Effects of Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae) Larval Defoliation, Clone, and Season on *Populus* Foliar Phagostimulants

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ABSTRACT The cottonwood leaf beetle, *Chrysomela scripta* F., is a serious defoliator of plantation *Populus* in the United States. Current control methods include biorational and synthetic chemicals as well as selecting *Populus* clones resistant or tolerant to *C. scripta* defoliation. Specific ratios of long-chain fatty alcohols to alpha-tocopherylquinone (α-TQ) on the leaf surface of *Populus* spp. act as phagostimulants to adult *C. scripta*. The chemical concentrations and ratios vary among *Populus* clones; however, the effect of defoliation on the subsequent production of these chemicals is unknown. We investigated the effects of defoliation, clone, and season on *Populus* leaf surface chemical production. Chemical concentrations and ratios were monitored in 1998 and 1999 on eight *Populus* clones with and without larval *C. scripta* defoliation. Chemicals were extracted from the leaf surface and analyzed via gas chromatography. Larval *C. scripta* defoliation rarely caused changes in leaf surface chemistry at the defoliation levels tested; however, the production of these phagostimulants did vary by clone and season. Foliar alcohol and α-TQ concentrations and α-TQ:total alcohol ratios differed significantly among clones. Furthermore, α-TQ concentrations and α-TQ:total alcohol ratios varied temporally in some, but not all, clones. In general, foliar alcohol and α-TQ concentrations either did not vary or increased, but α-TQ:total alcohol ratios declined throughout the growing season. This research illustrates that the production of leaf surface phagostimulants is not a function of defoliation, but is most likely controlled by genetic and physiologic processes. Additionally, because *Populus* clones vary in their foliar chemistry, this variation could be exploited in tree breeding programs.

KEY WORDS *Chrysomela scripta*, resistance mechanisms, short-rotation forestry, temporal variation

TREES IN THE GENUS *Populus* (Salicales: Salicaceae) possess characteristics that make them candidates for a myriad of applications, including pulpwood and sawlog production, phytoremediation, soil water quality control, and riparian land stabilization (Dickmann and Stuart 1983, Isebrands and Karnosky 2001, Stanton et al. 2002). Commercial plantations currently occupy more than 22,000 ha in the Pacific Northwest alone (Stanton et al. 2002). However, arthropod pests and pathogens can compromise the economic viability of such plantations. The cottonwood leaf beetle, *Chrysomela scripta* F., is the most economically important defoliator of plantation *Populus* in the eastern United States (Mattson et al. 2001; Coyle et al. 2002a, 2002b). Its high reproductive capability and its multivoltine biology makes this pest a serious problem in young stands (Burkot and Benjamin 1979, Coyle et al. 1999).

Insect herbivory can elicit both indirect and direct chemical defense responses from plants (Karban and Baldwin 1997). Indirect defenses include the emission of volatile compounds from damaged leaves that attract natural enemies of the attacking herbivore (Turlings et al. 1995, De Moraes et al. 1998). Direct defenses can include morphological changes (Dalin and Björkman 2003) and elevated production of tannins and phenolics in fast growing deciduous tree species (Bryant et al. 1991, Tscharntke et al. 2001) and increased monoterpene synthesis in conifers (Litvak and Monson 1998). For example, herbivory by adult chrysomelids (*Phratora* spp.) on willows, *Salix* spp. (Salicales: Salicaceae), induced defensive changes in both leaf structure (via increased trichome densities; Dalin and Björkman 2003), as well as chemical composition (via increases in salicylate production; Ruuhola et al. 2001) of subsequently produced foliage.

Decreased leaf nutritional quality (Robison and Raffa 1997) and increases in phenolic glycoside production (Clausen et al. 1989, Picard et al. 1994, Lindroth and Kinney 1998) are common induced defenses in *Populus*. Simulated herbivory in *P. tremuloides* induced significant increases in foliar defensive compounds (Clausen et al. 1989, Osier and Lindroth 2001). Studies have shown that lepidopteran herbivory can alter *Populus* foliar chemical production,

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thus inducing resistance mechanisms (Robison and Raffa 1997, Havill and Raffa 1999). Picard et al. (1994) showed that larval *C. tremulae* herbivory induced a defensive chemical response in the foliage of *P. tremula* × *P. tremuloides* hybrids.

Populus phytochemistry can be highly variable among clones (Lindroth and Hwang 1996, Osier et al. 2000a). *Populus* clones with high tremulacin concentrations were less preferred by *C. scripta* for oviposition, whereas high amounts of salicin or salicortin did not seem to influence *C. scripta* oviposition or feeding (Bingaman and Hart 1993).

Temporal variation in *Populus* foliar chemistry also has been demonstrated. Foliar phenolic glycoside concentrations declined throughout the growing season in *P. grandidentata* (Lindroth et al. 1987), and concentrations of certain other phytochemicals decline with age in certain members of the Salicaceae (Palo 1984). The decline in foliar water and nutrient content is a general trend for most plant species (Scriber and Slansky 1981).

Recently, a suite of nonvolatile chemicals consisting of C_{22} through C_{30} alcohols and alpha-tocopherylquinone (α-TQ) was discovered on the *Populus* leaf surface (Lin et al. 1998a). α-TQ has been found in relatively low concentrations in both plant and animal tissues (Kruk and Strzalka 1995) and had not previously been reported on the leaf surface. It is known to occur in chloroplasts and has a role in photosynthesis, although this role has not yet been concretely identified (Bucke et al. 1966, Kruk and Strzalka 1995, Kruk et al. 2000). Plants produce α-TQ from α-tocopherol oxidation and from light stimulation (Kruk and Strzalka 1995). Although the exact physiological role of α-TQ in plants is still unknown, it is recognized that it plays an important role in plant metabolism (Kruk and Strzalka 1995).

Chemicals on the leaf surface are important in insect feeding ecology and host recognition, as they are the first encountered when an insect comes in contact with a leaf (Eigenbrode and Espelie 1995, Müller and Hilker 2001). Foliar lipids (Städler 1986) and alcohols (Mori 1982, Adati and Matsuda 1993) have been shown to stimulate insect feeding. *Populus* foliar chemicals also can act as phagostimulants. For instance, salicin and populin stimulated feeding by adult *C. virgintipunctata costella* (Marsuel) (Matsuda and Matsuo 1985). The aforementioned C_{22} through C_{30} alcohols and α-TQ, in specific ratios, act as phagostimulants for adult *C. scripta* (Lin et al. 1998a). The highest phagostimulation (measured as bite marks in a laboratory study) of adult *C. scripta* was observed when the α-TQ:total alcohol ratio was 1:150; feeding activity decreased when the ratios were higher or lower. Chemical amounts present in the leaf surface varied among *Populus* species and hybrids, and this variation affected adult *C. scripta* feeding preference (Lin et al. 1998b). However, it is not known whether prior defoliation or time of season influence the production of these chemicals in *Populus.*

We made three hypotheses based on previous research demonstrating seasonal and defoliation-induced changes in foliar chemistry and genetic control of these responses. First, we hypothesized that larval *C. scripta* defoliation would elicit an inducible resistance mechanism in *Populus* species and hybrids, altering the amounts of leaf surface phagostimulants produced, and thus rendering the tree less desirable for subsequent *C. scripta* feeding. Second, we hypothesized that clones would differ greatly in their phagostimulant production and that amounts in the parent and F_1 selections would be similar to those found previously (Lin et al. 1998b). Third, we hypothesized that leaf surface phagostimulant concentrations would decline throughout the growing season, a trend also seen with other foliar chemicals (Scriber and Slansky 1981, Lindroth et al. 1987).

Table 1. *Populus* clones used in the leaf surface chemistry study (modified from Coyle et al. 2001)

Clone	Generation	Source
ILL-129	Parent	Pure *P. deltoides* of southern Illinois origin
93-968	Parent	Pure *P. trichocarpa* originating in the Pacific Northwest
53-242	F_1	*P. deltoides* × *P. trichocarpa* hybrid
53-246	F_1	*P. deltoides* × *P. trichocarpa* hybrid
1130	F_2	Offspring of 53-242 × 53-246
1140	F_2	Offspring of 53-242 × 53-246
1073	F_2	Offspring of 53-242 × 53-246
1162	F_2	Offspring of 53-242 × 53-246

Materials and Methods

Insects. Adult *C. scripta* from a laboratory colony, renewed twice yearly with field-collected insects, were used to infest trees in 1998 and in generation one of 1999. Beetles were fed greenhouse-grown *Populus euramericana* variety 'Eugenei' (a *P. deltoides* × *P. nigra* hybrid) and were reared under a 24:18°C temperature regime with a photoperiod of 16:8 (L:D) h (Coyle et al. 2001). Generations 2 and 3 in 1999 used *C. scripta* collected from Eugenei border rows on the study site.

Plant Material. University of Washington pedigree family 331 clones were used in this study (Table 1; Coyle et al. 2001); molecular marker data were used to estimate the F_2 parentage (Bradshaw and Stettler 1994, H. D. Bradshaw, personal communication). Previous research on these clones showed a range of adult *C. scripta* feeding preferences (Lin et al. 1998b) and variation in larval performance (Coyle et al. 2001). The ILL-129 parent presents a high concentration of α-TQ on the leaf surface and is a preferred food source of adult *C. scripta* over the other parent, 93-968, which has nearly no α-TQ present (Lin et al. 1998b). The F_1 selections present intermediate α-TQ amounts on the leaf surface (Lin et al. 1998b) and both were preferred over either parent tree in adult *C. scripta* feeding trials. F_2 clones contain varying amounts of α-TQ, some below (1130 and 1162), some within to above (1140), and some above (1073) the preferred feeding range of adult *C. scripta* (Lin et al. 1998b).

Field Sites and Procedure. Two trials during the summer of 1998 (hereafter referred to as 1998 generations 2 and 3) were conducted concurrently with the second and third naturally occurring *C. scripta* generations observed in the field. Three trials were conducted in 1999 (hereafter referred to as 1999 generations 1, 2, and 3) in concurrence with the three natural *C. scripta* generations observed in the field. The 1998 generation 2 study was performed at the Iowa State University (ISU) College of Agriculture Farm located southwest of the ISU Institute for Physical Research and Technology, Ames, IA. This Clarion series soil had a moderate clay content mixed with loam. Previous cover was alfalfa that was killed by an application of glyphosate (Roundup, Monsanto Company, St. Louis, MO) in the fall of 1996. The field plot was 24.4 m × 23.2 m and had a row width of 3.1 m with trees planted at 1.2-m intervals. Rooted hardwood cuttings were transplanted from the greenhouse to the field in June 1997. The site was disked and kept relatively weed free throughout the growing season.

Clones were arranged in a randomized complete block, split plot design. Clone 1162 was not planted in the 1998 generation 2 trial. Each of five blocks consisted of two trees per clone for a total of 14 experimental trees, hence, a total of five trees per treatment per clone were sampled. One tree was chosen randomly to be the control and the other to receive the defoliation treatment for each clone plot per block. Two rows of Eugenei trees were used as border trees on all sides of the study. This clone is used as a standard in all *Populus* field trials at ISU.

The remainder of this study (1998 generation 3 and 1999 generations 1–3) was performed at the ISU Moore Farm north of the ISU Institute for Physical Research and Technology. In May 1998, rooted hardwood cuttings were transplanted from the greenhouse to the Spillville Loam soils on the field site. The field plot was 24.4 × 32.9 m, with 3.1-m between-row and 1.2-m within-row spacing. Each of five blocks contained eight plots, with each plot consisting of three trees of the same clone (five trees per treatment per clone). Trees were selected randomly to receive the control or defoliation treatment or serve as a substitute tree. Only in the spring of 1999, when one of the experimental or control trees used in 1998 generation 3 was dead, was an extra tree used as a replacement. Experimental and control trees were not changed after 1999 generation one to maintain experimental integrity in data collection and analysis. Trees were arranged in a randomized complete block, split-plot design. Two rows of Eugenei trees were used as borders.

Study trees from all trials were enclosed in 1.12 × 1.12 × 1.42 m cages consisting of a PCV frame and mesh hardware cloth for the duration of each summer. Trees were pruned in winter 1998 to ensure their fit in the cages the following year. Defoliation treatments were implemented by allowing mated *C. scripta* pairs to oviposit on the trees, then culling the neonates to allow only 10 individuals to survive (Coyle et al. 2001). *Chrysomela scripta* larvae were reared to pupation in sleeve cages (20 cm diameter × 50 cm) on one tree per clone × block (Coyle et al. 2001). Chemical and mechanical weed control was performed when necessary.

Chemical Extractions. Approximately 1 wk after pupae were removed from the defoliation trees, 10–12 leaves of leaf plastochron index (LPI; Larson and Isebrands 1971) 3–4 were collected from both the terminal and axial shoots on both control and defoliation trees for leaf surface chemical analysis. The LPI is a leaf numbering system whereby the newest fully expanded leaf on a terminal is termed LPI 0. Leaves are given positive numbers moving down the branch toward the stem. Therefore, LPI 3 and 4 are the fourth and fifth newest leaves on a leader, respectively. Leaves of this age are rapidly growing on the tree, and have not yet developed a waxy cuticle. LPI 5 leaves were used if sufficient LPI 3–4 material was unavailable. Foliage on the leader used to rear *C. scripta* larvae was not sampled, as we desired to measure the impact larval defoliation would have on previously uneaten foliage. These leaves were chosen because they are preferred by adult *C. scripta* for feeding (Bingaman and Hart 1992). Leaf collection was delayed 1 wk to allow new leaves, reflecting any new phytochemistry, to develop and expand on the trees. Hand contact with the leaf surface was avoided by picking leaves at the petiole. Leaves were placed in paper bags, cooled on ice, and transported to the laboratory. The procedure from Lin et al. (1998b) was used for the chemical extraction. In this procedure, leaves were dipped in hexane to isolate leaf extracts, which subsequently were dissolved in ethyl ether in hexane. Five micrograms of erucyl alcohol (*cis*-13-docosen-1-ol) (Sigma), ≈99% by capillary gas chromatograph (GC), was added to each sample as an internal standard. Samples were cleaned via solid phase extraction using a capillary column, concentrated, and redissolved in chloroform. We deviated from the procedure of Lin et al. (1998b) in that hexane rather than chloroform was used as a solvent for the leaf residues because hexane is equally effective and less damaging to the GC column. After the leaf surface chemical extraction procedure, leaf area was recorded for all leaves using a Delta T area meter (Decagon Devices, Pullman, WA). Chemical samples were concentrated to one-tenth their original volume by evaporating hexane from the sample under a stream of nitrogen gas. A 1-μl aliquot was injected into a 30-m × 0.25-mm ID, 0.25-μm film thickness, DB-5, 5% phenyl methyl silicone column (J&W Associates, Folsom, CA) in an HP 5890 series II GC. Gas flow rate for He: 1.3 ml/min; temperature program: 100°C for 1 min, then to 295°C at 10°C per min., held at 295°C for 15 min; flame ionization detector: detector temperature, 300°C, injector temperature 250°C; split ratio 100:1.

Standard Compounds. Alcohols (C_{22}-C_{30}) (Sigma-Aldrich Co., St. Louis, MO) and synthetic α-TQ were used as standards (Lin et al. 1998a). Synthetic α-TQ was prepared from ($\pm$)-α-tocopherol (Sigma) after the procedures of Issidorides (1951) and Weng and Gordon (1993) with the substitution of hexane for

Table 2. ANOVA of *Populus* response to *C. scripta* feeding for 1998 generations 2 and 3

	Source	*F*	df	*P*
1998 Generation 2	Total alcohol concentration:			
	Clone	3.86	6,32	0.0052
	Treatment	6.58	1,32	0.0152
	Clone × treatment	0.69	5,32	0.6369
	α-TQ concentration:			
	Clone	4.26	6,32	0.0029
	Treatment	0.72	1,32	0.4036
	Clone × treatment	0.84	5,32	0.5307
	α-TQ:total alcohol ratio:			
	Clone	1.14	6,32	0.3596
	Treatment	2.38	1,32	0.1327
	Clone × treatment	0.63	5,32	0.6794
1998 Generation 3	Total alcohol concentration:			
	Clone	1.38	7,35	0.2638
	Treatment	0.00	1,35	0.9457
	Clone × treatment	0.14	6,35	0.9808
	α-TQ concentration:			
	Clone	1.11	7,35	0.3937
	Treatment	0.08	1,35	0.7863
	Clone × treatment	0.14	6,35	0.9800
	α-TQ:total alcohol ratio:			
	Clone	2.84	7,35	0.0288
	Treatment	0.09	1,35	0.7702
	Clone × treatment	0.13	6,35	0.9828

Table 3. Repeated measures ANOVA of *Populus* response to *C. scripta* feeding for 1999

Source	*F*	df	*P*
Total alcohol concentrations			
Clone	7.27	7,127	<0.0001
Treatment	0.00	1,127	0.9549
Time	1.38	2,127	0.2543
Clone × Treatment	1.50	7,127	0.1721
Clone × Time	1.80	14,127	0.0456
Treatment × Time	1.41	2,127	0.2469
Clone × Treatment × Time	0.88	14,127	0.5792
α-TQ concentrations			
Clone	7.12	7,127	<0.0001
Treatment	0.01	1,127	0.9057
Time	3.06	2,127	0.0504
Clone × Treatment	2.70	7,127	0.0123
Clone × Time	2.47	14,127	0.0040
Treatment × Time	1.53	2,127	0.2207
Clone × Treatment × Time	2.08	14,127	0.0170
α-TQ:total alcohol ratio			
Clone	4.43	7,127	0.0002
Treatment	1.11	1,127	0.2951
Time	13.63	2,127	<0.0001
Clone × Treatment	1.32	7,127	0.2460
Clone × Time	6.88	14,127	<0.0001
Treatment × Time	0.66	2,127	0.5184
Clone × Treatment × Time	2.11	14,127	0.0151

chloroform as a solvent. A solution containing the internal standard and all six reference compounds at a concentration of 500 ng/μl per standard was injected into the gas chromatograph daily before sample injection. A standard curve was used to identify alcohol and α-TQ amounts in the samples. Internal standard peak areas of known concentration were used to calculate the amount of chemical present in each sample.

Statistical Analyses. Because trees from 1998 generations 2 and 3 were at different locations and were of different ages, they were analyzed separately using a two-way analysis of variance (Proc GLM, SAS Institute 2000) to determine the effects of defoliation treatment, clone, and the interaction of defoliation treatment and clone on total alcohol concentrations, α-TQ concentrations, and α-TQ:total alcohol ratios. Clone 1162 was not planted in the 1998 generation 2 trial. A repeated-measures analysis of variance (Proc Mixed, SAS Institute 2000) was used to determine the overall effects of defoliation treatment, clone, time, and all interactions among the three for 1999 generations 1–3 on total alcohol concentrations, α-TQ concentrations, and α-TQ:total alcohol ratios. Means were compared using the Tukey-Kramer *t*-test ($\alpha = 0.05$).

Results

Defoliation Effects. In 1998, only the leaf surface alcohol concentrations in generation 2 were affected significantly by the defoliation treatment (Table 2). All trees receiving the defoliation treatment had significantly lower alcohol concentrations (mean ± SE, 80.9 ± 14.5 ng/cm^2) than the controls (129.7 ± 21.6 ng/cm^2). No significant differences occurred as a result of clone × treatment interactions in 1998 generations 2 or 3 (Table 2).

Defoliation alone did not significantly affect any chemical parameter in 1999 (Table 3). Also, no significant differences occurred among the interactions between defoliation treatment and clone with respect to total alcohol concentrations and α-TQ:total alcohol ratios (Table 3). However, significantly higher (Tukey-Kramer *t*-test; $P < 0.025$) α-TQ concentrations occurred in clone 1130 control trees (mean ± SE, 6.7 ± 2.0 ng/cm^2) than in those receiving the defoliation treatment (1.9 ± 0.6) (Table 3).

Clone Effects. Total alcohol (Fig. 1A) and α-TQ concentrations (Fig. 1B) differed significantly among clones in 1998 generation 2 (Table 2). Clones differed significantly in α-TQ:total alcohol ratios in 1998 generation 3 (Table 2), with higher ratios in parent clone 93–968 than in one F_1 and three F_2 clones (Table 4).

In 1999, total alcohol concentrations (Fig. 2A), α-TQ concentrations (Fig. 2B), and α-TQ:total alcohol ratios (Fig. 2C) all differed significantly among clones (Table 3). Total alcohol concentrations were greatest in both parent clones, whereas some of the lowest concentrations occurred in the F_1s (Fig. 2A). α-TQ concentrations were highest and lowest in parent clones ILL-129 and 93–968, respectively (Fig. 2B). α-TQ:total alcohol ratios were much higher in clone 93–968 than in any other clones (Fig. 2C).

Season Effects. Time of year had a significant effect on α-TQ concentrations and α-TQ:total alcohol ratios, but not on total alcohol concentrations in 1999 (Table 3). α-TQ concentrations were greatest in 1999 generation 3 (5.45 ± 1.12 compared with 2.90 ± 0.66 and 3.89 ± 0.63 in generations 1 and 2, respectively), whereas ratios were highest in generation 1 (75.8 ± 13.7 compared with 32.5 ± 3.6 and 21.1 ± 4.1 in generations 2 and 3, respectively).

Significant interactions between clone and time occurred in alcohol concentrations, α-TQ concentra-

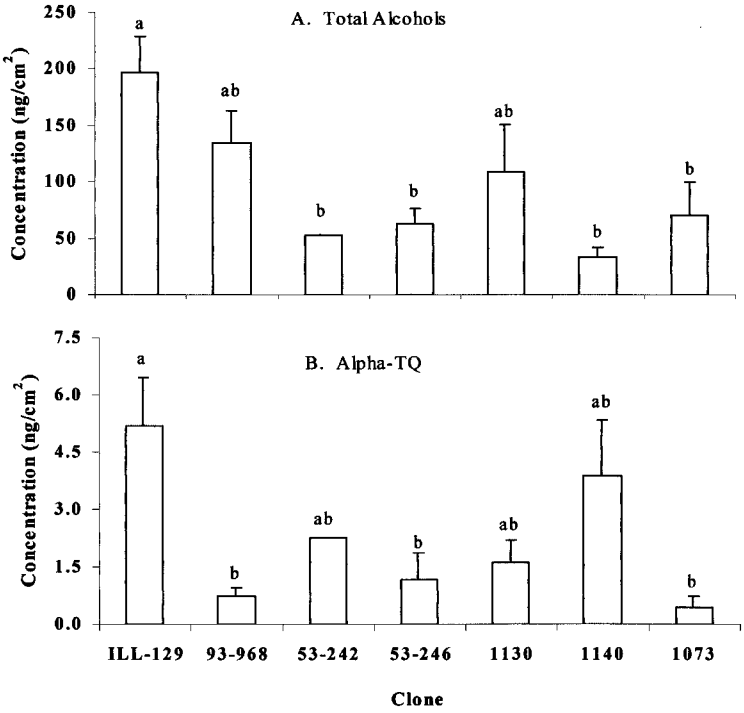

Fig. 1. 1998 generation 2 (A) total alcohols and (B) α-TQ concentrations on leaves from eight *Populus* clones in central Iowa. Means (±SE) sharing the same letter are not significantly different ($\alpha = 0.05$).

tions, and α-TQ:total alcohol ratios in 1999 (Table 3). Clones 93-968, 1130, and 1140 showed significant temporal changes in total alcohol concentrations (Fig. 3A), with concentrations in 1999 generation 2 being the lowest. Clones 1140 and 1162 both had significantly higher α-TQ concentrations in 1999 generation 3 (Fig. 3B). α-TQ:total alcohol ratios were significantly higher in 1999 generation 1 than in generations 2 or 3 in clones 93-968, 53-246, and 1130 (Fig. 3C). Defoliation treatment and time interactions were not significant in 1999 (Table 3).

Interactions between clone, defoliation treatment, and time were significant for α-TQ concentrations and α-TQ:total alcohol ratios, but not alcohols (Table 3). F_1 clone 53-242 receiving the defoliation treatment, and the F_2 clone 1073 control, had significantly higher α-TQ concentrations in 1999 generation 2 than in other 1999 generations (Fig. 4). A different pattern was evident in the F_2 clone 1130 control and the F_2 clone 1162 defoliation treatment, with highest α-TQ concentrations observed in 1999 generation 3 (Fig. 4). α-TQ concentrations in F_2 clone 1140 were highest in 1999 generation 3 in both the control and defoliation treatment (Fig. 4). Fewer individual interactions occurred in α-TQ:total alcohol ratios, although the overall trend was for ratios to decline throughout the growing season (Fig. 5). Parent clone 93-968 had the highest α-TQ:total alcohol ratios in 1999 generation 1 in both the control and defoliation treatment (Fig. 5). F_2 clone 1130 also had the highest α-TQ:total alcohol ratios in 1999 generation 1, but these ratios were not different from 1999 generation 2 in the defoliation treatment (Fig. 5).

Table 4. 1998 generation 3 α-TQ:total alcohol ratios (means ± SE) in eight *Populus* clones

Clone	α-TQ:total alcohol ratio[a]
ILL-129	48.34 ± 15.49ab
93-968	201.58 ± 60.80a
53-242	37.78 ± 13.76b
53-246	51.61 ± 19.31ab
1130	18.62 ± 12.74b
1140	27.68 ± 13.49b
1073	39.21 ± N/Ab
1162	43.37 ± 6.90ab

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$; Tukey-Kramer *t*-test).

Discussion

Defoliation Effects. The foliar phagostimulants discovered by Lin et al. (1998a) were postulated to be part of a defense induction mechanism in *Populus*, and we hypothesized that chemical amounts and ratios would be altered in response to larval *C. scripta* herbivory. However, defoliation induced significant changes in *Populus* phagostimulants in only two cases in our study. Lower total alcohol concentrations were found in trees receiving the defoliation treatment in 1998 generation 2 and lower α-TQ concentrations were observed in the clone 1130 defoliation treatment in 1999. This suggests that *C. scripta* defoliation does

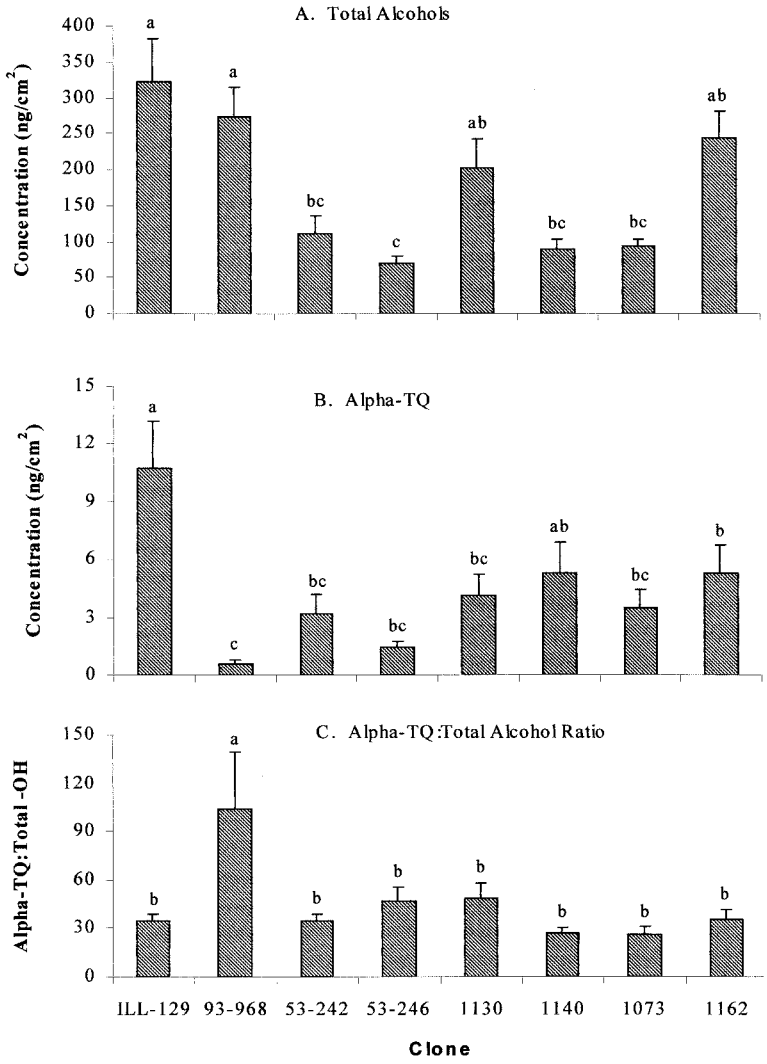

Fig. 2. (A) Total alcohol concentrations, (B) α-TQ concentrations, and (C) α-TQ:total alcohol ratios on leaves from eight *Populus* clones grown in central Iowa in 1999. Means (±SE) sharing the same letter are not significantly different ($\alpha = 0.05$).

not consistently influence leaf surface phagostimulant production.

Results from this study, coupled with those of Lin et al. (1998b) suggest that these *Populus* foliar phagostimulants are not part of a defense mechanism, but rather a naturally occurring host chemical that acts as a phagostimulant to a specialist herbivore. Many such examples exist among plants in the family Salicaceae. Salicin, a phenolic glycoside found in *Populus* and *Salix* leaves, stimulated adult *C. aeneicollis* to feed in laboratory trials (Rank 1992). Phenolic glucosides from *Salix* species stimulated feeding by three adult chrysomelids (Kolehmainen et al. 1995). Furthermore, increased feeding was observed when several phenolic glucoside extracts were combined, as compared with individual extracts, demonstrating the synergistic effects certain chemicals can have on herbivores. This parallels the relationship between foliar alcohols and α-TQ on the leaf surface of *Populus* in that the chemicals alone have little phagostimulatory activity, but when combined have great influence on the feeding behavior of *C. scripta* (Lin et al. 1998a).

Natural *C. scripta* herbivory levels on individual *Populus* trees vary greatly in nature, depending on time of season, amount of suitable foliage present for feeding, and beetle population levels. Julkunen-Tiitto et al. (1995) suggested that low levels of defoliation might not be enough to induce chemical changes in Salicaceous plants. We did not measure percent defoliation in our study, but with only 10 larvae per tree it is doubtful that we achieved 10% defoliation. These defoliation levels are within the range of those naturally encountered by *Populus;* however, our study may not have exerted enough defoliation pressure to induce measurable chemical changes. Additional stud-

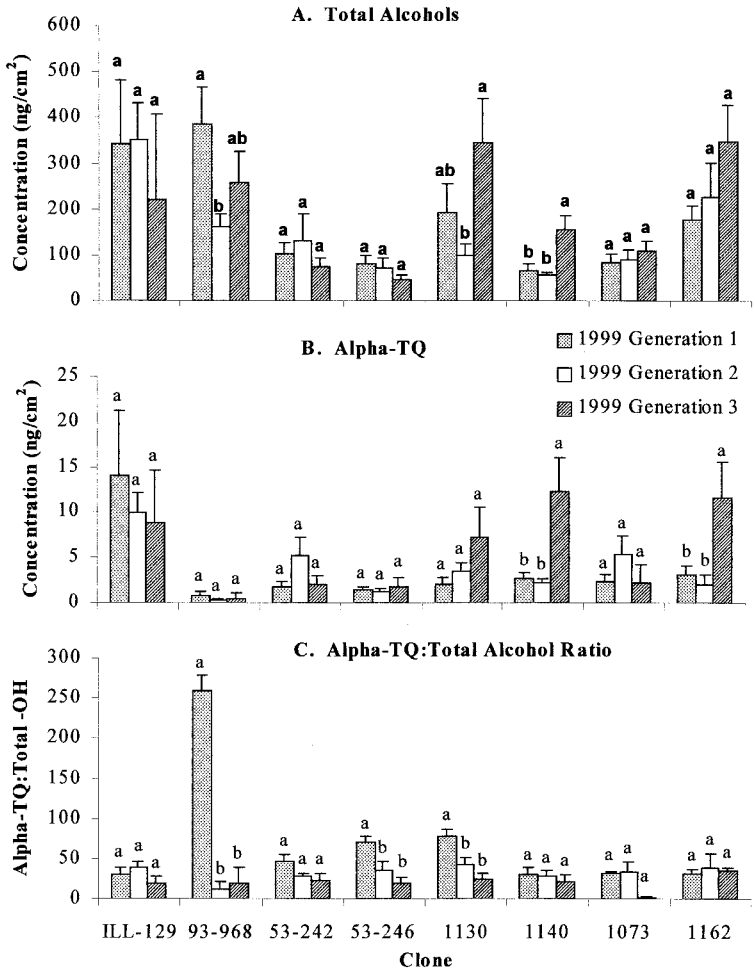

Fig. 3. (A) Total alcohol concentrations, (B) α-TQ concentrations, and (C) α-TQ:total alcohol ratios on leaves from eight *Populus* clones grown in central Iowa in 1999. Means (±SE) within a clone sharing the same letter are not significantly different ($\alpha = 0.05$).

ies to test the effects of defoliation rate may be warranted.

Clonal Effects. Prior studies have shown considerable differences in foliar chemistry among both pure species and hybrid *Populus* clones (Dickson and Larson 1976; Bingaman and Hart 1993; Robison and Raffa 1994; Lindroth and Hwang 1996; Hwang and Lindroth 1997; Gruppe et al. 1999; Osier et al. 2000a, 2000b). Bingaman and Hart (1993) and Lin et al. (1998b) examined foliar chemistry in *Populus* and found clonal variations in the quantities of chemicals present. We hypothesized that *Populus* clones would differ in foliar phagostimulant production, and our results support this hypothesis. We found significant variation in foliar chemistry among *Populus* clones on any single sample date with the exception of 1998 generation 3 (Figs. 1, 4, and 5).

One of our objectives was to compare the foliar phagostimulant production of both the parent and F_1 clones (Table 1) that also were used in a study by Lin et al. (1998b). We found total alcohol concentrations to be much lower than those reported previously, in that Lin et al. (1998b) found concentrations ranging between 1187 and 1773 ng/cm^2 in clones 93-968 and ILL-129, respectively. Total alcohol values in our study never exceeded 500 ng/cm^2 (Fig. 3). α-TQ concentrations in our study paralleled those of Lin et al. (1998b) in clones ILL-129 and 53-246, but were generally lower in clone 53-242. Also, whereas Lin et al. (1998b) reported no detection of α-TQ on clone 93-968, we found small amounts (<1.15 ng/cm^2) on that clone in all generations evaluated. With the exception of clone 93-968, lower total alcohol amounts accounted for lower α-TQ:total alcohol ratios in our study than those reported by Lin et al. (1998b). Also, with the exception of clone 93-968, ratios did not differ between clones.

The differences between our results and those of Lin et al. (1998b) may have arisen as a function of environment, time of season, or cutting source. Both studies were conducted within a few hundred meters of each other on the same field site. Our foliage was

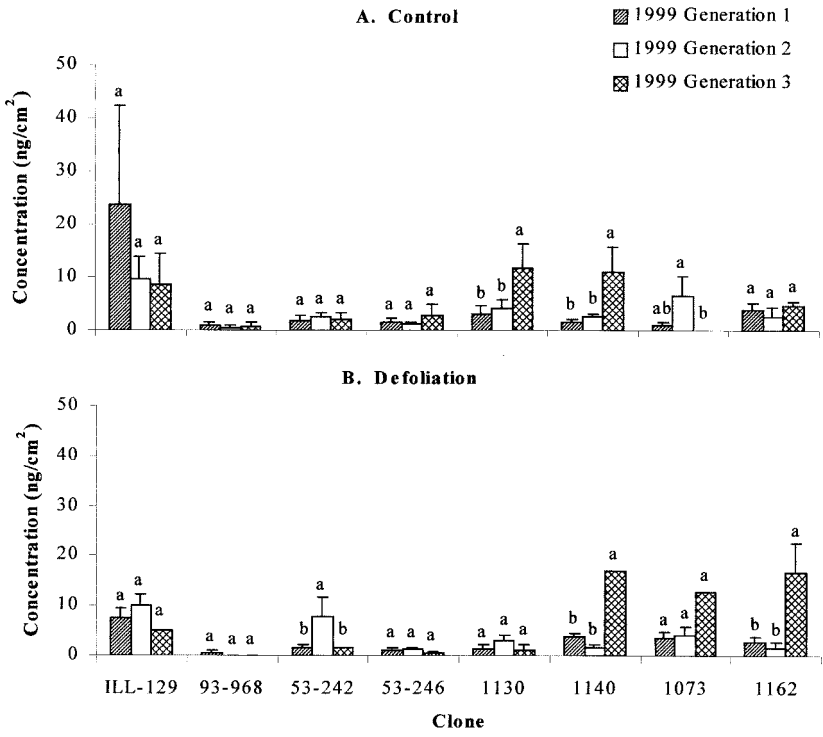

Fig. 4. α-TQ concentrations on leaves from eight *Populus* clones grown in central Iowa in 1999. (A) Control. (B) Defoliation treatment. Means (±SE) within a clone sharing the same letter are not significantly different at ($\alpha = 0.05$).

collected throughout the 1998 and 1999 growing seasons; Lin et al. (1998b) conducted their study in September 1994. Lin et al. (1998b) obtained their cuttings from greenhouse stock, whereas we obtained ours from dormant 4-yr-old trees in the field. Genetic differences among ramets within a clonal stand have been documented in *Populus*, suggesting that slight variation may exist within individual clones (Tuskan et al. 1996). Any or all of these factors may have contributed to the differences between our studies.

Seasonal Effects. Few field studies have examined temporal changes in *Populus* foliar chemistry. Seasonal decline in the foliar phenolic glycoside content of some *Populus* spp. has been reported previously (Palo 1984). Lindroth et al. (1987) found that phenolic glycoside concentrations either declined or remained constant throughout the growing season on three different *Populus* species. Depending on the individual *P. tremuloides* clone and phenolic glycoside measured, concentrations were highest at the beginning, middle, or end of the sampling period (Osier et al. 2000b). The large range of interclonal variation observed for phenolic glycosides is similar to the interclonal variation in leaf surface phagostimulant concentrations found in our study (Fig. 3).

Total alcohol amounts on clones in our study exhibited much temporal variation (Fig. 3A). Only clones ILL-129 and 53–246 followed the expected pattern of chemical decline throughout the growing season. Other clones had the highest alcohol concentrations in the beginning, middle, or end of the growing season in no predictable pattern (Fig. 3A). Overall α-TQ concentrations tended to decline throughout 1999 on parent clones, remain constant or greatest in 1999 generation 2 on F_1 clones, and increase throughout 1999 on F_2 clones (with the exception of clone 1073; Fig. 3B). Similar patterns occurred for most control clones, whereas clones receiving the defoliation treatment showed more deviation from these trends (Fig. 4). α-TQ:total alcohol ratios generally decreased throughout the 1999 growing season (Fig. 5).

It appears that environmental conditions affect the production of α-TQ, however, it is not known if environmental conditions affect *C. scripta* feeding or host plant preference. Previous research evaluated *C. scripta* larval performance on these same clones and no relationships were found between α-TQ amounts and larval performance (Coyle et al. 2001). However, general larval performance declined throughout the growing season, as did α-TQ:total alcohol ratios. These trends were not correlated, but increasing α-TQ concentrations may have altered α-TQ:total alcohol ratios enough to have affected *C. scripta* larval performance. Alternately, a general decline in foliage quality (Scriber and Slansky 1981), including water and nitrogen content, may have affected larval *C. scripta* performance (Coyle et al. 2001). Multiple choice feeding trials (using foliage from previously defoliated and nondefoliated plants)

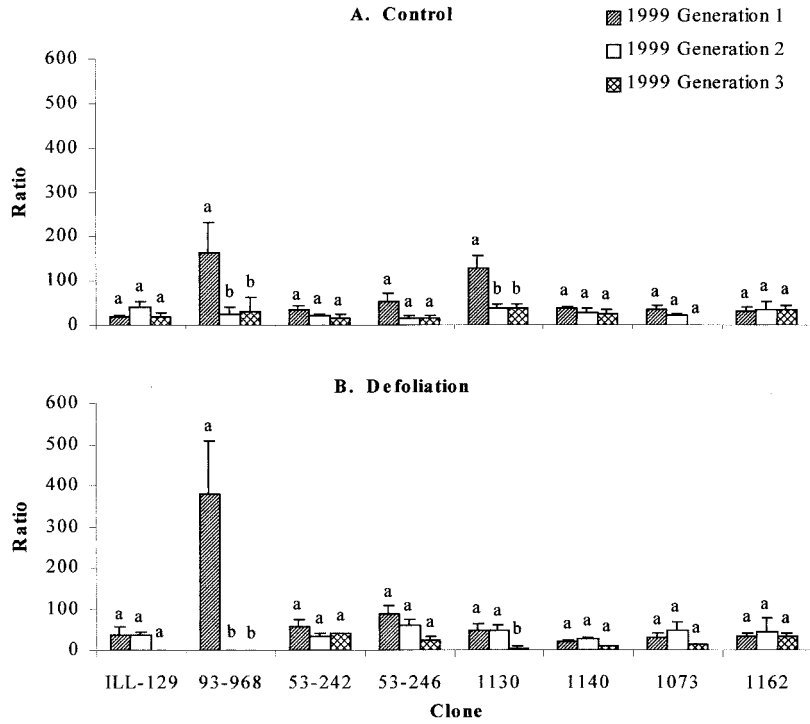

Fig. 5. α-TQ:total alcohol ratios on leaves from eight *Populus* clones grown in central Iowa in 1999. (A) Control. (B) Defoliation treatment. Means (±SE) within a clone sharing the same letter are not significantly different ($\alpha = 0.05$).

may provide some information regarding *C. scripta* feeding preference.

Conclusion

We know that adult *C. scripta* are positively affected by phagostimulants on the *Populus* leaf surface regarding their feeding behavior (Lin et al. 1998a, 1998b). This study showed that larval *C. scripta* defoliation does not consistently alter the production of these phagostimulants. We do not know whether larval *C. scripta* are stimulated to feed in the same manner as adults, nor do we know the deposition mechanisms or function of α-TQ on the leaf surface. The environment seems to play a major role in α-TQ production, yet it is not known how environmental conditions affect *C. scripta* development. Knowledge of *Populus* phagostimulant concentrations and production patterns may help identify when clones are most susceptible to *C. scripta* defoliation. Such information could be incorporated into an effective integrated pest management plan for *C. scripta* in plantation *Populus* (Coyle et al. 2002b).

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