Outline for constructing hypovirulent C. parasitica strains for tailored biocontrol of chestnut blight on individual trees.
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1). Recover C. parasitica from individual cankers.

a). Extract bark plugs from each canker using bone biopsy instrument Flame sterilize instrument between sampling of canker. Sampling best after leaves fall.

b). Surface sterilize bark plugs and transfer to 2% Bacto Agar/water. C. parasitica will grow out faster than most contaminating fungi. Transfer C. parasitica to potato dextrose agar (PDA).

2). Determine number of vegetative compatibility (VC) types represented.

a). Pair combinations of isolates from the same and different cankers on Bromocresol Green agar to determine the number of vegetative compatibility (VC) types represented in the different cankers.

3). Prepare spheroplasts from isolates of each VC type.

a). Remove cell walls and isolate spheroplasts. This process takes one full day. Difficult to process more than two VC types per day.

4). Introduce hypovirus RNA into spheroplasts by transfection.

a). Synthesize synthetic coding RNA strand from the full-length hypovirus cDNA clone and test for yield and integrity.
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