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## Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior

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**Abstract** We examined the effects of various wounding treatments and genotypic variation on induced resistance in *Populus* (Salicales: Salicaceae) against herbivory by the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Second-instar larvae grew and consumed less on leaves from induced than non-induced trees. Likewise, larvae preferred leaf disks from non-induced trees. Among induction treatments, gypsy moth feeding had the strongest and most consistent effect in behavioral choice tests. Mechanical wounding of leaves and mechanical wounding plus application of gypsy moth regurgitant had intermediate effects, while application of jasmonic acid had the weakest overall effect. Under no-choice conditions, there were no consistent trends across clones in the ability of various treatments to elicit plant responses affecting the herbivore. Levels of constitutive and inducible resistance to herbivory varied significantly among 12 *Populus* clones. Larvae grew up to 30-fold more, and consumed up to 250-fold more on the most suitable than the least suitable clone. Prior feeding by gypsy moths reduced larval feeding up to 71.4% on the most highly inducible clone, but it had little or no effect for the least inducible clones. There was no evidence for a relationship between levels of inducible and constitutive resistance, or between inducible resistance and phylogenetic relatedness among clones. We discuss implications for the ecology and evolution of plant-insect interactions and the management of insect pests.

**Key words** Induced resistance · Herbivory · Genotypic variation · *Populus* · *Lymantria dispar*

### Introduction

Induced plant responses to insect herbivores can reduce the nutritional quality of plant tissue to subsequent herbivory (e.g., Haukioja and Niemela 1977; Haukioja and Hanhimaki 1985; Broadway et al. 1986; Baldwin 1988; Krause and Raffa 1992; Wold and Marquis 1997; Litvak and Monson 1998; Rieske and Raffa 1998). Such responses have been demonstrated in a wide variety of plant-herbivore systems (Tallamy and Raupp 1991; Karban and Baldwin 1997), with various levels of specificity of response and elicitation (Hartley and Lawton 1987; Geervliet et al. 1997; McAuslane and Alborn 1998; Stout et al. 1998). The quality of a plant as a food source can be reduced through both behavior-modifying and development-inhibiting effects on herbivores (Karbon and Myers 1989), and induced responses can increase herbivore susceptibility to natural enemies (Vet and Dicke 1992).

Despite the numerous instances in which induced plant responses have been demonstrated, their ecological and evolutionary significance remain unclear (Hunter and Schultz 1993). Two critical considerations which merit further study are the extent to which herbivores and pathogens elicit a specific response versus generalized stress reactions, and the role of genetic and environmental variation in induction.

Induced responses can be experimentally elicited by a diverse array of external sources of stress and injury (Koricheva et al. 1998). Simple mechanical damage to plant tissues and cells can elicit induced responses in some systems. However, responses to artificial defoliation often differ qualitatively and/or quantitatively from responses to actual herbivory (e.g., Hartley and Lawton 1987; Krause and Raffa 1992; Turlings et al. 1995; Stout et al. 1998). Resistance to herbivores is frequently enhanced by specific elicitors associated with insects or pathogens (Bloch et al. 1984; Raffa 1991; Alborn et al. 1997; Korth and Dixon 1997; Sticher et al. 1997; Inbar et al. 1998). For example, application of herbivore

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regurgitant to artificially damaged plant tissues can more closely simulate actual feeding, due to chemical elicitors present in the saliva (Lin et al. 1990; Hartley and Lawton 1991; Alborn et al. 1997; Korth and Dixon 1997; McCloud and Baldwin 1997).

The process of elicitation is known to be regulated by a series of both exogenous and endogenous cues (Chen et al. 1995; Baldwin 1996; O'Donnell et al. 1996). Following initial plant or cell wall injury, chemical signals regulate the subsequent systemic expression of an induced response. For example, jasmonic acid (JA) occurs in many plants, and is considered an important component of the octadecanoic acid wound-signaling pathway (Creelman and Mullet 1997; Wasternack and Parthier 1997). The use of JA as an elicitor of plant wound responses has been proposed as a method to study more effectively the biochemical and evolutionary framework of induced responses (Baldwin 1996). JA has also been proposed as a means of uncoupling wound responses from actual wounding, and stimulating plant resistance in the field (Baldwin 1996; Thaler et al. 1996).

The extent to which variation among induced plant responses is under genetic control is central to our ability to interpret their ecological and evolutionary importance. A wide variety of environmental factors have been shown to affect the expression, rate, and extent of inducible responses (Karban and Baldwin 1997). Induced responses to insect herbivores have been shown to be heritable in some systems (Zangerl and Berenbaum 1990; van Dam and Vrieling 1994; English-Loeb et al. 1998). However, the role of genetic variation in induced responses to herbivory has not been intensively explored, in contrast to the work done on pathogens. Information on the heritability of induced responses to herbivores is especially lacking for trees. Insight into the relative importance of genetic versus environmental components of induced responses could improve our understanding of their ecological and evolutionary significance, and assist in implementing pest management.

The purpose of this study was to compare the effects of different methods of eliciting rapidly induced resistance across a variety of related but genetically distinct tree clones.

### System of study

We selected 12 clones representing a broad taxonomic background within the genus *Populus* (Salicales: Salicaceae). The parentage of clones is listed in Robison and Raffa (1994), except DN34 (= Eugenei) *Populus deltoides* × *P. nigra*, and NE308 *P. nigra* 'Charkowiensis' × *P. incassata*. Induced responses in *Populus* appear to be important components in its resistance to herbivory (Williams and Whitham 1986; Mattson and Palmer 1988; Reichenbacher et al. 1996). Phenolic glycosides and proteinase inhibitors have been shown to increase rapidly in response to wounding, and prior herbivory can cause a decline in plant suitability to

Lepidoptera (Bryant et al. 1987; Clausen et al. 1989; Lindroth and Hemming 1990; Bradshaw et al. 1991; Robison and Raffa 1997).

The gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), is a polyphagous folivore, which prefers *Quercus* spp. and *Populus* spp. (Liecihold et al. 1995). Previous studies have shown gypsy moth larval development and feeding preference to be sensitive to *Populus* defensive chemistry (Lindroth and Hemming 1990).

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## Materials and methods

### Plant and insect culture

*Populus* clonal material originated from sources growing at the University of Wisconsin West Madison and Arlington Agricultural Research Stations. To minimize possible environmental effects on stock trees growing at different sites, cuttings for our experiments were taken from trees that had been subsequently established in pots together in a greenhouse and moved outdoors into a cold frame under common environmental and cultural conditions. Dormant hardwood cuttings (20 cm long) were vegetatively propagated by dipping them in Hormex (Brooker Chemical, North Hollywood, Calif.) no. 8 rooting powder (0.8% indole-3-butyric acid), and placing them in saturated Fafard no. 2 potting soil in 15.12-l plastic pots. Trees were fertilized with 15 g/plant Osmocote (Sierra Chemical, Milpitas, Calif.) 19-6-12 slow-release fertilizer plus micronutrients, and flood irrigated daily. Trees were grown in the University of Wisconsin-Madison Biotron in a shaded greenhouse at 24°C and a 16:8 (L:D) light regime. Because herbivore performance can vary significantly according to leaf age in *Populus* (Bingaman and Hart 1992; Robison and Raffa 1997), all experiments were standardized for leaf position. The most apical fully unfolded leaf was designated as leaf no. 1 and numbering continued sequentially down the stem. All trees had 18–24 leaves when used in experiments. Trees were arranged in the greenhouse as a split-plot design. A randomized 12 × 5 factorial combination of clone and induction method was arranged as treatments within the whole and subplots, respectively.

Gypsy moth egg masses of strain NJSS were obtained from the Otis Methods Development Center (USDA, APHIS, Otis AFB, Massachusetts). Prechilled egg masses were surface sterilized in a sodium hypochlorite solution (2060 ml dH<sub>2</sub>O, 21 ml polyoxyethylene sorbitan monooleate and 40 ml bleach) for 5 min, rinsed three times with dH<sub>2</sub>O, and allowed to dry. Egg masses were then placed in circular 14.0 × 3.9 cm clear plastic containers (Tri-State Plastics, Dixon, Ky.) and kept under a 16:8 (L:D) photoperiod at 24°C and 50–70% relative humidity in an environmental growth chamber. Upon hatching, the larvae were provided with two 2-cm<sup>3</sup> cubes of artificial diet (ICN Biomedicals, Aurora, Ohio), which were replaced every 2–3 days as needed until larvae were used for experiments.

### Wound induction treatments

Five plants of each clone were randomly selected and subjected to one of four induction treatments, or left undamaged as non-induced controls. The induction treatments were: (1) larval gypsy moth feeding; (2) mechanical wounding of leaves; (3) mechanical wounding plus application of gypsy moth regurgitant; (4) application of JA. All induction treatments were performed on leaf positions no. 7–12, and upper, undamaged leaves (no. 4–5) were used for assays.

The gypsy moth feeding treatment was performed by enclosing six fifth-instar larvae on leaf nos. 7–12, within white nylon mesh sleeves. Larvae were allowed to feed for 24 h, and then removed. In previous studies, herbivores showed similar preference for leaves

7–12 across different poplar clones (Bingaman and Hart 1992; Robison and Raffa 1997), therefore selective feeding on different leaves within this group should not be significant and should have little or no effect on the overall induced response.

Mechanical wounding was administered by crushing designated leaves with pliers. Twenty plier wounds (12 teeth/cm) were made per leaf at each handling (see Bradshaw et al. 1991). Some wounds on each leaf were applied to damage the midvein. Treatments were administered at 0, 2, 4, 8, 18, and 22 h to provide continuous wounding over time and more closely simulate herbivory (Baldwin 1990). After 24 h, approximately 80% of each leaf surface had been wounded.

Mechanical wounding plus gypsy moth regurgitant incorporated the same treatment as above, except 20  $\mu$ l per leaf of regurgitant was evenly applied to the wounded portions of the leaf surface with a syringe at each handling. Regurgitant was collected from fourth-instar larvae reared on *Populus* foliage by gently squeezing them with forceps until regurgitation was induced. Drops of regurgitant were collected with a syringe, placed in a vial, and stored at 10°C until use.

JA was applied to plants using methods similar to those of Baldwin (1996). Briefly, JA (96% pure, Sigma, St. Louis, Mo.) was dissolved in acetone and suspended in lanolin. Lanolin paste (0.055 ml) containing 100 ng of JA was gently applied with a spatula in a thin layer over the distal half of the underside of each treated leaf. While the methylated form of JA is highly volatile (Farmer and Ryan 1990), the relatively low vapor pressure of the molecule precludes it affecting neighboring plants at normal environmental conditions (Farmer et al. 1992).

Foliage from induced and non-induced trees was assayed to determine the extent of induction with developmental no-choice and behavioral choice assays 72 h following initiation of induction treatments.

#### Development assay

We performed no-choice assays to examine gypsy moth development among induction treatments and *Populus* clones. Immediately following the molt to the second stadium, each larva was starved for 24 h and placed in a 9-cm petri dish. Larvae were subsequently fed only foliage from a plant subjected to one of the four induction treatments or a control plant. One leaf from each plant (position no. 5) was disinfected with 10% bleach solution, rinsed with dH<sub>2</sub>O and placed in each rearing dish with a water pick containing distilled water. Leaves were electronically scanned (Hewlett Packard Scanjet 4c) before and after feeding and consumed leaf area was measured using the software MacFOLIA (Regent Instruments 1996). Larval relative consumption rate (RCR) was calculated as leaf area consumed, divided by initial larval weight, divided by time (units cm<sup>2</sup>/g per day). Relative growth rate (RGR) was calculated as larval weight after 4 days of feeding minus initial weight, divided by initial weight, divided by time (units g/g per day). Larvae that died prior to the 4th day were assigned an RGR value of zero.

Effects of induction treatment and clonal variation on RCR and RGR were analyzed as a split-plot analysis of variance (PROC GLM; SAS 1990) with induction treatment as the whole-plot error, and clone as the subplot error to correspond with tree arrangement in the greenhouse. Mean separations were performed using least square means. Effects of induction within clones, and effects of clone within treatments were analyzed as one-way analysis of variance, and mean separations were performed with Fisher's protected LSD. Residuals for all tests were examined (PROC UNIVARIATE; SAS 1990) for normality and both RCR and RGR were log transformed to achieve homogeneity of variance. The data for RCR and RGR were not normally distributed because of a disproportionately high number of zero values. However, a non-parametric test consisting of a rank transformation followed by split-plot analysis of variance resulted in nearly equivalent *P*-values, and the conclusions were no different from those using the conventional split-plot analysis (Havill 1998).

#### Choice assay

We assayed larval preference among clones and induction treatments using a standard leaf disk choice procedure. The arena consisted of a 9-cm-diameter plastic petri dish with a thin coat of paraffin wax on the bottom. The dishes contained a filter paper moistened with distilled water to prevent leaf desiccation and shrinkage. Leaf disks (16 mm diameter) were cut intervenally with a cork borer. Three disks were taken from the same leaf (position no. 4) for each tree, and three dishes (subsamples) were assembled for each combination of induction treatment and clone. Single leaf disks from plants subjected to each of the four induction treatments, and from a non-induced plant, were randomly arranged evenly around the periphery of each dish. Leaf disks were anchored into dishes with minuten pins.

Six second-instar gypsy moth larvae were starved for 24 h and placed in the center of each dish. Dishes were covered and sealed with Parafilm (American National Can, Greenwich, Conn.), and the larvae were allowed to feed for 24 h at 16:8 (L:D) and 24°C. After 24 h, each leaf disk was electronically scanned and consumption was measured as before. For each dish, percent feeding reduction due to host induction was calculated as consumption of the control minus consumption of the gypsy-moth-induced disk, divided by consumption of the control.

Effects of induction treatment and clonal variation on larval choice were analyzed as a split-plot analysis of variance (PROC GLM; SAS 1990), with clone as the whole-plot error and treatment as the subplot error to correspond with leaf disk arrangement in petri dishes. Mean separations of induction treatments within clones, and clones within treatments were performed using least square means with PROC MIXED (Milliken and Johnson 1984; SAS 1990). Differences among clones were analyzed as one-way analysis of variance, and mean separations were performed with Fisher's protected LSD. Residuals for all tests were examined (PROC UNIVARIATE; SAS 1990) for normality and disk consumption was log transformed to achieve homogeneity of variance.

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## Results

### Development assay

The RGRs of gypsy moth larvae feeding on foliage from trees with various treatments are shown in Table 1. Within each clone there were no significant differences (*P* > 0.05) among individual induction methods, so values for all induction methods were pooled for comparison with controls. Analysis of control versus all induction treatments performed as a contrast resulted in nearly equivalent *P*-values, and the conclusions did not change compared to the pooled analysis (Havill 1998).

RGRs were significantly higher in control than treated foliage for 4 clones. Of the 8 clones that did not show significant effects of induction, 7 showed a general trend for more growth on constitutive trees, with the exception showing equivalence. Figure 1 shows the RGR of larvae fed foliage from induced versus constitutive trees for each induction treatment. The dashed line represents values where RGRs on induced and constitutive trees are equal. Points which fall to the right of this line indicate reduced growth due to induction.

The RCRs of gypsy moth larvae are shown in Table 2. Again, within clones there were no significant differences among individual induction treatments, so values for all induction treatments were pooled for comparison with

**Table 1** Effects of induction treatment and *Populus* clone on second-instar gypsy moth relative growth rate over 4 days. *P*-values refer to comparisons between controls and pooled induction treatments. Significant differences for the overall split-plot analysis ( $P < 0.05$ ) are represented by different uppercase letters among treatments (across all clones), and lowercase letters among clones within a treatment. Data were log transformed prior to analysis. Original untransformed values (means  $\pm$  SE) are presented

Clone	Relative growth rate (g/g per day) <sup>a</sup>							
	Control <sup>b</sup>	All induction treatments <sup>c</sup>	<i>P</i>	Jasmonic acid	Mechanical and regurgitant	Gypsy moth feeding	Mechanical wounding	Overall <sup>d</sup>
DN34	0.318 $\pm$ 0.109 a	0.177 $\pm$ 0.042 a	0.183	0.110	0.195	0.177	0.226	0.205 $\pm$ 0.041 a
NE332	0.296 $\pm$ 0.109 ab	0.165 $\pm$ 0.028 a	0.147	0.191	0.218	0.198	0.059	0.188 $\pm$ 0.031 a
NC11382	0.171 $\pm$ 0.071 abcde	0.166 $\pm$ 0.032 a	0.963	0.226	0.240	0.062	0.122	0.168 $\pm$ 0.029 ab
NE308	0.306 $\pm$ 0.059 a	0.119 $\pm$ 0.028 ab	0.008	0.137	0.173	0.159	0.006	0.156 $\pm$ 0.029 ab
NC11004	0.187 $\pm$ 0.015 abcde	0.138 $\pm$ 0.041 a	0.471	0.301	0.084	0.087	0.082	0.148 $\pm$ 0.033 ab
NC5271	0.212 $\pm$ 0.030 abc	0.122 $\pm$ 0.038 ab	0.225	0.203	0.049	0.165	0.073	0.140 $\pm$ 0.032 abc
NM6	0.104 $\pm$ 0.043 cde	0.104 $\pm$ 0.014 ab	0.956	0.084	0.103	0.114	0.116	0.104 $\pm$ 0.014 bc
NC5260	0.129 $\pm$ 0.061 bcde	0.061 $\pm$ 0.021 bc	0.198	0.172	0.001	0.014	0.057	0.074 $\pm$ 0.021 c
NC11396	0.055 $\pm$ 0.037 cde	0.001 $\pm$ 0.006 cd	0.017	0.018	0.005	-0.007	-0.013	0.012 $\pm$ 0.010 d
NC5331	0.016 $\pm$ 0.012 e	0.011 $\pm$ 0.013 cd	0.837	0.010	-0.014	-0.002	0.049	0.012 $\pm$ 0.011 d
DTAC2	0.053 $\pm$ 0.019 cde	-0.004 $\pm$ 0.009 cd	0.013	0.008	-0.025	0.001	0.000	0.007 $\pm$ 0.009 d
NC11505	0.031 $\pm$ 0.031 de	-0.012 $\pm$ 0.006 d	0.030	-0.009	0.001	-0.022	-0.018	-0.004 $\pm$ 0.008 d
Overall <sup>e</sup>	0.159 $\pm$ 0.021 A			0.119 $\pm$ 0.020 B	0.087 $\pm$ 0.018 C	0.081 $\pm$ 0.017 C	0.063 $\pm$ 0.015 C	

<sup>a</sup>  $n = 5$  for all treatment  $\times$  clone combinations, except  $n = 4$  for NC5271, NC11004, NC11396, and DTAC2

<sup>b</sup> Clone effect among controls significant at  $P = 0.002$

<sup>c</sup> Clone effect among treatments significant at  $P = 0.0001$

<sup>d</sup> Overall clone effect (split-plot) significant at  $P = 0.0001$

<sup>e</sup> Overall unpooled (split-plot) treatment effect significant at  $P = 0.0001$

control trees, and a contrast yielded very similar results (Havill 1998). RCRs were significantly reduced on foliage from treated relative to control trees in clones NE308 and NC11396. Of the 10 clones that did not show significant effects of induction, 6 showed a trend for more consumption on constitutive trees.

Three clones, NC11382, NM6 and NC5331, showed no trend for better larval RGR or RCR on constitutive trees. There were no significant interactions between treatment and clone for analyses of pooled treatments versus control for RGR ( $P = 0.554$ ), or RCR ( $P = 0.446$ ).

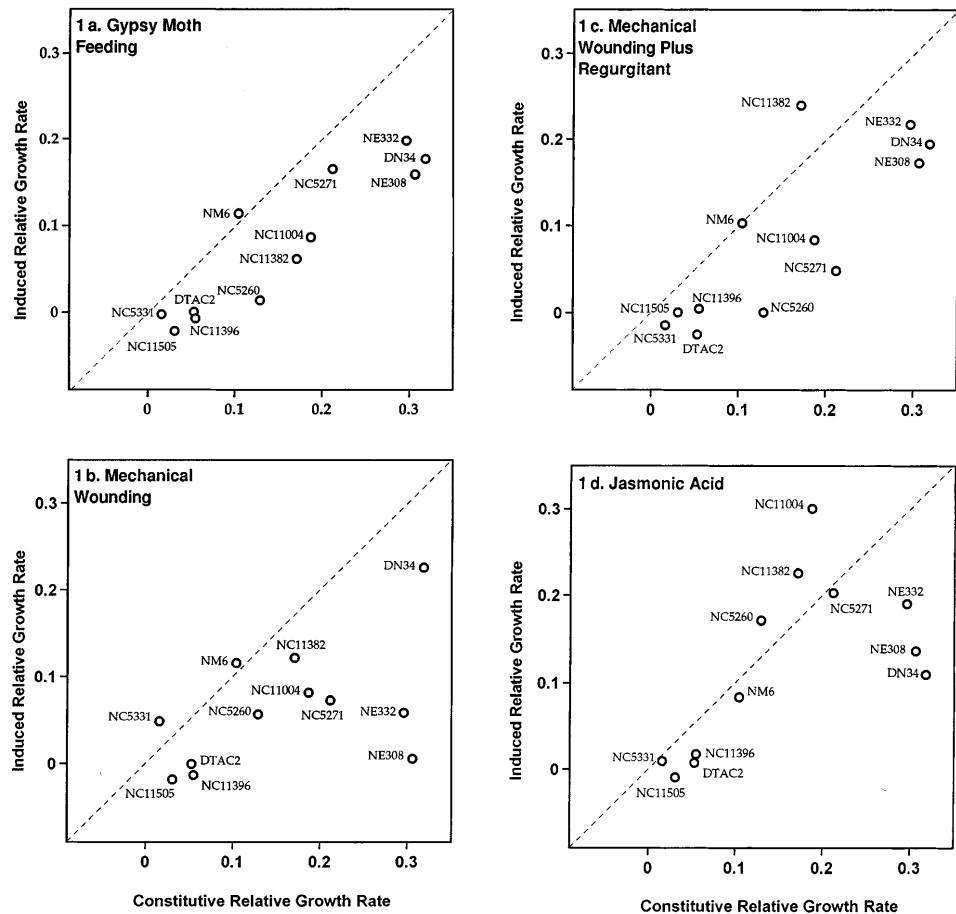
When all clones were pooled within the split-plot analysis, overall larval performance was significantly different among the various induction treatments. Larvae grew (Table 1) and consumed (Table 2) more on leaves from undamaged trees than on any of the induction treatments. Among induced trees, JA had the least effect on larval RGR, while mechanical wounding plus regurgitant, gypsy moth feeding, and mechanical wounding had equivalent effects. Larval RCR was most affected by mechanical wounding and mechanical wounding plus regurgitant, and least affected by gypsy moth feeding and JA. There was no significant treatment by clone interaction in the split-plot analysis of unpooled treatments for RGR ( $P = 0.306$ ). There was a significant clone by treatment interaction for RCR ( $P = 0.031$ ), but this arose almost entirely from variation among clones in their overall inducibility (i.e., control vs all induction methods), rather than variation among induction methods.

Constitutive resistance to larval feeding differed widely among clones (Tables 1, 2). Larvae exhibited growth loss and very little feeding on the least suitable clones, while the most suitable clones supported appreciable larval growth and consumption. These results support previous comparisons of constitutive resistance among these clones to the forest tent caterpillar, *Malacosoma disstria* Hubner (Robison and Raffa 1994). Larvae grew up to 30-fold and consumed up to 250-fold more on the most suitable compared to the least suitable clones. When clonal differences were analyzed within control and pooled induction treatments, larval growth and feeding tended to be more similar within the category of pooled induction treatments.

### Choice assay

Five of the 12 clones (Table 3) exhibited induced responses that reduced larval feeding relative to control trees. The highest reduction in feeding occurred in response to gypsy moth feeding on clones NC5271 and NE308. The effects of various induction treatments on larval feeding preference varied among clones. Larvae showed reduced preference for feeding on foliage from trees subjected to all four induction treatments on clone NC5271. On clone DN34, the direction was toward reduced feeding for all four treatments, but it was only

**Fig. 1a–d** Relative growth rates of gypsy moth larvae fed foliage from induced versus constitutive poplar trees. Each graph represents a separate induction treatment; plot points and labels represent feeding on a particular poplar clone. Dashed lines indicate values where the relative growth rate on induced trees equals relative growth rate on constitutive trees. Points that fall to the right of the line indicate induction expressed within a particular poplar clone



significant for mechanical wounding plus regurgitant. Larvae were not affected by the JA treatment on clone NC5260, but were affected by the other induction treatments. Larvae were affected by gypsy moth feeding, but not other treatments, on clone NC11004. On clone NC5331, larvae preferred the JA and mechanical wounding treatments over the mechanical wounding plus regurgitant, and gypsy moth feeding treatments. The remaining 6 clones showed no significant effect of induction on larval choice.

When all clones were pooled in the split-plot analysis, there were significant differences among induction treatments ( $P=0.0008$ ). Gypsy moth feeding had the strongest effect on larval choice, mechanical wounding and mechanical wounding plus regurgitant had intermediate and equivalent effects, and JA had the weakest effect. Larval feeding differed considerably among clones in the 24-h choice assay ( $P < 0.001$ ). These results generally parallel the consumption values in the development assay (Table 2). Clones that were highly suitable for larval consumption in the development assay tended to support more feeding in the choice assay.

The magnitude of herbivore-induced effects on gypsy moth larval feeding among clones is shown in Fig. 2. The relative extent of induction varied widely among clones that have considerably different levels of constitutive resistance. Clone NC5271 demonstrated the most

pronounced bioassay response to induction, and all clones, except NC11396, showed an absolute trend towards higher larval feeding on leaf disks from control trees.

There was no significant relationship ( $P > 0.05$ ) between constitutive and induced resistance among clones for either the developmental or behavioral parameters among the four induction treatments (Tables 1, 2, and 3; Figs. 1, 2). Clone NC11396 showed no response to induction.

## Discussion

We found significant variation in constitutive and inducible resistance among *Populus* clones, and various elicitation treatments. Genotypic variation in an inducible trait has been observed in several systems (Haukioja and Hanhimaki 1985; van Dam and Vrieling 1994; English-Loeb et al. 1998) including *Populus* (Robison and Raffa 1997). In the results reported here, induction had a clear systemic effect on foliar suitability for gypsy moth growth and consumption that varied widely among *Populus* genotypes (Fig. 1). Herbivory on the most highly inducible clones reduced larval feeding up to 71.4% while on the least inducible clones it had no (or perhaps a negative) effect on larval preference.

**Table 2** Effects of induction treatment and *Populus* clone on second-instar gypsy moth relative consumption rate over 8 days. *P*-values refer to comparisons between control and pooled induction treatments. Significant differences for the overall split-plot analysis ( $P < 0.05$ ) are represented by different uppercase letters among treatments (across all clones), and lowercase letters among clones within a treatment. Data were log transformed prior to analysis. Original untransformed values (means  $\pm$  SE) are presented

Clone	Relative consumption rate (cm <sup>2</sup> /g per day) <sup>a</sup>						Overall <sup>d</sup>	
	Control <sup>b</sup>	All induction treatments <sup>c</sup>	<i>P</i>	Jasmonic acid	Gypsy moth feeding	Mechanical and regurgitant		Mechanical wounding
NE308	245.23 $\pm$ 29.02 a	85.87 $\pm$ 19.44 ab	0.032	80.05	136.97	121.55	4.92	117.74 $\pm$ 20.90 a
NC5271	122.78 $\pm$ 18.70 ab	94.08 $\pm$ 24.33 a	0.206	120.36	103.47	27.59	124.91	99.82 $\pm$ 19.80 ab
NC11004	80.44 $\pm$ 5.77 abc	104.30 $\pm$ 32.20 a	0.379	189.32	114.96	47.48	65.46	99.53 $\pm$ 25.70 ab
NE332	91.42 $\pm$ 11.31 abc	74.54 $\pm$ 15.18 a	0.286	76.34	93.82	89.09	39.25	77.47 $\pm$ 12.67 b
NC11382	80.87 $\pm$ 33.29 abc	65.28 $\pm$ 10.15 a	0.837	74.22	50.18	80.98	53.49	68.82 $\pm$ 10.52 bc
DN34	87.54 $\pm$ 26.31 abc	59.58 $\pm$ 13.08 ab	0.214	45.91	53.88	64.99	73.55	65.17 $\pm$ 11.69 bc
NC5260	40.87 $\pm$ 18.50 cd	46.20 $\pm$ 19.71 bc	0.436	132.81	35.72	0.21	16.06	45.13 $\pm$ 16.05 bc
NM6	42.75 $\pm$ 18.36 bcd	39.94 $\pm$ 6.59 a	0.796	27.05	41.60	35.87	3.63	40.52 $\pm$ 6.26 c
DTAC2	48.92 $\pm$ 25.39 bcd	29.56 $\pm$ 15.05 c	0.229	13.47	65.26	0.00	0.04	33.43 $\pm$ 12.91 cd
NC11396	60.09 $\pm$ 42.07 cd	1.01 $\pm$ 0.92 d	0.001	3.69	0.33	0.00	0.04	12.83 $\pm$ 9.26 cd
NC5331	7.86 $\pm$ 5.45 de	8.07 $\pm$ 4.93 cd	0.487	25.51	0.63	2.79	3.37	8.04 $\pm$ 4.17 cd
NC11505	0.39 $\pm$ 0.25 e	0.49 $\pm$ 0.35 d	0.766	1.38	0.02	0.31	0.25	0.47 $\pm$ 0.28 d
Overall <sup>e</sup>	76.56 $\pm$ 10.55 A	64.50 $\pm$ 12.15 AB	57.27 $\pm$ 10.69 BC	43.14 $\pm$ 8.00 CD	34.86 $\pm$ 8.11 D			

<sup>a</sup>  $n = 5$  for all treatment  $\times$  clone combinations, except  $n = 4$  for NC5271, NC11004, NC11396, and DTAC2

<sup>b</sup> Control clone effect significant at  $P = 0.0001$

<sup>c</sup> Treatment clone effect significant at  $P = 0.0001$

<sup>d</sup> Overall clone effect (split plot) significant at  $P = 0.0001$

<sup>e</sup> Overall unpooled (split-plot) treatment effect significant at  $P = 0.015$

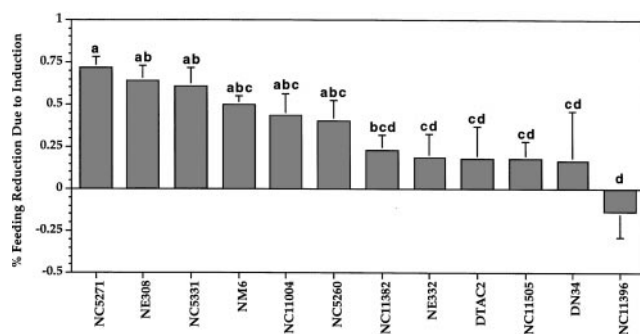
**Table 3** Feeding preference of second-instar gypsy moth larvae among induction treatments in five-choice leaf disk bioassay for 12 *Populus* clones. Significant differences ( $P < 0.05$ ) are represented by different uppercase letters among treatments, and lowercase letters among clones within a treatment. Consumption was log transformed prior to analysis. Original untransformed values (means  $\pm$  SE) are presented

Clone	Consumption (mm <sup>2</sup> ) <sup>a</sup>						Overall <sup>b</sup>
	Control	Jasmonic acid	Mechanical and regurgitant	Mechanical wounding	Gypsy moth feeding	<i>P</i>	
NC11382	66.1 $\pm$ 17.0 A abcd	64.4 $\pm$ 19.3 A a	52.6 $\pm$ 15.7 A a	45.8 $\pm$ 12.4 A a	37.9 $\pm$ 6.1 A a	0.757	53.37 $\pm$ 6.44 a
DN34	92.9 $\pm$ 16.9 A a	40.4 $\pm$ 12.9 AB ab	33.3 $\pm$ 7.5 B abc	41.7 $\pm$ 17.5 AB a	42.9 $\pm$ 17.2 AB a	0.043	50.23 $\pm$ 7.51 ab
NC5260	62.9 $\pm$ 16.4 AB abcd	62.0 $\pm$ 10.2 A a	34.7 $\pm$ 3.3 BC ab	33.1 $\pm$ 2.4 BC a	29.9 $\pm$ 4.5 C ab	0.029	44.51 $\pm$ 4.76 ab
NE308	86.3 $\pm$ 19.3 A ab	31.1 $\pm$ 8.8 BA bc	27.4 $\pm$ 12.6 BA abcd	20.0 $\pm$ 6.7 BA bc	24.1 $\pm$ 9.0 BA abc	0.057	37.76 $\pm$ 7.03 abc
NC11004	37.9 $\pm$ 5.1 A cdef	54.4 $\pm$ 11.7 A a	35.4 $\pm$ 4.1 A abc	38.9 $\pm$ 4.2 A a	18.4 $\pm$ 5.4 B bc	0.025	37.02 $\pm$ 3.74 abc
NE332	31.2 $\pm$ 5.7 A f	35.0 $\pm$ 8.1 A abc	47.1 $\pm$ 12.1 A a	29.6 $\pm$ 4.9 A ab	22.9 $\pm$ 4.3 A abc	0.233	33.15 $\pm$ 3.48 abc
NC5271	74.0 $\pm$ 17.6 A abc	19.8 $\pm$ 2.6 B c	18.3 $\pm$ 1.4 B bcd	28.4 $\pm$ 13.4 B abc	14.4 $\pm$ 3.0 B c	0.004	30.97 $\pm$ 6.44 bc
NC5331	46.3 $\pm$ 8.5 A bcde	24.7 $\pm$ 4.0 B bc	14.3 $\pm$ 3.2 C d	25.3 $\pm$ 4.6 B abc	14.4 $\pm$ 2.1 C c	0.0006	25.00 $\pm$ 3.13 c
NC11396	25.1 $\pm$ 3.1 A f	29.5 $\pm$ 5.5 A abc	24.8 $\pm$ 4.0 A abcd	20.5 $\pm$ 3.9 A abc	24.6 $\pm$ 4.2 A abc	0.711	24.88 $\pm$ 1.79 c
DTAC2	23.4 $\pm$ 7.3 A f	45.8 $\pm$ 18.7 A ab	17.1 $\pm$ 3.3 A cd	21.5 $\pm$ 3.2 A abc	13.9 $\pm$ 2.3 A c	0.181	24.34 $\pm$ 4.49 c
NM6	31.9 $\pm$ 8.6 A ef	21.4 $\pm$ 7.3 A c	24.3 $\pm$ 5.7 A abcd	19.3 $\pm$ 5.9 A bc	16.6 $\pm$ 3.7 A bc	0.540	22.70 $\pm$ 2.85 c
NC11505	34.9 $\pm$ 11.7 A def	19.6 $\pm$ 3.4 A c	15.8 $\pm$ 3.4 A cd	16.2 $\pm$ 4.1 A c	15.7 $\pm$ 3.0 A c	0.196	20.44 $\pm$ 2.90 c
Overall <sup>c</sup>	51.9 $\pm$ 4.6 A	37.4 $\pm$ 3.5 B	29.09 $\pm$ 2.67 C	28.4 $\pm$ 2.5 C	23.3 $\pm$ 2.2 D		

<sup>a</sup>  $n = 5$  for all treatment  $\times$  clone combinations, except  $n = 4$  for NC5271, NC11004, NC11396, and DTAC2

<sup>b</sup> Overall clone effect significant at  $P = 0.0001$

<sup>c</sup> Overall treatment effect significant at  $P = 0.0008$



**Fig. 2** Relative magnitude effect of host induction by gypsy moth feeding on larval choice among *Populus* clones in a leaf disk choice bioassay. Percent feeding reduction due to host induction was calculated as consumption of the control disk minus consumption of the gypsy-moth-induced disk, divided by consumption of the control. Means  $\pm$  SE with different letters are significantly different (Fisher's protected LSD,  $P < 0.05$ )

Induced responses also varied significantly among experimental induction treatments. In larval choice tests, the application of JA elicited the weakest overall response, and in two clones (NC5260 and NC11004), JA had no effect while other induction treatments did. This may be due to differences in foliar characteristics that prevented elicitation with JA. Herbivore feeding induced the strongest and most consistent response in larval choice tests. All clones that showed a significant response in larval preference included reduced feeding due to the gypsy moth feeding treatment. This is consistent with results from other studies in which actual herbivory elicited a stronger response than artificial induction treatments (Karban and Baldwin 1997). Under no-choice conditions, this trend was absent. Therefore, conclusions on whether or not artificial treatments simulate actual herbivory need to be based on the particular assay conditions and parameters involved.

It has been suggested that plants with high levels of constitutive resistance will consequently have low levels of inducible resistance (Herms and Mattson 1992). We did not find such a tradeoff between constitutive and inducible resistance in *Populus*. However, the available evidence from other systems is mixed. Researchers have found positive, negative, or no trend in the relationship between constitutive and inducible resistance in different plant-herbivore systems (Zangerl and Berenbaum 1990; Raffa 1991; Brody and Karban 1992). In addition, there was no apparent relationship between induction and phylogenetic relatedness among clones. Clones that shared parental lineages did not show a similar incidence or magnitude of induction. For example, of the six clones that share *P. nigra* as a parent species, two were highly inducible (NE308, NC5271), two were moderately inducible (DN34, NC5331), and two were poorly inducible (NC11382, NM6).

Levels of defensive chemicals between (Whitham et al. 1996; Lindroth and Hwang 1996; Hwang and Lindroth 1997) and within (Bingaman and Hart 1993;

Jones et al. 1993; Robison and Raffa 1994) *Populus* individuals are known to be highly variable. Variation in resistance to herbivory may benefit plants by making it difficult for herbivores to track host defenses in ecological and evolutionary time (Whitham 1983), and by increasing the effectiveness of nutritional investment in defense (Karban et al. 1997). The results reported here demonstrate that both constitutive and induced defenses are important sources of variation in *Populus* resistance to herbivory. Variation in response to different eliciting agents may be an additional level of heterogeneity confronting herbivores, which could benefit plants over evolutionary time.

These results also have implications for management of insect pests. Hybrid *Populus* can provide a fast-growing source of wood, and renewable biofuel in short-rotation plantations. *Populus* plantations are vulnerable to a large number of pests (Ostry et al. 1989), and therefore resistance breeding and judicious deployment of clonal cultivars are necessary for successful management. The number, and resistance characteristics of clones are important considerations for short-rotation clonal forestry (Roberds and Bishir 1997). In particular, resistant host plants can exert selective pressure on herbivores to evolve resistant biotypes. This is of particular concern for *Populus* plantations where trees are propagated vegetatively and there is potential for large numbers of genetically identical plants. To avoid evolution of resistant pest biotypes, it is recommended that managers maximize heterogeneity to provide refugia for susceptible herbivore genotypes within a population (Raffa 1989). Genotypic and functional heterogeneity in induced resistance among poplar clones could therefore be important traits to consider when selecting cultivars for deployment.

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