Testing wetland plants for susceptibility to *P. ramorum*

Constructed wetlands are being used to mitigate pollutants in agricultural runoff. These pollutants can include biological ones, such as plant pathogens. In this study we will test several wetland plants for susceptibility to *P. ramorum* to assess their usefulness in removing this organism from contaminated nursery runoff. Characters examined will include symptom expression, asymptomatic infection, and inoculum production.

Plant species used:

Dagger-leaved rush	*Juncus ensifolius*
Slender rush	*Juncus tenuis*
Small-flowered bulrush	*Scirpus microcarpus*
Common tule	*Scirpus acutus*
Slough sedge	*Carex obnupta*
Walter's sedge	*Carex striata*

Controls:
Inoculum only, no plant
Plant only

5 plants in each treatment
65 samples total

Week 1: wash plant roots of soil and media in running water, then measure length of longest root. Place each plant in cup and add tap water. Add leaf disc baits (10) to each cup to test for resident *Phytophthora* species. After 72 hrs, plate baits on PARPHV8 and check plates for *Phytophthora* after 2-3 days. Allow plants to establish in BCU for one week.

Week 2: Inoculate each plant by adding 10 6 mm plugs of an actively growing culture of *P. ramorum* PR98 NA2 strain. 1 day after inoculation, bait each plant to test for presence of *P. ramorum*. After 72 hrs, plate baits on PARPHV8 and check plates for *P. ramorum* after 2-3 days.

Week 3: Bait each plant to test for presence of *P. ramorum*. After 72 hrs, plate baits on PARPHV8 and check plates for *P. ramorum* after 2-3 days.

Week 4: Repeat inoculation as in Week 2. 1 day after inoculation, bait each plant to test for presence of *P. ramorum*. After 72 hrs, plate baits on PARPHV8 and check plates for *P. ramorum* after 2-3 days.

Week 5: Bait each plant to test for presence of *P. ramorum*. After 72 hrs, plate baits on PARPHV8 and check plates for *P. ramorum* after 2-3 days.

Week 6: Remove plants from cups and measure length of longest root. Wash roots in running water and cut 10 1 cm long sections of symptomatic roots (blackened and collapsed). Surface sterilize a portion of the roots in 10% bleach with 2 rinses of water and cut 10 1 cm long sections. Blot root pieces dry and plate each set of 10 on PARPHV8. Observe for *P. ramorum* after 2-3 days.

For each plant, separate the tops from the root systems and place in labeled paper bags. Dry in drying oven for 48-72h at 80C. Take dry weights of plant material. All plant material will then be further decontaminated by autoclaving.

Analyze the data – were there differences among plant species in amount of Pr+ baits? Did this amount increase or decrease over time? Were the roots infested (Pr isolated from unsterilized roots) or infected (Pr isolated from surface sterilized roots)? Did the treatments with Pr cause growth loss in the plants when compared with untreated plants? Which of these wetland plant species would be suitable for a constructed wetland to remove Pr in contaminated water?