Hydroponics: A Novel Approach to Culturing Aphanomyces *euteiches*.

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ABSTRACT:

Aphanomyces *euteiches* is a soil-borne, economically impactful pathogen that afflicts pulse crops. However, culturing it from environmental soil samples is made difficult by the presence of other microorganisms in field soil, particularly *Rhizopus*, that outcompete it on selective media. Nevertheless, by isolating and culturing A. *euteiches* from pea plants grown in a hydroponics apparatus, this can be ameliorated to allow for enhanced observation of A. *euteiches.*

INTRODUCTION:

Aphanomyces *euteiches* is a pathogen that affects legumes and pulse crops.[1] Since it arrived in Saskatchewan in 2012, it has become a serious problem for producers.[1] A. *euteiches* spores have also been found to have the ability to persist in infected field soil for up to ten years and have no known treatment.[1] It is thus evident that isolating and culturing A. *euteiches* field isolates is of high importance to the agri-food sector. MVB selective plates have long been established as the primary means to isolate A. *euteiches*.[2] However, many other microorganisms are co-selected with A. *euteiches* on MVB plates: including *Rhizopus* and *Pythium.*[2] Of these, *Rhizopus* is of particular concern, as it is visually identical to A. *euteiches* until after it must be isolated from the MVB plate. Based on laboratory experience, it also makes A. *euteiches* impossible to isolate due to its competitiveness.[2] To eliminate these unwanted contaminants, soil known to contain A. *euteiches* was used to infect pea plants growing in a Kratky hydroponic system. The hypothesis is that this would dilute the *Rhizopus* while enriching A. *euteiches,* preventing it from outcompeting A. *euteiches* on the MVB plate. This method was refined using an iterative process until a protocol allowing for the successful isolation of A. *euteiches* was developed.

MATERIALS AND METHODS:

The hydroponic system was contained in a Neptune 1000ul filtered micropipette tip container (4.5 inches x 3.25 inches x 4 inches). The bottom of the container was covered in Aluminum foil, and the sides of the container were wrapped in VWR laboratory tape to prevent light from entering. A small closable window was made into the tape to allow for viewing of the roots. This created the base of the hydroponic system. The pipette tip holder tray was cut according to the design shown in Figure 1 to create the removable seed tray. Lastly, the top of the lid of the container was cut so that the pea plants could grow, and a strip of tape was added to reduce the amount of light that reached the seedlings, thus creating the upper chamber of the hydroponics system. Figure 2 shows the completed setup after germination.

To begin seed growth: six AC Carver pea seeds were allowed to incubate in a 250mL flask filled with 30mL of sterile water overnight (approximately 24 hours). Then, 1.5mL of stock nutrient solution (2.55g Potassium Nitrate, 1.98g Calcium Phosphate, 1.70g Magnesium Sulfate, 0.43g Ammonium Sulfate, and 0.050g Ferric Citrate dissolved in 25mL sterile water) was added to 500mL of sterile water and placed into the base of the hydroponic system by removing the seed tray.[3] 2g of soil infected with Aphanomyces *euteiches* and a stir bar were then added, and the seed tray was placed into the system. Six pre-soaked AC Carver Pea seeds were then added to their appropriate positions on the seed tray, as shown in Figure 1. To increase efficiency roots of germinating seeds were placed pointing into the nutrient solution when applicable. Peat Moss broken into pieces measuring approximately 2 cm^3 was then added on top of empty gaps in the seed tray. Small grain vermiculite was then used to fill the upper chamber of the system, but this was found to be purely cosmetic and had no effect on seed growth. Sterile water (approximately 100mL) was then slowly added to the top of the system until the peat moss and vermiculite were wet. The system was then placed by a window on a stirring plate set at low. Because the experiment occurred during the summer, light conditions were satisfactory. After two days, the stirring plate was removed and the plants were allowed to grow. The species of isolate was confirmed by CPN60 site-specific DNA sequencing.

RESULTS:

Effect of Hydroponic Design:

Throughout the experiment, the hydroponic apparatus was manipulated with the hopes of increasing the speed at which Aphanomyces *euteiches* could be isolated. Through this process, it was found that A. *euteiches* can not infect plants unless a stir bar is initially present. It was also found that if a stir bar was left spinning for too long, damage to the roots of the pea seedlings could occur, reducing the yield of culturable material. Seed germination time was also found to be a bottleneck for culturing, as it varies between individuals. Initially, seeds were placed into the hydroponic apparatus without pre-soaking, and this resulted in an unsatisfactorily slow germination rate (up to four to five days) and necessitated that the seed be held in place, as it was initially too small to fit into the seed tray. Pre-soaking seeds drastically increased the rate of germination, in many cases to as little as one day. It also allowed peas to germinate within a narrower timeframe, allowing a more accurate measurement of when they should be harvested for culturing. Twenty-four hours was found to be the optimal time for soaking the seeds. Longer soaks were found to promote the growth of microorganisms that could stunt later seed development.

Findings of Culturing:

Because each seed was unique, there was no ideal day to harvest the pea roots for plating. However, the optimal time for attempting to culture was found to be around six days after placement into the apparatus, when the plants had a solid taproot with 4-6 developing lateral roots. At this time, the roots of the seedlings would appear mostly white, with a few patches of minor browning. If the seedling was allowed to continue to grow, the roots would

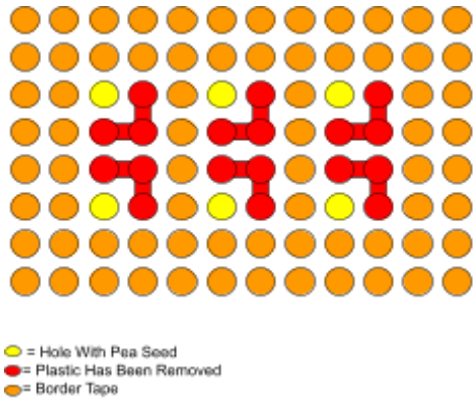
become a deep honey brown, as shown in Figure 3. Highly infected roots such as this were found to be not ideal for culturing A. *euteiches* due to the presence of other pathogens that could outcompete A. *euteiches* on the MVB plate, including *Rhizopus*. Using the procedure described in the material and methods section, up to seven unique A. *euteiches* individuals can be harvested per hydroponic apparatus. To test the effectiveness of the apparatus, five suspected Aphanomyces Isolates of drastically different phenotypes were selected for CPN60 sequencing. All five of these had a 100% match to the GenBank sequence CU365658.1 from base pair 411 to 752.

Limitations of the Growth Apparatus:

When infected soil was not added, the pea plants could grow for up to five weeks so long as nutrient solution was continually added when depleted. Uninfected pea plants did not have any discoloration on their roots until four weeks after planting. At this time, leaf discoloration also began to appear. At week five, further discoloration of the roots was visible, and more leaves began to turn yellow. It is unknown why this occurred. Figure 4 shows the stages of pea development in the absence of infected soil.

FIGURES AND DIAGRAMS:

Figure 1:

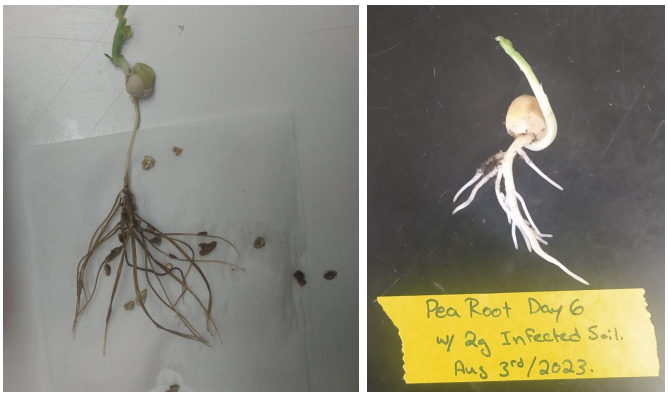


The layout of the seed tray.

Figure 2:

A completed hydroponic system.

Figure 3:

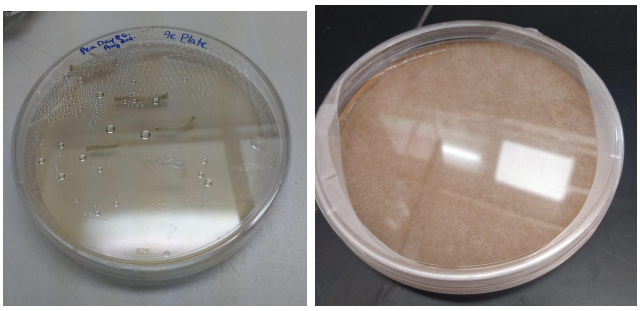


(Left) an Infected plant from the hydroponics system, only Rhizopus was obtained during culturing. (Right) an infected plant from the hydroponics system that yielded a potential Aphanomyces isolate.

Figure 4:

Timeline of the negative control experiment over five weeks. Note the whiteness of the roots.

Figure 5:



An example of how the pea roots were placed onto the selective media (Right), and a successfully sequenced Aphanomyces isolate (Left).

DISCUSSION:

The results indicate that when soil known to contain Aphanomyces *euteiches* spores is added to a hydroponic system containing a susceptible host, infection will occur. Infection is severe enough that infected host root tissue can then be used to isolate A. *euteiches* on an MVB plate.[2] Plants grown in the hydroponic system were found to be much more efficient in creating opportunities to isolate A. euteiches than those grown in soil, in part due to the ability to view the growth progress of each plant and harvest it at the ideal time, but also due to the lack of contaminated soil clinging to the hydroponically grown plants.

Another issue with culturing Aphanomyces *euteiches* this hydroponic apparatus may ameliorate is its ability to survive storage. Currently, A. *euteiches* spores do not store well in artificial media. However, they are known to last up to ten years in field soil.[1] The ability to easily isolate A. *euteiches* from soil may present a new opportunity for storage. Future research may consider how sterile soil can be inoculated with oospores of a particular culture, stored, revived using the hydroponic system, and then isolated from the resulting infected pea roots.

Works Cited:

1. Bogdan, Jennifer. “Aphanomyces Root Rot in Pulse Crops.” *Saskatchewan Pulse Growers*, 2 June 2023, saskpulse.com/resources/aphanomyces-root-rot-in-pulse-crops/.

2. Pfender, William F. et al. “A medium to enhance recovery of Aphanomyces from infected plant tissue.” Plant Disease 68 (1984): 845-847.

3. Stephens, Oscar. “DIY Hydroponic Nutrients: 6 Cheap Homemade Recipes.” *The Hydroponics Planet*, 19 Sept. 2022, thehydroponicsplanet.com/diy-hydroponic-nutrients-6-cheaper-homemade-recipes/.

I confirm that I do not have a conflict of interest.