Influence of *Apis mellifera* in-hive conditions on germination capacity of rapeseed pollen (*Brassica napus*)

Influencia del ambiente interno de la colmena de *Apis mellifera* sobre la capacidad germinativa del polen de colza (*Brassica napus*)

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Abstract

Brassica napus L. (rapeseed, canola) ranks third in worldwide importance among oilseeds. The production of hybrid rapeseed seed requires an androsterile female parent; therefore, fertilization is possible through pollinators carrying viable pollen from an androfertile line. To ensure high pollinator populations, hives are used. However, little is known about the risk of transporting viable pollen into hives. The *in vitro* germinability of pollen exposed to in-hive conditions was evaluated. Samples of rapeseed pollen obtained from potted plants were placed in four hives of *Apis mellifera* L. In-hive conditions are unfavorable for rapeseed pollen germinability. Brood areas with the highest temperatures showed no germinated pollen grains within 24 h. Starting at 48 h, germinability decreased significantly, with germinated grains showing atrophied tubes. At 72 h, pollen placed away from brood areas lost germinability.

Keywords
canola • germinability • honey bee • pollination

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Resumen

Brassica napus L. (colza, canola) es la tercera en importancia mundial de las oleaginosas. La producción de semilla híbrida de colza requiere de una línea parental androestéril; por lo tanto, su fertilización es posible a través de los polinizadores que portan polen viable desde una línea androfértil. Para lograr una alta población de polinizadores se colocan colmenas. Sin embargo, poco se conoce sobre el riesgo de transportar polen viable en el interior de las mismas. Se evaluó la germinabilidad *in vitro* del polen expuesto a las condiciones ambientales dentro de la colmena. A partir de plantas de colza cultivadas en macetas, se obtuvieron muestras de polen que se colocaron dentro de cuatro colmenas de *Apis mellifera* L. Las condiciones dentro de las colmenas son desfavorables para la germinabilidad de los granos de polen de colza. En el área de cría con las temperaturas más altas en 24 horas no se registraron granos de polen capaces de germinar. A partir de las 48 horas, la germinación decreció significativamente y los granos germinados mostraron tubos atrofiados. El polen ubicado más lejos del área de cría mantuvo su germinabilidad por menos de 72 horas.

Palabras claves
colza • germinabilidad • abeja melífera • polinización

Introduction

The productivity of cultivated plants, especially those that bear fruit, seed, or grain, is highly correlated with pollen production and viability. This viability can be strongly influenced by non-optimal environmental conditions, such as drought, heat, and solar radiation (13, 24, 25), causing reduced fruit set and yield, as reported for rapeseed crops exposed to high temperatures during flowering (2, 33).

Brassica napus L. (rapeseed, canola) ranks first among Brassicaceae and third among oilseeds, after palm and soybean (19, 31). The seed production of hybrid rapeseed requires an androsterile female parent and pollinators that transport viable pollen from an androfertile line (6, 23, 32). Female line productivity, pollen production, and floral-synchrony management between parental lines are key aspects for maximizing yield. Under this production scheme, the potential contamination with foreign pollen poses a major risk. In rapeseed, cross-pollination decreases with the increase in distance between receptive stigma and pollen source (11). Therefore, in hybrid seed production, the spatial distribution of plots is carefully defined to prevent the androsterile line from being fertilized by foreign pollen (32). Typically, such production considers natural pollen transfer by wind, water, or insects rather than human-mediated unintentional transfer (21, 30).

Unlike naturally occurring pollen transfer, the anthropic movement of pollen has scarcely been studied. In the case of entomophily, there is limited information on the duration of pollen viability on insect bodies (12, 20). Colonies dedicated to pollination in hybrid seed production are commonly moved from one field to another in the same crop. Pollen in hives or on bees could contaminate production if the androfertile lines in successive fields are different. Pollen already processed by bees (corbicular pollen, pressed pollen, or bee bread) is of no concern; rather, pollen grains that remain on their bodies or are free in the hive are in question (20).

The production of hybrid rapeseed seed requires large quantities of pollinators in a relatively short period, specifically during crop flowering. Native pollinators are highly efficient in pollen transfer, but their low and unpredictable population density requires incorporation of human-managed pollinators. The species most commonly used for pollinating crops, including rapeseed, is *Apis mellifera* L. (32).

Despite its global presence, honey bees maintain consistent colony traits, notably brood nest temperature (14). For proper development, *A. mellifera* larvae need a stable temperature, which adults keep between 32 and 36°C (4, 5, 8, 27, 28, 29). Unlike temperature, worker bees have limited control of hive humidity. The optimal humidity varies across the brood nest and fluctuates with external conditions. Because temperature regulation takes priority, it inevitably impacts humidity levels (9, 10).

Pollen grains on bee bodies are exposed to in-hive conditions. Temperature can affect canola pollen germinability during pre-anthesis (17), and germinability and pollen tube length can be reduced when germinated at 33°C (18). Beekeepers must follow strict hive movement rules to prevent potential contamination, even without clear evidence of whether the pollen in the hives retains its germination capacity. To date, there are no records of rapeseed pollen germinability under actual in-hive conditions. This study aimed to determine how long rapeseed pollen maintained germination capacity in the hive, helping to establish safe intervals before moving hives between hybrid rapeseed fields, thus minimizing the risk of contamination.

Materials and Methods

Experimental site

To assess *in vitro* pollen germinability under hive conditions, experiments were conducted once each spring (October/November) of 2017, 2018, and 2019 at the Laboratorio de Estudios Apícolas, Universidad Nacional del Sur, Bahía Blanca, Argentina (-38.694944, -62.253293). Four Langstroth-type *A. mellifera* hives were selected each year, with nine frames per brood chamber. The seven central frames held brood, while the two outer frames stored honey. No signs of disease were observed. The queen was ovipositing prolifically.

Pollen samples

Pollen samples were obtained from 20 Hyola 433 rapeseed plants grown in 10-liter pots. When plants reached full bloom, mature flower buds (close to opening) were labeled. The following day, at 7:30 AM (-3 GMT), 80 of these marked flowers were harvested, ensuring that anthers had been fully developed, as pollen is most fertile immediately after the flower opens (16). Anthers from all harvested flowers were pooled into one sample. Three random anthers were placed in 60 tubes (0.5 ml) and crushed with a histological needle to release pollen. Tubes were left open in the hives, secured with two pins to prevent movement due to bee activity (figure 1).

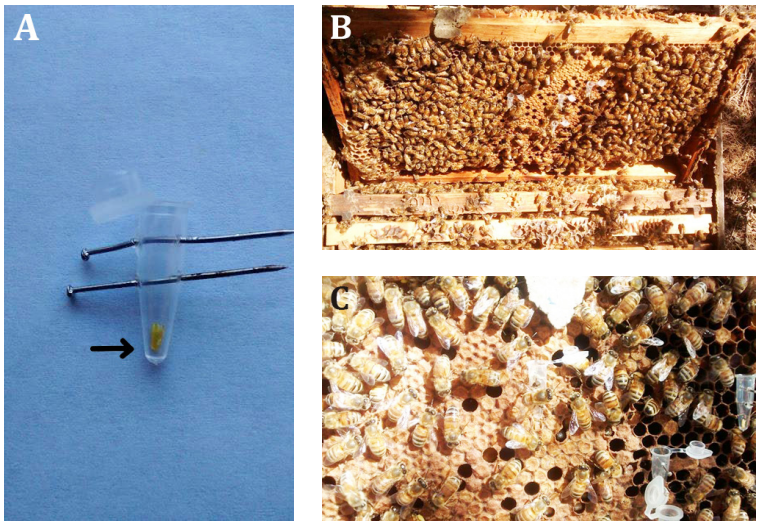


Figure 1. A: Tube with *Brassica napus* anther tissue and pollen samples (arrow) secured with pins to prevent movement from bee activity; B: Distribution of pollen samples in an *Apis mellifera* hive; C: Detail of pollen samples on a brood frame (Frame 5).

Figura 1. A: Tubo con tejido de anteras y muestras de polen (flecha) de *Brassica napus*, sujetado con alfileres para evitar su movimiento por la actividad de las abejas; B: Distribución de muestras de polen dentro de una colmena de *Apis mellifera*; C: Detalle de muestras de polen en un cuadro de cría (Cuadro 5).

Treatments

Out of 60 samples, the germinability of 12 freshly harvested pollen samples was analyzed as the 0 h treatment. The remaining 48 tubes were randomly grouped in fours and placed in three different frames in each hive, with 12 samples per hive per year. Frame 5, in the center of the brood chamber, had abundant brood. Frame 7, located between the edge and the center, had brood in the center and honey and pollen on the margins. Frame 9, on the outer edge, contained only honey (figure 2). Each year, temperature sensors (Onset HOBO UA-002-64 Temperature/Light Data Logger 64K spa) were placed to record in-hive conditions every hour. During 2019, two sensors (HOBO Onset H08-032-IS Temperature/HR Data Logger 64K HOBO), one in the brood area and another in the honey reserves, were added to record humidity hourly in one of the hives. Over three consecutive days, one tube per frame was removed every 24 h from each of the hives, totaling 12 tubes per day, for analysis.

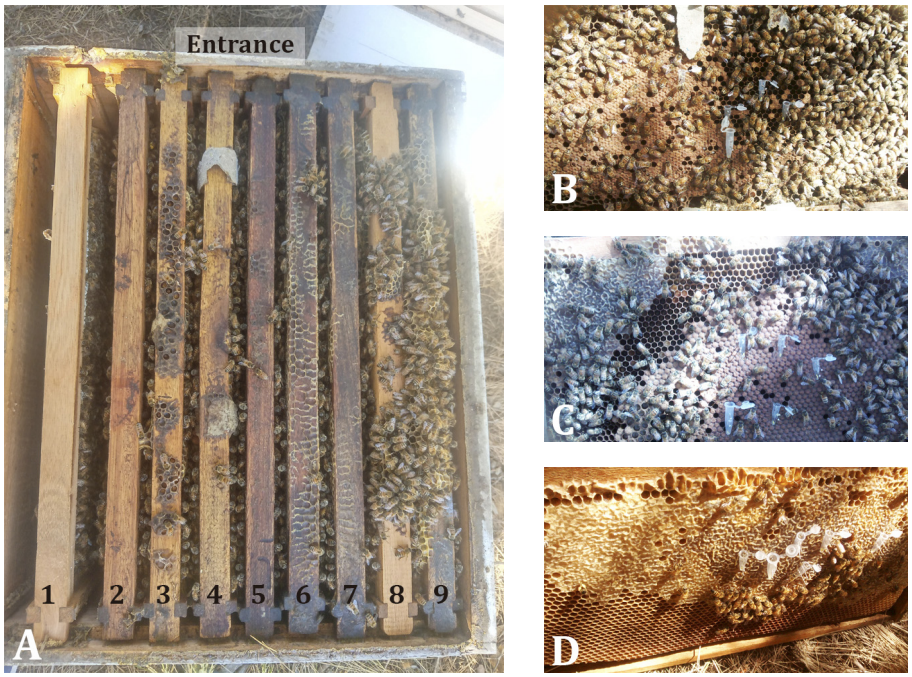


Figure 2. A: Identification of frames in one of the four *A. mellifera* hives used for the experiment; B: Frame 5, center of the brood chamber, completely covered by brood; C: Frame 7, with brood in the center and stored honey and pollen on the edges; D: Frame 9, outer edge of the brood chamber only with stored honey.

Figura 2. A: Identificación de los cuadros de una de las cuatro colmenas de *A. mellifera* utilizada para el ensayo; B: Cuadro 5, centro de la cámara de cría, cubierto completamente por cría; C: Cuadro 7, compuesto por cría en el centro, miel y polen en los bordes; D: Cuadro 9, externo de la cámara de cría compuesto por reservas de miel.

Technique to determine germinability

The hanging drop technique was used to assess the percentage of *in vitro* pollen germination (25). *Brassica napus* pollen grains were incubated in a culture medium at 25°C and 90% relative humidity for two hours. A drop of culture medium was placed on a glass slide, and pollen samples were added. The glass slide was then inverted in a sealed humid chamber with wet absorbent paper at the bottom. After two hours, a cover slip was placed over the drop, and 500 pollen grains per sample were counted under a microscope at 400X magnification. Germination was determined by the percentage of grains with a pollen tube longer than grain diameter (15).

Culture medium

The culture medium used to measure *in vitro* pollen germinability, originally described for sunflower by Astiz (2012), proved suitable for rapeseed pollen. It contained 150 g/L polyethylene glycol 6000 (PEG6000) in distilled water, 100 g/L sucrose, 240 mg/L calcium nitrate, and 100 mg/L boric acid. The pH was maintained between 6.5 and 7.0, adjusted with 0.1 N sodium chloride. PEG6000, which is inert to pollen metabolism and unable to enter cells (22), was included to enhance the development of pollen tubes by regulating plasma membrane permeability and providing stability to the pollen tube membrane (22).

Statistical analysis

The experimental design consisted of randomized complete blocks with four replicates. Data were subjected to analysis of variance (ANOVA), and if differences were detected at p-values < 0.05, means were compared using Fisher's LSD test. Statistical analyses were performed using Infostat software (7).

Results

In-hive temperature and humidity

Over the three years and during the experiments (three days), differences in hourly temperature were observed among the hive sectors studied ($p < 0.0001$; $n = 72$). Frame 5 had the highest temperatures (around 35°C), followed by Frame 7 and 9 (figure 3).

Throughout the period of pollen exposure to in-hive conditions, humidity showed no significant differences between brood and honey storage areas ($p = 0.1099$; $n = 138$). In the area with stored honey, the average (± SD) relative humidity was 42.09% (±10.47), and in the brood area it was 44.23% (± 5.29).

Bars show mean values (±SD).

Equal letters indicate no significant differences (p > 0.05) between the groups analyzed.

Las barras muestran valores medios (± DE).

Letras iguales indican que no se detectaron diferencias significativas (p > 0,05) entre los grupos analizados.

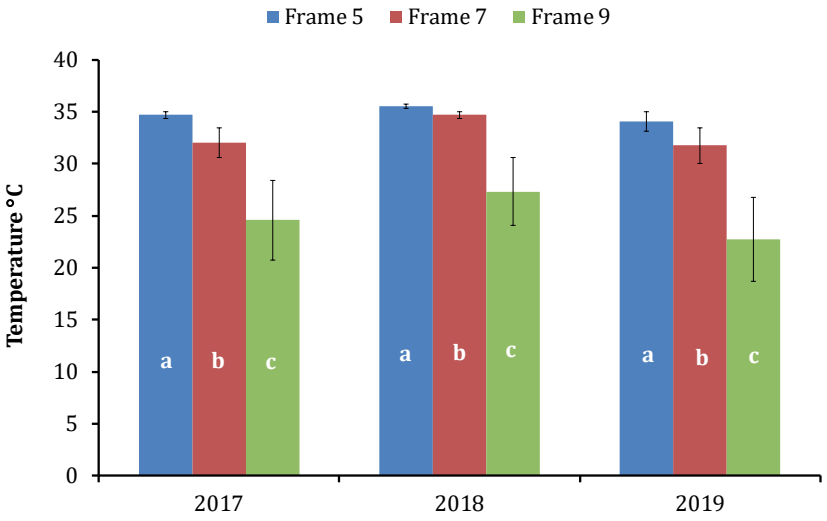


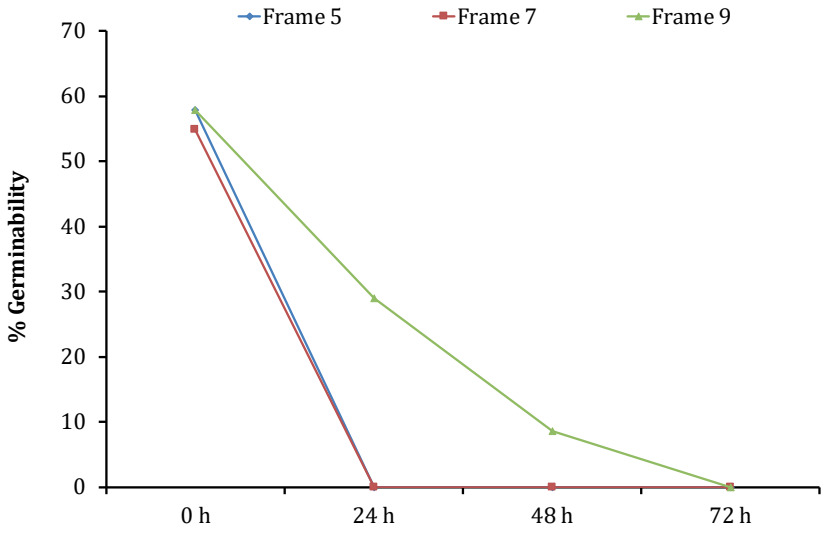
Figure 3. In-hive temperature variation in Frames 5, 7, and 9.

Figura 3. Variación de la temperatura dentro las colmenas experimentales, Cuadro 5, 7 y 9.

Pollen germinability

In all years, the highest germination percentages ($\bar{X} = 57.9\%$) were obtained with freshly collected pollen (0 h) ($p < 0.01$). In-hive conditions reduced rapeseed pollen germinability, with pollen in brood areas losing its germination ability within 24 h. No significant differences were found between brood frames (5 and 7) ($p > 0.05$).

Pollen in Frame 9, near honey reserves, retained its germination capacity longer than that in the brood areas ($p < 0.05$) (figure 4). Across all trials, germination capacity dropped similarly, with some pollen in Frame 9 still viable at 48 h, but falling below 20%. After 72 h, no pollen germinated, in most cases with a significant reduction occurring within 24 h.



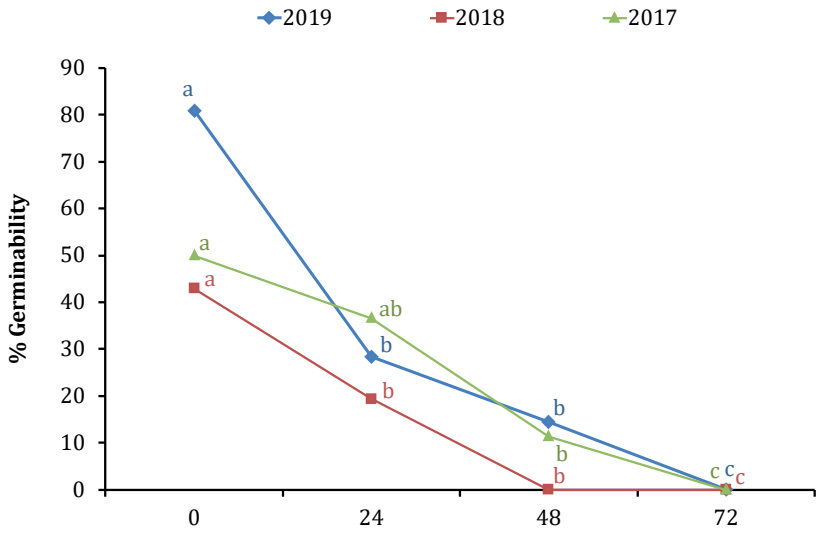
The curve corresponding to Frame 5 overlaps with that of Frame 7.

La curva correspondiente al Cuadro 5 se superpone con la del Cuadro 7.

Figure 4. Mean percentages of *in vitro* germinability of rapeseed pollen grains after storage in different locations in an *A. mellifera* hive.

Figura 4. Porcentajes promedios de la germinabilidad *in vitro* de los granos de polen de colza luego de permanecer en diferentes ubicaciones dentro de una colmena de *A. mellifera*.

Temperature averages in Frame 9 were 24.6 ± 3.8°C, 27.3 ± 3.2°C, and 22.7 ± 4.0°C in 2017, 2018, and 2019, respectively. Despite these differences, pollen germinability decreased substantially after 48 h of exposure to in-hive conditions (figure 5). Pollen germination capacity was completely lost after 72 h.



Equal letters indicate no significant differences ($p > 0.05$) between the groups analyzed.

Letras iguales indican que no se detectaron diferencias significativas ($p > 0,05$) entre los grupos analizados.

Figure 5. Percentage of pollen germinability in Frame 9 in 2017, 2018, and 2019.

Figura 5. Porcentaje de germinabilidad de los granos de polen en el Cuadro 9 en 2017, 2018 y 2019.

Fresh pollen had the thickest and most developed pollen tubes. After 24 h in the hive, pollen grains had thinner, shorter, and convoluted pollen tubes. After 48 h, pollen tubes were atrophied (figure 6).

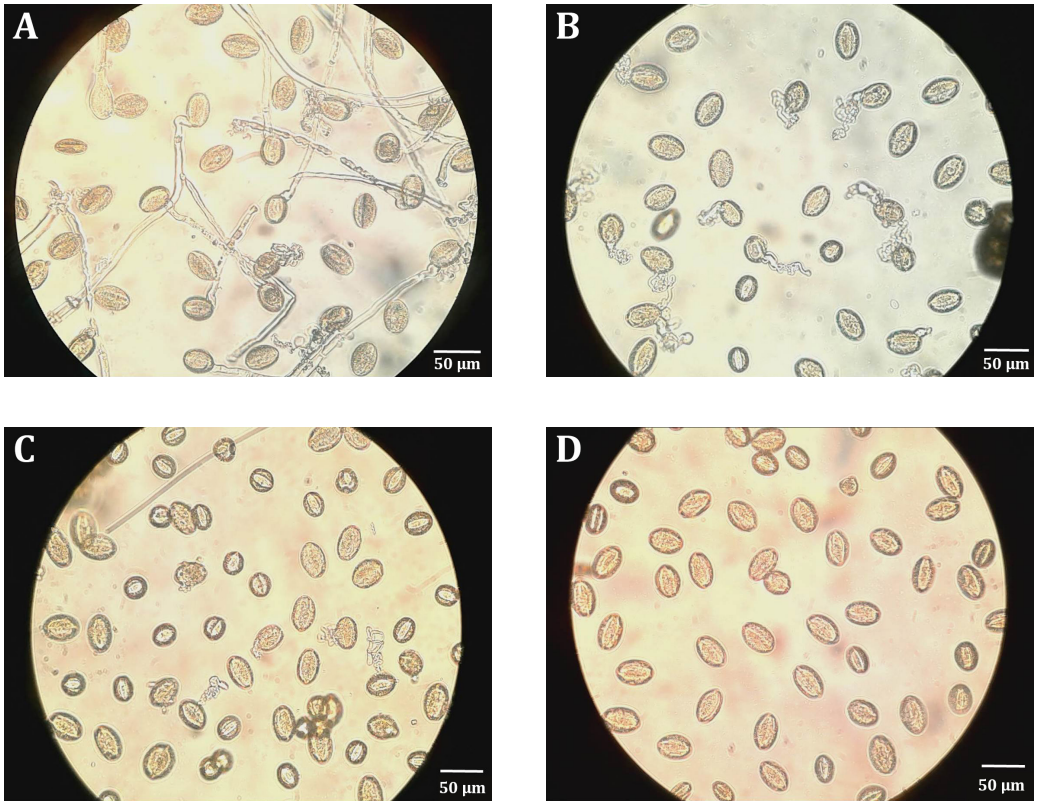


Figure 6. Development of pollen tubes from rapeseed pollen grains in Frame 9 after different in-hive exposure periods (400X). A: 0 h; B: 24 h; C: 48 h; D: 72 h.

Figura 6. Desarrollo de los tubos polínicos de granos de polen de colza en el Cuadro 9 luego de diferentes períodos de exposición en una colmena (400X). A: 0 h ; B: 24 h; C: 48 h; D: 72 h.

Discussion

The outer frames of the brood chamber showed wider temperature variations and lower average temperatures than those of the central frames. This was expected because the brood is mainly in the center, while the outer frames, typically filled with honey, have less temperature regulation from bees. Honey acts as an insulator against external temperature variations, and nurse bees concentrate on controlling the temperature of brood frames. Humidity levels showed no significant differences between honey and brood areas, consistent with findings reported by Alburaki & Corona (2021) and Human (2006).

Pollen grains in the brood area lost their germination capacity after 24 h of exposure to in-hive conditions, markedly reducing the risk of contaminating new seed production plots. Temperature affects pollen not only during transport to the stigma but also during its Temperature affects pollen not only during transport to the stigma but also during its development in the anther (17, 24, 26). Notably, pre-anthesis temperature affects canola pollen germinability (17), with significant reductions in germinability and pollen tube length at 33°C (18). Our findings support this, as the temperature of the brood area remained close to 35°C.

Pollen tubes from fresh pollen and those exposed to in-hive conditions showed similar results to those reported by Young *et al.* (2004), who found that pollen germinated at 35°C had abnormal growth, being thinner and shorter than those germinated at 23°C. These abnormalities in pollen tubes could hinder proper ovule fertilization and, even if successful, would be in significant disadvantage compared to fresh pollen grains in the new plot.

Conclusions

In-hive conditions at the experimental site significantly reduced the germination capacity and pollen tube development of rapeseed. To minimize contamination risks, hives should remain outside the new production plot for 72 h.

These studies provide a starting point for understanding the potential risks of *Apis mellifera* hives carrying viable pollen and contaminating hybrid rapeseed seed production plots. Further research is recommended to confirm the required waiting period before relocating hives between plots. Similar studies on other seed crops would also be beneficial.

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