**Year 2: Management of Phytophthora blight on Cucurbit and Solanaceous species**

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Phytophthora blight causes the most devastating disease of vegetable crops in Ontario. The causal agent is *Phytophthora capsici* and it has a very broad host range affecting solanaceous (i.e. tomatoes and peppers) and cucurbit (i.e., squash and cucumbers) crops. The disease can cause symptoms in all parts of the plant including fruits, crowns, and roots.

*Phytophthora capsici* has two mating types and populations can undergo either sexual or asexual reproduction. The movement and implications for management practises is related to the type of reproduction carried out by the pathogen, either sexual or asexual (Kalischuk et al. 2012). The disease can not be controlled by chemicals alone and metalaxyl resistance has been reported in some fields (Hausbeck and Lamour 2004). Understanding the population structure of this pathogen will provide valuable information about the best management practises and integrated pest management strategies that can be used to successfully control phytophthora diseases in vegetables (Hwang et al. 2014). The objectives of the project are to:

**1.** Isolate *Phytophthora capsici* to facilitate fungicide sensitivity and mating type testing
**2.** Evaluate sensitivity of isolated *Phytophthora capsici* to fungicides
**3.** Evaluate pathogenicity of isolated *Phytophthora capsici* on Solanaceae and Cucurbitaceae species

**Year 2 Progress:**

On July 23, 2025, two 500 g bulk soil samples were collected from commercial vegetable fields. The first field was a tomato field located near Chatham Kent. The second field was a pumpkin field located near Alymer. At each field site, bulk soil samples were collected by combining 100 g soil from 5-6 locations within each field. A probe was used to collect soil at 10 Cm below the surface. Soil collection locations were depressional and showed signs of Phytophthora blight. Soil was stored at 4℃ until it could be processed. In the lab, four procedures were tested to isolate Phytophthora species. The hemp seed protocol was the most effective method for isolating *Phytophthora capsica* from soil (**Table 1**).

**Table 1** Fungal isolation method tested

| Methods | Number of times | Result |
|---|---|---|
| Serial dilution (1: 10, 1:100. 1:1000) | 3 | unsuccessful |
| Apple bait | 2 | unsuccessful |
| Cucumber bait | 2 | unsuccessful |
| Hemp seeds from Amazon | 3 | unsuccessful |
| Hemp seed bait (collected from Max's Lab)<br>Pumpkin field<br>(not repeated, lack of seeds) | 1 | successful |
| Hemp seed bait (collected from Max's Lab)<br>Tomato field | 4 | unsuccessful |

**Hemp seed baiting protocol**

Four grams of soil were added to 355 mL of sterile water in a glass beaker and stirred for two minutes. Three hemp seeds were split into halves using a scalpel and added to the soil water mixture, which was then incubated in the dark at 12 °C for one week. After seven days, floating hemp seed showing mycelial growth were directly plated onto V8 juice agar with antibiotics and incubated at 25 °C under fluorescent light for five days. Following incubation, under a sterile laminar flow hood, white-tipped hyphae surrounding the hemp seeds were transferred to fresh V8 juice agar plates using a sterile scalpel, and the plates were wrapped. These plates were incubated at 25 °C in the dark for approximately five days to allow full fungal growth.
After incubation, a small amount of mycelium was scraped onto a microscope slide under sterile conditions. A drop of sterile distilled water was added, a coverslip was placed, and the morphological characteristics of the fungus were observed using a compound microscope at 40× magnification.

**Determining Mating Type Using Pathogen Morphology**

A 4 mm diameter plug of the pumpkin field isolate was placed on one side of a V8 juice agar plate, and a 4 mm plug of the known tester isolate (pure *Phytophthora capsici* received from the AFL lab) was placed on the opposite side. The plate was incubated in the dark at 25 °C for 14 days. After incubation, the interaction zone between the isolates was examined under a compound microscope at 40× magnification to observe the formation of oospores and determine the mating type.

**Determining Mating Type using PCR**

Total genomic DNA was extracted from pure cultures and the pumpkin field isolate using the DNeasy Plant Mini DNA Extraction Kit. DNA concentration and purity were quantified using a NanoDrop spectrophotometer, and the extracted DNA was used for PCR amplification.PCR was conducted in a 50 µL reaction volume that consisted of 25 ul 10X PCR master mix, 19 ul of nuclease free water, 2 µL of template DNA, 2 ul of 0.1 mM forward (ACGAGTACGAGTGCTTGGT) and reverse (TGAGTCTCGAGACAGAGAG) primers. Thermal cycling parameters were as follows: Initial denaturation at 94 °c for 5 min, 30 cycles of denaturation at 94 °c for 30 s, annealing at 54 °c for 30 s, extension at 72 °c for 1 min, and final extension at 72 °c for 10 min (Ref: Li et al., 2017).PCR products were electrophoresed through 1% agarose gels.

**Results**


Fig 1: Morphology of pumpkin field *Phytopthara capsica isolate*

Fig 2.: *Phytopthara capsici* pure culture (AFL lab, Guelph) and pumpkin field isolate after mating

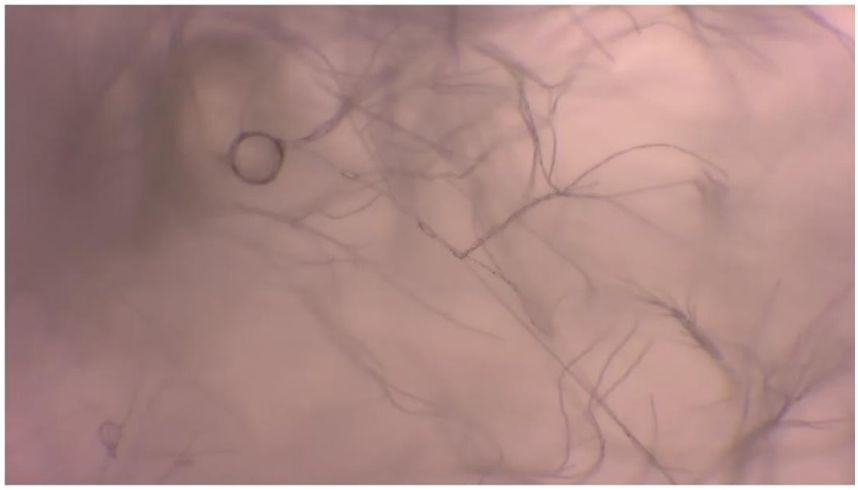

Fig 3: Oospore of *Phytopthara capsici* after mating

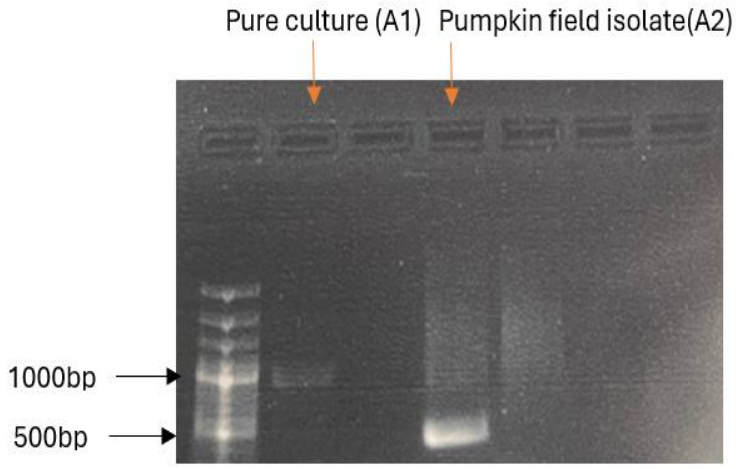


Fig 4: Gel Electrophoresis of PCR Products. The pure culture isolate from the AFL lab in well 2, and the pumpkin field isolate in well 4.

Reference image (Li et al., 2017)

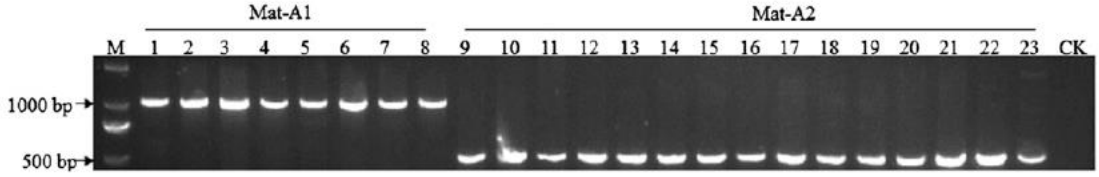


Fig. 3. Polymerase chain reaction profiles demonstrating mating type determination of *Phytophthora capsici* isolates using Pacp-1/Pcap-2 primers. Lane M, DL 2000 marker; lanes 1–8, *P. capsici* A1; lanes 9–23, *P. capsici* A2 (lane CK = no DNA template). Isolates of *P. capsici* are listed in Table 1.

**Conclusion**

The hemp seed baiting method can be used to isolate *Phytophthora c*apsici from infected field. Mating type can be determined using both morphological observations and PCR-based methods. *Phytophthora capsica*, mating type A2 was isolated from a pumpkin field in southern Ontario. Fungicide resistance testing and pathogenicity experiments are underway.

**Anticipated benefits:** Mating type, metalaxyl/fungicide sensitivity and pathogenicity of Ontario Phytophthora blight pathogens characterized and shared with OPVG (and the specific grower that submits a sample).

## References

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