

***Aphanomyces euteiches* Root Rot of Alfalfa: Improving Storage of Cultures in the Lab for Long-Term Experimental Success**

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Aphanomyces euteiches is an important pathogen of alfalfa and other legumes, causing root rot in seedlings as well as chronic root disease in established stands. Current storage methods for *A. euteiches*, such as making fresh culture transfers or storing in sterile water tubes, result in limited viability up to a few months. We have discovered a straight forward method of long-term storage that takes advantage of the survival structure of *A. euteiches*: oospores. A flat of vermiculite was planted with alfalfa and inoculated with a known isolate of *A. euteiches* with a mycelial suspension. Presence of oospores in the plant roots was microscopically confirmed, and all the material was scooped out of the growing cells and into 50ml plastic centrifuge tubes. These tubes were placed in a -20° freezer, and removed at different time intervals. The frozen samples were removed and tested for viability for periods as short as one week and as long as four years. To recover *A. euteiches*, the tubes were thawed and put into a flat, and new seeds were planted on the same material to bait out *A. euteiches*. Presence of oospores was again microscopically confirmed in plant roots, which were then placed onto MBV and then onto PDA with streptomycin, and finally pure cultures were obtained on normal PDA. To date, isolates have been recovered in pure culture from samples frozen up to 15 months. These recovered isolates were used to inoculate flats to see if there was a difference in virulence in material that had been frozen for over a year, frozen for only a week, and never frozen. This process is being repeated with more frozen tubes from 2018, 2017, and 2014. Molecular testing to ensure that the *same* isolate was actually cultured back out after being frozen is on-going, and would provide additional strength to the results.