

Insect herbivory on native and invasive honeysuckle in an American northeast forest

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Abstract: The spread of invasive species can dramatically change the natural landscape. *Lonicera morrowii* is one of the most abundant invasive plants in the northeastern United States. In this study insect herbivory, diversity and abundance on *L. morrowii* were compared to that on native honeysuckle, *Lonicera hirsuta*. Herbivory was divided into damage types present on the foliage: hole feeding, free feeding, leaf mining, skeletonizing and discoloration. Percent leaf damage estimates showed that *L. morrowii* encountered more total leaf damage as well as damage from free feeding insects. *L. hirsuta* leaves displayed greater percentages of leaf mining, skeletonizing and discoloration. Insect collections were taken via pit traps and branch beating for both the native and the invasive. A difference of insect species richness between the two plant species was not found however different abundances of Collembola, Lepidoptera larvae and several insect families were found suggesting different herbivorous pressures between the two *Lonicera* species. Further investigation into insect herbivory could contribute to targeted biocontrol to minimize the negative effects brought about by *L. morrowii*.

Keywords

Insect Herbivory, Invasive species, *Lonicera morrowii*, *Lonicera hirsuta*, Enemy Release Hypothesis, New Association Hypothesis, Biocontrol, Invasive Bush Honeysuckles, Morrow's Honeysuckle, Hairy Honeysuckle

Introduction

The introduction of invasive species is one of the largest problems in natural habitats (Lonsdale 1999, Pimental et al. 2000, Evans et al. 2001, Lovett et al 2006). Over the course of a 50-year period from 1950 to 2002, Rooney et al. (2004) observed an increase of six times the original amount of invasive shrubs on observed plots in Wisconsin. When an invasive species enters a new environment their success depends on many factors, including the traits of the invader (reproduction rate, defense, organism size, etc.), the density of the invader, and the environment's susceptibility (strength and abundance of native plants, drought, soil disturbance, etc.) (Lonsdale 1999). The balance of natives and invasive plants is complicated; both groups have a share of advantages and disadvantages creating a mechanistic relationship between the two (Davis and Pelsor 2001).

The Enemy Release Hypothesis (ERH) is a classical theory stating that invaders' success is due to their release from co-evolved natural enemies in their native range (Darwin 1859, Elton

1958). Of these natural enemies insect herbivory plays a large role in plant survival. The ERH has been tested in different systems to see if invasive plants released from their natural insect herbivores experience less insect herbivory in their new environment relative to their native counterparts. A study conducted both in Europe and North America on European and North American *Hypericum perforatum* showed that the native species at each location experienced more insect herbivory than their invasive counterparts, supporting the ERH (Vilà et al. 2004).

Conversely, the New Association Hypothesis (NAH) claims that due to lack of co-evolution with the native predators, invasive species do not have the appropriate defensive abilities to protect themselves from the native insects and are therefore less successful (Hokkan and Pimentel 1989, Parker and Hay 2005). Cogni (2009) studied the effects of the herbivore *Utetheisa ornatrix* on the native *Crotalaria incana* and introduced *Crotalaria pallida* and found that the invader was more susceptible to herbivory thereby supporting the NAH.

The relationship between invaders and herbivores is complicated and depends on very specific traits. This study examined *Lonicera hirsuta* (Hairy Honeysuckle), a native to the Upper Peninsula of Michigan, and *Lonicera morrowii* (Morrow's Honeysuckle), an invader from Asia. *L. morrowii* is one of the most abundant invasive and problematic woody exotic in the northeastern American ecosystem and has potential for an ongoing landscape-altering invasion (Hunter et al. 2002). A previous study on *L. morrowii* herbivory versus that of a native unrelated species, *Viburnum recognitum* (southern arrow-wood), found that the rate of herbivory on the native species was ten times that of the invasive while invertebrate biomass was less for the native species (Love 2006). Further investigation is required to understand this relationship with more closely related species to minimize other influences on herbivory.

This study furthered an the understanding of herbivory on introduced invasive by comparing herbivory and associated insect diversity and abundance between two honeysuckle species, one native and one invasive. The insect abundance indicates whether the similar insects are feeding on the native and invasive honeysuckle. Diversity will show if a more varied group of insects are affecting on plant species over the other. This study investigated the extent to which insect herbivory, diversity and family group abundance differed between native *L. hirsuta* and invasive *L. morrowii*. The investigated hypothesis is that the invasive *L. morrowii* would experience less herbivory than the native *L. hirsuta*. Additionally, insect diversity between these two species was expected to differ with *L. morrowii* having less than the native, because the abundance suggests a possible minimized herbivory. This study may further support the original ERH and was chosen because of its congruence with many past studies such as Love (2006).

Methods:Sites:

This study took place at the University of Notre Dame Environmental Research Center (UNDERC), which is located primarily in Gogebic County in the Upper Peninsula of Michigan. Six sites of no more than 100 m² were chosen for observation. Three sites had four or more *L. hirsuta* shrubs, and the other three plots had four or more *L. morrowii*. The sites were observed four times between May 29th and July 13th.

Herbivory:

Two plants in each plot were chosen randomly for observation. Three random heights on each plant were randomly selected by height. I sampled every leaf on branches with 16 or fewer leaves. For branches with more than 16 leaves alternating pairs of leaves were sampled until 16

had been collected. The collection for these branches started from the outer most leaf and worked inward. The percent leaf damage was estimated in five categories that were observed on the leaves: hole feeding, free feeding, leaf mining, skeletonizing and discoloration (Currano 2008).

Insect Diversity:

Insect diversity was measured by using multiple insect collection techniques at each site. Two additional plants that were not being sampled for herbivory were randomly chosen at every site, and three pit traps were placed under each. Each pit trap was a 710 ml cup, filled with 4-6 cm of soapy water and left for two nights. At each observation time, branch beating was also used to collect insects and arthropods on the shrub. A tarp was placed under the shrub and 3 branches were beaten 5 times such that specimens fell off the branches and onto the tarp (Zalucki 1991). For both methods the specimens were collected, preserved in 70% ethanol, and later identified to the level of family. Each collection method was run on the same day for all plants as to avoid temperature and weather differences.

Statistical Analysis:

Percent herbivory for each damage category was compared between the two species using a two-sampled t-test. A Shannon-Weiner diversity index was calculated and used compare family diversity between the two species of shrub via two-samples t-test. Abundance of insects by family was tested using a Friedman test and the data were further analyzed with a Wilcoxon test to analyze the insect families that differed.

Results

Herbivory:

For each type of leaf herbivory (total damage, hole feeding, free feeding, leaf mining, skeletonizing and discoloration) a Shapiro-Wilk test showed that the data was not normal (Total damage $W=0.534$ $n=2299$, $df=2298$, $p<0.05$; hole feeding $W=0.195$ $n=2299$, $df=2298$, $p<0.05$; free feeding $W=0.486$ $n=2299$, $df=2298$, $p<0.05$, leaf mining $W=0.037$ $n=2299$, $df=2298$, $p<0.05$, skeletonizing $W=0.107$ $n=2299$, $df=2298$, $p<0.05$; discoloration $W=0.198$ $n=2299$, $df=2298$, $p<0.05$). Thus a two-sample Wilcoxon test was run on each type. With the exception of hole feeding, each herbivory type showed a significant difference in percent leaf damage between *L. hirsuta* and *L. morrowii* (total damage $t=753670$, $n=12$, $df=11$, $p<0.0001$; free feeding $t=795740$, $n=12$, $df=11$, $p<0.0001$; hole feeding $t=671230$, $n=12$, $df=11$, $p=0.238$; leaf mining $t=655370$, $n=12$, $df=11$, $p=0.026$; skeletonizing $t=624660$, $n=12$, $df=11$, $p<0.0001$; discoloration $t=595260$, $n=12$, $df=11$, $p<0.0001$) (Figures 1-6). *L. morrowii* had higher percent total damage and free feeding damage. *L. hirsuta* had more skeletonizing, leaf mining, and discoloration. In order to calculate a percent of leaf damage at least part of the leaf was required to be present. For this reason, leaves with complete damage were not included in the results and the damage estimates on both species may therefore be underestimated

Insect Diversity:

For each sampled plant a Shannon-Wiener diversity index was calculated. The data were normally distributed (Shapiro-Wilk, $W=0.9$, $n=12$, $df=11$, $p=0.159$) so a Welch two-sample t-test was used. There was no difference in insect family biodiversity between the two species *L. hirsuta* and *L. morrowii* ($t=-1.480$, $n=12$, $df=11$, $p=0.174$). A two-sample Wilcoxon t-test showed that total abundance of insects found on each species was significantly different with a mean on *L. morrowii* of 22.25 ± 8.703 individuals and *L. hirsuta* of 7.292 ± 1.065 individuals ($T=384$, $n=47$, $df=46$, $p=0.048$) between *L. hirsuta* and *L. morrowii*. Insects were identified as

one of 40 insect families, 3 larvae types, or as Collembola (Table 1). Larva were identified to order if there were either Lepidoptera or Coleoptera or to suborder if Symphyta due to the difficulty of identification. A Friedman rank sum test was run for testing any difference in each family's abundance between on *L. hirsuta* or *L. morrowii*. The Friedman test showed a significant difference (FM = 104.93, n= 47, df= 46, p<0.0001). Then a Wilcoxon two-sample t-test was run for each family to determine the significantly different abundances. Six families and one larvae type had significantly different abundances between *L. hirsuta* and *L. morrowii* (*Acrididae* T=322, n=47, 6=45, p=0.045; *Anthribidae* T=240, n=47, df=46, p=0.041; *Ceratopogonidae* T= 202, n=47, df=46, p=0.016; Collembola T=149, n=47, df=46, p=0.0004; *Formicidae* T=395, n=47, df=46, p=0.021; Lepidoptera larvae T=361.5, n=47, df=46, p=0.04548, *Pentatomidae* T=372.5, n=47, df=46, p=0.01059) (Table 1). *Acrididae*, *Formicidae*, Lepidoptera larvae and *Pentatomidae* were more abundant on *L. morrowii* while *Anthribidae*, *Ceratopogonidae* and Collembola were more abundant on *L. hirsuta*.

Discussion

While free feeding was greater on *L. morrowii* (Figure 2), leaf mining, skeletonizing and discoloration were more abundant in *L. hirsuta* (Figure 4, 5 and 6). Plant defenses can differ between species leading to different herbivory (Johnson 2009). Trichomes, outgrowths from the leaf's epidermis (e.g. hairs), are found on *L. morrowii* leaves but less so on *L. hirsuta* (Petrides 1958). Larvae in stage L1 development are repelled by trichomes (Hoeck 2010), which would suggest less Lepidoptera larvae on *L. morrowii*. However *L. morrowii* had a greater number of Lepidoptera larvae than *L. hirsuta* suggesting the trichomes of *L. morrowii* are not proper deterrents for the North American larvae. Lepidoptera larvae are general defoliators, and thus

their greater abundance on *L. morrowii* could contribute to significant difference in free feeding (Shetlar 2004). The *L. hirsuta* leaves have a more rigid epidermis (Petrides 1958) suggesting more difficult access for general defoliant insects (e.g. free feeders, skeletonizing and hole feeders). However only free feeding was significantly increased in *L. morrowii*. By leaf mining an insect can avoid the plants defense of a strong epidermis as well as use the ridged layer for protection from predators (Connor and Taverner 1997). *L. hirsuta* exhibited less free feeding but more leaf mining showing a possible preference by internal feeding insects. Sampling was done in between May and July and herbivory types in may change at different stages of the growing season (Shetlar 2004).

There was no difference in species diversity between the two honeysuckle species but there was a difference in abundance of identified groups. Between *L. morrowii* and *L. hirsuta* there was a significantly difference in abundance of in Collembola, *Anthribidae*, *Ceratopogonidae*, *Pentatomidae* and *Acrididae* (Table 1). *L. morrow* had a greater abundance of *Acrididae*, *Formicidae*, Lepidoptera larvae and *Pentatomidae* while *L. hirsuta* had greater abundance of *Anthribidae*, *Ceratopogonidae* and Collembola. While *Anthribidae*, *Ceratopogonidae* and Collembola were more abundant on *L. hirsuta* their presence should not have affected the amount of herbivory. Collembola feed on fungus and decaying plant material (Salamon et al. 2004), and therefore their presence should not have affected the leaf damage. *Anthribidae* also feeds mainly on fungus so it would not contribute to herbivory (Wheeler and Blackwell 1984). *Ceratopogonidae* larvae require standing water and as adults they are mostly hematophagic (Anjos-Santos et al. 2005). Therefore this family would have little effect on herbivory on the honeysuckle shrub. *Pentatomidae*, *Acrididae* and *Formicidae* were more abundant on *L. morrowii* and could have impacted herbivory damage on the shrub. *Pentatomidae*

feed by stylet insertions which were not calculated into total damage but may still injure the plant fitness (Apriyanto 1989). *Formicidae* can be herbivorous by free feeding which could contribute to the increased free feeding on *L. morrowii* (Milne and Milne 1995). *Acrididae* can be on the scale of monophagous to polyphagous but most of them are either oligophagous or polyphagous (Joern 1979). Based on this, it is possible that the *Acrididae* were feeding on *L. morrowii*.

L. morrowii encountered less overall insect herbivory than the native *L. hirsuta* (Figure 1). This supports the NAH that more insect damage occurs on invasive plants. With a thorough knowledge of insect herbivory, biocontrol can be considered as a useful tool to limit the spread of the invasive species. This study shows that an increase of free feeders could impact the *L. morrowii* more than the *L. hirsuta*. General defoliants are often not host specific and impacts on surrounding fauna are probable (Shetlar 2004). Further, herbivorous interactions can differ for similar plant species. For example, another study of an Asia-originating honeysuckle *Lonicera japonica* showed that invasive had less herbivory than its native counterpart, *Lonicera sempervirens* (Schierenbeck et al. 1994). Further investigation of insect herbivory in the invasive's native range may give insight into more targeted biological controls. Large impact biocontrol is often caused by the introduction of specific enemies from the invasive species' native geographical range (Vila et al. 2004). Introduction of an insect from Southeast Asia range (same as *L. morrowii*) may minimize the growth of the invasive without impacting North American natives. Thus more investigation of invasive plants original enemies could be beneficial to limiting invasive spread.

Limiting *L. morrowii* growth, and therefore minimizing negative implications to native ecosystems, could have beneficial impacts on conservation of native species in the Northeastern American forest. The growth of a variety of native plants is limited because *L. morrowii*

decreases light availability and lower soil moisture and nutrients (Luken et al. 1996, Munger 2005). In addition, shrub negatively impacts bird populations because the dense branches and early leaf production provide desirable nesting sites but also increase nest mortality due to easy predator access (Munger 2005). These impacts are widespread as the invasive can be found across a large range reaching from Minnesota to Arkansas and east to Maine and South Carolina (Schmidt and Whelan 1999). Minimizing these impacts is possible but current eradication efforts of *L. morrowii* are costly as well as time and labor intensive (Hoffman and Kearns 1997). Biocontrol could provide an alternative method of eradication and knowledge of insect herbivory and damage can help guide the way. With more investigations like this one we can organize a host specific biocontrol that would specifically target the invasive honeysuckle while not limiting the native population. This could help conserve the native ecosystems in the northeastern United States.

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Tables

Table 1: Mean with standard error of number of individuals of Collembola and insect groups collected on *L. morrowii* and *L. hirsuta*. Insects were collected on 6 plants of each species by branch beating and pit falls traps.

Plant Species	Identified Arthropod Group						
	<i>Acrididae</i>	<i>Alydidae</i>	<i>Anthribidae</i>	<i>Asilidae</i>	<i>Cantharidae</i>	<i>Carabidae</i>	<i>Cecidomyiidae</i>
<i>L. morrowii</i>	0.458 ± 0.390	0.375 ± 0.388	0.000 ± 0.000	0.083 ± 0.082	0.000 ± 0.000	0.500 ± 0.269	0.083 ± 0.082
<i>L. hirsuta</i>	0.000 ± 0.000	0.042 ± 0.034	0.292 ± 0.125	0.000 ± 0.000	0.042 ± 0.034	0.250 ± 0.113	0.000 ± 0.000
	<i>Cerambycidae</i>	<i>Ceratopogonidae</i>	<i>Chironomidae</i>	<i>Cicadellidae</i>	<i>Cicadidae</i>	<i>Coccinellidae</i>	Collembola
<i>L. morrowii</i>	0.000 ± 0.000	0.083 ± 0.082	0.083 ± 0.118	0.542 ± 0.282	0.042 ± 0.059	0.125 ± 0.098	0.083 ± 0.082
<i>L. hirsuta</i>	0.042 ± 0.034	0.542 ± 0.155	0.000 ± 0.000	0.458 ± 0.130	0.000 ± 0.000	0.083 ± 0.047	1.208 ± 0.260
	<i>Coreidae</i>	<i>Culicidae</i>	<i>Curculionidae</i>	<i>Dascillidae</i>	<i>Elateridae</i>	<i>Formicidae</i>	<i>Halictidae</i>
<i>L. morrowii</i>	0.042 ± 0.059	0.708 ± 0.764	6.292 ± 4.401	0.000 ± 0.000	0.000 ± 0.000	9.750 ± 5.546	0.042 ± 0.059
<i>L. hirsuta</i>	0.000 ± 0.000	0.125 ± 0.056	0.833 ± 0.281	0.042 ± 0.034	0.000 ± 0.034	1.292 ± 0.376	0.000 ± 0.000
	<i>Hesperiidae</i>	<i>Ichneumonidae</i>	Lepidoptera Larvae	<i>Meloidae</i>	<i>Miridae</i>	<i>Muscidae</i>	<i>Papilionidae</i>
<i>L. morrowii</i>	0.042 ± 0.059	0.125 ± 0.129	0.417 ± 0.168	0.000 ± 0.000	0.167 ± 0.163	0.375 ± 0.238	0.042 ± 0.059
<i>L. hirsuta</i>	0.042 ± 0.034	0.167 ± 0.080	0.125 ± 0.056	0.042 ± 0.034	0.083 ± 0.070	0.167 ± 0.082	0.042 ± 0.034
	<i>Pentatomidae</i>	<i>Pteromalidae</i>	<i>Reduviidae</i>	<i>Rhagionidae</i>	<i>Rhizophagus</i>	Symphyta Larvae	<i>Scarabaeidae</i>
<i>L. morrowii</i>	0.458 ± 0.255	0.083 ± 0.149	0.042 ± 0.255	0.042 ± 0.059	0.042 ± 0.059	0.083 ± 0.082	0.000 ± 0.000
<i>L. hirsuta</i>	0.042 ± 0.034	0.000 ± 0.000	0.000 ± 0.034	0.000 ± 0.000	0.000 ± 0.000	0.208 ± 0.085	0.042 ± 0.034
	<i>Silphidae</i>	<i>Simuliidae</i>	<i>Sphecidae</i>	<i>Staphylinidae</i>	<i>Tabanidae</i>	<i>Tenebrionidae</i>	<i>Tenthredinidae</i>
<i>L. morrowii</i>	0.000 ± 0.000	0.083 ± 0.082	0.083 ± 0.082	0.458 ± 0.307	0.083 ± 0.083	0.083 ± 0.082	0.208 ± 0.120
<i>L. hirsuta</i>	0.042 ± 0.034	0.208 ± 0.170	0.042 ± 0.034	0.333 ± 0.145	0.000 ± 0.000	0.000 ± 0.000	0.458 ± 0.246

Figures:

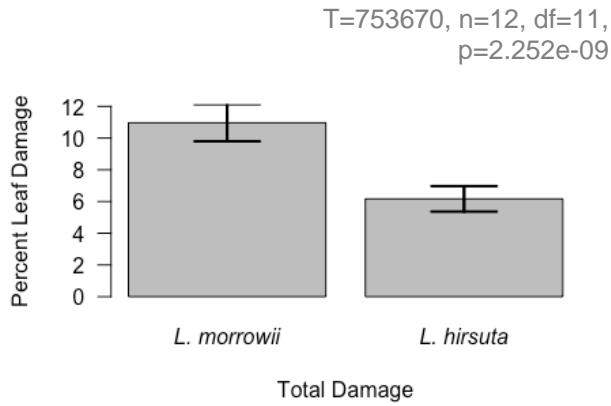


Figure 1: Mean total leaf damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.

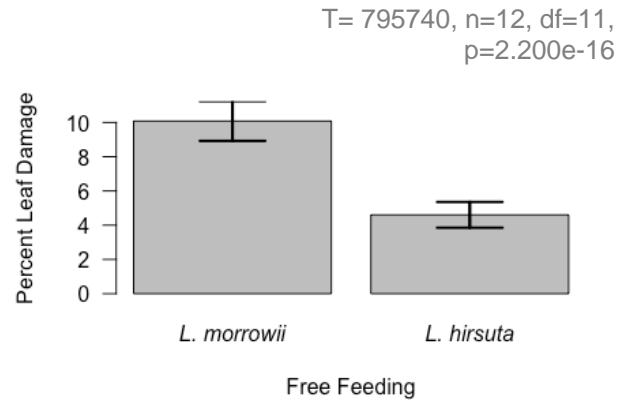


Figure 2: Mean free feeding damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.

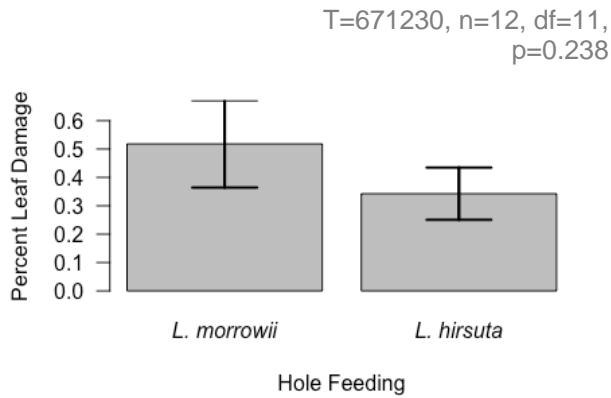


Figure 3: Mean hole feeding damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.

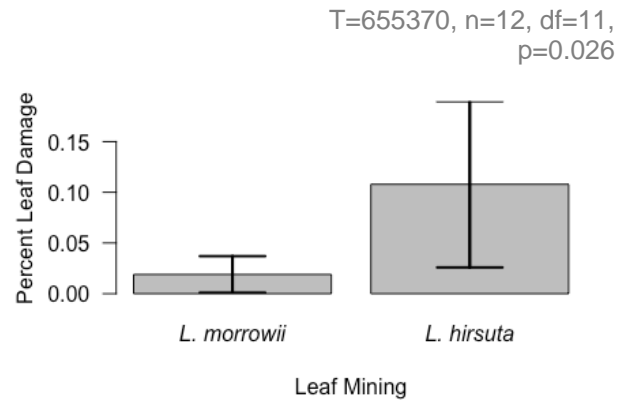


Figure 4: Mean leaf mining damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.

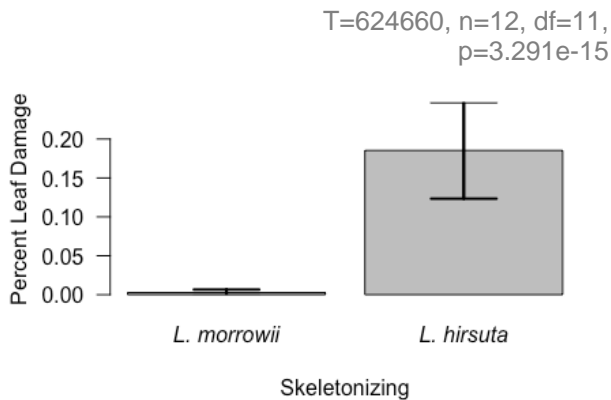


Figure 5: Mean skeletonizing damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.

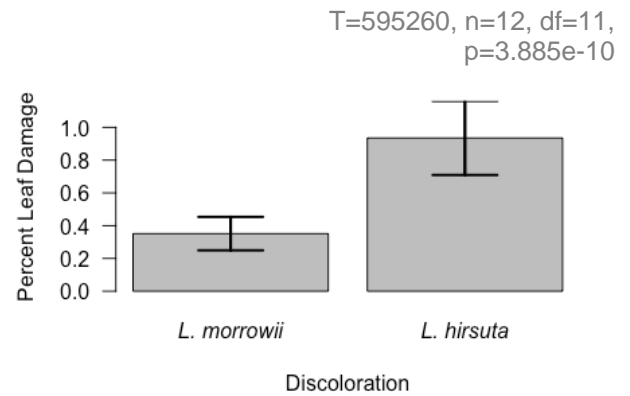


Figure 6: Mean discoloration damage on *L. morrowii* and *L. hirsuta*. Error bars show standard error.