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## The Effect on the Invasive Process of Phenotypic and Genetic Differences Among *Melaleuca quinquenervia* (Cav.) S.T. Blake Populations

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### *Abstract*

Exotic plants often expand into a wide range of habitats, some very different from native habitats. Characteristics of both the species and the habitat it is invading are likely to determine success. As a measure of invasive potential, the phenotypic plasticity and genetic variation of *Melaleuca quinquenervia* (Cav.) S.T. Blake populations was determined. This wetland tree, introduced into Florida from Australia beginning in the late 1800s, now covers more than 500 000 ac. Seeds were collected from populations in Florida and half-sib seedlings' phenotypic responses to moisture and pH conditions were determined in the greenhouse. These variables were chosen because these are two of the characteristics that vary greatly among melaleuca habitats, and because the hydrologic restoration plans for the southern Florida region involves increasing water levels in the Everglades ecosystem. All populations exhibited high levels of phenotypic plasticity, but they generally grew tallest and produced the greatest biomass with water levels 3 cm below soil surface and moderate pH (pH 7). There were significant differences among populations, indicating genetic differences among populations. The high plasticity of the plants probably influences their colonizing ability, but the genetic differences among populations should also be taken into consideration in developing management strategies.

### **Introduction**

This study examined genetic variation and phenotypic plasticity in populations of melaleuca, *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), in southeastern Florida. Melaleuca provides a useful test species for examining the role of these genetic factors on invasiveness. Melaleuca currently invades a wide range of novel habitats in south Florida, much different from its native habitats in eastern Australian wetlands. There is also relatively reliable information on the times and sources of the introductions of melaleuca to southern Florida.

Genetic variation and phenotypic plasticity influence the range of phenotypes melaleuca could express. Phenotypic plasticity can be defined as the amount of change expressed in a trait of a particular genotype under different environments (Bradshaw 1965). Phenotypic plasticity may be favored in environments with high variability or unpredictability, and could enable a plant to grow in a wide range of new environments (West-Eberhard 1989). Plasticity was originally considered an alternative strategy to having high levels of genetic variation, but organisms can have both high levels of genetic variation and phenotypic plasticity (Scheiner and Goodnight 1984; Quinn 1987).

My efforts focused on determining the genetic variation and phenotypic plasticity of four melaleuca populations from a single introduction in southeastern Florida. A moderate amount of genetic divergence and variation, among and within populations, respectively, was anticipated. Melaleuca was introduced to the Miami area in 1907 (Rothra 1972) and probably underwent a severe genetic bottleneck during its introduction. However, given that trees can produce seed after only 2-3 yr, the melaleuca in Florida has produced from 30 to 45 generations, with hundreds of thousands of trees in each generation. When a population is able to expand rapidly after introduction, it is unlikely to lose much of its initial genetic variation (Nei et al. 1975). There may be a shift in allele frequencies in the founding population, as compared to the source population, due either to genetic drift or to new selection pressures favoring different genotypes (Nei et al. 1975; Barton and Charlesworth 1984). New selection pressures can also favor the spread of any favorable genetic mutations in the founding population, resulting in genetic change in the new population (Slatkin 1987). The four populations grow under different environmental conditions in the field, e.g., different soil types and hydroperiods, and that could have led to genetic divergence. Alternatively, there could be little or no genetic divergence, indicating little effect of selective differences among habitats.

Populations growing close together were expected to be more similar to each other phenotypically than those growing farther apart. Habitats of populations growing close together share more features and, consequently, more similar selective pressures on phenotype. No pattern in phenotypic similarity would suggest that the differences in habitats do not impact these populations.

Finally, most of the phenotypic variation were expected to be due to phenotypic plasticity, rather than solely to genetic variation. In Australia, melaleuca grows under a wide range of environmental conditions, and the native habitats can undergo significant short-term environmental fluctuations within populations. These conditions can favor the evolution of phenotypic plasticity (Scheiner and Goodnight 1984; Quinn 1987). If genetic variation is high and phenotypic plasticity low, it would indicate that genetic variation within populations was very important in enabling plants to survive under the range of environmental conditions encountered. However, populations also could differ in amounts of phenotypic plasticity due to genetic divergence among the populations.

*Melaleuca quinquenervia* is native to eastern Australia, New Zealand, and New Caledonia, where it generally grows in seasonally flooded coastal and riverine habitats in open stands, either in mixed or monospecific populations. People introduced the species into Florida, California, Hawaii, Puerto Rico, and Malaysia; the species is now considered invasive in Florida and Hawaii. In Florida, it primarily occupies anthropogenically disturbed sites, sawgrass prairies, pinelands, and cypress domes. It generally grows in very dense, monospecific stands, with few understory plants.

In Florida, though peak flowering occurs in summer and fall, trees flower and produce seeds year-round. Trees can flower up to five times per year. Insects pollinate the flowers, and flowers can self-pollinate (Vardaman 1994). Woody capsules contain abundant seeds and release them when the capsules desiccate, as happens after a fire or when a branch breaks. Each capsule contains 200-300 seeds 1 mm long each (Woodall 1982; Bodle et al. 1994). Wind and possibly water disperse the seeds (Bodle et al. 1994).

## Methods

### *Study sites*

Seed was collected from four sites in southern Florida (Fig. 1). Populations East1 and East1a were located in the northeastern corner of Everglades National Park, along the west side of the L31 canal, 0.5 km and 0.8 km south of Tamiami Trail (U.S. Route 41), respectively. These sites remain constantly flooded. Population East2 was located along the west side of State Road 997, across from Thompson Park. The older portion of the stand at this site floods seasonally, and the younger portion is constantly flooded. Population Okeechobee was located along the Lake Okeechobee rim canal in Clewiston. This site ranges from always dry in the older portion of the stand to constantly flooded in the younger portion of the stand. The populations at these four sites probably originated from a teaspoonful of seeds introduced by John Gifford to his estate in Coral Gables in 1907 (Rothra 1972). All of these populations colonized land that was disturbed when the canals were built. Populations East1 and East1a are now invading relatively undisturbed sawgrass prairies, but populations East2 and Okeechobee are invading more disturbed sawgrass areas; the latter are more confined by surrounding canals and roads.

### *Seed collection and germination*

Seed capsules were collected from at least 50 open-pollinated trees in populations East1, East2, and Okeechobee, and from ten trees in East1a, during January 1996. The trees selected were at least 5 m apart and ranged in size from 2-30 m tall. Seed capsules were collected by hand or with the aid of a pruning pole and placed in labeled plastic bags. The seed capsules were allowed to dry in the sun until they

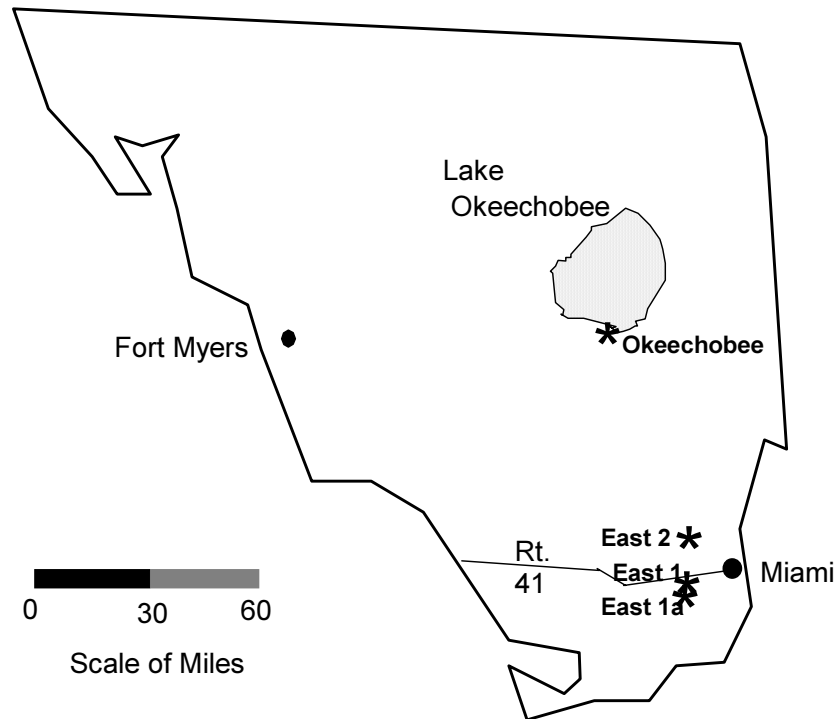


Figure 1. Location of study sites (\*) in southern Florida.

opened. The seeds germinated in the greenhouse at Rutgers University in June 1996 in trays filled with potting soil (Metromix 360). The seedlings were maintained in the greenhouse until they were at least 3 cm tall (about 3 weeks).

#### *Experimental design*

Seedlings were grown from mother trees of different populations under a range of environmental conditions, to determine the reaction norms of several traits of the seedlings. In this experiment, I could partition phenotypic variation into both genetic and environmental components.

Thirty-six seedlings were used from each mother tree, eight mother trees from each of the four populations (a total of 1152 seedlings). There were four treatment combinations: low water and low pH, low water and high pH, high water and low pH, and high water and high pH. For the low pH treatments, seedlings at least 3 cm tall were transplanted into a 3:1 sterilized sand:Metromix 360 mixture. For the high pH treatments, seedlings were transplanted into a 12:4:1 sand:Metromix:crushed limestone mixture. Low pH treatments had a pH of approximately 6.9 and high pH treatments had a pH of approximately 7.3. For the high

water treatment, the water levels were maintained at 10 cm, the level of the soil mixture in the pot, so the roots were always flooded. For the low water treatment, the water level was kept at about 2 cm above the bottom of the pot, so the soil was constantly moist. Three seedlings from each tree were placed in each treatment combination. Each treatment was replicated three times.

#### *Data collection and analysis*

Any characteristic of a species that allows it to colonize rapidly will increase invasiveness. Since seedling establishment is a critical phase of melaleuca's invasive process, seedling characteristics were measured. The initial heights of the seedlings were measured on 21 July 1996, and again on 30 July, 7 August, 14 August, 27 August, and 15 December. The fourth leaf below the apical meristem was collected in December and its length and width measured. Above-ground biomass of each seedling was harvested during the week of 9 December and oven-dried and weighed using a top-loading balance.

Leaf length was log-transformed to width ratios and biomass to improve normality. Growth rate was calculated from log-heights. All water and pH effects were treated as fixed effects. Population and family (tree) effects were considered random, since there are thousands of populations, each with thousands of trees, in southern Florida. The data was analyzed using SAS PROC GLM and Type III sums of squares (SAS 1985). F-tests were done based on the expected mean square calculations of the Scheffé model for mixed effects (Ayres and Thomas 1990). This model is useful for estimating genetic and phenotypic variances in populations occupying a defined set of environments (Fry 1992). The total phenotypic variation is partitioned into variance components for population effects, families nested within populations, treatment differences (water and pH), and interactions by equating observed mean squares with the expected mean squares for each component.

A significant effect of population indicates genetic differences among populations, and significant differences among families (within populations) reflect within-population genetic variance. Water and pH effects represent phenotypic plasticity in populations, in response to changes in those important environmental variables. Significant population (or family) by environment interactions indicate that at least two populations (or families) differ from each other in amounts of plasticity. The pattern of plasticity is seen in any changes in the rank order of populations (or families) in different environments.

## **Results**

Among populations, all traits except biomass showed significant differences (Table 1). Variance among populations accounted for up to 15.82% of the total pheno-

Table 1. Environmental experiment variance (var) components and percentages of variation explained by population, family (nested within population), seedling (error term), environment (water and pH), and interactions. Variables include the ratio of leaf length to width (l/w), biomass, height at 150 days, and overall growth rate from 0-150 days. P-values from univariate Analysis of Variances are reported (\* =  $p < 0.05$ , \*\* =  $p < 0.001$ , \*\*\* =  $p < 0.0001$ ).

Component	Leaf l/w		Height		Growth rate		Biomass	
	var	%	var	%	var	%	var	%
Population	0.00078**	<b>15.82</b>	472.08*	<b>4.87</b>	1.12E-07*	<b>4.47</b>	.00350	2.52
Family	0.00076***	<b>15.24</b>	1256.81***	<b>12.96</b>	1.31E-07***	<b>5.21</b>	0.01884***	<b>13.59</b>
Water	0.00022***	<b>4.40</b>	2104.53***	<b>21.70</b>	8.45E-07***	<b>33.67</b>	0.03599***	<b>25.97</b>
Pop x water	-0.00002	-0.39	-38.15	-0.39	1.41E-08	0.56	.0001	0.11
Fam x water	0.00003	0.66	167.26**	<b>1.72</b>	1.08E-07***	<b>4.28</b>	0.00349***	<b>2.52</b>
pH	0.00003*	<b>0.59</b>	1174.26***	<b>12.11</b>	1.82E-07***	<b>7.23</b>	0.01466***	<b>10.59</b>
Pop x pH	0.00000	0.01	-11.17	-0.12	-1.76E-08	-0.70	-0.00081	-0.59
Fam x pH	0.00003	0.55	386.20***	<b>3.98</b>	1.16E-07***	<b>4.63</b>	0.00567***	<b>4.09</b>
Water x pH	-0.00003	-0.53	500.91**	<b>5.17</b>	4.38E-09	0.17	-0.00059	-0.43
Pop x water x pH	-0.00008	-1.56	-64.01	-0.66	2.08E-08	0.83	-0.00188	-1.35
Fam x water x pH	0.00011	2.22	14.16	0.15	-3.33E-09	-0.13	-0.00063	-0.46
error	0.00312	62.97	3734.12	38.51	1.00E-06	39.84	0.06020	43.43

Table 2. Trait means of populations from south (East1b) to north (Okeechobee), across treatments, in environmental study. Traits include leaf length to width ratio (l/w), height at 150 days (cm), log growth rates (mm/day), and log biomass (mg). Letters indicate significant differences between populations for that trait as measured by Duncan's Multiple Range test. (Between population analysis of biomass was not conducted because population was not a significant effect in the ANOVA for biomass).

Trait	Population			
	East1a	East1	East2	Okeechobee
Leaf l/w	4.97a	4.69b	4.39c	4.23d
Height	171.08c	164.72c	193.95b	216.87a
Growth rate	0.0054c	0.0057b	0.0053c	0.0061a
Biomass	2.36	2.31	2.42	2.47

typic variance (Table 1). Okeechobee plants had the smallest leaf length to width ratios, were the tallest, and had the highest growth rate in all treatment combinations (Fig. 2). Populations that were further apart had greater differences between their traits (Table 2). For example, East1 and East1a did not show any significant differences in height, whereas both differed greatly in height from Okeechobee. Among families (within populations), however, every characteristic measured showed significant differences. Variance among families accounted for up to 15.24% of the total phenotypic variance. Variance within sibships (error variance) accounted for the highest proportion of phenotypic variance, ranging from 39-63% (Table 1). The error variance is due to micro-environmental differences and differences between siblings from the same family.

In response to both pH and water levels, all of the characteristics showed significant differences, demonstrating a high level of phenotypic plasticity in these plants (Fig. 2). In general, the magnitude of the phenotypic response to pH was lower than the differences due to water level. Plants generally had smaller leaves in the high water/high pH treatment. Low water treatments tended to result in larger leaves and taller plants, with a higher growth rate. Height demonstrated a significant interaction effect between the water and pH treatment, indicating that the combination of the pH and water depth affected height differently than would be expected from the separate responses. Trees within populations differed significantly in plasticity (tree x treatment interactions) of characteristics, in both water and pH treatments (Table 1).

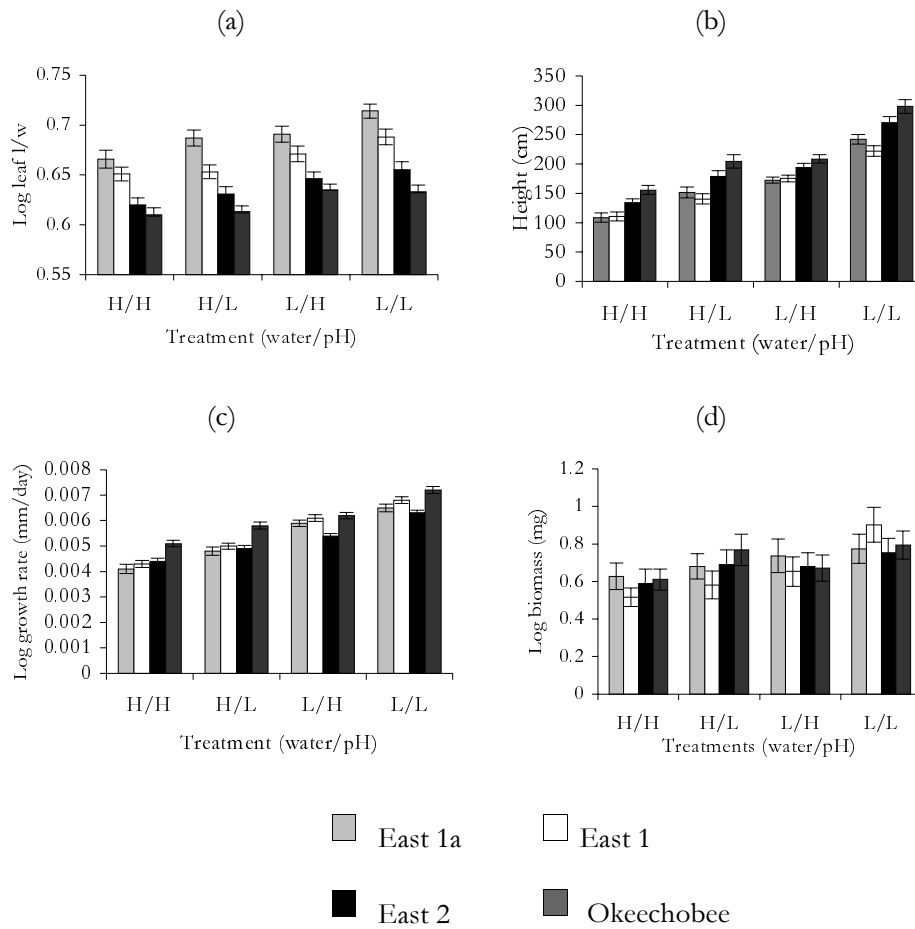


Figure 2. Reaction norms for (a) log leaf length/width (l/w), (b) average height (cm), (c) log growth rate (mm/day), and (d) biomass (mg) for each population. Bars are standard errors.

## Discussion

Examining the spread of invasive exotics in an ecological and evolutionary context provides a framework for identifying factors influencing the pattern and predictability of spread. Characteristics of the species that make it a good colonizer, habitat invasibility, and the adaptive response of organisms to new habitats will all influence invasiveness (Fig. 3) (Jain and Martins 1979; Moran et al. 1981; Bennington and McGraw 1995; Williams et al. 1995). This information should be used when planning management strategies. For example, the effectiveness of herbicides and biological control agents are known to vary among genotypes of many

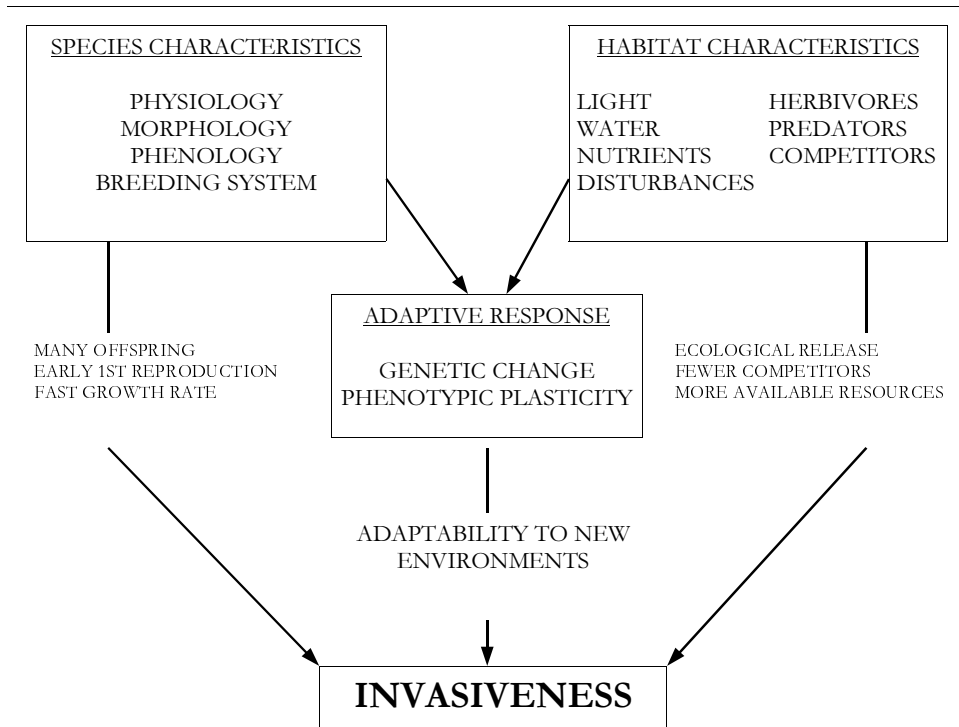


Figure 3. Model of species, habitat, and adaptive response effects on invasiveness.

plant species (Weis and Berenbaum 1989; Rayachhetry et al. 1996).

If there are genetic differences among these melaleuca populations derived from a single introduction, populations from other introductions are expected to differ even more genetically. Current studies on a biological control agent for melaleuca, *Oxyops vitiosa* Pascoe, a weevil, in populations from different introductions, may yield important information on the response of at least one biological control agent to population differences (A. Dray, pers. comm., 1998). Control agent effectiveness could also be affected by the phenotypic plasticity of the species. If morphological characteristics change under different environmental conditions, it is likely that physiological changes occur as well. Both types of changes could impact a plant's resistance to herbicides or biological control agents.

This study demonstrated significant genetic differences among melaleuca populations for several traits that could contribute to invasiveness. It is also notable that traits also seemed to follow a latitudinal gradient, with leaf width and plant height increasing, from south to north. Analysis of more than four populations along the gradient would be needed to confirm this trend, but the observation is worth pursuing because it implies adaptive radiation since initial colonization. Adaptation to

local environmental conditions could lead to this pattern of genetic variation if some environmental condition or set of conditions is changing along the same latitudinal gradient.

Despite real genetic differences among populations, within population genetic variation was usually greater. The amount of within population variance was somewhat unexpected because of the colonization pattern of melaleuca. Generally, one or a few trees establish and offspring are dispersed relatively close to the parent trees. Consequently, trees within a population are expected to be closely related. Genetic variation may remain high since populations expand rapidly, assuming progeny are usually outcrossed (Nei et al. 1975).

The amount and pattern of phenotypic plasticity did not differ significantly among populations, but families within populations differed in amounts of phenotypic plasticity under both water and pH treatments for growth rate, height, and biomass. Phenotypic plasticity in response to water and pH varies more within than among populations.

All of the traits measured were plastic in their response to water level and pH, and the water by pH interaction affected height as well. Plants generally achieved greater heights and had a higher growth rate under the low water/low pH treatment. The magnitude of the response to pH was surprising because, in the eastern Everglades, plants tend to grow under higher pH conditions ( $\text{pH} > 7$ ) than were represented in this experiment. In melaleuca's native habitats in Australia, pH is usually 6 or less (S. Kaufman pers. obs.). The two pH levels in this experiment only differed by 0.4, indicating that melaleuca's growth patterns may be quite sensitive to small pH differences.

The response to water level was expected. At the high water level, the plant's roots were constantly flooded. In the Everglades, populations can grow under constantly flooded conditions, but seedlings are more commonly found under lower water conditions.

For all traits, except leaf length to width, the environmental variance accounted for a larger proportion of the total phenotypic variance than did the genetic variance (Fig. 4). This could indicate that phenotypic plasticity in melaleuca plays an important role in its invasiveness. If it can grow under a wide range of environmental conditions, particularly under the fluctuating water levels encountered in wetlands, it should have little trouble invading new habitats. The small genetic differences among populations may enable seedlings from one population to perform somewhat better than another population under particular environmental conditions, but under field conditions, the genetic variance seen in the greenhouse may be less important than phenotypic plasticity under highly variable field conditions. It would be worthwhile to determine the importance of the genetic differences among populations and to further investigate the phenotypic plasticity of me-

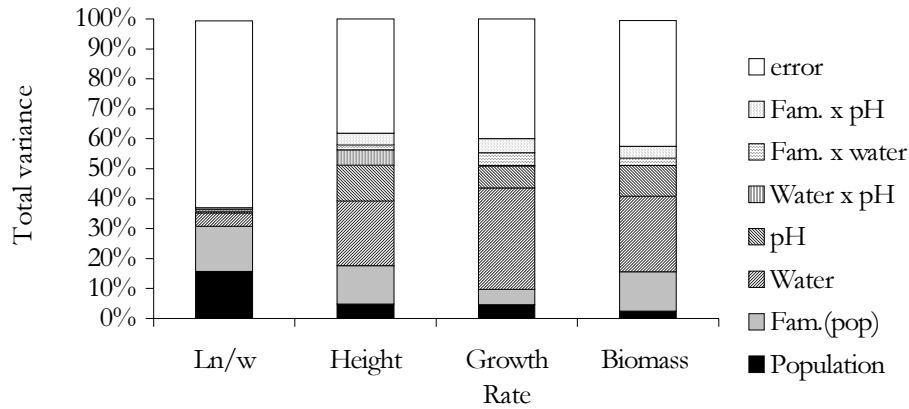


Figure 4. Components of total phenotypic variance by trait. Population and family components are genetic; water, pH, and water x pH are environmental. Family by treatment interactions represent differences among families in amounts of phenotypic plasticity.

laleuca populations in the field, where plants would encounter a wider range of environmental conditions.

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