HERBICIDE TOLERANCE

PEER REVIEWED

Development of glyphosate-tolerant hybrid cottonwoods

RICHARD MEILAN', KYUNG-HWAN HAN, CAIPING MA,
ROSALIND R. JAMES, JAMES A. EATON, BRIAN J. STANTON,
ERNIE HOIEN, RON P. CROCKETT, AND STEVEN H. STRAUSS

ABSTRACT

High levels of weed control in young cottonwood plantations are required to assure survival and rapid growth. Herbicide-tolerant trees should enhance the efficacy and cost-effectiveness of weed control, increasing the profitability of cottonwood fiber farming. Using Agrobacterium-mediated transformation and indirect shoot organogenesis, we produced 79 lines of transgenic poplars that contain glyphosate tolerance genes. Results indicate high levels of tolerance to Roundup' in a substantial number of these lines. When Roundup Pro" was sprayed at a rate of 4.7-9.4 Uha (2-4 qt/acre), only minor adverse effects on growth were detected in the highly tolerant lines during the first year of growth.

Application:

This paper demonstrates that the genetic engineering tools that have been widely used with herbaceous agronomic corps are equally applicable to trees. With a modest transformation effort, we were able to produce transgenic poplars with commercially useful levels of herbicide tolerance.

CONVENTIONAL BREEDING HAS LED to the development of hybrid cottonwood clones that have rapid growth rates (12-15 ft/year). However, rigorous weed control is required during the first 2-3 years of plantation development for successful establishment and maximal growth (l, 2). Effective weed control can also reduce water usage and help avoid rodent damage (3). Because cottonwood is susceptible to popular broad-spectrum, post-emergent herbicides, broadcast applications cannot be used after leaf emergence. Most growers now rely on sheltered sprays and repeated tilling to control weeds after planting; both methods are costly and only partially effective.

Genetically engineered herbicide-tolerant plants are now widely utilized in North America, and many more are under development. Examples of crops that have recently been engineered and field-tested for glyphosate tolerance include sugarbeet, maize, cotton, lettuce, poplar, potato, rape, soybean, tobacco, tomato, and wheat (4). Herbicide tolerance is expected to facilitate the level of weed control that is needed for high rates of cottonwood survival and productivity (3), and this tolerance represents one of the most immediate ways in which forestry can benefit from biotechnology.

The Tree Genetic Engineering Research Cooperative (TGERC) at Oregon State University has been working on herbicide tolerance since its inception in 1994. TGERC is a consortium of ten paper and timber companies, the U.S. Department of Energy; Monsanto Co., and Shell. TGERC emphasizes genetic engineering of poplars for short-rotation intensive culture, focusing on environmental effects of transgenes and near-term commercial uses of transgenic trees (5). In this paper, we describe the results of the first field trial in which transgenic trees generated by TGERC were screened for tolerance to glyphosate, the active ingredient in Roundup'.

METHODS

Using combined *Agrobacterium* mediated transformation and indirect organogenesis, we developed methods (6) for producing a population of poplar hybrids containing two genes that impart tolerance of the herbicide Roundup. One gene, CP4, encodes a version of Senolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19), to which glyphosate binds much more weakly than to the native version of the enzyme. The other gene encodes an enzyme, glyphosate oxidoreductase (GOX), that degrades glyphosate (7). Thirty-nine transgenic lines were derived from a hybrid aspen clone (section *Populus*, INRA 717-1134; *Populus tremula x P. alba),* and 40 lines were derived from four triploid *P, trichocarpa x P. deltoides* hybrid cottonwood clones (184-402, 24305,19-53, and 189-434); clones 184402 and 24-305 are in commercial production in western Oregon plantations.

Three field screening trials were established. The hybrid aspen lines were planted near Corvallis in late summer 1995. The transgenic cottonwood hybrids were planted on industry land, both east (Potlatch, Boardman, OR) and west (Fort James, Clatskanie, OR) of the Cascade mountain range. Trees at the Boardman site were planted on May 28, 1996; those near Clatskanie were planted on June 11, 1996. Single trees of each cottonwood line, plus non-transgenic versions of each clone, were placed in 6-9 blocks at each site. Blocks were either left unsprayed or treated twice with 4.7-9.4 L/hectare (L/ha) (2-4 qt/acre) of Roundup Pro^{TM} in 1996 and 1997.

Corresponding author (phone +I 541 737-6097. fax +I 541 737-1393, e-mail meilanr@FSL.orst.edu)

Score	Description
0	No damage
1	Some leaf chlorosis, no apical damage
2	Necrosis or discoloring in apical region
3	Some top dieback and leaf discoloration
4	Entire tree substantially damaged
5	Death; no green foliage present

I. Rating system used to score glyphosate damage in poplars

Basal caliper and height measurements, and damage ratings, were taken for each tree before and after each spraying. A simple scoring system was used to assess herbicide damage (see **Table I).**

RESULTS AND DISCUSSION

When sprayed at a rate of either 2 or 4 qt/acre, all nontransgenic control trees were killed (Fig. 1). However, a substantial proportion (38-82%) of the transgenic lines showed complete or near-complete tolerance to the spray, while a few lines (0-12°/; Fig. 2) were completely susceptible.

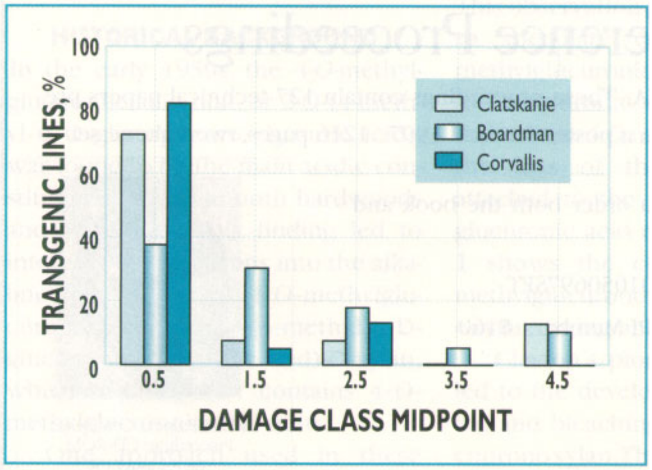
For a preliminary determination of the effect of glyphosate on the growth of the transgenic trees, we compared unsprayed controls to plants with minor damage (mean damage scores of 0-1.0). Glyphosate treatment did not significantly affect relative volume growth of the transgenic trees with high levels of tolerance at either Boardman or Corvallis (Fig. 3); however, there was a small reduction in growth at the Clatskanie site. This may be a consequence of poorer visual expression of damage at the Clatskanie site, and thus nearly twice as many lines are included in the "minor damage" class compared to Boardman (Fig. 2).

I.Transgenic hybrid cottonwoods in a demonstration block at the Potlatch field test site near Boardman. Photo taken I month after spraying with 2 qt/acre o f Roundup Pro.

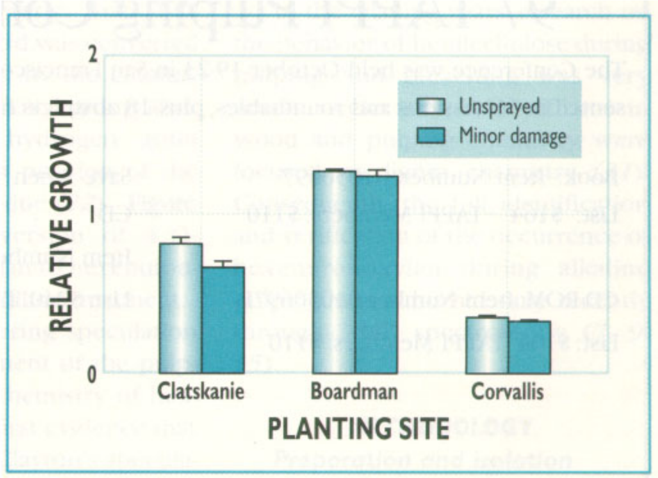
Second-year growth and herbicide damage results were similar to what was seen during the first year of this study. Enzyme-linked immunosorbent assays (ELISAs) are also being conducted to determine what levels of protein expression are required for high levels of herbicide tolerance. Finally, we are comparing the performance of transgenics containing both glyphosate tolerance genes (CP4 and GOX) with those containing only CP4. This work will allow us to determine whether CP4 alone will confer commercially useful levels of herbicide tolerance.

CONCLUSION

Herbicide tolerance holds considerable promise for vegetation management in poplar plantations, but additional work is needed to demonstrate its commercial value. TGERC is now conducting a series of large-scale, longterm management trials in which we are trying to determine the value of this trait to growers. In these studies, we will compare the effects of various conventional weed-control regimes to those that capitalize on glyphosate tolerance.



2. Mean damage (Table I) to transgenic poplars following the second spraying in 1996 (2 qt/acre of Roundup Pro). Damage class 0.5 includes mean ratings of 0 to 1.0; class 1.5 includes ratings of >1.0 to 2.0, etc.



3. Effects of Roundup Pro spraying on relative volume growth of transgenic lines with high tolerance to glyphosate (29 lines at Clatskanie and 15 lines at Boardman, damage class 0.5; see Fig. 2)

TGERC is also field testing poplars that have been engineered for insect resistance and reproductive sterility. The former is being done to minimize pest-control costs for growers. Sterility is being sought because federal regulators are likely to require a strategy to minimize the risk of an introduced gene's escape before transgenic trees can be commercialized. One way to reduce this risk is to engineer sterility (8). We have been working in this area for the past 5 years, and we are experimenting with several approaches (9). The technique that proves most effective will be combined with other traits of interest in a final commercial product.TJ

Meilan is an assistant professor and associate director, *TGERC; Ma* is a faculty research assistant, James is a faculty research associate, and Strauss is a professor and director, *TGERC,* at Oregon State University, Department of Forest Science, A001 Peavy Hall, Corvallis, *OR* 97331-7501. Han is a faculty research associate at Michigan State University, Department of Forestry, 126 Natural Resources Bldg., East Lansing, MI 48824. Eaton is plant materials manager at Potlatch Corp., PO. Box 38, Boardman, OR 97878. Stanton is a research associate at Fort James Corp., 347 NW 7th Ave., Camas, WA 98607. Hoien is coordinator, Technology/Stewardship, at Fort James Corp., 79114 Collins Rd., Clatskanie, OR 97016. Crockett is technical development manager at Monsanto Agricultural Co., 17004 NE 3 7th Cir., Vancouver, WA 98682.

Meilan and Han made equivalent contributions to this paper and are, therefore, considered senior coauthors. We thank the members *of* the Tree Genetic Engineering Research Cooperative for their support. Roundup' is a registered trademark and Roundup Pro™' is a trademark *of* Monsanto Co., St. Louis, MO 63167.

Received for review April 26, 1997. Accepted July 2, 1999.

LITERATURE CITED

I. Hansen, E. A., Netzer, D.A., and Rietveld,W J., Res. Note NC-317, USDA North Central Forest Experiment Station, St. Paul, MN, 1984.

2. Hansen, E. A. and Netzer, D.A., Res. Pap. NC-260, USDA North Central Forest Experiment Station, St. Paul, MN, 1985.
3. Strauss, S. H., Knowe, S.A., and Jenkins,J., J. Forestry 95(5): 12(1997).
4. Saroha, M. K., Sridhar, P, and Malik,V S.,J. Plant Biochem. and Biotech. 7(2): 65(I 998).
5. Strauss, S. H., Han, K.-H., James, R. R., et al., Tree Genetic Engineering Research Cooperative (TGERC) Annual Report: 1995-1996, Forest Research Laboratory, Oregon State University, Corvallis, OR, 1996.
6. Han, K.-H., Meilan, R., Ma, C., et al., Plant Cell Reports, in press.
7. Barry, G., Kishore, G., Padgette, S., et al. in Biosynthesis and Molecular Regulation *of* Amino Acids in Plants (B. K. Singly H. E. Flores, and J. C. Shannon, Eds.),Am. Soc. of Plant Physiologists, Rockville, MD, 1992, p. 139.
8. Strauss, S. H., Rottmann. W H., Brunner, A. M., et al., Mol. Breeding I : 5(I 995).
9. Skinner, J. S., Meilan, R., Brunner,A. M., et al. in Molecular Biology *of* Woody Plants (S. M. Jain and S. C. Minocha, Eds.), Kluwer Academic Publishers, Dordrecht, Netherlands, 1999, in press.