

Fungal Contamination does not Stop Micropropagation of *Cypripedium* or *Platanthera*

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Background Information

- Sterilization and cleanliness are very important when making orchid propagation plates in order to avoid contamination
- To my knowledge, most people throw out these plates at the sight of contamination
- This is not very practical since micro propagation of orchids takes a long time and some of these may be endangered species that cannot afford to be lost
- Maybe orchids can tolerate contamination more than we think (especially fungal)

Background Info

- *Cypripedium reginae*- Showy Lady Slipper (MN state flower!)



- *Cypripedium acaule*- Pink Lady Slipper



- *Platanthera obtusata*- Blunt-leaf Orchid



Orchid Conservation

- Why study orchids?
 - About 20% of MN native orchids are listed by the state
 - Orchids are usually hard to restore because of their complex connection to their preferred environment
 - Research needs to be done so that we can learn how to get propagated plants into their natural environment

Orchid Conservation

- Why micro propagate?
- We do micro propagation (sterile lab propagation from seed in petri dishes) of MN native orchids because orchid seeds can't usually be grown in a pot
- Orchids need a good nutrient medium with their fungal symbiotic relationship (orchid and fungus help each other) met in sterile conditions

Purpose

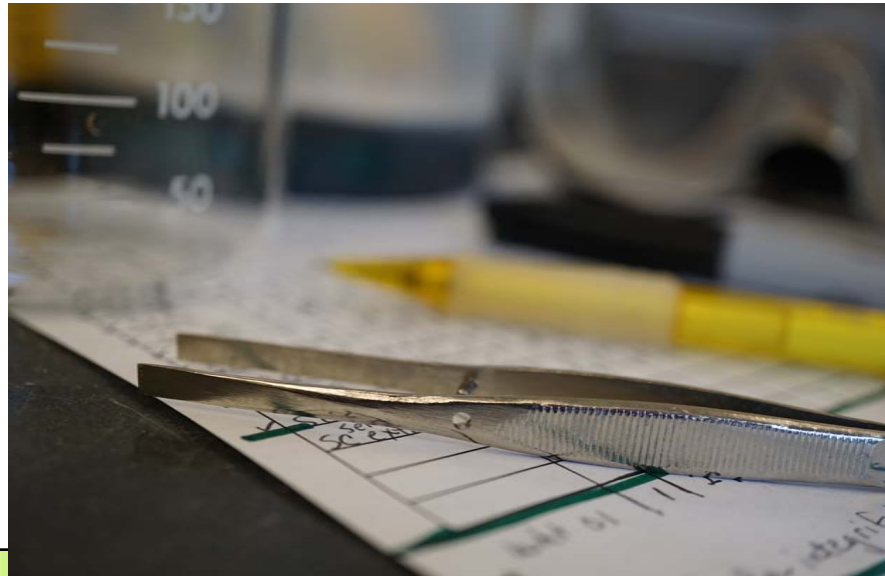
- See if it is possible to salvage contaminated orchids in the micropropagation stage
- In the future, we may be able to salvage contaminated orchids instead of throwing them out right away

Objectives

- Treat established orchid protocorms (young seedling of orchids) with fungal contamination that has already developed in micropropagation plates and keep another set as controls
- Keep track of growth with pictures over time
- See if the introduced fungus has any effect on the growth of the orchid species

Hypothesis

- The orchids will continue normal growth when fungal contamination from *Platanthera obtusata* is introduced



Procedure

1. Choose the three species to test: *Cypripedium acaule* (Pink Lady Slipper), *Cypripedium reginae* (Showy Lady Slipper), and *Platanthera obtusata* (Blunt-leaf Orchid)
2. Set up a control (uncontaminated) and a treatment (fungal contamination from *Platanthera obtusata*) for each species
3. Contaminate one set of orchid propagation plates with fungus that has developed in the *Platanthera obtusata* plate

Procedure cont.

- a. Use sterile tweezer under the hood to take chunks of fungus from *P. obtusata* plate and transfer into other two species (*Cypripedium acaule* and *Cypripedium reginae*)
 - b. Let fungus spread
4. Leave one set of orchid propagation plates uncontaminated
5. Observe growth and phenology of orchids by taking fixed pictures weekly over seven weeks

Data

Platanthera obtusata
contamination plate



6/23/2016



8/3/2016

Platanthera obtusata
uncontaminated plate



6/16/2016



8/2/2016

Data

Cypridium reginae
contamination plate



7/8/2016



8/3/2016

Cypridium reginae
uncontaminated plate



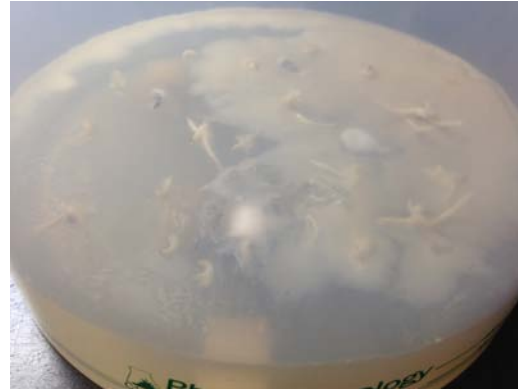
7/8/2016



7/21/2016

Data

Cypridium acaule
bacterial contamination



6/16/2016



8/2/2016

Cypridium acaule
uncontaminated plate



6/23/2016



8/2/2016

Results

- *Platanthera obtusata*
 - looked healthy
 - showed growth
 - turned more green over time
- *Cypripedium reginae*
 - looked healthy
 - showed growth
 - new sprouting appeared
- *Cypripedium acaule*
 - appeared taken over by bacteria
 - fungus never appeared to take over
 - most protocorms turned brown



Error Analysis

- One known error from this experiment is that I took pictures the first day without the cover on the plate
 - This could explain the bacterial contamination of *Cypripedium acaule*
 - Bacteria particles could have dropped off camera into the plate



Cypripedium acaule contamination plate (6/9/16)

Cypripedium acaule contamination plate (7/21/16)

Conclusion

- *Cypripedium reginae* and *Platanthera obtusata* seemed unaffected by the presence of fungal contamination (continued in growth and sprouting)
- *Cypripedium acaule* seemed to be contaminated by bacteria as the fungus did not ever appear to take over in the propagation plate (results from this plate cannot be compared)
- I conclude that orchid propagation plates should not be thrown out right away at the sight of contamination
- This could save a lot of time and resources in the future knowing that orchid contamination has potential for survival

References

- Special thanks to David Remucal for his insight and direction on this project
- *Native Orchids of Minnesota*
- *Orchids of the North Woods*
- *Terrestrial Orchids: from seed to mycotrophic plant*

Photo Credits

- Michaela Finley
- Peter M. Dziuk (Minnesota Wildflowers website)

Thanks!

