#115

DossierTècnic

Innovation and Knowledge Transfer

February 2022

Natural enemies of bees

Generalitat de Catalunya
Departament d'Acció Climàtica,
Alimentació i Agenda Rural

Rural
Cat

Carmel Mòdol Bresolí
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Research and training of beekeepers is essential to the sustainability of beekeeping

Beekeeping is a unique animal husbandry activity. It consists in the breeding of a single species of the *Insecta* class. This is quite different from other animal husbandry practices, which see to mammals, birds, fish and others. Here, the colony is the only possible herding unit, in keeping with bees' highly social behaviour, and it cannot be stabled or domesticated. This characteristic also implies a comparative lack of knowledge about its functioning and behaviour as an organism, and, consequently, greater difficulty in its care and management. Beekeeping is an agricultural activity from which, like the others, we obtain direct food products, with a value, however, lower than that created by bees' active role in producing many other plant products. These products will become food both for direct human consumption as well for other livestock. This close interaction with the environment also strongly conditions the activity and makes its life and health depend to an extraordinary degree on the conditions in the natural environment where it is located.

In recent decades, the living conditions of bees have become much more dire. The emergence of powerful enemies such as varroosis in its time and, more recently the Asian hornet (*Vespa velutina*) have added to the diseases bees already faced. Atmospheric pollution, the accumulation of toxic waste from cultivated plant species and the effects of treatments on the bees themselves, the effects of climate change on blooms and weather conditions in general, the loss of spaces with wild flora and other phenomena have caused significant difficulties for the life of these insect colonies and have tested the survival capacity of this essential species.

Research, education and training of beekeepers and other professionals who work in rural environments is essential for the sustainability of beekeeping, agricultural activity and healthy natural environments. Thus, the DACC, and specifically the Can Xifra Forestry Agricultural School, have identified the need to strengthen the reach and transfer of knowledge in beekeeping. For this reason, we are launching a series of *Dossiers Tècnics* that will address different high-impact topics within the world of beekeeping. We begin in the only way we could given the seriousness of the matter, with a *Dossier* dedicated to the enemies of bees. Dealing with the enemies of bees is another unique characteristic of this branch of animal husbandry, as surely no other farmed species has such a wide range of enemies that affect their lives.

On behalf of all authors who took part, we hope you find it useful.

Dossier Tècnic. No. 115
Natural Enemies of Bees
February 2022.

Publisher
General Directorate of Agri-Food Companies, Quality and Gastronomy.

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Printing
EADOP

Legal Deposit
B-16786-05.
ISSN: 1699-5465.

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Cover:
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ENEMIES
of bees

European bee-eater. Photo: Toni Sirera Moreno.

01. Introduction

Beekeeping is an animal husbandry activity that consists of raising the *Apis mellifera* bee to obtain products from it. We believe that this activity began at the time the human species obtained a container in which to put the colonies and thus be able to more easily care for them. Whether these first traditional hives were grouped in fixed-place apiaries, or whether they were transported during seasonal livestock movements, beekeeping activity did begin and that involved a certain management and protection of bees against their natural enemies. Bees have certainly always had enemies -diseases, parasites, predators and others- but bees have also become a species with a very high capacity for adaptation and survival. Old familiar foes, depending on the conditions of a given year, produced higher or lower losses in beekeeping activity, but they did not compromise a certain capacity for recovery and natural recolonisation. We can say that in the past there was a certain balance in bee populations in this territory.

Bees are a vital part of the ecosystem. They contribute to the preservation of biodiversity thanks to their pollinating activity.

The arrival of the varroa mite to Spain (*Varroa destructor*) in 1985-86 brought about a drastic change in the existing bee populations and, consequently, in the development of beekeeping. Until then, losses could be recovered with relative ease thanks to the potential for multiplication and swarming in the following spring, at which point bee colonies would reach their previous numbers. Except in some specific cases, such as foulbrood (*loque*), for some beekeepers it meant a certain healing of the hive, by completely renewing the old and deteriorated wax of the combs.

> Beekeeping is a unique part of the livestock sector that works for the preservation of bees, generates high-quality primary foods of outstanding nutritional value and is an economic pillar in more remote areas.

But the varroa mite changed everything. Losses in hives increased exponentially and, in the colonies that did not die, production fell dramatically due to the lack of a healthy population. Varroa mites reproduced the most in the most productive boxes. Furthermore, wild swarms practically disappeared within two or three years due to not receiving any kind of treatment. Additionally, there were no cleaning products specifically targeted against varroa mites, which meant entering a period of ‘home-style trials’ with other products used in the care for other animals being applied in all types of doses and supports. The varroa mite did not come alone either, but acted as a transmitter of other problems, including some viral diseases that significantly affect bees.

The fight against the varroa mite has improved a great deal, but it remains the main health problem for most beekeepers. Viral problems associated with this mite have also become increasingly apparent. American *loque* (fouldbrood), nosema, the appearance of the Asian hornet in some areas, among others, are examples of enemies with a high incidence in bee colonies. The incidence of these enemies plus the environmental problems mean that the average annual loss has gone from 5-10% before the varroa mite to proportions above 30-40% on bee farms currently, a state of affairs that pushes beekeeping to its limit in some places. In general, this situation forces beekeepers to reseed a large number of new colonies every year and to allocate a part of the farm to producing bees. This leads to a loss of production, more time spent working and requires more specialised training.

Table 1 provides an overview of the main enemies of bees today.

02. Bee health actions by the DACC

The Department of Climate Action, Food and Rural Agenda (DACC, by its Catalan initials) recognises and values the benefits that beekeeping and bees bring to the natural environment, agriculture, fruit growing and the rural world. For this reason, the DACC promotes actions that support bee preservation, improving the capacity of farms to face these problems and ensure environmental sustainability.

Since the late 1990s, there has been a decline in population of these insects worldwide. In Catalonia, the situation is no different and is also critical. Beekeepers reported mortality cases of up to 50% in 2021.

The DACC Prevention Service for Animal Health works toward the main objective of combating this population decline. For this reason, the service drafted the guidelines of the Bee Health Programme (PSAP, by its Catalan initials), a programme tailored to honey bee health, which includes all the mandatory regulatory requirements and is be the basis for preservation and care for bee health throughout Catalonia. This programme will be applicable to all types of farms, from the most specialised and professional operations to home consumption and hobbyist beekeepers.

The health programme is dynamic and will be adjusted based on the results of its implementation, the data obtained from farm visits and feedback from within Catalonia and the sector.

The main lines we want to work on are based on obtaining annual mortality data, with the possible attribution of causes and while raising awareness of the fight against varroosis and other diseases, by conducting periodic self-checks to assess the state of the apiary at the most critical moments.

To achieve this, the participation and support of the sector through the Associació de Defensa Sanitària (Health Defence Association, or ‘ADS’ by its Catalan initials) and the beekeepers' associations are essential. The role of both groups is fundamental as intermediary figures between the public administration and beekeepers. Fulfilling the task of the ADS to guarantee the health programme is being applied and collect data is also one of the objectives of the DACC. The health programme set up as part of the Beekeeping Technical Commission (Comissió Tècnica Apícola) was created with this aim and with that of learning the position and opinion of the sector through its representatives.

However, the reporting of transhumant movements and the locations of all settlements, as well as their registration with the DACC, makes it possible to have a map of the beehives in the event that a disease with a high risk of spread appears and containment measures and immediate notification must be undertaken.

At the same time, the DACC is collaborating in a study organised by the University of Valencia and the Ministry of

	Pest or disease	**Harms/Effects**
Mites	**Varroa mite** (*Varroa destructor, Varroa jacobsoni*)	It feeds mostly on the bees' fat bodies. It parasitises both adult bees and larvae during their development process.
	Acarapisosi mites (*Acarapis woodi*)	It settles in the tracheae of adult bees, in worker bees, drones and queens.
Bacteria	*American loque* (foulbrood) (*Paenibacillus larvae*)	Affects bee pupae, causing them to rot, desiccate and die.
	European *loque* (foulbrood) (*Melissoccocus pluton*)	Attacks the larvae and kills them before capping. Causes infection of their digestive tract and death by starvation.
Viruses	Up to twenty viruses have been identified as possible causes of bee diseases.	They are considered to be in a latent state inside the bee so that, in the proper conditions, they trigger a virosis.
Fungi	Nosema (*Nosema apis, Nosema ceranae*)	They parasitise adult bees by settling in the digestive tract and destroying the mucosa of the middle intestine.
	Other mycoses (mainly *Ascosphaera apis*)	They affect the brood, killing the pupae.
Insects	Coleoptera: *Trichodes apiarius*, some beetles of the genus *Meloe*, *Cetonia* sp.	Can cause assorted harm to the colonies. Cetonia can be found in abundance among hives. Bees have a hard time defending themselves because of their strong chitin armour.
	Hymenoptera: Wasps, European hornet (*Vespa crabro*), Asian hornet (*Vespa velutina nigritorax*)	Common predators of bees. The European hornet has never been a major problem. Vespa velutina is an invasive species that arrived in 2005 and causes great harm to bee colonies that have no self-defence system.
	Ants	Ants try to nest in some parts of the hives. Some species damage the beehive wood.
	Lepidoptera: The greater wax moth or honeycomb moth (*Galleria mellomella* and *Achoria grisella*) Death's-head hawk-moth (*Acherontia atropos*)	When, for other reasons, the bee population is dramatically reduced, the moth larvae destroy the combs and quickly devour the wax. The Death's-head hawk-moth is an eager consumer of honey, although it often ends up being killed by the bees.
	Diptera: The bee louse (*Braula coeca*) *Senotainia tricuspis*	The bee louse lives inside the hive, attaching itself to the bees, from which it takes the honey the bees feed on. It deposits a small larva on the bee, which will feed on it until the bee is killed.
Birds	Insectivorous species: swallows, black-tailed godwit, green woodpecker, honey buzzard, European bee-eater	Most are protected species and in the vast majority of cases do not cause significant harm. Of particular note is the European bee-eater, an extraordinary hunter, which comes to breed in southern Europe and fattens its young to prepare for the migratory return to Africa in September. As for the honey buzzard, it can be a good ally for bees when it comes to attacking Asian hornet nests.
Reptiles	Lizards and some snakes	It is common to see them around hive entrances occasionally eating bees.
Mammals	Bears	Notable predator for its appetite for brood and honey. Beekeepers install electric shepherd fencing systems in apiaries.
	Mice	They settle in very loose hives, especially in winter to make nests. They feed on pollen and honey, and destroy the combs.
	Martens	They are able to find weak or damaged spots (holes larger than 4 cm) in old hives to gain entry and feed on honey and pollen.
	Badgers	Thanks to the powerful claws on their forelegs, badgers are able to open holes in the wooden walls of hives and also access the honey.

Table 1. *Main enemies of bees. Source: own work. In yellow are the enemies of the bees that are discussed in this Dossier Tècnic*

Agriculture, Fisheries and Food (MAPA) to assess the effectiveness of certain antiparasitic drugs used to control the varroa mite. Thus, by assessing treatment effectiveness, it will be possible to check if there are resistances that require reorienting the control method by using molecules other than those authorised by the Agency for Medicines and Health Products, with the aim of controlling and reducing the incidence of varroa mites on farms.

Likewise, the DACC participates in MAPA's national Winter Colony Loss Monitoring Programme, which aims to study the main causes of bee mortality during the winter, detect the diseases that affect them and see the development of varroa mites in apiaries from season to season and in unfavourable environmental conditions. We visit up to eight apiaries every spring and autumn to take samples. The PSAP and other stakeholders aim to expand these visits to increase the sampling and data obtained each year.

At the same time, the aim is to facilitate more affordable access to sample analysis through the Lleida Livestock Health Laboratory to encourage beekeepers to carry out self-checks.

Additionally, to minimise the risk of entry of exotic parasites *Aethina tumida* or *Tropilaelaps*, we have designed a protocol for the control of imports of queen bees from third countries. Proper management is also promoted by strengthening beekeeper training and ensuring good beekeeping practices that see to proper biosecurity and the optimisation of the health and nutritional status of beekeeping operations.

Regarding *Vespa velutina*, PSAP is part of the Commission for the Fight and Control of the *Vespa velutina*. The Service also takes into account the harm it causes at bee farms, since it preys upon worker bees leaving to look for food.

Ants that have made their nests inside the boxes on top of the honeycomb panels. Photo: Àlex Sirera.

Poisoning by plant protection products is also a serious threat, and it is necessary to combat the use of prohibited plant protection products and, likewise, promote applying only authorised products and doing so at the appropriate times.

Finally, the DACC manages two beekeeping support programmes: the first is support to the beekeeping sector for pollination (POL), since this favours the conservation of certain wild plant species and the increase in yields of other cultivated species; and support aimed at improving the production and marketing of beekeeping products in sector for pollination (PIC), with the aim of improving the production and sale of beekeeping products in Catalonia.

03. Conclusions

The fragmentation of habitats, monocultures, high incidence of diseases such as varroosis and nosema, the introduction of exotic species such as the Asian hornet, climate change and the use of pesticides are the main causes of the decline in bee population.

Other critical factors that put bee populations at risk are the impacts caused by human activity and certain climatic and environmental conditions that endanger their survival.

Throughout this *Dossier Tècnic* you will find an extensive and accurate discussion of the enemies of bees that were highlighted in Table 1. These are the specific enemies that cause the most problems in the practice of beekeeping today, with which bees and beekeepers must contend.

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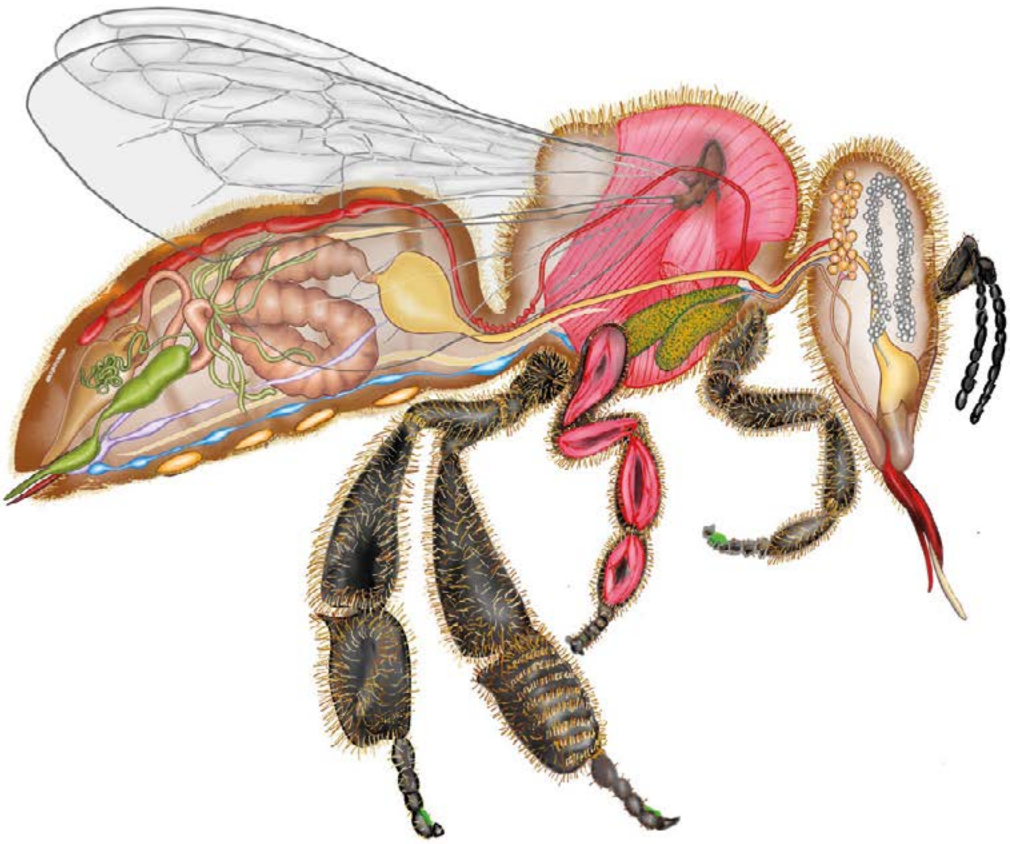
BEES:
anatomy, immune system and unique characteristics of the species



Internal anatomy of the bee. Designer: Àlex Sirera

01. Introduction

The honey bee (*Apis mellifera* sp.) is known for being one of the greatest pollinators there is. In recent years, its population has suffered a decline for various reasons and causes, which have resulted in what is known as CCD (*Colony Collapse Disorder*). Knowing and understanding the bee as an individual and as a colony is essential to preventing and stopping the main diseases that affect it.

02. Anatomy

a) External anatomy.

A bee's external anatomy consists of head, thorax and abdomen:

Head

The head houses two compound eyes that play an important role in the external vision of the honey bee. In addition, the head has three ocelli or simple eyes. They are believed to aid in stability during flight and to regulate entrances and exits from the hive. Bees have two antennae. Each is a movable jointed appendage. Thanks to these antennae, bees smell, touch and perceive chemical substances.

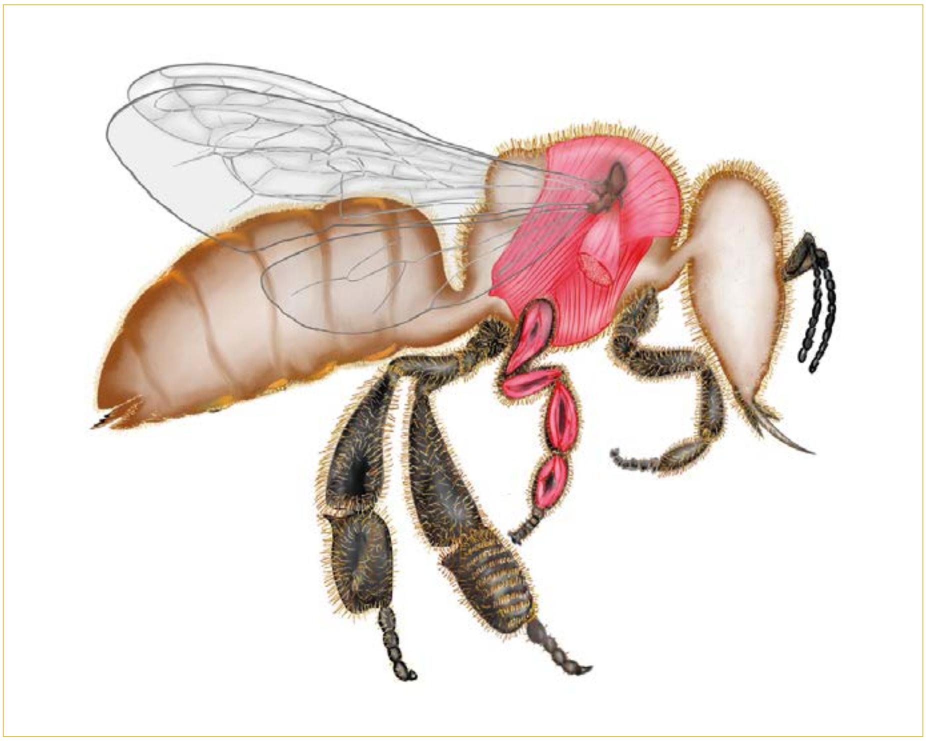
The mouth has jaws and a proboscis. The mandibles of the worker bee are used to bite through the cell caps in hives, mould wax and eat pollen. The group of mouth appendages of the honey bee form what is commonly called the proboscis. Among its functions are absorbing fluids such as nectar, licking surfaces and performing trophallaxis (fluid exchange between bees).

Thorax

The thorax is responsible for almost all of bees' locomotor functions.

The wings are attached to the thorax by sets of large muscles that provide strong lift. Wings are criss-crossed by many small nerves, whose pattern is useful in identifying bee breeds.

> The proboscis has functions which include absorbing fluids such as nectar, licking surfaces and performing fluid exchanges between bees.



Bee wings Credit: Àlex Sirera

Honey bee legs are highly modified. The front legs have an 'antenna cleaner' function. The middle legs have a thorn-like structure whose function is still debated. The hind legs form a basket whose function is to hold pollen harvested from the flowers and collect propolis.

Abdomen

It is a structure of great importance as it contains the haemolymph, the fat body, the digestive organs and other internal organs.

Its exoskeleton cover is divided into dorsal plates, called tergites, and ventral plates, or sternites, between which the *Varroa destructor* parasite establishes itself. In addition, the far end of the abdomen houses a structure of great importance for the bee: its stinger.

b) Internal Anatomy

Circulatory system

Bees have an open circulatory system, consisting of a heart and a large blood vessel, the aorta. The transport of nutritional substances and waste products is done through cells or haemocytes. Haemocytes carry out this transport of substances through haemolymph, analogous to blood in vertebrates.

Respiratory system

The bee's respiratory system is made up of spiracles, or holes that allow the entry and exit of gases. The first spiracle has traditionally been known to be the gateway for tracheal mites.

The respiratory system is structured in a series of ducts (tracheas) that take air from the spiracles to chambers (tracheal sacs).

Digestive system

It is divided into three sections: foregut, midgut and hindgut.

The foregut is made up of the mouth, the oesophagus and the crop or 'honey stomach'. The crop expands to allow the transport of honey, nectar and water collected by bees, while contracting to regurgitate its contents. Food passes into the middle stomach or ventricle, which is the main site for nutrient absorption, digestive enzyme secretion, food absorption and excretion of waste products. It is covered with a film called peritrophic membrane, which acts as a barrier against pathogens. This portion, however, is semi-permeable, which is why it can sometimes be the gateway for pathogens such as the *Nosema* sp. virus and *Paenibacillus larvae*. The end of the midgut is connected to the beginning of the hindgut through the Malpighian tubules, which serve to remove waste products.

The posterior portion is composed of the ileum and the rectum. The rectum is responsible for the reabsorption of water, salt and other elements collected by the Malpighian tubules. Bees, like other insects, do not urinate, but rather excrete a semi-liquid faeces.

Glandular system

- Corpora allata

The corpora allata are a pair of glands that produce hormones that control growth and development. They are behind the brain and are responsible for the secretion of the juvenile hormone that is involved in bee development, reproduction and type determination.

- Prothoracic gland

The prothoracic gland secretes ecdysone, a hormonal substance that regulates the moulting process.

- Nasanov's gland

This is an odoriferous gland located on the dorsal part of the abdomen that is used to call other bees and show them the entrance to the hive.

- Hypopharyngeal glands

Located on the head, they are especially developed in nurse bees. They are responsible for producing royal jelly to feed the larvae in their first three days of life and the queen throughout her life.

- Mandibular glands

They also participate in royal jelly production, along with the hypopharyngeal glands. Located in the head, they are found in workers and queens. In queens, their functions include inhibiting the breeding of new queens, ovarian inhibition of workers, attracting workers to the group and attracting drones outside the hive, among others.

- Wax glands

Located under the abdominal sternites, they are used to produce wax for the construction of honeycombs. A prior input of nectar is required to stimulate them.

Reproductive apparatus

Among the three individuals that make up the bee colony (worker, queen and drone), only two are considered reproductive.

The queen has two ovaries composed of numerous ovarioles that contain eggs at different stages of maturation. Eggs that are ready to be laid move into the middle oviduct. Once there, the queen can control whether or not the egg is fertilised with sperm provided by the drone. In addition, the queen has a spermatheca - that is, an organ used to store sperm collected during the mating flight with multiple drones.

The reproductive system of drones is made up of two testicles and two seminal vesicles. Drones reach sexual maturity 12-13 days after birth. Drones have an endophallus (penis), which is stored upside down inside its abdomen and turns out during copulation. At the end of copulation, it detaches from the drone.

Bee at flower. Photo: Àlex Sirera

03. Immune system

In bees, there are three main types of immunity: cellular, humoural and social, the last of these being especially prominent. Bees have only a third of the genes involved in the immune system that other insects do. This reduction is counteracted by a larger amount of genes involved in the collective behaviour of the colony. This gives an idea of the importance of social immunity in bees.

Mechanisms of cellular immunity

Cellular immunity works through the haemocytes. The amount of haemocytes varies between the different stages of bee development (larvae, pupae, nurses and foragers), and is higher in young bees than in adults. The response to pathogen encapsulation (phagocytosis and nodule formation) is reduced in drones and adult worker bees.

Cellular immunity plays a fundamental role in the response to nutritional deficiencies, pesticide poisoning, *Varroa destructor* and viruses, among other ailments. Haemocytes play key roles in wound healing and encapsulation responses of *Apis mellifera.*

Bees have only a third of the genes involved in the immune system that other insects do. This gives an idea of the importance of social immunity in bees.

Cellular immunity through haemocytes acts synergistically with other nutritional factors. This demonstrates, in short, the importance of nutrition for the health status of the honey bee colony.

Humoural immunity mechanisms

Humoural immunity acts through chemicals and antimicrobial peptides (AMPs), which are produced by the haemolymph in response to infections. Bees have four families of AMPs: defensive, abaecin, apidaecin, and hymenoptaecin.

These AMPs work such that each increases the activity of the others, and they work in concert with other physical barriers that prevent or resist pathogen entry, such as the exoskeleton cuticle or the peritrophic membranes of the digestive tract. The fat body acts as the main site of synthesis of antimicrobial peptides.

The phases of the immunological response can be summarised as follows:

1a Recognising pathogens.
2a Signalling: this is the activation of an intracellular signalling cascade that initiates activation of biochemical responses.
3a Action of cellular and humoural agents to eliminate the pathogen: culminates in the appearance of proteins that eliminate the pathogens.

Propolis on breeding boxes. Photo: Victoria Gámiz.

Hygienic behaviour Photo: Victoria Gámiz.

Nectar storage. Photo: Victoria Gámiz.

Social immunity mechanisms:

Collection of propolis

Bees collect propolis, which consists of resins collected from the buds of various trees and plants. Bees use them to coat small animals that reach the interior of the colony and mummify the bodies. These substances have traditionally been known for their antibacterial effects.

Hygienic behaviour

These consist in the ability of bees to, first, detect, and then extract, sick or parasitised brood cells. This behaviour is hereditary and is passed through the queen. It comes from the action of seven genes and is key in controlling the main diseases of the hive: ascospheriosis, American *loque* (foulbrood) and varroosis.

Grooming

Grooming consists using the jaws and legs to brush the head, thorax and abdomen. Bees may perform grooming on themselves (which is typical) or they may groom other bees.

Raising brood chamber temperature

This activity is unusual, as it requires a great deal of energy. It is used to control *Ascosphaera apis*.

Cannibalism

This is consumption of brood by the workers in the colony. Cannibalism is a survival mechanism in times of scarcity of protein-rich food so as to avoid the collapse of the colony. This behaviour also appears when, due to excess cold or heat, the larvae die, and the workers eat them to avoid the attack and appearance of pathogens such as *Ascosphaera apis*.

04. Bee colonies functioning as an individual

The behaviour of the bee colony as a superorganism has been the subject of numerous studies. Thus, in the hive each individual specialises in its jobs for the collective functioning of the bee colony as an individual.

Temperature regulation mechanisms

Bees maintain the temperature of the brood chamber at around 33-36º C.

> In the hive, each individual specialises in its jobs for the collective functioning of the bee colony as an individual.

On the one hand, when temperatures are low, bees carry out the so-called ‘winter cluster’ or grouping of individuals forming a rough ball shape. Another behaviour to generate heat is continuous and rapid abdominal muscle contractions.

On the other hand, in heat stress situations, temperature regulation mechanisms include the dispersal of individuals, ventilation and water evaporation. To prevent overheating, bees move water droplets to the upper walls of the brood cells and spread them out.

Food source search mechanisms

The promise of a nectar source is evaluated according to many variables. The storage bees are responsible for measuring and evaluating the nectar concentrations of a given honey source. Together with the storage bees, forager bees provide information about the distance from the hive to the source, the abundance of nectar at the source, the difficulty of feeding at the source, and its direction relative to the wind direction and speed.

Larvae feeding

Done by nurse bees, it begins with inspections by moving the antennae. The inspections may or may not be accompanied by administering food to the larvae. These inspections reveal brood cell contents, larval condition, age and location.

Construction of honeycombs and cell capping

Created by the wax glands (see section 01), the workers form a chain of wax , moving their heads back and forth to spread the wax string between their jaws. The workers also create wax flakes. Both scales and strings are used in the construction of honeycombs, which consist of a series of turns and combined movements of head and antennae. The process of closing or capping brood cells is done by measuring the thickness with the antennae, and the formation of the cocoon begins before the cell is completely capped.

Nectar and pollen storage

Nectar is regurgitated from the worker's stomach to the upper cell wall and falls into the cell due to gravity.

Pollen storage is done by letting the legs with pollen hang down, followed by cleaning the legs of the remaining pollen. The process ends with pushing the pollen into the cell with head movements. During this process, the pollen can be hydrated by the addition of saliva, nectar and honey, which creates bee bread.

05. Influence of diet on health

Influence of the Amount and Variety of Pollen on the Physiological Development of Nurse Bees

Significant reductions in the survival of workers are evident with just a 10% reduction in the amount of available pollen. Additionally, this affects the nurses' ability to care for the brood, because hypophaeryngeal gland development is highly dependent on protein intake.

Pollen and its Effects on Tolerance to *Nosema* sp.

> Numerous studies have confirmed the importance of pollen in the survival of workers and in the capacity of nurses to care for brood.

Recent scientific research concludes that the quality and diversity of the pollen consumed by bees has an effect on the tolerance to *Nosema ceranae*, with greater survival among bees whose diet had been with more varied and contained higher-quality pollen.

Micronutrients and their importance (lipids, vitamins and minerals)

Although the main groups of nutrients in bees come from nectar (carbohydrates) and pollen (proteins), bees also need to ingest lipids, vitamins and minerals.

Lipids are an energy source present in plant pollen in amounts that can vary between <1% and 18.9% (Roulston and Cane, 2000). The sterols in pollen are essential for bees, as they contribute to their survival and the production of brood. Nurses have the ability to synthesise small amounts of sterols without getting them from their diet, but this could lead to a depletion of internal sterol stores.

Most of the water-soluble vitamins are found in pollen in sufficient quantity. Among them, pyridoxine is considered essential for larval development.

Minerals are primarily obtained from pollen, but other sources such as nectar, water or even endogenous stores in adults are thought to exist.

06. Conclusions

Honey bees are complex insects whose specialisation has led to collective behaviour at the colony scale. That is why it is vital to be familiar with the particular features of their anatomy, biology, mechanisms of immunity and nutrition. All this will result in improved knowledge and, therefore, performance of our bee colonies.

To learn more

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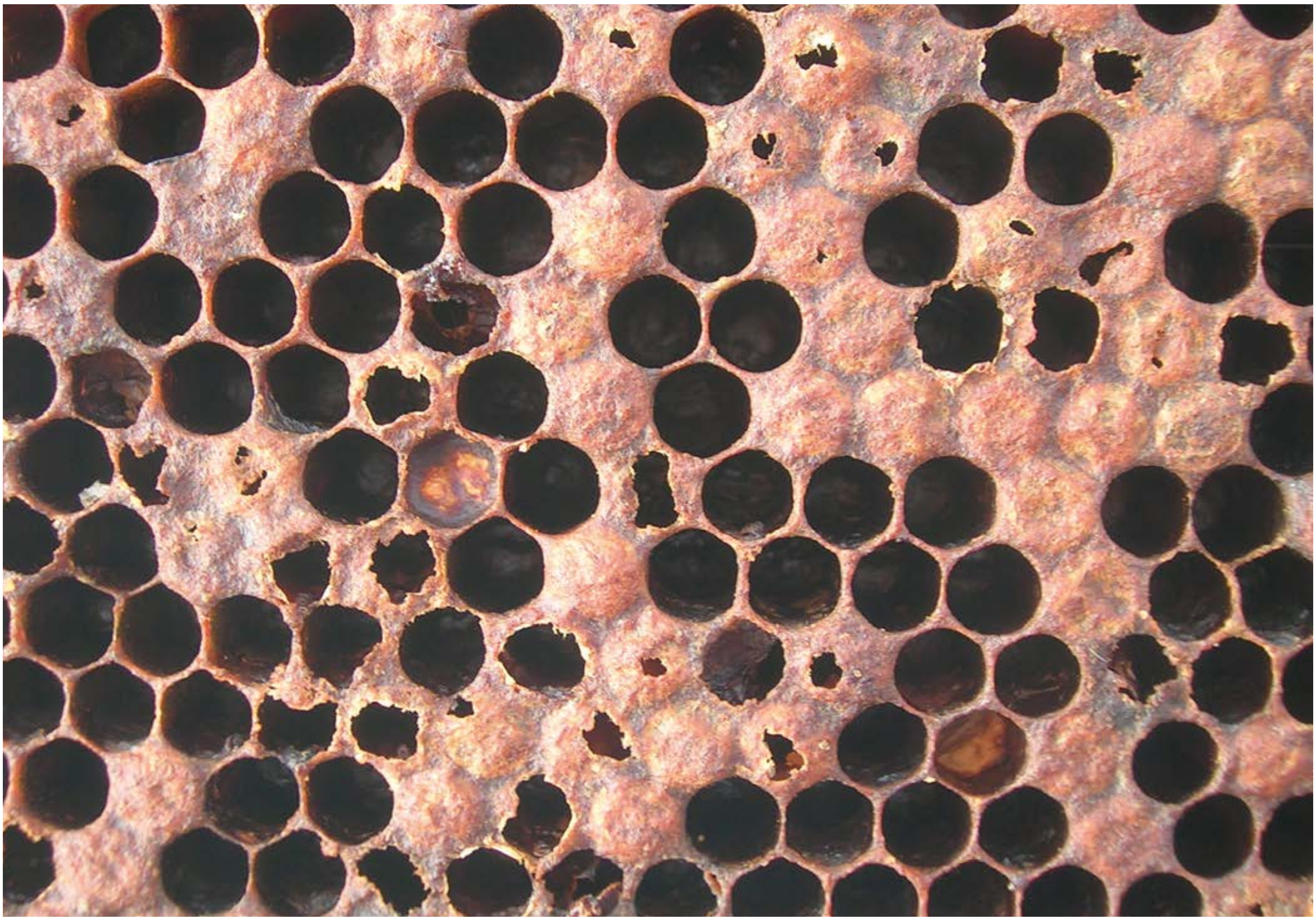
MAIN
bacterial and viral diseases of bees



Advanced American loque (foulbrood): 'splattered' brood chamber, dark, sunken, cracked caps. Photo: Antonio Gómez Pajuelo.

01. Bacterial diseases

Bacteria are more complex microorganisms than viruses. They are single-celled, but without many internal organs, so they take over the organelles of the cells they infect to complete their cycles of development and reproduction.

In a hive, a number of bacteria coexist with the bees. Some are beneficial, such as those from bee digestive systems, and bacteria from the fermentation of stored pollen. Others can cause diseases.

To combat these diseases, bees have an individual defence made up of 70 genes, which, among other things, code for the production of antimicrobial peptides, as part of the bee immune system. They also have a collective defence, based on a series of hygienic behaviours by the workers who expel sick or dead individuals from the hive. When the immune system and defensive behaviours do not work well enough, harmful bacteria can appear and cause disease. The most frequent and dangerous diseases are American *loque* (American foulbrood) and European *loque* (European foulbrood).

01.01 *American* loque (American foulbrood)

American foulbrood is caused by the bacterium *Paenibacillus larvae* (formerly *Bacillus larvae*). It produces spores that can remain in cysts in hives for years and will develop only when conditions are favourable. It appears when there are spores and a decrease in effectiveness of the bees' immune system due to poor nutrition (often stemming from varroosis), poisoning or lack of hygienic behaviour, which is often the result of bad genetics or imbalances in the young bee/brood relationship. The spores enter the larvae with feeding and do not develop until they cap and begin pupation. This change in environment causes the growth of these bacteria, which pass from the digestive tract to the other tissues and end up turning the pupa into a gummy mass. That mass is initially light brown, and then darkens over time and ends as a dry crust attached to the bottom of the cell.

Symptoms of this process are that the cap sinks and becomes a darker colour. Later, the cap cracks, or is opened laterally and asymmetrically by the bees that will try to clean the cell. Pupae mortality gives the capped brood chamber a splattered appearance.

All these symptoms are clearly visible. For a more certain diagnosis, a stick can be inserted into the cells with suspicious caps and rubbed along the lower wall. Upon removing it, the gummy mass of the affected pupa will be apparent and will stretch about 2.5 to 3 cm. The smell is disgusting, like something rotting.

Bees with highly hygienic behaviour detect affected pupae early and quickly expel them from the hive. In doing so, they carry millions of spores from their mouths to the larvae they feed, to the bees they exchange food with, to the food they handle (such as honey and pollen from the cells) and to the honeycombs and the floor of the hive where the pupae have been dragged.

When the bacteria affects enough brood, the hive gets weaker and usually dies. Sometimes bees can perform an 'abandonment for health', leaving the hive and infected combs behind and fleeing to a new location to start over.

For reasons of hygiene, affected hives, even if only one cell is detected, must be separated from the apiary to avoid contagion by drift or pillage. Strict disinfection measures must also be taken, as the spores can withstand temperatures of approximately 120° C for about 20 minutes, and survive for more than 15 years at room temperature.

American foulbrood diagnosis. Photo: Antonio Gómez Pajuelo.

When the bees' immune system and defensive behaviour do not work well enough, harmful bacteria can develop and cause disease.

If the hives keep good population numbers, measures to treat the affected hives can include:

- Asking a veterinarian for a prescription for antibiotic treatment, but the following collection of honey must be analysed for residues of the treatment. The dose and number of applications must be set in the prescription. It is considered that the disease is under control if no new cases appear in a month, although it is advisable to keep an extremely close eye on these hives during the following year.
- Completely eliminating the brood chamber by burning it.
- Transferring the colony to a new, clean and disinfected container.
- Do not use honey, combs or pollen from these hives for others hives.
- It is advisable to mark the boxes for these hives, set them aside and destroy them by burning them when they are removed from storage.
- The containers can be reused if they are scraped and disinfected with a gas blower until the wood has a straw tone. They can also be disinfected with water with 20% commercial bleach and a splash of detergent, and left to act for about 15 minutes.
- These hives will have to be marked to avoid breeding them and thus eliminate these sensitive genotypes from the farm.
- After handling affected cells, it is necessary to disinfect the material we have used by applying isopropyl alcohol, or by flaming them.

It is advisable to completely destroy the highly affected hives, close the hive at dusk and take them to storage. At best, the container can be recovered using the disinfection procedures mentioned.

As a preventative measure, hives that have had American *loque* (foulbrood), or those located in a high-risk area due to contagion with other apiaries, can be given a feed that contains nutritional supplements on 2 or 3 occasions. These supplements can be grapefruit seed oil (dose according to flavonoid content; it is necessary to have 0.1 g of flavonoids/kg of syrup), or propolis (1 litre of 20% propolis extract in 100 litres of syrup).

01.02 *European* loque (European foulbrood)

Caused by another bacterium, *Melissoccocus pluton*, European foulbrood is much less infectious than American *foulbrood*. It enters the larvae with food, but can only develop when their diet changes from royal jelly to honey and pollen, which comes on the third day after hatching. European foulbrood infection is usually followed by that of other associated bacteria.

Noticeable symptoms are that the affected larvae become opaque, losing the pearly colour of healthy larvae around them, and they fall to the bottom wall of the cell. Over time, their colour may turn ivory, or they may develop black spots on their skin. The death of these larvae gives the capped brood chamber a spattered appearance.

When an affected larva is cleaned by the bees, its mouth becomes contaminated, and the bacteria is passed on to the others it feeds on.

European *loque* is wholly connected to poor nutrition.

In recent years, there have been significant deaths attributable to this bacterium in hives using blueberry pollination, which is poor-quality pollen, and that try to develop very early on only sugary feed.

The larva that reaches capping, and thus the pupa stage, has overcome the disease, so in this *loque* disease (foulbrood) pupae are not affected. It also does not usually affect large numbers of larvae, and it does not give off a bad smell.

European foulbrood is entirely linked to poor nutrition. When it appears only in some larvae in the spring, this is not cause for concern, since it is easy for some larvae not to receive all necessary attention. But, if it appears in readily visible quantities, it is advisable to correct the feeding of the bees by moving to blooms or providing a feed that covers the deficiencies. In recent years, there have been significant deaths attributable to this bacterium in hives using blueberry pollination, which is poor-quality pollen, and that try to develop very early on only sugary feed.

For reasons of hygiene, it is advisable to disinfect hives where there is a significant infection and not to breed them, as in the case of American *loque* (foulbrood).

02. Viral diseases

Viruses are the smallest living things. They consist of just a strand of DNA or RNA in a protective coating, which takes over infected cells, changing their manufacturing orders to force them to produce only more viral particles, which eventually kills the cells.

In hives, a series of viruses that can cause diseases coexist with bees.

As mentioned above, to combat them, bees have an individual defence system in the form of their immune systems, which work as long as their diet of pollen provides the corresponding amino acids. Furthermore, it is necessary that these genes not be ‘switched off’ (epigenetic) by toxic agents such as acaricides or agricultural pesticides. They also have a collective defence system, formed by another series of genes that can cause the emergence of hygienic behaviours in the workers, which expel individuals who fall ill or die from the hive.

When the immune system and hygienic behaviours are not enough, viruses can develop and cause disease.

Harm from viruses is wholly linked to the presence of the parasitic varroa mite and poor nutrition, especially with respect to pollen. In recent years, the increase in prob-

Ivory-coloured larva affected by European loque (foulbrood), ‘splattered’ brood chamber. Photo: Antonio Gómez Pajuelo.

Dead larvae of European loque (foulbrood), fallen to the bottom of the cells, with the beginning of necrosis, ‘splattered’ brood chamber. Photo: A. Gómez Pajuelo.

lems with varroa and climate change have made their presence more common, and more harmful to bees.

The most frequent and dangerous ones are: in brood, deformed wing virus (DWV), sacbrood virus (SBV) and black queen cell (BQCV); and, in adult bees, there are two viruses associated with paralysis:*Acute paralysis virus and chronic paralysis virus* (APV and CPV, respectively).

02.01 Most common viroses in brood

Deformed Wing Virus (DWV)

This virus reproduces on the pupal tissue where the wings will emerge, so those wings are not fully developed. It is entirely associated with the presence of varroa mites, which transmit it from one bee to others with its bite.

It is effectively combated by controlling varroa, and, if necessary, with supplementary feeding to compensate for deficiencies in the hive diet.

Sacbrood Virus (SBV)

Until recently, this virus was very rare, but now it is becoming more common.

Sacbrood virus affects the internal tissues of the pupa, but not its skin. This leads to a final appearance of a sack filled with a dirty, more or less pasty brown liquid. The bees clean up the dead pupae, so they spread the virus when they feed other larvae. Cleaning up dead pupae makes the brood chamber look ‘splattered’.

Sacbrood virus is closely linked to the presence of varroa mites, like all viruses, but it is also linked to nutritional deficiencies due to poor flowering or an excessive load of hives in the settlement.

Bee with varroa mite and DWV. Photo: Antonio Gómez Pajuelo.

Bee heavily affected by DWV. Photo: Antonio Gómez Pajuelo.

Sacbrood virus (SBV). Photo: Antonio Gómez Pajuelo.


Black queen cell virus (BQCV). Photo: Antonio Gómez Pajuelo.


Black bee, acute or chronic paralysis virus (APV or CPV). Photo: Antonio G. P.

> Hives attacked by viruses must not reproduce. And, when taken to storage, the hives must be properly disinfected before being used again.

Black Queen Cell Virus (BQCV)

This virus has also become more common in recent years.

It only attacks the pupae chosen to become queens, which die before completing their development. Affected pupae turn yellow, then dark brown and finally to black, leaving dark-coloured spots on the interior of the cell wall. Queen breeders remove these hives from the production process.

02.02 Most common viruses in adult bees

Black Bee Virus, Acute Paralysis Virus (APV) and Chronic Paralysis Virus (CPV)

This is easily identified because affected bees are expelled from the hive, so they are seen at the hive entrance or nearby. They are also seen on the honeycombs. Initially, the hair on their bodies falls out and they leak fluids through their pores, giving them a glossy-leather appearance. Later, they begin to lose mobility in their hind legs, which spreads to their other legs. It usually appears in the most populated hives and is linked to poor nutrition. It most often occurs in the spring, though it can appear at other times.

It is advisable to isolate the affected hives and move them to a good bloom, or feed them to make sure they have a well-rounded diet. If a settlement is afflicted with CPV, then the settlement should be rejected.

02.03 Hygiene and preventative measures

When symptoms of any of these viruses appear in a hive, that hive should be isolated and removed from the apiary to prevent the viruses from being spread by drift or robbing.

Inside the hive, the APV and CPV will spread by rubbing between the bees; SBV and BQCV by cleaning the affected pupae; and, DWV, of course, by bites from varroa mites. Depending on the circumstances, it may be necessary to remove the affected brood chamber, as in the case of SBV or BQCV, control the varroa mites (especially when dealing with DWV) and, in any event, adequately provision the hive.

Hives attacked by viruses must not reproduce. And, when taken to storage, the hives must be properly disinfected before being used again. Recommended cleaning procedures are: scraping and flaming with a gas blower, or scraping and washing with water with 20% commercial bleach and a splash of detergent for 15 minutes.

At the moment, there are no effective treatments against bee viruses.

To learn more

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VARROA MITES

Bee parasitised by Varroa destructor. Photo: Antonio Gómez Pajuelo.

01. Introduction

The varroa mite is the major concern of the Spanish beekeeping sector, and with good reason. Since it was first detected in Spain in 1985, its current distribution throughout the country reflects how complicated it is to control it. Varroosis is the great health problem of Spanish beekeeping. And controlling it, with the tools currently available to beekeepers and veterinarians, is extremely complicated.

02. The Varroa mite: what is it?

Varroa destructor is a parasitic mite whose original host is the Asian bee *Apis cerana*. This mite has managed to colonise the hives of *Apis mellifera*, so that it is currently present in most of them.

From the perspective of biological time scales, the jump of this mite from one species to another has been very recent, coming around 1950, so our bees have not had time to establish a balanced relationship with the parasite. Therefore, controlling it depends exclusively on elements external to the hives.

This parasite has been studied for years, but its biology is not fully understood. In fact, it has recently been found that the mite feeds mostly on bee fat bodies, and an exclusively haematophagous (that is, blood-based) diet has been ruled out.

Varroa destructor has recently been found to feed mostly on bee fat bodies, and an exclusively blood-based diet has been ruled out.

Varroa destructor is an obligate parasite, requiring a suitable host to complete its life cycle. Its life cycle is closely linked to that of bees. The reproductive phase, one of two it goes through, takes place in the capped brood. The other phase, called the phoretic phase, is that of dispersion, in which it uses bees and drones as vectors to colonise other hives, and, in addition, to expand its distribution space within the hive itself.

The life cycle of the varroa mite is simple: a fertilised female penetrates a soon-to-be-capped brood cell of a worker or drone. The the first egg the mite lays is male, and the rest are female. When the females mature, they will be fertilised by the male inside the cell itself. And, when these females leave that cell, they can colonise other cells and start the cycle again.

Typically, the 'mother' female is thought to lay up to five eggs on worker brood and up to six on drone brood, although usually no more than two complete their development in worker cells and no more than four in drone cells. At this reproduction rate, varroa growth can be very rapid, and it can cause a hive to collapse within a year if there is no interruption of brooding. In climates with a winter hibernation pause, collapse can come in two to three years.

But the virulence of the attack depends on many factors, such as the mite's reproductive capacity and lifespan, and others related to the host (availability of worker and bee brood, propensity to swarming, bee cleaning and defence instincts), and others relating to the climate of the region where the bee colony is located.


Test for varroasis mites on uncapped brood and extracting brood on the cover of a hive. Photo: Àlex Sirera

03. Varroa-bee-hive: an unhealthy relationship

The interaction between *Varroa destructor* and bees is wholly asymmetric. Obviously, in this relationship, the parasite wins, because it causes extreme damage to the bees and can cause hives to collapse. The mite achieves its ultimate purpose, which is the expansion of its population and the transmission of its genes to future generations.

The damage caused by varroa mites to individual bees is clear: loss of weight of bees which appears in their larval and pupal phases (inside the brood cells), loss of body fat and reserves, open wounds on the body of the bee, shortened life expectancy, impaired flight, and, lastly, the transmission of other bee pathogens, such as different viruses. The virus that is easiest to spot is deformed wing virus.

Varroa also acts on the bee colony and on the general behaviour of the swarm. The increase in sick bees alters the normal behaviour of the colony; and we can note the decrease in nectar and pollen input, mosaic pattern breeding chamber or dispersed breeding nest, abnormal replacement of queens, alteration of hive hygienic behaviours, population decrease and weakened immune system. This vulnerability of the hive favours the appearance of pathologies that take the opportunity to develop, such as 'plastered lice', different viral diseases and some bacterial diseases.

04. Bees' adaptation to Varroa: a slow process

It is clear that bees can acquire the abilities to deal with varroa mites, but these characteristics currently do not appear equally among colonies.

Cleaning and hygiene behaviours have been observed in some hives, and some of them in an apiary have, notably, appeared to tolerate the varroa mite attacks.

Both the cleaning behaviour of the bees -namely grooming- and the ability to detect alarm odours of brood in capped cells, and then the hygienic behaviours of opening and cleaning the cells, are highly desirable characteristics in our hives. Hygienic behaviours seem to be triggered by the worker bees' olfactory ability; only some workers will detect odorous emissions from pupae and respond appropriately by uncapping, cleaning, and in some cases recapping problem cells. In different countries, there are ongoing selection programs for bee lines with elevated rates of anti-varroa behaviour, called 'varroa sensitive hygiene' or VSH.

Likewise, since the life cycle of varroa is intimately related to the days in which the bees are in the pupa and nymph phase (capped cells), selecting bees with a short post-capping period development cycle could reduce the virulence of varroa mites, as happens with the South African bee, *Apis mellifera capensis.*

It should be noted that we are currently far from achieving the selection and reproduction of this type of bee, but we know that many beekeepers manage to preselect hives with desirable characteristics such as low aggression, high productivity and low disease sensitivity. Beekeepers selectively reproduce these hives, in order to preserve these characteristics in the hives originating from their own beekeeping operation.

05. Detection of Varroa mites in hives: degree of parasitism

One of the challenges faced by field technicians upon detecting varroa mites in hives is quantifying the degree of infestation. There are different methods that can help tell if a hive is parasitised by varroa mites:

- Inspection of the sanitary bottoms installed in the hives (seeing how many mites fall over the course of 4 consecutive days is the most reliable method to correlate the fall with the total varroa population in the hives).
- The method of powdered sugar, soapy water or alcohol applied to adult bees (indicates the presence of phoretic varroa and not an accurate value on the percentage of infestation).
- Inspection of the brood nest to check for mosaic-looking breeding chamber (indicates the possible presence of varroa).
- Inspection of bees to detect specimens affected by deformed wing virus (indicates the presence of varroa or having survived parasitisation).
- Direct inspection of the bees to check for phoretic varroa mites (indicates the presence of varroa in the phoretic phase).
- Uncap a drone or worker brood to check if there is varroa in the reproductive phase (indicates the presence of varroa and even some value on the percentage of infestation).

The challenge with all these varroa detection methods lies in linking field observations and data with the actual degree of parasitism, and determining

Authorised medicines. April 2021.	**Active ingredients**
MAQS formic acid 68.2 g strips for beehives	Formic acid
VARROMED 5 mg/ml + 44 mg/ml dispersion for bee hives	Formic acid, oxalic acid dihydrate
OXYBEE 39.4 Mg/ml powder and solution for dispersion for beehives	Oxalic acid dihydrate
API-BIOXAL 886 mg/g powder for use in hives	Oxalic acid dihydrate
APITRAZ 500 mg strips for beehives	Amitraz
AMICEL varroa	Amitraz
APIVAR	Amitraz
POLYVAR 275 mg strips for hives	flumethrin
BAYVAROL 3.6 mg strips for hives	flumethrin
ECOXAL	Oxalic acid
APPISTAN	Tau fluvalinate
THYMOVAR	Thymol
APIGUARD	Thymol

Table 1. *Authorized acaricides to combat varroasis. Source: https://cimavet.aemps.es*

whether or not there is a need to apply control measures.

The degree of parasitism varies according to the presence or absence of worker and drone brood, the degree of development of the colony, and other factors. The same result from a given detection method can have a different interpretation depending on the time of year.

After studying the literature, the conclusion is that the best method to quantify the percentage of infection is the use of sanitary bottoms to count the varroa that naturally fall in a certain period of time.

Degrees of infestation can be extrapolated and linked to the need for treatment according to the following method (Vandame R, 2004):

- Diagnosis in capped worker brood: if the infestation rate is less than 10% (10 varroa mites per 100 pupae), the colony does not need urgent treatment. If the rate is higher than 10%, treatment must be applied to the colony.
- Diagnosis in adult bees: if the infestation rate is less than 5% (5 varroa mites per 100 bees), the colony does not need urgent treatment. If the rate is higher than 5%, treatment must be applied to the colony.
- Diagnosis via sanitary bottom: if less than 10 varroa have fallen in 24 hours, the colony does not need urgent treatment. If more than 10 varroa have fallen in 24 hours, treatment must be applied to the colony. This method is the most recommended and reliable for calculating the degree of varroa mite infestation.

06. Varroa mite control: the geat challenge, the great failure

Another major challenge we have to face with regard to varroa mites is, without a doubt, getting them under control.

Between 2020 and 2021, the Spanish beekeeping sector has suffered a serious crisis, the most obvious manifestation of which is the death of a large number of hives that could not survive the winter. Adverse weather conditions, together with the lack of effective varroa mite control methods, have dealt a major blow, plunging beekeeping operations into an unprecedented crisis.

The tools we currently have for the control of the *Varroa destructor* parasite can be placed into three groups:

1. Application of authorised acaricide treatments, which are synthetic and organic chemical products.
2. Management practices and application of physical methods.
3. Decision making.

1. Regarding authorised medicines, there are two issues to be considered. The first is the availability of active ingredients, and the second is their effectiveness (Table 1).

If we study the licensed medicines for beekeeping available in April 2021 (Ta-

Checking the moths. Photo: ADS APICAL and APIVAL.

ble 1), we will see that there are very few active ingredients available: two organic acids (formic and oxalic), an essential oil (thymol), an amidine (amitraz) and two pyrethroids (flumethrin and tau fluvalinate). This looks like an interesting range for a minor operation, but this list has several drawbacks that we'll discuss below.

During the 2018 and 2019, the beekeeping health defence groups of the Valencian Community, in collaboration with the Eri-Biotecmed of the University of Valencia, carried out a study financed with funds from the National Apiculture Plan. Varroa mites taken from the hives of 190 professional and amateur bee farms were studied to detect varroa resistance to amitraz, pyrethroids and coumaphos (which is currently withdrawn due to a decision by the Spanish Medicines and Health Products Agency as a treatment against varroa mites). The efficacy of amitraz and coumaphos were measured by bioassays, while pyrethroid's efficacy was assessed with a TaqMan®.

> Coexistence with Varroa is possible, but both technicians and beekeepers must change the way we relate to them in order to reach a balance that allows a healthy, sustainable and future beekeeping.

The results were devastating. The bioassays carried out with the varroa mites and the coumaphos strips showed some variability between the different samples. Average varroa mite mortality ranged from 50% to 54%, indicating that this product was less effective than expected, justifying its withdrawal from the market.

The estimated average efficacy of pyrethroids fluctuated between 36% and 41%, but farms where all mites were resistant to pyrethroids were identified, and, conversely, varroa populations were also found where 97% effectiveness was reached.

For amitraz, bioassay results from the three different drugs available showed mortality between 74% and 81%. We note that in the technical data sheets of these products a higher efficacy is estimated.

The results of the study show that pyrethroids are currently not a reliable control alternative. It has already been shown that resistance to these substances goes back three years since the last application of this type of acaricide. But, since beekeeping is transhumant animal husbandry, re-infestation with varroa mites from other farms can cloud the results. We believe that if applying a TaqMan® at every apiary became a viable technique, we could detect the operations in which the varroa mites' susceptibility to pyrethroids is high enough to be able to carry out control measures. However, given the ease with which pyrethroids generate resistance, this would have a very limited use over time.

On the other hand, we know that there are already farms with varroa populations with a marked resistance to amitraz. In fact, we recently analysed samples from farms where amitraz was 46% effective, and on that same farm, only 33 out of 100 varroa mites


Hives. Photo: ADS APICAL and APIVAL.

were killable with pyrethroids. Detecting hives with varroa mite populations with higher or lower sensitivity to this amidine is also key to applying an appropriate treatment.

In conclusion, with coumaphos withdrawn from the market, pyrethroids with enormous variability in effectiveness and amitraz facing marked resistance to treatment in the field, the control alternatives are pointing to formic and oxalic acids, thymol and management techniques.

Applying organic acids and thymol does not guarantee the persistence of the active ingredients inside the hives. For this reason, they are recommended as occasional treatments to reduce the percentages of infestation, but not to control them. In addition, hives located in areas of Spain with a Mediterranean climate generally breed throughout the year, a fact that reduces the effectiveness of treatments.

On the other hand, the preparation and application of currently registered products with bases of formic acid, oxalic acid or thymol is cumbersome and, in some cases, complex. Applying them is complicated when it is necessary to calculate the amount of product to apply based on the vigour of the hive. It is similarly difficult to administer them when it is necessary to anticipate the most favourable temperature conditions for the treatment. Even so, we have to take into account that some of the authorised products have restrictions on the hive model they can be applied to. We have found that if the application guidelines for these ‘natural’ products are not strictly followed, there can be serious adverse reactions in the colonies.

2. In terms of beehive management, there are practices with which the sector is more familiar, and others which, either because of the cost or the level of technology, are more complex to implement:

- Forcing the queen to stop laying, either by caging, or by swarming the affected hives.
- Placing wax sheets with drone cells to force the queen to lay brood where the varroa mites are concentrated, then removing these sheets before hatching.
- In transhumance, delaying transfers from the coldest areas to the warmest areas to keep the amount of breeding to a minimum, and thus be able to apply a treatment with greater effectiveness.
- Installing sanitary bottoms in the hives that prevent fallen mites from being able to climb back into the colonies.
- Introducing purified wax into hives having below-average levels of lipophilic acaricides.

All these management methods help to control varroa mite infestation with varying degrees of success. However, they are very imprecise methods and their mite-killing effect will depend on several factors, such as amount of breeding, weather, degree of parasitism before its application, ability of the beekeeper to perform them, etc.

3. A third group of control measures, which we think are extremely important, are those that influence the people who make the decisions about varroa control:

- Extensiveness of training in beekeeping for technicians and beekeepers.
- Compliance with acaracide technical data sheets when applying the products.
- Knowledge about the influence of the settlement location on the annual development of hives.
- Carrying out a proper combination of chemical and physical treatments which is appropriate for the time of year.
- Knowledge about the number of apiaries that are installed nearby, and the type of controls carried out by fellow beekeepers.

07. Varroa mite control: new times and new measures

A paradigm shift in the management of bee farms is imminent. Adaptation to online procedures (new apiary management systems) and compliance with increasingly demanding legislation on records and farm management will be essential to a beekeeping with a future, and for proper control of the varroa mite.

Urgent measures are needed to facilitate the production of new molecules ready for use in beekeeping.

The public authorities must do their part by assessing and anticipating the lack of effective treatments against varroa. The sector has reported this fact through all the means at its disposal, and we believe that the health authorities are aware of the health crisis that is upon us.

The regulations that govern veterinary treatments are very demanding, and the registration of new products is expensive and complicated. The active ingredients currently on the market are insufficient and ineffective.

For a new active substance to be part of a veterinary medicine intended for production animals, a Maximum Residue Limit (MRL) must first be established for this substance and then the Spanish Medicines and Health Products Agency or the European Commission must authorise the medication. The authorisation will be granted if a very complex scientific evaluation of the product is carried out to verify that it meets the minimum official standards of quality, safety (for users, bees, consumers and the environment), effectiveness, identification and information, and this, given the serious crisis the sector is experiencing, is impossible to carry out. Establishing new MRLs for beehive products requires complex studies.

We believe that the key to making this process easier would be through a correct extrapolation. That would involve being able to authorise active ingredients used as phytosanitary agents through the laboratories involved in the production of veterinary bee medicines, without reducing the products' quality, safety and good effect on health for bees and consumers. Given the resistance seen in official results, beekeeping faces extinction if current policy continues.

Currently, the beehives are managed in a way that brings them a high degree of stress (80% are transhumant, according to the Spanish Ministry of Agriculture), and, in addition, some of the farms are oversized. Therefore, carrying out a comprehensive and thorough varroa mite control programme by farm owners is very complicated. This is what we mean when we saw we consider a paradigm shift in beehive management to be necessary.

Our proposals to improve the health situation with regard to varroa mite fall into three categories, one addressing beekeepers individually, another on the beekeeping sector and a third for organisations and public authorities:

Beekeepers
- Resize bee farms.
- Standardise management processes, especially in treatments, feeding, cutting comb honey, etc.
- Resuming rest periods for hives, not getting caught up in efforts to increase yields that result in exhausted hives.
- Sampling and continuous monitoring of hives.
- Replace queens

The beekeeping sector
- Rotate available active ingredients.
- Treat the hives respecting the dose and duration of treatment, as indicated in the medication technical data sheets.
- Check the quality of waxes.
- Coordinate the location of beehives to avoid reinfestations.

Organisations and public authorities
- Collaboration between the sector and researchers to find and register new veterinary medicines.
- Encourage research, not only for new acaricides, but for substances such as pheromones and specific allelochemicals that interfere with the life cycle and behaviour of Varroa destructor.

In conclusion, coexistence with the varroa mite is possible, but both technicians and beekeepers must change the way we relate to those creatures in order to reach a balance that allows for healthy, sustainable beekeeping with a bright future.

To learn more

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FUNGAL DISEASES

01. Introduction

The *Fungi* kingdom includes a series of organisms that we popularly call ‘fungus’ (pl. ‘fungi’ or ‘funguses’) . From a beekeeping perspective, we are interested in moulds, yeasts and a special type of fungi, from the class *Microsporidia*, which are characterized by being obligate parasites of animal cells. We will find fungi that do not affect the colony in any way. We’ll also see beneficial fungi such as yeasts, which ferment the pollen the bees store and allow it to be preserved. There are also fungi that form part of the microbiome in the bees' gut, and others fungi still that are pathogenic, capable of causing diseases in our bee colonies. These fungal diseases of bees are, in general, much more important than beekeepers usually think.

The importance of this type of disease lies, first of all, in the fact that fungi are able to produce spores, which are particles that fungi use to reproduce and resist adverse conditions. Thanks to spores, fungi can resist relatively harsh conditions for a long time and maintain their infectious capacity. Moreover, some fungi produce spores in large quantities and are able to spread through the air. Secondly, and thanks to this, the pathogenic fungi of interest to beekeepers have a great capacity to infect colonies and remain there over time. So much so that approximately 70% of our hives are affected by at least one type of fungus that can cause diseases. While true that the relationship of these fungi with bee colonies usually keeps a certain balance, fungi can multiply due to certain stressful factors and cause serious symptoms in the bees and even the death of the colony . Due to the health and economic importance of fungal diseases, we will delve deeper into the two most important ones: ascospherosis and nosema. However, there is another -very rare- disease known as ‘stonebrood’, which is produced by some fungi of the *Aspergillus* genus. Of this disease, it can only be said that the pathogenesis, clinical signs, and control are very similar to ascospherosis, which we examine below.

70% of our hives are affected by at least one type of fungus with pathogenic capacity.

Mummies of A. apis *inside cells. Photo: Pajuelo Archive.*

02. Ascospherosis

Ascospherosis (popularly known as ‘plasterbrood’, among other names) is produced by *Ascosphaera apis*, a filamentous fungus of the Ascomycota division. This division is characterized by forming asci, or cells of sexual reproduction, which contain the spores through which the fungus will multiply. We can find this disease all over the world, and its importance has increased in recent years.

Pathogenesis

The disease originates when the spores of *Ascosphaera apis* are ingested by the bee larvae when they are fed by the nurse bees. When the spores are in the larva's digestive tract, they germinate and begin the production of filaments, or hyphae, which will eventually cross through the intestinal walls. Through these hyphae, the fungus feeds on the bodily fluids of the larva. In the pre-pupal stage, these hyphae are able to cross the body surface, and the larva dies from dehydration and takes on a dry, spongy and dusty appearance, like a piece of chalk inside the cell, hence the name by which this disease is popularly known. After a while, the surface of the larva begins to darken due to the production by production of fruiting bodies, which are the reproductive part of the fungus. From this moment, when the remains of the larva (or mummies) acquire a grey or black colour (centre column photo), this is when new spores will be released. They are very resistant and can be reactivated after years, at which point they can spread throughout the hive and infect new larvae.

However, ascospherosis depends to a great deal on the existence of certain predisposing factors, the most important of which is a drop in temperature in the brood area. As can be seen in Figure 1, a normal bee colony is able to maintain a constant temperature of approximately 35° C inside the brood area throughout the year. However, for certain reasons, the brood area can become cold, which interferes with the proper functioning of the immune system of the larvae, and therefore allows the development of *Ascosphaera apis*. Another factor that can favour an ascopherosis outbreak is poor nutrition of the larvae. This is usually the result of the workers using pollen that is old or poor in amino acids. This poor nutrition can also lead to weakened defences in the digestive system of the larvae, which also makes it easier for the fungus to spread.

> Larvae affected by ascospherosis acquire the typical appearance of a mummy or piece of plaster.

With this in mind, there are two periods during the year when cases of ascospherosis will often occur in the Mediterranean area: early spring and mid-autumn. In both cases, we find colonies with a significant population increase in which the queen has started to lay eggs at a brisk pace with a view to the spring blooms or the hibernation respectively, while the population of adult workers is not very high At this point, faced with the arrival of a sudden cold wave, we may find that the number of workers is not high enough to maintain proper temperature conditions in the brood nest, which leads the appearance of ascospherosis Also, at these times of year, workers often fall back on old pollen reserves, as there may still not be enough flowers in the field, which can further aggravate things. Likewise, another predisposing factor can be an increase in the number of hives, either naturally by swarming or artificially by the beekeeper. Either way, in both situations we will again have colonies with lots of brood and few adult bees, which makes them sensitive to the drop in temperatures and therefore to contracting this disease.

Diagnosis

Field diagnosis is very effective and quite simple, so a laboratory diagnosis is often not necessary. Even before opening the hive, if we look at the ground in front of it, we may see the mummies (white or black) mentioned above, which have been expelled from the honeycombs by the workers. If we look at the honeycombs, we will be able to see how, in the brood areas, we find scattered uncapped cells and dispersed larvae that have succumbed to the action of the fungus and acquired the typical appearance of a mummy or a piece of plaster. Here we should note that, to get an idea of the seriousness of the case, it is not enough to note only the percentage of larvae affected is not enough because the colour of the mummies must also be taken into account. Thus, if most of the mummies have a whitish colouration, that means the fungi are not yet reproducing, and therefore the disease-fighting measures we apply will be more effective. Conversely, if there are many dark coloured mummies, *A. apis* will have already released a large amount of spores throughout the hive. It will be more difficult to recover it, and we will even have to consider eliminating the hive to avoid spread of the disease of other colonies.

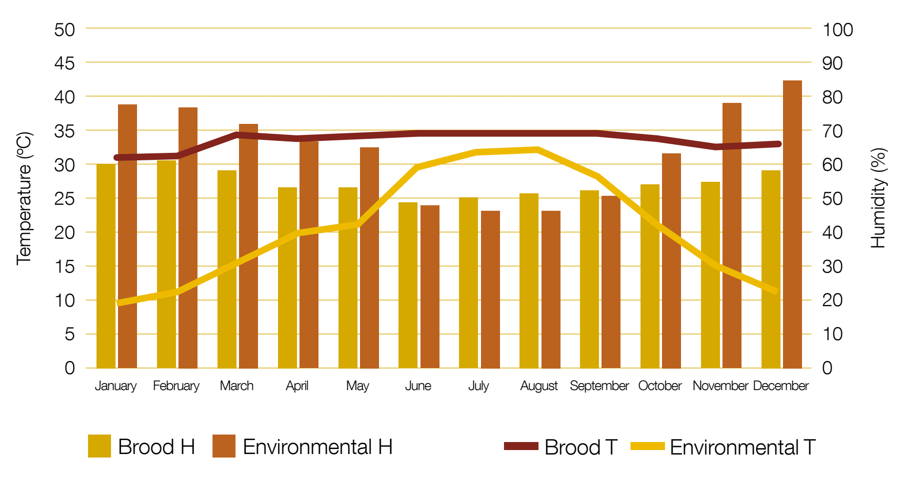

Figure 1. *Average evolution of temperature (Tª) and humidity (H) in beehives located in Córdoba, both in the breeding area and in the environment. Source: own work.*

Control

To prevent the appearance of ascospherosis, the most effective thing is to take management measures. A first measure must be to avoid these imbalances in the population. Thus, it would be advisable not to give the colonies supplementary liquid food at the start of the season, at least until we can be reasonably certain that there will be no new waves of cold. Likewise, when dividing hives, we will have to pay attention to maintaining a proper balance between brood and workers, and also join weak hives in the fall for wintering, as it is always better to have one strong hive than three weak ones. Also, we should try to have young queens, as they will produce a more compact brood, which will make the job of warming the brood area easier for the workers. In terms of genetics, it is advisable to select for bees (or buy selected queens) to improve hygienic behaviour. When bees exhibit this type of behaviour, the workers will detect affected larvae earlier and remove them from the hive before the fungus begins to produce spores and spread.

Another very useful measure to prevent ascospherosis is the use of open sanitary bottom boards. With this management measure, in addition to helping us fight against *Varroa destructor*, we promote proper ventilation of the hive and thus make it harder for fungal spores to accumulate. This proposal may lead to questions about whether leaving the hive with an open bottom could lead a cooling of the brood area in winter, and, consequently, proliferation of *A. apis* . However, in tests we carried out at the University of Córdoba a few years ago, it was observed how hives with open bottoms maintained higher and more stable temperatures in the centre of the brood nest compared to those equipped with wooden bottoms (fig. 2). It is also helpful to replace old frames, which can be reservoirs of spores. When melting the frames to make new sheets, we must take into account that the spores can survive at temperatures of 80º C, so it would be necessary to work with very high temperatures. Likewise, adding certain organic acids, propolis extracts and probiotics (such as *Saccharomyces cerevisiae* yeast) to food supplements can help strengthen the immune system of the larvae and, therefore, hinder proliferation of the fungus.

When facing the appearance of an outbreak in our hives, the first thing to be clear about is that there is no licensed veterinary medicine for this type of disease in Spain. In any case, it can be controlled quite well with handling measures. Thus, when *A. apis* is detected in a hive, we remove the affected frames and incinerate them. In addition, it is advisable to isolate these hives from healthy ones and combine the sick hives into one, so that we get a higher proportion of adult bees. We can also try to reinforce them with workers from strong hives and make sure they get proper nutrition, including the supplements mentioned above. Finally, in severe cases, it is best to remove the colony and perform a thorough cleaning and disinfection of the hive to remove the spores and prevent them from spreading to the rest of the beehive.

03. Nosema

The other main fungal disease, nosema, is caused by the species *Nosema apis* and *Nosema ceranae*. The first is native to Spain area, while the second was detected for the first time here in 2006 and is specific to the Asian bee *Apis cerana*, despite the fact that both affect adult bees. Nosemas, as mentioned in the introduction, belong to a special type of fungi known as microsporidia. Microsporidia are very small unicellular beings and obligate parasites of animal cells. They do not have mitochondria -the cellular structure responsible for producing energy- so they must use that of the cells they parasite.

Pathogenesis

The spores of *Nosema* spp. enter bees orally by feeding either on resources stored in the hive or directly on the nectar of flowers previously contaminated by another infected worker. In the digestive tract, gastric juices gradually weaken the protective covering of the spore, which eventually allows it to enter the cells of the intestinal walls. Once there, it uses the parasitised cell's machinery to produce new spores, to the point of breaking the cell walls due to the accumulation of these spores and releasing them back into the digestive tract. Thus begins a new cycle of parasitisation of the digestive cells, or their exit through the faeces, which can lead to spread to new bees. In addition, as an increasing number of intestinal cells are destroyed, there will be a decrease in the intestinal absorption capacity, leading to malnutrition. There will also be episodes of dysentery and diarrhoea.

This disease, which, as we said earlier, affects adult bees, has a pronounced effect on their immune capabilities. Thus, immunosuppressive factors such as sudden drops in

Nosema apis will mainly have productive effects, and colony loss will be relatively rare.

Nosema ceranae has a much more acute evolution, mainly due to the lack of adaptation.

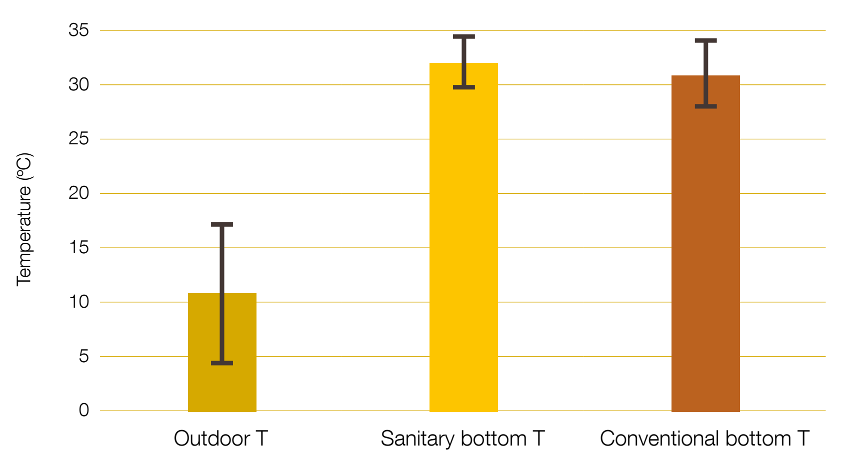

Figure 2. *Comparison of average temperatures and standard deviation (ºC) of beehives during a winter in Córdoba, according to whether they are equipped with an open sanitary bottom or a conventional wooden bottom, compared to the outside temperature. Source: University of Córdoba.*

temperature, nutritional deficits (recall that not only the lack of food, but also low-quality food can produce these deficits) or the concurrence of other diseases such as varroosis or certain viruses will favour nosema development, and therefore the presentation of the most serious forms. Certain environmental factors such as locating apiaries in cold, dim or humid areas can also promote this immunosuppression, as can long periods of continuous rain, as such periods prevent bees from defecating and alter the movement of matter through their intestines. These are the similarities of the nosemosis produced by *N. apis* and *N. ceranae*. Despite these things in common, each will present very different symptoms and development.

Diagnosis

Classic nosema, produced by *N. apis*, usually develops with milder signs. Since bees in Spain have been living with it for centuries, so they have been able to adapt to it. It is typically a seasonal disease of late winter and early spring, or early autumn in drier areas. These are times when there will normally be a lack of pollen in the hives and waves of cold. The most typical clinical signs will be workers, especially the older ones, with a slightly dilated abdomen and signs of dysentery (which we will see in the form of brown splashes both at the heads of the brood frames and in the area outside the entrance platform) (photo p . 29). It can also be common to find bees with a short abdomen (we will see how the abdomen does not protrude below the wings) due to malnutrition. In the most serious cases, we will find trembling workers and dead bees around the hive.

From the colony's point of view, there is some population imbalance due to the loss of adult bees. This results in less brood and generally slower colony growth during the spring. Therefore, it will mainly have effects on production, and colony loss will be relatively rare.

The nosems caused by *N. ceranae* (which in recent years has become the predominant nosema in Spanish apiaries), or nosems type C, has a much more acute evolution than classic nosema, mainly due to the lack of adaptation of bees in Spain to this new pathogen. The clinical diagnosis in bees will be much more complicated, since it will quickly kill the oldest worker bees, almost without symptoms. Although, we can see weak malnourished bees, as evident from their shorter abdomen. Intestinal fragility is also quite characteristic of the disease, which we can see by sacrificing a worker bee by pinching its head and gently pressing and pulling on the last segments of the abdomen to remove its digestive system. In healthy bees, the cloaca, small intestine and large intestine will come out, while in sick bees the digestive tract usually splits at the level of the small intestine.

Abdomen shrunken by nosema. Photo: Pajuelo Archive.

At the colony scale, a gradual depopulation of adult bees and a significant increase in mortality can be observed in autumn and winter, until only the queen remains with a handful of young bees. This is typical of warmer areas, where there is very little seasonal variation, while in colder European countries the incidence has so far been much lower.

In both types of nosema, it can be difficult to make an confident clinical diagnosis, since the signs can be shared with other pathologies. As such, making a laboratory diagnosis may be the better option. To do so, we must take a sample of bees, ideally foragers when they return to the hive, since they are the ones most likely to have the disease. If there are not enough flying bees, we can resort to taking bees from the honey boxes or the supers. To send the bee sample to the laboratory, it is best to keep it in 70° alcohol or frozen.

Control

Currently, there are no authorised veterinary treatments for this disease either, although it appears that some plant extracts can inhibit the prolif-

Parts of the digestive system of bees. Photo: Pajuelo Archive.

Hygienic behaviour of bees: on the ground, A. apis mummies expelled from the hive. Photo: Àlex Sirera

eration of *N. ceranae* spores. Faced with an outbreak, we must remove the dead bees, thoroughly disinfect the hives with a blower or with chemical disinfectants and never transfer the combs to other hives. It is possible, however, to reuse the wax once melted, as the spores are quite sensitive to high temperatures. As a preventative measure, we must not keep apiaries that are wet, cold or located in dark areas. We do need to ensure that the hives receive a sufficient supply of good quality pollen, or, where appropriate, an adequate protein supplement. We can also use commercial nutraceuticals, which help us restore healthy intestinal flora in our bees.

There are no licensed veterinary treatments to treat fungal diseases in bees.

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CONTROL OF THE ASIAN HORNET (*Vespa velutina*) in the beekeeping sector

Asian hornets (also called 'Asian wasps' and 'yellow-legged hornets)' start to appear mostly from the end of summer among plants that produce nectar, such as ivy in autumn (left) or on rare occasion capturing insects (right). With patience and luck, you can see how, once a catch is made, they hang it from a branch to process the protein and take it to the wasp nest. Their favourite prey is honey bees, but in this case it is a wild bee (male Halictus sp.). Text and photos: Narcís Vicens.

01. Introduction

Arrivals of exotic species have increased in recent decades due to the large-scale transport of people and goods. Although not all exotic species have negative impacts, some have significant economic and ecological repercussions. The management costs for the prevention, control and eradication of invasive species represent significant expenses for both public administrations and individuals.

The Asian hornet (*Vespa velutina*) is a species native to Asia that has recently been introduced to Europe (top photo). It was first observed in France in 2004, where it arrived through a shipment of earthenware imported from China bound for the port of Bordeaux. Since then, it has spread to numerous countries, including Italy, Spain, Portugal and England. The first specimens of the Asian wasp in the Iberian Peninsula were detected in 2010 in Navarre and the Basque Country. Next was Portugal in 2011, and Galicia and Catalonia followed in 2012. In 2014, its presence was confirmed in Cantabria, Asturias, La Rioja and Castilla y León. Currently, the species is present in a large part of the Atlantic coast region and and along the Mediterranean coast in the east, but it is expanding towards the interior of the peninsula (Rojas-Nossa et al., 2021).

The success of the Asian hornet's expansion stems from various factors, notable among them being climate, the presence of food sources, the absence of predators and direct competitors and the hornet's high capacity for adaptation and predation (Rodríguez-Flores et al., 2019). The temperate subtropical climate is the most favourable for the Asian hornet, while the most frequented environments are forestry and agriculture, small cities and areas just outside larger cities. They usually build their nests in the highest branches of larger trees, although they can also be found at lower heights. The most important predators that have been identified are the European honey buzzard (*Pernis apivorus*), which partially destroys wasp nests to feed on the larvae (Macià et al., 2019), and bee-eater birds (*Merops apiaster*). Even so, their effectiveness as biological control agents is limited due to their distribution and seasonality.

Although the Asian wasp is associated with human health problems, the most important impact is on the honey bee (*Apis mellifera*) and, consequently, the beekeeping sector. Although the wasp is a generalist and opportunistic predator, bees represent 38% of its diet and are its main food source (Rome et al., 2021). In addition to the direct impact of predation on the bees, it also exerts high pressure on the hive, which in turn causes a decrease in the activity of the bees. This phenomenon is known as food paralysis and it seriously affects the production of honey and pollen collection, and also the performance of the beekeeping activity, since many colonies are seriously affected and their survival in the winter is compromised (Requier et al., 2019). To this we must also add the impact of different diseases that affect bees such as varroosis, a disease caused by the *Varroa destructor* mite which acts as an ectoparasite of bees and can cause high mortality in the colony. Although there are no studies that show the combined action of the Asian wasp with other threats to bees, more and more beekeepers believe that the honey bee colonies that reach the end of summer in a better state of health and vigour will be able to better resist the effects of the Asian hornet.

02. Control methods

Worldwide, the first research on the Asian hornet dates to 1991, while the first European article was published in 2009, coinciding with the expansion of the species in Europe. As of 2011, the publication of articles on the Asian wasp has followed an upward trend both globally and in Europe. Of the 155 papers published to date, as per a Web of Science query on 20 November 2020, only 38 deal with control methods to reduce the pressure from this species.

The control methods that have received the most research focus are those related to chemical control and biological control, followed by those that seek to locate and destroy Asian wasp nests (Turchi & Derijard, 2018) (fig. 1). Although physical control methods have received less attention, they are the ones that see greater application among the beekeeping sector. In Table 1, you will find a brief description of the different methods, as well as their most importat pros and cons according to the literature reviewed.

Chemical control: This is based on the use of chemicals to the control of the Asian hornet. There are different variants of this method: traps to catch workers, traps to catch queens, pheromone traps, the use of workers as carriers of poison to the nest and poisoned baits. Of this variety of methods, one of the most promising is that of pheromone traps. There are based on the use of the sexual pheromones that the wasps themselves generate to attract each other and reproduce. On the other hand, traps to catch workers and queens are less expensive, but they have a greater environmental impact because they are not selective in terms of the species they capture and can spread toxic products into the environment (Wen et al., 2017).

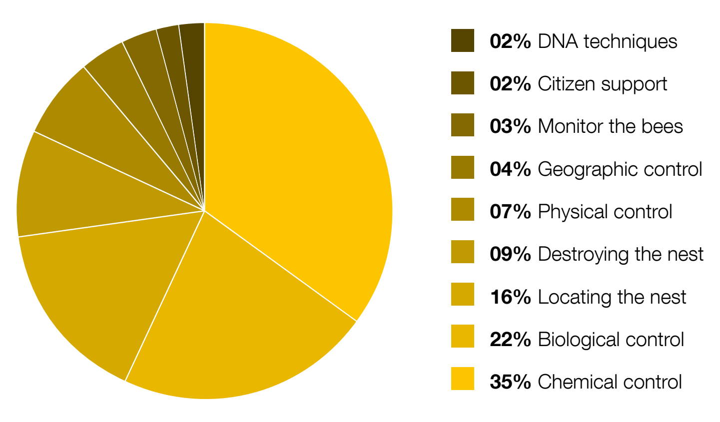

Figure 1. *Percentage of scientific articles that deal with different control methods of the Asian wasp; the chemical and biological control methods are the ones that have received more attention. Source: own work.*

Biological control: Methods based on the use of living organisms or viruses with the aim of controlling the Asian hornet. The variants of this control method differ depending on which organism is used to control the invasion. Some of the options are insects, nematodes, fungi, birds, mites and viruses). The main problem with this method is the difficult implementation so as not to disturb or destabilise the ecosystem with the entry of new species. If it is done in an appropriate way, giving preference to native species to deal with invasive species, it would be a very environmentally friendly control method (Beggs et al., 2011).

Physical control: Control methods based on the use of physical mechanisms to eliminate hornets or hinder their movement in a given area. The variants of these methods are: muzzles, passive traps, electric traps and harps and using badminton rackets to hit the wasps. A muzzle consists of placing a mesh at the entrance to the beehive to prevent the passage of wasps, but allow the passage of bees. It is an economical, environmentally friendly and selective method, although its effectiveness is limited. With regard to passive traps, they are traps formed by a funnel-shaped mesh that is arranged below the bee hive and allows wasps to enter but not exit. Its particular feature is that the smell of the bees themselves acts as an attractant for the wasps. The problem with this trap is that it creates stress on the beehive above the trap. On the other hand, electric traps and harps are the methods with greater effectiveness, efficiency and selectivity, purchasing and maintaining them is very expensive. These two devices aim to electrocute the wasps to kill them or make them fall into a container where they will be trapped (Bonnefond et al., 2020; Requier et al., 2020).

Control method	Specific control method	Effective	Selective	Respectful of the environment	Maintenance	Economic cost
Chemical control	Traps to catch queens	★★	★	★★★	★★	★
	Traps to catch workers	★★	★	★★★	★★	★
	Pheromone traps	★★★★★	★★★★★	★★★★★	★	★★★★
	Wasps as poison carriers	★★	★★★★★	★★★	★	★★
	Baits in a bucket	★★★	★★	★★	★	★★
	Poisoned baits in feed	★★★	★★	★★★	★	★★
Biological control	Insects	★★★	★★★	★★★★★	★	★★
	Nematodes	★★★	★★★	★★★★★	★	★★
	Fungi	★★★	★★★	★★★★★	★	★★
	Birds	★★★	★★★	★★★★★	★	★★
	Mites	★★★	★★★	★★★★★	★	★★
	Viruses	★★★	★★★	★★★★★	★	★★
Physical control	Muzzles	★★	★★★★★	★★★★★	★★★	★
	Passive traps	★★	★★★	★★★★	★★	★★★
	Electric traps	★★★★	★★★★	★★★★★	★★★★★	★★★★★
	Electric harps	★★★★	★★★★	★★★★★	★★★★★	★★★★★
	Badminton racket	★	★★★★★	★★★★★	★	★
Locating the nest	Harmonic radar	★★★★★	★★★★★	★★★★★	★★★★★	★★★★★
	Drones	★★★★★	★★★★★	★★★★★	★★★★★	★★★★★
	Triangulation	★	★★★★★	★★★★★	★	★
	Visually tracking tagged wasps	★★	★★★★★	★★★★★	★★	★
Destroying the nest	Biocides	★★★★★	★★★★★	★	★	★
	Steam	★★★★★	★★★★★	★★★★★	★★★	★★★
	Shotgun blasts	★	★★★★★	★	★★	★★
DNA techniques	SiRNA	★★★★	★★★★★	★★★★★	★	★★★★★
	CRISP-Cas9	★★★★	★★★★★	★★★★★	★	★★★★★
Geographic control	Geological study	★	★	★★★★★	★	★
Monitor the bees	Feeding of bees	★	★★★★★	★★★★★	★★★	★★
Citizen support	Educatingthe public	★★★★	★★★★★	★★★★★	★	★★

Table 1. *Characteristics of the different control methods and variants of these methods for dealing with the Asian wasp that have been found in the scientific literature: Source: own work.*

Methods based on nest location: Methods whose main purpose is to identify the hornet nest. The implementations of this method vary in how the nest is detected: harmonic radar, drones, triangulation and visually tracking wasps back to the nest. The most promising methods are harmonic radar and the use of drones, as they have very favourable ratings in terms of effectiveness and selectivity (Kennedy et al., 2018; Lioy et al., 2019).

Methods based on nest destruction: Methods where the goal is to destroy the Asian hornet nest once it has been identified using one of the methodologies presented above. Variants of this method have to do with how the nest is destroyed: biocide injection, steam injection or shotgun blasts. Both biocide injection and steam injection are very effective, although steam injection is a much more environmentally friendly method (Ruiz-Cristi et al., 2020).

DNA-Based techniques: Based on genetic modifications, these techniques are intended to propagate the recessive infertility gene among the Asian hornet for population control of the invasion. A negative of this method is that it is very recent and still being researched. Moreover, gene modification can cause severe damage if applied without proper consideration (Turchi & Derijard, 2018).

Others: In this category, we find more diverse methods, which, although they do not directly address controlling the species, can help with early detection or with applying adaptive measures to promote the resilience of beekeepers.

- Control based on geography: method based on the use of geographic data to predict the areas most likely to be occupied by the Asian wasp and thus adopt control measures or take other action. Its effectiveness and efficiency are directly related to complementary control methods (Lioy et al., 2019; Rodríguez-Flores et al., 2019).
- Monitoring the bees: it consists of monitoring the needs of the bees and providing the bee boxes with supplementary food so that they can make it through the winter without a food deficit (Requier et al., 2020).
- Citizen support: this is based on educating the public so that they actively participate in the detection of the Asian hornet and subsequently notify the competent authorities. This is a very important method to control the wasp because it does not generate negative impacts, and the cost of educating the public is minimal when compared to the benefits of having a well-informed population (Leza et al., 2018).

According to consultations in the Catalan beekeeping sector, the most used control methods among beekeepers are, in order of importance, traps with an attracting liquid (chemical control) and muzzles and electric harps (physical control). The first two methods enjoy a good reputation for being inexpensive and easy to apply. Despite being widely accepted, the long-term effectiveness of traps with attractant liquid has not been proven by any studies and is considered a non-selective method that may cause harm to other species. Muzzles do not generate adverse effects on other species, but the effectiveness, according to beekeepers themselves, is highly variable according to each situation. Electric harps are the third most used method in the sector, possibly because of their high cost, but there is unanimity in affirming that it is a method with good results (bottom photo).

03. Conclusions

To date, no completely effective control method has been found to control and/or eradicate Asian hornet populations. According to the scientific literature, the most promising methods for reducing pressure from Asian hornets are pheromone traps and DNA-based techniques, although the latter are still in their infancy and their utility remains to be determined. However, physical control methods such as muzzles and electric harps and the use of early detection methods in certain situations -such as on islands- also are and will be very important in the near future. The best strategy is probably to use several control methods at once.

Examples of physical control methods to reduce Asian wasp infestation in honey bee hives during September through November, the months of peak Asian hornet activity. Muzzles are intended to limit the access of the Asian wasp to the hive by using mesh that prevent their passage, but there are variants of this method that also serve to capture hornets (as seen in the photograph). Electric harps are frames surrounded by electrified wires that electrocute Asian wasps when they come into contact with two wires which are separated by a distance of approximately 2 cm. Text and photo: University of Girona.

An example of effective control of the invasion of the Asian wasp can be found in the Balearic Islands, where several methods were deployed to eradicate this species. The control strategy focused on: early detection of individuals with baited and toxic traps, citizen participation through detection of Asian hornet nests and individuals, queen traps set in the spring, nest detection by triangulation of individuals and the extraction and destruction of wasp nests (Leza et al., 2021). The procedures mentioned above are an example of success, although it is important to note that any control strategy should be adapted to the circumstances of the territory.

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WELL NOURISHED BEES:
healthy bees

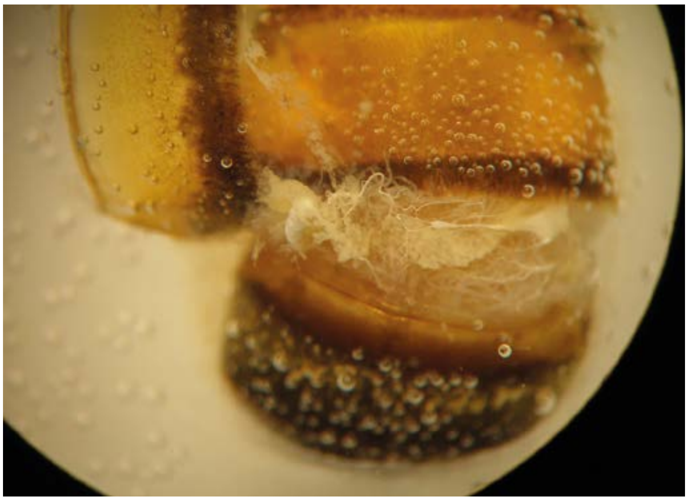

Photo 1. *Storage of body reserves in a worker. Photo: Pajuelo Archive.*

Photo 2. *Fat bee (top) / lean bee (bottom). Photo: Pajuelo Archive.*

Photo 3. *Storage of collective reserves. Good 'brood pattern', with a border of pollen and honey. Photo: Pajuelo Archive.*

Photo 4. *Pollen stored in a honeycomb. Photo: Pajuelo Archive.*

01. Introduction

When looking for the causes of low production and beehive mortality in a bee farm, poor nutrition often stands out, either as the main cause or as a trigger for certain pathologies.

In the recent history of Spanish beekeeping, there have been several years with high winter mortality of hives. The winters of 2004 and 2005 should be highlighted. We saw this situation again in 2019 and 2020. It was not to the same degree as in 2004 and 2005, but many farms still saw double or triple their normal losses.

In addition to the losses of hives, this generates a significant impact on the profitability of the operations, since some of the resources for the following year must be used to recover the losses, which decreases the productivity.

There have been hive deaths of these types everywhere, which has generated a large number of scientific publications on the possible causes. Suggested reasons include the impact of pesticides on bees, bacterial, viral or fungal diseases, the impact of the varroa parasite and predators, among other things. But, as usually happens, there is no single cause, but the sum of several things, and malnutrition is one of them.

Since 2010, malnutrition and its impacts on health have begun to be seen as one of the direct or indirect possible causes, which brings a new approach to the search for possible solutions.

02. Diet

The colony is a super-individual where food must fulfil three functions:

- Royal jelly feeding for the young larvae and the queen
- Feeding of honey and pollen of the older larvae and of the workers and drones
- Storage of body reserves in bees, and collective reserves in the colony's honeycombs.

The lack of some functions affects the others, which in turn impacts the survival of the colony.

Bee diet consists of 80% nectar (sugars and minerals) and 20% pollen (proteins, lipids, vitamins, minerals), and water.

The amount of reserves in an adult bee can be assessed by the length of the abdomen in relation to the wings. If there is accumulated fat (photo 1), the body will be longer than the wings, and if not the body will be shorter (photo 2). In the honeycombs, you can see the accumulation of collective reserves of honey and pollen (photo 3).

A sufficient contribution in quantity and quality of nutrients will ensure a colony size that guarantees its survival, and adult bees with a long life and resistance to diseases and toxic substances.

If protein nutrition fails, nurse bees may cannibalise young larvae and stunted, poorly developed pupae (photo 5) and recycle their nutrients. This will give an image of the capped brood area with empty spots (photo 6). Fewer adult bees will be born and they will be of poorer quality. They will not have well-developed hypopharyngeal glands, so they will feed the next generation of larvae more poorly. This will continue to impact the quality and quantity of adult bees, who will not be able to search for enough nutrients and the hive will collapse .

In the laboratory, this situation can be measured by the level of a protein in the bees' bodies called vitellogenin, which is related to their longevity.

The consequences may not be immediate. Bees can overcome a lack of nutrients in autumn by using body reserves (photo 1), but this situation will take its toll at the end of winter by generating a significant impact on their survival.

Climate change is causing changes in temperatures and precipitation and increasing the frequency of extreme events. All this is altering bloom times and forcing us to be more attentive to the amount of nutrients these blooms bring us.

More and more, when analysing honey, we find wrinkled, deformed, shrivelled pollen, which indicates that the plant has suffered a drought, a deficiency that has not allowed it to produce pollen with all the necessary nutrients (photo 7).

Changes in land use, more extensive agriculture, the abandonment of forest areas and other factors also influence the decrease in biodiversity available to bees.

Bees are generalist pollinators, collecting a large amount of pollen to feed their young.

Each pollen has a composition in proteins and fats, a profile of amino acids and other components, such as vitamins and antioxidants. It has been shown that polyfloral diets, -or diets of some very complete pollens such as bramble- improve the survival of bees, while others, lacking in some component, decrease bee survival. The same applies to N osema (Di Pasquale, G. et al., 2015).

For example, sunflowers are nutrient poor, having two essential amino ac-

Photo 5. *Cannibalized pupa. Photo: Pajuelo Archive.*

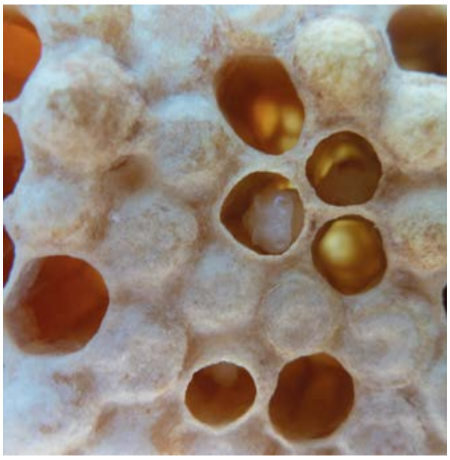

Photo 6. *Brood with uncapped stunted pupae and empty cells. Photo: Pajuelo Archive.*

Photo 7. *Unsuitable heather pollen (top) and more normal (bottom). Photo: Pajuelo Archive.*

Pollen from:	Protein (%)	Fats (%)	Sugars (%)	Amino acids (g)	Antioxidants (µmol)
Cistus , steppe cistus	12	6.9	5.2	11.9	103
Erica, heather	14.8	7.4	4.8	16.27	196
Castanea, chestnut tree	21.6	6.6	5	18.68	399
Rubus, brambles	22	6.4	6.7	19.98	475

Pollen proteins, lipids and sugars are expressed as percentage of pollen dry matter. The antioxidant power is expressed in µmol of Trolox equivalent/g of pollen. Amino acids are expressed in g/100g of pollen. Source: Di Pasquale, G. et al., (2013). 'Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter"

ids, methionine and tryptophan, below the minimum requirements for bees.

The same happens in areas with large plantings of eucalyptus, whose pollen has a low percentage of crude protein, which decreases as flowering progresses. Eucalyptus also has a low lipid content, and is deficient in the amino acid isoleucine . Branchicela et al., demonstrated in 2019 the impact of this nutritional stress in bees and related it to the level of nosema spores.

Hives brought to pollinate blueberry crops also tend to have more problems with European *loque* (foulbrood) (Higo, 2019).

The species of bee found in Spain, Apis mellifera iberiensis, has a high level of tolerance to toxins such as pesticides, a characteristic it loses if it is not well nourished and does not have adequate reserves in its fat body (Barascaou et al., 2021).

Malnutrition can also be the result of some pathogens:

- Varroa mites, which feed on the fat of bees, cause a nutritional deficit, a shorter adult life, poorly developed hypopharyngeal glands, and other negative effects.
- Nosema, which destroys the cells of the bee's intestinal wall and hinders the absorption of nutrients.

And we cannot forget the negative impact on the nutrition of the colony generated by predators such as the Asian hornet (Vespa velutina) and the bee-eater bird. The former actively hunts bees, but both depress the collection in autmn, a critical time in preparation for wintering.

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A Conversation With: RAQUEL MARTIN

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(Guadalajara).

> "In recent years, an upward trend has been observed in bee mortality"

Dr Raquel Martín Hernández graduated in veterinary medicine from the Complutense University of Madrid (UCM). There she also completed her doctoral thesis studying the host's immune response to tick infestation. In 1999, she began her relationship with the Regional Beekeeping Centre (now CIAPA), located in Marchamalo, Guadalajara. Since that time, her research activity has focused mainly on the field of bee health. Currently, she is part of the INCRECYT programme (co-financed by the European Social Fund) of the Castilla-La Mancha Science Park and continues to conduct her research work at the same centre, as part of the IRIAF of Castile-La Mancha. She has actively contributed to research on numerous pathogens that affect bees such as *Nosema ceranae*, varroa mites and viruses. In recent years, she has focused on the pathogen-host-environment interaction, including the effect of the microbiome and the coexistence of infection by multiple pathogens.

How did you become interested in beekeeping?

Some time after obtaining my doctorate in veterinary medicine, I applied for a postdoctoral grant from the INIA to do research at what was then called the Centro Apícola Regional de Castilla-La Mancha. The grant was to study methods of varroa mite controlusing natural products. Since my PhD had been based on the study of ticks, I thought my experience might be applicable to *Varroa destructor,* as both are mites. So in 1999 I started working with bees and since then it has been my main line of research.

> "Currently, I lead a line of research on the interaction between honey bees, their pathogens and the intestinal microbiota"

What is Marchamalo currently researching?

Currently, I lead a line of research on the interaction between honey bees, their pathogens and the intestinal microbiota. In addition, I coordinate a European project to learn about the adaptation of Mediterranean bees to climate change.

Are there other Spanish research groups on bee health issues?

Yes, as far as I know, in addition to our centre (CIAPA), which belongs to Castille-La Mancha, there are several research groups working on bee health and other topics related to beekeeping in Murcia, Andalusia, Galicia, Madrid, Valencia, Castille-León, the Balearic Islands and Catalonia.

> "In Spain, beehive losses of over 30% have been recorded and this high mortality has been occurring for years"

How do you rate the health of bees at the moment?

According to the data collected in the COLOSS group survey on winter losses in 2020-2021, hive losses of more than 30% have been recorded in Spain, and this high mortality has been occurring for years. Although the data obtained in the COLOSS survey that we coordinate at CIAPA are different from those published by the Ministry in its monitoring programme, in both cases an upward trend in mortality in recent years has been observed.

Test with 100 cells to assess the hygienic behaviour of a colony. Photo: Alex Sirera

What issues do you see as crucial to improving bee health?

Varroa mite control remains the main challenge for beekeepers. Although it has been with us for a long time and all beekeepers are already familiar with it and know the importance of applying treatments, it seems that the mite is adapting to acaricides and developing resistance, so it constitutes a serious health problem. In addition, beekeepers should be aware that there are other pathogens such as *Nosema ceranae* and viruses that are causing serious losses. The problem is that these pathogens are not visible at first glance and often go unnoticed until it is too late.

Do you think there will be new developments in veterinary products?

Yes, I think so. I believe that there are currently active pharmaceutical companies that see the importance of investing in this sector. Also, I know that there is a lot of research going on internationally to find new molecules and forms of administration. For this reason, I am optimistic and believe that in the future (hopefully in the near future) there will be new medicines to control the most important diseases.

Is there a relationship between the beekeeping sector and the research centres, and if so, is it fluid? Is there a good transfer of knowledge?

I can only speak about the relationship CIAPA has with beekeepers and I think it is fluid. Both Dr Higes and I try to help them in everything they ask of us, so we are always open to answer their inquiries and analysis requests when they have a health problem or because they want to check their hives . The same goes for Dr González Porto, who is in charge of the hive products laboratory. All of us are in direct contact both with associations throughout the country and with veterinarians and we regularly participate in numerous activities such as courses and conferences that aim to offer training to beekeepers in different topics related to beekeeping as well as disseminating the latest research in which we have participated or that carried out by other research groups in our country and abroad. Many of these conferences take place directly at our centre in Marchamalo, but we also regularly attend conferences organized by associations and those to which we are invited.

> "I am optimistic and believe that in the future (hopefully soon) there will be new drugs to control the diseases of greatest interest"

> "Varroa mite control remains the main challenge facing beekeepers"

What do you think of the future of beekeeping from the point of view of bee health?

In general, I have an optimistic view and believe that if the research centres, the beekeepers and the other interested parties continue to work together, we will eventually manage to control the main health challenges.

Generalitat de Catalunya
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