

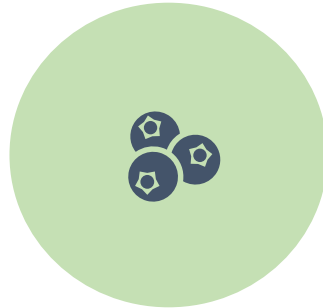
The Buzz Around Blueberries:

Determining the field efficacy of BVT CR-7
vectored by honeybees against three
pathogens of highbush blueberry

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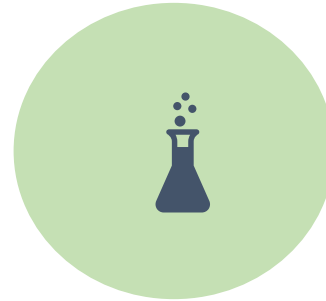
Outline



BACKGROUND



TARGET PATHOGENS



METHODS



RESULTS

Background



- In British Columbia highbush blueberries have grown into a \$7 billion industry. Which accounts for 96% of Canada’s production.
- Conventional agriculture can sometimes rely upon chemical fungicides as a last resort. The public demand for sustainable food, deregistration of certain fungicides, and the occurrence of natural resistance is driving a need for change.
- BVT is a leading technology company that delivers a sustainable and effective endophytic fungus called *Clonostachys rosea* (strain CR-7). Through solar-powered dispensers attached to commercial beehives, the powdered fungi is distributed by the bees and essentially “crowds the parking lot” by not leaving enough room for harmful pathogens to infiltrate plant tissue.

A close-up photograph of several mushrooms with light brown, cup-shaped caps and dark, textured stems, growing on a dark, moist surface. The mushrooms are arranged in a cluster, with some in the foreground and others slightly out of focus in the background. The lighting is soft, highlighting the texture of the mushroom caps and stems.

Target Pathogens



Mummy Berry

Monilinia vacciniae-corymbosi

Characterized by the blighting of new shoots, and a dry rot that mummifies the fruit. As the fruit matures, the berries turn pink to, shrivel and fall to the ground.



Gray Mold

Botrytis cinerea

A common blueberry pathogen. All plant parts can become infected with a fuzzy, gray fungal growth. Mature berries turn tan or pale brown.




Anthracnose – Ripe Rot

Colletotrichum acutatum

A salmon or rust-coloured berry rot. The blossom clusters will turn black or brown, and the infected fruit will show pink-orange spore masses at the blossom end.



Methods

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- Field trials were carried out at an organic blueberry farm in the Lower Mainland.
 - Four sets of BVT CR-7 treated honeybee hives were placed at the furthest points away from the central control hives.
 - The geographical disease data will be used to create annual “heat” maps for the target pathogens across the field to provide a visual representation of the impact of CR-7 over time.



Control Hives

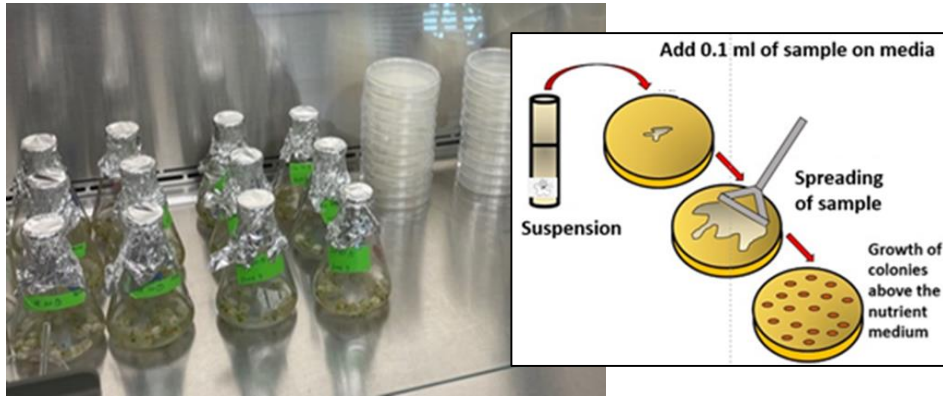
Field scoring for Mummy berry

- A preliminary field inspection was conducted to gain an understanding of disease occurrence at the site for the four varieties located in the field: Duke, Draper, Chandler and Liberty.
- Blueberry bushes were scored for Mummy berry due to the high frequency of pinkish, wrinkled, fruit and blighted new growth initially observed.



Blueberry blossom and fruit sampling

Blueberry bushes were flagged at certain intervals so that the flowers and berries were collected from the same plant.



- Blueberry blossoms were collected in the test field while in full bloom. The flowers were washed with a Tween 20 solution, and the suspension was spread on Petri dishes containing PDA media.
- The Petri dishes were incubated and the colony forming units (CFUs) were inspected for CR-7, Mummyberry, gray mold and anthracnose.

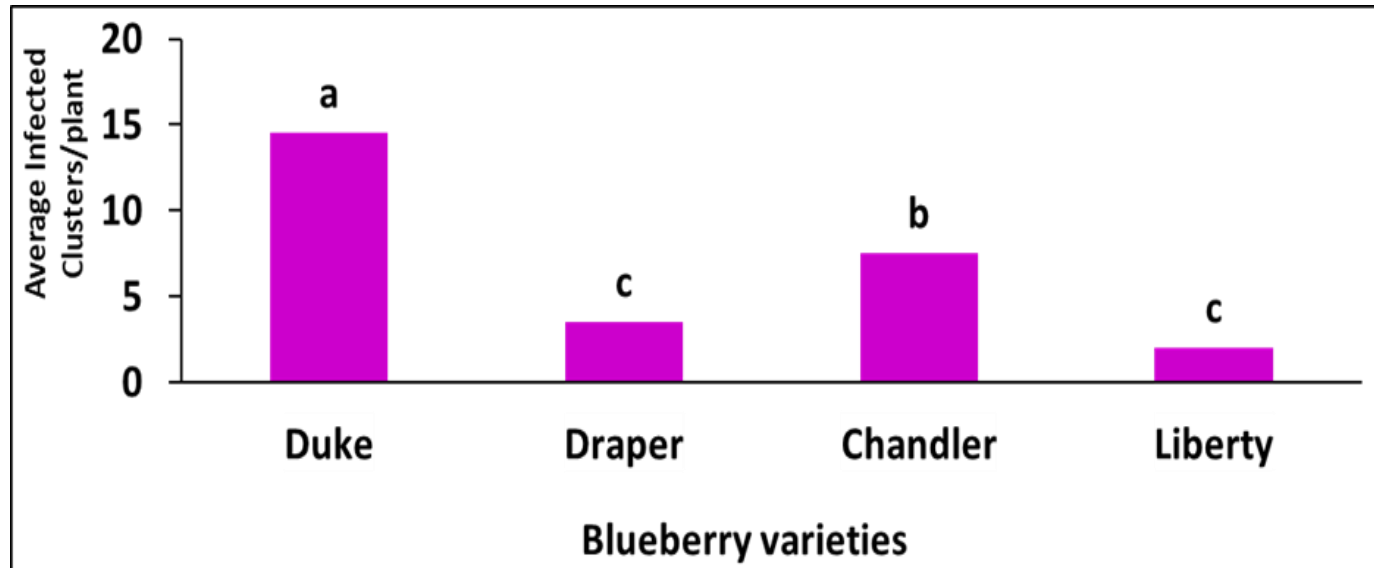


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- Berries were collected from the same locations as the blueberry blossoms
 - The surface of the fruit was sterilized, cut in half and placed incision-side down on PDA media. The petri dishes were incubated, and pure cultures were made for DNA extraction and PCR testing.



Results

Field scoring for Mummy berry



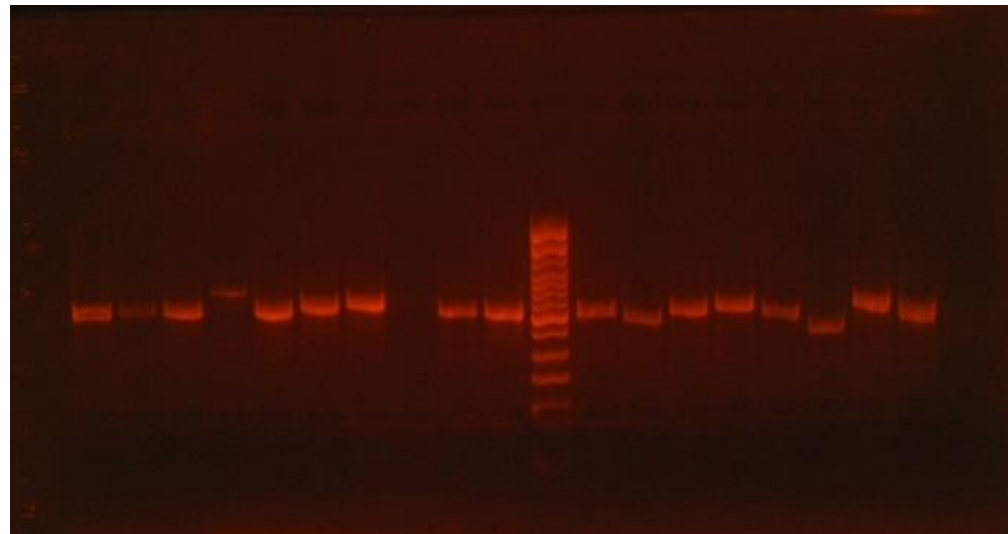
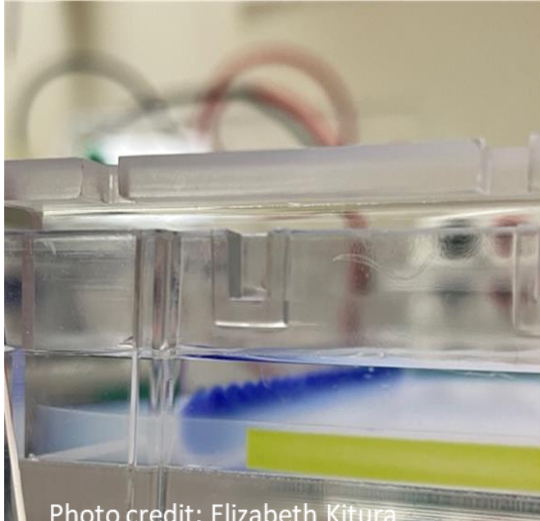
The variety Duke showed the highest frequency of mummy berry during the field scoring. However, Draper did not produce many clusters. Further field trials need to be conducted in order to confirm this data.

Polymerase chain reaction (PCR)

C. rosea was not found in any of the blueberry blossom solutions. A rapid molecular assay to selectively identify and quantify BVT CR-7 in environmental samples is currently in development.

C. rosea was found in 0.1% of the berries collected and tested in the lab. Small numbers, but the data shows that there is a possibility for the *C. rosea* to travel from flower to berry tissue.

More testing needs to be conducted. During the trial we experienced dispenser malfunction for a number of units which has been corrected ahead of this season.



Blueberry blossom sampling

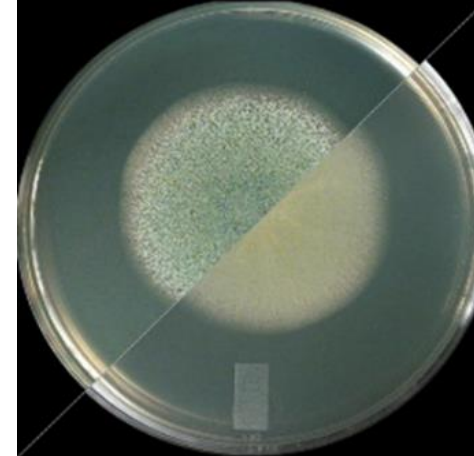
C. Rosea was not found in the blossoms, microorganisms that were identified from the flowers included:



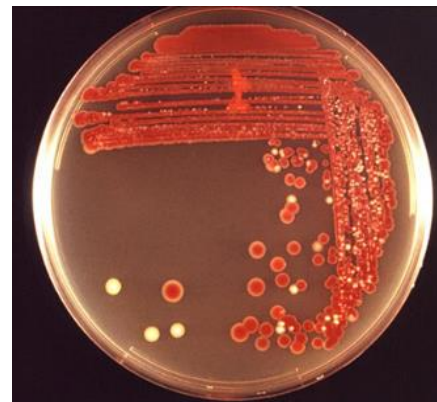
Epicoccum nigrum



Aureobasidium pullulans



Penicillium sp.



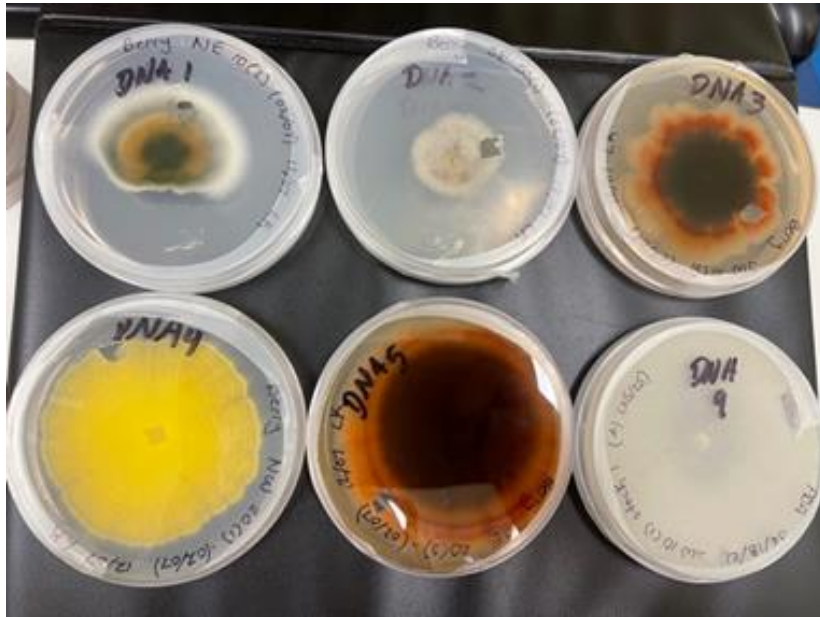
Serratia sp.



Clostridium sp.

Blueberry fruit sampling

C. Rosea was found in 2 berry samples collected from the field, the microorganisms that were identified from the fruit included:

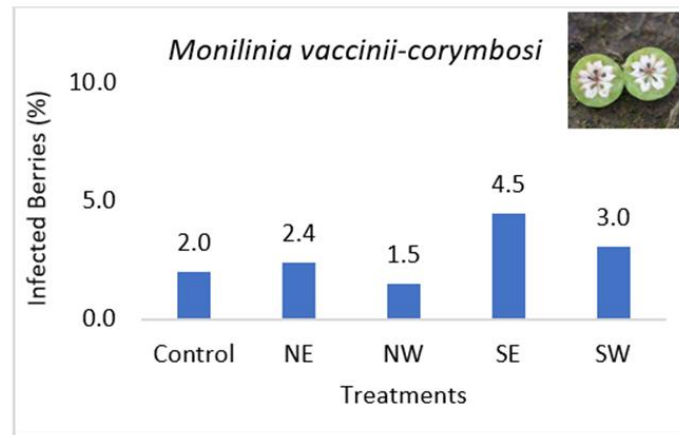


#	Location	Scientific Name	Common Name
1	NE	<i>Godronia cassandriae</i>	Godronia Canker
2	NE	<i>Monilinia vaccinii-corymbosi</i>	Mummy Berry
3	NE	<i>Penicillium sp. Penicillium</i>	
4	NE	<i>Botrytis cinerea</i>	Gray Mould
5	NE	<i>Epicoccum nigrum</i>	Red Blotch of Grain
6	NW	<i>Penicillium sp.</i>	Penicillium
7	NW	<i>Monilinia vaccinii-corymbosi</i>	Mummy Berry
8	NW	<i>Epicoccum nigrum</i>	Red Blotch of Grain
9	NW	<i>Clonostachys rosea**</i>	
10	NW	<i>Clonostachys rosea**</i>	
11	NW	<i>Mucor hiemalis</i>	Mucor Fungus
12	SE	<i>Monilinia vaccinii-corymbosi</i>	Mummy Berry
13	SE	<i>Epicoccum nigrum</i>	Red Blotch of Grain
14	SE	<i>Aureobasidium pullalans</i>	Blue Stain of Wood
15	SW	<i>Epicoccum nigrum</i>	Red Blotch of Grain
16	SW	<i>Botrytis cinerea</i>	Gray Mould
17	SW	<i>Cladosporium sp.</i>	Black Mold
18		Control	Control

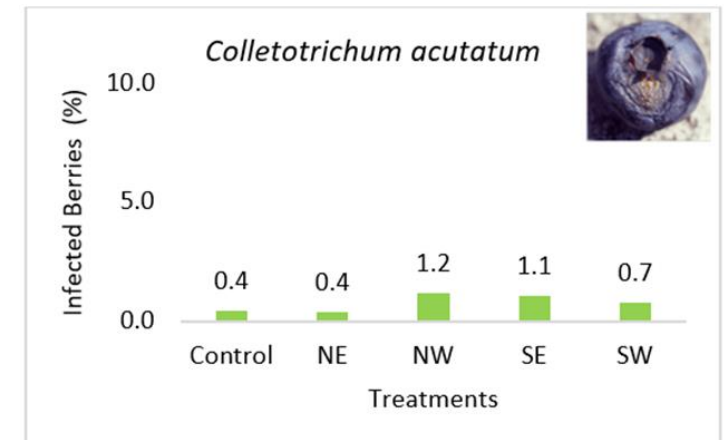
Comparison of the percentage of fungi identified in berries collected from treatment sites

Mummy berry showed the highest frequency within the field, but there was no significant difference between treatments.

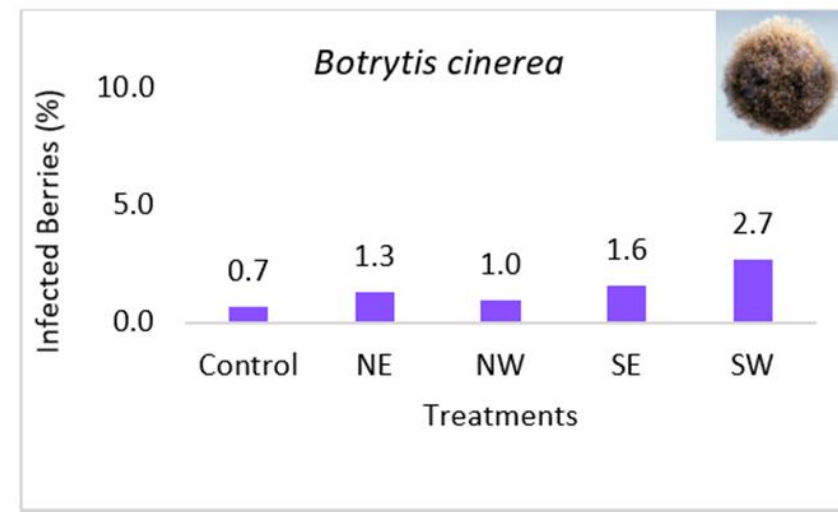
Mummy berry



Anthraco



Gray Mold



Conclusion



The honeybees involved in this trial were seemingly unaffected by CR-7



The trial demonstrated BVT CR-7's ability to colonize in flowers and then berries and persist once the fruit develops. But the low frequency of CR-7 due to dispenser malfunction leaves more room for further testing



This opens another door for developing organic bioproducts for managing diseases in blueberries while reducing the need for chemical fungicides, minimizing machinery damage to soil, and decreasing the cost of agricultural production.



Going Forward

- Continue the field efficacy of BVT CR-7 delivered by honeybees through repetition of the previous trials
- In the process of developing a rapid molecular assay to selectively identify and quantify BVT CR-7 in environmental samples
- Through this we hope to better understand the endophytic behaviour of BVT CR-7 in highbush blueberry, including persistence and ability to protect new growth from primary Mummy berry infection
- Investigate other novel approaches to pathogen management with BVT CR-7 in highbush blueberries through combined management approaches with organic products.

Acknowledgements

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Questions?

