Original article

Honey bee age-dependent resistance against American foulbrood

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Abstract – American foulbrood is a fatal disease of honeybee larvae. Larvae become infected by swallowing spores of *P. larvae larvae* that contaminate their food. Adult bees who transfer the spores and have close contact with larvae never become infected. Resistance to this bacterium was investigated in various larval stages and in adults of different ages. Substances inhibiting the growth of *P. larvae larvae* could be demonstrated in 4 day old larvae and, to a lesser extent, in 1 day old larvae. No such substances could be shown in 6 day old larvae. Extracts of midguts of adult bees generally showed a stronger ability to inhibit growth of the bacteria than did extracts of larvae. It was discovered that the midguts of 8 day old adult bees show a higher growth-inhibiting potential against *P. larvae larvae* than midguts of freshly emerged adult bees or foragers.

Apis mellifera / *Paenibacillus larvae larvae* / **age-dependent resistance / honeybee larvae**

1. INTRODUCTION

American foulbrood is a fatal disease of honeybee (*Apis mellifera* L.) larvae. The causative agent is the spore-forming bacterium *Paenibacillus larvae* subsp. *larvae* (formerly *Bacillus larvae*) (Heyndrickx et al., 1996). American foulbrood has a serious negative economic impact on the beekeeping industry and on agriculture (Hansen and Brødsgaard, 1999). Early detection of infection by investigating honey samples for spores (Hansen, 1984; Shimanuki and Knox, 1988; Hornitzky and Clark, 1991; Hornitzky and Karlovskis, 1998) or with PCR (Alippi and Aguilar, 1998; Govan et al., 1999) is desirable, but the disease is usually identified through recognition of advanced clinical signs by beekeepers. For protection and treatment, beekeepers have

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become increasingly dependent on the use of antibiotics (Peng et al., 1996; Alippi et al., 1999), which presents toxicological hazards to bees and risks contaminating hive products (Lehnert and Shimanuki, 1981; Peng et al., 1992). In countries where the use of antibiotics for bees is forbidden, often the colonies have to be killed.

Larvae become infected by swallowing spores of *P. larvae larvae* that contaminate their food. After spores germinate in the larval midgut, the vegetative forms penetrate the tissue of the intestine and multiply, which finally kills the larva (Ritter, 1996; Gregorc and Bowen, 1998). It has been known for a long time that young honeybee larvae are highly susceptible but older larvae and adult bees, even adults that transfer the spores and have close contact with young larvae, never become infected (Woodrow, 1942; Woodrow and Holst, 1942; Brødsgaard et al., 1998) Schulze-Langner (1955) reported that spores germinate in the rectum of the adult bee and are egested as non-infectious vegetative cells. However, Wilson (1971) reported that spores fed in large numbers to adult bees remained viable in the alimentary canal and, when then fed to young larvae, caused American foulbrood, although he did not find any pathological effects on the adults. Larvae from different lines of honey bees showed different mortality rates following inoculation with spores of *P. larvae larvae* (Rothenbuhler and Thompson, 1952). Feeding spores to larvae of different ages, Bamrick and Rothenbuhler (1961) found that larvae of a resistant line were no longer susceptible after about 36 hours of age, while larvae of a susceptible line did not become resistant until the age of about 48 hours. Vegetative stages of the bacterium were found in a higher percentage of young infected larvae than in larvae of intermediate age or older following approximately the same period for spore germination (Bamrick, 1967).

Riessberger-Gallé et al. (unpublished data) found that one or more non-induced,

temperature-stable substances in the midgut of adult bees have the potential to suppress the germination of spores and the growth of the vegetative stage of *P. larvae larvae*. Differences in the amounts of such substances in larvae from different colonies might contribute to the colonies' differences in resistance or susceptibility to American foulbrood.

The aim of the present work was to investigate if larvae have growth-inhibiting substances, and to compare their effectiveness with those substances in adult bees of different ages. To learn more about the origin of these non-induced substances, and if they are synthesised by the bee or incorporated with food, we also investigated the extent to which the larval food, honey, pollen and jelly inhibit the growth of these bacteria.

2. MATERIALS AND METHODS

2.1. *Paenibacillus larvae larvae*

2.1.1. Identification

For our experiments we used a wild strain of *P. larvae larvae* from Styria (Austria) (Riessberger-Gallé et al., unpublished data). Identification of *P. larvae larvae* was carried out by incubation on Columbia sheep blood agar (27.3 g Columbia agar base (Oxoid), 700 ml distilled water, 50 ml sheep blood), followed by the catalase and the "Plagemann" tests (giant whips could be identified in the liquid part of Columbia sheep blood slant agar) (Plagemann, 1985).

2.1.2. Cultivation

Bacteria were cultivated for two days in brain-heart-infusion (Oxoid) and frozen at $-70\ ^\circ$C in 1 ml aliquots until used. 40 ml of brain-heart-infusion was then inoculated with 1ml of defrosted suspension (see above), heated at 77 $^\circ$C for 10 min to eliminate rods that might have survived freezing and to activate spores to germinate, and

incubated at 37 °C for about 48 hours. The culture was in the beginning of the exponential phase of growth when it was used for inoculation, standardised by dilution at an optical extinction of 0.20–0.21 measured at 546 nm (von der Ohe et al., 1996), path length 1 cm. Optical extinction was measured as a quantification of the cloudiness caused by the growth of the bacteria *P. larvae larvae* (method: Bast, 1999).

2.2. Sampling and colonies

Samples of larvae and adult bees of different ages were taken from two full sized colonies (each with 20 combs and about 35 000 bees, which were estimated by screening the colonies) around the end of April 1999. Samples of royal jelly from queen larvae were taken near the end of May; samples of worker larvae 30–36 hours old and samples of pollen and worker jelly were taken during July; and honey samples came from honey extracted at the end of summer. The two colonies (A and B), standing side by side, differed in their behaviour towards intruders: one was very calm, the other was very aggressive. Whenever brood combs were drawn from a hive to obtain bees emerged in an incubator, the emerged bees and the combs were returned to the colony from which they originated. Samples of the food stores of both colonies were collected, and analysis confirmed the absence of spores of *P. larvae larvae* (von der Ohe and Dustman, 1997) before we started investigations.

2.3. Samples of larvae

The queen of each colony was caged on a brood comb in her colony for 6 hours to obtain brood of known age. Larvae at the age of 30–36 hours, of 4 days and 6 days (just capped) were taken out of their cells, washed twice in distilled water and touch-dried lightly on filter paper. Eppendorf vials were filled with 8 larvae each. After the addition of distilled water in the amounts of 160 μl (for 30–36 h larvae), 100 μl (for 4 day larvae) or none (for 6 day larvae), the contents were homogenised by ultrasonic treatment. Then each vial was filled with 560 μl (for 30–36 h larvae) or 650 μl (for 4 day and 6 day larvae) ethanol and stored at 4 °C overnight. The next day the suspension was centrifuged, and the supernatant was lyophilised and stored at 4 °C until used.

For another series of investigations, Eppendorf vials were each filled with 50 larvae 30–36 hours old, so that the combined weight in one vial corresponded to the weight of one 4 day larvae, and further treatment was carried out as described above.

2.4. Samples of adult bees

We investigated freshly emerged adult bees, 8 day old adults and foragers. The freshly emerged bees (0 day old) were taken out of their brood cells before they had any possibility of being fed by nurses or feeding themselves. The 8 day old adults were bees that had emerged in an incubator during a 12 hour period, had been marked, and had then been introduced into their original colonies and allowed to live normally for 8 days. Bees in this age class are fully physiologically equipped to function as nurse bees (Crailsheim, 1986; Moritz and Crailsheim, 1987; Crailsheim, 1990; Hrassnigg and Crailsheim, 1998) and are frequently observed nursing brood (Rösch, 1925; Lindauer, 1952; Riessberger and Crailsheim, 1997). Forager bees were collected in front of the hive entrance, returning from flights with pollen loads on their corbiculae.

The midguts of bees from each of these three temporal castes were dissected, washed twice in distilled water and lightly touch-dried. Eppendorf vials were filled with 8 midguts each; after addition of 160 μl distilled water, the contents were homogenised, the vials were filled with 560 μl ethanol, and analysis proceeded as described above for samples of larvae.

2.5. Samples of food

Samples of *Zea mays* pollen from three different sources were investigated: 1: untreated pollen picked directly from blooming *Zea mays*; 2: pollen pellets taken from bees that were caught on the flowers of *Zea mays*; and 3: bee bread removed from pollen cells, made by the bees from *Zea mays* pollen (as confirmed by microscopic analysis). Each Eppendorf vial was filled with 32 mg of pollen, because about 4 mg of pollen is typically found in an adult midgut (Crailsheim et al., 1992), and analysis proceeded as described above for 8 midgut samples from adult bees.

Similarly, we analysed 32 mg-samples of royal jelly (colony B only), worker jelly from 30–36 h old larvae (colonies A and B) and extracted honey.

The amount of pollen in the gut of a 4 day old larvae was determined by counting the pollen grains (Crailsheim et al., 1992; Loidl and Crailsheim, unpublished data). Twelve 4 day old larvae of two colonies, different from colonies A and B, were investigated.

2.6. Experimental set-up

To test for antibacterial activity, the lyophilised powder of each sample of larvae, adult midguts, pollen, royal jelly, worker jelly, or honey was dissolved in sterile distilled water, and different concentrations of the solutions were added to test tubes filled with 1 ml of brain heart infusion. We tested nine different concentrations of each larval extract or midgut extract, corresponding to 2 – 0.00781 larvae or midguts of adult bees. In addition, for larvae 30–36 hours old, we tested nine other concentrations, corresponding to 12.5 – 0.0488 30–36 h larvae. We also tested nine different concentrations of "food" extract, corresponding to 8 – 0.03125 mg pollen, royal jelly, worker jelly or honey. Sterile H_2O was used as control.

In each test, two test tubes were supplied with the same concentration of extract from the same pool of larvae, adult midguts or food samples. One of the tubes was inoculated with 30 µl of bacteria suspension, and the other one served as the blank during later photometrical measurement (564 nm). As the blank remained clear (no cloudiness) after incubation, there were no spores or vegetative forms of bacteria or fungi present in the added extracts. Tubes were incubated aerobically at 37 °C for 24 hours and shaken before measurement.

The method of inoculation, incubation and measurement was the same in all tests.

2.7. Statistics

Means and standard deviations are given. Six independent pools were tested in each series. Significance was tested with repeated measures analysis of variance (SPSS). Differences between concentrations of the extract, differences between age classes (and interactions between concentration differences and age class) and differences between the two colonies (and interactions between concentration differences and colonies) were tested. Differences in the weight of the pooled larvae (30–36 h) between colonies A and B were tested with the Mann-Witney U-Test. The level of significance was set at $P < 0.05$.

3. RESULTS

3.1. Effects of larval age (Figs. 1 and 2)

Extracts of larvae of different ages differed in their effect on the growth of the vegetative stages of *P. larvae larvae*. In these tests we compared extracts from equal numbers of larvae of different ages (one 30–36 h larva vs. one 4 day larva vs. one 6 day larva and so on), even though their weights differed greatly.

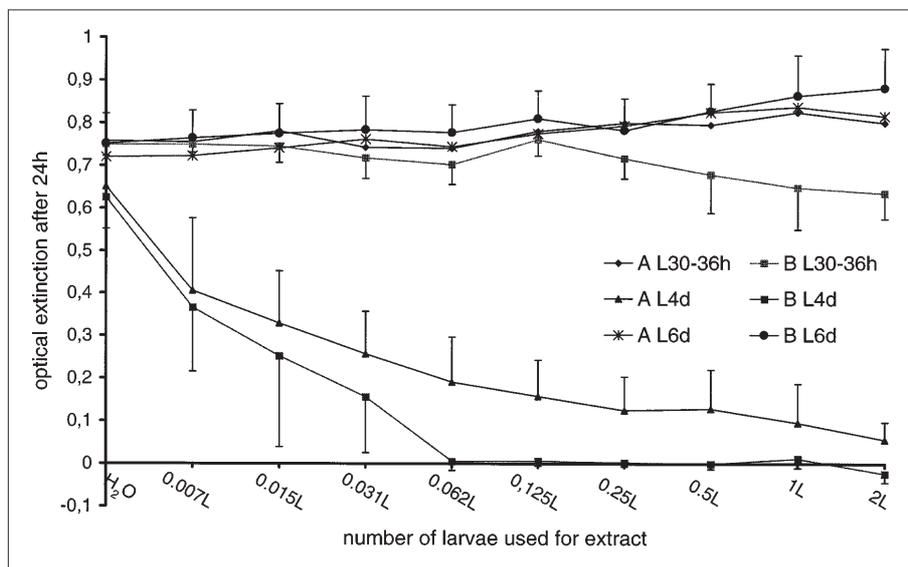An extract equivalent of one 4 day larva, that contained not more than 0.2 mg pollen

Figure 1. Growth-inhibiting effect (measured at E_{546} of bacterial suspension after 24 hours of aerobic incubation at 37 °C) of extracts of larvae (L) of different ages from two different colonies on the vegetative stage of *P. larvae larvae*. For optical reasons standard deviations are not given for the 30–36 h and 6 day larvae of colony A, SD does not exceed 13.1% of the mean (for statistical significance, see results).

at most (mean 0.069 mg, + 0.05) inhibited bacterial growth almost 100%. As the dilution of the larval extract increased (that is, the concentration of larval tissue decreased), the growth-inhibition decreased. In contrast, extracts from larvae 30–36 h old and extracts from 6 day old larvae did not greatly inhibit bacterial growth at any concentrations. There was no significant interaction between concentration and larval age for the youngest larvae and for the 6 day larvae, but when the 4 day larvae are also considered there was a significant age-concentration interaction. No significant differences between the two colonies were found (Fig. 1).

We also investigated pools of 50 larvae 30–36 hours old. The total amount of tissue in 50 of these very young larvae (combined weight: 62.9 mg +6.8 in colony A; 55.1 mg +9.5 in colony B) was similar to that in one 4 day larva (53.5 mg +2.7); similar weights were reported (Wang, 1965). In our

experimental set-up the highest extract concentration was equivalent to 12.5 30–36 h larvae, with a weight about equal to the weight of 0.25 4 day larvae. In both colonies, the extract concentration had a significant effect: the more diluted the larval extract, the greater the growth of the bacteria. The growth-inhibiting effect on *P. larvae larvae* was significantly stronger in colony A; this was the only significant difference we found between the two colonies (Fig. 2).

3.2. Effects of adult age (Fig. 3)

The extract of midguts of each adult age group (0 day old = freshly emerged bees; 8 day old bees; and foragers) inhibited the growth of *P. larvae larvae* rods, and in all age groups the higher the extract concentration the stronger the inhibition of growth. The freshly emerged bees showed significantly
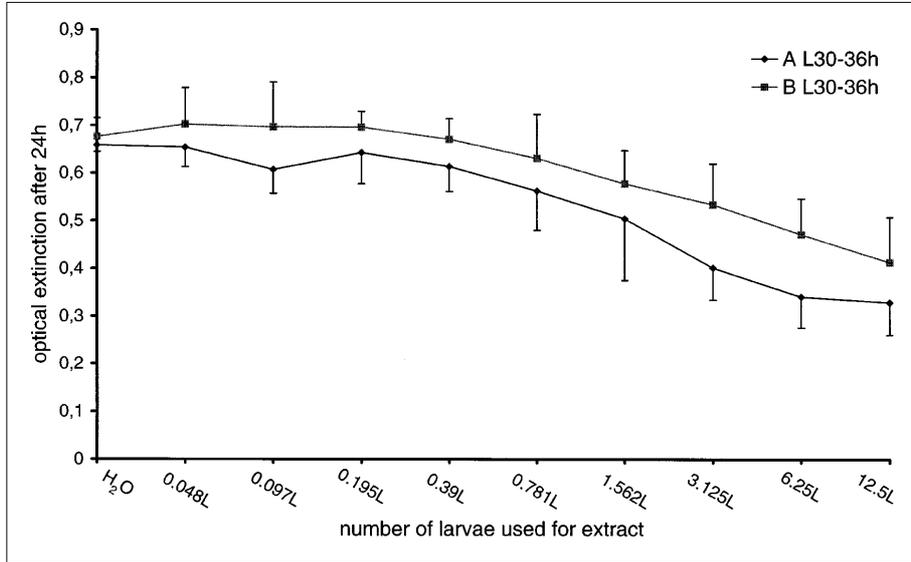
Figure 2. Growth-inhibiting effect (measured at E_{546} of bacterial suspension after 24 hours of aerobic incubation at 37 °C) of extracts of larvae (L) 30–36 hours old from two different colonies on the vegetative stage of *P. larvae larvae* (for statistical significance, see results).
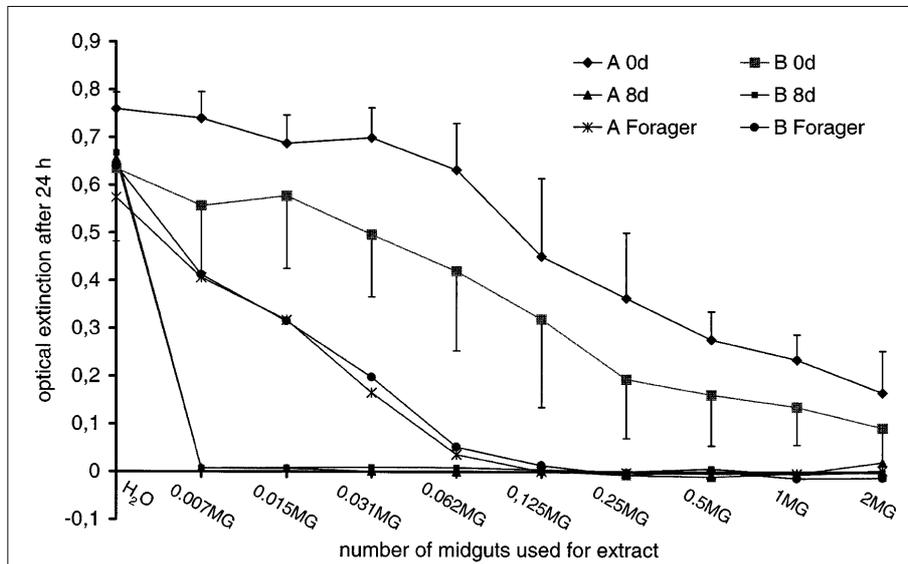

Figure 3. Growth-inhibiting effect (measured at E_{546} of bacterial suspension after 24 hours of aerobic incubation at 37 °C) of midgut extracts (MG) of adults of different ages from two different colonies on the vegetative stage of *P. larvae larvae*. For optical reasons standard deviations are only given for the freshly emerged bees (for statistical significance, see results).

less bacteria-inhibiting potential than the other two age groups. Midgut extracts from 8 day old bees totally inhibited bacterial growth for all concentrations, and those from foragers were equally effective except when diluted to 0.125 midguts and below. Overall, the difference between foragers and 8 day bees was significant, due to the difference at low concentrations. No significant differences were found between the colonies (Fig. 3).

3.3. Effects of worker jelly and royal jelly (Fig. 4)

For both worker jelly and royal jelly, as with the other substances tested, the different extract concentrations were significantly different in the extent of their inhibition of bacterial growth. Royal jelly (from colony B) had a significantly stronger growth- inhibiting effect than worker jelly from either colony; no differences were found between

the worker jellies from the two colonies (Fig. 4).

3.4. Effects of honey, pollen and beebread (Fig. 5)

Extracts of *Zea mays* pollen of all three types (collected directly from flowers; from pollen pellets collected from honeybee corbiculae; and from beebread) all inhibited bacterial growth, and for all three types the effect was significantly stronger at higher concentrations. Considering pollen pellets and bee bread, there was no significant interaction between concentration and pollen type, but when the untreated pollen was also considered there was a significant type-concentration interaction. The extract of untreated pollen (taken directly from flowers) showed a significantly poorer inhibiting effect on bacterial growth than the extracts of pollen pellets or beebread. The extract of honey did not show any growth-inhibiting
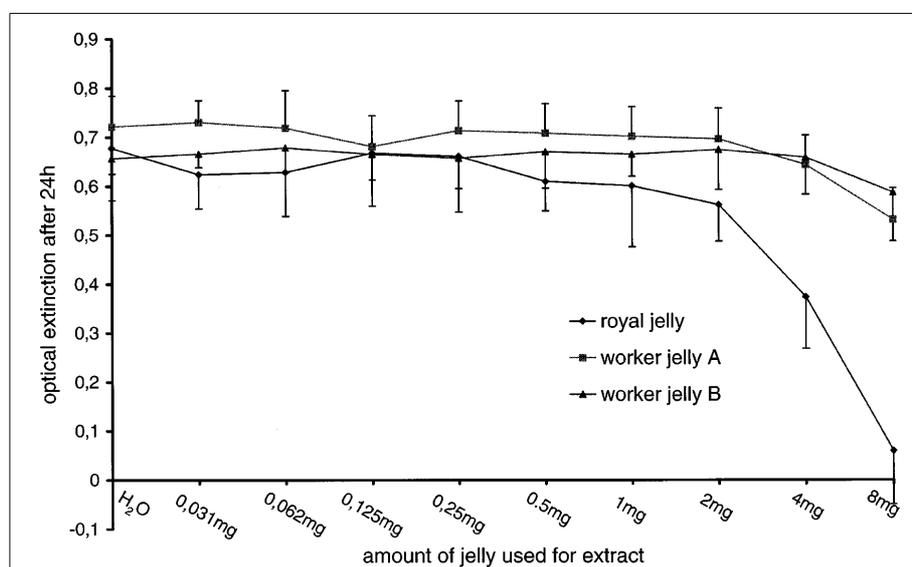

Figure 4. Growth-inhibiting effect (measured at E_{546} of bacterial suspension after 24 hours of aerobic incubation at 37 °C) of royal jelly and worker jelly from two different colonies on the vegetative stage of *P. larvae larvae* (for statistical significance, see results).
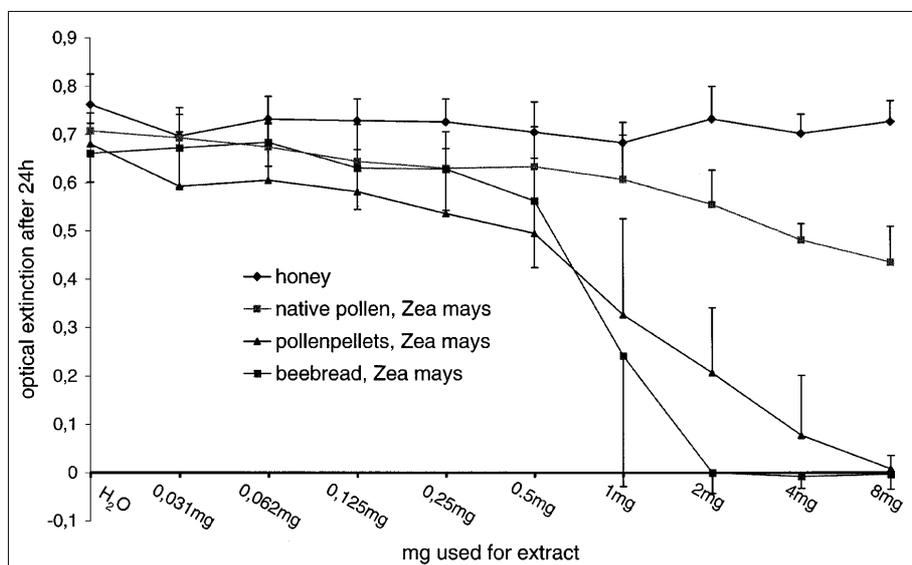
Figure 5. Growth-inhibiting effect (measured at E_{546} of bacterial suspension after 24 hours of aerobic incubation at 37 °C) of honey of investigated colonies after extraction and different qualities of pollen from *Zea mays* on the vegetative stage of *P. larvae larvae* (for statistical significance, see results).

effect, and the dilution effect was not significant (Fig. 5).

4. DISCUSSION

As we have stated with regard to nurse bees and winter bees (Riessberger-Gallé et al., unpublished data), we cannot say if the observed growth-inhibition of *P. larvae larvae* is caused by one or more substances. We are nevertheless using the term "substances."

The youngest and oldest larvae we tested did not show an inhibiting effect on the growth of *P. larvae larvae*. However the 4 day old larvae did demonstrate a highly significant inhibition of bacterial growth even with extracts up to the equivalent of two individuals (Fig. 1).

The lack of inhibiting potential in the youngest larvae might be due to their very low weights (on average a larva 36 h old

weighs 1.17 mg; a 4 day larva weighs 53.5 mg). If the youngest larvae had the same relative amount of inhibiting substance as those 4 day old, our methods would not be able to detect any growth-inhibiting effect. Therefore we used a more concentrated extract of the youngest larvae, equivalent to the weight of 0.25 4 day larvae. This extract did produce significant growth-inhibition (Fig. 2), but not as strong as produced by 4 day larvae (compare Figs. 1 and 2). This difference corresponds very well with the fact that the youngest larvae are highly susceptible to infection and those a few days older show a much reduced susceptibility or none at all (Bamrick and Rothenbuhler, 1961; Bamrick, 1967; Brødsgaard et al., 1998; Gregorc and Bowen, 1998).

The young larvae from colony A differed in weight from those in colony B, but the differences were not significant. This weight difference might have been caused by different rates of development (possibly caused

by better nursing or higher brood nest temperature in one colony, or perhaps by genetic differences). Another reason might be our sampling technique. We had caged the queen for 6 hours and then calculated the age of our larvae. An earlier onset of egg-laying of one of the two queens during the caging period might cause somewhat older larvae (closer to 36 h) in one colony and somewhat younger ones in the other case (closer to 30 h). Small age or weight differences might be the reason for the higher growth-inhibiting potential of the young larvae in colony A. On the other hand, it is possible that this observed difference between colonies (only demonstrated in one example so far, Fig. 2) might suggest that larvae from different colonies can differ in the time of onset of a resistance mechanism. An early onset and/or a higher rate of production of growth-inhibiting substances could cause greater resistance against an infection of *P. larvae lavae*. If so, our method of testing those substances in larvae would be a useful tool in measuring resistance in colonies used for breeding.

The oldest larvae that are already capped in their cells are no longer susceptible to infection (Bamrick and Rothenbuhler, 1961; Bamrick, 1967) but these larvae did not show inhibiting effects in our tests (Fig. 1). This might be because at this stage the gut (where infection by *P. larvae larvae* starts) begins to transform into the final adult gut (Snoodgrass, 1956). Also, larvae at this age (6 day) might not need this protection as the cell is already sealed and pathogens cannot enter.

In a recent paper (Riessberger-Gallé, unpublished data) we demonstrated the presence of bacterial growth-inhibiting substances in the midguts of nurse bees and winter bees, the two groups of adult bees that are maximally exposed to the bacterium in infected colonies. As at the end of the larval development (in 6 day larvae) this inhibiting effect was not found. What is the course of development of resistance against P. *larvae larvae* in the life of an adult bee?

Adult individuals pass through a sequence of behavioural phases during their life, and every stage of life is characterised by performance of a set of tasks (Rösch, 1925; Lindauer, 1952; Winston, 1987). Duties like cleaning cells, nursing or also cannibalising of brood, and performing foraging flights are correlated with different risks of contacting a pathogen like *P. larvae larvae* (Rembold,1965; Riessberger and Crailsheim, 1997). In the literature it is said that adult bees are totally resistant against infection by *P. larvae larvae* (Wilson, 1971). We investigated an extract of midguts of freshly emerged honey bees that had no possibility of being fed or of feeding themselves. A high growth-inhibiting effect was found but it was significantly weaker than the effect of midgut extracts from 8 day bees or foragers. Eight day bees, whose duties include nursing the brood, can have close contact with infected larvae. They cannibalise diseased or dead larvae and therefore ingest vegetative stages and spores of *P. larvae larvae*. We found a 100% growth-inhibiting effect in the midguts of this age class at all dilutions tested. This finding demonstrates the high adaptation of the individual to its task. The high ability to resist *P. larvae larvae* in bees of this age might be caused by a heightened production of the growth-inhibiting substances, perhaps intensified by ingesting large numbers of pollen grains, which cause a growth-inhibiting effect on *P. larvae larvae* themselves (see below and Fig. 5). In foragers we found a 100% growth-inhibiting effect at all dilutions corresponding to more than 0.125 midguts, a result significantly different from that for 8 day bees. This might be due to the lack of pollen in the guts of foragers or to a lower production rate of the inhibiting substances. Furthermore foragers do not have contact with this pathogen to the same extent as 8 day bees do.

After demonstrating the strength of resistance of different larval and adult stages against *P. larvae larvae*, we still had to test whether these inhibiting substances are bee-produced or are present in the bees' food.

The youngest and middle-aged larvae are mainly fed with worker jelly, and also consume some honey and some bee bread (Haydak, 1943). Our results show that worker jelly and bee bread do contain some growth-inhibiting substances. Bee-processed pollen had a considerably stronger effect at concentrations of about 1 mg in our experimental setup. We found only amounts up to 0.2 mg per 4 day larvae, but a much higher activity against *P. larvae larvae* in 4 day old larvae than could be attributable to that amount of consumed pollen. Nevertheless we cannot exclude an involvement of food consumed as larvae in the development of bacterial resistance, or perhaps consuming large amounts of food amplifies the effect. However, the fact that in the oldest larvae there is no growth-inhibiting effect argues against such an amplifying effect.

Royal jelly and worker jelly are known to have different compositions (Rembold, 1965). We found a striking difference between these two foods in their effectiveness against *P. larvae larvae*, consistent with the extremely high value of queen larvae in a honeybee colony (Sakogawa et al., 1999).

Freshly hand-harvested pollen inhibited growth of *P. larvae larvae*, but the effect was greatly increased when the bees processed the pollen. Perhaps when they form pollen pellets, bees add substances that inhibit growth of *P. larvae larvae* in regurgitated liquids from the honey sac. Another possibility is that the fermentation of pollen that takes place in the storage cells in a hive (Stanley and Linskens, 1985) progressively liberates substances that are already in the pollen. However, we investigated pollen from only one plant, *Zea mays*. Investigating more pollen species and collecting pollen at different stages of storage can clarify this question.

In general, our results strongly indicate that honeybee larvae produce substances that inhibit growth of the bacterium that causes American foulbrood. In fact, we can conclude that such substances are produced by the imagos. Although the oldest larvae do not show this resistance, it was present in freshly emerged adult bees that had not had any chance to ingest food. Also, bees fed only with artificial food for the first 8 days of adult life showed midgut-located resistance (Riessberger-Gallé, unpublished data). In addition, foragers that have only traces of pollen in their midguts (Crailsheim et al., 1992; Loidl and Crailsheim, unpublished data) and mainly feed on honey and nectar, show almost the same level of resistance as is found in nurse bees.

ACKNOWLEDGEMENTS

Résumé – Résistance des abeilles domestiques (*Apis mellifera*) à la loque américaine en fonction de leur âge. La loque américaine est une maladie du couvain largement répandue et très infectieuse, provoquée par *Paenibacillus larvae* subsp. *larvae*. Les spores représentent le stade infectieux. Si le couvain absorbe des spores se nourrissant, les spores germent dans l'intestin moyen de la larve et les bâtonnets, forme végétative très mobile, traversent la paroi de l'intestin et pénètrent dans la cavité abdominale. Là ils se multiplient rapidement et provoquent la mort de la larve. Les abeilles adultes ne sont que des vecteurs, elles-mêmes ne contractent pas la maladie. Pour nos expériences nous avons utilisé une souche sauvage de *P. l. larvae*. La résistance à la bactérie a été étudiée chez les larves aux divers stades de développement et chez les adultes à divers âges. Des tubes tests, remplis d'un milieu liquide (brain heart infusion, Oxoid) ont été inoculés avec une suspension de bactéries qui a été standardisée par dilution jusqu'à extinction optique de 0,20–0,22 mesurée à 546 nm. Des extraits de larves ou d'adultes ont été obtenus par mise

en suspension dans l'éthanol de larves ou d'intestins moyens d'abeilles adultes homogénéisés, puis par lyophilisation et dissolution à l'eau distillée. Ces extraits ont été ajoutés dans les tubes tests à différentes concentrations. De l'eau a été utilisée comme témoin. Après incubation, on a mesuré l'extinction optique pour quantifier l'état trouble du milieu liquide, utilisé comme mesure de la croissance bactérienne. Des substances inhibant la croissance de *P. l. larvae* ont pu être mises en évidence chez des larves de 4 jours et, dans une moindre mesure, chez celles d'un jour. De telles substances étaient absentes chez les larves de 6 jours, qui avaient atteint le stade de l'operculation. Des extraits d'intestins moyens d'abeilles adultes ont généralement montré une plus forte capacité que les extraits de larves à inhiber la croissance de la bactérie. Les intestins moyens de nourrices (adultes âgées de 8 jours) ont montré un potentiel inhibiteur plus élevé que ceux des adultes fraîchement écloses ou des butineuses.

Après avoir mis en évidence les diverses résistances des stades larvaires et adultes, on s'est posé la question de savoir si ces substances ne provenaient pas de la nourriture. On a testé le pouvoir inhibiteur de divers types de nourriture (pollens traités différemment, gelée d'ouvrière, gelée royale) ; ils inhibaient eux aussi la croissance bactérienne, bien qu'à un moindre degré que les extraits de larves ou d'adultes ; mais le miel dilué n'avait, lui, aucune action. Comme l'on pouvait s'y attendre, la gelée royale a montré une action inhibitrice forte, ce qui souligne l'importance des larves royales pour la colonie d'abeilles.

Apis mellifera / Paenibacillus larvae larvae / **loque américaine / résistance en fonction de l'âge**

Zusammenfassung – Alters-abhängige Resistenz der Honigbiene gegen die amerikanische Faulbrut. Die Amerikanische Faulbrut ist eine weltweit verbreitete, hochinfektiöse Erkrankung der Bienenbrut. Sie wird durch das sporenbildende Bakterium *Paenibacillus larvae larvae* hervorgerufen. Sporen repräsentieren das infektiöse Stadium; werden sie an Larven verfüttert so keimen sie im larvalen Darm zu Stäbchen aus. Diese vegetative, begeiβelte Form ist aktiv beweglich, durchdringt die Darmwand und gelangt so in die Leibeshöhle. Hier kommt es schlieβlich zu einer rapiden Vermehrung des Erregers was zum Tod der Larve führt. Im zersetzten larvalen Gewebe kommt es erneut zur Sporulation von *P. larvae larvae*. Adulte Bienen dienen dem Erreger als Vektor, sie selbst erkranken an der Amerikanischen Faulbrut nicht.

Die Untersuchungen wurden mit einem Wildstamm von *P. larvae larvae* durchgeführt. Die Resistenz unterschiedlicher Larvenstadien und adulter Bienen unterschiedlichen Alters gegen den Erreger der Amerikanischen Faulbrut wurde getestet. Teströhrchen, gefüllt mit einem flüssigen Medium (Brain Heart Infusion, Oxoid), wurden mit Bakteriensuspension beimpft, welche durch Verdünnung auf eine optische Extinktion von 0,20–0,22 bei 546 nm standardisiert wurde. Anschlieβend wurden Extrakte aus Larven oder adulten Bienen (homogenisierte Larven oder Mitteldärme adulter Bienen wurden in Ethanol suspendiert, lyophilisiert und in H_2O gelöst) zugegeben. H_2O wurde als Kontrolle verwendet. Nach der Inkubation wurde die optische Extinktion, die Trübung des flüssigen Mediums als Maβ für das Bakterienwachstum bestimmt.

Eine oder mehrere Substanzen die das Wachstum von *P. larvae larvae* hemmen, konnten in 4 Tage alten Larven und, in geringerem Ausmaβ auch in 30–36 Stunden alten Larven nachgewiesen werden. Bei den bereits verdeckelten 6 Tage alten Larven konnte kein hemmender Effekt gemessen werden. Der Extrakt aus Mitteldärmen adulter Bienen zeigte allgemein eine stärkere hemmende Wirkung auf das Wachstum von *P. larvae larvae* als der Extrakt aus Larven. Der Extrakt aus Mitteldärmen von Ammenbienen

verursachte eine signifikant stärkere wachstums-hemmende Wirkung auf *P. larvae larvae* als der Extrakt aus Mitteldärmen von frischgeschlüpften Bienen oder Sammlerinnen.

Nachdem wir unterschiedliche Resistenzen in Larven und adulten Bienen nachweisen konnten, stellte sich die Frage, ob diese Substanzen mit der Nahrung aufgenommen werden. Unterschiedliche Arten von Futter (unterschiedlich behandelter Pollen, Futtersaft für Arbeiterinnen und Gelee Royal) zeigten einen hemmenden Effekt auf das Wachstum von *P. larvae larvae*, dieser war jedoch wesentlich geringer als der von Larven oder Mitteldärmen adulter Bienen. In Honig konnte kein hemmender Effekt nachgewiesen werden. Wie angenommen werden konnte, zeigte Gelee Royal einen starken hemmenden Effekt, was die Wichtigkeit der Königinnenlarven für das Bienenvolk unterstreicht.

Apis mellifera / Paenibacillus larvae larvae **/ Alter-abhängige Resistenz / Larve**

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