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Researching ways to combat the negative effects of pathogen spillover for commercial bees and products

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Introduction

Bees pollinate over 80% of the agricultural crops produced in Europe. Honey Bees (Apis mellifera) are used as a commercial pollinator for many agricultural crops globally*(Commission, 2018)*. However there is evidence that commercial pollinators and the pollen they feed on can be contaminated with parasites (*Graystock et al., 2013*) which results in a spill over to native bee species (*Graystock, Goulson and Hughes, 2015)* such as *Bombus terrestris*. While parasites, viruses and mites may not be the sole cause of bee decline, there is growing evidence that they can act in synergy with pesticides to multiply the negative effects. There are other factors that contribute to the decline of bee colonies, such as climate change and habitat loss *(Tong et al., 2018) (Runckel et al., 2011) (Reeves et al., 2018)(Peso et al., 2018*), however, nutrient is a key factor in helping to negate some of the harmful effects of parasites and viruses (*Cedrix Alaux, Christelle Dantec, Hughes Parrinello, 2011)(Huang, 2012*) which is why ensuring good quality pollen that is parasite free is vital to the success of bees.

Current methods to control the spread of parasites on pollen involves the use of gamma irradiation. This can be time consuming (25kGy for 9hrs 45mins)and involves transporting to and from a sterilizing facility as it can't be carried out insitu *(Simone-Finstrom et al., 2018*) . This also increases the risk factor of becoming contaminated again on the way back to colony.

Pulse UV (PUV) is a system that uses short bursts of UV light to introduce irreparable damage to cellular organisms. It offers the benefits of been able to be housed insitu while also speeding up the process of reducing pathogen loads *(Naughton et al., 2017)* . However PUV has failed to show any efficacy of reducing the bioburden in pollen so far. We are currently exploring new methods to try reduce microbial loads in commercial pollen.

Methods

Bacillus cereus initial used as a test organism due to its ability to form spores. Concentrations of *B. cereus* were then subjected to Pulse UV (PUV) treatment at 1 pulse per second (p/s) for 150 seconds and then allowed to incubate with either nutrient agar or broth to aid recovery. A 10e6 reduction was observed. Pollen was spiked with *B.cereus* and subjected to PUV for 150s. The effects of PUV was monitored with plates counts and Flow cytometry.

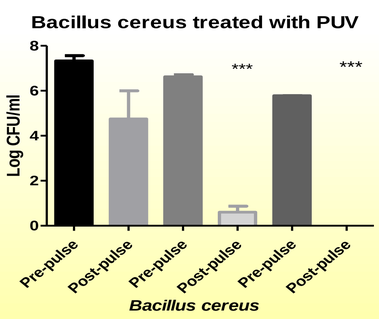
Microbial communities were extracted from pollen via filtration. These communities were treated with PUV and the effects observed with Flow cytometry and haemocytometers.

PUV Machine

Pollen

Vacuum flask filtration setup

A



B

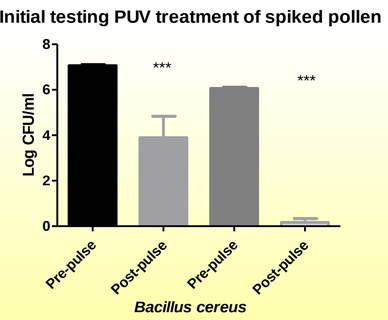


Fig A, shows the powerful effects PUV has on vegetative bacterial cells. In both cases large reductions of B. cereus were observed, even with the pollen solution present.

C

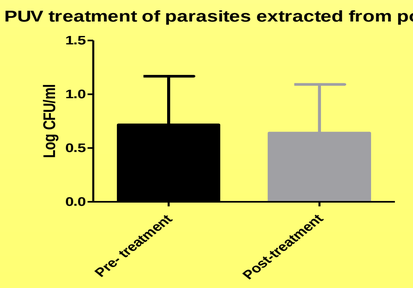
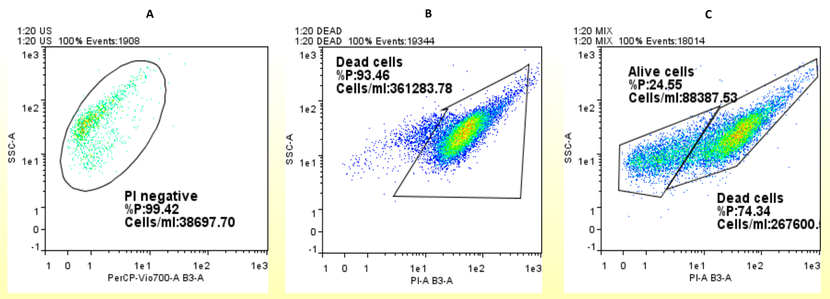


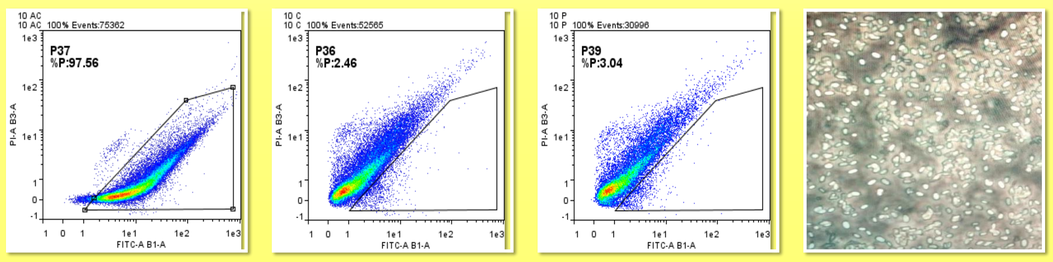
Fig C: suspected parasite communities were subjected to PUV treatment @ 800V for 150s (n = 9). Using a 2-way Anova and a Mann-Whitney U test, no significant difference between untreated and PUV treated samples. There is a 71% chance of randomly observing these events.

D



In Fig D: (A) depicts an unstained negative control, (B) a heat killed positive control & (C) a mix of both alive and dead *B.cereus* cells. Propidium iodide is a stain used that only fluoresces in damaged cells so is used to determine viability.

E



Microbial samples extracted from pollen and autoclaved for 20 minutes (A) is shown next to an untreated control (B) sample and a PUV treated sample(C). Sample (A) was used as positive controls to try measure the effectiveness of PUV treatment (C).The shift to the right in shows the percentage of Sybr that has bonded with DNA. This is not present in B & C. The lack of bound Sybr in both (B) & (C) hint at the population being spores, and at PUV not being effective in treating the community within the pollen. The image (D) depicts the microbial community residing inside commercial pollen, viewed under a light microscope at 40x.

Discussion

Previous experiments had shown the efficacy of reducing the spread of *Crithidia bombi* using PUV *(Naughton et al., 2017)*. We planned to build upon this discovery by using PUV to treat commercial pollen. Preliminary results indicated that PUV had potential to be a useful tool in reducing pathogen loads on commercial pollen, that is used to fed commercial pollinators like the honey bee. A 10e6 reduction of *B. cereus* was witnessed, when treated with PUV, with similar effects witnessed with pollen spiked with the bacterial strain. However when the integrity of the pollen was maintained, PUV treatment was ineffective, most likely due to a shading effect by the pollen.

Experiments carried out on the bioburden extracted from the commercial pollen observed no significant difference in microbial loads when treated with PUV technology. This leads us to believe that PUV treatment while effective for vegetative cells such as B. cereus on flat surfaces, PUV lacks the penetrative power needed to be effective in pollen sterilization.

The project will now focus on other sterilization techniques such as e-beam that might help halt the pathogenic spread that occurs with the use of commercial pollen.

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