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Acceptance of Mated Queens and Queen Cells in Colonies of Russian and Italian Honey Bees

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Summary: Requeening colonies is a standard beekeeping practice with both mated queens and queen cells. More beekeepers are requeening with Russian honey bee queens because of their significantly higher resistance to *Varroa* and tracheal mites, their good honey production and their overwintering abilities. However, some beekeepers report difficulties when attempting to requeen colonies with Russian queens. This experiment investigated the scope of that problem. In an experiment having 120 requeening attempts, no differences were found between requeening with Russian or Italian queens. Similar results were obtained when requeening with Russian or Italian queen cells.

Introduction

Queen honey bees (*Apis mellifera*) may live for a year or more (Seeley, 1978) but more often are replaced by colonies after a few months (Sugden and Furgala, 1982). Colonies naturally rear new queens in colonies when old queens are failing or lost (supersedure) and prior to swarming. These events are regulated by a variety of circumstances such as reduced levels of pheromones and broodnest crowding (Velthuis, 1970).

Beekeepers have taken advantage of the natural processes of queen replacement and developed methods to produce queens at will, allowing them to put a queen of their choosing into a colony. Periodic queen replacement is an important beekeeping practice (Guzman-Nova et al., 1998). Beekeepers replace queens in colonies to assure that colonies will have vigorous queens that are less likely to fail at a critical time in the annual colony cycle (Furgala and McCutcheon, 1992) or to improve the genetic stock. Beekeepers also introduce queens to queenless colonies they have made by dividing existing colonies to increase the number of colonies they own. Generally, new queens are placed in colonies either as mated adult queens or as queen cells that contain pupae almost old enough to emerge.

Russian honey bees are resistant to *Varroa* mites (Rinderer et al. 2001a, Harris and Rinderer, 2004), tracheal mites (de Guzman et al., 2002), overwinter well (de Guzman et al., 2006) and are good honey producers (Rinderer et al., 2001b). Many beekeepers have purchased or produced Russian queens to change the stock in their colonies. However, some of these beekeepers have reported difficulties when attempting to introduce Russian queens to Italian colonies. We conducted comparative experiments with both mated queens and queen cells to identify the magnitude and source of these queen introduction problems.

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Materials and Methods

Experiment 1. Acceptance of Introduced Queens

Thirty Russian and 30 Italian colonies were made as divides of established colonies. These divides were composed of three to four frames of brood (Langstroth frames 16.8 cm deep) with 1.4-1.6 kg of worker bees. The divides were arranged randomly in a single apiary.

Two days later, mated Russian and Italian queens were introduced into the colonies. For each colony the type of queen (Russian or Italian) was randomized. Seventeen Russian and 13 Italian queens were randomly introduced to the 30 Russian colonies; 17 Russian queens and 13 Italian queens were introduced to the 30 Italian colonies on May 24, 2004.

Unattended paint-marked queens in plastic queen cages were placed between brood frames near the top bars of the frames. The tubes of the cages were capped. Brood frames were inspected and any queen cells found were destroyed. Five days after the queens were placed in the colonies, they were hand released into the colonies from the cages. Seven days and 24 days (four weeks) after the queens were hand released the colonies were inspected. The presence of a marked queen, eggs, and all stages of larvae were interpreted as evidence of a successful queen introduction.

A second replication of the queen introduction experiment was conducted with the same colonies. When introduction success in the first replication of the experiment was determined, the queens were removed and placed in a populous queenless colony for storage. Likewise, frames with unsealed brood were removed from the colonies, randomly mixed within the stock groups and returned to colonies. Russian colonies received equal numbers of frames of Russian brood and Italian colonies received equal numbers of Italian brood frames.

Two days later, queens were introduced to the colonies. The type of queen (Russian or Italian) for each colony was randomized without reference to the first replication. Introduction and evaluation procedures were the same as they were for the first replication.

Data concerning the initial acceptance of the introduced queens and their continued presence were analyzed by Fisher's exact tests.

Experiment 2. Acceptance of Queen Cells

We also compared rates of acceptance of queen cells between Russian and Italian colonies. Thirty-three queenless Russian and 30 queenless Italian divisions were made. The divides were composed of five to seven frames of brood, two to 2.3 kg of worker bees and one frame of honey (Langstroth frames 16.8 cm deep). Making divisions was done when queen cells we produced contained pupae that were expected to emerge within two to three days.

Both Russian and Italian queen cells were produced. The 33 Russian divisions were given Italian cells and the 30 Italian divisions were given Russian cells. The cells were introduced the day after divisions were made by pressing plastic cell bases into comb just above the brood. Cell protectors were not used.

Colonies were inspected two to three days after cell introduction. Virgins were paint marked. Colonies were again inspected at nine to 10 days and then 15 days after cell introductions for the presence of marked queens and brood. A final colony evaluation was made 6 weeks later.

The presence of virgin queens was used to indicate that cells were accepted. Continued acceptance through time was based on the presence of the marked queen with a brood nest having all stages of brood. Data were analyzed by Fisher Exact Tests.

Colonies in which the first cell did not apparently produce an accepted virgin queen were given a second cell of the same type two to three days after the colonies were determined to be queenless. The success of these "secondary" introductions was monitored and evaluated by the same methods and criteria used for the "primary" introduction group of introductions.

Results

Experiment 1. Acceptance of Introduced Queens

By week four all colonies that accepted introduced queens had brood nests with all stages of brood. Overall, 80% of the colonies retained the original introduced queen: 82% for Russian colonies and 78% for Italian colonies. Additionally, each replication of the experiment produced similar results for Russian and Italian colonies. Differences between colony types for each replication and the combined replications were not significant (Table 1).

Similar results were obtained when acceptance was classified by queen type. About 78% of the Russian queens remained in their colonies while 82% of the Italian queens remained. This overall difference between queen types was not significant nor were differences between queen types for each replication (Table 1).

Results differed significantly between the two replications ($P = 0.003$). The replication that began in May (Replication 1) had 92% of the queens after three weeks while the replication that began in June (Replication 2) had 68% of the queens surviving.

Experiment 2. Acceptance of Queen Cells

Cell introductions were equally successful (Table 2). For the "primary" introductions, numerically fewer Russian cells produced queens that remained in Italian colonies 14 weeks later but this difference was not significant ($P = 0.13$). The "secondary" introductions provided similar non-significant results ($P = 0.57$).

The success rate (23%) of the secondary introductions was about a third of the success rate of the primary introductions after 14 weeks (Table 2). This difference was significant ($P = 0.01$) even though only 13 secondary introductions were monitored.

Discussion

We conclude that re-queening success does not depend on the stock of the colony.

Overall, no evidence was found which indicated that either Russian queens or Russian queen cells were more difficult to introduce to either Russian or Italian colonies. There was an early minor suggestion of Russian colonies

Table 1. Acceptance of Russian and Italian queens in Russian and Italian colonies for two replications of introductions.

Replication	Colony Type	Queen Type	Number Introduced	Number Released (5 days)	Percentage Released	Initial Acceptance (12 days)	Percentage Accepted	Week 4* Acceptance	Percentage Acceptance
1	Russian	Russian	17	16(1)	94	15(2)	88	13(4)	76
		Italian	13	13(0)	100	13(0)	100	13(0)	100
	Italian	Russian	17	17(0)	100	17(0)	100	16(1)	94
		Italian	13	13(0)	100	13(0)	100	13(0)	100
	Difference between colony types:			$P = 1.00$, NS		$P = 0.49$, NS		$P = 0.35$, NS	
	Difference between queen types:			$P = 1.00$, NS		$P = 0.49$, NS		$P = 0.35$, NS	
2	Russian	Russian	14	12(2)	85	11(4)	78	10(4)	71
		Italian	16	11(5)	68	11(5)	68	10(6)	62
	Italian	Russian	15	14(1)	93	14(1)	93	10(5)	66
		Italian	15	14(1)	93	13(2)	86	11(4)	73
	Difference between colony types:			$P = 0.15$, NS		$P = 0.18$, NS		$P = 1.00$, NS	
	Difference between queen types:			$P = 0.47$, NS		$P = 1.00$, NS		$P = 1.00$, NS	
1 & 2	Russian	Russian	31	28(3)	90	26(5)	83	23(8)	74
		Italian	29	24(5)	83	24(5)	82	23(6)	83
	Italian	Russian	32	31(1)	93	31(1)	97	26(6)	81
		Italian	28	27(1)	96	26(2)	93	24(4)	86
	Difference between colony types:			$P = 0.09$		$P = 0.07$		$P = 0.49$, NS	
	Difference between queen types:			$P = 0.74$		$P = 0.77$		$P = 0.63$, NS	
	Difference between replications:			$P = 0.008$**		$P = 0.008$**		$P = 0.003$**	

Introduction Group	Colony Type	Cell Type	Number Introduced	Number Accepted (Day 3)	Percentage (Day 3)	Number Accepted (Week 14)	Percentage (Week 14)
Primary	Russian	Italian	33	26	70	24	73
	Italian	Russian	30	24	80	16	53
	Difference between Colony/Cell Combinations			$P = 1.00$, NS		$P = 0.13$, NS	
Secondary	Russian	Italian	7	6	86	1	14
	Italian	Russian	6	3	50	2	33
	Difference between Colony/Cell Combinations			$P = 0.28$, NS		$P = 0.56$, NS	
	Difference between Primary and Secondary Acceptance			$P = 0.76$, NS		$P = 0.01$**	

Table 2. Rates of successful introductions of Russian queen cells into Italian colonies. For colonies that failed to accept cells in the first (primary introduction group) introduction second cells were introduced (secondary introduction group).

being generally more difficult to re-queen from day 12 data ($P = 0.07$). However, this proved not to be the case by week four. We found no evidence that Italian colonies were less accepting of Russian queens.

There were differences between the rates of queen acceptance between the two replications. The second replication had 68% acceptance while the first replication had 92% acceptance. A variety of seasonal and other environmental causes may have caused this difference, including the intense colony management we used to accomplish a second replication. However, the difference was not the result of either the stock of the queens or the stock of the colonies. Likely, the conditions that resulted in lower acceptance rates in the second replication should have created conditions for subtle stock differences to become more apparent. That there were no differences between stocks even in the more difficult conditions of the second replication further suggests that there are no differences in the acceptance rates of Russian or Italian queens in Russian or Italian colonies.

In a study involving larger numbers of colonies in many different environments and using different queen introduction procedures, packages of Italian bees were slightly less able (7%) to accept Russian queens (Tarpy, personal communication). Although we did not detect problems in our study and only a small difference was detected by Tarpy, it is possible that some larger problems of requeening with Russian queens may occasionally occur. It may be that some Italian stocks, at least under some conditions, have difficulty accepting Russian queens. However, such difficulties are not common. The difference detected by Tarpy, while statistically significant, is small and probably for most beekeepers is acceptable when balanced with having mite resistant stock.

Re-queening with cells was also equally successful for Russian and Italian colonies. However, there was a large difference between primary and secondary introductions. The colonies were reasonably large splits that had not been queenless long enough to have laying workers (Page and Erickson, 1988). There was no apparent reason why they rejected queen cells or would not accept a second cell. However, having failed once to accept a cell appears to be an excellent indication that a colony is unlikely to accept a second cell.

Overall, there is no evidence suggesting that it is more difficult to introduce Russian queens or cells to colonies. However, it may be that problems do exist which are probably infrequent and minor. The economic advantage of having colonies with mite resistant stock is considerable. These advantages certainly outweigh minor and infrequent queen introduction problems encountered with changing stock. BC

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