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Disease-mitigating innovations for the pollination service industry: Challenges and opportunities

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Abstract

Commercially reared bumblebees are often deployed for fruit, vegetable, and seed crop pollination. Commercial bumblebee pollination contributes significantly to economic and nutritional security; thus, maintaining healthy stocks should be a priority for bumblebee producers. Honey bee–collected pollen is used as a nutritional source for bumblebee rearing, but potential contamination of pollen with pathogens requires mitigation to limit spread of infectious diseases. Gamma irradiation is the primary means of sterilizing pollen, but limitations, including off-site access to cobalt-60, warrant exploration into alternatives. Sterilization technologies used in the food safety and medical device sectors, such as pulsed UV and electron beam, offer options with the potential to deliver safe, effective, and less restrictive mitigation. Adopting these alternatives could ultimately support healthy bumblebee stocks and reduce pathogen transmission to other bees.

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Keywords

Decontamination, Sterilization, Emerging infectious diseases, Insect viruses, Pathogenic microbes.

Introduction

Contamination of pollen with pathogens: a source of opportunity

Bumblebees reared commercially, mainly *Bombus impatiens* and *Bombus terrestris*, are essential contributors to global food production. Visitation of greenhouse, high tunnel, and field crops such as tomatoes, peppers, cucurbits, and soft fruits by bumblebees results in highly efficient pollination. This pollination efficiency is partly explained by the ability of bumblebees to buzz pollinate or produce thoracic vibrations that trigger the release of pollen held tightly within the anthers of these flowering plants [1]. Moreover, bumblebee colonies can be produced year-round in commercial facilities, and containment of individual colonies in small, transportable units simplifies deployment to meet growers' demand. There are more than one million bumblebee colonies reared globally every year, and pollination by commercially produced bumblebees increases crop yield and quality, promoting economic and nutritional security [2–4].

Initiating bumblebee colonies artificially requires that queens be confined to small nesting boxes provisioned with food (Figure 1). Diet quality and quantity are essential for queen nesting success and subsequent colony growth [5–8]. Unlike managed honey bees (e.g. *Apis mellifera*), artificial diets are not available to successfully rear bumblebee colonies [3]. Queens cannot forage freely during rearing confinement; therefore, their diet is provided to them and consists of sugar solution, which serves as a source of carbohydrates, and pollen harvested from honey bee colonies, which provides proteins, lipids, and micronutrients (Figure 2).

One concern of feeding commercially reared bumblebees honey bee–collected pollen is pathogens in pollen. Honey bee–collected pollen can be contaminated with viruses (e.g. black queen cell virus and deformed wing virus), bacteria (e.g. *Paenibacillus larvae*, the causative agent of American foulbrood), fungi (e.g. *Ascosphaera apis*, the causative agent of chalkbrood disease), Microsporidia (e.g. *Nosema* spp.), and protozoa (e.g. *Crithidia* spp.) [9–12]. Pathogens found in honey bee–collected pollen can infect bumblebees, which may pose a risk of transmission among managed and wild bee populations [9,10,13–18]. Although our understanding of the impact of pathogens on bee health is best characterized in managed bees [19,20], much remains unknown about their effects on several thousand species of wild bees [13,21–24]. As pathogens are a leading contributor to declining populations of both managed and wild bees [25–27], there is a precedent for mitigating infection and transmission in honey bee–collected pollen provisioned to commercial bumblebee colonies.

Figure 1

An early stage in the development of a bumblebee colony reared artificially. A queen incubates the brood raised atop a mass of honey bee–collected pollen. Two of the first workers have emerged to the adult stage and will assist the queen in caring for the brood. Photo Credit: Elaine Evans.

Figure 2

Collected pollen dislodged from the corbicula of honey bee foragers that have returned to their colony. A pollen-trapping device placed on the colony restricts the passage of returning pollen foragers into their nest, causing the pollen to become dislodged from their corbiculae. Significant quantities of pollen 'pellets' are harvested using this mechanism. Trapped pollen is the primary source of nutrition for rearing bumblebees. Photo Credit: University of Minnesota Bee Laboratory.

Challenges and potentially disruptive pollen sterilization technologies

Reducing the incidence and spread of pathogens among bumblebee colonies reared commercially is a priority for producers. Goulson and Hughes [3] illustrate critical control points in the flow of pathogens among managed bees where abatement is possible and that could reduce transmission to other bees. Honey bee–collected pollen is a point for control in this scheme [3]. The most common approach to sterilizing pollen is exposure to gamma irradiation [28]. Although effective, there are drawbacks to this technology (see the following section). Limitations of gamma treatment prompt exploration of alternative technologies, especially those used in the medical device and food production sectors (Table 1), for their efficacy in inactivating bee pathogens. Before technologies are adopted to treat honey bee–collected pollen, studies should establish effective doses and determine whether there are adverse effects on nutritional quality and associated dietary microbiota [29–32].

Biological surrogates and complementary techniques to optimize sterilization processes for honey bee–collected pollen

Researchers have historically approached sterilization efficacy through biological surrogates, such as *Bacillus* spp. endospores [33–35] or oocysts of waterborne protozoa [36,37]. Biological surrogates are innocuous microbes exhibiting greater resistance to applied inactivation stresses and provide a safe substitute over intended target pathogens for validating sterilization processes [38]. For example, a biological surrogate is exposed to conditions of a sterilization process, and the inability of the surrogate to grow in culture after treatment confirms the process is effective. Biological surrogates used in the food safety and healthcare sectors could serve as calibrators for adapting sterilization processes against complex pathogens that affect bees [39,40]. Biological surrogates would help resolve factors mediating inactivation of target pathogens, such as highly infectious *P. larvae* spores. These factors are multifaceted and include operational (e.g. applied dosage, system configuration, nonthermal modality), environmental (e.g. temperature, pH, water activity), and biological considerations (e.g. amount of organic matter, diversity and abundance of parasites present, inclusion of recalcitrant life stages) [41,42]. The addition of highly sensitive and specific molecular techniques, such as quantitative polymerase chain reaction (qPCR), and cell culture could complement the use of surrogates and permit reliable post-treatment quantification of the pathogen load and reduction in viability and infectivity [38,41]. The appropriateness of complementary *in vitro* systems will depend on the cell line selected. In the case of bees, demonstrating inhibition of infectivity and growth of treated pathogens using cell lines established from bee tissues could be highly useful [43]. Moreover, modeling inactivation kinetics of treated-bee pathogens by flow cytometry would help evaluate sterilization modalities as it will provide real-time cellular and molecular mechanistic information underpinning the killing process [35,44].

Table 1

Properties of different decontamination approaches considered for treating honey bee–collected pollen.[a]

Process considerations	Hydrogen peroxide vapor (VH_2O_2)	Ethylene oxide (EO)	Pulsed UV light	Moist heat	Electron beam	Gamma irradiation
Methodology	Penetration of sterilant gas	Penetration of sterilant gas	Surface irradiation	Penetration by uniform heating	Ionizing energy from electron beam	Irradiation using photons from radioisotopes
Efficacy of process	Efficacy confirmed by biological indicators and/or process monitoring	Efficacy confirmed by biological indicators and/or process monitoring	Variable, but efficacy confirmed by biological indicators or dosimetry	Efficacy confirmed by biological indicators and/or process monitoring	Efficacy confirmed by biological indicators	Process parameters confirmed using dosimetry
Penetration	Limited penetration; gas-permeable packaging/ product design required	Gas-permeable packaging and product design required	Limited penetration	Suitable for treatment of packaged products but depends on material sensitivity	Efficient penetration at bulk densities between 0.05 and 0.03 g/cc	Penetration at high densities (>0.4 g/cc)
Material compatibility	Good material compatibility except cellulose-based materials	Broad material compatibility	Broad material compatibility	Broad material compatibility, but heat can affect nutrients in pollen	Negative effects are less pronounced or eliminated based on packaging.	Broad material compatibility except plastics such as acetals, PTFE, polypropylene
Turnaround time	One-day processing	Conventional treatment requires 9–10 days.	Relatively short, typically $\leq$1 h depending on the dose	Relatively short, typically $\leq$1 h	Very short, several minutes depending on the dose	Relatively short, several hours depending on the dose
Process	Complex process that introduces VH_2O_2 under vacuum or aerosol	Complex process; variables include time, temperature, humidity, and [EO].	Simple, rapid process; delivery of UV (J/cm^2) in an enclosed chamber	Simple, rapid process; duration depends on time, temperature, and RH.	Complex process; variables include scan height, processing speed, number of passes, beam-product alignment	Simple process; variables include time and isotope load.
Putative mechanisms of pathogen inactivation	Potent oxidizer of proteins, but mechanism is not fully understood.	Proteins, enzymes, and nucleic acid alkylation (targets sulfhydryl groups)	Irreversible damage to RNA affecting replication and infection	Thermal aggregation of viral nucleocapsid and membrane proteins	Extensive degradation of RNA and DNA — but yet to elucidate mechanisms properly	Extensive degradation of RNA and DNA molecules
Limitation	Complex process requiring monitoring and control, not for *in situ* application	Toxic residuals (carcinogenic and teratogenic), not recommended for *in situ*	Operator safety due to UV exposure, shading issues, can be used *in situ*	Limited by thermal sensitivity of materials (e.g. pollen)	Not often used *in situ* but more as an external contract service	Adversely affects material, not recommended for *in situ*

PTFE, polytetrafluoroethylene; RH, Relative humidity.

[a] Modified from the study by McEvoy and Rowan [31*].

Gamma irradiation

Although various sterilization technologies are applied toward mitigating pathogens found in honey bee–collected pollen and equipment, gamma irradiation using cobalt-60 is the current standard [28]. Gamma irradiation causes irreparable breaks in nucleic acids and has been reported to inactivate several bee pathogens, including some but not all bee viruses [45–48]. Gamma treatment has been evaluated as safe for food production for more than 30 years (US Food and Drug Administration; URL: https://fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know), and direct exposure of bees or nest materials does not affect bee survivorship [28,49]. Gamma treatment improves food safety and extends the shelf life by reducing or eliminating microorganisms. Furthermore, treatment does not make foods radioactive, compromise nutritional quality, or noticeably change taste, texture, or appearance (US Food and Drug Administration; URL: https://fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know). Gamma irradiation facilities can accommodate large batch sizes, and treatment is compatible with high-density materials, with excellent penetration into nonuniform packaging [33]. However, treatment must be conducted at regulated facilities, requires relatively long processing periods (hours), and potentially degrades products through the release of heat. Owing to the shortage of cobalt-60 supply, medical devices are given priority for gamma treatment, making it prudent to investigate alternative approaches for pollen sterilization.

Hydrogen peroxide in vapor form

Vaporized hydrogen peroxide (VHP) is an environmentally gaseous process used for sanitation of hospitals and health-care facilities [51,49]. The mode of action stems from the generation of free hydroxyl radicals that cause oxidation of DNA, proteins, and lipids [52]. It is effective against adenovirus and avian flu virus [53] and sporicidal when distributed evenly into areas where manual cleaning is impractical [54]. There are two types of VHP sterilization: exposure to 30–35% vapor produced by heating hydrogen peroxide (H_2O_2) or evaporation of H_2O_2 droplets from a 5–7% aerosol. These treatments have long been explored for use in factories for packaging and machinery sterilization [55] and decontamination of meat processing facilities, with varying, but potential, efficacy, against *Listeria monocytogenes* [51]. VHP is most efficacious on inanimate objects but would likely be unsuitable for pollen treatment as exposure to condensate or heat (55–60 °C) would cause structural damage to pollen [55] and nutrient degradation (Eakins and Rowan, personal communication, December 9, 2020).

Moist heat

Moist heat uses either plant, process, or pure steam [56] and is used in the pharmaceutical industry for vaccine and medical device sterilization and in the food industry for pasteurization. Most vegetative microorganisms are inactivated between 55 and 65 °C using moist heat, with more resistant microbes and spores requiring temperatures ≥70 °C and 100 °C, respectively, to achieve inactivation [56]. Owing to pollen's organic nature and denaturation of matrix proteins at >60 °C [57], moist heat could be an obstacle, but further investigation is warranted. As mentioned previously, pollen will form a dough-like mass after exposure to condensate, which may provide opportunistic microbes a substrate for growth that leads to nutrient degradation and spoilage. Studies should determine if bees are attracted to pollen treated with moist heat.

Ethylene oxide gas

Ethylene oxide (EO) is a gaseous process traditionally used for sterilization of spices and now predominately for medical devices [58,59]. EO effectively diffuses through solid matter without causing damage to heat- or moisture-sensitive materials [58,60]. EO is an explosive, highly flammable gas and is highly toxic, carcinogenic, and mutagenic. It is an alkylating agent that interacts with biomolecules, such as nucleic acids and proteins. The addition of alkyl groups to these structures prevents regular cellular activity and inhibits microbial reproduction [61]. The compatibility of EO with moisture-sensitive products is of potential interest for pollen treatment. However, the generation of toxic byproducts, such as ethylene glycol, when EO interacts with water, would require further safety considerations [58,61]. Other potential drawbacks of EO include cost and cycle length [58]. Despite the compatibility with a broad range of materials, this modality will likely be reduced or replaced because of ongoing environmental and sustainability considerations.

Pulsed UV light

Pulsed UV (PUV) technology dissipates stored energy in ultrashort bursts of broad-spectrum light. Currently, PUV is used for high-throughput sterilization of packaging for the food industry [38]. PUV inactivates various complex pathogens [39,40], including those associated with bees [62]. Brief PUV exposure reduces the viability of surrogate oocysts of the trypanosome *Cryptosporidium parvum* [37] and the trypanosome *Crithidia bombi*, a common bumblebee parasite [62]. PUV is considered nontoxic and environmentally friendly based on an increased understanding of the relationship between the UV dose and inactivation of cellular mechanisms [38,41,63]. PUV can be delivered from a fixed source *in situ* or in an adjustable configuration via a handheld device to achieve maximum exposure; however, penetration depth is limited by nontarget materials obstructing the flow of UV radiation [38]. These drawbacks could restrict usage to surface disinfection, but PUV has several advantages compared with gamma irradiation, including *in situ* application and

relatively short processing time. Further studies are required to determine the potential of PUV for pollen sterilization.

Electron beam

High-energy electrons emitted from an accelerator (E-beam) are an alternative to gamma irradiation [33]. E-beam operates through standard electricity, negating the need for radioactive isotopes [33], and is a continuous process technology for sterilizing medical devices and pharmaceuticals [64]. E-beam reduces bacterial pathogens on fresh foods, including *Bacillus cereus* endospores using doses of 3.65 kGy (broccoli) and 4.8 kGy (red radish) [65]. It also reduces porcine epidemic diarrhea virus in contaminated feed [66] and causes minimal changes to powdered infant formula [67]. E-beam lacks the penetrative power of gamma sterilization, and as an *in situ* process, there is potential for recontamination of treated products during redistribution [65]. Despite these drawbacks, E-beam has several advantages compared with gamma irradiation and includes short exposure periods (minutes), fast cycle time, flexible batch size, even distribution of dose, simple validation, no quarantine, and real-time monitoring. Rapid processing of low-density materials and greater operational flexibility can make E-beam a cost-effective approach for pollen treatment.

Conclusions

Development and application of effective, nonthermal sterilization of contaminated pollen would be a potentially powerful tool to help sustain the health of commercial bumblebee stocks and reduce pathogen transmission to other managed and wild bees. Currently, there is a lack of efficacy data for emerging sterilization technologies, and research that addresses the complex morphology and culture requirements of bee pathogens is needed. This review highlights the potential benefits of alternatives to gamma irradiation for pollen treatment, but additional studies should address appropriate dosage, treatment configurations, and mechanistic information underpinning cellular and molecular damage to pathogenic microorganisms and viruses. There remains a reliance on using live bees to confirm treatment effect; however, advances in *in vitro* diagnostics may enable surrogate approaches as a screening tool. Novel processes will be informed by applying technology, policy, and society readiness level framework that considers the intended environment and sustainability of innovation. Ultimately, the deployment of sustainable decontamination technologies to treat honey bee–collected pollen used to rear bumblebees would contribute a vital countermeasure to reduce pollinator decline.

Author contributions

MG, JE, and NJR conceived and proposed the topics of the manuscript. MG, JE, and NJR wrote the article. MG and NJR provided funding and resources.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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