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Field study: Factors influencing virgin queen bee acceptance rate in *Apis mellifera* colonies

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Abstract: Regular replacement of old and problematic queen bees is necessary to maintain the strength and productivity of bee colonies. Beekeepers replace queen bees using queen cells, virgin queens, or mated laying queens. In this study, we explored factors influencing the acceptance of the virgin queens. A comprehensive field study was conducted in the summer of 2023, involving the introduction of 754 virgin queens of different age and 194 queen cells into mating nuclei of various states using three different methods. The article aims to provide recommendations to beekeepers for introducing virgin queens, thereby increasing the frequency of successful requeening. The duration of the colony being queenless has a significant impact on the acceptance of queens. If the colony is prepared in advance, at least five days prior, immature and mature virgin queens will be accepted equally well (60–77%). Conversely, suppose queen replacement is done without preparation for a colony that has been queenless for one day only. In that case, the result will be significantly lower due to the short queenless period (41%), and the age factor of queen bees will come into play. If queen replacement is done with preparation, the chosen timing within the season will play a significant role, as weather conditions can influence acceptance and mating, varying from 48% to 89%. Suppose a bee colony has long lost its queen and has developed laying worker bees. In that case, there is still a 55% chance of successfully introducing a virgin queen into such a colony without using time-consuming methods.

Keywords: bee breeding; beekeeping; honey bee; introduction success; requeening; virgin queens

Bee colonies are superorganisms consisting of a queen bee, worker bees, and drones. The queen bee is the only one capable of laying fertilised eggs which develop into the next generation of worker bees. On average, in Europe, annual winter losses of bee colonies reach 16.4%, with 4.3% attributed to issues with the queen bee (Gray et al. 2023). Queen bees typically live for about three years (Page and Peng 2001; Oxley and Oldroyd 2010).

However, their lifespan is influenced by the number of sperm stored in the spermatheca due to mating. Naturally mated queen bees have an average of 4 to 5 million sperm cells stored in their spermathecae (Yaniz et al. 2020). If the remaining sperm count drops below 0.5 million, there is an increased risk that the queen bee will start laying only unfertilised eggs (Paillard et al. 2017), which threatens the existence of the bee colony. Reduced egg-laying and fertility in queen bees are recognised by worker bees, prompting them to initiate a supersedure process. The decrease in queen bee productivity can impact the strength and viability of bee colonies, further complicating their ability to combat diseases and pesticides and

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adapt to changing climate. To prevent a decline in bee colony productivity and introduce desired characteristics (such as good wintering ability, disease resistance, low swarming tendency, high productivity, and gentleness), beekeepers regularly perform queen bee replacement themselves. The beekeeper can raise queen bees or purchase them from queen breeders. Queen bee replacement can be done by introducing a queen cell, a virgin queen, or a mated queen into the colony. Queen bee replacement is often carried out when a problem arises within the colony. Many prefer to introduce mated queens rather than virgin queens, as mated queens have higher acceptance rates (Butler and Simpson 1956; Rhodes et al. 2004; Perez-Sato et al. 2008). However, finding mated queens can be challenging due to high demand and limited availability, so queen bee replacement or rescuing queenless colonies is often done with virgin queens (Laidlaw 1981; the Author's experience).

Several factors influence the acceptance rate of queen bees: the duration of queenlessness in the bee colony (McCutcheon 2001; Perez-Sato et al. 2008), the type of queen bee (virgin, mated), the age of the queen bee (Szabo 1974; Rhodes et al. 2004; Wossler et al. 2006), weather conditions, nectar flow (McCutcheon 2001; Buchler et al. 2013), the ratio of young and old worker bees in the colony (Laidlaw 1981). Various methods of introducing queen bees are used to increase the acceptance rate of queens:

1. The newspaper method is considered one of the most successful methods. First, the queen bee is introduced into a nucleus or "artificial swarm". The nucleus with the queen bee is then placed on top of a strong colony, separating them with newspaper and allowing the bees to unite by chewing through it (Laidlaw 1981; Buchler et al. 2013).
2. The introduction of the queen with a shipping cage is also very popular. There are many types of cages available. The cage entry can be closed with beeswax (Laidlaw 1981) or a paste of honey and beeswax (Perez-Sato et al. 2007). The cage is then placed on top of the combs within the colony, where the queen is intended to be introduced. Worker bees then chew through beeswax or paste to release the queen into the colony.
3. It is possible to directly release the queen in a bee colony on top of the brood combs (Butler and Simpson 1956). Usually, just before introduction, the entire colony is sprayed with sugar syrup (McCutcheon 2001), blown with smoke (Perez-Sato et al. 2008), or shaken off combs, causing chaos (McCutcheon 2001).

The basis of all the above methods is to give time for the scent (pheromones) of the new queen bee to spread in the colony.

The condition of the colony significantly affects the outcome of the acceptance rate of queens or queen cells. First, before introducing a new queen bee, it is necessary to check if there are old queens, supersedure or emergency queen cells, or newly emerged queens in the colony. This is done because worker bees recognise the presence of a foreign queen very well and will not accept it while they have their queen (Pankiw 2004; Cobey 2007). In a study by Szabo (1982), queen cells were placed in colonies with laying queens to induce queen replacement, similar to how it occurs naturally. However, successful queen replacement occurred in 12.7% of cases only (Szabo 1982). Furthermore, the newly emerged queen and the old queen attempt to kill each other, which may result in the loss of both; for example, in Szabo's (1982) study, 7.4% of colonies were left queenless in this way. Significantly better results are obtained when queen cells are placed in queenless colonies, achieving an acceptance rate of 70% (Perez-Sato and Ratnieks 2006). The use of queen cells is primarily a privilege of queen breeders, as the queen cells are susceptible to external factors (temperature, pressure) and can only be introduced into colonies 1–2 days before the queen emerges from the cell.

For beekeepers who do not rear queens, it is possible to purchase virgin or laying queen bees. Laying queen bees are easier to introduce into queenless colonies with high success rates; for example, by directly introducing a laying queen with smoke blowing, a 100% success rate has been reported in 2-day queenless colonies (Perez-Sato et al. 2008) or by introducing a queen with a cage with success rate of 95–100% (Butler and Simpson 1956). Laying queens are easier to introduce than virgin queens because the amount of the queen mandibular gland pheromone (QMP) emitted by queens varies depending on their age and fertility, and worker bees recognise their queen based on this pheromone, as well as workers become selective in accepting new queens (Pankiw 2004; Cobey 2007). Younger immature queens (1–4 days old) will emit the

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least pheromone. In comparison, queens that have reached sexual maturity after five days (Page and Peng 2001), recently mated queens, and long-term laying queens will emit more QMP (Cobey 2007). Therefore, worker bees prefer a queen bee that will quickly restore the strength of the bee colony. When comparing virgin queens, many bees surround older virgin queens (Szabo 1974). No differences in pheromone levels were observed when comparing virgin queens aged 7 or 14 days (Wossler et al. 2006). The acceptance of virgin queens has a lower success rate than in naturally mated queen bees or queen cells, so it is essential to consider the queen's age and the queenlessness duration in the target colony.

In the literature, the acceptance rate of virgin queens varies widely from 15.4% (Rhodes et al. 2004) to 92% (Butler and Simpson 1956; Perez-Sato et al. 2008). If the queenless period in the colony is only one day, virgin queens are accepted very poorly. Better results are achieved when the queenless period in target colonies is 5–6 days (Perez-Sato et al. 2008). The longer the colony is queenless, the easier it is to introduce virgin queens (Butler and Simpson 1956; Perez-Sato et al. 2008). Introducing queens aged 0– 24 h with a cage to a 3-day queenless nucleus resulted in 65% of laying queens. Conversely, using the same method to introduce queens aged four days, the acceptance was 55% (Perez-Sato and Ratnieks 2006). These results indicate that the queenless period in the target colony and the queen bee's age are essential factors.

Different acceptance results were obtained also using various introduction methods. For example, introducing virgin queens aged 1–28 days with a cage to a 5-day queenless colony achieved 92% acceptance (Butler and Simpson 1956). Introducing queens aged four days to 2-day queenless nuclei using four different methods (with the queen in a natural queen cell, with the queen in an artificial queen cell, with a queen cage and holding the queen in a cage for two days) resulted in 95, 93, 47 and 73% acceptance, respectively (Perez-Sato et al. 2007). However, when directly introducing queens with smoke blowing, the acceptance was 83% (Perez-Sato et al. 2008), which is much less laborious when compared with the other four methods. In a study by Rhodes et al. (2004), the acceptance rate for seven-day queens ranged from 15.4% to 28.2%, while for older queens aged 14–35 days, it was 66–95%. However, there is a lack of information on the target nucleus or colony into which the queens were introduced, and some of the older queens were already mated as they were taken from open-mated nuclei (Rhodes et al. 2004), which could significantly offset the data. The acceptance results of virgin queen bees vary greatly from one source to another. Some studies lack details such as the age of the queens, the number of queens studied, and the condition of the target colonies (queenlessness, presence of emergency queen cells, presence of laying workers), which makes it more difficult to explain the results. Sometimes, the results were related to accepted queens, not successfully mated queens, or results of virgin and laying queens were combined.

There are as many methods used for introducing queens as beekeepers who practice it, resulting in various outcomes. Virgin queens are more readily available to beekeepers, and each successful introduction is crucial in small apiaries. Therefore, data-driven recommendations are necessary to help beekeepers increase the frequency of successful queen introductions into bee colonies, reduce the risk of losing colonies due to problematic or missing queens, and minimise the time spent rescuing colonies and expenses on queen bee purchases. This article describes and analyses a comprehensive field study conducted in the summer of 2023 involving the introduction of 754 virgin queens of different ages and 194 queen cells into mating nuclei of various states using three different methods. The article aims to provide precise recommendations to beekeepers for the introduction of virgin queens, thereby increasing the frequency of successful requeening. To the author's knowledge, this is one of the most extensive field studies dedicated to improve the results of introducing virgin queen bees.

MATERIAL AND METHODS

Field study location and period

The field study was conducted in central Latvia, in the Sigulda municipality, at a commercial beekeeping enterprise (Llc Smilga Spalvina) from May 1 to August 31, 2023. The summer was hot (Table 1) with little precipitation. The surroundings of the mating apiaries consisted of forests and natural meadows.

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Table 1. Temperature fluctuations during the 2023 season, collected by the Prohiver beehive monitoring system at the location of the mating apiary

Month	T min (°C)	T max (°C)
May	+5 to +12	+12 to +28
June	+2 to +18	+15 to +34
July	+9 to +17	+19 to +32
August	+10 to +19	+19 to +34

Mating nuclei (new colonies)

Mating units were created from four combs: two brood combs, one honeycomb, and one empty comb. The dimensions of the frames were 435 mm × 300 mm (Dadant-Blatt). Each mating unit was dated for the queenless period, the time between removing the laying queen and introducing the new queen, and the presence of laying workers. Before the queen introduction, the mating nuclei were inspected, and any emergency queen cells were removed.

Virgin queens

Queen bees were reared using larvae grafting as described by Buchler et al. (2013). Emerged caged queens were stored in a "queen bank" colony until the day of queen introduction. The queen's date of birth was written on the cages. In the records, the date of the queen bee addition to mating nuclei and the queen's age on the day of introduction were also recorded. Queens from different races were used in the study: *Apis mellifera ligustica* (*n* = 198), *Apis mellifera carnica* (*n* = 299), and Buckfast (*n* = 257).

Introduction methods

Three methods were used in the study:

1. Spraying the mating nucleus from above with a mixture of honey, ethanol, and water, then spraying the queen, directly introducing the queen from the top of the combs, then blowing smoke at the entrance of the nucleus.
2. The same as the first method, but the queen was kept in the queen's cage above the combs for one day and then released
3. It was the same as the first method but without using smoke.

The spray was prepared by dissolving 300 g of honey in 1 l of warm water and adding 20 ml of 70% ethanol. In addition, 194 queen cells were added during the study for comparison purposes.

Data used for result analysis

Data used for result analysis: queenless period of the nucleus and presence of laying workers in the mating nucleus; age of virgin queens; method of queen bee introduction; date of queen bee introduction; status of queen bees after introduction – not accepted/accepted and mated, laying queen (acceptance rate). Successful acceptance was recorded only when the laying queen was observed in the nucleus. If the queen was not accepted, accepted but not laying or was accepted but it did not return from a nuptial flight, all such introduction attempts were recorded as unsuccessful.

Data processing

Data processing was done in the following order:

1. Factor: introduction method. The three queen bee introduction methods grouped the entire dataset (*n* = 754), and it was determined whether there were statistically significant differences between the groups in the method selection. If there were no statistically significant differences between the groups, then further analysis used all the data.
2. Factor: queenless period of the mating nucleus. Data (*n* = 754) was grouped by queenless periods in the mating nucleus: 1 day (worker bees detect queenlessness), 2–4 days (eggs and young larvae are still present in the nucleus, the building of emergency queen cells has started), 5–8 days (open brood and emergency queen cells are present in the nucleus), 9–20 days (only capped brood is present in the nucleus), 21 days and more (the nucleus is broodless). If there were statistically significant differences between the groups, further analysis was conducted separately for each group or by combining groups with no statistically significant differences.
3. Factor: age of virgin queens. Data (*n* = 754) was grouped by the maturity of virgin queens: 1–4 days (immature queens), and five days and more (queen bees reaching sexual maturity).

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Differences between the groups of queens were evaluated for each queenless period (refer to the second factor description). Groups of queens' ages and queenless periods of mating nuclei without statistically significant differences were combined in one dataset for further analysis in the following steps.

4. Factor: date of queen bee introduction. Data (n = 432) was grouped by the week of queen bee introduction during the 2023 season from May 1 to August 31 to determine if there were statistically significant differences between the selected introduction periods.
5. Factor: presence of laying worker bees in the mating nucleus. Data (n = 227) was grouped by the status of the queenless nucleus for 21 days and more, whether laying worker bees were present or not, and it was determined whether there were statistically significant differences between the groups regarding the queen bee acceptance.

Statistical analysis methods

The Chi-square test (χ^2) and P-value were used to determine statistically significant differences between the groups in the data analysis, with the P-value compared to alpha values of 0.05, 0.01, or 0.001. The Pearson correlation coefficient was determined for the queenless period and the age of virgin queen bees. Data were processed in the Microsoft Excel software.

RESULTS AND DISCUSSION

From May 1, 2023, to August 31, 2023, 754 virgin queens of different age were introduced into queenless mating nuclei using three different methods. Additionally, 194 queen cells were added for comparison and included in the analysis of acceptance by queen's age.

Factor: Queen bee introduction method

With the first method, honey-ethanol spray and direct release and smoke, 276 virgin queens were introduced, of which 149 queens, i.e. 54%, were accepted (Figure 1). With the second method, honey-ethanol spray and cage one day and smoke, 394 virgin queens were introduced, of which 238 queens, i.e. 60%, were accepted. On the other hand, with the third method, honey-ethanol spray and direct release, 84 virgin queen bees were introduced, of which 36, i.e. 43%, were accepted. Although dividing the results of introduced queen bees according to the used methods shows that acceptance and mating rates ranged from 43% to 60% depending on the method, there were no statistically significant differences between the groups (χ^2 = 2.89, P = 0.24). Therefore, the choice of methods was not considered in further data analysis. This analysis did not include any queen cells because they are not a comparable queen bee introduction method with the three above-mentioned groups.

Factor: A queenless period of the mating nucleus

When queen bees were added to nuclei with a queenless period of 1 day, 41% of queen bees were accepted (Figure 2). With a queenless period of 2–4 days 48% of queens were accepted. With a queenless period of 5–8 days it was 68%. With a queenless period of 9–20 days the acceptance rate was 69%, and with a queenless period of 21 days and more it was 64%. There was a very high positive correlation between the results: the longer the queenless period, the higher the results of accepting queen bees (r = 0.83, R^2 = 0.69, P < 0.05). The

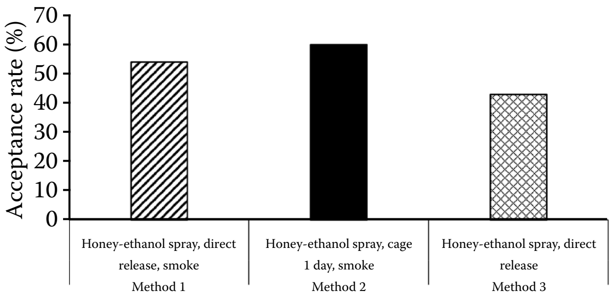

Figure 1. Dependence of the queen bee acceptance rate on the chosen queen bee introduction method

Only laying queens were counted as successfully accepted. Method 1: Honey-ethanol spray and direct release and smoke (n = 276) resulted in 54%. Method 2: honey-ethanol spray and cage one day and smoke (n = 394) resulting in 60%. Method 3: honey-ethanol spray and direct release (n = 84) resulted in 43%. No statistically significant differences between groups: χ^2 = 2.89, P = 0.24

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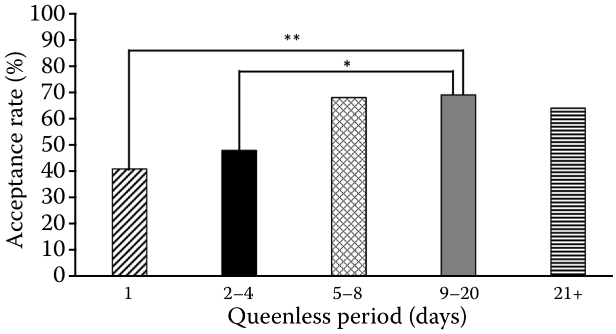

Figure 2. Dependence of the queen bee acceptance rate on the queenless period of the nucleus

Only laying queens were counted as successfully accepted. In nuclei with a queenless period of 1 day (n = 228) 41% of queen bees were accepted. With a queenless period of 2–4 days (n = 94) it was 48%. With a queenless period of 5–8 days (n = 104) 68% were accepted. With a queenless period of 9–20 days (n = 100) it was 69%. With a queenless period of 21 days and more (n = 228) 64% of queen bees were accepted. There were statistically significant differences between groups (χ^2 = 11.14, P = 0.025) *P < 0.05, **P < 0.01

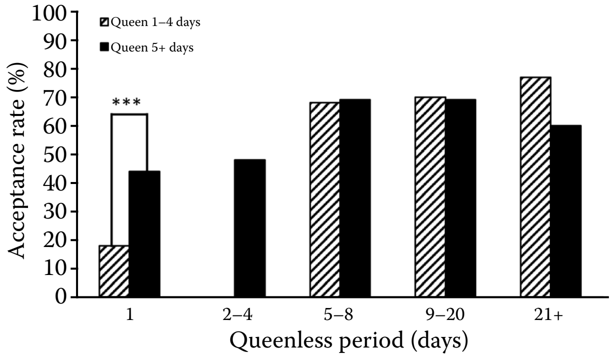

Figure 3. Dependence of the queen bee acceptance rate on the age of introduced queens in nuclei with different queenless periods (n = 742)

Only laying queens were counted as successfully accepted. In 1-day queenless nuclei, 18% of queens aged 1–4 days (n = 40) and 44% of queens aged five days and more (n = 175) were accepted. The difference between these results was statistically significant. In 2–4 day queenless nuclei, 48% of queens aged five days and more (n = 94) were accepted. In 5–8 day queenless nuclei, 68% of queens aged 1–4 days (n = 34) and 69% of queens aged five days and more (n = 70) were accepted. In 9–20 day queenless nuclei, 70% of queens aged 1–4 days (n = 20) and 69% of queens aged five days and more (n = 80) were accepted. In 21-day and more queenless nuclei, 77% of queens aged 1–4 days (n = 52) and 60% of queens aged five days and more (n = 176) were accepted. Statistical significance was tested between datasets of 1–4 days old queens and those aged 5 days and more ***P < 0.001

number of accepted queen bees was statistically significantly lower in nuclei that were queenless for 1–4 days compared to those that were queenless for a more extended period. Perez-Sato et al. (2008) also drew this conclusion when testing queenless periods from 1–6 days. Results showed no statistically significant differences if queen bees were added to nuclei that were queenless for 5–8 days, 9–20 days, or 21 days and more (χ^2 = 0.23, P = 0.89). If the beekeeper eliminates the emergency queen cells before adding the queen, then in more extended queenless periods, worker bees no longer have the opportunity to choose to rear a queen themselves because the larvae will be too old (Buchler et al. 2013). By the ninth day, all the brood will be capped (Page and Peng 2001).

Factor: Age of virgin queens

Initially, virgin queens were grouped into immature queens (1–4 days) and sexually mature virgin queens (5 days and more). This division was chosen because a queen reaches sexual maturity at the age of 5 days (Page and Peng 2001), emitting more pheromones than an immature queen (Pankiw 2004; Cobey 2007), which can affect the acceptance of queen bees. If queens of both age groups were introduced into a 1-day queenless nucleus, immature queens were successfully accepted only in 18% of cases (n = 40). In comparison, sexually mature queens were accepted in 44% of cases (n = 176) (Figure 3). Statistically significant differences in results between queens aged 1–4 days and queens aged 5 days and more appeared specifically in 1-day queenless nuclei (χ^2 = 10.90, P < 0.001). Unfortunately, there was a lack of data on the acceptance results of immature queens in 2–4 day queenless nuclei. However, starting from 5-day queenless nuclei, there was no difference in acceptance between immature and sexually mature queen bees, with successful acceptance ranging from 60% to 77%. Perez-Sato et al. (2008) also revealed the poor acceptance of virgin queen bees in 1-day queenless nuclei, while significantly higher acceptance was observed in 5–6-day queenless nuclei. The data showed that even in queenless nuclei for a more extended period (over six days),

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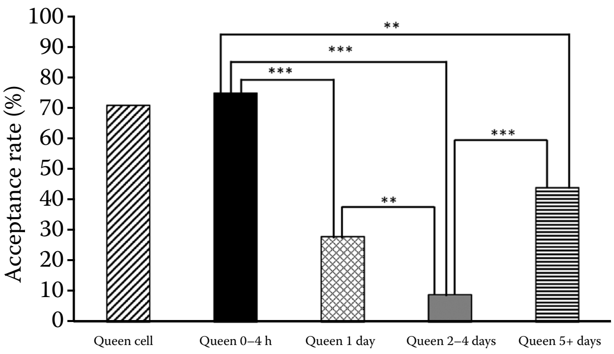

Figure 4. Dependence of the queen bee acceptance rate on the age of introduced queens into 1-day queenless nuclei (n = 228)

Only laying queens were counted as successfully accepted. Queen bees aged 0–4 h had an acceptance rate of 75% (n = 12), one-day-old queens 28% (n = 18), 2–4 days-old queens 9% (n = 22), and five-days-old queens and more had an acceptance rate of 44% (n = 176). The success rate for queen cells was 71% (n = 160). There were statistically significant differences between the groups of queen bees (0–4 h, 1 day, 2–4 days, 5 days and more) (χ^2 = 60.05, P < 0.001). For queen cells, significance was tested with 0–4 hours-old queens

*P < 0.05, **P < 0.01, ***P < 0.001

the acceptance rates of virgin queen bees remained high regardless of the queen's age.

Based on the author's experience, many beekeepers prefer introducing virgin queen bees not older than one day to increase acceptance rates. Another study also found that when introducing a younger queen (0–24 h) to a 3-day queenless nucleus, 65% acceptance was reported, whereas with an older queen (4 days) the success rate was 55% (Perez-Sato and Ratnieks 2006). To investigate the specific difference between 1-day queens and queens of other ages, a detailed analysis was conducted on the influence of queen age on acceptance in 1-day queenless nuclei, revealing statistically significant differences (Figure 4). Additionally, an analysis of queen cell results and results from very young (0–4 h) queen bees which emerged at the time of the cell introduction to numerous nuclei was carried out.

The results showed that newly emerged queens aged 0–4 h were accepted best in one-day queenless nuclei – 75% (n = 12). There were no statistically significant differences between the introduction of queen cells (resulting in 71%, n = 160) and the introduction of newly emerged queens (χ^2 = 0.11, P = 0.74). However, as the queens reached one day of age, the acceptance rate significantly decreased to 28% (n = 18), further being reduced to 9% (n = 22) at 2–4 days of age. Conversely, as the queens matured or reached five days of age and more, the acceptance rates increased to 44% (n = 176). There were statistically significant differences between newly emerged queens aged 0–4 h and other queen groups (P < 0.001). Similarly, there was a significant difference between queens aged one day and those aged 2–4 days (χ^2 = 9.76, P < 0.01) and between queens aged 2–4 days and those aged 5 days and more (χ^2 = 23.11, P < 0.001). However, no statistically significant difference existed between queens aged one day or those aged 5 days and more. There was a positive medium correlation between the queen's age (1 day, 2–4 days, 5 days and more) and the acceptance results: the older the queen, the higher the acceptance results in 1-day queenless nuclei (r = 0.43, R^2 = 0.19, P < 0.001). In newly established colonies or when removing the old queen for one day, if beekeepers rear the queens themselves, very good results will be obtained by introducing queen cells or newly emerged queens. However, if beekeepers purchase queens from breeders, they often receive queens by courier, so obtaining a queen aged one day or less is impossible. Still, it would be desirable to determine the queen's birth date to introduce a queen into the colony when it is at least five days old. Of course, in the timely preparation of queenless colonies, the queen's age is not significant, only that the older queen will mate and start laying eggs sooner.

A dataset was used for acceptance results by race of queens with no statistically significant differences between groups. Queenless nuclei for 5 days and more were selected (Figures 2 and 3), and all queens aged one day and more (n = 432). The acceptance rate for Buckfast queens was 61% (n = 142), for *Apis mellifera ligustica* queens 63% (n = 131), and for *Apis mellifera carnica* queens 74% (n = 159). No statistically significant differences existed between the races of introduced queens (χ^2 = 1.52, P = 0.47).

Factor: Date of queen bee introduction

A dataset with no statistically significant differences between different groups was used. Queenless nuclei for 5 days and more were selected (Figure 2), and all queen bees were one day old and more (Figure 3)

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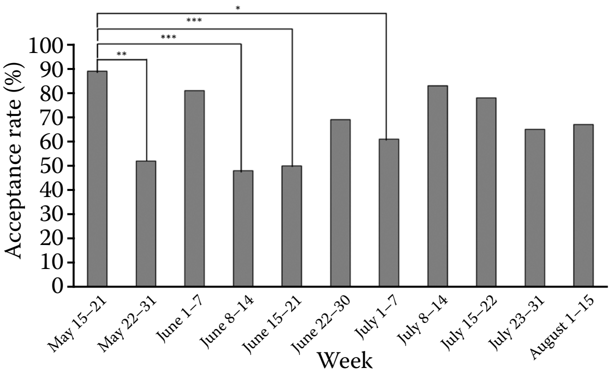

Figure 5. Dependence of successful acceptance and mating of queen bees on the week of queen bee introduction during the 2023 season (n = 432)

**P* < 0.05, ***P* < 0.01, ****P* < 0.001

(n = 432). During the 2023 season, from May 15 to August 15, the successful acceptance of virgin queens varied weekly from 48% to 89% (Figure 5). All groups showed statistically significant differences (χ^2 = 29.18, P < 0.01). The significantly lower acceptance and mating results during the 2023 season occurred on May 22–31, June 8–21, and July 1–7, compared to the highest result of 89% from May 15–21. Here, the influence of weather conditions was evident. For example, from July 1 to 7, it was a rainy week, the dearth of nectar began, and the bees became aggressive, which could have affected the acceptance of new queens. It should be noted that the acceptance rate was tied to the week of the introduction of the virgin queens, but mating took place in the next weeks. Queen bees mate on days with an air temperature of +20 °C at least and without strong wind and rain (Buchler et al. 2013). Therefore, if the beekeeper has such an opportunity, it is advisable to follow the weather forecast and to make the replacement in more favourable weather conditions. However, when it is often revealed that a colony has a queen no longer, new queens are added when necessary. Then, the impact of the absence of nectar flow can be mitigated by feeding the colony with sugar syrup (Laidlaw 1981; McCutcheon 2001), and it should be expected that the acceptance rate of virgin queen bees will be lower. Nowadays, monitoring systems implemented in hives help to monitor changes in weather conditions and nectar flow (Zacepins et al. 2015). Due to incomplete weather and nectar data in this study, it was not possible to conduct a deeper analysis of changes in acceptance results depending on the timing of introduction during the 2023 season.

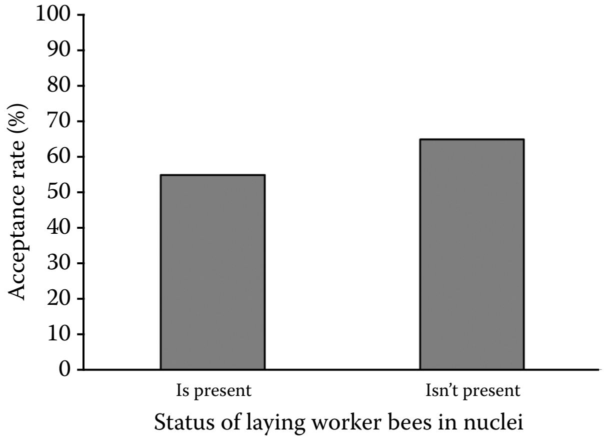

Figure 6. Dependence of the queen bee acceptance rate on the presence of laying worker bees

Only laying queens were counted as successfully accepted. Duration of queenlessness in the nucleus: 21 days and more; age of virgin queens: 2 days and more. With laying worker bees, the acceptance rate was 55% (n = 29); without laying worker bees it was 65% (n = 198). There were no statistically significant differences between the groups (χ^2 = 0.83, P = 0.36)

Factor: Presence of laying worker bees in the mating nucleus

If a bee colony has laying worker bees, it is recommended to shake off all the combs of bees outside the hive, followed by introducing a new queen bee (McCutcheon 2001). In this field study, a small amount (n = 29) of nuclei with laying worker bees was detected after an unsuccessful queen bee introduction. Laying worker bees were detected starting from an 11-day queenless period, but in most (70%) of the nuclei, laying workers appeared in 21 days of queenlessness, when the colony entered a broodless state. The acceptance rate of virgin queens was compared in 21 days and more of queenless nuclei with and without the presence of laying worker bees (Figure 6).

Queen bees were introduced into both cases without the recommended shaking-off method. Queens were successfully accepted in nuclei with laying workers at a rate of 55% (n = 29), while in nuclei without laying workers the rate was 65% (n = 198). There were no statistically significant differences between the two groups (χ^2 = 0.83, P = 0.36). Thus, we recommend introducing a queen bee even in the presence of laying worker bees without using the shaking-off method, significantly reducing labour and saving time.

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CONCLUSION

In this field study, conducted in the summer of 2023, the successful acceptance and mating of virgin queen bees were examined based on various factors:
- the method of introduction,
- the queenlessness periods of the nuclei,
- the age of virgin queens,
- the week of the queen bee introduction,
- the presence of laying worker bees in the nucleus.

Among the methods used (honey-ethanol spray and direct release and smoke; honey-ethanol spray and cage one day and smoke; honey-ethanol spray and direct release), there were no differences in the queen bee acceptance rate. Significantly fewer queens were accepted in nuclei with a queenless period of 1 day (41%) or 2–4 days (48%), while queens had a higher acceptance rate starting from 5 days of a queenless period (64–69%).

Therefore, it is worth preparing target colonies before introducing queen bees. If the virgin queen is introduced into a 1-day queenless colony, the age of the virgin queen influences the result. Accordingly, the highest result will be achieved by introducing newly emerged queens aged 0–4 h (acceptance rate 75%).

However, no statistically significant differences exist whether a queen bee is one day old (acceptance rate 28%) or a sexually mature queen bee aged 5 days and more (acceptance rate 44%) is added in such cases. It is necessary to avoid adding immature queen bees aged 2–4 days (acceptance rate 9%). Since beekeepers may not be able to purchase one-day-old queen bees considering the time spent in shipping, it is essential to know the date of the queen bee birth to add a queen bee to the new colony at the age of 5 days and more. However, if a bee colony is adequately prepared for the acceptance of a new queen bee, then the age of the virgin queen bee is irrelevant (a positive acceptance rate of 60–77% was achieved). The colony is considered adequately prepared if it has been without a queen bee for five days at least, preferably eight days because then there are no emergency queen cells present.

If a colony has been queenless for a long time and has developed laying worker bees, then it is possible to introduce a virgin queen bee with a 55% success rate without applying time-consuming methods. Because of weather conditions, a positive outcome of queen introduction can also vary seasonally, even by weeks (acceptance rate of 48–89%). Performing the queen bee replacement in warm weather conditions with good nectar flow is recommended.

If a beekeeper systematically performs the queen replacement, it is advisable to prepare the colony for the acceptance of new queen bees in advance, at least five days before the addition of the new virgin queen bee, which will guarantee a better result of queen bee acceptance regardless of the age of the received queen bee.

Conflict of interest

The authors declare no conflict of interest.

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