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Introduction

Disease cannot only be considered as the consequence of the multiplication or of the action of a pathogen. Pasteur often repeated: "The pathogen is nothing, but the ground makes all", the ground being the bee colony for us. Considering the bee colony as a social organisation, rather than thousands of bees, living in the hive independently of each other, various factors influence its activity and its phenology. Some of them act in favour of maintaining the good health of the colony while others cause weakness or stress, making the colony more susceptible to diseases. It seems more intelligent to know when and why a bee colony is suffering from exposure to a pathogen, rather than to detect the presence of a serious disease, which may have developed over several months. Some of the factors affecting the seriousness of disease outbreaks depend on the physical surrounding of the hive, others depend on the living environment such as plants, insects, and man. More precisely these factors are classified as follows: (i) meteorological factors; (ii) alimentary factors; (iii) hive management factors; (iv) genetic factors; and (v) presence of pollutants. Although the factors will be independently described and discussed, they often interact.

Meteorological factors

These factors are the first to be described, because it is impossible for beekeepers to influence them. Under cold and temperate climates, colonies always have difficulty in initiating growth after a long, cold and wet winter, because the bee population comprises predominantly old individuals and because provisions, stored before wintering, are lacking. The starved colonies are slow to recover a good foraging activity and often the underfed brood displays symptoms of diseases. Under the Mediterranean climate, this type of winter is less to be feared than a prolonged period of dryness during the summertime; this reduces yields from nectariferous and polliniferous plants, but maintains exploration and water storage flights, which means energy costs without benefits (food storage) for the colony. A timely and well managed migration of apiaries is sometimes the best solution to avoid the adverse effects of a dry season.

Alimentary factors

The quantity and quality of the nourishment of a colony greatly depend on the weather. However, the beekeeper can feed the colony in order to counteract the hazards of the weather. In the hive one can find four categories of food or harvest: (i) honey stored in cells; (ii) pollen stored in cells; (iii) royal jelly as food for the larvae before cell capping; and (iv) propolis, collected mainly by foraging on tree buds.

Honey

As defined in the Codex Alimentarius (1989), "honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature". Honey is essentially a concentrated solution of sugars (around 80% w/w, primarily fructose and glucose) at pH 4.

Honey provides the carbohydrate (energy) part of the nourishment of the bee colony. In relation to its source, the nutritional value can be quite different. Honey from nectar is the most suitable for the bee. In contrast, honeydew honey, when a unique food source, would be considered as irritating for the alimentary canal, which means the food favours the emergence of digestive diseases or the entrance of pathogens such as chronic bee paralysis virus, through the intestinal epithelium. Honey which has fermented in the cells (heather honey) or which is given back to the colony as complementary food in fermented solution, causes similar disadvantages. To replace honey, beekeepers often use concentrated sucrose solution (70% w/w) as supplementary food for wintertime or a more dilute solution (50% w/w) to stimulate the queen to commence egg-laying at springtime. However, it should be kept in mind that nectar honey is the best food for the adult bee, whatever the season. Excess feeding with sucrose solutions progressively weakens the colony, often correlated with the outbreak of mycosis. The addition of antibiotic to the feeding syrup as a preventive treatment also seems to favour mycosis.

Pollen

The protein content of pollen ranges between 7.5% to 35%. The sugar component ranges between 15% to 50%. Lipids are also abundant. Ten to eleven amino acids are regularly found. Many enzymes are present, particularly in the beebread. Fatty acids, palmitic acid being the most important, sterols and mono-, di- and triglycerides have also been identified.

Foragers carry pollen pellets to the hive, made of pollen grains gathered from the flowers of a single plant species. The pellets are packed tightly in cells and may remain there for two weeks or more, before their use as a proteinaceous food for the adults. During this storage period, the pollen undergoes a lactic fermentation, making it more digestible. However, the nutritive quality of this food, named bee bread, depends firstly on the nutritive quality of the pollen from the source plant species, and secondly, on the course of the fermentation. Some polliniferous species are of great value for bees, for instance *Papaver rhoeas*, *Castanea sativa*, *Erica* spp., *Trifolium* spp., *Cystus* spp., fruit trees, etc. Conversely, the less nutritive are *Betulus*, *Populus*, pine trees, etc. The bee bread must meet the protein requirements of the nurse bees, which are responsible for the production of royal jelly. The shortage of nutritious bee bread often finds expression in the bee brood with the appearance of symptoms of European foulbrood, which persist as long as the deprivation.

When bee bread is lacking, some proteinaceous flours or yeast extracts can be fed to the colony as a substitute. However, at the present time, no commercial substitute can totally replace the bee bread, because the balance between the protein concentration, the amino acid pattern and the other components is not well known for honeybees. Mouldy bee bread is not suitable for feeding to bees.

Royal jelly

Royal jelly is a secretion both of the thoracic glands and the mandibular glands of nurse bees, which is given directly as food to the queen or to young larvae. The proportion of the two secretions varies in relation to the age of the larva to be fed, and that of the nurse bee. The younger the larva, the richer the royal jelly is in hypopharyngeal gland secretions. The composition of royal jelly is thus variable. The water content is 60-70%, the protein content is 17-45% of the dry weight, the sugar content is 18-52% of the dry weight, the lipid content 3-19% of the dry weight and the mineral content 2-3%. The presence of short chain fatty acids and the concentration of various fat-soluble vitamins are remarkable. The nurse bees include a small amount of bee bread in the food given to the last larval instar.

In the colony, a minimum number of nurse bees of the right age and adequately nourished are required to ensure the optimal feeding of larvae. Only one weak link in this feeding chain causes inadequate nutrition of larvae, which favours the outbreak of European foulbrood. Inadequate feeding of larvae does not occur only in weak colonies, but also in strong ones. Early in spring a prolific queen is able to lay a very large number of eggs and the larvae that hatch from these eggs may not receive an adequate amount of royal jelly because of an imbalance between the number of nurse bees and the number of larvae to be fed.

Propolis

Propolis is a sticky, pliable substance collected by bees from trees, particularly leaf buds and bark and other vegetation. It is a mixture of wax and resins, mainly composed of flavonoids and phenolic acids or their esters. Propolis also contains around 10% of essential oils. The foragers carry propolis in their corbicula in the same way as pollen pellets. Depending on the plant origin, some propolis has good antibacterial properties, which may be particularly relevant in cases of European or American foulbrood. When a significant amount of freshly collected antibacterial propolis is stored in the hive, these diseases may be effectively suppressed. *Betulus* spp., *Populus* spp., and *Castanea sativa* provide propolis with antibacterial properties.

Bee colony management

The aim of this section is not to evaluate the benefits and the drawbacks of each of the innumerable techniques of beekeeping, practised around the world, but to propose reasoned schemes, valid for every type of beekeeping.

Moritz and Southwick (1992) considered the bee colony as a homeothermic animal; i.e. the temperature of some parts of the hive, or of the colony, need to be regulated. In particular, the uncapped brood must be maintained at a constant temperature of 35°C. Thermoregulation is achieved by the release of metabolic heat by the muscular activity of adult bees, finding the energy in the stored sugars. In order to save energy, the density of the adult bees has to be maximal in the area containing the uncapped brood; heat is conserved by the insulation property of the clustered bees' bodies. The illustrative saying used by beekeepers is: "the bees cover the comb surface". In practice saving energy means:

(i) Firstly, that the apiary or the hive must be located in a sunny place, protected from cold winds and moisture, when wintering. On the contrary, the apiaries will be in shaded and windy places for summertime.

(ii) Secondly, that the amount of space available to the colony, has to be adapted to the number of bees, so that the bees do not have to make the effort to heat a large volume without brood.

(iii) Thirdly, to maintain the temperature of the brood, it is necessary to have an appropriate number of bees and honey or syrup stores. Failure to observe these rules will result in the chilling of the brood which is generally followed by a sudden rise in the death of young larvae due to mycosis or directly, in the case of serious chilling.

One individual, the queen, ensures the perenniality of the colony from one year to the next and its harmonious growth within favourable surroundings. This individual is able to lay more than 2000 eggs a day, so a protein-rich diet necessary to sustain egg production is particularly important. The physiological exhaustion of the queen begins in its second year of life. Consequently, the eggs laid by a failing queen, contain a smaller amount of vitellogenin, which give rise to larvae less resistant to several stresses including the action of pathogens. An essential, basic rule of beekeeping for disease management is to maintain colonies only with young queens.

Another basic rule for maintaining the health of colonies is the provision of good quality wax foundation, which is drawn out by the bees to build up the comb cells. Worker bees are more reluctant to build comb if more than 5% paraffin wax is added to the wax foundation. Moreover, old drawn comb is often contaminated by bee pathogens such as bacterial or fungal spores, increasing the possible risk of disease transmission. Thus it is in the interest of the beekeeper to change the wax foundation in at least one year out of three.

Recently, the adaptation of an haematophagous mite, *Varroa jacobsoni,* to the honeybee (*Apis mellifera*), has created another permanent factor interacting with the diseases of bees. The number of mites in the colony tends to increase during the active season, forcing the beekeeper to treat regularly to control the mite population. Whatever the efficacy of the treatment, some mites always survive. They deplete the bee larvae and pupae of proteins, disrupting their development. Moreover, the feeding activities of mites are a means of transmission of pathogens within the colony because the parasite moves between hosts and thus could contaminate several bees. Mites also induce the multiplication of viruses, such as acute bee paralysis virus, if these are present as a latent infection in the bee colony. Acaricide treatments cannot be repeated frequently without risking the development of mite resistance and the contamination of hive products; the colonies must therefore accommodate parasites the whole year long, which means a permanent danger of increasing the impact of diseases.

Genetic factors

These factors play an important role in the resistance of the bee colony to diseases. From a practical point of view, the importance of the factors can be considered at two levels: (i) the race; and (ii) the strain.

(i) In respect of ascophaerosis, *Apis mellifera mellifera* is very susceptible. In contrast, *A. m. carnica* is never severely affected by the disease. Within a race, selection can be made on the basis of cleaning behaviour, which means the ability of the adult bees to detect and eject diseased larvae from the colony. In the case of foulbrood, *A. m. caucasica* is generally more resistant, but this important advantage is counteracted by its susceptibility to nosematosis. Obviously, bees resistant to all the diseases cannot be found at the present time.

(ii) Resistance against viral diseases seems to be related to the strain level. Kulincevic and Rothenbuhler (1975) demonstrated that increased resistance to hairless black syndrome, due to the multiplication of chronic bee paralysis virus in adult bees, could be gained after a selection attempt on four generations of queens. An epidemiological study of sacbrood leads to a similar conclusion (Giauffret, 1968). In practice, the queen breeders, who sell large numbers of selected queens from a particular strain, take great care to choose "parental" colonies without any viral symptoms.

The existence of strains of *Apis mellifera* tolerant or resistant to the mite *Varroa jacobsoni* is now proved, but the biological basis of the resistance is still unresolved (see chapter on Varroosis).

Residues in the hive

The origin of the residues is twofold: (i) external, when hive contamination is due to atmospheric pollution or to the persistence of agrochemicals on plants visited by the bees; and (ii) internal, which means post-therapeutic residues.

Although no direct evidence is available on the relationship between the presence of residues and the triggering of a bee disease, we believe that residues can adversely affect colony health. As to therapy, the repeated administration of antibiotics or of acaricides induces resistance in bacteria or mites, making future treatment ineffective and more hazardous. Because honeybees forage in various crops, they can bring different residues of agrochemicals back to the hive. Therefore the risk of a synergy between two agrochemicals, even when present at low concentrations, may be considerable and could lead to bee mortality or to a disruption of the normal social organisation of the colony. In addition, this disruption of social organisation can indirectly increase the severity of a disease, for instance, if the thermoregulation of the colony is disturbed or if the protein supply is inadequate.

In conclusion, the diagnosis of a bee disease depends not only on the detection and precise identification of a pathogen in the bee or in the hive, but also on an understanding of how the pathogen has the ability to multiply and spread and to affect the development or the strength of the colony. It is of fundamental importance to keep in mind these two features of the diagnosis: medical and practical, because some pathogens are not dangerous if the colony has not been weakened. We consider that the frequent outbreak of a disease in an apiary is not a holy punishment, but the symptom of a deficiency in good beekeeping practice.

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