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Scientific Note

Feasibility and effects of a nano-scale feed supplement on honey bee colony performance during blueberry pollination

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Abstract. Highbush blueberry (*Vaccinium* spp.) production relies on pollination by the European honey bee (*Apis mellifera* (Linnaeus, 1758), Hymenoptera: Apidae). Yet, recruiting honey bees to blueberry flowers can be challenging due to floral traits that limit access to nectar and pollen. Beekeepers have also become increasingly reluctant to rent colonies for blueberry pollination because of concerns about post-pollination declines in colony health associated with brood diseases and pesticide exposure. Nutritional supplements formulated at nano-scale that can be readily consumed and shared through trophallaxis offer a promising yet understudied approach to improving colony performance and overall health during pollination in open-field settings. This exploratory pilot trial evaluated the feasibility and potential impact of an easily deployable nano-scale feed supplement, Nano-B-Feed™, on colony growth during blueberry pollination and returning forager activity. The trial occurred in a commercial 'Duke' blueberry field in northwest Washington, USA, using 30 colonies distributed equally between a Nano-B-Feed™ treatment and an untreated control. Colony strength was estimated using cluster counts before and after the pollination period, and returning forager activity was quantified on two dates. Nano-B-Feed™-treated colonies exhibited greater increases in cluster counts compared with untreated colonies, suggesting enhanced brood production and adult worker population growth during the pollination period. No treatment differences were detected in returning forager counts. The product was simple to apply without opening colonies, offering practical advantages for beekeepers. These preliminary findings support further replicated trials of nano-scale feed supplements like Nano-B-Feed™ as a tool to improve honey bee colony performance during crop pollination.

Keywords: *Apis mellifera*, bee health, nanomaterials, pesticide detoxification, *Vaccinium corymbosum*.

Most cultivars of northern highbush blueberry *Vaccinium corymbosum* L. (Ericales: Ericaceae) and southern highbush blueberry (complex hybrids of *V. corymbosum* and low-chill *Vaccinium* species) depend on or benefit from insect-mediated pollination (MacKenzie 1997; Retamales & Hancock 2018). Although a diversity of insects and occasionally birds contribute to blueberry pollination (De Groot et al. 2015; Martins et al. 2018; Graham et al. 2021a; Cortés-Rivas et al. 2023; Santos et al. 2023), the European honey bee *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae) is the most widely used and economically important pollinator in commercial production systems worldwide (Eeraerts et al. 2023). However, recruiting honey bees to blueberry flowers can be challenging due to several floral traits, including morphological features that may restrict access to nectar and pollen and relatively low quantity and quality of floral rewards (Courcelles et al. 2013; Vaudo et al. 2020; Cortés-Rivas et al. 2023 and references therein). Poor recruitment of honey bees in the absence of alternative pollinators can lead to reductions in pollination with downstream impacts on yield and fruit quality attributes (Danka et al. 1993; MacKenzie 1997; Dogterom et al. 2000; Ramírez-Mejía et al. 2024). An additional barrier to effective honey bee pollination is that commercial beekeepers in some regions have become increasingly reluctant to rent colonies for blueberry pollination due to concerns about post-pollination declines in colony health. These concerns include elevated risk of brood diseases such as European foulbrood *Melissococcus plutonius* (White, 1912) (Lactobacillales: Enterococcaceae) and exposure to fungicides that may contribute to colony weakening or increased overwintering losses (Grant et al. 2021; Thebeau et al. 2023). As a result, blueberry growers may face challenges securing adequate numbers of honey bee colonies or may encounter higher rental costs as beekeepers attempt to offset increased management expenses.

To address these challenges, a wide range of pheromones and attractants have been developed to increase honey bee recruitment to blueberry flowers, although evidence for their efficacy remains limited (as reviewed in DeVetter et al. 2022). Nutritional and therapeutic supplemental feeds delivered to honey bee colonies have also been explored, with ingestible micro- or nanoparticle formulations representing a particularly promising avenue for bees exposed to pesticides (Chen et al. 2021; Caserto et al. 2024). The objective of this trial was to evaluate the feasibility and potential impact of an easily deployable nano-scale supplement on honey bee colony growth indicators and foraging activity during blueberry pollination. Findings from this preliminary trial will guide future research and support scaling up to larger studies.

An exploratory pilot trial was conducted on a commercial blueberry farm in Mount Vernon, Washington, USA, using the northern highbush blueberry cv. Duke. Honey bee colonies were supplied by a commercial beekeeper, and the site was fully stocked on 25 April 2025 at approximately 15 hives per hectare using eight-frame hives. Two treatments were evaluated between 29 April and 16 May 2025: 1) Nano-B-Feed™ (manufactured by H4Bees Ltd., D.N Hof Ashkelon, Sha'ar HaNegev Regional Council, Israel) and 2) an untreated control. Nano-B-Feed™ is a proprietary powdered supplement formulated with mineral, organic, and pheromonal components dispersed at nano-scale levels within the matrix, designed to enhance honey bee colony strength by improving bioavailability and triggering internal defense mechanisms, including enhanced activity, pollination, and increased pesticide-degradation capacity, when delivered via a sugar-based carrier and distributed through trophallaxis. The study used 30 colonies in total, with 15 colonies assigned to each treatment. Treatment groups were clustered together and separated by 300 meters to minimize the possibility of Nano-B-Feed™ transfer between groups. Colonies assigned to the Nano-B-Feed™ treatment received 5 g of the product twice weekly, for a total of 10 g per week, during the pollination period. The product was applied at the hive entrance during morning hours when precipitation was absent. All colonies were maintained by the beekeeper following standard commercial practices.

Colony strength was assessed using cluster counts on 28 April 2025 prior to treatment application. The same hives were evaluated again on 26 May 2025 after the pollination period. The change in cluster count was used to indirectly estimate changes in adult colony population, consistent with the established relationship between cluster counts and adult bee numbers described by Nasr et al. (1990). Returning forager activity was assessed using video recordings collected on 13 and 16 May 2025. Trained employees subsequently counted the number of returning foragers visible in each one-minute video segment, following established noninvasive methods for estimating colony strength described by Grant et al. (2021) and Sagili & Burgett (2011).

Analyses were conducted using R (R Core Team 2025). Because treatments were applied at the cluster level and individual colonies therefore represent pseudoreplicates rather than independent replicates, statistical comparisons are considered exploratory. Due to the lack of replication, along with non-normality and unequal variance in the data, treatment comparisons were performed using the Wilcoxon Rank-Sum Test, with significance defined as $P < 0.05$.

The change in colony population growth, estimated indirectly using the cluster count method, differed between treatments, with Nano-B-Feed™-treated colonies exhibiting greater increases than untreated colonies (Tab. 1; P-value = 0.0461). This result suggests that colonies receiving the Nano-B-Feed™ treatment produced more brood and adult workers during the experimental period, leading to a greater increase in cluster counts from the initial to the final assessment. All other measured variables including initial (baseline) and final cluster counts and return foragers measured on two dates showed no difference between treated and untreated colonies (P-value = 0.8822, 0.1578, 0.1347, and 0.465, respectively). Although no treatment differences were detected in returning forager counts, data were collected only on two dates due to inclement weather, and additional sampling may have revealed more nuanced effects on foraging activity.

Despite the small scale and limitations of this pilot trial, the observed improvements in colony growth are noteworthy and may reflect the supplement's capacity to enhance honey bee detoxification of pesticides encountered during foraging (Chen et al. 2021; Caserto et al. 2024). This aligns with an independent observation by a commercial beekeeper in Israel, who reported that colonies exposed to clothianidin showed 83% recovery when treated with Nano-B-Feed™, compared with 50% recovery in untreated colonies (n = 23 and 8, respectively; Yonatan Ha'klay, personal communication, 2025). Although pesticide application records for the study field were not available, the site was conventionally managed with routine fungicide treatments targeting common regional diseases such as mummy berry *Monilinia vaccinii-corymbosi* (Reade) Honey (Helotiales: Sclerotiniaceae) and botrytis blight *Botrytis cinerea* Pers. (Helotiales: Sclerotiniaceae). Independent research has demonstrated that both pesticides used in conventional blueberry production and those applied in nearby non-blueberry crops contribute to exposure in managed bees. For example, Graham et al. (2021b) found that blueberry pollen collected by managed honey bees and bumble bees *Bombus impatiens* Cresson, 1863 (Hymenoptera: Apidae) contained numerous pesticides, with higher concentrations from farms embedded within more extensive blueberry cultivated area. Furthermore, many pesticides detected in honey bee–collected pollen were highly toxic insecticides originating from adjacent non-blueberry fields, highlighting off-farm exposures are another important and significate route to pesticide exposure (Graham et al. 2022). These findings reinforce the importance of pesticide stewardship, a central component of the integrated crop pollination framework (Isaacs et al. 2017). This framework could be expanded to include enhanced management of commercial bee colonies through the use of supplements with demonstrated nutritional or therapeutic benefits, particularly in agricultural landscapes where pesticide exposure risk is elevated. Nonetheless, improving pesticide stewardship at a landscape scale remains essential, and continued research on bio-intensive farming practices that reduce the reliance on toxic pesticides should be encouraged.

This pilot trial was conducted to evaluate the feasibility and potential impact of an easily deployable nano-scale supplement on honey bee colony growth and performance indicators during blueberry pollination. The study design did not permit evaluation of floral visitation, and expanding the trial to include multiple replicated sites that would allow robust measurements of honey bee visitation to blueberry flowers was cost-prohibitive for a preliminary study. Despite these limitations, the increase in colony size indicated by higher cluster counts suggests that Nano-B-Feed™-treated colonies experienced greater brood production and adult worker population growth during the pollination period. These patterns in turn could support enhanced foraging activity, improved pollination services, and longer-term colony health. Individual honey bee foragers adjust their effort in response to colony state, with larger or higher-brood colonies eliciting greater pollen load collection by pollen foragers and longer, higher-yield nectar trips by nectar foragers compared with smaller colonies (Eckert et al. 1994). An additional practical observation was the ease of deploying the product. Many therapeutic or nutritional feeds require opening hives to access colonies, which is invasive and time-consuming, whereas Nano-B-Feed™ could be applied directly to the bottom board without disturbing the hive, representing a meaningful time savings and potentially reducing stress on the bees. Given these positive indications and the practical deployability of the product, expanded trials across multiple sites with true replication are warranted to inform commercialization and deployment strategies for beekeepers servicing the blueberry industry. Furthermore, future studies should apply metabolomic and transcriptomic approaches to identify the pathways driving enhanced colony growth and increased resilience to environmental stressors, including pesticide detoxification, and relate these mechanisms to key outcomes such as crop pollination and honey production.

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Authors' Contributions

LWD: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Data curation; AE: Conceptualization, Methodology, Writing – reviewing & editing; YE: Conceptualization, Methodology, Writing – reviewing & editing; YL: Conceptualization, Methodology, Project administration, Writing – reviewing & editing.

Table 1. Means (± standard error) of honey bee colony variables measured in Nano-B-Feed™ -treated and untreated (control) hives placed in a commercial northern highbush blueberry field in northwest Washington during the 2025 pollination period (n = 15 hives per treatment).

Treatment	Initial cluster count	Final cluster count	Cluster count difference (Δ)	Return foragers_1 (bees/min)	Return forager_2 (bees/min)
Nano-B-Feed™ treated	15.4±0.63	22.0±1.2	6.6±0.8	26.2±3.1	9.2±2.6
Untreated (control)	15.7±0.57	18.0±2.1	2.3±1.6	32.9±2.4	4.9±0.8
P-value[i]	0.8822	0.1578	0.0461	0.1347	0.465

[i]Treatment effects were evaluated with the Wilcoxon Rank-Sum Test (P < 0.05). Colonies are pseudoreplicates due to group-level treatment application.

Conflict of Interest Statement

The lead and corresponding author (LWD) has no conflict of interest nor financial associations with H4Bees Ltd. and Nexgen Biopharma Solutions Inc. Co-authors AE, YE, and YL are involved in the development of the Nano-B-Feed™ formulation evaluated in this study. The study design, data collection, analysis, and interpretation were conducted independently.

Ethical Approval

Ethics approval was not required for this trial, as it did not involve human participants nor vertebrate animals. No collection permits were necessary either because the work did not include sampling of wild species from natural parks or protected areas.

Data Availability

Data are available from the corresponding author upon reasonable request.

Generative AI Statement

Microsoft Copilot was used to confirm analytical methods, assist with R syntax, and support grammar and clarity checks during manuscript preparation.

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