Year 1: 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 1 Progress:
A procedure for isolation and quantification of *Phytophthora* from soil is being developed and tested. The isolation methods being tested include serial dilution plating, leaf disc baiting, and submersion leaf disc baiting as previously described by Larkin et al. (1995). The serial dilution method is performed by flooding soil samples with 10x their weight

with distilled water to create a 10^{-1} dilution, repeating the process to create 10^{-2}-10^{-4} dilutions. The dilutions are then spread onto a petri dish containing selective media and allowed to grow for 4-7 days at 26^0C in the dark. Baiting is completed by placing cucurbit leaf tissue on top of soil in a beaker which is filled with water and left at 10^0C for 2-4 hours. The leaves are moved to a moist chamber for 3-7 days or until lesions have developed, which are plated on selective media. The submersion baiting procedure is similar to the leaf disc baiting procedure but with an incubation step of 5 days at room temperature. To determine if the isolated fungus is *Phytophthora*, a double antibody enzyme linked immunosorbent assay (DAS-ELISA) is used for identification to genus and quantification and PCR to identify to species.

Currently the only method to result in isolated *Phytophthora* is the serial dilution plating methods, yielding nine isolates out of 27 replicates (**Table 1**). The submersion method has yielded six out of 18 isolates that are visually similar isolates to *Phytophthora* that have yet to be tested using DAS-ELISA and PCR. The serial dilution plating method is likely to be the most effective method as it is one of the most consistently successful methods for isolating *Phytophthora*. The existing soil samples were collected from five fields containing solanaceous species with known phytophthora history. The next step will be to inoculate sterile soil with a known quantity of *Phytophthora* propagules (i.e., sporangia, zoospores, mycelium, and oospores) to determine the most effective isolation technique for quantification using procedures described by Larkin et al. (1995) and Papavizas et al. (1981). The most effective method will be used to isolate *Phytophthora* from soil samples in years 2 and 3.

In years 2 and 3, soil and plant tissue will be collected from commercial fields to isolate *Phytophthora capsici* (Parada-Roas et al. 2021). Mating type will be determined by growing paired isolates under conditions conducive to sexual oospore development. Soil and plant samples will be collected (up to 10 sites per year; 3-10 samples per site; samples will be collected from OPVG Member/Grower fields – Crop Types include squash, cucumber, tomato, and other vegetables).

Fungicide sensitivity testing: Once isolated, EC50 values will be calculated to metalaxyl and novel RNAi molecules of *Phytophthora capsici*. The exposure and dilution of metalaxyl on the development of fungicide resistance and the ability of *Phytophthora* to maintain insensitivity in the absence of fungicide will be determined.

Pathogenicity: Fungicide sensitive and resistant *Phytophthora* isolates will be used to inoculate solanaceous and cucurbit susceptible hosts in the laboratory to determine pathogenicity and fitness of isolates. Cucurbit and solanaceous hosts of *P. capsici* display a wide range of disease responses between susceptible and resistant cultivars for fruit, root, and crown rot. Susceptible ‘Ylaspik’ cucumber and ‘Sugar Baby’ watermelon fruits will be surface sterilized and inoculated by placing a 5-mm agar plug form a 4-day old actively growing isolate of *P. capsici* with the mycelium side touching the fruit surface and without causing injury. The fruits will be placed in a humidity

chamber (>95%). Four days after inoculation, lesion diameter, sporulation diameter and intensity of sporulation will be measure along with intensity of sporulation measured on a 0–5-point scale (Parada-Roas et al. 2021). Pathogenicity screening of root and crown rot symptoms will be completed on 4-week-old susceptible 'Red Knight' pepper and 'Hunt100' tomato. Plants will be grown in a peatmoss/vermiculite potting medium in a greenhouse. Pathogen inoculation will include 5 mL zoospore suspensions (10,000 zoospores/mL) placed at the crown base and plants will remain in water saturated condition for 3 days. At 7 days post inoculation, plants will be scored for Phytophthora blight symptoms according to a 0-5-point scale (Parada-Roas et al. 2021). To ensure reproducibility, 10 plants per isolate will be tested and resistant checks will be used as controls. Pathogenicity of resistant vegetable cultivars and vegetable types (i.e., onion and carrot) will be tested to identify those that reduce disease incidence and severity in crop rotations.

Anticipated Benefits/Outcomes
Reduce incidence and severity of Phytophthora blight.
Improve yields and quality of product.
Increase competitiveness resulting from a reliable high-quality crop.

The specific benefits include: Mating type, metalaxyl/fungicide sensitivity and pathogenicity of Ontario Phytophthora blight pathogen shared with OPVG (and the specific grower that submits a sample). Information will be used to create a list of vegetable cultivars that can be used in crop rotations to reduce phytophthora blight.

Table 1: Isolation of Phytophthora from soil (2024 samples)

Isolation Method	Site	# plates from bulk soil sample	# Phytophthora isolations
SDP	Solanaceous S1-2024	12	0
SDP	Solanaceous S2-2024	12	0
SDP	Negative NC-2024	12	0
SDP	Negative NC-2024	12	0
SDP	Solanaceous S1-2024	9	4 (ISC)
SDP	Solanaceous S2-2024	9	5 (ISC)
SDP	Positive PCH-2024	9	0
Leaf disc bait	Solanaceous S1-2024	9 (4 abandoned)	0
Leaf disc bait	Solanaceous S2-2024	9	0
Leaf disc bait	Negative NC-2024	6	0
Leaf disc bait	Negative NC-2024	6	0
Leaf disc bait	Positive PCH-2024	9	0
Leaf disc bait	Solanaceous S1-2024	2 (1 abandoned)	0
Leaf disc bait	Solanaceous S2-2024	2	0
Leaf disc bait	Negative NC-2024	2	0
Leaf disc bait	Negative NC-2024	1	0
Leaf disc bait	Positive PCH-2024	2	0
Submerged	Positive PCH-2024	14 (2 abandoned)	3 (IS – not com.)
Submerged	Solanaceous S1-2024	3	4 (IS – not com.)

Isolation methods: SDP = serial dilution plating
Abandoned = plate was contaminated
ISC = Agdia Phytophthora Immuno-strip confirmed
IS = remains to be Agdia Phytophthora Immuno-strip confirmed

Literature Cited

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