The possible role of honey bees in the spread of pollen from field trials

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Colophon

Title:	**The possible role of honey bees in the spread of pollen from field trials**
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Preface

In field trials with genetically modified plants, visits by honey bees can contribute to the dispersal of pollen of such plants to areas outside the field trial area. If this pollen could lead to hybridization between the GM-plant and wild relatives in the surroundings of the field trial, or if pollen could end up in honey produced by local beekeepers, pollen dispersal by honey bees should be considered in the risk assessment. The GMO Office of The Netherlands and the Netherlands Commission on Genetic Modification (COGEM) have therefore commissioned a study on the role of honey bees in the dispersal of pollen with an emphasis on the distances over which pollen can be transferred in relation to cross-hybridization and the concentrations of pollen in honey. This report contains valuable background information on the foraging behaviour of honey bees, the distances over which they fly, and the practices of beekeepers in The Netherlands that influence the transport of pollen. It also includes a new survey on the concentrations of pollen in honeys manufactured in The Netherlands. I am convinced that these data will strengthen the risk assessment procedures presently done for field trials of GM-crops.

Nico M. van Straalen
Chairman advisory committee

Contents

Glossary of terms

Anther	–	The pollen-bearing part of the stamen of a flower
Aphid	–	Small, soft-bodied insects of the family Aphididae (Homoptera) that have mouthparts specially adapted for piercing and feeding by sucking phloem sap from plants; also known as plant louse
Brood	–	The egg, the larva and the pupa stages in the life of honey bees
Brood comb	–	The breeding area of the comb that contains larvae and pollen
Comb	–	A mass of hexagonal wax cells built by honey bees to contain larvae and store honey and pollen
Corbicula	–	A flat or hollow area bordered with stiff hairs on the hind legs (tibia) of a bee, suited to collect and transport pollen; also known as pollen basket
Cross-pollination	–	The transfer of pollen from the male reproductive organ (an anther or a male cone) of one plant to the female reproductive organ (a stigma or a female cone) of another plant
Dioecious	–	Plants that develop male and female flowers on separate individuals
Flower constancy	–	The tendency of individual pollinators to exclusively visit certain flower species or morphs within a species, bypassing other available flower species that could potentially be more rewarding (i.e. contain more nectar)
Genetic modification	–	The process of using recombinant DNA (rDNA) technology for the purpose of altering and controlling the genetic makeup of an organism, by introducing new or reinforced traits to a crop that are agronomically or otherwise desired; also known as genetic engineering
Gland	–	A specialized organ that produces a substance and secretes this for further use
Honeycomb	–	The area of the comb in which honey is stored
Honeydew	–	A sugar-rich sticky liquid, secreted by aphids and some other small scale insects when they feed on phloem sap from plants
Honey flow	–	Period in which honey bee colonies can collect a very large amount of honey
Honey super	–	Part of a commercial beehive that is used to collect honey
Hypopharyngeal gland	–	Glands that synthesize and secrete for a short period of the adult worker's life the so-called royal jelly; located in the head of the honey bee below the pharynx
Imaginal disc	–	One of the parts of an insect larva that will become a portion of the adult insect during pupal transformation
Mandible	–	A pair of appendages near the insect's mouth
Micropyle	–	A small opening at the broad end of the egg of a honey bee, which allows for passage of sperm

Monoecious	–	Plants that develop male and female flowers on the same individual, or carry bisexual flowers
Moulting	–	The manner in which insects and other arthropods routinely cast off their cuticle during larval stages
Nectar	–	A sugar-rich liquid secreted by nectaries in- or outside flowers of various plants, consumed by pollinators and gathered by honey bees as energy source; honey bees process the nectar into honey and store this as energy reserve for the winter time
Nectary	–	The sugar-secreting gland on plants, inside or outside a flower, that attracts and feeds pollinators (and defenders)
Phloem	–	In vascular plants, phloem is the living tissue that carries organic nutrients, particularly carbohydrates (sugars), synthesized in the green tissues, to all parts of the plant where needed
Pollen	–	The male germ cells produced by all flowering plants for fertilization and plant embryo formation
Proboscis	–	The straw-like tongue of the honey bee consisting of modified mouthparts, among others, used for sucking liquids
Propolis	–	A polyphenol-rich resinous substance with antibacterial and antifungal properties, collected by honey bees from botanical sources, to be used as a sealant to cover unwanted open spaces, cells of the comb and the inner walls of the nest
Proventriculus	–	A kind of valve between the honey stomach and the real stomach (ventriculus) of a honey bee, which filters solid particles from the contents of the honey stomach
Skewed distribution	–	A set of scores that is not equal on both sides: this results from a few scores in a data set falling farther to one end or the other
Stigma	–	The receptive tip of the carpel (i.e. the ovule and seed producing reproductive organ in flowering plants), which receives pollen at pollination and on which the pollen grains germinate

Executive summary

Honey bees are important pollinators in agricultural crops, home gardens, orchards and wildlife habitats. As they fly from flower to flower in search of nectar and pollen, they transfer pollen from plant to plant, thus fertilizing the plants and enabling them to bear fruit. In light of this, honey bees could be a factor in spreading pollen grains derived from genetically modified (GM) plants in field trials. The extent to which pollen dispersal occurs and the distances achieved depends on many factors. Knowledge of these factors may be important for (future) risk assessments of GM plants.

An overview of relevant information concerning the relationship between honey bees and pollen is presented, based on a literature survey, a database of pollen composition of Dutch honeys and a concise laboratory experiment. More in particular, this report encompasses:

- Information about the spread of pollen by honey bees, in connection with determining the possibility of pollen transport and potential cross-pollination with related (wild) plants in the surrounding environment
- Information about the presence of pollen in honey
- An experimental estimation of the pollen content of honeys (in µg pollen/g honey)
- Information about beekeepers and beekeeping in The Netherlands

Honey bees

Among the many species of bees, honey bees represent bees from the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax. In The Netherlands, the European-, Western-, or Common honey bee (*Apis mellifera*) is a native species. The subspecies *Apis mellifera mellifera*, which exists in The Netherlands, is also known as the European dark bee.

Two sexes (male (i.e. drones) and female) and two castes of female bees (queens and workers) make up the population of a beehive, each having its own characteristics, roles, and tasks within the hive. The vast majority of honey bees are worker bees. The worker bees sequentially take on a series of specific tasks during their lifetime, such as cleaning and foraging. Foragers take care of bringing from the environment everything that the colony needs to the hive: pollen, nectar, water and propolis:

- *Pollen*

  Pollen is the plant protein source for the larvae and young bees, providing nitrogen, phosphorus, amino acids, and vitamins essential for development of the bees. Pollen is collected in the fur of the bee and stored for transport in pollen baskets (corbicula) on the tibiae of the workers' rear legs.

– *Nectar*
  Nectar, obtained from floral nectaries, sometimes deep inside flowers, or from extrafloral nectaries, provides an almost pure carbohydrate source for honey bees.
– *Water*
  Honey bees collect only little amounts of water, because their muscles produce a lot of water when burning sugars, and the nectar collected contains a lot of moisture. Sometimes extra water is needed for cooling the hive.
– *Propolis*
  Propolis, another substance brought into the hive, is also called 'bee glue'. It is a plant resin used to cover the hive walls and combs to close cracks and holes. It has anti-bacterial and anti-fungal properties.

Foraging of honey bees is highly organized: through trophallaxis (i.e. the exchange of food) and the communication by the bee dance (waggle dance and round dance), there exists awareness of the needs and the spatial disposition of resources in the environment among the workers in a colony.

Pollination by honey bees
Pollination is the transfer of pollen grains, the male sex cells of a plant, from the anther where they are produced to the receptive surface, or stigma, of the female organ of a flower. Honey bees are among the insects that transfer pollen between flowers and between plants and are often regarded as being the most important pollinators, hence the word 'pollination' is habitually used to describe the service of providing bees to pollinate crop plants.

Honey bees are efficient pollinators for several reasons. Their hairy bodies easily trap pollen and carry it between flowers. The body size of honey bees enables them to pollinate flowers of many different shapes and sizes; however, sometimes no pollination occurs despite nectar or pollen removal, for instance because stigmas are not touched. The pollination potential of honey bees is also high because they can be managed to develop large populations.

Pollen in the pellets in the bees' pollen basket may remain viable for several hours, but are lost for pollination. Some pollen on the bee body is also able to germinate after several hours, and so can be transferred in viable state over large distances, but most of it is deposited nearby flowers of the same plant or on other plants closeby. Typical numbers of pollen grains found on the bee's body amount to 1,000 to 10,000 grains per bee. Pollen longevity varies considerably between plant species.

Powder box

The air inside the beehive, as well as its inhabitants, appears to contain pollen from all sources visited by the bees. Due to this 'powder box effect', individual bees may carry significant amounts of pollen from plants not actually visited by them. Although the number of pollen grains exchanged inside the hive very low compared to the number directly deposited on foraging bees, it may be of some significance for cross-pollination between plants in different spots that are not in direct flight connection of individual bees. This pathway with connecting flights, through which pollen is carried into the hive by one individual may be carried to a different flower patch by another individual might imply that the maximum pollen flow distance actually can be twice the maximum flight distance of the individual honey bees.

Pollen ending up in honey

Natural honey always contains pollen grains, and often spores of fungi as well. Pollen grains can end up in honey through three routes:

1. Pollen from the flower that is visited by the bee falls into the nectar and is harvested by the bee;
2. Pollen on bees and in the air inside the hive contaminate the nectar during processing by the bees (packing, drying, conserving); and
3. Pollen packed in cells in the same comb as the honey may be mixed with the honey by the beekeeper during centrifugation or pressing of the honey from the combs. Especially pressing of honeycombs may add a lot of pollen; however, this method is seldom applied in commercial honey production.

Honey flows

In some periods very rich 'honey flows' are available, from which bee colonies can easily harvest a lot of honey and pollen, and which boost the development of colonies. These honey flows allow the beekeeper to harvest honey. In between honey flows, the bees partly rely on their reserves and forage on several plant species that just deliver enough resources to cover the maintenance of the colony.

Most honey flows occur during spring, fewer during summer and the beginning of autumn. The opportunistic nature of the honey bees results in different limits for a source in order to be attractive for the bees.

Foraging and pollen transport

Finding rewarding spots

Honey bees are very well equipped to locate places in all directions around the beehive where plants are flowering and lots of pollen and nectar is to be found. As soon as scouts have found a rewarding foraging place, this is communicated in the beehive, which enables nectar foragers and pollen foragers to visit these spots as well.

Nearby or far away?

When enough pollen and nectar is available near the beehive or food is concentrated in a small area, bees tend to fly as little as possible and bring their pollen or nectar to the colony right away, As this is often the case, most of the time there is no need to fly further than a few hundred meters. However, when nearby food is limited and good patches are available at larger distances, honey bees are known to fly more than 5, up to even 10 kilometres.

The distance over which pollen are transported depends not only of the potential flying capacity of honey bees, but is also dependent on the complex foraging behaviour of bees. The foraging behaviour depends on a) the time of the year, b) the state of the colony, c) the distribution of food patches in the landscape surrounding the beehive, and d) on the variation in activity between individual bees. The quantification of pollen transport in terms of amounts in space and time is therefore much more complex than only measuring flying distances.

Spatial activity patterns

The range and distance covered by bees is extremely variable. The way in which bees distribute their activity in the environment around the beehive largely depends on how the most rewarding food sources are situated in the landscape. Only a few studies on bees and honey bees have analysed these activity patterns in detail. More recently, models have been developed to describe these patterns to predict the transport of pollen from flower to flower and from field to field.

Transport of pollen and cross-pollination

When visiting flowers the foraging bees continuously take up, but also lose pollen. When flying to another patch or to the hive, thousands of pollen grains are transported. In practice, most pollen is transported over small distances and dropped again. Often only a small fraction (less than 1%) is carried long enough to mediate pollen transfer between plants more than 1 km apart. It is difficult to quantify these events and their impact on gene flow. The probability of pollen transport and potential cross-pollination is expected to decrease exponentially with distance, not only because only few bees tend to fly long distances, but also because pollen is lost and replaced by new local pollen and because of loss of pollen viability. Experiments and modelling approaches give interesting and encouraging results, but simulating pollination patterns in heterogeneous landscape remains an elusive goal.

Pollen in honey

Real honey always contains pollen grains, originating from contamination of the nectar inside the flower, contamination of nectar and honey by the bees during processing and from contamination by the beekeeper during honey harvest from the honey combs. Contamination by the bees during processing inside the hive is the predominant route.

The botanical origin of the honey is to some extent reflected in the pollen profile of a honey. To be qualified as a unifloral honey, a minimum share of specific pollen species need to be met. Almost 200 honey samples from a 2008 survey all over The Netherlands were analysed for their botanical pollen profiles. More than 50 plant families were represented. In this report an overview is presented, focussing on the presence of pollen from Salicaceae, Fabaceae, Rosaceae, Brassicaceae, Solanaceae, Chenopodiaceae and maize. Pollen from maize was found in two samples (1.5 – 4.2 % of the grains). Both Rosaceae and Brassicaceae were well represented, but it is not known which part of these represent apple and oilseed rape. The family Solanaceae, to which potato (*Solanum tuberosum*) belongs, was represented in several honeys. However, it cannot be determined whether potato pollen was involved. Chenopodiaceae pollen was relatively rare in the honey samples, and the chance of it being pollen of beet (*Beta vulgaris*) is very small since beet is not attractive to bees and because it hardly ever flowers.

Five honey samples of the above-mentioned survey were used to quantitatively determine the abundance of pollen grains, using a cytometric counting chamber. The numbers ranged from a few hundred to 28,000 per gram of honey. In the literature data <2000 to 10.000 are mentioned. Further research is necessary and the method for sample preparation and counting still needs some improvement.

To express pollen abundance on a mass basis, a calibration experiment was conducted and the specific weight of pollen grains was estimated for five plant species. It was found that individual pollen grains of the chosen species weighed from 0.0043 µg to 0.067 µg. Together with the counted number of pollen grains in honey, this implies that a honey may contain 43 to 670 µg pollen per gram of honey. For unifloral oilseed rape honey and fruit blossom honey, the content of the specific pollen (oilseed rape, apple and pear) would be about 60 µg/g.

Conclusions

The main conclusions of the report, clustered per topic, are the following:

Beekeeping in The Netherlands

- Both hobbyist- and professional beekeepers practice beekeeping in The Netherlands. Of the approximately 8,000 Dutch beekeepers, about 6,900 are organised in three beekeeping associations, of which the Dutch Beekeepers Association (*Nederlandse Bijenhoudersvereniging* – NBV) is by far the largest with 6,000 members.
- Hobbyist beekeepers have an average of five colonies, professional beekeepers well over one hundred.
- Around ten beekeeping companies are professionally involved in the pollination of crops with honey bees and make a living out of it. Overall, it is estimated that something like 32,000 honey bee colonies are utilized for pollination in The Netherlands by approximately 1,700 beekeepers.
- Hobbyist beekeepers seldom move their hives (except when hired for pollination in fruit orchards), whilst professionals regularly move their hives (every 4 weeks on average).
- In The Netherlands, honey bees are being utilized in fruit orchards, in the horticulture industry and for seed production. Professional beekeepers or beekeeping companies cover nearly all pollination in closed greenhouses and polytunnels. However, regular collaboration takes place with hobbyist beekeepers, in order to guarantee the supply of bee colonies. Pollination in the fruit sector is mostly covered by non-commercial, hobbyist beekeepers.
- The annual value of pollination fees is roughly estimated at 4 million Euro for outdoor cultivation, and 7 million Euro for cultivation in greenhouses and polytunnels. However, the indirect economic value or benefit of pollination by honey bees and other insect pollinators is considered to be much higher.
- Honey bees are not only utilized for pollination. Several products that are produced by bees can be harvested by the beekeeper. Obviously, the most recognizable product made by honey bees is honey. Other products include beeswax, propolis, royal jelly, bee pollen and bee venom. All of these bee products (could) contain (trace amounts of) pollen.
- Most honey on the Dutch market (92%) is imported from amongst others China and Argentina; the two largest honey-producing countries in the world.

Foraging and pollen transport

- Honey bees visit flowers for pollen (their protein, fat and mineral source) and nectar (energy source). Individual bees specialize and do not forage for nectar and pollen simultaneously and devote their foraging to only one plant species at a time.
- Foraging is not only optimized at the individual forager level, but on the whole colony level. This is achieved through communication and task division.

- Only a small part of the pollen that is collected on flowers is available for pollination: bees use more than 99% as food.
- The distance up to which bees forage for water, pollen and nectar may range up to 2, 5, 10-14 km respectively. However, if a rich resource is nearby, most of the foraging trips will be much shorter (0.5-1 km). If food nearby the colony is scarce and very rewarding patches are further away, large flight distances are likely to become frequent.
- Inside the hive of honey bees, pollen is exchanged unintended between individuals: this may lead to pollen transport between distant flower patches that are not visited by the same individual forager.
- For oilseed rape, it has been shown that a) bees may fly longer distances to forage on such fields, and b) bees tend to stay close to their hive if the hive is located next to an oilseed rape field.
- Apple is a very attractive crop for honey bees to forage on. However, it is not documented which distances honey bees fly to forage on apple, because it is common practice to place colonies in (or very near) apple orchards in order to optimize pollination.
- Honey bees are not attracted to the crop potato, unless aphids feed on the potato plants. However, farmers rarely tolerate aphids that are attractive to honey bees, and even if aphids are present, aphid densities often peek before flowering.
- Sugar beet crops are not meant to flower and are therefore not attractive to honey bees.
- Maize, although it is a wind pollinated plant, is also visited by honey bees. However, no data is available about the distances bees cover for maize. In addition, hardly any of the maize pollen collected may result in pollination, since honey bees are not at all interested in female maize flowers.
- Though potential flight distances of honey bees have been measured and estimated in a number of detailed studies, it is almost impossible to set up a general model that predicts activity- and pollination patterns for specific cases, as the number of conditions that can influence such patterns is almost unlimited.
- It should be kept in mind that besides honey bees, also bumble bees and many other insects are involved in pollen transport. Therefore, much information on what this means at a landscape scale is needed to estimate its impact on the out-crossing phenomena.

Pollen in honey

The presence of pollen in honey

- Honey contains small amounts of pollen. This pollen has entered the honey through three pathways: direct contamination of nectar at the flower, contamination by bees inside the hive, and contamination by the beekeeper during honey harvesting and processing. Contamination by the bees during processing inside the hive is the most important route of pollen into honey.

- Although the botanical origin of the pollen grains in honey can be an indication of the nectar source of the honey, this is not straightforward due to the contamination paths mentioned above. Blending of commercial honeys may also be an additional obstacle to traceability.
- About 200 honey samples, from a survey throughout the country in The Netherlands (2008), carried pollen grains of at least 50 plant families, wind pollinated as well as insect pollinated species.
- Rosaceae pollen (including apple), Brassicaceae pollen (including oilseed rape) were very abundant. Chenopodiaceae (to which Beta belongs) and Solanaceae (to which potato *Solanum tuberosum* belongs) and Poaceae (grass family, including maize) were also represented in the honeys. Maize pollen was only encountered twice (out of 200) and in very small quantities.

Experimental estimation on the pollen content of honeys

- The numbers of pollen grains, counted in five of the Dutch honeys from the survey, corresponded well with those reported in literature, generally topping at 10,000 grains per gram honey.
- The reliability of pollen counting depends on the dilution of the suspension used in counting chambers and needs to be further improved.
- A strong and significant relationship was found between the amount of pollen (grams) added to an artificial honey, and the counted number of grains in these honeys. The highly significant calibration factors were used to estimate real pollen concentrations (µg/g) of real honey. Preliminary results indicate that only trace amounts of pollen are present in honey, ranging from 43 µg to 670 µg pollen/g honey.
- Based on the calibration curves, oilseed rape honey and fruit blossom honey were calculated to contain at least 60 and 58 µg of the specific pollen (oilseed rape, apple and pear) per gram of honey respectively.

Samenvatting

Honingbijen bestuiven landbouwgewassen, planten in tuinen, boomgaarden en planten in de wilde natuur. Als ze zich verplaatsen van bloem naar bloem, op zoek naar nectar en stuifmeel (ook wel aangeduid met het oorspronkelijk Engelse woord pollen, in het Nederlands *het* pollen), wordt er stuifmeel van de ene naar de andere plant overgebracht waardoor bevruchting kan plaatsvinden. In het licht hiervan zouden bijen een rol kunnen spelen in de verspreiding van stuifmeel afkomstig van veldproeven met genetisch gemodificeerde (GG) planten. De mate waarin deze verspreiding kan optreden en de door bijen te behalen foerageerafstanden zijn van vele factoren afhankelijk. Kennis van deze factoren kan van belang zijn voor (toekomstige) risicobeoordelingen van GG-planten.

In dit rapport wordt een overzicht gegeven van relevante informatie met betrekking tot de relatie tussen honingbijen en pollenverspreiding, gebaseerd op een literatuurstudie, een database met stuifmeelgegevens van Nederlandse honing en een beknopt laboratoriumexperiment. Er is ondermeer gekeken naar de volgende onderwerpen:

- Informatie over de verspreiding van pollen door honingbijen, in verband met het bepalen van de mogelijkheid van pollentransport en eventuele kruisbestuiving met verwante (wilde) planten in de omgeving.
- Informatie over de aanwezigheid van pollen in honing.
- Een experimentele schatting van de stuifmeelinhoud van honing (in µg pollen / g honing).
- Informatie over de imkers en de bijenteelt in Nederland.

Honingbijen

Binnen de vele bijengeslachten vertegenwoordigen honingbijen het geslacht *Apis*. Dit geslacht onderscheidt zich van andere door de productie en opslag van honing en het bouwen van nesten van was. In Nederland is de Europese- of westerse honingbij (*Apis mellifera*) een inheemse soort. De ondersoort *Apis mellifera mellifera* die in Nederland voorkomt wordt ook wel Europese zwarte bij genoemd.

Twee geslachten (mannelijk (darren) en vrouwelijk) en twee kasten van vrouwelijke bijen (koninginnen en werksters) vormen samen een bijenvolk. De overgrote meerderheid van honingbijen zijn werksters en deze zijn gedurende hun leven verantwoordelijk voor een aantal specifieke taken, zoals schoonmaken en foerageren. Foerageerders verzamelen alles wat het volk nodig heeft vanuit de omgeving van de kast: pollen, nectar, water en propolis.

- *Pollen*

  Stuifmeel of pollen is de plantaardige eiwitbron voor de larven en jonge bijen. Het levert essentiële bouwstoffen voor de ontwikkeling van bijen, zoals eiwitten, vetten, mineralen en vitamines. Pollen wordt verzameld in de vacht van de bij en opgeslagen voor transport in stuifmeelkorfjes (corbicula) op de achterpoten van de werksters.
- *Nectar*

  Nectar wordt verkregen uit de honingklieren van bloemen en biedt een bijna zuivere bron van koolhydraten voor honingbijen.
- *Water*

  Honingbijen verzamelen doorgaans slechts kleine hoeveelheden water, aangezien hun spieren veel water produceren bij de verbranding van suikers. Daarnaast bevat de verzamelde nectar veel vocht. Echter, soms is er extra water nodig voor het koelen van de kast.
- *Propolis*

  Propolis is een lijmachtige substantie, verzameld door honingbijen van de schubben van knoppen van bomen, meestal populieren. Propolis wordt door bijen gebruikt om ongewenste kieren en openingen in het nest te dichten. Propolis remt de groei van bacteriën en schimmels.

Het foerageren van honingbijen is tot op zekere hoogte georganiseerd: via trophallaxis (het uitwisselen van voedsel) en door communicatie, onder andere de bijendans (kwispeldans en rondedans), verkrijgen de werksters informatie met betrekking tot de behoefte van het bijenvolk en de omliggende voedselbronnen waarop gefoerageerd kan worden.

Bestuiving door honingbijen

Bestuiving is de overdracht van stuifmeelkorrels (waarin de mannelijke geslachtscellen of de spermakernen worden gevormd) uit de helmhokjes van de meeldraad naar de stempel van de stamper. Honingbijen behoren tot de insecten die stuifmeel overbrengen tussen bloemen en planten en worden vaak beschouwd als de belangrijkste bestuivers. Vandaar dat het woord 'bestuiving' doorgaans wordt gebruikt voor het inzetten van bijenvolken bij het bestuiven van gewassen.

Honingbijen zijn geschikt voor bestuiving om verschillende redenen. Hun behaarde lichaam vergemakkelijkt het opnemen van pollen en het transporteren ervan tussen bloemen. Door hun lichaamsgrootte zijn bijen in staat bloemen te bestuiven van verschillende vorm en grootte. Echter, soms vindt er geen bestuiving plaats ondanks het feit dat nectar of pollen zijn meegenomen, bijvoorbeeld doordat de stigma's niet zijn aangeraakt. Het bestuivingspotentieel is tevens hoog, doordat honingbijen in grote kolonies leven.

Pollen in de stuifmeelkorfjes van bijen kan enige uren levensvatbaar blijven, maar is verloren voor bestuiving. Stuifmeel op het lichaam van de bij is ook in staat om na een paar uur nog te kiemen; het is echter waarschijnlijker dat het wordt afgezet op een nabijgelegen bloem van dezelfde plant dan op een bloem van een andere plant op een grotere afstand. De aantallen pollenkorrels die zijn aangetroffen op het bijenlichaam liggen gemiddeld tussen de 1.000 en 10.000 korrels per bij. De levensduur van stuifmeel kan aanzienlijk verschillen tussen plantensoorten.

Poederdoos

De lucht in bijenkasten en het haarkleed van de bijen, blijkt stuifmeel te bevatten van ongeveer alle door de kolonie bezochte voedselbronnen uit de omgeving. Door dit 'poederdooseffect' kunnen individuele bijen aanzienlijke hoeveelheden stuifmeel met zich meedragen van planten die niet door henzelf bezocht zijn. Hoewel het aantal stuifmeelkorrels dat wordt uitgewisseld in de kast zeer laag is in vergelijking met het aantal dat direct terechtkomt op foeragerende bijen, kan het van enige betekenis zijn voor kruisbestuiving tussen planten op verschillende locaties, die niet op de directe vliegroutes van individuele bijen liggen. Het kan dus voorkomen dat het pollen van de ene bij in de kast wordt opgepikt door een andere bij en vervolgens wordt getransporteerd naar een veld in de tegenovergestelde richting. Dit zou betekenen dat de afstand waarover pollen verplaatst kan worden, in feite tweemaal de maximale vluchtafstand van individuele bijen is.

Hoe pollen terechtkomt in honing

Natuurlijke honing bevat altijd pollenkorrels en vaak ook sporen van schimmels. Pollenkorrels kunnen op drie manieren in honing terecht komen:

1. Pollen van de bloem waarop wordt gefoerageerd belandt in de nectar en deze nectar wordt vervolgens geoogst door de bij;
2. Pollen op de bijen en in de lucht van de kast verontreinigt de nectar als deze wordt verwerkt door de bijen (verpakken, drogen, opslaan);
3. Pollen dat is opgeslagen in de cellen van dezelfde raat als waarin de honing is opgeslagen kan in de honing terecht komen bij het slingeren of persen van de raat door de imker. Vooral het persen van de raat zorgt ervoor dat veel pollen in de honing belandt; deze methode wordt echter nauwelijks meer toegepast bij commerciële honingproductie.

Dracht

In perioden van goede dracht zijn drachtplanten aanwezig die op dat moment grote hoeveelheden nectar, stuifmeel en/of honingdauw bieden. Een goede dracht is over het algemeen bevorderlijk voor de gezondheid van het bijenvolk en stelt de imker in staat de honing te oogsten. Tussen drachten maken bijen deels gebruik van hun reservevoorraden en foerageren ze op bepaalde plantensoorten die net genoeg voedsel leveren om de kolonie te kunnen onderhouden.

De meeste goede honingdrachten vinden plaats in het voorjaar en in mindere mate tijdens de zomer en het begin van de herfst. Het opportunistische karakter van de honingbijen resulteert in verschillende ‘drempelwaarden’ voor gewassen om aantrekkelijk te zijn voor de bijen: in het voorjaar een hoge, in de zomer een lagere drempel.

Foerageergedrag en pollentranport

Hoe worden de beste voedselplekken gevonden?

Honingbijen zijn heel goed in staat om plekken rondom de kasten te vinden met veel bloeiende planten waar stuifmeel en nectar te halen is. Zodra de verkenners een voedselrijke plek hebben gevonden vliegen ze terug naar de kast waar ze aan de andere bijen via de bijendans laten weten waar deze plekken zich bevinden zodat andere pollen en nectar verzamelende bijen deze gaan bezoeken.

Dichtbij of ver weg?

Om energie te sparen en efficiënt voedsel te verzamelen vliegen bijen het liefst zo min mogelijk zodat alle tijd benut kan worden voor het foerageren. Dit kan echter alleen als er voldoende voedsel aanwezig is nabij de kast of als voedsel voldoende geconcentreerd is op één plek. Omdat de kasten veelal op gunstige plekken worden geplaatst zal de vliegafstand doorgaans niet verder zijn dan een paar honderd meter. Als de hoeveelheid voedsel in de nabijheid van de kast echter onvoldoende is en er goede plekken op afstand aanwezig zijn blijken honingbijen probleemloos afstanden van 5 tot 10 km te kunnen overbruggen.

De afstand waarover stuifmeel wordt getransporteerd hangt niet alleen af van de vliegcapaciteit van de bijen maar wordt mede bepaald door andere factoren rond het fourageergedrag. Hoever en waarheen bijen vliegen hangt onder andere af van a) de tijd van het jaar, b) de gezondheidstoestand / fitheid van de kolonie en c) de verdeling van voedselrijke plekken in de omgeving. Er is ook sprake van verschillen tussen individuen. Het bepalen van de mate van stuifmeeltransport in ruimte en tijd is veel moeilijker dan het meten van alleen vliegafstanden.

Ruimtelijke activiteitspatronen

Uit het voorgaande moge blijken dat de afstanden die bijen overbruggen om voedsel te verzamelen in de praktijk erg variabel zijn. De manier waarop bijen vanuit de kast hun omgeving exploiteren hangt vooral af van de ligging van de beste voedselplekken in het omringende landschap. Er zijn slechts een paar publicaties bekend waarin de foerageerpatronen gedetailleerd zijn onderzocht en beschreven. Recent zijn modelstudies gedaan om de vliegpatronen te voorspellen op zowel kleine schaal (van bloem tot bloem) als over grotere afstanden (tussen percelen).

Stuifmeeltransport en kruisbestuiving

Terwijl bijen van bloem tot bloem vliegen wordt steeds meer stuifmeel opgenomen maar een deel gaat daarbij ook weer verloren. Tijdens langere vluchten worden duizenden stuifmeelkorrels getransporteerd. In de praktijk wordt vooral veel stuifmeel over korte afstanden verplaatst. Slechts in uitzonderlijke gevallen zal een kleine fractie (minder dan 1%) over grotere afstanden (> 1 km) worden verspreid. De kans dat stuifmeel wordt verspreid en tot kruisbestuiving kan leiden neemt exponentieel af met de afstand, niet alleen omdat slechts weinig bijen grote afstanden overbruggen maar ook omdat veel stuifmeel onderweg verloren gaat. Experimenten en modelstudies bieden steeds meer inzicht, maar echt goede voorspellingen over de mate waarin stuifmeeltransport in complexe landschapssituaties tot kruisbestuiving kan leiden zijn op basis van de beschikbare gegevens niet mogelijk.

Stuifmeel in honing

Echte honing bevat altijd stuifmeelkorrels, die om diverse redenen in de honing terecht kunnen zijn gekomen: al in de bloem in de nectar, 'onbedoeld' tijdens de honingbereiding door de bijen zelf, of tijdens de honingoogst door de imker. Het meeste stuifmeel in de honing komt van de bijen zelf in de kast tijdens de honingbereiding.
De botanische samenstelling van de stuifmeelkorrels in de honing weerspiegelt tot op zekere hoogte de diversiteit van plantensoorten waarvan de nectar verzameld is. Om te worden gekwalificeerd als plantspecifieke honing dient er een bepaald minimum aandeel van het stuifmeel van die plant in de honing voor te komen. In 2008 is door bijen@wur in 200 honingmonsters de aanwezigheid van verschillende soorten stuifmeel geanalyseerd waarbij vooral gekeken is naar stuifmeel van Salicaceae, Fabaceae, Rosaceae, Brassicaceae, Solanaceae, Chenopodiaceae en maïs.
Maïs pollen werd in slechts twee honingmonsters aangetroffen (max. 1.5 – 4.2% van de korrels). Pollen van Rosaceae en Brassicaceae werd veel gevonden maar het was niet vast te stellen in welke mate dit pollen van appel, respectievelijk koolzaad, was. Pollen van de Solanaceae, waartoe ook aardappel behoort, werd ook in verschillende monsters gevonden maar ook hier is niet zeker of er aardappelstuifmeel bij zat. Stuifmeel van Chenopodiaceae werd vrijwel niet aangetroffen en de kans dat het suikerbietstuifmeel betrof is erg klein omdat deze plant nauwelijks bloeit en ook niet aantrekkelijk is voor bijen.

In vijf van de bovengenoemde monsters werden de aantallen stuifmeelkorrels door middel van de cytometrische telkamer zo nauwkeurig mogelijk geschat. Het aantal korrels per gram honing bleek te variëren van een paar honderd tot 28.000. Dit stemt redelijk overeen met de literatuur waarin een range van <2.000 tot 10.000 wordt beschreven. Er is meer onderzoek nodig om de monsterbereiding en telmethoden te verbeteren.

Om de stuifmeelhoeveelheden in honing niet alleen in aantal korrels te kunnen uitdrukken maar ook in een gewicht aan stuifmeel, werd het gewicht per stuifmeelkorrel vastgesteld voor 5 plantensoorten. Het gewicht per korrel loopt tussen deze vijf soorten sterk uiteen van 0.0043 µg to 0.067 µg.

Wanneer deze getallen worden gecombineerd met de aantallen korrels per gram honing, blijkt dat honing 43 tot 670 µg stuifmeel per gram kan bevatten. Voor plantspecifieke koolzaadhoning of fruithoning wordt geschat dat per gram ongeveer 60 µg koolzaad- respectievelijk appelstuifmeel aanwezig zal zijn.

Conclusies

Hieronder volgen de belangrijkste conclusies van het rapport, geclusterd per onderwerp.

Bijenteelt in Nederland

- In Nederland zijn zowel hobbymatige als professionele imkers actief. Van de ongeveer 8.000 imkers zijn er circa 6.900 aangesloten bij drie bijenhoudersverenigingen. De Nederlandse Bijenhoudersvereniging (NBV) is verreweg de grootste met 6.000 leden.
- Een hobbyimker heeft gemiddeld vijf bijenvolken, professionele imkers meer dan honderd.
- Er zijn ongeveer tien imkerbedrijven actief op de bestuivingmarkt en verdienen hier hun brood mee. Er wordt geschat dat circa 32.000 bijenvolken worden ingezet voor bestuiving in Nederland door ongeveer 1.700 imkers.
- Hobbyimkers verplaatsen zelden of nooit hun kasten, tenzij ze worden ingezet voor de bestuiving in de fruitteelt (boomgaarden). Professionele imkers verplaatsen hun kasten echter zeer regelmatig: gemiddeld elke vier weken.
- In Nederland worden bijen ingezet voor de bestuiving van boomgaarden, in de tuinbouwsector en in de zaadteelt. Professionele imkers nemen nagenoeg alle bestuiving onder glas (kassen) en in tunnels voor hun rekening. Niettemin wordt er regelmatig samengewerkt met hobbyimkers om de aanlevering van bijenvolken te garanderen. Bestuiving in de fruitteelt wordt traditioneel vooral verzorgd door de hobbyimkers.
- De jaarlijkse omzet van de bestuivingsgelden wordt grofweg geraamd op 4 miljoen euro voor buitenteelt en 7 miljoen euro voor teelt in kassen en tunnels. De indirecte economische waarde of de bijdrage van bestuiving door bijen (en andere bestuivers) wordt vele malen hoger geschat.
- Bijen worden niet enkel ingezet voor bestuiving. Verschillende producten die door bijen worden geproduceerd kunnen door de imker worden geoogst. Het meest herkenbare product is honing. Andere producten zijn bijenwas, propolis, Koninginnegelei, pollenkorrels en bijengif. Al deze bijenproducten kunnen sporen van pollen bevatten.
- De meeste honing op de Nederlandse markt (92%) wordt geïmporteerd uit onder andere China en Argentinië, de twee grootste honingproducerende landen in de wereld.

Foerageergedrag en pollentransport

- Honingbijen bezoeken bloemen voor stuifmeel (dat dient als bron voor eiwit, vet en mineralen) en nectar (als energiebron). Individuele bijen zoeken ofwel nectar ofwel stuifmeel en richten zich daarbij op één plantensoort gedurende de dag.

- Niet alleen foerageren bijen als individu zo efficiënt mogelijk maar ook het op kolonieniveau is het foerageergedrag geoptimaliseerd. Dit wordt bereikt door communicatie tussen de bijen en door een optimale taakverdeling.
- Slechts een klein deel van het verzamelde stuifmeel wordt gebruikt voor de bestuiving van bloemen. Meer dan 99% wordt door de bijen als voedsel gebruikt.
- De afstand die bijen kunnen overbruggen om water te halen is 2 km, voor stuifmeel 5 km en voor nectar 10 - 14km. Als er echter goede voedselbronnen nabij zijn blijven de meeste foerageervluchten van de bijen binnen een straal van 0,5-1km rondom de bijenkast. Als er echter weinig voedsel in de buurt is en er goede plekken op grotere afstanden aanwezig zijn, dan worden steeds vaker langeafstandsvluchten gemaakt.
- Binnen de bijenkast wordt onbedoeld stuifmeel tussen bijen uitgewisseld. Dit kan leiden tot pollentransport tussen verder afgelegen plaatsen die door verschillende bijen worden bezocht.
- Voor koolzaad is aangetoond dat bijen langere afstanden overbruggen door de aantrekkelijkheid van zulke velden. Wanneer kasten nabij bloeiende koolzaadvelden staan zullen de bijen daar vlak in de buurt foerageren.
- Appel is tijdens de bloei een erg aantrekkelijk gewas. Het is echter niet bekend hoever ze voor de boomgaarden zullen vliegen omdat bijenkasten meestal in of nabij de boomgaard worden geplaatst om de bestuiving te optimaliseren.
- Aardappel is geen aantrekkelijk gewas voor honingbijen, tenzij er veel luizen op voorkomen. Boeren laten echter zelden een dusdanige luizenbelasting van hun gewas toe dat het voor honingbijen aantrekkelijk wordt. Bovendien pieken de meeste luizensoorten voor de bloei van aardappel.
- Suikerbieten komen normaal gesproken niet tot bloei en zijn daarom niet attractief voor bijen.
- Hoewel mais een door via de wind bestoven gewas is, worden de bloeiende planten wel door bijen bezocht vanwege de rijkdom aan stuifmeel. Er werden in de literatuur echter geen harde data gevonden over de afstanden waarop maisvelden bijen aantrekken. Ook bij maispollentransport is de kans op langere afstand bestuiving klein omdat de vrouwelijke bloeiwijzen totaal niet aantrekkelijk zijn voor bijen.
- In verschillende diepgaande studies zijn vliegafstanden van bijen gemeten en via verschillende methoden geschat. Het is echter vrijwel onmogelijk om modellen te maken om de activiteits- en bestuivingspatronen precies te voorspellen in concrete situaties daar de complexiteit van het landschap en het aantal beïnvloedende factoren groot is.
- Het is belangrijk in gedachten te houden dat er naast honingbijen en hommels er nog veel andere insecten in staat zijn stuifmeel te transporteren en bij te dragen aan bestuiving. Mede daarom is er meer informatie nodig op landschapsschaal om het belang van stuifmeeltransport op het proces van uitkruising goed te kunnen inschatten.

Stuifmeel in honing

De aanwezigheid van stuifmeel in honing

- Honing bevat kleine hoeveelheden stuifmeel. Dit stuifmeel kan via drie wegen in de honing terecht zijn gekomen: directe verontreiniging van de nectar al in de bloem, verontreiniging veroorzaakt door de bijen in de kast, en verontreiniging door de imker bij het oogsten en verwerken van de honing. Het meeste stuifmeel komt in de honing terecht door de activiteit van de bijen in de kast met het bewerken en opslaan van de honing.
- De botanische herkomst van het stuifmeel in honing kan een aanwijzing geven over de nectarbron waarvan de honing afkomstig is. Toch is deze aanwijzing niet één op één, gedeeltelijk door de andere bovengenoemde routes waarmee stuifmeel in honing terecht komt. Doordat honingpakkers en handelaren verschillende soorten mengen wordt het soms nog moeilijker om iets van de oorsprong te zeggen.
- Ongeveer 200 honingen, afkomstig van een monitoring in 2008 over heel Nederland, bevatten stuifmeelkorrels van minstens 50 plantenfamilies. Dat waren zowel wind-bestuivende als insectenbestuivende plantensoorten.
- Stuifmeel van Rosaceae (inclusief appel) en Brassicaceae (inclusief koolzaad) kwamen heel frequent en veel voor. Chenopodiaceae (waartoe (suiker-) biet behoort) en Solanaceae (waartoe aardappel *Solanum tuberosum* behoort) and Poaceae (grassen familie, inclusief mais) werden ook in honing aangetroffen. Stuifmeel van mais werd slechts in 2 van de 200 monsters aangetroffen en in heel lage percentages.

Experimentele vaststelling van het stuifmeelgehalte van honing

- De aantallen stuifmeelkorrels in de vijf Nederlandse honingen van de monitoring in 2008 kwamen goed overeen met aantallen in de literatuur. Meestal maximaal 10.000 korrels per gram honing.
- De betrouwbaarheid van het tellen van stuifmeelkorrels hangt af van de verdunning van de suspensie die wordt gebruikt in de telkamers. De methode moet daarom nog verbeterd worden.
- Er werd een sterk en significant verband aangetoond tussen de hoeveelheid (grammen) stuifmeel die werd toegevoegd aan een kunstmatige honing, en het daarna via tellen teruggevonden aantal korrels in die honing. De sterk significante ijk-factoren werden gebruikt om de hoeveelheid stuifmeel in echte honing (µg/g) vast te stellen. De eerste resultaten geven aan dat er slecht heel kleine sporen stuifmeel in honing voorkomen, variërend van 43 µg tot 670 µg stuifmeel/g honing.
- Op grond van de ijklijnen werd uitgerekend dat een koolzaadhoning en een fruitbloesemhoning minstens 60 en 58 µg van het soortspecifieke stuifmeel (koolzaad en appel) bevatte per gram honing.

1 Introduction

1.1 Background

Honey bees are flying insects that are known for their role in gathering nectar to produce honey. In addition to this, honey bees pollinate agricultural crops, home gardens, orchards and wildlife habitat. As honey bees travel from flower to flower in search of nectar and pollen, they transfer pollen from plant to plant, thus fertilizing the plants and enabling them to bear fruit. Many crops rely on honey bees and other bees for pollination.

In light of this, honey bees could be a factor in spreading pollen grains stemming from genetically modified (GM) plants in field trials. This could possibly lead to cross-pollination of wild relatives, and pollen grains could end-up in the honey of these bees. Furthermore, if a newly produced protein in a GM plant has a toxic or allergenic effect, this activity could be expressed in the pollen as well. The extent to which the dissemination can occur and the distances achieved depends on many factors, including the type of crop, the distance over which honey bees fly and the time that pollen grains are viable. Knowledge of these factors may be important for (future) risk assessments of GM plants.

1.2 Objective of the report

This report presents an overview of relevant information concerning the relationship between honey bees and pollen, based on a literature survey, a database of pollen compositions of Dutch honeys and a concise laboratory experiment. More in particular, this encompasses:

- Information about the spread of pollen by honey bees, i.e. foraging distances and the specific crops that are visited, in connection with determining the possibility of pollen transport and potential cross-pollination with related (wild) plants in the surrounding environment.
- Information about the presence of pollen in honey, i.e. from which crops can pollen be found in honey and in what amounts and percentages, and which factors influence these numbers. In this regard, the focus is on honey produced in The Netherlands and on the following (GM) crops, relevant for The Netherlands: corn, potato, oilseed rape, sugar beet and apple.
- An experimental estimation on the pollen content of honeys (in µg pollen/g honey).
- Information about beekeepers and beekeeping in The Netherlands, both from a hobbyist and a professional (or commercial) point-of-view.

The above-mentioned information will be used in the context of both field trials and commercial cultivation. Accordingly, COGEM will draw conclusions and make recommendations to The Netherlands Ministry of Infrastructure and Environment.
Please note that the purpose of the research is not to map-out possible risks of GM crops for honey bees. Hence, the authors will not make recommendations regarding this subject.

1.3 Structure of the report

An introduction to honey bee biology is given in Chapter 2 of the report, illustrating amongst other its life stages. In Chapter 3, beekeeping in The Netherlands is elaborated by describing its history, the two kinds of beekeepers (i.e. hobbyists and professionals), and the most relevant beekeeping associations. In addition, the way in which honey bees are utilized for the pollination of crops is explained and a description is given about honey and other bee products in The Netherlands.

Chapter 4 presents aspects that play a role in foraging of honey bees and pollen transport. Chapter 5 portrays the outcomes of the laboratory experiment on pollen in honey. Finally, Chapter 6 presents some overall conclusions that can be drawn from the research.

2 Honey bees

2.1 Introduction

Honey bees are a subset of bees in the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax. Honey bees are the only extant members of the tribe Apini, which contains only one genus: *Apis* (see Picture 1). Currently, there are seven recognised species of honey bee with a total of 44 subspecies, though historically, anywhere from six to eleven species have been recognised.

Picture 1 – *Apis mellifera, one of seven recognised species of honey bee (Photo by Phil Bendle)*

Honey bees represent just a small fraction of the approximately 20,000-30,000 known species of bees. Several other bees produce and store some kind of honey, but only members of the genus *Apis* are true honey bees.

Two attributes of the honey bee that have been essential to its evolution and biology are its aggregation behaviour and, particularly in the case of the cavity-nesting species, its ability to cool the nest by evaporation of water collected outside. These features enable honey bee colonies to achieve a marked degree of temperature constancy within the nest irrespective of the external temperature. For this reason, the genus *Apis* was able to colonise a wide variety of environments, ranging from tropical to cool temperate (Milner 1996).

Another behavioural character of honey bees is the communication of information about food sources via the 'dance language'. The accurate dissemination of information concerning direction and distance of forage areas leads to efficient exploitation of food sources (Milner 1996).

2.1.1 The European honey bee

In The Netherlands, the European-, Western-, or Common honey bee (*Apis mellifera*[1] / *A.m.*) is a native species. The subspecies *Apis mellifera mellifera*, which exists in The Netherlands, is also known as the European dark bee. About 8,000 years ago, after the last ice age, this species spread over the whole of Europe from the Mediterranean (see Figure 1).

[1] The genus *Apis* is Latin for 'bee' and *mellifera* comes from Latin *melli* 'honey' and *ferre* 'to bear' – hence the scientific name means 'honey-bearing bee'.

It can be assumed that, in The Netherlands and large parts of Europe, the native dark bee does currently not exist as a pure subspecies in the wild anymore (Blacquière et al. 2009). This because beekeepers have brought in genetic material (i.e. queens) from subspecies *A.m. ligustica* and *A.m. carnica*. Nevertheless, most of the genes of the bees in The Netherlands still are those of an *A.m.mellifera* subspecies.

Figure 1 – *Distribution of the various subspecies of the European honey bee (Apis mellifera), such as Apis mellifera iberica (Spain and Portugal) and Apis mellifera mellifera (western and northern Europe)*

In addition, not many bee colonies actually live in the wild anymore, but it is difficult to say when the wild population of honey bees vanished. Presumably, the numbers gradually declined during the last century, through the reduction of wildlife habitats. This parallels the expansion and intensification of the agricultural sector, through which the availability of flowering plants in semi-natural and functional systems (e.g. meadows and fields) diminished, as well as the presence of nest cavities (hollow trees).

The accidental introduction of the *Varroa destructor* mite in the early 1980s gave the final blow to wild colonies of the honey bee in Europe. *Varroa* mites are external parasites and are the most important pest of honey bees around the world. The mites, which are about the size of a pinhead (approx. 1,5 x 1,1 mm, see Picture 2), use specialised mouthparts to attack developing bee larvae or adult bees, resulting in deformed bees, reduced lifespan and ultimately the destruction of the colony (DAFF 2011).

Picture 2 – *A Varroa mite on a bee pupa (Photo by Bram Cornelissen)*

From 1850 until now, the number of bee colonies in The Netherlands has steadily decreased from 200,000 to approximately 80,000 in summer and 40,000 in winter. These colonies, kept by beekeepers, essentially represent the continuation of the original population of wild honey bees (Blacquière et al. 2009).

2.1.2 Two sexes and two castes of honey bees

Two sexes (male (i.e. drones) and female) and two castes of female bees (queens and workers) make up the population of a beehive, each having its own characteristics, roles, and responsibilities within the hive. Upon closer examination, the three types of honey bees have a different appearance (see Picture 3).

Picture 3 – *Three types of honey bees: worker (l), drone (m) and mature queen (r) (Photo by Zach Huang)*

1. Queen

Within a hive, there is only one queen. It is a female bee with a fully developed reproductive system. The queen mates only once with several drones, and then remains fertile for life. A queen can live for 3-5 years and can lay up to 2,000 eggs per day. Fertilized eggs become female (workers) and unfertilized eggs become male (drones). When the queen dies or becomes unproductive, the other bees will initiate the development of a new queen. For queen bees, it takes 16 days from egg to emergence (BYBA 2011).

2. *Worker*

A worker is a female bee of which the reproductive organs are undeveloped, due to a specific diet during its development stage and through the activity of queen pheromone in the colony. The vast majority of honey bees are worker bees. Worker bees may live for 4-9 months during the winter season, but only 6 weeks during the demanding summer months. For worker bees, it takes 21 days from egg to emergence (BYBA 2011).

The worker bees sequentially take on a series of specific tasks during their lifetime, as depicted in Figure 2. The activities of young bees start in the centre of the brood nest with the cleaning of cells and tending the brood. Subsequently, the workers go to the outer edges of the nest in order to pack pollen and store nectar. Until after about three weeks, workers become foragers for another 10-20 days. Foragers take care of bringing from the environment everything that the colony needs in the hive: pollen, nectar, water and propolis (see also Section 2.2). Some activities can be executed lifelong (e.g. patrolling, resting, and ventilating the nest).

3. Drone

Drones are fertile male bees that are kept on standby during the summer for mating with a virgin queen. Because a drone has a barbed sex organ, which cannot be pulled out of the female genital opening, mating is followed by death of the drone. For drones, it takes 24 days from egg to emergence (BYBA 2011). Because drones are of no use in the winter, they are expelled from the hive in the autumn.

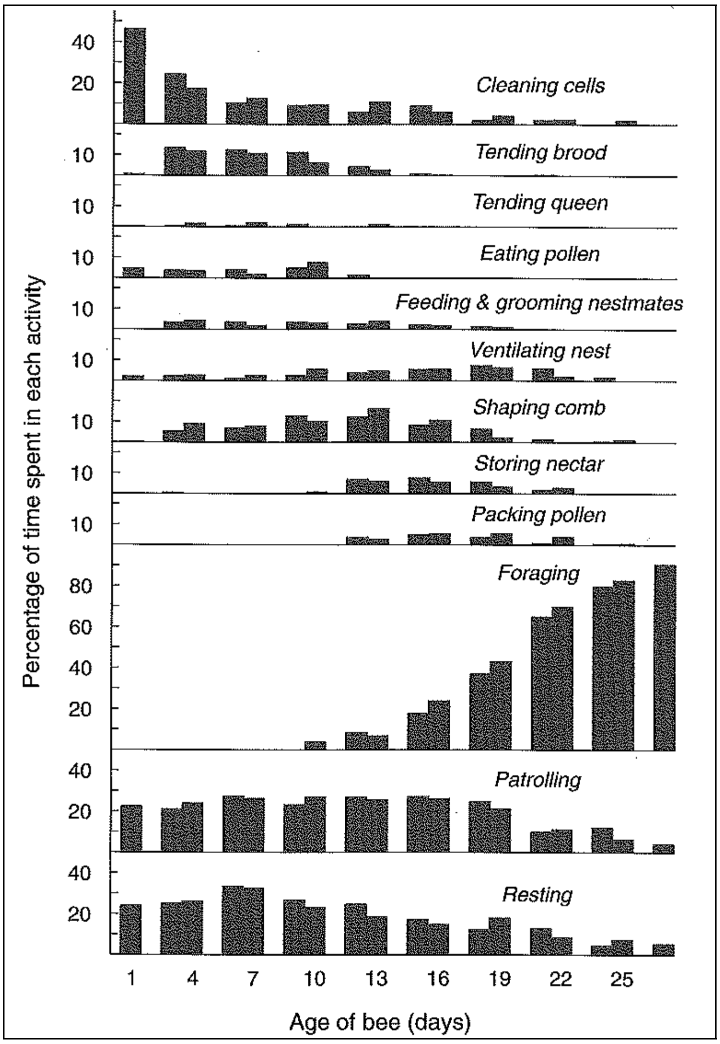


Figure 2 – *The changing tasks during the life of a worker honey bee (from Seeley 1995)*

2.1.3 Honey bee life stages

As with most advanced insects, honey bees exhibit a complete development or metamorphosis during their life: the young and the adults look very different. The life stages of a honey bee are egg, larva, pupa and adult (see Figure 3). Note that the cells are depicted vertically, but in reality, they are oriented horizontally. The first three stages are also referred to as brood. Development from egg to adult in general takes two to three weeks (Stone 2005).

Egg

The eggs are described as having an appearance similar to sausage-shaped poppy seeds. Each egg has a small opening at the broad end of the egg, the micropyle, which allows for passage of sperm. Hatching takes place three days after egg laying.

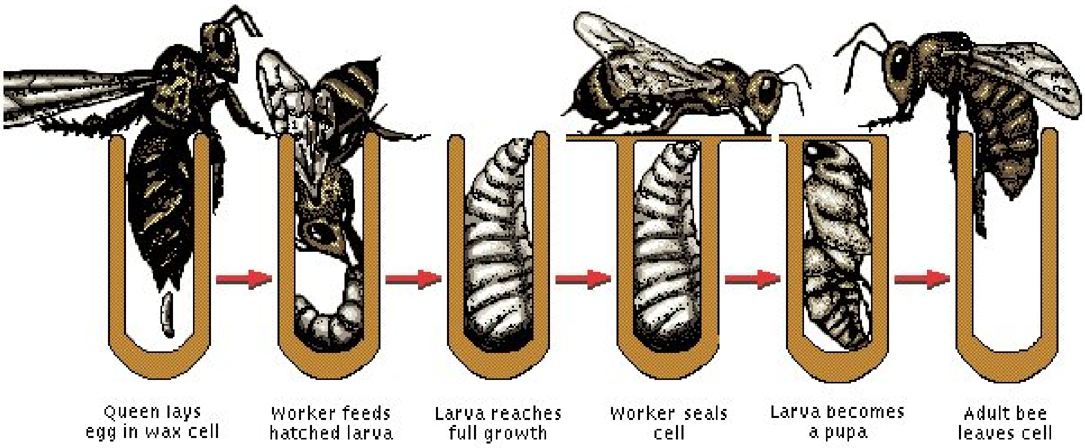


Figure 3 – *Honey bee life stages: from egg to larva, then to pupa and finally to an adult bee (Picture from getbuzzingaboutbees.com)*

Picture 4 – *Workers caring for larvae (Photo by Zach Huang)*

Larva

From hatching of the egg, the larval stage lasts six days. Upon hatching, the larva is almost microscopic, resembling a small, white, curved, segmented worm lacking legs and eyes. It lies coiled on the bottom of the cell. Larvae are fed royal jelly and later bee bread, i.e. nutritional granules of pollen with added honey or nectar prepared by the workers (see Picture 4). Each larva receives an estimated 10,000 feedings during this stage. Larval weight increases 5.5 times during the first day and approx. 1,500 times in 6 days.

The process of feeding and growing takes place while the cells are uncapped; the larvae spin their cocoons and change into pupae after workers have capped their cells (Winston 1987). Larval stage durations vary: 5.5 days for queens, 6 days for workers, and 6.5 days for drones. Regardless of whether the larva is male or female, it moults five times during its larval stage (Stone 2005).

Pupa

The pupal stage is a stage of massive reorganization of tissues: the adult tissues develop from the imaginal discs carried by the larva. Organs also undergo a complete transformation; while the body changes from the wormlike larval body shape to the adult body shape with three distinct body regions (see Picture 5). The pupal stage lasts about 8-9 days for workers and drones, and 4-5 days for queens. It is followed by the final moult to the adult stage (Winston 1987).

Picture 5 – *Worker and queen pupae; note the larger size of the queen pupa below (Photo by Zach Huang)*

Adult
As stated above, adult honey bees are either queens, workers or drones. The majority of honey bees that one sees outside of a hive are workers. A typical colony in mid summer consists of up to 20,000-30,000 workers, 500 to 1,000 drones, and one queen.

2.2 The forage of honey bees

The forage of honey bees consists of four main constituents, namely pollen, nectar, water and propolis:

– *Pollen*

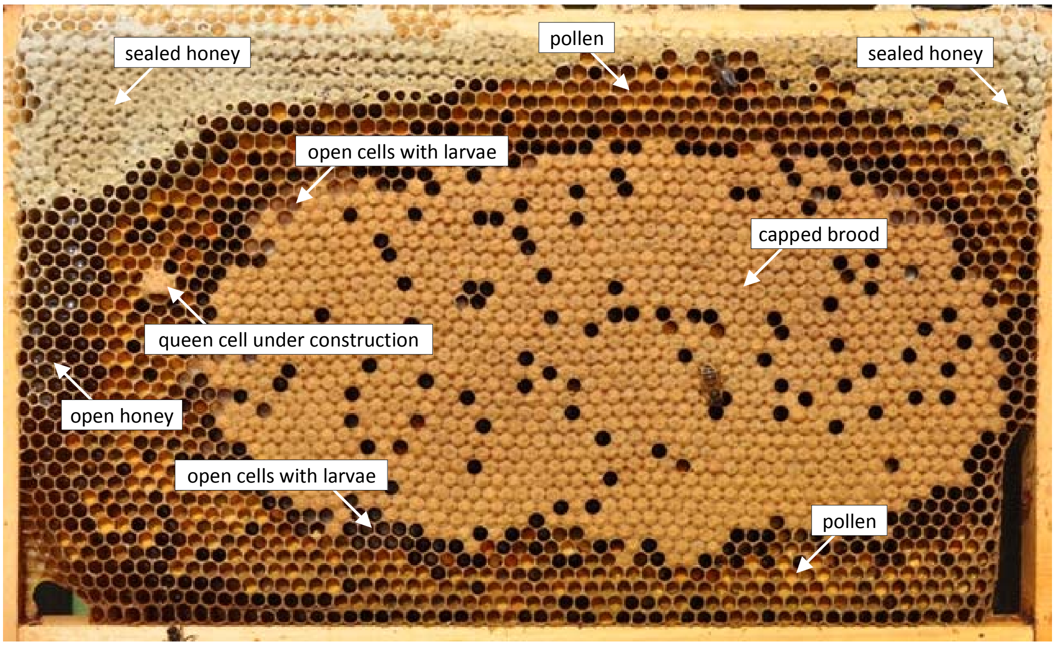
  Pollen is the plant protein source for the larvae and young bees, providing nitrogen, phosphorus, amino acids, and vitamins essential for development of the bees. Pollen is collected in the fur of the bee and stored for transport in pollen baskets (corbicula) on the workers' rear legs (tibia). As a rule, honey bees do not collect pollen and nectar on the same trip. For clarity, it needs to be stressed that although nectar foragers aim for nectar, they also do wipe the pollen grains that adhere to their body into the corbiculae, and deposit their pollen pellets in the hive, in the cells of the comb, around the developing brood (see Picture 6).

– *Nectar*

  Nectar, obtained from floral nectaries, sometimes deep inside flowers, or from extrafloral nectaries, provides an almost pure carbohydrate source for all honey bee life stages. Each worker fills its honey sac or crop of its digestive track by using its proboscis, increasing its weight by up to one half. The nectar is sucked from the plant and the worker will dilute it with saliva containing secretions from several glands such as the hypopharyngeal gland that add enzymes: invertase, diastase, and glucose oxidase (Stone 2005).

  Upon arrival at the hive, the worker regurgitates the contents of the honey sac to the younger workers within the hive. Usually the receiving worker will manipulate this small nectar load in its mouthparts. The worker unfolds and refolds its proboscis exposing the nectar to the air circulating within the hive. During this process, more glandular secretions are added by the worker.

  As the nectar is exposed to the air of the hive, it slowly thickens and it is then suspended from the upper surface of a cell wall. Here it will continue to lose moisture (dry out) and when the moisture content has been reduced to around 18.6%, the honey is ripe and the cell is capped by the workers. Pollen and honey are stored in the comb concentrically around the cells containing the brood (eggs, larvae and pupae), first the pollen and outwards the honey (see Picture 6). During a strong ‘honey flow’ (i.e. much nectar is available) honey is also stored further away from the brood nest. The beekeeper can utilize this storage behaviour by placing a special ‘honey super’ on top of the hive, of which the combs then become filled with honey only and no pollen or brood. The latter makes an easy and clean honey harvest possible, without any disruption of the brood nest of the colony (see also Section 3.5).



Picture 6 – *A brood comb of honey bees (Photo by Bram Cornelissen)*

Besides genuine nectar, honey bees also collect the exudates that aphids (or plant lice) and other scale insects leave on plants, called honeydew. These insects exploit the phloem of the plants to obtain sugars and amino acids as their protein source, but excrete the sugar surplus.

– *Water*

  Honey bees collect only little amounts of water, because their muscles produce much water when burning sugars, and the nectar collected contains a lot of moisture too. However, the need for water rises in hot weather: water is then collected and spread inside the brood comb, to cool the brood. Honey bee brood needs a constant temperature of around 35˚C.

– *Propolis*

  Another substance brought into the hive is propolis or 'bee glue'. This is a plant resin used to cover the hive's walls and combs, in order to close cracks and holes. It has anti-bacterial and anti-fungal properties (see also Section 3.5).

As stated before, foraging for the mentioned resources is aimed at fulfilling the demand of the whole colony, consisting of about 30,000 workers and an almost equal number of larvae and pupae. It is obvious that foraging for so many cannot be effective if every forager just starts at the entrance of the nest foraging on its own. Therefore, the foraging is to some extent organized. Through trophallaxis (i.e. the exchange of food) and the communication by the bee dance (waggle dance and round dance), there exists awareness of needs and resources among the workers in a colony. Again, within the task 'forager', bees specialize, although this specialization is not strict nor forever. About one third of the worker bees of a colony is active as forager.

10,000 Foragers: scouts, nectar foragers, pollen foragers, water collectors

Scouts

During morning time, a small number (a few hundred) of experienced forager bees fly out every day, and survey the wide area around the colony, in search for good resources. These bees are scouts, which bring to the colony what they found and start advertising this through the dance language. Recruits that observe the dances learn about the resource(s): its quality, its distance and its precise direction. Only those resources advertised that are really needed, will result in a recruit following up and going to forage. For example, if a scout has found water, but there is no need for water, no forager will follow up its dance. Many feedback signals in the colony guarantee that potential foragers are aware of the actual needs of the colony. In case the scouts were not able to find any proper resources at all, no foragers will depart. In this way, the colony avoids wasting energy with ineffective foraging.

Pollen foragers

If there is a need for pollen, a recruit will be triggered by a dance in which a scout advertised a pollen resource. Subsequently, it will leave the hive to find that specific resource. It will collect pollen and learn how to handle that specific flower species most effectively. It will specialize on that specific flower species. During handling of the flower and its anthers, the forager will collect the pollen grains on its body and wipe these to its corbicula. Pollen that has been collected in the corbiculae is a food source for the colony, but has no value for the plant anymore. Only pollen grains that are scattered on the body and in the fur of the bee can be deposited on a stigma of another plant and result in fertilization.

Upon return to the hive, the pollen forager will also dance while showing its harvest, to recruit more foragers for its successful resource. Of course, many other returning foragers also dance for their resource, and since the dance includes information on the profitability of a resource, new recruits can choose for the most profitable target. For instance, a flower patch near the hive will be more strongly advertised than a remote patch. Pollen foragers become very much devoted to their flower species, and show strong 'flower constancy' (Grüter et al. 2011). Pollen is generally harvested up to a range of 6 kilometres. The annual need for pollen of an average ('ten-frame') colony has been measured at 13-18 kg (Brodschneider and Crailsheim 2010), while a colony may collect a total of 10-26 kg per year.

Nectar foragers

Foraging for nectar is one of the toughest tasks in the colony, which takes a lot of energy from the forager (see Rortais et al. 2005). The annual need for nectar by a colony is estimated as 125 kg in a temperate climate (Seeley 1995). Of course, foraging for nectar is only viable if the gain in energy from the harvested nectar is greater than the energy consumption of the trip. Depending on the sugar concentration, the amount of nectar per flower and the abundance of the specific flowering plant, bees can fly up to more than 14 km for nectar (Beekman and Ratnieks 2000). Although nectar foragers solely aim at collecting nectar, contact with the anthers and/or stigmata of a plant is not or cannot be avoided. Therefore, nectar foragers contribute to pollen exchange between plants as well.

Generally, pollen foragers are more important for pollination than nectar foragers are (Free 1972), but in some plant species, such as alfalfa, sunflower, plum, peach, apricot, and sweet cherry, nectar collectors are very important pollinators too (Jay 1986). In dioecious plants like willows, only nectar foragers will visit both male and female plants and therefore are important for pollination. This would also be the case in monoecious plants with separate male and female flowers, like courgette.

Although in apple the pollen foragers are more efficient pollinators than the nectar foragers (Dag et al. 2005), the latter do contribute significantly to pollination, since apple flowers produce a high sugar nectar (>45%; Stern et al. 2001). Nevertheless, it was shown that pollination of apple trees (cultivar 'Delicious') by honey bees was improved if the colonies were placed in the orchard sequentially instead of simultaneously. Namely, inexperienced foragers had not yet learned how to forage optimally for nectar: only after some time, bees learn to get around the anthers. This 'side working' helps them to forage more efficiently (see Picture 7), but it reduces the changes on pollination (Stern et al. 2001).

Picture 7 – A *honey bee 'side working' on an apple flower (Photo from Stern et al. 2001)*

Water collectors

In temperate regions, a honey bee colony collects around 25 kg of water on an annual basis (Seeley 1995). Because the water collected is carried in the honey stomach, a water forager cannot take a lot of nectar on its trip (i.e. this has to be stored in the honey stomach too). Therefore, water collectors only fly short distances of up to 3 kilometres. When the situation arises that, for several days, the weather is not suitable for foraging, the need for water in the hive increases. Namely, water is then necessary to dilute the feed for the larvae. In addition, as mentioned earlier, in hot weather the need for water raises as well: water is then utilized to cool the brood nest (Nicolson 2009).

2.3 Pollination by honey bees

Pollination is the transfer of pollen grains, the male sex cells of a flower, from the anther where they are produced to the receptive surface, or stigma, of the female organ of a flower. Fertilisation occurs when the pollen grains on the stigma germinate and grow down the stem (style) of the stigma to fertilize the ovule. Honey bees are among the insects that transfer pollen between flowers and between plants, and are often regarded as being the most important pollinators. Hence, the word 'pollination' is habitually used to describe the service of providing honey bees to pollinate crop plants.

Honey bees are suitable for pollination for several reasons. Their hairy bodies easily trap pollen and carry it between flowers (see Picture 8). Since honey bees require large quantities of nectar and pollen to raise their young, they visit flowers regularly in large numbers to obtain these foods. In doing so, individual honey bees show flower constancy, which also contributes to the pollination efficiency.

The body size of honey bees enables them to pollinate flowers of many different shapes and sizes. Then again, sometimes no pollination occurs despite nectar or pollen removal, for instance because stigmas are not touched. The pollination potential of honey bees is also high because they can be managed to develop large populations. The number and sizes of colonies can further be increased by beekeepers according to the needs, and the colonies can be moved to the most desirable location for pollination purposes (Jaycox 1985).

Picture 8 – A *honey bee collecting pollen of a Dactylis glomerata, a wind pollinated species (Photo by Bram Cornelissen)*

Not all crops need pollination by insect pollinators such as honey bees though: some can produce fruit without fertilisation of the flower. In addition, some flowers are self-pollinated, which means that pollen is transferred from the anther to the stigma of the same flower or to other flowers of the same individual. Although this transfer can be achieved by wind or rain, insect pollinators are the most effective in transferring pollen.

2.4 The fate of pollen as managed by honey bees

Honey bees and plants have conflicting interests during pollination: honey bees aim at the highest possible reward per flower, while the plant aims at the highest possible number of successful transportations of pollen to other flowers and/or plants, against the lowest possible costs of energy (nectar) and nutrients (lost pollen grains)). The evolved compromise is acceptable to both parties.

However, conflicting interests may still be present, as was illustrated by the learning behaviour of foragers on apple (Stern et al. 2001) – although in this case of a men bred cultivar, no natural selection is going on. On the other hand, the mentioned flower constancy (Grüter et al. 2011) benefits the bees (more efficient handling of the flowers through specialization) as well as the plant (no pollen wasted on foreign stigmata).

When visiting a flower for pollen, bees actively use their legs, hairs and mandibles to manipulate the anthers in order to catch the pollen on their bodies, and to immediately transport it into their corbiculae. Despite their efficiency in cleaning the fur from pollen grains, many grains are found on the bees' bodies during foraging, and the numbers were the highest on bees that also carried large pollen loads in the corbiculae (Free 1993). Typical figures amount to 1,000 to 10,000 grains per bee. If a bee carries pollen pellets of 25 mg together, which would correspond with 375,000 to 16.8 million pollen grains (see Chapter 5), the fraction found on the bees' body might be 0.06 to 27 pro mille of the total pollen load of a bee.

Pollen in the pellets in the corbiculae may remain viable for several hours (Free 1993), but are lost for pollination. Pollen on the bee body is also able to germinate after several hours (Free, 1993), but more probably may be deposited to a nearby flower of the same plant than later on a flower of another plant. Pollen longevity may vary considerably between plant species. For instance, it was shown that after 3 hours, maize pollen was still viable and resulted in kernel formation. The same was found for *Raphanus sativus* after 5 days, and pollen grains of *Brassica campestris* still germinated for 50% after 6 days (Dafni and Firmage 2000). In these cases, it is not clear if and how much longer the pollen grains would have remained viable.

Powder box

Honey bees that left the hive were found to carry many pollen grains of different plant species on their bodies (Free and Williams 1972). Depending on the pollen sources, between 4,000 and 13,000 grains were already present at the start of the foraging trip: 80-90% of these were of the dominant crop visited. Young bees, as well as drones, also carried equal numbers of pollen grains on their bodies. Upon placing colonies inside a greenhouse, Paalhaar et al. (2008) were able to show that in-hive (young) bees rapidly carried pollen grains of the specific greenhouse crops on their bodies, but only < 1% of the load of foraging bees.

Using a two entrances hive (with one opening inside a cage with a few mango trees, the other (main) entrance outside the cage to the mango orchard), Dag et al. (2001) showed that the trees inside the cage carried 12% fruits fertilized by pollen from outside sources. These pollen grains must have been exchanged between bees inside the hive, since different bees foraged inside and outside the cage.

So the air inside the hive appears to contain pollen from all sources visited by the bees. Due to this 'powder box effect', individual bees may carry significant amounts of pollen from plants not actually visited by them. This possibility of pollination through exchange in the 'powder box' may especially be significant for self-incompatible and dioecious trees.

Although the number of pollen grains exchanged inside the hive may not be very high compared to the number directly deposited on foraging bees, it may be relevant for pollen transport between plants in different spots that are not in direct flight connection of individual bees. This pathway with connection flights might indicate that the maximum pollen flow distance actually can be twice the maximum flight distance of the honey bees. Because the quantitative importance of this effect is not clear from the literature, further research is needed to quantify the possible role of the 'powder box effect'.[2]

Pollen ending up in honey

Already inside the flower, some pollen grains may fall in the nectaries and subsequently mix with the nectar. In hanging flowers, this will be less compared to upright cup-shaped flowers. In both cases, most of the fallen pollen grains will be lost for pollination. Due to the behaviour of the honey bees, more pollen grains can be brought into the nectar during harvesting. Finally, only a very small fraction of the pollen grains in nectar will end up in the honey produced (see Chapter 5). Naturally, all of these grains will be lost for pollination.

Honey flows

In certain periods, when very rich or strong 'honey flows' are available, honey bees can easily harvest much honey and pollen. This boosts the development of the colony. Such honey flows allow the beekeeper to harvest honey by means of the earlier mentioned 'honey super' (Section 2.2). In between honey flows, the bees forage on several plant species that just deliver enough resources to cover the preservation of the colony, or they rely on stored honey from earlier honey flows.

Most honey flows occur during spring and some during summer and the beginning of autumn. The opportunistic nature of honey bees results in different limits for a source (e.g. nectar or pollen) in order to be attractive for the colony. Figure 4 shows the threshold sugar concentrations for which honey bees were dancing from May through July (research of Lindauer (1949), from Michener (1974)). While in July, 0.2 M sucrose (about 7% w/v) of the resource was sufficient to induce dancing by a forager bee, it was far from sufficient in May, when the tenfold concentration was needed (almost 70% w/v).

[2] Whether or not of proven significance, professional beekeepers supplying pollination services to seed producing companies are sometimes requested to take a waiting period (i.e. keeping the beehive closed) of 24 hours before utilizing the hive for pollinating the companies' seed crops (J. Calis, personal communication).

Note that in the Figure below, several spring honey flow crops are named: red clover (*Trifolium pratensis*), snowberry (*Symphoricarpos spp.*), rape (oilseed rape or rape seed, *Brassica napus* or *Brassica rapa*), charlock (*Sinapis arvensis*), and summer and winter lime (*Tilia spp*.). A similar list of honey flows can be made for The Netherlands, starting in spring with willows (*Salix spp.*), and dandelion (*Taraxacum officinalis)* in April, fruit trees (cherry, plums, apple), horse chestnut (*Aesculus hippocastanum*) and oilseed rape (*Brassica napus*) in May, raspberry and blackberry (*Rubus spp*.) and lime trees (*Tilia ssp*.) in June, and finally heather (*Calluna vulgaris*) in August. In some places in The Netherlands, there may be also strong honey flows of white clover (*Trifolium repens*) and sea aster (*Aster tripolium*) during summer, black locust (*Robinia pseudoaccacia*) in June, and the neophyte Himalayan balsam (*Impatiens glandulifera*) in August. During a strong honey flow, honey bee foragers may be less prone to visit other flowering plant species.

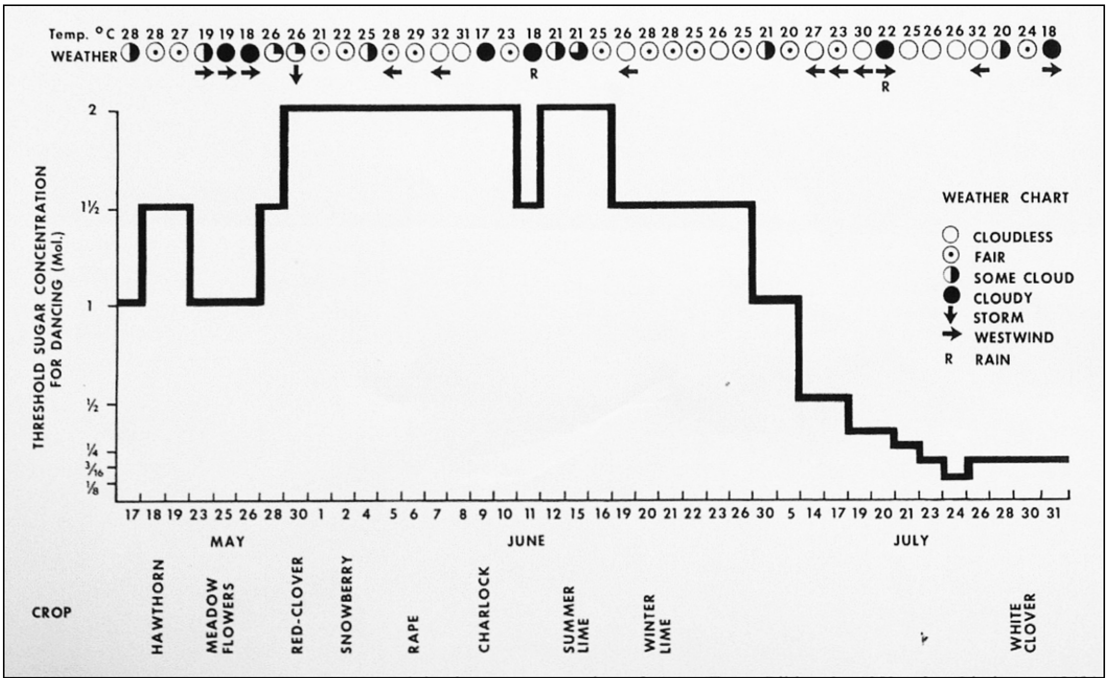


Figure 4 – *Seasonal variation in the minimal concentration of sugar needed to stimulate dancing (Lindauer (1949) from Michener (1974)). 1 Molar sugar is about 34% w/v sugar concentration (Mol weight sucrose is 342)*

3 Beekeeping in The Netherlands

3.1 History in brief

Beekeeping is one of the oldest known forms of food production. Some of the earliest known evidences of beekeeping is from a rock painting, dating to around 13,000 BC. Beekeeping was particularly well developed in Egypt and was discussed by the Roman writers Virgil, Gaius Julius Hyginus, Varro and Columella (Romanov 2005). Traditionally beekeeping was done for the bees' honey harvest, although nowadays crop pollination services can often provide a greater part of a beekeeper's income. For the most part, beekeeping is a hobby activity in The Netherlands, carried out by roughly 8,000 beekeepers (Blacquière et al. 2009).

3.2 Dutch beekeepers: hobbyists and professionals

Hobbyists

The majority of Dutch beekeepers are non-commercial hobbyists that aim to harvest some honey each year and possibly a few other products, mostly for family and friends, and at times for selling these products to shops or at markets. Motives for keeping honey bees are broader though, namely devotion to nature, spending time outdoors, passion for the old craft, and fellowship within an association (Blacquière et al. 2009). Non-commercial beekeepers regularly have only a few bee colonies (average of five), but they live all over the country and hence provide a 'nationwide coverage' of natural green areas with their colonies, which is an important factor in the role of the honey bee as pollinator. Overall, hobbyist beekeepers seldom move their hives, except the ones that provide pollination services in the fruit sector (see also Section 3.4).

Lately, Dutch society in general shows some more interest in bees and beekeeping (see also Box 1). For example, the largest beekeeping association in The Netherlands (NBV, see below) has welcomed many new members in the past few years and their 'beginner-courses' have never been visited so well. However, on the long run, the number of active beekeepers in The Netherlands is expected to further decrease. This negative trend could, amongst other reasons, be caused by the fact that people are increasingly occupied in modern society and, on the whole, make available less and less time for hobby activities.

Professionals

Professional or commercial beekeepers active in The Netherlands are mainly involved in pollination activities in the agricultural sector. These beekeepers regularly move their hives (every 4 weeks on average), and their main working areas are indoors (greenhouses and polytunnels). Commercial beekeeping only for the production of honey or other bee products is virtually non-existent in The Netherlands, although some professional beekeepers do sell their harvested honey. More on commercial beekeeping in Section 3.4.

Box 1 – *A new development: urban beekeeping*

During the past decade – within the movement for a small scale, traditional-artisanal and biological approach to beekeeping – the trend 'urban beekeeping' has developed in cities like Paris, Berlin, London, Tokyo and New York, but also in cities in The Netherlands, where more and more people are taking up beekeeping. Bees are being kept everywhere from small suburban backyards to high-rise rooftops and balconies.

(Photo by David Gleason)

Besides the fact that the bees pollinate the cities' green areas, urban beekeeping has the additional positive effect that this natural form of honey production is brought closer to the consumer again: people are able to experience a beekeeper at work in their own neighbourhood.

3.3 Dutch beekeeping associations

Of the approximately 8,000 beekeepers in The Netherlands, about 6,900 are organised in the following associations:

- *Nederlandse Bijenhoudersvereniging (NBV)*
  Dutch Beekeepers Association — 6,000 members
  www.bijenhouders.nl
- *Algemene Nederlandse Imkersvereniging (ANI)*
  General Dutch Association of Beekeepers — 500 members
  www.anibijen.nl
- *Imkersbond ABTB*
  Beekeepers Association ABTB — 400 members
  www.imkersbondabtb.nl

Below, a general outline of each association is given. More information can be found on the respective websites of the associations.

– The *'Nederlandse Bijenhoudersvereniging' (NBV)* was established on 1 January 2006, as a result of a merger of several smaller associations. The objective of the association is to endorse beekeeping in its broadest sense. The association is committed to the promotion of beekeeping, to increase the availability of honey plants for honey bees and other pollinating insects, and to provide support to both hobbyist and professional beekeepers. Furthermore, the NBV represents Dutch beekeepers towards the national government and it maintains contacts with organizations of beekeepers in neighbouring countries. The NBV also represents The Netherlands at *Apimondia*, the International Federation of Beekeepers' Associations.

  The NBV has a national coverage, whilst the two other associations tend to have a regional focus, on the Veluwe and Betuwe areas respectively. Since 2007, the NBV publishes its own monthly magazine *'Bijenhouden'* ('Beekeeping').

– The *'Algemene Nederlandse Imkersvereniging' (ANI)* is an organization dedicated to beekeeping in all its aspects, and promotes and defends the interests of beekeepers. The association was founded in 1934 and has branches in several places.

– The *'Imkersbond ABTB'* was founded in 1947 with the aim to represent the interests of its members – both commercial and non-commercial – in the field of beekeeping. Within the association, a commission on pollination is active, which aims to promote understanding and knowledge of pollination amongst beekeepers and growers, it offers guidance on pollination matters, and it provides bee colonies – via beekeepers – to growers for pollination services.

  The *'Algemene Nederlandse Imkersvereniging'* and the *'Imkersbond ABTB'* publish the magazine *'Mijn bijen'* ('My bees') (7 times per year).

3.4 Utilization of honey bees for pollination of crops

In The Netherlands, honey bees are being utilized in fruit orchards, in the horticulture industry and for seed production. Pollination in closed greenhouses and polytunnels (i.e. vegetables and seed production[3]) is mainly covered by professional beekeepers. However, much collaboration takes place between professional and hobbyist beekeepers, in order to guarantee the supply of bee colonies in the seed production sector (Calis and Boot 2009).

[3] For (hybrid) seed production, honey bees are utilized to maintain parent plants of certain crop varieties and to produce hybrid seeds. Nowadays, this predominantly happens in greenhouses or polytunnels, due to the unreliable climate in The Netherlands.

Traditionally, pollination in the fruit sector is mostly covered by non-commercial, hobbyist beekeepers. This latter group receives a fee of 40-60 Euro per colony (20-40 thousand honey bees), per 3 weeks or less (ABTB 2011a).

It is estimated that around 32,000 honey bee colonies are utilized for pollination in The Netherlands by approximately 1,700 beekeepers (NCB 2011). The annual value of pollination fees is roughly estimated at 4 million Euro for outdoor cultivation, and 7 million Euro for indoor cultivation (Blacquière et al. 2009).

In The Netherlands, around ten beekeeping companies are professionally involved in the pollination of crops with honey bees and make a living out of it. These commercially operating beekeepers, mostly part of the seed production companies themselves, often have more than one hundred colonies. In order to provide an insight in professional Dutch beekeeping companies, the main features of one of these companies are summarized in Box 2.

The provision of honey bees for pollination of crop plants is a specialized practice, not just a sideline of honey production, and in many ways, the work of such beekeepers requires a different approach towards the handling of bee colonies. For example, the decline of colonies that are used for pollination needs to be compensated for beforehand, by continuously creating new (pollination) colonies for the upcoming season. As a result, the colonies are usually relatively young and in their growing, or maturing phase. This is one aspect in keeping bees healthy and in good condition, which is essential in order to be able to provide a large ‘force’ of worker bees needed to do the job of transferring pollen (Jaycox 1985), and for the bees to be able to cover greater distances if needed.

Due to the further professionalization in agriculture and horticulture, hobbyist beekeepers appear to be less and less interested in providing their colonies for pollination (Blacquière et al. 2009). This provides extra opportunities for commercial beekeepers to fill that gap and it may well lead to growth in that sector.

Box 2 – *Main features a Dutch beekeeping company*

- Full-time business, two staff
- Approximately 1,000 colonies
- On average 50 to 150 colonies deployed at a time
- Typically, colonies are utilized 2-3 times per year for a period of approx. 4 weeks
- Composition of colonies varies greatly, because colonies are combined and split up regularly
- Apiaries (bee yards) on approx. 15 different locations in the central- and western part of The Netherlands
- The whole of The Netherlands can be serviced, as well as some parts of Germany

Bees are utilized for:

1. *Seed production* (predominantly)
   - in greenhouses and polytunnels
   - cabbage- (red, white, Brussels sprout), carrot-, union-, leek-, rocket-seeds
   - flower-seeds
   - seeds are highly valuable, so high ‘bee density’: easily 20 colonies per hectare
   - between April and the end of July
2. *Cultivation of vegetables*
   - in greenhouses
   - courgette, egg plant, bell pepper
   - number of colonies varies
   - year round
3. *Cultivation of fruits*
   - in orchards
   - pear, cherry, apple, blueberry, raspberry
   - approximately three colonies per hectare, but varies greatly
   - during flowering season

For a selection of crop plants, the following table (Table 1) indicates the minimum and optimal number of honey bee colonies, required in the field, per hectare. The number of colonies needed per crop varies, due to several variables, such as the number of flowers of a plant (some plants have many flowers, others have not) and the number of actual bee visits necessary for fertilization (some plants need more visits than others due to the plant’s specific morphology).

Table 1 – *The minimum and optimal number of honey bee colonies required per crop, per hectare, in the field* (ABTB 2011b)

Crop	***Number of colonies required***	
	minimum	***optimal***
Apple, pear	2	3
Cherry, sour cherry	4	6
Peach, plum, mirabelle plum	5	5-10
White currant, red currant, gooseberry	4	6-8
Blueberry	5	5-15
Strawberry	2	2-4
Blackberry, raspberry	4	4-6
Pickles, courgette	2	
Blue poppy, caraway, rapeseed, radish, mustard seed, field bean, evening-primrose	3	4-10
Ornamental plants, such as berry shrubs	3	4

Within greenhouses or polytunnels, the number of required colonies and the size of the colonies depend on the crop and on the size of the compartment in which they are placed (Calis and Boot 2009). The following guideline (Table 2) provides an insight in the number of colonies needed indoors for a selection of crops. It should be noted that honey bees generally have the opportunity to fly outside the greenhouse or polytunnel in which they are located.

Table 2 – *Average number of square metres per indoor crop that can be covered by one honey bee colony* (PPO 2004)

Crop	***One colony per***
Egg plant, bell pepper	5,000 m^2
Melon, pickle, courgette	1,500 m^2
Strawberry, raspberry, blackberry	1,000 m^2

3.5 Honey and other bee products

Honey

Honey is the most recognizable product made by honey bees. It is produced from nectar and in the course of this production process – due to the many interactions between bees within the hive – trace amounts of pollen end up in honey. In addition, airborne pollen, not specifically collected by bees, can also end up in honey.

Honey is used as an energy source for adult bees and brood. In cold weather, or when fresh food sources are scarce, bees use stored honey as their source of energy. Beekeepers can harvest this honey from the honeycomb, by means of a honey extractor.

A honey extractor is a mechanical device that extracts the honey from the honeycomb without destroying the comb. These extractors work by centrifugal force: a drum or container holds a frame basket that spins, flinging the honey out. With this method, the wax comb stays intact within the frame and the bee bread stays put: the bees can reuse it. An alternative method is to put a honeycomb into a honey press to squeeze out the honey. This method is seldom applied in commercial honey production, because the comb cannot be used again.

In The Netherlands, the production of honey is not a relevant market sector anymore. Nowadays, honey is predominantly harvested by hobbyist beekeepers and sold on a small scale as local produce in organic food stores, rural farm shops and on local fruit markets. In 2009, the estimated total 'home-made' honey production in The Netherlands was 1,422 tons (NCB 2011). This accounts for approximately 8% of the honey on the Dutch market. The remaining 92% is imported from amongst others China and Argentina; the two largest honey-producing countries in the world (see Table 3).

Table 3 – *Top 10 honey-producing countries in the world (2009)* (FAOSTAT 2009)

Country	***Production in tonnes***
1. China	407,367
2. Argentina	83,121
3. Turkey	82,003
4. Ukraine	74,000
5. USA	65,366
6. Mexico	56,071
7. Russia	53,598
8. India	43,865
9. Ethiopia	40,688
10. Brazil	38,765

Note: China alone produces more honey than the rest of the top-6 combined.

Beeswax

Besides honey, beeswax is won as well. Honey bees use beeswax to build honeycomb cells in which their young are raised and honey and pollen are stored. Hence, trace amounts of pollen can be found in beeswax. When beekeepers extract the honey, they cut off the wax caps from each honeycomb cell. Its colour varies from nearly white to brownish, but most often a shade of yellow, depending on purity and the type of flowers visited by the bees.

Wax from the brood comb of the beehive tends to be darker than wax from the honeycomb, since impurities accumulate more quickly in the brood comb. Due to these impurities, beeswax has to be rendered down before further use. The leftovers are called ‘slumgum’.

The extracted beeswax can be sold or for a small fee supplied to companies that reuse the wax to make honeycomb foundations. Purified and bleached beeswax is used in the production of food, cosmetics, pharmaceuticals, candles and wood polish.

Propolis

Propolis is a polyphenol-rich resinous substance honey bees collect from tree buds (especially poplars), sap flows, or other botanical sources. It is used as a sealant to cover unwanted open spaces, cells of the comb and the inner walls of the nest. Furthermore, propolis has an antibacterial and antifungal function within the beehive. Its colour varies depending on its botanical source, the most common being dark brown.

Propolis is sticky at and above 20° Celsius; at lower temperatures it becomes hard and very brittle. Typical northern temperate propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%), waxes (30%), essential oils (10%), and pollen (5%). Propolis can be harvested and processed into tinctures and ointments, although this not often happens in The Netherlands.

Royal jelly

Royal jelly is used by honey bees in the nutrition of their larvae, as well as adult queens. Royal jelly is secreted from the hypopharyngeal glands of worker bees, and is fed to all bee larvae in the colony, whether they are destined to become drones (males), workers (sterile females) or queens (fertile females). Between queen cells and worker cells the balance between sugar and protein components differ, as well as some specific determining components that have been identified recently (Kamakura 2011).

Royal jelly could contain trace amounts of pollen and is sometimes harvested (from the queen cells only) and added to foods (e.g. honey), because of its alleged positive effects to health. Royal jelly can also be found in some beauty products.

Bee pollen

Bee pollen is plant pollen carried by bees to the hive where it is gathered by placing brushes at the hive's entrance knocking the pollen off the bees as they enter. Bee pollen is used in naturopathic medicine traditions and as a nutritional supplement, although exposure may trigger allergic or anaphylactic reactions in sensitive people. Bee pollen available in The Netherlands is mainly imported from Spain, but also from South America.

Bee venom

Apitoxin, or honey bee venom, is a bitter colourless liquid. The active portion of the venom is a complex mixture of proteins, which causes local inflammation and acts as an anticoagulant. The venom is produced in the abdomen of worker bees from a mixture of acidic and basic secretions. It could contain trace amounts of pollen. Bee venom therapy is used by some as a treatment for rheumatism and joint diseases, due to its anti-coagulant and anti-inflammatory properties.

Bee venom is also used to desensitize people allergic to insect stings and can be delivered in the form of bee venom balm as well, although this may be less potent than using live bee stings.

4 Foraging and pollen transport

4.1 Introduction

This Chapter presents research data on foraging ranges and -distances of honey bees. This information can be used to estimate the minimal distance that should be maintained between beehives and crops when contamination of honey with pollen from these crops or cross-pollination should be avoided. It should be stressed that flight distances or flight activity patterns in general is only one aspect in pollen transport and pollination phenomena.

The distance over which pollen are transported depends not only of the potential flying capacity of honey bees, but it is dependent on the complex foraging behaviour of bees and the fate of pollen related to the activity patterns. The foraging behaviour depends on a) the time of the year, b) the condition of the colony, c) the distribution of food patches in the landscape surrounding the beehive, and d) on the variation in activity between individual bees. The quantification of pollen transport in terms of amounts in space and time is therefore much more complex than only measuring flying distances. The local configuration of crops, the natural environment, the position of the beehive and the nutritional state of the colony may trigger the behaviour and communication patterns among individual behaviour of bees from the colony. Pollen carry-over from bees to and from flowers and among bees themselves is an additional factor in pollen flows.

It is known that honey bees and other social insects strongly benefit from the communication between individuals to locate favourable food sources. By the so-called ‘waggle dance’, which is performed inside the beehive, direction and distance of nectar and pollen sources is effectively communicated. The recruitment of part of the honey bees to explore new sources further away from the nests allows them to collect food at considerable distances. The waggle dance behaviour is in particular effective to optimize the colony’s ability to exploit the most favourable foraging patches in the environment (Beekman and Lew 2007).

The ability to detect and exploit pollen and nectar sources make bees very effective as pollinators and collectors of nectar and pollen, as they can cover large areas from beehives placed at a single spot. However, this ability also allows bees to switch easily to find the most rewarding place. For farmers who want to optimize pollination of their crops this switching to other plant may be unprofitable. One general concern in the use of bees for pollination is that honey bees may effectively transport unwanted pollen types into the beehive or to crops that should be void of cross-pollination. The only way to prevent foraging of honey bees on unwanted pollen is to keep beehives at a safe distance from those places. To minimize risk for undesired pollen flows by honey bees, knowledge on foraging behaviour can provide cues about key factors. Foraging distances and covered area around beehives are such key factors. The final resulting undesired cross-pollination or contamination of unwanted pollen in honey however, depends on many other factors.

Even though it is long known that bees can collect food at several kilometres from the hives, it is only from recent studies under what circumstances they travel that far and to what extent they do so. In practise, such studies are more complicated than expected. This is mainly because bees forage in a heterogeneous landscape where the quality of different patches for food varies throughout the season. Bees will forage nearby when good sources are locally abundant, in order to save energy and to reduce mortality risk. However, when high quality sources are further away, flying over large(r) distances may be rewarding. It is obvious for example, that bees will more easily fly large distances to exploit a flowering crop of oilseed rape, which is of high food value, than to forage on distant flowering potato that is of low value. The patchiness and quality of patches in the agricultural landscape is likely to affect the estimates of ranges and flying distances in specific studies.

Therefore, data from literature on such estimates are interpreted in the context of the environmental setting. At least these give cues about the chance of large distance foraging and the magnitudes that should be dealt with when taking management decisions. In Section 4.2, a summary of studies is given which shows that results from different studies are very variable, but distances covered by the majority of the bees are in the range of 1 to 2 km. However, in some cases, this can increase to 5 or even 10 km.

Methods of estimating foraging distances

The following methods are available to estimate foraging distances of honey bees:

- Training of bees for a particular rich food source, situated at different distances from the colony.
- Theoretical estimates from optimal foraging theory and optimal energy use.
  Optimal foraging theory predicts that bees should minimize flight time and energy expenditure while maximising the energy it gains from the food collected.
  Theoretical models, predicting forager distributions for 'Central Place Foragers' (de Vries and Biesmeijer 1998; Dukas and Edelstein-Keshet 1998; Cresswell, Osborne and Goulson 2000, Visscher and Seeley 1982), differ between those for species considered 'social foragers', e.g. honey bees that communicate information about resource location to nest mates, and those relying on individual exploration to find resources, e.g. bumble bees. However, note that the extra energy and time for flying larger distances is relative and often compensated by the gain of finding a good patch.
- Interpreting waggle dance information from beehives. A typical example for this approach is the paper of Visscher and Seeley (1982), who extensively analysed foraging distributions in a

complex landscape during various time frames showing the complexity of the colonies foraging behaviour and distances up to 10 km.

- Measurements on transmission of marked pollen loads.
- By mark and observation studied in individuals (Osborn et al. 1999).
- Tracking by harmonic radar (Osborn et al. 1999).
- Evidence from gene flow measurements.

All methods used so far have bias and limitations for obtaining sufficient and accurate estimates on the range of variability in bee flight patterns. Spatial processes in ecology are an emerging field that is likely to result in better technologies for and data from such studies on insect movements.

4.2 Key studies

Beekman and Ratnieks (2000) studied long range foraging by honey bees by decoding waggle dance information from honey bees foraging at large blooming heather fields – these can be very attractive for bees – in England and concluded that the median distance foraged was 6.1 km, and the mean 5.5 km. Only 10% of the bees foraged within 0.5 km of the hive whereas 50% went more than 6 km, 25% more than 7.5 km and 10% more than 9.5 km from the hive. This study shows that bees are able to cover large distances in the particular case. Earlier studies showed smaller distances (average about 1 km). They assume that such distances are only found in situations where food quality per patch varies much and patches are large. Only in such cases large distance travelling can be rewarding.

Also by decoding waggle dances, Visscher and Seeley (1982) showed that honey bees regularly fly several kilometres from the hive (see Figure 5). In their study, the most common distance was 600-800 m. The mean was 2.3 km and the range enclosing 95% of the colony's foraging activity had a radius of 6 km.

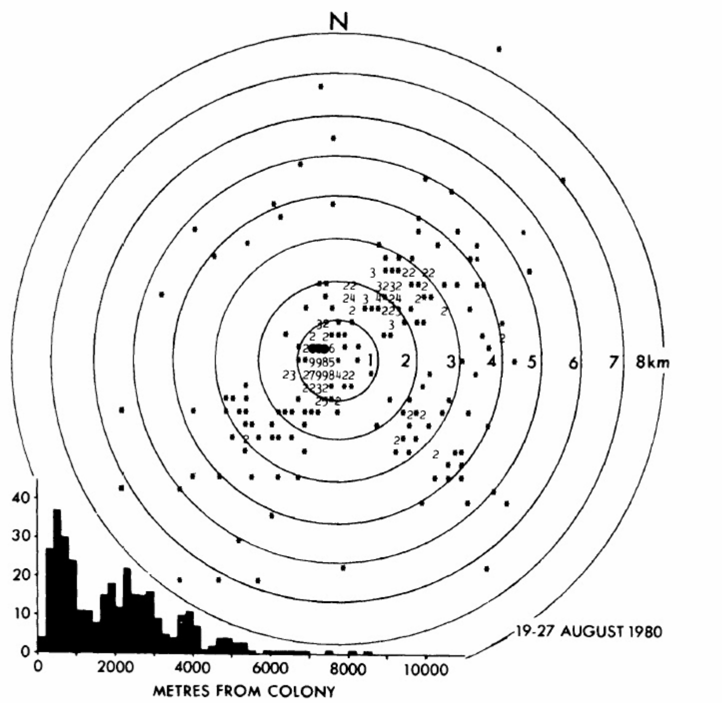


Figure 5 – *Distribution of foraging activities as analysed by waggle dance decoding (Visscher and Seeley 1982)*

Also much shorter distances were found in a study by Waddington et al. (1994), where the foraging range was 745-1,413 m. In patchy landscapes, where food richness varies, temporally and spatially recruitment of foragers that explore larger distances can be profitable (depending on scarcity and patchiness of pollen nectar density).

Ramsay et al. (1999) placed beehives at a distance of 800 m from a GM oilseeds rape field. Over 50% of the bees had GM pollen in their pollen loads, showing that this distance is easily covered by the majority of the bees. From this and other studies, they conclude that pollen is easily collected from this favoured crop at ranges up to 2 km.

Steffan-Dewenter and Kuhn (2003) observed and decoded over a thousand honey bee waggle dances from colonies in simple and complex landscapes in different seasons. Overall, the mean distance was about 1.5 km and ranged from 60 m to 10 km.

Williams (2001) reviewed the role of bees in pollen and gene flow from GM plants. Referring to maximum flight ranges up to 10 km for honey bees, there is ample evidence that by far most pollen are deposited on nearby plants during foraging or brought to the colony within the range of a few hundred meters. Typically mean distances are around 300 m. Because of the skewed distribution (frequency is exponentially decreasing with increasing distance, see Figure 3) of flight distances and pollen transport, occasional transport of pollen over large distances is possible. For example, the majority of bees may forage within a range of 500-1000 meter but a small fraction (that is hard to quantify) may forage at a distance of 5 km or more.

Of course, this affects the fraction of pollen transported over such distances but for some settings that can be relevant. In particular, when attractive patches such as flowering oilseed rape is within reach of the colony and other good food sources nearby are scarce.

4.3 Crop-specific information

Apple

The major role of insects including honey bees in pollinating fruit trees has long been recognized (Free 1993) Therefore, most apple and other fruit orchards apply honey bee pollination with colonies to improve fruit setting . Vice versa, for beekeepers and honey bees apple and other fruit trees are an important source for pollen and nectar in particular in springtime.

Even though many other wild bees, bumble bees and syrphids can play an important role in a varied landscape, pollination and pollen transport from and to orchards will be largely honey bee-mediated when beehives are applied at a large scale for orchards. As apple flowers are a preferred foraging target bees from a colony located in the orchards will mainly stay within the orchard (Free 1993) or forage at smaller distances in adjacent orchards when available. Because of massive flowering and abundant food the bees tend to stay where they are. The strategy of placement and number of beehives per orchard suggest that honey bees restrict their foraging range to a small area when being active in flowering orchards.

The researchers have not found any experimental data about the attractiveness of orchards for distant colonies, although it seems likely that flowering orchards could attract bees from a large distance because of the abundant high quality food. However, at the same time of the year other flowers may be equally attractive when available such as willows and dandelion. Though much information is available for optimizing apple fruit setting and necessary cross-pollination, it is remarkable that no information could be found on long distance apple pollen transport.

Oilseed rape

Oilseed rape is one of the most preferred crops for honey bees and possibly for other pollinating insects as well. When mass flowering, it attracts pollinating insects from over large distances. Due to the importance of this crop and suitability for experimental studies, several investigations have been done on the foraging activity and pollen transports from oilseed rape and similar cruciferous crops

and weeds. Most recent studies of Rader (2011) and Chifflet et al. (2011) have shown that these crops attract bees easily from distances of at least 500 m to 1,000 m.
Because of the significant role of honey bees and bumble bees in the pollination and potential unwanted cross-pollination between fields, or related Brassica species outside the field, much research have been done on pollination transport and gene flow patterns (e.g. Smith-Kleefsman et al. (2005), Cresswell et al. (2002), Damgaard and Kjellsson (2005), Luyten and De Jong (2011) and many articles these authors are referring to).

The results from empirical data or modelling data all point to the conclusion that pollen transport by flight and probability of cross-pollination exponentially decreases with distance from the pollen source. Therefore, even though honey bees or bumble bees may cover large distances under particular circumstances, the major activity and resulting pollination occurs within a distance of a few hundred meters (Beckie and Hall 2008). On the other hand, potential cross-pollination can occur over large distances up to 3 or 4 kilometres, even though the probability of such a pollination is in the order of 0.01 to 0.001 %. Depending on the spatial arrangement of fields and the size of the bee population and the number of flowers to be fertilized, this still may result in a significant absolute number of cross-pollinated flowers even though this may be a very small fraction of the total flower population.

Potato

Generally Potato is considered as a less relevant crop for honey bees because many cultivars do not flower at all or have a less abundant flowering with very little pollen and no nectar being available for insects (such as honey bees). Sanford and Hannemann (1981) already showed that honey bees have no real cue to visit potato flowers. Only a few bumble bee species tend to exploit potato flowers. Potato apparently is unattractive for honey bees and hence no information could be found for the distance over which potato pollen is transported.

Sugar beet

Although bees are known to visit flowering sugar beets (Free 1993), it is certainly not a preferred or a rewarding crop for honey bees. No data could be found on honey bee foraging patterns or flight distances in relation to this crop. Normally, cultivated sugar beet is kept vegetative, so the only possible visited and pollinated flowers are of the few bolting plants that occur in fields, and production fields of beet seeds (the latter will be inside greenhouses or polytunnels). Moreover, honey bees generally do not visit scattered individual plants, but specialize on rich patches of highly rewarding plants.

Maize

Maize is a major crop in many countries that are suitable for growing. Although it is a wind-pollinated crop, which is not dependent on insect pollination, it produces large amounts of pollen. Though maize pollen is not preferred as a food source, there is accumulating evidence that honey bees regularly feed on maize pollen when it is abundant and no more rewarding crops or flowers are available (Keller et al. 2005). The nutritional value of maize pollen is low due to its low protein content (Hörcherl et al. 2010).

During its flowering time in late summer maize pollen may be a substantial part (80%) of the food collection when maize is abundant and few other sources are available (Odoux 2004). Also in a mixed landscape maize pollen may can be one of the high-ranking food sources when available (Keller 2005), in particular when beehives are located near flowering maize fields. There are no experimental data on how far bees will fly to forage on maize with and without other food sources nearby, but it seems not likely that they fly longer distances for a less preferred pollen type such as maize compared to, for example, oilseed rape. It should be noted that oilseed rape is also very attractive for its nectar.

4.4 Other pollinating insects

According to Osborne et al. (1999), who studied flight distances of bumble bees, the ability to optimize flight distances under different circumstances is essential for predicting colony success. Foragers were mass-marked as they left the colony and pollen from foragers returning to the colonies was analysed. In this way, the spatial distribution of foraging could be mapped and foraging distances and forage availability for the bumble bees could be estimated. The bumble bees foraged at least up to 1.5 km from their colonies, and the proportion of foragers flying to one field declined, approximately linearly, with radial distance.

Other field studies have shown that some bumble bee species (including *B. terrestris*) forage at least several hundred metres (Dramstad 1996; Osborne et al. 1999); and even kilometres from the nest (2.2 km in Kreyer et al. 2004). Walther-Hellwig and Frankl (2000) found 25% of re-sightings between 1500 and 1750 m from the colony.

Chifflet et al. (2011) studied the presence of pollen on many different insects at various distances from a GM crop plot having marked pollen. Many potential pollinating insects had viable pollen with them at a distance of 500 m, but at 1,000 m the incidence was low (0-5%) and only one solitary bee species was found at 1,500 m. They conclude that a distance of 1 km for isolation is not enough to avoid pollen dispersal outside this range.

Though honey bees and bumble bees are very effective pollinators and carry more pollen per individual, many other insects can carry pollen with them and easily overcome large distances. For example syrphid and stratomyid flies that also have a high capacity to transport pollen were found to be more mobile and transport pollen very quickly over several hundreds of meters (Rader et al. 2011). However, they also note that there is general lack of data concerning the transport of pollen by such species over large distances.

4.5 Concluding remarks

There is much evidence that honey bees can cover and hence transport pollen over large distances up to 10 km or more. However, in many cases, colonies are put in place near nectar and pollen rewarding places – and where food is abundant individuals tend to stay in a favourite site – and after collecting enough food will return to the colony. This common pattern will lead to dominant pollination patterns that occur within a range of a few 100 meters or even less (e.g. when colonies are placed in a flowering orchard). The whole issue of the impact of long range flights on pollination over larger distances depends on too many factors to draw one general conclusion. In an extreme case scenario, two isolated but attractive fields at a large distance with a bee colony in between can be visited by the same individual bees at the same day, taking maximum pollen loads with them. In such a case, significant pollen transport could occur at a distance of two times 10 km.

As Beekman and Ratnieks (2000) have shown for heather fields, which can be very attractive for bees, more than 50% of the bees of a colony could focus on such distant fields. However, no experimental evidence, sufficient data or field validated models are available that can give clues about the final quantitative impact for different crops. Handling low probabilities in a variable landscape context is extremely difficult and more experimental and modelling research is needed to get a better understand on what is really going on (Beckie and Hall 2008). Direct measurement of labelled pollen transport and subsequent pollination has not been researched in an experimental setting covering more than a few kilometres.

The spatial arrangement of fields, bee colonies during the season, the variable flight activity of bees makes it very hard to determine a relation between distance and pollination probabilities that is valid for many different conditions (Steffan-Dewenter and Kuhn 2003). Hence, setting distance criteria for preventing undesirable out-crossing always includes a political decision in addition to ecological arguments (Lezaun 2011), especially as long as no more hard and convincing data is available.

5 Pollen in honey

5.1 Introduction

Natural honey always contains pollen grains, and often spores of fungi as well. Pollen grains can end up in honey through three routes:

1. Pollen from the flower that is foraged on by the bee falls into the nectar and is harvested by the nectar forager;
2. Pollen on bees and in the air inside the hive contaminate the nectar during processing by the bees (packing, drying, conserving); and
3. Pollen packed in cells in the same comb as the honey may be mixed with the honey by the beekeeper during centrifugation or pressing of the honey from the combs.

When a nectar foraging bee has collected a full honey stomach, it returns to the hive to turn over its nectar load to a younger worker bee. Although a foraging bee generally gets loaded with pollen from the flower anthers while collecting nectar (Free 1993), its honey stomach hardly ever contains pollen grains. In the honey stomach, pollen grains are rapidly filtered out and are loaded into the 'real stomach' (ventriculus), big pollen grains faster than small ones. It takes about 10 minutes for the honey stomach to filter out all pollen grains, which means that the travel time back to the hive is important. For some very good nectar sources, bees may fly as far as 9 km or even more (Beekman and Ratnieks 2000), which gives enough time to filter out the grains during flight, nectar collected nearby may still contain more pollen grains upon return. In the hive, the receiving worker bee will pass on the clean nectar to other bees immediately (for instance nursing bees that use it for feeding the larvae) or it will start to store and process it. Hence, it is the storing and processing of the nectar inside the hive that is the main source of pollen in the honey.

When a beekeeper extracts the honey from the comb by centrifugation (see also Section 3.5), hardly any pollen – apart from the grains dissolved in the honey – will be added, despite the fact that cells with beebread may be included in the honey combs. This beebread very strongly adheres to the comb and is not extracted. Only when the beekeepers harvests honey by pressing of the honey comb, the pollen from the cells containing beebread may be mixed with the honey. However, pressing of honey is abandoned as a way to extract honey, apart from rural beekeeping in for instance Africa.

To act as a conserved energy reserve, the nectar has to be dehydrated (so that it will not be susceptible to fermentation anymore) and enzymes need to be added. Processing and dehydration of honey is done by regurgitating the nectar several times and fanning with the wings to evaporate water. Only when it has a water content below 20%, the fully loaded honey cell can be sealed with a wax cap. Most of the pollen grains that are found in honey have generally entered unintended during the fanning, since the air inside the hive is loaded with pollen grains of several plant species, reflecting the availability of collected pollen species in the environment. Pollen grains of wind-pollinated plants might be well represented in the fanning air, since these generally are less sticky and easily float. A honey from Sinderhoeve (near Heelsum, The Netherlands) in 2007 contained as much as 74% of Hypericum pollen, though this plant does not produce nectar and was obviously just visited for its pollen (Blacquière et al. 2008).

Pollen determination in honey

Pollen determination in honeys is a way to determine to some extent the botanical origin of the honey. Such pollen profiles are generally made by microscopical determination of the family, genus or species of plant of 500 pollen grains in the honey. A dominant pollen species is then supposed to be an important donor of nectar, and thus candidate to be the nominator of the honey. However, it is not always that straightforward, since different pollen types have very different probabilities to end up in honey. This depends largely on pollen size and morphology, abundance, stickiness but also on flower shape and arrangement of the flowers in inflorescences. These factors together determine to which extent the bees, and eventually the honey, get ‘contaminated’ with airborne pollen grains.

Qualitative and quantitative pollen determination

The above-mentioned pollen profiles of honeys do inform about the relative numbers of different pollen species in honey: this is a relative measure only. Since pollen species do not show up equally easy in honey a more quantitative approach to determine the botanical origin of honey has been adopted, in which the specific pollen traits have been taken into account. For instance, a honey may carry the name of ‘chestnut honey’ only if more than 90% of the pollen in the honey originates from chestnut. Nectar of chestnut is already rich in pollen grains. The grains are relatively small, which retards the removal from the nectar in the honey stomach by the proventriculus. Moreover, bees foraging on chestnut are overloaded with the abundant (and small grained) pollen grains. Some other honeys can already be attributed to a certain species with only 20% representing the specific plant species. By using a ‘pollen coefficient’ (the number of pollen grains per 10 grams of monofloral honey divided by 1000), a more realistic share of the contributing plant species to honeys can be obtained (Kerkvliet 2011a, 2011b).

Although this way of determining the ‘honey species’ on the pollen present in the honey is called ‘quantitative pollen determination’ (Crane 1975) it is actually not quantitative but just relative: it does not give an assessment of the number of pollen grains nor the weight of the pollen load in honey. When pollen profiles of honey need to be used to predict the presence and concentration of certain compounds associated with it, for instance allergens, toxins, secondary plant substances (e.g. pyrrolizidine alkaloids, see Kempf et al. 2011) or pesticide residues, real quantification is needed.

According to the very scarce literature found about the amount of pollen present in honeys the share is typically lower than 0.5% (0.25%, Graham 1992) or 0-15,000 grains per 1 g honey (Lieux 1972; Persano Oddo et al. 1995). It is not easy to recover the pollen grains from honey and then quantify them. Therefore, in the small laboratory experiment below, which is part of this review on pollen in Dutch honeys, the researchers chose an opposite approach: samples were spiked of artificial honey (no natural pollen) with known amounts of different pollen species. Subsequently, pollen in these artificial honeys were determined in the usual way, by counting them microscopically in a counting chamber (Bürker). Hence, a calibration curve was made to relate the micrograms of pollen to the counted pollen numbers found in natural honeys.

In this Chapter, an overview of the pollen profiles of a survey of Dutch honeys from 2008 is presented, focussing on the pollen of Rosaceae, Brassicaceae and of maize (*Zea mays*). From five of these honeys the number of pollen grains present per gram honey was determined. Finally, an experiment was performed to estimate the amount of pollen present in honeys by weight. This was done by making calibration curves of known quantities of pollen added to an artificial honey without pollen linked to the counted pollen numbers in these 'honeys'.

5.2 Materials and methods

Pollen data of Dutch honeys

During spring and summer of 2008, bees@wur carried out a monitoring program among 172 Dutch beekeepers to gain some knowledge about the prevalence of several diseases and parasites in the apiaries. Thanks to the chosen set up of the programme, a proper distribution of the contributing beekeepers over the country was obtained. In June, samples of bees were taken from five hives per apiary, which were analysed not only for diseases but also for heavy metals. In addition to the bee samples, the beekeepers were asked to deliver one or a few pots of honey harvested that spring and summer. These 190 honeys have been analysed for the presence of the American Foulbrood bacterium *Paenibacillus larvae*, but the pollen profile was determined as well.

In 2008 and 2009, from the obtained honeys pollen, extracts were made according to Van der Ham et al. (1999): 10 gram of honey was diluted with 20 ml demi water, thoroughly mixed and then centrifuged for 10 min. at 2,200 rpm. The liquid was decanted and the pellet was suspended in two steps in 10 ml demi water, and again centrifuged at 1,000 rpm for 10 min. After decanting, the resulting pellet (or part of it) was suspended in a droplet of demi water and mixed. The suspension was brought onto a microscopy glass slide in two aliquots and dried. A droplet of glycerol- gelatine-

fuchsine was added and the two aliquots were closed with two cover slides. The slides were microscopically examined systematically, until 500 pollen grains had been determined. The determination was done using a collection of reference preparations and literature and website collections, up to the plant genus level.
Data of the honey samples from the 2008 monitoring have been used to show the presence of pollen of Brassicaceae, Rosaceae and maize pollen in Dutch honeys. In this report, especially the presence of pollen from Rosaceae (because of the focus on apple) and from the Brassicaceae (because of oilseed rape *Brassica napus*) is considered, as well as samples containing pollen grains of maize, beet and potato. The pollen in honey data were sorted into five classes of presence (0-20%; 20-40%; 40-60%; 60-80% and 80-100%) allocated to the home addresses of the beekeepers by GIS.

Pollen counting and calibration

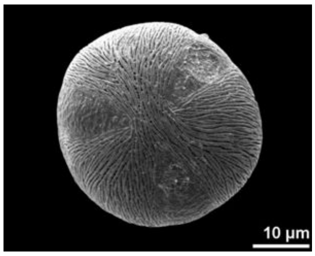
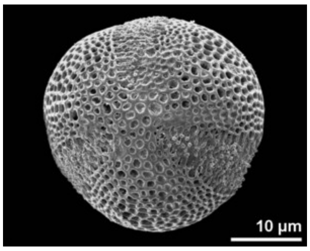
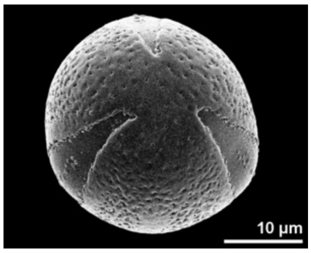
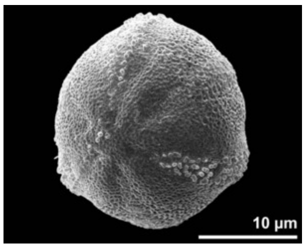
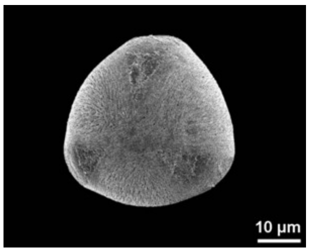
Pollen counts in five honeys from the survey

A selection of five honeys from the 2008 survey known to include pollen from Rosaceae and Brassicaceae (see Tables A1-A3) were used to prepare new pollen sediments. 2 g honey was taken and diluted with 4 ml water, mixed and centrifuged, and washed again with 2 times 2 ml water and centrifuged again. After centrifugation, the pollen pellets were suspended in an end volume of 10 as well as 100 µl. In both aliquots, the number of pollen grains was counted in duplicate in a 'Bürker' cytometer counting chamber. For each counting, the number of pollen grains was counted in 20 counting chambers. A Bürker counting chamber has a volume of 0.004 µl. The five honeys used (numbered 132, 133, 171, 181, 186) have been marked blue in the Tables A1- A3 giving the data about the 2008 honey survey (Annex 1).

Calibration of pollen numbers using artificial honey

Beefit sugar syrup (sugar content 70%) was used as the artificial honey (without pollen contamination). Beefit is commercially available as bee feed. To 18 grams of this artificial honey, in 2 ml water, standard quantities of pollen were added in a concentration range: 50; 5; 0.5 and 0.05 mg pollen per sample, ending up in the same amounts in 20 g of 'honey'. Sugar content of the artificial honey was reduced to 66% after adding the pollen, because the pollen was suspended in water. Bee collected pollen pellets were used as pollen sources. Pollen species were chosen that are often found in honey. These pollen pellets were collected from bees using a pollen trap at the hive entrance in the summer of 2010 by Moos Blom. The pollen pellets were homogenous and the pollen had been determined on to genus level. The pollen samples had been stored in plastic bags in a refrigerator at -20˚C since then. The pollen species selected for the experiment are shown in Table 4.

Table 4 – *Pollen species used in artificial honey* (pictures from www.paldat.org). *Pollen grains have been determined to the level of the plant families. The species mentioned between brackets refer to the pollen specimens in the pictures*

Pollen species	***code***	***size[µm]***	***form***	***picture***
Aceraceae (*Acer campestris*)	A	30 x 32	tricolpate (pollen grain with 3 long (colpate) apertures)	
Brassicaeae (*Brassica napus*)	B	31x32	tricolpate	
Fabaceae (*Trifolium repens*)	K	24x26	tricolpate	
Phacelia (*P. tanacetifolium*)	P	20x22	heterocolpate (pollen grain with both long as well as combined long + porous apertures)	
Rosaceae (*Malus sylvestris*)	R	31x35	tricolporate (pollen grain with 3 colpi with one pore each)	
Mixture of the 5 species	M			

The washing and concentration of the pollen pellets from this artificial honey was performed as with the natural honeys (see above) used for the counting of pollen grains. Pollen counts were made in duplicate. From each sample, the number of pollen grains was counted in 20 counting chambers, these 20 counts were averaged and the number of pollen grains was calculated. Subsequently, the number of pollen grains was related to the amount (µg) of pollen added to the sample.

Water content of pollen pellets was determined by weighing and re-weighing after drying.
As bees may use some nectar to stick together the pollen grains in their corbiculae, which might contribute to the mass of the pollen pellets used it was decided to determine the sugar content in the pollen pellets. To estimate the sugar (nectar origin) content of the dry pallet, repeated washing (and re-weighing) was used.

It was found that pollen pellets had a water content of 9.7% (SD < 0.1%), and the sugar content was 0.01%. This indicates that the samples had already been desiccating to some extent, since fresh pollen generally has a water content of 20-30% (Campos et al. 2008). In this case, using fresh pollen pellets does not introduce a very big aberration from using dry weights. It can be concluded that the sugar content is negligible.

Statistics
For natural honeys, differences between pollen counts in natural honey were tested, using a two-way ANOVA for the different end volumes (10 or 100 µl) and the different natural honeys tested. A Sidak posthoc test was used to test differences between means (interaction).

For artificial honey, the average pollen per gram honey was determined and it was tested whether the calibration curve crossed the origin. Therefore, linear regression models were used for all honeys separately.[4] To test differences in slopes of the calibration curves between honeys, the researchers used a general linear model with the counted pollen (#) per sample as a function of the pollen input per sample (co-variable; gram pollen per sample of artificial honey, and the different artificial honeys (fixed factor).

For this test, both the pollen input as the counted pollen were log10-transformed, in order to obtain a normal distribution of the residuals. A Sidak posthoc test on the interaction between pollen input and honey type was used to test differences between means (only the differences at the highest concentrations were used).

[4] The researchers could not log-transform the data for the first analysis, as it has to be tested whether the calibration lines crossed the origin (not possible with log transformation on data ranging between 0-1). Residuals were normally distributed without transformation of the data. A point of argument would be that the without transformation, the data of the highest concentration has a relatively large impact on the curve estimation.

5.3 Results

Honey survey 2008

Figure 6 to 12 show the locations where, for example, 80-100% (red dots) of the pollen grains in the honey belonged to the indicated botanical group. When more than one sample per location was available, the highest value is shown. The full data of the honeys is listed in Annex 1 in Tables A1 to A3. Honeys from the survey throughout the country carried pollen grains of at least 50 plant families, wind pollinated as well as insect pollinated species. Some families are very abundant, like Salicaeae, Rosaceae, Brassicaceae and Fabaceae.

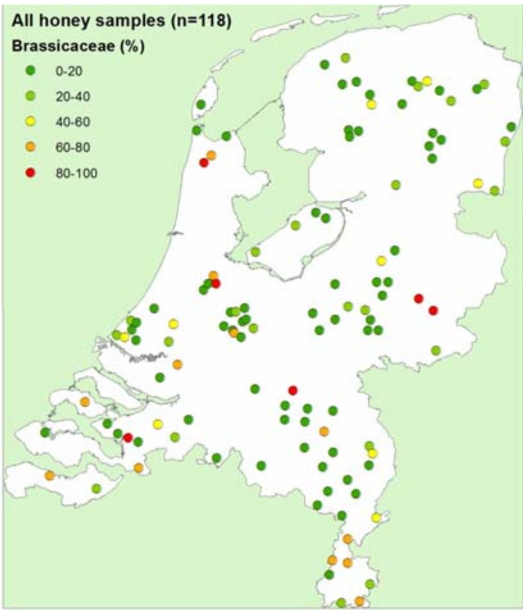


Figure 6: Distribution and percentage Brassicaceae-pollen out of 500 grains in honey in The Netherlands

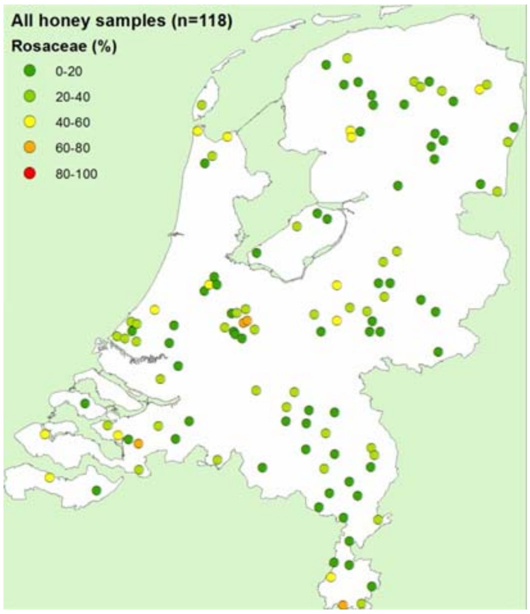


Figure 7: Distribution and percentage Rosaceae-pollen out of 500 grains in honey in The Netherlands

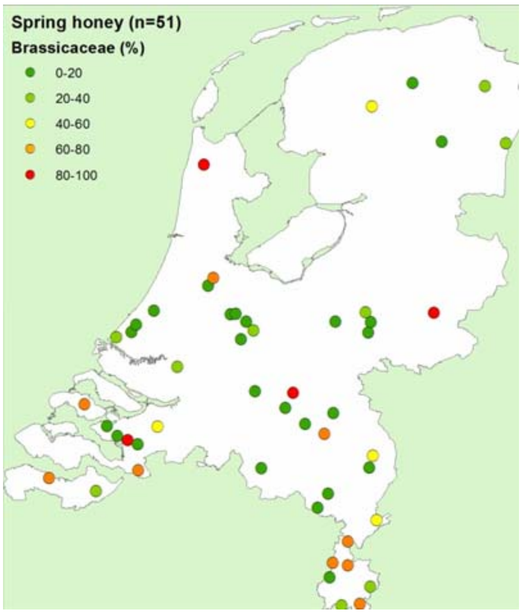


Figure 8: Distribution and percentage Brassicaceae-pollen out of 500 grains in spring harvested honey in The Netherlands

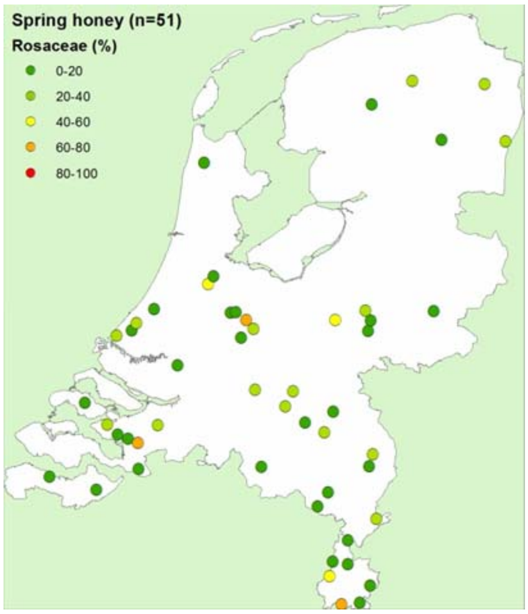


Figure 9: Distribution and percentage Rosaceae-pollen in spring harvested honey in The Netherlands

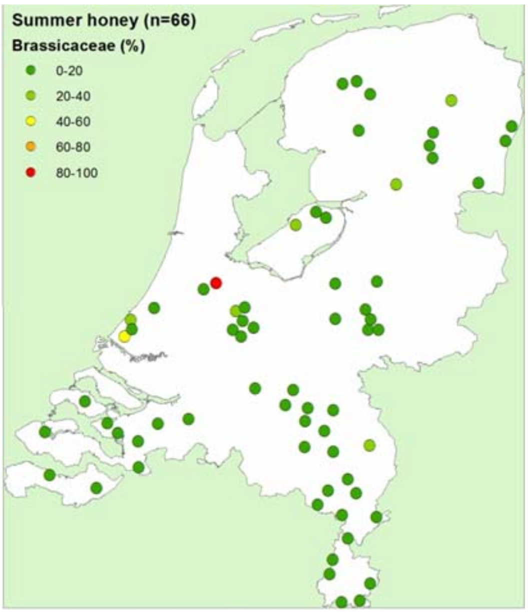


Figure 10: Distribution and percentage Brassicaceae pollen out of 500 grains in summer collected honey in The Netherlands

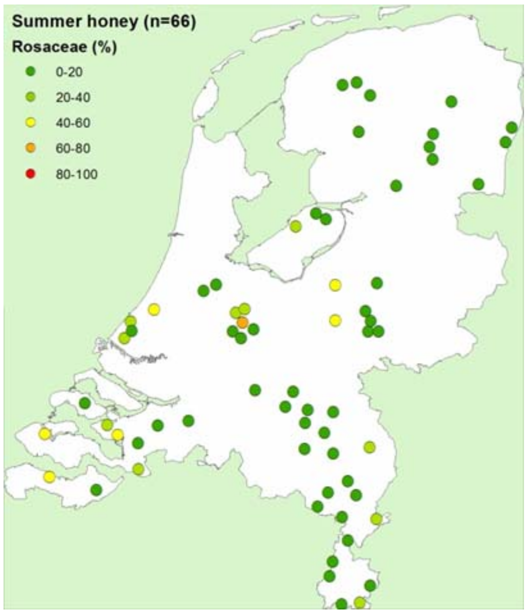


Figure 11: Distribution and percentage Rosaceae pollen out of 500 grains in summer collected honey in The Netherlands

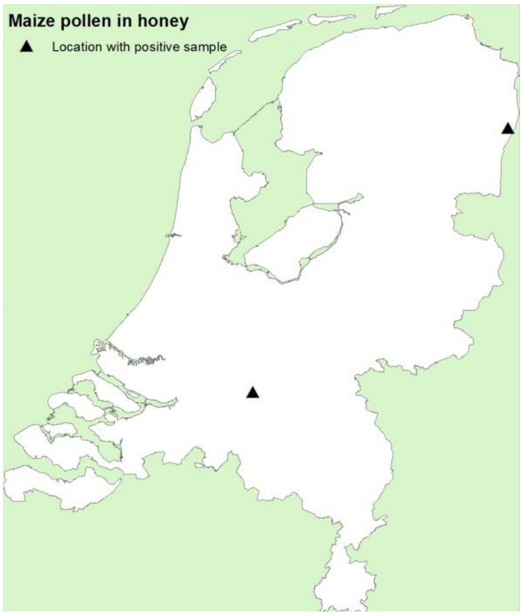


Figure 12: Distribution of samples positive for Maize pollen found in collected Dutch honey, summer 2008

The figures clearly show that both honeys rich in Brassicaceae pollen (> 80%) and Rosaceae pollen (60-80%) are quite abundant, especially in spring. The category of 0-20% may be a little rude, but real zeros are very rare for Rosaceae and Brassicaceae (see Tables in Annex 1). Spring honey (April through June) includes the flowering time of both oilseed rape (*Brassica napus*) as well as apple (*Malus domesticus*). Summer honey and late summer/autumn honey still may contain a lot of pollen from both Brassicaceae and Rosaceae, but the samples with very high shares are few (see Table A3). During the summer season, apart from Lime (*Tilia sp.*) and Heather (*Calluna vulgaris*), honeys generally become more multifloral (with individual shares declining).

Maize pollen was only found in two samples out of ~190, the percentage maize pollen was 1.5% and 4.2% (~500 grains counted). Pollen of Chenopodiacea is sometimes present and it cannot fully be excluded to be partly from beet, although honey bee foraging on beet is very improbable. Solanaceae is more often represented and potato might be included in very few cases. Pollen of potato was found in honeys when bees collect aphid derived honey dew from potato fields (van der Ham et al. 1999).

Number of pollen grains in five honeys

Natural honeys showed different pollen counts, ranging from less than 1,000/g honey to more than 25,000/g honey (Figure 13) but within two of these honeys (honey 177 and 186) there were also differences between the two end volumes in which the pollen numbers were counted (suspended in either 10 or 100 µl) (honey $F_{4,10}$=239.0, P<0.001; pollen input $F_{1,10}$=154.2, P<0.001; interaction $F_{4,10}$=64.1, P<0.001; Figure 13). The natural honeys with the highest pollen count, showed an additional higher pollen count for the 100 µl end volume. This may result from too high numbers of grains in the counting chambers at the 10 µl end volume causing some grains to be overlooked.

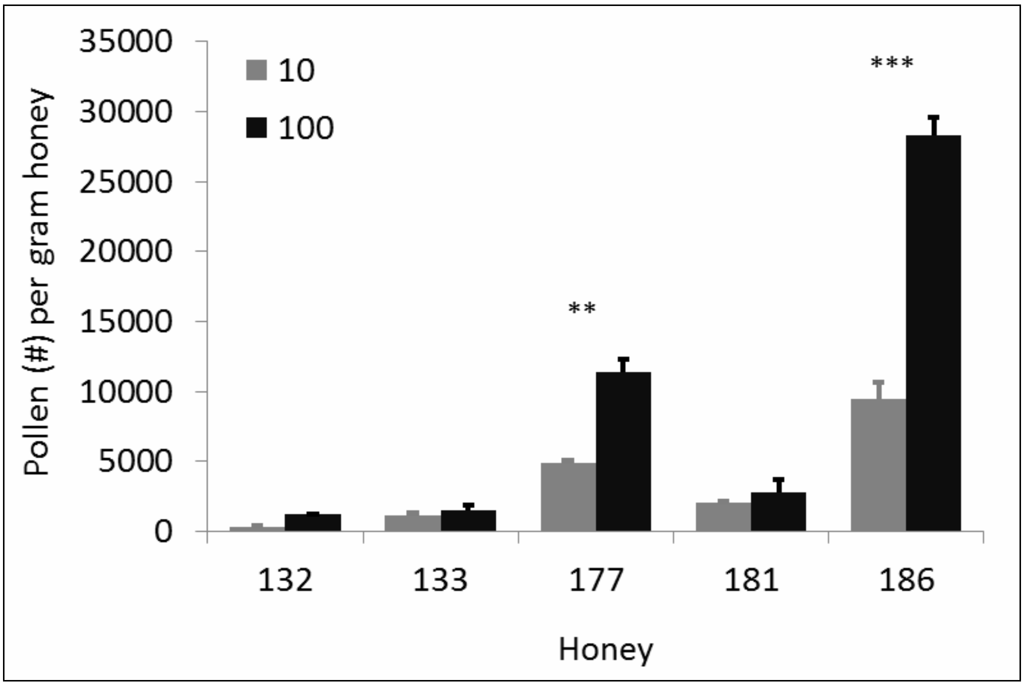


Figure 13 – *Pollen count per gram natural honey for 10µl and 100 µl input. A Sidak posthoc was used to test for differences between means. Asterisks show differences between groups (** P<0.01; *** P<0.001)*

Amount of pollen added versus numbers determined in counting chambers

In all artificial honeys, the calibration curves crossed the origin (P values Constant>0.05, Table 5, see also Figure 15)). The average number of added pollen grains counted per gram added pollen varied between $1.5 \times 10^7 \pm 1.1 \times 10^6$ for Aceraceae and $2.3 \times 10^8 \pm 7.0 \times 10^6$ for Phacelia (slope pollen input, Table 5). The calibration slopes differed between all artificial honeys (honey $F_{5,36}$=5.7, P=0.001; pollen input $F_{1,36}$=1830.6, P<0.001; interaction $F_{1,36}$=7.2, P<0.001; Figure 14). Omitting the lowest input concentration, because some samples gave low counts (which may affect the reliability), did not significantly alter the slopes nor the statistical significance.

Table 5 – *The test results of the linear regression models of the different artificial honeys, where β shows the estimated value for the number of pollen measured at 0g of pollen input, and slope shows the average number of pollen grains counted per gram pollen added*

Honey	**R^2**	**Constant**				**Pollen input (g per sample)**			
		β	**s.e.m. (β)**	**t**	**P**	**slope**	**s.e.m. (slope)**	**t**	**P**
Aceraceae	0.966	$-3.45 * 10^3$	$2.92 * 10^4$	-0.12	0.910	$14.9 * 10^6$	$1.14 * 10^6$	13.08	,000
Brassicaeae	0.996	$-40.34 * 10^3$	$5.00 * 10^4$	-0.81	0.451	$74.7 * 10^6$	$1.99 * 10^6$	37.48	,000
Rosaceae	0.998	$-40.29 * 10^3$	$3.65 * 10^4$	-1.10	0.312	$78.5 * 10^6$	$1.45 * 10^6$	54.15	,000
Fabaceae	0.941	$-18.25 * 10^3$	$7.88 * 10^4$	-0.23	0.824	$30.7 * 10^6$	$3.13 * 10^6$	9.80	,000
Phacelia	0.995	$-89.19 * 10^3$	$17.5 * 10^4$	-0.51	0.629	$232 * 10^6$	$6.98 * 10^6$	33.17	,000
Mixture	0.995	$-10.73 * 10^3$	$3.72 * 10^4$	-0.29	0.783	$52.0 * 10^6$	$1.48 * 10^6$	35.12	,000

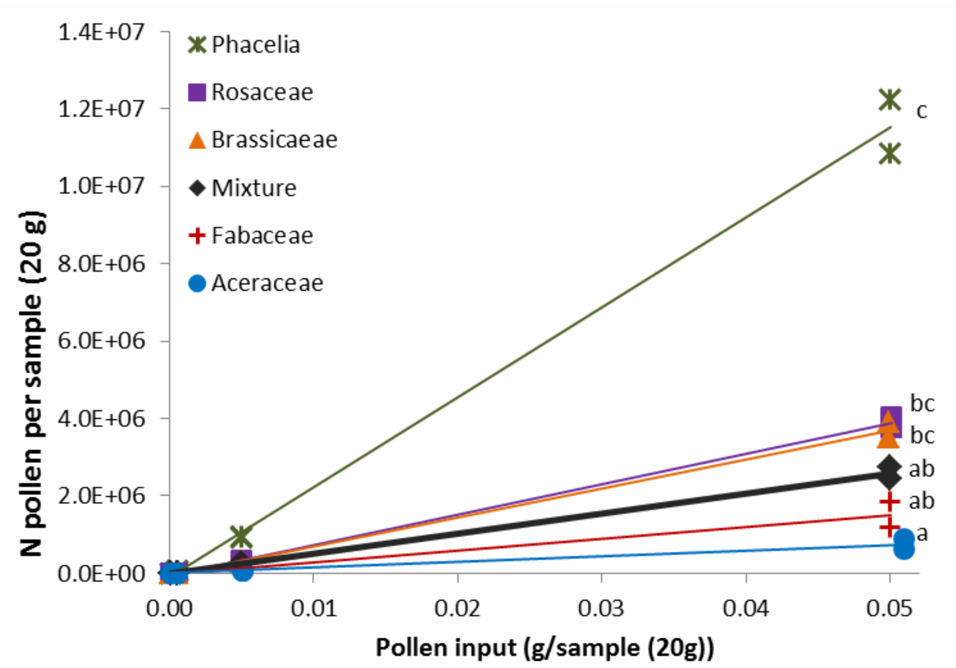


Figure 14 – *Calibration curves for the different artificial honeys (see legend). At the highest input (0.05), all honeys differed from each other (Sidak posthoc test P<0.001 for all combinations)*

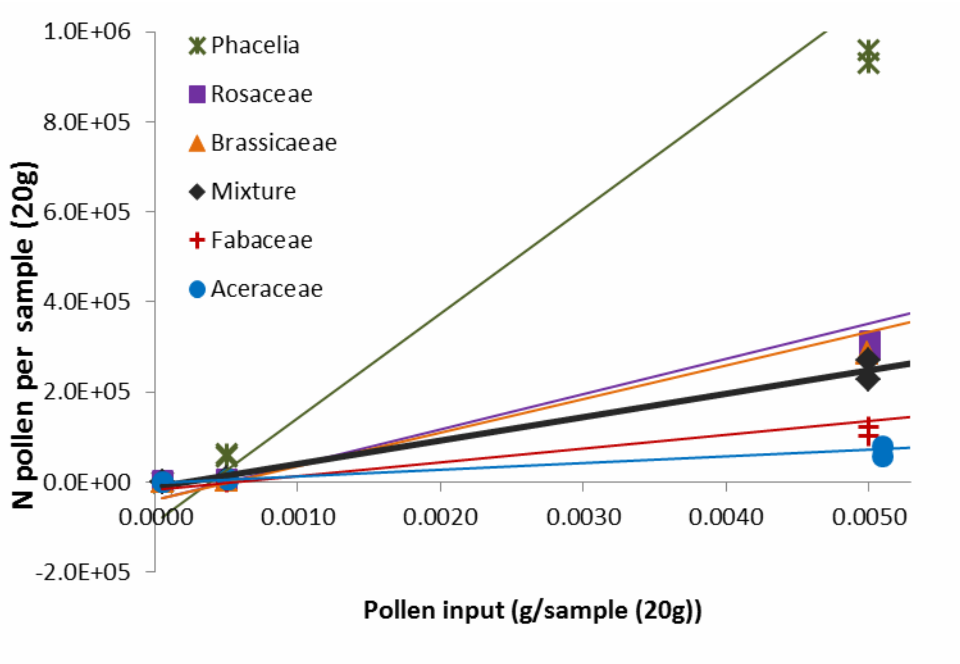


Figure 15 – *Detail of calibration curves (Figure 14) for the different artificial honeys (see legend) to show proximity of calibration curves to origin.*

5.4 Discussion

Botanical origin of pollen in honey

In the 2008 honey survey, many honeys with very high share of Brassicaceae pollen were found throughout the country, sometimes even higher than 80%. Brassicaceae are especially abundant in spring honey, corresponding to the flowering times of oilseed rape (*Brassica napus*) and charlock (*Sinapis arvensis*). Since not so much *B. napus* is grown in the western part of the country (provinces Utrecht, South Holland and parts of North Holland) and feral populations of it are rare (Luijten and De Jong 2010), most of the Brassicaceae pollen in honeys there may originate from feral populations of *B. rapa* and *Sinapis arvensis*. Both species can be very abundant on ruderal places (Luijten and De Jong 2010). The one > 80% Brassicaceae honey in the north of North Holland (Figure 6) may well be of *B. napus*, since the location corresponds with a large production area of B. napus in 2008 (Luijten and De Jong 2010). Most of the *B. napus* is grown in the Eastern part of The Netherlands, especially Eastern Groningen, while *B. rapa* and *S. arvensis* are more rare there (Luijten and De Jong, 2010). This might indicate a greater chance that Brassicaceae pollen in these honeys includes *B. napus*. Similarly, to Brassicaceae, also Rosaceae pollen are more abundant in spring honey than in summer honeys. In spring these pollen grains represent hawthorn, wild cherry, cultivated fruit trees (apple, pear, cherry, plum) as well as rose shrubs, in summer roses and black berries (*Rubus fruticosus*).

The only two found examples of pollen of maize (*Zea mays*) in honey seem to conflict with reports that state that maize pollen can be a major part of the pollen intake of a colony in France (Odoux et al. 2004). In the US state of Indiana, maize pollen comprised over 50% of the pollen collected by bees (by volume) in 10 out of 20 samples (Krupke et al. 2012). The researchers do not know to which extent honey bees forage for maize pollen in The Netherlands. Despite a sometimes high intake of maize pollen by bees, in honey only in few cases and with low percentages maize pollen is found. In our case, the occurrence of maize pollen in honey was rare and, additionally, the share was very low (max. 4%). In Germany in about 10% of the honeys maize pollen was detected, but generally the share was around 0.2% of the pollen grains only (W. von der Ohe, personal communication). Also in France, the share of maize pollen in honey was low: only 10% of the honeys contained maize pollen in low numbers (Patricia Beaune (Laboratory Famille Michaud Apiculteurs (France)) personal communication).

Possible differences between for instance France and The Netherlands may be the different maize varieties (with differing pollen production), as well as the poor honey flow during maize flowering time in The Netherlands. During the time maize flowers, Dutch beekeepers rarely harvest their honey, because during this period of the season honey flow in The Netherlands is poor. Although maize pollen may often be collected by bees, the low honey flow possibly results in maize pollen hardly ever showing up in harvested honey by beekeepers. Honeydew honey collected on potato crops may contain low numbers of maize as well as potato pollen, blown into the sticky honeydew by wind (J.D. Kerkvliet, personal communication), in real flower honeys these pollen species are not found.

Some care should be taken when interpreting the regional implications of the pollen profiles, since it is not absolutely certain that the beehives have actually been all the time on the spot where the researchers localized them. The researchers used the home addresses of the beekeepers, and some of them may have been travelling with their bees, although this is not very common practice. However, as most of the fruit orchards are pollinated by honey bee colonies of hobbyist beekeepers in The Netherlands (Blacquière et al. 2009), especially in spring a significant part of the hives may have been moved. Therefore, this survey can only be used as a first indication, and it would be very worthwhile to initiate a more focused survey.

Quantification of pollen numbers

In the five honeys from the survey used for absolute quantification of the numbers of pollen grains, the numbers varied from less than 1,000 to 28,000 per gram of honey. These figures nicely correspond to data from the literature (most honeys: 2,000 to 10,000 grains; Van der Ham et al. 1999; Von der Ohe et al. 2004; Persano Oddo and Piro 2004), although a honey with 25,000 grains per gram of honey is considered as a honey with a high pollen number (Von der Ohe et al. 2004). In a ring test (Von der Ohe et al. 2004); it was also found that the reproducibility (between laboratories) of pollen counting was better in honeys with high numbers of pollen than in honeys containing low numbers of pollen. In our case, in the honeys with the highest numbers of pollen grains the counts were higher when the pollen pellet was re-suspended in 100 µl than in 10 µl, suggesting that pollen grain recovery was better with a higher dilution, i.e. with lower dilution the number of pollen grains was systematically underestimated. This may moderate the absolute value of pollen counts.

The ranges of absolute numbers of pollen of several unifloral honeys are known (Persano Oddo and Piro 2004), so if the researchers have honeys among the five analysed with a high share of one of the 'unifloral' pollen types, the researchers can compare the absolute numbers between their honey and the unifloral honey. The 'spring' honey Nr. 177 has 50% Brassicaceae, and contained 12,000 grains per gram, which is very much comparable to real unifloral oilseed rape honey with 7,570 grains per gram (Persano Oddo and Piro 2004). The other four chosen honeys did not have a high share of any of the unifloral types and therefore no comparison could be made.

Calibration of recovered pollen numbers to the weight added to artificial honey

The numbers of pollen grains per gram of artificial honey varied between 74,000 (Aceraceae) and 1.15 million (Phacelia). This means that our artificial honeys outranged natural honeys several times, since the latter may contain generally 10,000 grains per gram at most (Van der Ham et al. 1999).

Numbers in the range of millions per gram can only be reached sometimes for pressed honeys. These numbers of 74,000 through 1.15 million had been recovered from an addition of 0,05 gram pollen per 10 gram of 'honey', i.e. 0,005 g/g (0,5% w/w). Looking back the highest addition was out of range of natural honeys, however it was based on the reported 0,25% mentioned by Graham (1992). The other additions were in the range of natural honeys. Moreover, inside and outside the natural range the relationship was similarly linear. The 0.25% mentioned by Graham would indicate 37,500 to 570,000 grains per gram of honey. In that case, the numbers of 0-15,000 grains per 1 g honey reported by Lieux (1972) and Persano Oddo et al. (1995) would be at the lower end of the range.

Despite being out of the natural range, our estimated linear calibration curves showed very high values for the coefficient of variation[5] (which means that the model is a very good predictor for future outcomes) and did cross the origin, which means the slope can be used to relate pollen counts to pollen weights. Deduced from the calculated slopes, the researchers conclude that 1 gram of pollen in honey corresponds with 14.9 million pollen grains (Aceraceae) to 232 million pollen grains (Phacelia). Ten thousand pollen grains then weigh 43 µg (Phacelia) to 670 µg (Aceraceae). Recalculating this to the expected pollen percentage in honey (10,000 grains per gram honey (Van der Ham et al. 1999)), this means that a 'normal' honey would contain 43 µg to 670 µg pollen/g honey (or 43 mg/kg to 670 mg/kg). This corresponds to 0.043 to 0.670 ‰. The latter is only 4 times lower than the 0.25% of Graham (1992). If honey 27 (Figure 12) with a share of maize pollen of 4.2% was a 'normal' honey with 10,000 grains per gram, the maize pollen content of the honey would be only 0.042 X 670 = 28 µg/g. However, the weight share of the maize pollen might be higher than 4%, since maize pollen is very big and heavy: one pollen grain weighs 250-350 µg (Fonseca et al. 2003). According to Van der Ham et al. (1999), honeys of both oilseed rape and fruit trees carry pollen numbers up to around 10,000 per gram honey, and need to carry at least 45% of the name giving pollen species: 4,500 grains per gram honey. From Table 5 (slope column) it can be deduced that 1 µg pollen of Rosaceae represents 78 grains resulting in a weight of 0.0128 µg per grain. 4,500 grains per gram honey would weigh 58 µg. For oilseed rape, 1 µg pollen is 74.7 grains, resulting in a weight per grain of 0.0134 µg per grain, and 4,500 grains weighing 60 µg. Another approach to estimate the weight of a pollen grain was used by Kerkvliet (personal communication): by calculating the volume of a pollen grain from its measured diameter ($4/3 \times \pi \times r^3$) and assuming a specific gravity of 1,0, this would result in one million pollen grains weighing:

- Phacelia: diameter = 20 µm: 4.2 mg
- Acer: diameter = 30 µm: 14.1 mg

[5] These very high values for the coefficient of determination did hold or even increased when the linear regression was done using the log-transformed data for both the dependent in independent variable. This means that the high values for the non-transformed data were not only due to the fact the most data points (except for the highest concentration) clustered near the origin.

These figures very well match with our estimation: 4.3 and 67 mg respectively. Although our approach appears reliable, it is just the first step; more research in this direction has to be done.

6 Conclusions

This report presented an overview of relevant information concerning the relationship between honey bees and pollen dispersal, based on a literature survey, a database of pollen compositions of Dutch honeys and a concise laboratory experiment. The main conclusions of the report, clustered per topic, are the following:

Beekeeping in The Netherlands

- Both hobbyist- and professional beekeepers practice beekeeping in The Netherlands. Of the approximately 8,000 Dutch beekeepers, about 6,900 are organised in three beekeeping associations, of which the Dutch Beekeepers Association (*Nederlandse Bijenhoudersvereniging* – NBV) is by far the largest with 6,000 members.
- Hobbyist beekeepers have an average of five colonies, professional beekeepers well over one hundred.
- In urban areas in The Netherlands, more and more people are taking up beekeeping. Bees are being kept everywhere from small suburban backyards to high-rise rooftops and balconies. Yet, this group represents a small part of the total number of beekeepers in The Netherlands.
- Around ten beekeeping companies are professionally involved in the pollination of crops with honey bees and make a living out of it. Overall, it is estimated that something like 32,000 honey bee colonies are utilized for pollination in The Netherlands by approximately 1,700 beekeepers.
- Hobbyist beekeepers seldom move their hives (except when hired for pollination in fruit orchards), whilst professionals regularly move their hives (every 4 weeks on average).
- In The Netherlands, honey bees are being utilized in fruit orchards, in the horticulture industry and for seed production. Professional beekeepers or beekeeping companies cover nearly all pollination in closed greenhouses and polytunnels. However, regular collaboration takes place with hobbyist beekeepers, in order to guarantee the supply of bee colonies. Pollination in the fruit sector is mostly covered by non-commercial, hobbyist beekeepers.
- The annual value of pollination fees is roughly estimated at 4 million Euro for outdoor cultivation, and 7 million Euro for cultivation in greenhouses and polytunnels. However, the indirect economic value or benefit of pollination by honey bees and other insect pollinators is considered to be much higher.
- Honey bees are not only utilized for pollination. Several products that are produced by bees can be harvested by the beekeeper. Obviously, the most recognizable product made by honey bees is honey. Other products include beeswax, propolis, royal jelly, bee pollen and bee venom. All of these bee products (could) contain (trace amounts of) pollen.
- Most honey on the Dutch market (92%) is imported from amongst others China and Argentina; the two largest honey-producing countries in the world.

Foraging and pollen transport

- Honey bees visit flowers for pollen (their protein, fat and mineral source) and nectar (energy source). Individual bees specialize and do not forage for nectar and pollen simultaneously and devote their foraging to only one plant species at a time.
- Foraging is not only optimized at the individual forager level, but on the whole colony level. This is achieved through communication and task division.
- Only a small part of the pollen that is collected on flowers is available for pollination: bees use more than 99% as food.
- The distance up to which bees forage for water, pollen and nectar may range up to 2.5, 10 and 14 km respectively. However, if a rich resource is nearby, most of the foraging trips will be much shorter (0.5-1 km). If food nearby the colony is scarce and very rewarding patches are further away, large flight distances are likely to become frequent.
- Inside the hive of honey bees, pollen is exchanged unintended between individuals: this may lead to pollen transport between distant flower patches that are not visited by the same individual forager.
- For oilseed rape, it has been shown that a) bees may fly longer distances to forage on such fields, and b) bees tend to stay close to their hive if the hive is located next to an oilseed rape field.
- Apple is a very attractive crop for honey bees to forage on. However, it is not documented which distances honey bees fly to forage on apple, because it is common practice to place colonies in (or very near) apple orchards in order to optimize pollination.
- Honey bees are not attracted to the crop potato, unless aphids feed on the potato plants. However, farmers rarely tolerate aphids that are attractive to honey bees, and even if aphids are present, aphid densities often peek before flowering.
- Sugar beet crops are not meant to flower and are therefore not attractive to honey bees.
- Maize, although it is a wind pollinated plant, is also visited by honey bees. However, no data is available about the distances bees cover for maize. In addition, hardly any of the maize pollen collected may result in pollination, since honey bees are not at all interested in female maize flowers.
- Though potential flight distances of honey bees have been measured and estimated in a number of detailed studies, it is almost impossible to set up a general model that predicts activity- and pollination patterns for specific cases, as the number of conditions that can influence such patterns is almost unlimited.
- It should be kept in mind that besides honey bees, also bumble bees and many other insects are involved in pollen transport. Therefore, much information on what this means at a landscape scale is needed to estimate its impact on the out-crossing phenomena.

Pollen in honey

The presence of pollen in honey

- Honey contains small amounts of pollen. This pollen has entered the honey through three pathways: direct contamination of nectar at the flower, contamination by bees inside the hive, and contamination by the beekeeper during honey harvesting and processing. Contamination by the bees during processing inside the hive is the most important route of pollen into honey.
- Although the botanical origin of the pollen grains in honey can be an indication of the nectar source of the honey, this is not straightforward due to the contamination paths mentioned above. Blending of commercial honeys may also be an additional obstacle to traceability.
- About 200 honey samples, from a survey throughout the country in The Netherlands (2008), carried pollen grains of at least 50 plant families, wind pollinated as well as insect pollinated species.
- Rosaceae pollen (including apple), Brassicaceae pollen (including oilseed rape) were very abundant. Chenopodiaceae (to which Beta belongs) and Solanaceae (to which potato *Solanum tuberosum* belongs) and Poaceae (grass family, including maize) were also represented in the honeys. Maize pollen was only encountered twice (out of 200) and in very small quantities.

Experimental estimation on the pollen content of honeys

- The numbers of pollen grains, counted in five of the Dutch honeys from the survey, corresponded well with those reported in literature, generally topping at 10,000 grains per gram honey.
- The reliability of pollen counting depends on the dilution of the suspension used in counting chambers and needs to be further improved.
- A strong and significant relationship was found between the amount of pollen (grams) added to an artificial honey, and the counted number of grains in the honey. The highly significant calibration factors were used to estimate real pollen concentrations (μg/g) of real honey. Preliminary results indicate that only trace amounts of pollen are present in honey, ranging from 43 μg to 670 μg pollen/g honey.
- Based on the calibration curves, oilseed rape honey and fruit blossom honey were calculated to contain at least 60 and 58 μg of the specific pollen (oilseed rape, apple and pear) per gram of honey respectively.

7 List of references

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8 List of interviewed persons

- Boot, Willem. Owner Inbuzz, professional beekeeping company. 8 September 2011.
- Dommerholt, Jan. Chairman Dutch Beekeepers Association (NBV). 15 July 2011.
- Rietveld, Aat. Vice-chairman Dutch Beekeepers Association (NBV). 15 July 2011.
- Vorstman, Jeroen. Manager 'Bijenhuis', Dutch Beekeepers Association (NBV). 15 July 2011.

9 Annexes

Annex 1 – Pollen determination tables

Table A1: list of pollen in 'spring' honeys, harvested in May-June 2008

Table A2: list of pollen in 'summer' honeys, harvested in July-August 2008

Table A3: list of pollen in 'autumn' honeys, harvested in September – October 2008

Annex 1. pollen determination tables

Table A1

List of the 'spring' honeys from the survey of 2008. These honeys were harvested in May and June.

- The honey samples are listed with an identification number. Of each honey about 500 pollen grains were determined up to family level the exact number determined per sample is in column 2 ("Totaal aantal pollen"). The pollen families are listed in the top header of the table.
- ∞: this pollen was over-represented, and not included in the determination of the 500 grains.
- The columns with the pollen of Brassicaceae, Rosaceae, Solanaceae and Chenopodiaceae have been marked yellow.
- The honeys used for the pollen counting (Chapter 5) have been marked as blue coloured rows.
- "Gemiddelden" in the lowest row means: average. This average is of the positive samples only (zeros are not included).

Honingmonsternummer	Totaal aantal pollen	Aceraceae	Apicaceae	Aquifoliaceae - hulst (ilex)	Asteraceae	Balsaminaceae	Berberidaceae - berberis	Betulaceae	Boraginaceae	Boraginaceae - vergeet-me-nietje	Brassicaceae	Campanulaceae	Caprifoliaceae	Caryophyllaceae	Celastraceae - kardinaalsmuts	Chenopodiaceae	Cistaceae	Cupressaceae - Juniperus communis	Elaeagnaceae	Ericaceae	Fabaceae	Fagaceae	Fagaceae - eik	Fagaceae - Castana sativa	Grossulariaceae	Hippocastaneaceae	Hyacintaceae	Hydrophyllaceae - phacelia	Juncaceae	Lamiaceae	Liliaceae	Loranthaceae	Lythraceae	Malvaceae	Menyanthaceae - Nymphaea	Oleaceae	Oleaceae -ligustervulgaris	Onagraceae	Papaveracea	Pineaceae	Plantaginaceae	Poaceae	Polemoniaceae - Gilia capitata	Polygonaceae	Polygonaceae - rumex, zuring	Primulaceae	Ranunculaceae	Rhamnaceae Rhamnus frangula	Rosaceae	Rubiaceae -Gallium	Rutaceae	Salicaceae	Saxifraga	Simaroubaceae - hemelboom	Solanaceae	Taxus baccata	Tiliaceae - linde	Vitaceae	onbekend
2	520		1								325											4				43					35											2						9	97			4							
3	500		41		3						40										299												3				3						1						93			16							1
5	504							5			286		6								3					25																						3	129			47							
6	511							1			57										40			13		5				1										1		3						17	360			6					7		
8	523		1		1						10										25			405	2	27				1	7						5					1						1	22			7					6		2
12	523		1		2			2			432														3	15				1																			19				43						5
13	509		4			2		5			402										11		1			6				1	7						1			1	17								28			23							
21	508										5										105			336						1		1																	9			3					48		
24	503				2			6			199										1		4			36					2																	4	101		6	138							4
26	569		7		3	2		1			219										7		6							14																			140			170							
28	526		1					1			383									1	3		1																			1							29			29							77
30	539		3					1	1	14	28									1	20		5			30											1				1			11				9	216			136							62
33	532				1			1			159						2				161			86		3				4							8					7							83			12		1			2		2
35	565		1						4	1	4		17								21				30	136	3			1												10						1	231	6		96							3
38	528		8		5			7		∞	100									2	10			2		31					7											1						7	136			205							7
41	543				5			1		?	401										13					4				1																4			55			56							3
42	511		9					3			349		4								10		2	1		26					7										3							1	61			35							
47	531		1		2			24		∞	127							1		1	41			21		10					8											8						9	137			139					2		
48	525				3			4		2	26										57			15		107				2	1						1	2							5		1	18	191			57							33
55	551		2		1			2	2		21										54					12									1														163		13	272				1			7
57	502	9	1								421										1		1			3																2							56			7							1
58	507		4					5			278										9																				1	2			10				87			111							
60	543				12						26									1	12					6					1						2									8			52		6	414							3
66	534		7		24			2			62										82			5		51					19						1										1	68	148			62							2
77	515		4		5						30										75			345		2		1									4											22	23			4							
78	542		2		1			6			430									5	5			8		5		1			6										1				1				49			17							5
80	545				4			5			319									2	48		1	3		10					3											5						11	116			18							
82	548		11		15			5			340										9			38		9		2			8						1					1			5				70			19							15
84	543				2			12			169										10																				1							3	341			4					1		
86	503	2	1		2			10			333		1								10			32																									67			38		4					3
88	583	6	5					43		156	215										14					33					1											8							65			33		1					3
90	543				2						63			1						2	22		1	116		6		2			4														21		4	2	250			46							1

Honingmonsternummer	Totaal aantal pollen	Aceraceae	Apicaceae	Aquifoliaceae - hulst (ilex)	Asteraceae	Balsaminaceae	Berberidaceae - berberis	Betulaceae	Boraginaceae	Boraginaceae - vergeet-me-nietje	Brassicaceae	Campanulaceae	Caprifoliaceae	Caryophyllaceae	Celastraceae - kardinaalsmuts	Chenopodiaceae	Cistaceae	Cupressaceae - Juniperus communis	Elaeagnaceae	Ericaceae	Fabaceae	Fagaceae	Fagaceae - eik	Fagaceae - Castana sativa	Grossulariaceae	Hippocastaneaceae	Hyacintaceae	Hydrophyllaceae - phacelia	Juncaceae	Lamiaceae	Liliaceae	Loranthaceae	Lythraceae	Malvaceae	Menyanthaceae - Nymphaea	Oleaceae	Oleaceae -liguster vulgaris	Onagraceae	Papaveracea	Pineaceae	Plantaginaceae	Poaceae	Polemoniaceae - Gilia capitata	Polygonaceae	Polygonaceae - rumex, zuring	Primulaceae	Ranunculaceae	Rhamnaceae Rhamnus frangula	Rosaceae	Rubiaceae -Gallium	Rutaceae	Salicaceae	Saxifraga	Simaroubaceae - hemelboom	Solanaceae	Taxus baccata	Tiliaceae - linde	Vitaceae	onbekend
91	0		5					5			351										3																					1			7			1	90			67							3
94	533	11	9		12						208										19		4			11														1		3						18	212			108					5		3
98	624		5		2			14			17										145			206		8					2						7								1			3	79			30		2				59	
101	580				3			9		296	39										13			4		22					5											1			3			23	65			9		5					5
105	502				4					412	1										13					13					15											3						9	7			29			1				
108	507		1		3			1			331									12	15		12			1																3		3				7	140			7		3					6
110	545										7										111		1	256							1						7					3		3					14			3			13		84		
114	503		1		17					164	72										21		7			75					6						1								10			13	87			45		1					
115	520		6		1			9		∞	51										114		37			28					3									3		3						72	161			67			7				2
117	564		6								453										9		1	2		4					3																			32			5		3			1		
118	519		1		9			13		56	70												12	24		29					1																			64			249			1				4
119	533				1			94	15	∞	62										5		5			60					1								1	1								13	164			100							1
120	523							3			11									4	50					7					1						21					3							336			30		30	27		3		
121	526		3		43						36			1							300		3			3					1						3				3	9			7			7	54			11		5	10				3
123	502				1			3		276	19										73		9			22																						34	88			13		12			1		4
128	555										282									10	1							1			1										10							119		10	68							
129	502				2						27					1				1	13			177				2			410						1													14			1					1		1
130	651	3	18		1			1			168										21		1	11		40					27			1			9					5		13				22	66			90			5	1			3
137	506				5															7	80		38	2		33					1													1				10	305			54							
142	536		8		3			1			212									5	40		5																		5	3		89				11	208			8			11				
144	609				5					453	36									1	3		10			5																							33			87			7				
146	640				1			38		168	38									3	1		1			5																	1						1	50			243			3				1
149	554				5			16		84	36									4	19		1			28				1															10				11	102			198			14				2
156	531	3	20					19	1	34	19										48		5			86				1	1					1				1				3			5	1	227			98			6				2
159	581				24					4	3										38		17	25		38											215					7		14				61	39					44	1		24		
166	554		23		3	3		1			250									2	1		6	2		16																	5		1				2	16			175		1	9				3
172	519		1								18			1						5	85		1	17		3		380														3						1	13			7			11		1		1
174	548	3	1		1			1			426										26		7			5																			1					18			7			2				7
177	560	3			5			5			280													47		15			18													5		7			1	131			26				11				6	
181	539	7	7		5			1		62	31		1					3						8		8	1		13			1				19										232		17	99				12				12	
185	505	3	9		3			9		6	9								1		15		17			212											19				1	1			13				145			13		1	1		1		22
186	512		5		3			1	1		33		4									1		17		1			1	17	1	1						3			1		17		1		164		15	150			1	13				61	
189	501				1			23		98	107										9		9			6										64				3					1			59	46				73	1	1				
194	501	8			3		3			226	47									15	11		7			42														1		3		5				15	65		5	54							7
197	517		7		3	1		10	9	56	18	3								75	75		13	17		27																		11		19				17	103		1	73	9	5	3				9
202	564	5		3	11					∞	87				1					3	17		11	12					6								10				1				15			115	121			74			9				7
Gemiddelden	528	5	6	3	6	2	3	10	5	135	151	3	6	1	1	1	2	2	1	7	41	3	7	73	12	27	2	56	10	4	19	1	3	1	1	28	16	3	1	2	3	4	8	13	7	4	77	18	104	70	7	69	32	8	7	1	12	35	8

Table A2

List of the 'summer' honeys from the survey of 2008. These honeys were harvested in July and August.

- The honey samples are listed with an identification number. Of each honey, about 500 pollen grains were determined up to family level. The exact number determined per sample is in column 2 ("Totaal aantal pollen"). The pollen families are listed in the top header of the table.
- ∞: this pollen was over-represented, and not included in the determination of the 500 grains.
- The columns with the pollen of Brassicaceae, Rosaceae, Solanaceae and Chenopodiaceae have been marked yellow. Sample 27 and 56 show a yellow marked cell for Poaceae: these pollen grains are from maize (*Zea mays*).
- The honeys used for the pollen counting (Chapter 5) have been marked as blue coloured rows.
- "Gemiddelden" in the lowest row means: average. This average is of the positive samples only (zeros are not included).

Honingmonsternummer	Totaal aantal pollen	Aceraceae	Apicaceae	Aquifoliaceae - hulst (ilex)	Asteraceae	Balsaminaceae	Berberidaceae - berberis	Betulaceae	Boraginaceae	Boraginaceae - vergeet-me-nietje	Brassicaceae	Campanulaceae	Caprifoliaceae	Caryophyllaceae	Celastraceae - kardinaalsmuts	Chenopodiaceae	Cistaceae	Ericaceae	Eucalyptus	Fabaceae	Fagaceae	Fagaceae - eik	Fagaceae - Castana sativa	Hippocastaneaceae	Hydrophyllaceae - phacelia	Iridaceae
1	565		1								65									56			266	1		
4	505										28									52			357			
7	503				2				5		13									35			420			
10	573		5		7			16			26									14				38		
11	528		101		11			1	3		7									122		1	5		3	
22	524		1		1			3			5						2			15			371	13		
23	548				11			8			48					1		1		36		286		44		
25	517		4		10			3			82									73		188		13		
27	643		1		42			6			55					4		7		228	1		37	23		
29	509		4		1	2		1			421									3				18		
31	468	7	2					36			85	14	4				2			11		4		10		1
32	542		1		3						6						2			255			182			
36	573		1		1				2		6		5				2			267				15		
39	503									∞	27						9			155			3	7		
40	548	5	2		5					11	43		30				3			144			2			
44	514		14								16	1	1							378			1			
45	516			1	1						9						1			102			305			
54	506		6		11						17									400						
56	519		6		13			3			36									272			24			
61	503		18								32							1		27	400					
67	534		1		3			25			13									72			343		7	
71	508				5						100									64					17	
75	503				2	375														11			114			
76	701										28									14			403	2	1	
83	531		8		30			7			82									202			108		1	
85	550							6			10									88			342			
89	687		2		1			1			22									71			459	2	1	
92	590				7						18									54			270	20		
93	509	1	21		1	1					15									128			264			
95	503		4					7			12									241			17			
99	548							3			13							1		50			71	21	7	
100	500		1								15					17				21			213			
102	614		1								1									125			185	7		
103	549				1						1									134			74	4		
104	501																			111		3	75			
106	529										13										13		64			
107	504							63		378												1	3			
109	533		1					1			17									41		1	355			

Honingmonsternummer	Lamiaceae	Liliaceae	Listaceae	Loranthaceae	Lythraceae	Malvaceae	Oleaceae	Oleaceae -ligustser vulgaris	Onagraceae	Pineaceae	Plantaginaceae	Poaceae	Polemoniaceae - Gilia capitata	Polygonaceae	Polygonaceae - rumex, zuring	Ranunculaceae	Rhamnaceae Rhamnus frangula	Rosaceae	Rubiaceae -Gallium	Rutaceae	Salicaceae	Saxifraga	Simaroubaceae - hemelboom	Solanaceae	Taxus baccata	Tiliaceae - linde	Violaceae	onbekend diversen
1								30		3							11	119			1					11		1
4		14						33				2					2	8								9		
7								9									1	12								3		3
10	8	6			2												18	263			158							12
11	7	1						1						126			1	41			47		6	3	3			38
22	4	6						8			1	4					7	28			21		14			20		
23	11	3			1			5									2	24			47		6			11		3
25		2						11			1		3					83			13		1			25		5
27	7							9			4	27		8	12			72			83					10		7
29										1		1						21			36							
31	3	2										5			9		8	58			200							7
32	16	7						19										44								6		1
36								1									3	251			17			2				
39	6	5								1		1						179	7		101							2
40	2	20	3					1		1	1	3					2	222			37			5		6		
44		1					31					4					1	30			6			30				
45		5			4						1	3					1	18			8		17			22		18
54											2	5			5		22	25			9							4
56								1			6	8						31			118					1		
61												1						19			5							
67		18						9			6	3				12	1	10			11							
71	293	15																14										
75														1														
76		3						8			1	2			1		15	125			57		8			10		23
83		19						6		7							4	41			7		4					5
85								3									4	83			5		6			2		1
89		18						3				3			2		2	72			20		6					2
92								7						7				180			11		16					
93	1							2				6			5		2	35					10			12		5
95								57				7					2	97			10		17					32
99		256															4	85			24							13
100		160						18				4					10	1								35		5
102	1	147						8									15	17					65			33		9
103		300						1									5	10					10			9		
104		268						5										18			1		11			9		
106		394												3			9	24			1		1			7		
107		4										1						25			24							5
109		17						3							3		5	15			1					73		

Honingmonsternummer	Total aantal pollen	Aceraceae	Apicaceae	Aquifoliaceae - hulst (ilex)	Asteraceae	Balsaminaceae	Berberidaceae - berberis	Betulaceae	Boraginaceae	Boraginaceae - vergeet-me-nietje	Brassicaceae	Campanulaceae	Caprifoliaceae	Caryophyllaceae	Celastraceae - kardinaalsmuts	Chenopodiaceae	Cistaceae	Ericaceae	Eucalyptus	Fabaceae	Fagaceae	Fagaceae - eik	Fagaceae - Castana sativa	Hippocastaneaceae	Hydrophyllaceae - phacelia	Iridaceae	Lamiaceae	Liliaceae	Listaceae	Loranthaceae	Lythraceae	Malvaceae	Oleaceae	Oleaceae -liguster vulgaris	Onagraceae	Pineaceae	Plantaginaceae	Poaceae	Polemoniaceae - Gilia capitata	Polygonaceae	Polygonaceae - rumex, zuring	Ranunculaceae	Rhamnaceae Rhamnus frangula	Rosaceae	Rubiaceae -Gallium	Rutaceae	Salicaceae	Saxifraga	Simaroubaceae - hemelboom	Solanaceae	Taxus baccata	Tiliaceae - linde	Violaceae	onbekend diversen	honingsoort
111	516							5			3									61			335		3			40												1				21			14			14		12		7	zomer, aug
112	566										3							1		38			152					159						16									3	3			7		4			180			zomer, juli
113	502									33	45									7		3	396					6										2						7					1			2			zomer, juli
116	536		2		1					220										13			188											5				3					3	3			1					96		1	zomer, juli
122	518		3		3			11			141			5								21		69				14								1		7					23	128			89							3	zomer, aug
124	539		8		9			5										56		60		50		1										17				1						134			53		125			16		4	zomer, juli
125	535				3			3		∞	33							3		31		9	1					3															1	381			51			13				3	zomer, aug
127	520				1			1			110							3		23		66		19	1			12										1			1		63	129			78		1	7				4	zomer, aug
132	540	42				111		1		105	15													160			15		1	1		3					3				1		3			5	21			13			33		
133	515				3	143					47													111					3	17		10				1		1								11	137			9			11	2	
135	529	4	1		1						75							11		25		17	8	8			1	5		1								3			9		15	256			78			1		5		5	zomer
138	543										1							3		54		5	80	7				44					3					5			20		12	295			8					6			zomer
140	534										29							1		40		7	424					1										1		5				16			6			1		1		2	zomer
143	509										5									132			263		3								48					1					1	11					27			15		3	zomer, juli
145	525		8		1			1		237	39							2		16		5	158	3			1														2		7	19			7		1	3		1		14	zomer
147	532		6		1			3		1	17							1		26			403	1														1			13			32			21		1	1		1		3	zomer
151	540		3		24	1				15	40	3							11	170		3	6	45			1	1					21							7			11	76			1	7	1	21		1		71	zomer, juli
152	352	6	1		3			3	11	15	23	1	30							66			19	13	1			1			1			27						5			13	45										68	zomer, juli
153	521	3				10		7	1	∞	231							21		17				21			1	5										1		3			11	132			47				1			9	zomer, juli
154	563	1	8		3						12									178		12	31	20										28				1		10			12	131			76			14		26			zomer, juli
155	502		2			1		2		85	148									6		2		6				3													3		1	47			195							1	zomer, juli
158	534		8		3			1										3		11		8		144			1	1										3		7			9	253			11			7				64	zomer
160	541		3		9						82							7		193		1	21	19	1		8	18									1	1		13		3	5	45			102			8				1	zomer, aug
161	534				3			3			9							243	29	133			39									8						1		1				3			45		1	1				15	zomer, juli
162	538				7		3	11		61	62	1								91		13	54	43	1								7					1		3			7	73			88			7		5			zomer, juli
170	505		13		3			5			13	5			1					108		16	162		29			17						75	10			1		3			5	15			5		6	4				9	zomer
171	562		16					1			120							5		73		19	5	3	32													1		13			164	37			46			23				4	zomer, juli
173	561		14		5			9			190		7					3		143		3	5	11	11												1			4			7	70			64			6				8	zomer, juli
190	583	1	1		5	1		7	1	96	33							24		40		13	72	30			3	3					6							1			1	174			27	3	1	23		9		8	zomer
192	558							7	3		7							1		117		3	194	5	1								3					5		1			3	180	9		3			9				7	zomer, aug
195	500		15		39		3	5	5	36	102							5		99		5	11	47									1					1		1			6	39			56			7		5	1	11	zomer
200	509							1	1		150									80			1										9	1				7		81			75	42			47		11					3	zomer, aug
201	514				3						15									42		9	433					8						1																		3			zomer, juli
203	559		3								16									11		6	440											5									25	27			13			1	1	10		1	zomer, juli
204	519	1			5	469			1		11									10			7					2						2				1		4				4										2	zomer, aug
205	523	1	1		5	27					46									215				1				6										1		7			7	168			27			11					zomer, juli
206	503		5		1	470					5									12																							1	9											zomer, aug
207	514		11			74				1	79							11		39		1	90		11			27						1					3	3				52			67			3		7		34	zomer, juli
208	539		1								25	1								106		4	63	14																	22			214			51			1		7		30	zomer, aug
Gemiddelden	533	7	8	1	7	130	3	7	3	92	47	4	13	5	1	7	3	18	20	91	138	25	162	26	7	1	20	45	2	6	2	7	14	12	10	2	2	3	3	13	7	8	12	78	7	8	42	5	14	9	2	18	15	12	

Table A3

List of the 'autumn' honeys from the survey of 2008. These honeys were harvested in September-October.

- The honey samples are listed with an identification number. Of each honey about 500 pollen grains were determined up to family level the exact number determined per sample is in column 2 ("Totaal aantal pollen"). The pollen families are listed in the top header of the table.

- ∞: this pollen was over-represented, and not included in the determination of the 500 grains.

- The columns with the pollen of Brassicaceae, Rosaceae, Solanaceae and Chenopodiaceae have been marked yellow.

- "Gemiddelden" in the lowest row means: average. This average is of the positive samples only (zeros are not included).

Honingmonsternummer	Totaal aantal pollen	Aceraceae	Apicaceae	**Asteraceae**	Balsaminaceae	Betulaceae	Boraginaceae - vergeet-me-nietje	Brassicaceae	Campanulaceae	Caprifoliaceae	Caryophyllaceae	Carnaceae	Cistaceae	Ericaceae	**Fabaceae**	Fagaceae - eik	Fagaceae - Castana sativa	Grossulariaceae	Hippocastaneaceae	Hydrophyllaceae - phacelia	Iridaceae	Lamiaceae	Liliaceae	Lythraceae	Malvaceae	Myrtaceae	Oleaceae	Oleaceae -liguster vulgaris	Pineaceae	Plantaginaceae	Poaceae	Polemoniaceae - Gilia capitata	Polygonaceae	Polygonaceae - rumex, zuring	Rhamnaceae Rhamnus frangula	Rosaceae	Rutaceae	Salicaceae	Saxifraga	Simaroubaceae - hemelboom	Solanaceae	Tiliaceae - linde	onbekend
9	528		3	9	251			34							15		193					1	8					2		1				1	1	8						1	
34	503				1			20	1						105			3	10									6		3	1				3	320		21				1	8
37	569		1	40	3	2	∞	79		2	1				56				5			2	44	5		225			1		6	17	3			63			9			2	3
43	518		35	1				87					35		235		69					4						1								28		23					
52	531			437	2			2						5	49					28										1	2					3	2						
69	536		1	243		17		18							139							1					61						1	2	11	17		24					1
70	533		10	15		7		128							126	1				1					1										70	139		28					7
72	559		60	35				102							4					52		305								1													
73	537		8	57		1		62							170		33		23	48		10									3					87		27					8
74	517			1	419										7		56			5	3							2			9		5		3	2				5			
79	635	1	8	11	48	3		41						6	52		26						229												3	191		14					2
81	549			1		1		41							365		68						12								2					58		1					
87	555		18			6	59	23							240		161			3			6					11		2	8					13				1			4
136	500							3						462	7		13		7				1											6									1
148	503		14	25		2	2	8							86	2			4	230								19					19		6	24		21		5	7	26	3
150	516		7	3		2		4	10			3		124	72	1	1			242			1					7			11		10		5	1						6	6
Gemiddelden	537	1	15	**68**	121	5	31	43	6	2	1	3	35	149	**108**	1	69	3	10	76	3	54	43	5	1	225	61	7	1	2	5	17	8	3	13	68	2	20	9	4	7	7	4