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ORIGINAL PAPER

Down-regulation of gibberellic acid in poplar has negligible effects on host-plant suitability and insect pest response

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Abstract Endogenous levels and signaling of gibberellin plant hormones such as gibberellic acid (GA) have been genetically down-regulated to create semi-dwarf varieties of poplar. The potential benefits of semi-dwarf stature include reduced risk of wind damage, improved stress tolerance, and improved wood quality. Despite these benefits, modification of growth traits may have consequences for nontarget traits that confer defense against insect herbivores. According to the growth-differentiation balance hypothesis, reductions in growth may shift allocation of carbon from growth to chemical resistance traits, thereby altering plant defense. To date, host-plant suitability and pest response have not been comprehensively evaluated in GA down-regulated plants. We quantified chemical resistance and nitrogen (an index of protein) in GA down-regulated and wild-type poplar (*Populus alba* × *P. tremula*) genotypes. We also evaluated the performance of both generalist (*Lymantria dispar*) and specialist (*Chrysomela scripta*) insect pests reared on these genotypes. Our evaluation of resistance traits in four GA down-regulated genotypes revealed increased phenolic glycosides in one modified genotype and reduced lignin in two modified genotypes relative to the non-transgenic wild type. Nitrogen levels did not vary significantly among the experimental genotypes. Generalists reared on the four GA down-regulated genotypes exhibited reduced performance on only one modified genotype relative to the wild type. Specialists, however, performed similarly across all genotypes. Results from this study indicate that although some nontarget traits varied among GA down-regulated genotypes, the differences in poplar pest susceptibility were modest and highly genotype-specific.

Keywords Genetically modified plants · Gibberellic acid · Nontarget plant defense · Plant–insect interactions · Cottonwood leaf beetle · Gypsy moth

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Introduction

Gibberellins are naturally occurring plant hormones that promote cell growth as well as aspects of flowering and fruiting (Paspatis 1990; Edwards et al. 1993; Yıldırım et al. 2010). Gibberellic acid (GA) is the first gibberellin to be structurally characterized (Takahashi et al. 1955) and is often utilized for crop enhancement. Exogenous application of bioactive GAs increases plant growth and improves fruit quality and yield (Marth et al. 1956; Riley 1987; Wolf and Loubser 1992). Endogenous levels of bioactive GAs, or their signal cascades, may be artificially altered via up- or down-regulation of associated biosynthetic, catabolic, or signaling genes (Ye et al. 2012). Down-regulation of GA action has been used to create dwarf plant varieties. Such plants typically exhibit reduced biomass production but may also exhibit increased root-to-shoot ratio, altered leaf morphology and/or canopy architecture (Busov et al. 2006; Han et al. 2010; Zawaski et al. 2011). Successful down-regulation of GA action has been achieved in trees such as poplar, which are important forestry and biofuel crops. The

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benefits of dwarf tree varieties for use as forestry crops include reduced risk of lodging and reaction wood formation (Busov et al. 2003). Dwarf trees may also be cultivated more densely and harvested more easily, facilitating their use for short-rotation coppice biofuel crops (Busov et al. 2003). Despite the potential benefits of GA down-regulation, however, there is also the potential for negative consequences for nontarget traits.

Consistent with the growth-differentiation balance hypothesis (GDBH), modification of target traits could alter resource trade-offs with nontarget traits, resulting in unanticipated changes in nontarget trait expression (Hjältén et al. 2007; Joshi et al. 2011; Kosonen et al. 2012). The GDBH states that carbon not allocated to growth is available for other traits, such as resistance to herbivores (Loomis 1932; Herms and Mattson 1992). According to this hypothesis, if photosynthesis remains constant carbon may accumulate in plants with reduced growth requirements (e.g., GA down-regulated plants). Once carbon requirements are satisfied for growth, 'excess' carbon may then be shifted toward resistance traits. Net photosynthesis may be altered by exogenous application of GA, although photosynthetic rate is not altered and is thus predicted to be unchanged when GA is genetically down-regulated (Haber and Tolbert 1957; Alvim 1960; Little and Loach 1975). The mechanism of GA action is not fully understood, but clearly involves the induction and inhibition of numerous transcription factors, thus with effects on many downstream metabolic processes (Taiz and Zeiger 2006; de Lucas et al. 2008). The growth-differentiation balance hypothesis is supported by evidence of higher levels of phenolic resistance traits in leaves and roots of GA down-regulated poplar genotypes relative to wild types (Busov et al. 2006). These phenolics confer resistance against many generalist pests, although they may serve as attractants for some specialist pests (Boeckler et al. 2011).

The primary chemical resistance traits utilized by poplar are the phenolic secondary metabolites condensed tannins and phenolic glycosides. Condensed tannins (CTs) deter vertebrate herbivory and have been correlated with reduced larval growth rate, pupal mass and survival of some invertebrate pest species (Bryant et al. 1983; Donaldson and Lindroth 2004; Salminen and Karonen 2011). Phenolic glycosides (PGs) inhibit growth, development and fecundity for many invertebrate pest species (Hemming and Lindroth 1995; Roth et al. 1997; Boeckler et al. 2011). Other limiting factors for insect growth that may be altered by GA down-regulation are lignin, which inhibits digestion, and nitrogen (an index of protein) (Mattson 1980; Scheirs et al. 2001; Ikonen 2002; Ohkuma 2003; Zehnder et al. 2009). As with CTs and PGs, lignin is a carbon-based compound that may be directly affected by changes in carbon allocation due to down-regulation of GA levels. Nitrogen uptake may be affected by changes in root-to-shoot ratio or root architecture, mediated by GA down-regulation (Luis and Guardiola 1981). Alteration of CT, PG, lignin or nitrogen levels, as a by-product of GA down-regulation, may have implications for the performance of key insect pests of poplar.

Poplar is highly susceptible to both generalist and specialist insect pests (Dickmann and Stuart 1983; Robinson et al. 2012). Generalists may feed on a variety of plants and are generally less adapted than specialists to specific chemical resistance traits, whereas specialists feed within a small range of chemically similar plant taxa that they have adapted to (Bowers and Puttick 1988; Bidart-Bouzat and Kliebenstein 2011). Two of the most damaging pests of poplar are the generalist caterpillar gypsy moth (*Lymantria dispar*) (Hu et al. 2001; Coyle et al. 2005) and the specialist cottonwood leaf beetle (*Chrysomela scripta*) (Burkot and Benjamin 1977; Caldbeck et al. 1978; Ye et al. 2012). Gypsy moths are not adapted to high levels of PGs, which have been correlated with negative effects on growth rate and development time (Montgomery 1986; Hemming and Lindroth 2000; Osier et al. 2000). Gypsy moths are not deterred by high levels of CTs (Appel and Maines 1995; Barbehenn and Constabel 2011), however, and may tolerate them by way of high pH or CT-inactivating surfactants in the gut (Salminen and Karonen 2011). In contrast, cottonwood leaf beetle performance correlates negatively with CT concentrations and positively (or not at all) with PG concentrations (Bingaman and Hart 1993; Donaldson and Lindroth 2004; Hjältén et al. 2007). Cottonwood leaf beetles have evolved the ability to sequester PGs, such as salicin, which they convert to salicylaldehyde to repel predators (Pasteels et al. 1983; Bingaman and Hart 1993).

To date, several studies have evaluated biomass production and its allocation among plant tissues in GA down-regulated *Populus* genotypes (Busov et al. 2006; Gou et al. 2010; Zawaski et al. 2011). Few studies have evaluated GA down-regulated genotypes for the expression of nontarget traits that influence resistance or pest performance (Biemelt et al. 2004; Busov et al. 2006; Coyle et al. 2011). If genetic modification of GA levels is to be utilized in crops for enhancement of desirable traits, potential effects on both plant defense and pest response should first be explored. Our study evaluated the impact of GA modification on the principal chemical resistance traits in poplar as well as on the performance of generalist and specialist insect pests of poplar. We predicted that: (1) transgenic genotypes with reduced biomass production would have increased levels of chemical resistance traits and altered levels of nitrogen (i.e., host-plant suitability), and (2) generalist and specialist insect pest performance would vary across genotypes in concert with altered host-plant suitability.

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Materials and methods

We conducted two independent studies in which we bioassayed generalist and specialist poplar pests reared on GA down-regulated and wild-type hybrid poplar. We quantified resistance compounds and nitrogen, and measured insect performance, to determine whether host-plant suitability and insect fitness varied among genotypes. We used chemical profiles, insect performance data and correlations to identify potential mechanisms underlying the performance of each pest on the experimental genotypes.

Plant materials

Our experimental model was hybrid poplar (*Populus alba* × *P. tremula*) clone 717-IB4. Poplar is one of the few taxa that have been genetically modified for altered GA levels (Etherington et al. 2007; Jansson and Douglas 2007). Additionally, the principal chemical resistance traits in *Populus*, CTs and PGs, have been well studied (Philippe and Bohlmann 2007; Chen et al. 2009; Lindroth and St. Clair 2013). Hybrid poplar trees were transformed to create four GA down-regulated genotypes; control trees were non-modified wild types (WT) (Table 1). Genes encoding *gai* (MTG and XG genotypes), *rgl* (RGL) and *GA2-oxidase* (C17) proteins were transformed using a method similar to that described by Busov et al. (2006). The *gai* protein acts as a constitutive repressor of GA signaling, expressed by moderate dwarfing (Peng et al. 1997; Busov et al. 2006). The function of *rgl* proteins is similar to that of *gai,* but with a stronger dwarfing effect (Busov et al. 2006). *GA2-oxidase* catabolizes active GA and can induce severe dwarfing (Busov et al. 2006; Zawaski et al. 2011). Each allele was combined with a 35S promoter except for one *gai* allele (MTG), which was combined with a native *Arabidopsis GAI* promoter. Transformations using the 35S promoter result in stronger phenotypic expression than transformations using the native *Arabidopsis* promoter (Etherington et al. 2007; Elias et al. 2012). We analyzed single transformation events (i.e., gene insertions) from each construct based on previous growth data showing mild but statistically significant semi-dwarfism and modification of GA levels in trees grown in Oregon (Elias et al. 2012).

Table 1 *Populus alba* × *P. tremula* gibberellic acid down-regulated and wild-type (WT) experimental genotypes produced at Oregon State University

Genotype	Promoter	Transgene (origin)	Replicate trees
WT	None	None	8, 7
MTG	Native (*A. thaliana*)	*gai* (*Arabidopsis thaliana*)	7, 7
XG	35S	*gai* (*A. thaliana*)	8, 8
RGL	35S	*rgl* (*A. thaliana*)	8, 6
C17	35S	*GA2-oxidase* (*Populus* sp.)	8, 6

The 'replicates' column indicates number of trees used in gypsy moth and cottonwood leaf beetle bioassay experiments, respectively

Plant chemistry

We chemically analyzed five fully expanded, undamaged leaves within leaf plastochron (development) index of 4–8 on the central leader. Leaves were collected midway through each bioassay. Leaves were clipped at the petiole, vacuum-dried and ground to a coarse particle size in a Wiley Mill (mesh size #20). Coarse-ground leaves were used for lignin analyses, and remaining tissue was ground further by ball milling and used for all other analyses. We quantified CTs spectrophotometrically via a modified acid-butanol method (Porter et al. 1986). Standards used in CT analyses were purified via adsorption chromatography (Hagerman and Butler 1980) from wild-type *P. alba* × *P. tremula* leaves. Qualitative and quantitative variation in PGs was assessed via an ultra high-performance liquid chromatography method (modified from Abreu et al. 2011) with standards purified from *Populus* and *Salix* spp. wild-type leaves.

We report individual PGs (i.e., salicin, salicortin, tremuloidin, hydroxycyclohexane-on-oyl salicortin [HCH-salicortin], tremulacin and 2′-cinnamoyl-salicortin) present in concentrations >0.5 % dry mass and the sum of these values as total PGs. We quantified lignin levels gravimetrically via sequential extraction in a hot acid-detergent solution in an Ankom 200 digestor and incubation in 72 % sulfuric acid bath (Rowland and Roberts 1994). Nitrogen levels were quantified via combustion analysis using a Flash EA1112 C/N analyzer. Levels of chemical compounds are reported as concentrations (percent dry mass).

Bioassays

Generalists (gypsy moths) and specialists (cottonwood leaf beetles) were reared on the various genotypes in independent bioassay experiments. For each bioassay experiment, greenwood cuttings from each genotype were planted outside in 12-l pots containing a 50/50 mixture of silt-loam field soil and sand. We added Nutricote 3–4 month slow release fertilizer (13:13:13 N–P–K + micronutrients) to each pot at a rate of 4.5 g/l of soil and hand-watered pots daily. Gypsy moth bioassay trees were planted in summer 2011, then overwintered and brought into the greenhouse in early spring 2012 to induce leaf flush and initiate bioassays. Larvae were added to trees a week after initial flush to replicate natural phenological synchrony between poplar

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flush and gypsy moth herbivory. Beetle bioassay trees were planted in summer 2012 and then moved to the greenhouse a month after planting to start bioassays. Pots for each bioassay experiment were arranged in a randomized design in a greenhouse at the University of Wisconsin–Madison. The greenhouse environment consisted of natural lighting and a daily ambient temperature range of 20–25 °C.

The gypsy moth experiment consisted of five hybrid poplar genotypes (four modified and one wild type) each with 7–8 replicate trees and five gypsy moths per tree. We obtained late first instar gypsy moth larvae from USDA-APHIS (Otis Air National Guard Base, Buzzards Bay, MA). One week after initial leaf flush we placed five second instars on each tree. Larvae were enclosed in fine mesh nylon bags and allowed to feed until pupation. Pupae were collected from each tree, weighed and kept in 0.24-l plastic deli cups at room temperature until eclosion. Eclosed adults were sexed and paired by host genotype and emergence date. Gypsy moths typically produce their entire complement of eggs within about 24 h. Each pair was allowed to mate for 24 h, after which we counted number of eggs produced by each female. We quantified survival from first instar to adult, development time (second instar to adult eclosion), larval growth rate ([(ln pupal mass−ln average first instar mass)/development time]), pupal mass and fecundity (eggs/female).

The cottonwood leaf beetle experiment consisted of five hybrid poplar genotypes (four modified and one wild type) each with 6–8 replicate trees and five beetles per tree. Beetles used in bioassays were the F_1 generation of individuals collected in the summer of 2012 from *P. deltoides* clone S7C15 in Aiken, SC, by Dr. David Coyle. We enclosed ten first instars on each tree in fine mesh nylon bags following leaf expansion, to mimic natural poplar and cottonwood leaf beetle synchrony. Larvae were allowed to feed until pupation. Pupae from each replicate tree were collected and kept in petri dishes in an incubator (15:9 L:D at 25 °C) until eclosion. Freshly enclosed adults were weighed and sexed, and male/female pairs that were reared on the same poplar genotype with the same emergence date were moved to plastic rearing boxes (19 × 14 × 10 cm). Each mated pair was kept in the incubator and continuously supplied with fresh foliage for 11 days after which we counted number of eggs. We quantified development time (first instar to adult eclosion), larval growth rate, adult mass and fecundity (eggs/day/female during the first 11 days of mating).

Statistical analysis

We tested the effect of genotype on plant chemical traits using a fixed effects model analysis of variance (ANOVA) in JMP Pro 9 (SAS Institute, Inc, Cary, NC, USA). Chemical concentration data were arcsine square root-transformed (arcsine[$\sqrt{}$(% dry mass/100)]) to adjust for non-normality before running ANOVA. We tested the individual and interactive effects of tree genotype and insect sex on insect performance variables using a fixed effects model ANOVA. Insect sex was nested by genotype in ANOVAs. Satterthwaite approximation was used to calculate degrees of freedom for all ANOVAs. An alpha level of 0.05 was considered significant and $0.05 < 0.10$ was considered marginally significant for all statistical analyses. For each significant ANOVA result, we used Tukey's honestly significant difference post hoc tests to determine which modified genotypes differed from the wild type. We identified correlations between means of plant chemical traits and arthropod pest abundance using partial least-squares regression (PLSR) in JMP Pro 10 (Buhl 2013). We determined the number of latent factors for maximum predictive power using predictive residual sum of squares values. The partial least-squares regression model was tested via linear regression of predicted by observed values for each response variable in JMP Pro 10.

Results

Genotype and phenotype expression

Here, we summarize GA expression for each of our modified genotypes as found in other studies. Quantitative real-time PCR analysis of genotype expression indicated low expression of GA down-regulation in MTG and XG relative to the higher levels of expression in RGL and C17 (Elias et al. 2012). Tree stem height and total dry weight biomass were measured to determine phenotype expression. Both height and biomass were lower in RGL and XG relative to MTG and C17, although XG and RGL were the only genotypes to exhibit significantly different stem height ($F_{4,57} = 24.79$, $P = <0.001$) and biomass ($F_{4,57} = 18.99$, $P = <0.001$) relative to the wild type (Buhl 2013). Differences between genotype and phenotype expression within constructs were likely a result of differences in environment, growing conditions and phenological stages of trees at the time of each analysis.

Plant chemistry

Expression of some, but not all, resistance traits varied among GA-modified and wild-type genotypes used in each bioassay experiment (Table 2). In the gypsy moth study, CT levels did not vary significantly among genotypes, although trends indicated 56 % lower CTs in XG and RGL than in the wild type. Variation in total PG levels was marginally significant among genotypes; trends indicated

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Table 2 Summary of analysis of variance (ANOVA) examining the effect of poplar genotype on chemical resistance and nitrogen levels in gypsy moth and cottonwood leaf beetle bioassay experiments

	Condensed tannin			Phenolic glycoside			Lignin			Nitrogen		
	$Df_{n,d}$	F	P	$Df_{n,d}$	F	P	$Df_{n,d}$	F	P	$Df_{n,d}$	F	P
Gypsy moth experiment	4,27	1.45	0.245	4,27	2.38	0.076	4,31	3.61	**0.016**	4,27	2.39	0.079
Cottonwood leaf beetle experiment	4,26	1.44	0.250	4,25	3.13	**0.032**	4,27	1.50	0.229	4,26	0.79	0.544

Statistically significant values are in bold

Table 3 Summary of phenolic glycosides (PGs) found in poplar genotypes used in gypsy moth and cottonwood leaf beetle bioassays

	Salicin	Salicortin	Tremuloidin	HCH-salicortin	Tremulacin	2′-Cinnamoyl-salicortin
Gypsy moth experiment						
WT	0.43 ± 0.22	2.26 ± 0.74	0.20 ± 0.06	0.36 ± 0.23	2.55 ± 0.60	0.09 ± 0.01
MTG	0.36 ± 0.08	1.75 ± 0.25	0.20 ± 0.02	0.24 ± 0.06	1.98 ± 0.18	0.09 ± 0.00
XG	0.33 ± 0.10	2.60 ± 0.35	0.28 ± 0.01	0.23 ± 0.06	3.13 ± 0.38	0.09 ± 0.01
RGL	0.18 ± 0.06	2.09 ± 0.27	0.29 ± 0.02	0.23 ± 0.06	3.03 ± 0.24	0.09 ± 0.01
C17	0.58 ± 0.10	2.18 ± 0.40	0.19 ± 0.02	0.43 ± 0.07	2.85 ± 0.38	0.09 ± 0.00
Cottonwood leaf beetle experiment						
WT	0.49 ± 0.10	1.73 ± 0.26	0.15 ± 0.03	0.52 ± 0.07	2.93 ± 0.42	0.09 ± 0.01
MTG	0.67 ± 0.07	1.87 ± 0.32	0.20 ± 0.03	0.63 ± 0.06	3.55 ± 0.37	0.09 ± 0.00
XG	0.83 ± 0.13	2.85 ± 0.33	0.23 ± 0.02	0.57 ± 0.14	4.14 ± 0.34	0.09 ± 0.00
RGL	0.54 ± 0.09	1.60 ± 0.59	0.16 ± 0.01	0.57 ± 0.09	2.64 ± 0.64	0.09 ± 0.00
C17	0.72 ± 0.06	2.24 ± 0.42	0.20 ± 0.01	0.67 ± 0.07	3.67 ± 0.38	0.09 ± 0.00

Mean percent dry weight and standard errors are reported for PGs within each line for each bioassay

22 % lower total PGs in MTG than in the wild type. Post hoc tests indicated that levels of HCH-salicortin varied significantly among genotypes ($F_{4,23} = 4.17$, $P = 0.011$) but not between modified and wild-type genotypes. Levels of individual PGs in each genotype for each insect experiment are listed in Table 3. Lignin levels varied significantly among genotypes; post hoc tests indicated 32 % lower lignin in MTG and RGL relative to the wild type (Fig. 1). Variation in nitrogen levels was marginally significant among genotypes; trends indicated 21 % higher nitrogen in RGL relative to the wild type.

In the cottonwood leaf beetle study, levels of CTs did not vary significantly among genotypes (Table 2). Variation in total PG levels was significant among genotypes; total PG levels were 34 % higher in XG than in the wild type (Fig. 1). Similar to the gypsy moth study, levels of HCH-salicortin varied significantly among genotypes; post hoc tests indicated that HCH-salicortin levels were 59 % higher in XG than in the wild type ($F_{4,21} = 2.81$, $P = 0.052$). Levels of lignin and nitrogen did not vary significantly among genotypes.

Insect performance

All gypsy moth performance variables, except for survival and fecundity, varied significantly among genotypes (Table 4). Survival did not vary significantly among genotypes, but trends indicated 35 % higher survival in gypsy moths reared on XG relative to those on the wild type (Fig. 2). Post hoc tests indicated 19 % lower larval growth rate, 26 % lower pupal mass and 9 % longer development time in gypsy moths reared on C17 relative to those on the wild type (Fig. 3). Fecundity did not vary significantly among moths reared on different genotypes, but moths reared on C17 had 33 % lower fecundity relative to those on the wild type (Fig. 4). Sexual dimorphism of gypsy moths was consistent with expected differences between sexes; females exhibited larger pupal mass and longer development time than males (Fig. 3). Gypsy moth performance on the various tree genotypes did not differ significantly between females and males (no significant genotype × sex interactions). Most gypsy moth performance variables correlated negatively with CTs, PGs and lignin and positively with nitrogen (Table 6), although predictive ability (i.e., R^2 values) of PLSR tests was low.

Cottonwood leaf beetle performance did not vary significantly among genotypes (Table 5). Cottonwood leaf beetles exhibited predicted differences between the sexes; larval growth rate and adult mass were higher in females than in males (Fig. 3). Cottonwood leaf beetle performance on the various tree genotypes did not differ significantly between females and males (no significant genotype × sex interactions). Most cottonwood leaf beetle performance

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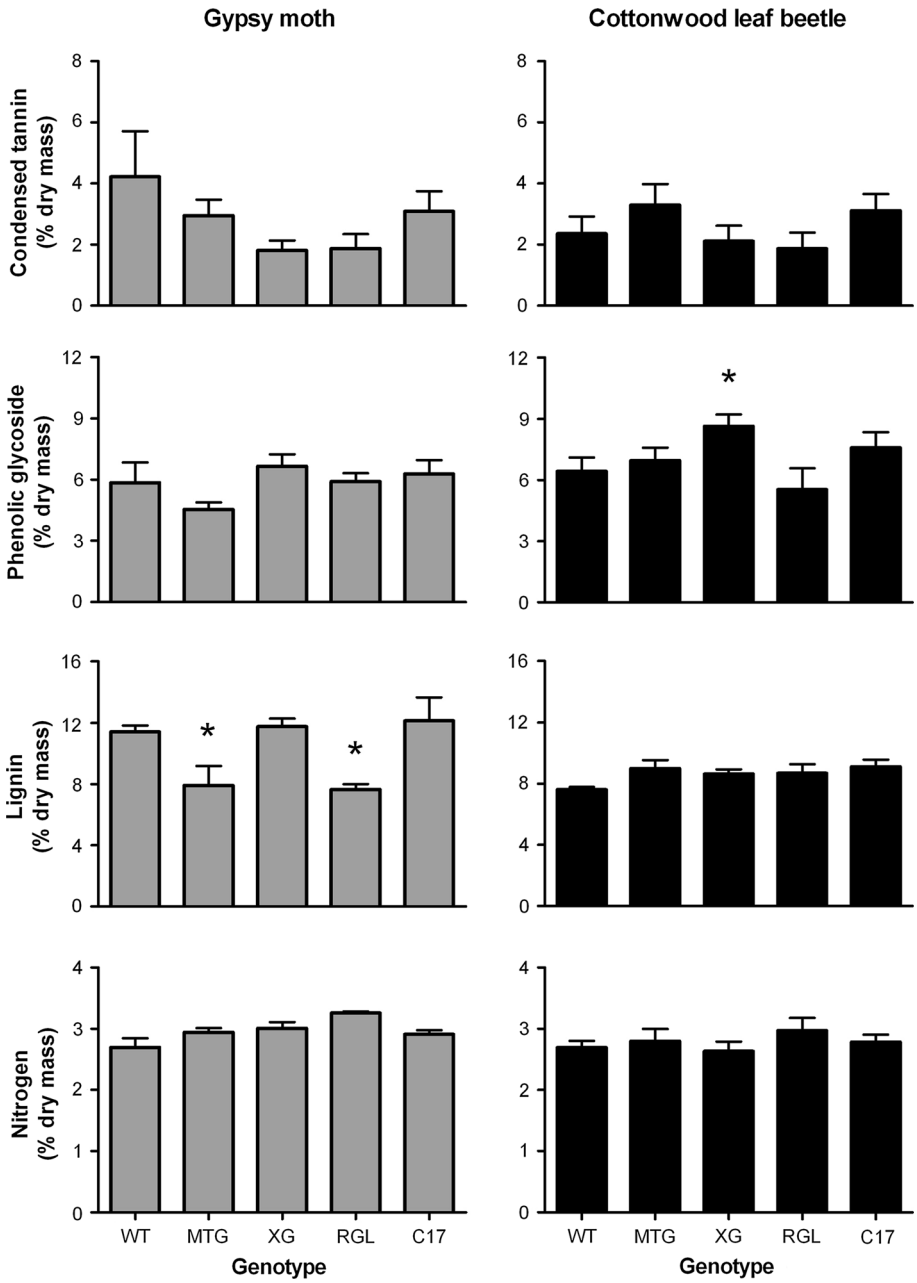

Fig. 1 Chemical profiles of experimental poplar genotypes used in each insect bioassay experiment. *Bars* represent mean levels of plant chemical traits in each genetically modified or wild-type (WT) tree genotype ($n = 7$–8 replicate trees, *error bars* represent +1 SE). *Asterisks* denote modified genotypes that vary significantly from the wild type, as indicated by post hoc tests

Table 4 Summary of ANOVAs examining the effects of poplar genotype, insect sex and their interaction (G × S) on gypsy moth performance

	Survival			Development time			Pupal mass		Larval growth rate		No. eggs/female	
	$Df_{n,d}$	F	P	$Df_{n,d}$	F	P	F	P	F	P	F	P
Genotype	4,32	0.65	0.632	4,4	2.64	**0.050**	2.66	**0.048**	4.14	**0.007**	1.78	0.189
Sex				1,1	19.66	**<0.001**	51.23	**<0.001**	2.27	0.140		
G × S				4,4	0.90	0.473	0.51	0.727	0.42	0.797		

Statistically significant values are in bold

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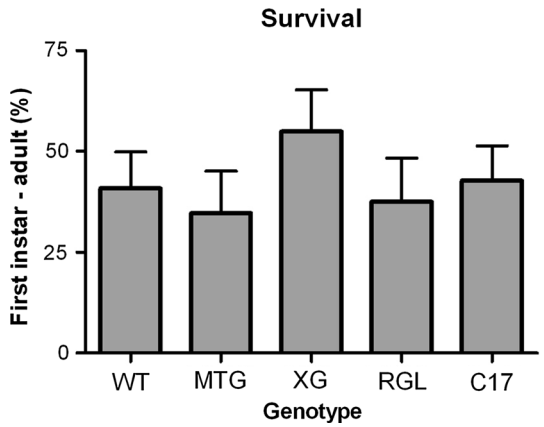

Fig. 2 Gypsy moth survival on the various experimental poplar genotypes. *Bars* represent mean percent survival of insects reared on each genetically modified or wild-type (WT) poplar genotype ($n = 7$–8 replicate trees, *error bars* represent +1 SE)

variables related negatively to CTs, PGs and lignin, and positively to nitrogen (Table 6). As with PLSR tests of gypsy moth performance, predictive ability of PLSR tests for leaf beetles was low.

Discussion

Down-regulation of GA had modest and inconsistent effects on plant chemical traits and insect pest performance. Levels of PGs and lignin were altered in some, but not all, modified genotypes relative to the wild type. Generalist performance was lower on one modified genotype relative to the wild type, but specialist performance did not differ significantly among genotypes. Effects of GA down-regulation on host-plant suitability were minimal, and therefore, pest response was also negligible.

GA down-regulation was expected to result in reduced biomass production. Only XG and RGL, however, had significantly reduced biomass production relative to the wild type. We predicted that down-regulation of GA levels would result in reduced biomass production and thus shift allocation of carbon from growth to carbon-based resistance traits. Contrary to that prediction, most GA down-regulated genotypes with reduced biomass production did not express increased herbivore resistance or significantly altered nitrogen levels. In further contradiction to our prediction, we found that biomass production and herbivore resistance did not vary between C17 and the wild type, but insect response did vary. C17 has been shown to express lower GA levels than our other modified genotypes as well as the wild type (Elias et al. 2012). The absence of phenotypic expression in C17 may indicate gene silencing by the 35S promoter (Mishiba et al. 2005), and therefore, changes in herbivore resistance would not be expected. As we discuss below, however, insect response to C17 did in fact vary from response to the wild type.

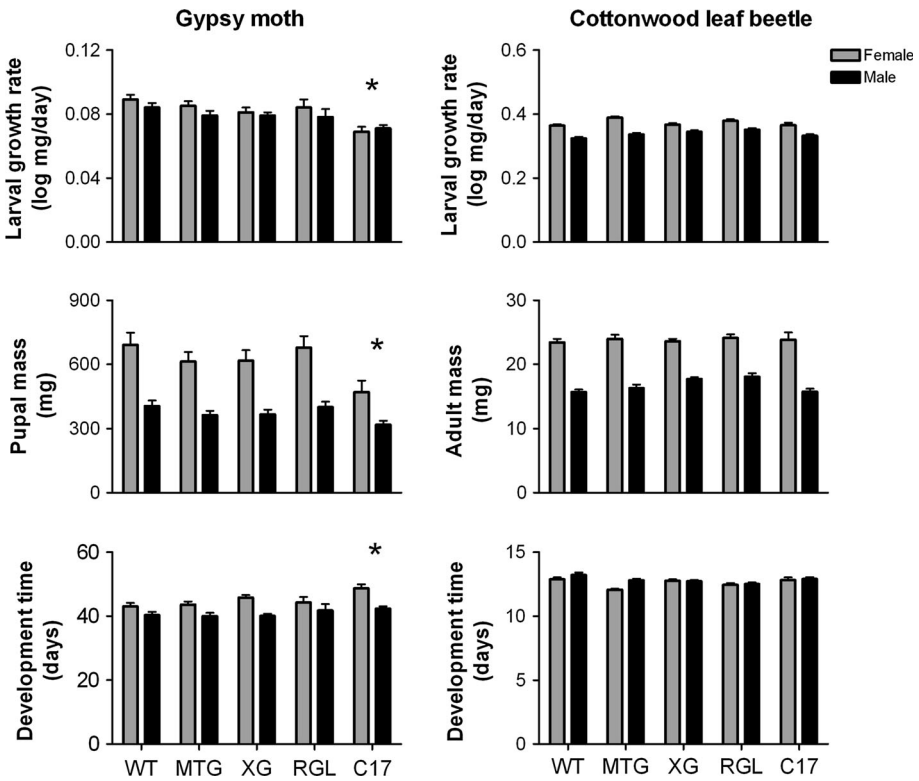

Fig. 3 Gypsy moth and cottonwood leaf beetle performance on the various experimental poplar genotypes. *Bars* represent mean performance of insects reared on each genetically modified or wild-type (WT) poplar genotype ($n = 6$–8 replicate trees, *error bars* represent +1 SE). *Asterisks* denote modified genotypes that resulted in significantly different insect performance from performance on the wild type, as indicated by post hoc tests

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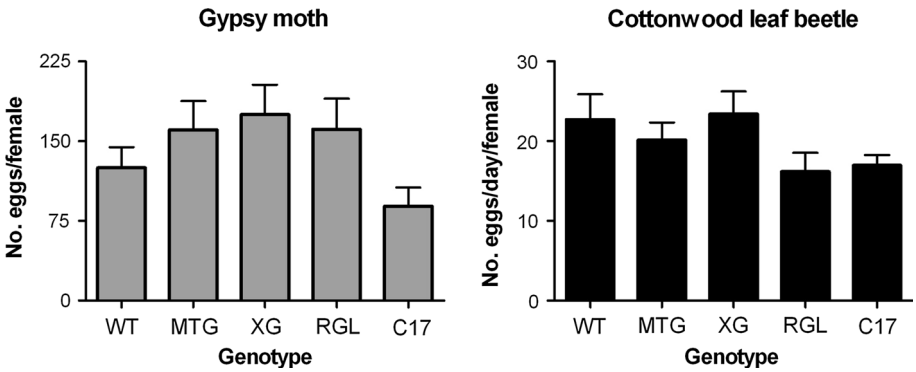

Fig. 4 Gypsy moth and cottonwood leaf beetle fecundity on the various experimental poplar genotypes. *Bars* represent mean fecundity of insects reared on each genetically modified or wild-type (WT) poplar genotype ($n = 6$–8 replicate trees, *error bars* represent +1 SE)

Table 5 Summary of ANOVAs examining the effects of poplar genotype, insect sex and their interaction (G × S) on cottonwood leaf beetle performance

	Development time			Adult mass		Larval growth rate		No. eggs/day	
	$Df_{n,d}$	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Genotype	4,4	1.18	0.335	1.33	0.274	1.77	0.155	1.42	0.255
Sex	1,1	1.68	0.202	180.87	**<0.001**	49.36	**<0.001**		
G × S	4,4	0.63	0.644	0.54	0.708	1.01	0.414		

Statistically significant values are in bold

Table 6 Summary of weighted coefficients calculated by partial least-squares regression (PLSR), relating poplar chemical traits to insect performance in the bioassay experiments

	Gypsy moth				
	Survival	Larval growth rate	Pupal mass	Development time	Fecundity
Condensed tannin	0.014	−0.220	−0.221	0.187	−0.068
Phenolic glycoside	0.006	−0.090	−0.090	0.076	−0.028
Lignin	0.031	−0.467	−0.470	0.398	−0.145
Nitrogen	−0.024	0.361	0.363	−0.308	0.112
	Cottonwood leaf beetle				
	Larval growth rate	Adult mass	Development time	Fecundity	
Condensed tannin	−0.251	−0.281	0.225	0.291	
Phenolic glycoside	−0.151	−0.169	0.135	0.175	
Lignin	0.169	0.189	−0.151	−0.196	
Nitrogen	0.233	0.260	−0.208	−0.269	

Negative and positive values indicate direction of relationship

Our first prediction was prompted by observations of increased PGs (salicin and tremulacin) in *GAI* and *RGL* down-regulated genotypes (MTG, XG and RGL) relative to the wild type (Busov et al. 2006). We observed higher PG levels in only XG relative to the wild type. Contrary to results in Busov et al. (2006), HCH-salicortin, but not salicin or tremulacin, was higher in XG than in the wild type. Altered lignin levels have also been identified in plants with modified GA levels. Biemelt et al. (2004) reported that up- and down-regulated GA levels were paralleled by increases or decreases, respectively, in lignin levels of tobacco (*Nicotiana tabacum*). Our results support that finding; we identified lower levels of lignin in MTG and RGL relative to the wild type. Biemelt et al. (2004) also found parallel expression in enzymes that catalyze synthesis of lignin but no change in an enzyme (phenylalanine ammonia-lyase) near the branchpoint between GA and lignin pathways. This last finding may indicate that carbon trade-offs are not occurring between GA (i.e., methylerythritol phosphate) and phenylpropanoid pathways. Overall, we found little evidence that down-regulation of GA levels also results in substantially altered chemical resistance or nitrogen levels.

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Contrary to our second prediction, pest response did not substantially vary among genotypes as a result of altered plant chemistry. We predicted poor gypsy moth performance on genotypes expressing high levels of PGs or lignin and superior performance on genotypes expressing high levels of nitrogen. Gypsy moth performance was significantly altered on only one modified genotype relative to the wild type. Larvae reared on C17 exhibited lower growth rate and pupal mass and longer development relative to those reared on the wild type. Fecundity was also lower in gypsy moths reared on C17 relative to those reared on the wild type, although not significantly. C17 expressed moderate PGs and high lignin; although not significant, these resistance traits may have contributed to poor gypsy moth performance. Our results indicate that C17 is the least suitable host-plant genotype for gypsy moths, although performance was not dramatically reduced. Correlation analyses across all genotypes revealed inverse relationships between PGs and lignin with gypsy moth performance. Previous studies have identified reduced performance in gypsy moths reared on host plants with higher levels of PGs and/or lignin relative to controls (Hemming and Lindroth 1995; Osier et al. 2000; Osier and Lindroth 2001; Couture et al. 2012). Another study that evaluated the performance of a generalist weevil (*Polydrusus sericeus*) on these genotypes also found little effect of GA down-regulation on performance (Coyle et al. 2011). Lastly, we were surprised to find that although phenotypic expression of altered GA levels was similar between C17 and the wild type, pest response differed between the two. Gypsy moth performance may have been affected by factors other than the resistance traits and nutritive factors we measured, such as moisture content and green leaf volatiles.

Cottonwood leaf beetle performance was more consistent across the various genotypes than that of gypsy moths. We predicted poor cottonwood leaf beetle performance on genotypes expressing high levels of CTs or lignin and good performance on genotypes expressing high levels of PGs or nitrogen. Results from the beetle experiment indicate that all of the experimental genotypes are equally suitable host-plants for cottonwood leaf beetles. Beetle performance did not respond to resistance and nutritional traits as predicted. Trends indicated higher CTs in MTG and C17, but beetle performance was not lower on those genotypes relative to the wild type. Correlation analyses across all genotypes, however, supported predictions that CTs would reduce performance and nitrogen would increase performance.

Independent correlation analyses conducted for gypsy moths and cottonwood leaf beetles indicated several relationships that contradict our predictions and results from previous studies. For example, we predicted a null relationship between CTs and gypsy moth performance, but observed a negative relationship. Poplar typically expresses CT levels within the range of 1–18 % dry mass (Lindroth and Hwang 1996). Even when CT levels are at the higher end of this range, gypsy moths are not deterred (Hwang and Lindroth 1997; Osier et al. 2000). Our trees expressed CT levels at the low end of that range (1.5–4 % dry mass), which suggests that CTs had little influence on gypsy moths and perhaps another factor was responsible for variation in performance. Levels of CTs are often inversely correlated with levels of PGs, which can confound correlations. In our study, however, there was no significant correlation between levels of CTs and PGs. We also predicted that PGs would relate positively and lignin would relate negatively to beetle performance, but we observed the opposite. Gypsy moth and cottonwood leaf beetle performance did not substantially vary among genotypes, and therefore, performance could not be reliably related to chemical resistance.

Modification of target plant traits has the potential to alter nontarget traits that determine plant defense and/or pest performance in trees (Hjältén et al. 2008; Vauramo et al. 2006). In this study, we evaluated the influence of genetic modification on nontarget traits that influence host-plant suitability of poplar, a forestry and bioenergy crop species. Alteration of plant resistance traits is particularly relevant in crops wherein reduced resistance can result in decreased yields or increased pesticide use (Oerke 2006; Post and Parry 2011). Results from this research demonstrate that genetic modification of growth traits in young *Populus* may affect several important nontarget traits such as lignin, nitrogen and herbivore deterrent chemicals. We found, however, that down-regulation of gibberellic acid did not result in appreciably altered host-plant suitability or pest performance.

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