Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae) Larval Performance on Eight *Populus* Clones

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ABSTRACT The cottonwood leaf beetle, *Chrysomela scripta* F., is the most serious defoliator of young plantation-grown *Populus* in the eastern United States, yet there is a paucity of data on larval feeding performance across *Populus* clones used in tree breeding. Field experiments were conducted in 1998 and 1999 to determine the overall feeding performance of larval *C. scripta* on 8 *Populus* selections from a pedigree family. Using a randomized complete block design, entire trees were enclosed in mesh cages, and female *C. scripta* were allowed to oviposit in sleeve cages on each tree. Larval cohorts were culled to 10 upon hatch and allowed to develop to pupation within each sleeve cage. Larval survival, pupal weight, adult emergence, and total mortality were recorded as performance parameters. Significant but inconsistent performance differences were found among clones, suggesting that some clones were less suitable for *C. scripta* larval development in some insect generations. Larval performance was generally poorer on clones with higher *P. trichocarpa* parentage. *C. scripta* performance was significantly poorer in 1999 and showed a decline throughout the 1999 growing season, but it is not clear whether this was caused by conditions of the host plants, weather patterns, or changes made in the source of insects for infestation. Clones with foliar characteristics detrimental to larval *C. scripta* performance could be used for plantings or in developing *C. scripta*-resistant hybrid *Populus* selections for short-rotation woody crop systems.

KEY WORDS *Chrysomela scripta*, *Populus*, larval performance, short-rotation forestry

TWENTY-FIVE PERCENT OF the world's wood harvest is used for the production of paper and paper products; this represents a 20-fold increase since the early 1900s (Bryant 1999). Woody biomass makes up nearly 3% of the energy used in the United States (Lazarz 2000). As efforts to develop new energy production techniques based on wood succeed, the demand for wood will increase substantially. Short-rotation woody crop systems have the potential to become an effective alternative to traditional forestry, because large amounts of wood for fiber and energy can be produced in a relatively short amount of time (Graham and Walsh 1999). Of the many species used in short-rotation woody crop systems, trees in the genus *Populus* seem to have excellent potential, especially in the north-central region of the United States (Dickmann and Stuart 1983). *Populus* selections in short-rotation woody crop systems have been shown to reduce pollution, pesticide runoff, and erosion compared with agricultural systems (Stettler et al. 1996; Zsuffa et al. 1996; Dix et al. 1997; Newman et al. 1997). On some sites, short-rotation woody crops also have the potential to be a better financial investment than some traditional agricultural crops. For instance, in the up-

per Midwest (Iowa, Minnesota, and Wisconsin) the average annual cash value of corn and soybeans in 1999 was $262.58 and $202.46 per acre, respectively (USDA 2000a, b, c, d). The average yield on *Populus* pulpwood can be up to 10 tons/acre/yr (R. J. Rousseau, Westvaco Corp., personal communication). Assuming a value of $20 per cord for *Populus* pulpwood, these yields would equate to an annual gross value of $200/acre/yr (Riemenschneider et al. 1997). Over a 10-yr rotation, this might be a better financial investment than traditional row crops. Establishment costs per acre are slightly lower in traditional field crop systems (USDA 2000b, c, d); however, over a 10-yr period, there is a much smaller total establishment cost with short-rotation woody crop systems because they do not need to be replanted each year. Thus, the yearly net financial gain per acre of short-rotation woody crops can increase as the crop matures by virtue of the unnecessary establishment costs.

Several *Populus* clones currently are being used in short-rotation woody crop plantations (Zsuffa et al. 1993). These plantations are generally established as monoclonal block plantings with different clones used in adjacent blocks (DeBell and Harrington 1994). Because of their reduced genetic variability, these types of plantings are particularly susceptible to pest outbreaks (Zsuffa et al. 1993).

The cottonwood leaf beetle, *Chrysomela scripta* F. is the most serious defoliator of young plantation *Populus* in the eastern United States (Burkot and Benjamin 1979; Harrell et al. 1981). Both larvae and adults of this

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multivoltine insect can damage *Populus.* During the establishment period (the first 1–3 yr), *Populus* trees bear a high percentage of succulent leaves throughout the growing season; this foliage is the most suitable for larval *C. scripta* consumption (Bingaman and Hart 1992). Feeding damage can result in loss of photosynthetic material, reduced growth rates, and damaged or dead terminal leaders or shoots (Kulman 1971; Bassman et al. 1982; Bingaman and Hart 1992). Reichenbacker et al. (1996) found that high levels of simulated *C. scripta* defoliation reduced *Populus* biomass production significantly during early establishment. Current control methods for the beetle include both broad-spectrum insecticides (Page and Lyon 1976; Abrahamson et al. 1977) and *Bacillus thuringiensis* (Berliner) formulations (Coyle et al. 2000).

Development of host plant resistance mechanisms for *C. scripta* control also should be a priority because trees in the genus *Populus* are easily bred, propagated, and genetically transformed (Dickmann and Stuart 1983; Klopfenstein et al. 1997). By crossbreeding and backcrossing, *Populus* hybrids can be produced that express a large range of the parent trees' traits. One such trait is susceptibility to *C. scripta.* Varied degrees of susceptibility to *C. scripta* feeding damage exist among *Populus* clones (Caldbeck et al. 1978; Harrell et al. 1981; Haugen 1985; Bingaman and Hart 1992, 1993). One goal of *Populus* breeding is to produce trees that are less desirable to *C. scripta*, yet retain good form, rooting ability, and rapid growth capabilities without increasing susceptibility to other pests.

Preceding chrysomelid preference and performance studies evaluated multiple host plant selections. Their results showed varying preference and performance depending on host plant. Larval and adult *Phratora vitellinae* L. (Coleoptera: Chrysomelidae) showed a wide range of preference and performance on *Salix* spp. (Salicales: Salicaceae) (Rowell-Rahier 1984; Rank et al. 1998). Orians et al. (1997) attained similar results using *Plagiodera versicolora* (Laicharting) (Coleoptera: Chrysomelidae). Studies using *Chrysomela* spp. on *Salix* spp. (Horton 1989; Rank 1992; 1994; Orians et al. 1997) and *Populus* spp. (Augustin et al. 1993, 1994; Floate et al. 1993) also found varying degrees of plant susceptibility and larval performance.

Previous studies have been concerned primarily with effects of foliar chemistry on adult feeding and oviposition (Bingaman and Hart 1993; Lin et al. 1998a, b), or made no distinction between adult and larval feeding when evaluating multiple clones (Oliveria and Cooper 1977, Caldbeck et al. 1978). Although adult *C. scripta* do consume more leaf tissue per insect, the larval defoliation pattern usually results in more serious damage to young *Populus* trees (Harrell et al. 1982). To our knowledge, no studies have explicitly examined *Populus* clonal effects on *C. scripta* larval performance. We hypothesized that some clones were more suitable for larval growth and development. Less suitable clones could be used immediately in short-rotation systems, or could serve as genetic contributors to other fast-growing *Populus* hybrids. Our major objective of this study was to determine if there were differences in *C. scripta* larval performance on eight *Populus* clones from a hybrid pedigree family that had been shown to influence adult *C. scripta* feeding behavior (Lin et al. 1998a; b). Significant variation in insect performance can occur both within and between years. Therefore, our secondary objective was to examine *C. scripta* larval performance among successive generations and between consecutive years.

Table 1. ***Populus* clones used in *C. scripta* larval performance study**

Clone	Generation	Source	% species composition[a]
ILL-129	Parent	Pure *P. deltoides* of southern Illinois origin	100 D
93-968	Parent	Pure *P. trichocarpa* of Pacific Northwest origin	100 T
53-242	F_1	*P. deltoides* × *P. trichocarpa* hybrid	50 D; 50 T
53-246	F_1	*P. deltoides* × *P. trichocarpa* hybrid	50 D; 50 T
1130	F_2	Offspring of 53-242 × 53-246	64 D; 36 T
1140	F_2	Offspring of 53-242 × 53-246	51 D; 49 T
1073	F_2	Offspring of 53-242 × 53-246	35 D; 65 T
1162	F_2	Offspring of 53-242 × 53-246	34 D; 66 T

[a] D, *P. deltoides*; T, *P. trichocarpa.*

Materials and Methods

Insects. Adult *C. scripta* from a laboratory colony were used in 1998 and in generation 1 of 1999. The colony was started in September 1997 from a local wild population obtained as larvae from a poplar plantation near the Ames Municipal Water Pollution Control Facility, Ames, IA, (41° 56′ 29″ N, 93° 33′ 28″ W). Beetles were reared in plastic crisper boxes (27 by 19 by 10 cm) under a constant 24 with 18°C temperature regime with a photoperiod of 16:8 (L:D) h. They were fed field-collected and greenhouse-grown *Populus* x *euramericana* variety 'Eugenei' (a *P. deltoides* x *P. nigra* hybrid) leaves of leaf plastochron index (LPI) 1–8 (Larson and Isebrands 1971). The laboratory colony was renewed each spring before the larval experiments with field-collected insects. Colony renewal was done primarily to minimize selection for laboratory-adapted insects. Generations 2 and 3 in 1999 used *C. scripta* collected from plot border trees (border trees were Eugenei) at the field site. Voucher specimens are held in the primary author's personal collection.

Plant Material. Material from the University of Washington/Washington State University Poplar Program was used in this study (Bradshaw and Stettler 1993, 1994; Table 1). Genetic composition of the F_2 clones was estimated using molecular marker data (H. D. Bradshaw, Jr., personal communication). We chose these clones because in vitro studies have shown that adult *C. scripta* prefer increased alpha-tocoph-

erylquinone (α-TQ) concentrations (Lin et al. 1998a). However, this phenomenon is not as stable in the field (Lin et al. 1998b). Lin et al. (1998b) found that 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 miniscule amounts of α-TQ present on its leaf surface. The F_1 selections, 53–242 and 53–246, present intermediate amounts of α-TQ (Lin et al. 1998a, b). Both are preferred over either parent tree in adult feeding trials. Clones 1130, 1140, 1162, and 1073 were selected from the F_2 plantings. These hybrids present varying amounts of α-TQ; some below, some within, and some above the preferred feeding range of adult *C. scripta* (Lin 1997).

Field Sites and Procedure. Experimental generations coincided with wild *C. scripta* generations on our study sites. During the 1998 *C. scripta* generations 1 and 2, study trees were not large enough to support larval defoliation. Therefore, the study was initiated coinciding with the third wild *C. scripta* generation in 1998. The 1998 generation 3 field study was performed near the Iowa State University Institute for Physical Research and Technology, Ames, IA, (42° 02′ 43″ N, 93° 39′ 40″ W). This site was primarily an alluvial floodplain of Squaw Creek. The soil was well suited for *Populus*, as it was close to the water table and maintained high moisture content. Previous ground cover (alfalfa) was killed in the fall of 1997 by an application of Roundup (Rohm and Haas, Philadelphia, PA). The field plot was 24.4 by 32.9 m. Row width was 3.1 m with trees planted at 1.2-m intervals. Trees were obtained from hardwood cuttings started in the greenhouse. Rooted hardwood cuttings were transplanted from the greenhouse to the field in May 1998. Weeds were controlled throughout both growing seasons by disking, mowing, and hand weeding.

Clones were planted in 5 blocks in a randomized complete block design. Each block consisted of 24 trees (8 clones x 3 trees per clone). Three trees of each clone were planted to ensure survival of at least one. Each clone was considered one treatment. Two rows of Eugenei trees were used as border trees around the entire study area. This clone, preferred by the cottonwood leaf beetle, is used as a standard in all *Populus* field trials at Iowa State University.

Tree cages were constructed of 18-mesh polyester screening (Balson Hercules, Pawtucket, RI) to minimize outside interference from defoliators, predators, and parasitoids, yet allow ambient weather to act as much as possible on the experimental units. Cortilet (1998) found no significant differences in plant growth resulting from this mesh. Tree cages (1.12 by 1.12 by 1.42 m) were constructed to fit over a PVC frame. Each frame was set on 2 rerod sections pushed halfway ($\approx$25 cm) into the ground for stabilization. These cages remained over two of the three trees for the duration of each growing season. From each clonal pair of caged trees, one was chosen at random as the experimental tree.

Sleeve cages (20 cm diameter by 50 cm) were placed within the tree cage on a growing terminal at the beginning and remained there for the duration of each *C. scripta* generation. Larval performance studies were initiated by placing a gravid female or mated pair of *C. scripta* into each sleeve cage. Preliminary greenhouse data had shown that female *C. scripta* would oviposit on each of the eight experimental clones used when given no choice. Adult(s) were removed after oviposition and the eggs were allowed to hatch. Larval cohorts were culled (using a forceps or metal probe) to 10 larvae per terminal. Sleeve cages were moved apically on the terminal as needed to provide the larvae with a constant supply of leaves. Larvae were allowed to develop to pupation within the sleeve cage. Pupae were collected daily and taken to the laboratory for weighing to the nearest 1×10^{-4} g on a Mettler AE 100 Analytical Balance (Mettler Instrument, Hightstown, NJ). Larval survival (from neonate to viable pupa), pupal weight, adult emergence (the percentage of viable pupae that emerged), and total mortality were recorded as performance parameters.

Trees were pruned during winter dormancy and after each *C. scripta* generation to ensure their fit inside the mesh tree cages. Trees were sprayed in November 1998 with TreeGuard deer repellent (Nortech Forest Technologies, St. Louis Park, MN) to discourage deer from eating the buds during winter. The area around the base of each tree was sprayed in April 1999 with Roundup, Goal (Rohm and Haas, Philadelphia, PA), and Pendulum (American Cyanamid, Wayne, NJ) for weed control. Care was taken to avoid herbicide contact with the trees. In 1999, PVC and mesh cages were installed over the same trees as in the 1998 generation 3. Only when one of the trees used in the 1998 generation 3 was dead was an extra tree used. Extra trees were not used after the first generation of 1999 because they were uncaged and sustained extensive feeding damage from natural *C. scripta* infestations.

Statistical Analyses. Individual generations were analyzed to determine if larval performance differences existed among clones. Generations 3 of 1998 and 1999 were analyzed together to compare the effect of year on larval performance. These two generations were conducted on the same site and trees each year. All three *C. scripta* generations in 1999 were analyzed together to evaluate seasonal changes in larval performance. All four generations were analyzed together to obtain overall performance differences among clones. Furthermore, interactions between clone and generation also were examined. All data were analyzed using the General Linear model procedure (SAS Institute 1998). Means for each clone were analyzed using the Least Squared Difference test (SAS Institute 1998). Data also were log-transformed; this resulted in no changes in significance in any of the parameters evaluated. For this reason, all values reported herein are from the General Linear model procedure.

Results

Generational Variation in Larval Performance. Significant differences were not evident in any of the

Table 2. *Chrysomela scripta* larval performance parameters (mean ± SEM) from four generations conducted in central Iowa, 1998 and 1999

Parameters	Generation[a]	Clone							
		ILL-129	93-968	53-242	53-246	1130	1140	1073	1162
Larval survival (%)	1	76 ± 18.2	54 ± 30.5	52 ± 33.5	72 ± 16.4	94 ± 8.9	74 ± 27.2	88 ± 16.4	56 ± 36.5
	2	74 ± 25.1	80 ± 23.5	88 ± 13.0	82 ± 14.8	92 ± 13.0	76 ± 32.1	76 ± 21.9	82 ± 17.9
	3	50 ± 24.5	52 ± 41.2	60 ± 15.8	84 ± 15.2	74 ± 5.5	63 ± 20.8	74 ± 23.0	54 ± 11.4
	4	26 ± 35.8	36 ± 41.0	42 ± 37.7	55 ± 48.0	84 ± 23.0	25 ± 35.4	36 ± 30.5	63 ± 55.1
	All gens.	57 ± 32.2	56 ± 37.6	61 ± 30.5	74 ± 25.9	86 ± 15.4	66 ± 30.9	69 ± 29.4	64 ± 30.3
Pupal weight (mg)	1	41.8 ± 3.6	41.8 ± 1.7	45.7 ± 5.2	41.7 ± 3.9	44.2 ± 5.4	43.4 ± 2.9	43.1 ± 2.5	41.3 ± 2.3
	2	42.7 ± 2.5	42.7 ± 7.3	46.7 ± 3.6	46.8 ± 4.3	46.6 ± 3.6	43.8 ± 2.8	41.1 ± 6.0	46.9 ± 5.2
	3	39.2 ± 3.7	41.6 ± 1.6	43.0 ± 5.2	42.4 ± 3.8	38.9 ± 4.7	40.2 ± 2.7	40.8 ± 2.6	40.3 ± 3.5
	4	37.5 ± 6.4	35.8 ± 4.4	36.8 ± 6.1	36.9 ± 5.9	36.8 ± 4.8	38.4 ± 0.0	35.9 ± 2.2	33.3 ± 2.3
	All gens.	40.8 ± 3.8	40.9 ± 5.1	43.3 ± 6.0	42.5 ± 5.2	41.6 ± 5.9	42.5 ± 3.1	40.2 ± 4.6	41.8 ± 5.6
Adult emergence[b]	1	96 ± 6.2	89 ± 14.0	97 ± 6.3	80 ± 18.6	82 ± 34.8	80 ± 44.7	88 ± 25.0	76 ± 42.7
	2	82 ± 27.8	72 ± 40.7	61 ± 33.2	66 ± 38.1	78 ± 32.5	91 ± 14.5	56 ± 40.8	52 ± 31.8
	3	59 ± 39.6	47 ± 39.6	33 ± 23.8	43 ± 37.2	30 ± 20.7	58 ± 52.0	54 ± 18.6	39 ± 30.3
	4	42 ± 58.9	0 ± 0.0	66 ± 26.9	62 ± 29.7	50 ± 38.1	60 ± N/A	63 ± 4.7	17 ± 33.4
	All gens.	74 ± 34.3	57 ± 42.7	63 ± 32.6	63 ± 32.6	60 ± 36.8	78 ± 35.8	64 ± 29.5	47 ± 38.5
Total mortality (%)	1	28 ± 14.8	52 ± 28.6	50 ± 31.6	42 ± 20.5	24 ± 33.6	46 ± 37.8	34 ± 39.7	52 ± 34.2
	2	34 ± 32.1	38 ± 36.3	46 ± 31.3	48 ± 32.7	28 ± 31.1	32 ± 30.3	64 ± 16.7	54 ± 37.1
	3	78 ± 8.4	74 ± 37.8	80 ± 17.3	68 ± 22.8	78 ± 14.8	63 ± 40.4	76 ± 13.4	72 ± 8.4
	4	90 ± 22.4	100 ± 0.0	76 ± 18.2	67 ± 34.0	52 ± 40.3	85 ± 40.3	96 ± 8.9	60 ± 34.6
	All gens.	58 ± 33.9	66 ± 36.5	63 ± 28.1	56 ± 28.0	46 ± 36.3	50 ± 35.7	68 ± 31.3	59 ± 28.8

[a] 1 = generation 3, 1998; 2 = generation 1, 1999; 3 = generation 2, 1999; 4 = generation 3, 1999; all gens. = all generations combined.
[b] Equals the percent of viable pupae.

performance parameters when individual *C. scripta* generations were analyzed. As a result, individual generation data are not addressed further.

Yearly Variation in Larval Performance. With few exceptions, all larval performance parameters were lower in generation 3, 1999 compared with generation 3, 1998 (Table 2). Larval survival differed significantly between years ($F = 10.87$; df = 1, 58; $P = 0.002$) and decreased in 1999 on all but the F_2 clone 1162 (Fig. 1A). Mean pupal weight was significantly lower in 1999 compared with 1998 ($F = 38.26$; df = 1, 45; $P < 0.001$), and was the lowest on the F_2 clones 1162 and 1073 (Fig. 1B), both having a high proportion of *P. trichocarpa* parentage. A significant decline also occurred in adult emergence in 1999 compared with 1998 ($F = 32.59$; df = 1, 58; $P < 0.001$; Fig. 1C). The greatest reduction in adult emergence occurred on 2 clones with high *P. trichocarpa* parentage, the pure *P. trichocarpa* parent clone (93–968) and the F_2 clone 1162 (66% *P. trichocarpa* parentage). Total mortality in generation 3, 1999 was significantly higher than in 1998 ($F = 32.59$; df = 1, 58; $P < 0.001$) on all clones evaluated (Fig. 1D). No significant clone x generation interactions were found among any of the parameters tested.

Seasonal Variation in Larval Performance. Overall, performance declined throughout the 1999 growing season (Table 2). Larval survival decreased steadily throughout the season ($F = 14.19$; df = 2, 88; $P < 0.001$), especially on the parent clones (Fig. 2A). F_1 and F_2 clones were split in this regard; larval performance showed a constant decline as the growing season progressed on the F_1 clone 53–242 and the F_2 clones 1140 and 1073; this decline was not apparent on the other clones. Clone also had a significant effect on larval survival throughout the season when data for the three generations were combined ($F = 2.13$; df =

7, 88; $P = 0.049$). Overall, larvae performed better on clone 1130 during the 1999 generations. Higher larval survival occurred on most clones during generation 1. Although not significant, larvae on the F_2 clones 1130 and 1162 had lower survival rates in the second generation than in the third generation. Of all the F_2 clones evaluated, these two clones contained the lowest and one of the highest percentages of *P. trichocarpa* parentage, respectively.

Overall mean pupal weight decreased steadily throughout the 1999 growing season ($F = 28.18$; df = 2, 76; $P < 0.001$; Fig. 2B). However, no differences were found in pupal weight among clones.

Adult emergence decreased significantly throughout the growing season ($F = 7.47$; df = 2, 76; $P = 0.001$; Fig. 2C), yet clonal differences were not statistically significant ($F = 1.25$; df = 7, 76; $P = 0.286$). Only *C. scripta* on the parent clones and the F_2 clone 1162 exhibited a steady decline in emergence throughout the 1999 growing season (Fig. 2C). A higher percentage of adult emergence occurred on the F_1 clone 53–242 and the F_2 clone 1073 in generation 3 compared with generation 1. Also, with the exception of clone 1162, adult emergence was lower in generation 2 on every other F_1 and F_2 clone.

Total *C. scripta* mortality increased significantly throughout the growing season ($F = 20.6$; df = 2, 88; $P < 0.001$; Fig. 2D), but clonal differences were not significant ($F = 1.28$; df = 7, 88; $P = 0.269$). No significant clone x generation interactions were evident in any of the parameters tested.

Overall Variation in Larval Performance. Larval survival differed significantly among generations ($F = 10.6$; df = 3, 104; $P < 0.001$) and clones ($F = 2.78$; df = 7, 104; $P < 0.011$). Overall larval survival was lowest on the two parent clones (Table 2). Larval survival was particularly high on clone 1130, an F_2 with a high
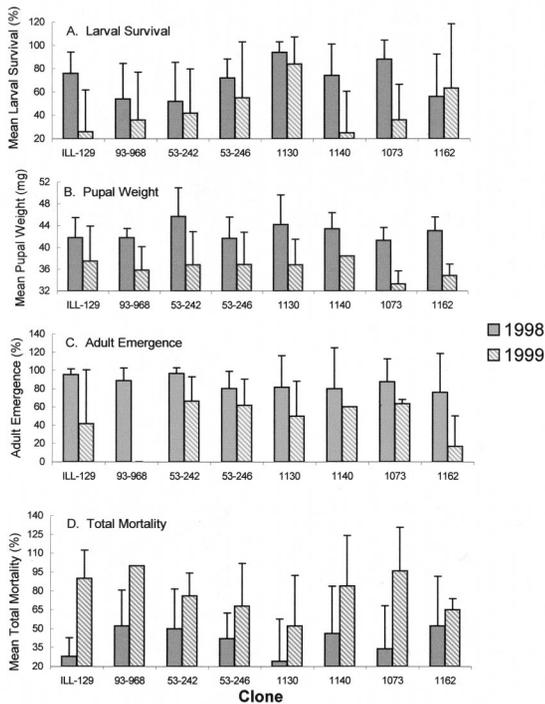
Fig. 1. Third-generation *C. scripta* larval performance parameters in 1998 compared with 1999. (A) Percentage larval survival. (B) Mean pupal weight (mg) of all pupae collected from each clone. (C) Percentage adult emergence from pupae collected. (D) Percentage total mortality (this value combines both larval and pupal mortality). Although it is understood that the actual percentage values in A, C, and D cannot exceed 100%, Y-axis labels have been extended to show true statistical SE values.

percentage of *P. deltoides* parentage. Mean pupal weight differed significantly among generation ($F = 24.51$; df = 3, 89; $P < 0.001$) but not clone ($F = 1.33$; df = 7, 89; $P = 0.245$). Adult emergence also was significantly different among generation ($F = 17.28$; df = 3, 89; $P < 0.001$). Clonal differences in adult emergence were not significant ($F = 1.35$; df = 7, 89; $P = 0.235$). Total mortality differed significantly among generations ($F = 24.84$; df = 3, 104; $P < 0.001$) but not clones ($F = 1.57$; df = 7, 104; $P = 0.151$). No significant clone x generation interactions were found among any of the parameters examined.

Discussion

Clonal Variation in *C. scripta* Larval Performance. We hypothesized that some *Populus* clones used in this study would be less suitable for *C. scripta* larval growth and development than others. An examination of individual generation data in our study did not support this hypothesis. Because there were essentially no clonal effects on larval performance in individual generations, it suggests that these effects may take >1 generation to manifest on *C. scripta.* This also suggests that there was not a strong resistance mechanism to
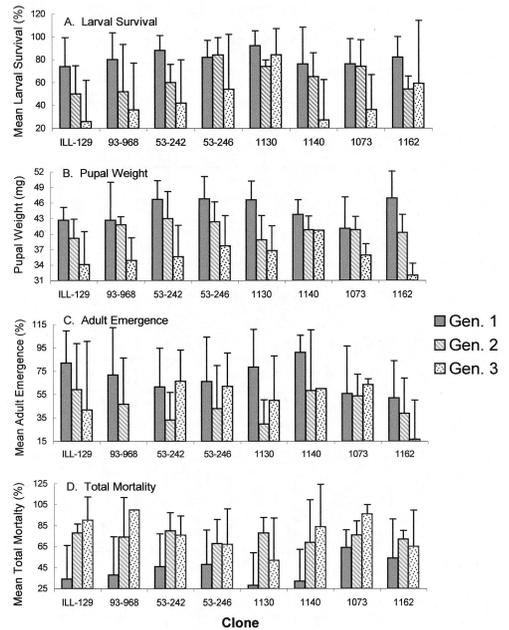

Fig. 2. *Chrysomela scripta* larval performance parameters during the 1999 growing season. (A) Percentage larval survival. (B) Mean pupal weight (mg) of all pupae collected from each clone. (C) Percentage adult emergence from pupae collected. (D) Percentage total mortality (this value combines both larval and pupal mortality). Although it is understood that the actual percentage values in A, C, and D cannot exceed 100%, Y-axis labels have been extended to show true statistical SE values.

larval *C. scripta* in the clones tested. However, when data from all generations were examined together, clonal differences in larval survival were evident. Contrary to our hypothesis, significant clonal differences in pupal weight, adult emergence, and total mortality were not found. Previous *C. scripta* performance studies have found clonal differences in larval survival and pupal weight (Augustin et al. 1993, 1994, 1997). Also, several other studies have shown adult *C. scripta* preference and performance to vary among *Populus* clones and leaf age classes (Caldbeck et al. 1978; Harrell et al. 1981; Dickmann and Stuart 1983; Bingaman and Hart 1992, 1993).

The genus *Populus* is divided into 5 sections, each containing several species. *P. deltoides* is in the section Aigeiros, and *P. trichocarpa* is in the section Tacamahaca. Sectional comparisons are often made when comparing pest resistance and other growth characteristics among *Populus* clones because trends exist between these sections. These trends between parentage and performance parameters were not as clear as expected in our study. We expected larval performance to be better on section Aigeiros clones compared with intersectional and section Tacamahaca clones. Larval performance was generally lower but not significantly different on clones with higher *P. trichocarpa* parentage; several examples occurred throughout the four generations conducted (Table 2).

Also, performance on the hybrid clones in our study was quite variable, and comparisons with the pure species clones are difficult. Caldbeck et al. (1978) and Harrell et al. (1981) found that defoliation was greater on clones with 100 or 75% section Tacamahaca parentage. Conversely, Bingaman and Hart (1992) found pure Tacamahaca clones to be the least consumed clone. However, the three aforementioned studies were choice tests, the first involving adult and larval feeding (Caldbeck et al. 1978), the second involving adult feeding only (Harrell et al. 1981), and the third involving adult feeding and oviposition (Bingaman and Hart 1992), whereas our study was not. In addition, although Aigeiros x Tacamahaca hybrids were used in the previous and current studies, the parents were different. Therefore, the individual clones used in our study cannot be compared directly with different clones used previously. Although Bingaman and Hart (1992) did not measure *C. scripta* larval performance, consumption of less leaf tissue may correspond to lower performance. However, lower consumption can also mean that there was more nutrition per unit of leaf area. Therefore, less leaf area would need to be consumed to gain adequate nutrition. Monitoring foliage consumption in this study may have provided more insight into the effects of these clones on larval *C. scripta* growth and performance.

Populus trichocarpa has desirable growth characteristics for short-rotation forestry. However, it is not native to the northcentral region of the United States. One reason larval performance on this clone was lower may be that the *C. scripta* populations in the northcentral United States are not fully adapted to this food source. Because of their susceptibility to *Septoria* canker, the *P. trichocarpa* and hybrid clones in this study would not be used in a breeding program unless it involved backcrossing to *P. deltoides* parents to gain resistance to the canker disease. However, because ILL-129 originated from a known family pedigree, we do not know if performance on this clone would be consistent with what would occur in a native *P. deltoides* stand. A study comparing *C. scripta* performance on native *P. deltoides* and *P. trichocarpa* stands is necessary.

Larval performance on clone ILL-129 (pure *P. deltoides*) varied greatly among generations. Overall performance on ILL-129 ranged from poor to average. Other studies have shown the best larval performance (Augustin et al. 1994, 1997) and heaviest defoliation (Caldbeck et al. 1978, Bingaman and Hart 1992) on section Aigeiros clones. Our results indicate that at least for specific clones, these relationships may not hold true. A wide range of performance occurred on all other clones used in our study, particularly the hybrid selections. Several studies have shown hybrid plants to be more susceptible to insect attack (Whitham 1989, Floate et al. 1993, Kruse and Raffa 1996).

Floate et al. (1993) showed higher *Chrysomela confluens* Rogers larval survival on hybrid poplars compared with 2 pure species. Furthermore, Whitham (1989) showed increased aphid populations in *Populus*

hybrid zones compared with pure species. In general, insects were much more abundant and performed better in hybrid zones. Hybrids may not possess the natural genetic resistance demonstrated in the pure species because these traits may have been lost, suppressed, or had important combinations of alleles broken up through hybridization. Conversely, hybridization also can create selections with increased pest resistance; this is one goal of selective breeding for selections to be used in short-rotation woody crop systems. A wide range of larval performance occurred on F_1 and F_2 intersectional clones in our study. With the exception of larval survival on clone 1130, we did not find any clear cases of increased or decreased larval performance on hybrid clones. Although other differences were not significant, overall larval performance on clones 1073 and 1162 was generally lower, while performance on clone 1130 was higher (Table 2). One reason for *C. scripta*'s apparent indifference to hybrid foliage may have been that parentage was estimated for the F_2 selections, thus potentially misrepresenting the actual parental composition. Parental percentage was estimated using only a sampling of the total number of genes available. Therefore, certain genes important to insect resistance may not have been sampled.

Seasonal Variation in *C. scripta* Larval Performance. Although only 1 generation for each year was analyzed, larval performance was much better on 1-yr-old trees than on 2-yr-old trees. Other chrysomelid larvae have been shown to prefer and perform better on younger trees (Brown 1956; Petrenko 1987; Kearsley and Whitham 1989); this may be the case for *C. scripta* as well. Decreased beetle performance on older trees may be a function of physiological changes as trees age, specifically, leaves on older trees having a lower water percentage and subsequently being tougher (Larsson and Ohmart 1988, Kearsley and Whitham 1989). However, all egg masses in our study were laid on leaves LPI 1–8, and after hatching most larvae migrated to and stayed near LPI 2–6, *C. scripta*'s preferred feeding range (Bingaman and Hart 1992, Augustin et al. 1997).

Changes in foliar chemistry occurred throughout the course of our study, possibly affecting larval performance (Coyle 2000). Martinsen et al. (1998) found that *Populus* spp. resprout growth (resulting from cutback stems and branches) contained increased amounts of phenolic glycosides and nitrogen. This correlated positively with increased *C. confluens* performance. We did not measure phenolic glycoside or foliar nitrogen concentrations in this study. Phenolic glycosides have been shown to negatively affect adult *C. scripta* feeding and oviposition (Bingaman and Hart 1993), yet to our knowledge no studies have examined their effect on larval *C. scripta* performance. Earlier studies using this pedigree material have shown that α-TQ and long-chain alcohol content and ratios affected adult *C. scripta* behavior; these chemicals varied among parent and offspring trees (Lin et al. 1998a, b). However, no studies have been done to measure alcohol or α-TQ content in *Populus* resprouts.

Amounts of these chemicals declined over the course of the growing season on the trees used in this study (Coyle 2000). Generation 1, 1999, took place on trees that had not experienced defoliation or artificial cutback that growing season. Subsequent damage to these trees may have played a part in the decline of insect performance during the 1999 growing season.

Insufficient moisture, elevated CO_2 levels, and light availability can induce changes in leaf chemistry that may reduce the foliage quality for insect defoliators (Horton 1989, McDonald et al. 1999). Insect herbivory also has been shown to induce chemical changes in *Populus*, thereby making foliage less suitable for subsequent herbivores (Petrenko 1987, Robison and Raffa 1997, Lindroth and Kinney 1998, Roth et al. 1998). Coyle (2000) showed changes in tree chemistry over the course of the 1999 growing season. Generation 3 larvae in 1998 fed on trees that had never been defoliated. Changes in leaf chemistry, toughness, or moisture caused by the defoliation effects of 2 prior leaf beetle generations may have attributed to the decline in 1999 generation 3 larval performance.

In contrast, herbivory has been shown to increase the susceptibility of hybrid poplars to disease (Klepzig et al. 1997). Also, trees infected with disease may have altered nutrient or energy allocation to help control the disease. Trees in this study were subjected to repeated defoliation, and several diseases may have affected larval performance. Infestations of Septoria leaf spot, *Septoria musiva* Peck (Deuteromycotina: Coleomycetes), and *Melampsora* spp. leaf rust were found on both sites. These diseases occurred primarily on older leaves (Coyle, unpublished data) and partially defoliated some trees. Disease did not affect the larvae directly, as larval *C. scripta* prefer to feed on young leaves (Bingaman and Hart 1992). In no treatment were larvae observed consuming foliage with visible signs of disease. We did not quantify the impact, if any, these diseases had on larval performance.

We cannot rule out the possibility of change in egg viability and subsequent larval performance resulting from using different sources of adult *C. scripta* in our study. Food availability, exposure to disease, and temperature variation may stress adult beetles, subsequently causing a reduction in resource allocation to reproduction and oviposition. The *C. scripta* generation in 1998 and generation 1, 1999 used offspring from a laboratory colony that was renewed each spring and fall with field-collected insects. These factors may explain why, in general, larval performance in these generations was better than in the latter two generations of 1999 that used offspring of wild adults.

Overall Variation in *C. scripta* Larval Performance. The clones tested did not exhibit strong resistance mechanisms to larval *C. Scripta* because survival was >55% for all clones. Only larval survival was significantly different among clones, and was highest on the hybrid clone 1130. Coyle et al. (1999) showed that larval survival and adult longevity may be the most dependable indicators of potential fecundity for multivoltine leaf feeding beetles. The poorest larval performance in 2 previous studies occurred on a pure

section Aigeiros species (Augustin et al. 1994, 1997). Our study did not find any significant reduction in larval performance on pure species compared with intersectional hybrids.

General Conclusions. Multiple factors, including cultural treatment, understanding the fundamental mechanisms of production, and pest control, govern the advancement of short-rotation woody crop system production. *C. scripta* is one of the most severe defoliators of plantation *Populus;* currently, only chemical means are known to provide adequate control. Creating fast-growing pest-tolerant selections is an important aspect in the advancement of short-rotation woody crop systems. Several clones, particularly those in the *Populus* section, are known to be resistant to *C. scripta* damage (Caldbeck et al. 1978, Bingaman and Hart 1992). The potential exists to combine the pest-resistant traits of certain *Populus* species with the growth capabilities of others to create selections optimal for use in short-rotation woody crop systems. A combination of pest-resistant clones subsequently planted in clonal mosaics or other deployment strategies (Zsuffa et al. 1993) may control *C. scripta* in plantation *Populus* to sub-economic thresholds.

More research needs to be conducted on the factors affecting pest resistance in hybrid poplars to support efforts on improving the host plant's insect resistance mechanisms. Laboratory or greenhouse studies are needed to further investigate the mechanisms that drive host-plant resistance, as well as insect responses to these factors. Furthermore, field studies are the most accurate in predicting what will happen when pest-resistant clones are deployed for large-scale use. Studies examining the interactions between defoliators, natural enemies, and plantation *Populus* in large-scale field settings are needed.

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