INITIAL FIELD TESTING OF TRANSGENIC GLUFOSINATE AMMONIUM-RESISTANT SUGARCANE

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Abstract

The South African sugar industry has recognised that benefits may be obtained from genetically transformed sugarcane. Herbicide resistance is seen as a potential advantage. In a model study, sugarcane variety NCo310 was genetically modified for resistance to the herbicide Buster® (hereafter referred to as Buster) (glufosinate–ammonium 200 g a.i./L). In preliminary studies under semi-controlled field conditions, young transgenic sugarcane plants were asymptomatic when sprayed with up to 7.0 L/ha of Buster, while untransformed plants were killed at 4.0 L/ha. A field trial to assess possible physiological differences between transformed and untransformed sugarcane and to compare different Buster applications was established. This trial included a plant and first ratoon crop. Buster was either used alone as a repeated 'chemical hoe' application, or in conjunction with pre-emergence herbicides. Further treatments included an industry standard programme without Buster, and a hand-weeded control.

The transformed sugarcane expressed complete tolerance to Buster, while phytotoxicity in the untransformed cane was severe. Due to weed competition, yields of hand-weeded and Buster only treatments were inferior to those of treatments that included pre-emergence herbicides. The results also confirmed the economic advantages of early weed control. It is therefore unlikely that only one product would be recommended for use with herbicide resistant sugarcane. Yields of untransformed cane were significantly higher than transformed cane in non-Buster treatments. In addition, the transformed cane had thinner stalks and higher fibre % cane levels, which suggested that there were indirect physiological effects of the transformation process.

Introduction

The development of herbicide resistance in crop plants using genetic engineering was one of the first applications of this technology to a commercially important objective (Mullineaux, 1992; Tsaftaris, 1996; Freyssinet and Cole, 1999). In addition, the use of herbicide resistance has served as a model system for developing many of the methods that are now routine in plant transformation experiments. It is in this context that sugarcane was genetically engineered with the *pat* gene, conferring resistance to glufosinate ammonium (Snyman *et al*., 1998). Although evaluations of herbicide resistant crop plants in the field have been reported in tobacco and potato (de Greef *et al*., 1989), sugarbeet (Buckmann *et al*., 2000) and rice (Jiang *et al*., 2000), little published information is available on field trials of herbicide resistant sugarcane.

For the purposes of the model study, cultivar NCo310 was transformed with the *pat* gene (Aventis, France) by microprojectile bombardment (Snyman *et al*., 1996). Preliminary investigations showed that transgenic plants were resistant to Buster at rates of 4.0 and 7.0 L/ha in glasshouse and small scale field trials respectively (Snyman *et al*., 1998). A more comprehensive field trial was needed to determine:

- the economic benefits of controlling weeds with Buster in genetically engineered sugarcane,
- the optimum use of Buster in weed control programmes,
- whether growth characteristics or other phenotypic differences occur between transformed and untransformed NCo310.

Approach

The following four treatments were conducted on transformed and untransformed plants of variety NCo310, which were planted 45 days prior to application. Grass seed was disced into the soil to increase weed pressure to the level normally found under industry conditions. The trial had a randomised block design with split plots, each with 6 rows, 7.5 m in length and spaced at 1.2 m. Treatments were replicated eight times. Records were kept of labour use per hectare, operational times and amounts of herbicide used.

Treatment 1: *Repeated Buster application*

Buster at 5.0 L/ha was applied to each cane interrow once weed emergence was complete. Three applications in the plant crop and two in the first ratoon were necessary to keep weeds under control. This method of 'chemical hoeing' has the potential to replace conventional handweeding practices currently used by small-scale growers.

Treatment 2: *Pre-emergence herbicides followed by repeated Buster application*

The plant crop was given a pre-emergence treatment comprising 2.0 L/ha Visor (thiazopyr 240 g a.i/L) + 1.5 L/ha Harness (acetochlor 900 g a.i/L) + 2.5 L/ha diuron 800 SC (diuron 800 g a.i/L) followed by two Buster applications of 5.0 L/ha. The first ratoon was hand-weeded once using 8 labour units (man/days/ha) before receiving 2.0 L/ha Har-i-cane (acetochlor 960 g a.i/L) + 2.5 L/ha Diuron 800 SC. One Buster application at 5.0 L /ha was necessary.

Treatment 3: *Conventional weed control*

The plant crop received a pre-emergence application of 2.0 L/ha Visor + 1.5 L/ha Harness + 2.5 L/ha Diuron 800 SC, followed by a post-emergence application of 3.0 L/ha MCPA (400 g a.i/L) + 4.0 L/ha Gesapax (ametryn 500 g a.i/L) + 0.5% Reverseal 10 (adjuvant). One hand-weeding using 4 labour units was necessary. The first ratoon was hand-weeded with 8 labour units and then sprayed with 2.0 L/ha Har-i-cane + 2.5 L/ha Diuron 800 SC. A final hand-weeding was carried out with 8 labour units two months later.

Treatment 4: *Hand hoeing only*

Five operations using a total of 78 labour 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 labour units).

Tillers were counted and stalk height and diameter measured. Inspections were conducted to record eldana damage and the incidence of smut and mosaic diseases. The trial was harvested when the plant crop was 12.5 months old and the first ratoon when 10 months old. The cane was cut by hand, weighed and sampled for direct analysis of cane.

General observations of field performance of transgenic sugarcane

In both crops, stalk heights and populations of untransformed cane were significantly reduced in the two Buster treatments (results not shown). No significant differences were found for the two non-Buster treatments. Stalk diameters in the ratoon crop appeared normal and conformed to information provided by van Dillewijn (1952). However, untransformed cane had thicker stalks than the transformed cane in the non-Buster treatments (results not shown). This appears to be an effect of *in vitro* culture, as a similar observation was made previously when comparing vegetatively propagated and tissue culture-derived plants of varieties NCo310, NCo376 and N12 (Bailey and Bechet, 1989). These differences could be attributed to somaclonal variation that has been observed previously in long-term sugarcane cultures (Irvine *et al*., 1991). Phenotypic differences in transgenic field-grown sugarcane have been observed more recently by Australian researchers (Birch, personal communication[1]).

Variety NCo310 is susceptible to both smut and mosaic (Bailey, 1979). Although there were between 1.4 and 5.1% plants infected with smut and 1.3 to 5.9% with mosaic, there were no differences between the reactions of transformed and untransformed cane. There was little damage from eldana in the plant crop with 0.8% internodes bored in transformed cane and 1.1% in the untransformed cane. Borer damage increased in the first ratoon, in which untransformed cane had significantly more internodes bored (6.9% compared with 4.7% in transformed cane (P=0.05)).

Yield comparisons between transformed and untransformed cane in plant and first ratoon crops

Cane analysis at harvest showed the transformed cane in both plant and first ratoon crops to have significantly higher fibre % cane (17.4 and 16.4%, respectively) compared with the untransformed cane (16.9 and 15.6%, respectively). Higher fibre % cane can be attributed to the lower incidence of eldana observed in the transformed cane (Leslie, personal communication[2]).

Table 1. Cane and sucrose yield for plant and first ratoon crops in transformed herbicide resistant and untransformed NCo310.

Treatment	Category	Plant crop		First ratoon crop	
		Cane yield tons/ha	Sucrose yield tons /ha	Cane yield tons/ha	Sucrose yield tons /ha
Treatment 1 Repeated Buster application	Untransformed	6.6	0.7	0	0
	Transformed	37.8	4.9	59.8	9.0
Treatment 2 Pre-emergence herbicides followed by Buster	Untransformed	39.5	5.7	56.3	8.4
	Transformed	58.3	8.1	68.0	10.4
Treatment 3 Conventional weed control programme	Untransformed	50.5	7.3	67.5	10.6
	Transformed	46.5	6.6	60.1	9.1
Treatment 4 Hand-weeding only	Untransformed	32.1	4.2	60.5	9.0
	Transformed	27.6	3.7	53.9	8.1
Comparing transformed and untransformed cane					
Standard error		1.5	0.3	1.8	0.3
LSD (0.05)		3.1	0.6	3.8	0.7
Comparing treatments					
Standard error		4.6	0.8	3.7	0.7
LSD (0.05)		9.5	1.6	7.9	1.4

Statistically significant (P=0.05) reductions in cane and sucrose yields were recorded for untransformed NCo310 in both crops where Buster was used (Table 1). This suggests that high levels of phytotoxicity can occur from multiple Buster applications on untransformed cane. This was not the case, however, in Treatments 3 and 4 where Buster was omitted. Here, transformed cane generally yielded less than the untransformed equivalent.

Plant crop and first ratoon yields were significantly lower in non-transformed cane compared with transformed cane in Treatments 1 and 2 (Table 1). It is important to note that severe weed competition occurred in Treatment 1, where Buster was sprayed repeatedly only after weeds reached an advanced stage of growth. The untransformed cane in Treatment 1 yielded poorly because it suffered from both Buster phytotoxicity and severe competition from weeds. None of this cane had survived by the end of the first ratoon. In contrast, weed competition was avoided for approximately three months by the use of pre-emergence herbicides in Treatment 2, which led to improvements in the yield of transformed cane of 20 tons/ha in the plant crop and 8 tons/ha in the first ratoon (Table 1). Transformed cane yields in Treatment 2 were higher than those in Treatment 3. It is likely that mechanical damage gave rise to the lower yields observed in Treatment 3.

Repeated hoeing in Treatment 4 resulted in relatively poor yields for both the transformed and untransformed cane. This indicates the extent to which yields may be affected by mechanical damage caused by this method of weed control.

Weed control economics

Weed control costs for each treatment are summarised in Table 2. Costs were comparable for Treatments 1 and 2 where Buster was used. The conventional weed control programme (Treatment 3) was by far the least expensive. Continuous hand-weeding proved to be most expensive, particularly in the plant crop. Table 2 shows the monetary value for sucrose from the transgenic cane in each treatment. This income was offset by weed control costs and the return per Rand spent is calculated. Transformed cane in Treatment 1 produced a low return for expenditure on weed control, which was also the case for Treatment 4. The high cost of Buster also resulted in only moderate returns on investment for transformed cane in Treatment 2. The best returns on investment for transformed cane were obtained from Treatment 3. This highlights the importance of using a cheaper herbicide than Buster (R153 per L). The return per Rand spent on weed control could be increased by six times if a cheaper herbicide were used.

Conclusions

This is the first report that evaluates transgenic sugarcane in terms of agronomic performance under field conditions and simultaneously assesses the novel, introduced trait.

Table 2. Value of sucrose, weed control costs and income per Rand spent on weed control in the plant and first ratoon crops of herbicide resistant transformed cane.

Treatment	Crop	Sucrose yield (tons/ha)	Value of sucrose (Rands/ha) based on R865/ton in 1999	Total weed control costs (Rands/ha)	Return per Rand spent on weed control (Rands)
Treatment 1 Repeated Buster	Plant	4.9	4 239	2 427	1.8*
	First ratoon	9.0	7 785	1618	4.8*
Treatment 2 Pre-emergence herbicides + Buster	Plant	8.1	7 007	2 668	2.6
	First ratoon	10.4	8 996	1 542	5.8
Treatment 3 Conventional weed control	Plant	6.6	5 709	1 581	3.6
	First ratoon	9.1	7 872	1 085	7.3
Treatment 4 Hand-weeding only	Plant	3.7	3 201	3 432	0.9
	First ratoon	8.1	7 007	1 496	4.7

* these figures could be increased by six times if a herbicide cheaper than Buster® was used

Untransformed cane treated with Buster died, whereas the transformed cane yielded a moderate tonnage of cane (59.8 t/ha). The treatment which resulted in the highest yield in the first ratoon crop (68.0 tons cane/ha) was transformed cane sprayed with conventional pre-emergence herbicides followed by Buster. Pre-emergence herbicides play an important role in reducing weed competition during the early growth stage of the crop, and prevent a possible build-up of resistance to a herbicide in weed populations. As conventional herbicides are competitively priced, the cost of Buster would have to be cheaper for it to be used in a weed control programme in transgenic cane.

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