Title

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Keywords

Abstract

**Introduction**

Plant genetic effects can have important effects on plant phenotypes and associated communities.

While plant genotype effects have documented their importance, there is still an important call for research addressing the relative importance of plant genotype vs. the environment in shaping phenotypes and resulting community responses.

- Review GxE studies done at various scales? Mostly on a single arthropod species or community?

Here, we address this knowledge gap by examining the relative importance of willow genotype vs. the environment in a coastal dune ecosystem in shaping willow phenotypes and the associated arthropod community. Based on prior work (Barbour et al. 2015), we expected that plant architecture would be more influenced by the environment than leaf quality, due to its tendency to have lower heritability.

We sought to address how do plant genotype and the environment affect plant phenotypes and associated communities? Which plant phenotypes are associated with community responses?

Predicting phenotype and community responses from common garden experiment.

Methods

*Study Site*

We conducted this research at Lanphere Dunes (40**°** 53'29.85"N, 124**°** 8'49.06"W), a pristine coastal dune ecosystem managed by US Fish and Wildlife service in Humboldt County, California. Coastal willow (*Salix hookeriana* ex Barratt ex Hooker) naturally occurs in nearshore dune swales – seasonal freshwater wetlands that form in depressions between dune ridges (Pickart 2009). Aside from coastal willow (hereafter willow), the dominant vegetation in these swales consists of beach pine (*Pinus contorta* ssp. *contorta*) and slough sedge (*Carex obnupta*).

During preliminary surveys, we qualitatively identified two important sources of environmental variation in the dune swale habitat. First, wind exposure…We often observed noticeable reduction in willow growth in naturally occurring wind exposed willows, therefore, we hypothesized that willow growth would be reduced in wind exposed areas. Plant size was an important determinant of arthropod abundance (Barbour et al. 2016), so we expected that wind exposed willows would have a lower probability of being colonized by herbivores, due to reduced growth.

Second, the presence of the aphid, *Aphis farinosa*. When aphids feed on willow phloem they secrete honeydew, which attracts ants (citation). The dominant ant species in these coastal dune ecosystems is *Formica obscuripes* (citation), which we’ve observed actively tending *A. farinosa* in this system. *Effects of aphids and/or ants on willow phenotypes? Inducible defenses?* We hypothesized that this ant-aphid interaction could affect the associated arthropod community in two ways. First, aphids may present a source of competition (either through induced defenses or reduction in available resources for other herbivores). Both of these mechanisms of competition we would expect to reduce the probability of colonization by other herbivores. Second, aphids may present a reliable resource for other predators and therefore attract them. In contrast, we expected the presence of *F. obscuripes*, since they are also large generalist predators on the willows,would reduce the probability of colonization from both herbivores and predators.

*Experimental Design*

Prior to bud burst in February 2012, we took shoot cuttings (40 cm length & ~0.5 cm diameter) from one to two replicates of 10 different willow genotypes from a pool of 26 locally collected willow genotypes planted in a large common garden experiment. Details about the establishment of this common garden are given in Barbour et al. (2015). These 10 genotypes displayed substantial variation in both leaf quality and plant architectural traits (Barbour et al. 2015). Shoot cuttings were soaked in water overnight and then planted in a mixture of 80% perlite, 20% peat moss and \_\_\_ dolomite lime inside 'cone-tainers'. We grew cuttings under ambient weather conditions outside the greenhouse at Humboldt State University.

Wind experiment – In late May 2012, we planted 200 willow cuttings in a split-plot experimental design at Lanphere Dunes. At 10 different willow patches (blocks), we established an 'exposed' and 'unexposed' common garden. Each garden consisted of one replicate cutting of each of 10 genotypes randomly planted in 2 m by 0.5 m grid with 0.5 m spacing between plants. The center of exposed and unexposed gardens within each block were the same distance (2 m) from the edge of the willow patch to control for insect accessibility; however, exposed gardens faced prevailing winds during the growing season. To estimate the maximum amount of wind speed (km/h) experienced by exposed vs. unexposed plants, we went out on a windy afternoon in September 2012 (weather station estimated wind speeds of 22 km/h during this period) and used an anemometer (MAKE and MODEL) to measure wind speed at a height of 37 cm aboveground (approximate height of tallest plants in the garden) in each plot of our experiment. Maximum wind speed measurements were taken over a 30 s period and haphazardly started on either an exposed or unexposed site. On this windy day, exposed treatments experienced 5-fold higher wind speeds on average compared to unexposed treatments (paired t-test: *t9* = 13.7, *P* < 0.001).

Ant-aphid experiment – We established common gardens around 5 different ant mounds (blocks) in late May of 2012. Within each block, we randomly planted 20 cuttings (aphid and control treatment of each of 10 genotypes) with 0.5 m spacing at each distance of 1, 6, and 12 meters from the edge of the ant mound, for a total of 60 cuttings per ant mound (300 cuttings for entire experiment). We removed all arthropods on the willows at the time of planting. On May 22, we collected aphids (*Aphis farinosa*) 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. We checked plants for aphids on June 6, June 13, June 24, July 4, July 14, and July 20, 2012. 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. Up until May 27, we supplemented planted cuttings with water to promote the survival of cuttings. The ant-aphid experiment was restricted to the summer of 2012, because in the summer of 2013 there was high willow mortality (\_% of plants died by DATE) and *Aphis farinosa* was not in high enough abundance on naturally occurring willows to repeat the aphid treatment.

*Measuring willow phenotypes*

Plant architecture – To measure plant architecture, we measured plant height, the number of shoots produced, and total shoot growth at the end of each growing season (\_\_ 2011 and \_\_ 2012). We quantified plant height as the distance (mm) from the ground to the tip of the tallest shoot. We quantified total shoot growth by measuring every shoot on each plant to the nearest millimeter and summing the total shoot growth for each plant.

Leaf quality – We measured leaf quality in several ways.

We measured plant quality in several ways. First, we have correlational data on the concentration of 29 phenolic glycosides, total condensed tannins and carbon:nitrogen ratios and the abundance of one of a dominant herbivore Caloptilia pruniella that specializes on willows from the willow common garden in which we took cuttings from. We knew that \*Caloptilia pruniella\* abundance varied across these genotypes [NEED TO CONTROL FOR PLANT SIZE AND CONVERT TO DENSITY] so we looked for correlations between these plant traits and the abundance of this herbivore. Furthermore, we examined whether the rank average abundance of *C. pruniella* on these 10 genotypes in the common garden matched our observed variation in *C. pruniella* abundance on genotypes in unexposed plots.

Second, we conducted leaf quality assays using Spodoptera exigua. We replicated this experiment twice, once with late first instars (4 day duration) and another with 2nd instar larva (3 day duration). For both experiments, we added a single freshly collected leaf to a 30 mL plastic transport vials (loosely capped at end) with moist cotton ball, and then we added one, randomly selected larva to each vial using a small paintbrush. We used wet body mass at the end of each experiment as our measure of larva performance and therefore leaf quality (i.e. small larva = low quality plant tissue

We measure several putatively important traits that could shape leaf quality for herbivores, including water content, trichome density, percentage carbon (C) and nitrogen (N), and C : N. For water content and trichome density, we excised a single fully expanded and undamaged leaf from each plant in MONTH YEAR. We placed leaf samples into separate plastic bags with a moist paper towel, within a cooler and immediately brought them back to the laboratory. We then weighed leaves to obtain fresh mass (g), and oven-dried them at 60 °C for 72 h to obtain dry weight (g) (Cornelissen et al. 2003). We calculated leaf water content as the (Munns & PrometheusWiki Contributors 2010). To measure trichome density, we counted the number of trichomes along an 11 mm by 1 mm transect in the centre of the leaf, halfway between the leaf edge and the mid-vein, under a dissecting scope. To measure percentage C and N, we collected up to 3 fully expanded and undamaged leaves from each plant.

Leaves were air-dried and grounded to a fine powder using a ball mill (Mixer/Mill 8000D, SPEX SamplePrep; Metuchen, NJ, USA). Subsamples of each material were then analysed for percentage C and N on an elemental analyser (ECS 4010; Costech Analytical Technologies, Valencia, California, USA) using atropine (4.84% N and 70.56% C) as a reference standard.

*Quantifying Aboveground Community*

Given the small size of our plants (max plant height = \_\_ cm) and to avoid sampling arthropods on surrounding vegetation, we visually surveyed plants for arthropods to determine the abundances of different (morpho)species. Given the relatively low abundances of individual morphospecies, we grouped arthropods at the Family-level for insects and at the Order-level for other arthropods. JUSTIFY GROUPINGS. For the wind experiment, we surveyed arthropods once at the end of July 2012 and then once a month in May, June, and July of 2013. For the ant-aphid experiment, we surveyed arthropods on 5 different occasions between early June and late July 2012. ALSO MENTION HOW I’M TAKING THE MAXIMUM OBSERVED ABUNDANCE OF EACH ARTHROPOD SAMPLED OVER THE COURSE OF EACH YEAR.

*Quantifying Belowground Community*

At the end of the growing season in 2013, we dug up the willows from the wind experiment in order to sample the mychorrizal and bacterial communities associated with the willow roots. We did not sample the belowground communities of plants in the ant-aphid experiment due to the high mortality of plants in 2013. To sample these belowground communities, we carefully removed dirt until we found all of the living root tissue. We then placed the root tissue of each plant into separate plastic bags that went into a cooler, which were immediately transported back to the lab and kept at TEMPERATURE until further processing.

ASK SONYA FOR DETAILS ON QUANTIFYING THE BELOWGROUND COMMUNTIES.

*Soil characteristics*

Soil nutrients, total organic matter, and moisture may all influence the assembly of mychorizzal and bacterial communities on plant roots (cite). Moreover, we expected that wind exposure to affect these soil characteristics (cite); therefore, we measured soil nutrients, total organic matter, and moisture within each replicate common garden.

To estimate soil nutrient uptake by willows, we installed Plant Root Simulator (PRS) Probes (Western Ag Innovations, Saskatchewan, Canada) at three randomly selected locations within each garden for 11 days in September 2012. PRS Probes estimate nutrient supply rates to roots by continuously adsorbing charged ionic elements over the burial period. For our study, we estimated potential root uptake of total N, NO3+, NH4-, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd.

To measure total organic matter content (TOM), we used a trowel to collect soil (depth = 0 – 15 cm) adjacent to the randomly positioned PRS probes in September 2012. Soils were transported back to the lab in plastic bags, sieved into fragments less than 2 mm, randomly subsampled using a soil splitter, and dried at 105 **°**C for 72 hours. We then weighed a subsample of the oven dried soil into an oven dried crucible and placed the crucible and soil into a furnace to be combusted at 375 **°**C for 16 hours. We then weighed the combusted samples, placed them in a desiccator for 20 minutes, and weighed them again. We calculated percent organic matter as .

To measure soil moisture (volumetric water content, m3/m3), we used a 5TE soil sensor coupled to an EM50 Digital/Analog Data Logger (Decagon Devices, Pullman, Washington, USA). In September 2012, while PRS probes were in the ground, we measured soil moisture at a depth of 5 cm in three random locations within each garden on three different days between 1100 – 1500 hours. We repeated this same sampling scheme in early July 2013.

*Statistical Analyses*

For both experiments, we used mixed-effect models to analyze responses measured at the plant level (e.g. willow phenotypes, above- and below-ground communities). For the wind experiment, wind exposure, willow genotype, and their interaction were set as fixed effects, whereas block and wind exposure nested within block were set as random effects. For the ant-aphid experiment, aphid presence, distance to ant mound, willow genotype, and all possible interactions were treated as fixed effects, whereas block and distance to ant mound nested within block were treated as random effects.

Willow phenotypes – To examine how wind exposure and willow genotype affected plant architecture and leaf quality, we used permutational ANOVA (PERMANOVA) (Anderson 2001). We standardized traits (mean = 0, SD = 1) to give them the same weight in our analyses. We then conducted separate PERMANOVAs on the pairwise Euclidean distances of traits associated with plant architecture (X traits) and leaf quality (Y traits).

Above- and below-ground communities – As with the willow phenotypes, we used PERMANOVA. Specifically, we conducted separate PERMANOVAs on the pairwise Bray-Curtis dissimilarities of the composition of arthropod, myrchorrizal, and bacterial communities.

To examine the effect of wind exposure on soil nutrients, we first standardized nutrients (mean = 0, SD = 1) to give them equal weight in the analysis. We then performed a PERMANOVA on pairwise Euclidean distances, where wind exposure was a fixed effect and ‘block’ was a random effect. To test for an effect of wind exposure on soil organic matter and moisture, we used separate paired t-tests (paired by block).

All analyses were conducted in R version \_\_ (R Core Team YEAR).

**Results**

*Wind experiment*

Phenotypes:We found that plant architecture was influenced by willow genotype, wind exposure, and GE (Table \_). By the end of the experiment, willows in wind-exposed plots were 49% shorter in height (F1,11.92 = 15.53, P = 0.002), produced 40% fewer shoots (F1,9.96 = 7.63, P = 0.020), and tended to grow shorter shoots (F1,9.92 = 3.83, P = 0.089). Willow genotype had a pronounced effect on plant height, but only in plots protected from wind exposure (GE, 2 = 10.31, P = 0.006). In contrast to plant height, willow genotype was an important predictor of the number (1 = 4.56, P = 0.032) and length (1 = 15.63, P < 0.001) of shoots regardless of wind exposure. In terms of relative importance, willow genotype and GE tended to have a bigger effect than wind exposure in 2012, explaining 23% of the variance in plant growth traits (on average) vs. 13% for wind exposure. This pattern switched by the end of the experiment though, with wind exposure explaining 18% of the variance in plant growth traits, on average, vs. 9% for willow genotype and GE (Table\_).

In contrast to plant architecture, willow genotype was the primary factor in determining leaf quality across both years of the experiment (Table \_). For example, willow genotype explained 36% and 17% of the variance in trichome density (1 = 39.13, P < 0.001) and water content (1 = 15.13, P < 0.001), respectively, in 2012. Similarly, willow genotype explained 25% of the variance for both specific leaf area (1 = 23.02, P < 0.001) and carbon-to-nitrogen ratio (1 = 13.29, P < 0.001) in 2013. However, growth rates of the generalist caterpillar, *S. exigua*, appeared to depend on an interaction between willow genotype and wind exposure (GE = 22%, 2 = 4.84, P = 0.089).

Communities: The responses of the arthropod community mirrored those of plant architecture, depending on both willow genotype and wind exposure in 2012 and 2013 (Table \_).

The odds of finding an arthropod on a willow in a wind-exposed plots was always lower (\_x in 2012; 2.6x in 2013)

The probability of finding a herbivore or a predator on a willow was always lower in wind-exposed plots, although predators seemed to be more sensitive in their response compared to herbivores (Table \_; Table \_). Willow genotype was also an important predictor explaining \_ and \_% of the variance in herbivore and predator responses, respectively. Similar to plant architecture, willow genotype was relatively more important than wind exposure in predicting both herbivore and predator responses in 2012 and 2013 (Table \_).

In contrast to the above-ground arthropod community, the below-ground fungal and bacterial communities were influenced solely by wind exposure. For example, mycorrhiza abundance and richness were \_x and \_x lower in wind-exposed plots, respectively. Similarly, bacterial abundance and richness were \_x and \_x lower in wind-exposed plots, respectively. Fungal community responses were influenced primarily by \_\_\_\_ and \_\_\_\_ taxonomic groups, which were \_x and \_x lower in wind-exposed plots, respectively. Bacterial community responses were influenced primarily by \_\_\_\_ and \_\_\_\_ taxonomic groups, which were \_x and \_x lower in wind-exposed plots, respectively.

*Ant-aphid experiment*

Phenotypes:In contrast to the wind experiment, we found that both plant architecture and leaf quality were influenced solely by willow genotype (Table \_). Specifically, willow genotype explained \_, \_, and \_% of the variance in plant height, number of shoots, and shoot length. Similarly, willow genotype explained \_, \_, and \_% of the variance in trichome density and water content.

Communities: In contrast to the plant traits, the responses of the arthropod community were contingent on the presence of aphids, distance from mounds of *F. obscuripes*, and willow genotype (Table \_). Specifically, the probability of finding a herbivore increased by \_% with every 1m increase in distance from ant mounds. Similarly, the probability of finding a predator increased by \_% with every 1m increase in distance from ant mounds. Still, willow genotype was the most important predictor of arthropod responses explaining \_ and \_% of the variance for herbivores and predators, respectively.

*Structural Equation Models*

*Idea: Conduct piecewise SEMs, with predator and herbivore responses converted into presence absence and conduct binomial GLMMs. Predictors are the treatments (genotype, plots, and blocks as random effects), presence/absence of herbivores and predators, principal components of willow architectural and leaf quality traits. For the ants-aphids, I’ll additional include the presence/absence of F. obscuripes.*

*In fact, we found that plant height was the best predictor of both herbivore and predator responses. Specifically, with every X cm increase in plant height, the probability of finding an arthropod increased by \_% for herbivores and \_% for predators.*

in 2012, the probability of finding the leaf-mining moth, *Caloptilia* sp., decreased \_x in wind-exposed plots; however, willow genotype still explained \_% of the variance in the moth’s response. Similarly, the probability of finding a predatory spider decreased \_x in wind exposed plots.

Willow genotype had consistent effects on plant growth across both experiments, explaining between \_ and \_% of the variance in plant architecture PC1. In terms of the environment, only wind exposure had a strong effect on plant growth traits, while we did not detect any effect of the presence of aphids or the distance to ant mounds.

In both 2012 and 2013, willow genotype and wind exposure had strong, but independent effects on plant growth (plant architecture PC1).

Plant height, number of shoots, and total shoot growth varied \_x, \_x, and \_x, respectively, among the most disparate willow genotypes.

Plants were smaller (\_x), produced fewer shoots (\_x) and exhibited less total shoot growth (\_x) in plots exposed to wind.

In contrast to plant growth, willow genotype was the only factor that had a detectable effect on leaf traits (leaf quality PC1: 2012, ; 2013,).

For example, in 2012, the leaves of willow genotypes varied \_x in water content and \_x in trichome density.

Similarly, in 2013, the leaves of willow genotypes varied \_x in water content, \_x in C:N, and \_x in the growth rate of *Spodoptera* larva.

Discussion

Acknowledgements

References

Figure Legends

**Tables**

**Table 1**: Wind experiment – Permutational multivariate ANOVAs on willow phenotypes (plant architecture and leaf quality) and willow community responses (arthropods, mycorrhizal, bacteria).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 2012 | | | 2013 | | |
| Response | Treatment | *df* | *F* | *P* | *df* | *F* | *P* |
| Plant architecture PC1 | Genotype (G) | 9,148.76 | 6.29 | <0.001 | 9,128.24 | 3.59 | <0.001 |
|  | Wind exposure (E) | 1,8.99 | 24.90 | <0.001 | 1,9.32 | 10.27 | 0.010 |
|  | G x E | 9,148.76 | 1.61 | 0.116 | 9,128.20 | 0.74 | 0.673 |
| Leaf quality PC1 | Genotype (G) | 9,114.54 | 6.22 | <0.001 | 9,61.57 | 6.85 | <0.001 |
|  | Wind exposure (E) | 1,9.18 | 0.39 | 0.550 | 1,7.78 | 0.03 | 0.868 |
|  | G x E | 9,114.54 | 1.21 | 0.294 | 9,61.27 | 1.24 | 0.288 |
| Arthropod community | Genotype (G) |  |  |  |  |  |  |
|  | Wind exposure (E) |  |  |  |  |  |  |
|  | G x E |  |  |  |  |  |  |
| Mychorrizal community | Genotype (G) | -- | -- | -- |  |  |  |
|  | Wind exposure (E) | -- | -- | -- |  |  |  |
|  | G x E | -- | -- | -- |  |  |  |
| Bacterial community | Genotype (G) | -- | -- | -- |  |  |  |
|  | Wind exposure (E) | -- | -- | -- |  |  |  |
|  | G x E | -- | -- | -- |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Traits | Year | G | E | GxE | Plot | Block |  |  |  |
| Plant height | 2012 | X | F |  |  |  |  |  |  |
|  | 2013 |  |  |  |  |  |  |  |  |
| Shoot count | 2012 |  |  |  |  |  |  |  |  |
|  | 2012 |  |  |  |  |  |  |  |  |
| Leaf trichome density |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |

**Table 2**: Ant-aphid experiment: Permutational multivariate ANOVAs on willow phenotypes (plant architecture and leaf quality) and willow community responses (arthropods, mycorrhizal, bacteria).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | 2012 | | |
| Response | Treatment | *df* | *F* | *P* |
| Plant architecture | Genotype (G) | 9,204.19 | 4.52 | <0.001 |
|  | Aphids (Eaphid) | 1,204.66 | 0.10 | 0.750 |
|  | Ant mound distance (Eant) | 1,9.13 | 0.01 | 0.912 |
|  | G x Eaphid | 9,204.38 | 0.65 | 0.754 |
|  | G x Eant | 9,204.32 | 0.98 | 0.456 |
|  | Eaphid x Eant | 1,204.53 | 0.05 | 0.817 |
|  | G x Eaphid x Eant | 9,204.48 | 0.46 | 0.898 |
| Leaf quality | Genotype (G) |  |  |  |
|  | Aphids (Eaphid) |  |  |  |
|  | Ant mound distance (Eant) |  |  |  |
|  | G x Eaphid |  |  |  |
|  | G x Eant |  |  |  |
|  | Eaphid x Eant |  |  |  |
|  | G x Eaphid x Eant |  |  |  |
| Arthropod community | Genotype (G) |  |  |  |
|  | Aphids (Eaphid) |  |  |  |
|  | Ant mound distance (Eant) |  |  |  |
|  | G x Eaphid |  |  |  |
|  | G x Eant |  |  |  |
|  | Eaphid x Eant |  |  |  |
|  | G x Eaphid x Eant |  |  |  |

Plant survival: end of 2012 and 2013

Plant architecture traits: height (2012 and 2013), total shoot growth (2012 and 2013), mature leaf count (2012 and 2013), number of shoots (2012 and 2013), mean shoot length (2012 and 2013). Need to think about whether I should set a lower limit on shoots for contributing to branching architecture…Look for correlations and a composite measure of plant architecture.

Plant quality traits: SLA (2012 and wind 2013), water content (2012 and wind 2013), trichome density (2012), spodoptera leaf quality (wind 2013), C:N (wind 2013)

Arthropod community responses: Total spider abundance, Total predator abundance, total ant abundance, total arthropod abundance, Caloptilia sp. (LTF, tent mines, etc.), tortricid moths (leaf-edge silk and rolls into leaf bundles), sawfly larva, multiple aphid species, psyllids, leaf hoppers, gallers, spiders, ants, other.

Herbivory: wind 2013 (includes details from different sources as well).

Fungal community responses: Ask Sonya (wind 2013).

Soil: organic matter content (wind 2012), nutrients (wind 2012), moisture/temperature/EC (wind 2012 and both 2013). Can only test for environmental treatment effects here. Look for correlations in wind 2012 dataset.

What to do with perfectly correlated random effects or zero variance parameters?

Experimental designs and modeling points.

Wind experiment is a split-plot design with wind exposure as the whole-plot factor and genotype as the split-plot factor. Note also that I attempted first to fit a random slope and intercept model (treatment | genotype), but that the correlation between the intercept and slope was +/- 1, suggesting that the model is more complex than the data can support. I am a bit surprised though, because I didn’t think I had too few levels of random effects. According to <http://glmm.wikidot.com/faq#singular_fits>, I should treat genotype as a fixed effect, but doesn’t that make the model more complex? Right now, it just seems to make more sense to treat it as a fixed effect and cross it with wind. Importantly though, since wind exposure is the whole-plot factor, I need to nest treatment within block (1 | block/treatment) in order to calculate the appropriate degrees of freedom for the effect of wind exposure.

Lastly, we measured several other plant traits that may influence plant tissue quality, including: specific leaf area, leaf dry matter content, trichome density, and percent leaf desiccation (i.e. browned portion at end of leaf tip). To measure these traits, we picked a single fully expanded leaf that appeared to be of the highest quality for that plant (method in summer of 2011).