varroa Destructor in Kazakhstan. Arch. Razi Inst. 76:1163–1171. doi:10.22092/ari.2021.355621.1704.

Almaguer, C., C. Schönberger, M. Gastl, E. K. Arendt, and T. Becker. 2014. Humulus lupulus - a story that begs to be told. A review. J. Inst. Brew. 120:289–314. doi:10.1002/jib.160.

Anderson, D. L., and J. W. H. Trueman. 2000. Varroa jacobsoni (Acari: Varroidae) is more than one species. Exp. Appl. Acarol. 24:165–189. doi:10.1023/A:1006456720416.

Annoscia, D., F. Del Piccolo, and F. Nazzi. 2012. How does the mite Varroa destructor kill the honeybee Apis mellifera? Alteration of cuticular hydrcarbons and water loss in infested honeybees. J. Insect Physiol. 58:1548–1555. doi:10.1016/j.jinsphys.2012.09.008.

Bahreini, R., M. Nasr, C. Docherty, O. de Herdt, S. Muirhead, and D. Feindel. 2020. Evaluation of

potential miticide toxicity to Varroa destructor and honey bees, Apis mellifera, under laboratory conditions. Sci. Rep. 10:1–14. doi:10.1038/s41598-020-78561-2. Available from: https://doi.org/10.1038/s41598-020-78561-2

Bartlett, L. J. 2022. Frontiers in effective control of problem parasites in beekeeping. Int. J. Parasitol. Parasites Wildl. 17:263–272. doi:10.1016/j.ijppaw.2022.03.003. Available from: https://doi.org/10.1016/j.ijppaw.2022.03.003

Berry, J. A., L. J. Bartlett, S. Bruckner, C. Baker, S. K. Braman, K. S. Delaplane, and G. R. Williams. 2021. Assessing Repeated Oxalic Acid Vaporization in Honey Bee (Hymenoptera: Apidae) Colonies for Control of the Ectoparasitic Mite Varroa destructor. J. Insect Sci. 22:15–16. doi:10.1093/jisesa/ieab089. Available from: https://doi.org/10.1093/jisesa/ieab089

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. doi:10.1051/apido:2002029.

Brodschneider, R., A. Gray, N. Adjlane, A. Ballis, V. Brusbardis, J. Charrière, R. Chlebo, M. F. Coffey, B. Dahle, D. C. De Graaf, M. M. Dražić, G. Evans, I. Forsythe, A. Gregorc, U. Grzęda, A. Hetzroni, P. Kristiansen, M. Martikkala, R. Martín-hernández, A. Ryzhikov, N. Simon-delso, J. Stevanovic, A. Uzunov, R. Brodschneider, A. Gray, N. Adjlane, and A. Ballis. 2018. Multi-country loss rates of honey bee colonies during winter 2016 / 2017 from the COLOSS survey. J. Apic. Res. 57:452–457. doi:10.1080/00218839.2018.1460911. Available from: https://doi.org/10.1080/00218839.2018.1460911

Calderone, N. W. 2005. Integrated Pest Management for Varroa Destructor in the Northeastern United States using Drone Brood Removal and Formic Acid. Cornell Univ.

DeGrandi-Hoffman, G., F. Ahumada, R. Curry, G. Probasco, and L. Schantz. 2014. Population growth of Varroa destructor (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids. Exp. Appl. Acarol. 64:171–186. doi:10.1007/s10493-014-9821-z.

DeGrandi-Hoffman, G., F. Ahumada, G. Probasco, and L. Schantz. 2012. The effects of beta acids from hops (Humulus lupulus) on mortality of Varroa destructor (Acari: Varroidae). Exp. Appl. Acarol. 58:407–421. doi:10.1007/s10493-012-9593-2.

Giacobino, A., A. Molineri, N. B. Cagnolo, J. Merke, E. Orellano, E. Bertozzi, G. Masciángelo, H. Pietronave, A. Pacini, C. Salto, and M. Signorini. 2015. Risk factors associated with failures of Varroa treatments in honey bee colonies without broodless period. Apidologie. 46:573–582. doi:10.1007/s13592-015-0347-0. Available from: www.satscan.org

Gray, A., N. Adjlane, A. Arab, A. Ballis, V. Brusbardis, J. D. Charrière, R. Chlebo, M. F. Coffey, B. Cornelissen, C. Amaro 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. doi:10.1080/00218839.2020.1797272. Available from: https://doi.org/10.1080/00218839.2020.1797272

Imdorf, A., and C.- Berna. 1999. Prontuario per la rilevazione della forza della colonia. 1–4.

Ivana Smodiš Škerl, M., J. Rivera-Gomis, I. Tlak Gajger, J. Bubnič, G. Talaki, G. Formato, A. Baggio, F. Mutinelli, W. Tollenaers, D. Laget, V. Malagnini, L. Zanotelli, and M. Pietropaoli. 2021. Efficacy and Toxicity of VarroMed ® Used for Controlling Varroa destructor Infestation in Different Seasons and Geographical Areas Toxicity of VarroMed ® Used for. doi:10.3390/app11188564. Available from: https://doi.org/10.3390/app11188564

Jack, C. J., and J. D. Ellis. 2021. Integrated Pest Management Control of Varroa destructor (Acari: Varroidae), the Most Damaging Pest of (Apis mellifera L. (Hymenoptera: Apidae)) Colonies. J. Insect Sci. 21:6–7. doi:10.1093/jisesa/ieab058. Available from: https://doi.org/10.1093/jisesa/ieab058Review

Jones, G., C. A. M. Campbell, J. Hardie, J. A. Pickett, B. J. Pye, and L. J. Wadhams. 2003. Integrated management of two-spotted spider mite Tetranychus urticae on hops using hop beta-acids as an antifeedant together with predatory mite Phytoseiulus persimilis. Biocontrol Sci. Technol. v. 13.

Karabín, M., T. Hudcová, L. Jelínek, and P. Dostálek. 2016. Biologically Active Compounds from Hops and Prospects for Their Use. Compr. Rev. Food Sci. Food Saf. 15:542–567. doi:10.1111/1541-4337.12201.

Llorente, J., and M. Suárez. 1999. Original article Negative long-term effects on bee colonies treated with oxalic acid against Varroa jacobsoni Oud . Mariano a Aránzazu Meana a. Apidologie. 30:289–292.

Lodesani, M., and C. Costa. 2008. Maximizing the efficacy of a thymol based product against the mite Varroa destructor by increasing the air space in the hive. J. Apic. Res. 47:113–117. doi:10.1080/00218839.2008.11101436.

Macedo, P. A., J. Wu, and M. D. Ellis. 2002. Using inert dusts to detect and assess varroa infestations in honey bee colonies. J. Apic. Res. 41:3–7. doi:10.1080/00218839.2002.11101062.

Maddaloni, M., and D. W. Pascual. 2015. Isolation of oxalotrophic bacteria associated with Varroa destructor mites. Lett. Appl. Microbiol. 61:411–417. doi:10.1111/lam.12486.

Manino, A., and A. Patetta. 2004. Bee space: social behaviour and basis of rational beekeeping. Redia. LXXXVII:203–206.

Manzano Sánchez, L., M. J. Gómez Ramos, M. del M. Gómez-Ramos, P. Parrilla Vazquez, J. M. Flores, and A. R. Fernández-Alba. 2021. Presence, persistence and distribution of thymol in honeybees and

beehive compartments by high resolution mass spectrometry. Environ. Adv. 5. doi:10.1016/j.envadv.2021.100085.

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. doi:10.1126/science.1220941.

Milani, N. 1999. The resistance of Varroa jacobsoni Oud. to acaricides. Apidologie. 30:229–234. doi:10.1051/apido:19990211.

Moškrič, A., J. Bubnič, M. I. S. Škerl, and J. Prešern. 2018. Potential positive effects of hop beta acids on parasitic mite varroa destructor control in honey bee colonies. J. Hyg. Eng. Des. 24:21–28.

Paudel, Y. P., R. Mackereth, R. Hanley, and W. Qin. 2015. Honey Bees (Apis mellifera L.) and Pollination Issues: Current status, impacts and potential drivers of decline. J. Agric. Sci. 7:93–109. doi:10.5539/jas.v7n6p93.

Piccirillo, G., and D. De Jong. 2003. The influence of brood comb cell size on the reproductive behavior of the ectoparasitic mite Varroa destructor in Africanized honey bee colonies. Genet. Mol. Res. 2:36–42.

Pietropaoli, M., and G. Formato. 2019. Acaricide efficacy and honey bee toxicity of three new formic acid-based products to control Varroa destructor. J. Apic. Res. 58:824–830. doi:10.1080/00218839.2019.1656788.

Poonia, A., R. Gulati, and S. Sharma. 2009. Efficacy of Screen Floor and Powdered Sugar against Varroa destructor Anderson and Trueman in Apis mellifera L. Colonies. Biopestic. Int. 5:0.

Porrini, C. 2007. Le api come bioindicatori dell'inquinamento ambientale. Dip. di Sci. e Tecnol. Agroambientali (DiSTA), Entomol. Univ. di Bol. 3.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25:345–353. doi:https://doi.org/10.1016/j.tree.2010.01.007. Available from: https://www.sciencedirect.com/science/article/pii/S0169534710000364

Rademacher, E., and M. Harz. 2006. Oxalic acid for the control of varroosis in honey bee colonies – a review. Apidologie. 37:98–120. doi:10.1051/apido:2005063. Available from: http://10.0.4.27/apido:2005063

Rademacher, E., M. Harz, and S. Schneider. 2015. The development of HopGuard® as a winter treatment against Varroa destructor in colonies of Apis mellifera. Apidologie. 46:748–759. doi:10.1007/s13592-015-0363-0.

Reams, T., J. Rangel, and M. Lopez-Uribe. 2022. Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of Varroa destructor, the Parasitic Mite of Apis mellifera. J. Insect Sci.

22:18–19. doi:10.1093/jisesa/ieab101. Available from: https://doi.org/10.1093/jisesa/ieab101Review

Rice, N. D., M. L. Winston, and H. A. Higo. 2004. Integrated pest management for the parasitic mite Varroa destructor (Anderson and Trueman) in colonies of honey bees (Apis mellifera). Am. Bee J. 144:791–795.

Rinderer, T. E., J. W. Harris, G. J. Hunt, and L. I. De Guzman. 2010. Breeding for resistance to Varroa destructor in North America. Apidologie. 41:409–424. doi:10.1051/apido/2010015.

Shumkova, R., R. Balkanska, and P. Hristov. 2021. The herbal supplements nozemat herb® and nozemat herb plus®: An alternative therapy for n. ceranae infection and its effects on honey bee strength and production traits. Pathogens. 10:1–19. doi:10.3390/pathogens10020234.

Underwood, R., and M. Lopez-Uribe. 2019. Methods to Control Varroa Mites: An Integrated Pest Management Approach. Pennsylvania State Univ. Coll. Agric. Sci. 1–5. Available from: https://extension.psu.edu/methods-to-control-varroa-mites-an-integrated-pest-management-approach

Vandervalk, L. P., M. E. Nasr, and L. M. Dosdall. 2014. New miticides for integrated pest management of Varroa destructor (Acari: Varroidae) in honey bee colonies on the Canadian prairies. J. Econ. Entomol. 107:2030–2036. doi:10.1603/EC14048.

Wantuch, H., and D. Tarpy. 2009. Removal of Drone Brood From Apis mellifera (Hymenoptera: Apidae) Colonies to Control Varroa destructor (Acari: Varroidae) and Retain Adult Drones. J. Econ. Entomol. 102:2033–2040. doi:10.1603/029.102.0603.

Ward, R., M. F. Coffey, and K. Kavanagh. 2022. Exposure of Apis mellifera to anti-Varroa destructor formic acid treatment induces significant proteomic alterations. J. Apic. Res. 0:1–9. doi:10.1080/00218839.2022.2038055. Available from: https://doi.org/10.1080/00218839.2022.2038055

Zar, J. H. 2014. Biostatistical Analysis.

4. GEOGRAPHICAL DISCRIMINATION OF CITRUS HONEYS FROM MEDITERRANEAN BASIN (CASE STUDY 3)

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Introduction

Honey is a natural viscous, aromatic and sweet substance produced by the honey bees (*Apis mellifera* L.) from the nectar of flowers, usable by humans without having to be transformed (Bettar et al. 2019). Honey is a complex foodstuff, contains more than 200 different organic and inorganic compounds (Alvarez-Suarez et al. 2010). Among which, being a type of carbohydrate, sugars predominate (40% fructose, 30% glucose), water (17%), and in a much lower percentage with other sugars, carbohydrates, little of vitamins and minerals, and complex patterns of phytochemicals (Alvarez-Suarez et al. 2010; Kasiotis et al. 2023; Sollid 2020).

Relevant difference in some physical-chemical traits such as colour, electrical conductivity, pH, moisture content, proline content, HMF content, invertase, diastase activity and free acidity can help to separate the different kind of honey (Pita-Calvo and Vázquez 2017; Waykar Bhalchandra 2022). Many of these traits, which are also honey' quality indicators, can depend on several factors, such as: i) production season; ii) honey harvesting and processing; iii) honey conservation; iv) botanical composition; v) geographical origin (Al-Mamary, Al-Meeri, and Al-Habori 2002; Andersen and Markham 2005; Beretta et al. 2005; Bertoncelj et al. 2007; Khalil et al. 2012; Saxena, Gautam, and Sharma 2010).

Honey contains minerals in percentage that varies according to the type, and the mineral content can encompass some undesired trace elements (sometimes called "heavy metals", as lead) which are relevant marker for assessing the honey quality under the food safety standpoint (Hernández

et al. 2005; Solayman et al. 2016). Toxic elements in honey can come from pollution sources such as industrial plants, environmental disasters, urbanization, anthropic areas or pesticides usage, and can cause health problems (Bogdanov 2006; Mititelu et al. 2022; Perna et al. 2021; Solayman et al. 2016). Notwithstanding, the consumers' expectations for honey as natural, unmanipulated and high-quality food are usually high. Overall, the authenticity of honey lies on two main aspects (Bogdanov and Martin 2002): i) according to its origin (*i.e.*, geographical, botanical); ii) with respect to its description (*e.g.*, natural, organic, raw and unheated). It should be emphasized that there is often a strong relationship between botanical and geographical origins; some nectar producing species are closely linked to a specific geographical area, making it rather easy to identify the geographical origin of honey given its botanical composition, or at most to reduce the number of possible origins (Trifković et al. 2017).

One of the most appreciated honeys in the Mediterranean region is the citrus honey, a honey produced by nectars of various *Citrus* species and related hybrids. In the liquid state it is almost colorless to straw yellow, but it becomes from white, sometimes pearly, to light beige in the crystalline state (Persano Oddo and Piro 2004). Its aroma is characterized by the occurrence of caffeine (Jerković et al. 2016). In monofloral citrus honey, citrus pollen (highly relevant for the melissopalinological characterization) is usually underrepresented, reaching a quota of 10% of total pollens, at the most, unless there are only nectar sources of the genus *Citrus* genus in the nectar collection area (Louveaux, Maurizio, and Vorwohl 1978; Mendes et al. 1998).

As far as the botanical origin, in Europe more than 100 botanical species can potentially give monofloral honeys (Persano Oddo and Piro 2004). Monofloral honey is a food that is often subject to adulteration, mainly carried out through the fraudulent addition of exogen sugars, different kind of syrups, or the addition of poor-quality honey (Ballabio et al. 2018; Everstine, Spink, and Kennedy 2013; Padovan et al. 2003; Zhu et al. 2010). In Europe there is a legislative frame protecting the quality of honey (Council & European Parliament, 2002; European Council & European Parliament, 2014), but the importation of honey with qualitative characteristics and origins different from those set by law and reported on the label is still a major problem. The problem is particularly relevant for monofloral honeys, which are more expensive and thus frequently objects of counterfeiting. In general, despite the legislative efforts of the European Commission, the adulteration of honey in European Union (EU) still appears to be a relevant issue, quantifiable with a value greater than 14% of the marketed honey in the EU (Aries et al. 2016; European Council and European Parliament 2014).

For the determination botanical and geographical origin of honey, several methods have been developed, applied and evaluated in recent decades (Danieli and Lazzari 2022; Fernández-Torres et al. 2005; Soares et al. 2017). The honey composition, its physical and organoleptic properties depend mainly on the types of floral nectars or insects' sweet secretions gathered by the honey bees. In principle, different types of honey having different chemical profiles, such as the mineral profile, should be rather easy to distinguish, and to identify. However, in some circumstances, the elemental compositions of different monofloral honeys are similar, making the discrimination work difficult. Likewise, the geographical discrimination of monofloral honeys with the sole use of the mineral profile can be difficult, due to similarities in the soil and climatic conditions between the various regions of origin (I.K. Karabagias et al. 2017; M. Shantal Rodríguez-Flores et al. 2019). However, a large number of studies have confirmed the possibility of characterizing honey samples by quantifying selected elements in combination with other physical-chemical traits which allow for significant improvements of the discriminant power of the analysis (Jovetić et al., 2017; Bergamo et al., 2018; Karabagias et al., 2018; Danieli and Lazzari, 2022). The analysis of isotopic ratios for stable elements (*e.g.*, C, N) is mostly used to discover fraudulent add of exogen sugars in honey (C. T. Chen et al. 2019; Luo et al. 2016; Padovan et al. 2003), but some studies in recent years have also proven the validity of the technique to support the investigation of the geographical and botanical origin of honey (Bontempo et al. 2017; She et al. 2019). As a single parameter is insufficient to trace the geographical origin of honey (Danieli and Lazzari, 2022), a number of methods in combination are routinely to get more accurate and standardized results (Schiassi et al., 2021). In this context an integrated evaluation of mineral profiling, chemical-physical analysis and isotopic ratios can be useful for assessing the quality and traceability of the monofloral honeys. In particular by comparing the data with threshold values indicated by current legislation, such as for example the threshold value of lead or humidity as regards European legislation (European Council & European Parliament, 2014). Or compare the data obtained with the data resulting from the bibliographic research, focusing on the area of origin of the samples.

In this work, the analysis of the mineral profile of *Citrus* honey, coming from three countries of the Mediterranean basin (Italy, Greece and Egypt), and from three different harvest seasons (2020-2021-2022), were associated with analysis of chemical-physical parameters and isotope ratios of carbon and nitrogen. As a first aim, the quality and safety of citrus honeys were evaluated against the European legislative frame for honey quality (moisture, pH, electrical conductivity, free acidity and colour) and food safety (lead concentration). For the other honey traits, in particular for the potentially toxic elements (*i.e.*, As, Cd, Cr, Hg) for which there are no European legal limits, a comparison was made with the data coming out of the bibliographic

research. As the secondary, though not less important, aim of this study was also to evaluate whether the association of different characterizing analytical methods can be able to discriminate the geographical origin of the *Citrus* honeys, in the light of finding which set chemical-physical traits can lead to the better discrimination.

Materials and Methods

Honey samples

For this research, were used a total of 110 honey samples of citrus honey from the Plant-B case study areas, of 2020, 2021 and 2022 harvesting seasons, coming from Greece, Italy and Egypt (Table 1). All the samples respected the national regulatory frames to be commercialized as "citrus honey". The honey samples were stored in 50 mL centrifuge tubes or in glass jars away from sunlight and maintained at a temperature of 23-25 °C. For each harvesting season, samples' internal traceability was ensured by assigning them univocal codes which included the nation, the region of origin and the harvesting year.

Table 1 Samples of citrus honey divided by harvesting season, country and region of provenance

Harvesting season	Nation	Region	Number of samples
2020	Italy	Sicily	24
2020	Italy	Sardinia	4
2020	Italy	Campania	6
2020	Greece	Peloponnesus	7
2021	Italy	Sicily	14
2021	Italy	Sardinia	6
2021	Italy	Campania	5
2021	Greece	Peloponnesus	10
2021	Egypt	Qalyubia Governorate	5
2022	Italy	Sicily	14
2022	Greece	Peloponnesus	10
2022	Egypt	Qalyubia Governorate	5

Phisico-chemical analysis

The physicochemical traits investigated were: free acidity (FA), electrical conductivity (EC), water content (WC), pH and colour. To determine the FA value of the honey samples the titration method at pH 8.30 was used according to the method established by the International Honey Commission (Bogdanov 2009). Briefly, in a 250 mL beaker, 10.00 ± 0.05 g of honey, diluted in 75 mL of NIST Type I water, was kept in slow stirring by means of a magnetic stirrer during the titration with 0.1M of NAOH until the end-point of pH 8.3, by monitoring the pH changes with a HI2210 pH meter (Hanna Instruments Italia Srl, Italy) pre-calibrate on two points (pH 4.01 and pH 10.01) before each analytical session. The EC was measured by means of a Conductivity Meter (Hanna Instruments Italia Srl, Italy), calibrated before each measurement session by using a 0.1 M potassium chloride (KCl). Aliquots of 10.00 ± 0.20 g of honey was dissolved in NIST Type I water water up to 50 mL final volume. A refractometer Lega HB90 (Lega Srl, Italy) was used to evaluate the percentual WC of the honey samples. The refractometer, able to measure the honey WC in the range 10-32%, was zeroed with distilled water according the manufacturer's operative instructions. The pH measurement of the samples under examination was carried out using a pH 5 FOOD Tester Kit (XS Instruments, Italy), after calibration at pH 4.01 and pH 7.01 before each measurement session. To distinguish the shades of yellow of the different honey samples, a HI 96785 digital colorimeter (Hanna Instruments Italia Srl, Italy) was used. Before analysis, the instrument was calibrated with glycerol according to the manufacturer's instructions. For crystallized honey samples, it was necessary a heat treatment at 37 °C for 10 minutes aimed at melting the sample in order to prevent interference with the bright beam.

Mineral profiling – chemicals and reagents

If not otherwise stated, all the reagents were of analytical grade. Ultrapure water (NIST Type I) was used for all dilutions and glassware/plastics cleaning procedures. Nitric acid (67-69%) for trace analysis (VWR International bvba; Belgium) was used both for cleaning all the instrumentation and for serial dilutions. All glassware and plastics were cleaned by overnight soaking in 10% HNO_3 before a final rinse with ultrapure water. Standard stock solutions (1000 mg/L in 2% NaOH) for Pd, Li, Na, Pb, Al, Co, Ni, Ca, K, Fe, Sn were purchased from Carlo Erba Reagents (Italy); those for Cu, Mg, V, Mn, Hg, As, Cd, Mo, B, Cr were from VWR International bvba (Belgium).

Mineral profiling - Microwave mineralisation

Aliquots of 0.70 ± 0.01 g of honey were mineralized with 6 mL of 65% HNO_3 and 1 mL of ultrapure water by using a microwave Multiwave GO Plus (Anton Paar Italia Srl, Italy). A Reagent Laboratory Blank (RLB) was included in each mineralization cycle (1 out of 8 positions per run). The mineralization program was as follow: a 15-min' ramp to reach 100 °C; 10 min' at 100 °C; a ramp to reach 180 °C in 15 minutes; a decreasing 10-min' ramp to reach the internal vessels' temperature of about 40 °C. Before mineralisation, all the samples and the reagents were handled with non-metallic materials carefully washed with 10% HNO_3.

With the operative aim of avoiding any possible memory effect of one mineralization cycle on the following one, after each mineralization run a standard cleaning procedure for all the vessel was performed with 1 mL of ultrapure water in 4 mL of HNO_3 by running a 10 min' ramp up to an internal temperature of 180 °C, that was then held for 5 min'. Once the cleaning procedure was completed, the vessels were emptied and then left to dry in a static stove at 65°C for two hours.

Mineral profiling - Recovery trials

Due to the lack of suitable Standard Reference Material (SRM) to assess the whole-method' accuracy for the investigated elements in honey, two different SRM from the National Institute of Standards & Technology (Department of Commerce, USA) were used: i) spinach leaves (SRM 1570a) for As, Ni, Hg and V; ii) non-fat milk powder (SRM 1549) for Pd, Na, Pb, Al, Co, Ca, K, Fe, Sn, Cu, Mg, V, Mn, Cd, Mo, Cr. The min and max recoveries were recorded for Mo (69%) and Rb (132%), respectively the recovery value was then applied to the raw data from the elemental analysis. Supplementary Table 1).

Mineral profiling – Atomic absorption spectrophotometry (AAS) analysis

The quantitation of the Ni, Na, Fe, Ca, Mg, K, Zn, Rb, and Al concentrations in honey was carried out by flame atomic absorption spectroscopy (F-AAS), by using an AA 7000 (Shimadzu Italia Srl, Italia) atomic absorption spectrophotometer equipped with an ASC-7000 autosampler (Shimadzu Italia Srl, Italia). Acetylene 6.0 pure grade (AirLiquide, Italy) was used as combustible and compressed air as comburent. For Pb, Mo, Co, Mn, Cd, Cr, Sn, V, Li, Cu the analysis were performed by graphite furnace atomic absorption spectroscopy (GF-AAS), by using a GFA 7000A (Shimadzu Italia Srl, Italia) with an ASC-7000 (Shimadzu Italia Srl, Italia) autosampler. For the graphite furnace operations, argon 6.0 pure grade (Air Liquide, Italy) and pyrolytic coated graphite tubes (Shimadzu Italia Srl, Italia) with a platform were used. A 100 mg/l palladium solution (Carlo Erba Reagents, Italy) was used as a matrix modifier for the analysis of Pb and Cd, applying it to all the solutions analysed, blank solutions and standards

included. The analysis of Hg and As were carried out according to the hydride vapor techniques (HVG-AAS) by means of a HVG-1 generator (Shimadzu Italia Srl, Italia) linked to the burner of the F-AAS. For the generation of the hydrides vapor, HCl 67 % (VWR international, Italia), sodium borohydride 98% and sodium hydroxide 98% (Sigma Aldrich, Italy) were used as reactants. Multi-element (for Fe, Cu, Mn, Cd, K, Na, Mg, Co, Ca, Cr and Al) or single element (for Pb, Mo, Ni, Zn, Rb, Sn, V, Li, As and Hg) hollow cathode lamps (Hamamatsu Photonics K.K., Japan), were used as radiation sources.

The standard solutions used for the AAS calibration were daily prepared by diluting standard stock solutions of each element to 10 mg/l , and then further diluted in HNO_3 (67%) the desired calibrators (Supplementary Table 2). As far as the elements analyzed by GF-AAS, the serial dilutions were carried out through the autosampler, and the calibration ranges varied between 0.2 to 100 µg/l with minimum fitting (R^2) of 0.995 (Co). For the elements analysed by F-AAS the calibration ranges varied between 0.06 and 10.00 mg/l with a minimum value of R^2 of 0.995 (Mg). For those elements analysed by HGV-AAS, the calibration ranges varied from 0.4 to 5.0 mg/l, with a minimum value of R^2 equal to 0.996 (As). Only for some elements (*i.e.*, Ca, Zn, Co, Sn and Mo) 2nd order calibration curves were used to get satisfying curves' fittings.

Isotope ratio mass spectrometry (IRMS)

The analysis of the C and N isotopic ratios of the honey samples were performed at the Laboratory of the Research Institute on Terrestrial Ecosystems (IRET) of the National Research Council (CNR) of Porano (TR, Italy). Before the analysis, the samples were kept for 5 days in a freezer at a temperature of -85°C. Subsequently, after placing them in a rack with the cap open, they were placed in a LIO5P lyophilizer (5 PASCAL S.r.L., Italy) at a temperature of about -52°C and a pressure of 0.06 mbar and maintained in the freeze dryer for a week. After that, the samples were taken and cleaned of any bubbles, possibly formed during their stay in the lyophilizer, using a special scalpel suitably cleaned passing from one sample to another. Before carrying out the encapsulation and before IRMS analysis, some test runs were made to test how much sample to insert in the capsule in order to obtain an acceptable chromatographic peak (*i.e.*, 2 - 15 nA). This procedure was done both for the analyzes on ^{13}C and on ^{15}N. Once the suitable mass has been identified according on the percentage of analyzed element occurring in the sample (max 1 mg for carbon and about 6-8 mg for nitrogen), the sample was placed in a 5 × 9 mm tin capsule. The capsules with samples, and the appropriate reference standards were placed in the sampler of a NA 1500 elemental analyzer (Carlo Erba Instruments, Italy), connected to the IsoPrime isotope ratio mass spectrometer (GV Instruments Ltd., UK).

Data processing and statistical analysis

All the data were analysed by factorial ANOVA through Statistica 10 (StatSoft Inc., Tulsa, USA). The significance of the differences in the comparison between means was assayed through the Tuckey's post hoc test. Significance was declared at $p<0.05$ and $p<0.01$. To get insights about which were the main factors able to potentially discriminate the citrus honey between the different geographical origins, a Principal Component Analysis (PCA) was performed.

Results and discussions

With regard to the chemical-physical traits of citrus honeys (Tab. 2), all samples analysed for the three different nations, complied with EU Regulation 2001/110/EC values for water content (< 20%) and electric conductivity (0.8 mS/cm).

Also, with regard to the legal limits required by EU Regulation 2015/1005, which indicates a value of less than 0.1 mg/kg as the limit value for lead, our samples showed lower values for all three source nations (Table 2)

Univariate results

Talking about the statistical analysis, and the univariate results (Tab. 2), the C effect was significant ($p<0.01$) on the variables pH (3.75 ± 0.25, overall mean), EC (0.31 ± 0.17 mS/cm) and FA (5.51 ± 1.23 meq/kg), with the values of the Italian samples that were greater than those of the Egyptian samples for the three parameters analysed, the C effect was significant ($p<0.01$) also for the colour (30.47 ± 10.24 mm Pfund), with values for the Egyptian samples greater than those of the Greek samples. The Y effect was significant ($p<0.01$) for WC, with higher values found in the 2022 samples. On the other hand, no significant C × Y interactions were found.

Table 2. Effect of the harvesting year (Y) and the country of origin (C) on the physio-chemical parameters (mean ± SE) measured in Citrus honey samples.

Parameter	**Country (C)[1]**			**Year (Y)**		***p*-level**		
	ITA	**EGY**	**GRC**	**2021**	**2022**	**C**	**Y**	**C × Y**
pH	3.8 ± 0.3^{A}	4.0 ± 0.3^{B}	3.9 ± 0.1^{AB}	3.9 ± 0.3	3.7 ± 0.2	<0.01	n.s.	n.s.
WC (%)	17.1 ± 1.0	16.7 ± 1.5	17.2 ± 1.2	16.4 ± 0.9^{B}	16.7 ± 0.9^{A}	n.s.	<0.01	n.s.
EC (mS/cm)	0.4 ± 0.2^{A}	0.3 ± 0.3^{B}	0.2 ± 0.1^{B}	0.3 ± 0.2	0.4 ± 0.3	<0.01	n.s.	n.s.
Colour (mm Pfund)	31.2 ± 0.1^{AB}	35.9 ± 14.0^{A}	26.0 ± 8.3^{B}	32.2 ± 12.0	27.9 ± 7.9	<0.01	n.s.	n.s.
FA (meq/kg)	5.8 ± 0.9^{A}	4.2 ± 1.6^{B}	5.6 ± 1.6^{A}	5.6 ± 1.4	5.4 ± 1.2	<0.01	n.s.	n.s.

ITA=Italian honey samples, EGY=Egyptian honey samples, GRC=Greek honey samples; different capital letters above the means (± standard error) denote values significantly different at p<0.01.

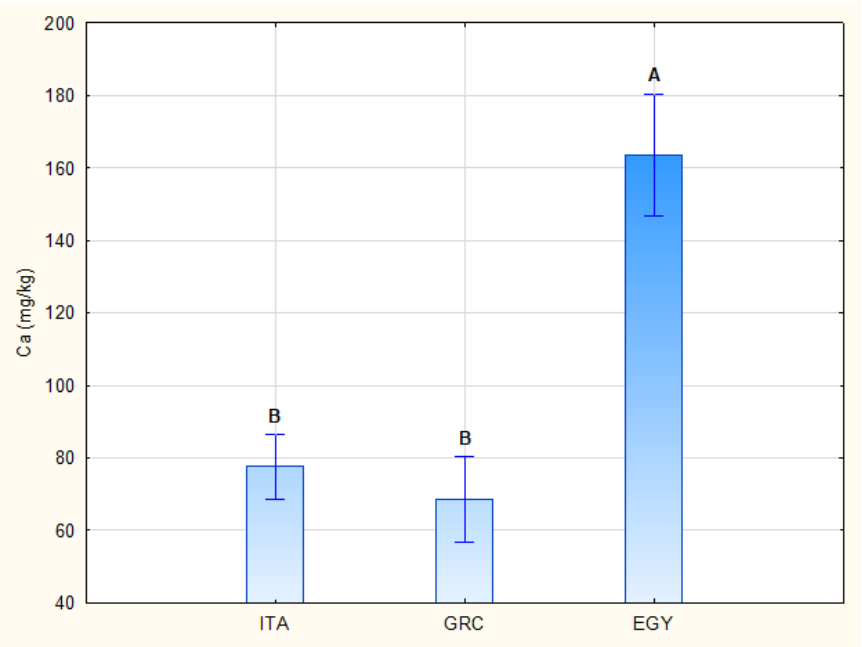
With regard to the macro-elements' concentrations in honey samples (Tab. 3), the C effect was significant ($p<0.01$) for Ca (99.57 ± 58.01 mg/kg, overall means), with The Egyptian honeys showing higher levels than the Greek and Italian samples (Fig. 1). The year of collection affected ($p<0.01$) the honey K content (785.07 ± 536.13 mg/kg) with values for 2021 samples higher than for the 2022 samples. The C × Y interaction was also significant ($p<0.01$) for Ca. No significant differences depending on C or Y were found for the elements Mg and Na.

Table 3. Effect of the harvesting year (Y) and the country of origin (C) on the macro-elements (mean ± SE) analysed in Citrus honey samples.

Element	**Country (C)**			**Harvesting year (Y)**		***p*-level**		
	ITA	**EGY**	**GRC**	**2021**	**2022**	**C**	**Y**	**C × Y**
Ca	88.8 ± 72.2^{B}	163.5 ± 65.8^{A}	68.5 ± 31.5^{B}	113.0 ± 62.2	67.2 ± 68.7	<0.01	n.s.	<0.01
K	853.3 ± 515.3	823.5 ± 657.3	555.1 ± 283.3	910.7± 549.8^{A}	558.3± 318.8^{B}	n.s.	<0.01	n.s.
Mg	47.7 ± 34.3	51.0 ± 26.6	38.3 ± 20.4	46.5 ± 33.6	44.1 ± 24.0	n.s.	n.s.	n.s.
Na	96.4 ± 118.6	70.7 ± 29.2	60.9 ± 37.9	96.3 ± 117.3	63.2 ± 33.7	n.s.	n.s.	n.s.

ITA=Italian honey samples, EGY=Egyptian honey samples, GRC=Greek honey samples; different capital letters above the means (± standard error) denote values significantly different at p<0.01

Figure 1. Effect of the country of origin (C) on the Ca (mg/kg) in citrus honey samples.



ITA=Italian honey samples, EGY=Egyptian honey samples, GRC=Greek honey samples; different capital letters above the bars denote mean value significantly different at p<0.01.

Looking at the micro-elements quantified in citrus honey (Tab. 4), the C effect was significant ($p<0.01$) for Fe (1.73 ± 1.20 mg/kg), with the highest values found in the Egyptian samples, Ni (0.10 ± 0.25 mg/kg) and Sn (0.01 ± 0.02 mg/kg) of which the Greek honeys were particularly rich in comparison to both Egyptian and Italian honey samples, which, in turn, were richer ($p<0.05$) in Mn (0.15 ± 0.11 mg/kg) than the Greek ones. The year of harvesting had significant effect ($p<0.01$) on the content Fe, Mn but also of Zn (0.70 ± 0.60 mg/kg), with the values for the 2021 samples being higher than for the 2022 samples) and of Ni that exhibited an opposite temporal pattern. The Y effect was also significant ($p<0.05$) for Cu (0.33 ± 0.48 mg/kg), with the values of the 2021 samples being higher than the 2022 ones. For the elements Mn, Ni and Fe, but not for Mn, significant Y × C interactions were observed ($p<0.05$).

Table 4. Effect of the harvesting year (Y) and the country of origin (C) on the micro-elements (mean ± SE) analysed in Citrus honey samples.

Element	Country (C)			Harvesting year (Y)		*p*-level		
	ITA	**EGY**	**GRC**	**2021**	**2022**	**C**	**Y**	**C × Y**
Al	0.1 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.4	n.s.	n.s.	n.s.
Cu	0.3 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.4^{A}	0.1 ± 0.04^{B}	n.s.	<0.01	n.s.
Fe	1.9 ± 1.2^{B}	3.2 ± 2.2^{A}	1.3 ± 0.8^{B}	2.3 ± 1.5^{A}	1.4 ± 1.1^{B}	<0.01	<0.01	<0.05
Mn	0.2 ± 0.1^{A}	0.1 ± 0.1^{A}	0.1 ± 0.4^{B}	0.2 ± 0.1^{A}	0.1 ± 0.1^{B}	<0.01	<0.01	<0.01
Ni	0.1 ± 0.1^{B}	0.1 ± 0.0^{B}	0.2 ± 0.2^{A}	0.1 ± 0.0^{B}	0.1 ± 0.2^{A}	<0.01	<0.01	<0.01
Rb	1.3 ± 3.5	0.6 ± 0.6	0.1 ± 0.3	1.2 ± 3.4	0.5 ± 0.1	n.s.	n.s.	n.s.
Sn	0.1 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{A}	0.1 ± 0.1	0.1 ± 0.0	<0.01	n.s.	n.s.
Zn	0.8 ± 0.6	0.5 ± 0.5	0.6 ± 0.7	0.8 ± 0.4^{A}	0.6 ± 0.8^{B}	n.s.	<0.01	<0.01

ITA=Italian honey samples, EGY=Egyptian honey samples, GRC=Greek honey samples; different capital letters above the means (± standard error) denote values significantly different at p<0.01

As far as the toxic trace elements (Tab. 5), the country of origin of honey samples (C) significantly (p<0.01) influenced the Pb content (0.01 ± 0.01 mg/kg, overall mean), with the highest values found in Italian samples if compared to honeys from Greece or Egypt. On the other hand, also the year of collection (Y) affected the Pb, with the value for the 2021 samples being higher than the 2022 (p<0.01). On the contrary the As level (0.05 ± 0.11 mg/kg), was lower in the 2021 samples than the 2022 ones (p<0.01). For such elements, the Y × C interactions were not significant.

Table 5 Effect of the harvesting year (Y) and the country of origin (C) on the undesired-elements (mean ± SE) analysed in Citrus honey samples.

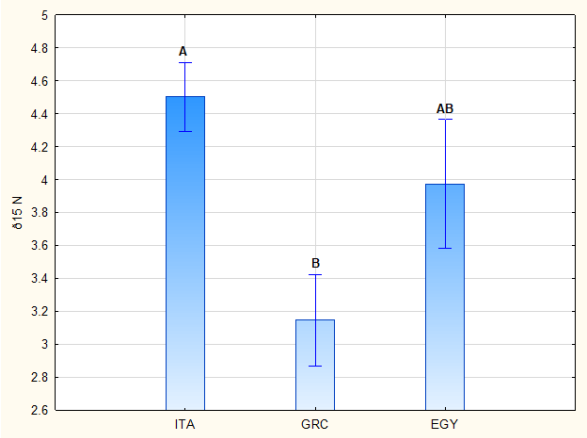
Element	Country (C)			Harvesting year (Y)		*p*-level		
	ITA	**EGY**	**GRC**	**2021**	**2022**	**C**	**Y**	**C × Y**
As	0.04 ± 0.05	0.04 ± 0.05	0.03 ± 0.03	0.01 ± 0.04^{B}	0.08 ± 0.01^{A}	n.s.	<0.01	n.s.
Cd	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.03	0.00 ± 0.00	n.s.	n.s.	n.s.
Cr	0.02 ± 0.03	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.03	0.03 ± 0.02	n.s.	n.s.	n.s.
Hg	0.14 ± 0.16	0.12 ± 0.11	0.07 ± 0.05	0.12 ± 0.17	0.10 ± 0.01	n.s.	n.s.	n.s.
Pb	0.02 ± 0.01^{A}	0.01 ± 0.01^{B}	0.01 ± 0.01^{B}	0.02 ± 0.01^{A}	0.01 ± 0.01^{B}	<0.01	<0.01	n.s.

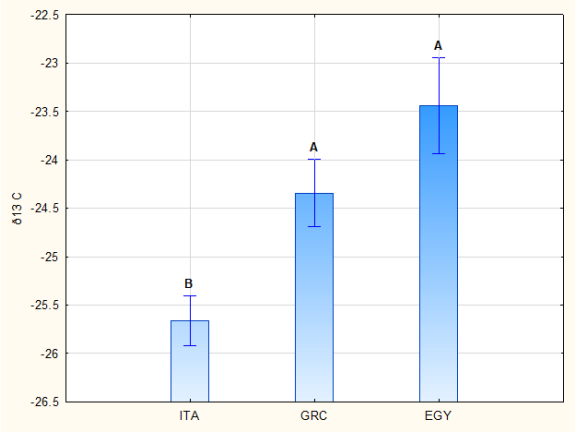
ITA=Italian honey samples, EGY=Egyptian honey samples, GRC=Greek honey samples; different capital letters

above the means (± standard error) denote values significantly different at p<0.01

With regard to IRMS analysis (Tab. Supplementary material), the geographical origin (C) effect was significant (p<0.01) on both isotope ratios considered (Fig. 2-3). With regard to $\delta^{15}N$ (4.05 ± 1.43‰, overall mean), the highest values were found in the Italian samples compared to the Greek ones, while with regard to the $\delta^{13}C$ (-25.05 ± 1.51‰) both Greek and Egyptian citrus honeys had higher values (p<0.01) if compared to the Italian honeys. Both the harvesting year (Y) and the Y×C interactions showed no significant effects on the N and C isotopic abundances.

Figure 2-3. Effect of the country of origin (C) on the nitrogen (left) and carbon (right) isotopic abundances in citrus honey samples.



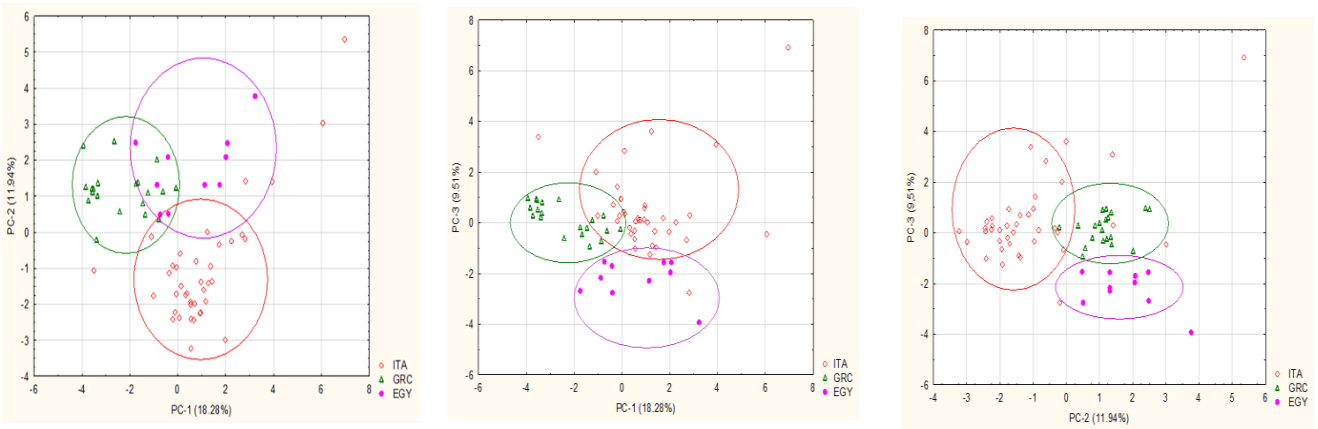


Multivariate analysis

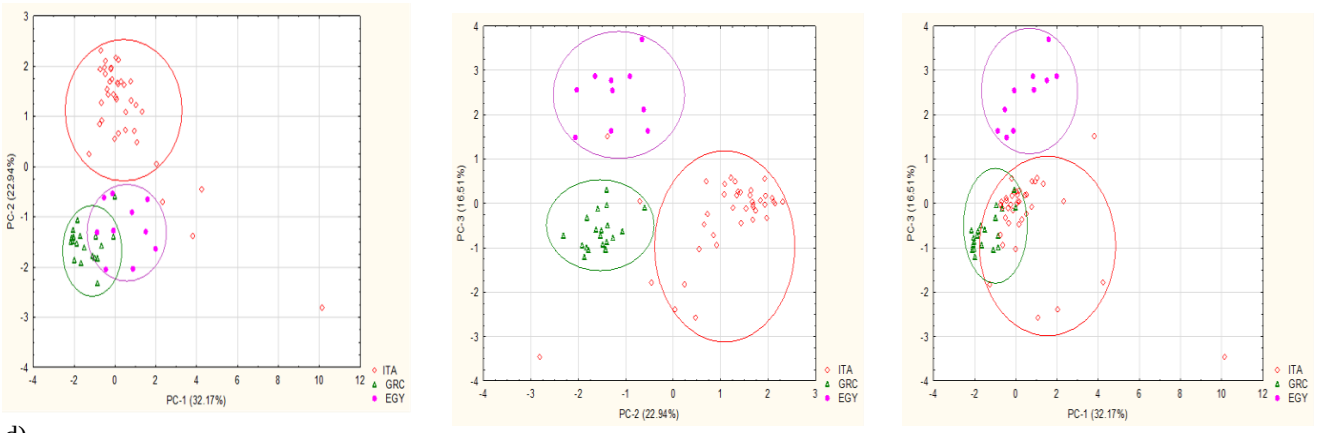
To test whether there was a systematic correlation structure underlying the data, a principal component analysis (PCA) was performed on all the variables measured in the Citrus honey samples. The first component explained 18.28% of the variance, and the second 11.94%, with a total of 30.22% explained variance, the third component explained 9.51%. At this point, the first and second components were considered. In the 1st principal plane (Fig. 3a), the honey samples clustered fairly separated according to the County of origin, though with some overlap between the Italian and Greek samples, and between the Greek and Egyptian samples. In addition, three Italian hone samples were outside the area were lied the great part of the plotted cases. Italian honey samples are mostly located in the III and IV planes with a few samples in quadrant II. In

contrast Greek samples are predominantly found of the I floor. At the same time three Egyptian samples are found placed in the II floor, while all the rest are placed in the I quadrant.

c)



a) b)



d) e) f)

Figure 3, a-b-c: Graph of principal component analysis (PCA) scores of honey samples. The variance explained by PC1, PC2 and PC3 is reported within brackets.
d-e-f: Graph of the PCA scores of honey samples taking into account the 7 variables with highest importance. The variance explained by PC1, PC2 and PC3 is reported within brackets.

In order to have a clearer view in the discrimination of the three nations, a PCA was developed on the basis of the variables that had greater importance in the spatial discrimination of honeys, based on the loadings described in the general PCA (Tab. 5). The variables chosen were δ^{15} N, pH, Cd, Fe, Mg, Rb and Pb. The result was that the first component explained 32.27% of the

variance, the second component 22.94%, and the third component 16.51%, for a total of 71.72% of the variance explained (Fig 3d-e-f).

Table 5 PC1, PC2, PC3 loadings matrix for all the variables analysed

	PC-1	**PC-2**	**PC-3**
pH	0.41	-0.63	-0.02
WC (%)	-0.39	0.36	-0.05
EC (mS/cm)	0.49	0.34	-0.11
COLOUR (mm Pfund)	0.54	0.00	0.11
FA (meq/kg)	0.03	0.26	-0.41
Al	-0.28	-0.12	-0.39
As	-0.18	0.27	-0.06
Ca	0.31	0.03	0.63
Cd	0.43	-0.35	-0.51
Cr	0.23	-0.30	-0.24
Cu	0.16	0.40	0.15
Fe	0.74	-0.09	0.23
Hg	0.07	0.15	0.43
K	0.40	0.26	0.09
Mg	0.72	-0.27	-0.15
Mn	0.23	0.35	0.09
Na	0.55	-0.03	-0.04
Pb	0.70	-0.25	-0.35
Ni	-0.11	-0.10	0.04
Rb	0.30	-0.08	-0.27
Sn	-0.19	0.16	-0.02
Zn	0.26	0.29	0.00
$\delta^{13}C$	-0.05	-0.51	0.38
$\delta^{15}N$	0.27	0.59	0.20

Conclusions

The 81 honey samples from the three different nations of the Mediterranean basin were characterized according to their elemental composition, physicochemical parameters and isotope ratios. The honey samples analyzed met all legal Eurpoean limits for toxic elements (Pb) and physicochemical parameters (WC and EC). The results of the analyses were used to discriminate the origin of the honey samples by applying both univariate and multivariate analysis of the data. The PCA model based on the analyzed parameters was able to partially discriminate the geographical origin of honey samples. Although some elements and parameters showed considerable discriminating potential (pH, Pb, Mg, Fe, Rb, Cd, δ15 N).

References

A., de Ruijter. 1987. “Reproduction of Varroa Jacobsoni during Successive Brood Cycles of the Honeybee.” *Apidologie* v. 18.

Al-Mamary, Mohamed, Ali Al-Meeri, and Molham Al-Habori. 2002. “Antioxidant Activities and Total Phenolics of Different Types of Honey.” *Nutrition Research* 22(9): 1041–47. www.elsevier.com/locate/nutres.

Alaux, Cédric, Christelle Dantec, Hughes Parrinello, and Yves Le Conte. 2011. “Nutrigenomics in Honey Bees: Digital Gene Expression Analysis of Pollen’s Nutritive Effects on Healthy and Varroa-Parasitized Bees.” *BMC Genomics* 12(June 2014).

Aliano, Nicholas P., and Marion D. Ellis. 2009. “Oxalic Acid: A Prospective Tool for Reducing Varroa Mite Populations in Package Bees.” *Experimental and Applied Acarology* 48(4): 303–9.

Aljadi, A. M., and M. Y. Kamaruddin. 2004. “Evaluation of the Phenolic Contents and Antioxidant Capacities of Two Malaysian Floral Honeys.” *Food Chemistry* 85(4): 513–18.

Allabergenova, A., G. Turganbayeva, and M. Nurseitova. 2021. “Control of Varroa Destructor in Kazakhstan.” *Archives of Razi Institute* 76(5): 1163–71.

Almaguer, Cynthia et al. 2014. “Humulus Lupulus - a Story That Begs to Be Told. A Review.” *Journal of the Institute of Brewing* 120(4): 289–314.

Alvarez-Suarez, Jose Miguel et al. 2010. “Contribution of Honey in Nutrition and Human Health: A Review.” *Mediterranean Journal of Nutrition and Metabolism* 3(1): 15–23.

Andersen, Øyvind M., and Kenneth R. Markham. 2005. “Flavonoids: Chemistry, Biochemistry and Applications.” *Flavonoids: Chemistry, Biochemistry and Applications*: 1–1239.

Anderson, D. L., and J. W.H. Trueman. 2000a. “Varroa Jacobsoni (Acari: Varroidae) Is More than One Species.” *Experimental and Applied Acarology* 24(3): 165–89.

Anderson, D L, and J W H Trueman. 2000b. *Varroa Jacobsoni (Acari: Varroidae) Is More than One Species*.

Anklam, Elke. 1998. “A Review of the Analytical Methods to Determine the Geographical and Botanical Origin of Honey.” *Food Chemistry* 63(4): 549–62.

Annoscia, Desiderato, Fabio Del Piccolo, and Francesco Nazzi. 2012. “How Does the Mite Varroa Destructor Kill the Honeybee Apis Mellifera? Alteration of Cuticular Hydrcarbons and Water Loss in Infested Honeybees.” *Journal of Insect Physiology* 58(12): 1548–55.

Ansari, Mohammad Javed et al. 2018. “Validation of Botanical Origins and Geographical Sources of Some Saudi Honeys Using Ultraviolet Spectroscopy and Chemometric Analysis.” *Saudi Journal of Biological Sciences* 25(2): 377–82. https://linkinghub.elsevier.com/retrieve/pii/S1319562X17302280.

Aries, Eric et al. 2016. “Scientific Support to the Implementation of a Coordinated Control Plan with a View to Establishing the Prevalence of Fraudulent Practices in the Marketing of Honey.” *JRC Technical Reports*: 27. https://ec.europa.eu/jrc.

Attali, Myriam et al. 2007. “Control of β-Cell Differentiation by the Pancreatic Mesenchyme.” *Diabetes* 56(5): 1248–58.

Bahreini, Rassol et al. 2020. “Evaluation of Potential Miticide Toxicity to Varroa Destructor and Honey Bees, Apis Mellifera, under Laboratory Conditions.” *Scientific Reports* 10(1): 1–14. https://doi.org/10.1038/s41598-020-78561-2.

Ballabio, Davide et al. 2018. “Chemical Profiling and Multivariate Data Fusion Methods for the Identification of the Botanical Origin of Honey.” *Food Chemistry* 266: 79–89.

Balog, Julia et al. 2010. “Identification of Biological Tissues by Rapid Evaporative Ionization Mass Spectrometry.” *Analytical Chemistry* 82(17): 7343–50.

Barbattini, Renzo, and Stefano Fugazza. 2006. “L’ape Nell’arte Antica.” *Apitalia* 32: 12–17.

Bartlett, Lewis J. 2022. “Frontiers in Effective Control of Problem Parasites in Beekeeping.” *International Journal for Parasitology: Parasites and Wildlife* 17(March): 263–72. https://doi.org/10.1016/j.ijppaw.2022.03.003.

Beinert, Helmut, Mary Claire Kennedy, and C David Stout. 1996. “Aconitase as Ironminus SignSulfur Protein, Enzyme, and Iron-Regulatory Protein.” *Chemical reviews* 96(7): 2335–74.

Bellucci, V. et al. 2019. “Monitoring Honey Bee Healthin Five Natural Protected Areas in Italy.” *Veterinaria italiana* 55(1): 15–25.

Beretta, Giangiacomo et al. 2005. “Standardization of Antioxidant Properties of Honey by a Combination of Spectrophotometric/Fluorimetric Assays and Chemometrics.” *Analytica Chimica Acta* 533(2): 185–91.

Bergamo, Greici et al. 2018. “Mineral Profile as a Potential Parameter for Verifying the Authenticity of Bracatinga Honeydew Honeys.” *LWT* 97: 390–95.

Berry, Jennifer A et al. 2021. “Assessing Repeated Oxalic Acid Vaporization in Honey Bee (Hymenoptera: Apidae) Colonies for Control of the Ectoparasitic Mite Varroa Destructor.” *Journal of Insect Science* 22(1): 15–16. https://doi.org/10.1093/jisesa/ieab089.

Bertoncelj, Jasna, Urška Doberšek, Mojca Jamnik, and Terezija Golob. 2007. “Evaluation of the Phenolic Content, Antioxidant Activity and Colour of Slovenian Honey.” *Food Chemistry* 105(2): 822–28.

Bettar, Ismail et al. 2019. “Characterisation of Moroccan Spurge (Euphorbia) Honeys by Their Physicochemical Characteristics, Mineral Contents and Colour.” *Arabian Journal of Chemistry* 12(8): 2052–60.

Blacquière, Tjeerd, Gertraut Altreuther, and Klemens J. Krieger. 2017. “Evaluation of the Efficacy and Safety of Flumethrin 275 Mg Bee-Hive Strips (PolyVar Yellow®) against Varroa Destructor in

Naturally Infested Honey Bee Colonies in a Controlled Study." *Parasitology Research* 116: 109–22.

Bogdanov, Stefan et al. 2002. "Determination of Residues in Honey after Treatments with Formic and Oxalic Acid under Field Conditions." *Apidologie* 33: 399–409.

———. 2006. "Contaminants of Bee Products." *Apidologie* 37(1): 1–18.

———. 2009. "Harmonised Methods of the International IHC." *Bee Product Science,* (5): 1–62.

Bogdanov, Stefan, V Kilchenmann, and A Imdorf. 1998. "Acaricide Residues in Some Bee Products." *Journal of Apicultural Research* 37: 57–67.

Bogdanov, Stefan, and Peter Martin. 2002. *Honey Authenticity: A Review*.

Boi, Michela et al. 2016. "A 10 Year Survey of Acaricide Residues in Beeswax Analysed in Italy." *Pest Management Science* 72(7): 1366 – 1372. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84969792521&doi=10.1002%2Fps.4161&partnerID=40&md5=d0d0e717d38d9ebfe35ad1616f656b61.

Bontempo, Luana et al. 2017. "Isotopic and Elemental Composition of Selected Types of Italian Honey." *Measurement: Journal of the International Measurement Confederation* 98: 283–89.

Bonzini, Sara et al. 2011. "Predicting Pesticide Fate in the Hive (Part 1): Experimentally Determined τ-Fluvalinate Residues in Bees, Honey and Wax." *Apidologie* 42(3): 378–90.

Bormetti, Matteo. 2014. "Api e Miele Nel Mediterraneo Antico." *Acme* 66(1–2): 7–50.

Bortolotti, Laura et al. 2009. "Salute Delle Api : Analisi Dei Fattori Di Rischio . Il Progetto Apenet." (September 2014).

Bowen-Walker, P L, Stephen Martin, and Alan Gunn. 1999. "The Transmission of Deformed Wing Virus between Honeybees (Apis MelliferaL.) by the Ectoparasitic MiteVarroa JacobsoniOud." *Journal of invertebrate pathology* 73: 101–6.

Brodschneider, Robert et al. 2018. "Multi-Country Loss Rates of Honey Bee Colonies during Winter 2016 / 2017 from the COLOSS Survey." *Journal of Apicultural Research* 57(3): 452–57. https://doi.org/10.1080/00218839.2018.1460911.

Brodschneider, Robert, and Karl Crailsheim. 2010. "Nutrition and Health in Honey Bees." *Apidologie* 41(3): 278–94.

Brunner, Franziska S, Paul Schmid-Hempel, and Seth M Barribeau. 2014. "Protein-Poor Diet Reduces Host-Specific Immune Gene Expression in Bombus Terrestris." *Proceedings. Biological sciences* 281(1786).

Burkert, W. 2003. *La Religione Greca Di Epoca Arcaica e Classica*. Milano: Jaca Book.

Cajka, T., M. R. Showalter, K. Riddellova, and O. Fiehn. 2016. "Advances in Mass Spectrometry for Food Authenticity Testing: An Omics Perspective." *Advances in Food Authenticity Testing*: 171–200.

Calderone, Nicholas W. 2005. “Integrated Pest Management for Varroa Destructor in the Northeastern United States Using Drone Brood Removal and Formic Acid.” *Cornell University*.

Calzoni, G. L., Rondinini, T. 2012. “Api, Impollinazione e Miele.” *Al Sas*: 52–59.

Carmen Gigliotti, Roberto Verga. 2007. *Biotecnologie Alimentari*. Piccin-Nuova Libraria.

Carreck, Norman, Brenda Ball, and Stephen Martin. 2010. “Honey Bee Colony Collapse and Changes in Viral Prevalence Associated with Varroa Destructor.” *Journal of Apicultural Research* 49: 93–94.

Cevallos-Cevallos, Juan M. et al. 2009. “Metabolomic Analysis in Food Science: A Review.” *Trends in Food Science and Technology* 20(11–12): 557–66.

Chen, Chun Ting et al. 2019. “Novel Inspection of Sugar Residue and Origin in Honey Based on the 13C/12C Isotopic Ratio and Protein Content.” *Journal of Food and Drug Analysis* 27(1): 175–83.

Chen, Y P et al. 2004. “Detection of Deformed Wing Virus Infection in Honey Bees, Apis Mellifera L., in the United States.” *American Bee Journal* 144: 557–59.

Chen, Yan Ping, and Reinhold Siede. 2007. “Honey Bee Viruses.” *Advances in Virus Research* 70: 33 – 80. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34548304773&doi=10.1016%2FS0065-3527%2807%2970002-7&partnerID=40&md5=3855e3c8328cc0f225d2e0f46b624059.

Chua, Lee Suan, Norul Liza A. Rahaman, Nur Ardawati Adnan, and Ti Tjih Eddie Tan. 2013. “Antioxidant Activity of Three Honey Samples in Relation with Their Biochemical Components.” *Journal of Analytical Methods in Chemistry* 2013.

Chuda-Mickiewicz, Bożena, J Prabucki, J Kazimierczak, and Jerzy Samborski. 2007. “The Varroacidal Efficacy of Apitraz.” *Medycyna Weterynaryjna* 63: 110–12.

Coffey, Mary, and John Breen. 2016. “The Efficacy and Tolerability of Api-Bioxal ® as a Winter Varroacide in a Cool Temperate Climate.” *Journal of Apicultural Research* 55: 1–9.

Commission of the European Communities. 1988. *European Research on Varroatosis Control*. 1ST ed. ed. CRC.

Connolly, Joanne B, Antonietta Wallace, and Sara Stead. 2016. “Discrimination of Honey of Different Botanical Origins Using an Untargeted High-Definition.” : 2016.

Consonni, Roberto, and Laura Ruth Cagliani. 2019. “The Potentiality of NMR-Based Metabolomics in Food Science and Food Authentication Assessment.” *Magnetic Resonance in Chemistry* 57(9): 558–78.

Contessi, A. 2016. *Le Api. Biologia, Allevamento, i Prodotti*. Bologna: Edagricole.

Conti, Marcelo Enrique et al. 2007. “Characterization of Italian Honeys (Marche Region) on the Basis of Their Mineral Content and Some Typical Quality Parameters.” *Chemistry Central Journal* 1(1).

Corvucci, Francesca, Lara Nobili, Dora Melucci, and Francesca Vittoria Grillenzoni. 2015. “The Discrimination of Honey Origin Using Melissopalynology and Raman Spectroscopy Techniques Coupled with Multivariate Analysis.” *Food Chemistry* 169: 297–304.

Council, European, and European Parliament. 2002. “COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 Relating to Honey.” *Official Journal of the European Communities* L10.

Crane, E., Graham, A. J. 1985. *No TitleBee Hives of the Ancient World (Part I)*.

Cubero-Leon, Elena, Rosa Peñalver, and Alain Maquet. 2014. “Review on Metabolomics for Food Authentication.” *Food Research International* 60: 95–107. http://dx.doi.org/10.1016/j.foodres.2013.11.041.

De D’Aubeterre, Judith Principal, David D. Myrold, Lynn A. Royce, and Philippe A. Rossignol. 1999. “A Scientific Note of an Application of Isotope Ratio Mass Spectrometry to Feeding by the Mite, Varroa Jacobsoni Oudemans, on the Honeybee, Apis Mellifera L.” *Apidologie* 30(4): 351–52.

Dainat, Benjamin et al. 2012. “Dead or Alive: Deformed Wing Virus and Varroa Destructor Reduce the Life Span of Winter Honeybees.” *Applied and Environmental Microbiology* 78(4): 981–87.

Danieli, Pier Paolo, and Filippo Lazzari. 2022. “Honey Traceability and Authenticity. Review of Current Methods Most Used to Face This Problem.” *JOURNAL OF APICULTURAL SCIENCE* 66(2): 101–19.

David Aston, Sally Bucknall. 2021. *Good Nutrition-Good Bees*. Hebden Bridge: Northern Bee Books.

DeGrandi-Hoffman, Gloria et al. 2014. “Population Growth of Varroa Destructor (Acari: Varroidae) in Commercial Honey Bee Colonies Treated with Beta Plant Acids.” *Experimental and Applied Acarology* 64(2): 171–86.

DeGrandi-Hoffman, Gloria, Fabiana Ahumada, Gene Probasco, and Lloyd Schantz. 2012. “The Effects of Beta Acids from Hops (Humulus Lupulus) on Mortality of Varroa Destructor (Acari: Varroidae).” *Experimental and Applied Acarology* 58(4): 407–21.

Delaplane, Keith. 1999. “Bee Products: Properties, Applications, and Apitherapy.” *American Entomologist* 45.

Desneux, Nicolas, Axel Decourtye, and Jean-Marie Delpuech. 2007. “The Sublethal Effects of Pesticides on Beneficial Arthropods.” *Annual Review of Entomology* 52: 81 – 106. https://www.scopus.com/inward/record.uri?eid=2-s2.0-33846354297&doi=10.1146%2Fannurev.ento.52.110405.091440&partnerID=40&md5=1fd845edca244ffdac1bccfd3a5ca597.

Devillers, J., M. Morlot, M. H. Pham-Delègue, and J. C. Doré. 2004. “Classification of Monofloral Honeys Based on Their Quality Control Data.” *Food Chemistry* 86(2): 305–12.

Dietemann, Vincent et al. 2012. “Varroa Destructor: Research Avenues towards Sustainable Control.” *Journal of Apicultural Research* 51(1): 125–32.

Döke, Mehmet Ali, Maryann Frazier, and Christina M Grozinger. 2015. “Overwintering Honey Bees: Biology and Management.” *Current opinion in insect science* 10: 185–93.

Dzugan, Małgorzata, Monika Tomczyk, Patrycja Sowa, and Dorota Grabek-Lejko. 2018. “Antioxidant Activity as Biomarker of Honey Variety.” *Molecules* 23(8): 2069.

Ebert, T A et al. 2007. “Oral Toxicity of Essential Oils and Organic Acids Fed to Honey Bees (Apis Mellifera).” *Journal of apicultural research* v. 46.

Elzen, P J, and David Westervelt. 2002. “Detection of Coumaphos Resistance in Varroa Destructor in Florida.” *American Bee Journal* 142: 291–92.

Engwa, Godwill Azeh, Paschaline Udoka Ferdinand, Friday Nweke Nwalo, and Marian N Unachukwu. 2019. “Mechanism and Health Effects of Heavy Metal Toxicity in Humans.” In *Poisoning in the Modern World*, eds. Ozgur Karcioglu and Banu Arslan. Rijeka: IntechOpen. https://doi.org/10.5772/intechopen.82511.

ENRICO ZAGNOLI, ILARIA BIGANZOLI CORAZZA. 2013. *Una Speranza per Le Api*. Agri.Bio.Edizioni, Cissone, Cuneo,.

EU COMMISSION. 2023. “Honey Market Presentation.” (April).

European Council, and European Parliament. 2014. “Directive 2014/63/EU of the European Parliament and of the Council of 15 May 2014 Amending Council Directive 2001/110/EC Relating to Honey.” *Official Journal of the European Communities* L164(May): 1–5.

Everstine, Karen, John Spink, and Shaun Kennedy. 2013. “Economically Motivated Adulteration (EMA) of Food: Common Characteristics of EMA Incidents.” *Journal of Food Protection* 76(4): 723–35.

Faucon, J.-P, P Drajnudel, Marie-Pierre Chauzat, and M Aubert. 2007. “Control of the Efficacy of APIVAR ND against Varroa Destructor, a Parasite of Apis Mellifera.” *Revue de Medecine Veterinaire* 158: 283–90.

Fernández-Torres, Rut et al. 2005. “Mineral Content and Botanical Origin of Spanish Honeys.” *Talanta* 65(3): 686–91.

Ferrer-Dufol, M, A I Martinez-Viñuales, and C Sanchez-Acedo. 1991. “Comparative Tests of Fluvalinate and Flumethrin to Control Varroa Jacobsoni Oudemans.” *Journal of Apicultural Research* 30(2): 103–6. https://doi.org/10.1080/00218839.1991.11101241.

Fries, Ingemar, and Scott Camazine. 2001. “Fries_2001_Vertical Trasmision.” *Apidologie* 32: 199–214.

Frilli, F. 1983. “Varroa Jacobsoni: The Situation in Italy.” *Varroa jacobsoni Oud. affecting honey bees :*

present status and needs: proceedings of a meeting of the EC Experts' Group, Wageningen, 7-9 February 1983 / edited by R. Cavalloro.

Frost, Elisabeth H., Dave Shutler, and Neil Kirk Hillier. 2013. "Effects of Fluvalinate on Honey Bee Learning, Memory, Responsiveness to Sucrose, and Survival." *Journal of Experimental Biology* 216(15): 2931–38.

Fuchs, S. 1990. "Preference for Drone Brood Cells by Varroa Jacobsoni Oud in Colonies of Apis Mellifera Carnica." *Apidologie* 21(3): 193–99.

Garedew, Assegid, Erik Schmolz, and Ingolf Lamprecht. 2004. "The Energy and Nutritional Demand of the Parasitic Life of the Mite Varroa Destructor." *http://dx.doi.org/10.1051/apido:2004032* 35.

Gashout, Hanan. 2009. "Acute Toxicity of Essential Oils and Other Natural Compounds to the Parasitic Mite, Varroa Destructor, and to Larval and Adult Worker Honey Bees (Apis Mellifera L.)." *Journal of Apicultural Research - J APICULT RES* 48: 263–69.

Genersch, Elke, and Michel Aubert. 2010. "Emerging and Re-Emerging Viruses of the Honey Bee (Apis Mellifera L.)." *Veterinary research* 41(6): 54.

Genersch, Elke, Constanze Yue, Ingemar Fries, and Joachim R de Miranda. 2006. "Detection of Deformed Wing Virus, a Honey Bee Viral Pathogen, in Bumble Bees (Bombus Terrestris and Bombus Pascuorum) with Wing Deformities." *Journal of invertebrate pathology* 91(1): 61–63.

Giacobino, Agostina et al. 2015. "Risk Factors Associated with Failures of Varroa Treatments in Honey Bee Colonies without Broodless Period." *Apidologie* 46(5): 573–82. www.satscan.org.

Giacomelli, Alessandra et al. 2016. "Combination of Thymol Treatment (Apiguard®) and Caging the Queen Technique to Fight Varroa Destructor." *Apidologie* 47(4): 606–16.

Giovenazzo, Pierre, and Pascal Dubreuil. 2011. "Evaluation of Spring Organic Treatments against Varroa Destructor (Acari: Varroidae) in Honey Bee Apis Mellifera (Hymenoptera: Apidae) Colonies in Eastern Canada." *Experimental & applied acarology* 55: 65–76.

Girisgin, Ahmet, and Levent Aydin. 2010. "Efficacies of Formic, Oxalic and Lactic Acids Against Varroa Destructor in Naturally Infested Honeybee (Apis Mellifera L.) Colonies in Turkey." *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 16: 941–45.

Giuliani, T. 2017. "LE LACRIME DI RA – L'apicoltura e l'importanza Delle Api Nell'antico Egitto." *Mediterraneo Antico*.

Goodwin, M ., and C. Van Eaton. 2001. New Zealand Ministry of Agriculture and Forestry *Control of Varroa (A Guide for New Zealand Beekeepers)*. http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:CONTROL+OF+VARROA+A+Guide+for+New+Zealand+Beekeepers#2.

Gray, Alison et al. 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.” *Journal of Apicultural Research* 59(5): 744–51. https://doi.org/10.1080/00218839.2020.1797272.

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 | Zastosowanie Preparatów Zawieraja{ogonek}cych Tymol Oraz Amitrazy I Kwasu Szczawiowego W Zwalczaniu Roztoczy Varroa W .” *Journal of Apicultural Science* 56(2): 61–69.

Gregorc, Ales, and Ivo Planinc. 2001. “Original Article Acaricidal Effect of Oxalic Acid in Honeybee (Apis Mellifera) Colonies.” 32: 333–40.

Harbo, John, and Jeffrey Harris. 2004. “Effect of Screen Floors on Populations of Honey Bees and Parasitic Mites (Varroa Destructor).” *Journal of Apicultural Research* 43.

Heimpel, George E., and Jetske G. De Boer. 2008. “Sex Determination in the Hymenoptera.” *Annual Review of Entomology* 53(January 2014): 209–30.

Hernández, O. M. et al. 2005. “Characterization of Honey from the Canary Islands: Determination of the Mineral Content by Atomic Absorption Spectrophotometry.” *Food Chemistry* 93(3): 449–58.

Honey source. 2023. “The 5 Largest Honey-Producing Countries in the World.” *honeysource.com*.

Van Hoorn, M. Ter Horst, R. 2014. “(12) Patent Application Publication (10) Pub . No .: UUS 2014/0045407 A1 Patent Application Publication.” 1(19): 33. https://patents.google.com/patent/US20140331942A1/en.

Howis, Maciej, and Piotr Nowakowski. 2009. “Varroa Destructor Removal Efficiency Using Beevital Hive Clean Preparation.” *Journal of Apicultural Science* 53: 15–20.

Huang, Zachary. 2010. “Honey Bee Nutrition.” *American Bee Journal* 150(8): 773–76.

Imdorf, Anton et al. 2003. “Alternative Strategy in Central Europe for the Control of Varroa Destructor in Honey Bee Colonies.” *Apiacta* 38.

Imdorf, Anton, and Ch- Berna. 1999. “Prontuario per La Rilevazione Della Forza Della Colonia.” : 1–4.

IPBES. 2019. “Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.” https:/www./ipbes.net/.

Iqbal, Javaid, and Uli Mueller. 2007. “Virus Infection Causes Specific Learning Deficits in Honeybee Foragers.” *Proceedings of the Royal Society B: Biological Sciences* 274(1617): 1517 – 1521. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34447325877&doi=10.1098%2Frspb.2007.0022&partnerID=40&md5=4147aafed873d8e18e9cb5874777f6c5.

ISMEA. 2019. “Il Settore Apistico Nazionale.” : 1–24.

ISPRA. 2020. News.Ge *Il Declino Delle Api e Degli Impollinatori*.

———. 2021. *Piante E Insetti Impollinatori*. 350/2021. ISPRA.

Ivana Smodiš Škerl, Maja et al. 2021. “Efficacy and Toxicity of VarroMed ® Used for Controlling Varroa Destructor Infestation in Different Seasons and Geographical Areas Toxicity of VarroMed ® Used For.” https://doi.org/10.3390/app11188564.

Jack, Cameron J., and James D. Ellis. 2021a. “Integrated Pest Management Control of Varroa Destructor (Acari: Varroidae), the Most Damaging Pest of (Apis Mellifera L. (Hymenoptera: Apidae)) Colonies.” *Journal of Insect Science* 21(5).

Jack, Cameron J, and James D Ellis. 2021b. “Integrated Pest Management Control of Varroa Destructor (Acari: Varroidae), the Most Damaging Pest of (Apis Mellifera L. (Hymenoptera: Apidae)) Colonies.” *Journal of Insect Science* 21(5): 6–7. https://doi.org/10.1093/jisesa/ieab058Review.

Jamal, Muhmmad et al. 2020. “Detection of Flumethrin Acaricide Residues from Honey and Beeswax Using High Performance Liquid Chromatography (HPLC) Technique.” *Journal of King Saud University - Science* 32.

Janmaat, A. F., and M. L. Winston. 2000. “The Influence of Pollen Storage Area and Varroa Jacobsoni Oudemans Parasitism on Temporal Caste Structure in Honey Bees (Apis Mellifera L.).” *Insectes Sociaux* 47(2): 177–82.

Jerković, Igor et al. 2016. “Traceability of Satsuma Mandarin (Citrus Unshiu Marc.) Honey through Nectar/Honey-Sac/Honey Pathways of the Headspace, Volatiles, and Semi-Volatiles: Chemical Markers.” *Molecules* 21(10).

Jiménez, Juan, José Bernal, María Nozal, and Maria Martín. 2005. “Residues of Organic Contaminants in Beeswax.” *European Journal of Lipid Science and Technology* 107: 896–902.

Johnson, Reed M., Lizette Dahlgren, Blair D. Siegfried, and Marion D. Ellis. 2013. “Acaricide, Fungicide and Drug Interactions in Honey Bees (Apis Mellifera).” *PLoS ONE* 8(1).

Johnson, Reed M et al. 2010. “Pesticides and Honey Bee Toxicity - USA * To Cite This Version :” *Apidologie* 41(3): 312–31.

Johnson, Reed M, Henry S Pollock, and May R Berenbaum. 2009. “Synergistic Interactions between In-Hive Miticides in Apis Mellifera.” *Journal of economic entomology* 102(2): 474–79.

Jones, G et al. 2003. “Integrated Management of Two-Spotted Spider Mite Tetranychus Urticae on Hops Using Hop Beta-Acids as an Antifeedant Together with Predatory Mite Phytoseiulus Persimilis.” *Biocontrol science and technology* v. 13.

De Jong, David, Roger Morse, and George Eickwort. 2003. “Mite Pests of Honey Bees.” *Annual Review of Entomology* 27: 229–52.

Jovetić, Milica et al. 2017a. “Mineral Content as a Tool for the Assessment of Honey Authenticity.” *Journal of AOAC International* 100(4): 862–70.

———. 2017b. “Mineral Content as a Tool for the Assessment of Honey Authenticity.” *Journal of AOAC International* 100(4): 862–70.

Kadar, Melinda et al. 2011. “Volatile Fraction Composition and Physicochemical Parameters as Tools for the Differentiation of Lemon Blossom Honey and Orange Blossom Honey.” *Journal of the Science of Food and Agriculture* 91(15): 2768–76.

Kanost, Michael, and Maureen Gorman. 2008. “Phenoloxidases in Insect Immunity.” In *Insect Immunology*, , 69–iv.

Karabagias, I.K. et al. 2017. “Characterization and Classification of Commercial Thyme Honeys Produced in Specific Mediterranean Countries According to Geographical Origin, Using Physicochemical Parameter Values and Mineral Content in Combination with Chemometrics.” *European Food Research and Technology* 243(5): 889–900.

Karabagias, Ioannis K. et al. 2018. “Characterization and Botanical Differentiation of Monofloral and Multifloral Honeys Produced in Cyprus, Greece, and Egypt Using Physicochemical Parameter Analysis and Mineral Content in Conjunction with Supervised Statistical Techniques.” *Journal of Analytical Methods in Chemistry* 2018.

Karabín, Marcel, Tereza Hudcová, Lukáš Jelínek, and Pavel Dostálek. 2016. “Biologically Active Compounds from Hops and Prospects for Their Use.” *Comprehensive Reviews in Food Science and Food Safety* 15(3): 542–67.

Kasiotis, Konstantinos M et al. 2023. “Fingerprinting Chemical Markers in the Mediterranean Orange Blossom Honey: UHPLC-HRMS Metabolomics Study Integrating Melissopalynological Analysis, GC-MS and HPLC-PDA-ESI/MS.” *Molecules (Basel, Switzerland)* 28(9).

Kavanagh, Saorla et al. 2019. “Physicochemical Properties and Phenolic Content of Honey from Different Floral Origins and from Rural versus Urban Landscapes.” *Food Chemistry* 272: 66–75.

Khalifa, Shaden A. M. et al. 2021. “Crop Production.” : 1–23.

Khalil, Md Ibrahim et al. 2012. “Physicochemical and Antioxidant Properties of Algerian Honey.” *Molecules* 17(9): 11199–215.

Koeniger, N, and S Fuchs. 1989. “Eleven Years With Varroa—Experiences, Retrospects and Prospects.” *Bee World* 70(4): 148–59. https://doi.org/10.1080/0005772X.1989.11099011.

Korta, E et al. 2003. “Determination of Amitraz and Other Acaricide Residues in Beeswax.” *Analytica*

Chimica Acta 475(1–2): 97 – 103. https://www.scopus.com/inward/record.uri?eid=2-s2.0-0037414672&doi=10.1016%2FS0003-2670%2802%2901221-7&partnerID=40&md5=a563143a732e8748f6b247c97173147f.

Koulis, Georgios A. et al. 2021. "Honey Phenolic Compound Profiling and Authenticity Assessment Using Hrms Targeted and Untargeted Metabolomics." *Molecules* 26(9): 1–21.

Kralj, Jasna, Axel Brockmann, Stefan Fuchs, and Jürgen Tautz. 2007. "The Parasitic Mite Varroa Destructor Affects Non-Associative Learning in Honey Bee Foragers, Apis Mellifera L." *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 193(3): 363–70.

Kraus, Bernhard, and Stefan Berg. 1994. "Effect of a Lactic Acid Treatment during Winter in Temperate Climate upon Varroa Jacobsoni Oud. and the Bee (Apis Mellifera L.) Colony." *Experimental and Applied Acarology* 18(8): 459–68.

Krupke, Christian H. et al. 2012. "Multiple Routes of Pesticide Exposure for Honey Bees Living near Agricultural Fields." *PLoS ONE* 7(1).

Kuenen, L P S, and N W Calderone. 1997. "Transfers of Varroa Mites from Newly Emerged Bees : Preferences for Age- and Function-Specific Adult Bees (Hymenoptera : Apidae)." 10(2).

Kuterbach, D A, and B Walcott. 1986. "Iron-Containing Cells in the Honey-Bee (Apis Mellifera). II. Accumulation during Development." *The Journal of experimental biology* 126: 389–401.

Laube, I. et al. 2010. "Development of Primer and Probe Sets for the Detection of Plant Species in Honey." *Food Chemistry* 118(4): 979–86.

Leida, Barbara, Giorgio Della Valle, and Maria Lucia Piana. I quaderni dell'apicoltore *Flora Apistica*. www.bombus.it.

Leza, M. Mar, Gregori Llado, and Miguel A. Miranda-Chueca. 2015. "Comparison of the Efficacy of Apiguard (Thymol) and Apivar (Amitraz) in the Control of Varroa Destructor (Acari: Varroidae)." *Spanish Journal of Agricultural Research* 13(3): 1–5.

Llorente, Jesús, and Miguel Suárez. 1999. "Original Article Negative Long-Term Effects on Bee Colonies Treated with Oxalic Acid against Varroa Jacobsoni Oud . Mariano a Aránzazu Meana A." *Apidologie* 30(4): 289–92.

Lodesani, Marco, and Cecilia Costa. 2008. "Maximizing the Efficacy of a Thymol Based Product against the Mite Varroa Destructor by Increasing the Air Space in the Hive." *Journal of Apicultural Research* 47: 113–17.

Louveaux, J., Anna Maurizio, and G. Vorwohl. 1978. "Methods of Melissopalynology." *Bee World* 59(4): 139–57.

Luo, Donghui et al. 2016. “Hydrogen (2H/1H) Combined with Carbon (13C/12C) Isotope Ratios Analysis to Determine the Adulteration of Commercial Honey.” *Food Analytical Methods* 9(1): 255–62.

M. Carmen, Seijo-Coello, Olga Escuredo, and María Fernández-González. 2011. “Fungal Diversity in Honeys from Northwest Spain and Their Relationship to the Ecological Origin of the Product.” *Grana* 50: 55–62.

M. L. Winston. 1991. “The Biology of the Honey Bee.” In *Harvard University Press. - USA,*.

Macedo, P. A., J. Wu, and M. D. Ellis. 2002. “Using Inert Dusts to Detect and Assess Varroa Infestations in Honey Bee Colonies.” *Journal of Apicultural Research* 41(1–2): 3–7.

Maddaloni, M., and D. W. Pascual. 2015. “Isolation of Oxalotrophic Bacteria Associated with Varroa Destructor Mites.” *Letters in Applied Microbiology* 61(5): 411–17.

Magdas, Dana Alina et al. 2021. “Applications of Emerging Stable Isotopes and Elemental Markers for Geographical and Varietal Recognition of Romanian and French Honeys.” *Food Chemistry* 334(July 2020): 127599. https://doi.org/10.1016/j.foodchem.2020.127599.

Manino, Aulo, and Augusto Patetta. 2004. “Bee Space: Social Behaviour and Basis of Rational Beekeeping.” *Redia* LXXXVII: 203–6.

Manning, Rob. 2001. “Fatty Acids in Pollen: A Review of Their Importance for Honey Bees.” *Bee World* 82(2): 60–75. https://doi.org/10.1080/0005772X.2001.11099504.

Manzano Sánchez, Lorena et al. 2021. “Presence, Persistence and Distribution of Thymol in Honeybees and Beehive Compartments by High Resolution Mass Spectrometry.” *Environmental Advances* 5.

Maria Rizelio, Viviane et al. 2012. “Fast Determination of Cations in Honey by Capillary Electrophoresis: A Possible Method for Geographic Origin Discrimination.” *Talanta* 99: 450–56.

Martano, M., Maiolino, P., Power, K. 2016. Ateneapoli editore *Gestione Igienico Sanitaria Degli Apiari a Salvaguardia Dell’ambiente e Della Biodiversità*.

Martin, Stephen J et al. 2012. “Global Honey Bee Viral Landscape Altered by a Parasitic Mite.” *Science (New York, N.Y.)* 336(6086): 1304–6.

Martin, Stephen J, Brenda V Ball, and Norman L Carreck. 2010. “Prevalence and Persistence of Deformed Wing Virus (DWV) in Untreated or Acaricide-Treated Varroa Destructor Infested Honey Bee (Apis Mellifera) Colonies.” *Journal of Apicultural Research* 49(1): 72–79. https://doi.org/10.3896/IBRA.1.49.1.10.

Mendes, E., E. Brojo Proença, I. M.P.L.V.O. Ferreira, and M. A. Ferreira. 1998. “Quality Evaluation of Portuguese Honey.” *Carbohydrate Polymers* 37(3): 219–23.

Milani, N. 2001. “Activity of Oxalic and Citric Acids on the Mite Varroa Destructor in Laboratory Assays.” *Apidologie* 32(2): 127–38.

Milani, Norberto. 1999. “The Resistance of Varroa Jacobsoni Oud. to Acaricides.” *Apidologie* 30(2–3): 229–34.

Milko Sinacori. 2014. “Analisi Delle Popolazioni Microbiche Ricorrenti Nel Miele.” Università degli studi di Palermo.

Minaei, Saeid et al. 2017. “VIS/NIR Imaging Application for Honey Floral Origin Determination.” *Infrared Physics and Technology* 86: 218–25.

de Miranda, J R, and I Fries. 2008. “Venereal and Vertical Transmission of Deformed Wing Virus in Honeybees (Apis Mellifera L.).” *Journal of invertebrate pathology* 98(2): 184–89.

de Miranda, Joachim R, and Elke Genersch. 2010. “Deformed Wing Virus.” *Journal of invertebrate pathology* 103 Suppl: S48-61.

Mititelu, Magdalena et al. 2022. “Quality Control of Different Types of Honey and Propolis Collected from Romanian Accredited Beekeepers and Consumer’s Risk Assessment.” *Crystals* 12(1): 1–18.

Möckel, Nadine, Sebastian Gisder, and Elke Genersch. 2011. “Horizontal Transmission of Deformed Wing Virus: Pathological Consequences in Adult Bees (Apis Mellifera) Depend on the Transmission Route.” *The Journal of general virology* 92(Pt 2): 370–77.

Molan, P.C. 1998. “The Limitations of the Methods of Identifying the Floral Source of Honeys.” *Bee World* 79(2): 59–68.

Moškrič, Ajda, Jernej Bubnič, Maja Ivana Smodiš Škerl, and Janez Prešern. 2018. “Potential Positive Effects of Hop Beta Acids on Parasitic Mite Varroa Destructor Control in Honey Bee Colonies.” *Journal of Hygienic Engineering and Design* 24: 21–28.

Al Naggar, Yahya et al. 2016. “Effects of Treatments with Apivar ® and Thymovar ® on V. Destructor Populations, Virus Infections and Indoor Winter Survival of Canadian Honey Bee (Apis Mellifera L.) Colonies.” *Journal of Apicultural Research* 54: 1–7.

Nanetti, Antonio et al. 2003. “Oxalic Acid Treatments for Varroa Control (Review).” *Apiacta* 38.

Neumann, Peter, and Norman L. Carreck. 2010. “Honey Bee Colony Losses.” *Journal of Apicultural Research* 49(1): 1–6.

Oddo L. P., Piana L., Sabatini A. G. 2007. *Conoscere Il Miele*. 5th ed. ed. Avenue Media.

Oddo, L. Persano, MG G. Piazza, AG G. Sabatini, and M. Accorti. 1995. “Characterization of Unifloral Honeys.” *Apidologie* 26(6): 453–65.

Von Der Ohe, Werner et al. 2004. “Harmonized Methods of Melissopalynology.” *Apidologie* 35(Suppl.

1): S18–25.

Oldroyd, Benjamin P. 1999. “Coevolution While You Wait: Varroa Jacobsoni, a New Parasite of Western Honeybees.” *Trends in Ecology & Evolution* 14(8): 312–15. https://www.sciencedirect.com/science/article/pii/S0169534799016134.

Ongus, Juliette R et al. 2004. “Complete Sequence of a Picorna-like Virus of the Genus Iflavirus Replicating in the Mite Varroa Destructor.” *The Journal of general virology* 85(Pt 12): 3747–55.

Oruc, H H et al. 2012. “Determination of Acute Oral Toxicity of Flumethrin in Honey Bees.” *Journal of economic entomology* 105(6): 1890–94.

Padovan, G. J., D. De Jong, L. P. Rodrigues, and J. S. Marchini. 2003. “Detection of Adulteration of Commercial Honey Samples by the 13C/12C Isotopic Ratio.” *Food Chemistry* 82(4): 633–36.

Paleolog, Jerzy et al. 2023. “Imidacloprid Pesticide Causes Unexpectedly Severe Bioelement Deficiencies and Imbalance in Honey Bees Even at Sublethal Doses.” *Animals* 13(4).

Paudel, Yagya Prasad, Robert Mackereth, Rodney Hanley, and Wensheng Qin. 2015. “Honey Bees (Apis Mellifera L.) and Pollination Issues: Current Status, Impacts and Potential Drivers of Decline.” *Journal of Agricultural Science* 7(6): 93–109.

Perna, Anna Maria, Giulia Grassi, Emilio Gambacorta, and Amalia Simonetti. 2021. “Minerals Content in Basilicata Region (Southern Italy) Honeys from Areas with Different Anthropic Impact.” *International Journal of Food Science and Technology* 56(9): 4465–72.

Persano, Livia, and Maria Lucia. “Miele e Territorio.”

Persano Oddo, Livia, and Stefan Bogdanov. 2004. “Determination of Honey Botanical Origin: Problems and Issues.” *Apidologie* 35(Suppl. 1): S2–3. http://www.apis.admin.ch/host/honey/.

Persano Oddo, Livia, and Roberto Piro. 2004. “Main European Unifloral Honeys: Descriptive Sheets.” *Apidologie* 35(Suppl. 1): S38–81.

Piccirillo, Giancarlo, and David De Jong. 2003. “The Influence of Brood Comb Cell Size on the Reproductive Behavior of the Ectoparasitic Mite Varroa Destructor in Africanized Honey Bee Colonies.” *Genetics and molecular research : GMR* 2: 36–42.

Pickard, R. S., and G. Y. Kither. 1983. “An Evaluation of Shaking and Displacement Methods for Separating a Sample of Honeybees with Large Hypopharyngeal Glands from the Remainder of a Colony.” *Journal of Apicultural Research* 22(2): 101–6.

Pietropaoli, Marco. 2013. “Verifica Dell’efficacia Acaricida Di Trattamenti Estivi Con Acido Formico in Gel (Mite Away Quick Strips®).” *Lapis* 4: 8–12.

Pietropaoli, Marco, and Giovanni Formato. 2019a. “Acaricide Efficacy and Honey Bee Toxicity of Three

New Formic Acid-Based Products to Control Varroa Destructor.” *Journal of Apicultural Research* 58(5): 824–30.

———. 2019b. “Acaricide Efficacy and Honey Bee Toxicity of Three New Formic Acid-Based Products to Control Varroa Destructor.” *Journal of Apicultural Research* 58(5): 824–30. https://doi.org/10.1080/00218839.2019.1656788.

Pinzauti, M., & Frediani. 1998. “Appunti Di Apicoltura.” *ervizio Editoriale Universitario di Pisa.*

Pita-Calvo, Consuelo, and Manuel Vázquez. 2017. “Differences between Honeydew and Blossom Honeys: A Review.” *Trends in Food Science and Technology* 59: 79–87.

Ponisio, Lauren C. et al. 2015. “Diversification Practices Reduce Organic to Conventional Yield Gap.” *Proceedings of the Royal Society B: Biological Sciences* 282(1799).

Poonia, Asha, Rachna Gulati, and S Sharma. 2009. “Efficacy of Screen Floor and Powdered Sugar against Varroa Destructor Anderson and Trueman in Apis Mellifera L. Colonies.” *Biopesticides International* 5: 0.

Porrini, Claudio. 2007. “Le Api Come Bioindicatori Dell’inquinamento Ambientale.” *Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Entomologia, Università di Bologna*: 3.

Potts, S.G. et al. 2010. “Declines of Managed Honey Bees and Beekeepers in Europe.” *Journal of Apicultural Research* 49(1): 15–22.

Potts, Simon G et al. 2010. “Global Pollinator Declines: Trends, Impacts and Drivers.” *Trends in Ecology & Evolution* 25(6): 345–53. https://www.sciencedirect.com/science/article/pii/S0169534710000364.

Prisco, Gennaro Di et al. 2011. “Dynamics of Persistent and Acute Deformed Wing Virus Infections in Honey Bees, Apis Mellifera.” *Viruses* 3(12): 2425–41.

Ptaszyńska, Aneta A., Grzegorz Borsuk, Marcin Anusiewicz, and Wieslaw Mułenko. 2012. “Location of Nosema Spp. Spores within the Body of the Honey Bee.” *Medycyna Weterynaryjna* 68(10): 618–21.

Quigley, Tyler P, Gro V Amdam, and Gyan H Harwood. 2019. “Honey Bees as Bioindicators of Changing Global Agricultural Landscapes.” *Current opinion in insect science* 35: 132–37.

R., Oliver. 2010. *The Economy of the Hive, Part 1*. American Bee Journal.

Rademacher, Eva, and Marika Harz. 2006. “Oxalic Acid for the Control of Varroosis in Honey Bee Colonies – a Review.” *Apidologie* 37(1): 98–120. http://10.0.4.27/apido:2005063.

Rademacher, Eva, Marika Harz, and Saskia Schneider. 2015. “The Development of HopGuard® as a Winter Treatment against Varroa Destructor in Colonies of Apis Mellifera.” *Apidologie* 46(6): 748–59.

Ramsey, Samuel D et al. 2019. “Varroa Destructor Feeds Primarily on Honey Bee Fat Body Tissue and

Not Hemolymph.” 116(5).

Reams, Taylor, and Juliana Rangel. 2022. “Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of Varroa Destructor, the Parasitic Mite of Apis Mellifera.” *Journal of insect science (Online)* 22(1).

Reams, Taylor, Juliana Rangel, and Margarita Lopez-Uribe. 2022. “Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of Varroa Destructor, the Parasitic Mite of Apis Mellifera.” *Journal of Insect Science* 22(1): 18–19. https://doi.org/10.1093/jisesa/ieab101Review.

Reza, By et al. 2008. “The Efficacy of Apivar ® and Bayvarol ® and CheckMite +® in the Control of Varroa Destructor in Iran.” *October*.

Rice, Nathan D., Mark L. Winston, and Heather A. Higo. 2004. “Integrated Pest Management for the Parasitic Mite Varroa Destructor (Anderson and Trueman) in Colonies of Honey Bees (Apis Mellifera).” *American Bee Journal* 144(10): 791–95.

Richards, E H, Benjamin Jones, and Alan Bowman. 2011. “Salivary Secretions from the Honeybee Mite, Varroa Destructor: Effects on Insect Haemocytes and Preliminary Biochemical Characterization.” *Parasitology* 138(5): 602–8.

Rinderer, Thomas E., Jeffrey W. Harris, Gregory J. Hunt, and Lilia I. De Guzman. 2010. “Breeding for Resistance to Varroa Destructor in North America.” *Apidologie* 41(3): 409–24.

Rodríguez-Flores, M. Shantal, Olga Escuredo, Montserrat Míguez, and M. Carmen Seijo. 2019. “Differentiation of Oak Honeydew and Chestnut Honeys from the Same Geographical Origin Using Chemometric Methods.” *Food Chemistry* 297: 124979.

Rodríguez-Flores, M.S., O. Escuredo, A. Seijo-Rodríguez, and M.C. Seijo. 2019. “Characterization of the Honey Produced in Heather Communities (NW Spain).” *Journal of Apicultural Research* 58(1): 84–91.

Rosenkranz, Peter, Pia Aumeier, and Bettina Ziegelmann. 2010. “Biology and Control of Varroa Destructor.” *Journal of invertebrate pathology* 103 Suppl: S96-119.

Sabahi, Qodratollah et al. 2020. “Evaluation of Dry and Wet Formulations of Oxalic Acid, Thymol, and Oregano Oil for Varroa Mite (Acari: Varroidae) Control in Honey Bee (Hymenoptera: Apidae) Colonies.” *Journal of Economic Entomology* 113(6): 2588–94.

Sahinler, Nuray, Aziz Gül, and Ahmet Şahin. 2005. “Vitamin E Supplementation In Honey Bee Colonies To Increase Acceptance Rate and Royal Jelly Production.” *Journal of Apicultural Research* 44: 58–60.

Sakla, Rasha, and Samah El-shafeiy. 2022. “Evaluation of Royal Jelly Quality and Queens Production by Using Natural Food Supplements in Honeybee Colonies.” *Egyptian Journal of Agricultural*

Research 0(0): 0–0.

Salima, Koumad, and Nizar Haddad. 2015. “Resistance of Varroa Destructor to Apistan and Bayvarol.”

Saxena, Sudhanshu, Satyendra Gautam, and Arun Sharma. 2010. “Physical, Biochemical and Antioxidant Properties of Some Indian Honeys.” *Food Chemistry* 118(2): 391–97. http://dx.doi.org/10.1016/j.foodchem.2009.05.001.

Schaepman, Michael E. 2007. “Spectrodirectional Remote Sensing: From Pixels to Processes.” *International Journal of Applied Earth Observation and Geoinformation* 9(2): 204–23.

Schievano, Elisabetta et al. 2019. “NMR Assessment of European Acacia Honey Origin and Composition of EU-Blend Based on Geographical Floral Markers.” *Food Chemistry* 288: 96–101.

Semkiw, Piotr, Piotr Skubida, and Krystyna Pohorecka. 2013. “The Amitraz Strips Efficacy in Control of Varroa Destructor After Many Years Application of Amitraz in Apiaries.” *Journal of Apicultural Science* 57: 107–21.

She, Seng et al. 2019. “Discrimination of Geographical Origins of Chinese Acacia Honey Using Complex 13C/12C, Oligosaccharides and Polyphenols.” *Food Chemistry* 272(August 2018): 580–85.

Shen, Miaoqing, Xiaolong Yang, Diana Cox-Foster, and Liwang Cui. 2005. “The Role of Varroa Mites in Infections of Kashmir Bee Virus (KBV) and Deformed Wing Virus (DWV) in Honey Bees.” *Virology* 342(1): 141–49.

Shumkova, Rositsa, Ralitsa Balkanska, and Peter Hristov. 2021. “The Herbal Supplements Nozemat Herb® and Nozemat Herb Plus®: An Alternative Therapy for n. Ceranae Infection and Its Effects on Honey Bee Strength and Production Traits.” *Pathogens* 10(2): 1–19.

Siddiqui, Amna Jabbar, Syed Ghulam Musharraf, M. Iqbal Choudhary, and Atta ur Rahman. 2017. “Application of Analytical Methods in Authentication and Adulteration of Honey.” *Food Chemistry* 217: 687–98. http://dx.doi.org/10.1016/j.foodchem.2016.09.001.

Skorbiłowicz, Elżbieta, Mirosław Skorbiłowicz, and Izabela Cieśluk. 2018. “Bees as Bioindicators of Environmental Pollution with Metals in an Urban Area.” 19(3): 229–34.

Soares, Sónia, Joana S. Amaral, Maria Beatriz P.P. Oliveira, and Isabel Mafra. 2015. “Improving DNA Isolation from Honey for the Botanical Origin Identification.” *Food Control* 48: 130–36.

———. 2017. “A Comprehensive Review on the Main Honey Authentication Issues: Production and Origin.” *Comprehensive Reviews in Food Science and Food Safety* 16(5): 1072–1100.

Solayman, Md et al. 2016. “Physicochemical Properties, Minerals, Trace Elements, and Heavy Metals in Honey of Different Origins: A Comprehensive Review.” *Comprehensive Reviews in Food Science and Food Safety* 15(1): 219–33.

Sollid, Kris. 2020. “What Is Honey?” *food insight*. https://foodinsight.org/what-is-honey/%22.

Somerville, Doug. 2005. “FAT BEES SKINNY BEES -a Manual on Honey Bee Nutrition for Beekeepers-.” *Australian Government Rural industries Research and Development Corporation* (05): 1–142. http://library.wur.nl/WebQuery/clc/1766764.

STANIMIROVIC Z, TODOROVIC DAJANA, STEVANOVIC JEVROSIMA, MLADENOVIC M, and JANKOVIC LJILJANA. 2003. “Cymiazole Hydrochloride Is an Iminophenyl Thiazolidine Derivative, Active against Acari (Mites and Ticks). In Veterinary Medicine, Cymiazole Hydrochloride Is Recommended for the Control of Varroasis in Honey Bees. The Intended Pharma-Ceutical Formulation.” *Acta Veterinaria Beograd* 53(1): 47–55.

Staveley P.J. 2014. “A Causal Analysis of Observed Declines in Managed.”

Stevanovic, Jevrosima et al. 2012. “Stimulating Effect of Sugar Dusting on Honey Bee Grooming Behaviour.” *Entomologia Experimentalis et Applicata* 143: 23–30.

Sumpter, D. J.T., and S. J. Martin. 2004. “The Dynamics of Virus Epidemics in Varroa-Infested Honey Bee Colonies.” *Journal of Animal Ecology* 73(1): 51–63.

Svoboda, J A, M J Thompson, E W Herbert, and H Shimanuki. 1980. “Sterol Utilization in Honey Bees Fed a Synthetic Diet: Analysis of Prepupal Sterols.” *Journal of Insect Physiology* 26(5): 291–94. https://www.sciencedirect.com/science/article/pii/0022191080901365.

Teeters, Bethany S, Reed M Johnson, Marion D Ellis, and Blair D Siegfried. 2012. “Using Video-Tracking to Assess Sublethal Effects of Pesticides on Honey Bees (Apis Mellifera L.).” *Environmental toxicology and chemistry* 31(6): 1349–54.

Thakur, Mamta, and Vikas Nanda. 2020. “Composition and Functionality of Bee Pollen: A Review.” *Trends in Food Science and Technology* 98: 82 – 106. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85079864385&doi=10.1016%2Fj.tifs.2020.02.001&partnerID=40&md5=3a352ca25cad5b79d87e768048d98dba.

Tosun, Murat. 2013. “Detection of Adulteration in Honey Samples Added Various Sugar Syrups with 13C/12C Isotope Ratio Analysis Method.” *Food Chemistry* 138(2–3): 1629–32. http://dx.doi.org/10.1016/j.foodchem.2012.11.068.

Al Toufailia, Hasan, Luciano Scandian, and Francis 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.” *Journal of Apicultural Research* 54(2): 108–20. http://dx.doi.org/10.1080/00218839.2015.1106777.

Traynor, Kirsten S et al. 2020. “Varroa Destructor: A Complex Parasite , Crippling Honey Bees Worldwide.” *Trends in Parasitology* 36(7): 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

Trifković, Jelena et al. 2017. “Analytical Methods in Tracing Honey Authenticity.” *Journal of AOAC International* 100(4): 827–39.

Underwood, Robyn, and Margarita Lopez-Uribe. 2019. “Methods to Control Varroa Mites: An Integrated Pest Management Approach.” *The Pennsylvania State University: College of Agricultural Sciences*: 1–5. https://extension.psu.edu/methods-to-control-varroa-mites-an-integrated-pest-management-approach.

Valentini, Alice, Christian Miquel, and Pierre Taberlet. 2010. “DNA Barcoding for Honey Biodiversity.” *Diversity* 2(4): 610–17.

Valverde, Silvia et al. 2023. “Mineral Composition of Bee Pollen and Its Relationship with Botanical Origin and Harvesting Period.” *Journal of Food Composition and Analysis* 119: 105235.

Vandervalk, L. P., M. E. Nasr, and L. M. Dosdall. 2014. “New Miticides for Integrated Pest Management of Varroa Destructor (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies.” *Journal of Economic Entomology* 107(6): 2030–36.

Vanlalhmangaiha, R et al. 2022. “Impact of Insect Pollination on the Quantitative and Qualitative Characteristics of Sweet Orange, Citrus Sinensis (L.) Osbeck.” *Journal of Apicultural Research*: 1–10. https://doi.org/10.1080/00218839.2021.2013401.

Vaudo, Anthony, John Tooker, Christina Grozinger, and Harland Patch. 2015. “Bee Nutrition and Floral Resource Restoration.” *Current Opinion in Insect Science* 10.

Vesco, U. 2013. “Varroa e Patogeni, Accoppiata Mortale.” *L’Apis* 2: 5–9.

VETO-PHARMA. 2020. *Guía de Varroa*. second.

Vilarem, Caroline, Vincent Piou, and Fanny Vogelweith. 2021. “Varroa Destructor from the Laboratory to the Field: Control, Biocontrol and IPM Perspectives—A Review.”

Wallner, Klaus. 1999. “Varroacides and Their Residues in Bee Products.” *Apidologie* 30(2–3): 235–48.

Wang, H. et al. 2019. “A Novel Methodology for Real-Time Identification of the Botanical Origins and Adulteration of Honey by Rapid Evaporative Ionization Mass Spectrometry.” *Food Control* 106.

Wantuch, Holly, and David Tarpy. 2009. “Removal of Drone Brood From Apis Mellifera (Hymenoptera: Apidae) Colonies to Control Varroa Destructor (Acari: Varroidae) and Retain Adult Drones.” *Journal of economic entomology* 102: 2033–40.

Ward, Rachel, Mary F. Coffey, and Kevin Kavanagh. 2022. “Exposure of Apis Mellifera to Anti-Varroa Destructor Formic Acid Treatment Induces Significant Proteomic Alterations.” *Journal of Apicultural Research* 0(0): 1–9. https://doi.org/10.1080/00218839.2022.2038055.

Waykar Bhalchandra, Mahesh A Joshi and Nilesh Jawalkar. 2022. “Effect of Storage on Various Honey

Quality Parameters of Apis Mellifera Honey Harvested from Kannad Region, Aurangabad." *Journal of Pharmacognosy and Phytochemistry* 11(1): 239–46.

Wilde, Jerzy, Stefan Fuchs, Janusz Bratkowski, and Maciej Siuda. 2005. "Distribution of Varroa Destructor between Swarms and Colonies." *Journal of Apicultural Research* 44: 190–94.

Yang, X, and D Cox-Foster. 2007. "Effects of Parasitization by Varroa Destructor on Survivorship and Physiological Traits of Apis Mellifera in Correlation with Viral Incidence and Microbial Challenge." *Parasitology* 134(Pt 3): 405–12.

Yue, Constanze, and Elke Genersch. 2005. "RT-PCR Analysis of Deformed Wing Virus in Honeybees (Apis Mellifera) and Mites (Varroa Destructor)." *The Journal of general virology* 86(Pt 12): 3419–24.

Yue, Constanze, Marion Schröder, Sebastian Gisder, and Elke Genersch. 2007. "Vertical-Transmission Routes for Deformed Wing Virus of Honeybees (Apis Mellifera)." *The Journal of general virology* 88: 2329–36.

Yves, L C, E Marion, and R Wolfgang. 2010. "Review Article Varroa Mites and Honey Bee Health : Can Varroa Explain Part of the Colony Losses ?*." 41: 353–63.

Zar, Jerrold H. 2014. 13 Nucl. Phys. *Biostatistical Analysis*.

Zhou, Xiaoteng, Mark Patrick Taylor, Helen Salouros, and Shiva Prasad. 2018. "Authenticity and Geographic Origin of Global Honeys Determined Using Carbon Isotope Ratios and Trace Elements." *Scientific Reports* 8(1): 1–11. http://dx.doi.org/10.1038/s41598-018-32764-w.

Zhu, Xiangrong et al. 2010. "Detection of Adulterants Such as Sweeteners Materials in Honey Using Near-Infrared Spectroscopy and Chemometrics." *Journal of Food Engineering* 101(1): 92–97.

SUPPLEMENTARY MATERIALS

Supplementary table 1 Average percent recoveries for the elements analyzed in citrus honeys

Element	No. of replicate	Average recovery (%)	NIST SRM
Al	3	110	1549
As	3	116	1570a
Ca	3	86	1549
Cd	3	97	1549
Co	3	78	1549
Cr	3	103	1549
Cu	3	106	1549
Fe	3	101	1549
Hg	3	113	1570a
K	3	96	1549
Mg	3	115	1549
Mn	3	90	1549
Mo	3	69	1549
Na	3	89	1549
Ni	3	81	1570a
Pb	3	107	1549
Rb	3	132	1549
Sn	3	98	1549
V	3	96	1570a
Zn	3	106	1549

Supplementary table 2 Calibration data for all the elements analysed, divided by atomization system: continuous flame (FL), graphite furnace (GF) and hydride generator (HVG). The calibration ranges have been chosen on the basis of the Shimadzu manual for the AAS 7000 and adapted to our range needs

ELEMENT	AT. SYSTEM	CALIBRATORS RANGE	UoM	R^2 MEAN	CAL. CURVE ORDER
Pb	GF	1.00-20	µg/kg	0.9986	1st
Hg	HVG	0.65-10	µg/kg	0.9993	1st
Cd	GF	0.10-2	µg/kg	0.99795	1st
Cr	GF	0.40-5	µg/kg	0.9975	1st
Cu	GF	1.00-10	µg/kg	0.99935	1st
Mn	GF	0.50-5	µg/kg	0.99855	1st
Co	GF	1.00-20	µg/kg	0.99715	1st
Ni	GF	2.00-20	µg/kg	0.99865	1st
Mo	GF	1.00-20	µg/kg	0.9984	1st
Zn	FL	0.10-2	mg/kg	1.0000	2nd
Fe	FL	0.06-2	mg/kg	0.9998	1st
Ca	FL	0.62-10	mg/kg	0.99885	1st
Mg	FL	0.01-2.5	mg/kg	0.99745	1st
Na	FL	0.06-2	mg/kg	0.9993	1st
Sn	GF	5.00-50	µg/kg	0.99905	2nd
Al	GF	8.00-40	µg/kg	0.99965	1st
K	FL	0.31-3	mg/kg	0.99965	1st
Li	GF	1.00-10	µg/kg	0.9986	1st
As	HVG	0.50-2	mg/kg	0.99705	1st
Rb	FL	0.10-2	mg/kg	1.0000	1st
V	GF	2.00-20	µg/kg	0.9988	1st

5. GENERAL CONCLUSION

The work done in this thesis targeted two important issues affecting the beekeeping world.
The first part of this paper showed how bees are important in many ways and how seeking innovative solutions to increase their viability should be a priority. It was also shown how the Varroa destructor mite is an increasingly important problem in modern beekeeping. To do this, they went on to develop an IPM strategy that does not involve the use of synthetic acaricides,and can be used as a back-up to other treatments throughout the year. Although not both trials yielded optimal results, hop beta acids, tested for two consecutive years at the same time of year, proved to be a viable alternative to the use of common oxalic acid. Unfortunately, hop alpha acids, at the concentrations tested, proved to be inefficient, although in the future perspective one might consider increasing these concentrations and evaluating their tolerability by bees. Mussi Space, on the other hand, used as an alternative strategy to support acaricide treatments, with the goal of increasing varroa fall in treated hives, did not prove to be a consistently useful tool, generally failing to increase varroa fall.
On the other hand, the work done attempted to verify safety and traceability, understood as verification of geographical origin, on citrus honey samples from the Mediterranean basin. Regarding safety, all samples met the European legal limits. Regarding traceability, a multifactorial strategy was tested to arrive at a precise and accurate discrimination of the geographical origin of citrus honeys, from the Mediterranean basin, from three different harvest seasons. The study involved a considerable number of elements and parameters analyzed. In this case, a number of parameters and elements were identified that have been shown to have important discriminating power between different geographical origins. These data can be an interesting starting point for a subsequent study, going to carry out analyses on other parameters and elements, which could be added to the list of potential discriminating factors of the geographical origin of honeys from Italy, Greece and Egypt.

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7. GENERAL REFERENCES

A., de Ruijter. 1987. “Reproduction of Varroa Jacobsoni during Successive Brood Cycles of the Honeybee.” *Apidologie* v. 18.

Al-Mamary, Mohamed, Ali Al-Meeri, and Molham Al-Habori. 2002. “Antioxidant Activities and Total Phenolics of Different Types of Honey.” *Nutrition Research* 22(9): 1041–47. www.elsevier.com/locate/nutres.

Alaux, Cédric, Christelle Dantec, Hughes Parrinello, and Yves Le Conte. 2011. “Nutrigenomics in Honey Bees: Digital Gene Expression Analysis of Pollen’s Nutritive Effects on Healthy and Varroa-Parasitized Bees.” *BMC Genomics* 12(June 2014).

Aliano, Nicholas P., and Marion D. Ellis. 2009. “Oxalic Acid: A Prospective Tool for Reducing Varroa Mite Populations in Package Bees.” *Experimental and Applied Acarology* 48(4): 303–9.

Aljadi, A. M., and M. Y. Kamaruddin. 2004. “Evaluation of the Phenolic Contents and Antioxidant Capacities of Two Malaysian Floral Honeys.” *Food Chemistry* 85(4): 513–18.

Allabergenova, A., G. Turganbayeva, and M. Nurseitova. 2021. “Control of Varroa Destructor in Kazakhstan.” *Archives of Razi Institute* 76(5): 1163–71.

Almaguer, Cynthia et al. 2014. “Humulus Lupulus - a Story That Begs to Be Told. A Review.” *Journal of the Institute of Brewing* 120(4): 289–314.

Alvarez-Suarez, Jose Miguel et al. 2010. “Contribution of Honey in Nutrition and Human Health: A Review.” *Mediterranean Journal of Nutrition and Metabolism* 3(1): 15–23.

Andersen, Øyvind M., and Kenneth R. Markham. 2005. “Flavonoids: Chemistry, Biochemistry and Applications.” *Flavonoids: Chemistry, Biochemistry and Applications*: 1–1239.

Anderson, D. L., and J. W.H. Trueman. 2000a. “Varroa Jacobsoni (Acari: Varroidae) Is More than One Species.” *Experimental and Applied Acarology* 24(3): 165–89.

Anderson, D L, and J W H Trueman. 2000b. *Varroa Jacobsoni (Acari: Varroidae) Is More than One Species*.

Anklam, Elke. 1998. “A Review of the Analytical Methods to Determine the Geographical and Botanical Origin of Honey.” *Food Chemistry* 63(4): 549–62.

Annoscia, Desiderato, Fabio Del Piccolo, and Francesco Nazzi. 2012. “How Does the Mite Varroa Destructor Kill the Honeybee Apis Mellifera? Alteration of Cuticular Hydrcarbons and Water Loss in Infested Honeybees.” *Journal of Insect Physiology* 58(12): 1548–55.

Ansari, Mohammad Javed et al. 2018. “Validation of Botanical Origins and Geographical Sources of Some Saudi Honeys Using Ultraviolet Spectroscopy and Chemometric Analysis.” *Saudi Journal of Biological Sciences* 25(2): 377–82.

https://linkinghub.elsevier.com/retrieve/pii/S1319562X17302280.

Aries, Eric et al. 2016. “Scientific Support to the Implementation of a Coordinated Control Plan with a View to Establishing the Prevalence of Fraudulent Practices in the Marketing of Honey.” *JRC Technical Reports*: 27. https://ec.europa.eu/jrc.

Attali, Myriam et al. 2007. “Control of β-Cell Differentiation by the Pancreatic Mesenchyme.” *Diabetes* 56(5): 1248–58.

Bahreini, Rassol et al. 2020. “Evaluation of Potential Miticide Toxicity to Varroa Destructor and Honey Bees, Apis Mellifera, under Laboratory Conditions.” *Scientific Reports* 10(1): 1–14. https://doi.org/10.1038/s41598-020-78561-2.

Ballabio, Davide et al. 2018. “Chemical Profiling and Multivariate Data Fusion Methods for the Identification of the Botanical Origin of Honey.” *Food Chemistry* 266: 79–89.

Balog, Julia et al. 2010. “Identification of Biological Tissues by Rapid Evaporative Ionization Mass Spectrometry.” *Analytical Chemistry* 82(17): 7343–50.

Barbattini, Renzo, and Stefano Fugazza. 2006. “L’ape Nell’arte Antica.” *Apitalia* 32: 12–17.

Bartlett, Lewis J. 2022. “Frontiers in Effective Control of Problem Parasites in Beekeeping.” *International Journal for Parasitology: Parasites and Wildlife* 17(March): 263–72. https://doi.org/10.1016/j.ijppaw.2022.03.003.

Beinert, Helmut, Mary Claire Kennedy, and C David Stout. 1996. “Aconitase as Ironminus SignSulfur Protein, Enzyme, and Iron-Regulatory Protein.” *Chemical reviews* 96(7): 2335–74.

Bellucci, V. et al. 2019. “Monitoring Honey Bee Healthin Five Natural Protected Areas in Italy.” *Veterinaria italiana* 55(1): 15–25.

Beretta, Giangiacomo et al. 2005. “Standardization of Antioxidant Properties of Honey by a Combination of Spectrophotometric/Fluorimetric Assays and Chemometrics.” *Analytica Chimica Acta* 533(2): 185–91.

Bergamo, Greici et al. 2018. “Mineral Profile as a Potential Parameter for Verifying the Authenticity of Bracatinga Honeydew Honeys.” *LWT* 97: 390–95.

Berry, Jennifer A et al. 2021. “Assessing Repeated Oxalic Acid Vaporization in Honey Bee (Hymenoptera: Apidae) Colonies for Control of the Ectoparasitic Mite Varroa Destructor.” *Journal of Insect Science* 22(1): 15–16. https://doi.org/10.1093/jisesa/ieab089.

Bertoncelj, Jasna, Urška Doberšek, Mojca Jamnik, and Terezija Golob. 2007. “Evaluation of the Phenolic Content, Antioxidant Activity and Colour of Slovenian Honey.” *Food Chemistry* 105(2): 822–28.

Bettar, Ismail et al. 2019. “Characterisation of Moroccan Spurge (Euphorbia) Honeys by Their Physicochemical Characteristics, Mineral Contents and Colour.” *Arabian Journal of Chemistry* 12(8): 2052–60.

Blacquière, Tjeerd, Gertraut Altreuther, and Klemens J. Krieger. 2017. “Evaluation of the

Efficacy and Safety of Flumethrin 275 Mg Bee-Hive Strips (PolyVar Yellow®) against Varroa Destructor in Naturally Infested Honey Bee Colonies in a Controlled Study." *Parasitology Research* 116: 109–22.

Bogdanov, Stefan et al. 2002. "Determination of Residues in Honey after Treatments with Formic and Oxalic Acid under Field Conditions." *Apidologie* 33: 399–409.

———. 2006. "Contaminants of Bee Products." *Apidologie* 37(1): 1–18.

———. 2009. "Harmonised Methods of the International IHC." *Bee Product Science*, (5): 1–62.

Bogdanov, Stefan, V Kilchenmann, and A Imdorf. 1998. "Acaricide Residues in Some Bee Products." *Journal of Apicultural Research* 37: 57–67.

Bogdanov, Stefan, and Peter Martin. 2002. *Honey Authenticity: A Review*.

Boi, Michela et al. 2016. "A 10 Year Survey of Acaricide Residues in Beeswax Analysed in Italy." *Pest Management Science* 72(7): 1366 – 1372. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84969792521&doi=10.1002%2Fps.4161&partnerID=40&md5=d0d0e717d38d9ebfe35ad1616f656b61.

Bontempo, Luana et al. 2017. "Isotopic and Elemental Composition of Selected Types of Italian Honey." *Measurement: Journal of the International Measurement Confederation* 98: 283–89.

Bonzini, Sara et al. 2011. "Predicting Pesticide Fate in the Hive (Part 1): Experimentally Determined τ-Fluvalinate Residues in Bees, Honey and Wax." *Apidologie* 42(3): 378–90.

Bormetti, Matteo. 2014. "Api e Miele Nel Mediterraneo Antico." *Acme* 66(1–2): 7–50.

Bortolotti, Laura et al. 2009. "Salute Delle Api : Analisi Dei Fattori Di Rischio . Il Progetto Apenet." (September 2014).

Bowen-Walker, P L, Stephen Martin, and Alan Gunn. 1999. "The Transmission of Deformed Wing Virus between Honeybees (Apis MelliferaL.) by the Ectoparasitic MiteVarroa JacobsoniOud." *Journal of invertebrate pathology* 73: 101–6.

Brodschneider, Robert et al. 2018. "Multi-Country Loss Rates of Honey Bee Colonies during Winter 2016 / 2017 from the COLOSS Survey." *Journal of Apicultural Research* 57(3): 452–57. https://doi.org/10.1080/00218839.2018.1460911.

Brodschneider, Robert, and Karl Crailsheim. 2010. "Nutrition and Health in Honey Bees." *Apidologie* 41(3): 278–94.

Brunner, Franziska S, Paul Schmid-Hempel, and Seth M Barribeau. 2014. "Protein-Poor Diet Reduces Host-Specific Immune Gene Expression in Bombus Terrestris." *Proceedings. Biological sciences* 281(1786).

Burkert, W. 2003. *La Religione Greca Di Epoca Arcaica e Classica*. Milano: Jaca Book.

Cajka, T., M. R. Showalter, K. Riddellova, and O. Fiehn. 2016. "Advances in Mass Spectrometry for Food Authenticity Testing: An Omics Perspective." *Advances in Food*

Authenticity Testing: 171–200.

Calderone, Nicholas W. 2005. “Integrated Pest Management for Varroa Destructor in the Northeastern United States Using Drone Brood Removal and Formic Acid.” *Cornell University*.

Calzoni, G. L., Rondinini, T. 2012. “Api, Impollinazione e Miele.” *Al Sas*: 52–59.

Carmen Gigliotti, Roberto Verga. 2007. *Biotecnologie Alimentari*. Piccin-Nuova Libraria.

Carreck, Norman, Brenda Ball, and Stephen Martin. 2010. “Honey Bee Colony Collapse and Changes in Viral Prevalence Associated with Varroa Destructor.” *Journal of Apicultural Research* 49: 93–94.

Cevallos-Cevallos, Juan M. et al. 2009. “Metabolomic Analysis in Food Science: A Review.” *Trends in Food Science and Technology* 20(11–12): 557–66.

Chen, Chun Ting et al. 2019. “Novel Inspection of Sugar Residue and Origin in Honey Based on the 13C/12C Isotopic Ratio and Protein Content.” *Journal of Food and Drug Analysis* 27(1): 175–83.

Chen, Y P et al. 2004. “Detection of Deformed Wing Virus Infection in Honey Bees, Apis Mellifera L., in the United States.” *American Bee Journal* 144: 557–59.

Chen, Yan Ping, and Reinhold Siede. 2007. “Honey Bee Viruses.” *Advances in Virus Research* 70: 33 – 80. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34548304773&doi=10.1016%2FS0065-3527%2807%2970002-7&partnerID=40&md5=3855e3c8328cc0f225d2e0f46b624059.

Chua, Lee Suan, Norul Liza A. Rahaman, Nur Ardawati Adnan, and Ti Tjih Eddie Tan. 2013. “Antioxidant Activity of Three Honey Samples in Relation with Their Biochemical Components.” *Journal of Analytical Methods in Chemistry* 2013.

Chuda-Mickiewicz, Bożena, J Prabucki, J Kazimierczak, and Jerzy Samborski. 2007. “The Varroacidal Efficacy of Apitraz.” *Medycyna Weterynaryjna* 63: 110–12.

Coffey, Mary, and John Breen. 2016. “The Efficacy and Tolerability of Api-Bioxal ® as a Winter Varroacide in a Cool Temperate Climate.” *Journal of Apicultural Research* 55: 1–9.

Commission of the European Communities. 1988. *European Research on Varroatosis Control*. 1ST ed. ed. CRC.

Connolly, Joanne B, Antonietta Wallace, and Sara Stead. 2016. “Discrimination of Honey of Different Botanical Origins Using an Untargeted High-Definition.” : 2016.

Consonni, Roberto, and Laura Ruth Cagliani. 2019. “The Potentiality of NMR-Based Metabolomics in Food Science and Food Authentication Assessment.” *Magnetic Resonance in Chemistry* 57(9): 558–78.

Contessi, A. 2016. *Le Api. Biologia, Allevamento, i Prodotti*. Bologna: Edagricole.

Conti, Marcelo Enrique et al. 2007. “Characterization of Italian Honeys (Marche Region) on the Basis of Their Mineral Content and Some Typical Quality Parameters.” *Chemistry Central*

Journal 1(1).

Corvucci, Francesca, Lara Nobili, Dora Melucci, and Francesca Vittoria Grillenzoni. 2015. "The Discrimination of Honey Origin Using Melissopalynology and Raman Spectroscopy Techniques Coupled with Multivariate Analysis." *Food Chemistry* 169: 297–304.

Council, European, and European Parliament. 2002. "COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 Relating to Honey." *Official Journal of the European Communities* L10.

Crane, E., Graham, A. J. 1985. *No TitleBee Hives of the Ancient World (Part I)*.

Cubero-Leon, Elena, Rosa Peñalver, and Alain Maquet. 2014. "Review on Metabolomics for Food Authentication." *Food Research International* 60: 95–107. http://dx.doi.org/10.1016/j.foodres.2013.11.041.

De D'Aubeterre, Judith Principal, David D. Myrold, Lynn A. Royce, and Philippe A. Rossignol. 1999. "A Scientific Note of an Application of Isotope Ratio Mass Spectrometry to Feeding by the Mite, Varroa Jacobsoni Oudemans, on the Honeybee, Apis Mellifera L." *Apidologie* 30(4): 351–52.

Dainat, Benjamin et al. 2012. "Dead or Alive: Deformed Wing Virus and Varroa Destructor Reduce the Life Span of Winter Honeybees." *Applied and Environmental Microbiology* 78(4): 981–87.

Danieli, Pier Paolo, and Filippo Lazzari. 2022. "Honey Traceability and Authenticity. Review of Current Methods Most Used to Face This Problem." *JOURNAL OF APICULTURAL SCIENCE* 66(2): 101–19.

David Aston, Sally Bucknall. 2021. *Good Nutrition-Good Bees*. Hebden Bridge: Northern Bee Books.

DeGrandi-Hoffman, Gloria et al. 2014. "Population Growth of Varroa Destructor (Acari: Varroidae) in Commercial Honey Bee Colonies Treated with Beta Plant Acids." *Experimental and Applied Acarology* 64(2): 171–86.

DeGrandi-Hoffman, Gloria, Fabiana Ahumada, Gene Probasco, and Lloyd Schantz. 2012. "The Effects of Beta Acids from Hops (Humulus Lupulus) on Mortality of Varroa Destructor (Acari: Varroidae)." *Experimental and Applied Acarology* 58(4): 407–21.

Delaplane, Keith. 1999. "Bee Products: Properties, Applications, and Apitherapy." *American Entomologist* 45.

Desneux, Nicolas, Axel Decourtye, and Jean-Marie Delpuech. 2007. "The Sublethal Effects of Pesticides on Beneficial Arthropods." *Annual Review of Entomology* 52: 81 – 106. https://www.scopus.com/inward/record.uri?eid=2-s2.0-33846354297&doi=10.1146%2Fannurev.ento.52.110405.091440&partnerID=40&md5=1fd845edca244ffdac1bccfd3a5ca597.

Devillers, J., M. Morlot, M. H. Pham-Delègue, and J. C. Doré. 2004. "Classification of Monofloral Honeys Based on Their Quality Control Data." *Food Chemistry* 86(2): 305–12.

Dietemann, Vincent et al. 2012. “Varroa Destructor: Research Avenues towards Sustainable Control.” *Journal of Apicultural Research* 51(1): 125–32.

Döke, Mehmet Ali, Maryann Frazier, and Christina M Grozinger. 2015. “Overwintering Honey Bees: Biology and Management.” *Current opinion in insect science* 10: 185–93.

Dzugan, Małgorzata, Monika Tomczyk, Patrycja Sowa, and Dorota Grabek-Lejko. 2018. “Antioxidant Activity as Biomarker of Honey Variety.” *Molecules* 23(8): 2069.

Ebert, T A et al. 2007. “Oral Toxicity of Essential Oils and Organic Acids Fed to Honey Bees (Apis Mellifera).” *Journal of apicultural research* v. 46.

Elzen, P J, and David Westervelt. 2002. “Detection of Coumaphos Resistance in Varroa Destructor in Florida.” *American Bee Journal* 142: 291–92.

Engwa, Godwill Azeh, Paschaline Udoka Ferdinand, Friday Nweke Nwalo, and Marian N Unachukwu. 2019. “Mechanism and Health Effects of Heavy Metal Toxicity in Humans.” In *Poisoning in the Modern World*, eds. Ozgur Karcioglu and Banu Arslan. Rijeka: IntechOpen. https://doi.org/10.5772/intechopen.82511.

ENRICO ZAGNOLI, ILARIA BIGANZOLI CORAZZA. 2013. *Una Speranza per Le Api*. Agri.Bio.Edizioni, Cissone, Cuneo,.

EU COMMISSION. 2023. “Honey Market Presentation.” (April).

European Council, and European Parliament. 2014. “Directive 2014/63/EU of the European Parliament and of the Council of 15 May 2014 Amending Council Directive 2001/110/EC Relating to Honey.” *Official Journal of the European Communities* L164(May): 1–5.

Everstine, Karen, John Spink, and Shaun Kennedy. 2013. “Economically Motivated Adulteration (EMA) of Food: Common Characteristics of EMA Incidents.” *Journal of Food Protection* 76(4): 723–35.

Faucon, J.-P, P Drajnudel, Marie-Pierre Chauzat, and M Aubert. 2007. “Control of the Efficacy of APIVAR ND against Varroa Destructor, a Parasite of Apis Mellifera.” *Revue de Medecine Veterinaire* 158: 283–90.

Fernández-Torres, Rut et al. 2005. “Mineral Content and Botanical Origin of Spanish Honeys.” *Talanta* 65(3): 686–91.

Ferrer-Dufol, M, A I Martinez-Viñuales, and C Sanchez-Acedo. 1991. “Comparative Tests of Fluvalinate and Flumethrin to Control Varroa Jacobsoni Oudemans.” *Journal of Apicultural Research* 30(2): 103–6. https://doi.org/10.1080/00218839.1991.11101241.

Fries, Ingemar, and Scott Camazine. 2001. “Fries_2001_Vertical Trasmision.” *Apidologie* 32: 199–214.

Frilli, F. 1983. “Varroa Jacobsoni: The Situation in Italy.” *Varroa jacobsoni Oud. affecting honey bees : present status and needs : proceedings of a meeting of the EC Experts’ Group, Wageningen, 7-9 February 1983 / edited by R. Cavalloro*.

Frost, Elisabeth H., Dave Shutler, and Neil Kirk Hillier. 2013. “Effects of Fluvalinate on Honey

Bee Learning, Memory, Responsiveness to Sucrose, and Survival." *Journal of Experimental Biology* 216(15): 2931–38.

Fuchs, S. 1990. "Preference for Drone Brood Cells by Varroa Jacobsoni Oud in Colonies of Apis Mellifera Carnica." *Apidologie* 21(3): 193–99.

Garedew, Assegid, Erik Schmolz, and Ingolf Lamprecht. 2004. "The Energy and Nutritional Demand of the Parasitic Life of the Mite Varroa Destructor." *http://dx.doi.org/10.1051/apido:2004032* 35.

Gashout, Hanan. 2009. "Acute Toxicity of Essential Oils and Other Natural Compounds to the Parasitic Mite, Varroa Destructor, and to Larval and Adult Worker Honey Bees (Apis Mellifera L.)." *Journal of Apicultural Research - J APICULT RES* 48: 263–69.

Genersch, Elke, and Michel Aubert. 2010. "Emerging and Re-Emerging Viruses of the Honey Bee (Apis Mellifera L.)." *Veterinary research* 41(6): 54.

Genersch, Elke, Constanze Yue, Ingemar Fries, and Joachim R de Miranda. 2006. "Detection of Deformed Wing Virus, a Honey Bee Viral Pathogen, in Bumble Bees (Bombus Terrestris and Bombus Pascuorum) with Wing Deformities." *Journal of invertebrate pathology* 91(1): 61–63.

Giacobino, Agostina et al. 2015. "Risk Factors Associated with Failures of Varroa Treatments in Honey Bee Colonies without Broodless Period." *Apidologie* 46(5): 573–82. www.satscan.org.

Giacomelli, Alessandra et al. 2016. "Combination of Thymol Treatment (Apiguard®) and Caging the Queen Technique to Fight Varroa Destructor." *Apidologie* 47(4): 606–16.

Giovenazzo, Pierre, and Pascal Dubreuil. 2011. "Evaluation of Spring Organic Treatments against Varroa Destructor (Acari: Varroidae) in Honey Bee Apis Mellifera (Hymenoptera: Apidae) Colonies in Eastern Canada." *Experimental & applied acarology* 55: 65–76.

Girisgin, Ahmet, and Levent Aydin. 2010. "Efficacies of Formic, Oxalic and Lactic Acids Against Varroa Destructor in Naturally Infested Honeybee (Apis Mellifera L.) Colonies in Turkey." *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 16: 941–45.

Giuliani, T. 2017. "LE LACRIME DI RA – L'apicoltura e l'importanza Delle Api Nell'antico Egitto." *Mediterraneo Antico*.

Goodwin, M ., and C. Van Eaton. 2001. New Zealand Ministry of Agriculture and Forestry *Control of Varroa (A Guide for New Zealand Beekeepers)*. http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:CONTROL+OF+VARROA+A+Guide+for+New+Zealand+Beekeepers#2.

Gray, Alison et al. 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." *Journal of Apicultural Research* 59(5): 744–51. https://doi.org/10.1080/00218839.2020.1797272.

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 | Zastosowanie Preparatów Zawieraja{ogonek}cych Tymol Oraz Amitrazy I Kwasu Szczawiowego W Zwalczaniu Roztoczy Varroa W .” *Journal of Apicultural Science* 56(2): 61–69.

Gregorc, Ales, and Ivo Planinc. 2001. “Original Article Acaricidal Effect of Oxalic Acid in Honeybee (Apis Mellifera) Colonies.” 32: 333–40.

Harbo, John, and Jeffrey Harris. 2004. “Effect of Screen Floors on Populations of Honey Bees and Parasitic Mites (Varroa Destructor).” *Journal of Apicultural Research* 43.

Heimpel, George E., and Jetske G. De Boer. 2008. “Sex Determination in the Hymenoptera.” *Annual Review of Entomology* 53(January 2014): 209–30.

Hernández, O. M. et al. 2005. “Characterization of Honey from the Canary Islands: Determination of the Mineral Content by Atomic Absorption Spectrophotometry.” *Food Chemistry* 93(3): 449–58.

Honey source. 2023. “The 5 Largest Honey-Producing Countries in the World.” *honeysource.com*.

Van Hoorn, M. Ter Horst, R. 2014. “(12) Patent Application Publication (10) Pub . No .: UUS 2014/0045407 A1 Patent Application Publication.” 1(19): 33. https://patents.google.com/patent/US20140331942A1/en.

Howis, Maciej, and Piotr Nowakowski. 2009. “Varroa Destructor Removal Efficiency Using Beevital Hive Clean Preparation.” *Journal of Apicultural Science* 53: 15–20.

Huang, Zachary. 2010. “Honey Bee Nutrition.” *American Bee Journal* 150(8): 773–76.

Imdorf, Anton et al. 2003. “Alternative Strategy in Central Europe for the Control of Varroa Destructor in Honey Bee Colonies.” *Apiacta* 38.

Imdorf, Anton, and Ch- Berna. 1999. “Prontuario per La Rilevazione Della Forza Della Colonia.” : 1–4.

IPBES. 2019. “Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.” https:/www./ipbes.net/.

Iqbal, Javaid, and Uli Mueller. 2007. “Virus Infection Causes Specific Learning Deficits in Honeybee Foragers.” *Proceedings of the Royal Society B: Biological Sciences* 274(1617): 1517 – 1521. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34447325877&doi=10.1098%2Frspb.2007.0022&partnerID=40&md5=4147aafed873d8e18e9cb5874777f6c5.

ISMEA. 2019. “Il Settore Apistico Nazionale.” : 1–24.

ISPRA. 2020. News.Ge *Il Declino Delle Api e Degli Impollinatori*.

———. 2021. *Piante E Insetti Impollinatori*. 350/2021. ISPRA.

Ivana Smodiš Škerl, Maja et al. 2021. “Efficacy and Toxicity of VarroMed ® Used for

Controlling Varroa Destructor Infestation in Different Seasons and Geographical Areas Toxicity of VarroMed ® Used For.” https://doi.org/10.3390/app11188564.

Jack, Cameron J., and James D. Ellis. 2021a. “Integrated Pest Management Control of Varroa Destructor (Acari: Varroidae), the Most Damaging Pest of (Apis Mellifera L. (Hymenoptera: Apidae)) Colonies.” *Journal of Insect Science* 21(5).

Jack, Cameron J, and James D Ellis. 2021b. “Integrated Pest Management Control of Varroa Destructor (Acari: Varroidae), the Most Damaging Pest of (Apis Mellifera L. (Hymenoptera: Apidae)) Colonies.” *Journal of Insect Science* 21(5): 6–7. https://doi.org/10.1093/jisesa/ieab058Review.

Jamal, Muhmmad et al. 2020. “Detection of Flumethrin Acaricide Residues from Honey and Beeswax Using High Performance Liquid Chromatography (HPLC) Technique.” *Journal of King Saud University - Science* 32.

Janmaat, A. F., and M. L. Winston. 2000. “The Influence of Pollen Storage Area and Varroa Jacobsoni Oudemans Parasitism on Temporal Caste Structure in Honey Bees (Apis Mellifera L.).” *Insectes Sociaux* 47(2): 177–82.

Jerković, Igor et al. 2016. “Traceability of Satsuma Mandarin (Citrus Unshiu Marc.) Honey through Nectar/Honey-Sac/Honey Pathways of the Headspace, Volatiles, and Semi-Volatiles: Chemical Markers.” *Molecules* 21(10).

Jiménez, Juan, José Bernal, María Nozal, and Maria Martín. 2005. “Residues of Organic Contaminants in Beeswax.” *European Journal of Lipid Science and Technology* 107: 896–902.

Johnson, Reed M., Lizette Dahlgren, Blair D. Siegfried, and Marion D. Ellis. 2013. “Acaricide, Fungicide and Drug Interactions in Honey Bees (Apis Mellifera).” *PLoS ONE* 8(1).

Johnson, Reed M et al. 2010. “Pesticides and Honey Bee Toxicity - USA * To Cite This Version :” *Apidologie* 41(3): 312–31.

Johnson, Reed M, Henry S Pollock, and May R Berenbaum. 2009. “Synergistic Interactions between In-Hive Miticides in Apis Mellifera.” *Journal of economic entomology* 102(2): 474–79.

Jones, G et al. 2003. “Integrated Management of Two-Spotted Spider Mite Tetranychus Urticae on Hops Using Hop Beta-Acids as an Antifeedant Together with Predatory Mite Phytoseiulus Persimilis.” *Biocontrol science and technology* v. 13.

De Jong, David, Roger Morse, and George Eickwort. 2003. “Mite Pests of Honey Bees.” *Annual Review of Entomology* 27: 229–52.

Jovetić, Milica et al. 2017a. “Mineral Content as a Tool for the Assessment of Honey Authenticity.” *Journal of AOAC International* 100(4): 862–70.

———. 2017b. “Mineral Content as a Tool for the Assessment of Honey Authenticity.” *Journal of AOAC International* 100(4): 862–70.

Kadar, Melinda et al. 2011. “Volatile Fraction Composition and Physicochemical Parameters as Tools for the Differentiation of Lemon Blossom Honey and Orange Blossom Honey.” *Journal of the Science of Food and Agriculture* 91(15): 2768–76.

Kanost, Michael, and Maureen Gorman. 2008. “Phenoloxidases in Insect Immunity.” In *Insect Immunology*, , 69–iv.

Karabagias, I.K. et al. 2017. “Characterization and Classification of Commercial Thyme Honeys Produced in Specific Mediterranean Countries According to Geographical Origin, Using Physicochemical Parameter Values and Mineral Content in Combination with Chemometrics.” *European Food Research and Technology* 243(5): 889–900.

Karabagias, Ioannis K. et al. 2018. “Characterization and Botanical Differentiation of Monofloral and Multifloral Honeys Produced in Cyprus, Greece, and Egypt Using Physicochemical Parameter Analysis and Mineral Content in Conjunction with Supervised Statistical Techniques.” *Journal of Analytical Methods in Chemistry* 2018.

Karabín, Marcel, Tereza Hudcová, Lukáš Jelínek, and Pavel Dostálek. 2016. “Biologically Active Compounds from Hops and Prospects for Their Use.” *Comprehensive Reviews in Food Science and Food Safety* 15(3): 542–67.

Kasiotis, Konstantinos M et al. 2023. “Fingerprinting Chemical Markers in the Mediterranean Orange Blossom Honey: UHPLC-HRMS Metabolomics Study Integrating Melissopalynological Analysis, GC-MS and HPLC-PDA-ESI/MS.” *Molecules (Basel, Switzerland)* 28(9).

Kavanagh, Saorla et al. 2019. “Physicochemical Properties and Phenolic Content of Honey from Different Floral Origins and from Rural versus Urban Landscapes.” *Food Chemistry* 272: 66–75.

Khalifa, Shaden A. M. et al. 2021. “Crop Production.” : 1–23.

Khalil, Md Ibrahim et al. 2012. “Physicochemical and Antioxidant Properties of Algerian Honey.” *Molecules* 17(9): 11199–215.

Koeniger, N, and S Fuchs. 1989. “Eleven Years With Varroa—Experiences, Retrospects and Prospects.” *Bee World* 70(4): 148–59. https://doi.org/10.1080/0005772X.1989.11099011.

Korta, E et al. 2003. “Determination of Amitraz and Other Acaricide Residues in Beeswax.” *Analytica Chimica Acta* 475(1–2): 97 – 103. https://www.scopus.com/inward/record.uri?eid=2-s2.0-0037414672&doi=10.1016%2FS0003-2670%2802%2901221-7&partnerID=40&md5=a563143a732e8748f6b247c97173147f.

Koulis, Georgios A. et al. 2021. “Honey Phenolic Compound Profiling and Authenticity Assessment Using Hrms Targeted and Untargeted Metabolomics.” *Molecules* 26(9): 1–21.

Kralj, Jasna, Axel Brockmann, Stefan Fuchs, and Jürgen Tautz. 2007. “The Parasitic Mite Varroa Destructor Affects Non-Associative Learning in Honey Bee Foragers, Apis Mellifera L.” *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 193(3): 363–70.

Kraus, Bernhard, and Stefan Berg. 1994. “Effect of a Lactic Acid Treatment during Winter in Temperate Climate upon Varroa Jacobsoni Oud. and the Bee (Apis Mellifera L.) Colony.” *Experimental and Applied Acarology* 18(8): 459–68.

Krupke, Christian H. et al. 2012. “Multiple Routes of Pesticide Exposure for Honey Bees Living near Agricultural Fields.” *PLoS ONE* 7(1).

Kuenen, L P S, and N W Calderone. 1997. “Transfers of Varroa Mites from Newly Emerged Bees : Preferences for Age- and Function-Specific Adult Bees (Hymenoptera : Apidae).” 10(2).

Kuterbach, D A, and B Walcott. 1986. “Iron-Containing Cells in the Honey-Bee (Apis Mellifera). II. Accumulation during Development.” *The Journal of experimental biology* 126: 389–401.

Laube, I. et al. 2010. “Development of Primer and Probe Sets for the Detection of Plant Species in Honey.” *Food Chemistry* 118(4): 979–86.

Leida, Barbara, Giorgio Della Valle, and Maria Lucia Piana. I quaderni dell’apicoltore *Flora Apistica*. www.bombus.it.

Leza, M. Mar, Gregori Llado, and Miguel A. Miranda-Chueca. 2015. “Comparison of the Efficacy of Apiguard (Thymol) and Apivar (Amitraz) in the Control of Varroa Destructor (Acari: Varroidae).” *Spanish Journal of Agricultural Research* 13(3): 1–5.

Llorente, Jesús, and Miguel Suárez. 1999. “Original Article Negative Long-Term Effects on Bee Colonies Treated with Oxalic Acid against Varroa Jacobsoni Oud . Mariano a Aránzazu Meana A.” *Apidologie* 30(4): 289–92.

Lodesani, Marco, and Cecilia Costa. 2008. “Maximizing the Efficacy of a Thymol Based Product against the Mite Varroa Destructor by Increasing the Air Space in the Hive.” *Journal of Apicultural Research* 47: 113–17.

Louveaux, J., Anna Maurizio, and G. Vorwohl. 1978. “Methods of Melissopalynology.” *Bee World* 59(4): 139–57.

Luo, Donghui et al. 2016. “Hydrogen (2H/1H) Combined with Carbon (13C/12C) Isotope Ratios Analysis to Determine the Adulteration of Commercial Honey.” *Food Analytical Methods* 9(1): 255–62.

M. Carmen, Seijo-Coello, Olga Escuredo, and María Fernández-González. 2011. “Fungal Diversity in Honeys from Northwest Spain and Their Relationship to the Ecological Origin

of the Product.” *Grana* 50: 55–62.

M. L. Winston. 1991. “The Biology of the Honey Bee.” In *Harvard University Press. - USA,*.

Macedo, P. A., J. Wu, and M. D. Ellis. 2002. “Using Inert Dusts to Detect and Assess Varroa Infestations in Honey Bee Colonies.” *Journal of Apicultural Research* 41(1–2): 3–7.

Maddaloni, M., and D. W. Pascual. 2015. “Isolation of Oxalotrophic Bacteria Associated with Varroa Destructor Mites.” *Letters in Applied Microbiology* 61(5): 411–17.

Magdas, Dana Alina et al. 2021. “Applications of Emerging Stable Isotopes and Elemental Markers for Geographical and Varietal Recognition of Romanian and French Honeys.” *Food Chemistry* 334(July 2020): 127599. https://doi.org/10.1016/j.foodchem.2020.127599.

Manino, Aulo, and Augusto Patetta. 2004. “Bee Space: Social Behaviour and Basis of Rational Beekeeping.” *Redia* LXXXVII: 203–6.

Manning, Rob. 2001. “Fatty Acids in Pollen: A Review of Their Importance for Honey Bees.” *Bee World* 82(2): 60–75. https://doi.org/10.1080/0005772X.2001.11099504.

Manzano Sánchez, Lorena et al. 2021. “Presence, Persistence and Distribution of Thymol in Honeybees and Beehive Compartments by High Resolution Mass Spectrometry.” *Environmental Advances* 5.

Maria Rizelio, Viviane et al. 2012. “Fast Determination of Cations in Honey by Capillary Electrophoresis: A Possible Method for Geographic Origin Discrimination.” *Talanta* 99: 450–56.

Martano, M., Maiolino, P., Power, K. 2016. Ateneapoli editore *Gestione Igienico Sanitaria Degli Apiari a Salvaguardia Dell’ambiente e Della Biodiversità*.

Martin, Stephen J et al. 2012. “Global Honey Bee Viral Landscape Altered by a Parasitic Mite.” *Science (New York, N.Y.)* 336(6086): 1304–6.

Martin, Stephen J, Brenda V Ball, and Norman L Carreck. 2010. “Prevalence and Persistence of Deformed Wing Virus (DWV) in Untreated or Acaricide-Treated Varroa Destructor Infested Honey Bee (Apis Mellifera) Colonies.” *Journal of Apicultural Research* 49(1): 72–79. https://doi.org/10.3896/IBRA.1.49.1.10.

Mendes, E., E. Brojo Proença, I. M.P.L.V.O. Ferreira, and M. A. Ferreira. 1998. “Quality Evaluation of Portuguese Honey.” *Carbohydrate Polymers* 37(3): 219–23.

Milani, N. 2001. “Activity of Oxalic and Citric Acids on the Mite Varroa Destructor in Laboratory Assays.” *Apidologie* 32(2): 127–38.

Milani, Norberto. 1999. “The Resistance of Varroa Jacobsoni Oud. to Acaricides.” *Apidologie* 30(2–3): 229–34.

Milko Sinacori. 2014. “Analisi Delle Popolazioni Microbiche Ricorrenti Nel Miele.” Università degli studi di Palermo.

Minaei, Saeid et al. 2017. “VIS/NIR Imaging Application for Honey Floral Origin Determination.” *Infrared Physics and Technology* 86: 218–25.

de Miranda, J R, and I Fries. 2008. “Venereal and Vertical Transmission of Deformed Wing Virus in Honeybees (Apis Mellifera L.).” *Journal of invertebrate pathology* 98(2): 184–89.

de Miranda, Joachim R, and Elke Genersch. 2010. “Deformed Wing Virus.” *Journal of invertebrate pathology* 103 Suppl: S48-61.

Mititelu, Magdalena et al. 2022. “Quality Control of Different Types of Honey and Propolis Collected from Romanian Accredited Beekeepers and Consumer’s Risk Assessment.” *Crystals* 12(1): 1–18.

Möckel, Nadine, Sebastian Gisder, and Elke Genersch. 2011. “Horizontal Transmission of Deformed Wing Virus: Pathological Consequences in Adult Bees (Apis Mellifera) Depend on the Transmission Route.” *The Journal of general virology* 92(Pt 2): 370–77.

Molan, P.C. 1998. “The Limitations of the Methods of Identifying the Floral Source of Honeys.” *Bee World* 79(2): 59–68.

Moškrič, Ajda, Jernej Bubnič, Maja Ivana Smodiš Škerl, and Janez Prešern. 2018. “Potential Positive Effects of Hop Beta Acids on Parasitic Mite Varroa Destructor Control in Honey Bee Colonies.” *Journal of Hygienic Engineering and Design* 24: 21–28.

Al Naggar, Yahya et al. 2016. “Effects of Treatments with Apivar ® and Thymovar ® on V. Destructor Populations, Virus Infections and Indoor Winter Survival of Canadian Honey Bee (Apis Mellifera L.) Colonies.” *Journal of Apicultural Research* 54: 1–7.

Nanetti, Antonio et al. 2003. “Oxalic Acid Treatments for Varroa Control (Review).” *Apiacta* 38.

Neumann, Peter, and Norman L. Carreck. 2010. “Honey Bee Colony Losses.” *Journal of Apicultural Research* 49(1): 1–6.

Oddo L. P., Piana L., Sabatini A. G. 2007. *Conoscere Il Miele*. 5th ed. ed. Avenue Media.

Oddo, L. Persano, MG G. Piazza, AG G. Sabatini, and M. Accorti. 1995. “Characterization of Unifloral Honeys.” *Apidologie* 26(6): 453–65.

Von Der Ohe, Werner et al. 2004. “Harmonized Methods of Melissopalynology.” *Apidologie* 35(Suppl. 1): S18–25.

Oldroyd, Benjamin P. 1999. “Coevolution While You Wait: Varroa Jacobsoni, a New Parasite of Western Honeybees.” *Trends in Ecology & Evolution* 14(8): 312–15. https://www.sciencedirect.com/science/article/pii/S0169534799016134.

Ongus, Juliette R et al. 2004. “Complete Sequence of a Picorna-like Virus of the Genus Iflavirus Replicating in the Mite Varroa Destructor.” *The Journal of general virology* 85(Pt 12): 3747–55.

Oruc, H H et al. 2012. “Determination of Acute Oral Toxicity of Flumethrin in Honey Bees.” *Journal of economic entomology* 105(6): 1890–94.

Padovan, G. J., D. De Jong, L. P. Rodrigues, and J. S. Marchini. 2003. “Detection of Adulteration of Commercial Honey Samples by the 13C/12C Isotopic Ratio.” *Food Chemistry* 82(4): 633–36.

Paleolog, Jerzy et al. 2023. “Imidacloprid Pesticide Causes Unexpectedly Severe Bioelement Deficiencies and Imbalance in Honey Bees Even at Sublethal Doses.” *Animals* 13(4).

Paudel, Yagya Prasad, Robert Mackereth, Rodney Hanley, and Wensheng Qin. 2015. “Honey Bees (Apis Mellifera L.) and Pollination Issues: Current Status, Impacts and Potential Drivers of Decline.” *Journal of Agricultural Science* 7(6): 93–109.

Perna, Anna Maria, Giulia Grassi, Emilio Gambacorta, and Amalia Simonetti. 2021. “Minerals Content in Basilicata Region (Southern Italy) Honeys from Areas with Different Anthropic Impact.” *International Journal of Food Science and Technology* 56(9): 4465–72.

Persano, Livia, and Maria Lucia. “Miele e Territorio.”

Persano Oddo, Livia, and Stefan Bogdanov. 2004. “Determination of Honey Botanical Origin: Problems and Issues.” *Apidologie* 35(Suppl. 1): S2–3. http://www.apis.admin.ch/host/honey/.

Persano Oddo, Livia, and Roberto Piro. 2004. “Main European Unifloral Honeys: Descriptive Sheets.” *Apidologie* 35(Suppl. 1): S38–81.

Piccirillo, Giancarlo, and David De Jong. 2003. “The Influence of Brood Comb Cell Size on the Reproductive Behavior of the Ectoparasitic Mite Varroa Destructor in Africanized Honey Bee Colonies.” *Genetics and molecular research : GMR* 2: 36–42.

Pickard, R. S., and G. Y. Kither. 1983. “An Evaluation of Shaking and Displacement Methods for Separating a Sample of Honeybees with Large Hypopharyngeal Glands from the Remainder of a Colony.” *Journal of Apicultural Research* 22(2): 101–6.

Pietropaoli, Marco. 2013. “Verifica Dell’efficacia Acaricida Di Trattamenti Estivi Con Acido Formico in Gel (Mite Away Quick Strips®).” *Lapis* 4: 8–12.

Pietropaoli, Marco, and Giovanni Formato. 2019a. “Acaricide Efficacy and Honey Bee Toxicity of Three New Formic Acid-Based Products to Control Varroa Destructor.” *Journal of Apicultural Research* 58(5): 824–30.

———. 2019b. “Acaricide Efficacy and Honey Bee Toxicity of Three New Formic Acid-Based Products to Control Varroa Destructor.” *Journal of Apicultural Research* 58(5): 824–30. https://doi.org/10.1080/00218839.2019.1656788.

Pinzauti, M., & Frediani. 1998. “Appunti Di Apicoltura.” *ervizio Editoriale Universitario di Pisa.*

Pita-Calvo, Consuelo, and Manuel Vázquez. 2017. “Differences between Honeydew and Blossom Honeys: A Review.” *Trends in Food Science and Technology* 59: 79–87.

Ponisio, Lauren C. et al. 2015. “Diversification Practices Reduce Organic to Conventional Yield Gap.” *Proceedings of the Royal Society B: Biological Sciences* 282(1799).

Poonia, Asha, Rachna Gulati, and S Sharma. 2009. “Efficacy of Screen Floor and Powdered Sugar against Varroa Destructor Anderson and Trueman in Apis Mellifera L. Colonies.” *Biopesticides International* 5: 0.

Porrini, Claudio. 2007. “Le Api Come Bioindicatori Dell’inquinamento Ambientale.” *Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Entomologia, Università di Bologna*: 3.

Potts, S.G. et al. 2010. “Declines of Managed Honey Bees and Beekeepers in Europe.” *Journal of Apicultural Research* 49(1): 15–22.

Potts, Simon G et al. 2010. “Global Pollinator Declines: Trends, Impacts and Drivers.” *Trends in Ecology & Evolution* 25(6): 345–53. https://www.sciencedirect.com/science/article/pii/S0169534710000364.

Prisco, Gennaro Di et al. 2011. “Dynamics of Persistent and Acute Deformed Wing Virus Infections in Honey Bees, Apis Mellifera.” *Viruses* 3(12): 2425–41.

Ptaszyńska, Aneta A., Grzegorz Borsuk, Marcin Anusiewicz, and Wieslaw Mułenko. 2012. “Location of Nosema Spp. Spores within the Body of the Honey Bee.” *Medycyna Weterynaryjna* 68(10): 618–21.

Quigley, Tyler P, Gro V Amdam, and Gyan H Harwood. 2019. “Honey Bees as Bioindicators of Changing Global Agricultural Landscapes.” *Current opinion in insect science* 35: 132–37.

R., Oliver. 2010. *The Economy of the Hive, Part 1*. American Bee Journal.

Rademacher, Eva, and Marika Harz. 2006. “Oxalic Acid for the Control of Varroosis in Honey Bee Colonies – a Review.” *Apidologie* 37(1): 98–120. http://10.0.4.27/apido:2005063.

Rademacher, Eva, Marika Harz, and Saskia Schneider. 2015. “The Development of HopGuard® as a Winter Treatment against Varroa Destructor in Colonies of Apis Mellifera.” *Apidologie* 46(6): 748–59.

Ramsey, Samuel D et al. 2019. “Varroa Destructor Feeds Primarily on Honey Bee Fat Body Tissue and Not Hemolymph.” 116(5).

Reams, Taylor, and Juliana Rangel. 2022. “Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of Varroa Destructor, the Parasitic Mite of Apis Mellifera.” *Journal of insect science (Online)* 22(1).

Reams, Taylor, Juliana Rangel, and Margarita Lopez-Uribe. 2022. “Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of Varroa Destructor, the Parasitic

Mite of Apis Mellifera.” *Journal of Insect Science* 22(1): 18–19. https://doi.org/10.1093/jisesa/ieab101Review.

Reza, By et al. 2008. “The Efficacy of Apivar ® and Bayvarol ® and CheckMite +® in the Control of Varroa Destructor in Iran.” *October*.

Rice, Nathan D., Mark L. Winston, and Heather A. Higo. 2004. “Integrated Pest Management for the Parasitic Mite Varroa Destructor (Anderson and Trueman) in Colonies of Honey Bees (Apis Mellifera).” *American Bee Journal* 144(10): 791–95.

Richards, E H, Benjamin Jones, and Alan Bowman. 2011. “Salivary Secretions from the Honeybee Mite, Varroa Destructor: Effects on Insect Haemocytes and Preliminary Biochemical Characterization.” *Parasitology* 138(5): 602–8.

Rinderer, Thomas E., Jeffrey W. Harris, Gregory J. Hunt, and Lilia I. De Guzman. 2010. “Breeding for Resistance to Varroa Destructor in North America.” *Apidologie* 41(3): 409–24.

Rodríguez-Flores, M. Shantal, Olga Escuredo, Montserrat Míguez, and M. Carmen Seijo. 2019. “Differentiation of Oak Honeydew and Chestnut Honeys from the Same Geographical Origin Using Chemometric Methods.” *Food Chemistry* 297: 124979.

Rodríguez-Flores, M.S., O. Escuredo, A. Seijo-Rodríguez, and M.C. Seijo. 2019. “Characterization of the Honey Produced in Heather Communities (NW Spain).” *Journal of Apicultural Research* 58(1): 84–91.

Rosenkranz, Peter, Pia Aumeier, and Bettina Ziegelmann. 2010. “Biology and Control of Varroa Destructor.” *Journal of invertebrate pathology* 103 Suppl: S96-119.

Sabahi, Qodratollah et al. 2020. “Evaluation of Dry and Wet Formulations of Oxalic Acid, Thymol, and Oregano Oil for Varroa Mite (Acari: Varroidae) Control in Honey Bee (Hymenoptera: Apidae) Colonies.” *Journal of Economic Entomology* 113(6): 2588–94.

Sahinler, Nuray, Aziz Gül, and Ahmet Şahin. 2005. “Vitamin E Supplementation In Honey Bee Colonies To Increase Acceptance Rate and Royal Jelly Production.” *Journal of Apicultural Research* 44: 58–60.

Sakla, Rasha, and Samah El-shafeiy. 2022. “Evaluation of Royal Jelly Quality and Queens Production by Using Natural Food Supplements in Honeybee Colonies.” *Egyptian Journal of Agricultural Research* 0(0): 0–0.

Salima, Koumad, and Nizar Haddad. 2015. “Resistance of Varroa Destructor to Apistan and Bayvarol.”

Saxena, Sudhanshu, Satyendra Gautam, and Arun Sharma. 2010. “Physical, Biochemical and Antioxidant Properties of Some Indian Honeys.” *Food Chemistry* 118(2): 391–97. http://dx.doi.org/10.1016/j.foodchem.2009.05.001.

Schaepman, Michael E. 2007. “Spectrodirectional Remote Sensing: From Pixels to Processes.” *International Journal of Applied Earth Observation and Geoinformation* 9(2): 204–23.

Schievano, Elisabetta et al. 2019. “NMR Assessment of European Acacia Honey Origin and Composition of EU-Blend Based on Geographical Floral Markers.” *Food Chemistry* 288: 96–101.

Semkiw, Piotr, Piotr Skubida, and Krystyna Pohorecka. 2013. “The Amitraz Strips Efficacy in Control of Varroa Destructor After Many Years Application of Amitraz in Apiaries.” *Journal of Apicultural Science* 57: 107–21.

She, Seng et al. 2019. “Discrimination of Geographical Origins of Chinese Acacia Honey Using Complex 13C/12C, Oligosaccharides and Polyphenols.” *Food Chemistry* 272(August 2018): 580–85.

Shen, Miaoqing, Xiaolong Yang, Diana Cox-Foster, and Liwang Cui. 2005. “The Role of Varroa Mites in Infections of Kashmir Bee Virus (KBV) and Deformed Wing Virus (DWV) in Honey Bees.” *Virology* 342(1): 141–49.

Shumkova, Rositsa, Ralitsa Balkanska, and Peter Hristov. 2021. “The Herbal Supplements Nozemat Herb® and Nozemat Herb Plus®: An Alternative Therapy for n. Ceranae Infection and Its Effects on Honey Bee Strength and Production Traits.” *Pathogens* 10(2): 1–19.

Siddiqui, Amna Jabbar, Syed Ghulam Musharraf, M. Iqbal Choudhary, and Atta ur Rahman. 2017. “Application of Analytical Methods in Authentication and Adulteration of Honey.” *Food Chemistry* 217: 687–98. http://dx.doi.org/10.1016/j.foodchem.2016.09.001.

Skorbiłowicz, Elżbieta, Mirosław Skorbiłowicz, and Izabela Cieśluk. 2018. “Bees as Bioindicators of Environmental Pollution with Metals in an Urban Area.” 19(3): 229–34.

Soares, Sónia, Joana S. Amaral, Maria Beatriz P.P. Oliveira, and Isabel Mafra. 2015. “Improving DNA Isolation from Honey for the Botanical Origin Identification.” *Food Control* 48: 130–36.

———. 2017. “A Comprehensive Review on the Main Honey Authentication Issues: Production and Origin.” *Comprehensive Reviews in Food Science and Food Safety* 16(5): 1072–1100.

Solayman, Md et al. 2016. “Physicochemical Properties, Minerals, Trace Elements, and Heavy Metals in Honey of Different Origins: A Comprehensive Review.” *Comprehensive Reviews in Food Science and Food Safety* 15(1): 219–33.

Sollid, Kris. 2020. “What Is Honey?” *food insight*. https://foodinsight.org/what-is-honey/%22.

Somerville, Doug. 2005. “FAT BEES SKINNY BEES -a Manual on Honey Bee Nutrition for Beekeepers-.” *Australian Government Rural industries Research and Development Corporation* (05): 1–142. http://library.wur.nl/WebQuery/clc/1766764.

STANIMIROVIC Z, TODOROVIC DAJANA, STEVANOVIC JEVROSIMA, MLADENOVIC M, and JANKOVIC LJILJANA. 2003. “Cymiazole Hydrochloride Is an Iminophenyl Thiazolidine Derivative, Active against Acari (Mites and Ticks). In Veterinary Medicine, Cymiazole Hydrochloride Is Recommended for the Control of Varroasis in Honey Bees. The Intended Pharma- Ceutical Formulation.” *Acta Veterinaria Beograd* 53(1): 47–55.

Staveley P.J. 2014. “A Causal Analysis of Observed Declines in Managed.”

Stevanovic, Jevrosima et al. 2012. “Stimulating Effect of Sugar Dusting on Honey Bee Grooming Behaviour.” *Entomologia Experimentalis et Applicata* 143: 23–30.

Sumpter, D. J.T., and S. J. Martin. 2004. “The Dynamics of Virus Epidemics in Varroa-Infested Honey Bee Colonies.” *Journal of Animal Ecology* 73(1): 51–63.

Svoboda, J A, M J Thompson, E W Herbert, and H Shimanuki. 1980. “Sterol Utilization in Honey Bees Fed a Synthetic Diet: Analysis of Prepupal Sterols.” *Journal of Insect Physiology* 26(5): 291–94. https://www.sciencedirect.com/science/article/pii/0022191080901365.

Teeters, Bethany S, Reed M Johnson, Marion D Ellis, and Blair D Siegfried. 2012. “Using Video-Tracking to Assess Sublethal Effects of Pesticides on Honey Bees (Apis Mellifera L.).” *Environmental toxicology and chemistry* 31(6): 1349–54.

Thakur, Mamta, and Vikas Nanda. 2020. “Composition and Functionality of Bee Pollen: A Review.” *Trends in Food Science and Technology* 98: 82 – 106. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85079864385&doi=10.1016%2Fj.tifs.2020.02.001&partnerID=40&md5=3a352ca25cad5b79d87e768048d98dba.

Tosun, Murat. 2013. “Detection of Adulteration in Honey Samples Added Various Sugar Syrups with 13C/12C Isotope Ratio Analysis Method.” *Food Chemistry* 138(2–3): 1629–32. http://dx.doi.org/10.1016/j.foodchem.2012.11.068.

Al Toufailia, Hasan, Luciano Scandian, and Francis 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.” *Journal of Apicultural Research* 54(2): 108–20. http://dx.doi.org/10.1080/00218839.2015.1106777.

Traynor, Kirsten S et al. 2020. “Varroa Destructor : A Complex Parasite , Crippling Honey Bees Worldwide.” *Trends in Parasitology* 36(7): 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

Trifković, Jelena et al. 2017. “Analytical Methods in Tracing Honey Authenticity.” *Journal of AOAC International* 100(4): 827–39.

Underwood, Robyn, and Margarita Lopez-Uribe. 2019. “Methods to Control Varroa Mites: An

Integrated Pest Management Approach." *The Pennsylvania State University: College of Agricultural Sciences*: 1–5. https://extension.psu.edu/methods-to-control-varroa-mites-an-integrated-pest-management-approach.

Valentini, Alice, Christian Miquel, and Pierre Taberlet. 2010. "DNA Barcoding for Honey Biodiversity." *Diversity* 2(4): 610–17.

Valverde, Silvia et al. 2023. "Mineral Composition of Bee Pollen and Its Relationship with Botanical Origin and Harvesting Period." *Journal of Food Composition and Analysis* 119: 105235.

Vandervalk, L. P., M. E. Nasr, and L. M. Dosdall. 2014. "New Miticides for Integrated Pest Management of Varroa Destructor (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies." *Journal of Economic Entomology* 107(6): 2030–36.

Vanlalhmangaiha, R et al. 2022. "Impact of Insect Pollination on the Quantitative and Qualitative Characteristics of Sweet Orange, Citrus Sinensis (L.) Osbeck." *Journal of Apicultural Research*: 1–10. https://doi.org/10.1080/00218839.2021.2013401.

Vaudo, Anthony, John Tooker, Christina Grozinger, and Harland Patch. 2015. "Bee Nutrition and Floral Resource Restoration." *Current Opinion in Insect Science* 10.

Vesco, U. 2013. "Varroa e Patogeni, Accoppiata Mortale." *L'Apis* 2: 5–9.

VETO-PHARMA. 2020. *Guía de Varroa*. second.

Vilarem, Caroline, Vincent Piou, and Fanny Vogelweith. 2021. "Varroa Destructor from the Laboratory to the Field: Control, Biocontrol and IPM Perspectives—A Review."

Wallner, Klaus. 1999. "Varroacides and Their Residues in Bee Products." *Apidologie* 30(2–3): 235–48.

Wang, H. et al. 2019. "A Novel Methodology for Real-Time Identification of the Botanical Origins and Adulteration of Honey by Rapid Evaporative Ionization Mass Spectrometry." *Food Control* 106.

Wantuch, Holly, and David Tarpy. 2009. "Removal of Drone Brood From Apis Mellifera (Hymenoptera: Apidae) Colonies to Control Varroa Destructor (Acari: Varroidae) and Retain Adult Drones." *Journal of economic entomology* 102: 2033–40.

Ward, Rachel, Mary F. Coffey, and Kevin Kavanagh. 2022. "Exposure of Apis Mellifera to Anti-Varroa Destructor Formic Acid Treatment Induces Significant Proteomic Alterations." *Journal of Apicultural Research* 0(0): 1–9. https://doi.org/10.1080/00218839.2022.2038055.

Waykar Bhalchandra, Mahesh A Joshi and Nilesh Jawalkar. 2022. "Effect of Storage on Various Honey Quality Parameters of Apis Mellifera Honey Harvested from Kannad Region, Aurangabad." *Journal of Pharmacognosy and Phytochemistry* 11(1): 239–46.

Wilde, Jerzy, Stefan Fuchs, Janusz Bratkowski, and Maciej Siuda. 2005. “Distribution of Varroa Destructor between Swarms and Colonies.” *Journal of Apicultural Research* 44: 190–94.

Yang, X, and D Cox-Foster. 2007. “Effects of Parasitization by Varroa Destructor on Survivorship and Physiological Traits of Apis Mellifera in Correlation with Viral Incidence and Microbial Challenge.” *Parasitology* 134(Pt 3): 405–12.

Yue, Constanze, and Elke Genersch. 2005. “RT-PCR Analysis of Deformed Wing Virus in Honeybees (Apis Mellifera) and Mites (Varroa Destructor).” *The Journal of general virology* 86(Pt 12): 3419–24.

Yue, Constanze, Marion Schröder, Sebastian Gisder, and Elke Genersch. 2007. “Vertical-Transmission Routes for Deformed Wing Virus of Honeybees (Apis Mellifera).” *The Journal of general virology* 88: 2329–36.

Yves, L C, E Marion, and R Wolfgang. 2010. “Review Article Varroa Mites and Honey Bee Health : Can Varroa Explain Part of the Colony Losses ?*.” 41: 353–63.

Zar, Jerrold H. 2014. 13 Nucl. Phys. *Biostatistical Analysis*.

Zhou, Xiaoteng, Mark Patrick Taylor, Helen Salouros, and Shiva Prasad. 2018. “Authenticity and Geographic Origin of Global Honeys Determined Using Carbon Isotope Ratios and Trace Elements.” *Scientific Reports* 8(1): 1–11. http://dx.doi.org/10.1038/s41598-018-32764-w.

Zhu, Xiangrong et al. 2010. “Detection of Adulterants Such as Sweeteners Materials in Honey Using Near-Infrared Spectroscopy and Chemometrics.” *Journal of Food Engineering* 101(1): 92–97.

Photographic bibliography

apacame.org.br.(05/2020)

https://www.apicolturaonline.it/peste.htm (06/2022)

https://www.izslt.it/apicoltura/peste-europea/ (06/2022)

Ptaszynska, A.A.; Borsuk, G.; Anusiewicz, M.; Mułenko, W. Location of Nosema spp. spores within body of honey bee. Med. Weter. 2012

(https://www.blog-veto-pharma.com/. 01/2022

https://unaapi.it/sanita-dell-alveare/varroatosi/varroa-acido-ossalico. (12/2014)

https://www.quartiitaly.it/en-EN/beekeeping-shop/58_langstroth/beekeeping_r11_1058_honey- gate-o-mm-40-1990www.unaapi.it (06/2022)

https://agriculture.ec.europa.eu/system/files/2023-05/market-presentation honey_spring2023_en.pdf (04/02023)

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