



Evaluating Peanut Germ Plasm for Resistance to *Pythium myriotylum* using Sorghum Seeds

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

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ABSTRACT

Using sorghum colonized by *Pythium myriotylum* to evaluate the peanut germ plasm resistance of peanut seedlings in a greenhouse.

PROTOCOL REFERENCES

- Stirling, G. R., Turaganivalu, U., Stirling, A. M., Lomavatu, M. F., & Smith, M. K. (2009). Rhizome rot of ginger (*Zingiber officinale*) caused by *Pythium myriotylum* in Fiji and Australia. *Australasian Plant Pathology*, 38(5), 453–460. <https://doi.org/10.1071/AP09023>
- Jones, B. L., & Woodard, K. E. (1983). A Technique for Evaluating Peanut Germ Plasm for Resistance to *Pythium myriotylum*. *Plant Disease*, 67(10), 1093–1094. <https://doi.org/10.1094/PD-67-1093>
- Boote, K. J. (1982). Growth Stages of Peanut (*Arachis hypogaea* L.). *Peanut Science*, 9(1), 35–40. <https://doi.org/10.3146/i0095-3679-9-1-11>. [\[link\]](#)

Greenhouse Materials:

- potting soil
- sand
- pots (~0.5 L)
- trays (individual or large)
- heat mat & temperature controller
- peanut seeds (shelled)

Lab materials

- Erlenmeyer flasks (~125 mL)
- sorghum seeds
- clean culture of Pythium myriotylum on CMA or V8
- autoclave sterilizer
- weigh scale
- roH₂O or dH₂O

Prep and Seed Planting

1

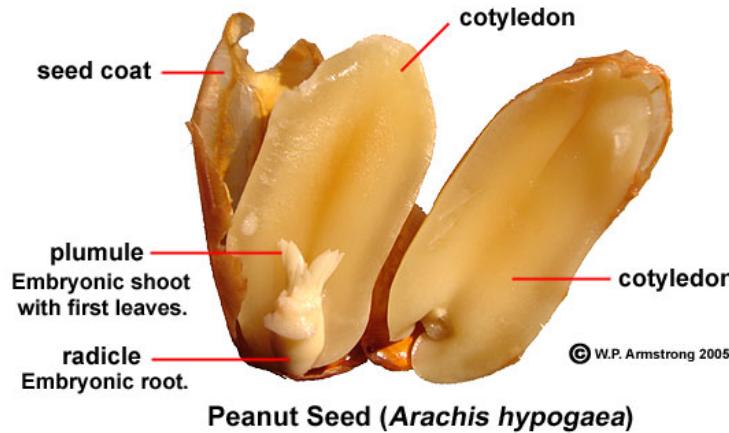
- 1. Make a 1:1 sand-soil (v/v) potting mixture for the required number of pot replicates. Include 1-2 additional replicates for each treatment, in case seedling emergence is inconsistent.
- 2. Place pots in trays and water to soak medium.
- 3. Let pots soak overnight.



Planting (Day 0)

2

- 1. Check that the potting mixture is thoroughly hydrated.
- 2. Use a clean plastic or bamboo/plastic rod to create two holes in the center of each hemisphere, at a depth of 1".
- 3. Plant 2 seeds per pot, with the radicle (pointy end) facing down.
- 4. Cover holes and water as needed.
- 5. Label pots with planting date and seed variety.



- 2.1** Place pots with trays under fluorescent lights at recommended greenhouse conditions. Water as needed until emergence (typically 7-12 DAP).

Sorghum Prep and Inoculation (~2 DAP)

1d 0h 20m

- 3** At ~2 DAP, weigh out sorghum seeds for each pot, where each pot receives rlen 0.5-1 g
Place sorghum seeds in two labeled flasks, one for *Pythium* inoculation, one for control.

- 3.1** Add water to completely soak seeds overnight for rlen 24:00:00 in flasks, covering tops with rlen 1d aluminum foil.

- 4** Decant water and cover opening with two layers of aluminum foil. Add autoclave tape and label flasks with rlen 20m initials and date.

Autoclave on liquid cycle at rlen 121 °C for rlen 00:20:00 and let cool overnight.

- 4.1** Autoclave a second time and let cool overnight.



5 Under a sterilized workbench, inoculate with *Pythium myriotylum* strain(s).

5.1

Place flasks in an incubator at  25 °C and monitor growth until the oomycete has fully colonised the substrate (7-10 days).

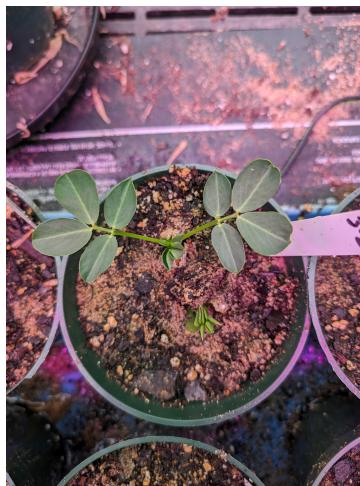
- Check for white mycelial growth in *Pythium* flasks and ensure the Control flasks remain clean.



Seedling Preparation

6

1. Monitor seedlings for emergence and growth until the V-1 vegetative stage, where the first pair of trifoliate leaves are unfurling or completely unfurled.
2. Remove the second seedling from each pot by clipping just below the cotyledons above the root. If there are pots without viable seedlings, remove them and replace with the extra replicate pots.



6.1

Using a clean plastic rod, create 4 holes at a depth of 3" around the perimeter of each pot, slightly wider than the size of a sorghum seed.



Sorghum Preparation

- 7 Prepare *Pythium* inoculant in flasks by mixing the media with a sterile metal double-ended spatula. Spatulas can be autoclaved in advance or the morning-of, or thoroughly sterilized using 100% EtOH and a flame.
- 7.1 Mix by breaking up sorghum mats so that individual seeds can be separated. Try not to puncture seeds excessively.
- 7.2 Weigh required amount of inoculum for each pot in clean plastic weigh boats. A recommended range is between 0.5-1 g per 500 mL potting mixture.
 - Adding the same total weight of sand into the flask may help break up mycelial mats.

Note

Stirling et al. (2009) used 4L pots, and 40 g of inoculum mixed with 40 g of potting soil.

Seedling Inoculation with Pythium

- 8 Inoculate seedlings in pots by dropping sorghum seeds down each of the four holes until all holes are evenly filled.
- 9 Cover holes and water gently. Bottom watering is recommended to keep the top of the soil from being disturbed, and it helps moves Pythium up towards the roots with the flow of water.

Monitoring Growth

- 10 Maintain a wet-moist watering schedule, where plants are kept wet for 4 days, and moist for 3 days (watering every day vs watering every other day).
 - 10.1 For the first 4 days of watering, keep a tray below the plants to maintain a flooded root zone.
- 11 Monitor plant growth for 15-20 days, counting the appearance of wilt or necrosis. Maintain greenhouse temperatures  $>24\text{ }^{\circ}\text{C}$.
 - Brown rot may appear from 4 to 6 days after infection.
 - Wilt symptoms may appear from 6 to 18 days after infection.
 - Note V-# vegetative stages or any R-# reproductive stages if visible.

Seedling Wilt and Necrosis

- 12 At harvest, carefully remove plants from potting mix without pulling on the stem.

- 13 Bait *Pythium* from infected parts using protocol: [Baiting *Pythium myriotylum* from infested soil.](#)