Altered *GAI* activity of hybrid aspen has minimal effects on the performance of a polyphagous weevil, *Polydrusus sericeus*

David R. Coyle[1]*, Angela M. Allred[2], Kevin R. Kosola[2†] & Kenneth F. Raffa[1]

[1]Department of Entomology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA, and [2]Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706, USA

Abstract

Genetically modified plants can have unintended effects on herbivores, even when the transgene is not explicitly devised for pest protection. These effects may lead to altered ecological relationships in areas where transgenic plants are deployed. We tested several genetically-modified hybrid aspen clone INRA 717-IB4 [*Populus tremula* L. × *P. alba* L. (Salicaceae)] lines expressing transgenic constructs with altered gibberellic acid expression for their effects on a polyphagous insect, *Polydrusus sericeus* (Schaller) (Coleoptera: Curculionidae). Longevity, egg production, and leaf consumption were measured in laboratory assays. Only male longevity was negatively affected by foliage from genetically modified trees, and in no case was oviposition or leaf consumption significantly altered. The importance of males in population dynamics varies among species, but we do not have evidence it is a major driver in this system. Contrary to our hypothesis of increased performance on modified foliage, adult *P. sericeus* were rarely affected by GM foliage, suggesting that not all genetic modifications that alter secondary metabolism in plants will necessarily have a strong effect on specific insect-plant relations, supporting the view that evaluations continue to require case by case analysis.

Introduction

Trees in the genus *Populus* (Salicaceae) are excellent candidates for transgenic modification, due to a variety of physiological characteristics (Cheng & Tuskan, 2009; Ralph, 2009), operational considerations (Stanturf et al., 2001), and a recently sequenced genome (Tuskan et al., 2006). Several traits have been expressed through genetic modification, beginning with the *Bacillus thuringiensis* endotoxin (McCown et al., 1991; Kleiner et al., 1995), each with the potential to address particular problems confronting short rotation plantation forestry. Genetically modifying trees can result in increased energy resources through pulp and biofuel production, increased pest resistance (Delledonne et al., 2001; Frankenhuyzen & Beardmore, 2004), and

phytoremediation of contaminated soils (Strauss et al., 2001; Gartland et al., 2003; Henderson & Walter, 2006).

Recently, genes that control the gibberellic acid (GA) response in *Populus* spp. have been modified to generate traits deemed desirable for short rotation plantation systems, including dwarfing, changes in crown architecture, and altered carbon allocation and partitioning (Busov et al., 2003, 2006; Etherington et al., 2007). Semi-dwarf trees could be planted at high density with reduced etiolation (Busov et al., 2003). Increased biomass allocation to roots and increased concentrations of secondary metabolites in roots or leaf litter could increase the residence time of plant carbon (C) in the soil, and potentially increase the rate of C flux into recalcitrant soil C pools (Walmsley & Godbold, 2010). Collectively, these traits could provide substantial improvements in C sequestration potential.

The potential to affect non-target organisms is a factor of concern regarding the large-scale deployment of transgenic plants (Cowgill & Atkinson, 2003). Non-target effects are often difficult to predict. For instance,

*Correspondence: David R. Coyle, D.B. Warnell School of Forestry and Natural Resources, 180 Green St., Athens, GA, 30602, USA. E-mail: drcoyle@uga.edu
†Current Address: Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA

transgenic maize containing the *B. thuringiensis* gene for pest control had slower decomposition rates than their non-transformed counterparts (Flores et al., 2005), and glyphosate-resistance in soybean [*Glycine max* (L.) Merrill] led to an increased susceptibility to sudden death syndrome caused by the soil pathogen *Fusarium solani* Snyder & Hansen (Sanogo et al., 2000). Few studies have examined how transgenes that were not intended for pest resistance might affect ecological interactions with herbivorous species. The glyphosate-resistance trait in soybean has been shown to increase susceptibility to the root knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood (Colyer et al., 2000). Brodeur-Campbell et al. (2006) measured gypsy moth herbivory on transgenic aspen foliage that had been modified for low levels of lignin production in stem wood. Their results were mixed, as one of four transgenic lines reduced gypsy moth larval survival, and caterpillars showed no preference between transformed and nontransformed trees. Thorough testing is required to fully elucidate the potential environmental effects of transgenic plants (O'Callaghan et al., 2005; Henderson & Walter, 2006).

We investigated the effects of GA modifications in *Populus tremula* L. × *P. alba* L. (Salicaceae) leaves on adult performance of the weevil *Polydrusus sericeus* Schaller (Coleoptera: Curculionidae). *Polydrusus sericeus* is a common invasive herbivore in aspen stands and northern hardwood forests in the Great Lakes region of North America. Adult *P. sericeus* emerge from the soil in late spring, mate, and oviposit in the soil. Larvae are rhizophagous, and develop belowground through the winter. Adults live for several weeks and feed on several host species (Coyle et al., 2008, 2010b). Altered GA in our study plants led to increased foliar concentrations of several phenolic compounds, including tremulacin, salicin, and salicylic acid (Busov et al., 2006). Some folivores, especially beetles, have shown an increased feeding preference or fitness on foliage containing elevated levels of phenolic compounds (Tahvanainen et al., 1985; Ikonen, 2002; Donaldson & Lindroth, 2004). Therefore, we hypothesized

that *P. sericeus* would exhibit increased performance and leaf area consumption on trees expressing a gene blocking GA signaling compared with control trees and trees overexpressing a wild-type GA signaling gene.

Materials and methods

Plant material

Agrobacterium tumefaciens Smith & Townsend strain C58 was used to transform *Arabidopsis* genes into the hybrid aspen clone INRA 717-IB4 (*P. tremula* × *P. alba*). The transgenic constructs were prepared at Oregon State University using a method similar to that described in Busov et al. (2006). Three transgenic constructs with altered *GAI* activity were prepared by combining one of two *GAI* (*GAI* and *gai*) alleles with one of two promoter sequences (CaMV 35S and the native *Arabidopsis* promoter; Table 1). *GAI* encodes a putative transcriptional regulator that suppresses GA signaling in the absence of GA and allows GA signaling in the presence of GA (Peng et al., 1997; Boss & Thomas, 2002; Petty et al., 2003). The mutant allele, *gai*, lacks the DELLA region, is insensitive to GA, and therefore has previously been shown to constitutively repress GA signaling (Peng et al., 1997; Phillips, 2004; Busov et al., 2006). Because an ultimate downstream effect of GA signaling is an increase in plant height, *GAI* plants were expected to be 'normal,' and *gai* plants were expected to be dwarfed (Busov et al., 2006).

Of the three constructs, only one (D1) contained the wildtype *Arabidopsis GAI* gene. Under control of the constitutive 35S promoter, this construct overexpressed the wildtype *GAI*. The two other constructs expressed the *gai* allele, one (XG) under control of the 35S promoter and the other (Mtg62) under control of the native *Arabidopsis* promoter. Each event is a unique DNA recombination that took place in a single plant cell. These cells, or events, were then used to generate entire transgenic plants. Each transgenic plant was then used to generate multiple clone plants from the parent plant, and we then tested these clones in our assays (Table 1).

Table 1 Constructs and events used in the experimental plant material. Construct D1 contains the native *Arabidopsis GAI* gene. Constructs XG and Mtg62 both contain the mutated *GAI* gene lacking the DELLA region. All constructs were inserted into an INRA 717-IB4 background. n represents the number of experimental trees from each event used in the study

Group	Construct (promoter::gene)/Construct ID	Event	n
Transformed	35S::*GAI* (*Arabidopsis*)/D1	135	2
Transformed	35S::*gai* (*Arabidopsis*)/XG	115	2
Transformed	35S::*gai* (*Arabidopsis*)/XG	10-2	3
Transformed	Native *GAI* (*Arabidopsis*):*gai*(*Arabidopsis*)/Mtg62	562-1	6
Control	None/Control	Control	4

Plants were established from dormant shoot cuttings taken from plants grown in raised beds for one season. Ten-cm long cuttings were prepared with two 2.5-cm long wounds at the base, dipped in 0.1% indole butyric acid solution, and placed in damp silica builder's sand. Cuttings were placed in a cold frame with bottom heat (21–26 °C) between 29 March and 3 May 2007, then repotted into 3.8-l containers filled with silica sand. Plants were placed outside to harden off, and fertilized weekly for 1 month with Peter's Professional 20-10-20 plus micronutrients fertilizer (The Scotts, Marysville, OH, USA) diluted to 200 p.p.m. N. Trees were then repotted into a Metro-Mix 350:peat medium (3:1) (Sun Gro Horticulture, Bellevue, WA, USA).

Insects

Mating pairs of adult *P. sericeus* were hand captured from paper birch (*Betula papyrifera* Marsh.) trees near Rhinelander, WI, USA (45°36′N, 89°30′W) on 26 June 2007. Each weevil pair was placed in a separate vial and starved for 24 h prior to the assay, and only active, apparently healthy weevils were used. These weevils likely had some feeding experience prior to collection, but this was minimized by timing the collection date to be shortly after the narrow window of adult emergence from the soil (Coyle, 2010). Although we cannot know the feeding or mating history of *P. sericeus* collected under natural conditions, the short emergence period exhibited by *P. sericeus* (Coyle, 2010) allowed us to focus our adult collection shortly after emergence, when adults have presumably had little opportunity to feed. This collection method does not appear to diminish subsequent ability to show inter- and intra-specific preference and performance in laboratory assays (Pinski et al., 2005; Coyle et al., 2010b; Hillstrom et al., 2010).

Experimental design

We conducted three individual Petri dish assays (subsamples) from each experimental tree (Table 1). Three leaves from each tree from the leaf plastochron index (LPI) 2-4 (Larson & Isebrands, 1971) were cut at the base of the petiole, placed in individual Ziploc® bags, and transported to the laboratory on ice. The leaf plastochron index states that the first fully expanded leaf at the terminal end of a shoot is termed LPI 1, with each successively older leaf on that shoot numbered consecutively. Leaves were digitally scanned before assays using WinFOLIA Pro software (Régent Instruments, Quebec, Canada). A moistened cotton ball was wrapped around the base of each petiole, and each leaf was placed in a Petri dish (15 cm diameter, 1.5 cm high) lined with moistened Whatman no. 1 filter paper. One apparently healthy pair of mating *P. sericeus* and a Kimwipe® tissue (to serve as an oviposition site;

Pinski et al., 2005) were placed into each Petri dish. Kimwipe® tissues and foliage were replaced every 5–7 days. Leaves were not noticeably desiccated upon removal, and appeared to retain adequate turgor pressure. Leaves were again digitally scanned upon removal from assays. All dishes were held at a regime of L16 (24 °C):D8 (18 °C) hours.

Dishes were checked at least every other day for weevil mortality and oviposition. In all cases, the female was larger than the male, allowing us to determine both female and male longevity. Leaf consumption measurements were recorded until both weevils had died. Leaf area was calculated as initial leaf area minus leaf area when removed from the dish. We ceased recording oviposition after the female died. Eggs on the Kimwipe® tissues were counted under a dissecting microscope.

Statistical analysis

Female and male longevities were recorded, and total longevity was the sum of the two. Total egg production was recorded, and egg production rate was calculated as the number of eggs divided by the number of days the female was alive. Leaf area consumption rate was the total amount of leaf area eaten divided by the number of days the female was alive. Longevity data, total egg production, and total leaf area consumed data did not require transformation to achieve normality. Prior to analysis, egg production rate and leaf area consumption rate were square-root and log transformed, respectively, to achieve normality. Data were analyzed using an ANOVA (Proc GLM; SAS Institute, Version 8.1, Cary, NC, USA) with tree group (control or transformed), construct, and event (Table 1) as fixed factors. Means were compared using Tukey's HSD test with a Bonferroni correction, with an α value of 0.05 to denote significance.

Results and discussion

Overall, there were little to no effects of transgenic foliage on the performance of adult *P. sericeus*. Males lived longer on control trees than on trees having construct XG (Table 2). Males can have a substantial effect on the population dynamics of a species (Rankin & Kokko, 2007), but we were unable to determine the population-level impact of a shorter male lifespan in our study. Increased male longevity in sexually reproducing species such as *P. sericeus* could indirectly contribute to increased population levels (Rankin & Kokko, 2007; Schmickl & Karsai, 2010). Increased male longevity was also observed for *Phyllobius oblongus* (L.), a contemporary species in this complex of invasive weevils (Coyle et al., 2010a). None of the measured variables differed significantly among events (Table 2). The effects of genetic transformation were

Table 2 *Polydrusus sericeus* adult performance parameters (mean ± SE) and statistical comparison of control vs. GM *Populus* trees. Three genetically modified constructs, Mtg62 (n = 18), XG (n = 13), and D1 (n = 6) were created by combining one of two mutant alleles with one of two promoters (Table 1). Control (n = 12) plants were not modified. All events (n = 49) were analyzed to determine whether any one particular tree affected weevil performance

Performance parameter	Construct				F	P	Event	
	Control	Mtg62	XG	D1			F	P
Total longevity (days)	33.9 ± 2.8	29.9 ± 3.2	22.9 ± 2.3	32.0 ± 9.8	1.58	0.21	1.32	0.28
Female longevity (days)	11.0 ± 1.8	11.6 ± 1.3	15.3 ± 2.4	19.5 ± 4.9	2.37	0.083	1.96	0.12
Male longevity (days)	22.9 ± 3.6a	18.3 ± 3.4ab	7.6 ± 2.8b	12.5 ± 6.3ab	3.22	0.031	2.38	0.066
Total egg production	219.7 ± 75.4	203.1 ± 41.0	341.1 ± 75.5a	307.0 ± 71.0	1.15	0.34	1.86	0.13
Egg production rate (eggs/day)	14.9 ± 4.4	15.4 ± 2.1	22.3 ± 3.1	20.0 ± 5.1	1.14	0.34	1.76	0.15
Total leaf area consumed (cm^2)	7.3 ± 1.2	6.9 ± 1.0	8.9 ± 1.5	8.9 ± 1.1	0.73	0.54	0.71	0.59
Leaf area consumption rate (cm^2/day)	0.3 ± 0.15	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1.72	0.18	1.27	0.30

Means within a row sharing a letter are not significantly different (Tukey's HSD test: P>0.05).

variable, but in general insects fed transgenic foliage had a trend towards longer longevity, and greater egg production and leaf consumption. Our data provide evidence that not all genetic modifications that alter secondary metabolism in plants will affect insect-plant relations.

Transgenic modification of poplars, and the subsequent influence on plant secondary metabolites, does not consistently affect herbivore performance. For example, poplar foliage containing a *B. thuringiensis* gene increased mortality in five lepidoteran species, but the efficacy of control varied greatly among insect species, insect life stage, and year tested (Yang et al., 2003). An increased feeding preference for or performance on foliage containing elevated levels of phenolic compounds has been demonstrated by some coleopteran herbivores (Martinsen et al., 1998) including the weevil *Phyllobius pyri* (L.) (Freiwald et al., 2008). However, neither over-expression of *GAI* nor over-expression of *gai* (GA insensitivity) in *P. tremula × P. alba* led to differences in *P. sericeus* survival, reproduction, or foliage consumption relative to unmodified plants, indicating that phenolic concentrations did not appear to influence *P. sericeus* performance.

Few studies other than this one have examined the effect of transgenes in *Populus* that were not intended for pest resistance on insects. Brodeur-Campbell et al. (2006) measured gypsy moth herbivory on transgenic aspen that had been modified for low levels of lignin production. Their results were mixed, as only one transgenic line resulted in reduced gypsy moth larval survival, and caterpillars showed no preference between transformed and non-transformed trees. Because the response of herbivores to transgenic plants depends on the insect species, parameter measured, and the transgene itself, our results cannot be extrapolated to other insect species or systems. Rather, these results contribute to a growing body of knowledge

on the applicability of transgenic approaches to short rotation plantation trees, and support the view that both target and non-target effects on insect performance need to be evaluated on a case-by-case basis.

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