

Stability of Gene Expression and Agronomic Performance of a Transgenic Herbicide-Resistant Sugarcane Line in South Africa

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ABSTRACT

Sugarcane (*Saccharum* hybrid) cultivar NCo310 was transformed with the *pat* gene, which confers resistance to the herbicide Buster (glufosinate ammonium; Bayer CropScience, Monheim am Rhein, Germany). The effect of the expression of this gene on the agronomic performance of a successful transgenic line, 22.2, was investigated. Field testing showed that the *pat* gene was stably expressed during three rounds of vegetative propagation. Morphological and agronomic characters such as stalk height, diameter, population, fiber, disease resistance, and yield, measured in the first ratoon, were not significantly different in the transgenic line and its untransformed counterpart. Of four weed treatments applied to transformed and untransformed plots, there were two scenarios in which significantly higher yields were observed: (i) untransformed cane treated using the conventional (non-Buster) herbicide protocol, widely used for weed control in the South African sugar industry, and (ii) transformed cane treated with a conventional preemergence cocktail, followed by two 5-L ha⁻¹ Buster applications. However, the most economical weed control treatment is dependent on the cost of the herbicide to which resistance has been engineered. Buster currently costs (in South African Rand) ZAR150 (≈US \$14.01) L⁻¹. For Buster treatments to be cost effective for commercial growers, the price would have to be considerably lower. However, since hand-hoeing is deleterious to the cane, small-scale growers currently controlling weeds manually would find herbicide use an advantage. If they were to adopt transgenic sugarcane, Buster costs would need to be reduced only slightly for them to increase their returns.

THE DEVELOPMENT of herbicide-resistant crop plants was one of the first commercial applications of plant genetic engineering (Mullineaux, 1992; Tsaftaris, 1996; Freyssinet and Cole, 1999). In genetically modified sugarcane, resistance to phosphinothricin (PPT) via the *bar* (bialaphos; Gallo-Meagher and Irvine, 1996; Enriquez-Obregon et al., 1998; Falco et al., 2000) and *pat* (phosphinothricin acetyltransferase; Snyman et al., 1998) genes has been reported. Although commercial application of herbicide resistance in the field has been recorded for tobacco and potato (de Greef et al., 1989), sugar beet (Buckmann et al., 2000), and rice (Jiang et al., 2000), little published information is available on the agronomic performance of herbicide-resistant sugarcane, which is a development priority in the South African sugar industry.

Commercialization of a transgenic crop usually requires stable expression of the introduced gene in a plant that retains its own genetic integrity and agronomic characteristics. Extensive field testing is essential. Stable inheritance of a transgene in field trials has been

reported in transgenic sugarcane previously (Gallo-Meagher and Irvine, 1996; Arencibia et al., 1999; Sala et al., 1999), as well as in other graminaceous monocotyledons such as barley (Horvath et al., 2001) and rice (Jiang et al., 2000). However, the importance of thorough screening of transgenic plants that are propagated vegetatively was stressed in a paper on transgenic tall fescue (Bettany et al., 1998). In that work, loss of gene expression was observed in some tillers in the plant and stable transgene expression could only be determined and selected after three to four rounds of vegetative propagation, an effect the authors suggested might be due to environmental factors. Underlining the need for field data is the fact that the integrity and stability of the plant genome as a whole may be affected by the tissue culture and transformation process. Genomic change resulting from transgenesis has been documented in various plants including rice (Jiang et al., 2000; Labra et al., 2001), barley (Bregitzer et al., 1998; Choi et al., 2000) and sugarcane (Arencibia et al., 1999, 2000; Sala et al., 1999).

In this study, the effect of *pat* herbicide-resistance expression on the agronomic potential of sugarcane cultivar NCo310 was investigated using a novel field approach. NCo310 was bred in South Africa and has been used as the recipient genotype and model system in several sugarcane transformation programs (Gallo-Meagher and Irvine, 1996; Snyman et al., 1998) as it responds favorably to in vitro culture. A single transformed line (22.2), characterized by nine copies of the *pat* gene inserted at separate loci, was selected for the trial experiments as it showed significant resistance to the herbicide Buster [monoammonium 2-amino-4-(hydroxymethylphosphinyl) butanoate] in glasshouse spraying trials.

The aims of the study were twofold. First, to characterize the transgenic line genetically and phenotypically by (i) establishing the stability of the gene across several ratoons, (ii) determining herbicide sensitivity at different application rates, and (iii) comparing morphological and agronomic characteristics such as stalk morphology, susceptibility to pests and diseases, and yields in relation to the untransformed parent cultivar NCo310. Second, to assess the economic significance and viability of herbicide-resistant sugarcane by measuring the efficacy of weed control using two different treatments involving the herbicide Buster and two weed control methods currently used in the South African sugar industry, namely hand-weeding and a cocktail of pre- and post-

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Abbreviations: *Bt*, *Bacillus thuringiensis*; *pat*, phosphinothricin acetyltransferase; PPT, phosphinothricin; SASEX, South African Sugar Association Experiment Station; SCMV, *Sugarcane mosaic virus*; WCT, weed control treatment; ZAR, South African Rand.

emergence herbicides used by small- and large-scale commercial growers, respectively. To address these aims, two field trials were devised, one a simple small-scale dosage trial conducted across two ratoons, the other a more complex weed control trial on a larger scale.

MATERIALS AND METHODS

The field experiments were performed at the South African Sugar Association Experiment Station (SASEX) at Mount Edgecombe, KwaZulu Natal, South Africa (29°42' S and 31°02' E) under natural rainfall conditions (1045 mm yr⁻¹). Soil at the site is a sandy loam categorized as an Alfisols Plinthusalfs in the USDA classification (Soil Survey Staff, 1999) and a Westleigh form in the South African classification (Soil Classification Working Group, 1991).

Trial to Establish Herbicide Dosage and Phenotype Stability

A small-scale field trial was performed to measure herbicide sensitivity of a single transformed line, and to determine the stability of the resistance gene across several rounds of vegetative propagation. Line 22.2 contains nine copies of the *pat* gene, and expressed herbicide-resistance to Buster (200 g L⁻¹ glufosinate ammonium a.i.) in glasshouse-based spraying trials. Sugarcane stalk sections (setts) from line 22.2 and from untransformed NCo310 were grown in polystyrene seedling trays for approximately three months, then transplanted into 7- by 18-m rows, separated by concrete strips 0.5 m wide. Supplementary irrigation was provided. Once the plants had tillered, Buster was applied to the foliage using a lever-operated knapsack fitted with a 110° Teejet (Minneapolis, MN) nozzle. Rates from 1 to 7 L ha⁻¹ of Buster were applied incrementally to Rows 1 to 7 using the same volume of aqueous solution in each case (≈200 L ha⁻¹). Damage was assessed 3 wk after application. The plant crop was cut back after 6 mo and allowed to ratoon (the regrowth of the crop after harvesting). The two subsequent ratoons each received a single application of Buster at 7 L ha⁻¹ after four months, resulting in one of the rows (Row 7) of transgenic cane having received a total of 21 L ha⁻¹ from planting to second ratoon.

Trial to Compare Phenotypic Responses of Herbicide-Resistant and Control Sugarcane in Four Weed Control Programs

A large-scale field trial was conducted to compare transformed with untransformed NCo310, for (i) stalk morphology (height, diameter, population density) and fiber content, (ii) response to smut disease (causal agent *Ustilago scitaminea* Syd. & P. Syd.), *Sugarcane mosaic virus* (SCMV), and the insect pest eldana (*Eldana saccharina* Walker; Lepidoptera: Pyralidae), and (iii) yield (both cane biomass and sucrose production). During field preparation, grass seeds of *Panicum maximum* Jacq. (1.7 kg ha⁻¹), *Sorghum bicolor* (L.) Moench (2.4 kg ha⁻¹) and *Rottboellia cochinchinensis* (Lour.) Clayton (3.1 kg ha⁻¹) were introduced to the soil to increase weed pressure to the level normally found under industry conditions. Sugarcane stalk sections (setts) were obtained from bulking plots grown for 12 mo, and were dipped in Panocrine fungicide [1% (v/v); a.i. guazatine 400 g L⁻¹; Rhone-Poulenc Agrichem, Durban, South Africa] before planting. Fertilizer (N-P-K at 160, 52, and 160 kg ha⁻¹, respectively) was applied in the planting furrows and as a top-dressing along the rows.

The following four weed control treatments were applied: (i) Repeated Buster application (WCT1); Buster (5 L ha⁻¹)

was applied to sugarcane once weed emergence was complete. Three applications in the plant crop and two in the first ratoon were necessary to keep weeds under control. (ii) Preemergence herbicides, followed by repeated Buster application (WCT2). The plant crop was treated with a preemergence herbicide *cocktail* comprising Visor [2 L ha⁻¹; a.i. thiazopyr [3-pyridine carboxylic acid, 2-(difluoromethyl)-5-(4,5 dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-methyl ester] 240 g L⁻¹; Rohm and Haas, Philadelphia, PA], Harness [1.5 L ha⁻¹; a.i. acetochlor (2-chloro-2'-methyl-6'-ethyl-N-ethoxymethyl-acetanilide) 900 g L⁻¹; Monsanto, St. Louis, MO] and Diuron 800 SC [2.5 L ha⁻¹; a.i. 1-(3,4-dichlorophenyl)-3,3-dimethylurea 800 g L⁻¹; Sanachem, Canelands, South Africa] followed by two Buster (5 L ha⁻¹) applications. The first ratoon was hand-weeded once using eight labor units (one labor unit = one laborer d⁻¹ ha⁻¹) before receiving Har-i-cane [2 L ha⁻¹; a.i. acetochlor (2-chloro-2'-methyl-6'-ethyl-N-ethoxymethyl-acetanilide) 960 g L⁻¹; Monsanto] and Diuron 800 SC (2.5 L ha⁻¹). One Buster application (5 L ha⁻¹) was necessary in the first ratoon. (iii) Conventional weed control program (WCT3). The plant crop received a preemergence application of Visor (2 L ha⁻¹), Harness (1.5 L ha⁻¹) and Diuron 800 SC (2.5 L ha⁻¹), followed by a postemergence application of MCPA (3 L ha⁻¹; a.i. 2-methyl-4-chlorophenoxyacetic acid 400 g L⁻¹; Sanachem), Gesapax [N-ethyl-N'-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine; 4 L ha⁻¹; 500 g ametryn per L⁻¹; Syngenta, Greensboro, NC] and Reverseal 10 [adjuvant; 0.5% (v/v); Revertex Chemicals, Moberi, South Africa]. The plant crop was hand-weeded using four labor units. The ratoon crop was hand-weeded with eight labor units and then sprayed with Har-i-cane (2 L ha⁻¹) and Diuron 800 SC (2.5 L ha⁻¹). Finally, weeds were removed by hand-weeding with eight labor units two months later. (iv) Hand-weeding (WCT4). Five operations using hand-hoes and a total of 78 labor units were required to keep weeds under control in the plant crop. Three hand-weeding operations were necessary in the first ratoon crop (total of 34 labor units).

The trial was laid out in a split plot design, with eight replicates, four treatments as whole plots (WCT1-WCT4) and two subplots (transformed and untransformed). The herbicide treatments were randomized between the whole plots, and the allocation of the transformed and untransformed sugarcane was randomized independently within each whole plot. Each split plot comprised six rows, 7.5 m in length and spaced at 1.2 m. The two outermost rows served as guard rows. Analysis of variance was performed on the full model (i.e., treatment + transformed + treatment × transformed). Comparisons were made using first ratoon data, on the basis that Bailey and Bechet (1989) found that growth characteristics of tissue culture (callus)-derived plants compared more favorably to conventional sugarcane in the first ratoon than the plant crop.

Records were kept of labor usage per hectare, operational times, and amounts of herbicide used in the four treatments. The number of stalks was counted in two inner rows in each plot to give an estimate of stalk population density per hectare. Stalk height (measured from base to the top visible dewlap) and diameter (measured at the center of the stalk) were recorded in each of 20 stalks per plot. The incidence of smut and SCMV was determined by field inspection performed when the ratoon crop was 10 mo old, directly before harvest. Damage by the borer eldana was measured as percentage internodes bored in each of 25 stalks per plot, sampled from harvested material. These assessments were performed by SASEX inspection teams from Pathology and Entomology departments, respectively.

Planting was performed in September 1998 and the trial

was harvested when the plant crop was 12.5 mo old and the ratoon 10 mo of age. The cane was cut by hand, weighed, and sampled for direct analysis of sucrose and fiber (Buchanan and Brokensha, 1974) at the SASEX mill room. The South African industry formula for the estimated recoverable crystal (ERC) % cane, used to calculate the recoverable value payment system, was calculated using the following formula:

$$\text{ERC} = a \times S - b \times N - c \times F,$$

where S = sucrose % cane, N = non-sucrose % cane, F = fiber % cane, a = losses other than molasses and bagasse (value of 0.978), b = losses in molasses (0.539), c = losses in bagasse, which is dependant on the fiber content (0.019).

RESULTS AND DISCUSSION

Level of Herbicide Resistance and Stability of *pat* Gene Expression across Repeated Ratooning

Plants of transgenic line 22.2 showed no signs of damage after Buster application of rates as high as 7 L ha^{-1} . Untransformed cane plants of parent cultivar NCo310 displayed phytotoxic symptoms at 1 L ha^{-1} . Damage was extreme at 4 L ha^{-1} and plants died after application of 5 to 7 L ha^{-1} (Fig. 1). Buster was applied to two ratoon crops at a rate of 7 L ha^{-1} , so that the transgenic cane in row 7, to which 7 L ha^{-1} Buster was applied in the plant crop, received a total dose of 21 L ha^{-1} of Buster. This repeated application of the herbicide with no phytotoxic symptoms indicates that the introduced gene is stable and expressed across successive ratoon crops. Such stability has been reported in sugarcane previously (Gallo-Meagher and Irvine, 1996). That work also was based on use of the cultivar NCo310.



Fig. 1. Buster herbicide application to transformed sugarcane line 22.2 and untransformed parent cultivar NCo310 in a small-scale field trial. Incremental amounts ($1\text{--}7 \text{ L ha}^{-1}$) were applied to successive rows in plant cane and two ratoon crops, so that the transgenic cane in Row 7, to which 7 L ha^{-1} Buster was applied in the plant crop, received a total dose of 21 L ha^{-1} of Buster. Phytotoxic symptoms were visible on untransformed cane at all the rates tested (center; far right and left) and rates $>4 \text{ L ha}^{-1}$ were lethal. No phytotoxic damage was evident in the transformed line (flanking center).

Comparative Agronomic Performance of Transformed and Untransformed Sugarcane under Four Weed Control Programs

Stalk Parameters

Early studies on sugarcane somaclonal variation reported changes in gross morphology (Heinz et al., 1977), isoenzyme profiles (Larkin and Scowcroft, 1981) and sucrose content (Liu, 1990). More recently, in sugarcane genetically modified with a *Bacillus thuringiensis* (*Bt*)

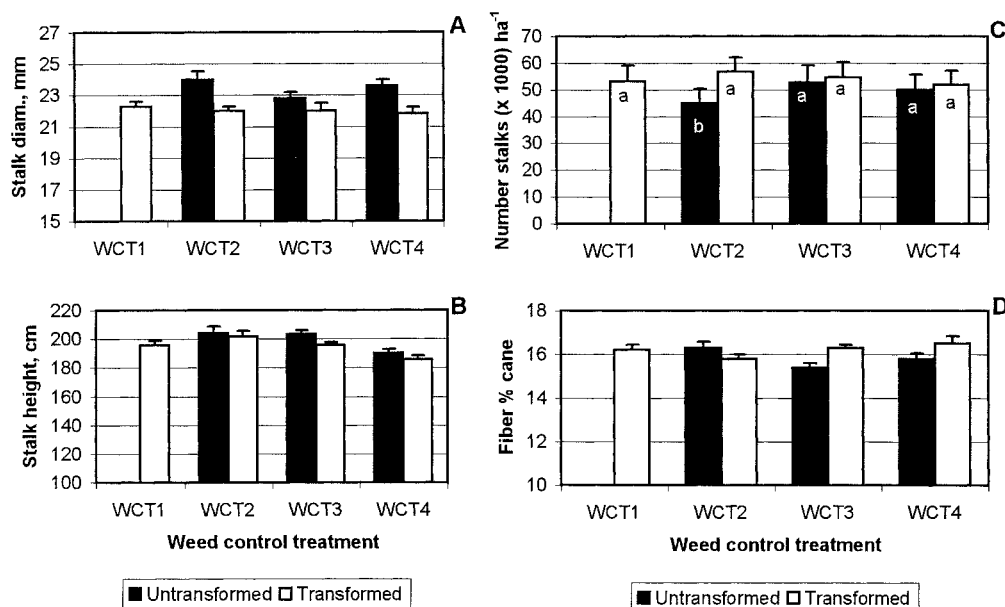


Fig. 2. A comparison of morphological stalk characteristics and fiber content in transformed line 22.2 and untransformed parent cultivar NCo310 under four weed control programs. Measurements were taken when the first ratoon crop was harvested at the age of 10 mo. Treatments are as follows: WCT1, Buster only; WCT2, preemergence and Buster application; WCT3, conventional herbicide-based (no Buster) weed control program; WCT4, hand-hoeing. Standard errors are shown by vertical lines above the bars ($n = 8$). (A) Stalk diameter. Measurements were taken around the center of stalks. (B) Stalk heights. (C) Stalk population. Bars with different alphabetical letters indicate significant differences (ANOVA SED = 3995; LSD0.05 = 8246). (D) Fiber content, the insoluble portion of a sugarcane stalk, expressed as a percentage of the fresh mass of the stalk.

transgene, stalk diameter and number of stalks per stool were found not to be significantly different when compared with conventionally propagated and untransformed in vitro derived material, but there was a significant increase in stalk height in the transformed plants (Arencibia et al., 1999, 2000; Sala et al., 1999). Comparisons of midstalk diameter, height, population density per hectare, and fiber content for first ratoon plants in this study are shown in Fig. 2A, 2B, 2C, and 2D. As untransformed cane treated with Buster in WCT1 died during the plant crop, no measurements could be taken from these plots in the ratoon crop. When ANOVA was applied to whole plot means, no significant effect of herbicide treatment was apparent for any stalk parameter measured. Hence, comparisons between transformed and untransformed cane (subplots) were seen as the important features of the morphological data.

Sugarcane stalk diameters can vary widely, depending on the cultivar and on growing conditions (van Dillewijn, 1952, p. 4). In this study, untransformed cane had thicker stalks than transformed cane in all treatments, but these differences were not significant (Fig. 2A) (ANOVA SED = 0.57; LSD0.05 = 1.17).

Stalk heights measured when the first ratoon was harvested ranged from 185.6 to 204.5 cm and there were no significant differences between untransformed and transformed cane in Treatments WCT2, WCT3, and WCT4 (Fig. 2B) (ANOVA SED = 4.63; LSD0.05 = 9.6). The shortest stalks (186–190 cm) were observed in WCT4, where weeds were removed by hand-hoeing. It is possible that mechanical damage to the roots caused by hoeing stunted the growth of the cane.

Only the treatment that involved preemergence herbicides followed by two Buster applications at 5 L h⁻¹ (WCT2) showed a significant difference in stalk populations between transformed and untransformed sugarcane (Fig. 2C) (ANOVA SED = 399; LSD0.05 = 824). This is because the untransformed cane is susceptible to Buster and some stalks did not survive the herbicide application. Although transformed cane in WCT3 and WCT4 contained more fiber than the untransformed plants from the same treatment, the differences were not significantly different (Fig. 2D) (ANOVA SED = 0.22; LSD0.5 = 0.46).

Pests and Diseases

Varying levels of smut resistance have been observed in previous field studies performed on tissue culture-derived (Bailey and Bechet, 1989) and transgenic (Arencibia et al., 2000) sugarcane. Cultivar NCo310 is susceptible to both smut and SCMV (Bailey, 1979). Directly before harvesting of the first ratoon crop, 1.4 to 5.1% of plants were infected with smut and 1.3 to 5.9% with SCMV. However, there were no significant differences in the ratings of transformed and untransformed cane in any of the treatments for both smut (ANOVA SED = 1.67; LSD0.05 = 3.44) (Fig. 3A) and SCMV (ANOVA SED = 1.74; LSD0.05 = 3.59) (Fig. 3B).

Stalks in hand-weeded plots (WCT4) had significantly more internodes damaged by eldana (7.8% compared

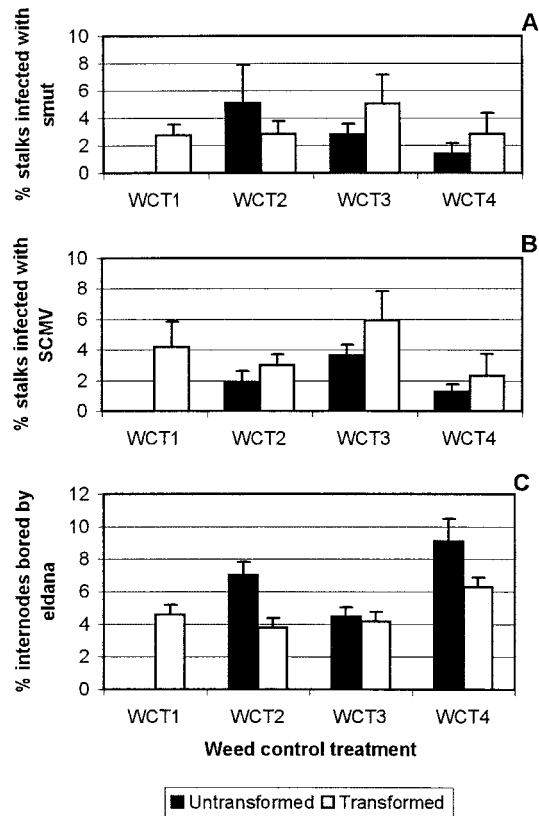


Fig. 3. Incidence of smut, *Sugarcane mosaic virus* (SCMV), and borer in transformed line 22.2 and untransformed parent cultivar NCo310 under four weed control programs. Assessments were performed at the harvest of the ratoon crop, at age 10 mo. (A) Differences in percentage stalks infected with smut (*Ustilago scitaminea* Syd. & P. Syd.). Smut inspections were performed by the South African Sugar Association Experiment Station Pathology inspection team at harvest. Vertical lines above bars indicate SE ($n = 8$). (B) Incidence of stalks infected with SCMV. Observations were performed by the Plant Pathology inspection team directly before harvesting. Vertical lines above bars are SE ($n = 8$). (C) A comparison of internodes bored by the stalk borer (*Eldana saccharina* Walker; Lepidoptera: Pyralidae). Vertical lines above bars represent SE ($n = 8$). Different alphabetical letters represent statistical differences (ANOVA SED = 1.3; LSD0.05 = 2.71).

with 4.6, 5.4, and 4.4% for WCT1, WCT2, and WCT3; ANOVA SED = 0.86; LSD0.05 = 1.83; $P = 0.0207$). Hand-hoeing may stress sugarcane by disturbing or damaging roots, which can cause the plants to be more susceptible to eldana (G. Leslie, 2001, personal communication). Untransformed cane in Treatments WCT2 and WCT4 had a greater proportion of damaged internodes compared with transformed cane, but the differences were not statistically significant (ANOVA SED = 1.3; LSD0.05 = 2.7). It is thought that an increase in fiber content of sugarcane may deter eldana from feeding on the plant (G. Leslie¹, 2001, personal communication), but there was no relationship between fiber content and eldana damage in this trial (Fig. 2D and 3C).

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Table 1. A comparison of sugarcane biomass, sucrose yields, and cane quality for the four weed control treatments in Buster-resistant line 22.2 and untransformed parent cultivar NCo310 in the ratoon crop (Buster, a.i. glufosinate ammonium, made by Bayer CropScience, Monheim am Rhein, Germany). Means are of eight replicates and are followed by different alphabetical letters if differences are significant (ANOVA).

Weed control treatment for first ratoon	Category	Cane mass	Sucrose yield, ERC†	Cane quality
		Mg ha ⁻¹		ERC % cane
WCT1 (Buster)	Untransformed	0c	0c	0
	Transformed	59.8b	8.2b	13.8
WCT2 (cocktail + Buster)	Untransformed	56.3b	7.7b	13.6
	Transformed	68.0a	9.5a	13.9
WCT3 (conventional herbicides, no Buster)	Untransformed	67.5a	9.7a	14.3
	Transformed	60.1b	8.3b	13.9
WCT4 (hand-hoeing)	Untransformed	60.5b	8.2b	13.6
	Transformed	53.9b	7.4b	13.7
Comparing subplot treatments in same whole-plot treatment (i.e., transformed and untransformed)				
	SED	1.8	0.31	0.21
	LSD0.05	3.8	0.65	0.44
	F value	28.5	24.0	328.0
	P value	<0.001	<0.001	0.04
Comparing treatments not in same whole-plot treatment (i.e., Weed Control Treatments 1 to 4)				
	SED	3.72	0.62	0.37
	LSD0.05	7.93	1.32	0.79
	F value	1.2	1.3	0.5
	P value	0.35	0.30	0.69

† ERC = estimated recoverable crystal.

Yield

Yields obtained under the four different weed control treatments were compared to see whether cane treated with Buster showed comparable yields with cane in the conventionally used weed control treatments. In addition, transformed and untransformed cane yields were examined to determine whether the growth and therefore yield capacity of the plants was affected by transgenesis. When comparing treatments not in same whole plots, there were no significant differences in any yield parameter tested (Table 1) (i.e., there was no effect of weed control treatment on yield). No significant differences in cane quality were observed between transformed and untransformed cane in any of the weed control treatments, indicating that overall C allocation and the proportion of sucrose stored in the sugarcane culm was not influenced by either transformation or method of weed control. This result is compatible with the lack of effect of these factors on fiber (Fig. 2D). However, some significant differences in biomass and sucrose yields of transformed and untransformed cane were observed. As would be expected, untransformed cane under Treatment WCT1 died because it was susceptible to Buster, and yields from untransformed cane under Treatment WCT2 were poor due to the phytotoxicity of Buster applications after initial use of other herbicides. The effect of the blank plots in the former treatment on other plots was counteracted by the presence of guard rows. The poorest performance was in transformed cane under the WCT4 treatment, suggesting that transgenic cane does not respond well to hand-weeding. This possibly reflects great variability of growth and vigor of any kind of cane under hoeing. It is also a possible result of transgenic cane having less sturdy stalks than the untransformed counterparts, although statistical analysis had shown that these differences were not significant (Fig. 2A).

There were two scenarios in which significantly higher

yields were observed. The first was untransformed cane treated using the conventional (non-Buster) herbicide protocol WCT3, widely used for weed control in the South African sugar industry. The second was transformed cane under the WCT2 treatment. Although only a single line was tested in this study, the results suggest that transgenic cane treated with conventional herbicides plus Buster has economic potential.

Recent reports dealing with yields in genetically engineered crops have shown a range of responses, from reduced yields in PPT-resistant barley (Bregitzer et al., 1998), to no effect in bacterial leaf blight-resistant rice (Tu et al., 2000a), and increased yields in herbicide-resistant flax (McHughen and Holm, 1991) and *Bt*-rice (Tu et al., 2000b). This emphasizes the need for thorough assessment of agronomic characteristics of transgenic crops.

Weed Control Costs

Economically viable transgenic sugarcane relies on a financial and/or management advantage to growers. To assess the financial advantage of herbicide-resistant sugarcane, a comparison was made between transgenic and conventional sugarcane based on the value of the crop and the cost of the herbicide treatment. From comparisons of yields in the previous section, no significant differences exist between transgenic cane using Herbicide Treatment WCT2 (the likely scenario) and untransformed cane using conventional herbicide treatment (WCT3), so the profit margin will be determined by the cost of the weed control treatment, and more specifically, by the herbicide to which resistance has been genetically engineered. The current cost of Buster in South Africa is high (ZAR 150 L⁻¹, ≈US \$14.01 L⁻¹), but indications from previously commercialized herbicide-resistant crops in the USA is that herbicide prices decrease with higher demand (Dunwell, 1999; Herrera-Estrella, 2000; Barboza, 2001; Bullock, 2002).

Table 2. A comparison of the return per South African Rand (ZAR) spent on weed control. Conventional weed control methods using pre- and postemergent herbicides (WCT3) and hand-weeding (WCT4) costs and returns were compared with transgenic sugarcane treatments incorporating Buster (glufosinate ammonium; Bayer CropScience, Monheim am Rhein, Germany) in WCT1 and WCT2. Sucrose value was calculated by multiplying sucrose yield by the sucrose price (ZAR 865 Mg ha⁻¹ in 2001 season). Total weed control costs in WCT1 and WCT2 were calculated using the current cost of Buster (ZAR 150 L⁻¹).

Weed control treatment	Sucrose value	Total weed control costs	New value of the crop	Return per ZAR spent on weed control†	Return per ZAR spent on weed control if cost of Buster is:	
					ZAR60 L ⁻¹	ZAR30 L ⁻¹
					ZAR ha ⁻¹	
WCT1 transformed	7093	1618	5475	4.4	10.3	18.3
WCT2 transformed	8218	2182	6036	3.8	6.6	8.6
WCT3 untransformed	8391	1004	7387	8.4	8.4	8.4
WCT4 untransformed	6401	1496	4905	4.3	4.3	4.3

† Buster priced at ZAR150 L⁻¹.

A costing exercise was performed using three different prices of Buster: ZAR150 L⁻¹, ZAR60 L⁻¹, and ZAR30 L⁻¹. The return per ZAR spent on weed control was calculated where the value of the crop and the cost of weed control were taken into account. The returns per ZAR spent on weed control in transgenic cane using the two Buster treatments most likely to replace current weed control methods (WCT1 and WCT2) were compared with those in untransformed cane using current industry methods of weed control (WCT3 and WCT4; Table 2). Calculations show that commercial grower returns using the standard industry herbicide treatment (WCT3) are not matched by returns using Buster-resistant cane with the herbicide at its current price of ZAR150 L⁻¹. If transgenic cane were to be grown, Treatment WCT1 would begin to be cost effective if the cost of Buster was half, and very advantageous if the cost of Buster were to drop to one-fifth. Treatment WCT2 would not offer any great advantage even at a low herbicide price. By contrast, because of the damaging effect of hand-hoeing, small-scale growers currently employing manual weed removal (WCT4) would find use of herbicides an advantage. If they were to adopt transgenic sugarcane and use only Buster (WCT1) to control weeds, they would maintain the same level of return on their crop at the present Buster price. Buster costs would need to be reduced only slightly for them to increase their returns. In view of the fact that Buster is not competitively priced, herbicide-resistance to a cheaper herbicidal compound may be an economically more viable approach.

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