JUL 21, 2023

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Protocol Citation: Nimalka M Weerasuriya 2023. Baiting Pythium myriotylum from Infested Soil . **protocols.io** https://protocols.io/view/baiting-pythium-myriotylum-from-infested-soil-cvxkw7kw

Protocol status: In development
We are still developing and optimizing this protocol

Created: Jun 19, 2023

Last Modified: Jul 21, 2023

PROTOCOL integer ID: 83660

Baiting Pythium myriotylum from Infested Soil

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ABSTRACT

Baiting Pythium from Ft. Cobb or other infested soil.

MATERIALS

P_5ARP plates
CMA/PDA+amp plates
Soil with *Pythium* infestation signs
Seeds of susceptible peanut cultivar; we used PI 378012 from the USDA Germplasm Collection
Fume hood and tools for sterile plating technique
Sieve and beakers for surface sterilization
RO water
Forceps and scalpel/blade
95% ethanol
Flame

For seedling baiting:
4" pots with catch tray
greenhouse or adequate outdoor conditions
Mature banker plants (Alyssum and Peppers) for IPM

Preparation

1 Prep P_5ARP Plates 1-2 days before plating.

Prepare working culture plates (CMA, PDA + amp) up to 1 week before plating.

Seed Pod or Seedling Baiting

8m

2 Select whether you want to bait from diseased hulls in soil or from seedlings grown in diseased soil.

Step 2 includes a Step case.

Peanut Seedlings

Diseased Hulls

step case

Peanut Seedlings

To bait from soil infested with Pythium using susceptible germinated seedlings.

3 Germinate susceptible seedlings using of Sample by wrapping in RO-dampened paper towels and incubating for 2-4 days at 28 °C in the dark.

Check daily to re-wet paper towels as necessary.

4 Plant germinated seeds in infested soil, in triplicate, in 4" diameter pots with a drip tray. Maintain

greenhouse at 70 % humidity with temperatures between 24 °C and 30 °C.

4.1 Water to maintain a level of 1-2" in drip tray for the first 2 days, and then to maintain soil moisture without excessive water until seedlings reach 4-6" in height.

5 At 4 DAP, trim to 1 seed/pot, or carefully repot in separate pots.

6 Grow for 15-20 days and check for rot symptoms.

> **Note**
>
> Brown rot will start at 4-6 DAP
> Wilt at 6-18 DAP

Those exposed to higher temperatures were more susceptible to rot.

Harvest and Surface-Sterilization

7 Harvest seedlings. Rinse plants thoroughly in RO water to remove soil particles.

8 Select appropriate samples with black rotted segments.

Rotted segments of peanut seedling suitable for plating

8.1 Cut up segments into 1/4 inch (1-2 cm) pieces.

9 Try with or without [M] 95 % (v/v) ethanol soak for 00:04:00 or rinse in sterile RO water. 4m
Use a sieve in a small beaker.

CITATION

Garren, K. H. (1996). Peanut (Groundnut) Microfloras and Pathogenesis in Peanut Pod Rot. Journal of Phytopathology. 55(4), 359–367.

LINK

https://doi.org/10.1111/j.1439-0434.1966.tb02238.x

Do not use bleach due to *Pythium* sensitivity.

CITATION

Stanghellini, M. E., & Kronland, W. C. (1985). Detrimental effect of surface sterilization on isolation of Pythium spp. from feeder roots (Abstr.). Phytopathology.

LINK

https://www.apsnet.org/publications/phytopathology/backissues/Documents/1987Articles/Phyto77n08_1192.pdf

9.1 Blot dry on sterile paper towels.

10 Plate on P_5ARP or other media with bacterial inhibition (CMA or PDA + amp).

CITATION

Jeffers, S. N., & Martin, S. B. . Comparison of Two Media Selective for Phytophthora and Pythium Species. Plant Disease. 70(11), 1038–1043.

LINK

https://doi.org/10.1094/PD-70-1038

Transfer Culture

11 Check plates after 24-48 h.

12 Hyphal tip transfer using sterile loop to clean plates of CMA (V8 or PDA).

Oospore Check

13 Check for "gold coin" oospores at plate edges to indicate *Pythium myriotylum*.

- Use CMA for oospore production
- Use full strength PDA to increase hyphal growth for DNA extraction
- Use V8 to increase hyphal growth for DNA extraction (untested)