Washington Mint Commission Field Day

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Management of Verticillium wilt

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OBJECTIVES

- Develop and validate tools for assessing risk of Verticillium wilt in mint fields.

PROGRESS

1. Soils from 30 commercial mint fields in Washington and Oregon were collected since 2015.
2. Soil samples are used to (i) develop a real-time quantitative PCR (qPCR) assay to detect, quantify, and differentiate strains of *Verticillium dahliae* from mint fields and (ii) validate the qPCR assay. Validation includes:
    i. Comparisons between traditional methods for detecting *V. dahliae* and the qPCR assay.
    ii. Scoring for wilt in commercial mint field from which soils were collected.
    iii. Ongoing greenhouse bioassays where susceptible and resistant mint varieties are grown in field soils and scored for wilt and pathogen presence.
    iv. Ongoing greenhouse trials where peppermint is grown in field soils artificially infested with strains of *V. dahliae* aggressive to mint and other crops and detected with the qPCR assay.
    v. Screening historical and contemporary *V. dahliae* collections with qPCR primers.
3. *V. dahliae* was quantified from soil samples with traditional plating methods, plant-parasitic and non-parasitic nematodes were counted and total genomic DNA was extracted at OSU.
4. The DNA extraction method for soil samples was optimized by OSU for the qPCR assay to enable detection of 0.1 pg of DNA

qPCR Assay:

- The qPCR assay is still being developed due to unforeseen diversity among strains which complicated the identification of a genomic region able to differentiate strains.
    - The intergenic spacer (IGS) region (100-1,746 bp) of *V. dahliae*'s genome was queried among 77 isolates for differences associated with strains aggressive to mint vs. other crops; however, formerly identified

differences were shared between strains aggressive to mint and strains aggressive to other crops.

- Other genomic regions were also investigated, primers were designed around single nucleotide polymorphisms (SNP) by OSU, and both laboratories used the primers to score our isolate collection. Differences between mint strains and strains from other hosts were not detectable with these primers across a range of conditions.
- Published primers for Random Amplified Polymorphic DNA (RAPDs) were used to score our isolate collection. Amplicons from these primers were used to differentiate strains of *V. dahliae* from mint in different growing regions in the Pacific Northwest.

qPCR Assay Validation:

- We are currently quantifying *V. dahliae* from soils collected in winter of 2016 and spring of 2017 with traditional methods. These data will subsequently be compared with estimates from the qPCR assay.
- During the 2017 growing season we will walk through the commercial mint fields sampled during winter of 2016 and spring of 2017 and score wilt in each field. These data will also be compared with estimates of *V. dahliae* inoculum from the qPCR assay.
- The greenhouse bioassay will be planted in early June once the soils from both Washington and Oregon are dried and prepared for planting. The disease and pathogen incidence data from this trial will be compared with data from the qPCR assay.
- Soils representative of the Columbia basin of Washington and Oregon are being collected. These soils will subsequently be artificially infested with strains of *V. dahliae* that can be distinguished with the qPCR assay and planted with peppermint. Wilt will be assessed throughout the growing season and inoculum will be estimated at the end of the growing season with a traditional method and the qPCR assay.
- Historical and contemporary strains of *V. dahliae* will be screened with eh qPCR primers during the summer of 2017.
- Data from the first 5 validation procedures and nematode counts are being used to create a model to predict wilt in commercial mint fields.