Assessment of the in-vitro inhibitory activity of HiveAlive™ against Ascosphaera apis, the causative agent of Chalkbrood infection in honeybees

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Abstract
Chalkbrood is one of the most common and widely recognised fungal diseases that affects honeybee brood, the causative agent of which is Ascosphaera apis. Currently, there are no approved treatments for the management of Chalkbrood infection in beekeeping operations. Anecdotal evidence from beekeepers suggests that use of the feed supplement HiveAlive may cause a reduction in Chalkbrood infection. Here, a number of in-vitro tests were carried out to assess this hypothesis including agar dilution assays and 96 well plate microdilution assays. A concentration of 0.25% inhibited the growth and sporulation of A. apis on agar plates. Results from the 96 well microdilution assays suggest that even lower concentrations may be effective at inhibiting growth. Further research is required to validate the findings presented here and to examine effects in a hive setting.

Introduction
Chalkbrood usually occurs in damp weather and is most common in the spring. Inadequate nutrition and exposure to other diseases or conditions such as chilled brood or colony stress may also promote Chalkbrood. Currently, there are no approved treatments for this fungal infection. Reports from beekeepers suggest that supplementing hives with HiveAlive, a feed for bees, decreases levels of Chalkbrood observed.

Materials & Methods
Plugs of agar from cultures of positive and negative mating strains (ARSEF7405 and ARSEF7406) of Ascosphaera apis were placed approximately 4cm apart from each other on Sabouraud Dextrose agar supplemented with varying concentrations of HiveAlive. Plates were subsequently incubated at 30°C and growth was monitored every 24 hours.

Spore solution was obtained from 7 day old plates containing both mating strains with visible spores present on the plate by flooding the plate with 5ml of sterile H₂O and swirling. The liquid was removed and placed in a tube to allow spore cysts (ascoma) to be ruptured to release spore balls (asci) by mechanical crushing. Spores balls (asci) contain ascospores which are the primary source of infection. This solution was added to a 96 well plate in SD media supplemented with varying concentrations of HiveAlive. Growth was monitored spectrophotometrically.

Results & Discussion
Results from agar dilution plates suggest that a concentration of 0.25% HiveAlive prevents growth of A. apis (see image 4). At a lower concentration of 0.05%, growth is slightly inhibited but is not inhibited at all when 0.005% HiveAlive is present. This would suggest that HiveAlive is suited to prevent sporulation of A. apis. A range of concentrations were also tested in the microdilution assay and it was found that even lower than 0.25% inhibited growth.

Further experiments are required to determine minimum inhibitory concentration in vitro and determine effects in a hive setting.

References: