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THE INFLUENCE OF *TROPILAELAPS CLAREAE* INFESTATION ON THE DEVELOPMENT OF HONEY BEE QUEENS

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Summary

We carried out our I[st] experiment on 288 *A. mellifera* queen cells and 756 *T. clareae* mites. Into each queen cell, 1, 2, 3, 4 or 5 *T. clareae* mites were introduced. Queen cells free of the parasite served as control groups: not opened queen cells and opened and then closed with bee wax. The queen cells from which the queens did not emerge were opened. In the IInd experiment we reared 968 queen cells in colonies (cell builders) without any open brood. Emerged queens and queen cells were clinically examined. From 288 control and experimental queen cells 219 queens emerged, out of which 206 were properly developed (71.5%). We found only 9 alive *T. clareae* in all experimental queen cells which were clinically examined. In IInd experiment 794 queens emerged and dead larvae or pupae were found in 174 queen cells. We found 27 alive *T. clareae* in all cells with dead queens and 31 alive mites on all emerged queens. Infestation by *T. clareae* decreases the number of queens emerging from queen cells. Presence of 5-6 *T. clareae* in the queen cells causes the death of 25.0-30.6% of queens. Body weight of emerged queens seems not to depend on the presence and number of parasites in queen cells.

Keywords: *Tropilaelaps clareae* infestation, queen rearing, queen cell, development of *T. clareae* in queen cell.

INTRODUCTION

Tropilaelaps clareae is characterised by high dynamics of development and quick proliferation. However, the reproductive rate of *T. clareae* is only slightly higher than that of *V. jacobsoni*, but the rate of population increase of *T. clareae* is geometrically greater, and this is attributable chiefly to shorter stay on adult bees and thus earlier entry of *T. clareae* mites into the brood cells (Woyke 1987d). Usually the number of parasites increases very fast during the first and second year of infestation. That causes that *Apis mellifera* honey bee colonies die out in the end of the second or in the third year (Burgett et al 1983, Woyke 1987a). *T. clareae* parasitises drone as well as worker brood in the same percentage, not like *Varroa jacobsoni* which prefers drone brood (Woyke 1995). The biology of reproduction of both mites mentioned above is different, too (Woyke 1989). Female of *T. clareae* starts to lay her

first egg 48-52 h after the cell with larva is sealed. She lies next eggs in a short time. Woyke (1987c) found the last eggs in 2 day-old pupae, 5 days after the cell was sealed. *T. clareae* lies 1-4 eggs (Woyke 1987d) and the development period of this mite takes 6 days (Woyke 1987b). The presence of large number of *T. clareae*, particularly in the bee brood causes the handicapped metabolism, bacterial and viral infections and even the death of the honey bee colony. After control of colonies destroyed by *T. clareae*, we found a queen with a low number of workers, often strongly infested by the parasites, and some uncapped pupae in each colony.

There are few papers only which discuss the infestation of queens and queen cells by *V. jacobsoni* (Burtov 1982, Bobrzecki and Wilde 1989, Muravskaya 1979, Romaniuk et al 1987) and no one described infestation by *T. clareae*. In Burtov's opinion (1982) the *Varroa* female very rarely enters the queen cells because the royal jelly contains substance which scares the parasites away. Muravskaya (1979) suggests that the queen development cycle is too short to permit the *Varroa* parasite to develop fully in closed queen cells. But according to Woyke's (1989 and 1987b) investigations it is possible that at least one *T. clareae* female can fully develop.

We decided to check the influence of *T. clareae* on the queens in queen cells in laboratory and in field conditions. The aims of our experiments were:

1. Investigation of the influence of *T. clareae* on:
   * development of honeybee queens
   * body weight of queens
2. Possibilities of development and reproduction of *T. clareae*.

MATERIALS AND METHODS

The research was carried out from February to July 2000 in Apiculture Centre in Jugedi, Nepal (Chitwan District). We carried out our Ist experiment on *A. mellifera* colonies using 288 queen cells (in three series of 96 sealed queen cells each) and 756 *T. clareae* mites. Queen cells built out and sealed by bees were transferred from rearing colonies to the laboratory and treated according to the Romaniuk's method (Romaniuk et al 1987). Next opening 2 mm in diameter was made in each cell (excluding the control group C1). *T. clareae* parasites collected from sealed worker brood shortly before being closed were introduced into cells through those openings. The openings were sealed with small pieces of wax foundation and melted with soldering gun after the mites' introduction. One mite was introduced into each queen cell in experimental group I, 2 mites in group II, 3 mites in group III, 4 mites in group IV, 5 mites in group V and 6 mites in group VI. Queen cells free of the parasites presented control groups - group C1 (not opened queen cells) and

group C2 (queen cells opened and then closed with bee wax). Afterwards the queen cells were put into Zander cages and transferred for 8 days into an incubator with temperature of 34.5°C and relative humidity of about 70%. Emerged queens were clinically examined and weighted on the 16th day of the development after the eggs were laid. The queen cells from which the queens did not emerge were opened. The age of the dead larvae, the number of alive and dead parasites and their development form were evaluated.

In the IInd experiment we reared 968 queen cells in colonies (cell builders) without any open brood. To control a natural infestation we put each sealed queen cell separately into the Zander cage which was protected with the gauze with small meshes to prevent *T. clareae* from leaving the cage after queen emerging. Emerged queens and queen cells were clinically examined like in the Ist experiment.

Significance of differences in weight of queens was determined by variance analysis (ANOVA) and multiple range test with least significant difference range (LSD). The values which differ significantly at p=0.05 are marked with small letters and those at p=0.01 are indicated with capitals.

RESULTS

Out of 288 control and experimental queen cells 219 queens emerged. Of those, 206 were properly developed (71.5%). This included: 34 (94.4%) queens of an average body weight of 209.7 mg emerged in the control group Cl (table 1 and 2) and 91.7% weighting 204.3 mg in control group C2. In experimental groups with the following number of mites: 1 - 88.9%, average weight 211.9 mg, 2 - 83.3% average weight 203.7 mg, 3 - 77.8% - 203.4 mg, 4 - 80.6% - 204.7 mg, 5 - 25.0% - 190.3 mg, and 6 - 30.6% - 181.0 mg. The differences in body weight in 2 groups (infested by 5 and 6 mites) were significantly lower (table 1). No significant differences in body weight of emerged queens in control groups and infested by 1, 2, 3 or 4 mites were observed. Highly significantly lower body weight was found in experimental groups of queens infested by 6 and 5 mites, respectively, than in all other groups.

The low percentage of queens died in the stage of pupa (6.6%) while in the stage of stretched larva - 12.2% (table 2). Out of 215 adult 9 (3.1%) from the 219 could not leave the queen cells on her own and 10 queens (4.9%) emerged without wings or with deformed wings. Legs were not properly developed in 3 (1.5%) queens. We found only 9 alive *T. clareae* in all experimental queen cells which were clinically examined. Among 69 dead queens which were artificially infested with the parasites only in 5 (7.3%) 1 larvae and 6 nymphs of *T. clareae* were observed.

Out of 968 queen cells in experiment II 794 queens emerged and 174 queen cells with dead larvae or pupae were found. We found: 27 alive *T.*

clareae in all cells with dead queens and 31 alive mites in all emerged queens. Among 174 dead queens which were naturally infested only in 3 cells (1.7%), 2 larvae and 3 nymphs of *T. clareae* were observed.

Table 1

Emerged queens and their body weight in control and experimental groups, artificially infested with *Tropilaelaps clareae*
Wygryzione matki i ich masa ciała w grupach kontrolnych i doświadczalnych sztucznie infekowanych *Tropilaelaps clareae*

No mites Liczba roztoczy	Number of queen cells Liczba mateczników	% of emerged queens % wygryzionych matek	Body weight in mg Masa ciała	
			Average - średnia	S
C1	36	94.4	**209.7** ^{B}	12.3
C2	36	91.7	**204.3** ^{B}	14.3
I	36	88.9	**211.9** ^{B}	17.1
II	36	83.3	**203.7** ^{B}	12.0
III	36	77.8	**203.4** ^{B}	13.8
IV	36	80.6	**204.7** ^{B}	11.9
V	36	25.0	**190.3**^{Ab}	19.2
VI	36	30.6	**181.0**^{Aa}	14.8

C1 and C2 control groups with queen cells not opened and opened and then closed with bee wax, respectively
C1 i C2 - grupy kontrolne z matecznikami, w których nie wykonano otworków (C1) oraz wykonywano,
a następnie zasklepiano je woskiem (C2)
Different small letters indicate significant differences at p = 0.05, capitals - at p = 0.01.
Różne małe litery oznaczają różnice istotne przy p = 0,05, zaś duże - przy p = 0,01.
s - standart deviation
- odchylenie standardowe

Table 2

Dead brood and emerged queens artificially infested by *Tropilaelaps clareae* in control and experimental groups
Zamarły czerw i wygryzione matki sztucznie w grupach kontrolnych i doświadczalnych sztucznie infekowane *Tropilaelaps clareae*

Materials Materiał	Number of parasitizing mites - Liczba roztoczy								Total - Ogółem	
	C1	C2	1	2	3	4	5	6	No	%
Total investigated queens or queen cells - Badane matki lub mateczniki ogółem										
Dead brood: Zamarły czerw:	36	36	36	36	36	36	36	36	288	100
Stretched larvae Larwa zwinięta	1	1	1	2	3	3	13	11	35	12.2
Prepupa Przedpoczwarka	1	1	2	1	2	1	6	5	19	6.6
Pupa - light eyes Poczwarka - jasne oczy	0	0	0	1	1	1	2	3	8	2.8
Pupa - violet eyes Poczwarka -oczy fioletowe	0	0	0	0	0	1	1	1	3	1.0
Pupa - black eyes Poczwarka -oczy czarne	0	0	1	1	2	0	2	2	8	2.8
Adult queens - cannot leave the queen cells - Matki nie mogące wygryźć się z matecznika										
	0	1	0	1	0	1	3	3	9	3.1
Adult emerged queens - Matki wygryzione										
	34	33	32	30	28	29	9	11	206	71.5 100
No wings or wings improperly developed Brak skrzydeł lub nieprawidłowo rozwinięte	1	0	1	1	1	1	2	3	10	4.9
Legs improperly developed Nieprawidłowo rozwinięte odnóża	0	0	1	0	0	0	2	0	3	1.5
Emerged queens properly developed - Matki wygryzione wykształcone prawidłowo										
	31	33	30	29	27	28	5	8	193	93.7

Explanation:
C1 and C2 control groups with not opened and opened and then closed with bee wax queen cells, respectively,
C1 i C2 - grupy kontrolne z matecznikami, w których nie wykonano otworków (C1) oraz wykonywano, a następnie zasklepiano je woskiem (C2)

DISCUSSION

The obtained results indicate that queen larvae in queen cells artificially or naturally infested by *T. clareae* mites are damaged in a few cases only. Some of them even die. The greatest number of queens die at the stage of stretched larvae and next of prepupa. The reason for that can be *T. clareae* infestation. The infestation by 5 and 6 mites was particularly dangerous for queen development. The parasites cause the death of larvae or morphological changes.

The body weight of emerged queens did not differ significantly between control uninfested queens and infested by 1 to 4 mites. It is similar to Romaniuk et al (1987) results on infestation by *V. jacobsoni* mites. But infestation by 5 and 6 mites resulted in the lowest body weight of emerged queens. The reason for that seems to be the influence of the parasites - disturbing the larvae and their feeding on the larvae haemolymph.

This results showed that *T. clareae* may also enter the queen cells, in the natural way and causes the changes to queen larvae. However, this occurs incidentally in rearing colonies without open brood in colonies severely infested by *T. clareae*. Those results are similar to observation made by Bobrzecki and Wilde (1989) on rearing colonies infested by *V. jacobsoni*. It seems just the same phenomenon occurs as with *V. jacobsoni* according to Burtov's (1982) opinion. Most probably some substance in royal jelly scares *T. clareae* as well as *V. jacobsoni* and inhibits the reproduction in queen cells.

CONCLUSIONS

1. Infestation by *T. clareae* decreases the number of queens emerging from queen cells.
2. Presence of 5-6 *T. clareae* in queen cells causes the death of 75.0 and 69.4% of queens, respectively.
3. Body weight of emerged queens seems not to be changed by the presence of 1-4 parasites in the queen cells.

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WPŁYW INWAZJI *TROPILAELAPS CLAREAE* NA ROZWÓJ MATEK PSZCZELICH

Wilde J.

Streszczenie

Badania przeprowadzono między lutym a lipcem 2000 roku w Centrum Pszczelarskim w Nepalu w rodzinach *A. mellifera*. W doświadczeniu I. użyto 288 mateczników (3 serie po 96 zasklepionych mateczników w każdej) i 756 osobników *T. clareae*. Do każdego matecznika poddawano 1, 2, 3, 4, 5 lub 6 roztoczy. Dwie grupy kontrolne stanowiły mateczniki bez pasożytów. W jednej nie otwierano matecznika, a w drugiej wykonywano otwór w mateczniku, a następnie zalepiano go woskiem. Mateczniki, z których nie wygryzły się matki rozcinano, badając klinicznie ich zawartość.

Podczas II. doświadczenia wyprodukowano 968 mateczników w rodzinach wychowujących nie posiadających czerwiu otwartego. Każdy zasklepiony matecznik izolowano w klateczce Zandera z siatką o drobnych oczkach, uniemożliwiających opuszczenie klateczki przez roztocze, po wygryzieniu się matki. Wygryzione matki lub zamarłe mateczniki badano klinicznie na obecność roztoczy, bądź ich stadiów rozwojowych.

Z 288 mateczników wygryzło się 206 matek, z których 193 było prawidłowo rozwiniętych. Masa ciała matek w 2 grupach doświadczalnych, do których dodawano 5 i 6 roztoczy była wysoko istotnie niższa niż w pozostałych grupach (tabela 1). Z 215 matek w 9 przypadkach (3,1%) matki nie były w stanie samodzielnie opuścić mateczników, w 10 przypadkach (4,9%) wygryzły się matki bez skrzydeł lub z ich deformacjami, a w 3 przypadkach (1,5%) odnóża matek były nie w pełni rozwinięte. We wszystkich grupach doświadczalnych na matkach lub zamarłych w matecznikach ich stadiach rozwojowych znaleziono tylko 9 żywych *T. clareae*. Spośród 69 mateczników z martwymi matkami, sztucznie porażanych roztoczami, tylko w 5 (7,3%) zaobserwowano 1 larwę i 6 nimf *T. clareae*.

W doświadczeniu II. zbadano 794 wygryzionych matek i 174 zamarłych mateczników. Znaleziono: 27 żywych roztoczy *T. clareae* we wszystkich zamarłych matecznikach i 31 żywych roztoczy na wszystkich wygryzionych matkach. Spośród 174 zamarłych mateczników tylko w 3 (1.7%) zaobserwowano rozwój pasożyta, znajdując 2 larwy i 3 nimfy *T. clareae*.

Uzyskane wyniki dowodzą, iż sztuczne lub naturalne porażenie mateczników roztoczami *T. clareae* tylko w niewielu przypadkach powoduje deformację rozwoju matek. Przyczyną zamarcia mateczników w grupach doświadczalnych mogła być inwazja roztoczy *T. clareae*. Masa ciała wygryzionych matek nie różniła się istotnie pomiędzy grupami kontrolnymi a doświadczalnymi, porażonymi przez 1-4 roztocze, a zmniejsza się dopiero gdy mateczniki były porażone przez 5-6 roztoczy. Inwazja *T. clareae* zmniejsza liczbę wygryzionych matek. Obecność w matecznikach 5-6 roztoczy *T. clareae* powoduje śmiertelność matek odpowiednio w 75,0 i 69,4%.

Słowa kluczowe: inwazja *Tropilaelaps clareae*, wychów matek, mateczniki, rozwój *T. clareae* w matecznikach.