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Separating ontogenetic and environmental determination of resistance to herbivory in cottonwood

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Abstract. We used narrowleaf cottonwood, *Populus angustifolia*, and the gall-forming aphid, *Pemphigus betae*, to determine the extent to which ontogenetic variation in resistance to herbivory is due to endogenous, stable genetic influences. In a three-year common garden trial using ramets propagated from the top, middle, and bottom of mature trees, we found that the resistance of trees to aphids was significantly higher in top vs. bottom source ramets, supporting the hypothesis of a stable, genetically programmed component to aphid resistance. The magnitude of ontogenetically based variation in resistance within an individual tree is comparable to the genetic variation in resistance among narrowleaf cottonwood genotypes or populations found in other studies. These ontogenetic-based findings have the potential to alter ecological interactions and evolutionary trajectories of plant–herbivore interactions.

Key words: development; environment; genetic; herbivory; ontogeny of resistance; Pemphigus betae; *Populus.*

INTRODUCTION

Despite the ubiquity of developmental variation in the phenotypes of long-lived plants, the evolutionary and ecological implications of this form of within-plant variation have received much less attention than has intra- or interspecies variation. Developmental variation within a single plant is often due to the simultaneous presence of vegetative structures of differing maturity, which can have pronounced affects on the distribution, survival, and composition of individual herbivore species and arthropod communities (Kearsley and Whitham 1989, 1998, Waltz and Whitham 1997, Karban and Thaler 1999, Boege 2005, Loney et al. 2006, Du et al. 2008). Developmental trait variation is frequently disregarded as "environmental noise" unless the developmental variation is itself of interest (Boege and Marquis 2005, Shiojiri and Karban 2006, Goodger et al. 2007). Here, we present an example in which developmental variation in resistance to herbivory in a foundation tree species has the potential to influence

both ecological and evolutionary interactions with other species.

Developmental variation in long-lived plants can result from endogenously programmed changes in gene expression in plant meristems, environmental heterogeneity, or from environmentally influenced changes in gene expression (Zagory and Libby 1985, Poethig 1990, Wiltshire et al. 1994, Lawrence et al. 2003). Regardless of the underlying mechanism, the same general outcome occurs: a gradient of age-related changes in ramet characters. This gradient, termed a developmental stream, occurs in cottonwoods and many other plant species (Lillie 1927, O'Rourke 1949, Kearsley and Whitham 1998). The developmental stream produces a pattern in which shoot age is a function of its degree of separation from the root crown. Shoots in the chronologically oldest regions of a plant (i.e., closest to the root crown) will express the most developmentally young phenotypes and are not reproductive, while the youngest regions of the plant, i.e., upper canopy, developmentally express the oldest phenotypes and are reproductively mature (Poethig 1990, 2003).

Separating the proportion of developmental variation in resistance that has a stable, genetically programmed basis (or that is a result of a predictable gene × environment interaction) is essential to understanding the evolution of plant defense. When selection imposed

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by herbivores on resistance traits varies with plant ontogeny, it creates a mosaic of selection pressures within a single plant or genotype.

Many studies that have noted developmental changes in plants have not identified their causal basis; most that have done so have attributed them to physiological changes via the gradual process of plant senescence (e.g., Nakamura et al. 2006). In this study, we use *Populus angustifolia* trees propagated from cuttings to disentangle stable, genetically programmed effects from environmentally influenced effects on developmental variation in resistance to the aphid, *Pemphigus betae*. Most differential effects of the local environment that a shoot was taken from in the parent tree (e.g., vasculature, position in the canopy, water stress as a function of height, sun vs. shade) were standardized by the use of rooted cuttings grown in the same environment. Using cuttings taken from different developmental modules within and across several *P. angustifolia* genotypes, we addressed the question: To what extent is the developmental variation in resistance to *P. betae* due to endogenous, stable genetic influences?

MATERIALS AND METHODS

P. angustifolia occupies riparian areas between 1500 and 3000 m in elevation across the southwestern United States. They reproduce asexually via root suckers and sexually via wind-dispersed seeds. The cuttings used in this study were taken from trees that were at least 8 m tall growing along the Weber River in Utah, USA. One year before cuttings were taken, trees at the collection site were prepared by removing all branches between the root crown and 7.5 m up the trunk to encourage growth from dormant buds (Kains and McQwestern 1947). This ensured that all of the cuttings collected the next year (from along the trunk) would be the same physiological age and would have no extensive history of herbivory, thus minimizing the effects of environmental factors on variation in cuttings within a particular tree (Tuomi et al. 1988, 1989).

Vegetative shoots were taken from four mature tree genotypes before bud break. We collected cuttings from a particular tree only if it met the following criteria: reproductively mature, vigorous, and lacking visible disease or physical damage. Cuttings for each genotype were collected from three "source positions" (less than 1.5, 2–4, and 4.5–7 m above the root crown). One of the genotypes (clone 6) did not have enough new vegetative growth in the highest source position to collect cuttings. The cuttings were wrapped in wet burlap, placed in coolers with snow, and transported to the greenhouse for rooting in coarse sand. Soluble balanced fertilizer was applied twice daily, and rooting hormone was applied once daily for 45 days. We then transferred the cuttings to 4-inch (~10 cm) pots, followed by transplants to 1 gallon (3.79 L) buckets, and finally to 5-gallon (18.93 L) buckets. They were then moved outdoors and one year later were randomly planted at 3 m spacing into a common garden along the Weber River in northern Utah. Cutting mortality was very low and the final sample size in the common garden was three to five cuttings per source position per source tree. We fertilized and watered the trees using drip irrigation until they were established.

Pemphigus betae, a leaf-galling aphid, has a complex life cycle with narrowleaf cottonwood as its primary host. In northern Utah, its secondary hosts include docks (*Rumex crispus*, *R. patientia*), and lambsquarter (*Chenopodium album*). *P. betae* stem mothers (fundatrices) emerge in the early spring from eggs that overwinter on cottonwoods. The stem mothers initiate gall formation along the midvein of developing leaves. If gall formation is successful, each stem mother parthenogenically produces 10–300 offspring that mature in the gall and then fly to the secondary host plants during the summer. Stem mother death often occurs during gall formation, but the failed attempt leaves a characteristic scar (Whitham 1978). Fall migrants return to cottonwoods from the secondary hosts in October and deposit wingless sexuales in bark crevices. The sexuales mate, and leave one overwintering egg per female (Whitham 1978, Kearsley and Whitham 1989).

When the trees were three years old, one target branch per tree was isolated with a sticky barrier (Tanglefoot, Grand Rapids, Michigan, USA) to prevent naturally colonizing aphids that had overwintered on the stem from colonizing target branches, and to keep experimentally transferred aphids restricted to the target branches. To standardize our source population, we collected overwintering eggs from a single naturally occurring tree in February and maintained them in refrigerators. In late March, we placed the eggs in plastic bins in a warm room at ~20°C to promote hatching. The first instar stem mothers emerged in April and were not allowed to feed. We collected the aphids into vials, placed the vials on ice, and transferred the aphids to trees in the common garden within 3 hours of collection. 15–40 aphids were delivered to each target branch, depending on the number of buds present on the branch.

In mid-June (after gall formation was complete but before the departure of the summer migrants from the gall), we measured the performance of the stem mothers. We calculated survivorship as the number of successful galls divided by the number of total galls (successful galls and unsuccessful/aborted galls) per branch. Because colonizing stem mothers have few predators this early in the growing season when the trees are just beginning to break bud, failure to produce a successful gall is largely due to plant traits rather than predation (Whitham 1978, 1989). Although competition among colonizing aphids can result in significant mortality (Whitham 1986), competition was standardized by equalizing the number of colonizing stem mothers per available bud on the target branches. We estimated an upper limit of fecundity by counting the number of progeny in each of the five largest galls per branch.

Expected total fitness (number of expected progeny) per stem mother was calculated as survivorship × fecundity. We arcsine-transformed the survivorship data prior to statistical analysis to conform to assumptions of normality.

Aphid performance was analyzed using a series of three two-way factorial ANOVAs. Tree source genotype, source position, and source genotype × source position interaction were fixed factors, and stem mother survivorship, fecundity, and expected total fitness were response variables. No significant interaction between tree source genotype and source position was found in any of the ANOVAs, so this term was dropped from further analyses.

RESULTS

Stem mother survival rates were significantly affected by the source height of the cutting used for tree propagation ($F_{2,30} = 12.36$; $P < 0.001$). Stem mothers placed on three-year-old trees derived from the highest source heights consistently had the highest survival rates, followed by those on trees derived from intermediate and lowest source heights, respectively (Table 1, Fig. 1, see also Appendix: Table A1). No significant effect of parent tree genotype on aphid stem mother survivorship was found ($F_{2,30} = 2.68$; ns). Source position of cuttings within a tree did not have a significant effect on aphid stem mother fecundity ($F_{2,30} = 0.03$; ns). In addition, no significant effect of parent tree genotype on aphid fecundity was found ($F_{2,30} = 0.03$; ns).

Aphid stem mother expected total fitness (survivorship × fecundity) was strongly affected by source position used in propagation ($F_{2,30} = 4.27$; $P < 0.05$). Aphids on trees derived from the highest sources had higher total expected fitness than those on trees derived from the lower zones (Table 1). In addition, parent tree genotype had a significant effect on aphid expected total fitness ($F_{3,30} = 3.40$; $P < 0.05$; Table 1).

DISCUSSION

Our common garden experiment, which largely eliminated environmental source effects, indicates a stable, genetically programmed basis to the well-documented pattern that the aphid, *P. betae*, survives significantly better on mature foliage in the upper crown relative to juvenile foliage at the base of the tree (Kearsley and Whitham 1989, 1998). This survivorship pattern is comparable with genetic-based differences in resistance observed in other studies of tree resistance to *P. betae* (e.g., Bailey et al. 2006), and is inconsistent with patterns expected if differences in resistance were due to environmental factors such as height, water relations, or sun/shade leaves. Resistance of narrowleaf cottonwood to *P. betae* decreased with increasing distance of the galling site from the root crown (Table 1), and these changes in resistance were in large part stably expressed in trees derived from cuttings. Aphid stem mother

TABLE 1. Aphid stem mother survivorship, fecundity, and total expected fitness for each tree genotype (clone) at each source position. One standard error (SE) for each mean is <2 for fecundity and expected fitness measurements and is <0.2 for each survivorship measurement.

	Height of source cutting (m)		
Trait and clone	<1.5	2–4	4.5–7
Survivorship†			
Clone 1	0.366	0.425	0.521
Clone 3	0.350	0.445	0.615
Clone 5	0.382	0.476	0.615
Clone 6	0.200	0.222	
Fecundity			
Clone 1	31.2	40.3	38.8
Clone 3	44.3	53.6	60.1
Clone 5	56.3	47.1	42.0
Clone 6	34.0	51.0	
Expected fitness†			
Clone 1	14.3	16.7	19.9
Clone 3	16.0	24.0	37.6
Clone 5	22.1	22.3	25.3
Clone 6	6.80	11.3	

Note: Empty cells are cells for which we have no data.

† Source height groups are significantly different from one another ($P \leq 0.05$) within this trait.

survivorship and total expected fitness was greatest on trees derived from the highest source heights (Table 1; Fig. 1). For example, on clone 3, expected fitness of aphids more than doubled on trees derived from the highest source heights relative to the lowest source heights (Table 1). If resistance to aphids were only environmentally influenced, differences in aphid fitness among trees derived from different source heights should have disappeared in our common garden trials.

While our common garden approach eliminates most environmental effects on the phenotype (e.g., water stress, light, vertical stratification due to herbivore behavior), it does not necessary exclude some maternal effects, such as fungal endophytes (Brown et al. 1997, Bailey et al. 2005). However, growing the cuttings for three years in the field prior to testing largely eliminated or standardized other factors such as hormonal differences and twig condition that might have initially differed in upper and lower canopy twigs (Whitham 1981).

We are aware of only one other study that has isolated stable, genetically programmed effects from environmental effects to quantify an ontogenetic trajectory of resistance to herbivory. Using common garden trials with synthetic crosses of *Eucalyptus globulus* × *E. nitens*, Lawrence et al. (2003) identified ontogenetic variation in resistance to a common leaf-feeding beetle, *Chrysophtharta agricola*. They also demonstrated that the magnitude of variation in resistance within individual trees was greater than among individual trees or between the parental tree species. Similarly, comparisons of our findings in the present study with other studies in the same cottonwood system (Kearsley and Whitham 1989,
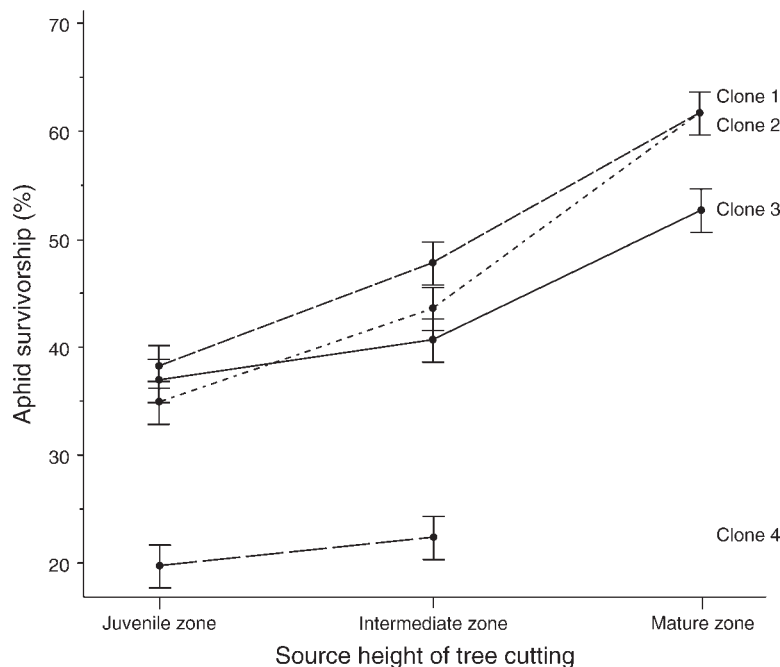
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FIG. 1. On replicated clones of the same four tree genotypes, we found that the survival rate of aphid stem mothers was a function of tree genotype (clone) and tree source position. Error bars indicate ±SE.

Whitham 1989, Bailey et al. 2006, Rehill et al. 2006) show that ontogenetic variation in phytochemistry and resistance to aphids within an individual cottonwood tree can equal or exceed that found among individual trees or cottonwood species.

Ontogenetic variation in resistance within individuals can influence community composition and dynamics. Previous studies of the effects of genetic variation on herbivore community structure have focused on variation among host genotypes (reviews by Whitham et al. 2006, Johnson and Stinchcombe 2007). These studies have shown that genetic variation in plant populations can affect the abundance of foundation herbivore species, which in turn can cascade to a diverse community. However, ontogenetic variation within individuals may be as ecologically relevant as genetic variation among individuals in terms of its effects on associated species and communities. For example, ontogenetic effects on *P. betae* directly and indirectly affect the distribution of diverse arthropods, fungi and vertebrates (Dickson and Whitham 1996, Waltz and Whitham 1997).

Although both environmental and genetic forms of developmental variation in resistance to herbivory can have important ecological effects, only stable, genetically programmed ontogenetic variation in resistance is subject to natural selection and can influence the evolutionary trajectories of plants and dependent species (Falconer and Mackay 1998). In addition, as plants evolve in response to herbivore selection pressures, their changes can feed back to affect herbivore species abundance, interactions among herbivore species, and herbivore community composition (Whitham et al. 2006, Johnson and Agrawal 2007).

Considering the impacts of ontogeny on resistance, the empirical and theoretical incorporation of ontogeny into defense theory is rare (but see Boege and Marquis 2005, Goodger et al. 2007). Our results support the conclusions of Boege and Marquis (2005) that a new synthesis of plant defense theory is necessary; only when we examine ontogentic-based resistance to *P. betae* across different zones of an individual tree do we get a clear picture of the selection pressures that have favored the evolution of aphid behaviors to be highly selective in colonizing the most susceptible tree genotypes and mature zone of these trees (Whitham 1989, Kearsley and Whitham 1998, Bailey et al. 2006).

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APPENDIX

Summary of the ANOVA results demonstrating the effects of source tree (genotype) and source height on aphid survivorship, fecundity, and expected fitness (*Ecological Archives* E090-212-A1).