# Characterization of fatty acids from blueberry and cranberry flowers and their effects on the fruit rotting pathogen *Colletotrichum fioriniae*

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AMERICAN CRANBERRY GROWERS association

NORTHEAST SARE Sustainable Agriculture Research & Education

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**Research Hypothesis:** Specific plant chemicals produced during bloom play a critical role in the infection process (appressorial formation) and sporulation (secondary conidiation) events of *C. fioriniae.* Characterization of cuticular waxes from blueberry and cranberry flowers will lead to identification of selectively stimulatory compounds. Qualification of this relationship will elucidate factors related to optimizing disease control / management strategies.

**Introduction and Objective:** Blueberry and cranberry floral extracts (FE) have been shown to dramatically alter lifecycle events of numerous fruit rotting pathogens, especially those related to infection and inoculum buildup (both aqueous and chloroform-based). Extracts collected during bloom are consistently more stimulatory than at any other growth stage. However, the exact chemicals responsible for this observation were unclear. Here blueberry and cranberry cuticular waxes were characterized using the GC-FAME technique, with identified fatty acids and their derivatives subjected to a glass coverslip bioassay to determine the bioactivity of each compound, mimicking the interaction of a pathogen once landing on the waxy surface of a host.

**Methodology:** Chloroform-based extractions were collected from multiple tissue types and developmental stages of blueberry and cranberry plants. An aqueous extraction of blueberry and of cranberry flowers was also collected and further extracted with chloroform. Extractions were saponified and methylated, then characterized via GC-FAME (Midi Inc.) for their fatty acid composition (C9:0 –C20:0). Identified compound concentrations were estimated using a standard curve for hexadecenoic acid. Identified compounds were screened in a glass coverslip bioassay to determine bioactivity.

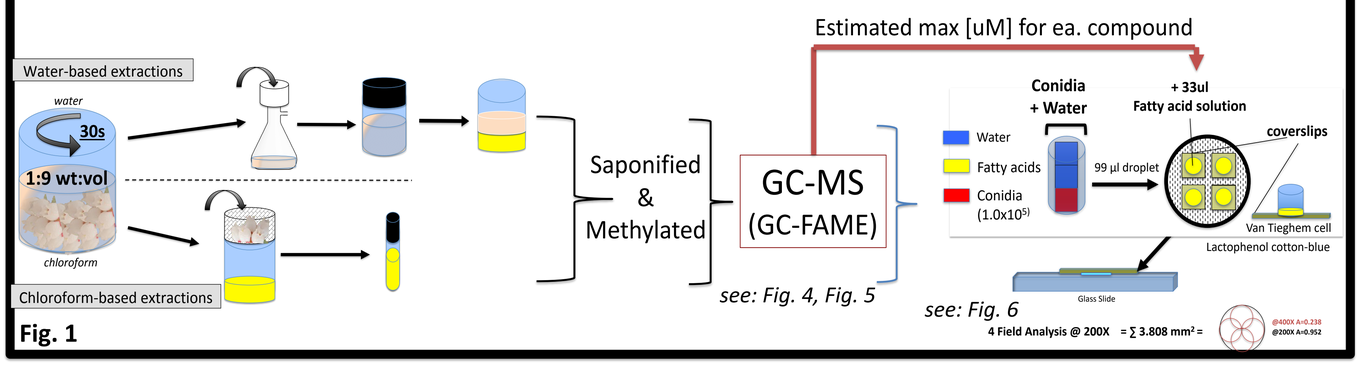



Fig. 1

Fig. 2

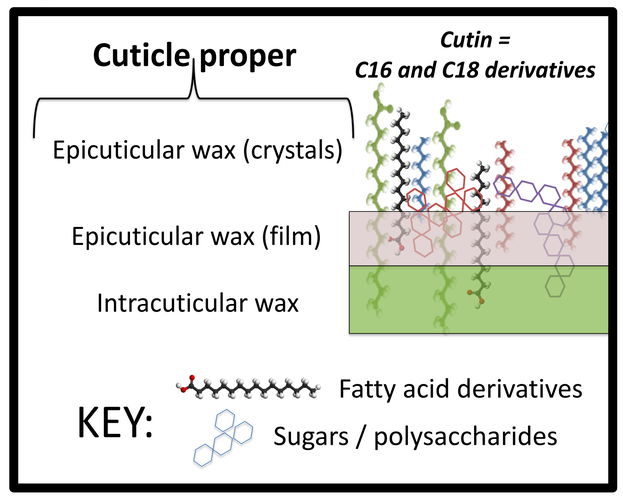



Fig. 3

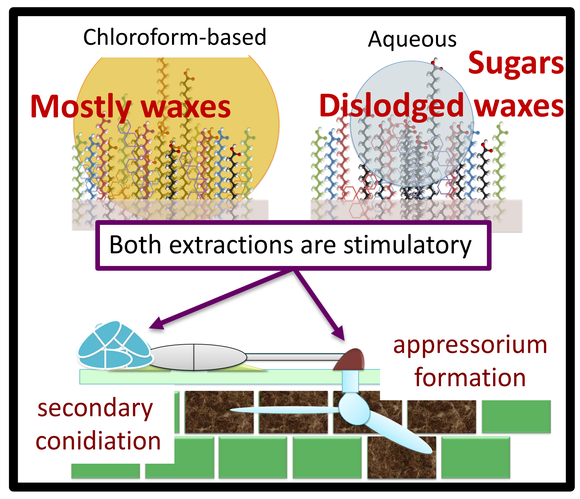



**Fig. 4.** Multiple tissue types - transition of fatty acids over development

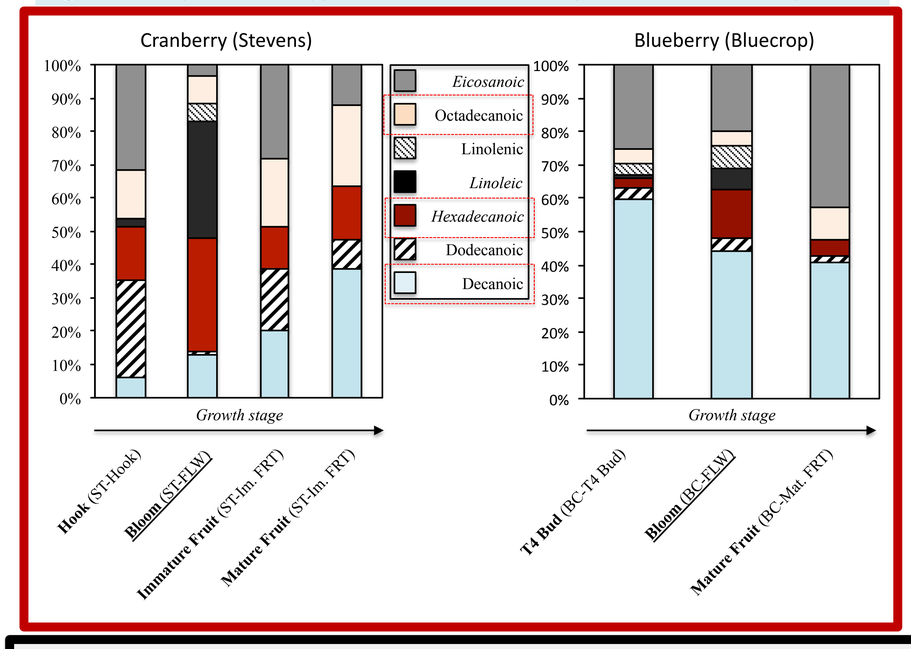



**Fig. 5.** Fatty acid composition from multiple tissues – [uM]

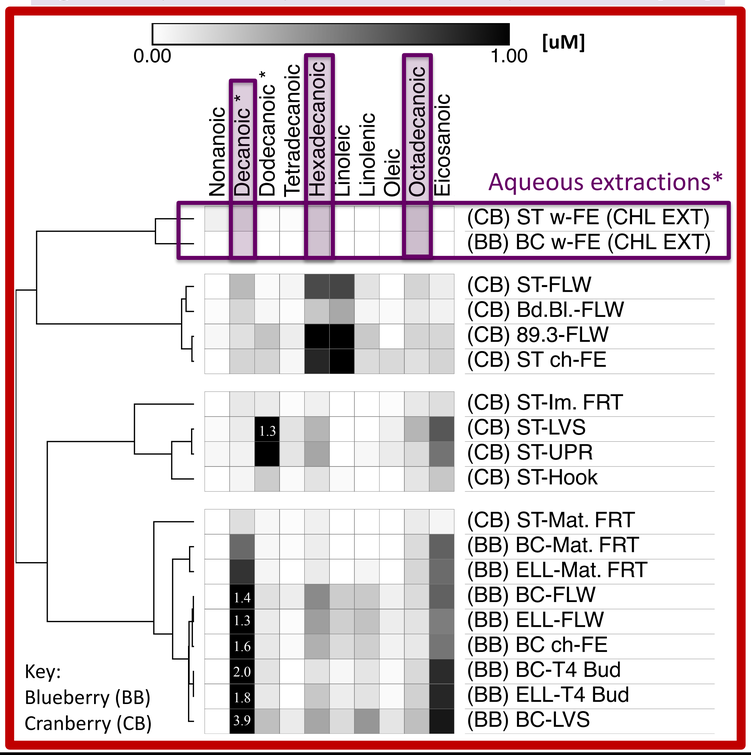
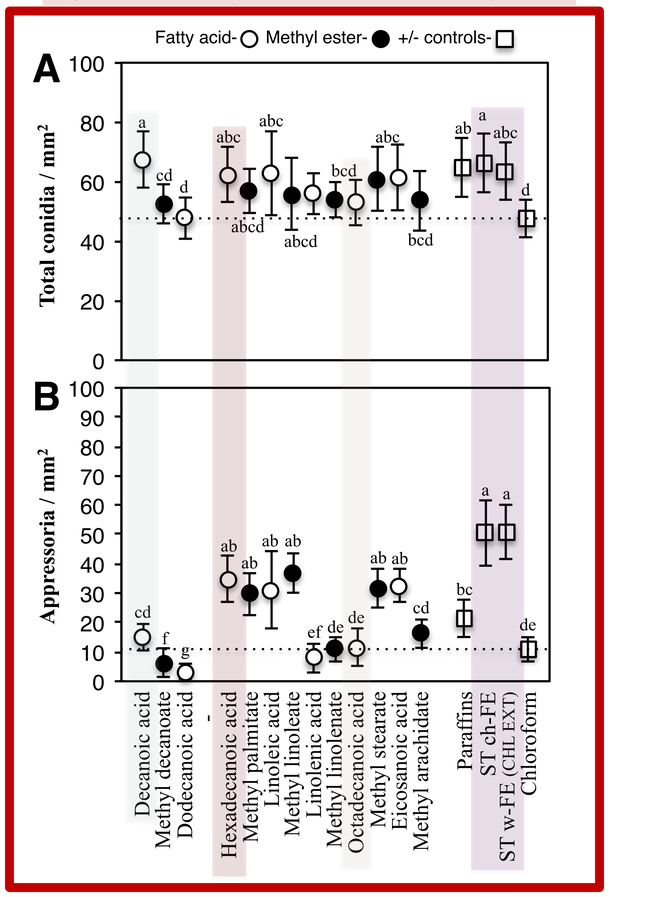



**Fig. 6.** Bioactivity of characterized fatty acids




## Results:

1. Fatty acid (FA) composition changes across plant development – C16:0 hexadecenoic acid (HEX) was most abundant during bloom. **Fig. 4**
2. C16:0 and C18:0 scaffolding FAs (**Fig. 2**) detected in both aqueous and chloroform-based extracts (**Fig. 3**) – indicates that surface signaling molecules can become mobilized or 'knocked' free into water. **Fig. 5**
3. FA composition groups by host, tissue, and extraction type. **Fig. 5**
4. HEX and decanoic acids stimulate *C. fioriniae* secondary conidiation **Fig. 6A**, whereas HEX stimulates appressorial formation. **Fig. 6B**
5. **HEX is present in both extraction types – stimulates secondary conidiation and appressorial formation – key plant signal for disease**

## Moving forward & Considerations:

- **Entire suite** of stimulatory and inhibitory signals likely explain observed discrepancies in cultivar stimulation – leading to tools for management
- **Weaponize HEX** – stimulate pathogens in presence of fungicides - Utilize stimulation when fruit are not present (*deregulate lifecycle transition*)
- Utilize these trap-sprays outside of bloom to safeguard pollinators from fungicides (*bloom applications = critical for disease control currently*)

Waller, T. et al 2018. Phytopathology. 108, 561-567.
Waller, T. et al 2019. JoVE 146:e58880.
Waller, T. et al 2020. Phytopathology 110, 1270-1279