Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae) Performance in Relation to Variable Phytochemistry in Juvenile Aspen (*Populus tremuloides* Michx.)

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ABSTRACT Larval performance of the cottonwood leaf beetle, *Chrysomela scripta* F., was evaluated in relation to genetic variation in phytochemical characteristics among first year micropropagated ramets of five aspen clones (*Populus tremuloides* Michx.). Foliage from the juvenile ramets used in this experiment exhibited moderate variation in nitrogen, phenolic glycosides, and condensed tannin concentrations among clones, and overall, had very high levels of phenolic glycosides (15–22% dry weight) and low levels of condensed tannins (4–6% dry weight). Results from performance assays indicate that genetic differences among aspen clones resulted in only marginal differences in larval performance of this specialist leaf beetle. Although tannin levels were quite low in the juvenile trees, larval growth rate was reduced by 30% with increasing condensed tannin concentrations ($R^2 = 0.209$). Recent evidence suggests that aspen undergoes ontogenetic shifts in foliar concentrations of secondary metabolites resulting in decreased phenolic glycoside and increased condensed tannin concentrations as trees age. The high phenolic glycoside and low condensed tannin phytochemical profile of juvenile aspen appears to make it an ideal host for cottonwood leaf beetles.

KEY WORDS *Chrysomela scripta*, condensed tannins, water, nitrogen, phenolic glycosides, performance assays

TREMBLING ASPEN (*Populus tremuloides* Michx.) is the most widely distributed and genetically variable tree species in North America (Perala 1990). Aspen exhibits marked interclonal variation in both foliar condensed tannin and phenolic glycoside concentrations (Lindroth and Hwang 1996, Lindroth et al. 1999), and recent studies also suggest that aspen exhibits significant intraclonal developmental changes in phytochemical profiles (Erwin et al. 2001, Donaldson et al. 2004). Aspen's considerable genetic variation, particularly in concentrations of the phenolic glycosides salicortin and tremulacin, has repeatedly been linked to variable performance of both generalist (Hemming and Lindroth 1995, Hwang and Lindroth 1997, 1998, Agrell et al. 2000) and specialist (Bryant et al. 1987, Auerbach and Alberts 1992, Hwang and Lindroth 1998) lepidopteran herbivores. However, whether this pattern extends to specialists from different insect orders and whether the phytochemicals important in determining insect distributions are similar or different are unknown.

The cottonwood leaf beetle (*Chrysomela scripta* F.) occurs over a wide geographical range and specializes on species of Salicaceae. It is one of many chrysomelid beetle species that is well adapted to high salicylate-containing species of *Populus* and *Salix*. Larvae use salicylaldehyde, a breakdown product of salicylates, as a defensive secretion, and the recovered glucose moiety makes an important contribution to the larval energy budget (Rowell-Rahier and Pasteels 1986). Depending on climate, multiple generations occur (four to six generations per year in the north central region) and localized population outbreaks are common, particularly in establishing hybrid poplar plantations (Gruppe et al. 1999, Coyle et al. 2001). During outbreaks, beetle larvae can cause severe defoliation, leading to significantly reduced growth and economic losses (Reichenbacker et al. 1996, Coyle et al. 2001).

Susceptibility to cottonwood leaf beetle feeding damage varies significantly among hybrid poplar clones (Harrell et al. 1981, Lin et al. 1998, Coyle et al. 2001), and many studies have examined the effects of *Populus* and *Salix* phytochemical characteristics in relation to leaf beetle feeding preferences. Considering the degree of specialization among leaf beetles, it is not surprising that most of these studies have found leaf beetle host selections and feeding preferences to coincide with phenolic glycoside distributions within their host species (Rowell-Rahier 1984, Tahvanainen et al. 1985, Rowell-Rahier and Pasteels 1986, Rank 1992). For example, Kolehmainen et al. (1995) tested beetle feeding preferences relative to phenolic extracts of host and nonhost willows. In their study, beetle species showed preferences for specific phe-

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nolic glycosides common to their respective host plants. In another study examining preferences of several species of beetles for hybrid willow, Orians et al. (1997) conclude that salicortin best explained preferences. However, their results also indicate that high concentrations of salicortin (>10%) may inhibit feeding. Tahvanainen et al. (1985) similarly concluded that salicin elicited feeding at low to moderate concentrations but slowed growth of *Plagiodera* at high concentrations.

Clearly, host specialization among leaf beetles is intricate, involving both qualitative and quantitative aspects of phenolic glycoside distributions among the Salicaceae, but also includes factors such as phenology, morphology, and other plant chemical traits. For example, Lin et al. (1998) have linked beetle preferences to leaf surface compounds such as α-tocopherylquinone. Another study by Ikonen et al. (2001) indicates that patterns of host use of three chrysomelid species are related primarily to variation in leaf nutritive traits, such as nitrogen content, rather than to secondary metabolites, including phenolic glycosides and condensed tannins.

Performance trade-offs may preclude insect herbivores adapted to high phenolic glycoside plants from thriving on plants containing high levels of condensed tannins. Negative relationships or tradeoffs in the concentrations of condensed tannins and phenolic glycosides have been documented among and within plant taxa, and herbivore distributions seem to be influenced by these trends in some systems (Orians et al. 1997, Hallgren et al. 2003, Rehill et al. unpublished data). For example, Gruppe et al. (1999) conclude that among-species distributions of condensed tannins best explain host use patterns for leaf beetles specializing on Salicaceae. Conversely, Ikonen et al. (2002) showed that a primarily alder feeding beetle is strongly inhibited by phenolic glycosides from a salicaceous plant.

If cottonwood leaf beetles are deterred by high levels of foliar condensed tannins and attracted to trees with high levels of phenolic glycosides (Bingaman and Hart 1993, Orians et al. 1997), regenerating or juvenile aspen may be an ideal host. We recently found that juvenile aspen have much lower foliar tannin and much higher phenolic glycoside concentrations than mature aspen from the same clone (Donaldson et al. 2004). In fact, this study was initiated in response to an "outbreak" of cottonwood leaf beetles among first year aspen cuttings in an experimental plot at the University of Wisconsin–Madison.

Although Perala (1990) reported that the cottonwood leaf beetles will defoliate trembling aspen, this species is not a preferred host and the beetles are not known to feed on mature trees (Brown 1956). Consequently, no research has examined cottonwood leaf beetle preference or performance among aspen clones. This study examined *C. scripta* larval performance on juvenile trees from five different aspen clones. Genetic-based variation in phytochemical characters was measured to relate beetle growth and development to aspen leaf water, nitrogen, phenolic glycoside, and condensed tannin concentrations.

Materials and Methods

Plant Materials. The five aspen clones used in this study, Dan 2, PG 1, PG 3, PI 12, and Wau 1, are maintained in a common garden at the University of Wisconsin–Madison and were originally collected from field populations occurring throughout south-central Wisconsin (Hwang and Lindroth 1997, 1998). Based on previous assessments of chemistry from 2- to 3-yr-old potted trees (Hwang and Lindroth 1997, Donaldson, unpublished data), these clones were selected to span the range of variation in condensed tannins and phenolic glycosides typical of natural aspen populations in south central Wisconsin.

We used a tissue culture–based micropropagation technique to generate replicated clones (Sellmer et al. 1989). Micro-cuttings were taken from culture in January and February of 2001, rooted in the greenhouse, and transplanted outside into 5-liter pots in May and June 2001. Growth medium was a mix of 40:40:20, field soil:sand:perlite amended with 4.5 g/liter 3–4 mo 14–14-14 (N-P-K) slow-release Osmocote fertilizer with micronutrients (Scotts Company, Marysville, OH). As is typical for first-year aspen seedlings, all trees had an unbranched, indeterminately elongating leader and were ≈0.7 m tall at the time of performance assays.

Chemical Analyses. At the same time as third stadium larvae were weighed, the leaves bagged for chemical analyses were clipped and weighed without petioles. Leaves were freeze-dried and reweighed to calculate water content. Leaf samples were ground in a dental amalgamator and analyzed for total N in a LECO elemental analyzer (St. Joseph, MI). Condensed tannins (CT) were quantified using the acid butanol assay described by Porter et al. (1986), using purified aspen condensed tannins as a standard. Aspen phenolic glycosides, including salicin, salicortin, tremuloidin, and tremulacin, were quantified using high-performance thin-layer chromatography methods described by Lindroth et al. (1993), using purified aspen phenolic glycosides (PG) as standards. Total PGs were calculated as the sum of salicin, salicortin, tremuloidin, and tremulacin. All phytochemicals concentrations are reported as percent dry weight.

Beetle Larvae. In the summer of 2001 a moderate *C. scripta* population outbreak occurred in an experimental plot containing ≈1,500 juvenile aspen cuttings. Three female and one male adult cottonwood leaf beetles were collected as soon as adults could be found in the plot. The beetles were caged together on several young aspen saplings, and each day, leaves containing egg masses were removed, petioles were inserted into water picks, and leaves were stored at 4°C. After 4 d, when we had collected a sufficient number of eggs, they were removed from the refrigerator and allowed to hatch at 25°C.

Beetle Performance Assays. We measured average larval growth rate (LGR), total development time for

males and females, and fresh and dry adult weight for males and females. Cottonwood leaf beetle performance and preference are strongly influenced by age of leaf tissue (Bingaman and Hart 1992). Thus, leaf age (as determined by relative position on an indeterminately growing shoot) was consistent among the five aspen clones in bioassays. The most apical fully unrolled leaf was designated as position no. 1 and leaves below no. 1 were numbered sequentially (Robison and Raffa 1997). Neonate larvae were reared on leaf position numbers 5 or 6, depending on maturity and leaf toughness of replicate trees (i.e., leaf toughness for a given leaf position varied among clones).

During preliminary trials, > 50% of neonates transferred to trees in the field died or escaped from mesh bags within 12 h. To reduce such losses, larvae were reared through the first stadium in a Percival environmental chamber set at 25°C with a 15:9 (light:dark) photoperiod. Leaves from each experimental tree were excised, and petioles were inserted into water picks and placed in individual petri dishes (90 mm by 15 mm) with moist filter paper to maintain humidity. As egg masses hatched, the larvae were combined, and 20 haphazardly selected larvae were placed on the underside of a single leaf. This procedure was repeated for a total of six replicates for each of the five clones. Thus, each experimental unit consisted of an assemblage of 20 individual larvae from two to three egg clutches (all hatched within a several-hour period). Egg clutches did not hatch simultaneously, so the replicates were staggered over a 3-d period.

Average larval growth rate from second to third stadium was measured over a 4-d period. Within a day of molting into the second stadium, the 20 larvae on each replicate were collectively weighed. Because we had limited plant material available for larvae development, 10 of the 20 larvae were haphazardly selected and transferred outside to leaf position no. 7 on their respective clones. Larvae were placed on the underside of a single leaf and caged in a fine mesh bag. Because leaf shading can affect leaf chemical attributes in aspen (Hemming and Lindroth 1999), at the same time as the larvae were transferred, an additional mesh bag was placed over the next younger leaf (no. 6) to be used for chemical analyses. After the larvae had consumed most of the leaf, the bag and larvae were moved to the next younger available leaf on the stem (e.g., no. 5).

During the third and final stadium of larval development, the 10 larvae (less any mortality) were again counted and weighed collectively for each of the 30 trees. Initial and final larval weights were taken when replicates were in a consistent developmental stage, and thus, replicate weights were staggered over several days. After weighing the larvae, we transferred them back onto the tree and placed a mesh sleeve over the tip of the shoot so that leaves no. 3–9 were enclosed in the sleeve. The apical bud and youngest portion of the trees protruded beyond the sleeve to allow for continued leaf development. Larvae were allowed to feed and disperse freely within the bag until they pupated. As necessary, the mesh sleeve was moved up the stem so that beetles were never food limited. Larvae and pupae were counted twice daily. Newly emerged adults were removed three times daily to minimize food consumption before being weighed. Adults were weighed within 24 h after emerging. They were sexed and freeze-dried to measure dry weight. At all stages, timing for replicates remained staggered over an ≈4-d period (coinciding with timing of egg hatch).

For insect performance measurements, each replicate was calculated as the average of subsamples (individual larvae). Initial weights for average LGR measurements included 20 larvae. Other performance variables, including final larval weights, were based on the remaining 10 haphazardly selected larvae (see above) minus any mortality. Sex-specific data were calculated based on the average of males and females per replicate (number of individuals per replicate depended on sex ratios).

Average LGR from second to third stadium (LGR) was calculated as (Ln final wt. − Ln initial wt.)/no. days. Development time was measured from egg hatch to adult emergence (for males and females). Adult fresh and dry weight data yielded identical statistical interpretation so only fresh weights are reported.

Statistical Analyses. The experiment included five clones each with six replicated potted trees arranged in a randomized latin square design for a total of 30 trees. We used analysis of variance (ANOVA; Proc GLM; SAS Institute 1998) to test for differences in insect performance and plant quality among clones with adult beetle sex nested within clone. Because replicates were staggered over time, replicate was included as a block effect in our statistical analyses. Male and female beetle development times did not differ; therefore, these values were pooled in the final statistical analyses. A correlation analysis was used to assess relationships among plant chemistry and beetle performance variables. We used regression analyses to relate quantitative variation in aspen phytochemistry to each beetle performance variable (SAS Institute 2001).

Results

Phytochemistry. Concentrations of chemical constituents varied among the five aspen clones, although marginally for some variables (Fig. 1). Water content was highly consistent among clones. Leaf N varied among clones, but the magnitude of variation was fairly small (e.g., PI-12 had 15% higher N than did other clones). Overall, total phenolic glycosides (TPG) were high and ranged from ≈15 to 22% of dry leaf weight among clones. Clone PI-12 had 20% lower concentrations of TPG compared with the average for the other clones. Total CT also varied among clones, and were generally low, ranging from 3.8 to 6.2% dry weight.

Several relationships among aspen phytochemical variables were significant. First, water concentration was weakly correlated with CT concentration (Pearson's correlation coefficient = 0.38, $P = 0.038$).

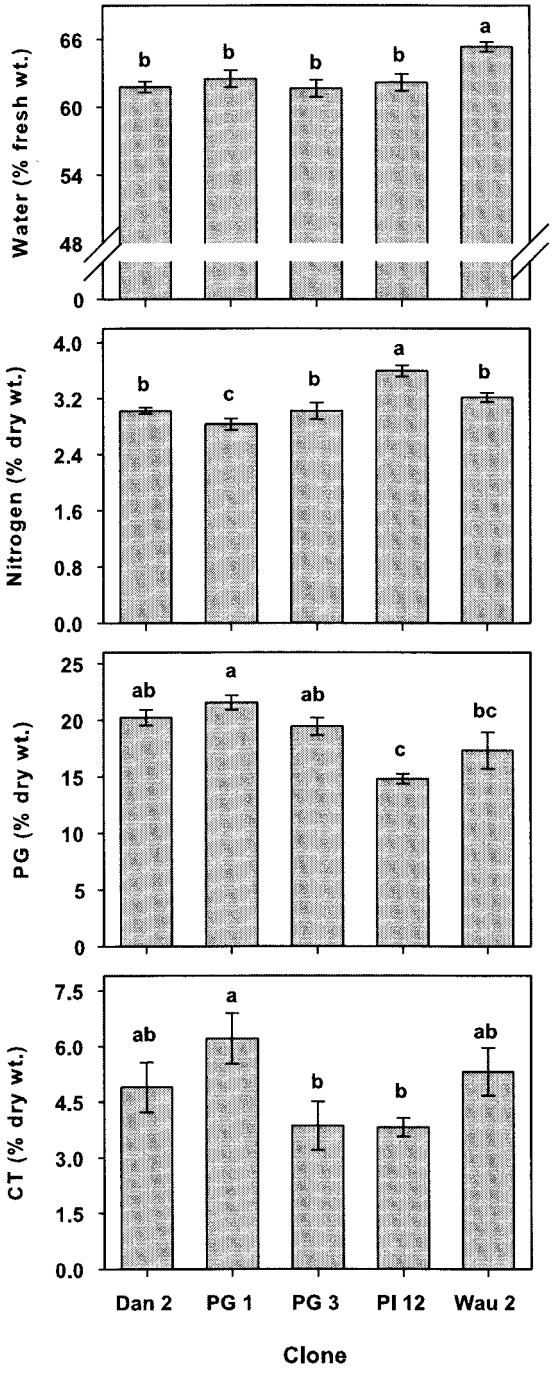

Fig. 1. Concentration of phytochemical constituents in juvenile aspen foliage of five clones; $n = 6$ replicates per clone (mean ± SE). Leaf samples for chemical analyses were collected during the third stadium of larval development. Bars bearing different letters are significantly different (Proc GLM; SAS Institute 1998, 2001; Tukey test, $\alpha = 0.05$). PG, total phenolic glycosides; CT, total condensed tannins.

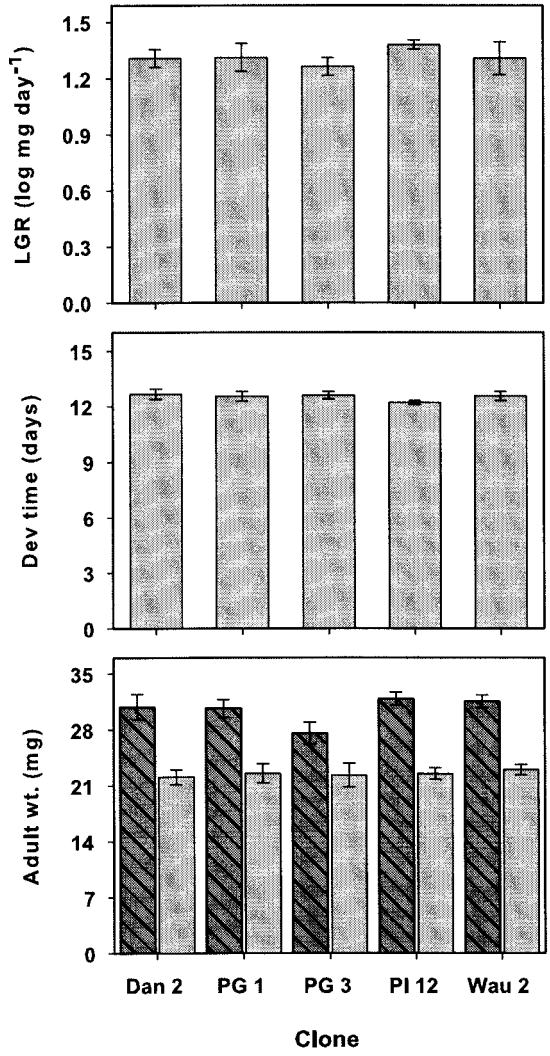

Fig. 2. Beetle performance among five juvenile aspen clones; $n = 6$ replicates per clone (mean ± SE). LGR was calculated as log final –log initial weight divided by time in days. Development time (Dev time) was measured from egg hatch to pupation. Female adult fresh weight was significantly greater than that of males ($P < 0.001$). None of the performance variables presented differed among clones at the $P = 0.05$ level (ANOVA; SAS Institute 2001). For adult weight, dark hashed bars represent means for females, and gray bars represent means for males.

Second, nitrogen concentration was negatively correlated with both TPG and CT concentrations (−0.78, $P < 0.001$, and −0.50, $P = 0.005$, respectively). And, finally, TPG and CT concentrations were positively correlated (0.64, $P < 0.001$).

Beetle Performance. Overall, larval growth rate averaged 1.3 mg/d and was similar among the five aspen clones (Fig. 2). Development time (DT) did not differ between males and females and was highly consistent among beetles on the different clones ($F_{4,29} = 3.05$, $P = 0.382$). Beetles reared on clone P12 had slightly increased LGR and decreased DT compared with those reared on the other clones, but this trend was not statistically significant. Adult weights did not differ among beetles on their respective aspen clones, but

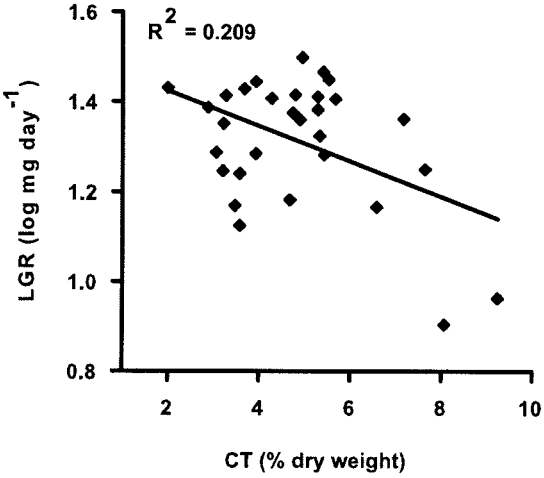

Fig. 3. LGR decreased with increasing concentrations of CT ($P = 0.011$). Each point represents the average LGR (average of ≈10 larvae per replicate).

female beetles were notably larger than males (average wet weight = 30.4 and 24.5 mg, respectively; $F_{1,58} = 25.25$, $P < 0.001$).

Regression Analyses. We used regression analyses to assess relationships between host quality (water, N, CT, salicortin, tremulacin, and TPG) and insect performance. The only statistically significant model we could fit was for larval growth rate, in which CT concentration explained 20% of the overall variation (Fig. 3; $R^2 = 0.201$ and $P = 0.011$; SAS Institute 2001). Given the highly consistent performance of beetles across the five clones, the absence of significant relationships with plant chemistry is not surprising.

Discussion

This study was prompted by observations of a rapidly growing cottonwood leaf beetle population in a newly established experimental aspen plot at the University of Wisconsin–Madison. We found phytochemical differences among the five aspen clones, but this genetic variation had marginal effects on cottonwood leaf beetle performance.

Previous research assessing insect performance relative to aspen phytochemistry has shown that phenolic glycosides almost always negatively impact growth and development of generalist aspen-associated lepidoptera (Bryant et al. 1987, Auerbach and Alberts 1992, Hemming and Lindroth 1995, Hwang and Lindroth 1997, Hwang and Lindroth 1998, Agrell et al. 2000). In this study, total phenolic glycoside levels varied among clones, but all were quite high (15–22% of dry leaf weight) relative to previous work with these and other aspen clones (Lindroth and Hwang 1996, Hwang and Lindroth 1997, Osier and Lindroth 2001). This range of variation in phenolic glycoside concentrations did not result in any differences in larval performance, and development times of 12–13 d, as observed in this study, are short relative to other studies with *C. scripta.* For example, Burkot and Benjamin (1979) report development times of 13–14 d for larvae growing on hybrid poplars at 28°C, and a field study by Augustin et al. (1997) measured development times of 16–19 d on several *Populus* species. Similar studies with willow and *Populus* have generally failed to find negative impacts of phenolic glycosides on chrysomelid beetles (Bingaman and Hart 1993, Lin et al. 1998, Wait et al. 1998). Moreover, other studies suggest that phenolic glycosides may stimulate chrysomelid feeding and oviposition behavior (Bingaman and Hart 1993, Orians et al. 1997) and enhance larval performance (Matsuki and MacLean 1994, Orians et al. 1997). In light of the negative effects phenolic glycosides on lepidopteran herbivores, it is interesting to note that aspen with high levels of these compounds may be particularly susceptible to attack from the cottonwood leaf beetle.

Gruppe et al. (1999) concluded that leaf tannins negatively affect performance of chrysomelid beetles. Because of aspen's usually high levels of tannins, it is not considered an important host for *C. scripta* (E. R. Hart, personal communication). In this study using juvenile aspen trees, CT levels were quite low (average of 3.8–6.2% leaf dry weight) but still were associated with decreased larval growth rates (Fig. 3). In field-collected aspen, condensed tannin concentrations can be as high as 25% (Lindroth and Hwang 1996, Hwang and Lindroth 1997, 1998) and are generally much higher than those measured in the juvenile experimental trees used here.

Recent work in our laboratory has revealed strong ontogenetic shifts in secondary chemistry of aspen foliage. Phenolic glycosides are accumulated at high concentrations in juvenile growth forms, but concentrations decrease sharply with age. Conversely, condensed tannin concentrations increase significantly with age (Donaldson et al. 2004). Trees in this study were <1 yr old, which likely explains the high levels of phenolic glycosides and low levels of condensed tannins found.

In other systems, ontogenetic shifts in chemical defenses are known to strongly influence insect performance and distributions. For example, Kearsley and Whitham (1989) found significant increases in *Chrysomela confluens* performance on, and preference for, juvenile versus mature cottonwood trees, and juvenile versus mature hybrid cottonwood "zones" support strikingly different insect assemblages in this system (Kearsley and Whitham 1989, Waltz and Whitham 1997, Rehill et al. unpublished data). Similarly, Andersen and Nelson (2002) showed that *C. scripta* oviposit and feed selectively on seedling versus sapling or mature Fremont cottonwoods.

This experiment involved a small number of parental beetles, and therefore, minimal genetic variation in the beetle population was tested. However, results from this work emphasizing the effects of genetic-based variation in aspen do suggest that among-clone genetic variation in juvenile aspen leaf chemistry may not be of sufficient magnitude to significantly affect cottonwood leaf beetle performance. Furthermore,

juvenile aspen trees seem to be a high-quality host for the cottonwood leaf beetle. Ontogenetic shifts in foliar chemistry, including decreased phenolic glycoside and increased CT concentrations, may preclude cottonwood leaf beetles from using mature aspen trees. Although aspen may not be a primary host of the cottonwood leaf beetle, environmental conditions that favor aspen establishment, and disturbances or management practices that result in widespread aspen regeneration could potentially have significant impacts on natural beetle populations.

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