Infection and transmission of Nosema bombi in Bombus terrestris colonies and its effect on hibernation, mating and colony founding

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Original article

Infection and transmission of *Nosema bombi* in *Bombus terrestris* colonies and its effect on hibernation, mating and colony founding*

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Abstract – The impact of the microsporidium *Nosema bombi* on *Bombus terrestris* was studied by recording mating, hibernation success, protein titre in haemolymph, weight change during hibernation, and colony founding of queens that were inoculated with *N. bombi* in the larval phase. Infection with *N. bombi* was diagnosed in 36% of *B. terrestris* queens exposed to *N. bombi*. Mating and hibernation of queens was not significantly affected by *N. bombi* infection but colony founding was reduced significantly. Haemolymph protein titre of *N. bombi* diseased queens was reduced, possibly indicating a disturbance of the metabolism. It was demonstrated that *N. bombi* infection was transmitted to the successive age cohorts in a colony and to the adults that were already in the colony prior to the introduction of the infection. The study showed a significant negative impact of *N. bombi* on *B. terrestris* colony development and indoor rearing.

***Bombus terrestris* / *Nosema bombi* / hibernation / colony founding / mating behaviour / protein / weight / transmission**

1. INTRODUCTION

Nosema bombi Fantham and Porter 1914 is a microsporidian parasite that infects the Malpighian tubules, the ventriculus, the fat tissue and nerve tissues, including the brain of bumble bees (Fries et al., 2001). *N. bombi* causes chronic rather than lethal effects on bumble bees (Macfarlane et al., 1995). Eijnde and Vette (1993) reported that the viability of infected bumble bees is decreased, their lifespan is shortened and fewer queens are produced in infected compared to uninfected colonies. Imhoof and Schmid-Hempel (1999) found no effect of *N. bombi* on performance of *Bombus terrestris* colonies although they did record increased production of males in infected colonies. As the bumble bee gyne is the only individual in the colony to hibernate, it is presumed that *N. bombi* overwinters in the queen. However, no data are currently available on how or where in the queen's body the parasite overwinters. The impact of *N. bombi* on the queen during hibernation and on colony founding after hibernation is not known. The exact transmission routes of *N. bombi* are unknown and remain to be determined. For indoor rearing and management of *B. terrestris* it is essential to understand the influence of *N. bombi* on colony founding, colony development and transmission of the parasite.

Theoretically a bumble bee colony can be infected by *N. bombi* via three routes: (1) directly via the infected hibernated queen, (2) indirectly via infected workers that enter the colony or, (3) indirectly via contaminated food collected during foraging.

In honey bees (*Apis mellifera* L.) *Nosema apis* Zander is transferred via contaminated

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food inside the hive. *N. apis* spores enter the young bees and cause an infection of the ventriculus. Infection in the larval phase is not possible. *N. apis* overwinters in worker bees. As no bumble bee worker or male hibernates, the transmission in *Bombus* species must occur via other routes. In the eusocial phase a similar route as in honey bees is possible. It has previously been shown that an *N. bombi* infection can be induced artificially through sprinkling *N. bombi* spores over the *Bombus* brood (Eijnde and Vette, 1993). Schmid-Hempel and Loosli (1998) successfully infected *B. terrestris* adults by feeding sugar water contaminated with *N. bombi* spores and infected larvae by sprinkling *N. bombi* spores over the brood. Both developmental stages were infected successfully and it appeared that there was no significant difference in susceptibility between adults and larvae.

The impact of *N. bombi* infection on mating, hibernation success, changes in total haemolymph protein titre and body weight during hibernation, and colony founding of *B. terrestris* queens was studied. Furthermore *N. bombi* transmission in a colony and the impact of an *N. bombi* infection on the development of a *B. terrestris* colony was studied. As an infection can be induced via the brood, the duration of the brood developmental stages of *B. terrestris* may have an effect on *N. bombi* infection, transmission, infection route and numbers of spores that can induce an infection. Therefore the normal duration of the larval and pupal developmental stages of queens and worker bees was recorded in a preliminary experiment in order to determine any effect on the duration of these stages by the experimentally induced infection. Colonies were infected with *N. bombi* via the infected queen and via infected workers. Eijnde and Vette (1993) and Schmid-Hempel and Loosli (1998) infected larvae and workers via mass feeding, but it was unknown how many spores induced infection, in what larval/adult stage the infection occurred, and the route infection (i.e., via direct ingestion of spores by larvae or via feeding by adults). In our experiments well-defined numbers of *N. bombi* spores were used to induce *N. bombi* infection directly in queen and worker larvae during known stages of development.

2. MATERIALS AND METHODS

Two separate experiments were conducted. Experiment I was conducted to determine the impact of *Nosema bombi* on mating, hibernation and colony founding by infected queens. In experiment II the transmission within the colony and the impact of an *N. bombi* infection on a *Bombus terrestris* colony was determined.

2.1. *Bombus terrestris*

All colonies used in the experiments were from Dutch *B. terrestris* that were reared indoors by Bunting Brinkman Bees in the Netherlands.

2.1.1. B. terrestris *queen larvae*

Ten *B. terrestris* colonies were prepared to produce queen cells from brood batches by a patented queen production method (patent nr 1000803). The newly emerged queens were removed from the "queen" colonies within one day after emergence.

2.1.2. Indoor rearing of B. terrestris

Colony founding was initiated by placing mated and hibernated queens individually in starter boxes of 2 × 10 × 10 cm. To stimulate oviposition, two, one-day old honey bees were added to each starter box one week after placing the queens in the starter box. The honey bees were removed five weeks after their introduction. Colonies that developed into the eusocial phase were housed in bumble bee boxes of 20 × 30 × 30 cm. The starter boxes and the colonies in the eusocial phase were kept in a climate room at 27 to 29 °C and RH 40 to 60%. The bumble bees were fed ad libitum with sucrose-solution 50% (w/v) and pollen patties. The pollen patties were made of honey bee collected pollen pellets mixed with a 50% (w/v) sucrose-solution into smooth dough. The pollen pellets were not pretreated for elimination of *N. apis* spores, possibly present in these pellets, because *N. apis* is not infectious to bumble bees (Eijnde and Vette, 1993). The handling of the colonies was carried out under red light in order to disturb the colonies as little as possible.

2.2. *Nosema bombi*

All experiments were conducted with *N. bombi* spores taken from infected *B. terrestris* workers.

2.2.1. N. bombi *spores*

The *N. bombi* spores used to infect *B. terrestris* queen larvae (experiment I) originated from the Malpighian tubules of worker bumble bees of an *N. bombi* diseased bumble bee colony. This colony was founded by a queen caught in the field in spring 2003 and reared by Bunting Brinkman Bees. The infected colony was transferred to our laboratory and stored in the freezer at –21 °C in June 2003. In January 2004 the Malpighian tubules of the worker bees were dissected, squashed and used immediately.

The *N. bombi* spores used to infect worker larvae (experiment II) originated from workers of a different *N. bombi* diseased colony (experiment I).The colony was stored in the freezer at –21 °C in October 2004. In January 2005 the Malpighian tubules of the workers were dissected, squashed and the *N. bombi* spores used immediately.

For both experiments the *N. bombi* spores were suspended in a 12.5% (w/v) sucrose-solution. The concentrations were recorded by counting the spores in a Bürker counting chamber using the light microscope (400 × magnification).

2.2.2. Introduction of an N. bombi *infection*

In the "queen" colonies (experiment I) and in starter colonies (experiment II), five μL of a 12.5% (w/v) sucrose-solution containing *N. bombi* spores was administered to all individual larvae in the open cell stage. For accurate administration, a Hamilton micro syringe was used. Immediately before the administration of *N. bombi* spores to the larvae, all adults were removed and kept in a box to prevent ingestion of the spores solution by the adults. Five minutes after administration the larvae had ingested the spores suspension (determined by observation) and the adults were replaced in the same colonies that they were removed from.

2.2.3. Detection of N. bombi *spores in* B. terrestris *adults*

B. terrestris adults were checked for *N. bombi* spores in the ventriculus and Malpighian tubules by light microscopy (400 × magnification). Also adults were sent to Queen's University Belfast for molecular detection of *N. bombi* (Klee et al., 2005).

2.3. Preliminary test. Duration of developmental stages of queens and workers

The successive development of *B. terrestris* brood begins with 5 to 10 eggs in a sealed egg cup and progresses to the sealed cell stage in which 5 to10 larvae share an enlarged sealed cell. This sealed larval cell is opened and reclosed by the workers to feed the larvae. Next, wax cells are constructed around each larva and in each cell the larva is fed individually through a permanent orifice; this is called the open cell stage. The last stage is the pupal stage when each pupa is individually enclosed in a sealed cocoon. The duration in days of the developmental stages of *B. terrestris* queens from one egg batch from a "queen" colony and from one egg batch from a normal colony with a queen and ten workers was recorded with an infra red video camera and time lapse recorder.

2.4. Experiment I. Impact of *N. bombi* on mating, weight change during hibernation, protein titre in haemolymph, and colony founding

All queen larvae in the open cell stage in each of the five "queen" colonies recevied 313 000 *N. bombi* spores; these were the "inoculated queens". At the same time, all queen larvae in the open cell stage in each of five "control queen" colonies received the 12.5% (w/v) sucrose-solution without *N. bombi* spores. The "inoculated queens" and "control queens" were maintained separately during the experiment. After emergence all queens could feed *ad libitum* for one week on 50% (w/v) sucrose-solution and pollen dough prepared in the same way as the pollen dough used in rearing of the colonies.

After this one week period had elapsed, the queens were placed in a mating box (mesh cage, 40 cm × 50 cm × 50 cm) with twice as many males as queens. The queens were given one hour to mate.

B. terrestris queens mate for about 15 minutes. During mating the couples were transferred to another mating box. After mating the mated queens were kept for one week at room temperature with 50% (w/v) sucrose-solution and pollen dough ad libitum in a mate box. The queens that did not mate were diagnosed for *N. bombi* microscopically.

One week after mating the hibernation process began. First, all queens were marked with a numbered coloured disk, which was glued to the thorax with insect glue used to mark honey bee queens. Then the queens were placed individually in empty match boxes. A pre-hibernation period of 7 days at 12 °C preceded storage at 5 °C in a refrigerator equipped with forced fresh air circulation for 22 weeks.

The weight of the inoculated and control queens was recorded at the start of the hibernation and after the 22nd week of hibernation.

After 12 and 22 weeks hibernation, the total haemolymph protein titre of ten inoculated and control queens was recorded. Haemolymph was tapped by inserting a capillary needle between the 4th and 5th abdominal segment into the heart. This procedure killed the queen. Haemolymph protein was determined photospectrometrically using the Bradford method (BSA reference protein and absorption at 595 nm).

After hibernation, the queens were housed individually in starter boxes to start ovipositing and colony founding. Queens that had not begun ovipositing within six weeks were considered an non-colony founding queens. The successful colony founding queens and their brood were transferred into bumble bee boxes when the first offspring emerged. At the end of the experiment, when the first males emerged, the inoculated and control queens and their offspring were diagnosed microscopically for *N. bombi* spores.

2.5. Experiment II. Transmission in the colony via diseased worker offspring and impact of *N. bombi* infection on colony development

N. bombi transmission within a colony was studied in six young colonies that each consisted of a queen and 10 to 20 workers and had brood at all stages of development at the start of the experiment. Two hundred thousand *N. bombi* spores in five μL 12.5% (w/v) sucrose-solution were administered to all worker larvae in the open cell stage in each colony. In six additional control colonies only the five μL sucrose-solution 12.5% (w/v) without spores was administered to all worker larvae in the open cell stage.

To identify age cohorts, workers were marked with a dot of acrylic paint on the thorax. Before the *N. bombi* spores were administered all adults present in the colony were marked white (age cohort A). The age cohort that was inoculated with *N. bombi* spores in the larval stage was marked orange (age cohort B). Based on duration of the development stages, derived from the preliminary study, the age cohort of newly emerged bees that were in the egg or sealed cell stage when the *N. bombi* spores were supplied was marked green (age cohort C). The age cohort that emerged four weeks after spore administration, not yet deposited as eggs at the time of application, was marked blue (age cohort D).

Periodically samples of adults were taken and the Malpighian tubules and ventriculus were checked microscopically for *N. bombi* spores. Nine weeks after the spore administration the number of workers, males and brood cells was recorded.

3. RESULTS

3.1. Duration of development

The open queen and worker larval cells from one egg batch were sealed within 24 hours. The queens and workers from these cells emerged within a 24 hours time frame after completion of the pupal phase. The duration of the larval and pupal phases of seven queens and of ten workers was recorded. The open cell stage and the pupal stage of a queen lasted approximately 6 and 13 days respectively. The egg stage, sealed cell stage, open cell stage and pupal stage of a worker lasted approximately 6, 8, 6 and 9 days respectively. As the duration of the larval and pupal stage of the groups were recorded and the sealing of the cells and emergence was both within a 24 hours time frame, the presented duration are plus and minus one day.

Table I. Average (sd) titre of haemolymph protein in μg μL^{-1} (sd) in *B. terrestris* queens after 12 and 22 weeks hibernation at 5 °C.

	"Inoculated" queens		"Control" queens		*t*-test
	protein	# queens	protein	# queens	P
12 weeks	347.2 (62.8)	7	314.5 (37.6)	9	0.22
22 weeks	206.8 (29.1)	10	254.1 (45.2)	8	0.02

Table II. Average weight (sd) in grams at the start of hibernation period and the decrease of weight during hibernation of "inoculated" queens in which an *N. bombi* infection was detected and in which no *N. bombi* infection was detected and of "control" queens.

Groups	Start hibernation		ANOVA		Decrease		ANOVA	
	weight	# queens	F	*P*	weight	# queens	F	*P*
"Inoculated" *N. bombi* infection	0.82 (0.09)	8			0.23 (0.05)	7		
"Inoculated", no *N. bombi* infection	0.83 (0.11)	50	1.71	0.18	0.22 (0.07)	39	0.11	0.89
"Control" queens	0.79 (0.10)	70			0.22 (0.07)	54		

3.2. Experiment I. Impact of *N. bombi* on mating, weight change during hibernation, protein titre in haemolymph and colony founding

3.2.1. Impact on brood development and mating of queens

In the five inoculated queen colonies and five control queen colonies, 167 and 162 queen larvae were treated and 113 and 117 queens emerged respectively. There was no statistical difference between the mean numbers of treated larvae (t-test for two means, $P = 0.87$) and emerged larvae (t-test for two means, $P = 0.85$).

All emerged queens were prepared for mating. Of the 113 and 117 queens from the test colonies and control colonies, 57 and 70 queens mated respectively. These numbers were not statistically different (GLM analysis binomial distribution, $P = 0.15$).

3.2.2. Haemolymph protein titre

The mean total protein titre in the haemolymph of the "inoculated" and "control" queens after 12 weeks of hibernation was not significantly different (t-test for two means $P = 0.22$) (Tab. I). After 22 weeks of hibernation the haemolymph protein titre of the "inoculated" queens was significantly lower (t-test for two means $P = 0.02$) (Tab. I).

3.2.3. Weight change

Because queens had to be dissected to diagnose *N. bombi*, the statistical analysis of the weight data was carried out only at the end of experiment I. The group of inoculated queens was split up into groups: inoculated queens in which an *N. bombi* infection was detected and those in which no *N. bombi* infection was detected. The mean weights of both inoculated queen groups and of the control queens at the start of the hibernation and the decrease in weight over the 22 weeks of hibernation were not statistically different (Tab. II).

3.2.4. Colony founding

Of the 57 and 70 mated inoculated queens and control queens, 38 and 45 queens were placed in starting boxes, respectively, to initiate colony founding. Oviposition was observed for 25 and 30 queens, respectively. There was no statistically significant difference in the number of queens that began ovipositing between the groups (binomial distribution GLM analysis, $P = 0.93$). However two inoculated queens and 19 control queens successfully founded a colony, which was a statistically significant difference (binomial distribution GLM analysis, $P < 0.01$). The brood of the inoculated queens was malnourished and died.

Table III. *N. bombi* infection detected microscopically in 98 "inoculated" queens.

Life stage "inoculated" queens	# queens	*N. bombi* infection
Dead during hibernation	2	2
Not mated	56	25
Mated, no colony founded	38	8
Mated, colony founded	2	0

3.2.5. N. bombi *infection*

Not all queens that mated and hibernated were diagnosed for *N. bombi*. Some queens were used to record the protein titre in their haemolymph. *N. bombi* infection was detected microscopically in 35 out of 98 inoculated queens (36%) (Tab. III). Of the two queens that founded a colony, in one colony no *N. bombi* spores were detected in the queen and in her offspring. The offspring of the other colony had microscopically detectable *N. bombi spores*. However no *N. bombi* spores in the Malpighian tubules and ventriculus of the queen were detected microscopically. No *N. bombi* infection was detected microscopically in 92 control queens and offspring.

3.3. Experiment II. Transmission in a colony and impact on colony development

3.3.1. N. bombi *infection transmission within a* B. terrestris *colony*

Inducing an *N. bombi* infection with 200 000 spores *N. bombi* spores administered to worker larvae in the open cell stage, resulted in an *N. bombi* infection in workers from those larvae (age cohort B, Tab. IV). The infection rates of the age cohorts were not determined. Transmission of *N. bombi* to younger age cohorts of brood (age cohort C and D, Tab. IV) and to adults that were in the colony at the moment of inoculation (age cohort A, Tab. IV) was recorded. *N. bombi* spores in age cohort A were detected six weeks after the start of the experiment and four weeks after the adults from the infected larvae emerged. In age cohort B, *N. bombi* spores were detected in the newly emerged adults. *N. bombi* spores were detected in age cohort C, two to three weeks after emergence. They were not checked for *N. bombi* spores immediately after emergence. The infection was also transmitted to age cohorts that were not yet in the egg stage when the infection was induced (age cohort D). These adults were not checked for *N. bombi* spores immediately after emergence but 3 weeks later.

3.3.2. Impact of N. bombi *on colony development*

The average number of adults in the six infected and six uninfected *B. terrestris* colonies, nine weeks after spore administration was 242 (sd: 57.3, n colonies = 6) and 270 (sd: 40.0, n colonies = 6) respectively. The numbers of adults were not statistically different (t-test for two means, $P = 0.34$). The average number of brood cells nine weeks after spore administration was 69 (sd: 46, # colonies = 5) and 125 (sd: 65, # colonies = 5) respectively. The number of brood cells was not statistically different (t-test for two means, $P = 0.15$).

4. DISCUSSION

4.1. Duration of brood development

The duration of the open larval and pupal phase of the *B. terrestris* queen recorded in the preliminary test was approximately 6 and 13 days respectively, which was confirmed by the recorded emergence of 230 queens in experiment I. The first queen cells in which the larvae were inoculated with *N. bombi* spores and the control queen cells were sealed within 24 hours after treatment. The first and last queens in experiment I, emerged 14 and 21 days after treatment respectively. Assuming that the larvae that were sealed first also emerged first, the pupal stage lasted 13 to 14 days and the preceding larval stage 6 to 7 days. The longer duration of the brood development of the queen compared to the development of the worker is due to the longer pupal phase.

Table IV. Brood developmental stage of the age cohorts when the *N. bombi* infection was introduced, the emergence (in weeks) of the age cohorts and the diagnosis of *N bombi* in workers during colony development (mixed squash preparation of workers, microscopical detection).

Brood development stage when infection was induced (identification age cohort)	Emergence in weeks after infection was induced	Result *N. bombi* diagnosis (weeks after the infection was induced) – = no *N. bombi* spores detected + = *N. bombi* spores detected
Adults (age cohort A)	–4 to 0	–* (2)
		–* (5)
		+* (6)
		+** (9)
Larvae in open cells (age cohort B)	2	+* (2)
	2	+* (5)
	2	+** (10)
Eggs and larvae in sealed cells (age cohort C)	3 to 4	+* (6)
	3 to 4	+** (9)
Egg 1 to 2 weeks after treatment (age cohort D)	5 to 6	+** (9)

* Squash preparation of five workers.
** Squash preparation of ten workers.

In experiment II, workers emerged every day and no detailed observations were made in experiment II to confirm the duration period of the open larval and pupal phases recorded in the preliminary experiment. The data recorded in the preliminary experiment was used to determine the age cohorts of the workers.

4.2. Impact of *N. bombi* on queen pupal development, mating, hibernation, haemolymph protein titre and weight course during hibernation and colony founding abilities

4.2.1. Pupal development and mating of queens

The experimental infection of the queen larvae with *N. bombi* spores, in the open cell stage, did not have an effect on the duration of the pupal stage. According to the statistical analysis mating was also unaffected by the experimental infection. However, as 45% of the inoculated queens that did not mate were diagnosed positive with an *N. bombi* infection (Sect. 3.2.5), and as 21% of the inoculated queens that did mate were diagnosed with an *N. bombi* infection, it is difficult to deny any impact of an *N. bombi* infection on mating. These numerical data tend to confirm the finding of Macfarlane et al. (1995) that an *N. bombi* infection has a negative impact on mating. The stage of brood development in which an infection started might play a role. Hibernation was not affected by *N. bombi.*

4.2.2. Haemolymph titre

The infection rate of the inoculated queens in which an *N. bombi* infection was detected was at least 36% (Sect. 3.2.5). Thus, the statistically significant decrease in haemolymph protein must be considered as an indication of the effect of *N. bombi* on metabolism. A parasite-host change in host resources is demonstrated in *Crithidia bombi* infection of *B. terrestris*; infected bees infested relatively more resources in their fat body than in their reproductive system (Brown et al., 2000). The decrease of haemolymph protein due to *N. bombi* infection might have had an impact

on colony founding as the nourishing of first brood depends on the queen.

4.2.3. Weight change

An *N. bombi* infection did not result in a significant decrease in weight change during hibernation. *N. bombi* infects the Malpighian tubules in *B. terrestris* and the function of the Malpighian tubules might be disturbed. During hibernation this did not affect the weight change of the queens.

4.2.4. Colony founding

We have shown that *N. bombi* infected queens can mate and start oviposition. However few colonies reached the eusocial phase. *N. bombi* infection has a negative impact on the colony founding abilities of *B. terrestris* queens as was shown previously by McFarlane et al. (1995). The same phenomenon was demonstrated by Brown et al. (2003). It was demonstrated that a *Crithidia bombi* infection of hibernated queens reduced the colony founding success significantly.

4.2.5. **N. bombi** *infection*

Feeding individual queen larvae with 313 000 *N. bombi* spores in sugar solution in the open cell stage resulted in a microscopically detectable *N. bombi* infection in at least 36% of the queens. Infections of the ovaries and even in the alimentary track might have been overlooked as was shown in four queens that were checked for *N. bombi* spores both microscopically and molecularly (see end of this section). No *N. bombi* infections were detected microscopically in the "control" queens. The infection rate was in the order of magnitude reported by Schmid-Hempel and Loosli (1998) who recorded infection rates of 19 to 29%. Both modes to induce an *N. bombi* infection, sprinkling *N. bombi* spores over the brood nest or individual administration of *N. bombi* spores to larvae in the open cell stage resulted in about the same infection rate. Rutrecht et al. (2007) demonstrated a dose-effect relationship in infection tests with adult bees. Administration of five times more spores per adult (100 000 and 500 000 spores) resulted in a 20 fold increase of prevalence of *N. bombi* infection. This dose-effect relationship was not studied in experiment I. The similar result of the sprinkling method (Eijnde and Vette, 1996; and Schmid-Hempel and Loosli, 1998) in which the larvae probably ingest different numbers of spores and the individual application used in this study indicate that lower numbers of spores may also produce an infection. As the queens were subsequently pooled into one "inoculated" and one "control" group immediately after emergence, it cannot be ascertained in hindsight whether the difference in susceptibility between queens was due to larval age at spore administration or to particular colonies or to an as yet unknown resistance mechanism.

Eight mated and hibernated "inoculated" queens were both diagnosed microscopically and molecularly. Although no *N. bombi* spores were detected microscopically in the Malpighian tubules and ventriculus of any of the eight queens, in four queens *N. bombi* spores were detected molecularly. Also despite the lack of visually detectable spores in the intestines of one queen that did not initiate oviposition, *N. bombi* was detected molecularly in the alimentary track. Seven queens started oviposition but did not found a colony. In three of these queens *N. bombi* spores could be detected molecularly in the alimentary track. In one queen *N. bombi* spores were detected molecularly in the ovaries.

The data show that diagnosis based on light microscopic detection of spores in the ventriculus and Malpighian tubules is not 100% reliable. The PCR diagnosis has the advantage of being able to detect the parasite in the vegetative phase as well as in the sporogenic phase. This appears to be a more reliable technique for *N. bombi* detection in adult hosts. The molecular diagnosis of *N. bombi* in the ovary suggests the possibility of a transovarian transmission route.

4.3. Transmission of *N. bombi* in a *B. terrestris* colony and the impact on colony development

4.3.1. Transmission in a B. terrestris *colony*

Once an *N. bombi* infection is introduced into a *B. terrestris* colony via workers that were infected in the larval stage, this infection is transmitted to both future generations and to adults that were previously not infected. The transmission of the infection to the adults that were in the colony before the *N. bombi* infection was induced indicates that there is a circulation of spores inside the colony and ingestion of the spores via food. It is shown that *B. terrestris* can be effectively infected with *N. bombi*, both in the larval and in the adult stage. This is in line with the infection results of Schmid-Hempel and Loosli (1998) and McIvor and Malone (1995) that both in larvae and adults an *N. bombi* infection can develop. Rutrecht et al. (2007) demonstrated an age dependent susceptibility to *N. bombi*; two days old bees are twice as susceptible than ten days old bees.

4.3.2. Impact on colony development

There was a trend toward the presence of fewer adults and brood cells in colonies in which the adults and the brood had an *N. bombi* infection compared to healthy colonies, however this trend was not statistically significant. Our results support the findings of Imhoof and Schmid-Hempel (1999) that *N. bombi* does not have a negative impact on colony development of *B. terrestris* but are in contrast with the finding of Eijnde and Vette (1993) who showed a negative impact on the colony development. However, because infected colonies are likely to produce infected queens, of which most queens can not found a colony, the reproductive fitness of the original colony is severly negatively affected.

The stage of development of a colony at the moment the *N. bombi* infection is introduced might play a role. Infected starting colonies might have less chance to develop into big colonies than colonies in which the infection was introduced later. This might influence the early male production and reduced queen production as recorded by Imhoof and Schmid-Hempel (1999) and Eijnde and Vette (1993). Otti and Schmid-Hempel (2007) demonstrated that an *N. bombi* infection of a colony in an early stage of development results in a strong reduction of functional fitness of males and young queens.

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Infection et transmission de *Nosema bombi* dans les colonies de *Bombus terrestris* et son effet sur l'hibernation, l'accouplement et la fondation de colonie.

***Bombus terrestris* / *Nosema bombi* / transmission agent pathogène / hibernation / fondation de colonie / comportement d'accouplement / protéine / poids corporel / Apidae**

Zusammenfassung – Infektion und Übertragung von *Nosema bombi* in *Bombus terrestris* Völkern und Auswirkungen auf die Überwinterung, die Paarung und die Koloniegründung. Wir untersuchten die Auswirkung des Mikrosporidiums *Nosema bombi* auf die Koloniegründung bei *Bombus terrestris*. Dazu registrierten wir die Faktoren Pupalentwicklung, Paarung, Überwinterung und Koloniegründung bei Königinnen, die in der Larvalphase mit *N. bombi* Sporen infiziert wurden. *N. bombi* Sporen wurden von *Nosema* infizierten *B. terrestris* Arbeiterinnen gewonnen und in einer 12.5 % (w/v) Sacharoselösung an 9–14 Tage alte Kökininnenlarven verfüttert. Bei mindestens 36 % der Hummelköniginnen führte dies zu einer *Nosema bombi* Infektion. Weder die Paarung noch die Überwinterung von Königinnen war durch die *N. bombi* Infektion beeinflusst. Die Koloniegründung hingegen erwies sich als signifkant reduziert. Das

Lebendgewicht vor, während und nach der Überwinterung sowie der Hämolymphproteingehalt von *B. terrestris* Königinnen waren durch die *N. bombi* Infektion deutlich beeinflusst, was auf eine Wirkung auf den Stoffwechsel hinweist.
Bezüglich der Detektion einer *N. bombi* Infektion bei *B. terrestris* erwies sich ein molekulares Verfahren als deutlich besser im Vergleich zur lichtmikroskopischen Erfassung von *N. bombi* Sporen im Darmtrakt.
Die Art und Weise wie *N. bombi* innerhalb von *B. terrestris* Kolonien übertragen wird, untersuchten wir durch Infektion von 9–14 Tage alten Arbeiterinnenlarven in Kolonien mit 10–20 Arbeiterinnen. Während der Kolonieentwicklung wurden in sukzessiver Weise Alterskohorten markiert und sowohl diese Alterskohorten als auch die bereits vorhandenen adulten Arbeiterinnen wurden später auf *N. bombi* Infektionen hin untersucht. Wir fanden eine Übertragung sowohl bei den sukzessiven larvalen Alterskohorten als auch bei den adulten Arbeiterinnen, die bereits vor Beginn der Infektion in der Kolonie waren. Damit zeigt sich, dass *N. bombi* sowohl auf Adulte als auch auf Larven erfolgreich übertragen werden kann.

***Bombus terrestris* / *Nosema bombi* / Überwinterung / Koloniegründung / Paarungsverhalten / Protein / Gewicht / Übertragung**

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