Invasion of *Varroa* mites into honey bee brood cells

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Invasion of *Varroa* mites into honey bee brood cells

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'He must be a dull man who can examine the exquisite structure of a comb, so beautifully adapted to its end, without enthusiastic admiration'

(Charles Darwin, The origin of species)

Stellingen

1. De strofe 'Oh, East is East, and West is West, and never the twain shall meet' kan niet geciteerd worden om aan te geven dat de Oosterse kultuur voor Westerlingen ondoorgrondelijk is. De hoofdrolspelers in Kipling's ballade blijken elkaar juist uitstekend te begrijpen.
(Rudyard Kipling, The ballad of East and West)

2. Omdat in een demokratie alleen het totaal aantal stemmen telt is een zwaar stemmenverlies geen goed argument om een partij een hoofdrol in de formatie te ontzeggen.
(Algemeen kommentaar na afloop van tweede kamer verkiezingen, mei 1994)

3. De onder imkers populaire bewering dat het gebruik van alkoholische versnaperingen de bijen steeklustig maakt is een voorbeeld van de menselijke eigenschap om eigen onhandigheid aan anderen toe te schrijven.

4. Voor een toekomst in de wetenschap verdient het aanbeveling geen of geen plaatsgebonden partner te hebben zodat het mogelijk is in het 'post-doc' circuit over de wereld te zwerven.

5. Promovendi kunnen rekenen op efficiënte begeleiding als eventuele kosten voor hun wachtgeld voor rekening komen van het begeleidende instituut.

6. Wanneer studenten hun studie sneller doorlopen zullen bij de huidige stand van de werkeloosheid de kosten voor de maatschappij stijgen.

7. De bij heeft het laatste woord.
(Van Dale, twaalfde uitgave 1992, pagina 3743)

Stellingen behorend bij het proefschrift:

Invasion of *Varroa* mites into honey bee brood cells

Wageningen, 1 februari 1995

Willem Jan Boot

Table of contents

General Introduction

Varroa jacobsoni Oudemans has become the most important pest of the Western honey bee, *Apis mellifera* L. It has spread almost all over the world (Matheson, 1993), and severe losses of colonies due to the *Varroa* mite have been reported (e.g. De Jong et al., 1982). Yet a few decades ago almost nobody had even heard of the *Varroa* mite.

Originally, the *Varroa* mite only occurred in colonies of the Eastern honey bee, *Apis cerana* Fabr., which species is restricted to Asia. In 1904, Oudemans described the *Varroa* mite as a parasite of Eastern honey bees in Indonesia, but in the fifty years that followed Oudemans' observations almost no reports were issued on this mite (Ritter, 1981). This lack of interest illustrates that the *Varroa* mite was not considered a problem in colonies of its original host. Although the actual damage inflicted on the Eastern honey bee has never been determined, this view probably still holds. How could this mite develop from a harmless curiosity into a universal threat to beekeeping? This happened because beekeepers moved the Western honey bee into the area of distribution of the Eastern honey bee. The *Varroa* mite started to parasitize the Western honey bee, wherever it had access to it (Ritter, 1981). The mite appeared to be a harmful parasite on its new host, but before beekeepers realized this it had been spread all over the world through shipments of colonies and queens (De Jong et al., 1982).

The immediate threat of *Varroa* to its newly acquired host resulted at first in a vigorous search for acaricides to prevent further losses. Many chemicals were screened and nowadays several effective acaricides are available (Koeniger & Fuchs, 1989; Ritter, 1990). They are applied by the beekeepers on a large scale. Using acaricides to control the mites has some drawbacks, however. Acaricides may contaminate bee products such as honey and wax (Lodesani et al., 1992). Even minimal contamination or the mere threat of contamination may decrease their value as a 'natural' product. Furthermore, in future mites may become resistant to the acaricides used, which implies the need for alternatives. Thus, new *Varroa* control methods in which application of acaricides is minimized or made redundant, should be developed.

To find new control methods against the *Varroa* mite, more knowledge about the biology of the mite and about its interaction with the honey bee is needed, because such knowledge may reveal the Achilles' heel of the mite. In this thesis I concentrate on invasion behaviour of the mites into brood cells. *Varroa* mites survive on adult bees, but they can only reproduce inside the brood cell (Ifantidis & Rosenkranz, 1988). Therefore, they have to leave the bees and invade drone or worker brood cells. Insight into the factors that play a role during invasion of mites may contribute to development of new control methods in two ways. Firstly, insight into the process of invasion may be used to manipulate the mites. For example, stimuli that attract mites into the brood cells may be used to trap them, or factors that make brood cells less attractive may be used to diminish their invasion into brood cells, which in turn diminishes reproduction of the mites. Secondly, insight into the process of invasion may enable a better application of current control methods, because the process of invasion into brood cells determines the distribution of mites over brood cells and adult bees, and because in current control methods either mites on the bees or mites in the brood cells are killed. For

example, biotechnical control methods have been developed in which mites are trapped in brood cells (e.g. Rosenkranz & Engels, 1985; Maul et al., 1988; Calis et al., 1993b; Fries & Hansen, 1993). Insight into the process of invasion may show how the number of mites trapped can be increased.

I have studied invasion into brood cells in different ways. Firstly, events occurring when a cell is invaded by a mite have been observed directly (chapter 1). The observations provide background information for interpretation of results described in the following chapters. In chapter 2-5, the population of mites on the adult bees has been studied with respect to invasion behaviour, by asking the following questions: when do the mites leave the adult bee and invade a worker or drone brood cell, which factors determine the rate of invasion, and what is the relation of the length of the period that mites stay on adult bees with mortality and reproduction of the mites? In chapter 6-8, attractiveness of brood cells to the mites has been studied. Why are drone cells more frequently invaded than worker cells, and why does the size of brood cells affect invasion by the mites? In other words: which factors of the brood cell play a role during invasion of brood cells? The effect of methyl palmitate, that has been claimed to be the factor determining the attractivity of cells to mites (Le Conte et al., 1989; Trouiller et al., 1992), has been tested in chapter 8. In chapter 9, results of the previous chapters have been integrated to develop a simple model of invasion by *Varroa* mites into honeybee brood cells. In this final chapter, population growth is taken as a fitness measure to increase insight into which selective forces may have shaped the various reproductive strategies used by honey bee mites.

Summary

The parasitic mite *Varroa jacobsoni* is one of the most serious pests of Western honey bees, *Apis mellifera*. The mites parasitize adult bees, but reproduction only occurs while parasitizing on honey bee brood. Invasion into a drone or a worker cell is therefore a crucial step in the life of *Varroa* mites. In this thesis, individual mites, the population of mites and characteristics of honey bee brood cells have been studied in relation to invasion behaviour. In addition, a simple model has been developed to study which selective forces may have shaped the strategies used by *Varroa* mites with respect to the allocation of invasion and subsequent reproduction over drone and worker brood cells of the honey bee.

Invasion behaviour of individual *Varroa* mites (chapter 1).

Preceding invasion, *Varroa* mites are carried close to a suitable brood cell by a honey bee. The mite moves directly from the bee into the selected brood cell, crawls between the larva and the cell wall, and moves on to the bottom of the cell. At the moment of leaving the bee, the mite cannot touch the larva. It still has to cover the distance from the cell rim to the larva, which measures 4-7 mm in cells that are attractive to the mites. Thus, information to decide whether to stay on the bee or to invade a brood cell is acquired at a distance from the larva, possibly by a volatile chemical or by differences in temperature. Since invasion only occurs when a bee brings a mite close to a suitable brood cell, the chance of being carried close enough may well limit the number of mites that invade. If so, population growth of the mites is limited in turn, because the mites reproduce exclusively inside brood cells.

Invasion of brood cells by a population of *Varroa* mites.

Invasion into worker brood cells (chapter 2 & 3)

Within a day after emergence from a brood cell, i.e. the moment when *Varroa* mites begin their residence period on adult bees, some mites invade a new brood cell. The percentage of mites on adult bees that invade per day depends on the number of cells suitable for invasion and on the number of bees in the colony, regardless of the time that the mites have stayed on adult bees. The more cells and the fewer bees, the higher is the percentage of mites that invade per day, as expected when invasion is limited by the chance of being carried close enough to a suitable brood cell. This can be understood as follows. Since only one or a few bees can be near a cell simultaneously, the chance of being carried close enough for invasion increases when the number of brood cells increases. In addition, in a smaller bee colony, but with the same number of brood cells, the mites are spread over a smaller number of bees. The number of bees that come close enough to a suitable cell stays the same, and therefore the mite's chance of invasion is increased.

The percentage of mites that invade per day decreases when young open brood, still too young to be suitable for invasion by mites, is present. This decrease in invasion rate may arise because the mites prefer to be carried by young adult bees, which are likely to stay in the brood nest area. Within the brood nest area these young bees are divided over areas with brood cells that are suitable and areas that are unsuitable for invasion by mites. Hence, an

increase in the amount of unsuitable open brood may keep part of the preferred young bees away from the suitable brood cells, and may thus decrease the invasion rate.

If invasion is limited by the chance of being carried close enough to a brood cell, the spatial distribution of the mites inside the colony may affect invasion. In the areas where invasion occurs, the mite density on the bees will decrease. The mites will redistribute spatially by movement of the bees that carry them and by moving from bee to bee, but depending on the rate of this phoretic process invasion will be more or less limited. However, the rate of invasion was similar in bee colonies in which either 600 brood cells were available for mite invasion during one day or three times 200 brood cells were available during three days, whereas the colonies were comparable in all other respects. Thus, on a time scale of days the process of redistribution of mites inside the colony seems to be fast enough to prevent a significant effect of the period of brood cell availability on the rate of invasion. This is important for application of biotechnical control methods in which brood combs are introduced into the colony to trap mites. The 'trapping combs' are removed from the colony and the mites inside the cells are killed. Our results have shown that the number of cells used for trapping the mites is crucial, whereas the period during which the cells are available to the mites is of minor importance.

Invasion into drone brood cells (chapter 4)

Invasion by a population of mites into drone brood cells is similar to invasion into worker cells, except that invasion into drone cells is a much faster process. When invasion is compared between colonies with either exclusively worker cells or exclusively drone cells, *Varroa* mites invade a drone cell about 12 times more frequently than a worker cell. Hence, when both types of brood cells are available a biased distribution of 12 times more mites in drone cells than in worker cells is expected based on the differential frequency of invasion. This expected bias is larger than the bias actually found in colonies with both types of brood cells, which measures on average 8 times more mites per drone cell than per worker cell. The lower actual bias when compared to the expected one may be understood as follows. In normal honey bee colonies invasion into drone and worker cells is probably more or less segregated in time. Since the frequency of invasion is much higher per drone cell than per worker cell, the number of mites on bees will decrease much faster during periods when drone cells are abundantly present. Fewer mites will invade drone cells than expected when a constant number of mites on bees is assumed. Hence, the actual distribution over drone and worker cells may be less biased than expected from the differential frequency of invasion per cell. In addition, the biased distribution is sufficiently explained by the differential frequency of invasion per cell alone. There is no reason to believe that mites respond to the presence of nearby drone brood cells by refraining from invasion into worker brood cells, thus causing the biased distribution over drone and worker cells. Since the rate of invasion into drone brood cells is high, a trapping method using drone combs may be very effective in controlling the *Varroa* mite. When no other brood is present, 462 drone cells were estimated to be sufficient to trap 95% of the mites in a colony of 1 kg of bees.

Effect of the period spent on adult bees on reproduction of the mites (chapter 5)

No correlation has been found between the length of the period that *Varroa* mites stay on adult bees (1-20 days) prior to invasion and the total number of offspring per mite, the

number of viable daughters per mite, the fraction of mites without offspring, and the fraction of mites that produces only male offspring. Thus, reproduction of the mites is apparently independent of the period that the mites reside on adult bees prior to invasion into brood cells.

Mortality of mites during the period spent on adult bees (chapter 5)

Mortality of *Varroa* mites, as measured by counting mites fallen on the bottom of the hive, occurs primarily right after emergence from the brood cell. When brood containing mites emerges during one day, 18% of the mites that have been present on the emerging brood is found on the bottom of the bee hive at the end of that day. Part of these mites may already have died inside the capped brood cells, and have fallen down after cleaning of cells by the bees. At the second and third day following emergence, respectively 4% and 2% of the mites on adult bees is found on the bottom, whereas from the fourth day on (up to 23 days) only 0.6% of the mites on adult bees is found on the bottom per day. Since the number of mites on the bottom of the hive will be strongly associated with the number of freshly emerged mites, counting the number of dead mites on the bottom may be a useful tool to estimate infestation levels in honey bee colonies.

Attractiveness of brood cells to *Varroa* mites

The attractive period of worker and drone brood cells (chapter 6)

Worker brood cells are attractive to *Varroa* mites from 15-20 hours preceding cell capping, whereas drone cells are attractive from 40-50 hours preceding cell capping. Since the attractive period of drone cells is 2-3 times longer than that of worker cells, drone cells are consequently expected to be invaded 2-3 times more frequently. Actually, a drone cell is invaded 12 times more frequently than a worker cell. Hence, more factors must be involved in causing this difference in frequency of invasion. When the frequency of invasion is proportional to the surface of a brood cell, more mites are expected per drone cell due to its 1.7 times larger surface than a worker cell. Taken together, this would result in a 3.4-5.1 times higher frequency of invasion, which is clearly much lower than the 12 times actually found. Therefore, the higher frequency of invasion into drone cells may be attributed for an important part to differences in the information mites use to select a cell for invasion, either quantitatively or qualitatively.

Effect of larva-cell rim distance on attractiveness of brood cells (chapter 7)

Varroa mites are not randomly distributed over different types of cell which contain similar larvae. Per cell, more mites invade into shorter and narrower cells than control cells, whereas fewer mites invade into longer and wider cells. The period during which cells are attractive to mites varies among the different cell types, and whether in a certain type of cell more or fewer mites are found in comparison to control cells, is correlated with the length of the attractive period of that type of cell. The type of cell also affects the distance from larva to cell rim in the period preceding cell capping. When this distance is larger in comparison to control cells with larvae of the same age, the attractive period of the brood cells is shorter and vice versa. Since in all cell types the distance from larva to cell rim continuously decreases preceding cell capping, this negative correlation suggests that there is a critical larva-rim distance under which brood cells are attractive to the mites. Then, the length of the attractive period of brood cells depends on the moment this critical distance is

reached. The distribution of mites over different cell types in turn results from differences in the attractive period. In normal drone and worker brood cells the critical larva-rim distance for invasion is 7-8 mm.

Effect of methyl palmitate on attractiveness of brood cells (chapter 8)

Since *Varroa* mites decide at some distance from the larva whether to stay on a bee or invade into a cell, they may well use a volatile chemical to select a brood cell. A few aliphatic esters, predominantly methyl palmitate, have been claimed to be this volatile signal for the mites for two reasons. The mites respond to the esters in an olfactometer (Le Conte et al., 1989), and the levels of the esters in worker and drone larvae may explain that drone cells are attractive during a longer period and are invaded more frequently than worker cells (Trouiller et al., 1992). However, invasion itself is unaffected by application of methyl palmitate to brood cells. In addition, analysis of headspace volatiles above attractive brood cells showed hundreds of components in the volatile blend, but in only 2 of 17 analyses a trace of methyl palmitate was found. Hence, there is no reason to believe that methyl palmitate is used as a signal for invasion by the mites.

Reproductive strategy of *Varroa* mites (chapter 9)

Since reproductive success of *Varroa* mites is higher in drone cells than in worker cells, the question arises why the mites do not restrict invasion to drone cells. Therefore, a simple model using population growth as a fitness measure has been developed to study under which circumstances specialization on drone brood would be a better strategy to adopt than reproduction in both types of cell. For European *A. mellifera*, the model suggests that if mites have to wait less than 7 days on average before they can invade a drone cell, specialization on drone brood would be a better strategy. This is close to the estimated waiting time of 6 days. Hence, small differences in reproductive success in drone and worker cells, and in the rate of mortality may determine whether specialization on drone brood will be promoted or not. In European *A. mellifera* colonies, *Varroa* mites invade both drone and worker cells, but specialization on drone brood cells seems to occur to some extent because a drone cell is more frequently invaded than a worker cell. In the parasite-host association of *V. jacobsoni* with African or Africanized *A. mellifera* or with *A. cerana*, the mites also invade both drone and worker cells, but the mites specialize on drone brood with respect to reproduction since a large percentage of the mites in worker brood do not reproduce. Only in the parasite-host association of *Euvarroa sinhai*, a mite closely resembling *V. jacobsoni*, and *A. florea* specialization is complete because these mites only invade drone brood.

Does current knowledge of invasion behaviour help in controlling the *Varroa* mite?

Our research on invasion behaviour did not result in a method in which *Varroa* mites are controlled using attractant or repellent chemicals. We still have to identify the signal the mite uses to invade a brood cell, although we know that mites perceive this signal at a distance from the larva and that the larva-cell rim distance affects the response of the mites to it. However, our results on invasion behaviour are useful to understand the possibilities and limitations for improvement of biotechnical control methods. We now know how many drone or worker cells are needed in a 'trapping comb' to catch a certain percentage of the mites in a colony. In theory, control methods that make use of 'trapping combs' are simple. In practice

however, the methods may become complicated because application is integrated with other activities by the beekeeper like building of new colonies and swarm prevention. In addition, application of biotechnical control methods is usually labour intensive. Our results can be applied to design the simplest method that is sufficiently effective. This will remain an important application in future. Since much research is nowadays directed to breed honeybees that are less susceptable to *Varroa* mites (Woyke, 1989b; Büchler, 1994; Moritz, 1994), the effectiveness of control methods needed for control may decrease, which allows simplification of control methods. By combining simple 'trapping comb' methods and breeding of *Varroa*-less susceptable honey bees, there is a clear perspective for beekeeping without the use of acaricides to kill *Varroa* mites.

1

Behaviour of *Varroa* mites invading honeybee brood cells.

Abstract

Invasion behaviour of *Varroa jacobsoni* into honeybee brood cells was studied using an observation hive. The mites were carried close to a suitable brood cell by the bees. Subsequently, the mites moved from the bees to the rim of the cell, walked quickly inside, crawled between the larva and the cell wall, and moved onto the bottom of the cell. *Varroa* mites were never seen walking across the comb, and entering and leaving brood cells as has been described for *Tropilaelaps clareae*. Differences in invasion strategies between *V. jacobsoni* and *T. clareae* are discussed.

Introduction

The parasitic mite *Varroa jacobsoni* is currently the most important pest of *Apis mellifera*, worldwide. *Varroa* mites parasitize both adult bees and bee brood. They feed on the haemolymph of adult bees and additionally use the bees as transport vehicles within the colony as well as for dispersal from colony to colony. Reproduction of the mites only takes place while residing on honeybee brood. Consequently, the mites have to leave the bees and invade the brood cells (Ritter, 1981; Ifantidis & Rosenkranz, 1988).

Since invasion into brood cells is indispensable for reproduction, knowledge about invasion behaviour and the factors affecting this behaviour may provide a basis for development of new control methods. For example, the finding that *Varroa* mites invade drone cells in larger numbers than worker cells (e.g. Fuchs, 1990), prompted a biotechnical control method in which mites are trapped in drone cells and subsequently removed from the colony (Schulz et al., 1983; Rosenkranz & Engels, 1985).

Invasion has been studied primarily at the population level. Studies have been done on the distribution of mites over different types of brood cells (e.g. Ruijter & Calis, 1988; Büchler, 1989; Fuchs, 1990), and on the distribution of mites over adult bees and brood cells (e.g. Woyke, 1987; chapters 2,3). In these studies the resulting distribution was studied, whereas the underlying behaviour was reconstructed indirectly by inference. Direct studies on behaviour of individual mites have been carried out in artificial settings (e.g. Otten & Fuchs, 1988; Le Conte et al., 1989; Rickli et al., 1992) because manipulations to see individual mite behaviour are difficult in a bee hive. Such studies are useful to provide answers to specific questions, e.g. whether mites react to certain odours (Le Conte et al., 1989; Rickli et al., 1992), but the relevance of these observations to the behaviour of mites under realistic conditions is not immediately obvious. In this study mite behaviour was observed in a more realistic setting to identify which factors and conditions may play a role during invasion of mites, and to help interpretation of observations in artificial settings.

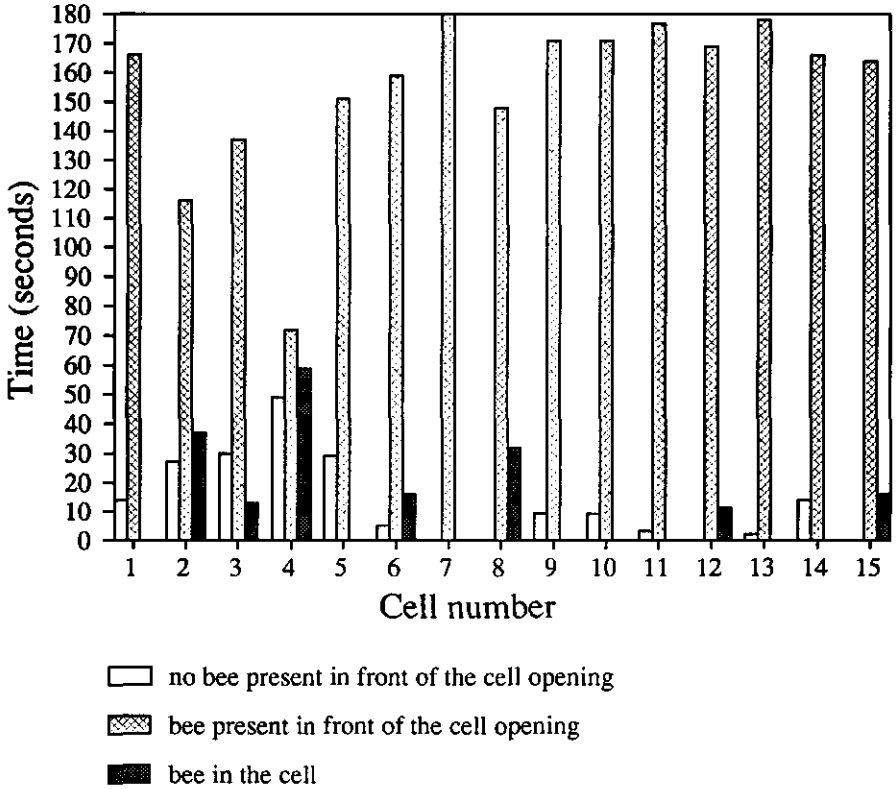

Figure 1: Position of the bees at the cell opening of 15 invaded cells during the three minutes preceding mite appearance at the bottom of these cells. The position of the bees was divided into three classes: (1) no bee present in front of the cell opening, (2) bee present in front of the cell opening and (3) bee in the cell. The time distribution over these three classes is shown.

Materials & Methods

Invasion behaviour was observed in small, heavily infested *Apis mellifera* colonies. A specially designed observation hive and two types of frames were used, the design of which was recently described in detail by Beetsma et al. (1993). Below, the frame types are described to clarify the nature of observations on mite behaviour.

Observations with a 'half-comb' frame

The cell walls of newly constructed worker combs were cut from the bottom of a comb and melted carefully onto a transparent sheet. Subsequently, this sheet was fixed into a frame of 22 x 35.5 cm. The frame thus held only half a comb, allowing observations through the cell openings as well as through the cell bottoms. Two movable cameras were

positioned on either side of the comb in such a way that events at the opening and at the bottom of the same cells were recorded simultaneously. The hive was illuminated with infra-red light, using a ring of 50 light-emitting diodes (LED's), placed at the side of the cell openings. At this side, the mites, being red brown under natural light, turned out to be white in the recordings with infra-red light, contrasting strongly to the dark bees on which the mites resided. The infra-red light transmitted through the bee larvae covering the cell bottoms caused the mites in the cells to be visible as dark spots on the video screen due to absorption of part of the light. The cell bottom side of the comb was not illuminated directly, because mites and bee brood are both white under infra-red light, and cannot be distinguished. Generally, two hive parts, both containing a 'half-comb' frame, were connected to each other because in a larger colony more brood can be nursed.

Observations using a frame of cells with transparent side walls

A considerable percentage of the mites reside on the ventral side of the bees (Kraus et al., 1986). When the 'half-comb' frame was used for observations, bees walking over the comb surface presented their dorsal aspect to the camera and the mites on the ventral side of the bee could not be recorded. In addition, bees blocked the view on the cell opening, thereby hindering observation of invasion behaviour. Hence, another frame type was constructed in which mite invasion could be observed through opposite transparent Perspex cell walls of vertical rows of cells (Beetsma et al., 1993). Larvae were grafted into the cells and, when the larvae were large enough, events were recorded with two cameras directed towards the same cells from opposite sides. Again the observation hive was composed of two hive parts, one containing the frame with the transparent side walls, and the other containing a 'half-comb' frame to provide ample space for honey and pollen storage, and for oviposition by the queen. The larvae in the cells with the transparent cell walls were nursed well by the bees, and therefore this setting was assumed to be realistic. However, manipulations of the brood may have interfered with invasion of the mites.

Results

Observations with a 'half-comb' frame

When *Varroa* mites invaded brood cells, they crawled between the cell wall and the larva until they reached the bottom of the cell, where they stayed immobilized (Ifantidis, 1988; chapter 6). Mites were visible from the moment they reached the cell bottom, but some time elapsed between their entering into the cell and their appearance at the cell bottom. Our observations suggested that this movement required about one minute. Therefore, the position of the bees present at the cell opening of the invaded cells was observed during three minutes preceding appearance of the mite at the cell bottom, because within that period the mite probably invaded the cell (Fig. 1).

Positions of the bees were divided into three classes: (1) no bee present in front of the cell opening, (2) bee at least partly covering the cell opening and (3) bee present in the invaded cell. Bees often moved their head towards the cell opening, apparently for a quick inspection, but this was classified as 'bee in front of the cell opening'. Only when the head and thorax of the bee were in the cell was it classified as 'bee in the cell'. In such cases the bee probably fed the larva, which takes 10 seconds up to a few minutes (Brouwers et al., 1987).

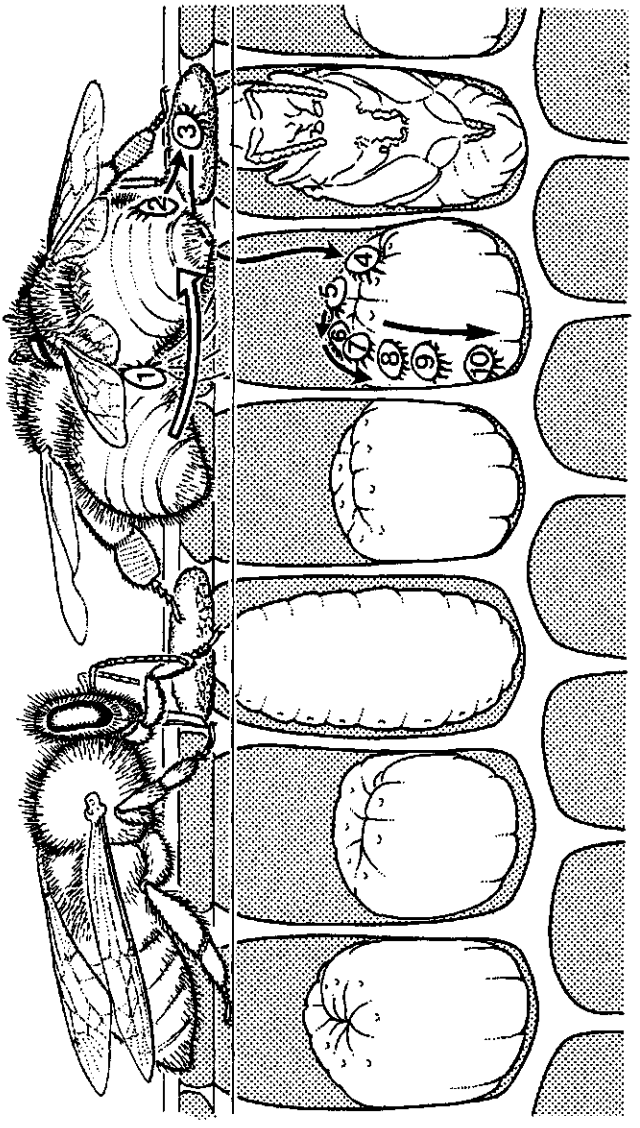

Figure 2: Invasion of a Varroa *mite into a brood cell, as observed with a frame containing cells with transparent side walls.*

Moving into the cell by the bee was not necessary for mite invasion to occur, because only in 7 out of 15 mite invasions was a bee present in the cell during part of the 3-minute-period preceding mite appearance (Fig. 1). The mites probably invaded the cells when a bee was present in front of the cell opening. In all 15 cases observed, this position was found most frequently during the 3-minute-period preceding mite appearance. Mites were never observed walking across the comb surface, suggesting that the mites went directly from the ventral side of the bee into the cells.

When the 'half-comb' frame was used, the dorsum of bees walking over the comb surface was recorded. Consequently, even a glimpse of the mites was rare at the cell opening, as the bees blocked the view. Mite invasion was observed once, however, when the bee on which the mite resided fortuitously moved away from the cell opening. This mite went from the bee to the cell rim, and immediately entered the cell, after which it could no longer be observed at the cell opening. After 67 seconds, the mite reappeared at the bottom of the cell.

Observations using cells with transparent side walls

With the transparent side wall type of frame, only 4-5 cells were observed simultaneously because a large magnification was needed. Additionally, not all cells observed were attractive to mites simultaneously, and since invasion into a particular cell was a rather rare event, only three mite invasions were recorded despite our efforts. One of these mite invasions could be followed clearly (Fig. 2). First the mite walked over the abdomen of the bee (1-2). The mite then left the bee and went to the cell rim/cap of the adjacent cell (2), moved quickly around over the cap and entered the cell (3). The mite subsequently walked for 3 seconds on the surface of the larva (4-6), before it crawled between the larva and the cell wall (7) and then moved on slowly to the bottom of the cell (8-10). After 34 seconds from the time of entering the cell the mite had covered half the distance from the surface of the larva to the cell bottom (9; this could easily be marked as the moment the mite moved in front of the gut of the larva), and 65 seconds after entering the cell the bottom was reached (10), which matches with the 67 seconds period needed to reach the cell bottom in the observation with the other frame type.

The other two observations of mite invasion were less clear. The mites could only be observed from the moment of cell invasion, because the large number of bees present near the cell opening hindered observation. After invasion, the mites walked for 4 and 2 seconds, respectively, over the larva before crawling between the larva and the cell wall. The sites chosen by the mites to crawl between the larva and the cell wall could not be observed clearly, and therefore the moment the mites reached the cell bottom was not determined. In one of the two recorded invasions, the mite had moved half-way the larva after 31 seconds since entering the cell.

Discussion

In principle, *Varroa* mites should invade brood cells as quickly as possible because they cannot reproduce while on adult bees. Still the mites may reside several weeks and longer on adult bees, depending on the number of brood cells that are available for invasion (chapters 2,3). Probably invasion into brood cells is relatively slow because the distance between the mite on the bee and the brood cell has to be small before invasion can occur. For instance, during the ten minutes after invasion of the single mite as described in Figure 2,

eight other mites were seen on the bees passing the recently invaded cell, but they did not invade the cell. Since infested cells seem to be just as attractive to mites as non-infested cells (Fuchs, 1985), the distance between mite and cell may have been too large for invasion. In addition, some of the mites on adult bees are positioned between the abdominal sternites (Kraus et al., 1986; Rath, 1993). Since they are positioned deep between the sternal plates, these mites may be unable to react to stimuli coming from the brood cell that play a role in invasion.

When mites leave a bee to invade a brood cell, they do so without having had prior contact with the larva. The mites have to cover the distance from the cell rim to the larva, which measures 4-7 mm in cells that are attractive to mites (Goetz & Koeniger, 1993). Thus, information to decide whether to stay on the bee or to invade a brood cell is acquired at a distance from the larva, possibly by a chemical signal or by differences in temperature. It has been shown that *Varroa* mites in artificial settings react to a number of chemicals (Rickli et al., 1992; Le Conte et al., 1989). However, reaction of mites to these chemicals should also be studied while the mites stay on bees, because the mites go directly from the bees into the brood cell. When not residing on a bee, mites may be motivated to find an adult bee rather than a larva. Kraus (1993) recently showed that *Varroa* mites indeed prefer the scent of adult bees over the scent of larvae when tested in an olfactometer.

The mites were never seen walking on the comb, or entering and leaving the brood cells to select a cell for invasion. This strategy is used by *Tropilaelaps clareae* (Laigo & Morse, 1968; Woyke, 1989), another parasitic mite of honey bees, which also has to invade brood cells for reproduction. Both mite species recently started to parasitize *A. mellifera*, *A. cerana* being the original host of *Varroa* and *A. dorsata* being the original host of *T. clareae* (De Jong et al., 1982). Walking across the comb to select a brood cell has two advantages for *T. clareae*. First, they can reach a new brood cell quickly enough to survive because, in contrast to *Varroa*, *T. clareae* cannot survive on adult bees for more than two days (Woyke, 1984). Secondly, by reaching a new brood cell faster, they can reproduce earlier than *Varroa*, which is probably one of the main causes of the higher growth rate of the *T. clareae* population when compared to *Varroa* in *A. mellifera* colonies (Woyke, 1987). However, in colonies of the original host of the *Varroa* mite, *A. cerana*, walking across the comb to select a brood cell for invasion may be a risky strategy. *A. cerana* was observed to kill *Varroa* mites while they were on adult bees (Peng et al., 1987; Büchler et al., 1992). Nevertheless, *Varroa* mites still survive for long periods outside brood cells in *A. cerana* colonies, probably by hiding in relatively safe sites on the adult bees (Rath, 1993). Possibly, *Varroa* mites minimize exposure to adult bees in not walking across the comb to select a brood cell.

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2

Invasion of *Varroa jacobsoni* into honey bee brood cells: a matter of chance or choice?

Abstract

Invasion of *Varroa* mites into honey bee brood cells was studied in three initially mite-free colonies. Frames with emerging worker brood, heavily infested with mites, were introduced into each colony and removed the next day. During the experiments ample worker brood was available for the mites to invade. Invasions into brood cells started immediately after the introduction of the mite-infested combs, showing that mites do not necessarily have an obligatory residence period on adult bees before invading a cell. However, as most mites stayed on the bees for several days or even several weeks, the average rate of invasion was rather low. In addition, replicate experiments in three colonies showed much variation: 50% of the mites invaded brood cells within 2.0 days in the first replicate, within 8.3 days in the second replicate and within 4.3 days in the third replicate. Possible causes for the low and variable invasion rates are discussed.

Introduction

The parasitic mite *Varroa jacobsoni* Oud. is currently a major pest of the Western honey bee, *Apis mellifera* L. Female mites parasitize both adult bees and brood, but they reproduce only in brood cells (Grobov, 1977; Ritter, 1981; De Jong, 1984; Ifantidis & Rosenkranz, 1988). When a young bee emerges from a cell, the mites and their offspring also leave the cell. Subsequently, the female mites disperse over the bee population (Le Conte & Arnold, 1987; Kovac & Crailsheim, 1987) and remain on adult bees until they invade new cells. The cycle of transport by bees, invasion and reproduction may be repeated several times (De Ruijter, 1987).

Varroa mites invade brood cells during the last day before cell capping (Ifantidis, 1988; Fuchs & Müller, 1988; chapter 6). A mite may well obtain information from a brood cell which determines whether it stays on the bee or invades the cell. However, when mites become receptive to information from brood cells is unknown. In this paper we focus on the length of the period that *Varroa* mites spend on adult bees between reproductive cycles, more specifically on how it affects invasion into brood cells.

When bees are used by the mites as no more than transport vehicles, it pays the mites to take the first opportunity to invade a suitable cell, because the sooner the mites invade a brood cell, the sooner they can reproduce. Thus, fitness of mites is increased by minimizing their stay on adult bees, especially if contact with adult bees is not required for subsequent reproduction, as found by De Ruijter (1987). However, it has often been suggested that the mites choose to stay for a certain period on adult bees (Schulz, 1984; Fuchs, 1985; Hänel & Koeniger, 1986; Moosbeckhofer et al., 1988; Beetsma & Zonneveld, 1992) Such a choice may increase fitness of the mites when feeding on the bees stimulates their reproduction later. In addition, freshly emerged young mites may have to complete maturation and may therefore stay longer on the bees before invading a new cell than older mites that have invaded a brood

Table 1: Estimated probability, P_t, for a mite present on an adult bee, to invade a brood cell in relation to the time spent on adult bees. P_t was calculated by dividing the number of mites that invaded during a certain day by the estimated number of mites on the bees at the beginning of the same day.

	Probability of invasion per day		
Time interval (days)	Replicate 1	Replicate 2	Replicate 3
1	0.28	0.03	0.14
2	0.30	0.03	0.24
3	0.32	0.13	0.08
4	0.26	0.08	0.11
5	0.37	0.07	0.21
6	0.37	0.12	0.17
7	0.17	0.11	0.03
8	0.15	0.08	0.10
9	0.24	0.05	0.08
10	0.05	0.06	0.22
11	0.17	0.05	
12	0.23	0.02	
13	0.09	0.06	
14	0.10	0.11	
15	0.21	0.03	
16	0.00	0.04	
17	0.07	0.00	
18		0.04	
19		0.04	
20		0.03	
Average probability per day	0.20	0.06	0.14

cell before. Differences in residence time between young and older mites have indeed been suggested (Schulz, 1984; Moosbeckhofer et al., 1988; Wendel & Rosenkranz, 1990).

The following hypotheses concerning invasion behaviour of *Varroa* mites can thus be formulated:

(1) *Varroa* mites are motivated to invade a new brood cell immediately after emergence from the previous cell, and thus invade brood cells as soon as the opportunity is given.

(2) *Varroa* mites choose to stay on adult bees before they become motivated to invade new brood cells.

In principle, mites could invade brood cells quickly, since they are very mobile. However, whether they do so is the subject of investigation in this paper. We tested the above hypotheses by introducing a group of mites, that were just emerging from brood cells, into a normal-sized honeybee colony that contained ample brood cells suitable for invasion by mites. The subsequent invasion by the mites into the brood cells was monitored.

Materials & Methods

Preparation of colonies

Dutch honey bee colonies were used for our experiments. These bees cannot be classified into a specific race of *Apis mellifera* because several different races have been imported. The exchange of mites between neighbouring colonies is possible (Sakofski & Koeniger, 1988; Rademacher et al., 1989), so the colonies were moved to an isolated place without other colonies within a radius of at least one km.

All brood was taken out of a colony in which invasion was to be studied later. Mites that were present on the bees were killed by 2-5 treatments with 15-25 ml 85% formic acid (Wachendörfer et al., 1985; Fries, 1989), until less than five mites were found on the bottom of the hive after treatment. Acaricides may affect the results of the experiments when residues are left in the colony. Formic acid was chosen because its residue is probably the most transient when compared with other available acaricides. When formic acid was applied, the queen was temporarily caged, as bees often kill the queen during application when no brood is present (personal observation).

Introduction of mites into the colony

Brood combs from which heavily infested bees were emerging were placed into the colony and removed the next day, thus ensuring that all mites in the colony had started their stay on adult bees on the same day.

Recapturing mites in introduced brood cells

Brood cells had to be permanently available to determine how many days mites stayed on adult bees before invading brood cells. Therefore, once a day a dated brood comb (Boot & Calis, 1991), containing about 500 worker larvae, three to four days old, was placed into the colony and removed after three days. When placed into the colony the brood was still too young to be invaded by the mites, because worker brood is invaded from 15-20 hours before cell capping (chapter 6), and the worker brood cells were capped when the larvae were five days old.

Table 2: Number of mites invading into brood cells per day, and number of available brood cells per day, in the three replicates.

Time interval (days)	Replicate 1		Replicate 2		Replicate 3	
	Number of available cells	Number of invading mites	Number of available cells	Number of invading mites	Number of available cells	Number of invading mites
1	441	202	267	38	207	135
2	532	153	189	36	610	202
3	657	112	585	145	322	53
4	539	64	272	75	218	67
5	495	66	332	60	467	110
6	654	42	457	107	337	68
7	422	12	428	84	300	12
8	474	9	479	54	360	32
9	543	12	260	28	55	24
10	640	2	555	37	448	61
11	411	6	137	30		
12	397	7	184	11		
13	541	2	424	31		
14	217	2	671	55		
15	225	4	227	12		
16	405	0	219	16		
17	302	1	75	1		
18			498	15		
19			328	14		
20			580	10		
Mites remaining on bees:		14		356		214

Three days before the introduction of the mites, the first dated brood comb was placed into the colony. This guaranteed that brood cells of the right age were available for the mites from the moment they emerged from the infested combs. Additionally, this guaranteed that many brood cells were capped before mite introduction and these cells were checked for mites that had invaded. The number of mites found in these cells indicated whether the formic acid treatments applied prior to introduction had been effective.

The capped brood cells were marked daily on transparent sheets to register the invasion times of mites that were recorded later in these cells. Because worker brood cells are invaded from 15-20 hours before capping, the number of marked cells at a certain day was assumed to represent the number of cells available for mite invasion during the previous day. After capping of the cells, the combs were taken out of the colony and the mites present in the capped cells were recorded. At the end of the experiments the mites remaining on the adult bees were killed. In the first replicate 15 ml 85% formic acid was applied four times. In the second and third replicate 1 ml of Perizin in 49 ml water (Bayer; active ingredient: coumaphos) was applied two times, to kill the mites faster and with higher precision. The dead mites that fell to the bottom of the hive were counted.

During the experiments the queen was allowed to oviposit. Eggs were laid almost exclusively into the combs with dated brood, and these combs were removed from the colony after three days. No other brood than the combs with dated brood was allowed, to keep conditions as constant as possible. Therefore, other combs in the hive were regularly inspected and when eggs were present the comb was removed.

Invasion of a group of mites into brood cells was studied three times: in June, in July/August and in August/September 1989.

Statistical analysis

The number of mites present on the bees at the beginning of every interval of one day, N_t, was estimated by summing the mites that had invaded brood cells in the following days and the number of mites remaining on the bees at the end of the experiment. Subsequently, the probability of invasion, P_t, was calculated by dividing the number of mites that invaded during a certain day, Y_t, by the estimated number of mites on the bees at the beginning of the same day.

We assumed a binomial model for the number of mites that invaded the brood cells per day, depending on the number of mites present on the bees and the probability of invasion. The probability of invasion was modelled using a logit link-function to obtain a continuous variable ranging from $-\infty$ to $+\infty$:

$$Y_t/N_t = P_t = \{1 + \exp(-L_t)\}^{-1}$$
$$L_t = \beta_0 + \beta_1 X_t + \beta_2 t$$

where t is the index of time, indicating the number of time intervals of one day; Y_t is the number of mites that invaded brood cells during time interval t; N_t is the number of mites present on the adult bees at the beginning of time interval t; P_t is the probability of invasion into brood cells during time interval t; L_t is the logit of P_t; and X_t is the number of brood cells available for invasion during interval t. The parameters β_0, β_1 and β_2 were estimated by multiple logistic regression, using maximum likelihood estimation (SAS Institute Inc., 1989).

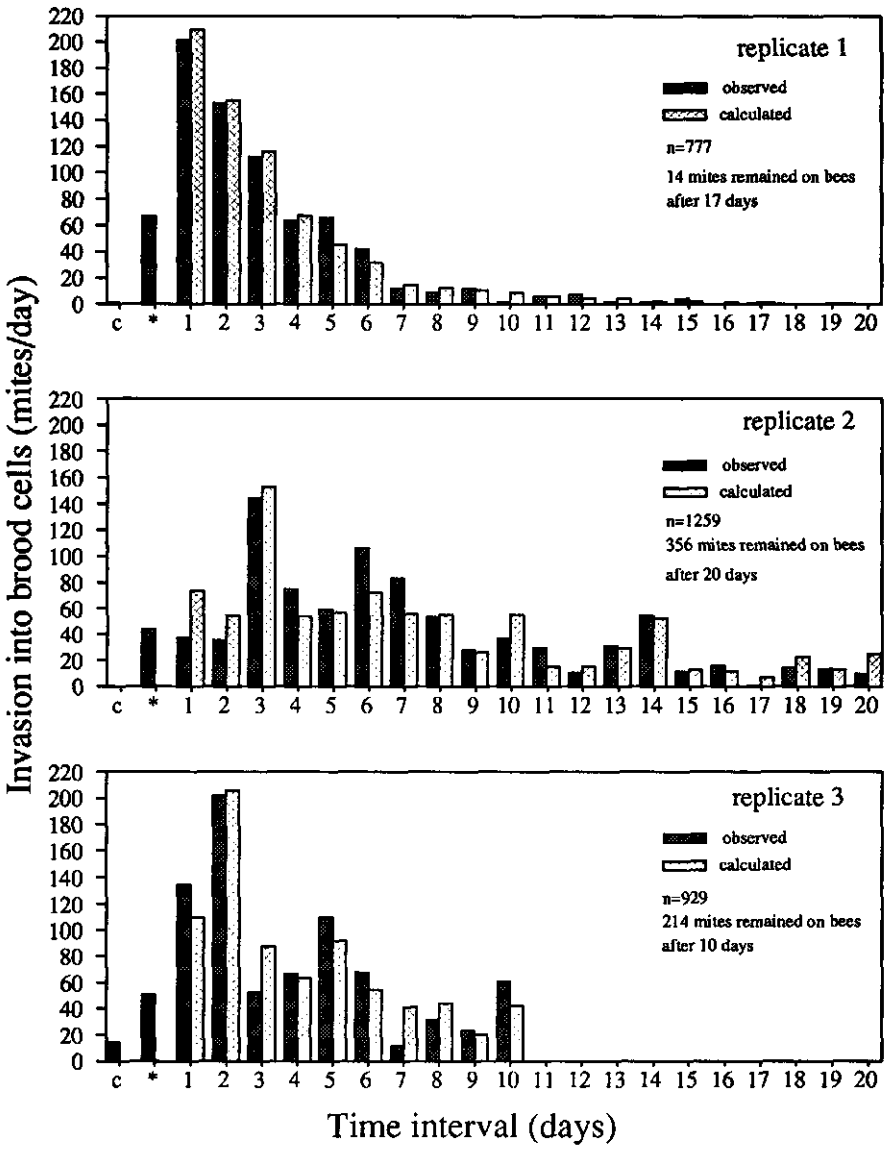

Figure 1: Observed and calculated invasion of mites into brood cells. Calculated values are based on estimates from table 3. Mites were introduced during the time interval marked with an asterisk. The interval marked with 'c' represents the mites found in the control combs before introduction.

Results

Mites started to invade brood cells within one day after emergence (Fig. 1). During the day of introduction, invasion was relatively low when compared to the next days, because emergence of bees and mites from brood cells was still in progress. Therefore, the average number of mites present on the bees was lower. Though the estimated probability for a mite to invade a brood cell varied considerably from day to day and between the replicates, it remained in the same order of magnitude in each of the separate replicates (Table 1). Therefore, invasion was assumed to be determined by the binomial probability of invasion into a brood cell, P_t, while variation in P_t is explained by the number of available brood cells and the time interval. Data are summarized in Table 2.

Invasion by mites was adequately described by this model, since observed invasion of mites and estimates from the model were similar (Fig. 1). However, the unexplained part of the variation in P_t was larger than expected using the binomial model ($P<0.001$ in all replicates using likelihood ratios as test for 'goodness of fit').

A larger number of brood cells available for invasion was correlated with a higher P_t, and a longer time on adult bees was correlated with a lower P_t (Table 3). In the third replicate the effect of time was not significant, probably because the data were collected during a relatively short period.

Qualitatively, the process of invasion into brood cells was similar in the three replicates, but large differences were found in the average rate of invasion (Fig. 2). Of the mites introduced into the colony at the start of the experiment, 50 % had invaded a brood cell after 2.0 days in the first replicate, after 8.3 days in the second replicate, and after 4.3 days in the third replicate. Part of the differences found may have been due to variation in the number of available brood cells. Therefore, invasion into brood cells was calculated based on the parameters from Table 2, and with a fixed number of 500 available brood cells per day instead of the real number. However, large differences in the rate of invasion were still found after this standardization of the number of available brood cells (Fig. 3). The estimated median time on bees before invasion into a new brood cell was 1.9 days in the first replicate, 6.3 days in the second replicate and 3.1 days in the third replicate.

Table 3: Multiple logistic regression of the available brood cells, X_t, and time elapsed since introduction, t (index of time), on the probability of invasion, P_t: $Logit(P_t)= \beta_0 + \beta_1 X_t + \beta_2 t$. Data from table 1 were used.

	Replicate 1	Replicate 2	Replicate 3
β_0	-1.39	-3.50	-2.54
β_1	0.0013^{*}	0.0030^{***}	0.0024^{***}
β_2	-0.068^{***}	-0.044^{***}	-0.023^{NS}

^{NS} = not significant
* = $P< 0.05$
*** = $P< 0.001$

Discussion

Our data show that *Varroa* mites started to invade brood cells within a day after emergence (Fig. 1). Thus, the hypothesis that mites are motivated to invade a brood cell immediately after emergence from the previous cell is supported. However, young mites that have freshly emerged may choose to stay on the bees at first, while older mites, that have invaded a cell before, start to invade immediately after emergence. Both young and older mites were introduced in our experiments, but in what ratio can they be expected to be present? In our experiments about 80% of the mites produced female offspring with an average of 1.2 viable daughters per mite, while 20% did not reproduce or produced male offspring only (chapter 5). Thus, 0.8*1.2=1.0 young mites can be expected for every older mite that has invaded before. This one to one ratio may be changed by differential mortality between young and older mites, but mortality of all mites amounted to only 20-30% (chapter 5). Thus, a significant change in the one to one ratio cannot be expected. Since young and older mites form groups of similar size, we would expect two groups in the invasion patterns found if young mites start invading brood cells later than older mites. However, in our data two groups could not be distinguished in the invasion patterns (Table 1; Fig. 1), suggesting that young and older mites behave similarly.

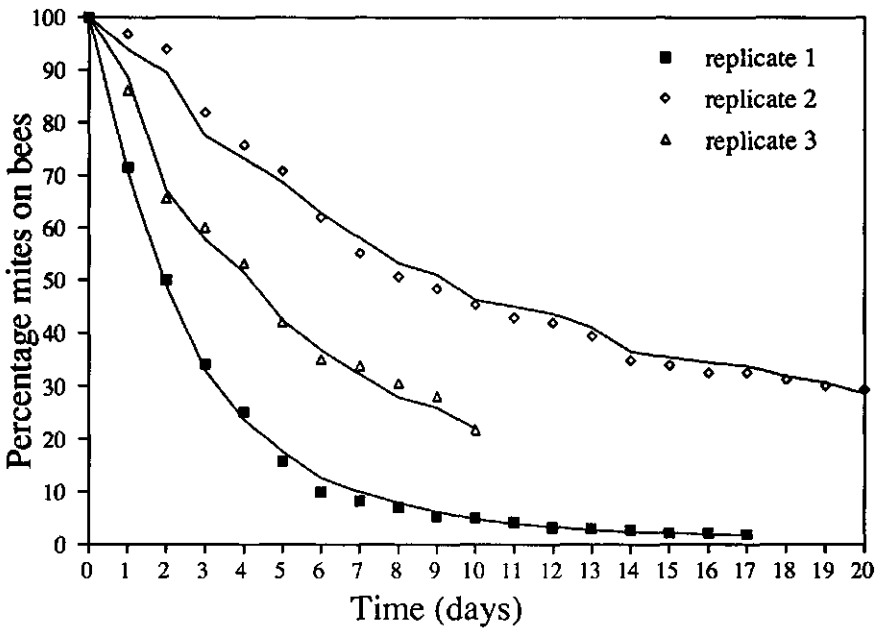

Figure 2: Percentage of mites still remaining on bees, starting from the moment all mites had been introduced. The lines represent the estimates as fitted by the model.

In our experiments, ample brood for invasion was available on each day, but the probability of invasion was still rather low: about 0.20 per day in the first replicate, about 0.06 per day in the second replicate, and about 0.14 per day in the third replicate. Even after 3 weeks a significant part of the mites still remained on the bees in replicate 2 (Fig. 1). It may be that even if mites invade brood cells as soon as possible, the opportunities to do so are limited. Observations of mite invasion behaviour showed that bees carrying a mite have to come close to the opening of the cell before the mite invades (chapter 1). Since only one or a few bees can be near a cell simultaneously, the number of bees that bring a mite close enough to a brood cell may limit invasion. If so, then the probability of invasion, P_t, will be higher when more brood cells are available, and this was indeed found (Table 3). Alternatively, not all mites may be motivated to invade brood cells at the same time, thus causing the rather low P_t found in our experiments. If so, then we would not expect the number of available brood cells and the number of bees to affect the motivation mites to invade brood cells, and thus to affect P_t. However, in subsequent experiments P_t was strongly correlated to the ratio of available cells and colony size (chapter 3). Hence the low P_t found was probably not caused by differences in motivation to invade.

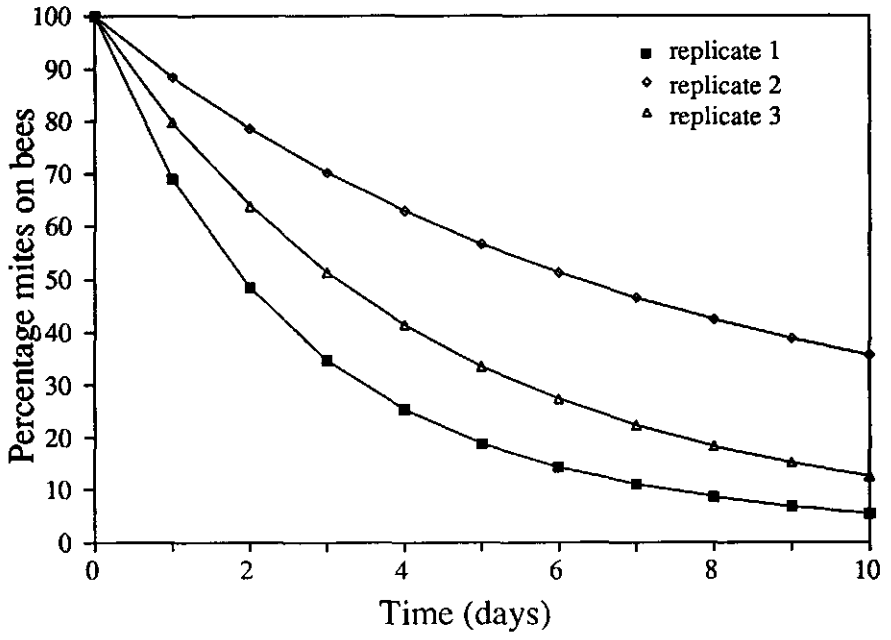

Figure 3: Calculated percentage of mites residing on bees, standardized according to the number of available brood cells (500), and based on estimates from table 3.

P_t was also negatively correlated to the time which had elapsed since the mites emerged from the brood cells. One possible explanation is that P_t decreases with time because some of the mites are present on bees that seldom come near brood cells that are available for invasion. Such mites would have a lower probability to invade. Therefore, they would represent an increasing proportion of the mites still present on the bees, resulting in a lower average probability to invade for all mites. A similar effect of time on P_t will occur when mites have a differential sensitivity to signals involved in invasion. Relatively insensitive mites would represent an increasing proportion of the mites remaining on the bees during the experiment.

Invasion of mites into brood cells was adequately described by the model (Fig. 1), but a large part of the variation in P_t remained unexplained. One of the reasons may be that for calculating P_t we excluded the mites that died during our experiments. About 20-30% of the introduced mites, were found dead on the bottom of the hive. These mites were excluded because 95% of the mortality occurred within three days after introduction of the mites into the colony (chapter 5). A large number of these mites may have died already inside the cell, and mites dying during the first days of the experiment may have been non-viable at emergence. In addition, mortality of mites may have been partly overlooked, because of mites dying outside the hive. Ignoring mortality results in an underestimate of the number of mites present at the beginning of a day (N_t), and thus in an overestimate of P_t. Variation in relative mortality per day may therefore cause variation of P_t.

Estimating the available cells from the number of capped cells, may be another reason for unexplained variation in P_t. Cells are invaded from 15-20 hours before cell capping. Because the number of capped cells was variable from day to day (Table 2), the actual number of available cells probably deviated from the estimated number. Additionally, on combs that were removed from the colony, a few cells were sometimes not capped. Mites found in these open cells were included in the number of mites that invaded during the next time interval, because these cells would have been capped within one day. However, only the capped cells were used as an estimate for the number of available cells, which was thus slightly underestimated. Other factors may also have affected P_t, and in addition variation may have been caused by a non-linear relationship of the logit of P_t with the number of available cells and time.

The rate of invasion into brood cells was different in the three replicates (Figs 2 & 3). Possibly, colony size is the main reason. Colony size was not measured, but the colony in the second replicate occupied about twice as many combs as the colonies in the first and the third replicates. If invasion is limited because only one or a few bees can be close enough to a brood cell simultaneously, a larger colony size results in a lower P_t, because the mite population is spread over a larger number of bees, while the number of bees that come close enough to a cell suitable for mite invasion stays the same. In the relatively large colony of replicate 2 a low P_t was indeed found.

Now that the invasion of *Varroa* mites into brood cells has been described qualitatively, more quantitative studies are needed. This could be done by experimentally manipulating the number of available brood cells and colony size, and studying their effect on invasion. This is important, because the invasion rate strongly affects the growth of the mite population. For instance, more than a quarter of the mites were still present on the bees

after 20 days in replicate 2. In that period another generation of mites could easily have been completed, because brood cells remain capped for about 12 days (Jay, 1963).

Knowledge about the factors affecting the rate of invasion into brood cells will also increase our knowledge about the distribution of mites over adult bees and brood. Control methods are generally based on killing either mites on bees or mites in brood cells. Knowledge of the distribution of mites over adult bees and brood may therefore enable control methods to be improved.

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3

Factors affecting invasion of *Varroa* mites into honeybee brood cells.

Abstract

To study the rate of invasion into brood cells, a group of *Varroa* mites was introduced into honeybee colonies. For each experiment one of two otherwise comparable colonies was treated, and the effect on the rate of invasion was assessed. The results showed that the rate of invasion increases with the number of available brood cells, and decreases with the size of the honey bee population. This is expected when the rate of invasion is limited because the bees have to carry the mites close to a brood cell for invasion to occur, and only a limited number of bees can come close enough to a brood cell simultaneously. In addition, the rate of invasion decreased when young brood was present. This counterintuitive result may arise because the mites prefer young honey bees, young bees are likely to stay in the brood nest area, and young bees in the brood nest are divided over areas with brood cells that are suitable for invasion and over areas with brood cells that are unsuitable for invasion by mites. Hence, an increase in the amount of young brood, which is not yet suitable for invasion by mites, may decrease the invasion rate. Differences in the period during which brood cells suitable for mite invasion were available to the mites, appeared not to affect the rate of invasion.

Introduction

The parasitic mite *Varroa jacobsoni* Oudemans endangers honey bee, *Apis mellifera* L., beekeeping all over the world. Female mites parasitize both adults and honey bee brood by feeding on haemolymph. (Ritter, 1981; De Jong, 1984; Ifantidis & Rosenkranz, 1988). Mite reproduction occurs only inside the capped brood cells of the host. Invasion of brood cells is therefore a crucial process, determining when the mites can reproduce and thus determining population growth. Moreover, the rate of invasion into brood cells determines how mites are distributed between adult bees and brood cells. This is important for control of the mites, as current methods are generally based on killing either mites on the bees or mites in the brood cells. Insight into the distribution of mites over bees and brood cells and the factors affecting this distribution, may therefore enable improvement of control methods.

Recently we found that within a colony the invasion rate of *Varroa jacobsoni* into brood cells is largely independent of the period that the mites have stayed on adult bees (chapter 2). However, differences in the rate of invasion can be large between colonies (Woyke, 1987; chapter 2). Thus, the question arises which factors affect the rate of invasion, and to what extent.

Observations on invasion behaviour of individual mites showed that a honey bee carrying a mite has to come close to the opening of a cell before the mite invades (chapter 1). Only some of the bees in a colony reside in the brood nest, and only some of the brood cells are suitable for mite invasion. Therefore, the number of bees that bring a mite close enough to a brood cell may limit invasion, since only one or a few bees can be near a cell

simultaneously. If this is so, then the rate of invasion will be higher when more brood cells are available. Additionally, in a larger bee colony, but with the same amount of brood cells, the rate of invasion will be lower, because the mites become diluted over a larger number of bees, and the number of bees that come close enough to a brood cell which is suitable for invasion stays the same.

If invasion is limited only by the number of bees that can be close enough to a brood cell simultaneously, then the rate of invasion is expected to double when twice as many brood cells are available, or the mites are residing on half the number of bees. However, mites may influence their probability of invasion by discriminating between the bees that carry them. Mites prefer young bees (1-15 days) as hosts (Schneider, 1985; Kraus et al., 1986; Le Conte & Arnold, 1987). This may increase their probability of invasion, because young bees are usually performing tasks inside the brood nest (Lindauer, 1953; Seeley, 1982). Possibly, mites even prefer nurse bees to young bees per se. When mites select nurse bees as hosts to carry them near a brood cell, the number of brood cells which are suitable for mite invasion and the number of adult bees may affect their probability of invasion relatively little, because in bee colonies the number of nurse bees is matched to the amount of brood to be nursed

Table 1: General data from the experiment to determine the effect of the number of brood cells on the rate of invasion by mites.

	Number of brood cells			
	Replicate 1		Replicate 2	
	low	high	low	high
Average number of brood cells suitable for mite invasion per time interval	255 ± 46	482 ± 115	189 ± 51	390 ± 74
Size of bee population (Kg bees) after composition of colonies:	1.14	1.19	1.38	1.40
after end experiment:	0.89	1.16	1.20	0.99
Total number of mites[1]	568	318	414	272
Total number of mites invaded within 7 days; % in parentheses	401 (71)	275 (86)	211 (51)	216 (79)

[1]*The total number of mites was calculated by summing the mites that had invaded brood cells and the mites that had remained on the bees after the experiment.*

(Robinson, 1992). Therefore, selection of a nurse bee may render the chance to come close enough for invasion relatively constant. Conversely, if mites prefer nurse bees, then their average probability of invasion may be lower when much young bee brood, which cannot be invaded by mites yet, has to be nursed, as this young brood will also attract the nurse bees.

If invasion is limited by the number of bees that come close enough to a brood cell simultaneously, it can also be limited by the spatial distribution of the mites inside the colony. In the areas where invasion occurs, the mite density on the bees will decrease. The mites will redistribute spatially by movement of the bees that carry them and by moving from bee to bee, but depending on the rate of this process, invasion will be more or less limited. Thus, a higher average rate of invasion is expected when not all brood cells that are suitable for mite invasion are available to the mites simultaneously. Because brood cell availability is then extended over a longer period, there will be more time for redistribution of the mites inside the colony.

We studied whether the above mentioned factors, i.e. the number of brood cells suitable for mite invasion, the size of the bee population, presence of young brood that is not yet suitable for mite invasion, and the period of brood cell availability to mites, affected the rate of invasion, and to what extent the rate was affected.

Table 2: General data from the experiment to determine the effect of the size of the bee population on the rate of invasion by mites.

	Size of the bee population			
	Replicate 1		Replicate 2	
	small	large	small	large
Average number of brood cells suitable for mite invasion per time interval	380 ± 64	352 ± 90	310 ± 115	300 ± 93
Size of bee population (Kg bees) after composition of colonies:	1.19	2.50	1.28	2.48
after end experiment:	1.01	2.16	1.16	1.95
Total number of mites[1]	468	385	376	214
Total number of mites invaded within 7 days; % in parentheses	315 (67)	163 (42)	243 (65)	121 (57)

[1]*The total number of mites was calculated by summing the mites that had invaded brood cells and the mites that had remained on the bees after the experiment.*

Materials & methods

Preparation of colonies

The commonly found honey bees in the Netherlands were used for our experiments. These bees cannot be attributed to a specific race of *Apis mellifera*, as many races have been imported. Two comparable colonies were obtained as follows. Three to five kg of bees from one or a few colonies were collected in a bucket and mixed by shaking the bucket. Subsequently these bees were carefully divided over two hives by weighing, and a queen was added. The colonies were moved to an isolated place without other bee colonies within a radius of at least one km, to prevent exchange of mites between the experimental colonies and other colonies (Sakofski & Koeniger, 1988; Rademacher et al., 1989). Ample honey and pollen stores were available in the colonies.

Invasion of mites was studied by introducing a group of mites into the colonies and by recapturing these mites in dated brood combs. During the week before introduction of mites, the colonies were prepared as follows. Firstly, mites already present on the bees were killed with three treatments of 15 ml 85% formic acid (Wachendörfer et al., 1985; Fries, 1989). It is assumed that only a negligible number of mites may have stayed alive in the colonies, because none or only a few (maximum five) mites were found dead on the bottom after the last formic acid treatment. Secondly, a similar amount of dated worker brood (Boot & Calis, 1991) as used later for recapture of the mites, was placed daily into the colonies. It

Table 3: General data from the experiment to determine the effect of the presence of young brood, not suitable for invasion by mites, on the rate of invasion.

	Presence of young brood, not suitable for invasion			
	Replicate 1		Replicate 2	
	yes	no	yes	no
Average number of brood cells suitable for mite invasion per time interval	316 ± 168	338 ± 180	372 ± 32	358 ± 36
Size of bee population (Kg bees) after composition of colonies:	2.65	2.70	1.23	1.24
after end experiment:	2.50	2.36	0.90	0.95
Total number of mites[1]	457	515	633	1341
Total number of mites invaded within 7 days; % in parentheses	182 (40)	296 (57)	459 (73)	1062 (79)

[1]*The total number of mites was calculated by summing the mites that had invaded brood cells and the mites that had remained on the bees after the experiment.*

is assumed that in the artificially composed colonies the balance between bees performing brood nursing tasks and other bees was restored. The time lag until a new balance is established may be a few days at most, because the hypopharyngeal glands, which produce the larval food, are fully activated in about three days after introduction of brood into a colony (Brouwers, 1983; Huang & Otis, 1989).

Release and recapture of mites

Mites were introduced by placing brood combs with emerging bees which were heavily infested with mites, into the colonies. The next day, these combs were removed. To recapture the mites, a dated brood comb with worker larvae aged three to four days old (Boot & Calis, 1991) was placed into the colony once a day and removed after capping. Worker brood cells are invaded from 15-20 hours preceding cell capping (chapter 6). Therefore, the invasion time of the mites later found in the cells could be registered by marking capped cells daily on transparent sheets. Additionally, the number of marked cells that were capped at a certain day was assumed to represent the number of brood cells suitable for mite invasion during the previous day. After capping of the brood cells on a comb, it was taken out of the colony. The cells were opened and the mites in the cells were counted. Combs with eggs had to be removed regularly, because the queen was allowed to oviposit freely, and because brood other than on the combs introduced by us was not allowed in the colonies in order to keep conditions as constant as possible.

Table 4: General data from the experiment to determine the effect of the period of brood cell availability on the rate of invasion.

	Period of brood cell availability			
	Replicate 1		Replicate 2	
	long	short	long	short
Average number of brood cells suitable for mite invasion per time interval	609 ± 37	583 ± 24	573 ± 38	597 ± 29
Size of bee population (Kg bees) after composition of colonies:	1.38	1.44	1.75	1.78
after end experiment:	0.82	0.38	1.34	1.33
Total number of mites[1]	272	560	962	799
Total number of mites invaded within 21 days; % in parentheses	253 (93)	542 (97)	856 (89)	737 (92)

[1]*The total number of mites was calculated by summing the mites that had invaded brood cells and the mites that had remained on the bees after the experiment.*

Invasion of mites was compared during seven time intervals. Afterwards, mites that still remained on the bees were killed by applying one ml of Perizin in 49 ml water (Bayer; active ingredient: coumaphos) twice. The dead mites, which had fallen down onto the bottom of the hive, were counted. Immediately after the experiments the actual size of the bee population (kg of bees) during the experiments was estimated by weighing the total hive and subsequently subtracting the weight of the hive after removal of all bees.

Types of experiments in which mite invasion was compared

One of the two bee colonies in each experiment was subjected to one of the following treatments:

1. Two-fold increase of the number of brood cells that are suitable for mite invasion.
2. Two-fold increase of the size of the bee population.
3. Presence of two to three combs with young worker brood, not yet suitable for mite invasion. The combs with young brood were positioned next to the combs placed into the colony to recapture the mites.
4. A three times longer period of brood cell availability to the mites. To achieve this, invasion of mites was compared during intervals of three days instead of one day, and into one of the colonies a comb with about 200 brood cells was introduced daily, while into the other colony a comb with approximately 600 brood cells was introduced every second day of a 3 day time interval. Because the brood combs were dated within one day, brood cells were available to the mites for a three times longer period, but the total number of brood cells was similar.

Statistical analysis

The rate of invasion into brood cells was assumed to depend on the number of mites present on the bees and the probability of invasion. The number of mites present at the beginning of every time interval was estimated by summing the mites that had invaded brood cells in the following time intervals and the number of mites remaining on the bees at the end of the experiment. Subsequently, the probability of invasion was calculated by dividing the number of mites that invaded during a certain time interval by the estimated number of mites on the bees at the beginning of the same time interval. A logit link-function was used to model the probability of invasion (chapter 2). Three parameters varied in the experiments, and were therefore incorporated in the model: the number of brood cells suitable for invasion in a time interval, the time elapsed since introduction of mites, and a parameter to account for the difference in composition between the two colonies in which invasion was compared directly:

$$P_t = \{1 + \exp(-L_t)\}^{-1}$$
$$L_t = \beta_0 + \beta_1 X_t + \beta_2 t + \beta_3 C$$

where t is the index of time, which indicates the number of time intervals of one or three days; P_t is the probability of invasion into brood cells during time interval t; L_t is the logit of P_t; X_t is the number of brood cells suitable for invasion during interval t; and C is -1 or +1 for the two colonies to be compared, respectively.

The parameters β_0, β_1, β_2 and β_3 were estimated by multiple logistic regression, using maximum likelihood estimation (SAS Institute Inc., 1989).

Results

Data concerning the number of brood cells, the size of the bee population and mite invasion in each experiment are summarized in tables 1-4. The size of the bee population decreased during the experiments by mortality of bees, which was only partly compensated for by emergence of young bees during the introduction of mites. On average the size of the bee population at the end of the experiments was 78% of the size at the time of composition. In the fourth experiment, however, mortality of bees was much higher, because invasion was compared in intervals of three days instead of one day (in total 21 days instead of 7 days). This resulted in colony sizes at the end of the experiment of only 26% and 59% compared with colony size at the time of composition (table 4).

The percentage of mites which had invaded after seven time intervals (tables 1-4) gives a first indication of the rate of invasion in the different experiments, but conclusions cannot be drawn. Therefore, the rate of invasion was studied per time interval, using multiple logistic regression on the probability of invasion, P_t (table 5).

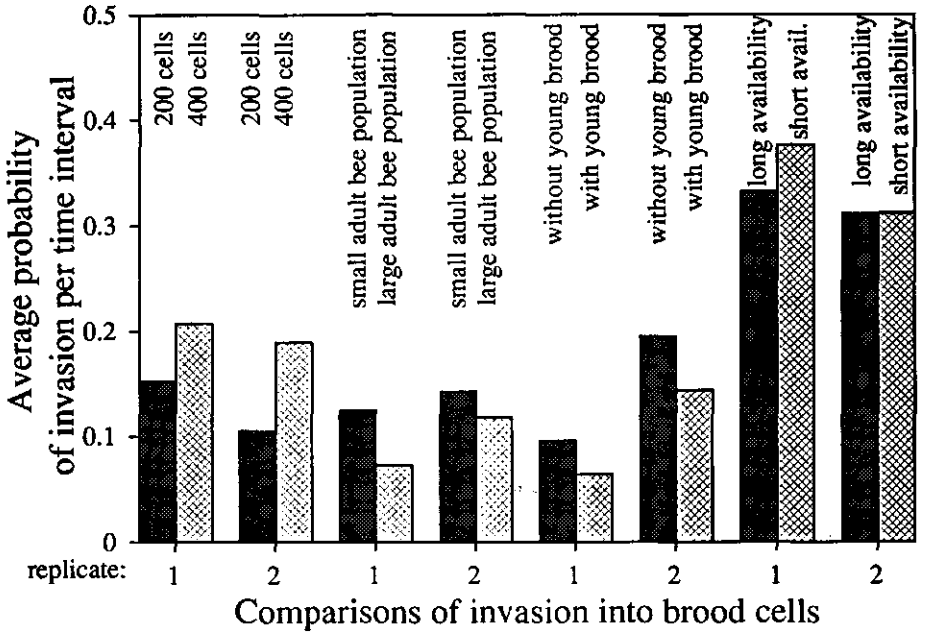

Figure 1: Modelled effect of the number of brood cells suitable for mite invasion, the size of the bee population, the presence of young brood and the period of brood cell availability, on the probability of invasion. Average probabilities of invasion per time interval were calculated using the parameters from table 5. In the calculations a constant number of brood cells was used: per time interval 200 versus 400 cells for comparison between different numbers of brood cells, 600 cells for the comparisons of the period of brood cell availability, and 350 cells for the other comparisons.

Table 5: Multiple logistic regression of the number of brood cells suitable for mite invasion, X_t, the time elapsed since introduction, t (index of time which indicates the number of time intervals of one or three days), and a colony effect, C, on the probability of invasion, P_t: $Logit(P_t) = \beta_0 + \beta_1 X_t + \beta_2 t + \beta_3 C$, where C= +1 or -1 for small versus large colonies, colonies without young brood versus colonies with young brood, and colonies with a long availability of brood cells versus colonies with a short availability of brood cells, respectively.

Type of experiment	β_0	β_1	β_2	β_3
Number of brood cells	-1.81	0.00191***	-0.075**	
	-2.63	0.00347***	-0.055*	
Size of the bee population	-3.77	0.00503***	-0.061*	0.297***
	-2.11	0.00117*	-0.054 NS	0.110 NS
Presence of young brood	-3.92	0.00301***	0.098 *	0.213***
	-2.97	0.00572***	-0.169***	0.189***
Period of brood cell availability	-4.89	0.00580***	0.198***	-0.101 NS
	-3.78	0.00467***	0.0456*	-0.001 NS

NS = *not significant*
* = *P< 0.05*
** = *P< 0.01*
*** = *P< 0.001*

In all experiments a significantly positive effect of the number of brood cells on the rate of invasion was observed (table 5). Thus, the more brood cells suitable for mite invasion, the larger the probability for a mite to invade. The effect of the time elapsed since introduction of mites on P_t was variable in the four times two replicates tested: four times negative, three times positive, and once no effect.

In part of the experiments a significant colony effect was present. Probably, such a colony effect mainly results from the factor that was manipulated to estimate its effect on the rate of invasion, i.e. the size of the bee population, the presence of young brood, and the period of brood cell availability. However, other differences may also have contributed to the colony effect; e.g., in experiments 1,3 and 4 the size of the bee population could not be kept totally equal in the two colonies to be compared (tables 1, 3 and 4). Assuming that other possible differences contributing to the colony effect can be ignored, however, both a larger bee population and the presence of young brood were correlated to a smaller P_t. The period of brood cell availability was not correlated to P_t.

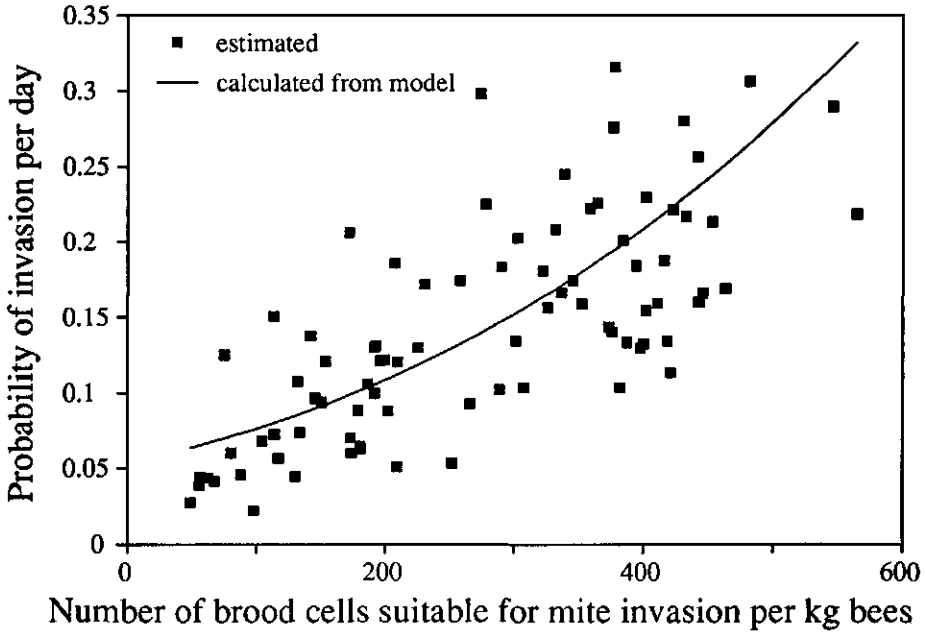

Figure 2: Relationship between the brood/bees ratio and the probability of invasion per day. The line has been drawn using parameters from Table 6: Logit(P)= -2.87 + 0.00385[brood/bees ratio], where P is the probability of invasion and [brood/bees ratio] is the number of brood cells suitable for mite invasion per kg of bees*

To visualize the extent to which P_t is affected by the different factors studied, the parameters from table 5 were used to calculate the average P_t over seven time intervals, while the number of brood cells was kept constant in the calculations. This constant number of brood cells was chosen close to the actual number of brood cells in the experiments (tables 1-4): per time interval 200 versus 400 cells for the comparisons of the number of suitable brood cells, 600 cells for the comparisons of the period of brood cell availability and 350 cells for the other comparisons (fig. 1).

If invasion is limited by the number of bees that come close enough to brood cells simultaneously, then the rate of invasion will be proportional to the number of brood cells available, and inversely proportional to the number of bees. Therefore the effect of the brood/bees ratio, i.e. the number of brood cells suitable for mite invasion per kg bees (in our colonies 1 kg represents about 8000 bees), was also considered with logistic regression

Table 6: Multiple logistic regression of the brood/bees ratio (number of brood cells suitable for mite invasion per kg of bees) and time elapsed since introduction, t (index of time which indicates the number of time intervals of one day) on the probabilty of invasion, P_t: $Logit(P_t) = \beta_0 + \beta_1[brood/bees\ ratio]_t + \beta_2 t$.

Model	β_0	β_1	β_2
$\beta_0 + \beta_1[\text{brood/bees ratio}]_t$	-2.87	0.00385***	
$\beta_0 + \beta_1[\text{brood/bees ratio}]_t + \beta_2 t$	-2.67	0.00388***	-0.064***

*** = $P < 0.001$

analysis (table 6; figure 2). The brood/bees ratio was calculated by dividing the number of brood cells suitable for mite invasion in each time interval by the size of the bee population at the end of the experiment. The data of all experiments were combined for this regression analysis, except the data from experiment 4, because the time interval was not comparable to the other experiments.

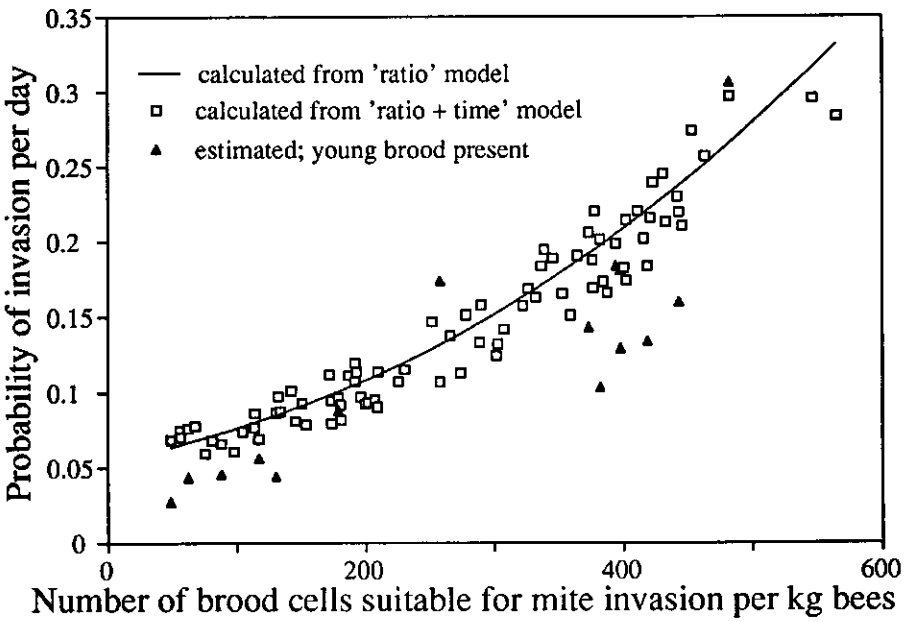

Figure 3: Comparison between the two regression models (Table 6), and the estimated probabilities of mite invasion into brood cells of the colonies in which young brood was present.

P_t was positively correlated to the brood/bees ratio and negatively correlated to the time elapsed since introduction of mites (table 6). Figure 2 shows the relationship found between the brood/bees ratio and the probability of invasion per day. The two models from table 6 are compared in figure 3. The squares represent the estimates of P_t when the time elapsed since introduction was also used as an explanatory variable in the regression analysis. In addition, observed data of the colonies in which young brood not yet available for invasion was present, are shown. These observed probabilities were clearly lower than average, again showing the negative effect of the presence of young brood on the rate of invasion.

Discussion

Effect of the brood/bees ratio and the presence of young worker brood on the rate of invasion

The number of brood cells suitable for mite invasion and the size of the bee population appeared to affect the rate of invasion of *Varroa* mites in such a way that the logit of P_t was proportional to the brood/bees ratio (table 6; figure 2). This is in agreement with the hypothesis that the rate of invasion is limited by the number of bees that simultaneously can come close enough to the brood cells for mites to invade. In addition, this suggests that the rate of invasion is not determined by a preference for nurse bees while these nurse bees bring the mites with a high probability to the brood cells. Such a preference is unlikely, because the number of bees performing brood nursing tasks is probably matched to the amount of brood to be nursed (Robinson et al., 1989; Robinson, 1992), despite that division of labour is related to the age of the bees (Lindauer, 1953; Seeley, 1982). Therefore, if preference for brood nursing bees plays a role in determining the rate of invasion, the size of the preferred group of nurse bees would be matched to the amount of brood to be nursed, and the brood/bees ratio would be expected to affect P_t relatively little. However, the presence of young brood was correlated to a lower rate of invasion (table 5; fig. 2), as is expected when the mites do prefer bees performing brood nursing tasks, because during nursing of the young brood the bees cannot bring the mites close to the brood which is suitable for mites. Therefore, there may be a preferred group of bees that is concentrated in the brood nest, while this group is not matched to the amount of brood to be nursed. Indeed mites seem to prefer young bees (Schneider, 1985; Kraus et al., 1986; Le Conte & Arnold, 1987) and they may prefer them independently of the tasks these bees perform. Because young bees are more likely to perform tasks inside the brood nest (Lindauer, 1953; Seeley, 1982), the mites may thus increase their probability to come close enough to suitable brood cells. Alternatively, the effect of the presence of young brood may be explained by a difference in clustering of the bees. Bees tend to cluster in the brood nest area. They possibly occupied more comb area in the colonies with young brood present than in the other colonies. Therefore, fewer bees may have come close enough to a brood cell that is suitable for mite invasion in the colonies with young brood, resulting in a lower rate of invasion.

Effect of time on the rate of invasion

When the data were taken together, the time elapsed since introduction of mites negatively affected the rate of invasion (table 6). This is in agreement with earlier results (chapter 2). The positive effect of time in the experiment concerning the period of brood cell availability (table 5), may well have been caused by a decreasing number of bees because of the long duration of this experiment. The rate of invasion may then increase with a decreasing

size of the bee population and not with time. In the first replicate of the experiment in which young brood was present, the number of brood cells which were suitable for mite invasion increased during the experiment, due to technical difficulties in brood dating. The increasing number of brood cells resulted in an increased probability of invasion. In the regression analysis the higher probabilities found during the experiment may subsequently be ascribed partly to the number of brood cells and partly to time, thus probably resulting in a positive effect of time on the rate of invasion.

Effect of the period of brood cell availability

Differences in the period of brood cell availability appeared not to affect the rate of invasion. However, there is evidence suggesting that in areas where invasion occurs, the mite density on the bees decreases, which may thus lower the average rate of invasion in the colony. Earlier we found that the cells capped first in a patch of brood were invaded by more mites than the cells capped last in another patch in the same colony, though the cells in both patches were capped within the same time interval of one day. In addition, the earlier cells are capped in a patch of brood, the more mites invade them (Calis et al., 1993a). These examples occur on a time scale of hours, however. On a time scale of days, as in our experiments, the process of redistribution of the mites inside the colony seems to be fast enough to prevent a significant effect of the period of brood cell availability on the rate of invasion.

Perspectives for improvement of Varroa *control methods*

The rate of invasion of *Varroa* mites into honeybee brood cells, seems to be limited by the number of bees that simultaneously can come close enough to brood cells which are suitable for mites to invade. The brood/bees ratio is therefore probably the main factor determining the rate of invasion into brood cells. Insight into the rate of invasion may be of direct use for *Varroa* control, because the rate of invasion affects the distribution of mites over adult bees and brood cells, and because control methods are based on either killing mites on the bees or mites in the brood cells. Control methods in which mites are trapped in worker brood combs have been developed and are being developed (Maul et al., 1988; Calis et al., 1993b). This study shows that the number of brood cells for trapping of mites is of main importance, and not the period during which trapping occurs, since the period of brood cell availability appeared not to affect the rate of invasion. Additionally, estimates of the number of brood cells in trapping combs needed for sufficient control of mites can be made. Fifteen percent of the mites present on adult bees is trapped with 300 worker brood cells in colonies with an adult bee population of one kg (fig. 2). This means that for instance 90 % can be trapped with 4200 brood cells in such colonies. Trapping comb methods vary according to the size of the bee population, the number of brood cells used for trapping and the percentage of the mites present on adult bees during trapping. The results from this study can be used to compare different trapping comb methods theoretically.

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4

Invasion of *Varroa* mites into drone brood cells of the honey bee.

Abstract

Invasion of *Varroa* mites into drone brood cells of honey bees was studied in colonies without worker brood. The probability for a mite to invade was dependent on the brood/bees ratio, which is defined as the number of drone brood cells capped per kg of bees. When compared with invasion in colonies with exclusively worker cells, *Varroa* mites invaded drone cells 11.6 times more frequently. This suggests that the biased distribution of mites over drone and worker cells in colonies with both types of brood cells, results predominantly from the higher rate of invasion into drone cells per se, when compared to that into worker cells per se. Since the rate of invasion is high in drone cells, a trapping method using drone combs may be very effective in controlling the *Varroa* mite. When no other brood is present, 462 drone cells are estimated to be sufficient to trap 95% of the mites in a colony of 1 kg of bees.

Introduction

The parasitic mite *Varroa jacobsoni* Oudemans is worldwide a harmful pest of the Western honey bee, *Apis mellifera* L. It parasitizes both adult and immature bees, but reproduction of the mites only occurs inside capped drone and worker brood cells (Ifantidis & Rosenkranz, 1988). More adult offspring are produced in drone cells when compared to worker cells (e.g. Fuchs & Langenbach, 1989). Therefore, one might expect that mites prefer drone brood over worker brood. Indeed, about 8 times more mites are generally found per cell in drone cells than in worker cells (e.g. Fuchs, 1990). In addition, mites prefer drone larvae to worker larvae when given the choice in laboratory tests (Rosenkranz et al., 1984; Otten & Fuchs, 1988; Le Conte et al., 1989).

Recently, Boot et al. (chapter 3) showed that the rate of invasion into worker cells (number of mites that invade per day) depends on the ratio of worker brood cells to adult bees: the brood/bees ratio (number of brood cells capped per day and per kg of bees). The larger the brood/bees ratio, the higher was the rate of invasion. This may be understood as follows. A mite has to be carried close to a brood cell by a bee before invasion occurs (chapter 1), and only some of the honey bees in a colony reside near a cell suitable for mite invasion. Therefore, the number of bees that bring a mite close enough to a brood cell may determine invasion and this number of bees will increase with a higher brood/bees ratio (chapter 3).

The higher number of mites found in drone cells in comparison to worker cells (Fuchs, 1990), may result from a higher rate of invasion into drone brood cells per se, when compared to that into worker brood cells per se. This higher rate of invasion may have three causes. Firstly, drone brood cells are invaded during a 2-3 times longer period than worker brood cells (Ifantidis, 1988; chapter 6). Hence, the chance for a mite on a bee to come close enough to a suitable brood cell is increased accordingly. Secondly, the surface of a drone cell

is 1.7 times larger than that of a worker cell, which also increases the chance for a mite on a bee to come close enough to a suitable brood cell. Thirdly, the presence of a drone larva instead of a worker larva may increase the rate of invasion. This may occur when a signal coming from drone larvae evokes a stronger response to invade than a corresponding signal from worker larvae. Alternatively, behaviour of the bees may be affected by the presence of either drone or worker larvae in such a way that the rate of invasion is affected too.

The biased distribution of mites over drone and worker cells may also arise because the mites refrain to invade worker cells when drone cells are nearby. If so, then the presence of drone cells will negatively affect invasion into worker cells. Consequently, the difference between the rate of invasion into drone cells per se and that into worker cells per se, will be too small to explain the biased distribution of mites when both cell types are present.

In this paper, the rate of invasion into drone cells per se is determined for two reasons. Firstly, it is compared with that into worker cells per se to test whether the difference between these rates is large enough to explain the biased distribution of the mites. Secondly, it is determined because it is a valuable parameter for development of *Varroa* control methods that apply trapping of mites in drone combs.

Materials and Methods

Experimental set-up

The rate of invasion into drone cells was studied in the Netherlands in 6 colonies of the Western honey bee, *Apis mellifera*. A similar set-up was used as in earlier experiments to determine the rate of invasion into worker cells (chapter 3).

The colonies in which invasion was studied were moved to an isolated place, at least 1 km away from other bee colonies, to prevent exchange of mites between experimental and other bee colonies (Sakofski & Koeniger, 1988; Rademacher et al., 1989). All brood was removed from the colonies and most of the mites that were already present on the bees were killed with 2 treatments of 20 ml 85% formic acid applied on pieces of cardboard (Wachendörfer et al., 1985; Fries, 1989). Ample honey and pollen stores were available in the experimental colonies.

Every day a comb with about 50 drone larvae was placed into the colonies. These drone larvae were 3-4 days of age, dated according to the method of Boot & Calis (1991). In the colonies that were used for brood dating, drone comb and worker comb were alternately offered for oviposition. When only drone comb was offered for oviposition, the queen started to lay worker eggs into the drone cells within a few days. In the experimental colonies, the queen was allowed to oviposit freely, and therefore combs with eggs had to be removed regularly to keep the colonies free of brood other than the 50 drone cells introduced daily. After 1 week, it was assumed that the bees in the experimental colonies had adapted to nurse the number of drone larvae introduced. Subsequently, a group of mites was introduced by placing brood combs with emerging bees heavily infested by mites into the colonies. After 1-2 days, these combs were removed again. To monitor invasion of the mites that had been introduced in this way, capping of the drone cells was registered daily by placing transparent sheets over the brood area and by marking capped cells. After capping of the cells, the comb was removed from the colony, cells were opened, and the number of mites per cell were counted.

Table 1: Data from the experiments to study the rate of invasion into drone cells by Varroa mites.

Colony number	1	2	3	4	5	6
Introduction date	28-5-92	28-5-92	13-6-92	13-6-92	9-7-92	9-7-92
Number of time intervals of 1 day	5	5	7	7	9	9
Average number of drone cells capped per time interval	46 ± 21	35 ± 16	54 ± 21	53 ± 20	51 ± 28	31 ± 16
Size of bee population[1] (kg bees)	0.659	0.473	2.233	1.475	1.319	1.022
Total number of mites[2]	40	92	480	524	417	630
Total number of mites invaded (% in parentheses)	33 (83)	77 (84)	385 (80)	473 (90)	392 (94)	541 (86)

[1]*The size of the bee population was estimated by weighing the bees at the end of the experiments.*
[2]*The total number of mites was calculated by summing the mites that had invaded the drone cells and the mites that had remained on the bees after the experiment.*

Invasion of mites into drone cells was monitored at intervals of one day during a period of 5-9 days. At the end, mites still on the bees were killed by applying twice 1 ml of Perizin[R] (Bayer; active ingredient: coumaphos) in 49 ml of water. The dead mites, which had fallen through the gauze bottom of the hive into a drawer, were then counted. Because after the second application of Perizin[R] dead mites were hardly found, it is assumed that all mites were killed. Subsequently, the actual size of the bee population (kg of bees) during the experiments was estimated by weighing the hive with the experimental colony and subtracting the weight of hive and combs, after bee removal.

Statistical analysis

The rate of invasion into drone cells was assumed to depend on the number of mites present on the bees and the probability of invasion. The number of mites present on the bees at the beginning of each time interval was estimated by summing the mites found in all drone cells that were capped after that moment and the number of mites remaining on the bees at the end of the experiment. Subsequently, the probability of drone cell invasion was calculated for each interval by dividing the number of mites found in drone cells that were capped in a certain interval by the estimate of mites on bees at the start of that interval. A logit link-

function was used to model the probability of invasion, which was assumed to depend on the number of drone cells capped during a time interval per kg bees (brood/bees ratio) and the index of time (chapter 3):

$$P_t = \{1 + \exp(-L_t)\}^{-1}$$
$$L_t = \beta_0 + \beta_1[\text{brood/bees ratio}]_t + \beta_2 t$$

where t is the index of time, indicating the number of time intervals of one day, P_t is the probability of invasion into drone cells capped during time interval t, L_t is the logit of P_t, and $[\text{brood/bees ratio}]_t$ is the number of drone cells capped during t per kg bees. The parameters β_0, β_1 and β_2 were estimated by multiple regression, using maximum likelihood estimation (SAS Institute Inc., 1989).

Comparison of the rate of invasion into drone cells with that into worker cells

When the probability of invasion per unit of time (t) is constant, the number of mites on bees (M) decay in a negative exponential way:

$$M_t = M_0 e^{-rt}$$

where r is the relative rate of invasion (day^{-1}). This relative rate of invasion was used to compare invasion into drone cells with that into worker cells by assuming a linear function through the origin, providing the relation between the number of brood cells capped during a time interval per kg of bees (brood/bees ratio) and r. For each time interval r was estimated from the probabilities of invasion found for drone cells (this study) and found for worker cells (chapter 3) using the following equation:

$$r = -\ln(M_{t+1}/M_t) = -\ln(1-P_t)$$

where, t is the index of time which indicates the number of time intervals of 1 day, M_t is the number of mites on bees at the beginning of time interval t, and P_t is the probability of invasion during time interval t.

Table 2: Multiple logistic regression of the brood/bees ratio (number of drone cells that were capped during one day per kg of bees) and the index of time which indicates the number of time intervals of one day, t, on the probabilty of invasion, P_t: $Logit(P_t) = \beta_0 + \beta_1[\text{brood/bees ratio}]_t + \beta_2 t$.

Model	β_0	β_1	β_2
$\beta_0 + \beta_1[\text{brood/bees ratio}]_t$	-2.02	0.0235^{***}	
$\beta_0 + \beta_1[\text{brood/bees ratio}]_t + \beta_2 t$	-1.94	0.0233^{***}	-0.024^{NS}

NS = *not significant*
*** = $P < 0.001$

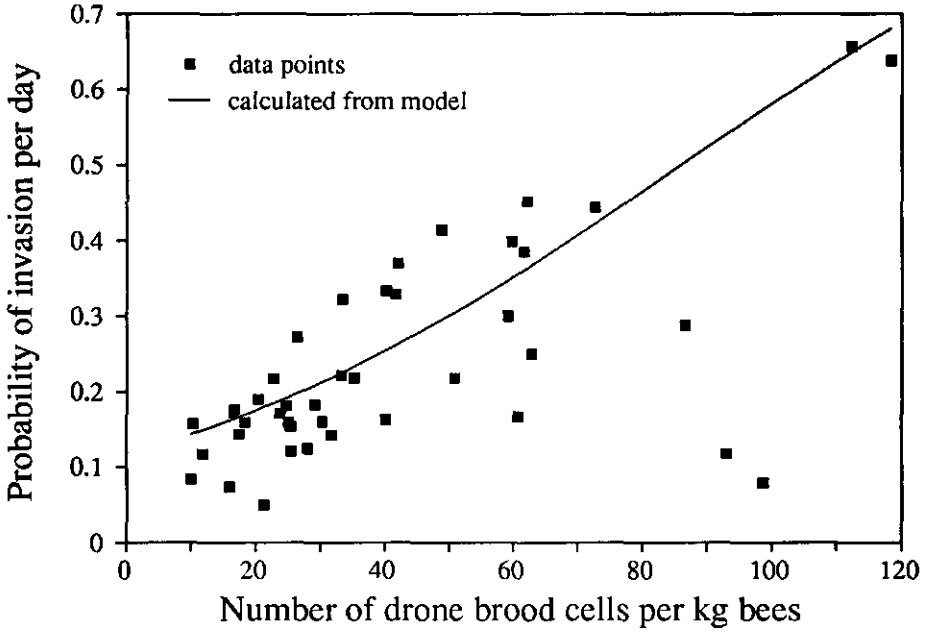

*Figure 1: Relationship between the brood/bees ratio and the probability of invasion per day. The line has been drawn using the parameters from table 2: Logit(P) = 0.0235 * [brood/bees ratio], where P is the probability of invasion and [brood/bees ratio] is the number of drone cells per kg of bees.*

Results

Data on invasion into drone cells are shown in Table 1. Drone brood proved to be very effective in trapping the mites present on adult bees. Daily introduction of about 50 drone cells during 5-9 days led already to invasion of a substantial part of the mitesintroduced: 80-94% of the mites invaded into 175-459 drone cells.

Invasion into drone cells was more precisely studied per time interval (chapter 3), using multiple regression on the probability of invasion, P_t, whereas P_t was assumed to depend on the brood/bees ratio (number of drone cells capped during one day per kg bees), and the time since introduction of the mites (Table 2). The data of all experiments were used for statistical analysis. P_t was positively correlated with the brood/bees ratio, whereas the time elapsed since introduction of the mites had no effect on P_t. Figure 1 shows the relationship found between the brood/bees ratio and the probability of invasion per day.

In case of equal brood/bees ratios, the rate of invasion into drone cells was much higher than that into worker cells (Fig. 2). The relationships between the relative rate of invasion and the brood/bees ratio per day were described by:

r_d (day^{-1}) = 0.00649 (kg bees/number of cells capped) * [brood/bees ratio] (number of cells capped/(kg bees * day)), for drone brood cells and:

r_w = 0.00056 * [brood/bees ratio], for worker brood cells.

Thus, *Varroa* mites invaded drone cells 11.6 times more frequently than worker cells.

Discussion

Similar to invasion into worker cells (chapter 3) the logit of P_t was proportional to the brood/bees ratio (Table 2; Fig. 1), but the probability for a mite to invade a drone cell was much higher than the probability to invade a worker cell. In case of equal brood/bees ratio's, *Varroa* mites invaded drone cells 11.6 times more frequently than worker cells (Fig.2). Part of this higher frequency of invasion may be due to the 2-3 times larger attractive period of drone cells (Ifantidis, 1988; chapter 6). When invasion in a cell depends on the frequency that a bee brings a mite close enough to invade, the number of mites that invade per cell is expected to be also 2-3 times higher, provided that the number of mites on the bees stays the same. In addition, when the frequency that a bee brings a mite close enough for invasion is proportional to the surface of a brood cell, 1.7 times more mites are expected per drone cell due to their 1.7 times larger surface. Combining these would result in 3.4-5.1 times more frequent invasion into drone cells than into worker cells. An 11.6 times higher frequency was found, however. Thus, invasion into drone cells is probably increased further due to the presence of a drone larva instead of a worker larva in the cell.

In a colony of a given size the relative rate of invasion, r, is constant per cell. When both drone and worker cells are available for mite invasion, and assuming that invasion into drone and worker cells are independent, the number of mites on adult bees decreases as follows:

$$dM/dt = -(r_d + r_w)M$$

where M is the number of mites on bees, t is time (days), r_d is the relative rate of invasion in drone cells (day^{-1}), and r_w is the relative rate of invasion in worker cells (day^{-1}). Because mites invading drone cells and mites invading worker cells both come from the same pool of mites on adult bees, M, the ratio of r_d per cell to r_w per cell is equal to the ratio of mites found per drone cell to their number found per worker cell (see Appendix). Hence, 11.6 times more mites are expected per drone cell than per worker cell, which is even more than the actual distribution of about 8 times more mites per drone cell. This suggests that differences in r are the most important cause of the biased distribution over drone and worker cells. Under the alternative hypothesis that mites refrain from invasion into worker cells when drone cells are nearby, one would expect a smaller ratio of r_d per cel to r_w per cell than 8:1, because r_d and r_w were determined in a situation with exclusively drone and exclusively worker brood, respectively. Hence, our results strongly suggest that the mites do not refrain from invasion into worker cells when drone cells are nearby.

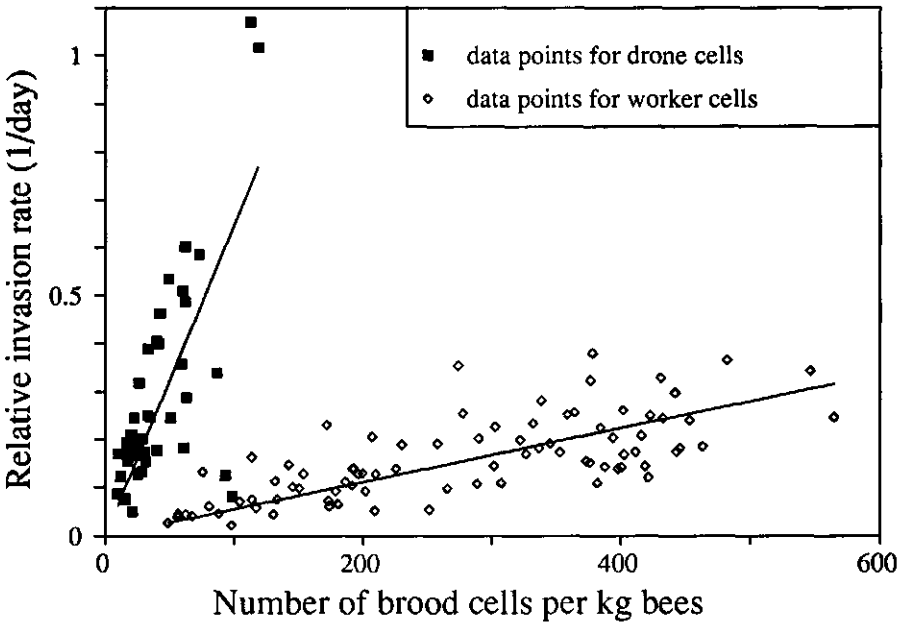

Figure 2: Comparison of the relative invasion rate into drone cells and that into worker cells in relation to the number of brood cells per kg of bees.

In studies of the distribution of mites over drone and worker cells, a large variation in the ratio between the number of mites per drone cell and that per worker cell is found. Fuchs (1990) found ratios ranging from 0.94 to 30.6 in 68 replicates, whereas Calis et al. (1993) found ratio's of 7.7 and 15.3 in two replicates. This variation probably occurs because drone and worker cells are never attractive to mites during exactly the same period, and because the number of mites that reside on the bees in a honey bee colony varies strongly over time, due to invasion of the mites into open brood cells and emergence of mites from capped cells. Though variation in the ratio of the number of mites per drone cell to the number of mites per worker cell is high, studies concerning the distribution over drone and worker cells have shown an average ratio of about 8. Fuchs (1990) found an average ratio of 8.3, whereas Schulz (1984) and Sulimanovic et al. (1982) reported ratio's of 8.6 and 7.2, respectively. These values are well below the ratio of 11.6, expected from the ratio of r_d per cell to r_w per cell. The distribution of mites over drone and worker cells may be less biased to drone cells than 11.6 times when invasion into drone cells and invasion into worker cells is more or less segregated in time, and this probably occurs in normal honey bee colonies. Since r is 11.6 times higher per drone cell than per worker cell, the number of mites on bees will deplete much quicker during periods when drone cells are available and therefore the

actual distribution over drone and worker cells may be less biased. Such an effect is expected to be stronger when the proportion of drone cells versus worker cells is higher, and indeed Fuchs (1990) found a less biased distribution with a higher proportion of drone cells.

Trapping of mites in drone cells as a biotechnical control method

Since the rate of invasion into drone cells is high, trapping mites in drone brood is a useful biotechnical method to control the *Varroa* mite (Schulz et al., 1983; Rosenkranz & Engels, 1985). Currently, trapping of mites in drone brood is applied in colonies with normally developing brood nests. This will decrease the effectiveness of the trapping method, because in normal colonies about 80% and more of the brood consists of worker brood (Page & Metcalf, 1984; Rowland & McLellan, 1987) and therefore a substantial part of the mites will invade worker cells. Additionally, mites may invade drone cells that are not removed from the colonies. A drone cell trapping method will be much more effective when it is applied during periods when no brood other than introduced for trapping is present in the colony. Using the regression line from figure 2, our results show that to reach a 95% trapping effectiveness per day, a relative invasion rate of $-\ln(0.05) = 3.00$ per day is needed. Thus, only 3.00 (day^{-1})/0.00649 (kg bees/number of cells capped) = 462 drone cells are needed to trap 95% of the mites in a colony of 1 kg bees per day. Trapping of mites in drone brood can therefore be an effective non-chemical method to control *Varroa* mites in honey bee colonies.

Acknowledgements

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Appendix

When both drone and worker cells are available for mite invasion, and when invasion into drone and worker cells are independent, the number of mites on adult bees decreases as follows:

$$dM/dt = -(r_d + r_w)M$$

where M is the number of mites on bees, t is time (days), r_d is the relative rate of invasion in drone cells (day^{-1}), and r_w is the relative rate of invasion in worker cells (day^{-1}).

In case of constant numbers of drone and worker cells available, r_d and r_d are also constant, and:

$$M_t = M_0 e^{-(r_d + r_w)t}$$

while the number of mites that invade drone cells increases as follows:

$$dM/dt = r_d M$$

Integration of M over t yields an expression, indicated by $M_{d,t}$:

$$M_{d,t} = -r_d/(r_d+r_w) \cdot M_0(e^{-(r_d + r_w)t} - 1)$$

Similarly the number of mites that invade worker cells is:

$$M_{w,t} = -r_w/(r_d+r_w) \cdot M_0(e^{-(r_d + r_w)t} - 1)$$

Thus,

$$M_{d,t}/M_{w,t} = r_d/r_w = r_{1,d}N_d/r_{1,w}N_w$$

where $r_{1,d}$ and $r_{1,w}$ are the relative rates of invasion per drone and per worker cell respectively, and N_d and N_w are the numbers of drone and worker cells. Then, the ratio of mites per drone cell to their number per worker cell is:

$$(M_{d,t}/N_d)/(M_{w,t}/N_w) = r_{1,d}/r_{1,w} = 11.6$$

Every small time step *t*, N_d and N_w can be considered constant and the ratio of mites per drone cell to their number per worker cell will be 11.6 in each time step, regardless whether N_d and N_w vary between time steps.

5

Does time spent on adult bees affect reproductive success of *Varroa* mites?

Abstract

Reproduction of *Varroa jacobsoni* Oudemans (Acari: Varroidae) and the number of *Varroa* mites that were found dead on the bottom board of the hive, were studied in relation to the period the mites spent on adult honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), prior to invasion into brood cells. The maximum period on adult bees was 23 days. To introduce mites, combs with emerging worker brood, heavily infested with mites, were placed into a colony and removed the next day. At the beginning of the first day following emergence from brood cells, 18% of the mites introduced into the colony was found on the bottom of the hive. Part of these mites may already have died inside the capped brood cells, and then fallen down after cleaning of cells by the bees. At the second and third day following emergence, respectively 4% and 2% of the mites on adult bees at the previous day was recovered on the bottom, whereas from the fourth day on only 0.6% of the mites on adult bees was recovered on the bottom per day. After invasion into brood cells, 8-12% of the mites did not produce any offspring. Of the mites that did reproduce, the total number of offspring was 4.0-4.4 per mite during one reproductive cycle, part of which may reach maturity resulting in 1.2-1.3 viable daughters, and 8-10 % of the mites produced only male offspring. Reproduction was independent of the period the mites had spent on adult bees prior to invasion into brood cells.

Introduction

Reproduction of *Varroa jacobsoni*, a parasitic mite on honey bees, only occurs on larvae and pupae in capped brood cells (De Jong et al., 1982; Ifantidis & Rosenkranz, 1988). Brood cells are invaded about 1-2 days preceding cell capping (Ifantidis, 1988; chapter 6). About 60 hours after cell capping the mites lay their first egg, which usually develops into a male. Subsequently, the mites continue to lay about 4-5 female eggs in 30-hr-intervals. One or a few of these female eggs reach maturity, the number depending on the development time of the bee pupa in the cell (Ifantidis, 1983; Ifantidis, 1984; Rehm & Ritter, 1989).

After emergence from brood cells, the female mites reside a certain period on adult bees in the colony before they invade a new brood cell (chapters 2,3). The length of this period strongly affects the population dynamics of the mites, because mites cannot reproduce while they reside on adult bees and therefore reproduction is delayed. In addition, the period on adult bees may affect the population dynamics of the mites in two other ways.

Firstly, part of the mites will die during their stay. Data on natural mortality of mites have been collected (Liebig et al., 1984; Rademacher, 1985; Imdorf & Kilchenmann, 1990), but in these studies the number of dead mites fallen down on the bottom of the hive has been studied in relation to the number of mites in the colony at the end of the experiments. During the experiments, the mites were distributed over adult bees and brood cells, and therefore data on relative mortalty of mites residing on adult bees are still needed.

Secondly, the length of the period on adult bees may affect subsequent reproduction of the mites. Young *Varroa* mites that had been deprived of a period on adult bees before introduction into a brood cell, laid fewer eggs and their oviposition was retarded (Beetsma & Zonneveld, 1992). In addition, Hänel & Koeniger (1986) found that after feeding on bees with relatively high Juvenile hormone titres, the percentage of reproducing mites was higher than after feeding on bees with low titres. They hypothesize that *Varroa* mites take up Juvenile hormone from the bees, which in turn stimulates invasion behaviour and subsequent reproduction of the mites. A longer period on adult bees may then well increase reproduction of the mites.

In this study we followed groups of *Varroa* mites starting at the moment of emergence from brood cells. Mortality and reproduction of the mites were determined in relation to the period the mites spent on adult bees.

Materials & Methods

All experiments were done at a location isolated from other bee colonies by at least one km, in order to prevent exchange of mites between colonies (Sakofski & Koeniger, 1988; Rademacher et al., 1989; Greatti et al., 1992). Honey bees as commonly found in the Netherlands, were used. These bees cannot be classified into a specific *Apis mellifera* race, because many races have been imported resulting in a mixed population. All brood was removed from the colonies and the mites present on bees were killed with 2-5 treatments 15-25 ml 85% formic acid (Wachendörfer et al., 1985; Fries, 1989), until less than five mites were found on the bottom of the hive after treatment. Formic acid was chosen because its residue is probably the most transient when compared with other available acaricides. Subsequently, a group of mites was introduced by placing heavily infested, emerging worker brood combs into the colony for one day. This group of mites was subsequently followed to determine mortality and reproduction in relation to the period spent on adult bees.

Mortality

Mortality was measured daily by counting of the mites fallen down on the bottom of the hive. At the end of the experiments the mites that had remained on the bees were killed by applying one ml of Perizin^{R} in 50 ml water (Bayer; active ingredient: coumaphos) twice, and the dead mites were counted. During the experiments, part of the mites invaded brood cells. Their number and the day at which they invaded were determined as well (chapters 2,3). Therefore, the number of mites present on the bees at any day could be calculated by summing (1) the mites that fell on the bottom board from that day until the end of the experiment, (2) the mites that invaded brood cells from that day until the end of the experiment, and (3) the mites that still remained on the bees at the end of the experiment. For each day, the relative mortality of the mites was calculated by dividing the estimated number of mites on adult bees by the number of mites fallen onto the bottom board of the hive at that day. Subsequently, relative mortality of the mites was related to the period they had spent on adult bees.

The experiment to assess mortality per day as a percentage of the number of mites on adult bees, was repeated 17 times. On average, 769 mites (range: 280 - 1730) were introduced at the start of each experiment, and mortality was measured during an average of 13 days

(range: 8 - 23). Mean mortality per day was calculated by weighing mortality in each experiment by the number of mites that were present on the bees.

Reproduction

A dated worker brood comb (Boot & Calis, 1991), containing worker larvae of 3-4 days old, was placed into the colony once a day to recapture the mites in brood cells. Worker brood cells are invaded from 15-20 hours preceding cell capping (chapter 6). Therefore, the period mites had spent on adult bees could be registered by marking capped cells daily on transparent sheets. After capping, the brood combs were moved into 'nursing' colonies for further development of the bee brood and the mites. Ten days after cell capping, when the honey bee brood was between 18 and 19 days old, the combs were taken out of the 'nursing' colonies and development of immature mites was stopped by putting the combs into the refrigerator. All cells were opened and the mites and their offspring were counted. Ten days after cell capping, young adult mites can still easily be distinguished from their mothers because their pigmentation is not completed yet (de Ruijter & Pappas, 1983). Subsequently, reproduction of the mites was related to the period that the mites had spent on adult bees.

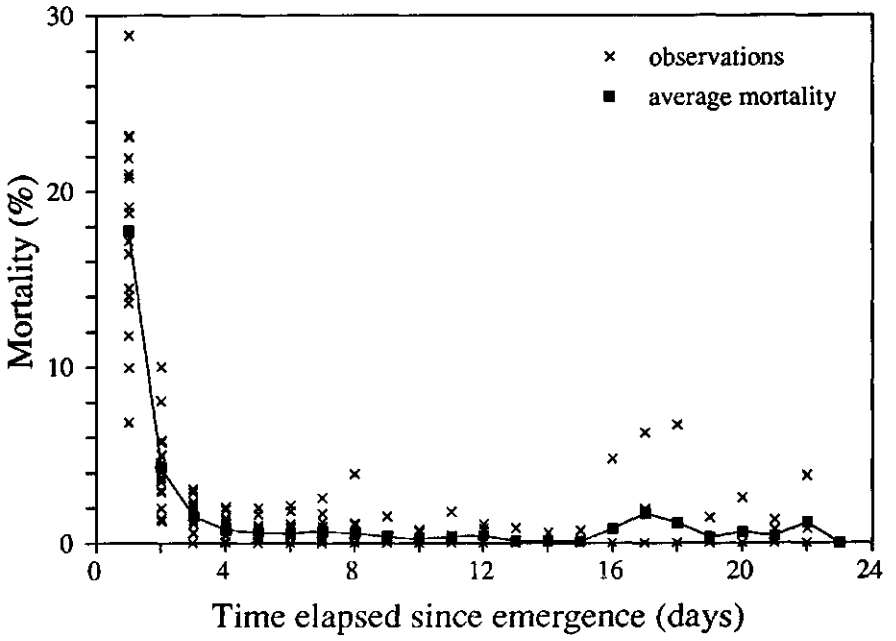

Figure 1: Daily mortality of Varroa mites (% of the number of mites that was estimated to be present on the bees at that day) in relation to the length of the period spent on adult bees after emergence from brood cells. Values are based on mite counts on the bottom of the hive in 17 colonies.

The experiment to assess reproduction of the mites in relation to the time they spent on bees was repeated three times. In the first, second, and third series, offspring were counted of mites that resided up to 16, 20 and 10 days on adult bees, and reproduction was measured of 546, 663 and 473 mites respectively.

Results

Mortality

The number of mites fallen on the bottom board of the hive was typically high during the first days after emergence from brood cells and then dropped to almost zero (Fig. 1). At the first day, during which the mites emerged from the brood cells introduced into the colony, 17.8 $\pm$ 5.4 % of the number of mites that were estimated to be present on the bees fell on the bottom. Subsequently, 4.3 $\pm$ 2.2 % fell during the second day and 1.5 $\pm$ 0.9 % fell during the third day after emergence. From the fourth day onwards an average of 0.6 $\pm$ 0.4 % fell on the bottom board per day.

Table 1: Comparison of four reproduction characteristics between the three series. Data within a row with the same letter are not significantly different (Mann-Whitney U test for differences in number of offspring; Chi-square test for differences in fractions).

	Series 1	Series 2	Series 3
Starting date	19-6-1989	31-7-1989	4-9-1989
Length of period measured (days)	16	21	11
Total number of mites invaded	756	892	801
Number of cells where 1 mite invaded	546	663	473
Fraction of mites without offspring	0.12[a]	0.11[a]	0.08[a]
Average number of offspring per mite that produced offspring	4.08[a]	4.00[a]	4.38[b]
Average number of viable daughters per mite that produced offspring	1.23[a]	1.24[a]	1.25[a]
Fraction of mites with only male offspring	0.08[a]	0.10[a]	0.08[a]

Reproduction

To compare reproduction of the mites only data from cells invaded by one mite were analysed because otherwise offspring of different mites cannot be distinguished, and reproduction of mites is negatively affected by mite density (Fuchs & Langenbach, 1989). Four characteristics of reproduction per brood cycle were analysed (Table 1): (1) the fraction of mites without offspring, (2) the total number of offspring per mite that produced offspring, (3) the number of viable daughters per mite that produced offspring, and (4) the fraction of mites with only male offspring. The number of viable daughters was estimated by summing the young adults and immobile deutonymphs found per cell ten days after capping, since immobile deutonymphs can develop into adults within the remaining two days of honey bee development (Ifantidis, 1983). No differences between the series were found, except for the higher number of offspring per mite that produced offspring in series 3 when compared with series 1 and 2 (Table 1).

In none of the three series a significant correlation (Kendall Rank Correlation test) between time spent on adult bees and the four characteristics for reproduction was found (Table 2; Fig. 2), except for a negative correlation with the number of offspring per mite that produced offspring in the first series (Table 2; Fig. 2). Detailed data are shown of the second series, because in this series offspring of mites was counted during a longer period than in the other series (Table 1).

Discussion

Mortality

Dead mites on the bottom of the hive are mainly found during the first days after emergence from brood cells. Part of these mites will already have died earlier inside cells, but they can only be recorded in the debris on the bottom from the moment the young bee has emerged and the cell is cleaned. Additionally, a considerable number of lightly pigmented adult mites were always found on the bottom during the first day after emergence. Such mites have probably just moulted into adults when the young bee emerges, and may be too young to survive on adult bees. After the first few days the number of mites that was daily found on the bottom board dropped to low levels. The few mites found may have died due to grooming activities by the bees (Ruttner & Hänel, 1992).

Mortality of mites on adult bees seems to have little effect on population growth, because only a few mites die per day, and because in colonies rearing brood the mean residence time of mites on adult bees is maximally 1-3 weeks, depending on the number of brood cells available for mite invasion (chapters 2,3). This may be different during winter in temperate climates, because long broodless periods may occur and mites may therefore have to stay on adult bees for several months. When the same percentage of mites dies per day during winter, even this low mortality may result in a high total mortality. Korpela et al. (1992) estimated a total mortality of 40% over a broodless period of 125 days during winter in Finland, which corresponds to a mean mortality of 0.4% per day. This is in the same order of magnitude as the 0.6% per day found in this study. Therefore, there is no reason to think that mortality of mites on adult bees depends much on the time of the year.

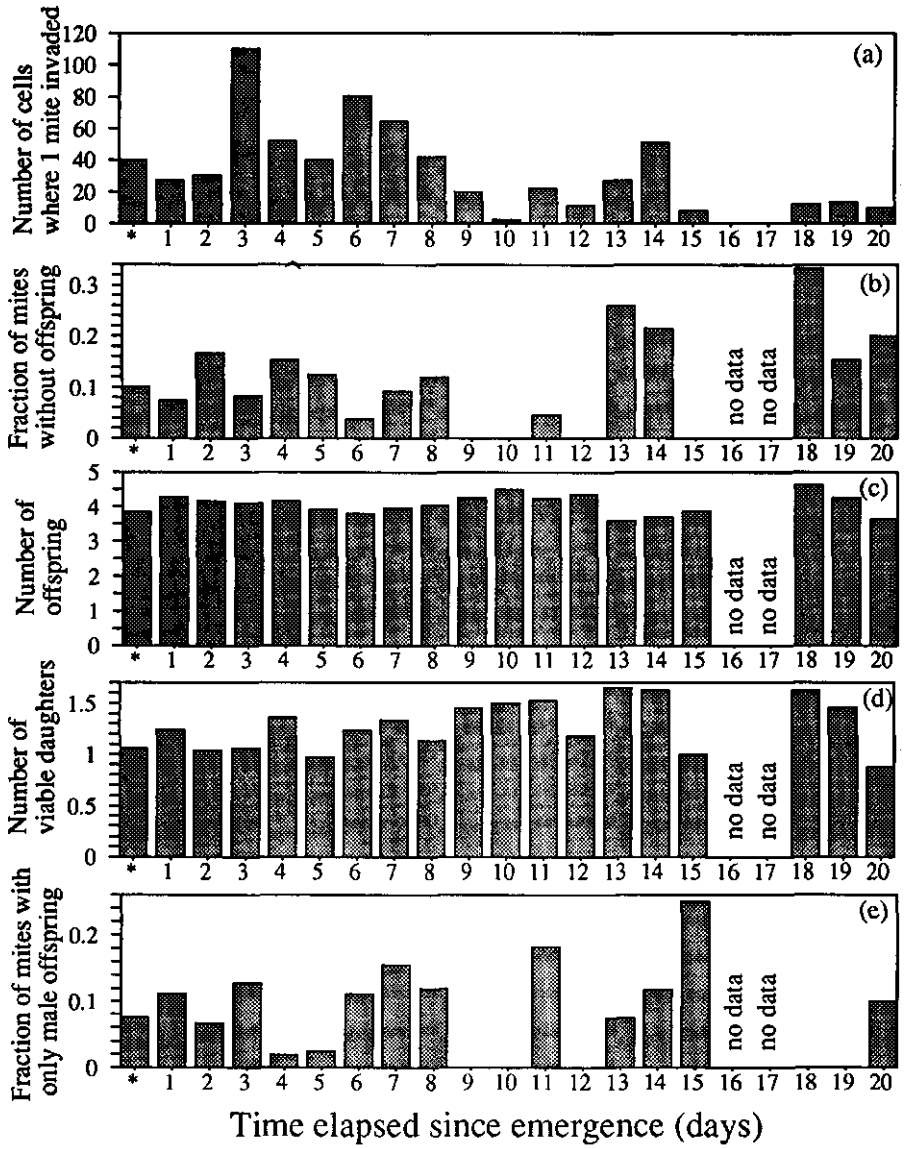

Figure 2: Number of cells where one mite invaded (a), fraction of mites without offspring (b), number of offspring per mite that produced offspring (c), number of viable daughters per mite that produced offspring (d), and fraction of mites with only male offspring (e), in relation to the period that the mites spent on adult bees. Only data from series 2. The asterisk indicates the day during which the mites were introduced into the colony.

When mortality of mites is determined, it is generally assumed that the number of mites fallen down on the bottom of the hive provides a good estimate of total mortality, mainly because it was never measured how many mites disappear from the colony. Mites may disappear when they reside on bees that die outside the hive and this may have influenced our results. The number of mites lost in this way may be small, because the mites prefer young bees (Kraus et al., 1986), which remain mostly inside the hive, and because only part of the bees that do not return to the colony carry a mite. It is unlikely that mites disappeared by removal of debris including dead mites, as a result of hygienic activities by the bees, because the debris fell through a gauze screen placed over the bottom of the hive. Therefore the bees could not reach the debris on the bottom.

Table 2: Correlation of the time that mites spent on adult bees prior to invasion into a brood cell with (1) the fraction of mites without offspring, (2) the average number of offspring per reproducing mite, (3) the average number of viable daughters per reproducing mite and (4) the fraction of mites with only male offspring (Kendall Rank Correlation Test)

	correlation coefficicient; significance level in captions		
	series 1 (n=16)	series 2 (n=19)	series 3 (n=11)
Fraction of mites without offspring	- 0.31 (0.06)	+ 0.07 (0.66)	+ 0.27 (0.24)
Average number of offspring per mite that produced offspring	- 0.54 (0.003)	- 0.01 (0.97)	- 0.20 (0.39)
Average number of viable daughters per mite that produced offspring	- 0.33 (0.08)	+ 0.28 (0.09)	+ 0.15 (0.52)
Fraction of mites with only male offspring	- 0.13 (0.42)	- 0.05 (0.76)	+ 0.20 (0.39)

Reproduction

Within a period up to three weeks after emergence from brood cells, reproduction of *Varroa* mites proved to be independent of the period the mites stayed on adult bees (Figure 2; Table 2). This is in agreement with Wendel & Rosenkranz (1990), who also found no effect. Consequently, the period mites needed to stay on adult bees to stimulate reproduction (Beetsma & Zonneveld, 1992) is either relatively short, or this period is variable and only mites that are sufficiently stimulated invade brood cells. In the latter case it is implicitly assumed that invasion into brood cells is limited because part of the mites is not motivated

to invade. However, recently we found that all mites present on adult bees are apparently motivated to invade within a day after emergence from a brood cell, and that invasion is limited by the number of brood cells available in a colony with a constant number of bees (chapters 2,3). Because mites are apparently motivated to invade within a day after emergence, and because reproduction of the mites was not affected by the time spent on adult bees, a short period on bees seems to be enough to stimulate reproduction. In addition, De Ruijter (1987) showed that contact with bees is by no means indispensible for reproduction of the mites. In his experiments, the same mites were repeatedly introduced into freshly capped brood cells (up to seven times). Despite the fact that the mites had no contact with adult bees between successive introductions, the number of eggs produced per mite remained the same.

Our results cannot refute nor confirm the hypothesis of Hänel & Koeniger (1986). They proposed a 2-step-model in which (1) mites are stimulated to invade cells by ingesting Juvenile hormone from adult bees with a high titre and (2) oviposition of the mites is initiated when they ingest Juvenile hormone from the larvae. However, assuming Juvenile hormone affects invasion and reproduction, the relative short residence on bees can only be explained when mites quickly reach bees with high enough titres. *Varroa* mites indeed change quickly from one-day-old bees to older bees (Kraus et al., 1986; Le Conte & Arnold, 1987), which usually have a higher Juvenile hormone titre (Fluri et al., 1982; Robinson et al., 1987).

The fraction of mites without offspring was in the same range as found in other studies from Western Europe. Schulz (1984), Moosbeckhofer et al. (1988) and Fuchs & Langenbach (1989) found 16, 7 and 7 % respectively, while in this study 8-12 % was found. The 8-10 % mites with only male offspring was rather high. In the above mentioned studies 6, 3 and 3 % was found, respectively. Mites that produced only male offspring probably had not mated, since haploid eggs of *Varroa* mites develop into males (De Ruijter & Pappas, 1983). Our estimates of viable daughters, 1.23-1.25 per mite that produced offspring, are low compared to the above mentioned studies: 1.6, 1.5 and 1.5 viable daughter per mite that produced offspring, respectively. Both estimates of this study and of the other studies are extrapolations however, because cells have to be opened before development has been completed. The developmental stage of the brood that was opened to determine reproduction of mites differs between the studies, and therefore the extrapolated values of viable daughters per mite that produced offspring are hard to compare.

Otten (1990) found seasonal variability in reproductive success of *Varroa* mites. In his experiments in Germany, reproductive success increased from January to June/July and decreased again from August to November. Our data, which were collected from the 19th of June to the 15th of September, show that reproductive success is constant in this part of the year (Table 1).

Effects of time spent on adult bees on population growth of the mites

The relation between the period that *Varroa* mites have spent on adult bees and mortality and reproduction, proved to be rather straightforward. In general, large numbers of dead mites are found on the bottom board only within the first three days after emergence from brood cells. In this period 22.2 $\pm$ 5.6 % of the mites that were originally present in the brood cells, was found on the bottom of the hive in our experiments. This may be a tool for beekeepers to estimate infestation levels in their hives, because the number of mites on the

bottom of the hive will be strongly associated with the number of freshly emerged mites. Thus, mortality is mainly related to emergence and not to the period mites spent on adult bees, and this period does not affect reproduction of the mite. Although reproduction is not enhanced, mites may still reside for several weeks or longer on adult bees, which evokes the question why *Varroa* mites not invade earlier. In selecting brood cells, the mites do not walk across the comb, but have to be carried close to a cell before invasion occurs (chapter 1). This limits the rate of invasion (chapter 3), which in turn limits population growth of *Varroa* mites, as they cannot reproduce while on adult bees.

Acknowledgements

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6

Differential periods of *Varroa* mite invasion into worker and drone cells of honey bees.

Abstract

Invasion of *Varroa* mites into honeybee brood cells was studied in an observation hive, using combs with cell openings at one side only. The cell bottoms had been replaced by a transparent sheet, through which mites were clearly visible after invasion into a cell. Mites invaded worker cells from 15-20 hours preceding cell capping, whereas they invaded drone cells from 40-50 hours preceding capping. The larger number of mites generally found in drone cells, when compared to worker cells, may be partly due to the longer period of mite invasion into drone brood.

Introduction

The parasitic mite, *Varroa jacobsoni* Oud., is an important pest of the honey bee, *Apis mellifera* L. (Grobov, 1977; Ritter, 1981; de Jong, 1984; Ifantidis & Rosenkranz, 1988). Female mites parasitize both adult bees and bee brood, but only reproduce in brood cells. Therefore the mites have to leave the adult bees and invade the brood cells. Both worker and drone cells are invaded, but more mites are found in drone cells when compared to worker cells (Schulz, 1984; Fuchs, 1990). It is often suggested that mites prefer drone brood to worker brood (de Jong, 1984; Rosenkranz et al., 1984; Otten & Fuchs, 1988), because reproductive success of *Varroa* mites is higher in drone cells (Schulz, 1984; Ifantidis, 1984; Fuchs & Langenbach, 1989). The larger number of mites generally found in drone cells is then thought to be due to selection for preference of the mites. Preference of mites for drone brood was indeed found in an artificial environment (Rosenkranz et al., 1984; Otten & Fuchs, 1988; le Conte et al., 1989), but whether mites can discriminate between drone and worker brood in a natural environment has not been shown.

Varroa mites are present on adult bees prior to invasion of brood cells. To decide whether to invade a brood cell or to stay on the bee, mites should obtain information about the content of the cell, e.g. a volatile chemical coming from the larva. Such chemical information could be different for worker and drone brood, enabling discrimination by the mites. Le Conte et al. (1989) suggested that some aliphatic esters might be involved in mite invasion of cells. In their study they used a four-arm airflow olfactometer (Vet et al., 1983), in which walking patterns of individual mites were observed. However, *Varroa* mites go directly from the adult bee into the brood cell (chapter 1). Sometimes mites are observed running over the comb surface, but these mites are believed to remount an adult bee and not to invade brood cells. Thus, the bioassay used by Le Conte et al. (1989) may have tested the mites for behaviour to find an adult bee, rather than for invasion behaviour. More work is needed to evaluate the importance of the various cues involved in cell invasion by *Varroa* mites.

The first step towards identification of cues involved in brood cell invasion by mites is to study the period during which brood cells are invaded. When mites use chemical

information, this is expected to be present during this period, which allows accurate sampling for subsequent research steps. Mites invade brood cells in a period just before cell capping (Fuchs & Müller, 1988; Ifantidis, 1988). However, the exact time of brood cell invasion has not been determined. Therefore we made direct observations of invasion by mites into worker and drone cells.

Materials & Methods

An observation hive containing two combs with cells at one side only (Brouwers et al., 1987) was used for the experiments. A comb was prepared by cutting the cell walls from their bottoms and melting it carefully onto a transparent sheet. The comb was then placed into a colony for a few days, during which time the bees repaired it and solidly fixed the cell walls to the sheet. Queens readily accepted these combs for oviposition.

Combs containing a patch of brood were put into the observation hive. A small bee colony, highly infested with *Varroa* mites, was added. Bee colonies predominantly kept in the Netherlands were used. This population consists of a mixture of *A. mellifera* races. The hive was placed into a dark room. Mites that invaded brood cells immediately crawled behind the larva and were clearly visible through the cell bottom, when the cell opening side of the comb was illuminated. No apparent reaction to this light was observed. Bees continued to nurse the larvae.

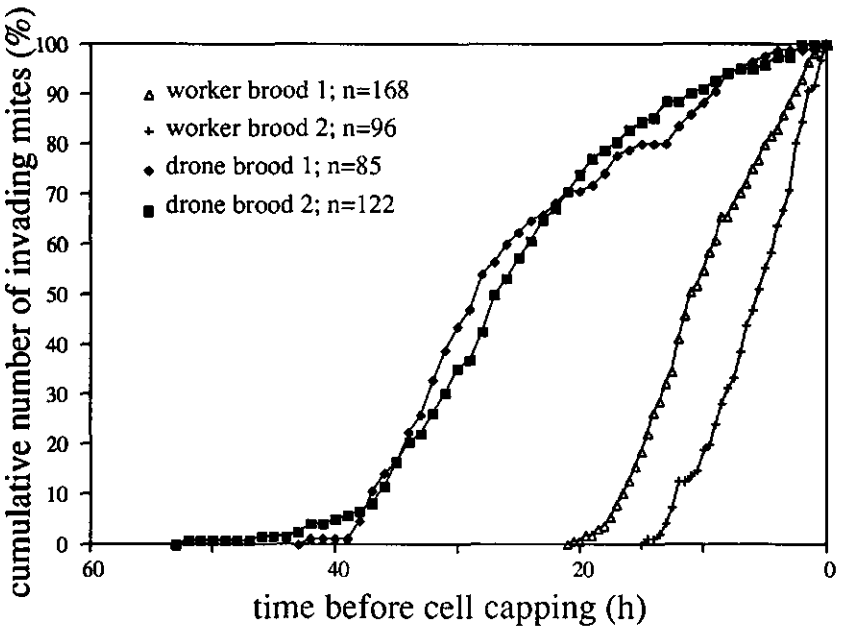

Figure 1: Cumulative relative number of mites invading worker and drone cells preceding cell capping, n represents the number of mites invaded.

Invasion of mites into worker brood was observed at 0.5-h intervals and invasion into drone brood at 1-h intervals. For each cell, records were made of the time that mites had appeared in a cell and the time at which the cell had been capped. After finishing the observations, the capped cells were opened and checked for mites. The number of mites found was compared with the number actually observed to have invaded a cell, to determine the percentage of unrecorded mites. Two series of observations of invasion into both worker cells and drone cells were made.

Results

Most mites that invaded brood cells crawled behind the larva until they reached the transparent cell bottom. However, a few mites did not reach the cell bottom and were sometimes not recorded. In the first series of observations of drone cells, 19% of the mites which were found in the cells had not been recorded, whereas the other series of observations had about 5% of the mites unrecorded. Brood cells were invaded by mites during a period of 15-20 hours and 40-50 hours preceding cell capping for worker and drone brood respectively (Fig. 1). The rate of invasion into worker brood, as illustrated by the tangent of the relevant curves in Fig. 1, was fairly constant during the whole period of invasion. The rate of invasion into drone brood, however, slowly decreased during the 20-25 h prior to the moment of cell capping.

Invasion of individual brood cells may be different when compared to invasion of the population of cells. In theory the period of invasion found for the population of cells could be the result of brood cells that were individually invaded for a relatively short interval only, while this interval would be different between cells. However, many brood cells were invaded by more than one mite and provide data on the period of invasion into individual cells. We recorded four worker cells and five drone cells, which had been invaded by five or more mites (Fig. 2). The period preceding cell capping during which individual cells were invaded, was in the same order of magnitude as found for that of the population of cells, suggesting that individual cells can probably be invaded during the whole period of invasion.

Discussion

Drone brood was invaded by mites during a period that was two to three times longer than that observed for the worker brood. This is in agreement with earlier studies based on indirect observations. Ifantidis (1988) used the weight of larvae as an estimate of their age, and concluded that mites invaded drone and worker brood cells 45 hours and 15 hours preceding cell capping, respectively. Fuchs & Müller (1988) found values of 60 and 30 hours preceding cell capping, by opening cells of dated brood combs. Furthermore, Wieting & Ferenz (1991) independently showed in a study similar to ours, that drone and worker cells were invaded by mites 36 hours and 14 hours preceding cell capping respectively. However, the number of mites observed was low: invasion of 24 mites into drone cells and 33 mites into worker cells was recorded.

The rate of invasion into worker cells was fairly constant during the entire period of invasion, but the rate of invasion into drone brood decreased when approaching the moment of cell capping. This may have been due to the limited number of mites present on the bees, and the long period before cell capping during which drone brood was invaded by mites. Probably, most mites had already invaded by the time the patch of drone cells was about to be capped.

Mite invasion of cells started when the bee larva completely covered the cell bottom, both in worker and drone cells. Chemical information from the larva may be emitted from this moment onwards. Alternatively, if chemical emissions begin earlier, their effect may be modulated by other factors, e.g. by the distance between larva and cell opening. From the moment the larva completely covers the cell bottom, this distance clearly decreases.

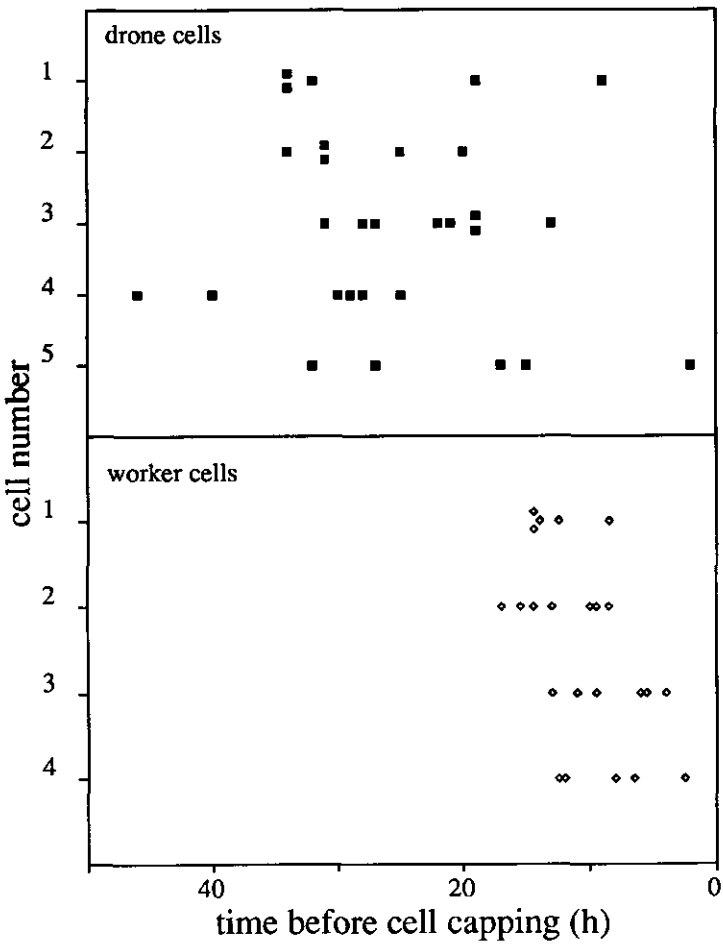

Figure 2: Course of mite invasion into nine cells, five drone and four worker cells, with a final infestation of at least 5 mites.

The number of mites invading worker cells was found to depend on the number of brood cells available. The more brood cells that are available, the higher the rate of invasion (chapters 2,3,4). Because the invasion period of drone cells is 2-3 times as long as that of worker cells, a similar ratio of numbers of invading mites may be expected. However, about eight times more mites are generally found in drone cells when compared to worker cells (Fuchs, 1990). Other factors must be involved in determining the distribution of mites over worker and drone cells. Differences in chemical information, qualitatively or quantitatively, could enable the mites to discriminate between worker and drone brood. However, discrimination by mites between worker and drone brood should not necessarily be expected. Other factors, e.g. the 1.7 times larger area of the cell opening of drone cells when compared to worker cells, and the number of bees visiting drone and worker cells during the period of invasion, also may be involved.

What can be the ultimate reason for the observed differential periods of mite invasion into cells? Why do mites not invade worker cells as early as drone cells, or why do mites not invade both cell types even earlier? Possibly, mites have a high risk of mortality when they invade a brood cell too early. Eastern honey bees, *A. cerana*, can detect and remove mites from open brood cells (Peng et al., 1987). Mites can even be removed from capped brood cells by the worker bees in *A. cerana* (Rath & Drescher, 1990) and *A. mellifera* (Boecking & Drescher, 1991). Invasion from the moment the cell bottom is completely covered by the larva may enable the mite to escape detection, thus reducing the risk of being removed. Selection for low mortality of mites may then explain the longer invasion period of drone cells, and may subsequently affect the distribution of mites over worker and drone cells. It appears that preference of mites for drone brood, as a result of the higher reproductive success in drone cells (de Jong, 1984; Rosenkranz et al., 1984; Otten & Fuchs, 1988), is not the only hypothesis explaining the unequal distribution of mites.

Acknowledgements

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7

Attractiveness of honey bee brood cells to *Varroa jacobsoni* is correlated to the distance from larva to cell rim.

Abstract

Varroa jacobsoni Oudemans (Acari: Varroidae) was studied with respect to invasion into different types of honeybee, *Apis mellifera* L., brood cells. Different cell types were obtained by shortening and elongating of cells, grafting worker larvae into drone cells and vice versa. The type of cell strongly affected the number of mites per cell, and the attractive period of the cells to the mites. The type of cell also affected the distance from larva to cell rim preceding cell capping. When this distance was larger in comparison to control cells of the same age, the attractive period of the brood cells was shorter and vice versa. Since in all cell types the distance from larva to cell rim continuously decreased preceding cell capping, this negative correlation is in agreement with the hypothesis that there is a critical larva-rim distance under which brood cells are attractive to mites. Then, the length of the attractive period of brood cells depends on the moment this critical distance is reached. The distribution of mites over different cell types in turn results from differences in the attractive period.

Introduction

Varroa jacobsoni Oudemans, worldwide a major pest of *Apis mellifera* L., parasitizes both adult bees and honeybee brood, but only reproduces in the capped brood cells (Ifantidis & Rosenkranz, 1988). Since invasion into brood cells is crucial for reproduction of the mites, this aspect has been studied frequently, especially the resulting distribution of mites over different cell types. A well known example is the more frequent occurrence of mites in drone cells when offered the choice between worker and drone cells (e.g. Fuchs, 1990). This phenomenon may be explained as a differential response to the two types of larvae. However, non-random distributions of mites over different cell types have also been found when the type of larva was the same. De Jong & Morse (1988) and De Ruijter & Calis (1988) found more mites in worker cells protruding above the comb surface than in neighbouring worker cells. Another example is due to Rosenkranz (Meeting EC-Experts Group, Thessaloniki 1984, unpublished), Calis et al. (1993a), and Ramon et al. (1993) who found more mites in the smaller cells, when brood attractivity to mites was tested in cells differing in diameter. Finally, Calis et al. (1993a) and Goetz & Koeniger (1993) found more mites in shortened worker cells.

Why do *Varroa* mites invade certain types of brood cells more frequently than others? The distribution of mites over cells depends on the way the mites select a cell for invasion. Brood cells are invaded during a certain period preceding cell capping: c. 1 day for worker brood cells, and c. 2 days for drone brood cells (Ifantidis, 1988; chapter 6). To find a suitable brood cell *Varroa* mites do not walk over the comb nor go in and out the cells. They move directly from the bee into the selected cell, and probably use a chemical or physical signal like heat production to decide whether to stay on the bee or to invade the cell (chapter 1). When signal strength decreases with increasing distance between larva and cell rim, the

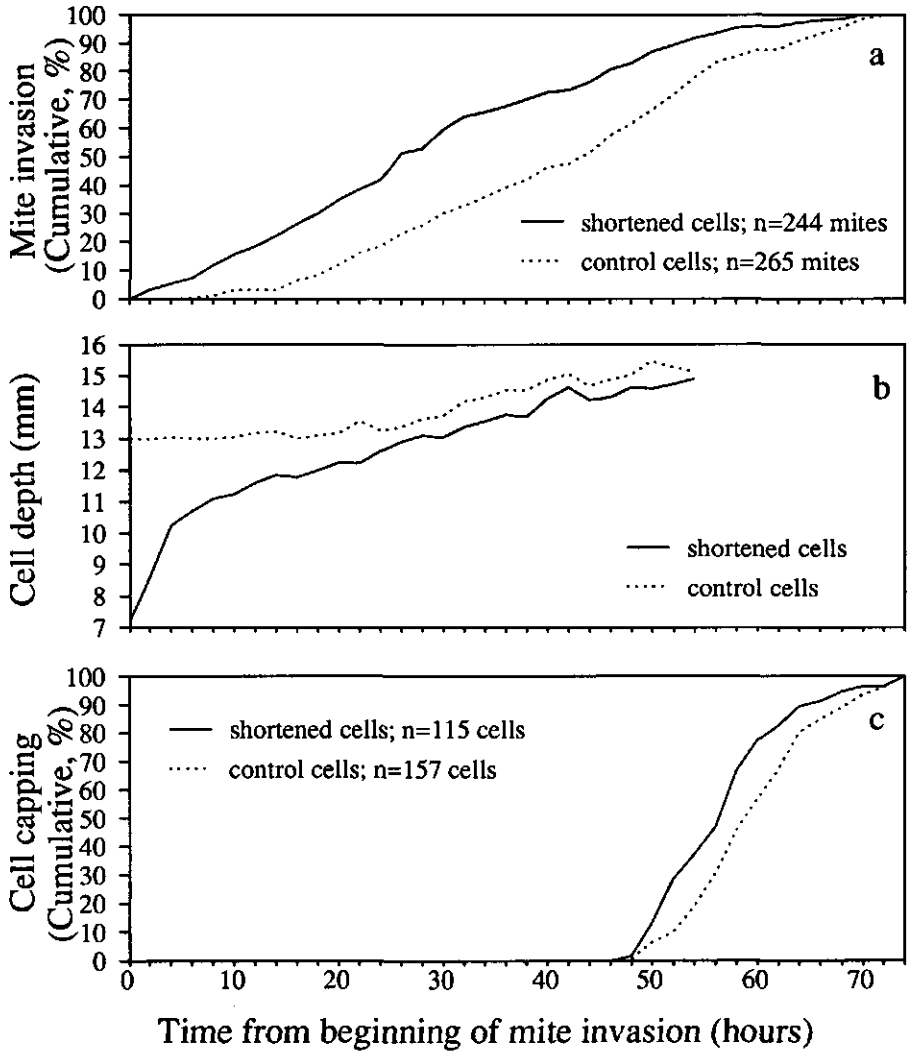

Figure 1: Invasion of mites into shortened and control drone brood cells. Example of the data collected during the course of the experiments to determine the attractive period of brood cells: cumulative number of mite invasions (A), cell depth (B), and cell capping (C).

response to a signal coming from the brood cell may well be correlated to this distance. Since larvae grow and the length of their cells remains more or less the same, the distance from larva to cell rim probably decreases during development of the larva. Hence, there may be a critical distance between larva and cell rim under which brood cells become attractive to the mites. The type of cell may determine how many hours before cell capping this critical distance is reached, thus defining the length of the attractive period. In addition, it is expected that more mites will invade per cell when cells are attractive for a longer time span, since the probability for a mite to invade a cell is constant per time unit (chapters 2,3).

In the above referred studies, the biased distribution of mites over various cell types possibly occurred because the type of cell affected the moment at which the critical distance between larva and cell rim was reached. As a result of shortening the cells (Calis et al.,1993a; Goetz & Koeniger, 1993), the distance between larva and cell rim was shortened as well. A critical distance would be reached earlier. Assuming that the moment of capping depends on the age of larvae and that the moment of capping is not much affected by shortening of the cells, the attractive period would thus be extended. In cells that protrude above the comb surface after cell capping (De Jong & Morse, 1988; De Ruijter & Calis, 1988), the critical distance may also have been reached earlier than in other cells. Protruding cells had possibly been lengthened by the bees just before capping, whereas the cells had already become attractive to the mites at that time. Hence, the distance from larva to cell rim may have been shorter in the period that the cells were about to become attractive, which resulted in the critical distance being reached earlier. Finally, in cells with a smaller diameter than others (Rosenkranz, unpublished; Calis et al., 1993a; Ramon et al., 1993), the distance between larva and cell rim was probably smaller as well, because after the larva has completely covered the cell bottom, its body can expand only in the direction of the cell opening. If so, then the critical distance would also be reached earlier in development of the larva.

These observations lead to the phenomenological hypothesis that the length of the attractive period depends on the moment a critical distance from larva to cell rim is reached, and that the distribution of mites over different cell types in turn results from differences in the attractive period. In this study we intend to subject this a posteriori hypothesis to a more precise test by studying not only the distribution of mites over various cell types, but also the length of the attractive period and the distance from larva to cell rim.

Materials & Methods

All experiments were carried out in 10-frame hives with common Dutch honeybees, *Apis mellifera* L. Three kinds of experiments were carried out: (1) to estimate the period that brood cells are attractive to mites, (2) to determine the distribution of mites over different cell types, and (3) to measure the distance from larva to cell rim of different cell types in relation to the time preceding cell capping.

Determination of the attractive period of brood cells

In these experiments 'half-combs' from which the bottoms of the cells had been replaced by a transparent sheet, were used (Beetsma et al., 1993; chapter 6). Two of these 'half-combs', one of them containing a patch of brood, were clamped together and placed in the middle of a bee colony, heavily infested with mites. Other combs containing open brood

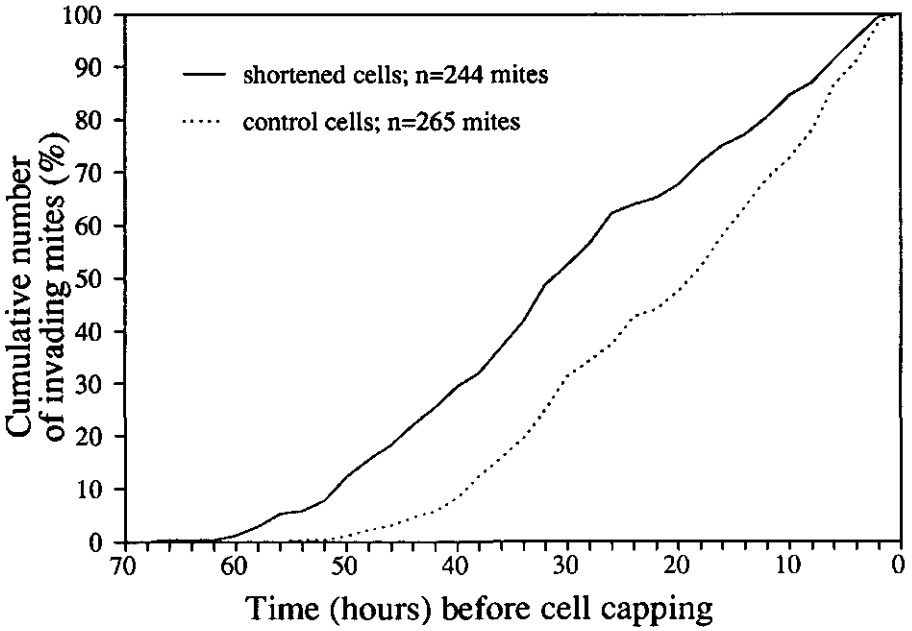

Figure 2: Cumulative relative number of mites invading shortened and control drone brood cells preceding cell capping; same example as figure 1.

were removed from the colony. Mites could therefore only invade cells of the patch of brood introduced for the experiment.

Invasion of mites was recorded by removing the test comb from the colony, brushing the adhering bees into a bucket, separating the two 'half-combs', and holding the 'half-comb' with brood against the light. Mites could easily be seen through the transparent cell bottoms. Invasion of mites was recorded every two hours. For each cell, records were made when a mite had appeared and when the cell had been capped. These data were later used to calculate how many hours preceding cell capping each mite had invaded into a cell. Subsequently, the 'half-combs' were clamped together, put into the colony again, and the bees collected in the bucket were returned to the colony by shaking them on top of the test comb. Other combs were kept covered with cloth during hive manipulations, to minimize disturbance of the colony. After finishing the observations, the capped cells were opened to check the number of mites in cells.

Invasion of mites was recorded in normal worker and drone cells, shortened worker and drone cells, elongated worker cells, drone cells with worker larvae, and worker cells with drone larvae. Shortened cells were made by carefully cutting the cell rims from one half of a patch of brood by using electrically heated wire as a knife. This was done about 10-20

hours before the cells were normally expected to become invaded by mites (chapter 6). The other half of the brood patch was used as a control. After cutting the cell rims, the bees started rebuilding the cells. This process was monitored by emptying 8 evenly distributed cells in the brood patch, 4 in the shortened part and 4 in the control part, and measuring cell depth every two hours during inspections. Elongated cells were made indirectly as follows. A droplet of melted wax was put into all six adjacent cells of the brood cell to be elongated. This droplet of wax raised the bottom of the cells about 3 mm. Subsequently, the comb was put into a colony and the queen was allowed to oviposit into the cells. Eggs in the cells to be elongated were removed. The bees elongated the cells containing brood due to the raised cell bottom. Consequently, the cells enclosed were elongated to the same height as that of the surrounding cells, but the cells were 3 mm deeper. Young larvae, less than one day old, were grafted into the elongated cells. Only one half of the 'half-comb' was used to record invasion into elongated cells. The other half was used as control, whereas the control brood cells were alternated with capped brood cells in the same pattern as for the elongated cells.

To monitor invasion of mites into drone cells with worker larvae and worker cells with drone larvae, young larvae, less than one day old, were grafted into the cells. Invasion into drone cells with worker larvae and vice versa was not directly compared with control cells, because when control cells were present the bees did not nurse the manipulated brood cells well enough, and many larvae died.

Distribution of Varroa mites over different cell types

The number of mites invading various brood cell types was determined and compared with control brood cells. A comb containing both a brood patch of a certain cell type and a patch of control brood cells, was placed into a heavily infested colony that had their own combs with open brood removed. Every four hours, capping of both manipulated and control cells was marked on transparent sheets. After capping of most of the brood cells, the comb was removed from the colony and the cells were checked for mites. To compare the number of mites per cell, only those 4-hour intervals were used during which 10 or more mites had invaded. Differences from a random distribution between manipulated and control cells were tested with the chi-square test. The ratio of mites per manipulated cell to their number per control cell was calculated for each interval. Subsequently, the average ratio was calculated from the ratio's in the separate intervals, weighed to the number of mites that had invaded.

The number of mites in shortened worker cells, elongated worker cells, and drone cells with worker larvae, were compared with the number in control worker cells. The number of mites in shortened drone cells and worker cells with drone larvae were compared with control drone cells. Brood patches of various cell types were obtained using the same methods as for the experiment to determine the attractive period of brood cells.

Distance from larva to cell rim

The distance from larva to cell rim was determined in relation to the time preceding cell capping. A brood comb with many old larvae, the cells of which were capped by the bees within 30 to 50 hours, was taken from a colony. The distances from these larvae to the cell rims were measured with a probe, as used by Goetz & Koeniger (1992). The brood comb was put into the colony again, and subsequently every two hours a transparent sheet was placed over the brood area on which newly capped cells were marked. In this way, the distance to

the cell rim in relation to the time preceding cell capping was measured for control worker and drone cells, worker larvae in elongated cells, worker larvae in drone cells, and worker larvae in an artificial worker comb (Wieting & Ferenz, 1991). This artificial comb has plastic cells of which the bottom is as wide as a drone cell and the opening as wide as a worker cell. Because of the conical shape, a different distance from larva to cell rim was expected. Worker larvae in elongated cells and worker larvae in drone cells were obtained as before.

Table 1: Differences in the period attractive to Varroa mites between various cell types containing worker larvae and control worker cells. The attractive period is defined as the period preceding cell capping during which 90% of the mites invaded.

Treatment	Number of mites	Percentage of mites overlooked	Attractive period[1] (hours)
Shortened worker cells	110	3.5	26***
Control	92	1.1	20
Shortened worker cells	70	2.8	24***
Control	31	11.4	14
Shortened worker cells	95	4.0	30***
Control	49	10.9	18
Elongated worker cells	16	42.9	6***
Control	114	6.6	14
Drone cells with worker larvae	45	13.5	12
Drone cells with worker larvae	24	29.4	3[2]
Drone cells with worker larvae	28	6.7	7[2]

[1]: *Differences in the distribution of invasions over time between shortened or elongated cells and control cells were tested with the Kolmogorov-Smirnov Two Sample test, *** = P<0.001*
[2]: *Invasion of mites was recorded every hour instead of every 2 hours.*

Results

Attractive period of brood cells

In the experiments to determine the attractive period of brood cells, records were made of mite invasion into brood cells and capping of cells. In addition, differences in cell depth between control and shortened brood cells were monitored. Figure 1 shows an example for the experiment with the highest number of mites invaded. Cell capping of control and manipulated cells always occurred during the same period (Fig. 1c), but mite invasions took place during different periods in control and manipulated cells (Fig. 1a). After cutting the rims from the cells in the experiments with shortened cells, the bees quickly started rebuilding, although the cells remained shorter than control cells for a long time (Fig. 1b). The cell rims were therefore cut for a second time when no mites had invaded after 6 hours, to maintain a marked difference between control cells and shortened cells at the moment at which invasion started.

For each mite the time of invasion was calculated in relation to cell capping (Fig. 2, same example as Fig. 1). Sometimes, one or a few mites invaded much earlier before capping than the others. to reduce the effect of an accidental early invasion on the estimate of the attractive period of brood cells, the attractive period was arbitrarily defined as the period preceding cell capping during which 90% of the mites had invaded. Tables 1 and 2 summarize the results.

Table 2: Differences in the period attractive to Varroa mites between various cell types containing drone larvae and control drone cells. The attractive period is defined as the period preceding cell capping during which 90% of the mites invaded.

Treatment	Number of mites	Percentage of mites overlooked	Attractive period[1] (hours)
Shortened drone cells	244	5.8	52***
Control	265	5.7	40
Shortened drone cells	143	3.4	48***
Control	79	9.2	34
Worker cells with drone larvae	35	10.3	64

[1]: *Differences in the distribution of invasions over time between shortened cells and control cells were tested with the Kolmogorov-Smirnov Two Sample test, *** = P<0.001*

Table 3: Number of mites which invaded various cell types containing worker larvae in comparison with control worker cells. The number of time intervals in which mite invasion was compared, the total number of brood cells that were capped, the total number of mites that invaded, and the average ratio of mites per cell of a type of cell to their number in control cells, are shown.

Treatment	Number of intervals	Total number of cells	Total number of mites	Average ratio to control cells[1]
Shortened worker cells	8	526	203	2.17***
Shortened worker cells	3	270	70	2.85**
Shortened worker cells	4	74	122	2.85***
Elongated worker cells	3	137	127	0.16***
Drone cells with worker larvae	3[2]	411	69	0.49**
Drone cells with worker larvae	4[2]	310	124	0.44***

[1]: *Differences from a random distribution over cells of various types and control cells were tested with Chi^2-test, ** = P<0.01, *** = P<0.001*
[2]: *Intervals of 6 hours instead of 4 hours*

Shortening of both worker and drone brood cells always resulted in a longer attractive period when compared to control brood cells. Elongated worker cells were attractive to the mites during a shorter period than the control ones. Drone cells with worker larvae seemed to be attractive to mites during a shorter period than control worker brood cells, and drone larvae in worker cells seemed to be attractive during a longer period than control drone brood cells, although the attractive period of these cell types could not be compared directly with that of control cells.

After assessing the real number of mites in the cells, the percentage of mites overlooked appeared to be less than 15% in 14 of the 16 brood patches recorded. In elongated worker cells however, the percentage of mites overlooked was very high: 43%. This may be due to the short attractive period of elongated cells. A larger part of the mites is then expected to invade shortly before capping. Because cells were inspected at 2-hourly intervals, the cells

Table 4: Number of mites which invaded various cell types containing drone larvae in comparison with control drone cells. The number of time intervals in which mite invasion was compared, the total number of brood cells that were capped, the total number of mites that invaded, and the average ratio of mites per cell of a type of cell to their number in control cells, are shown.

Treatment	Number of intervals	Total number of cells	Total number of mites	Average ratio to control cells[1]
Shortened drone cells	4	397	185	1.84^{**}
Shortened drone cells	7	271	542	1.68^{***}
Worker cells with drone larvae	4[2]	156	534	1.20^{NS}

[1]: *Differences from a random distribution over cells of various types and control cells were tested with Chi^2-test, NS = not significant, ** = P<0.01, *** = P<0.001*
[2]: *Intervals of 6 hours instead of 4 hours*

invaded during the preceding 2 hours were often already capped and the larva had stretched to start spinning (Jay, 1963). At this stage, mites do not stay on the bottom of the cell anymore. They may be hidden behind the larva, and the chance for a mite to be overlooked increases. The shorter attractive period may also be the reason for the relatively high percentages of mites overlooked in drone cells with worker larvae. Although in some cases a considerable part of the mites was overlooked, the estimates for the attractive period may still be reliable, provided that overlooked mites invaded within the same period as the others.

Distribution of Varroa mites over different cell types

The cell type strongly affected the number of mites that invaded. In shortened worker brood cells, 2 to 3 times as many mites were found per cell when compared with control cells (Table 3). In elongated worker cells and in drone cells with worker larvae, fewer mites were found per cell when compared with control cells: in elongated worker cells about 1/6, and in drone cells with worker larvae about 1/2 of the number in control cells. Shortening of drone cells also resulted in more mites per cell: about 1.5 to 2 times as many mites as in control drone cells (Table 4). Between worker cells with drone larvae and control drone brood cells no significant difference was found in the number of mites per cell.

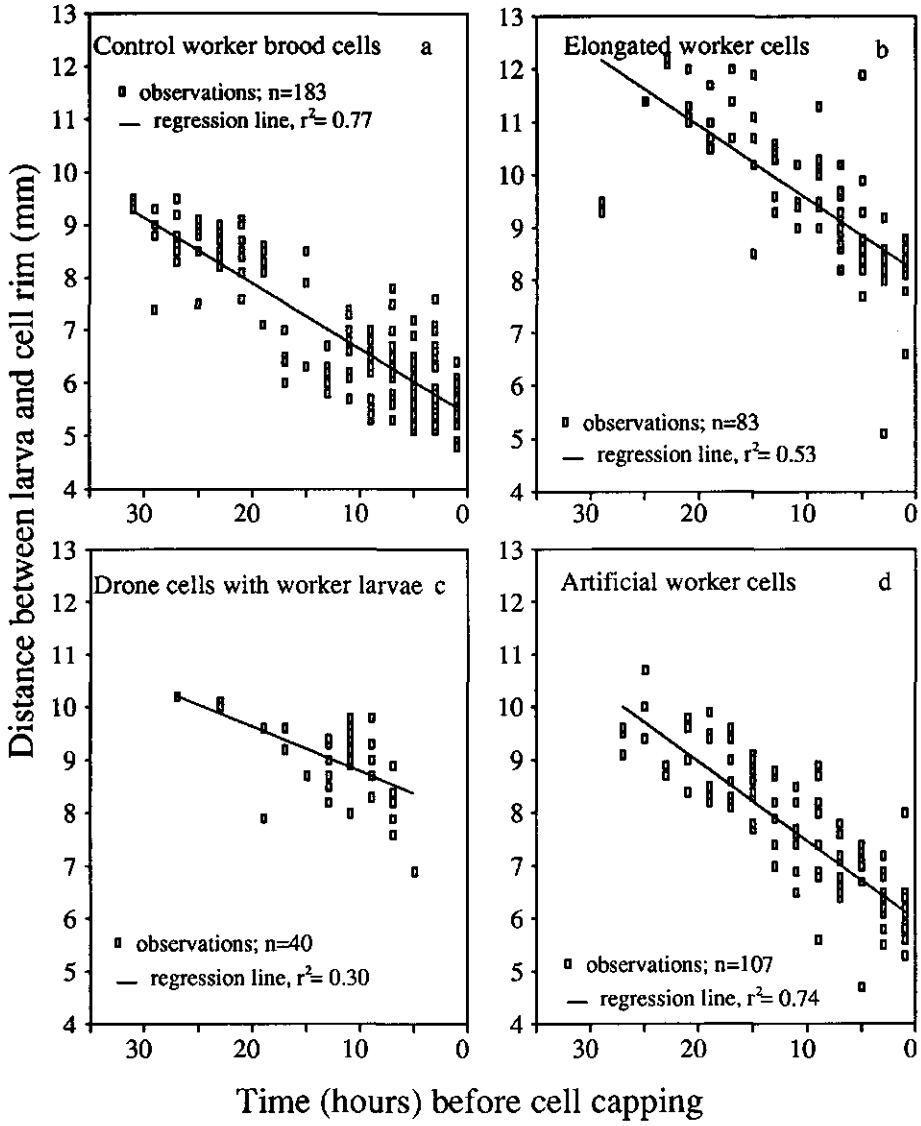

Figure 3: The distance between larva and cell rim in relation to the time before cell capping of worker larvae in control worker cells (A), in elongated worker cells (B), in drone cells (C), and in artificial worker cells (D).

The distance from larva to cell rim

In the 30 hours preceding cell capping, the distance from larva to cell rim decreased linearly in cells with worker larvae (Fig. 3). Control worker cells were capped when this distance was about 5.5 mm (Fig. 3a). In elongated worker cells the same relationship between time before capping and distance from larva to cell rim was found, but this distance was about 3 mm more than that of control cells at the same time before capping (Fig. 3b), corresponding to the distance by which the cells had been elongated. In drone cells with worker larvae, the distance from larva to cell rim was also much larger than in control worker cells: about 2 to 3 mm (Fig. 3c). In the artificial worker cells, the distance from larva to cell rim was about 0.5 to 1 mm more than in control worker cells.

Drone brood cells showed a different relationship between the distance from larva to cell rim and the time preceding cell capping (Fig. 4). From about 35 hours preceding cell capping, the distance from larva to cell rim remained on average about 7 mm. Only before 35 hours preceding cell capping a decrease of this distance was found in relation to time.

In general, the distance between larva and cell rim decreased with time. Hence, the critical distance at which mites start to invade the cells may be estimated by taking the distance found at the start of the attractive period. Figure 5 shows such estimates, based on the attractive periods found in this study and in two other studies, and based on the regression lines from figure 3 for cells with worker larvae and the average distance from larva to cell rim from figure 4 for cells with drone larvae. For worker brood cells attractive periods between 12 and 20 hours preceding cell capping were found, and for drone brood cells attractive periods between 33 and 40 hours preceding cell capping were found. Such attractive periods are related to critical distances from larva to cell rim between 6.9 and 7.9 mm for worker cells and between 7.2 and 7.8 mm for drone cells. For the artificial worker cells, an attractive period of 6 hours preceding cell capping was found (Wieting & Ferenz, 1991). This period is related to a critical distance of 6.9 mm, the same distance as estimated from Wieting & Ferenz's attractive period of 12 hours preceding cell capping for control worker brood cells. The critical distances for invasion into elongated worker cells and for invasion into drone cells with worker larvae were estimated to be larger: between 8.2 and 9.0 mm.

Discussion

The type of brood cell strongly affected the number of mites per cell (Tables 3&4), and the differences in the number of mites found per cell were related to the length of the attractive period to mites (Tables 1&2). When the attractive period was longer, more mites invaded. Because a longer attractive period implies that more cells are available, this positive relationship is in agreement with earlier results showing that more mites will invade when more brood cells are available (chapters 2,3). The results show that the size of cells has to be taken into account when attractivity of brood cells to *Varroa* mites is compared between various honey bee strains or races. Brood of small-sized bees may for instance be less attractive to the mites than brood of large-sized bees, when compared in standard combs. Standard combs will be relatively large for the small bees and relatively small for the large bees. Differences in brood attractivity may in such cases be solely caused by differential attractive periods of the brood cells due to relative differences in cell size.

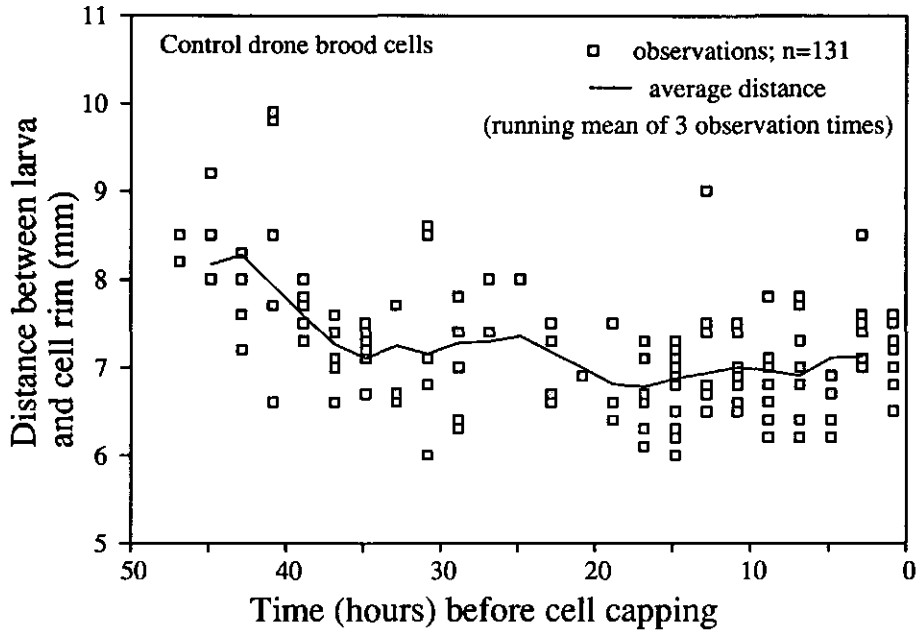

Figure 4: The distance between larva and cell rim in relation to the time before cell capping in control drone cells.

In addition to the relationship between the attractive period and the number of mites per cell, a relationship between the attractive period and the distance from the larva to the cell rim was found. Cell types with a larger distance in comparison to control cells of the same age, had a shorter attractive period and vice versa. Since the distance from larva to cell rim continuously decreased preceding cell capping, this negative relationship is in agreement with the hypothesis that mites start to invade cells when the distance from larva to cell rim drops below a threshold. In normal brood cells, this critical distance for invasion appeared to be 7 to 8 mm, both in worker and drone cells (Fig. 5). Goetz & Koeniger (1993) found a similar distance at which invasion into worker brood cells starts: between 7 and 7.5 mm. In Wieting & Ferenz's study (1991) a shorter attractive period was found for artificial worker cells than for control cells: 6 hours and 12 hours, respectively. No explanation was provided for this difference in attractive period. Our results show that at the same time before cell capping the distance from larva to cell rim is larger in the artificial cells than in control cells (Fig. 3a,d). Hence, in the artificial cells the critical distance is reached at a later moment in the development of the larva, resulting in a shorter attractive period. Based on the attractive periods of 6 and 12 hours for the artificial and control cells respectively (Wieting & Ferenz, 1991), both periods indeed give the same estimate for the critical distance: 6.9 mm (Fig. 5). Thus, the shorter attractive period of the artificial cells may be caused by the differential distance from larva to cell rim. This distance may be larger in the artificial cells, because

these cells are conical with a bottom that has the same width as a drone cell. Hence, the larva can expand more without being forced in the direction of the cell opening. The same explanation may be valid for the larger distance from larva to cell rim found in drone cells with worker larvae.

The effect of distance from larva to cell rim on invasion implies that this distance interferes with the way mites select brood cells. In theory, mites may measure the distance from larva to cell rim, whereas they use this distance as a correlate for cell suitability. However, this is unlikely because the mites cannot estimate the distance visually and because they do not select brood cells by walking in and out cells but select cells while they stay on an adult bee (chapter 1). Thus they probably use a signal coming from the larva like heat production or production of volatile chemicals, whereas the distance from larva to cell rim affects the strength of the signal as it reaches a mite on a bee.

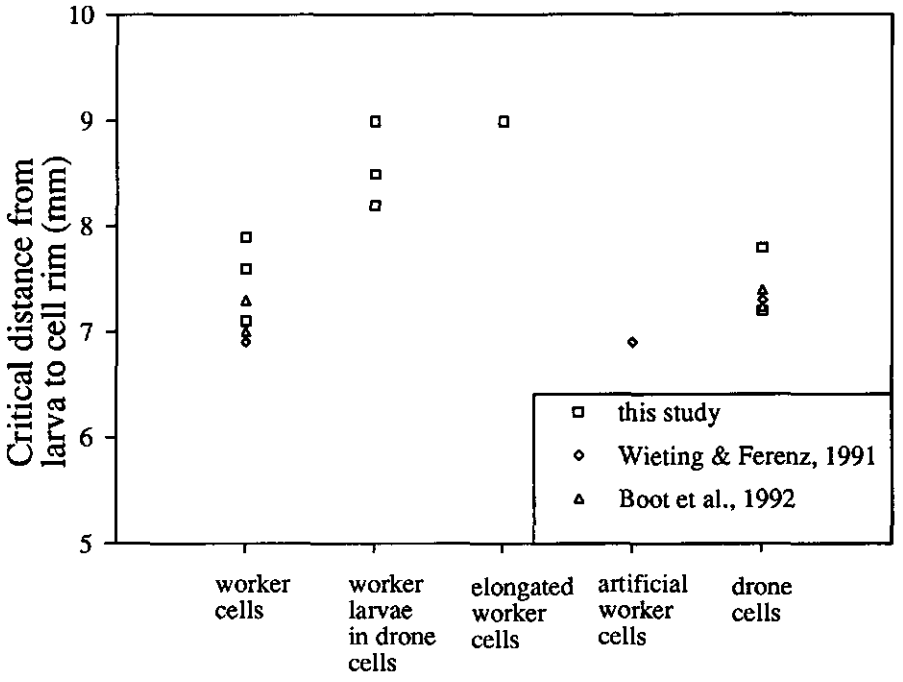

Figure 5: Critical distance from larva to cell rim at which invasion into brood cells is estimated to start in different cell types.

In elongated worker cells and drone cells with worker larvae, the critical distance at which invasion starts was estimated to be larger than in control brood cells (Fig. 5). Since the attractive period was shorter for elongated worker cells and for drone cells with worker larvae, the larva was older when invasion by mites began. Possibly, the critical distance for invasion is larger when the larva is older, because the strength of a signal coming from the larva may increase with larval age. This may also explain why invasion did not always start immediately after shortening of cells, though the distance from larva to cell rim was clearly shorter than 7 mm. The signal coming from these young larvae may have been too weak for invasion to occur.

Our results are in agreement with the hypothesis that the length of the attractive period depends on the moment a critical distance from larva to cell rim is reached, and that the distribution of mites over different cell types in turn results from differences in the attractive period. The number of mites that eventually invade in different cells is still difficult to quantify, however, because the number of mites present on the adult bees in a colony is never constant. This number varies strongly due to invasion into open brood cells and emergence from capped brood cells. In this study, the number of mites per cell were therefore compared between types of cells that had been capped during the same time interval. Since the attractive period of these cells was different however, the cells compared were still not exposed to the same number of mites on adult bees. Furthermore, the rate of invasion per cell may vary during the attractive period and between cells of different types. In any case, there seems to be an important difference in the rate of invasion into cells with worker larvae and cells with drone larvae. The rate of invasion per drone brood cell was found to be about 12 times as high as the rate of invasion per worker brood cell (chapter 4). This higher rate of invasion per cell is partly due to the 2-3 times longer attractive period of drone cells and, when the area of brood determines the rate of invasion rather than the number of cells, to the 1.7 times larger area of drone cells on the comb's surface in comparison with worker cells. Taken together this would result in a 3.4-5.1 times higher rate of invasion per drone cell, which is not enough to explain the 12 times higher rate found. Thus, the rate of invasion per cell is another 2-4 times increased by the presence of a drone larva versus a worker larva.

Acknowledgements

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8

Methyl palmitate does not elicit invasion of honey bee brood cells by *Varroa* mites.

Introduction

Varroa jacobsoni Oudemans, a major acarine pest of the honey bee, *Apis mellifera* L., can only reproduce in capped brood cells (Ifantidis & Rosenkranz, 1988). Outside the brood cells, *Varroa* mites stay exclusively on adult bees. The mites do not walk over the comb, nor do they leave a cell once they have entered it. They are carried close to a suitable cell by a bee, and move directly from the bee into the cell (chapter 1).

Varroa mites invade worker and drone cells during a period of 15-20 h and 40-50 h preceding cell capping respectively (chapter 6). They may obtain information about the content of the cell to determine whether to stay on the bee or to invade a brood cell, e.g. by using volatile chemicals. Recently, it has been found that the response of the mites is correlated with the distance from larva to cell rim (chapter 7). For example, cells that had been shortened were invaded earlier than control cells containing larvae of the same age. The results suggest that the distance from larva to cell rim affects the strength of a signal reaching the mites on bees, and that invasion occurs when the strength of this signal passes a threshold.

Mites invade drone cells with a 12 times higher rate than they invade worker cells (number of mites/(cell.day); chapter 4). This higher invasion rate partly results from the 2-3 times longer attractive period of drone cells. Additionally, a 1.7 times higher invasion rate is expected if the invasion rate is proportional to the area on the comb's surface of a brood cell. Taken together, this would result in a 3.4-5.1 times higher invasion rate, much less than the rate actually found which is 12 times higher (chapter 4). Thus, the higher invasion rate into drone cells may be mainly attributed to quantitave or qualitative differences in the signal to invade between worker and drone cells.

Le Conte et al. (1989) claimed that the signal for invasion of both worker and drone cells consists of the odour of a few aliphatic esters, especially methyl palmitate. They showed that each of these esters, which had been extracted from the larval cuticle, attracted mites in an olfactometer. A maximum of 17 ng and 320 ng of methyl palmitate can be extracted from the cuticle of a worker or drone larva respectively (Trouiller et al., 1991). Since drone larvae secrete much more of the esters, and these are secreted during a longer period preceding cell capping (Trouiller et al., 1992), secretion of the esters correlates with the differential invasion of worker and drone cells by the mites. Thus, methyl palmitate may well be a signal used by *Varroa* mites for invasion.

In the study of Le Conte et al. (1989), the response of mites to methyl palmitate was determined in an olfactometer in which the mites walked on a flat surface of 56 x 56 mm. Such a bio-assay only demonstrates that mites can respond to this ester, but not whether they use the ester to decide to enter a cell or stay on a bee. In the present study, methyl palmitate was applied to worker cells to determine whether it actually affects invasion by *Varroa* mites.

Both the number of mites found per treated cell, and the length of the attractive period of treated cells were compared with that of control cells. If methyl palmitate is a primary signal to invade, it is expected that application will increase the number of mites per cell and that cells will attract mites over a longer period.

Materials & Methods

Experiments were carried out in 10-frame hives with honey bees commonly used in the Netherlands. Special 'half-combs', from which the bottoms of the cells had been replaced by a transparent sheet, were used (Beetsma et al., 1993). Two 'half-combs' were clamped together and placed in the middle of a bee colony heavily infested with *Varroa* mites (chapter 7). One of the 'half-combs' contained a patch of open worker brood with larvae varying up to one day in age (Boot & Calis, 1991). No other combs with open brood were present in the colony.

Table 1: Attractive period of worker cells treated with acetone or methyl palmitate dissolved in acetone and control worker brood cells. The attractive period was defined as the period preceding cell capping during which 90% of the mites invaded. Differences in the distribution of invasions over time before cell capping between treated and control cells were tested with the Kolmogorov-Smirnov Two Sample test

Treatment	Number of mites that invaded	Attractive period[1] (hours)
Acetone	46	10^{NS}
Control	32	12
Acetone	21	14^{NS}
Control	123	12
Me-palmitate 1%	25	14^{NS}
Control	108	14
Me-palmitate 0.1%	64	12^{NS}
Control	108	14
Me-palmitate 0.1%	61	12^{**}
Control	40	12

[1]: *NS = not significant; ** = P<0.01*

To determine the attractive period of treated and control worker cells, the test comb was removed from the colony, the adhering bees were brushed gently into a bucket, the two 'half-combs' were separated, and the patch of brood was held against the light. Mites could easily be seen through the transparent cell bottoms. This inspection was repeated at two-hour intervals. For each cell, records were made of when a mite had appeared and when the cell had been capped. These data were later used to calculate when each mite had invaded in terms of the number of hours preceding cell capping. After inspection the 'half-combs' were clamped together, returned to the colony, and the bees collected in the bucket were replaced by shaking them on top of the test comb.

Treatment of cells commenced after one or a few cells of the patch of brood had been invaded. Into each of a group of about 100 cells a droplet consisting of 2 µl of 10, 1 or 0.1% methyl palmitate dissolved in acetone (v/v) was applied (Wirtz, 1973). This corresponds to an additional 172, 17.2 or 1.72 ng methyl palmitate in the treated worker cell respectively.

The number of mites invaded per cell were also compared between treated and control cells. The number of mites per cell should be compared between cells that were attractive to mites during the same period because the number of mites that reside on adult bees varies strongly over time in a bee colony. Therefore, the cells were grouped in intervals of 4 hours according to their capping time, and each interval was treated as a pseudo-replicate. Only those 4-hour intervals during which 10 or more mites had invaded were used to compare the number of mites per cell. This resulted in 4 intervals in all trials. For each interval the ratio of the number of mites per treated cell to the number per control cell was calculated. The average ratio was calculated from the ratios in the separate intervals, weighted to the number of mites that had invaded.

Results

Since methyl palmitate was dissolved in acetone, tests were first carried out to determine whether acetone itself affected invasion of the cells by the mites. A droplet of acetone (2 µl) had no effect on either the attractive period of the cells (Table 1) or the number of mites per cell (Table 2). Application of methyl palmitate did not affect invasion of the cells either (Table 1,2). The only difference between treated and control cells was found in one of the trials in which 0.1% methyl palmitate was applied. In this trial, a larger proportion of mites invaded 0-6 h preceding cell capping in comparison with the control, whereas the length of the attractive period was similar.

Applying high doses of methyl palmitate resulted in mortality of the larvae. When cells were treated with 10% methyl palmitate all larvae in the treated cells died, either directly because of the treatment or indirectly because the bees no longer nursed the brood. Treatment with 1% methyl palmitate also caused some mortality: 1/3 of the larvae died in one trial, and 73 out of 75 larvae died in the other. Treatment with 0.1% methyl palmitate or acetone alone did not cause mortality.

Table 2: Number of mites which invaded worker cells treated with acetone or acetone with methyl palmitate in comparison with control worker brood cells. The numbers were compared in 4 intervals of 4 hours. The average ratio of the number of mites per treated cell to the number per control cell was calculated from the ratios in the separate intervals, weighted to the number of mites that had invaded.

Treatment	Total number of treated cells capped	Total number of control cells capped	Total number of mites	Average ratio to control cells[1]
Acetone	77	120	70	0.93^{NS}
Acetone	31	142	152	$0.80^{NS;3}$
Methyl palmitate 1%[2]	49	127	103	0.56^{NS}
Methyl palmitate 0.1%	90	171	165	1.10^{NS}
Methyl palmitate 0.1%	44	48	94	$1.50^{NS;4}$

[1]: *Differences in number of mites per cell between treated and control cells were tested with Chi^2-test, NS = not significant.*
[2]: *1/3 of the larvae in treated cells were removed by the bees before capping. In another trial (not shown), 73 of 75 larvae were removed from the treated cells.*
[3]: *When the data from the two Acetone treatments are combined the average ratio is 0.82 and there is no significant difference in the number of mites per cell ($0.3<P<0.5$).*
[4]: *When the data from the two 0.1% Methyl palmitate treatments are combined the average ratio is 1.27 and there is no significant difference in the number of mites per cell ($0.3<P<0.5$).*

Discussion

The results do not support the suggestion that *Varroa* mites use methyl palmitate as a signal to invade brood cells of the honey bee, because application of this substance to brood cells did not increase the number of mites per cell, nor did it prolong the attractive period of the cells. In theory, methyl palmitate could still be one of the components of a composite signal, whereas it has no effect when offered alone. Furthermore, it cannot be excluded that the bioassay used was unable to show an effect. However, there is other evidence that methyl palmitate does not play a role in brood cell invasion. Mites select brood cells while on adult bees. Thus, a signal from the larva has to bridge at least the distance from larva to cell rim, which measures 5-8 mm in attractive brood cells (Goetz & Koeniger, 1993; chapter 7). Therefore, a chemical that is used by the mites as a signal is expected to be volatile. Analysis of the headspace volatiles above attractive brood cells (c.f. Dicke et al., 1990 for methodology), showed hundreds of components in the volatile blend, but in only 2 out of 17 analyses was a trace of methyl palmitate found (M.A. Posthumus & W.J. Boot, unpublished).

Thus, both the experiment described here and the analysis of volatiles give no reason to think that methyl palmitate is used as a signal for invasion.

Le Conte et al. (1990) showed that methyl palmitate is used by the bees as a pheromone evoking capping behaviour. When paraffin lures impregnated with methyl palmitate were placed in cells, these cells were capped by the bees. In the experiment described here there was no evidence to suggest that treated cells were capped earlier than control cells. The doses of methyl palmitate applied to the cells may have been too low.

The bio-assay described here may be used to test whether chemicals other than methyl palmitate do affect invasion of brood cells. Some components of the volatile blend coming from brood cells have been tested already (W.J. Boot, unpublished) but so far no attractant has been identified. However, application of two substances that were found in the headspace volatiles above drone larvae, capro-lactone and valero-lactone, resulted in a greatly reduced number of mites per cell.

The bio-assay method described here has some advantages over an olfactometer. It tests actual invasion behaviour of the mites whereas the olfactometer merely shows whether the mites respond to a specific odour. In addition, looked at the viewpoint of a *Varroa* mite, there are two separate problems concerning invasion of a cell: (1) how to identify whether or not a cell contains a larva, and subsequently (2) how to determine whether a cell is suitable to invade. Different stimuli may be involved in solving these two problems, and lack of stimuli indicating that a larva is present may prevent a response to stimuli indicating that a cell is suitable. If so, stimuli indicating that a cell is suitable to invade can still be tested with the bioassay described here, because substances are applied to brood cells and this implies that stimuli identifying larvae are present.

Acknowledgements

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9

Why do *Varroa* mites invade worker brood cells of the honey bee despite lower reproductive success?

Abstract

Varroa jacobsoni reproduces both in drone and worker brood cells of honey bees, but in drone cells reproductive success is higher than in worker cells. A simple model using clonal population growth as a fitness measure has been developed to study under which circumstances specialization on drone brood would be a better strategy than reproduction in both types of cell. For European *Apis mellifera*, the model suggests that if mites have to wait less than 7 days on average before they can invade a drone cell, specialization on drone brood would be a better strategy. This is close to the estimated waiting time of 6 days. Hence, small differences in reproductive success in drone and worker cells, and in the rate of mortality may determine whether specialization on drone brood will be promoted or not. In European *A. mellifera* colonies, *Varroa* mites invade both drone and worker cells, but specialization on drone brood cells seems to occur to some extent because a drone cell is more frequently invaded than a worker cell. In the parasite-host association of *V. jacobsoni* with African or Africanized *A. mellifera* or with *A. cerana*, the mites also invade both drone and worker cells, but the mites specialize on drone brood with respect to reproduction since a large percentage of the mites in worker brood do not reproduce. Only in the parasite-host association of *Euvarroa sinhai*, a mite closely resembling *V. jacobsoni*, and *A. florea* specialization is complete because these mites only invade drone brood.

Introduction

Varroa jacobsoni is currently one of the most important pests in *Apis mellifera* colonies. It parasitizes adult bees, but reproduction only occurs while parasitizing on capped honeybee brood. Therefore, mites have to invade either drone or worker brood cells before capping (Ifantidis & Rosenkranz, 1988). After invasion into a brood cell, *Varroa* mites lay up to 6-7 eggs in 30-hour intervals, the first one being a (haploid) male egg and the following (diploid) female eggs (Ifantidis, 1983; Rehm & Ritter, 1989). However, the last eggs laid will usually not reach maturity, because the developmental time of the immature bee in the capped cell is too short to allow for complete mite development. Since the capped stage of drone cells is about two days longer than that of worker cells (Jay, 1963), drone cells are in principle more rewarding in terms of mite reproduction than worker cells because more young mites can reach maturity. In West-European *A. mellifera*, mites produce on average 2-3 viable female offspring in drone cells and 1-2 viable female offspring in worker cells (Schulz, 1984; Fuchs & Langenbach, 1989).

The question arises why *Varroa* mites do not restrict invasion to drone cells, thus maximizing their reproductive success. *Euvarroa sinhai*, a species closely related to *Varroa jacobsoni*, parasitizes *Apis florea* and apparently uses this reproductive strategy (Mossadegh & Birjandi, 1986; Kapil & Aggarwal, 1989). However, invading only drone brood cells may be less successful when mite density is such that invasion per drone cell is high. Since

reproduction per mite is negatively correlated with the number of mites that invaded in the same cell (Fuchs & Langenbach, 1989), reproductive success in worker cells may become just as high as that in drone cells when the density of mites increases in the colony. Using a mathematical model in which reproductive success was negatively influenced by mite density, Fuchs (1992) simulated the effect of the annual cycle of brood cell availability in typical European colonies on reproductive success. The simulations suggested that at an average infestation level of 300 or more mites per colony a *Varroa* mite should invade also worker cells to maximize reproduction. A second reason why exclusive invasion of drone cells may be a less successful strategy is the risk of losing much time while searching for a drone cell to invade, especially since drone brood cells may be scarce or even absent in the colony (Page & Metcalf, 1984; Rowland & Mclellan, 1987). During the period mites stay on adult bees waiting for a drone cell they may die. Moreover, they might have used this period to produce offspring in worker cells.

In this paper we calculate the net rate of increase of a *Varroa* mite population assuming a strategy of invading exclusively drone brood cells. Subsequently, we evaluate under which circumstances invading both drone and worker brood cells will result in higher population growth. In *Varroa* mites sib-mating is the rule since mating takes place inside the capped brood cells and in most cases one mother mite is confined to a cell. Therefore daughters and granddaughters increase fitness of a mother mite equally and a mite clone that has the highest population growth will outnumber others.

Comparing different reproductive strategies is not a mere theoretical exercise to increase insight into the system. Different reproductive strategies do exist. A high percentage of *Varroa* mites that invade worker cells of Africanized honey bees in Southern America refrain from reproduction (e.g. Camazine, 1986; Rosenkranz et al., 1990), and the same is reported for *Varroa* mites invading worker cells of *Apis mellifera intermissa* (Ritter et al., 1990; Ritter, 1993). In *Apis cerana*, *Varroa* mites invade both worker and drone cells, but as a rule reproduce only in drone cells (Tewarson et al., 1992; Rosenkranz et al., 1993). Finally, *Euvarroa sinhai* invades and reproduces only in drone brood cells of *Apis florea* (Mossadegh & Birjandi, 1986; Kapil & Aggarwal, 1989). This study aims to increase insight into which selective forces may have shaped the various reproductive strategies used by honey bee mites.

A model for the net rate of population increase

Consider a mite specializing on reproduction in drone brood cells only. At the beginning of her adult life she emerges from a brood cell and she stays for a certain time on adult bees searching for a new drone brood cell to invade. Subsequently she invades a drone brood cell and then reproduces, after which a new cycle starts. The expected number of offspring, m(t), produced over the total period from emergence via invasion to emergence (i.e. the sum of the time spent searching, T_s, plus the time spent in development, T_d; $t = T_s + T_d$) is:

$$m(T_s + T_d) = f(T_s)N_d$$

where the time spent searching for a drone cell, T_s, is a stochastic variable with probability density function $f(T_s)$, T_d denotes the developmental time of capped drone brood, and N_d denotes the number of females emerging from the drone brood cell (N_d includes the mother if she survives, based on the assumption that the current reproductive effort is independent of the reproduction in the past). This parameter will also be determined by behaviour of the

bees (brood care, brood removal). When $t<T_d$, the time is too short to produce offspring, and when $t>T_d$ offspring is produced if a mite finds a drone cell in the time that is left for searching,
$T_s = t-T_d$:

$$m(t) = 0 \qquad (t<T_d)$$
$$m(t) = f(t-T_d)N_d \qquad (t \geq T_d)$$

After emergence a mite stays hitch-hiking on adult bees and waits until she encounters a drone brood cell, which she then enters. If the mite has to wait δ time units on average, and if she encounters drone brood cells at a constant rate, $\alpha = 1/\delta$, the probability density function for the expected time until encounter is described by

$$f(t) = \alpha e^{-\alpha t}$$

The per capita rate of encounter α is the same as the per capita rate of invasion into drone cells which we determined recently (chapter 4), because a mite should always accept a suitable drone cell upon encounter. In colonies where the ratio between the number of brood cells capped per day and the number of bees in the colony is constant, mites do invade brood cells at a constant rate (chapters 3,4).

Hitch-hiking on a bee, a mite experiences a constant mortality rate of μ (chapter 5), which will be affected by grooming behaviour of the bees. This implies a survivorship function, l(t), i.e. the probability to survive up to age t after emergence from a brood cell, of

$$l(t) = 1 \qquad (t<T_d)$$
$$l(t) = e^{-\mu(t-T_d)} \qquad (t \geq T_d)$$

Thus, a mite of age t has not experienced any risk of mortality during development and has been exposed to a constant mortality rate μ during searching, i.e. during $t-T_d$ time units. Note that mortality within the brood cell is incorporated in N_d.

The expected reproductive success of a mite per brood cycle is then given by

$$R_0 = \int_0^\infty l(t)m(t)dt$$

This could be used as a fitness measure. However, R_0 does not take into account that the mite population may be growing, and that offspring produced at an early age contribute more to population growth. This is incorporated by weighing offspring produced at age t by a factor e^{-rt}, where r is the net rate of population increase:

$$\int_0^\infty e^{-rt} l(t)m(t)dt$$

By setting this expression equal to 1, Lotka's equation is obtained (Yodzis, 1989), which can be used to calculate the net rate of population increase r. Substitution of m(t) and l(t) gives

$$\int_{T_d}^\infty e^{-rt} e^{-\mu(t-T_d)} \alpha e^{-\alpha(t-T_d)} N_d \, dt = 1$$

which can be solved, to yield

$$N_d e^{-rT_d} = 1 + (r+\mu)/\alpha \qquad (1)$$

As a control, note that if the encounter rate with drone cells becomes very large (i.e., $\alpha \rightarrow \infty$), the searching time becomes very small and the above expression approaches

$$N_d e^{-rT_d} = 1$$

which has an explicit solution in r, the familiar

$$r = \ln(N_d)/T_d$$

When α *is not infinite*, equation (1) *cannot* be solved explicitly. *Using a graphical method* (Fig. 1), it can be shown that r will decrease when α decreases, which is intuitively obvious since growth rate decreases when it takes more time to find drone brood cells.

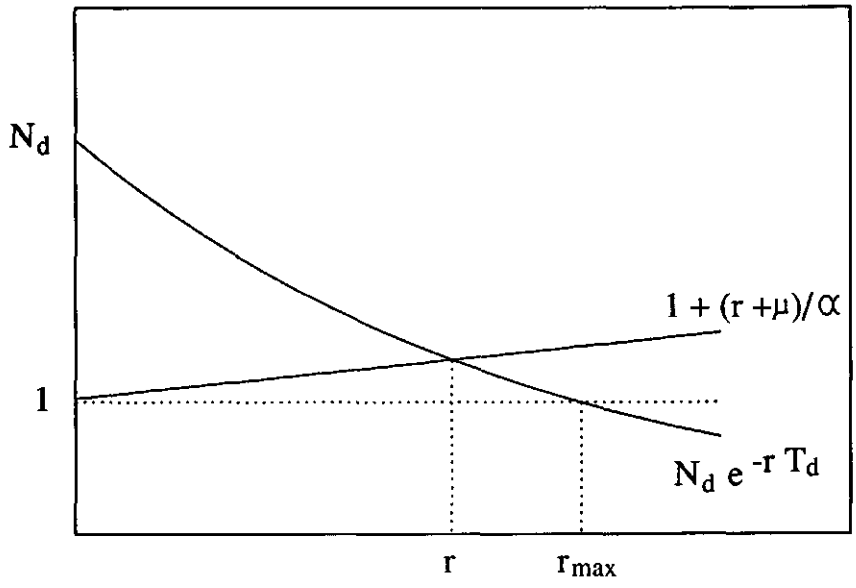

Figure 1: Graphical method to find the long-term rate of increase r, defined implicitly by equation 1. It can be seen that if the encounter rate with drone cells, α, decreases, the line becomes steeper and intersects the exponential function more to the left, implying a lower r.

When to invade a worker brood cell?

A mite hitch-hiking on a bee can also invade a worker brood cell and reproduce there. When drone brood cells are abundant, however, a mite will do best by ignoring opportunities to invade worker brood cells, since the number of mites emerging from a worker brood cell, N_w, is less than the number emerging from a drone brood cell. The optimal strategy (invade only drone cells, or invade both types of cell) depends on the net rate of population increase

r. Consider a mite on a bee encountering a worker brood cell. Should the mite invade, or should she remain on the bee? If she enters the worker brood cell, N_w mites emerge T_w time units later, where T_w is the developmental time of capped worker brood. If she remains on the bee, she may encounter a drone cell soon, but she may also have to wait for a long time. On average, a mite specializing on drone cells will have

$$e^{rT_w}$$

number of offspring after T_w time units, as follows from the expected rate of increase of mites specializing on drone cells (specified by equation [1]). Therefore it is best to ignore worker bee cells if

$$N_w < e^{rT_w}$$

or equivalently,

$$\ln(N_w)/T_w < r \qquad (2)$$

Estimates for input parameters are listed in Table 1. Reproductive success has been determined in many studies (see Fries et al. [1994] for a review). Here, five studies from Europe are listed in which the number of mites studied is relatively high in comparison to other studies on reproduction of *Varroa* mites. In our calculations, the number of viable daughters emerging from worker and drone cells is assumed to be 1.1 and 2.2 respectively. In addition, the mother's mortality during her stay in a brood cell is assumed to be 20%, which results in estimates for N_w and N_d of 1.9 and 3.0 respectively.

When the net rate of population increase is higher than the criterion for invading a worker cell, $r_{crit} = \ln(N_w)/T_w$ (equation 2), mites should not invade worker cells. With our set of parameters, this occurs when the average time that mites have to wait for a drone cell to invade, δ, is less than 7 days (fig. 2a). When δ is longer, mites will do better invading both drone and worker brood cells.

The actual time mites would have to wait on average for a drone cell can be calculated as the inverse of the per capita rate of invasion into drone cells, σ (chapter 4). The per capita rate of invasion depends linearly on the ratio between the number of drone brood cells capped daily and the number of bees in the colony:

$$\sigma = 51.9 \times \text{Number of drone cells capped daily/Number of bees } (\text{day}^{-1})$$

In a honey bee colony the ratio between the number of brood cells occupied and the number of bees present appears to be c. 1 on average (range 0.8-1.5), whereas c. 8% of the brood is drone brood (Table 1). If the ratio between the number of drone brood cells occupied and the number of bees is assumed to be 0.08, 0.08/24 drone cells will be capped daily per bee since total development time of drones is c. 24 days (Jay, 1963), and $\sigma = 51.9(0.08/24) = 0.173\ \text{day}^{-1}$. Thus mites would have to wait on average $1/0.173 = 5.8$ days for a drone cell to invade, which seems to be short enough to favour specialization of mites on drone brood, but also quite close to the critical value of 7 days.

Table 1: Estimates of input parameters.

Reproductive success of *Varroa* mites in worker brood cells:

Number of viable daughters per mite (F_w)	Number of mites on which estimate is based	Reference
0.7	238	Ifantidis, 1984
1.3	450	Schulz, 1984
1.4	316	Fuchs & Langenbach, 1989
1.1	1334	Martin, 1994
1.1	1682	chapter 5

Reproductive success of *Varroa* mites in drone brood cells:

Number of viable daughters per mite (F_w)	Number of mites on which estimate is based	Reference
1.7	155	Ifantidis, 1984
2.6	660	Schulz, 1984
2.2	279	Fuchs & Langenbach, 1989

Mortality related to staying in a brood cell (μ_{cell}) is c. 20% during one brood passage (chapter 5)

Mortality related to staying on adult bees (μ_{bee}) is c. 0.6% per day (chapter 5)

Developmental time of capped worker brood (T_w) is c. 12 days (Jay, 1963)

Developmental time of capped drone brood (T_d) is c. 14 days (Jay, 1963)

Average brood/bee ratio in an *A. mellfera* colony over a season:

Mean number of occupied brood cells per bee	Number of colonies on which estimate is based	Reference
0.8	16	McLellan, 1978
0.9	3	Omholt, 1986
1.1	30	Imdorf & Kilchenmann, 1987
0.8	2	Imdorf et al., 1987
1.5	3	Bühlmann, 1992

Average drone/worker brood ratio in an *A. mellifera* colony over a season (Page & Metcalf, 1984): 0.077 (Data from Allen, 1965); 0.079 (Data from Page, 1981)

Effect of different parameters on the period that mites can afford to wait for a drone cell to invade

Effect of the number of mites emerging from a drone cell, N_d

When the number of mites emerging from a drone cell increases, mites can afford to wait longer for a drone cell before invading both types of brood cells will be a better strategy. If the criterion (equation 2) for invading both types of brood is met, then

$$r = \ln(N_w)/T_w$$

Substitution of r in equation 1 gives a linear relationship between N_d and the average period mites have to wait for a drone cell, δ (Fig. 2b). Whether mites should specialize on drone brood or not, is sensitive to changes in N_d. When N_d is smaller than 2.1, both types of brood should always be invaded.

Effect of the mortality rate while on adult bees, μ

When μ increases, waiting for a drone cell to invade will incur a higher penalty. Invading both types of brood will be a better strategy at shorter average waiting times for a drone cell. Substitution of r by $\ln(N_w)/T_w$ in equation 1 gives an inverse relationship of μ with δ (Fig. 2c). Specialization on drone brood is most sensitive to changes in μ at low mortality rates. When $\mu = 0$ mites can afford to wait 7.8 days for a drone cell before invading both types of brood is a better strategy.

Effect of the number of mites emerging from worker cells, N_w

The criterion determining whether invading both types of brood cells is a better strategy or not, increases logarithmically with increasing N_w. Invading both types of brood will be a better strategy at shorter average waiting periods for a drone cell. Substitution of r by $\ln(N_w)/T_w$ gives the implicit relationship shown in Fig. 2d. When N_w approaches 1, the period mites can afford to wait for a drone cell rapidly increases before invading both types of brood wil be a better strategy.

Discussion

The model shows that *Varroa* mites can afford to wait on average c. 7 days for a drone brood cell before invasion of both brood cell types would be a better strategy to adopt. This period of 7 days is close to the actual period mites have to wait for a drone cell, which was estimated to be c. 6 days on average. Whether mites do better by specialization on drone brood or not, is therefore sensitive to small differences in the parameters (Fig. 2), and the result may well depend on local circumstances.

In the model the mite population is assumed to grow exponentially. Thus, mite density will increase in a colony and more mites will invade per cell. Since the number of viable daughters produced per mite is negatively correlated with the number of mites that invaded the same cell (Fuchs & Langenbach, 1989), N_d will slowly decrease with increasing mite density. On the short term, however, during which the mites should choose either to invade a worker cell or to wait for a drone cell, N_d may be assumed constant. When mite density is high, the model may therefore be applied by adjusting the number of viable daughters per mite produced in a drone cell, F_d, where $N_d = F_d$ + the fraction of mother mites that survive

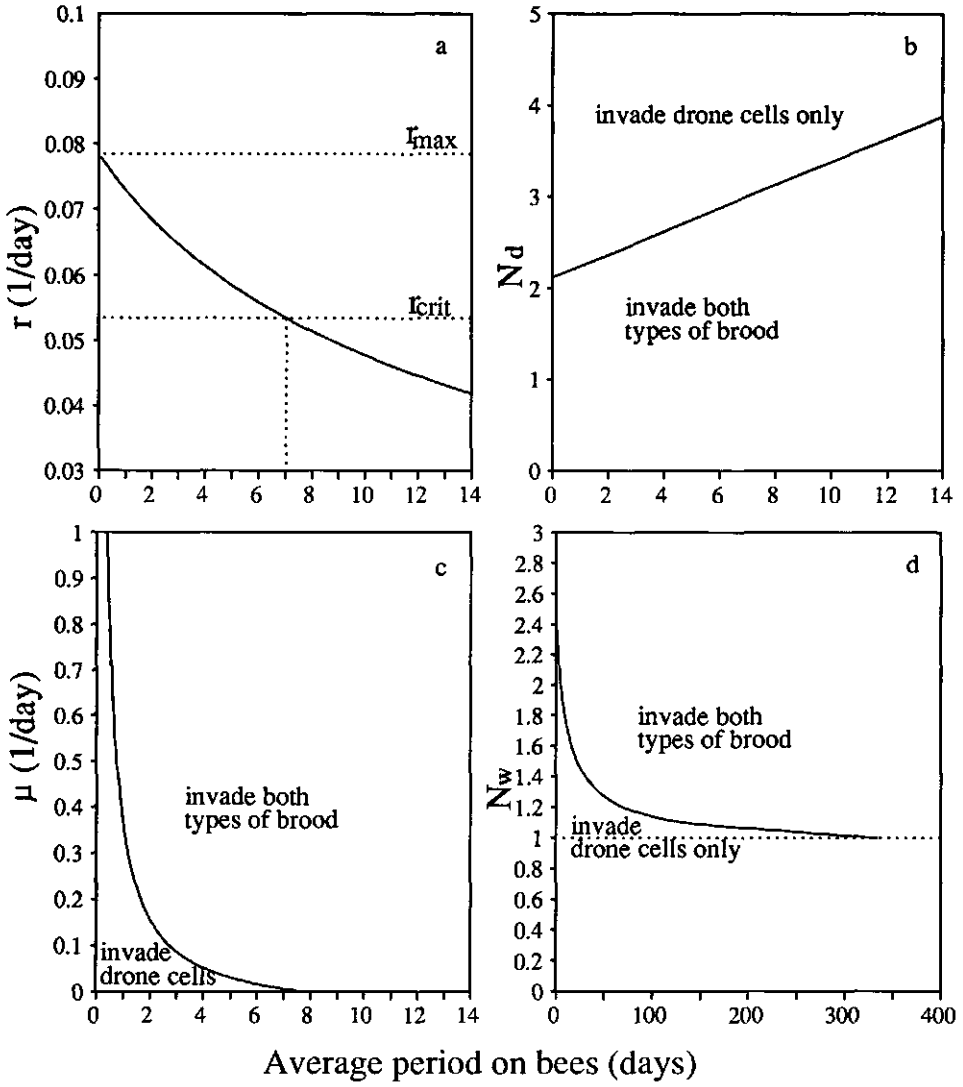

Figure 2: (a) Relation between the net rate of population increase, r, and the average period mites have to stay on adult bees waiting for a drone cell to invade, δ; N_d=3.0, N_w=1.9, T_d=14 days, T_w=12 days and μ=0.006 day^{-1}. (b-d) Sensitivity analysis of the average number of mites emerging from a drone cell, N_d (b), the relative mortality rate on adult bees, μ (c), and the average number of mites emerging from a worker brood cell, N_w (d) on the period that mites can afford to wait for a drone cell.

in the cell. Fuchs (1992) simulated to what extent F_d is lowered at different densities: at an average density of 2, 6, 10, 16 and 20 mites per drone cell, F_d decreases 18, 41, 58, 69 and 76%, respectively.

In European *A. mellifera* colonies, specialization for drone brood cells seems to occur to some extent, because a drone cell is invaded 12 times more frequently than a worker cell (chapter 4). Since the mites should always accept a suitable drone brood cell when they encounter one, the rate of invasion into drone cells is a measure for the encounter rate. One cannot expect that a suitable worker brood cell is encountered as often as a suitable drone brood cell, because drone brood cells are attractive to mites 2-3 times as long compared to worker brood cells, and will therefore be encountered 2-3 times more often. Moreover, the chance of encountering a cell may be proportional to its surface, which is 1.7 times larger for a drone cell when compared with a worker cell. By combining these effects on the encounter rate, a suitable drone brood cell is expected to be encountered 3.4-5.1 times more frequently than a suitable worker cell. However, since a drone cell is invaded 12 times more frequently, this suggests that suitable worker cells are not accepted in c. 70% of the encounters.

In the host-parasite relationship between *A. florea* and *E. sinhai*, specialization on drone brood cells is complete. In principle, mites could reproduce in worker brood cells of *A. florea*, because the development time of capped worker brood measures 11.2 days (Sandhu & Singh, 1960). By transferring *E. sinhai* to *A. mellifera* brood, which *A. mellifera* bees keep at the same temperature as *A. florea* bees do (Dyer & Seeley, 1991), Mossadegh (1990a,b) showed that 11.2 days should be long enough to produce viable daughters. Hence, *E. sinhai* has probably specialized on drone brood cells because waiting for drone cells is a better strategy to adopt than invading both types of cell.

In the host-parasite relationship between *A. cerana* and *V. jacobsoni* the mites do invade worker cells, but as a rule do not reproduce in these cells (Tewarson et al., 1992; Rosenkranz et al., 1993). In African or Africanized *A. mellifera* the same phenomenon occurs, albeit less explicit: a high percentage of the mites do not reproduce after invasion into worker cells (e.g. Camazine, 1986; Ritter, 1993). This is difficult to understand because the mites will be trapped for 11-12 days without any possibility to invade a drone cell. In this case our model only predicts invasion into worker cells during periods of negative population growth and if invasion into worker cells implies a less negative growth than staying on adult bees. This might occur during periods in which drone brood cells are absent or scarce, whereas mortality on adult bees is high. It has indeed been suggested that 'grooming' behaviour by the bees causes high mortality of the mites on adult bees in colonies of *A. cerana* (Boecking et al., 1993), Africanized *A. mellifera* (Moretto et al., 1993) and *A. mellifera intermissa* (Boecking & Ritter, 1993). In all of these hosts the phenomenon of a high percentage of mites that invade worker cells but do not reproduce, has been reported. Another reason why mites may invade worker cells in which they do not reproduce, is that by parasitizing a larva/pupa the mite enhances reproduction later. This implies that reproduction is negatively correlated with the period the mite stays on adult bees before. Al Ghzawi (1992) indeed found that after a period of more than 26 days on adult bees, reproduction in worker brood cells rarely occurred. However, when mites stayed 1 to 20 days on bees, Boot et al. (chapter 5) found no correlation between the period on bees and reproduction.

Whether or not a mite reproduces after invasion into a worker cell, she will be trapped for 11-12 days. Therefore, refraining from reproduction, as occurs in *A. cerana* and African

or Africanized honey bees, only makes sense if this results in a higher average number of emerging mites, N_w, than by reproducing. This may indeed be the case for the following reasons. Firstly, mites that reproduce may have a higher chance to die than mites that do not, because reproduction increases their physiological age. In addition, mortality may increase if the chance for an infested brood cell to be removed by the bees is increased by reproduction of the mite (Boecking et al., 1993). Secondly, developmental time of capped worker brood is only 11 days in *A. cerana* (Kapil, 1959; Tan et al., 1993) versus 12 days in European *A. mellifera* (Le Conte & Cornuet, 1989; Harbo, 1992). Hence, less daughters than in European *A. mellifera* are expected to reach maturity if mites would reproduce in worker brood. In the *A. mellifera* races *scutellata* and *capensis* developmental times of 11.2 and 9.7 days respectively will also decrease N_w in comparison to European *A. mellifera* (Moritz, 1985).

If the phenomenon of non-reproduction in worker cells occurs because the average N_w is lower by reproducing than by refraining from reproduction, this implies selection of the mites. Thus it may not result from a direct character of the honey bee strain. Depending on the mite population used for testing, mites may either reproduce or refrain from reproduction in worker brood when bee strains are tested for the character of non-reproduction by mites in worker brood. This is in agreement with Fuchs (1994), who recently showed that variation in the percentage of mites refraining from reproduction did not depend on the origin of the worker brood when 12 distinct bee lines were tested.

In our model, we use intracolonial population growth as a fitness measure and evaluate the effect of the average period on adult bees. On a larger scale however, there may be other factors selecting for a larger or shorter period on adult bees, for instance dispersal from colony to colony or absconding behaviour by the bees. Which reproductive strategy of mites is promoted under different circumstances is an important question for further research, because whenever tolerance of honey bees for *(Euvarroa)Varroa* mites has been reported, it is associated with an increased specialization on drone brood cells in comparison with susceptible honey bees. Therefore, insight into which factors promote specialization on drone brood may help in breeding more tolerant European *A. mellifera* in future.

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References

Al Ghzawi, A., 1993. Factors affecting the maturity of young females of *Varroa jacobsoni* Oudemans. PhD Thesis, University of Hohenheim.

Allen, M.D., 1965. The effect of a plentiful supply of drone comb on colonies of honeybees. Journal of Apicultural Research 4: 109-119.

Beetsma, J. & K. Zonneveld, 1992. Observations on the initiation and stimulation of oviposition of the *Varroa* mite. Experimental & Applied Acarology 16: 303-312.

Beetsma, J., W.J. Boot & J.N.M. Calis, 1993. Special techniques to make detailed observations of *Varroa* mites staying on bees and invading honeybee brood cells. In: L.J. Conner, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 510-520.

Boot, W.J. & J.N.M. Calis, 1991. A method to obtain dated brood in honeybee colonies. Bee World 72: 19-21.

Boecking, O. & W. Drescher, 1991. Response of *Apis mellifera* L. colonies infested with *Varroa jacobsoni* Oud. Apidologie 22: 237-241.

Boecking O. & W., Ritter, 1993. Grooming and removal behaviour of *Apis mellifera intermissa* in Tunisia against *Varroa jacobsoni*. Journal of Apicultural research 32: 127-134.

Boecking O., W. Rath & W. Drescher, 1993. Behavioral strategies of *Apis mellifera* and *Apis cerana* against *Varroa jacobsoni*. International Journal of Acarology 19: 173-177.

Brouwers, E.V.M., 1983. Activation of the hypopharyngeal glands of honeybees in winter. Journal of Apicultural Research 22: 137-141.

Brouwers, E.V.M., R. Ebert & J. Beetsma, 1987. Behavioural and physiological aspects of nurse bees in relation to the composition of larval food during caste differentiation in the honeybee. Journal of Apicultural Research 26: 11-23.

Büchler, R., 1989. Attractivity and reproductive suitability for the *Varroa*-mite of beebrood from different origin. In: R. Cavalloro (Editor), Present status of Varroatosis in Europe and progress in the Varroa mite control. Commission of the European Communities, Luxembourg, pp. 139-145.

Büchler, R., W. Drescher & I. Tornier, 1992. Grooming behaviour of *Apis cerana, Apis mellifera* and *Apis dorsata* and its effect on the parasitic mite *Varroa jacobsoni* and *Tropilaelaps clareae*. Experimental & Applied Acarology 16: 313-319.

Büchler, R., 1994. *Varroa* tolerance in honey bees - occurrence, characters and breeding. Bee World 75: 54-70.

Bühlmann, G., 1992. Volksentwicklung. Schweizerische Bienenzeitung 115: 583-587.

Calis, J.N.M., W.J. Boot & J. Beetsma, 1993a. Behavior of *Varroa* mites invading honey bee brood cells of different sizes. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 499-509.

Calis, J.N.M., J. Beetsma, W.J. Boot, J. van den Eijnde & A. de Ruijter, 1993b. Control of the *Varroa* mite by treatment of sealed honeybee brood with formic acid. Proceedings Experimental & Applied Entomology, N.E.V. Amsterdam 4: 217-222.

Camazine, S., 1986. Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). Annals of the Entomological Society of America 79: 801-803.

De Jong, D., R.A. Morse & G.C. Eickwort, 1982. Mite pests of honey bees. Annual Review of Entomology 27: 229-252.

De Jong, D., 1984. Current knowledge and open questions concerning reproduction in the honey bee mite *Varroa jacobsoni*. In: W. Engels, W.H. Clark Jr, A. Fischer, P.J.W. Olive & D.F. Went (Editors), Advances in invertebrate reproduction, 3. Elsevier Science Publishers B.V., Amsterdam, pp. 547-552.

De Jong, D. & R.A. Morse, 1988. Utilisation of raised brood cells of the honey bee, *Apis mellifera* (Hymenoptera: Apidae), by the mite, *Varroa jacobsoni* (Acarina: Varroidae). Entomologia Generalis 14: 103-106.

De Ruijter, A. & N. Pappas, 1983. Karyotype and sex determination of *Varroa jacobsoni* Oud. In: R. Cavalloro (Editor), *Varroa jacobsoni* Oud. affecting honey bees: present status and needs. A.A.Balkema, Rotterdam, pp. 41-44.

De Ruijter, A., 1987. Reproduction of *Varroa jacobsoni* during successive brood cycles of the honeybee. Apidologie 18: 321-326.

De Ruijter, A. & J.N.M. Calis, 1988. Distribution of *Varroa jacobsoni* female mites in honey bee worker brood cells of normal and manipulated depth (Acarina: Varroidae). Entomologia Generalis 14: 107-109.

Dicke, M., T. Van Beek, M.A. Posthumus, N. Ben Dom, H. Van Bokhoven & Æ. De Groot, 1990. Isolation and identification of volatile kairomone that affects acarine predator prey interactions: Involvement of host plant in its production. Journal of Chemical Ecology 16: 381-396.

Dyer, F.C. & T.D. Seeley, 1991. Nesting behavior and the evolution of worker tempo in four honey bee species. Ecology 72: 156-170.

Fluri, P., M. Lüscher, H. Wille & L. Gerig, 1982. Changes in weight of the pharyngeal gland and heamolymph titres of Juvenile hormone, protein and vitellogenin in worker honey bees. Journal of Insect Physiology 28: 61-68.

Fries, I., 1989. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. Swedish Journal of Agricultural Research 19: 213-216.

Fries, I. & H. Hansen, 1993. Biotechnical control of *Varroa* mites in cold climates. American Bee Journal 133: 435-438.

Fries, I., Camazine, S. & J. Sneyd, 1994. Population dynamics of *Varroa jacobsoni*: a model and a review. Bee World 75: 5-28.

Fuchs, S., 1985. Untersuchungen zur Quantitativen Abschätzung des Befalls von Bienenvölkern mit *Varroa jacobsoni* Oudemans und zur Verteilung des Parasiten im Bienenvolk. Apidologie 16: 343-368.

Fuchs, S. & K. Müller, 1988. Invasion of honeybee brood cells by *Varroa jacobsoni* in relation to the age of the larva. In: R. Cavalloro (Editor), European research on varroatosis control. A.A. Balkema, Rotterdam, pp. 77-79.

Fuchs, S. & K. Langenbach, 1989. Multiple infestation of *Apis mellifera* L. brood cells and reproduction in *Varroa jacobsoni* Oud. Apidologie 20: 257-266.

Fuchs, S., 1990. Preference for drone brood cells by *Varroa jacobsoni* Oud. in colonies of *Apis mellifera carnica*. Apidologie 21: 193-199.

Fuchs, S., 1992. Choice in *Varroa jacobsoni* Oud. between honey bee drone or workerbrood cells for reproduction. Behavioral Ecology and Sociobiology 31: 429-435.

Fuchs, S., 1994. Non-reproducing *Varroa jacobsoni* Oud. in honey bee worker cells - status of mites or effect of brood cells? Experimental & Applied Acarology 18: 309-317.

Goetz, B. & N. Koeniger, 1992. Structural features trigger capping of brood cells in honey bees. Apidologie 23: 211-216.

Goetz, B. & N. Koeniger, 1993. The distance between larva and cell opening triggers broodcell invasion by *Varroa jacobsoni*. Apidologie 24: 67-72.

Greatti, M., N. Milani & F. Nazzi, 1992. Reinfestation of an acaricide-treated apiary by *Varroa jacobsoni* Oud. Experimental & Applied Acarology 16: 279-286.

Grobov, O.F., 1977. Varroasis in bees. In: Harnaj (Editor), Varroasis: a honeybee disease. Apimondia, Bucharest, pp. 46-90.

Hänel, H. & N. Koeniger, 1986. Possible regulation of the reproduction of the honey bee mite *Varroa jacobsoni* (Mesostigmata: Acari) by a host's hormone: Juvenile Hormone III. Journal of Insect Physiology 32: 791-798.

Harbo, J.R., 1992. Breeding honey bees (Hymenoptera: Apidae) for more rapid development of larvae and pupae. Journal of Economic Entomology 85: 2125-2130.

Huang, Z.-Y. & G.W. Otis, 1989. Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). Insectes Sociaux 36: 264-276.

Ifantidis, M.D., 1983. Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honeybee brood cells. Journal of Apicultural Research 22: 200-206.

Ifantidis, M.D., 1984. Parameters of the population dynamics of the *Varroa* mite on honeybees. Journal of Apicultural Research 23: 227-233.

Ifantidis, M.D., 1988. Some aspects of the process of *Varroa jacobsoni* mite entrance into honey bee (*Apis mellifera*) brood cells. Apidologie 19: 387-396.

Ifantidis, M.D. & P. Rosenkranz, 1988. Reproduktion der Bienenmilbe *Varroa jacobsoni* (Acarina: Varroidae). Entomologia Generalis 14: 111-122.

Imdorf, A. & V. Kilchenmann, 1987. Entwicklung von Kunstschärmen und Muttervölkern. Schweizerische Bienenzeitung 110: 189-202.

Imdorf, A., G. Bühlmann, L. Gerig & V. Kilchenmann, 1987. Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in Freifliegenden Bienenvölkern. Apidologie 18: 137-146.

Imdorf A. & V. Kilchenmann, 1990. Natürlicher Milbenfall im Oktober. Schweizerische Bienenzeitung 113: 505-506.

Jay, S. C., 1963. The development of honeybees in their cells. Journal of Apicultural Research 2: 117-134.

Kapil, R.P., 1959. Variation in the developmental period of the Indian bee. The Indian Bee Journal 21: 3-6, 26.

Kapil, R.P. & K. Aggarwal, 1989. Observations on reproduction and seasonal population trends of *Euvarroa sinhai* (Mesostigmata: Varroidae) in India. In: G.P. Channabasavanna & C.A. Viraktamath (Editors), Progress in Acarology 2. Brill, Leiden, pp. 277-281.

Koeniger, N. & S. Fuchs, 1989. Eleven years with *Varroa* - experiences, retrospects and prospects. Bee World 70: 148-159.

Korpela, S., A. Aarhus, I. Fries & H. Hansen, 1992. *Varroa jacobsoni* Oud. in cold climates: population growth, winter mortality and influence on the survival of honey bee colonies. Journal of Apicultural Research 31: 157-164.

Kovac, H. & K. Crailsheim, 1987. *Varroa jacobsoni* Oud. changes its host. In: J. Eder & H. Rembold (Editors), Chemistry and biology of social insects. Verlag J. Peperny, München, pp. 681-682.

Kraus, B., N. Koeniger & S. Fuchs, 1986. Unterscheidung zwischen Bienen verschiedenen Alters durch *Varroa jacobsoni* Oud. und Bevorzugung von Ammenbienen im Sommerbienenvolk. Apidologie 17: 257-266.

Kraus, B., 1993. Preferences of *Varroa jacobsoni* for honey bees (*Apis mellifera* L.) of different ages. Journal of Apicultural Research 32: 57-64.

Laigo, F.M. & R.A. Morse, 1968. The mite *Tropilaelaps clareae* in *Apis dorsata* colonies in the Philippines. Bee World 49: 116-118.

Le Conte, Y. & G. Arnold, 1987. Influence de l'age des abeilles (*Apis mellifica* L.) et de la chaleur sur le comportement de *Varroa jacobsoni* Oud. Apidologie 18: 305-320.

Le Conte, Y. & J.M. Cornuet, 1989. Variability of the post-capping stage duration of the worker brood in three different races of *Apis mellifera*. In: R. Cavalloro (Editor), Present status of varroatosis in Europe and progress in the *Varroa* mite control. Commission of the European Communities, Luxembourg, pp. 171-174.

Le Conte, Y., G. Arnold, J. Trouiller, C. Masson, B. Chappe & G. Ourisson, 1989. Attraction of the parasitic mite *Varroa* to the drone larvae of honey bees by simple aliphatic esters. Science 245: 638-639.

Le Conte, Y., G. Arnold, J. Trouiller & C. Masson, 1990. Identification of a brood pheromone in honeybees. Naturwissenschaften 77: 334-336.

Liebig, G., U. Schlipf, W. Fremuth & W. Ludwig, 1984. Ergebnisse der Untersuchungen über die Befallsentwicklung der Varroa-Milbe in Stuttgart-Hohenheim 1983. Allgemeine Deutsche Imkerzeitung 18: 185-191.

Lindauer, M., 1953. Division of labour in the honeybee colony. Bee World 34: 63-73 & 85-90.

Lodesani, M., A. Pellacani, S. Bergomi, E. Carpana, T. Rabitti, P. Lasagni, 1992. Residue determination for some products used against *Varroa* infestation in bees. Apidologie 23: 257-272

Martin, S.J., 1994. Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. Experimental & Applied Acarology 18: 87-100.

Matheson, A., 1993. World bee health report. Bee World 74: 176-212.

Maul, V., A. Klepsch & U. Assmann-Werthmüller, 1988. Das Bannwabenverfahren als Element imkerlicher Betriebsweise bei starkem Befall mit *Varroa jacobsoni* Oud. Apidologie 19: 139-154.

McLellan, A.R., 1978. Growth and decline of honeybee colonies and inter-relationships of adult bees, brood, honey and pollen. Journal of Applied Ecology 15: 155-161.

Moosbeckhofer, R., M. Fabsicz & A. Kohlich, 1988. Untersuchungen über die Abhängigkeit der Nachkommensrate von *Varroa jacobsoni* Oud. vom Befallsgrad der Bienenvölker. Apidologie 19: 181-208.

Moretto, G., L.S. Gonçalves & D. De Jong, 1993. Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*. Brazilian Journal of Genetics 16: 71-77.

Moritz, R.F.A., 1985. Heritability of the postcapping stage in *Apis mellifera* and its relation to varroatosis resistance. The Journal of Heredity 76: 267-270.

Moritz, R.F.A., 1994. Selection for Varroatosis resistance in honeybees. Parasitology Today 10: 236-238.

Mossadegh, M.S. & A.K. Birjandi, 1986. *Euvarroa sinhai* Delfinado & Baker (Acarina: Mesostigmata): A parasite mite on *Apis florea* F. in Iran. American Bee Journal 126: 684-685.

Mossadegh, M.S., 1990a. Development of *Euvarroa sinhai* (Acarina: Mesostigmata), a parasitic mite of *Apis florea*, on *A. mellifera* worker brood. Experimental & Applied Acarology 9: 73-78.

Mossadegh, M.S., 1990b. In vitro observations on ontogenesis of the mite, *Euvarroa sinhai* Delfinado & Baker (Acari: Varroidae), in drone brood cells of the honeybee, *Apis mellifera* L. Journal of Apicultural Research 29: 230-232.

Omholt, S.W., 1986. A model for intracolonial population dynamics of the honeybee in temperate zones. Journal of Apicultural Research 25: 9-21.

Otten, C. & S. Fuchs, 1988. Individual differences in *Varroa jacobsoni* of preference for drone larvae to worker bee larvae. In: R. Cavalloro (Editor), European research on varroatosis control. A.A. Balkema, Rotterdam, pp. 69-71.

Otten, C., 1990. Reproduction and population dynamics of *Varroa jacobsoni* Oud. in colonies of *A. mellifera* L. of different origin. In: W. Ritter (Editor), Proceedings of the international symposium on recent research on bee pathology, Gent, Belgium. Apimondia, pp. 67-69.

Oudemans, A.C., 1904. On a new genus and species of parasitic acari. Notes from the Leyden Museum 24: 216-222.

Page, R.E., 1981. Protandrous reproduction in honey bees. Environmental Entomology 10: 359-362.

Page, R.E.Jr & R.A. Metcalf, 1984. A population investment sex ratio for the honey bee (*Apis mellifera* L.). The American Naturalist 124: 680-702.

Peng, Y.-S., Y. Fang, S. Xu & L. Ge, 1987. The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. Journal of Invertebrate Pathology 49: 54-60.

Rademacher, E., 1985. Ist eine Befallsprognose aus dem natürlichen Totenfall von *Varroa jacobsoni* möglich? Apidologie 16: 395-406.

Rademacher, E., H. Böttcher & M. Pekel, 1989. Control of Varroatosis in Berlin (West), a region exposed to high invasion pressure. In: R. Cavalloro (Editor), Present status of Varroatosis in Europe and progress in the Varroa mite control. Commission of the European Communities, Luxembourg, pp. 183-191.

Ramon, H., O. Van Laere & M. Ifantidis, 1993. Size of comb cell and reproduction of *Varroa jacobsoni*. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 521-529.

Rath, W. & W. Drescher, 1990. Response of *Apis cerana* Fabr. towards brood infested with *Varroa jacobsoni* Oud. and infestation rate of colonies in Thailand. Apidologie 21: 311-321.

Rath, W., 1993. Aspects of preadaptation in *V. jacobsoni* while shifting from its original host *A. cerana* to *A. mellifera*. In: L.J. Conner, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 417-426.

Rehm, S.-M. & W. Ritter, 1989. Sequence of the sexes in the offspring of *Varroa jacobsoni* and the resulting consequences for the calculation of the developmental period. Apidologie 20: 339-343.

Rickli, M., P.M. Guerin & P.A. Diehl, 1992. Palmitic acid released from honeybee worker larvae attracts the parasitic mite *Varroa jacobsoni* on a servosphere. Naturwissenschaften 79: 320-322.

Ritter, W., 1981. *Varroa* disease of the honeybee *Apis mellifera*. Bee World 62: 141-153.

Ritter, W., 1990. Drug therapy development of *Varroa jacobsoni* in the federal republic of Germany. Apiacta 25: 102-106.

Ritter, W., P. Michel, M. Bartholdi & A. Schwendemann, 1990. Development of tolerance to *Varroa jacobsoni* in bee colonies in Tunesia. In: W. Ritter, O. Van Laere, F. Jacobs & L. De Wael (Editors), Proceedings of the international symposium on recent research on bee pathology, September 5-7, 1990, Gent, Belgium. Apimondia, pp. 54-59.

Ritter, W., 1993. New results of the development of tolerance to *Varroa jacobsoni* in bee colonies in Tunisia. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire, CT, USA, pp. 463-467.

Robinson, G.E., A. Strambi, C. Strambi, Z.L. Paulino-Simoes, S.O. Tozeto & J.M. Negraes Barbosa, 1987. Juvenile hormone titres in European and Africanized honey bees in Brazil. General and Comparative Endocrinology 66: 457-459.

Robinson, G.E., R.E. Page Jr, C. Strambi & A. Strambi, 1989. Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246: 109-112.

Robinson, G.E., 1992. Regulation of division of labour in insect societies. Annual Review of Entomology 37: 637-665.

Rosenkranz, P., N.C. Tewarson & W. Engels, 1984. Optimal host selection by reproductive female *Varroa jacobsoni*. In: W. Engels, W.H. Clark Jr, A. Fischer, P.J.W. Olive & D.F. Went (Editors), Advances in invertebrate reproduction, 3. Elsevier Science Publishers B.V., Amsterdam, pp. 628.

Rosenkranz, P. & W. Engels, 1985. Konsequente Drohnenbrutentnahme, eine wirksame biotechnische Massnahme zur Minderung von Varroatose-Schäden an Bienenvölkern. Allgemeine Deutsche Imkerzeitung 19: 265-271.

Rosenkranz, P. A. Rachinsky, A. Strambi, C. Strambi & P. Röpstorf, 1990. Juvenile hormone titer in capped worker brood of *Apis mellifera* and reproduction in the bee mite *Varroa jacobsoni*. General and Comparative Endocrinology 78: 189-193.

Rosenkranz, P., N.C. Tewarson, A. Rachinsky, A. Strambi, C. Strambi & W. Engels, 1993. Juvenile hormone titer and reproduction of *Varroa jacobsoni* in capped brood stages of *Apis cerana indica* in comparison to *Apis mellifera ligustica*. Apidologie 24: 375-382.

Rowland, C.M. & A.R. McLellan, 1987. Seasonal changes of drone numbers in a colony of the honeybee, *Apis mellifera*. Ecological Modelling 37: 155-166.

Ruttner, F. & H. Hänel, 1992. Active defense against *Varroa* mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollmann). Apidologie 23: 173-187.

Sakofski, F. & N. Koeniger, 1988. Natural transfer of *Varroa jacobsoni* among honeybee colonies in autumn. In: R. Cavalloro (Editor), European research on varroatosis control. A.A. Balkema, Rotterdam, pp. 81-83.

Sandhu, A.S. & S. Singh, 1960. The biology and brood rearing activities of the little honeybee - *Apis florea* Fabricius. The Indian Bee Journal 22: 27-35.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 1. SAS institute Inc., Cary, NC, USA, 943pp.

Schneider, P., 1985. Befall von Sammlerinnen, Stockbienen, Flugdrohnen und Stockdrohnen mit *Varroa jacobsoni*. Apidologie 16: 209-211.

Schulz, A., N. Koeniger & F. Ruttner, 1983. Drohnenbrut als Varroafalle. Allgemeine Deutsche Imkerzeitung 17: 52-54.

Schulz, A.E., 1984. Reproduktion und Populationsentwicklung der parasitischen Milbe *Varroa jacobsoni* Oud. in Abhängigkeit vom Brutzyclus ihres Wirtes *Apis mellifera* L. Apidologie 15: 401-420.

Seeley, T.D., 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behavioral Ecology & Sociobiology 11: 287-293.

Sulimanovic, D., F. Ruttner & H. Pechhacker, 1982. Studies on the biology of reproduction in *Varroa jacobsoni* (in Japanese). Honeybee Science 3: 109-112.

Tan, N.Q., L.V. Huan & N.V. Dung, 1993. Development time of eggs, open brood and capped brood of *Apis cerana* and *Apis mellifera* in southern Vietnam. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire, CT, USA, pp. 243-248.

Tewarson, N.C., A. Singh & W. Engels, 1992. Reproduction of *Varroa jacobsoni* in colonies of *Apis cerana indica* under natural and experimental conditions. Apidologie 23: 161-171.

Trouiller, J., G. Arnold, Y. Le Conte & C. Masson, 1991. Temporal pheromonal and kairomonal secretion in the brood of honeybees. Naturwissenschaften 78: 368-370.

Trouiller, J., G. Arnold, B. Chappe, Y. Le Conte & C. Masson, 1992. Semiochemical basis of infestation of honey bee brood by *Varroa jacobsoni*. Journal of Chemical Ecology 18: 2041-2053.

Vet, L.E.M., J.C. van Lenteren, M. Heymans & E. Meelis, 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiological Entomology 8: 97-106.

Wachendörfer, G., J. Fijalkowski, E. Kaiser, D. Seinsche & J. Siebentritt, 1985. Labor- und Feldversuche mit der Illertisser Milbenplatte als neue Anwendungsform der Ameisensäure im Rahmen der Varroatose-Bekämpfung. Apidologie 16: 291-306.

Wendel, H.P. & P. Rosenkranz, 1990. Invasionsgeschwindigkeit und Fertilität von *Varroa*-Weibchen in aufeinanderfolgenden Reproduktionszyklen. Apidologie 21: 372-374.

Wieting, J. & H.-J. Ferenz, 1991. Behavioral study on the invasion of honeybee brood by the mite *Varroa jacobsoni* on wax combs and ANP combs. American Bee Journal 131: 117-118.

Wirtz, P., 1973. Differentiation in the honeybee larva. Mededelingen Landbouwhogeschool Wageningen 73-5, 155pp.

Woyke, J, 1984. Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. Apidologie 15: 421-434.

Woyke, J., 1987. Comparative population dynamics of *Tropilaelaps clareae* and *Varroa jacobsoni* mites on honeybees. Journal of Apicultural Research 26: 196-202.

Woyke, J., 1989a. Change in shape of *Tropilaelaps clareae* females and the onset of egg laying. Journal of Apicultural Research 28: 196-200.

Woyke, J., 1989b. Breeding of honey bees resistant to *Varroa jacobsoni*. American Bee Journal 129: 21-23.

Yodzis, P., 1989. Introduction to theoretical ecology. Harper & Row, New York, 384pp.

List of publications

Beetsma, J., W.J. Boot & J.N.M. Calis, 1993. Special techniques to make detailed observations of *Varroa* mites staying on bees and invading honey bee brood cells. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 510-520.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1990. Invasion of *Varroa* mites into honeybee brood cells: observation of *Varroa* mite behaviour. In: W. Ritter, O. Van Laere, F. Jacobs & L. De Wael (Editors), Proceedings of the international symposium on recent research on bee pathology, September 5-7, 1990, Gent, Belgium. Apimondia, pp. 43-44.

Boot, W.J. & J.N.M. Calis, 1991. A method to obtain dated brood in honeybee colonies. Bee World 72: 19-21.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1991. Invasion of *Varroa* mites into honeybee brood cells; When do brood cells attract *Varroa* mites? Proceedings Experimental & Applied Entomology, N.E.V. Amsterdam 2: 154-156.

Boot, W.J., O.P.J.M. Minkenberg, R. Rabbinge & G.H. de Moed, 1992. Biological control of the leafminer *Liriomyza bryoniae* by seasonal inoculative releases of *Diglyphus isaea*: simulation of a parasitoid-host system. Netherlands Journal of Plant Pathology 98: 203-212.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1992. Differential periods of *Varroa* mite invasion into worker and drone cells of honey bees. Experimental & Applied Acarology 16: 295-301.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1993. Invasion behavior of *Varroa* mites into honey bee brood cells. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 491-498.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1993. Invasion of *Varroa jacobsoni* into honey bee brood cells: a matter of chance or choice? Journal of Apicultural Research 32: 167-174.

Boot, W.J. & P. Rosenkranz, 1994. Discussion group on honey bee susceptibility. In: A. Matheson (Editor), New perspectives on varroa. IBRA, Cardiff, UK, pp. 151-152.

Boot, W.J., J. Beetsma & J.N.M. Calis, 1994. Behaviour of *Varroa* mites invading honey bee brood cells. Experimental & Applied Acarology 18: 371-379.

Boot, W.J., J.N.M. Calis & J. Beetsma, 1994. Possibilities for breeding honey bees resistant to varroa. In: A. Matheson (Editor), New perspectives on varroa. IBRA, Cardiff, UK, pp. 53-55.

Boot, W.J., D.J.A. Sisselaar, J.N.M. Calis & J. Beetsma, 1994. Factors affecting invasion of *Varroa jacobsoni* (Acari: Varroidae) into honeybee, *Apis mellifera* (Hymenoptera: Apidae), brood cells. Bulletin of Entomological Research 84: 3-10.

Boot, W.J., in press. Methyl palmitate does not elicit invasion of honeybee brood cells by *Varroa* mites. Experimental & Applied Acarology.

Boot, W.J., M. van Baalen & M.W. Sabelis, in press. Why do *Varroa* mites invade worker brood cells of the honey bee despite lower reproductive success? Behavioral Ecology and Sociobiology.

Boot, W.J., J.N.M. Calis & J. Beetsma, in press. Does time spent on adult bees affect reproductive success of *Varroa* mites? Entomologia Experimentalis et Applicata.

Boot, W.J., R.G. Driessen, J.N.M. Calis & J. Beetsma, in press. Further observations on the correlation between attractiveness of honey bee brood cells to *Varroa jacobsoni* and the distance from larva to cell rim. Entomologia Experimentalis et Applicata.

Boot, W.J., J. Schoenmaker, J.N.M. Calis & J. Beetsma, in press. Invasion of *Varroa* mites into drone brood cells of the honey bee. Apidologie.

Calis, J.N.M., W.J. Boot & J. Beetsma, 1990. Transfer from cell to cell. How long do *Varroa* mites stay on adult bees? In: W. Ritter, O. Van Laere, F. Jacobs & L. De Wael (Editors), Proceedings of the international symposium on recent research on bee pathology, September 5-7, 1990, Gent, Belgium. Apimondia, pp. 45-46.

Calis, J.N.M., W.J. Boot, J. Beetsma & D.J.A. Sisselaar, 1991. How long do *Varroa* mites stay on adult bees before invading a new worker brood cell? Mededelingen van de Faculteit voor Landbouwwetenschappen van de Rijksuniversiteit Gent 56/2a: 313-317.

Calis, J.N.M., W.J. Boot & J. Beetsma, 1993. Behavior of *Varroa* mites invading honey bee brood cells of different sizes. In: L.J. Connor, T. Rinderer, H.A. Sylvester & S. Wongsiri (Editors), Asian apiculture. Wicwas Press, Cheshire CT, USA, pp. 499-509.

Calis, J.N.M., W.J. Boot & J. Beetsma, 1993. A standardized test method to evaluate effects of pesticides on honeybee larvae. In: E.G. Harrison (Editor), Proceedings of the fifth international symposium on the hazards of pesticides to bees, October 26-28, 1993. Plant Protection Service, Wageningen, The Netherlands, pp. 55-60.

Calis, J.N.M., J. Beetsma, W.J. Boot, J. van den Eijnde & A. de Ruijter, 1993. Control of the *Varroa* mite by treatment of sealed honeybee brood with formic acid. Proceedings Experimental & Applied Entomology, N.E.V. Amsterdam 4: 217-222.

Calis, J.N.M., W.J. Boot & J. Beetsma, 1994. Evaluation of varroa control methods on trapping mites in honey bee brood. In: A. Matheson (Editor), New perspectives on varroa. IBRA, Cardiff, UK, pp. 106-108.

Van der Blom, J., W.J. Boot & H.H.W. Velthuis, 1994. Simultaneous queen raising and egg laying by workers in Africanized honeybee colonies (*Apis mellifera* L) in Costa Rica. Apidologie 25: 367-374.

Samenvatting

Inleiding

De mijt *Varroa jacobsoni* Oudemans is momenteel de belangrijkste plaag van de westerse honingbij, *Apis mellifera* L. De mijt heeft zich bijna over de hele wereld verspreid (Matheson, 1993) en heeft grote verliezen aan bijenvolken veroorzaakt (De Jong et al., 1982). Toch had enkele tientallen jaren geleden nog vrijwel niemand van de varroamijt gehoord.

Oorspronkelijk kwam de varroamijt alleen voor in volken van de oosterse honingbij, *Apis cerana* Fabr., een honingbij die alleen in Azië voorkomt. Oudemans beschreef de mijt in 1904 als een parasiet van de oosterse honingbij in Indonesië. In de volgende 50 jaar werd er echter vrijwel niets meer vernomen van de mijt (Ritter, 1981). Dit gebrek aan interesse illustreert dat de varroamijt niet als een probleem gezien werd in volken van haar oorspronkelijke gastheer en dit geldt eigenlijk nog steeds, hoewel de werkelijke schade die de mijten in volken van de oosterse honingbij kunnen aanrichten nooit gemeten is. Hoe kon de varroamijt zich ontwikkelen tot een wereldwijde bedreiging voor de bijenhouderij? Bijenhouders zelf zijn hiervoor verantwoordelijk. Ze namen volken van de westerse honingbij mee in het verspreidingsgebied van de oosterse honingbij. Overal waar de twee bijensoorten met elkaar in aanraking kwamen, begon de varroamijt ook de westerse honingbij te parasiteren (Ritter, 1981). In volken van haar nieuwe gastheer bleek ze een zeer schadelijke parasiet, maar voordat dit tot de bijenhouders doorgedrongen was had de mijt zich al over de wereld verspreid via verzendingen van bijenvolken en koninginnen (De Jong et al., 1982).

De nieuwe bedreiging voor de westerse honingbij had een intensieve zoektocht naar acariciden tot gevolg om verdere verliezen te kunnen voorkomen. Vele stoffen werden getest en momenteel is er een aantal effectieve acariciden beschikbaar (Koeniger & Fuchs, 1989; Ritter, 1981). Ze worden op grote schaal door de bijenhouders toegepast. Er zijn echter nadelen verbonden aan het gebruik van acariciden. Bijenprodukten zoals honing en was kunnen met deze stoffen besmet worden. Zelfs alleen de dreiging van besmetting is al genoeg om de waarde als natuurlijk produkt te kunnen verminderen. Bovendien zouden de mijten in de toekomst resistent tegen de acariciden kunnen worden en dit betekent dat er alternatieven voor varroamijtbestrijding beschikbaar moeten komen. Nieuwe bestrijdingsmethoden, waarin het gebruik van acariciden zoveel mogelijk teruggebracht is, of waarin zelfs helemaal geen acariciden gebruikt hoeven te worden, zouden daarom ontwikkeld moeten worden.

Om nieuwe bestrijdingsmethoden tegen de varroamijt te vinden is een grotere kennis van de biologie van de mijt en van de interaktie tussen de honingbij en de mijt van groot belang om de "Achilleshiel" van de mijt te kunnen vinden. In dit proefschrift richt ik me op het binnendringen van varroamijten in broedcellen. Varroamijten overleven op volwassen bijen, maar alleen in broedcellen kunnen ze zich voortplanten (Ifantidis & Rosenkranz, 1988). Ze moeten daarom de bijen verlaten en een darre- of werkstercel binnendringen. Inzicht in de faktoren die een rol spelen tijdens het binnendringen in broedcellen kan in principe op twee manieren bijdragen aan de ontwikkeling van nieuwe bestrijdingsmethoden. Ten eerste zou inzicht in het proces van binnendringen gebruikt kunnen worden om mijten te kunnen manipuleren. Stoffen die broedcellen voor mijten aantrekkelijk maken om binnen te dringen zouden bijvoorbeeld gebruikt kunnen worden om de mijten te vangen. Factoren die

broedcellen minder aantrekkelijk maken voor mijten zouden gebruikt kunnen worden om het binnendringen zelf te verminderen, wat betekent dat de mijten zich minder snel zouden kunnen voortplanten. Ten tweede zou inzicht in het proces van binnendringen de toepassing van de huidige bestrijdingsmethoden kunnen verbeteren, omdat het proces van binnendringen de verdeling van mijten over volwassen bijen en broedcellen veroorzaakt en omdat met de huidige bestrijdingsmethoden óf de mijten op de bijen, óf de mijten in de broedcellen gedood worden. Er is bijvoorbeeld een aantal biotechnische methoden ontwikkeld waarin de mijten gevangen worden in broedcellen (b.v. Rosenkranz & Engels, 1985; Maul et al., 1988; Calis et al., 1993b; Fries & Hansen, 1993). Inzicht in het proces van binnendringen zou kunnen aangeven hoe het aantal gevangen mijten verhoogd kan worden.

In dit proefschrift is gekeken naar het gedrag van individuele varroamijten, de hele mijtenpopulatie in een bijenvolk en de eigenschappen van broedcellen in relatie tot het proces van binnendringen in broedcellen. Bovendien is een eenvoudig model ontwikkeld om te bestuderen welke selectieve krachten varroamijten gevormd kunnen hebben in de strategie die ze volgen om zich te verdelen over darre- en werksterbroedcellen.

Gedrag van individuele mijten tijdens het binnendringen van een broedcel (hoofdstuk 1)

Een varroamijt wordt door een bij tot dicht in de buurt van een geschikte cel gebracht voordat ze die cel binnendringt. De mijt gaat direct van de bij in de geselecteerde broedcel, ze wringt zich tussen de larve en de celwand en kruipt langzaam verder tot de bodem van de cel. Op het moment dat de mijt de bij verlaat kan ze de larve niet aanraken. Ze moet dan nog de afstand van celrand tot larve overbruggen, welke 4-7 mm bedraagt in voor mijten aantrekkelijke broedcellen. De mijt krijgt de informatie die nodig is om te besluiten of ze de cel binnen zal dringen of niet dus op een zekere afstand van de larve, misschien door geurwaarnemingen of door verschillen in temperatuur. Omdat binnendringen in een cel alleen plaatsvindt als een bij de mijt tot dicht bij een geschikte cel brengt, wordt het aantal mijten dat een cel binnendringt wellicht beperkt door de kans dat een mijt dicht genoeg bij een cel gebracht wordt door een bij. Als dat zo is, zal de populatiegroei ook beperkt worden omdat mijten zich alleen in de broedcellen voortplanten.

Het binnendringen in broedcellen door een populatie van varroamijten

Binnendringen in werksterbroedcellen (hoofdstuk 2 & 3)

Binnen een dag nadat varroamijten uit een broedcel tevoorschijn gekomen zijn, d.w.z. het moment dat ze hun verblijf op volwassen bijen beginnen, dringt een gedeelte van deze mijten al weer een nieuwe cel binnen. Het percentage van de mijten op volwassen bijen dat per dag binnendringt hangt af van het aantal geschikte broedcellen en het aantal bijen in het volk, ongeacht de tijd die de mijten al op volwassen bijen doorgebracht hebben. Hoe meer cellen en hoe minder bijen, des te hoger is het percentage mijten dat per dag binnendringt. Dit is volgens verwachting als het binnendringen in broedcellen beperkt wordt door de kans die een mijt heeft om dicht genoeg in de buurt van een geschikte cel te komen, wat duidelijk wordt door de volgende redenering. Omdat er hooguit enkele bijen tegelijkertijd dicht genoeg bij een geschikte broedcel kunnen zijn, zal de kans voor een mijt om dicht genoeg bij een cel te komen toenemen met het aantal geschikte broedcellen in een volk. Bovendien zullen in een kleiner bijenvolk dat wel hetzelfde aantal voor mijten geschikte broedcellen heeft, de mijten

verspreid worden over een kleiner aantal bijen. Aangezien het aantal bijen dat dicht genoeg bij geschikte cellen komt gelijk blijft, zal daardoor de kans voor een mijt om een cel binnen te dringen toenemen.

Het percentage mijten dat per dag een cel binnendringt neemt af als er jong open broed, dat nog te jong is om voor de mijten aantrekkelijk te zijn, aanwezig is. Deze afname in de snelheid van binnendringen treedt mogelijk op omdat de mijten zich bij voorkeur laten dragen door jonge volwassen bijen en deze bijen zijn voornamelijk in het broednest aanwezig. Binnen het broednest zijn deze bijen verdeeld over gebieden waar de broedcellen geschikt zijn voor de mijten om binnen te dringen en over gebieden die ongeschikt zijn voor de mijten. Een toename van broed dat ongeschikt is voor mijten kan daarom een gedeelte van de jonge bijen, waarop de mijten bij voorkeur aanwezig zijn, weghouden van het geschikte broed en daardoor de snelheid van binnendringen verlagen.

Als het binnendringen in broedcellen beperkt wordt door de kans dat een mijt tot bij een geschikte broedcel gebracht wordt, kan de ruimtelijke verdeling van de mijten binnen het bijenvolk de snelheid van binnendringen in broedcellen beïnvloeden. In gebieden waar mijten cellen binnendringen, zal de mijtendichtheid op bijen afnemen. De mijten zullen zich herverdelen binnen het volk omdat de bijen waarop ze zitten zich verplaatsen en omdat de mijten overstappen van bij tot bij, maar afhankelijk van de snelheid van dit proces zal het binnendringen meer of minder beperkt worden. In bijenvolken waarin óf 600 broedcellen beschikbaar waren gedurende 1 dag, óf 3 keer 200 broedcellen beschikbaar waren gedurende 3 dagen, terwijl de volken in andere opzichten vergelijkbaar waren, was de snelheid van binnendringen over 3 dagen gezien gelijk. Op een tijdschaal van dagen gaat het proces van herverdeling van mijten binnen het volk blijkbaar zodanig snel dat geen effect op de snelheid van binnendringen optreedt. Dit is belangrijk voor de toepassing van biotechnische bestrijdingsmethoden waarin broedramen gebruikt worden om mijten te vangen. Deze vangramen worden uit het volk verwijderd en de daarin aanwezige mijten worden gedood. Onze resultaten geven aan dat het aantal broedcellen op een vangraam cruciaal is, terwijl the periode dat de cellen beschikbaar zijn voor de mijten van minder belang is.

Binnendringen in darrebroedcellen (hoofdstuk 4)

Het binnendringen van een populatie mijten in darrebroedcellen is vergelijkbaar met het binnendringen in werksterbroedcellen, behalve dat dit proces in darrecellen veel sneller gaat. Wanneer de snelheid van binnendringen vergeleken wordt tussen bijenvolken met óf alleen werksterbroed, óf alleen darrebroed, blijkt dat varroamijten een darrecel 12 keer zo vaak binnendringen als een werkstercel. Als beide typen broedcellen aanwezig zijn in een bijenvolk, wordt daarom verwacht dat er 12 keer zoveel mijten per darrecel gevonden worden dan per werkstercel uitgaande van de verschillen in snelheid van binnendringen per cel. Deze verwachte verdeling is schever dan de werkelijk gevonden verdeling van gemiddeld 8 maal meer mijten per darrecel dan per werkstercel. Dit verschil in verwachte en werkelijke verdeling van mijten kan als volgt begrepen worden. In normale bijenvolken zijn de perioden waarin mijten in darrecellen en in werkstercellen binnendringen waarschijnlijk min of meer gescheiden in de tijd. Omdat mijten veel vaker in darrecellen binnendringen dan in werkstercellen, zal het aantal mijten op de bijen snel afnemen in perioden waarin veel geschikte darrecellen aanwezig zijn. Daarom zullen minder mijten de darrecellen binnengaan dan in een situatie met een constant aantal mijten op de volwassen bijen. De werkelijke

verdeling van mijten over darre- en werkstercellen zal daarom minder scheef zijn dan verwacht op basis van de verschillen in snelheid van binnendringen per cel. Verder is duidelijk dat de scheve verdeling van mijten over darre- en werkstercellen voldoende verklaard wordt door de verschillen in snelheid van binnendringen per cel. Er is daarom geen reden om aan te nemen dat de scheve verdeling veroorzaakt wordt doordat mijten hun gedrag veranderen als er geschikte darrecellen in de buurt zijn en dan afzien van het binnendringen van werkstercellen. Aangezien de snelheid van binnendringen in darrecellen hoog is, kan een vangramen-methode waarin darrebroed gebruikt wordt zeer effectief zijn om varroamijten te bestrijden. Het aantal cellen dat nodig is om 95% van de mijten in een volk van 1 kg bijen te vangen terwijl er geen ander broed aanwezig is, werd geschat op 462 darrecellen.

Effect van de periode op volwassen bijen op de voortplanting van de mijten (hoofdstuk 5)

Er werd geen verband gevonden tussen de tijdsduur die de mijten op volwassen bijen doorgebracht hebben alvorens een cel binnen te dringen en het totaal aantal nakomelingen per mijt, het aantal levensvatbare nakomelingen per mijt, de fraktie mijten zonder nakomelingen en de fraktie mijten die alleen mannetjes produceert. Blijkbaar is de voortplanting van de mijten onafhankelijk van de periode die mijten op volwassen bijen doorbrengen voordat ze een cel binnendringen.

Sterfte van mijten tijdens hun verblijf op volwassen bijen (hoofdstuk 5)

Sterfte van varroamijten werd gemeten door de mijten die op de bodem van de bijenkast gevallen waren te tellen. Sterfte treedt vooral op direct nadat de mijten uit broedcellen tevoorschijn gekomen zijn met het uitlopen van jonge bijen. Wanneer de mijten gedurende een dag uit broed tevoorschijn komen, wordt 18% van de mijten die in het uitgelopen broed zat op de bodem gevonden. Een gedeelte van deze mijten was misschien al dood in de cel, en valt dan op de bodem na het poetsen van de cel door de bijen. Op de tweede en de derde dag nadat de mijten te voorschijn gekomen zijn, valt respectievelijk 4% en 2% van de mijten op de volwassen bijen op de bodem, terwijl vanaf de vierde dag (tot 23 dagen) slechts 0.6% van de mijten op volwassen bijen per dag op de bodem gevonden wordt. Aangezien het aantal mijten op de bodem van de kast sterk zal samenhangen met het aantal mijten dat net uit broedcellen tevoorschijn gekomen is, kan het tellen van dode mijten op de bodem van de kast een handige methode zijn om de grootte van de mijtenpopulatie in een bijenvolk te bepalen.

Aantrekkelijkheid van broedcellen voor varroamijten

De periode waarin werkster- en darrecellen aantrekkelijk zijn (hoofdstuk 6)

Werksterbroedcellen zijn vanaf 15-20 uur voorafgaande aan het sluiten van de cel aantrekkelijk voor varroamijten, terwijl darrebroedcellen vanaf 40-50 uur voor het sluiten aantrekkelijk zijn. Aangezien darrecellen 2-3 keer zo lang aantrekkelijk zijn dan werkstercellen, kunnen we verwachten dat de mijten ook 2-3 keer vaker binnendringen. In werkelijkheid dringen de mijten een darrecel 12 keer vaker binnen. Er moeten daarom meer factoren een rol spelen om het grote verschil in snelheid van binnendringen per cel te kunnen veroorzaken. Als de snelheid van binnendringen afhangt van de oppervlakte van een broedcel, worden er eveneens meer mijten per darrecel verwacht vanwege de 1.7 maal grotere oppervlakte in vergelijking met een werkstercel. Samen met de langere periode dat darrecellen

aantrekkelijk zijn, zou dit een 3.4-5.1 maal hogere snelheid van binnendringen per cel betekenen, wat duidelijk lager is dan de 12 keer die in werkelijkheid gevonden wordt. Daarom kan de hogere snelheid van binnendringen per darrecel waarschijnlijk voor een belangrijk deel toegeschreven worden aan verschillen in signalen, kwalitatief dan wel kwantitatief, die mijten gebruiken bij hun beslissing om een cel binnen te dringen.

Effect van de afstand van larve tot celrand op de aantrekkelijkheid van broedcellen (hoofdstuk 7)

Varroamijten zijn niet lukraak verdeeld over verschillende typen broedcellen met daarin vergelijkbare larven. Per cel dringen meer mijten binnen in kortere en smallere cellen dan controle cellen, terwijl minder mijten in langere en wijdere cellen binnendringen. De lengte van de periode waarin de cellen aantrekkelijk zijn voor de mijten varieert tussen de verschillen typen broedcellen. Of in een bepaald type cel meer of minder mijten gevonden worden in vergelijking met controle cellen, hangt samen met de lengte van de periode waarin dat type cel aantrekkelijk is voor mijten. Het type cel beïnvloedt ook de afstand van larve tot celrand. Als deze afstand groter is in vergelijking met controle cellen met larven van dezelfde leeftijd, zullen de cellen gedurende een kortere periode aantrekkelijk zijn en vice versa. In alle typen broedcel neemt de afstand van larve tot celrand voortdurend af voorafgaand aan het sluiten van de cel. Het verband tussen de afstand van larve tot celrand en de periode dat een cel aantrekkelijk is suggereert daarom dat er een kritische afstand tussen larve en celrand is, waaronder broedcellen aantrekkelijk worden voor mijten. De periode dat een cel aantrekkelijk is hangt dan af van het moment waarop deze kritische afstand bereikt wordt. De verdeling van mijten over verschillende celtypen hangt vervolgens af van de verschillen in de periode dat de cellen aantrekkelijk zijn. In normale darre- en werkstercellen is de kritische afstand tussen larve en celrand 7-8 mm.

Effect van methylpalmitaat op de aantrekkelijkheid van broedcellen (hoofdstuk 8)

Aangezien varroamijten op een zekere afstand van de larve beslissen of ze op een bij zullen blijven dan wel in de cel zullen binnendringen, zouden ze op de geur kunnen afgaan om een broedcel te selecteren. Er wordt beweerd dat de geur van enkele alifatische esters, voornamelijk methylpalmitaat, dit signaal voor de mijten zou zijn om de volgende redenen. De mijten reageren op de esters in een olfaktometer (Le Conte et al., 1989) en het verschil in de hoeveelheid esters in werkster- en darrelarven kan verklaren dat darrecellen gedurende een langere periode aantrekkelijk zijn en dat de mijten darrecellen vaker binnendringen dan werkstercellen (Trouiller et al., 1992). Het binnendringen van mijten in broedcellen wordt echter niet beïnvloed wanneer methylpalmitaat aan broedcellen toegediend wordt. Bovendien werden bij analyses van geurstoffen uit broedcellen die aantrekkelijk zijn voor de mijten honderden stoffen geïdentificeerd, maar in slechts 2 van de 17 analyses werd een spoortje methylpalmitaat aangetoond. Er is daarom nog geen reden om aan te nemen dat methylpalmitaat door de mijten als signaal gebruikt wordt.

Strategie van varroamijten met betrekking tot het binnendringen in darre- of werkstercellen (hoofdstuk 9)

Aangezien het voortplantingssucces van varroamijten groter is in darrecellen dan in werkstercellen, werpt de vraag zich op waarom de mijten zich niet beperken tot het

binnendringen van darrebroedcellen. Er is daarom een eenvoudig model ontwikkeld waarin populatiegroei van de mijten gebruikt wordt om te bestuderen onder welke omstandigheden specialisatie op darrebroed een betere strategie zou zijn dan voortplanting in beide typen broedcel. Voor Europese *A. mellifera* suggereert het model dat als mijten gemiddeld minder dan 7 dagen moeten wachten voor ze een darrecel kunnen binnendringen, specialisatie op darrebroed een betere strategie zou zijn. Dit is dicht bij de geschatte wachttijd van 6 dagen. Kleine verschillen in voortplantingssucces in darre- en werkstercellen en in sterftesnelheid kunnen daarom bepalen of specialisatie op darrebroed bevorderd wordt of niet. In Europese *A. mellifera* volken dringen varroamijten zowel darre- als werkstercellen binnen, maar er lijkt een zekere specialisatie op darrebroed op te treden omdat ze een darrecel veel vaker binnendringen dan een werkstercel. In de parasiet-gastheer relatie van *V. jacobsoni* met Afrikaanse of "geafrikaniseerde" *A. mellifera*, of met *A. cerana* dringen de mijten ook beide broedceltypen binnen. Met betrekking tot voortplanting specialiseren ze zich echter op darrebrood, aangezien in werksterbroed een hoog percentage van de mijten zich niet voortplant. Alleen in de parasiet-gastheer relatie van *Euvarroa sinhai*, een mijt die veel op *V. jacobsoni* lijkt, en *A. florea* is er een volledige specialisatie. Deze mijten dringen alleen darrecellen binnen.

Draagt de kennis over het binnendringen in broedcellen bij aan de bestrijding van varroamijten?

Het onderzoek naar het binnendringen van varroamijten in broedcellen heeft niet geleid tot een methode waarin de mijten bestreden kunnen worden door aantrekkelijke of afwerende stoffen toe te passen. We moeten nog altijd het signaal identificeren dat de mijten gebruiken om een cel te selecteren, hoewel we weten dat de mijten dit signaal op een zekere afstand van de larve waarnemen en dat de afstand tussen larve en celrand de respons van de mijt beïnvloedt. Onze resultaten zijn echter nuttig om de mogelijkheden en beperkingen voor verbetering van biotechnische bestrijdingsmethoden te begrijpen. We weten nu hoeveel darre- of werkstercellen er nodig zijn in een vangraam om een bepaald percentage van de mijten in een volk te kunnen vangen. In theorie zijn bestrijdingsmethoden die gebruik maken van vangramen eenvoudig. In de praktijk kunnen deze methoden echter ingewikkeld zijn omdat de bijenhouder andere aktiviteiten, zoals het maken van nieuwe volken of zwermverhindering, tegelijkertijd uitvoert. Bovendien is de toepassing van biotechnische bestrijdingsmethoden over het algemeen arbeidsintensief. Onze resultaten kunnen toegepast worden om de eenvoudigste methode die toch voldoende effectief is te ontwerpen. Dit zal ook in de toekomst een belangrijke toepassing blijven. Aangezien veel onderzoek tegenwoordig gericht is op het kweken van bijen die minder gevoelig zijn voor varroamijten (Woyke, 1989b; Büchler, 1994; Moritz, 1994), zou de effectiviteit van de bestrijdingsmethoden die nodig is voor voldoende bestrijding misschien omlaag kunnen en dit maakt een vereenvoudiging van de bestrijdingsmethoden mogelijk. Door combinatie van eenvoudige vangraammethoden en het kweken van minder gevoelige honingbijen is er een duidelijk perspektief voor bijenhouderij zonder het gebruik van acariciden om varroamijten te doden.

Nawoord

Als onderzoeker-in-opleiding (OIO) kreeg ik in de loop van de jaren regelmatig bezorgde vragen op me afgevuurd. Is de werkdruk niet verschrikkelijk hoog, sta je niet stijf van de stress, is het niet moeilijk om zo lang te werken aan het oplossen van een relatief kleine vraag, kan je het wel opbrengen voor dat salaris? Blijkbaar leeft het idee dat het bestaan van een OIO zwaar is. Medelijden lijkt gepast. En natuurlijk moet je soms hard werken, heb je soms last van stress, zou je je soms meer willen verbreden in plaats van verdiepen en is het salaris niet geweldig hoog. Maar nu ik terugkijk op de afgelopen jaren vind ik vooral dat het erg leuk was.

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Willem

Curriculum vitae

Op 12 september 1962 werd ik, Willem Jan Boot, in Kruiningen geboren. In 1980 behaalde ik het VWO-diploma aan het Christelijk Lyceum te Dordrecht. Daarna begon ik aan de studie Planteziektenkunde aan de Landbouwuniversiteit. In de doctoraal-fase verdiepte ik me in agrarische bedrijfseconomie en deed leeronderzoeken bij de vakgroep Entomologie (neuropeptiden in de coloradokever bij Dr C.A.D. de Kort; gedrag van mineervliegen bij Dr O.P.J.M. Minkenberg), de vakgroep Virologie (virustransmissie door bladluizen bij Dr D. Peters) en de vakgroep Theoretische Productie Ecologie (populatie-dynamica van mineervliegen en hun natuurlijke vijanden bij Prof. Dr R. Rabbinge). Mijn stage deed ik bij de 'Forschungsanstalt Pflanzenschutz', Zürich-Reckenholz (onderzoek naar de schadedrempel van maisboorders bij Dr F. Bigler). In juni 1988 behaalde ik mijn doctoraalexamen Planteziektenkunde. Van mei 1989 tot november 1993 werkte ik als onderzoeker-in-opleiding bij de vakgroep Entomologie van de Landbouwuniversiteit. Het onderzoek, beschreven in dit proefschrift, richtte zich op het binnendringen van varroamijten in broedcellen. Tijdens het onderzoek werd een projectvoorstel geschreven voor onderzoek in EU-verband naar honingbijen die minder gevoelig zijn voor varroamijten. Dit voorstel werd gehonoreerd en sinds november 1994 ben ik in dat kader weer als onderzoeker aangesteld bij de vakgroep Entomologie van de Landbouwuniversiteit.