Objective 1. Diagnosis of latent infection on foliage of strawberry propagation materials

Methods

Samples were shipped to the University of Maine Plant Disease Diagnostic Lab to test for the presence or absence of *Colletotrichum* spp. One sample represented one strawberry leaf. A strawberry leaf has 3 separate leaflets, each of which is split into 2 sections by a midrib. From left to right, the sections were numbered as 1-6 (Figure 1.) A subset of the sample was selected by using a random number generator from one to six to determine the location of the tissue used for molecular testing on each sample. A total of 0.20 g of plant tissue was used for DNA extraction.

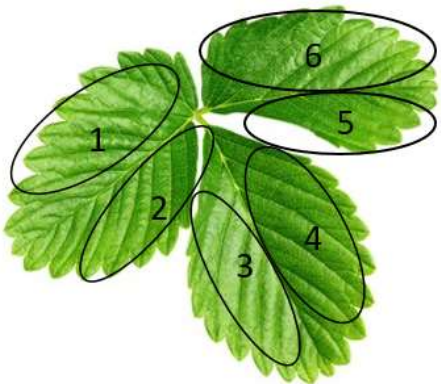

Figure 1. Strawberry leaves were divided into six sections to randomize the tissue selected for DNA extraction.

Modification were made to the Qiagen DNeasy mini plant kit protocol to address the low concentration of the organism in the tissue. The kit protocol was amended by using cetyltrimethyl ammonium bromide (CTAB) buffer in place of the kits AP1 buffer and RNase A. The AW1 buffer was increased to 1125 µL to increase the cleaning step.

Quantitative polymerase chain reaction (qPCR) was performed using a QuantStudio 5 with Biosystems MicroAmp EnduraPlate Optical 96-well Clear Reaction Plates. Each sample was duplicated and run at a denaturation temperature of 95°C for 3 minutes, followed by 45 cycles at an annealing temperature of 95°C for 15 seconds and an extension temperature of 60°C for 45 seconds. A master mix (table 1.) with 2µL of DNA template for each sample or control was used. The positive control of *Colletotrichum* (spp.) originated from a live culture obtained from the University of Virginia Diagnostic Lab and the negative control was nuclease-free distilled water. Each reaction had 3µL of the primer/probe (Table 2.)

Master mix reagents	Concentrations per well
Toughmix	5µL
p/p mix	3µL
Cy5.5	0.02µL

Table 1. Master mix ingredients and concentrations

Primer Name	Sequence	Probe	Final Concentration
Col_Gen_F1	CTA CGC AAA GGA GGC TCC G		300 nM
Col_Gen_R2	TGC CTG TTC GAG CTG CAT T		300 nM
Col_Gen_PB	ACC CCT CAA GCW CYG CTT GGY KTT GG	FAM	200 nM
Plant 18S-F	GAC TAC GTC CCT GCC CTT TG		48 nM
Plant 18S-R	AAC ACT TCA CCG GAC CAT TCA		48 nM
18S chr-P	ACA CAC CGC CCG TCG CTC C	HEX	24 nM

Table 2. Primers and probes used in qPCR