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Tolerance of cotton expressing a 2,4-D detoxification gene to 2,4-D applied in the field

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Abstract. The tolerance to 2,4-dichlorophenoxy acetic acid (2,4-D) of a genetically modified (transgenic) cotton (*Gossypium hirsutum*) expressing a 2,4-D detoxification gene was compared with conventional (non-transgenic) cotton over 2 seasons. The 2,4-D was applied over-the-top of cotton in the field at 7–17 nodes of crop growth at rates of 0.004–1.12 kg a.i./ha. The transgenic cotton displayed better tolerance to 2,4-D than conventional cotton at all growth stages and herbicide rates. Some damage was apparent on both types of cotton at 2,4-D rates of 0.07 kg/ha and above, with damage most pronounced when the plants were exposed at 7 nodes. The transgenic cotton also had some tolerance to MCPA. Commercial use of transgenic, 2,4-D-tolerant cotton has the potential to greatly reduce problems of 2,4-D damage in cotton from accidental spray drift and herbicide residues in spraying equipment, where plants are predominantly exposed to low rates of 2,4-D.

Additional keywords: genetically modified organism, herbicide resistance, MCPA, transgenic.

Introduction

Cotton (*Gossypium hirsutum*) is grown over a broad geographical region of Australia, from intensive mixed-cropping areas through to areas that previously had only extensive grazing enterprises. Historically, 2,4-dichlorophenoxy acetic acid (2,4-D) has been used through many of these areas to control broadleaf weeds in winter cereal crops such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) and summer crops such as sorghum (*Sorghum bicolour*) (Martin *et al*. 1988). The 2,4-D has also been widely used to control weeds in fallows (Storrie *et al*. 1998) and for controlling the noogoora burr complex (*Xanthium* spp.) and Bathurst burr (*X. spinosum*) in grazing areas (Medd and McMillan 1992).

Cotton is very sensitive to 2,4-D and is readily damaged by 2,4-D drift and residues. The problem of accidental 2,4-D damage to cotton was recognised as early as 1946 (Staten 1946) and remains a problem nearly 60 years later in Australia, the USA, China, and elsewhere. Damage symptoms have been observed from 2,4-D rates as low as 77 μg a.i./ha (Birch 2004), and rates as low as 10 mL/ha can reduce yields when applied to young cotton (Horowitz *et al*. 1976). Field rates, however, are typically much higher (0.5–1.0 kg a.i./ha), increasing the risk of damage from herbicide drift.

Continuing use of 2,4-D in cotton-growing areas has resulted in many instances of 2,4-D damage to cotton crops in Australia (Birch 2004). The use of 2,4-D during the warmer months while cotton is growing (especially the use of the ester formulation of 2,4-D) is actively discouraged in and around the cotton areas, but there have still been many instances where 2,4-D has been inappropriately applied too near to cotton fields, resulting in herbicide damage (Storrie *et al*. 1998). The 2,4-D has also been routinely used during autumn and winter on some fields to be planted to cotton. In some instances, 2,4-D residues remaining in the soil (Hammond 1992; Baker 1993) or in spraying equipment have damaged a subsequent cotton crop.

The development of genetically modified cotton tolerant to 2,4-D has the potential to protect cotton from accidental exposure to 2,4-D, greatly reducing what is an industry-wide problem in many cotton-producing countries, including Australia. The 2,4-D-tolerant cotton expressing a bacterial enzyme (2,4-D dioxygenase) that detoxifies this herbicide was developed in several laboratories (Bayley *et al*. 1992; Lyon *et al*. 1993; Chen *et al*. 1994; Zhang *et al*. 2001) and all have shown good tolerance to 2,4-D under greenhouse conditions.

The objective of this work was to assess the tolerance of the transgenic event produced by Lyon *et al*. (1993) to 2,4-D applied under field conditions. Although cotton is normally exposed to low levels of 2,4-D, exposure to higher levels was also examined in these experiments to better determine the potential of this material to tolerate 2,4-D. The tolerance of the transgenic, 2,4-D-tolerant cotton was compared with conventional cotton of the same genetic background, but without the tolerance gene.

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MCPA (4-chloro-2-methylphenoxy acetic acid) was also included in the second season, as later laboratory studies showed that the transgenic cotton had some tolerance to this herbicide that is structurally similar to 2,4-D.

Materials and methods

These experiments were undertaken using cotton cv. Coker 315, which had been transformed using *Agrobacterium*-mediated transformation to introduce a gene encoding a 2,4-D dioxygenase from *Ralstonia* (formerly *Alcaligenes*) *eutrophus*, which is expressed throughout the plant and breaks down 2,4-D to the considerably less toxic 2,4 dichlorophenol (Lyon *et al.* 1993). Plants were selected that contained a single copy of the gene in a homozygous state and seed was increased under greenhouse conditions. The transformed material was compared with conventional (non-transgenic) cotton cv. Coker 315. All experiments were carried out under contained conditions under permit from the Australian Genetic Manipulation Advisory Committee (GMAC) that at the time administered all research with genetically modified organisms in Australia.

The experiments were situated near Premer, NSW (31°26′S, 149°55′E). The location was selected to be remote from other cotton production, enabling 2,4-D to be used in-crop with safety. The soil was a self-mulching grey clay, pH 7.3, that had been fallowed for more than 12 months before the experiments and contained a full moisture profile at planting. Cotton was sown in November of each year, on raised beds 1.8-m wide, with rows 1-m apart and 2 rows per bed. Regular rain through most of the 1996–97 season ensured good soil moisture and plots were not irrigated in that season. Plots were furrow-irrigated on 3 occasions during the 1997–98 season.

The experiments were surrounded by a 20-m buffer of conventional cotton to act as a pollen sink (Llewellyn and Fitt 1996; Llewellyn *et al.* 2007) and were physically isolated from other cotton by many kilometers to ensure containment of the transgenic trait as required by Australia's guidelines for such experiments. The site was fallowed following the experiments and has not since been replanted to cotton.

The experiment was designed as a stratified, randomised complete block, with transgenic and conventional cotton sown in paired subplots within each herbicide and growth-stage main-plot treatment. Treatments were stratified to ensure that plots with the highest herbicide rates did not occur immediately adjacent to plots with low rates. Main plots were 4 rows wide by 10 m long, with subplots 2 rows by 10 m. Treatments were replicated 3 times. Four buffer rows were sown between each set of main plots in 1996 and 8 buffer rows between plots in 1997. An additional buffer plot was placed between each pair of main plots in 1997 to minimise within-crop spray drift.

Herbicide rates and application times are shown in Table 1. Herbicides were applied through a 4-m-wide hand-held boom, using 8001 flat-fan nozzles operated at 200 kPa with 100 L water/ha. Herbicides were applied in calm conditions, with the lowest rates applied first. The boom was cleaned between each herbicide and application time.

In 1997 the cotton was cut by an early heavy frost 149 days after planting (DAP), before reaching full maturity, and the crop was subsequently terminated. Plant-mapping measurements where taken from 2 randomly selected 1-m sections of row within each subplot. Plant node number, height, boll number, boll retention, and dry weight were recorded. The 1998 crop reached maturity and was machine-harvested 214 DAP. Node number and plant height were also recorded for 2 randomly selected 1-m sections of row within each subplot. Lint yields and ginning percentage were estimated from seed-cotton subsamples using a 20-saw gin.

Data were analysed using the REML routine in GENSTAT 6. Only statistically significant ($P < 0.05$) results are discussed. Plant mapping data were smoothed using moving averages.

Table 1. Summary of treatment combinations used in each season

Both the crop growth stage and corresponding average development stage are indicated. Herbicides were present as dimethylamine salts. The cotton was cut by frosts 149 and 167 days after planting (DAP) and harvested 155 and 214 DAP in 1997 and 1998, respectively

Herbicide rate (kg a.i./ha)		Application time		
2,4-D	MCPA	Nominal growth stage (nodes)[A]		DAP
		1996–97		
Nil		Vegetative	7	76
0.07		Flowering	14	113
0.28		Boll-fill	17	133
1.12				
		1997–98		
Nil	Nil	Vegetative	7	58
0.004	0.08	Square initiation	11	81
0.022	0.4	Flowering	15	109
0.11	2.0	Boll-fill	17	127
0.56				

[A] All herbicide rate × growth stage combinations were included in the experiment.

Results

2,4-D 1996–97 season

Visual observation indicated that the transgenic cotton plants were more tolerant of 2,4-D than were conventional plants at all growth stages and herbicide rates, with rates up to twice a typical field rate of 2,4-D (Table 2). Visual symptoms of 2,4-D damage to conventional plants included leaf reddening, petiole twisting, and leaf death. Plant death occurred following the highest 2,4-D rate applied to conventional plants at 7 nodes.

Herbicide damage symptoms were apparent on both conventional and transgenic cotton 1–2 weeks after application, although symptoms were far more severe on conventional cotton when compared at the same herbicide rate and application time. All new growth on the conventional cotton following 2,4-D applications was distorted, but no distorted growth was observed on transgenic plants. Growth of the transgenic cotton did appear to be delayed for up to 2 weeks after 2,4-D application, but new growth was free of visual symptoms of herbicide damage, regardless of herbicide rate or application time.

The 2,4-D applied to conventional plants at 7 nodes caused a reduction in most plant parameters at all herbicide rates (Table 3). Plants were severely stunted, produced few flowers, retained few bolls, and were unlikely to yield a harvestable amount of cotton, consistent with 2,4-D-induced death of most meristematic tissues. The effect at later growth stages was less

Table 2. Visual observations of plant damage 7 and 21 days after topical applications (DAA) of 2,4-D to conventional and transgenic 2,4-D-tolerant cotton plants during 1996–97

Growth stage at application	Herbicide rate (kg a.i./ha)	Conventional 7 DAA	Conventional 21 DAA	Transgenic 7 DAA	Transgenic 21 DAA
7	0.07	Reddening & leaf curling	Severe damage, plant stunting	No effect	No effect
	0.28	Reddening & twisting, leaf curling	Severe damage, leaf curling	No effect	Slight damage
	1.12	Severe reddening & twisting, leaf death	Severe reddening & twisting, leaf death	Reddening & leaf curling	Slight damage
14	0.07	Slight reddening & twisting	Slight reddening & twisting	No effect	No effect
	0.28	Reddening & twisting	Reddening & twisting	Slight reddening & twisting	Slight reddening
	1.12	Severe reddening & twisting, leaf death	Severe reddening & twisting, leaf death	Severe reddening & twisting	Moderate reddening & twisting
17	0.07	Mild reddening	Mild reddening	Slight reddening	Slight reddening
	0.28	Reddening	Reddening	Slight reddening	Slight reddening
	1.12	Heavy reddening	Heavy reddening	Mild reddening	Slight reddening

Table 3. Plant parameters at season end following topical applications of 2,4-D to conventional and transgenic 2,4-D-tolerant cotton plants during 1996–97

The herbicide was applied at 7, 14, and 17 nodes of plant growth. Parameters were: average node number, height and dry weight per plant, and average % boll retention in the first 3 fruiting positions. The maximum standard error is shown for each parameter

Growth stage at application	Herbicide rate (kg a.i./ha)	Node number		Height (m)		Dry weight (g)		Boll retention (%)	
		Conven.	Trans.	Conven.	Trans.	Conven.	Trans.	Conven.	Trans.
	Nil	18.1	17.4	0.75	0.70	593	512	23.6	21.4
7	0.07	16.7	16.8	0.48	0.65	313	518	1.1	28.2
	0.28	13.1	16.7	0.39	0.79	203	636	1.9	32.2
	1.12	10.3	15.1	0.29	0.73	69	707	0.3	45.4
14	0.07	15.9	16.8	0.49	0.55	377	450	7	27.5
	0.28	17.3	15.2	0.69	0.66	333	466	8.5	25.0
	1.12	15.6	15.7	0.55	0.67	198	363	3.3	18.6
17	0.07	16.8	16.2	0.61	0.58	457	391	25.3	20.0
	0.28	15.1	15.1	0.70	0.70	477	468	21.3	18.4
	1.12	18.0	15.1	0.85	0.75	531	386	21.4	19.7
	Max. s.e.	1.6		0.08		71		3.5	

severe and may have been masked by the production of distorted vegetative growth following the herbicide application.

The final number of nodes of conventional plants was reduced by the higher herbicide rates applied at 7 nodes. The 2,4-D had a much smaller effect on node number when applied to older plants, partly due to the production of vegetative growth following the herbicide application. The 2,4-D had little effect on node number of the transgenic plants.

The 2,4-D applications affected the internode lengths of both conventional and transgenic plants, with the largest effects following the herbicide application at 7 nodes (Fig. 1). There was a significant ($P < 0.05$) 4-way interaction between node, herbicide rate, growth stage, and gene. The 2,4-D applied at 7 nodes reduced the internode lengths of later nodes of the conventional plants at all rates, although there was a stimulation of growth at nodes 18 and 19 at the highest rate. In contrast, internode length was stimulated in transgenic plants sprayed at 7 nodes, with increases in length from nodes 2 to 11 at the highest rate. The 2,4-D had less effect on the internode lengths of plants sprayed at the later growth stages (applied at nodes 14 and 17), where most nodes had developed before the herbicide application. Some stimulation of growth of the lower inter-nodes was apparent in both conventional and transgenic plants sprayed at nodes 14 and 17 at the higher herbicide rates.

The reduction in node number and node length following the 2,4-D application at 7 nodes combined to give a large reduction in the height of conventional plants sprayed at all herbicide rates (Table 3). The height of conventional plants was also lower at the 0.07 and 1.12 kg/ha rates sprayed at 14 nodes and the low rate applied at 17 nodes. The 2,4-D did not reduce the height of the transgenic cotton, except at the low rate applied at 14 and 17 nodes. Reductions in node number at other rates and stages were compensated for by increases in internode length.

Dry-matter production of the conventional cotton was reduced by all 2,4-D applications except the 1.12 kg/ha rate applied at 17 nodes. Reductions were greater with increasing

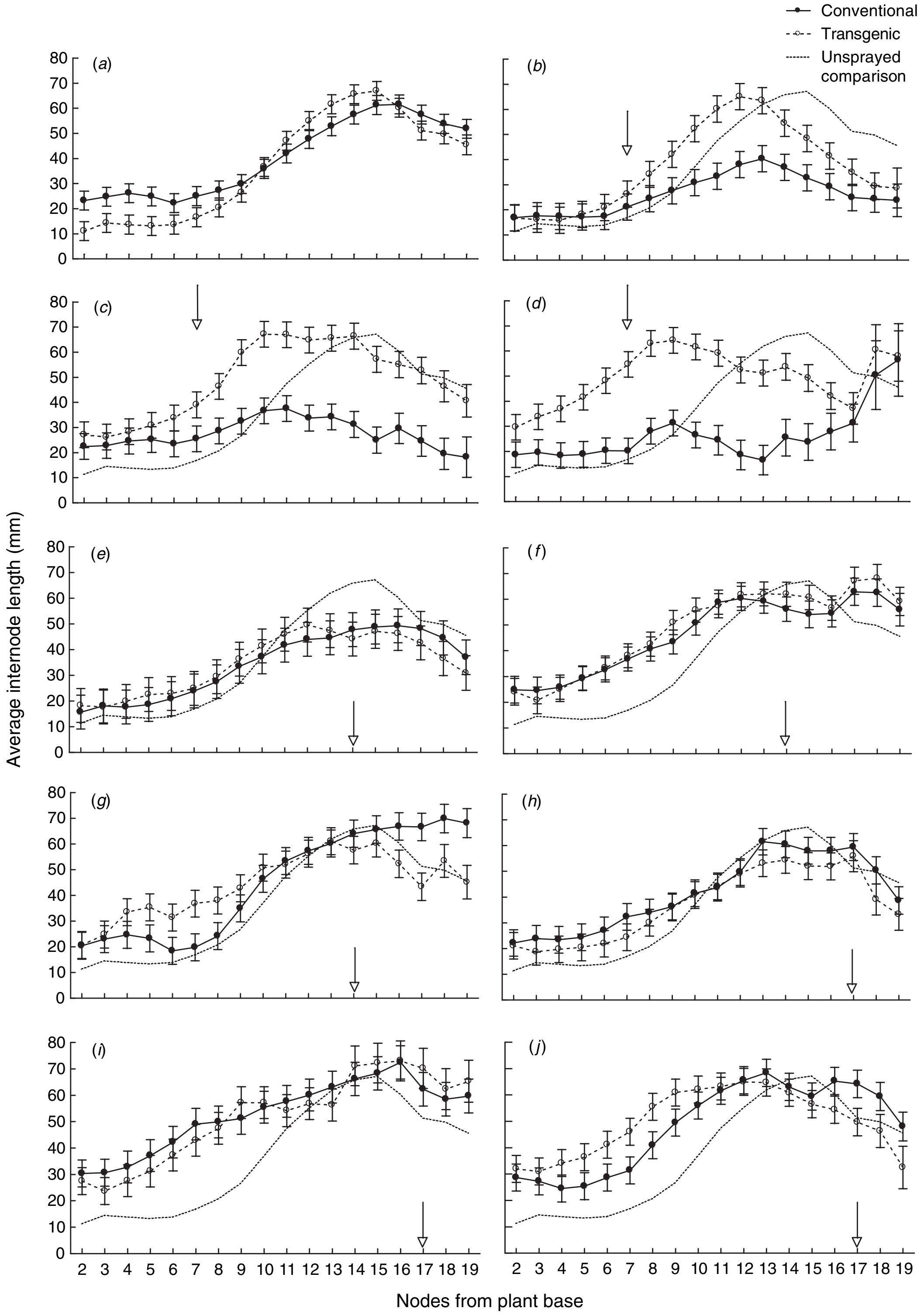

Fig. 1. Average internode length of conventional and transgenic cotton in the 1996–97 season starting at node 2, exposed to 2,4-D at 7, 14, and 17 nodes of plant growth, as indicated by arrows. Treatments were (*a*) untreated, (*b*) 0.07 kg a.i./ha at 7 nodes, (*c*) 0.28 kg a.i./ha at 7 nodes, (*d*) 1.12 kg a.i./ha at 7 nodes, (*e*) 0.07 kg a.i./ha at 14 nodes, (*f*) 0.28 kg a.i./ha at 14 nodes, (*g*) 1.12 kg a.i./ha at 14 nodes, (*h*) 0.07 kg a.i./ha at 17 nodes, (*i*) 0.28 kg a.i./ha at 17 nodes, and (*j*) 1.12 kg a.i./ha at 17 nodes. The internode length curve from the unsprayed transgenic treatment has been included in all sprayed treatments for ease of comparison. Curves were smoothed using moving averages. Standard error bars are shown.

herbicide rates at 7 and 14 nodes (Table 3). The transgenic cotton was less affected by the herbicide, although there were reductions in dry weight at the 1.12 kg/ha 2,4-D rate applied at 14 nodes and the 0.07 and 1.12 kg/ha rates applied at 17 nodes.

Percentage boll retention on the first 3 fruiting positions of the conventional cotton was substantially reduced by all 2,4-D applications at 7 and 14 nodes because of high levels of floral abortion (Table 3). Average percentage boll retention was not reduced by any of the applications to the transgenic cotton, although there were effects on total boll number at different nodes (Fig. 2). The large increase in boll retention of the transgenic plants sprayed with the highest 2,4-D rate at 7 nodes is probably an artefact of the design, where transgenic plants were able to gain a soil moisture advantage as they were paired with much smaller conventional plants damaged by the herbicide. The design used, with gene as a subplot treatment, was partly confounded by this effect, but this was the most satisfactory way to ensure that conventional and transgenic plants were equally exposed to the herbicide.

The final distribution and number of retained bolls on conventional and, to a lesser extent, transgenic plants were affected by all 2,4-D rates applied at all growth stages, most often with a reduction in the retention of early bolls (Fig. 2). The number of retained bolls on conventional cotton was reduced at nodes 10–16 by all 2,4-D applications at the 7 and 14 node stages, with most squares and flowers aborting following the herbicide applications. The 2,4-D applied at 17 nodes had less effect on boll number, but still caused some boll loss on conventional plants. Transgenic plants were much less affected, with some loss of bolls at nodes 10–12 following most applications and increased boll production on later nodes.

In the untreated plots, boll number at nodes 10–12 was lower on the conventional than on the transgenic plots, probably due to a small amount of spray drift. Evidence of this was also apparent in the internode length data, with increased internode lengths on nodes 2–6 of the conventional cotton (Fig. 2). The spray drift could have been from some of the higher application rates, but may have been from a more distant source.

2,4-D 1997–98 season

Visual observation again indicated that transgenic cotton plants were more tolerant of 2,4-D than were conventional plants at all growth stages and herbicide rates used. Damage symptoms were less apparent with the lower herbicide rates used in this season.

Complete plant mapping was not undertaken in this season as plants were able to grow through to maturity and lint yields were recorded. Plant height and node number were recorded.

Only the highest 2,4-D rate reduced the number of nodes per plant on conventional plants sprayed at 7 nodes (Table 4). Node number of the conventional plants was also reduced by the 0.11 kg/ha rate applied at 11 nodes and 0.022 and 0.11 kg/ha rates applied at 17 nodes. Node number was only reduced on the transgenic plants sprayed with the 0.004 kg/ha rate at 15 nodes and the 0.11 kg/ha rate at 17 nodes.

The height of conventional cotton was reduced by all 2,4-D rates applied at 7 nodes and by the higher rates sprayed at 11 nodes (Table 4).

The ginning percentage of conventional cotton was reduced by 2,4-D applications at the higher rates sprayed at 7 and 11 nodes (data not presented). The reductions were associated with very low yields and high trash content on these plots. The herbicide had no effect on the ginning percentage of the transgenic cotton.

Lint yields of the conventional plants were reduced by nearly all herbicide applications, regardless of growth stage, with large yield reductions from applications at 7 and 11 nodes (Table 4). Yield reductions generally increased as herbicide rate increased. Lint yield of transgenic cotton was not reduced by 2,4-D applied at 7 or 17 nodes, but was reduced by the heavier rates of 2,4-D applied at 11 nodes and the heaviest rate applied at 15 nodes, suggesting that developing flowers were the most susceptible to 2,4-D damage.

MCPA

Visual observations indicated that transgenic cotton was more tolerant of MCPA than was conventional cotton, although both cotton types were heavily damaged by MCPA applied at the highest rate, equivalent to a typical field rate of MCPA. Visual symptoms of MCPA damage were similar to symptoms of 2,4-D damage, with severe reddening, petiole twisting, and some leaf death.

The higher rates of MCPA reduced the number of nodes of conventional plants sprayed at 7 and 11 nodes (Table 5). There was also some reduction from the 15 and 17 node applications. There was a reduction in node number on the transgenic plants sprayed at the highest rate at 7 and 15 nodes.

MCPA reduced the height of conventional cotton sprayed at the higher rates at 7 and 11 nodes, but did not affect the plant height of transgenic cotton (Table 5).

The yield of conventional cotton plants was reduced by all MCPA rates (Table 6). The transgenic cotton was more tolerant of MCPA than was conventional cotton. Lint yield of the transgenic cotton was nearly double that of the conventional cotton at the higher MCPA rates. Time of application had no significant effect on lint yield.

Discussion

The topical 2,4-D applications damaged conventional cotton, regardless of application rate or crop growth stage, with rates as low as 4 g a.i./ha reducing the yield of young cotton. These results were consistent with previous observations (Porter *et al.* 1959; Horowitz *et al.* 1976; Banks and Schroeder 2002).

Overall, the transgenic cotton showed good tolerance to 2,4-D in the field at all growth stages, even at rates well above those expected from spray drift of ~10–30% of field rates (Al-Khatib *et al.* 1993). Where damage was observed, the transgenic plants were generally able to recover and reductions in yield were relatively small by comparison with the effects of comparable exposure to conventional cotton. Nevertheless, the transgenic cotton was damaged by the higher herbicide rates, with reductions evident in all plant parameters recorded. The degree of herbicide tolerance observed in the field was clearly less than was expected from greenhouse observations (Lyon *et al.* 1993). Consequently, the direct use of 2,4-D on this cotton for weed control could not be recommended even if this

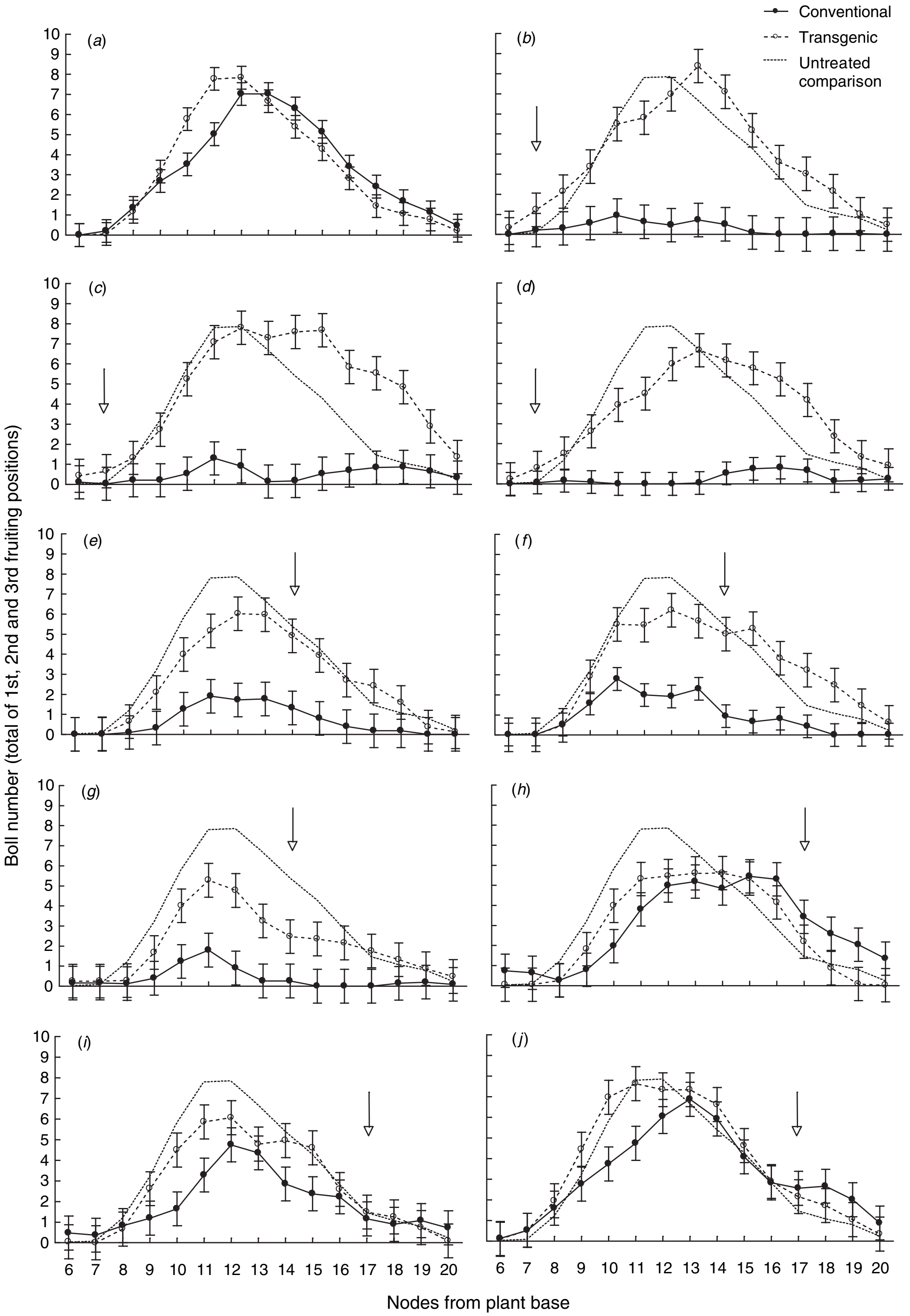

Fig. 2. Total boll number of the first 3 fruiting positions of conventional and transgenic cotton in the 1996–97 season, exposed to 2,4-D at 7, 14, and 17 nodes of plant growth, as indicated by arrows. Treatments were (*a*) untreated, (*b*) 0.07 kg a.i./ha at 7 nodes, (*c*) 0.28 kg a.i./ha at 7 nodes, (*d*) 1.12 kg a.i./ha at 7 nodes, (*e*) 0.07 kg a.i./ha at 14 nodes, (*f*) 0.28 kg a.i./ha at 14 nodes, (*g*) 1.12 kg a.i./ha at 14 nodes, (*h*) 0.07 kg a.i./ha at 17 nodes, (*i*) 0.28 kg a.i./ha at 17 nodes, and (*j*) 1.12 kg a.i./ha at 17 nodes. The boll production curve from the unsprayed transgenic treatment has been included in all sprayed treatments for ease of comparison. Curves were smoothed using moving averages. Standard error bars are shown.

Table 4. Plant parameters at maturity following topical applications of 2,4-D to conventional and transgenic 2,4-D-tolerant cotton plants during 1997–98

The herbicide was applied at 7, 11, 15, and 17 nodes of plant growth. Parameters were: average node number and height per plant and lint yield. The maximum standard error is shown for each parameter

Growth stage at application	Herbicide rate (kg a.i./ha)	Nodes		Height (m)		Lint yield (kg/ha)	
		Conven.	Trans.	Conven.	Trans.	Conven.	Trans.
	Nil	20.0	19.1	0.90	0.81	468	402
7	0.004	19.8	19.0	0.80	0.78	88	362
	0.022	20.7	20.3	0.73	0.85	97	403
	0.11	18.2	23.2	0.69	0.92	0	343
	0.56	11.5	20.5	0.32	0.99	0	377
11	0.004	22.8	18.3	1.04	0.89	190	386
	0.022	18.3	20.5	0.78	0.85	33	382
	0.11	17.2	23.2	0.65	0.92	6	243
	0.56	18.0	20.5	0.61	0.91	0	257
15	0.004	19.0	16.7	0.82	0.74	350	365
	0.022	22.5	20.8	0.95	0.86	402	417
	0.11	19.8	18.2	0.90	0.76	213	401
	0.56	18.8	19.5	0.79	0.82	24	280
17	0.004	20.5	20.3	0.94	0.81	295	417
	0.022	16.8	20.5	0.87	0.86	389	383
	0.11	15.0	15.2	0.84	0.72	313	317
	0.56	19.8	19.2	0.87	0.87	274	389
	Max. s.e.	1.3		0.05		74	

Table 5. Average node number and height per plant at maturity following topical applications of MCPA to conventional and transgenic 2,4-D-tolerant cotton plants during 1997–98

The herbicide was applied at 7, 11, 15, and 17 nodes of plant growth. The maximum standard error is shown for each parameter

Growth stage at application	Herbicide rate (kg a.i./ha)	Nodes		Height (m)	
		Conven.	Trans.	Convent.	Trans.
	Nil	21.1	19.8	0.91	0.84
7	0.08	20.8	18.7	0.84	0.93
	0.4	16.8	20.8	0.66	0.81
	2.0	13.7	15.2	0.41	0.76
11	0.08	20.0	16.3	0.80	0.73
	0.4	17.5	18.7	0.73	0.76
	2.0	12.3	19.2	0.63	0.87
15	0.08	19.2	18.7	0.99	0.82
	0.4	18.2	21.2	0.84	0.85
	2.0	21.2	17.3	0.86	0.87
17	0.08	17.7	20.3	0.93	0.93
	0.4	20.8	21.0	0.86	0.86
	2.0	17.7	19.2	0.83	0.76
	Max. s.e.	1.2		0.05	

Table 6. Cotton lint yield following topical applications of MCPA to conventional and transgenic 2,4-D-tolerant cotton plants during 1997–98

The time of herbicide application had no effect on lint yields. The maximum standard error is shown

Herbicide rate (kg a.i./ha)	Lint yield (kg/ha)	
	Conven.	Trans.
Nil	406	340
0.08	281	296
0.4	125	241
2.0	92	173
Max. s.e.	39	

transgenic material became commercially available in an elite cotton variety.

Even larger reductions in lint yield and other plant parameters of both transgenic and conventional cotton may have been observed in these experiments if lower water rates had been used and spray concentration rather than spray volume had been maintained at a constant across treatments (Banks and Schroeder 2002; Ellis *et al.* 2002). It is likely that our results underestimate the impact of spray drift from aerial and ground-rig applications where water rates are typically in the order of 25–60 L/ha at the point of application. Spray concentration at the point of deposition may be an order of magnitude higher again, with much of the carrier volume evaporating before deposition in hot conditions (typically 20–35°C). However, accurate application of very low spray volumes in these conditions in the field (down to 0.8 mL/ha if spray concentration was maintained) was not possible with the available equipment and would not have been conducive to accurate spray placement.

The lint yields obtained in these experiments were also lower than is normal in commercial Australian cotton production, due to the use of a poorly adapted variety (Coker 315) and the location of the experiments in a suboptimal growing area. Consequently, the experimental results cannot be directly extrapolated to the commercial cotton industry in Australia where water rates for herbicide applications are typically in the range of 25–60 L/ha and crop yields in the range of 1500–2500 kg lint/ha. However, it seems likely that this gene event would protect commercial cotton from most 2,4-D spray drift occurrences.

We were not able to compare this transgenic event with others developed elsewhere in the world (Bayley *et al*. 1992; Chen *et al*. 1994; Zhang *et al*. 2001), but it is possible that the field performance of one of these other gene events may give superior results to those reported here. The field expression of this tolerance may also be improved with another gene promoter.

Nevertheless, the transgenic plants displayed a high level of tolerance to 2,4-D and the commercial use of this material should greatly reduce problems of 2,4-D damage to cotton from spray drift and herbicide residues in spraying equipment.

While transgenic cotton displayed better tolerance to MCPA than did conventional plants, the tolerance was less robust than was the case for 2,4-D. This is consistent with the known differences in the ability of the 2,4-D dioxygenase enzyme encoded by the transgene to degrade phenoxyacetic acid herbicides. The additional tolerance afforded to MCPA is likely to be a bonus, but not a marketable attribute of the 2,4-D-tolerant transgenic cotton.

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