

# Preparing for Africanized Honey Bees: Evaluating Control in Mating Apiaries

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## SUMMARY

The queen production industry, as we know it today, is seriously threatened by the Africanized honey bee. In this study we collaborated with two commercial queen producers from Texas with the following objectives: 1) Develop techniques that will allow queen producers to assess mating control; and 2) Test the hypothesis that commercial queen producers can control at least 90% of the matings.

Cordovan queens and drones were used to assess control in mating apiaries (that is, the relative proportion of managed and feral drones that mate with queens). Three types of apiaries were measured: 1) Remote Non-commercial (used to establish a baseline of feral drone influence); 2) Central Commercial; and 3) Outlying Commercial. Mating control in July was approximately 93% in the Central Apiaries and 83% in the Outlying Apiaries. The estimated amount of mating control predicted for the April-May period, when most of the queens are produced, is 96-98% for the Central Apiaries and 93-96% for the Outlying Apiaries. We believe 90 to 95% is a realistic level of mating control that most queen producers will be able to attain without substantially modifying existing practices.

We predict that genetic material from Africanized bees, after rigorous screening and selection, will be used to improve stock. We suggest that the industry should adopt a new name for tolerable bees that are partially Africanized.

## INTRODUCTION

FIVE YEARS AGO a queen producer, only in a worst nightmare, would have imagined simultaneous threats from tracheal mites, Varroa mites and Africanized honey bees. Tracheal mites are now found in nearly every state; and Varroa mites are found in at least twenty states and are a threat to all the others (Shimanuki 1990). The presence of these parasitic mites and the subsequent border closings and quarantines have reduced the market for package bees and queens. Additionally, beekeepers are reluctant to buy queens or packages for fear of introducing mites to their bees.

The third participant in this nightmare, the Africanized honey bee (AHB), is expected to spread into southern Texas from Mexico during 1990 (Taylor 1988). Beekeepers and bee scientists believe that the threat from the AHB can be reduced by frequent requeening of colonies with desirable stock. This strategy, however, requires a reliable supply of acceptable queens — queens of gentle European stock that have been mated to non-Africanized drones. Presently such queens are provided to U.S. beekeepers by commercial queen producers located mainly in the Southern States and in California. Unfortunately, both these regions are susceptible to the Africanization process. If we were to adopt a zero-tolerance policy toward Africanization (i.e., all bees

must be of 100% European origin), no naturally mated queens could be produced for sale in these areas. Instrumental insemination is adequate for maintaining breeding stock; however, it is not yet practical for the commercial production of queens (Harbo & Szabo 1984, Harbo 1985). Thus, the queen production industry, because it depends on natural matings, is seriously threatened by Africanized bees.

One solution to this problem is the industry acceptance of low levels of Africanization. Queen producers may be able to control 90% or more of the matings of their production queens through drone saturation procedures (Hellmich *et al.* 1988). There is evidence that colonies with 90% European and 10% hybrid (A x E) workers are manageable and virtually indistinguishable in behavior from European colonies. Defensive behavior of a colony was not dominated by hybrids when they comprised about 10% of the population (Collins 1987). These data suggest that beekeepers should be able to tolerate colonies headed by queens that are "mismatched" with 10% or less Africanized drones.

Two U.S. queen producers likely to first encounter the Africanized bee are Howard Weaver & Sons (HWS) and Weaver Apiaries (WA) both from Navasota, Texas. About two years ago Morris Weaver (HWS) and Binford Weaver (WA) expressed an interest in collaborating with the ARS honey bee labs from Baton Rouge and Tucson. This paper reports the first results from this collaboration.

The objectives of the study were twofold: 1) Develop technologies that will allow queen producers to assess mating control; and 2) Test the hypothesis that commercial queen producers can control at least 90% of the matings.

## MATERIALS AND METHODS

### *Drone Equivalents — A measure for mating control*

Before starting the experiment we developed a system that allowed us to measure mating control. Our goal was simple, we wanted to establish the percentage of managed drones that mated with our queens. We did this with a measure we called the Drone Equivalent (DE). The Drone Equivalent essentially measures the effective population of drones within the mating range of the queen. The method resembles mark-and-recapture procedures that are commonly used by biologists to assess animal populations. In this section we describe the mark-and-recapture procedure and how it relates to the Drone Equivalent method.

In mark-and-recapture studies a specific number of animals are captured, marked and then released. Later some of these animals are recaptured along with non-marked

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animals. The ratio of non-marked and marked animals is used to estimate the number of animals in the population by solving for X in the following equation:

$$\frac{\% \text{ non-marked in sample}}{\% \text{ marked animals in sample}} = \frac{\# \text{ animals in population (X)}}{\# \text{ marked animals released}}$$

During such studies biologists assume that marked and non-marked animals behave in a similar manner and that the sample represents the real ratio of marked and non-marked animals in the population.

In our study cordovan, a single-gene recessive trait for light-brown (cordovan) cuticle, functions as the mark. Cordovan queens are mated from an apiary that has a known number of cordovan drones (this corresponds to # marked animals released). Recapture occurs when a queen mates with a cordovan drone. This is detected when the worker progeny of the queen are evaluated. Cordovan workers result from cordovan queens mating with cordovan drones; wild-type workers result from cordovan queens mating with wild-type drones. Since queens mate with several drones, they usually produce both types of workers.

If 10,000 cordovan drones are established in a mating apiary and 10% of the queens' progeny, on average, were cordovan, then an estimate of the wild-type (non-cordovan) drone population is derived from this:

$$\frac{\% \text{ wild-type progeny in samples}}{\% \text{ cordovan progeny}} = \frac{\# \text{ wild-type drones in population (X)}}{\# \text{ cordovan drones in apiary}}$$

$$\frac{90\%}{10\%} = \frac{X}{10,000} \quad , X = 90,000 \text{ drones}$$

The progeny ratio suggests that cordovan queens, on average, mated with nine wild-type drones for every one cordovan drone. The influence of non-cordovan drones is equivalent to 90,000 cordovan drones that are centrally located, or 90,000 Drone Equivalents (DE).

This DE determination, however, does not distinguish between feral and managed wild-type drones. In a commercial apiary this would mean that a producer still could not evaluate mating control because the feral baseline is unknown. An estimate of the feral drone population in terms of Drone Equivalents (feral<sub>DE</sub>) would solve this problem. Thus two types of DE determinations are necessary:

- 1) Commercial<sub>DE</sub> - conducted in commercial apiaries;
- 2) Feral<sub>DE</sub> - conducted in remote apiaries.

The remote apiaries have no managed drones, except the cordovan test drones, within a 5-mile (~8 km) radius. Only mating colonies with cordovan virgins and drone source colonies that produce only cordovan drones are present in a remote apiary.

The feral<sub>DE</sub> establishes a baseline of feral drone influence. A measure of the influence of managed drones, again in terms of DE, is calculated from:

$$\text{managed}_{DE} = \text{commercial}_{DE} - \text{feral}_{DE}$$

The managed<sub>DE</sub> measures the influence of drones from the mating apiary and drones from any other apiary that could mate with test queens. The ratio of managed<sub>DE</sub> to feral<sub>DE</sub> determines whether it is possible to attain acceptable matings in a particular apiary.

The reason for using the term Drone Equivalent rather than simply Drone is that a population of drones affects a mating apiary not only by their numbers but also by other factors, such as, distance from the mating apiary, weather at the time of matings, terrain and vegetation. As stated before, the DE measures the effective drone population within the mating range of the queen. By using this measure we focus on the result of a particular apiary and avoid the

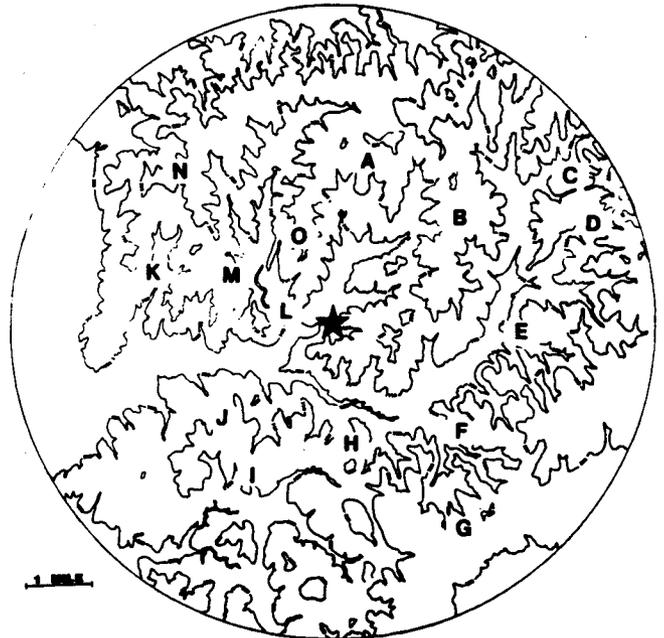


Figure 1. Contour map for area surrounding Central apiary 2 (star) with all managed colonies (A through O) within a five-mile radius. Number of full-sized colonies (FSC) and nucleus colonies (NC) at each of the apiaries during the April-May and July-August periods are designated as follows: [Period: Apiary (#FSC, #NC)]; [April-May: Central (52, 1280); A (0, 0); B (10, 0); C (20, 0); D (60, 2470); E (57, 1110); F (53, 2130); G (40, 0); H (56, 1310); I (35, 0); J (45, 0); K (60, 1680); L (17, 0); M (70, 2260); N (25, 0); O (25, 0)]; [July-August: Central (0, 300); A (56, 0); B (10, 0); C (6, 0); D (15, 400); E (21, 0); F (24, 400); G (12, 0); H (23, 0); I (0, 0); J (3, 0); K (50, 600); L (2, 0); M (40, 300); N (12, 0); O (10, 0)].

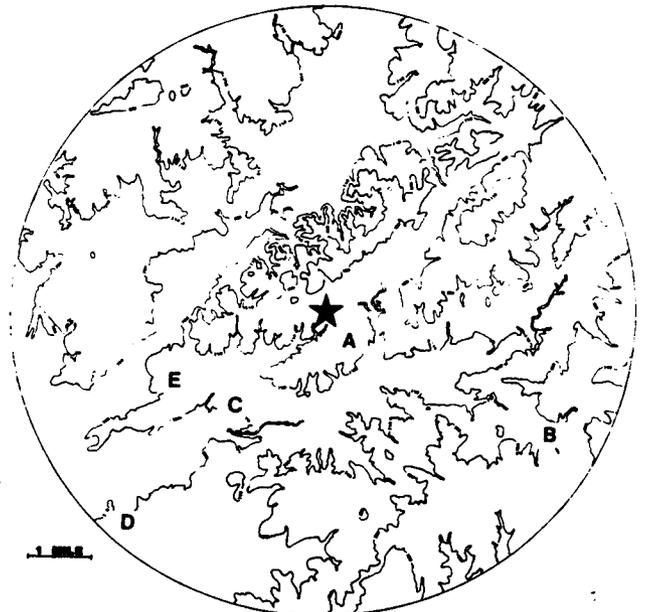


Figure 2. Contour map for area surrounding Outlying apiary 1 (star) with all managed colonies (A, B, C, D, E) within a five-mile radius. Number of full-sized colonies (FSC) and nucleus colonies (NC) at each of the apiaries during the April-May and July-August periods are designated as follows: [Period: Apiary (#FSC, #NC)]; [April-May: Outlying (60, 1500); A (49, 0); B (51, 0); C (46, 0); D (62, 0); E (48, 0)]; [July-August: Outlying (20, 500); A (0, 0); B (0, 0); C (0, 0); D (0, 0); E (0, 0)].

unnecessary and difficult determination of actual drone population. Consequently, the Drone Equivalent simplifies procedures for queen producers, and it uses a measure that is familiar to them, drones from drone source colonies.

We assumed that cordovan queens mated randomly with cordovan and wild-type drones, that is, no assortative mating (positive nor negative) occurred. This assumption is supported by tests in Kansas (Taylor et al. 1989). We also assumed that there was a low frequency of cordovan genes, preferably none, in the feral and managed populations. Another point to consider is that we cannot assume the DE is a static measure. It will vary yearly, seasonally and even daily as populations of drones fluctuate. The value of the DE will increase when it is determined during the season when most queens are produced. Furthermore, the accuracy of the DE should improve if an average value is generated by repeating the procedures one or more times.

#### Experimental Design:

The experiment was conducted in Grimes and Waller counties in Texas during July 1989. The low rolling hills of this area are interspersed with forests of deciduous and evergreen trees, farmland and permanent pastures. All colonies used in this study were either from the apiaries of Howard Weaver and Sons or Weaver Apiaries, who propagated test queens, established temporary apiaries, and provided experimental mating and drone source colonies.

Mating units were similar to those commonly used by commercial queen producers, small double-chambered hives (about five liters per chamber). Each chamber contained about a half pound of bees (227 g), three frames of drawn comb and a division-board feeder. Drone source colonies were comprised of two 10-frame Langstroth chambers with 6-9 lbs. (3-4 kg) of bees, all stages of brood and the equivalent of 2-3 frames of drone comb.

We established Drone Equivalent values for six apiaries which were divided into three categories:

1. Remote Non-commercial — Apiaries located five miles or more from known managed colonies. These apiaries were used to estimate contributions from feral drones. (Remote 1 and 2)
2. Central Commercial — Apiaries located near home operations which are clustered near several other apiaries. These apiaries were expected to have a high level of mating control. (Central 1 and 2)
3. Outlying Commercial — Isolated apiaries that are used to maintain and produce particular lines of queens. Mating control in these apiaries was expected to be lower than that of the Central Commercial Apiaries. (Outlying 1 and 2)

One of the Central Commercial apiaries (Fig. 1) and one of the Outlying Commercial apiaries (Fig. 2) used in the experiment are mapped with all colonies that were within a five-mile radius during April and July. (The maps for the other Central Commercial and Outlying Commercial apiaries are similar to those shown.)<sup>3</sup> The remote mating apiaries used in this study were located at least five miles from commercial colonies in areas with terrain and flora similar to that of the commercial mating apiaries. We assumed that the population of feral drones in these areas was similar to those in the commercial setting. These remote apiaries were used to establish a baseline estimate of the feral drone influence (feral<sub>DE</sub>). Only cordovan drones and queens were present in these apiaries, i.e., we eliminated all drone brood and wild-type drones in the mating nucs.

<sup>3</sup>Nucleus colonies are included on the maps because about 10-15% of these had drones. The relative contribution these drones had on the matings is unknown.

Cordovan breeder queens which produced only cordovan progeny were used to produce cordovan virgin queens. (Breeder queens had been instrumentally inseminated with semen from cordovan drones.) Two lines of unrelated breeders were selected in order to reduce inbreeding problems. One line was used to produce queens for the drone source colonies; and the other was used to produce test queens.

Drone source queens were naturally mated approximately three months prior to our mating experiment. Although these queens open mated randomly, viz to wild-type drones, they still produced only cordovan drones due to the drone's haploid nature. These cordovan drone source colonies were moved into the test apiaries approximately one week before our test matings commenced.

#### Drone Counts:

The number of cordovan drones flying in each experimental apiary was estimated with a drone trap placed on the bottom of each drone source colony. (Note that we counted only flying drones.) This trap utilized one-way exits and a queen excluder (Fig. 3). All drones left the colonies through one of four extended plastic tubes ( $\frac{3}{8}$  in. i.d.). When drones returned to the colonies they were trapped between the bottom board and a queen excluder. No drones were seen returning to the inside of these colonies through the exit tubes.

The number of drones trapped under these excluders was counted with a mechanical counter after supers and brood chambers were removed. Drone counts were made in the morning before drones started to fly after drones had flown with the drone trap in place for two or three days.

A survey for cordovan drones was made in the commercial apiaries prior to the experiment. No cordovan drones were found in Central Apiary 1 and Outlying Apiaries 1 and 2. A small number of cordovan drones (< 0.1%) were found in Central Apiary 2; such a low frequency was considered insignificant.

#### Mating Queens and Counting Progeny:

Mature queen cells that contained cordovan virgins about to emerge were introduced into 30 to 40 mating colonies at each mating apiary. These queens remained in

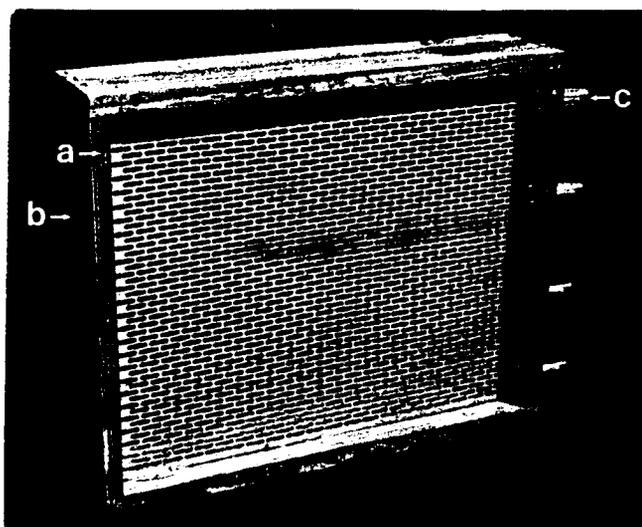


Figure 3: The drone trap is simply a queen excluder nailed between two wooden frames (a & b) that is put between the bottom board and the first brood chamber. Its function is similar to that of a pollen trap with drone escapes. Drones leave the colony through one of the plastic tubes (c); then, unable to locate tube entrances, they return to the cavity formed by the wooden frame (b) and the bottom board.

the mating nucs until a frame of sealed brood had been produced. About a week after the first cells were capped frames of brood were distinctively marked and placed above the brood chamber of a full-sized colony which served as an incubator colony. These brood frames were later put in nylon mesh bags immediately prior to worker emergence. After 100-200 workers had emerged, they were shaken into plastic bags and frozen. Later they were sorted into cordovan and wild type and counted.

## RESULTS AND ANALYSES

### Establishing a Feral Baseline:

The average percentage of cordovan progeny produced by cordovan test queens at each of the remote apiaries is presented in Table 1. The 25.1% value for Remote 1a, for example, suggests that queens mated with wild-type and cordovan drones in approximately a 3:1 ratio. This table also lists the estimated number of cordovan drones that were flying when the queens mated. This value for Remote 1a was 1400. Since we know that the queens mated to about three times more wild-type drones than cordovan drones, the influence of wild-type drones to the matings was equivalent to approximately 4200 (3.0 x 1400) cordovan drones, or  $feral_{DE} = 4200$ .<sup>4</sup> The average influence feral drones had on the matings at the remote apiaries was 7,800 DE  $\pm$  3700 (x  $\pm$  standard deviation; Table 1). Based on these measures we are 95% confident that feral drone influence in this part of Texas during July 1989 fell between 3,600 and 12,000 DE. This confidence interval is designated by two vertical hatched lines in Figures 4 and 5.

### Evaluating Commercial Apiaries:

The Central Apiaries had a significantly higher commercial<sub>DE</sub> (108,600  $\pm$  21,900; t = 4.04; df = 4; P < 0.01) than did the Outlying yards (Table 1, Figure 4). The mating control estimate for these apiaries (when  $feral_{DE} = 7,800$ ) was

<sup>4</sup>Drone Equivalents (DE) are calculated from  $DE = (PW/PC) \times D$ , where PW = % wild-type progeny, PC = % cordovan progeny, and D = # cordovan drones in test apiary.

93% (95% confidence interval 91 to 94%).<sup>5</sup> The Outlying Apiaries, on the other hand, had a commercial<sub>DE</sub> mean of 46,600  $\pm$  9,800. The influence the queen breeders had in this area in July 1989 (again when  $feral_{DE} = 7,800$ ) was approximately 83% (95% confidence interval 79 to 86%).

Mating control changes with the feral population of drones as depicted in Figure 4. This graph shows these changes for commercial<sub>DE</sub> means representing "outlying" and "central" apiaries. As mentioned previously, the two vertical hatched lines in this graph represent the 95% confidence interval for  $feral_{DE}$ .

## DISCUSSION

When Morris Weaver (HWS) was informed that he had better than 90% control at his home apiary his response was, "I knew that." We received a similar response from Binford Weaver (WA). Queen producers who rigorously select breeder queens, and carefully monitor their product, know that they have a high level of genetic control. But, the level of this control, until now, has largely been open to speculation.

This is the first study to document mating control for commercial queen producers and the results are encouraging. Mating control percentages were higher than 90% for Weavers' Central Apiaries. These apiaries, even if Africanized bees are present, should produce acceptable queens to head commercial colonies. As mentioned previously, European colonies in Venezuela that had 10% Africanized-European hybrids displayed acceptable levels of defensiveness (Collins 1987). Mating control in the Outlying Apiaries, however, fell below this arbitrary 90% level. These locations, too, might still produce acceptable queens if they are mated earlier in the season when more drone source colonies are still in place, or if the drone source colonies are not moved. The DE values established during this study were determined during July when 50 to 80% of the drone source colonies had been moved to nectar flows in other parts of

<sup>5</sup>mating control % =  $managed_{DE}/commercial_{DE}$  or  $(commercial_{DE} - feral_{DE})/commercial_{DE}$ .

Table 1.

Location	# Cord. Test Queens	% Cord. Workers	Wild-type/Cord.	Estimated # Cord. Drones in (x) col.	Drone Equivalents (DE)	x $\pm$ SD
					Feral <sub>DE</sub>	
Remote						
1 <sub>a</sub>	35	25.1	3.0	1,400 <sub>(10)</sub>	4,200	
1 <sub>b</sub>	17	15.3	5.5	1,400 <sub>(10)</sub>	7,700	
2	30	14.1	6.1	1,900 <sub>(10)</sub>	11,600	7,800 $\pm$ 3,700
					Commercial <sub>DE</sub>	
Central						
1 <sub>a</sub>	28	6.0	15.6	8,100 <sub>(44)</sub>	126,400	
1 <sub>b</sub>	15	8.8	10.4	8,100 <sub>(12)</sub>	84,200	
2	20	5.2	18.3	6,300 <sub>(50)</sub>	115,300	108,600 $\pm$ 21,900
Outlying						
1 <sub>a</sub>	24	4.3	22.3	1,800 <sub>(12)</sub>	40,100	
1 <sub>b</sub>	22	3.8	25.4	1,800 <sub>(12)</sub>	45,700	
2 <sub>a</sub>	26	4.3	22.1	1,800 <sub>(12)</sub>	39,800	
2 <sub>b</sub>	19	2.9	33.7	1,800 <sub>(12)</sub>	60,700	46,000 $\pm$ 9,800

Table 1. Mean percentage of cordovan workers counted from approximately 200 workers from each of the queens that were mated in Remote, Outlying and Central apiaries. (Measures that were repeated at a location are denoted by n<sub>a</sub> and n<sub>b</sub>. All measures were conducted during July.) These percentages were used to derive non-cordovan to cordovan ratios. Drone Equivalent (DE) values for Remote (feral<sub>DE</sub>), and Central and Outlying (commercial<sub>DE</sub> which is equal to feral<sub>DE</sub> and managed<sub>DE</sub>) apiaries were calculated by multiplying (ratio of non-cordovan to cordovan progeny) by (estimated # of cordovan drones). Mean DE ( $\pm$  standard deviation) were calculated for the three types of apiaries.

Texas or to North Dakota. The estimated amount of mating control predicted for the April-May period, when most of the queens are produced, is 96-98% for Central Apiaries and 93-96% for Outlying Apiaries. Weavers also influence, at least to some degree, the genetics of the area's feral population. Therefore, actual genetic control in these apiaries may be higher than the above figures.

The concentration of feral colonies may increase when Africanized bees spread into the Navasota area. Yet even if these populations double ( $15,000 > \text{feral}_{DE} < 20,000$ ), mating control should be acceptable for both outlying and central apiaries in April and May (Commercial<sub>DE</sub> in the 150,000 to 400,000 range; Figure 5).

Mating control can be improved either by increasing the number of managed drones or by decreasing the number of feral drones (Hellmich 1988). However, a point of diminishing returns is reached for both methods when control is in the 95 to 99% range (Figure 5). A queen producer, under these circumstances, may not be able to justify the cost of altering management practices for such a modest increase in control. We consider 90 to 95% to be a realistic level of mating control (Commercial<sub>DE</sub> in the 75,000 to 200,000 range) that most queen producers will be able to attain without substantially modifying existing practices.

When the control is 90% this does not mean that every queen will mate with 10% Africanized drones. Due to chance and the fact that queens mate with many drones, we know some of the queens will mate with a lower percentage and some with a higher percentage of Africanized drones. For example, when there is 90% control, we calculated that about 35% will mate only with European drones while 26% of queens will mate 20% or more with Africanized drones (Figure 6). With 95% control most queens will mate only with European drones, but about 9% will mate 20% or more with Africanized drones. Such distributions indicate a need to establish whether colonies with hybrid percentages greater than 10% are also acceptable, or if they can be detected early and requeened.

The question is, are low levels of Africanization acceptable? We think so, and with careful selection such stock could be used to benefit beekeepers. Africanized honey bees have many traits that are different from European honey bees, perhaps some could be used to improve stock. If these bees have no value, the gradual increase or introgression of high levels of Africanized genes into breeding stock can be avoided with rigorous screening for favorable traits. With the arrival of the Africanized bee, U.S. beekeepers will need to be more alert to acceptable and unacceptable bees, and will need to control their stock with more frequent requeening than is practiced today. Yet, all these practices, including some carefully selected material from AHB, should lead to better honey bee stock.

Another question to consider is, what will the Beekeeping Industry call these bees? We suggest that the stigma associated with Africanized bees and the inevitable reference by the media (and others) to Killer bees, should be counteracted. Thus, we recommend the adoption of a new name for tolerable Africanized bees. We should call these bees a name that might have public acceptance, such as: "Select European," "Select American," "Gentle American" or "Gentle Domestic."<sup>6</sup>

The reliability of the Drone Equivalent is only as good as the assumptions that are made. All managed colonies in these areas are assumed to be headed by acceptable queens. This assumption will be particularly important after Africanization occurs. Furthermore, as mentioned previously,

<sup>6</sup>"Domestic" was recommended by Dr. Eric Erickson, Carl Hayden Bee Research Center, Tucson, Arizona.

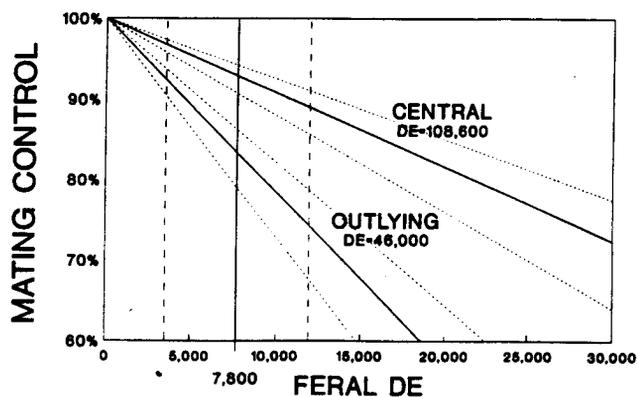


Figure 4. Estimated percentage of mating control in July for Outlying (average DE=46,000) and Central (average DE=108,600) apiaries when the feral population of drones (Feral<sub>DE</sub>) ranges from 0 to 30,000. The hatched lines (---) represent 95% confidence limits for the Central and Outlying DE averages. The vertical lines represent the 7,800 average and the 95% confidence limits (3,600 & 12,000 represented by — —) for Feral<sub>DE</sub> which were estimated at Remote Apiaries. For example, estimated control at the Central Apiaries when Feral DE = 7,800 is approximately 93% and when Feral DE = 20,000 is approximately 82%.

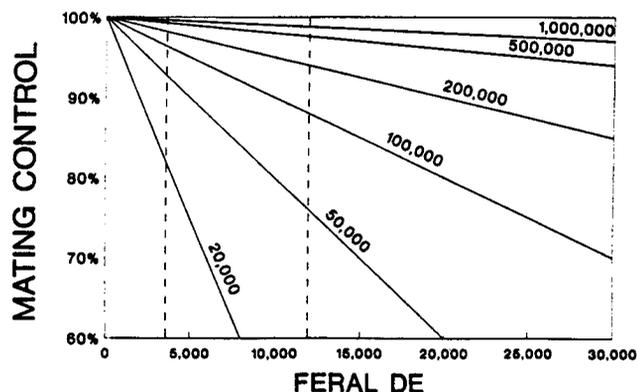


Figure 5. Estimated percentage of mating control when the feral population of drones (Feral<sub>DE</sub>) ranges from 0 to 30,000 and Commercial<sub>DE</sub> = 20,000, 50,000, 100,000, 200,000, 500,000 and 1,000,000. The hatched lines represent the 95% confidence limits (3,600 & 12,000) for Feral<sub>DE</sub> which were estimated at Remote Apiaries.

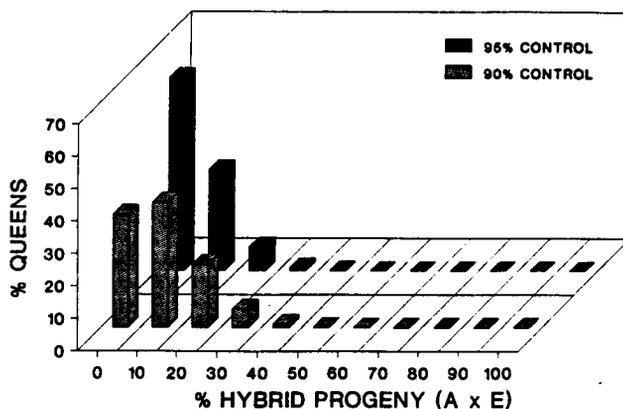


Figure 6. Percentage of European queens expected to produce a designated percentage of hybrid (A x E) progeny when 90% and 95% of the matings are controlled in an Africanized area. For example, when 95% of the matings are controlled, about 60% of the queens will produce all European progeny, and about 32% of the queens will produce 10% hybrid progeny. These values are based on the binomial sampling distribution. For these calculations, queens are assumed to mate randomly with 10 drones.

we also assume that our test queens mated randomly with test (cordovan) and non-test (wild-type) drones. The validity of this assumption will not be known until we better understand the mating behavior of queens and drones. If matings are nonrandom and the degree of nonrandomness can be measured, then DE values can be adjusted. A DE is underestimated when test queens preferentially mate with test drones, and overestimated when test queens preferentially mate with non-test drones.

This study should serve as a prototype for similar studies throughout North America. If queen producers establish DE measures in their regions, they will be able to: 1) assess mating control in their apiaries, and 2) adjust this control in predictable amounts either by increasing managed drones or by decreasing feral drones. Equipped with such information, queen producers will be better prepared to make decisions concerning Africanized honey bees.

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