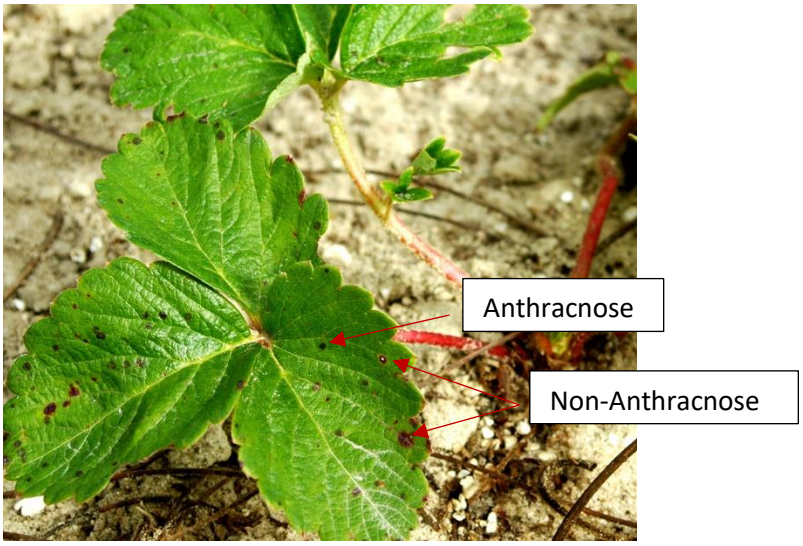
Strawberry sample collection guide and latent infection diagnosis

As soon as new shipment of tips/cuttings arrives, grower cooperators will check for suspected tips that may show black spots on leaves. Photo below was included to show them the difference between anthracnose and other leaf spots. The photo below has both Colletotrichum gloeosporioides anthracnose and other leaf spots (*Mycosphaerella*: common leaf spot; Scorch or Phomopsis). We included this in our curriculum for education part of the project.



However, in lower level of infection it is unlikely to find any anthracnose leaf spots. In the absence of symptomatic leaves, 10 tips from each box were collected randomly in a small bag and labeled as box #1, 2, 3 etc. and shipped to us for diagnostics. One leaf including petiole from each tip was separated and subjected to paraquat (gramoxone) protocol. Briefly, induction of senescence to surface-sterilized (1 minute in 70% Ethanol, rinse in sterile water, 1 minute in 10% bleach, rinse in sterile water, 1 minute dip in paraquat, rinse followed by 7 days of incubation on metal screens inside a humid chamber layered with moist paper towel to enhance acervular growth. Spore masses if available from the leaf surface were streaked on potato dextrose agar (PDA; Difco, Lawrence, KS) and single-spore colonies were transferred to separate slants. Each isolate was identified by microscopic study of the spore morphology as well as with PCR using a species-specific internal transcribed spacer region (ITS1) primer CgInt (GGCCTCCCGCCTCCGGGCGG) and the conserved universal primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') encoded in the 28S ribosomal subunit. Extraction of DNA and PCR amplification was performed according to the methods described by Rahman et al. (2019). Molecular identification can also be done with real time PCR or DNA macro array or micro tube hybridization. However, as morphology based diagnostic process produced reliable result, we avoided molecular methods. Similar sampling was made from plug plants. In that case, rather than sending plug trays, cooperators collected leaf samples and sent to us.

Reference: Rahman, M., Islam, T., Schwegel, R. and Louws, F. 2019. Simultaneous Detection of Colletotrichum acutatum and C. gloeosporioides from Quiescently Infected Strawberry Foliage by Real-Time PCR Based on High Resolution Melt Curve Analysis. American Journal of Plant Sciences, 10, 382-401.

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