Examining Dwarf Ginseng as a Research Model for *Ilyonectria* Pathogens

McKenna Campbell[1], Sean Westerveld[1], Amy Fang Shi [2]

1 Ontario Ministry of Agriculture, Food, and Agribusiness, Simcoe, ON
2 Ontario Ginseng Growers Association, Simcoe, ON

INTRODUCTION

One of the largest threats to American ginseng (*Panax quinquefolius* L.) is ginseng replant disease (GRD). When planting ginseng in a location that has had the crop before, GRD is likely to occur even 30 years after the initial crop. The plant pathogen *Ilyonectria mors-panacis (IMP)* is a causal agent of GRD. Despite it's importance, IMP is not well studied in the wild because of ginseng's threatened status under the Endangered Species Act.

Dwarf ginseng (*Panax trifolius L.*) is a closely-related species that could be a suitable research model for *Ilyonectria* plant-pathogen interactions in the wild.

Figure 1: Dwarf ginseng plant

OBJECTIVE

The objectives of this experiment are to determine whether and which *Ilyonectria* spp. are naturally associated with dwarf ginseng.

METHODS

Nine dwarf ginseng roots were harvested from a forest in Southern Ontario. The roots were severed from the stems and surface sterilized with 2% sodium hypochlorite. Three different treatments were applied to the roots:

- 1: Three roots were left unwounded
- 2: Three were wounded with a stainless-steel pin
- 3: Three were inoculated with one fungal plug of seven-day-old IMP culture on potato dextrose agar per root, then attached with a stainless-steel pin

All roots were incubated in a moist container at room temperature for 14 days, with additional watering when the bottom wipe dried.

To isolate fungi from the wounded, uninoculated roots, pieces about 2mm long were cut from the edges of rotten lesions using sterile tools. These pieces were rinsed in 70% ethanol, treated with 1% sodium hypochlorite for 60 seconds, and rinsed with distilled water. Four 1mm pieces were placed on a Petri dish with 2% potato dextrose agar. Seven days later, two subcultures were made from the dark brown mycelial growth, which were then sent to the University of Guelph's Laboratory Services for Histone gene sequencing using the CYLH3F and CYLH3R primer set.

Figure 2: Experiment treatments 1) control, 2 wounded, and 3) wounded and inoculated

RESULTS

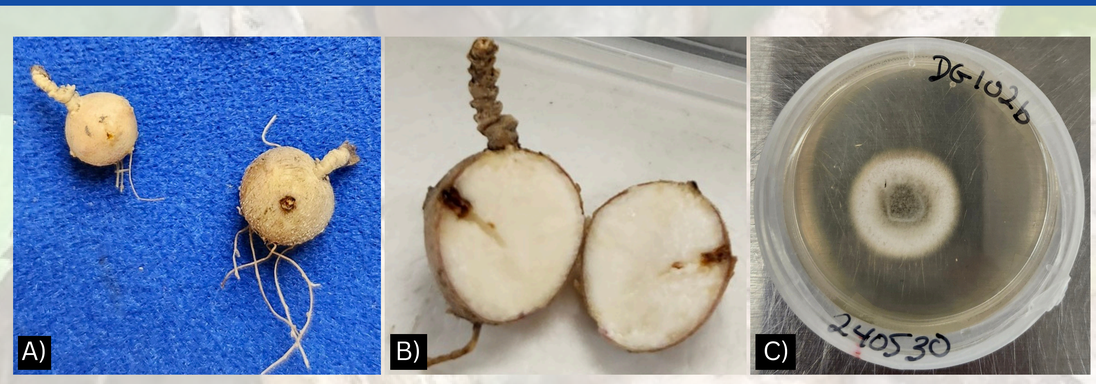

Figure 3: A) dwarf ginseng roots showing root rot symptoms from the wounding treatment B) wounded, un-inoculated dwarf ginseng root cut in half for pathogen isolation C) *Ilyonectria pseudodestructans* grown on PDA for seven days at 22-25C

The pathogen isolated from the wounded dwarf ginseng was identified as *Ilyonectria pseudodestructans* (Figure 3).

Ilyonectria mors-panacis also caused rot spots on the dwarf ginseng roots as seen in Figure 4.

Figure 4: IMP-inoculated roots showing root rot symptoms

DISCUSSION

Natural ginseng populations could be useful for studies comparing pathogen infection, soil characteristics and the natural microbiome, which could provide insights into ginseng replant disease. In this study we explored the potential to use dwarf ginseng as a model organism to be used in place of wild American ginseng.

The number of years required to grow a mature dwarf ginseng root is one consideration to make when deciding on a model organism. The roots harvested from a forest lot for this laboratory trial were anywhere from 15-23 years old based on the rhizome scars. This is a significant amount of time to grow a model organism for a trial in wild conditions, meaning that it might not be the easiest organism to use.

Ilyonectria pseudodestructans was found to exist in association with dwarf ginseng. It has been found in association with other perennial horticulture crops such as apple and grapevine, and can cause root rot in these hosts as well. This pathogen should be further researched to understand its role in ginseng.

MAIN CONCLUSIONS

- More reserach is needed to assess the fitness of dwarf ginseng as a model for *Ilyonectria* pathogens
- *Ilyonectria pseudodestructans* was found to cause root rot in dwarf ginseng

[1] Kernaghan, G., Releeder, R.D., and Hoke, S.M.T. 2007. Quantification of Cylindrocarpon destructans f.sp. panacis in soil by real-time PCR. Plant Pathol. 56: 508–516. https://doi.org/10.1111/j.1365-
[2] Farh, M.E.A., Kim, Y.J., Abbai, R., Singh, P., Jung, K.H., Kim, Y.J., and Yang, D.C. 2020. Pathogenesis strategies and regulation of ginsenosides by two species of Ilyonectria in Panax ginseng: power of speciation. J. Ginseng Res. 44: 332–340. https://doi.org/10.1016/j.jgr.2019.02.001. pmid:32148416
[3] Pielhop, T. P., Popp, C., Knierim, D., Margaria, P., & Maiß, E. (2022). First report of a chrysovirus infecting a member of the fungal genus Ilyonectria. Archives of Virology, 167(11), 2411–2415. https://doi.org/10.1007/s00705-022-05551-2
[4] Berlanas, C., Ojeda, S., López-Manzanares, B., Andrés-Sodupe, M., Bujanda, R., Del Pilar Martínez-Diz, M., Díaz-Losada, E., & Gramaje, D. (2020). Occurrence and Diversity of Black-Foot Disease Fungi in Symptomless Grapevine Nursery Stock in Spain. Plant Disease, 104(1), 94–104. https://doi.org/10.1094/PDIS-03-19-0484-RE