PERFORMANCE OF ARTIFICIALLY INSEMINATED HONEYBEE QUEENS THAT HAVE BODILY INJURIES

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S u m m a r y

The aim of the study was three-fold: 1) to examine the acceptance by the bees of artificially inseminated honeybee queens which have bodily injuries and 2) the effect of different kinds of injuries on the egg-laying abilities of the queens and 3) the propensity of the bees to supersedure. A total of 396 honeybee queens were studied. Their condition was examined under a microscope prior to and after introduction into bee colonies. Among the 330 queens introduced into the colonies, 31 had their claws and arolia injured. Their claws and arolia were injured prior to the introduction into the bee colonies. The bees did not accept 13.0% of the uninjured and 3.2% of the injured queens. The colonies with the uninjured queens occupied only slightly more combs (10.5) than did the colonies with queens, which had injuries to their claws and arolia (9.7) or with queens with paralyzed legs (10.2). The colonies with queens with missing parts of their legs and with injured antennae were weaker as they occupied significantly fewer combs; 8.6 and 7.6, respectively. The greatest average brood area in the first ten days of June was in colonies with uninjured queens (54.5 dm^2). The brood area was lower, although insignificantly, in the colonies where the queens had injured arolia and claws or paralyzed legs; 51.8 and 51.7 dm^2, respectively. There was significantly less brood area in the colonies where the queens had some leg parts missing or had injured antennae; 30.8 dm^2 and 34.6 dm^2, respectively.

Out of 396 examined queens, 18.7% were supersedured. Uninjured queens were significantly less frequently supersedured by bees (11.3%) than the injured ones. Supersedured queens with injuries to their arolia and claws accounted for 11.3%, those with paralyzed legs and missing leg parts for 33.3 and 37.5%, respectively. As many as 83.3% of queens with injured antennae were supersedured.

Keywords: honeybee queen, injured queens, insemination, supersedure, oviposition.

INTRODUCTION

Injury mainly concerns instrumentally inseminated queens. The issue of injury less frequently concerns naturally inseminated queens (Gerula and Bieńkowska 2002). Injuries may arise during the queen rearing period (Woyke et al. 1956, Woyke 1988, Jasiński 1987, 1995, Jasiński and Fliszkiewicz 1995, 1996, Loc et al.1996, Wilde and Loc 1997). Injuries to the queens may also arise when being introduced into the colonies (Gerula 2004, 2006). The hostile attitude of worker bees towards unfamiliar queens is the most frequent cause of injuries. The reason for this is that they smell differently (Yadawa 1971). Some of the workers become aggressive. They catch the queens with their mandibula and tear those body parts to which they have immediate access (Woyke et al. 1956, Szabo and Townsend 1974, Robinson 1982). The most frequent damage queens suffer is to their legs. Less frequently they suffer damage to their to antennae and wings.

Most beekeepers think that injured queens are worthless. Despite strict control in breeding apiaries such queens occur in

commercial apiaries (Czekońska 2001).

Injuries to the queen's body may disturb the transfer of nervous stimuli between the queens and the workers. It may interfere with the distribution of pheromones. This is especially true of those pheromones produced in the tarsal glands of the queens. These glands are located in the last tarsal segment. They have their outlet on the inner side where it joins the arolia. While walking on the combs and on smooth surfaces honeybee queens leave foot-prints in the form of secretion from those glands. Woyke (1988) noticed that queens with injured arolia do not leave such foot-prints. The injuries may interfere with the production of tarsal secretion.

Tarsal glands were described by Arnhart (1923), but their structure was examined in detail by Lensky and Slabesky (1981), Lensky et al. (1984, 1985). Queens, workers and drones all have those glands. The secretion of the tarsal pheromone in 6-month-old queens is more than 14 times higher than that in worker bees and 16 times higher than that in drones. In 2-year-old queens the secretion drops by nearly half (Lensky et al. 1985). The role performed by the tarsal gland secretion in the queens is to prevent the construction of swarm queen cells (Lensky and Slabesky 1981).

Queens with damaged legs continue to be attractive to bees. Injured queens were even better accepted than the uninjured ones; 97.3 % and 82.0 %, respectively, and the same number, ca. 92%, started egg-laying (Wilde and Loc 1997). However they did not investigate the impact of injuries on the ability of the queens to lay eggs.

Gerula and Bieńkowska (2002) and Gerula (2004) observed that queens with damaged arolia were supersedured twice as frequently as uninjured ones. The queens with paralyzed legs and missing parts of legs were supersedured four times more frequently than the uninjured queens. According to Forster (1971), other factors influencing the supersedure might be inappropriate transport conditions negatively affecting the condition of the queen. He found that such queens can be supersedured as early as the first year of life. The tendency of bees to supersedure can also be hereditary (Foti 1979 after Roussy 1959). Such supersedure prevents swarming. Roussy (1959) considers that late supersedure in August prevents the queens mating with drones with an inclination to swarming. This in turn keeps the disadvantageous trait from being passed on.

The aim of the study was to:

1. Investigate how the queens with body injuries are accepted by the bees.

2. Determine the impact of the queens' injuries on the queens performance in the colonies.

MATERIAL AND METHODS

The observation was carried out in Puławy, Poland at the apiary of the Department of Bee Breeding, Apiculture Division of the Institute of Pomology and Floriculture, Skierniewice, Poland.

Honeybee queens of four breeding lines: Puławska (Caucasian), and Marynka, Zosia and GR1 (Carniolan) were investigated. Queens were reared in queenless colonies with the single-grafting larva method.

The queens were inseminated when 6-8 days old with a single dose of 8 µl of semen. At the same time, the inseminated queens were treated for the first time with CO_2 for 3 minutes. The cages with queens and attendant bees were placed in incubators for 48-96 hours. Next the queens were treated for the second time with CO_2, for 3 minutes. After this they were introduced into two-chamber mailing cages without attendant bees. Then they were introduced into mating colonies composed of ca. 1000 worker bees. The colonies were settled in Styrofoam

trapezoid-shaped hives with 3 frames having a total surface area of 5.6 dm^2. The queens were kept there until oviposition.

Egg-laying queens in the mailing cages were introduced into colonies in Dadant hives in a stationary apiary. They were introduced immediately after the old queens were removed. The food chamber of the mailing cage filled with candy was opened after 6 to 9 days. This depended on bees' behavior in the colony and also on the possibility to build queen cells in the colony. Most frequently, the queens were released in the following 24-48 hrs after opening the mailing cage. In the years 2001-2005, altogether 330 queens were introduced into colonies. Another 106 queens reared in 2000 were included in the monitoring of supersedure.

Before and after introduction into colonies the queens were examined under the stereoscopic microscope for injuries to their bodies. Uninjured queens were treated as a control group (UNI). Injured queens were divided into groups according to the kind of injury related to the same part of body:

- IAC- injuries to arolia and claws
- PL- paralyzed legs
- MPL- missing parts of legs
- IA- injured antennae

Effect of injury to the queens' body on their performance was studied. This was studied in terms of the amount of reared brood and colony strength in the second year of the queens' life. No such investigations were done for the queens reared in 2005. Colony strength was expressed by the number of combs occupied by bees in the first ten days of June. The amount of reared brood was counted per 1 dm^2 of both capped and uncapped cells. Supersedure was monitored during the entire presence of queens in the colonies. The observations were done during colony examination for the presence of the old queen and queen cells. Queens reared in 2005 were monitored for the shortest time.

In the calculations of the effect of injuries on the queen acceptance data, the χ^2 test with Yates' correction was applied. Mean values for number of occupied combs, brood area and number of supersedured queens as related to injury and year of life were compared using ANOVA. Percentage data relating to supersedure were transformed according to Bliss' function. Homogeneous groups were singled out by using Duncan's test at a significance level of α=0.05.

RESULTS

The acceptance of injured and uninjured honeybee queens by the colonies.

Out of the 330 queens, which were introduced into colonies, 31 already had slight injuries to their legs before introduction and were classified to IAC. The remaining ones were uninjured. The percentage of the unaccepted queens

Table 1

The acceptance of injured and uninjured queens by the colonies. Years 2001-2005.

Kind of introduced queen	Number of introduced queens	Number (percentage) of unaccepted queens
Uninjured UNI	299	39 (13.04) a*
Injured IAC	31	1(3.2)a
Total	330	40 (12.1)

* different letters in columns indicate significant differences at $p \leq 0.05$.

among the uninjured ones was slightly higher than that among the injured ones (Table 1) but there were no statistical differences (χ^2 Yates' correction =1.64, df=1, p=0.2).

was statistically confirmed only for the colonies of the IA group.

In the experimental groups, the amount of reared brood varied (F=5.908, df=4, p<0.01). In the first ten days of June, the

Table 2

Honeybee colony strength and brood area (dm^2) in the colonies with uninjured vs. injured queens. Years 2001-2005.

Colonies with the queens of specific grooup	Average number of occupied combs		Average brood area	
	Number of colonies	Number of combs ± s.d.	Number of colonies	dm^2 ± s.d.
UNI (uninjured)	99	10.5 a* ± 3.36	95	54.5 a ± 17.72
IAC (injuries to arolia and claws)	83	9.7 a ± 2.18	53	51.8 a ± 19.49
PL (paralyzed legs)	9	10.2 a ± 1.78	6	51.7 a ± 8.89
MPL (missing parts of legs)	19	8.6 a, b ± 2.05	12	30.8 b ± 12.85
IA (injured antennae)	5	7.6 b ± 1.51	5	34.6 b ± 12.17

* different letters in columns indicate significant differences at p≤ 0.05.

Colony strength and brood area in colonies with uninjured vs. injured queens.

In the first ten days of June, honeybee colonies with observed queens occupied 9.3 combs on average (Table 2).

Colony strength was found to vary significantly between two experimental groups (F=4.921, df=4, p<0.01). The colonies with uninjured queens occupied slightly more combs than those with injured queens of the IAC group (injuries to arolia and claws) and of the PL group (paralyzed legs). The majority of queens, 92.7%, had only one leg paralyzed. In the group with uninjured queens the greatest variation was found. The colonies with the queens from the MPL group (missing parts of legs) and of the IA group (injured antennae) were the lowest strength. This colonies with queens of the UNI group (uninjured) had the greatest average brood area. The brood area was slightly lower, but not confirmed statistically, in the colonies of the IAC and PL groups. The queens of the MPL and IA groups had significantly less brood than those mentioned above (Table 2).

Supersedure

Throughout the study years, the bees supersedured 18.7% of the observed queens. Uninjured queens (UNI group) and injured queens of the IAC group were significantly less frequently supersedured; 11.3 and 19.1%.

The percentage of supersedured queens among those of the PL group (paralyzed legs) was 33.3 and 37.5%, respectively. In the group with injured antennae (IA group) the percentage of supersedured queens was

Table 3

Number and percentage of supersedured queens depending on kind of injury. Years 2001-2005.

Queen group	Number of queens accepted in colonies	Number (percentage) of supersedured queens
UNI (uninjured)	212	24 (11.3) a*
IAC (injuries to arolia and claws)	136	26 (19.1) ab
PL (paralyzed legs)	21	7 (33.3) b
MPL (missing parts of legs)	32	12 (37.5) ab
IA (injured antennae)	6	5 (83.3) c
Total	396	74 (18.7)

* different letters in columns indicate significant differences at $p \leq 0.05$ (after Bliss transformation).

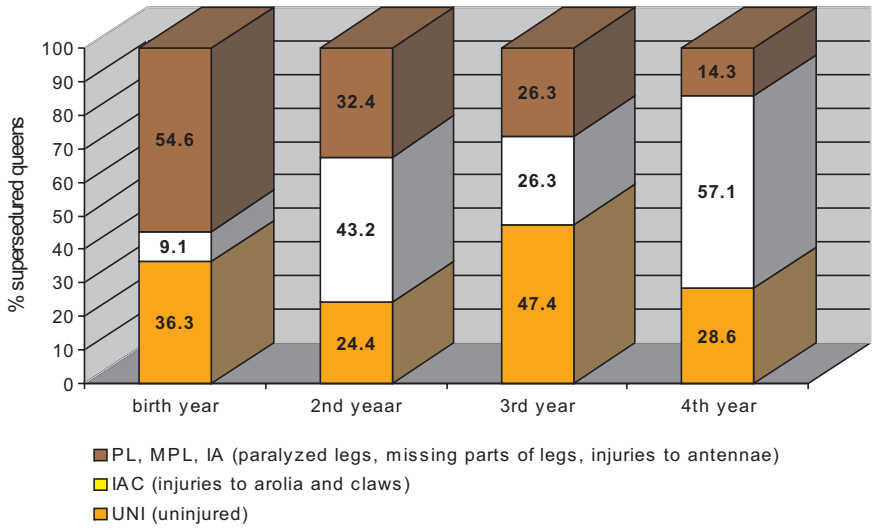

Fig. 1. Percentage of queens from individual groups supersedured in the first (birth), second, third and fourth year of their life.

significantly the highest; amounting to 83.3% of the total (Table 3).

Queens were supersedured in the first year of their life, some in the second, some in the third and some in the fourth year of their life. Regardless of the kind of injury as much as 50% out of 74 supersedured queens were lost in the second year of their life (Table 4). The frequency of supersedure as related to particular year of life was not found to be statistically valid ($F=1.690$, $df=3$, $p>0.05$).

The queens from the groups with more severe injuries (PL, MPL and IA) were the first to be supersedured. The majority of queens with less serious injuries of the arolia and claws (IAC group) and the uninjured queens (UNI group) were supersedured when older (Fig. 1).

Table 4

Number and percentage of queens superseded over consecutive years regardless of kind of injury.

Year of life	Number of superseded queens	Superseded queens as percentage of their total number (n=74)
First (birth) year	11	14.7 a*
Second year	37	50.0 a
Third year	19	25.7 a
Fourth year	7	9.5 a
Total	74	100

* different letters in columns indicate significant differences at $p \leq 0.05$ (after Bliss transformation).

DISCUSSION

Light injuries to the queens' legs did not affect the rate of their acceptance. Similar results were obtained by Wilde and Loc (1997). It may be thought that all injured queens would perform inferior egg-laying capability. However, in this study only colonies with queens with paralyzed legs and injured antennae had less brood than the remaining ones.

Every kind of injury significantly increased supersedure as predicted by Woyke (1988) and observed by Gerula and Bieńkowska (2002). Even minor leg injuries such as arolium injuries caused the percentage of superseded queens to be twice as high as that in the group of uninjured queens (Table 2). It could reflect a compromised effect on the bees of the tarsal gland secretion in the queens.

A large percentage of superseded queens in the IA group (injured antennae) proves the importance of that organ for the normal performance of the queen in the honeybee colony. Restricted communication of the queen with the bees due to mechanical injury to the antennae may be further increased by the injury to the first pair of legs. The tibial organ that performs the function of the aural organ is located on the first pair of legs.

Nevertheless, 11.3% of superseded queens among the uninjured ones points to some other factors influencing the inclination of bees to supersedure. The inclination can be hereditary as shown by Foti (1979 after Roussy 1959).

CONCLUSSIONS

1. Injuries to the queen's body do not affect the acceptance of queens in colonies.
2. Colonies with queens that have missing parts of leg or injured antennae rear less brood than those with queens that have no injuries, as well as with queens that have paralyzed legs or injured arolia.
3. Any injury to a queen's body starting with injury to arolia favours supersedure.
4. Regardless of the kind of injury, supersedure most often takes place in the second year of the queen's life.

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WPŁYW USZKODZEŃ CIAŁA SZTUCZNIE UNASIENIONYCH MATEK PSZCZELICH NA ICH WARTOŚĆ UŻYTKOWĄ

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S t r e s z c z e n i e

Obserwacje prowadzono w latach 2001-2005 w Puławach w pasiece Zakładu Biologii i Hodowli Pszczół Oddziału Pszczelnictwa, Instytutu Sadownictwa i Kwiaciarstwa w Skierniewicach. Celem pracy było sprawdzenie wpływu uszkodzeń ciała na przyjmowanie sztucznie unasienionych matek przez pszczoły oraz wpływu różnych rodzajów uszkodzeń na intensywność czerwienia matek w rodzinach i skłonność pszczół do cichej wymiany matek. Zbadano 396 matek pszczelich kontrolując pod mikroskopem ich stan, przed i po przyjęciu przez pszczoły w rodzinach pszczelich. Pośród 330 matek poddawanych do rodzin, 31 miało uszkodzone przylgi i pazurki (nabyte przed poddaniem). Pszczoły nie przyjęły 13,0% matek spośród nieuszkodzonych i 3,2% z uszkodzonych. Nie stwierdzono jednak istotnej różnicy między tymi średnimi. Rodziny z matkami nieuszkodzonymi, obsiadały tylko nieznacznie i nieistotnie więcej plastrów (10,5 szt.) niż rodziny z matkami z uszkodzonymi przylgami i pazurkami (9,7) i z matkami ze sparaliżowanymi nogami (10,2). Rodziny z matkami, które miały ubytki części nóg i uszkodzone czułki były słabsze, gdyż obsiadały mniej plastrów, odpowiednio 8,6 i 7,6. Największą średnią powierzchnię czerwiu w pierwszej dekadzie czerwca miały rodziny z matkami nieuszkodzonymi (54,5 dm^2). Nieco mniej, choć nieistotnie, miały rodziny z matkami z uszkodzonymi przylgami i pazurkami oraz ze sparaliżowanymi nogami odpowiednio 51,8 i 51,7 dm^2. Istotnie mniej czerwiu od wyżej wymienionych miały rodziny z matkami, którym brakowało części nóg i które miały uszkodzone czułki odpowiednio 30,8 dm^2 i 34,6 dm^2.

Pszczoły wymieniły na drodze cichej wymiany 18,7% z 396 obserwowanych matek. Matki nieuszkodzone były istotnie rzadziej wymieniane (11,3%) niż uszkodzone od 19,1 do 83%. Wśród matek z uszkodzeniami przylg i pazurków zostało wymienionych 19,1%, ze sparaliżowanymi nogami i z brakującymi częściami nóg odpowiednio 33,3 i 37,5%. Natomiast wśród matek z uszkodzonymi czułkami pszczoły wymieniły ich najwięcej, bo aż 83,3%.

Słowa kluczowe: matka pszczela, uszkodzenia matek, inseminacja, cicha wymiana, czerwienie.