**Goal:** To examine how a major biotic interaction (ants tending aphids) interacts with the genotype of a foundation tree species (dune willow) to influence insect food webs and plant fitness.

**Overarching Question:** How does the presence of aphids and distance from thatch-ant mounds alter the effects of willow genotype on insect communities and willow performance?

**Sub-questions:**

1. Is there significant heritable genetic variation in aphid performance on different willow genotypes? If so, which plant traits correlate with aphid performance?
2. How does willow genotype interact with distance from ant mounds to influence aphid performance?
3. How does willow genotype and ant-aphid aphid interactions influence ecological networks?
   1. Investigate putative willow traits and see whether any of them correlated with the outcome of these interactions
   2. Investigate all possible networks: spider-prey, plant-herbivore, and/or host-parasitoid networks
4. How does willow genotype and ant-aphid interactions influence willow growth?
5. How do ant-aphid interactions influence ecological networks?
   1. Investigate putative willow traits and see whether any of them correlated with the outcome of these interactions
   2. Investigate all possible networks: spider-prey, plant-herbivore, and/or host-parasitoid networks
   3. Figure out how best to sample density in willow patches
      1. Think about using the “throw-line” method typically used by Price and colleagues to sample gall densities on willow trees (may be ideal for sampling willow patches on the dunes).

**Materials & Methods**

*Study Site*

*Natural History*

*Genotype effect on aphid performance*

*Experimental Design*

On February 21, 2012 (double check date) we sacrificed 1-2 replicates from 10 of 27 genotypes that varied significantly in leaf carbon:nitrogen ratios (NEED STATS), a putative trait that influences aphid performance. COMMON GARDEN DETAILS. We took cuttings from shoots grown in the previous year and soaked cuttings in water overnight. On February 22, 2012 we planted cuttings in a mixture of 80% perlite, 20% peat moss and \_\_\_ dolomite lime. We grew cuttings under ambient weather conditions outside the greenhouse at Humboldt State University. (do I need to mention that they were exposed to herbivory?)

From May 20-22, 2012 we planted willow cuttings in the swales at the Lanphere Dunes Unit within Humboldt Bay National Wildlife Refuge in California (GPS). We removed all arthropods on the willows at the time of planting. On May 22, we collected aphids (GET SPECIES or GENUS name) and placed 5 adult apterate aphids on willow cuttings in the aphid treatment. We bagged aphids onto the apical shoots of cuttings using organza bags to promote aphid establishment in spite of oncoming inclement weather (wind and rain). We also placed organza bags on all control plants as well. On May 27, we checked aphid treatments to ensure there were 5 adult aphids and removed bags from all cuttings. If necessary, we added aphids to these treatments until there were 5 adults and we removed any aphid nymphs that were produced since initial establishment. On May 27, we double-checked willows to ensure that all arthropods (spiders and leaf rollers; except for a couple of stem galls) were removed.

**TO DO**

We planted cuttings around 5 different ant mounds (experimental blocks). We planted 20 cuttings (aphid and control treatment of each of 10 genotypes) at each distance of 1.5, 6.5 and 12.5 meters from the edge of the ant mound, for a total of 60 cuttings per ant mound. At each distance, we staggered cuttings at a distance of 25 cm around each distance measurement and ensured that all cuttings were spaced 50 cm apart from each other. Up until May 27, we supplemented planted cuttings with water to promote the survival of cuttings.

To assess the independent effects of willow genotype on aphid performance, we conducted a separate experiment (IN SOLARIUM OR GROWTH CHAMBERS) where we placed 2 adult apterate aphids on the apical shoots of willow cuttings on \_\_\_\_. 2 weeks later, we surveyed cuttings for aphid abundance and used Agrawal 2004 to calculate aphid population growth. We used the mean aphid population growth for each genotype as a continuous explanatory variable of genotype’s effect on ant-aphid interactions.

**TO DO**

To assess the effects of willow genotype and distance from ant mound on ant-aphid interactions, we surveyed all cuttings June 10, 24, and July 8 and 22 for aphid and ant abundance.

**TO DO**

To assess the effects of willow genotype and ant-aphid interactions on ecological networks, we surveyed all cuttings on June 24 and July 22 for the abundance and diversity of arthropods. For web-building spiders, we collected their webs and identified the abundance and diversity of their prey items. For herbivore larva, we collected them, brought them into the lab, and fed them leaves until they pupated. We reared pupa until either the original herbivore or an adult parasitoid emerged.

**TO DO**

To assess the effects of ant-aphid interactions on ecological networks, we also surveyed willow patches that occurred in proximity to thatch-ant mounds. We found \_\_ ant mounds and recorded its distance from the closest willow patch. We recorded whether or not ant-aphid was occurring, and if so, which ant and aphid species were involved (think about recording specific details of ant and aphid abundances…).

**TO DO**

To assess the effects of willow genotype and ant-aphid interactions on willow performance, we surveyed all cuttings on July 22 for percent leaf area damaged on the 5 most recent leaves of the most apical growing shoot. We scored leaves (0-10 damage score). In addition, we removed all growth from the current year, dried and weighed it to the nearest gram to assess total biomass production from that year.

**TO DO**

To assess the putative mechanisms by which willow genotype and ant-aphid interactions influence ecological networks, we quantified several traits for each willow cutting, including: specific leaf area (SLA), leaf area, water content, leaf toughness, trichome density, and above ground biomass (CURRENT YEAR SHOOT PRODUCTION OR DRY AND WEIGHT ENTIRE CUTTING?). (GIVE DETAILS IN APPENDIX FOR QUANTIFYING EACH PLANT TRAIT). **NEED TO DETERMINE HOW COST-EFFECTIVE IT WOULD BE TO CALCULATE C:N RATIOS OR DEFENSIVE CHEMISTRY FOR ALL PLANTS.**

*Effect of willow genotype and distance from ant mound on ant-aphid interactions*

*Effect of willow genotype and ant-aphid interactions on willow fitness*

*Effect of willow genotype and ant-aphid interactions on host-parasitoid or food web networks*

*Effect of ant-aphid interactions on host-parasitoid or food web networks*

**Field Experiment: Genotype by Ant-Aphid Interactions effect on insect communities**

Completely Randomized Block Experimental Design

* 6 treatments
  + Distance from ant mound: 1, 6, and 12 meters
  + Aphids presence: no addition or add 5 wingless individuals
    - Check that no new aphids have colonized “no addition” plants approximately once a week.
* 10 genotypes (60 cm cuttings)
  + Genotypes vary significantly in C:N ratios which may influence aphid performance.
  + More plant traits will be measured that likely exhibit heritable genetic variation.
* 5 blocks
  + 5 different ant mounds.
* Experimental unit: 1 willow cutting planted into the ground
* Summary of material needed
  + 300 cuttings (5 blocks \* 6 treatments \* 10 genotypes)
  + 750 aphids (5 blocks \* 3 treatments \* 5 aphids per cutting \* 10 genotypes)
* Bag all cuttings to prevent insect colonization. Allow aphids to establish for 24-48 hrs then unbag all plants.
* Survey cuttings approximately once a week to assess ant activity, aphid population growth and aphid parasitism.
* After 2-3 months, survey cuttings for insect abundance and diversity. Sample galls and leaf miners and rear them for their parasitoids.
* Before ending experiment, place one western tent caterpillar larva on each cutting as a bioassay of the effects of ant-aphid interactions on herbivory and survival of non-aphid insects (monitor herbivory and survival every 12-24 hrs).

**Greenhouse Study:** **Plant Traits and Aphid Performance**

- Completely Randomized Experimental Design

* Unbiased assessment of whether there is significant variation among genotypes in plant quality for aphids.
* 2 Treatments
  + Aphids and no aphids
  + 4 replicates of all 10 genotypes per treatment (80 cuttings total from 40 cm trees)
* 200 aphids
* Bag 5 aphids on each aphid treatment cutting and monitor aphid population growth
* Monitor aphid population growth approximately once per week.
* After 4 weeks, measure plant traits of both aphid and non-aphid grown cuttings.
  + Assess constitutive and induced plant traits

**Observational Study:**

* Assess gall and leaf miner densities on branches or trees with and without ants at various distances from thatch-ant mounds
* Rear subsample of galls and leaf miners to assess parasitism.