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Improved growth and weed control of glyphosate-tolerant poplars

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Abstract We studied the impact of glyphosate tolerance on weed control and tree growth in field-grown transgenic poplars. Using *Agrobacterium*-mediated transformation, we produced 94 transgenic transformation events in four hybrid genotypes (three *Populus trichocarpa* × *P. deltoides* and one of *P. trichocarpa* × *P. nigra*). These lines were screened for high levels of tolerance in two plantations in Oregon. Based on screening results, we propagated four lines from two hybrid genotypes to study their value for weed control and productivity in a 2-year management trial in eastern Oregon, comparing conventional weed control at the time of the study to methods that included over-the-top applications of glyphosate during the growing season. Herbicide tolerance was stable in all of the trees over the 2-year period. Weed control, based on weed abundance, was substantially improved in the over-the-top application. Growth of the trees, as measured by stem volume index, was correspondingly improved; transgenic trees grew approximately 20 % faster than the transgenic and non-transgenic control trees. An exploratory life-cycle analysis of the embodied greenhouse-gas benefits for a coppice bioenergy plantation

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suggested that over a 6-year rotation with three coppice cycles, the growth improvement could provide an ~8 % savings in greenhouse gas emissions per unit of wood produced. Despite the potential benefits, adoption of this technology will depend on compatibility with management regimes, regulatory and market acceptance, and probably also the development of a robust transgene containment system.

Keywords *Populus* · Roundup · Genetic engineering · Herbicide tolerance · LCA · Forest biotechnology

Introduction

To accommodate the growing demand for wood products and bioenergy, fast growing woody crops are being cultivated in short-rotation intensive culture (SRIC) plantations in many countries around the world (Hinchee et al. 2011; AHB 2014). Poplars are excellent for SRIC because they are fast growing under intensive management, amenable to coppice culture, and have diverse end-uses. For example, their short fibers can be used to produce high-quality paper, their wood has lower bleaching requirements than many other tree species, and their wood is suitable for production of bioethanol and other bioenergy products (Withrow-Robinson et al. 1995; Kauter et al. 2003). In addition, poplars lend themselves to innovative breeding and biotechnology because of their excellent genomic resources, including a high-quality reference genome, and their amenability to genetic transformation (Tuskan et al. 2006; Ellis et al. 2010).

High levels of growth and survival are expected from the trees grown under SRIC, which strongly depends on efficient weed management (Singh 2008). Poplar growth can be severely stunted by competing vegetation (Marino and Gross 1998). However, poplars are susceptible to many of the commonly used broad-spectrum, post-emergent herbicides, so growers generally rely on combinations of pre-emergent herbicides, hooded sprayers, and tilling to control weeds (Meilan et al. 2000). To help achieve cost-effective weed control, herbicide tolerance may be a useful trait in SRIC plantations.

The development of genetically modified (GM) herbicide-tolerant poplars would enable growers to select herbicides based on toxicity to target weeds, environmental safety, and cost, and may reduce the need for pre-emergence herbicides (Strauss et al. 1997). The herbicide glyphosate is popular among growers due to its effectiveness, low cost, and low environmental impact (Castle et al. 2004). Glyphosate is the active ingredient in Roundup®, an herbicide marketed by Monsanto. Its mode of action is to inhibit EPSP (5-enolpyruvateshikimate-3-phosphate) synthase, an enzyme involved in the production of aromatic side chain amino acids (Teichmann et al. 2007).

Many GM crops available today are tolerant of glyphosate or other herbicides. As of 2013, herbicide-tolerant crops accounted for a larger land area than any other type of GM crop (James 2013). In the United States, 10 different glyphosate-tolerant crops are currently approved for cultivation: alfalfa, canola, chicory, cotton, flax, maize, potato, rice, soybean, and sugar beet (ISAAA 2014). The economic advantages of glyphosate-tolerant crops include reduced herbicide expenses, reduced tillage, and increased yield (Gianessi 2008; Klümper and Qaim 2014). Meta-analysis of herbicide-tolerant soybean, maize, and cotton revealed an average reduction in pesticide costs of 25 % and a yield benefit of 9 % (Klümper and Qaim 2014). The adoption of glyphosate-tolerant maize in the US led to a

reduction in weed control costs by $24 per hectare, leading to a net aggregate benefit of $269 million in 2005 (Gianessi 2008). Beyond economic benefits, the use of herbicide-tolerant crops has been shown to have environmental benefits in some crop systems by reducing the total amount or ecotoxicity of herbicides used, and for promoting no- or low-till systems (Fernadez-Cornejo et al. 2014). However, the extent of these benefits may have been considerably reduced in recent years due to the evolution of glyphosate-tolerant weed communities (Service 2013). New types of herbicide resistance integrated weed management methods, may be able to mitigate these problems (USDA 2014). However, in forestry systems weed resistance evolution has been far less of a problem than in agricultural systems (Strauss et al. 1997; Service 2013).

The objective of this study is to determine the potential utility of glyphosate-tolerant poplars for SRIC. Although herbicide tolerance has been demonstrated in a number of previous studies of transgenic trees (Ye et al. 2011), these projects have been limited to in vitro, controlled environments, or simple field designs. For example, using similar materials to those presented here, Meilan et al. (2002) showed that glyphosate tolerance in poplars can be robust in diverse genotypes grown in the field. However, to our knowledge no studies have employed management conditions that are similar to those in commercial field plantings, nor have they examined weed control and growth impacts. Moreover, although it is well known that poplars have lower expected greenhouse gas (GHG) emissions than annual energy crops (Bonin and Lal 2012), we are unaware of studies that have used life cycle analysis (LCA) to evaluate the integrated GHG impacts of transgenic trees. LCA of glyphosate-tolerant sugar beet, oilseed rape, and maize suggest that glyphosate tolerant crops can, under some conditions, have lower environmental impacts than their conventionally grown counterparts (Bennett et al. 2004; Mamy et al. 2010).

The goal of this study was to evaluate the potential value of glyphosate-tolerance in management of high intensity poplar plantations. Our main objectives were to evaluate the frequency of highly resistant genotypes during an initial screening in the field, quantify the impacts on weed control and growth rate, and conduct an exploratory life-cycle assessment of potential greenhouse gas savings. We report that glyphosate tolerance was stable, led to much improved weed control, resulted in a substantial improvement in tree growth at 2 years of age, and appears to provide net greenhouse gas benefits.

Materials and methods

Plant material and transformation

For the purposes of this study, "clone" refers to a non-transgenic poplar genotype, "event" denotes an individual within a clone derived from an independent genetic insertion (commonly called a "line" in the literature), and "ramet" is a vegetative propagule of an event. Leaf discs from in vitro- and greenhouse-grown plants of four triploid clones of hybrid cottonwood (*Populus trichocarpa* × *P. deltoides* clones 50–197, 189–434, 195–529, and *P. trichocarpa* × *P. nigra* clone 311–93) were used for transformation (Fig. 1). All transgenic events were produced with the binary vector, pMON17204; the construct and transformation method was previously described in detail (Han et al. 2000). The vector, which was provided by the Monsanto Company, includes the GUS gene and two genes to impart glyphosate-tolerance. The CP4 gene encodes an enzyme which binds glyphosate much more weakly than its native counterpart, and the GOX gene encodes

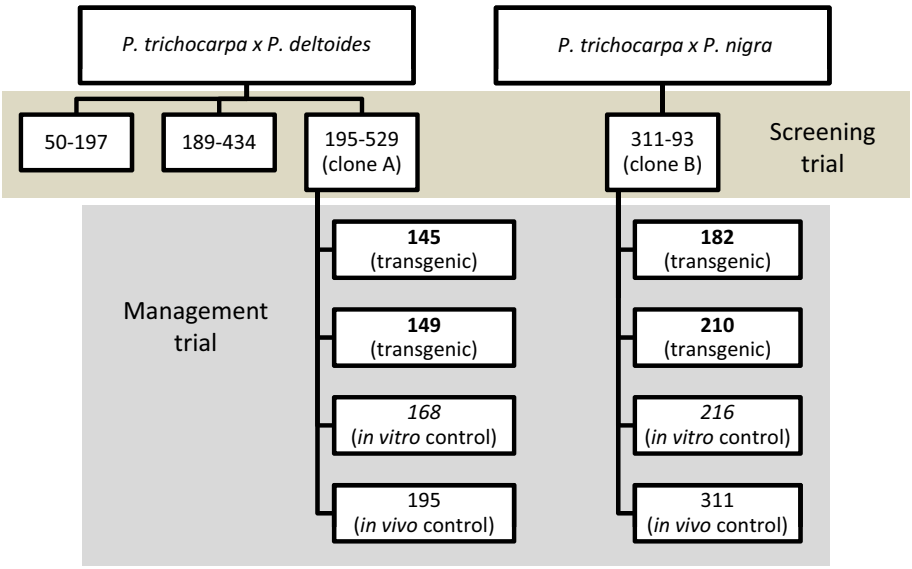

Fig. 1 Summary of plant materials studied. Transgenic events are in *bold*, in vitro controls are in *italics*, and in vivo controls are neither *bolded* nor *italicized*

glyphosate oxidoreductase that degrades glyphosate (Barry et al. 1992). Non-transgenic in vivo and in vitro controls, and 94 in vitro independent events were generated (15 in 50–197, 24 in 189–434, 27 in 195–529, and 28 in 311–93). Each transgenic event was propagated by rooting excised nodes from primary transformants grown on selection media. After roots were established in the presence of kanamycin, plants were transferred to an antibiotic-free, ½-strength M–S media (Han et al. 2000). The process by which plants were acclimated for growth in the greenhouse and the field was described previously (Meilan et al. 2002).

Verification

All transformants were rooted on media containing kanamycin (25 mg/L). Leaf tissue from each event was histochemically stained for GUS activity in a solution containing 1 g/L X-Gluc (Jefferson et al. 1987) and cleared with 95 % ethanol. Approximately half of the transgenic events were pre-tested by rooting on a glyphosate-containing medium (2 mg/L). The presence of both glyphosate tolerance genes (*GOX* and *CP4*) was confirmed using polymerase chain reaction (PCR). Southern blots were performed to confirm T-DNA insertion in events that were tolerant to the selection agents but from which a product could not be amplified via PCR. Sequence-specific primers and blotting conditions were described by Meilan et al. (2002).

Field study establishment

Initial screening trial

The screening trial was conducted to evaluate how rapidly and accurately highly resistant events could be identified in a field trial. Though not statistically analyzed, the results

inform practitioners of the level of effort (number of events and replicates) needed to produce highly herbicide tolerant events for commercial use.

Transgenic cottonwoods were planted under irrigation in Morrow County (eastern OR; mean annual rainfall [MAR], 8.6 cm; and mean annual temperature [MAT], 11.9 °C) in May 1996 and in Clatsop County (western OR; MAR, 146.9 cm; and MAT, 10.6 °C) in June 1996. Rooted stock were planted at 1.8 m × 1.8 m spacing. One ramet of each transgenic cottonwood event and the corresponding non-transgenic controls were randomly planted in each of six replicated blocks at both the Morrow and Clatsop sites. Herbicide treatments were randomly assigned to four blocks as described below. Different irrigation schemes were used in each area to compensate for their differences in rainfall. In Morrow County, plants were irrigated daily; in Clatsop County, trees were only irrigated until their survival was established. Thirty-cm wind screens were installed at the Morrow site immediately following establishment to shelter plants from the sun and wind; screens were removed after plants reached approximately 1/2 m in height (about 1 month after planting).

Management trial

The four transgenic events exhibiting high tolerance to glyphosate in the screening trial were propagated for the establishment of a 2-year management trial at the Morrow County site in the spring of 1998. These events included two each in clones 195–529 (events 145 and 149) and 311–93 (events 182 and 210; Fig. 2). Despite low damage scores, clone 189–434 was not chosen for the management trial due to low industry interest in the clone for east-side, irrigated plantations in Oregon. Hereafter clones 195–259 and 311–93 will be referred to as A and B, respectively. Two types of non-transgenic control plants were used— in vitro controls that had been micropropagated in tissue culture (#168 for clone A and #216 for B) and in vivo *controls* that were propagated directly from cuttings (event 195 for A and 311 for B; Fig. 1). Cuttings from the selected events were propagated by Broadacres Nursery in western Oregon (Marion County) for establishment of rooted stock for the field trials.

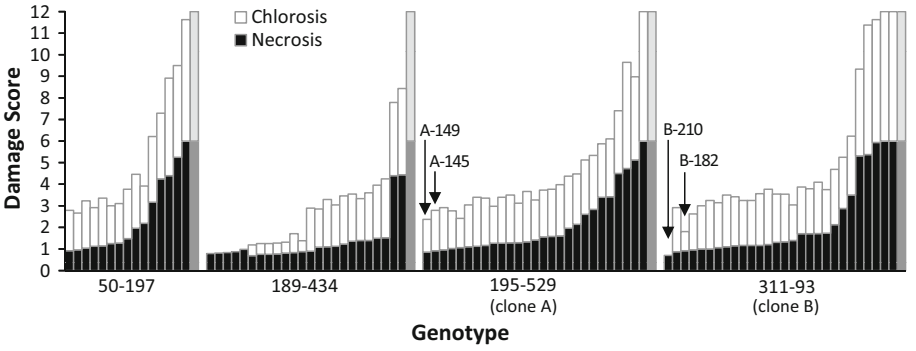

Fig. 2 Necrosis (*stacked top clear bars*) and chlorosis (*stacked bottom dark bars*) scores of the four genotypes tested during the screening trial. The controls are shown to the *right* in *gray*, and events chosen for the management trial are indicated with an *arrow*. *Each bar* represents the mean over 6–8 ramets. The first four events in clone 189–434 were not scored for chlorosis

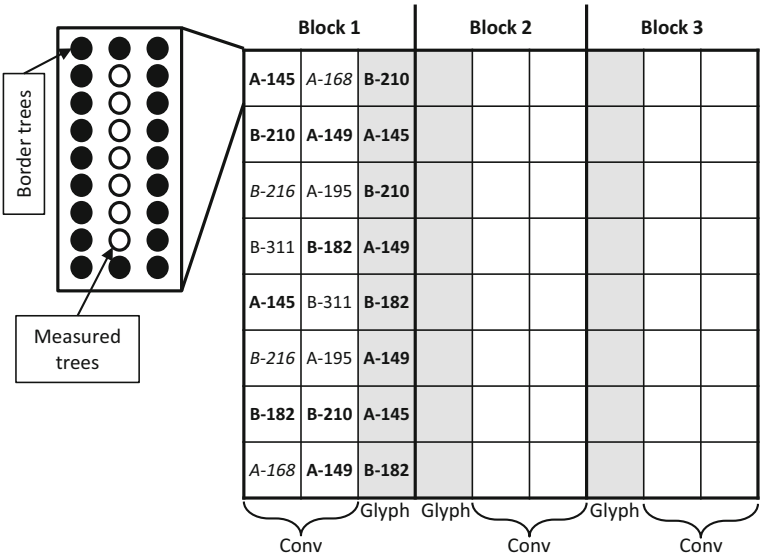

Fig. 3 Overview of field plot design for the management trial. "Conv" and "Glyph" denote conventional weed-control treatment and glyphosate weed-control treatments (also *shaded gray*), respectively. Transgenic events are in *bold*, in vitro controls are in *italics*, and in vivo controls are neither *bolded* nor *italicized*

Twenty-seven plants of each transgenic event or control were planted per plot, which included three rows of nine trees each (Fig. 3). Only the central seven trees were measured, leaving a one-tree border around the trees that were evaluated. There were two replicate plots per event or control for the conventional weed-control treatment in each of three replicated blocks. There were two replicate plots per block containing only the transgenic events in the glyphosate-only treatments. Non-transgenic controls were not exposed to the glyphosate-only treatments because of the certainty, based on prior work, that they would have rapidly succumbed to herbicide treatment.

Treatments

Initial screening trial

Roundup Pro^{TM} (41 % glyphosate, the active ingredient [ai]) was applied at a rate of either 4.7 L/ha (2.0 qt/ac; 2.0 kg ai/ha; 1×) or 9.5 L/ha (4.0 qt/ac; 3.9 kg ai/ha; 2×). At the Clatsop and Morrow County sites, each treatment was applied to two randomly selected replicate blocks; two blocks were left unsprayed as controls. Treatment dates for the Morrow County site were: July 8 and August 15, 1996, and May 6 and July 21, 1997; at the Clatsop County site they were: July 16 and August 29, 1996, and April 25 and July 25, 1997. Treatment methods were described by Meilan et al. (2002). Weeds in the unsprayed plots were controlled exclusively through cultivation. At the Clatsop site, a roto-tiller was driven in a grid between and within rows, whereas at the Morrow site tilling was only done within alleys between plant-rows. Hand hoeing was also done to remove persistent weeds near the base of each tree at both sites.

Management trial

Triflurilan (TN Treflan) was incorporated in all plots prior to planting at the rate of 4.7 L/ha (2 qt/ac) with a tractor-mounted rototiller. In late March, a pre-plant spray (0.47L [1 pt] of 2,4-D amine and 0.47 L [1 pt] of Roundup Pro^{TM} per acre) was broadcast sprayed from a tractor-mounted spray unit. In late June and late July 2000, Roundup Pro^{TM} (41 % glyphosate) was applied in the glyphosate treatment plots at a rate of 2.3 L/ha (1.0 qt/ac; 1.0 kg ai/ha). For the conventional treatment, hand weeding and hoeing around the trees was conducted in May and June. Additionally, in the conventional-treatment plots, spot-spraying was done with at 2 % (by volume) Roundup Pro^{TM} using backpack sprayers in July and August.

In the second growing season, both treatments included one spray of 0.71 L (1.5 pt) of 2,4-D amine and 0.71 L (1.5 pt) of Roundup Pro^{TM} per acre. They were broadcast sprayed from a tractor mounted spray unit on March 15, 2001, prior to bud break. In early July, the same herbicide mixture containing 2,4-D amine and Roundup Pro^{TM} was applied with a tractor-mounted hooded sprayer at the rate of 1.2 L/ha (1 pt/ac) to control weeds between the tree rows in the conventional-treatment plots. On May 16, 2001 Roundup Pro^{TM} (41 % glyphosate) was applied to the trees in the glyphosate treatment plots at a rate of 2.3 L/ha (1 qt/ac, 1 kg ai/ha) with a directed spray from two nozzles on a tractor-mounted sprayer. This application was done on both sides of each row to achieve full coverage of all foliage.

Measurements

Heights and basal diameters were taken on all trees immediately after planting and then again at the end of each growing season. Trees were evaluated for herbicide damage at least four weeks after glyphosate treatment, and scored for necrosis and chlorosis (Table 1). Percent weed cover was estimated in nine 1-m^2 plots randomly assigned to each plant-row. Weed coverage estimates were made at the time trees were evaluated for herbicide damage.

Calculations

Statistical analyses

A randomized complete block (RCB) split-plot analysis of variance was carried out using Proc Mixed in SAS (SAS Institute Inc 2008). The whole-plot effects were weed-control method and block. The split-plot effects were clone, events nested within clone, and the

Table 1 Scoring system used to categorize leaf damage from herbicide sprays

Score	Description
0	No apparent damage
1	<5 % of area affected
2	5–25 %
3	26–50 %
4	51–75 %
5	76–99 %
6	Tree dead (completely necrotic)

interaction between weed-control method and clone. The response variables were volume index (height × diameter^2), height, and diameter for years 1 and 2, and change in volume index, height, and diameter from year 1 to year 2. Residuals from ANOVA were examined to insure that assumptions were adequately met. When blocks were found to be non-significant, they were removed from the model and rerun for greater statistical power, but statistical differences remained the same. Likewise, the controls (in vitro and in vivo) were pooled as no significant differences were found between them. Because event A-149 was statistically distinct from other events based on examination of residuals, it was considered an outlier and excluded from analyses of transgenic performance. More details are provided under results. Type 3 sums of squares were used to test the null hypothesis of no significant effects. Contrast statements were used to compare weed-control methods, clone groups, and each event to its corresponding control. Due to their specific selection, weed-control method, clones, and event nested within clone were considered fixed effects.

In order to determine whether the difference in percent weed cover was statistically significant between the weed-control treatments on each of the three dates, a Kruskal–Wallis non-parametric ANOVA was performed using the NPAR1WAY procedure in SAS (SAS Institute Inc 2008). A Kruskal–Wallis test was chosen over one-way ANOVA because it was unlikely that the data would be normally distributed. Treatment was used as the class variable, while the response variable was percent weed cover.

Life cycle analysis

LCA is a systematic tool to estimate the energy use (fossil and total energy) and the environmental impacts (greenhouse gas emissions in this study) to produce a product. The purpose of this work was to create a limited cradle-to-gate LCA model using the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET) model (Argonne National Laboratory 2012). Life Cycle Analysis was performed using a cradle-to-gate model which assesses the environmental impact of a plant from initial cultivation to when the processed product leaves the factory.

The functional unit for this study was 1000 kg of bone dry poplar wood. The system boundary for the analysis was defined using the relative mass, energy, and economic value (RMEE) method with a 5 % cutoff. In this method the mass, energy content, and economic value of each input in the process is calculated for the production of one functional unit of product. If the ratio of mass, energy content, or economic value of any of the input to that of functional units exceeds the cut of value (5 % in this study), the upstream production of that input is included in the system boundary (Raynolds et al. 2000). Based on this boundary, inputs include all herbicide and insecticide applications, water and associated pumping energy, and mechanical inputs and associated fuels. Estimated pesticide, water, electricity, and fuel inputs from each treatment were taken from an established LCA for poplars grown for bioenergy (Hohenschuh et al. 2015). A detailed data inventory spreadsheet for the LCA including assumptions and calculations is provided in Online Resource 1. The complete GREET file used can be accessed at http://tinyurl.com/RRGREET. (The GREET database file can be opened in GREET as a new database. The data reported in the paper can be found under Farmed Trees product category.)

The only product produced was poplar wood. Growth for wood was based on experimental results over a 2-year coppice. Some mechanical inputs are required during the first coppice cycle, but would not be required in future coppice cycles. In two extrapolated scenarios, the GHG emissions from three successive coppice cycles were calculated. In these scenarios the growth benefit of GM trees for the second and third coppice cycle was

assumed to be 50 % of the benefit realized in the first coppice cycle (expected faster growth rate of coppice sprouts and thus earlier crown dominance over weeds compared to planted "sticks" in cycle one).

Results

Initial screening trial

Herbicide damage was much lower on the transgenic trees compared to controls. All controls scored 6 (dead), while the majority of transgenic events scored much lower with mean transgenic scores of 2.7, 1.7, 2.5, and 2.8 for chlorosis and 2.4, 1.3, 2.1, and 2.2 for necrosis among events in clones 50–197, 189–434, 195–529 (clone A), and 311–93 (clone B), respectively (Fig. 2). Events A-149, A-145, B-210, and B-182 were chosen to propagate for the management trial because they had the lowest average necrosis scores (<1) and very low chlorosis scores (0–2). Industry partners indicated that clone 189–434 was no longer of commercial interest, so it was excluded from the management trial in spite of high levels of tolerance in some events.

Management trial

Most of the selected events underwent propagation with rooted cuttings and grew normally. However, event A-149 and all of its vegetative propagules exhibited leaf mottling (Online Resource 2) and grew poorly after over-wintering at the propagation field and was excluded from further analysis as an outlier (see methods). Full results from ANOVA are provided in Online Resource 3.

Differences in weed cover between plants treated with glyphosate and those with conventional weed control could be observed with the naked eye (Fig. 4). A Kruskal–Wallis test at $\alpha = 0.05$ (95 % confidence limit) demonstrated that these differences in weed cover were not significant prior to glyphosate treatment ($P = 0.055$) on June 14, but they were highly significant after treatments ($P \ll 0.01$) on July 24 and September 18 (Fig. 5). Using the RCB split-plot model, block was found to be non-significant ($P < 0.05$), and was thus dropped from the model. The final split-plot ANOVA had weed-control method as the whole-plot effect and the split-plot effects were clones, events nested within clone, and the interaction between weed-control method and clone. Weed control method was associated with significant differences in mean growth increment from year 1 to year 2 as measured by volume index ($P = 0.04$) and height ($P = 0.05$). Transgenics subjected to glyphosate treatment had an average increase in volume index (height $\times$ diameter^2) of 23.4 and 24.5 % over those subjected to conventional weed control treatment and over their non-transgenic controls that were also given conventional weed control treatments, respectively (Fig. 6).

Contrasts were used to compare the growth of clones subjected to the same weed-control method. Statistically significant differences were obtained in year 1, with clone A having larger diameter under conventional weed control ($P = 0.03$), and clone B being taller under the glyphosate weed-control treatment ($P = 0.02$). Contrasts were also used to compare weed-control methods to each other for a given clone. Statistically significant differences were seen only for clone B, with diameter in year 1 ($P = 0.04$), and for volume index ($P = 0.03$), height ($P = 0.03$), diameter ($P = 0.05$) in year 2, and for increase in

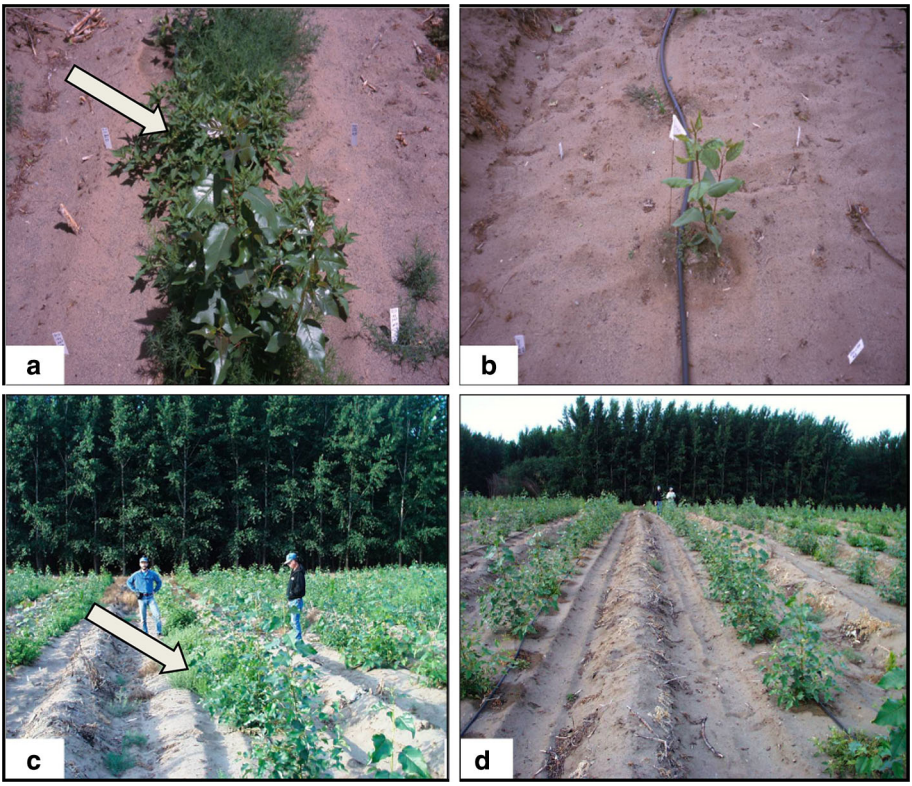

Fig. 4 Variation in weed control between the glyphosate-only and conventional weed-control regimes during the first growing season of the management trial. The response to conventional treatment is shown on the *left* (**a**, **c**) with *arrows* to indicate weed proliferation, and the result of treating with glyphosate over the *top* of the trees is shown on the *right* (**b**, **d**). *Top* images taken during the first week of July 2000, lower images during the last week of July 2000

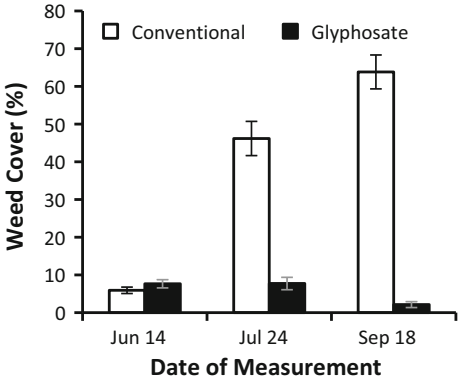

Fig. 5 Weed cover during the first year of growth (2000). Brackets denote one standard error of the mean. Measurements were taken before each glyphosate treatment

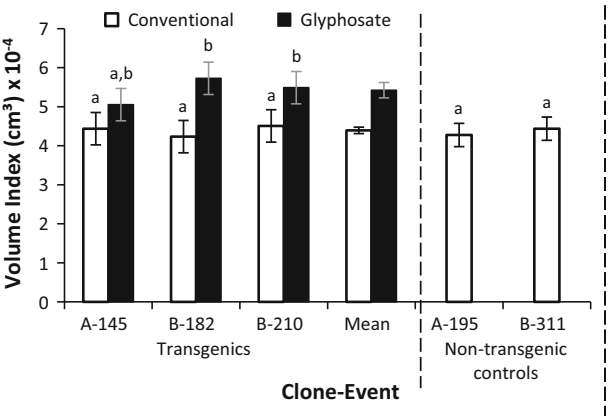

Fig. 6 Least square (LS) means for final size (volume index) during the second growing season under conventional and glyphosate weed control treatments. Brackets show one standard error of the mean and *letters* indicate significant differences ($P < 0.05$). In vitro and in vivo controls were pooled because they were not significantly different, and event A-149 was excluded as an outlier (discussed in text)

volume index and diameter in year 2 ($P = 0.02$ and 0.03, respectively) being significantly greater under the glyphosate weed-control treatment.

Life cycle analysis

LCA showed a reduction in GHG emissions of 19.3 % per 1000 kg of wood produced between the conventional and glyphosate-tolerant treatments over the course of the 2-year experiment (Fig. 7). When the LCA was extended to model three 2-year coppice cycles with an estimated growth benefit in subsequent cycles that was 50 % of that during the first cycle, GHG emissions were reduced by 8.3 % per 1000 kg of wood produced between the conventional versus glyphosate-tolerant treatments (Fig. 7). In both cases, this benefit was due predominantly to the increased production of wood per acre and a small decrease in

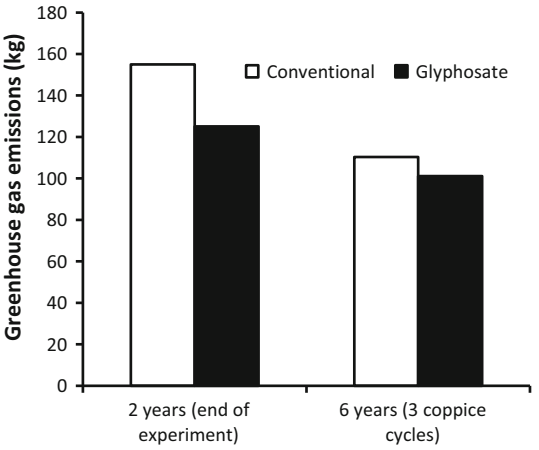

Fig. 7 Life cycle analysis results for greenhouse gas emissions produced per metric ton of wood. *Left* Predicted results after 2 years in the management trial. *Right* projected emissions during 6 years with three coppice cycles and growth benefit reduction of 50 % after the initial 2 years

fossil fuel input per acre. The decrease in fossil fuel use in glyphosate tolerant crops was limited to the year in which the trees were planted (2.8 gal/ac in glyphosate tolerant plots vs. 6.7 gal/ac in conventional plots).

Discussion

Despite low necrosis and chlorosis scores in the initial screening trial, event A-149 exhibited extensive leaf mottling and grew poorly in the management trial. Variation among in vitro regenerated organisms, known as somaclonal variation, though rare in poplar, is common in gene transfer and tissue culture systems (Kaeppler et al. 2000). The delayed, post-dormancy expression of leaf mottling in all of the vegetatively propagated ramets was surprising, and to our knowledge has not been reported before. This suggests an epigenetic change whose expression was elicited by dormancy, rather than a physical mutation, was the cause. The visibly poor growth of event A-149 in the management trial caused the event to be a statistical outlier, which led us to discard it from statistical analyses.

The variation in glyphosate tolerance among transgenics and controls, and among transgenic events, was striking. All controls treated with glyphosate died, illustrating the risk growers would face from pesticide drift if they used broadcast sprays or misapplied glyphosate to control weeds post-emergence. By contrast, the transgenics were almost completely unharmed. The minor and varying levels of chlorosis and necrosis exhibited may be due to the use of two glyphosate-tolerance genes. In a separate trial using plants from the same clones, plants with both *GOX* and *CP4* showed greater foliar damage than those with *CP4* alone (Meilan et al. 2002). The events with low damage scores that were selected for use in the management trials may have had lower expression of *GOX*. Future applications of this technology would likely avoid the GOX gene entirely.

In the management trial, weed control was visibly improved under glyphosate treatment, and glyphosate-tolerant trees grew better than their non-transgenic counterparts. Greater weed control likely allowed the transgenic plants to grow more with the same inputs because of reduced competition for soil nutrients and water (both provided in the irrigation system). The LCA demonstrated that increased growth with the same inputs led to substantially reduced GHG emissions per unit of wood, which might have a significant environmental impact on a commercial scale. We expected that the benefits of weed control for plant growth would diminish in subsequent years, but have no data on the extent of reduction. Thus, to try and account for this reduction we assumed growth benefit reduction of 50 % during three subsequent 2-year coppice cycles (6 years total). While we believe that this is a conservative assumption, it represents a potential weak point in this LCA and its applicability to an industrial farm setting. Under this scenario the GHG savings would be 6796 metric tons for a commercial tree farm of 10,117 hectares (25,000 acres), which is equivalent to taking 1,332 cars off the road for the 6 years of the coppice cycle (EPA 2011). In this study, irrigation and fertilization of glyphosate tolerant plants was the same as for conventionally produced poplar. The optimization of irrigation and fertilization for glyphosate tolerant trees could serve to further decrease GHG emissions.

One of the driving forces behind using short-rotation woody crops for biofuel production is the demand for environmentally sustainable energy sources. Poplars have several traits associated with lower GHG emissions when compared to annual biofuel crops such as corn. Lignocellulosic crops, including poplar, offset more GHG than corn (Bonin

and Lal 2012), and as a perennial, crop poplars require less nutritional inputs than annual feedstock options. By reducing the GHG profile of poplar feedstock even further with herbicide-tolerant plants—especially where they do not depend on irrigation (as they did in the current experimental system)—poplar has the potential to be one of the leading options for low GHG emitting biofeedstock crops.

Several herbicide-tolerant crops have been widely adopted in the last two decades, but despite the potential for economic and environmental gains no herbicide-tolerant trees have been approved for commercial use. Public opinion and regulatory hurdles for genetic engineering technology have likely reduced the appeal of GM trees to commercial growers (Strauss et al. 2009, 2011, 2015; Viswanath et al. 2012). The dominant wood-product certification system, Forest Stewardship Council (FSC), and affiliated certification systems (Costanza 2013), do not permit the use of any GM trees, which discourages industry from researching or investing in promising new technologies (Strauss et al. 2001; Meilan et al. 2012). One concern with GM trees is the potential for gene flow to wild or weedy relatives (DiFazio et al. 2012). Gene flow from glyphosate-tolerant, transgenic crops to non-transgenic crops has been observed, or is expected, in canola (*Brassica:* Hall et al. 2000), creeping bentgrass (*Agrostis:* Watrud et al. 2004), and alfalfa (*Medicago*: Van Deynze et al. 2011*)*. However, genetic containment technologies could be employed to reduce dispersal risk by including genes that impart complete male- and female-sterility in vegetatively propagated GM plants (Klocko et al. 2014). Even imperfect sterility of this kind could provide a strong means to avoid significant gene flow (DiFazio et al. 2012). Beyond the hurdles of regulation and markets, the adoption of herbicide-tolerant trees may complicate management, as such trees can be more challenging to kill when a plantation cycle has ended. However, most trees are highly susceptible to an array of herbicides, and can often be effectively killed without chemicals, by stump-mulching and tilling.

Conclusions

This study is the first to show the utility of glyphosate-tolerant tree crops under operational conditions that are similar to those of commercial plantations. The adoption of herbicide-tolerant trees has the potential to benefit growers with simplified management regimes, and also may reduce the environmental impact of growing a renewable fuel source. With the development of reliable containment technologies, herbicide-tolerant trees might become a part of sustainable plantation management systems.

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References

Advanced Hardwood Biofuels Northwest (AHB) (2014) Poplar (*Populus* spp.) Trees for biofuel production. http://www.extension.org/pages/70456/poplar-populus-spp-trees-for-biofuel-production. Accessed 6 Jun 2014

Argonne National Laboratory (2012) GREET v1.0.0.8079. Chicago, IL

Barry G, Kishore G, Padgette S, et al (1992) Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. In: Singh BK, Flores HE, Shannon JC (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, Md, pp 139–145

Bennett R, Phipps R, Strange A, Grey P (2004) Environmental and human health impacts of growing genetically modified herbicide-tolerant sugar beet: a life-cycle assessment: environmental impact of herbicide-tolerant sugar beet. Plant Biotechnol J 2:273–278. doi:10.1111/j.1467-7652.2004.00076.x

Bonin C, Lal R (2012) Agronomic and ecological implications of biofuels. Adv Agron 117:1–50. doi:10.1016/B978-0-12-394278-4.00001-5

Castle LA, Siehl DL, Gorton R et al (2004) Discovery and directed evolution of a glyphosate tolerance gene. Science 304:1151–1154. doi:10.1126/science.1096770

Costanza A (2013) Regulation, certification, and use of biotech trees. In: Institute of Forest Biotechnology. http://www.forestbiotech.org/biotech_tree_use/. Accessed 9 Jun 2014

DiFazio SP, Leonardi S, Slavov GT et al (2012) Gene flow and simulation of transgene dispersal from hybrid poplar plantations. New Phytol. doi:10.1111/j.1469-8137.2011.04012.x

Ellis Jansson S, Strauss SH, Tuskan GA (2010) Why and how Populus became a "Model Tree". Genetics and genomics of *Populus*. Springer Science Buiseness Media, LLC, New York, pp 3–14

Environmental Protection Agency (2011) Measuring greenhouse gas emissions from transportation. http://www.epa.gov/OTAQ/climate/measuring.htm

Fernadez-Cornejo J, Wechsler SJ, Lingingston M (2014) Adoption of genetically engineered crops by US farmers has increased steadily for over 15 years. USDA economic research service. http://www.ers.usda.gov/amber-waves/2014-march/adoption-of-genetically-engineered-crops-by-us-farmers-has-increased-steadily-for-over-15-years.aspx#.U5i_OijcBMB. Accessed 11 Jun 2014

Gianessi LP (2008) Economic impacts of glyphosate-resistant crops. Pest Manag Sci 64:346–352. doi:10.1002/ps.1490

Hall L, Topinka K, Huffman J et al (2000) Pollen flow between herbicide-resistant Brassi*ca napus* is the cause of multiple-resistant *B. napus* volunteers1. Weed Sci 48:688–694. doi:10.1614/0043-1745(2000)048[0688:PFBHRB]2.0.CO;2

Han K-H, Meilan R, Ma C, Strauss SH (2000) An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus *Populus*). Plant Cell Rep 19:315–320. doi:10.1007/s002990050019

Hinchee M, Rottmann W, Mullinax L et al (2011) Short-rotation woody crops for bioenergy and biofuels applications. In: Tomes D, Lakshmanan P, Songstad D (eds) biofuels. Springer, New York, pp 139–156

Hohenschuh W, Kumar D, Murthy GS (2015) Economic and cradle-to-gate life cycle assessment of poly-3-hydroxybutyrate production from plastic producing, genetically modified hybrid poplar leaves. J Renew Sustain Energy 6:063113

ISAAA (2014) International Service for the Acquisition of Agri-Biotech Applications (ISAAA), GM approval database. http://www.isaaa.org/gmapprovaldatabase/advsearch/default.asp?CropID=Any&TraitTypeID=1&DeveloperID=Any&CountryID=US&ApprovalTypeID=3. Accessed 9 Jun 2014

James C (2013) Global status of commercialized biotech/GM crops: 2013. ISAAA, Ithaca

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Kaeppler SM, Kaeppler HF, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Mol Biol 43:179–188. doi:10.1023/A:1006423110134

Kauter D, Lewandowski I, Claupein W (2003) Quantity and quality of harvestable biomass from *Populus* short rotation coppice for solid fuel use—a review of the physiological basis and management influences. Biomass Bioenergy 24:411–427. doi:10.1016/S0961-9534(02)00177-0

Klocko AL, Ault K, Ma C et al (2014) Targeting floral development genes for genetic containment of forest trees: PtLFY-RNAi causes sterility while allowing vigorous tree growth in field-grown poplar. Am Soc Plant Biol, Meeting Portland

Klümper W, Qaim M (2014) A meta-analysis of the impacts of genetically modified crops. PLoS One 9:1–7. doi:10.1371/journal.pone.0111629

Mamy L, Gabrielle B, Barriuso E (2010) Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops. Environ Pollut 158:3172–3178. doi:10.1016/j.envpol.2010.06.036

Marino PC, Gross KL (1998) Competitive effects of conspecific and herbaceous (weeds) plants on growth and branch architecture of *Populus xeuramericana cv. Eugenei*. Can J For Res 28:359–367. doi:10.1139/x97-223

Meilan R, Ma C, Cheng S, et al (2000) High levels of Roundup® and leaf-beetle resistance in genetically engineered hybrid cottonwoods. In: Blatner KA, Johnson JD, Baumgartner DM (eds) Hybrid poplars in the Pacific Northwest: culture, commerce and capability symposium proceedings, Pasco, Washington, 7–9 April 1999. Washington State University Cooperative Extension Bulletin MISC0272, Pullman, WA, pp 29–38

Meilan R, Han K-H, Ma C et al (2002) The CP4 transgene provides high levels of tolerance to Roundup® herbicide in field-grown hybrid poplars. Can J For Res 32:967–976. doi:10.1139/x02-015

Meilan R, Harfouche A, Nehra N (2012) Risk assessment of biotech trees: a path forward. J For 110:171–172

Raynolds M, Fraser R, Checkel D (2000) The relative mass-energy-economic (RMEE) method for system boundary selection part 1: a means to systematically and quantitatively select LCA boundaries. Int J Life Cycle Assess 5:37–46

SAS Institute Inc (2008) SAS release 9.2 TS Level1M0. SAS Institute Inc, Cary

Service RF (2013) What happens when weed killers stop killing? Science 341:1329. doi:10.1126/science.341.6152.1329

Singh B (2008) Nutrient uptake by hybrid poplar in competition with weed species under growth chamber and field conditions using the soil supply and nutrient demand (SSAND) model. Department of Soil Science, University of Saskatchewan

Strauss SH, Knowe SA, Jenkins J (1997) Benefits and risks of transgenic, roundup ready cottonwoods. J For 95:12–19

Strauss SH, Campbell MM, Pryor SN et al (2001) Plantation certification and genetic engineering: FSCs ban on research is counterproductive. J For 99:4–7

Strauss SH, Schmitt M, Sedjo R (2009) Forest scientist views of regulatory obstacles to research and development of transgenic forest biotechnology. J For 107:350–357

Strauss SH, Zhang C, Hinchee M (2011) Gene flow control in forest trees: approaches and progress. In: Proceedings of the science of gene flow in agriculture and its role in co-existence, Washington, DC. USDA-NIFA and U.C. Davis Department of Plant Sciences, Davis, CA, pp. 80–83. http://sbc.ucdavis.edu/files/198273.pdf

Strauss SH, Costanza A, Seguin A (2015) Genetically engineered trees: paralysis from good intentions. Science 349:794–795. doi:10.1126/science.aab0493

Teichmann T, Bolu WH, Polle A (2007) Transgenic trees. In: Kües U (ed) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, Gottingen, pp 117–139

Tuskan GA, DiFazio S, Jansson S et al (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596–1604. doi:10.1126/science.1128691

USDA (2014) USDA Announces measures to help farmers diversify weed control efforts. http://www.usda.gov/wps/portal/usda/usdamediafb?contentid=2014/10/0227.xml&printable=true&contentidonly=true

Van Deynze A, Bradford KJ, Daniell H, DiTomaso JM, Kalaitzandonakes N, Mallory-Smith C, Stewart NC, Strauss SH, Van Acker R (2011) The science of gene flow in agriculture and its role in co-existence. In: Proceedings of the science of gene flow in agriculture and its role in co-existence, Washington, DC. USDA-NIFA and U.C. Davis Department of Plant Sciences, Davis, CA. http://sbc.ucdavis.edu/files/198273.pdf

Viswanath V, Albrectsen B, Strauss S (2012) Global regulatory burden for field testing of genetically modified trees. Tree Genet Genomes 8:221–226. doi:10.1007/s11295-011-0445-8

Watrud LS, Lee EH, Fairbrother A et al (2004) Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. Proc Natl Acad Sci USA 101:14533–14538. doi:10.1073/pnas.0405154101

Withrow-Robinson BA, Hibbs DE, Beuter JH, Laboratory OSUFR (1995) Poplar chip production for Willamette Valley grass seed sites. Forest Research Laboratory, Oregon State University, Corvallis

Ye X, Busov V, Zhao N et al (2011) Transgenic *Populus* trees for forest products, bioenergy, and functional genomics. Crit Rev Plant Sci 30:415–434. doi:10.1080/07352689.2011.605737