Damir Šekulja [1]
Hermann Pechhacker [2]
Elisabeth Licek [3]

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# DRIFTING BEHAVIOR OF HONEY BEES (*APIS MELLIFERA CARNICA* POLLMAN, 1879) IN THE EPIDEMIOLOGY OF AMERICAN FOULBROOD[4]

## ABSTRACT

*The drifting of honey bees often occurs in apiaries where numbers of hives are kept close together and it is an important vector in the spreading of honey bee diseases. It occurs in high numbers, frequently and over long distances. Within this work, it was investigated whether drifting might play a role in the epidemiology of American foulbrood. To determine the presence and the number of P. larvae spores in samples of honey, pollen and honey sacs, three different methods were used: modified Ritter-Kiefer (1993) method, Columbia blood agar test (Plagemann, 1985) and catalase tests (Haynes, 1972). Although the level of drifting within the colonies with clinical symptoms of AFB was slightly higher than the one within the colonies without clinical symptoms, AFB infection did not significantly influence the drifting frequency. Even when drifting was compared between two groups of young bees at the age of orientation flights, AFB infection did not significantly influence the level of drifting. However, as the level of P. larvae spores detected both in the pollen and honey stored in the hives, as well as in the pollen found on the legs of the bees from infected colonies, was significantly higher compared with the one in colonies with no clinical symptoms of AFB, it can be concluded that drifting plays a role in the spreading of AFB, especially during the orientation flights of young bees.*

***Key words:*** *drifting, American foulbrood, epidemiology*

## 1. INTRODUCTION

Honey bee colonies affected by American foulbrood (AFB), a disease caused by the spore forming bacterium *Paenibacillus larvae* (Genersch et al., 2006, former *Bacillus larvae* (White, 1906)), normally die, if not treated, and therefore the disease can cause significant losses for beekeepers (Hansen,1984a).

As it is highly contagious, it can easily spread from the infected colony to other colonies of the same or even neighboring apiaries.

[1] PhD, College Professor, Polytechnic of Rijeka, Trpimirova 2, Rijeka, Croatia. E-mail: damir.sekulja@veleri.hr
[2] PhD, Dr., Austrian Carnica Association, Kleine Seeaustrasse 10, 3293 Lunz am See, Austria. E-mail: hc.pechhacker@aca.at
[3] PhD, Dr., profesor, Veterinärmedizinische Universität Wien, Veterinärmedizinische Universität Wien, Klinik für Bienen,Geflügel, Ziervögel, Reptilien und Fische, Veterinärplatz 1, 1210 Wien, Austria. E-mail: elisabeth.licek@vu-wien.ac.at
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In this study, the epidemiology of AFB is investigated from the aspect of the drifting behavior of honey bees. Drifting is defined as the movement of bees between colonies (Butler, 1939), when honey bees leave one colony and join another.

It can result in increased disease spread, and though the effects of drifting are known (Frasnaye, 1963; Free, 1958 a; b; c; Garifullina, 1960;1962;1963;1965; Jay,1965; 1966 a; b;1968;1969 a; b; c;1971; Lindström, 2006), beekeepers often do not take any steps to prevent it (Matheson, 1991).

Bees entering another hive after drifting are accepted without antagonism, regardless of their age. The greatest amount of drifting occurs when large numbers of bees are flying and the least during the spring build-up and at the end of the main flow (Poltev, 1968; Hooper, 2008). It occurs regardless of the position of the hive or location of the apiary (Sulimanović, 1985).

Heavy drifting could reduce colony populations and may also lead to robbing between colonies and, in extreme cases, to the destruction of weaker colonies (Frasnaye, 1963). It is one of the ways that bee diseases are spread over considerable distances, from hive to hive, or even from apiary to apiary.

The purpose of this work was to find out whether the AFB could be spread by drifting, and whether bees from the infected colonies drift more than those from the healthy ones. The aim was also to see if the drifting of young bees at the age of orientation flights is more intensive than that of adult bees.

It was also investigated whether the drifting bees have *P. larvae* spores in their honey sacs and in pollen on their legs, and the result was compared with the level of infection of the honey and pollen in their hives.

## 2. MATERIALS AND METHODS

All experiments concerning the spreading of AFB between the colonies were done in isolated areas with no risk of spreading the disease to neighboring apiaries. The material contaminated with *P. larvae* spores was obtained from heavily infected colonies. The methods used for determining the level of drifting between the group of colonies with and the one without clinical symptoms of AFB were modified according to methods described by Šekulja (1989,1991), and according to the previous experience of Pechhacker (1993). To determine the drifting behavior between the colonies with and without symptoms of AFB, ten colonies placed in LR hives (with approximately equal number of bees) were split into two groups. Five of those colonies were heavily infected with AFB, while the other five colonies had no clinical symptoms of the disease. The samples of the bees from each group were differently marked, to allow distinguishing the bees from different groups.

Experiments were organized in the spring, when the activity of the bees was high, due to the rich sources of nectar and pollen in the nature. Both groups of colonies were kept untreated and

the examinations were made in the same way and at the same time of the day for both groups of colonies. In this way, the results were comparable.

The drifting was monitored on the adult foraging bees, and on the young bees at the age of the orientation flights.

After the drifting experiments were done, samples of honey and pollen from the combs, and samples of the honey bees were taken from each of the colonies used in the experiments. From each colony three samples of about fifty bees were taken and euthanized in a different way. The first group of sampled bees was euthanized mechanically, the second by hypothermia at minus 18$^{O}$ C, and the third was treated with liquid nitrogen at minus 196$^{O}$ C. It was done in order to find out whether the way of killing the bees had any influence on the vitality of *P. larvae* spores, i.e. on their ability to form characteristic colonies on MYP - agar.

### 2.1 Layout of honey bee colonies with and without clinical symptoms of AFB

Ten nuclei were formed in LR hives, consisting of only one body. Five of them were formed (with brood combs and bees) from the heavily infected colonies, with clinical symptoms of AFB, while other five were formed from the colonies with no clinical symptoms of the disease. Newly introduced queens for all nuclei were of the same age and from the same strain of *A. mellifera carnica* P. Colonies were moved to the new location and placed in two rows of five hives each, at the same apiary. Before the beginning of the experiments, the colonies were left at the new site for about one week.

### 2.2 Marking the honey bees

Samples of honey bees were marked by fluorescent tempera colors, which have been proven to be convenient for such experiments (Sulimanović, 1985; Šekulja, 1989; 1991). Colors are quickly placed either on the thorax or on the abdomen of honey bees, which makes them easily distinguishable.

Some bees at the age of orientation flights were marked genetically. The so-called 'Cordovan mutants' - with brown instead of grey hair, were just added to the colonies. A total of 2,750 marked honey bees were used in the experiments.

### 2.3 Drifting experiments in honey bee colonies with and without clinical symptoms of AFB

In order to see whether there is a difference between the level of drifting in the colonies with and those without clinical symptoms of AFB, two parallel experiments were organized.

In the first one, from each colony (A, B, C, D and E) of the honey bees with signs of AFB, a sample of 200 honey bees was marked on the thorax. Each sample of bees was marked

in different colors, in order to make it possible to follow the drifting among the colonies within the same row.

At the same time, in each of the colonies (a, b, c, d and e), from the group of bees without clinical symptoms of AFB, a sample of 200 honey bees was also marked, but on the abdomen, according to their position in the row, using the same colors as in the first group. In this way, it was not only possible to observe the drifting among the colonies within the same row, but also between the two groups of colonies, i.e. it was possible to see whether the bees from the healthy colonies tend to drift to the ones with the clinical symptoms and vice versa.

For each group of colonies, 6 observations were done, during a period of three days. Colonies were first examined early in the afternoon, and then again in the evening, when most or sometimes all foraging bees were already back.

### 2.4 Drifting experiments with honey bees during their orientation flights

Drifting experiments with honey bees at the age of orientation flights were organized with the same layout of colonies, but a few days later. As many of the previously marked bees were still alive, it was necessary to mark the young bees in a different way. The most convenient way of marking honey bees is by recessive genus, i.e. by the genetically different color.

A few brood combs from the colonies of the so-called 'Cordovan mutants' were taken from the Institute in Lunz am See, to the Faculty of Veterinary Medicine in Zagreb, and placed in the incubator. A few days later, a considerable number of young honey bees hatched, but only 250 of them had a recessive gene for brown color. Other young bees were marked on the abdomen with a white color as it had not been used in the previous experiments. They had been marked on the abdomen before being placed in the middle hives in each group of colonies. The driftings were observed in all other colonies in both rows.

In the row of the colonies with clinical symptoms of AFB, 500 young bees were marked on the thorax and introduced into the central colony in the row. As soon as the bees had hatched, the new colony accepted them without any problems.

The same thing was simultaneously repeated in the row of the colonies without clinical symptoms of the disease, the only difference being that 250 young bees were 'Cordovan' mutants, and the other half of young bees were marked on the abdomen. In this way, it was again possible not only to observe the drifting among the colonies of the same group, but also between the two rows.

After the young honey bees had been introduced to the colonies, they were left, until they reached the age of the orientation flights (age of 8 to 12 days). It was not until then that the observations started.

Six observations were made during the period of three days (in the middle of May). The weather conditions were favorable and there was enough pollen and nectar in the nature, so no extra feeding was required.

### 2.5 Determining the presence and quantity of *P. larvae* spores in bee products of the colonies used in the experiments

After the experiments between the honeybee colonies had been performed, samples of the honey and pollen from the combs and living honey bees were collected.

Three samples of honey were taken from each colony, together with a sample of pollen and three samples of honey bees. The samples were separately packed, marked and placed in a refrigerated container until the time of the examinations.

Honey bees were taken both from the entrance of the hive and from the combs. From each colony, 3 samples of foraging bees, and one sample of the bees at the age of orientation flights were isolated. Honey bees were killed in various ways: by liquid nitrogen, mechanically (by squeezing the thorax), and simply by putting them at - 18° C. The last method has proven to be the best one.

The infected material was examined partly at the Tierhygienische Institut in Freiburg, and mainly at the Institut für Bienenkunde in Lunz am See.

Honey sacs of the bees and samples of honey were examined using the Ritter and Kiefer method (1993), while pollen was examined using the Columbia blood agar test (Plagemann, 1985).

## 3. RESULTS AND DISCUSSION

The level of drifting in colonies without clinical symptoms of AFB (Table 1) was 0.68% on average (in colony a - 0.44%, in colony b - 0.5%, in colony c - 1.63%, in colony d 0.71%, and in colony e - 0.15% ), while in colonies with clinical symptoms of AFB (Table 2) it was 0.85% on average (in colony A - 0.25%, in colony B - 0.65%, in colony C - 2.21%, in colony D - 0.94%, and in colony E - 0.21%). Drifting between the two groups of colonies was not observed.

The results represent the average of 6 observations during 3 days.

Table 1. Drifting of honey bees - colonies without clinical symptoms of AFB

| Date | Time | Colony | | | | |
|---|---|---|---|---|---|---|
| | | a-green | b-yellow | c-rose | d-orange | e-red |
| 4 May | 13:00-15:30 | 2 yellow | 1 rose | 3 orange<br>1 red | 2 rose | 1 orange |
| | 18:30-20:00 | 5 yellow | 5 rose | 9 orange<br>5 yellow | 2 rose | _ |
| 5 May | 13:00-15:30 | 4 yellow | 2 rose<br>1 orange | 5 orange<br>4 green<br>1 red | 4 rose<br>1 red | 2 orange |
| | 18:30-20:00 | 3 yellow | 3 rose<br>1 orange | 8 orange<br>4 green<br>2 red | 5 rose<br>1 red | 2 orange |
| 6 May | 13:00-15:30 | 4 yellow | 5 rose<br>1orange | 10 yellow<br>9 orange | 4 rose<br>3 yellow<br>2 red | 1 orange |
| | 18:30-20:00 | 3 yellow | 3 rose,<br>2 orange | 7 yellow<br>6 orange<br>4 red | 4 yellow<br>3 red<br>3 rose | 2 orange |
| Average number of drifted bees | | 3,5 | 4 | 13 | 5.67 | 1.17 |
| Percentage of drifting | From the same group | 0.44% | 0.50% | 1.63% | 0.71% | 0.15% |
| | Average | 0.68% | | | | |
| | From the group with clinical symptoms | - | - | - | - | - |
| | Average | - | | | | |

Source: authors

Table 2. Drifting of honey bees - colonies with clinical symptoms of AFB

| Date | Time | Colony | | | | |
|---|---|---|---|---|---|---|
| | | A-green | B-yellow | C-rose | D-orange | E-red |
| 4 May | 13:00-15:30 | 2 rose<br>2 yellow | 5 rose<br>1 orange | 8 yellow<br>4 orange<br>3 red | 4 rose<br>1 yellow<br>1 red | 2 orange |
| | 18:30-20:00 | 2 yellow | 5 rose | 12 yellow<br>8 orange<br>2 red | 4 yellow<br>3 rose | 3 orange |
| 5 May | 13:00-15:30 | 1 yellow | 4 rose<br>1 green | 8 yellow<br>5 orange<br>2 green | 4 rose<br>2 yellow<br>2 green | 2 orange |
| | 18:30-20:00 | 2 yellow | 5 rose<br>1 orange | 10 yellow<br>6 orange<br>2 green | 4 rose<br>3 yellow<br>2 green | 2 orange |
| 6 May | 13:00-15:30 | 2 yellow | 4 rose<br>1 green | 12 yellow<br>7 orange | 4 rose<br>3 yellow<br>1 green | 1 orange |
| | 18:30-20:00 | 1 yellow | 3 rose,<br>1 green | 9 yellow<br>6 orange<br>2 green | 5 rose<br>1 red<br>1 yellow | _ |
| Number of drifted bees | | 2 | 5.17 | 17.67 | 7.5 | 1.67 |
| Percentage of drifting | From the same group | 0.25% | 0.65% | 2.21% | 0.94% | 0.21% |
| | Average | 0.85% | | | | |
| | From the group without clinical symptoms | - | - | - | - | - |
| | Average | - | | | | |

Source: authors

Drifting during orientation flights in colonies without clinical symptoms of AFB was 0.71% on average (Table 3). As all marked young bees were placed in colony c, drifting was registered in 4 neighboring colonies (in colony a - 0.33%, in colony b - 1.46%, in colony d - 0.77%, and in colony e - 0.29%).

Table 3. Drifting of honey bees during orientation flights - colonies without clinical symptoms of AFB

| Date | Time | Colony | | | | |
|---|---|---|---|---|---|---|
| | | a | b | c | d | e |
| 14 May | 10:00-12:00 | 1 mutant<br>1 white abdomen | 6 mutants<br>2 white abd. | – | 4 mutants<br>1 white abd. | – |
| | 18:00-20:00 | 1 mutant<br>2 white abd. | 5 mutants<br>3 white abd. | – | 4 mutants | 1 white abd. |
| 15 May | 10:00-12:00 | 1 mutant<br>1 white abd. | 7 mutants<br>2 white abd. | – | 3 mutants<br>2 white abd. | 1 white abd. |
| | 18:00-20:00 | 1 mutant | 8 mutants<br>3 white abd. | – | 4 mutants<br>1 white abd. | 1mutant<br>1 white abd. |
| 16 May | 10:00-12:00 | 3 mutants<br>2 white abd. | 12 mutants<br>5 white abd. | – | 7 mutants<br>2 white abd. | 3 mutants<br>1 white abd. |
| | 18:00-20:00 | 1 mutant<br>2 white abd. | 10 mutants<br>7 white abd. | – | 6 mutants<br>3 white abd. | 2 mutants<br>4 white abd. |
| Average number of drifted bees | | 2.67 | 11.67 | - | 6.17 | 2.33 |
| Percentage of drifting | From the same group | 0.33% | 1.46% | - | 0.77% | 0.29% |
| | Average | 0.71% | | | | |
| | From the group with clinical symptoms | - | - | - | - | - |
| | Average | - | | | | |

Source: authors

Drifting during orientation flights in colonies with clinical symptoms of AFB was 1.52% on average (Table 4). Again, as all marked young bees were placed in colony C, drifting was registered in 4 neighboring colonies (in colony A - 0.46%, in colony B - 3.77%, in colony D - 1.38%, and in colony E - 0.48%).

Drifting between the two groups of colonies was registered only in colony B - 0.13%, and in colony C - 0.04%, 0.03% on average (drifting from colonies with clinical symptoms to other group was not observed).

The results represent the average of 6 observations during 3 days.

Table 4. Drifting of honey bees during orientation flights - colonies with clinical symptoms of AFB

| Date | Time | Colony | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| 14 May | 10:00-12:00 | 6 white thorax | 35 white th.<br>2 white abd.<br>1 mutant | _ | 7 white th. | 4 white th. |
| | 18:00-20:00 | 3 white th. | 37 white th.<br>2 white abd.<br>1 mutant | _ | 6 white th. | 2 white th. |
| 15 May | 10:00-12:00 | 2 white th. | 32 white th. | _ | 8 white th. | 3 white th. |
| | 18:00-20:00 | 2 white th. | 28 white th. | _ | 5 white th. | 1 white th. |
| 16 May | 10:00-12:00 | 4 white th. | 25 white th. | 1 white abd. | 18 white th. | 7 white th. |
| | 18:00-20:00 | 5 white th. | 24 white th. | 1 white abd. | 22 white th. | 6 white th. |
| Average number of drifted bees | | 3.67 | 31.17 | - | 11 | 3,83 |
| Percentage of drifting | From the same group | 0.46% | 3.77% | - | 1.38% | 0.48% |
| | Average | 1,52 | | | | |
| | From the group with clinical symptoms | 0.00% | 0.13% | 0.04% | 0.00% | 0.00% |
| | Average | 0.03% | | | | |

Source: authors

Concentration of *P. larvae* spores in samples of honey, pollen and honey bees from the colonies used in drifting experiments was examined according to the modified (Šekulja,1996) method described by Ritter and Kiefer (1993), according to which each grown *P. larvae* colony represents 119 +/- 15 spores (Table 5).

Table 5. Concentration of *P. larvae* spores in the samples of honey from the combs and from the honey sacs from the colonies used in drifting experiments

| Colony<br>Honey from the combs<br>1st experiment | | Concentration of P. larvae colonies | | |
|---|---|---|---|---|
| | | Honey (or nectar) from the honey sacs | | |
| | | 2nd experiment | Orientation flights (2nd experiment) | |
| Without clinical symptoms of AFB | A | 18.75 | 11.20 | -- |
| | B | 22.60 | 15.24 | -- |
| | C | 22.80 | 14.18 | 23.85 |
| | D | 23.66 | 17.32 | -- |
| | E | 27.00 | 15.02 | -- |
| | Average | 22.96 | 14.59 | 23.85 |
| With clinical symptoms of AFB | A | 75.00 | 29.43 | -- |
| | B | 53.14 | 45.86 | -- |
| | C | 65.55 | 31.12 | 64.60 |
| | D | 76.13 | 33.85 | -- |
| | E | 101.77 | 36.93 | |
| | Average | 74.32 | 35.44 | 64.60 |

Source: authors

From the honey of the colonies with clinical symptoms, 74.32 colonies had grown on the MYP agar, from the honey sacs of the mechanically killed bees, 34.82 *P. larvae* colonies and from the honey sacs of the bees killed by deep freezing at minus 18°C, 36.06 colonies. From the honey sacs of the bees at the age of orientation flights (killed by deep freezing at minus 18°C), 64.60 *P. larvae* colonies had grown on the MYP agar.

Pollen from the legs of the honey bees and from the combs has also proved to be positive. Columbia blood agar (Plagemann, 1985) and catalase tests (Haynes, 1972) were positive in all cases, except for samples treated by liquid nitrogen (Table 6).

Table 6. Concentration of *P. larvae* spores detected in the samples of combs (pollen/honey), and honey sacs from the colonies used in drifting experiments with different methods

| Colony | Samples | | Concentration of P. larvae spores | | |
|---|---|---|---|---|---|
| | | Method | | | |
| | | Modified Hansen/ Ritter | Columbia – Blut – Schrägagar | Catalase test | |
| | Honey from the combs | | 22.96 | + | + |
| Without clinical symptoms of AFB | Honey from the honey sacs | Mechanically killed bees | 14.49 | + | + |
| | | Bees killed at - 18°C | 14.69 | + | + |
| | | Bees killed by liquid nitrogen | negative | negative | negative |
| | Pollen from the combs | | n/a | + | + |
| | Pollen from the bee legs | | n/a | + | + |
| | Honey sacs (orientation flights) | | 23.85 | + | + |
| With clinical symptoms of AFB | Honey from the combs | | 74.32 | + | + |
| | Honey from the honey sacs | Mechanically killed bees | 34.82 | + | + |
| | | Bees killed at - 18°C | 36.06 | + | + |
| | | Bees killed by liquid nitrogen | negative | negative | negative |
| | Pollen from the combs | | n/a | + | + |
| | Pollen from the bee legs | | n/a | + | + |
| | Honey sacs (orientation flights) | | 64.6 | + | + |

Source: authors

*P. larvae* infection could be spread from one bee hive to another, or from one apiary to another by beekeepers (during manipulation with hives or frames), by various bee parasites (*V. destructor* or other mites) or by occasional visitors to the hives (wasps or other insects). However, it is mainly spread by the bees themselves. Honey bees can get in touch with *P. larvae* spores in the nature visiting the same places as the bees from the infected colonies (water, same sources of pollen and nectar, etc.). During periods of the year when there is not enough food in the nature, honey bees usually engage in robbing behavior, which is one of the most important ways of spreading the disease between colonies.

Another behavior pattern of honey bees which could significantly contribute to the spreading of *P. larvae* infection between colonies is drifting behavior. The drifting of honey bees is known

to be an important way of spreading many bee diseases. It occurs when honey bees leave one colony and join another.

Bees entering another hive after drifting are accepted without antagonism whatever their age. Although the greatest amount of drifting occurs when a large number of bees are flying (Poltev,1968), it is present always, regardless of the position of the hive or location of apiary (Cook, 1962; Free, Spencer-Booth, 1961; Jay, 1980; Jay, Dixon, 1988; Jay, Harris, 1979; Jay, Warr, 1984 ; Nekrasov, 1949; Sulimanović, 1985). All three members of the bee colony drift (Currie, Jay, 1988; 1989; 1991; Goetze, 1954, Hüttinger, Pechhacker 1988; Levenets, 1951; Šekulja, 1991), but under practical conditions of beekeeping, only the drifting of worker bees is significant (Withrell, 1965).

Drifting could also lead to robbing between colonies and, in extreme cases, to the destruction of the weaker colonies (Frasnaye, 1963).

Drifting behavior should be distinguished from robbing. Drifting occurs by mistake, and as the bees are convinced that they are entering their own hive, they are accepted by the host colony and many of them remain in it forever.

Robbing behavior occurs when bees enter a foreign hive in order to rob it. They usually kill the bees of the host colony and take the food to their own hive.

When honey bees drift into a foreign hive, they may have *P. larvae* spores on their bodies, in the pollen on their legs or in their honey sacs. They usually have no direct contact with the brood, but through social contact in the colony, *P. larvae* spores could find their way and infect the unsealed brood.

During the experimental part of this work, drifting occurred in both groups of colonies used in the experiments. The level of drifting within the colonies without clinical symptoms of AFB was lower than that within the colonies with clinical symptoms of AFB. However, the obtained difference was not considerable.

The obtained difference was slightly higher when the level of drifting was compared between two groups of young bees at the age of orientation flights. The results have also shown that the drifting of young bees was more intensive than the drifting of adult bees.

*P. larvae* spores were found both in honey sacs and in pollen on the legs of honey bees used in the experiments. The pollen taken from the combs was also positive. Although spores were detected in the honey sacs of both groups of colonies, a much lower concentration of *P. larvae* spores was detected among the bees from the colonies without clinical symptoms of AFB. In the honey from the combs a considerably higher concentration of *P. larvae* spores was detected in the honey from the colonies with clinical symptoms of AFB.

Liquid nitrogen probably kills all *P. larvae* spores given the fact that not a single sample treated by liquid nitrogen was positive.

It has also been observed that there was a significant difference between the concentration of *P. larvae* spores in the honey from the combs and the one in the honey sacs of the adult bees from the same hives, but at the age of orientation flights, the difference was not significant any more.

## 4. CONCLUSION

Based on various data about the level of drifting of worker bees, it could be concluded that high proportions of worker bees drift, drift frequently and drift over long distances.

It could not be concluded from the obtained results that the level of AFB infection has a significant influence on the drifting behavior of honey bees. Even in this case of young bees during their orientation flights, it could hardly be concluded that the level of infection has significantly influenced the level of drifting.

However, as the concentration of *P. larvae* spores found in pollen on the legs of infected honey bees, as well as in honey and pollen taken from the combs from the infected colonies, was significantly higher compared with the one in colonies with no clinical symptoms of the disease, it could be concluded that the drifting behavior of honey bees is one of the ways of spreading of *P. larvae* infection.

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Damir Šekulja [1]
Hermann Pechhacker [2]
Elisabeth Licek [3]

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# ZALIJETANJE PČELA (*APIS MELLIFERA CARNICA* POLLMAN, 1879) U EPIDEMIOLOGIJI AMERIČKE GNJILOĆE PČELINJEG LEGLA[4]

**SAŽETAK**

*Zalijetanje pčela predstavlja važan put u prijenosu pčelinjih bolesti i često se događa na pčelinjacima, gdje je veći broj košnica na okupu. Zahvaća velik broj pčela, javlja se učestalo i na većim udaljenostima. U ovom je radu istraženo može li zalijetanje imati ulogu i u epidemiologiji američke gnjiloće pčelinjeg legla. Za utvrđivanje prisutnosti i broja* P. larvae *spora u uzorcima meda, peluda i u mednim mjehurima, korištene su tri različite metode: modificirana Ritter-Kiefer metoda (1993), Columbia krvni agar test (Plagemann,1985) i katalaza test (Haynes,1972).*

*Iako je razina zalijetanja unutar pčelinjih zajednica s kliničkim znacima američke gnjiloće bila neznatno viša od one unutar zajednica bez kliničkih simptoma, zaraženost američkom gnjilоćom nije značajno utjecala na učestalost zalijetanja. Čak i kada se zalijetanje usporedilo između dviju grupa mladih pčela u dobi orijentacijskih letova, zaraženost američkom gnjilоćom nije značajno utjecala na razinu zalijetanja. No kako je razina spora* P. larvae *utvrđena u peludu i medu iz košnica, kao i na peludu nađenom na nogama mladih pčela iz zaraženih pčelinjih zajednica bila značajno veća u usporedbi s onom u zajednicama bez kliničkih znakova američke gnjiloće, može se zaključiti da zalijetanje pčela ima ulogu u širenju američke gnjiloće, naročito u vrijeme orijentacijskih letova mladih pčela.*

***Ključne riječi:*** *zalijetanje, američka gnjiloća, epidemiologija*

[1] Dr. sc., profesor v. š., Veleučilište u Rijeci, Trpimirova 2, Rijeka, Hrvatska. *E-mail:* damir.sekulja@veleri.hr
[2] Dr. sc., Austrian Carnica Association, Kleine Seeaustrasse 10, 3293 Lunz am See, Austria. *E-mail:* hc.pechhacker@aca.at
[3] Dr. sc., profesor, Veterinärmedizinische Universität Wien, Veterinärmedizinische Universität Wien, Klinik für Bienen,Geflügel, Ziervögel, Reptilien und Fische, Veterinärplatz 1, 1210 Wien, Austria. *E-mail:* elisabeth.licek@vu-wien.ac.at
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