EVALUATION OF DIFFERENT QUEEN INTRODUCTION METHODS IN HONEYBEE COLONIES IN URUGUAY[1]

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QUEEN INTRODUCTION METHODS IN URUGUAY

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Abstract

Four methods for introducing queens in productive honeybee colonies were evaluated: mated queens (MQ), virgin queens (VQ) and queen cells (QC) in dequeened colonies, and protected queen cells (PQC) in colonies with queens. Two requeening tests were done at the beginning of the spring and two at the end of the summer. Among the introduction methods used in dequeened colonies none of them oustanded consistently according to the number of accepted queens. Even so, method MQ was significantly more efficient in the spring requeenings when both requeening periods were analized together. On the contrary, the PQC method had almost no success, only replacing the queen in one out of 54 colonies in which it was employed. The maximum acceptance was of 80,8% and the minimum of 48,9%, both corresponding to the end of summer requeenings. The number of queenless colonies at the end of the evaluation was between 11,5% and 23,7%.

Key words: honeybees/queen introduction//Uruguay

Introduction

Most beekeepers in Uruguay do not replacement failing queens in their hives because of the cost of the queens, the cost of transport to the apiaries, the difficulty to find the queen in heavily populated colonies (in Uruguay queens are usually not marked), the low acceptance of the introduced queens and the loss of productive units when they remain queenless. The use of queens is normally reduced to the formation of new productive units from small bee nucleii, to replace the winter loss of colonies or to increase the number of hives. These are usually done at the beginning or at the end of the season so as not to weaken the colonies during the months of high nectar flow.

The most important benefit of requeening is the increase in honey production, as colonies are provided with young and prolific queens. In addition, the new queen stock can improve some brood disease conditions.

Requeening frequency is very variable and depends on the queen activity. Queens that maintain a high constant rate of egg laying can get exhausted after a year and are usually replaced (Furgala and McCutcheon, 1992). However, in regions with cold fall and winter, as in Uruguay, queens reduce drastically or interrupt egg laying during this period. In this situation queens can continue being very prolific for two or three seasons (Szabo and Lefkovitch, 1989; Szabo and Lefkovitch, 1991). Uruguayan beekeepers estimate that a queen can maintain a strong hive at least for two seasons.

Queen introduction constitutes a key stage of requeening because her acceptance by the colony is critical for success. The proportion of accepted queens has an important influence in beekeeping production, as the colonies that reject the new queen must develop another one from worker larvae of less than three days of age. This generates, among other problems, a delay in the development of the hive that diminishes honey production. If the colony does not have young workers larvae available because too many days have passed since its dequeening, the colony may remain as a laying worker colony (workers that lay unfertilized eggs), and in this case a complete productive unit is lost.

Ruttner (1983) describes the multiple factors that contribute to the success of the introduction of a new queen: condition of the resident queen (age, laying activity, etc.); condition of the queen to be introduced (mating, damage during transport, egg laying activity, production of pheromones, etc.); size and weight of the queen to be introduced; characteristics of the colony that receives the new queen (race, aggressiveness, seasonal development, ratio between young and old bees, presence of laying workers); external conditions (nectar flow, climatic conditions, season of the year). These large quantities of factors act simultaneously and are very difficult to control during research work.

In spite of the several queen introduction methods described (Johansson and Johansson, 1971; Ruttner, 1983), in Uruguay beekeepers usually use four introduction methods:

1) Introduction of queen cells in dequeened colonies: consists of placing a queen cell between the frames of the brood chamber 24 to 48 hours before the emergence of the queen. This method has several risks: the possibility that the queen dies inside the cell (for example, due to chilling), that it may emerge with

morphological problems (for example, incomplete development of the wings), the fact that it must still accomplish the mating flights, and that its egg laying pattern is unknown. On the other hand, queen cells introduction is the most economical option for the beekeeper.

2) Introduction of caged virgin queens in dequeened colonies: the virgin queen is kept inside a cage that has in an escape hole filled with candy. This cage is put among the brood frames and the bees free the queen after several hours by gnawing the candy. During this time the queenless workers get familiarized with the scent of the new queen (which in turn gets impregnated with the odor of the hive), they feed it, and finally accept it as queen of the colony. This introduction method shares many disadvantages with the former method. The benefits are the possibility of an early evaluation of the queen's morphology and the possibility of maintaining the queen for some days before it is introduced in the hive. The cost of virgin queens is higher than the queen cells (approximately twice as costly).

3) Introduction of caged mated queens in dequeened colonies: the process is similar to the former one, except that the queen is already fecundated. This fact gives the beekeeper the guarantee that the queen was previously evaluated with regard to its egg laying pattern, brood health and, eventually, the characteristics of its worker daughters in the mating nucleus. Moreover, queens start to lay eggs a few days after being introduced, so the population of the colony is not affected. This method seems to be the better option from the productive point of view; however, mated queens have a higher cost.

4) Introduction of protected queen cells in queenright colonies: this method is

similar to the introduction of queen cells in dequeened colonies, but with the advantage that is not necessary to dequeen the colony. In order to avoid that the queen destroy the queen cells (a frequent behavior when a queen cell is introduced in a colony that has a queen), a plastic tube is used to cover the cell that prevents the typical attack from the side walls. This method is based on the premises that the colony prefers a new queen instead of an old one, and that if both queens meet the young virgin queen will probably kill the old one. The practical advantages of this process are clear: there is no waste of time searching the old queen in order to dequeen the colony (one of the main difficulties that the former methods present), one or more visits to the apiary are avoided and the queen cells have a low cost. However, this method has the same risks detailed in point 1. In addition, the response of the workers when they find the newborn queen is not clear (as they have never been queenless). Several researchers have evaluated this introduction method moved by its operative advantages, obtaining very diverse results, surely due to the different methodologies employed (Forster, 1972; Reid, 1977; Peer, 1977; Boch and Avitabile, 1979; Jay, 1981; Szabo, 1982).

As it was formerly described, the different introduction methods vary between them in the economic values of the queens to be introduced, the apiary management requisites and the risks of introducing defective or low quality queens. However, in order to determine the most appropriate method, and in addition to this group of variables, it is essential to know the proportion of introduced queens that are accepted by the colonies with the different methods and regarding the different requeening periods. This work intends to elucidate this aspect by employing the usual methodology of professional beekeepers of Uruguay.

Materials and methods

The requeening experiments were carried out in Sarandí Grande, department of Florida, in a region with a high density of hives. Bees of this region are hybrids of *Apis mellifera mellifera* and *A. mellifera scutellata* (Burgett *et al.*, 1995; Diniz *et al.*, 2003). The production apiaries employed had between 15 and 25 Langstroth hives and were situated within a radium of 5 km, so that all of them were exposed to the same climatic conditions.

Four requeening experiments were made at the end (March) and the beginning (October) of the production seasons of 2002 and 2003. In March the colonies presented a bee population that exceeded widely the brood chamber and the brood was distributed among 6 to 9 frames. In October the colonies had a lower population, especially the ones that were used in 2003. Even though, the bees covered between 6 and 10 frames of the brood chamber and the brood was distributed among 3 to 5 frames. The age of the queens of the colonies was unknown. Weak or ill hives were not taken into account.

Four methods for queen introduction were employed.

In queenless colonies:

Queen cells (QC): the cells were located between the top bars of two frames in the middle of the brood chamber.

Virgin queens (VQ): the marked queens were kept alone inside a cage with an escape tube filled with candy. The cages were located with the escape tube pointing downwards between the top bars of the frames in the middle of the brood chamber.

Mated queens (MQ): the same as in the former method, except that the marked queen was kept with 5 to 6 workers to feed it.

In queenright colonies:

Protected queen cells (PQC): the cells protected by a plastic tube were situated between the top bars of two frames on the side of the brood chamber.

In the year 2002 the four methods for introducing queens were employed, while in the year 2003 the method PQC was not included.

In each apiary the different requeening methods were asigned to the colonies at random. The number of colonies for each treatment ranged from 24 to 37 in the four requeening periods.

The queen cells, virgin queens and mated queens were supplied by a queen breeder that obtained them from hybrid Italianized colonies. In the case of the queen cells, special care was taken in that the queens emerged 24 to 48 hours after situating the cells in the hives.

Dequeening the colonies was carried out 5 days before introducing the new queens. In order to find the queen the bees were strained by shaking the frames over a queen excluder, set over a device that allowed the collection of the strained bees and so helped them to return to the hive. In the four tests the dequeening of the colonies was carried out by the same 6 people in pairs to work with each hive. The time employed in finding the queen in each hive was calculated as the average time employed by the group in the inspection of all the hives in the different apiaries (the time spent in transport and equipping was not included). The efficiency of the work was determined by the number of queens found over the total number of hives inspected.

Before introducing the queen cells and the cages with virgin and mated queens in the queenless hives, all the brood combs were carefully inspected in order to destroy the emergency queen cells that the bees had constructed. However, the bees could construct new queen cells as they still had worker larvae of two days of age available, capable of developing as queens. These queen cells were not destroyed afterwards. In the case of the queenright hives (PQC method) the presence of the queen and the absence of queen cells were verified.

From the moment the queens were introduced the hives were inspected periodically, registering the different possible situations: the emergence of the queens, the release of the caged queens, the presence of emergency queen cells and their state (entire or opened by its sides), the presence or absence of the old queens in the colonies where the method PQC was used, the date of the beginning of egg laying of the introduced queens (determining by visual inspection the age of the brood; it was only registered in the requeenings of the year 2003), the emergence of queens from emergency cells, the beginning of egg laying of the queens reared by the bees.

Results

The methods QC, VQ and MQ used on queenless colonies did not present differences in the number of accepted queens in the four requeening periods (March 2002: G = 1,14; df = 2; ns; October 2002: G = 2,52; df = 2; ns; March 2003: G = 4,14; df = 2; ns; October 2003: G = 5,52; df = 2; ns) (Table I). The analysis of the total results also did not show significant differences between the three methods (G = 2,64; df = 2; ns). However, when the results of the two spring requeenings were analyzed together, method MQ appeared as the most efficient (G = 7,64; df = 2; $P < 0,05$) (Table I). Independent of the requeening method employed, the proportion of accepted queens was different in the four requeening periods (G = 10,48; df = 3; $P < 0,05$), being requeenings of year 2003 the least successful ones.

The PQC method employed in the two requeenings of the year 2002 showed almost no success. In March none of the 30 colonies employed accepted the queen and in October in only one of the 24 colonies the replacement of the queen was achieved.

The percent of queens that did not emerge (methods QC and PQC) or that were not set free from the cages (methods VQ and MQ) was low (4% in QC, 5,5% in PQC, 0,9% in VQ and 0,8% in MQ); these situations were considered as not acceptance of the queen.

The days elapsed between the introduction of the queen and the beginning of egg laying, evaluated in the requeening experiments of the year 2003, is shown in Table II.

The time spent in the inspection of the hives and the efficiency in finding the queens are shown in Table III.

Table I. Final situation of the colonies in the four requeening experiments, introduction of queen cells (QC), virgin queens (VQ) and mated queens (MQ).

Period	Method for introducing the queen	Colonies employed	Colonies that accepted the introduced queen	Colonies that di d not accept the queen and were left with a queen emerged from an emergency cell	Colonies that did not accept the queen and were left queenless (laying worker)
March 2002	QC	26	22 (84,6 %)	2 (7,7 %)	2 (7,7 %)
	VQ	25	21 (84,0 %)	1 (4,0 %)	3 (12,0 %)
	MQ	27	20 (74,1 %)	3 (11,1 %)	4 (14,8 %)
	Total	**78**	**63 (80,8 %)**	**6 (7,7 %)**	**9 (11,5 %)**
October 2002	QC	33	21 (63,6 %)	6 (18,2 %)	6 (18,2 %)
	VQ	25	15 (60 %)	4 (16,0 %)	6 (24,0 %)
	MQ	28	22 (78,6 %)	4 (14,3 %)	2 (7,1 %)
	Total	**86**	**58 (67,4 %)**	**14 (16,3 %)**	**14 (16,3 %)**
March 2003	QC	29	11 (37,9 %)	11 (37,9 %)	7 (24,1 %)
	VQ	30	19 (63,3 %)	3 (10,0 %)	8 (26,7 %)
	MQ	29	13 (44,8 %)	7 (24,1 %)	9 (31,0 %)
	Total	**88**	**43 (48,9 %)**	**21 (23,9 %)**	**24 (27,3 %)**
October 2003	QC	35	17 (48,6 %)	10 (28,6 %)	8 (22,9 %)
	VQ	37	19 (51,3 %)	7 (18,9 %)	11 (29,7 %)
	MQ	37	27 (73,0 %)	8 (21,6 %)	2 (5,4 %)
	Total	**109**	**63 (57,8 %)**	**25 (22,9 %)**	**21 (19,3 %)**
Total	QC	123	71 (57,7 %)	29 (23,6 %)	23 (18,7 %)
	VQ	117	74 (63,2 %)	15 (12,8 %)	28 (23,9 %)
	MQ	121	82 (67,8 %)	22 (18,2 %)	17 (14,0 %)
	Total	**361**	**227 (62,9 %)**	**66 (18,3 %)**	**68 (18,8 %)**

Table II. Days elapsed between the introduction of the queens and the beginning of egg laying in two requeening experiments introducing queen cells (QC), virgin queens (VQ) and mated queens (MQ).

Period of requeening	Method for introducing the queen	Mean ± Standard deviation	Range
	QC	13,8 ± 4,4	7 - 20

	VQ	11,8 ± 2,7	8 – 18
	MQ	9,0 ± 2,6	5 - 14
October 2003	QC	13,8 ± 1,3	11 - 17
	VQ	11,5 ± 2,6	6 – 19
	MQ	5,0 ± 4,6	2 – 14

Table III. Times employed in dequeen a colony (T) and efficiency of the operation (E) measured as number of queen found over the total number of hives inspected in the four requeening experiments.

March 2002	**October 2002**	**March 2003**	**October 2003**
T	**T**	**T**	**T**
13´30´´± 4´50´´	10´52´´± 3´13´´	6´0´´ ± 1´12´´	3´42´´± 2´12´´
E	**E**	**E**	**E**
85,5 %	89,8 %	92,7 %	93,5 %

Discussion

The periodic requeening in order to maintain strong, healthy and productive hives is a fundamental activity in professional apiculture. The economic cost of replacing queens directly in the production hives, and not in small nuclei of bees, is mainly determined by the acceptance of the introduced queen her rejection could lead to colony loss. All methods for introducing queens create conditions for the colony to accept a strange queen that they would normally reject (Johansson and Johansson, 1971).

The PQC method for introducing queens, which *a priori* seemed an economical method that did not put at risk the viability of the colony, showed very negative results with only one case of acceptance. With this method Reid (1977) obtained an acceptance of 75% and 58% replacing queens of two and one year of age, respectively, although these values may be over evaluated as the colonies that remained queenless, weak or that showed other problems were not considered as failures. Peer (1977) also achieved to replace the queens with a high success (80%) introducing the cells at the end of the honey season. However, other researchers only achieved very limited results using this method. Boch and Avitabile (1979) could only replace 10% of the queens of small nucleii, and 15% of the queens of production colonies. They also found that the success in the acceptance increases with the age of the resident queen. Szabo (1982) obtained a reduced acceptance (12,7%) by introducing queen cells without protection in queenright colonies. Jay (1981) only achieved to replace 17% and 31% of the queens in two experiments with similar conditions. This method can be employed by using a queen excluder in order to keep separated for a while the old and the introduced queen. In that way, Foerster (1972) introduced protected queen cells in the lower part of a hive with double brood chamber leaving the old queen isolated in the upper part. Once the introduced queen was mated both parts of the hive were reunited. By this way he achieved to replace 76% of the queens. However, Boch and Avitabile (1979) using a similar technique with a queen excluder only achieved to replace 4,5% of the queens. The acceptance grew significantly when they reunited the colony before the queens were mated.

Taking into account the former results it is difficult to explain the almost no success found for this method. A fight between both queens would hardly happen, something that beekeepers that advocate this method usually say, but most probably the workers, used to their own queen, when they suddenly find the strange queen they will rapidly kill it. Other explanation for the failure of this method lies on racial aspects of the bees. In the works previously cited european bees were surely employed, very different from the hybrid bees that are common in this part of Uruguay, mainly product of the matings of European and Africanized bees (Burgett *et al.*, 1995; Diniz *et al.*, 2003). These bees may react more aggressively when they find strange queens. Moreover, the fact that the introduced queens were Italian slightly hybridized with other bees, could increase the rejection response of the bees due to more acute racial differences.

When the results obtained with the introduction methods QC, VQ and MQ in previously dequeened colonies are analyzed together, the percentages of queen acceptance were very low and many colonies remained queenless. Only in the requeening of March 2002 the queens were introduced with a relatively high success (81%). A factor that may explain the high level of rejection towards the introduced queens is that in production colonies, in contrast to bee nuclei, bees of all ages are present. Szabo and Towsend (1974) found that workers of 14 days of age were more aggressive with virgin queens than the workers of 7 and 21 days of age. In contrast, bee nuclei are made of young bees (nurses) excluding the foragers. These small colonies with a different age composition may be more prone to accept a new queen when they are queenless.

The time elapsed from the dequeening of the colonies and the nectar income, two important factors for the success of requeening (Baribeau, 1976; Ruttner, 1983), do not seem to explain the high level of queen rejection. According to Boribeau (1976) five days of queenless condition is the optimum period for maximizing the acceptance, and is larger than what beekeepers usually use. On the other hand, during the period when the replacements were done there was a continuous nectar flow, even though it was of different intensity.

Considering the three methods as a whole, no association between the moment of the year when the requeenings took place and the acceptance of the introduced queens was found. As a proof of this the maximum and minimum acceptances were obtained in spring. There are no evident reasons for explaining that the two requeenings of year 2002 were more successful than those of year 2003.

Of the three methods for introducing queens in dequeened colonies none of them was consistently more successful than the others. On the contrary, the queen acceptance with any of the methods showed large variations in the different experiments. However, the good acceptance of mated queens stood out in the two spring experiments.

The failure of queen acceptance did not account for the fact that the queens did not emerge (method QC) or that the queens were not released (methods VQ and MQ) as these situations had a low occurrence. So, the failure of the different methods was mainly because the workers directly rejected the new queens or due to the loss of these during mating flights. Studying this last aspect Ratnieks (1990) and Tarpy and Page (2000) found that mating flights are relatively safe, registering losses of 3,85% and 6,25% of the queens, respectively. In both studies the colonies were very close to each other (as in the apiaries employed in this study), which makes it possible for the queen to enter the wrong hive when returning from a mating flight. These results would indicate that the main loss of queens is due to the direct rejection of the colony bees before mating. However, Moritz (1985) using mathematical models found that the risk that a queen is lost during the mating flights is of between 14% to 21% when it mates with 7 to 10 drones. Also Ruttner (in Moritz, 1985) indicates that the loss of queens during mating is of about 15% to 30%. According to these results, very different for the previous ones, the low acceptance of queens, at least in methods QC and VQ, could be explained to a high degree by the loss of queens during mating flights.

In the conditions that this work was carried out the dequeened colonies could construct queen cells from the moment that the queens were introduced. In the colonies that accepted the introduced queen, the queen killed the queens still held inside the cells by cutting small holes in the cell sides and stinging the occupants. Workers then completed cell destruction and destroyed the immature queen (Caron and Greve, 1979). Also, in some cases the queens emerged from these cells but did not substitute the introduced queen already accepted. In the cases where the introduced queen was quickly eliminated, with no time to kill the immature queen, the colony might remain with a queen raised by itself. On the contrary, if the queen was eliminated and at the same time the queens that should emerge from the queen cells were lost for some reason, the colony irremediably remained queenless and in a few days the workers started to lay eggs (laying worker colony). From the beekeeper's point of view, the difference between these two last results implies the maintenance or loss of the productive unit. In the two requeenings of the year 2003, apart from the low acceptance of the introduced queens, there were an important number of colonies that remained with laying workers (27% and 19%). Although it is possible to avoid these results trying to introduce again a new queen and destroying the queen cells immediately after being sure of the failure of the first introduction, the economic cost implied (buying new queens, transport, etc.) limits a lot this complementary management.

The high quantity of colonies with laying workers during spring and summer of recent years, possibly due to problems in natural requeening, is an issue that worries beekeepers.

With regard to the mean time elapsed from the introduction of the queens and the beginning of egg laying, we found, as it was expected, that with the mated queens this time was smaller than with the virgin queens and the queens emerged from queen cells. However, in the requeening of March the mated queens needed an average almost twice the time than in October to begin laying eggs (9 and 5 days respectively). A possible cause of this delay may be the lower nectar flow at the end of the season in comparison with the beginning of spring. As a response to this resources decrease the colonies reduce drastically the population since April and the queens maintain a minimum egg laying pattern that can be interrupted if it is too cold. On the other hand, the virgin queens and the ones emerged from queen cells showed almost identical time registries in both requeenings for the beginning of egg laying, being a bit less for the queens introduced with the VQ method. As the release of the caged queens coincides fairly well with the emergence of the queens, the factor that may affect the different start time of egg laying is the age of the queens, as virgin queens emerged about 24 hours before the moment they were introduced in the hive. However, the time ranges are very wide, what indicates that the moment of the fecundation and the beginning of egg laying do not follow a rigid pattern in the queens.

Among the most important practical problems that beekeepers declare for not doing massive requeenings of their hives is the difficulty in finding an unmarked queen in the hive. The time employed by each pair of researchers to find the queen with the bee straining system diminished drastically along the four requeening experiments, surely due to the acquired experience; meanwhile, the efficacy of the dequeening process (dequeened colonies/inspected colonies) slightly improved. It must be stated that none of the members of the research team was previously skilled in this task. The average time employed inspecting a colony and the efficiency in finding the queen achieved in the last experiment (3:42 minutes and 93,5% respectively) do not seem important obstacles among the stages implied in requeening, and there are no reasons for the beekeepers not to have the same performance.

Finally, in the light of the obtained results, the massive and systematic requeening of the production apiaries would not be advisable, at least with these queen introduction methods, mainly due to the low general acceptance and the loss of important quantities of productive units.

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