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Relative resistance of willow and poplar biomass production clones across a continuum of herbivorous insect specialization: Univariate and multivariate approaches

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Abstract

Short-rotation woody crops (SRWC) are being developed as a sustainable system that simultaneously produces a renewable feedstock for bioenergy and bioproducts and a suite of environmental and rural development benefits. However, damage from defoliating insects can significantly reduce the yield of SRWC and negatively impact their sustainability. Information regarding the relative resistance to defoliation of different SRWC clones is not only useful for deployment and breeding choices, but can also help elucidate ecological patterns of herbivore specialization. Laboratory feeding bioassays tested the resistance of 19 willow (*Salix* spp.) and six poplar (*Populus* spp.) biomass production clones to feeding by seven common folivorous insects. Defoliation was measured using a leaf area meter and results were standardized according to leaf area consumed per insect per day. Significant differences in resistance were found among clones ($p \leq .05$). The most folivore-resistant groups included the six poplar clones and willow clones SH3, SP3, S546 and S625. Willow clones with *S. eriocephala* or *S. dasyclados* parentage were generally less resistant to herbivory than those with other parentages. Comparisons of univariate and multivariate approaches found that the multivariate techniques are robust and conservative, and provide an efficient means of screening a large number of clones in a development program. The multivariate approach provided a clearer sorting of folivores along a continuum of insect specialization. Such sorting may be useful in selecting model folivores to use in tree genetic improvement screening systems to efficiently reveal which clones are more likely to be resistant to multiple insect pests.

Keywords: *Salix*; *Populus*; Short rotation woody crops

1. Introduction

Concerns about national energy security, environmental impacts associated with the use of fossil fuels,

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sustainability of natural resources, increasing demand for biodegradable products, and a need to revitalize rural economies have made the development of biomass for bioproducts and bioenergy a priority (National Research Council, 2000). Biomass can come from a number of sources including forests, agricultural crops, various residue streams, and short-rotation woody crops (SRWC). Interest in the development of SRWC has grown over the past few decades in Europe, North America and elsewhere because of the multiple environmental and rural development benefits associated with their production and use (Abrahamson et al., 1998; Börjesson, 1999). Willows (*Salix* spp.) and poplars (*Populus* spp.) grown at densities of 10,000–20,000 plants ha^{-1} are appealing as SRWC because of the high yields that can be obtained in just a few years, ease of propagation from dormant hardwood cuttings, broad genetic bases and ease of breeding, and ability to resprout (coppice) after multiple harvests (Christersson et al., 1993; Mitchell, 1995; Volk et al., 1999; Tharakan et al., 2005).

SRWC biomass crops are being developed around the world as sustainable systems that simultaneously produce a renewable feedstock for bioenergy and bioproducts and a suite of environmental and rural development benefits. Damage from defoliating insects, however, can significantly reduce the yield of SRWC (Kendall and Wiltshire, 1998; Peacock et al., 2002) and negatively impact their sustainability (Volk et al., 2004). Since susceptibility to insect damage varies widely among clones (Robison and Raffa, 1994, 1997; Kendall et al., 1996; Caldebeck et al., 1978; Bingaman and Hart, 1992, 1993; Peacock et al., 2002; Hodkinson et al., 1998), knowledge of their pest resistance can be used to make selections for breeding programs and large-scale deployment (Schipper, 1976; Meyers et al., 1976; Robison and Raffa, 1998). Establishing large areas of SRWC with limited genetic diversity may increase the risk of catastrophic loss due to pests (DeBell and Harrington, 1993; Robison, 2002). Planting insect pest-resistant clones, or a mixture of clones, may lead to reduced pest damage and pesticide use, lowering both operating costs and detrimental environmental effects while maintaining high biomass production. It may also decrease the risk of pest biotype evolution (Raffa, 1989).

The study's three objectives were to characterize the relative resistance of 19 willow and 6 poplar clones to 7 common insect defoliators, to investigate common feeding trends among insect pests along a continuum from specialist to generalist for the selection of model folivores, and to compare the utility of univariate and multivariate approaches for this type of characterization. Trials of high-density willow and poplar biomass production have been conducted in the northeastern United States since the mid 1980s (Volk et al., 1999) and Europe since the mid 1970s (Verwijst, 2001). Over 400 different willow and poplar clones, including those of North American, European and Asian origin, have been tested in replicated trials and several hundred other clones are being screened (Kopp et al., 2001).

The relative insect resistance of most of these clones, however, is unclear. All of the 25 clones tested in this study had previously been evaluated for production potential in the northeastern United States, and ranked high in this regard. The willow and poplar clones selected for this study varied in parentage and origin. Most of the willow clones were either pure or hybrids of the North American *Salix eriocephala* Michx. (diamond willow), but the study also included several clones of European willows (*S. purpurea* L.(purpleosier willow), *S. alba* L. (white willow), *S. dasyclados* Wimm), and another North American species (*S. discolor* Muhl. (pussy willow)). Poplar clones included pure species and hybrids of the North American *Populus deltoides* Bartr. ex Marsh. (eastern cottonwood), a European species, *P. nigra* L. (Lombardy poplar), and *P. maximowiczii* Henry (Japanese poplar). The insect species tested represented a suite of common species found in central New York and represented a variety of feeding guilds and specializations. Most of these insect species are native to New York State, though two have been introduced from Europe and Asia.

2. Materials and methods

Nineteen willow and six poplar clones with potential for commercial biomass production (Table 1) were assayed for resistance to generalist and specialist defoliators of willow and poplar in central New York (Table 2). The assays were also used to characterize the insect species along a continuum of herbivory from specialist to generalist with the goal of

Table 1
Nineteen willow and six poplar clones used in laboratory insect feeding bioassays

Clone	Parentage female × male
S19	*Salix eriocephala* × *eriocephala*
S185	*S. eriocephala* × *eriocephala*
S25	*S. eriocephala* × *eriocephala*
S287	*S. eriocephala*
S301	*S. interior* × *eriocephala*
S34	*S. eriocephala* × *eriocephala*
S365	*S. discolor*
S546	*S. eriocephala* × *eriocephala*
S557	*S. eriocephala* × *eriocephala*
S566	*S. eriocephala* × *eriocephala*
S599	*S. eriocephala* × *petiolaris*
S625	*S. eriocephala* × *interior*
S646	*S. eriocephala* × *eriocephala*
S652	*S. eriocephala* × *eriocephala*
S71	*S. petiolaris* × *eriocephala*
SA2	*S. alba*
SH3	*S. purpurea*
SP3	*S. purpurea*
SV1	*S. dasyclados*
DN74	*Populus deltoides* × *nigra* (“*P. euroamericana* cv. Stormont”)
NE299	*P. nigra* “Betulifera” × *trichocarpa*
NM5	*P. nigra* × *maximowiczii*
NM6	*P. nigra* × *maximowiczii*
Siouxland	*P. deltoides*
WIS5	*P. deltoides* × *nigra*

selecting model folivores for the efficient screening of clones. Clones were classified as resistant if the insect species refrained from feeding on them, compared with feeding on other clones. For the purposes of this study, specialist herbivores are defined as those species feeding on only one plant family. Generalists are those that feed on more than one plant family (Janz et al., 2001).

Leaf material for feeding assays was collected from field plants located at the State University of New York College of Environmental Science and Forestry’s (SUNY-ESF) Genetic Field Station at Tully, NY, 25 km south of Syracuse (42°47′30″N, 76°07′30″W). The soil at this site is a productive, well-drained to somewhat excessively well-drained Palmyra gravelly silt loam (Glossoboric Hapludalf) with slope of 0–3% (Hutton and Rice, 1977). Four branch tips, approximately 20 cm long, with little or no prior feeding were cut from each of the 25 clones immediately prior to bioassays, during July and August 1997. Once cut, the bottoms of the branch tips were immersed in water, placed in a cooler for not more than 4 h to keep the foliage fresh, and then were prepared for the bioassays immediately or stored overnight in a refrigerator at 10 °C for use the next day. Five centimeters of stem were trimmed and the lower leaves were removed from the bottom of the branch tips resulting in a branch tip with 7.5 cm of leaf material at the top and 7.5 cm of bare stem on the bottom. The stem was then inserted through a cover of parafilm into a vial of distilled water. The branch tip and vial were laid down inside a clear plastic box (21 cm × 7 cm × 6 cm) lined with a moist paper towel, which helped maintain a high relative humidity inside the box (Robison and Raffa, 1994).

Table 2
Insects used in laboratory foliage feeding bioassays with 19 willow and 6 poplar clones, grouped by host feeding specialization

	Size of life stage used in assay	Abbreviations used in tables
Generalists (insects that feed on more than one plant family)		
Nymphalis antiopa L. (Lepidoptera: Nymphalidae)	Larva, 50 mm[a]	NA
Popillia japonica Newman (Coleoptera: Scarabaeidae)	Adult, 12.5 mm[b]	PJ
Specialists (insects that feed only on Salicaceae)		
Nematus ventralis Say (Hymenoptera: Tenthredinidae)	Larva, 18 mm[b]	NV
Nematus salicisodoratus Dyar (Hymenoptera: Tenthredinidae)	Larva, 15 mm[c]	NS
Plagiodera versicolora (Laicharteg) (Coleoptera: Chrysomelidae)	Adult, 4 mm[d]	PV
Polydrusus impressifrons (Gyllenhall) (Coleoptera: Curculionidae)	Adult, 7 mm[c]	PI
Crepidodera nana Say (Coleoptera: Chrysomelidae)	Adult, 2.8 mm[e]	CN

[a] Johnson and Lyon (1991).
[b] Drooz (1985).
[c] Baker (1972).
[d] Wade and Breden (1986).
[e] Parry (1986).

Because of the different sizes (Table 2) and feeding rates of the various species, each assay differed in the number of insects used and the duration of feeding (see ''Specifics for Each Assay'' below). Insects were placed inside the box and the cover was closed to prevent escape and maintain humidity. Fluorescent lights maintained 16 h of light and the ambient (room) temperature ranged from 25 to 29 °C during the assays. Foliage from each clone tested was replicated three times (three boxes) with each insect species in a randomized complete block design. All replications were carried out simultaneously. The number of replications was limited because of the large number of clones being assayed. The same 25 clones were tested with each of the seven insect species (Table 2), except for *Nymphalis antiopa* L., for which willow clone S301 was not assayed due to a foliage collection error. The insects were allowed to feed until at least 10% of the leaf material was consumed, but the assays were stopped before leaves visibly lost turgor.

At each assay's conclusion, insect survival was noted and insects were either removed immediately or the entire box was placed inside a freezer. Only leaves with feeding damage were measured for leaf area consumption. The leaves were cut from the stem with a scalpel and taped to clear acetate sheets. Each sheet was labeled according to insect species, clone, replicate, and date. The acetate sheets with the leaves were photocopied onto white paper to provide a permanent record of the experiment. These sheets were then photocopied back on to acetate.

The remaining leaf area (cm^2) on each acetate sheet was measured with a Li-Cor 3100 area meter and recorded. Consumed portions of the leaf missing from the acetate copy were filled in using a black permanent marker to recreate the size and shape of the original, undamaged leaf. These leaf shapes are very predictable and regular for each clone. The acetates of reconstructed leaves were measured with the area meter and the difference between the two measurements represented the leaf area consumed. The amount of leaf material eaten was standardized to the area consumed (cm^2) per insect per day ($insect^{-1}\ d^{-1}$).

2.1. Specifics for each assay

All insects except the *Nymphalis antiopa* (see below) were collected one species at a time from a mix of the 19 willow clones used in the current study, at the Genetic Field Station in Tully, NY, throughout the summer of 1997. Insects were not collected from field-grown poplars because of the relatively low insect numbers on these plants. The insects were collected using a sweep net or hand picked off plants, and transported to the laboratory in a cooler. When the insects were captured with the sweep net, samples were sorted inside a cold room (0 °C).

Twelve *Crepidodera nana* Say adults were placed in each assay box. Because the tiny insect causes little leaf damage, the assay ran for 3.0 days (d) (72 h (h)). Five each of *Polydrusus impressifrons* (Gyllenhall) adults, *Nematus ventralis* Say late-instar larvae and *Nematus salicisodoratus* Dyar late-instar larvae were used in each box in their respective assays. The *Polydrusus impressifrons* assay lasted 2.79 d (67 h), the *N. ventralis* assay lasted 1.44 d (34.5 h) and the *N. salicisodoratus* assay lasted 2.0 d (48 h). Four *Plagiodera versicolora* (Laicharteg) adults per box were allowed to feed for 3.06 d (73.5 h), while three *Popillia japonica* Newman adults per box were allowed to feed for 1.69 d (40.5 h).

A feeding colony of 132 late-instar *Nymphalis antiopa* larvae was found on willow clone SA2 in the field. There were not enough larvae for two to be placed in each box for all three replications of the 24 clones (S301 was not included). Two whole replications and half of the third received two larvae in each box, while the other half of the third replication received one larva. The larvae fed for 1.0 d (24 h).

2.2. Data analysis

Mean leaf areas consumed $insect^{-1}\ d^{-1}$ among clones were compared using univariate ANOVA by each insect species, and when significant differences were found, means were separated by LSD. The Cochran *C*-statistic (StatSoft Inc., 1995) was used to test for homogeneity of variance for each of the bioassays, and where necessary, data were log transformed (Table 3). For each assay, the clones were ranked by mean leaf area consumed $insect^{-1}\ d^{-1}$. These ranks were tested for correlations with the other assays (Table 4).

Two distinct and sequential multivariate cluster analysis methods were also used to separate the clones into groups based on their relative insect resistance

Table 3
Mean (±S.E.) leaf area consumed ($cm^2\ insect^{-1}\ d^{-1}$) in laboratory no-choice bioassays for each clone-insect combination

Clone[a]	Insect species tested						
	PJ[b]	NA	NV[b]	NS	PI	PV[b]	CN
d.f.; *F*	24; 4.83	23; 2.57	24; 3.90	24; 2.51	24; 2.28	24; 1.78	24; 1.82
p-value	<.05	<.05	<.05	<.05	<.05	<.05	<.05
NE299	.00 ± .00 a	1.78 ± .91 ab	.77 ± .73 a	.01 ± .01 a	.01 ± .01 a	.08 ± .05 abc	.00 ± .00 ab
NM6	.00 ± .00 a	1.36 ± 1.36 a	1.12 ± .30 ab	.02 ± .02 a	.04 ± .03 a	.02 ± .02 ab	.00 ± .00 a
DN74	.03 ± .03 a	5.13 ± .97 abcd	2.82 ± .66 abcde	.02 ± .02 a	.06 ± .03 abc	.10 ± .10 abc	.03 ± .03 abcd
NM5	.04 ± .04 a	.81 ± .32 a	2.23 ± .40 abcd	.03 ± .03 a	.03 ± .02 a	.00 ± .00 a	.04 ± .02 abc
WIS5	.05 ± .05 a	2.50 ± 1.28 abc	1.62 ± .84 abcd	.54 ± .10 abcd	.00 ± .00 a	.04 ± .02 ab	.02 ± .02 ab
SIOUX	.06 ± .06 a	4.91 ± 1.17 abcd	4.19 ± .65 cdefghi	.54 ± .20 abcd	.17 ± .09 bcdef	.15 ± .09 abc	.02 ± .00 ab
SP3	.27 ± .16 b	1.96 ± .46 ab	1.58 ± .34 abc	.11 ± .03 ab	.12 ± .02 abcdef	.10 ± .01 abc	.09 ± .04 bcdefg
SA2	.58 ± .10 b	9.45 ± 1.44 de	5.53 ± .44 fghi	.76 ± .35 abcde	.02 ± .02 a	.22 ± .08 cd	.02 ± .01 ab
SH3	1.16 ± .85 bcd	.88 ± .55 a	1.61 ± .14 abcd	.07 ± .04 ab	.07 ± .06 abc	.05 ± .03 ab	.00 ± .00 a
S625	1.51 ± .18 bcde	5.32 ± 1.72 abcde	4.22 ± .17 defghi	1.21 ± .58 def	.21 ± .06 f	.00 ± .00 a	.08 ± .05 abcdef
S546	1.62 ± .40 bcdef	7.69 ± 5.05 bcde	3.65 ± .38 bcdefgh	.39 ± .32 abcd	.05 ± .02 def	.06 ± .04 abc	.02 ± .02 ab
S599	1.84 ± .30 bcdefg	11.48 ± 1.67 e	6.05 ± .21 ghi	.59 ± .33 abcd	.20 ± .04 ab	.13 ± .09 abc	.02 ± .02 ab
S71	2.26 ± .41 defgh	9.22 ± 1.01 de	6.59 ± 2.45 I	.32 ± .17 abc	.09 ± .03 abcde	.15 ± .12 abc	.12 ± .04 defg
S365	2.66 ± .33 efgh	8.89 ± 2.29 de	3.15 ± 1.19 abcdef	1.74 ± .28 f	.09 ± .03 abcde	.17 ± .02 bcd	.05 ± .02 abcd
S652	2.69 +.25 efgh	8.59 ± 1.78 cde	4.20 ± .77 cdefghi	.12 ± .11 ab	.17 ± .09 cdef	.07 ± .07 abc	.17 ± .03 g
S19	2.73 ± .33 efgh	7.53 ± 1.67 bcde	5.22 ± .67 efghi	.81 ± .36 abcde	.21 ± .07 ef	.09 ± .03 abc	.12 ± .02 defg
S646	2.86 ± .77 efgh	10.39 ± 3.40 de	5.44 ± .18 efghi	.38 ± .30 abcd	.15 ± .06 def	.13 ± .06 abc	.07 ± .05 abcdef
S301	2.94 ± .34 efgh	N/A	3.42 ± .88 bcdefg	.51 ± .30 abcd	.06 ± .04 abc	.14 ± .04 abc	.02 ± .02 ab
S34	3.36 ± .63 h	8.71 ± .91 de	5.79 ± 1.71 ghi	.82 ± .03 abcde	.08 ± .02 abcd	.04 ± .04 abc	.15 ± .06 efg
S566	3.46 ± .04 h	7.90 ± 3.40 bcde	5.18 ± 1.26 efghi	.75 ± .30 abcd	.05 ± .03 ab	.19 ± .05 bcd	.06 ± .06 abcdef
S557	3.79 ± 2.32 defgh	6.56 ± 4.07 abcde	6.05 ± 1.05 ghi	.34 ± .34 abc	.07 ± .01 abc	.15 ± .08 abc	.05 ± .03 abcd
S185	3.99 ± .80 h	10.63 ± 1.96 de	5.53 ± 1.53 fghi	1.04 ± .37 cdef	.05 ± .05 ab	.17 ± .15 abc	.06 ± .02 abcde
S287	4.04 ± .51 h	9.42 ± 1.33 de	5.16 ± .57 efghi	.88 ± .73 bcde	.01 ± .01 a	.01 ± .01 a	.04 ± .01 abcd
S25	5.17 ± 1.93 gh	9.35 ± 3.19 de	6.31 ± .91 i	.38 ± .30 abc	.11 ± .03 abcdef	.02 ± .02 ab	.16 ± .10 fg
SV1	5.35 ± 1.50 h	10.24 ± 1.91 de	2.00 ± .48 abcd	1.42 ± .11 ef	.07 ± .03 abc	.37 ± .10 d	.03 ± .01 ab

Means with same letter within a column not significantly different ($p \leq 0.05$; LSD test). See Table 2 for species abbreviations.

[a] Clones are listed in ascending order using the mean leaf area consumed by *P. japonica*.

[b] *P. japonica*, *Ne. ventralis* and *Pl. versicolora* LSD tests performed on log transformed data.

Table 4
Correlation coefficients among insect species for feeding assays on 24 willow and poplar clones

Insect species[a]	NA	NV	NS	PI	PV	CN
P. japonica (PJ)	.73*	.57*	.52*	.10	.33	.48*
N. antiopa (NA)	.78*	.56*	.17	.48*	.40	
Ne. ventralis (NV)	.26	.28	.13	.54*		
Ne. salisodoratus (NS)	.10	.45*	.06			
Po. impressifrons (PI)	−.10	.45*				
Pl. versicolora (PV)	−.15					

S301 not included.
[a] See Table 2 for insect species abbreviations.
* Indicates significant correlation ($p \leq .05$).

and to group insects by their feeding preferences. Because of the 10-fold difference in leaf area consumption between the large and small insects, the leaf area measurements for these analyses were standardized. For each of the seven insect feeding bioassays, the mean consumption of each clone was converted to a proportion of the total leaf area across all the clones consumed by that insect.

The unweighted pair-group average (UPGA) method was used to show graphically the resistance relationships among clones or insects (StatSoft Inc., 1995). The presence of a plateau on the linkage distance plot indicates that many clusters are formed at approximately the same linkage distance. This cutoff distance is then applied to a tree diagram to identify the number of clusters and the cluster members.

The UPGA results were then used to initiate a *k*-means clustering method to explore similarities among clones and insects. In this approach the user forces the clones or insects into a predetermined number of clusters, in this case the number identified in the UPGA analysis (StatSoft Inc., 1995). The two clustering methods act as a check on one another.

The cluster analysis methods used here do give equal weight to each insect species defoliation. The first objective of this study was to characterize resistance to defoliation by herbivorous insects. Since we are not attempting to model or estimate defoliation in the field by large populations of insects and since reliable data on the impact of these insects on willow in the field does not exist, there is no basis for weighting the insect defoliation based on the amount consumed by each species.

3. Results

3.1. Univariate ranking of relative clone resistance (ANOVA)

The 25 clones exhibited a wide range of differences in the leaf area consumed ($cm^2\ insect^{-1}\ d^{-1}$) among the seven insects tested (Table 3). The large late-instar *Nymphalis antiopa* larvae consumed the most leaf material of any insect tested, more than 10 $cm^2\ insect^{-1}\ d^{-1}$ from the most susceptible clones (S599, S646, S185, and SV1) (Table 3). These caterpillars ate more than 10 times the leaf area of the most susceptible clone (S599) compared to the most resistant clone (NM5). *Popillia japonica* adults refrained from feeding on all six poplar clones while consuming substantial amounts of willow leaf material. The *Nematus salicisodoratus* larvae consumed considerably less foliage than the similarly sized *Nematus ventralis* larvae, less than 2 $cm^2\ insect^{-1}\ d^{-1}$ for even the most susceptible clone. The adults of *Plagiodera versicolora*, *C. nana* and *Polydrusus impressifrons* all consumed small amounts of foliage, generally less than .2 $cm^2\ insect^{-1}\ d^{-1}$.

While the insects generally fed less on the six poplar clones as compared to the willow clones, the ''Siouxland'' cottonwood clone (*P. deltoides*) was preferred over other poplar clones by the two *Nematus* sawfly larvae and the adults of *Plagiodera versicolora* and *Polydrusus impressifrons*. Among the willow clones, SV1, S185, and S25 were the most susceptible to feeding by all insects except for the *C. nana* and *Polydrusus impressifrons* adults. SH3 and SP3, both *S. purpurea* clones, were typically the most resistant willow clones in the assays. No willow or poplar clone was completely immune to all the insects tested.

3.2. Correlations among insects

The feeding patterns of adult *Popillia japonica* and *Nymphalis antiopa* larvae, the two generalist herbivores tested (Johnson and Lyon, 1991; Janz et al., 2001), were significantly correlated ($r = .73$) (Table 4). The extent of feeding by both the *Nematus ventralis* and the *Nematus salicisodoratus* larvae were significantly correlated with that of *Popillia japonica* adults ($r = .57$, $r = .52$, respectively) and *Nymphalis antiopa* larvae ($r = .78$, $r = .56$, respectively), but not with each

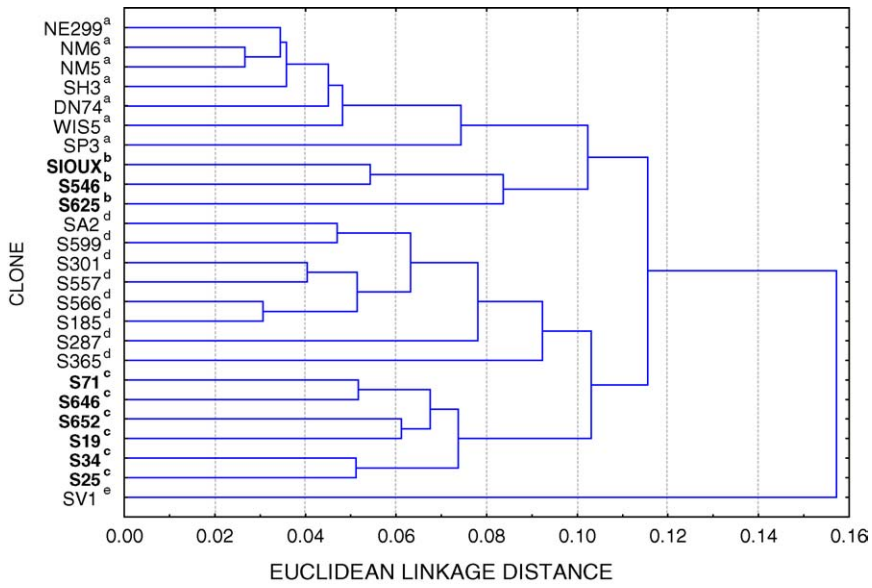

Fig. 1. Unweighted pair-group average (UPGA) clustering tree diagram for 25 willow and poplar clones with regard to consumption by seven insect folivores in no-choice laboratory feeding bioassays. Clones followed by the same letter superscript belong to the same cluster (a = most resistant to e = least resistant).

other. Feeding by *C. nana* adults was significantly correlated with that of *Nematus ventralis* larvae ($r = .54$) and *Polydrusus impressifrons* adults ($r = .45$). Adults of *Plagiodera versicolora*, a willow specialist, were significantly correlated with *Nematus salicisodoratus* larvae ($r = .45$) and *Nymphalis antiopa* larvae ($r = .48$). Only 30% of the specialist × specialist correlations tested (3 of 10) were significant, while 60% of the specialist × generalist correlations tested (6 of 10) were significant, and the single generalist × gener- generalist correlation was significant.

3.3. Multivariate ranking of relative clone resistance (cluster analysis)

The UPGA tree diagram (Fig. 1) shows resistance relationships among clones based on the unweighted pair-group average clustering method. Five principal clusters were identified in the tree diagram, based on a cutoff distance of .07 from the plot of linkage distances. Clones in clusters A and B were overall most resistant to feeding by all the insect species tested, those in cluster C were of intermediate resistance, and those in clusters D and E were least resistant. All insects are given equal importance in the analyses. Using the *k*-means clustering approach (Table 5), clusters 1 and 2 were identified as most resistant to defoliation, while clusters 4 and 5 were least resistant. Cluster 3 showed an intermediate amount of resistance. The two clustering methods, UPGA and *k*-means, provided similar groupings among the most resistant clones, but were increasingly different as resistance decreased (Fig. 1 and Table 5).

Table 5
k-means clustering for 25 willow and poplar clones with regard to consumption by seven insect folivores in no-choice feeding bioassays

Cluster	Clones	Resistance to feeding
1	NM5, SH3, NM6, NE299, WIS5, SP3, DN74	High resistance
2	S625, SIOUX, S546	Moderately high resistance
3	S34, S25	Intermediate resistance
4	S19, S652, S646, S71	Moderately low resistance
5	S301, S287, S557, S365, S185, S566, S599, SA2, SV1	Low resistance

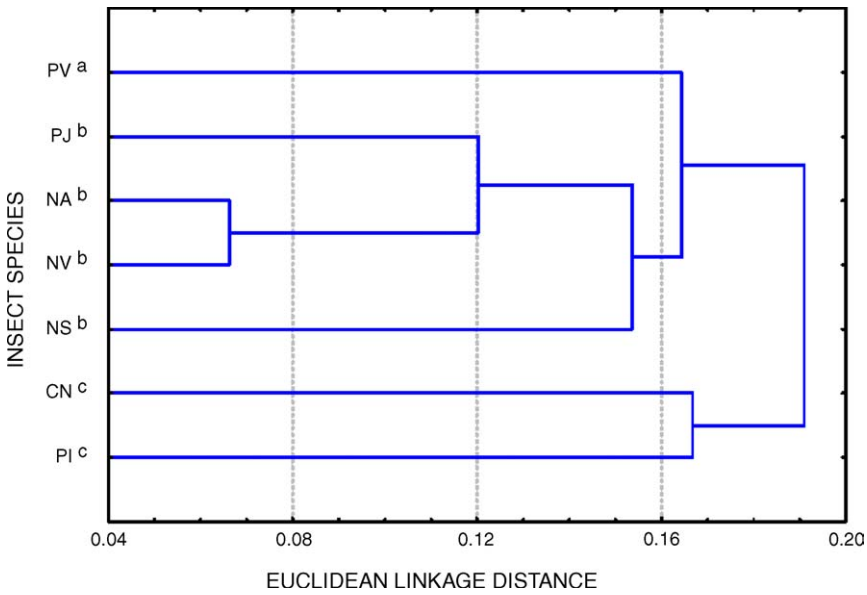

Fig. 2. Unweighted pair-group average (UPGA) clustering tree diagram for seven folivorous insects used in no-choice laboratory feeding bioassays on 25 willow and poplar clones. Insect species followed by the same letter superscript belong to the same cluster. See Table 2 for insect species abbreviation codes.

Three feeding specialization clusters were identified using cluster analysis to evaluate relationships according to insect feeding patterns with the same techniques described above and based on a cutoff distance of .16. In both the UPGA (Fig. 2) and *k*-means (Table 6) clustering methods, the generalist defoliators, *Popillia japonica* adults and *Nymphalis antiopa* larvae, were grouped together along with the *Nematus ventralis* larvae. Both methods grouped together the adults of the *C. nana* and the *Polydrusus impressifrons*. The difference lies in the placement of the *Nematus salicisodoratus* larvae. The UPGA method placed the *Nematus salicisodoratus* larvae in Group B with the *Popillia japonica* adults, *Nymphalis antiopa* larvae, and *Nematus ventralis* larvae. The *k*-means clustering placed the *Nematus ventralis* larvae in the same group as the *Plagiodera versicolora* adults (Group 1).

Table 6
k-means clustering for seven folivorous insects used in no-choice laboratory feeding bioassays on 25 willow and poplar clones

Cluster	Insect species
1	PV, NS
2	PJ, NA, NV
3	CN, PI

4. Discussion

4.1. Clonal variation

The genetics of an individual clone is a key factor determining resistance to feeding by insects. All six poplar clones were grouped by both methods in the two most resistant clusters, A and B for UPGA and #1 and #2 for the *k*-means. Both *S. purpurea* clones, SH3 and SP3, and two of the 14 clones with *S. eriocephala* parentage, S546 and S625, were grouped in the two most resistant clusters by both multivariate methods. The other clones with *S. eriocephala* parentage were scattered among the more susceptible clusters (#3, 4 and 5; C, D and E). Ten of the 13 clones in the two least resistant *k*-means clusters (#4 and 5) were pure or hybrids of *S. eriocephala*. Most of the clones with *S. eriocephala* parentage in this study were highly susceptible to feeding by the insects in this study. Only one clone each with *S. alba*, *S. dasyclados* and *S. discolor* parentage was included in this study, and they were all highly susceptible to insect feeding in this study.

While the two *S. purpurea* clones used in this study, SH3 and SP3, were found to be defoliation resistant,

these particular clones are highly susceptible to girdling by rabbits (*Sylvilagus* spp.) (Nordman, 1998) and are not recommended for inclusion in future plantings. Other clones of *S. purpurea* are not susceptible to rabbits and have been shown to have relatively good biomass production potential (Kopp et al., 2001; Tharakan et al., 2005), but have not been assayed against insect defoliation.

Our finding that parentage plays an important role in conferring resistance to defoliation supports research from the United Kingdom. Kendall and Wiltshire (1997) investigated the relative resistance of 106 SRWC willow clones to defoliation by three beetle species in the UK. Their results show that the beetles tested, *Phyllodecta vulgatissima*, *Phyllodecta vitellinae*, and *Plagiodera versicolora*, consistently selected and consumed certain clones over others in multiple choice tests. *Phyllodecta vulgatissima* consistently rejected unpalatable willow clones in no-choice feeding assays using a subset of 12 willow clones.

Only one species, *S. purpurea*, was tested in both our feeding assays and the Kendall and Wiltshire study. While our results show that clones with *S. purpurea* parentage (SH3 and SP3) are relatively resistant to defoliation by the insects tested, Kendall and Wiltshire found that *S. purpurea* is preferred by *Phyllodecta vitellinae*, but rejected by *Phyllodecta vulgatissima* and *Plagiodera versicolora*.

Interestingly, Kendall and Wiltshire found that *Plagiodera versicolora* largely rejected feeding on most of the 106 willow clones tested in the multiple choice assays. The beetle confined its feeding to just two or three willows. In our no-choice tests, the *Plagiodera versicolora* adults fed on all 19 willow clones tested.

In this study the Siouxland clone, a pure *P. deltoides* clone, was the most susceptible poplar, and NM6 was among the most resistant to feeding by the insects tested. These results are consistent with that of other studies on insect resistance of hybrid poplar. No-choice feeding tests using early instar forest tent caterpillars (*Malacosoma disstria* Hbn.), a generalist herbivorous insect, indicated that Siouxland and WIS5 foliage are preferred foods, NE299 is acceptable and NM6 is not preferred (Robison and Raffa, 1994, 1997). Our results here corroborate this finding for generalists. Field trials of these same clones demonstrated that the Siouxland, WIS5, and NM6 poplar clones were highly susceptible to defoliation by cottonwood leaf beetle (*Chrysomela scripta* Fabricius), while clone NE299 showed moderate resistant to defoliation by this insect (Robison and Raffa, 1998). The cottonwood leaf beetle is a specialist insect and an important pest of willow and poplar in eastern North America (Rose and Lindquist, 1982).

The univariate ANOVA technique for ranking relative clone resistance (Table 3) produced few distinct groups of clones with similar resistances. While the LSD means separation identified five to eight groups of clones for each insect tested, clones often belonged to more than one group. This considerable overlap in groupings makes it difficult for resource managers to make decisions with regard to selecting those clones with apparent broad, multiple-insect resistance, or eliminating those with broad susceptibility. The univariate approach also placed clones SA2, S599, S185, S301, and SV1 into both the resistant and susceptible categories among insects.

The multivariate approaches, on the other hand, provided a more integrated and concise classification of the clones. Neither the UPGA nor the *k*-means method ever put a Table 3 susceptible clone into a resistant category. However both the *k*-means and UPGA approaches did misclassify S301, placing it in a less resistant cluster when it should be among the more resistant.

Thus it appears that the cluster techniques used in this study are robust and conservative and rarely misclassify clones. The cluster methods provide good discrimination among groups with which to continue working (the resistant groups), those to eliminate, at least superficially (the susceptible groups), and a large intermediate group that would require further investigation. Thus the cluster techniques provide an efficient means to screen large numbers of clones in development programs, which is a continuing challenge (Robison, 2002).

4.2. Insect specialization

A comparison of the univariate (Table 4) and multivariate (Fig. 2 and Table 6) approaches to classifying insect specialization reveals trends similar to the clonal variation comparison. The results of the cluster analyses (Fig. 2 and Table 6) never imply a

correlation where there is not one (Table 4), but do ignore some correlations that exist. The clustering approaches are more conservative than the univariate approach and provide a clearer sorting of folivores than the univariate method.

The clear sorting is useful for explaining the ecological nature of specialization across a variety of closely related plant taxa. More practically, the multivariate approaches may provide a useful means of selecting surrogate or model folivores to use in genetic improvement screening systems to efficiently reveal which clones are likely to possess broad resistance to multiple insect pests (as in Tables 3 and 5 and Fig. 1) (Mattson et al., 1988; Robison, 2002). The results of this evaluation suggest that a small but carefully selected group of insects could have provided the same level of discrimination among clones as the seven-insect analysis. Nearly all the discrimination found among clones, with respect to the seven insects tested in the cluster analysis, could have been assumed from the assay results of three insects: the *Popillia japonica* adults, the *Nymphalis antiopa* larvae, and adults of either *Polydrusus impressifrons* or *C. nana*.

Based on the univariate correlation matrix (Table 4) and the multivariate cluster analyses (Fig. 2 and Table 6), adults of the two chrysomelid beetles, *C. nana* and *Plagiodera versicolora*, did not have similar consumption patterns. This indicates that phylogeny and taxonomy, at least among specialist insects, is not a reliable indicator of an insect's feeding pattern on willow and poplar. This finding that is consistent with Orians et al.'s (1997) work with *Plagiodera versicolora* and other chrysomelids feeding on willows.

The univariate (Table 4) and multivariate (Table 6 and Fig. 2) analyses show the similarity in feeding patterns between *Popillia japonica* adults and *Nymphalis antiopa* larvae. As generalists, these insects have similarly broad food preferences. In this study, both generalist insects avoided the same few clones and preferred others, possibly because of the kinds of leaf traits such as constitutive plant secondary compounds, leaf toughness, leaf pubescence or leaf nutritional quality which plant defense theory suggests are active against generalist herbivores (Feeny, 1976; Coley et al., 1985; Bryant et al., 1987; Mattson et al., 1988). However, some of these factors do not always influence herbivore feeding (Lower and Orians, 2002).

The results of the univariate and multivariate cluster methods suggest a continuum of specialization among the insects tested. *Popillia japonica* adults are clearly the most general folivores, followed by the *Nymphalis antiopa* larvae and *Nematus ventralis* larvae. The *Nematus salicisodoratus* larvae and *Plagiodera versicolora* adults exhibit relatively intermediate feeding specializations. Finally, *C. nana* and *Polydrusus impressifrons* adults are the most specialized of the insects tested. The correlation of the *C. nana* adults with the *Nematus ventralis* larvae and *Popillia japonica* adults, however, suggests that strict distinctions among specific types of specialists, with regard to feeding in no-choice laboratory bioassays, cannot be made.

The cluster analysis groupings highlight a basic division in the feeding patterns of generalist and specialist herbivores. These data concur with those of Robison and Raffa (1998), who found that the clonal preference of the cottonwood leaf beetle, a specialist on poplar, was significantly different from that of the forest tent caterpillar, a generalist herbivore. Orians et al. (1997) found differences in willow clonal preferences among specialist and generalist herbivores. Adults of *Popillia japonica*, a generalist herbivore, consistently preferred *S. eriocephala* leaves in multiple choice feeding assays. The specialist beetles *Calligrapha multipunctata bigsbyana* (Kirby) (larvae and adults), *Chrysomela scripta* (adults), *C. knabi* Brown (larvae and adults) all preferred *S. sericea* over the pure *S. eriocephala*. *Plagiodera versicolora* adults preferred the *S. sericea* × *eriocephala* hybrid over the two pure species. *S. eriocephala* leaves lack phenolic glycosides, while *S. sericea* and its hybrid are rich in these compounds (Orians and Fritz, 1995).

5. Conclusion

The feeding bioassays demonstrate the large range in resistance among willow and poplar clones to the seven common defoliators tested. The poplar clones generally showed more resistance to defoliation than the willow clones, though willow clones SH3, SP3, S546 and S625 ranked high in resistance. Future research should include investigations into the various resistance mechanisms these clones possess.

The feeding patterns displayed by the seven defoliating suggest a continuum from generalists to specialists. Clones, particularly the willow clones, resistant to feeding by the generalist insects were not necessarily resistant to feeding by specialist insects, and vice versa. Closely related insect taxa, such as the chrysomelid beetles tested, did not always share similar feeding patterns.

Our results show that multivariate cluster analysis methods are superior to univariate ANOVA for screening large numbers of clones for resistance to multiple insect pests. The robust and conservative multivariate cluster analysis techniques provided discrete groupings of clones based on their relative insect resistance, while the univariate method did not yield the clear sorting desired. Such clear distinctions among clones can provide guidance for breeding and planting programs.

Our study also demonstrated the utility of multivariate cluster analysis techniques for grouping folivorous insects by specialization. The bioassay results show that nearly all the discrimination found among clones, with regard to the seven insects tested, could have been assumed from three assays using *Popillia japonica* adults, *Nymphalis antiopa* larvae, and adults of either the *Polydrusus impressifrons* or *C. nana*. Screening clones using a limited number of model folivores identified in the cluster analysis may provide the needed information about relative insect resistance but with less cost and effort than using a larger suite of insects. Future screening work on willow and poplar clones also should focus on identifying leaf traits that confer broad insect resistance and then using this knowledge to make insect-clonal screening protocols more robust and efficient.

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