FINAL REPORT

Project Title: The influence of time of year and rearing environment on the acceptance of introduced European queens in Africanized honey bee colonies.

Project Number: 04-GD-01

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Objectives

1) **Are certain times of year better than others for introducing European queens into African colonies?**
2) **Do queens emit volatiles that stimulate workers to supersede them?**

Objective 1. Colonies of European honey bees become Africanized if they replace their queen and she mates with African males (drones). The only way to reverse the Africanization process is to introduce a European queen that has mated exclusively with European drones. However, it is difficult to introduce European matriline queens mated with European drones into Africanized colonies and have them accepted.

We designed an experiment to compare the success rate of queen introduction under three different colony conditions. The first queen introduction was during a honey flow in May when most of the comb in the colony was being filled with incoming nectar and space for the queen to lay eggs was limited. The second condition was when relatively little nectar was coming into the colony but the brood area was expanding (late June through July in Tucson). We supplied honey and pollen to the colonies so that brood rearing was not constrained by a lack of resources. The third condition was in the fall when brood rearing was declining and the colony was preparing for winter. We conducted the experiments using 5-frame nucleus colonies during Year-1, and full sized 9-frame colonies in Langstroth equipment during Year-2. We sampled the volatiles emitted by the queens just prior to their introduction and 6 weeks after they were introduced into the colonies. The experiment was run for 6 weeks because by that time the colony population was composed almost entirely of offspring from the introduced queen.

Results

Objective 1:

During Year-1, about 50% of the European queens we introduced into African colonies in either May or July were superseded within six weeks (Table 1). Supersedure rates of European queens in European colonies were only 25% in May and in July all the queens we introduced were accepted. Supersedure rates decreased to 12.5% when queens were introduced in the fall. In Year-2 a similar trend occurred where queens introduced in the fall had much lower risk of being superseded.

Table 1. Percentage of introduced European queens mated with European drones that were superseded in European and Africanized honey bee colonies.

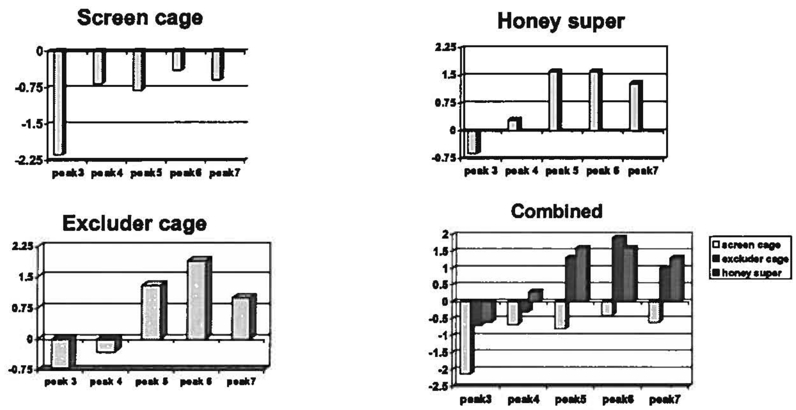
Year	Trial	Colony type	Supercedure (% of colonies)
1	May	EHB	25
		AHB	48
	July	EHB	0
		AHB	50
	September	EHB	0
		AHB	12.5
2	May	EHB	25
		AHB	50
	July	EHB	25
		AHB	50
	September	EHB	0
		AHB	0

Objective 2: Do queens emit volatiles that stimulate workers to supersede them?

We subjected laying European queens to three sets of conditions to determine factors that affected their volatile chemical profiles and the initiation of supersedure events. The first condition was to place the queen in a screen cage in the colony so that her movement among workers was restricted. The queen could not lay eggs and workers could not contact her except with their mouthparts. In the second treatment, we caged the queen using queen excluder material. Again, the queen's movement was limited and she could not lay eggs, but workers could physically contact her with their bodies. In the final treatment, we confined the queen to a honey super containing frames with honey or wax foundation. There were no cells in the honey super for the queen to lay eggs. The queen could move among the frames and fully contact the workers. Three full sized colonies housed in Langstroth equipment were used for each treatment. We exposed queens to each condition for two weeks. The volatiles produced by the queens were collected using solid phase microextraction devices and analyzed using gas chromatography and mass spectroscopy. We sampled the volatiles prior to the treatment (when the queens were unrestricted in movement and egg laying) and again immediately after the treatment. The number of supersedure cells constructed by the workers also was recorded.

We followed the changes in five compounds that occurred consistently in the volatile profiles of the queens. When queens were confined in cages and could not lay eggs or contact workers except with their mouthparts, they produced reduced amounts of all five compounds compared with when they were free running in the colony (Figure 1).

Figure 1. Relative amounts of five volatile compounds produced by honey bee queens before and after various treatments. Negative values indicate a reduction in the amount of compound after the treatment relative to the amount produced prior to it.



Queens confined in excluder cages where they could contact the workers had reduced amounts of compound represented by peaks 3 and 4 but had increased amounts of peaks 5-7. When queens were confined in the honey super, the only compound produced at lower levels then when the queen had unlimited space to lay eggs was the one represented by peak-3.

We tested for a relationship between the number of supersedure cells constructed by workers during each treatment and the amount of each of the five compounds produced by the queens.

The only compounds that were significantly related to the number of supersedure cells were those represented by peaks 5 and 6 (Peak 5: $R^2 = 74.7$; $p = 0.026$; queen cells $= -0.18 + (1.94 * p5)$; peak 6: $R^2 = 91.1$; $p = 0.012$; queen cells $= 0.04 + (0.44 * p6)$). Amounts of peak 7 were highly correlated with peak 6 ($p<0.0001$) suggesting that the two are related in their biosynthesis. When amounts of compounds represented by peaks 5 and 6 peaks were used to derive an equation predicting the number of supersedure cells constructed by workers, more than 98% of the variation in queen cells among treatments could be accounted for by the amounts of those compounds (Peaks 5 and 6: $R^2 = 98.6$; $p = 0.080$ peak 5, $p = 0.007$ peak 6; queen cells $= 0.14 - (0.29 * p5) + (0.50 * p6)$.

Conclusions

Results from this study indicate that colonies that have become Africanized can be requeened with a mated European queen, but the time of year will affect the success rate. We had the fewest supersedures of European queens introduced into Africanized colonies when we requeened in the fall.

We have found five compounds consistently produced by queens that might communicate their egg laying state. All of the volatile chemicals produced by queens decreased when the queens were confined and could not lay eggs or physically contact nestmates. The amounts of three compounds increased with worker contacts. The amounts of two other compounds were highly correlated with the number of supersedures produced by workers. We are in the process of identifying the compounds. The compounds correlated with supersedure events could provide a basis for selecting European queens that would be accepted by Africanized colonies. High amounts of those compounds in conjunction with introducing queens in the fall could significantly increase the success rate of queen introductions and prevent colonies used for almond pollination from becoming permanently Africanized.