FIELD EVALUATION OF TRANSGENIC 2,4-D TOLERANT COTTON

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Summary

The efficacy of transgenic, 2,4-D tolerant cotton, developed by CSIRO has been field tested over the past 2 seasons on an irrigated field at Premer, on the Liverpool Plains of NSW. The material tested was a single transgenic line in the Coker 315 cultivar containing an introduced 2,4-D degradation gene and it showed good tolerance to 2,4-D, even at high rates. The effects of spraying both transgenic and normal plants with 2,4-D were monitored in the 1996/97 season using visual damage symptoms and dry matter production. Dry matter cuts taken at the end of the season indicated complete tolerance of the transgenic line to 2,4-D at up to twice the normal field rate when applied during the vegetative growth stage. Good tolerance was also observed at flowering and boll-fill stages.

A similar trial was carried out with the same material in the 1997/98 season when conditions were more favourable and the plots were harvested to determine lint yield. Lint yields showed no negative effect of 2,4 -D on the transgenic cotton, except when the herbicide was applied at the squaring stage, where some yield depression occurred. Tolerance to MCPA was also assessed in this season. Lint yield showed no depression in the transgenic cotton due to MCPA at the lowest rate (4% of field rate), but significant yield depression occurred at the highest MCPA rate. The level of tolerance displayed by the plants warrants further evaluation at a commercial level if the 2,4-D tolerance gene were backcrossed into elite cultivars

Background

2,4-D is a phenoxy herbicide that is a potent growth regulator in many plants that is actively transported to the meristematic area (the area of new cell growth) where it causes deformation of the tissues and even plant death. Cotton is extremely sensitive to 2,4-D and even very low rates cause some visible damage. On cotton, damage normally shows up as deformed leaves, with leaf strapping, crinkling and cupping, and twisting of the petioles (leaf stems), stem and squares. Reddening of the leaves and petioles may also occur. In severe cases, 2,4-D damage can kill cotton plants.

2,4-D commonly comes in two formulations; 2,4-D amine, which is relatively non-volatile, and 2,4-D ester, which is volatile. Where 2,4-D is sprayed during the cotton-season there is always a risk of off-target movement, but this risk is much greater with the ester formulation. Movement can be in the form of spray drift, such as may occur in windy conditions, or through volatilisation of 2,4-D ester, where the herbicide is correctly applied to the target, but some then volatilises back into the air. These very fine herbicide droplets can be carried for long distances and may effect a cotton crop many kilometres from the site of herbicide application.

2,4-D damage can also occur when 2,4-D is applied to a fallow field in a dry winter and no significant rainfall occurs between herbicide application and the planting of cotton in that field. 2,4-D normally has a very short half-life in soil, its breakdown is depedent on the activity of soil bacteria, but under cold dry conditions microbial activity can be minimal and sufficient residual 2,4-D may remain in the soil to cause damage to the subsequent cotton crop.

2,4-D is also quite difficult to remove from spraying equipment and damage can occur if equipment is used to spray 2,4-D in winter and not adequately decontaminated before use on the subsequent cotton crop. This damage can occur as a result of the trace amounts of 2,4-D which remain in the equipment.

Despite these difficulties, 2,4-D is, nevertheless, an effective and relatively inexpensive herbicide which is often used for broad-leaf weed control in fallows in winter, often as a tank-mix with glyphosate. These fallows may follow a cotton crop or may precede a new crop. 2,4-D is also widely used for broad-leaf weed control in cereal crops and in summer for burr control, although this later practice should never occur in a cotton-growing region because of the sensitivity of cotton to this herbicide.

As one way to reduce the problem of 2,4-D damage in the cotton industry, CSIRO have developed a cotton plant which is genetically altered to make it tolerant to 2,4-D (Lyon *et al.,* 1989). A gene isolated from one of the bacteria that breaks down 2,4-D in our agricultural soils has been inserted into the plants to allow them the detoxify the 2,4-D to a less toxic chemical before any damage has occurred to the cotton. The gene was originally introduced into the Coker 315 cultivar, an American cultivar not suited to Australia's cotton production system and some breeding is being done to transfer the genes into our elite cultivars. As yet, all the field efficacy studies have been done on the Coker material. The Coker transformant has shown good tolerance to 2,4-D in the laboratory, as well as some tolerance to MCPA, but it was necessary to test the material's performance in the field to evaluate whether it had a useful level of tolerance. To do this a site was selected near Premer, on the Liverpool Plains. This site was too cool for commercial cotton production, but was sufficiently far away from commercial cotton to ensure that the 2,4-D could be used without danger to any neighbouring commercial crops.

The 1996/97 season

The cotton was first evaluated in the 1996/97 season in small plots within a commercial sorghum field. Sorghum is tolerant to 2,4-D. The material was planted on November 12 into 2 row by 10 m sub-plots (4 row by 10 m main-plots) with 3 replicates and surrounded by buffers of a Siokra cultivar as required by GMAC, the regulatory authority controlling the field testing of transgenic plants. The design was a split-plot, with transgenic and normal cotton side-by-side in each treatment. This design ensured that transgenic and normal plants were equally exposed to the herbicide, but gave the transgenic plants an advantage when the normal plants in the neighbouring rows were stunted or killed by the herbicide. Unfortunately, the cotton established poorly and unevenly due to variable soil moisture at planting and was subsequently treated as a rain-grown crop. 2,4-D was applied at vegetative, flowering and boll fill stages (27 January, 4 March and 24 March, respectively), at 4.5, 1.1 & 0.28 L/ha, corresponding to 2 X, 0.5 X and 0.125 X of normal field rates.

The experiment was terminated on 15 April, when plant mapping occurred and dry-matter cuts were taken to assess biomass production; there were no open bolls on the crop at this stage. The results for the first trial are shown below in Figure 1, where the dry matter yield of the normal cotton (Coker 315) is expressed as a percentage of the yield of the corresponding transgenic cotton treatment.

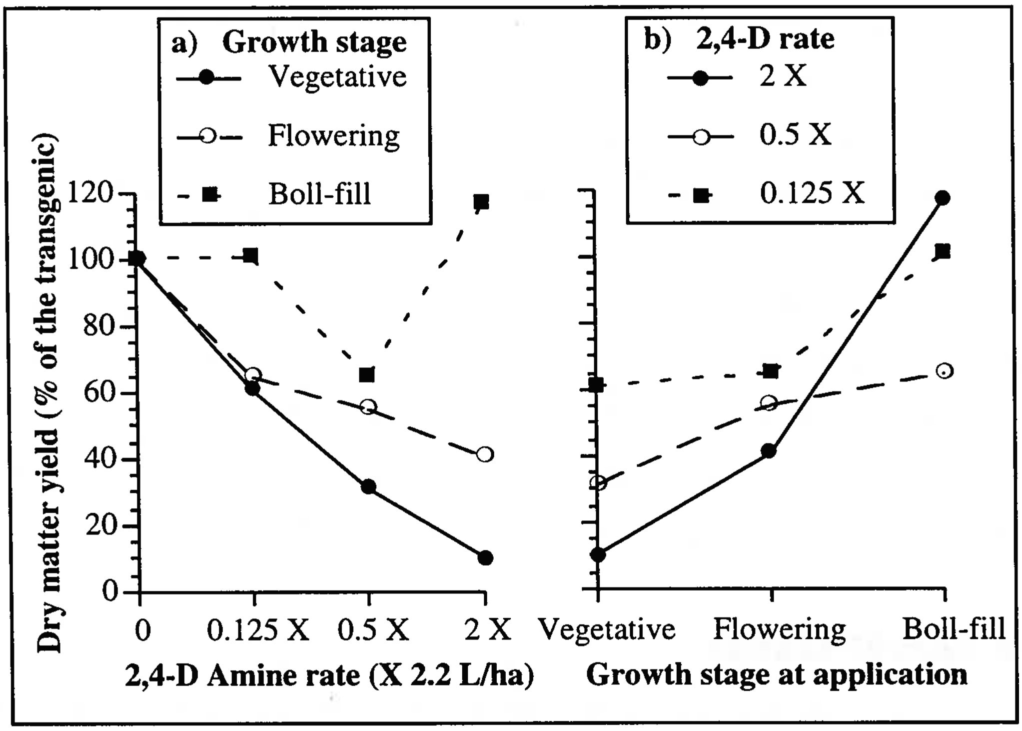

Figure 1. Cotton dry-matter production of normal cotton (Coker 315) relative to transgenic, 2,4-D tolerant cotton in 1996/97. 2,4-D was applied over-the-top of cotton at the growth stages and rates indicated.

The dry-matter yield was most effected when 2,4-D was applied at the vegetative growth stage (Figure 1a), with plant weight reduced by 90% at the highest 2,4-D rate (weight of the normal cotton compared to the transgenic cotton). The result from the boll-fill application was inconsistent, with plants already having achieved their mature size before the 2,4-D was applied. Figure 1b shows more clearly that the herbicides had less effect on plant weight later in the season.

Visual observations indicated that the transgenic material was mildly effected by the highest rate of 2,4-D, but they seemed to grow out of the effect after 7 to 10 days, with no apparent effect on square development, flowering or boll retention.The transgenic plants treated with the lower rates showed no visible effects of 2,4-D application.

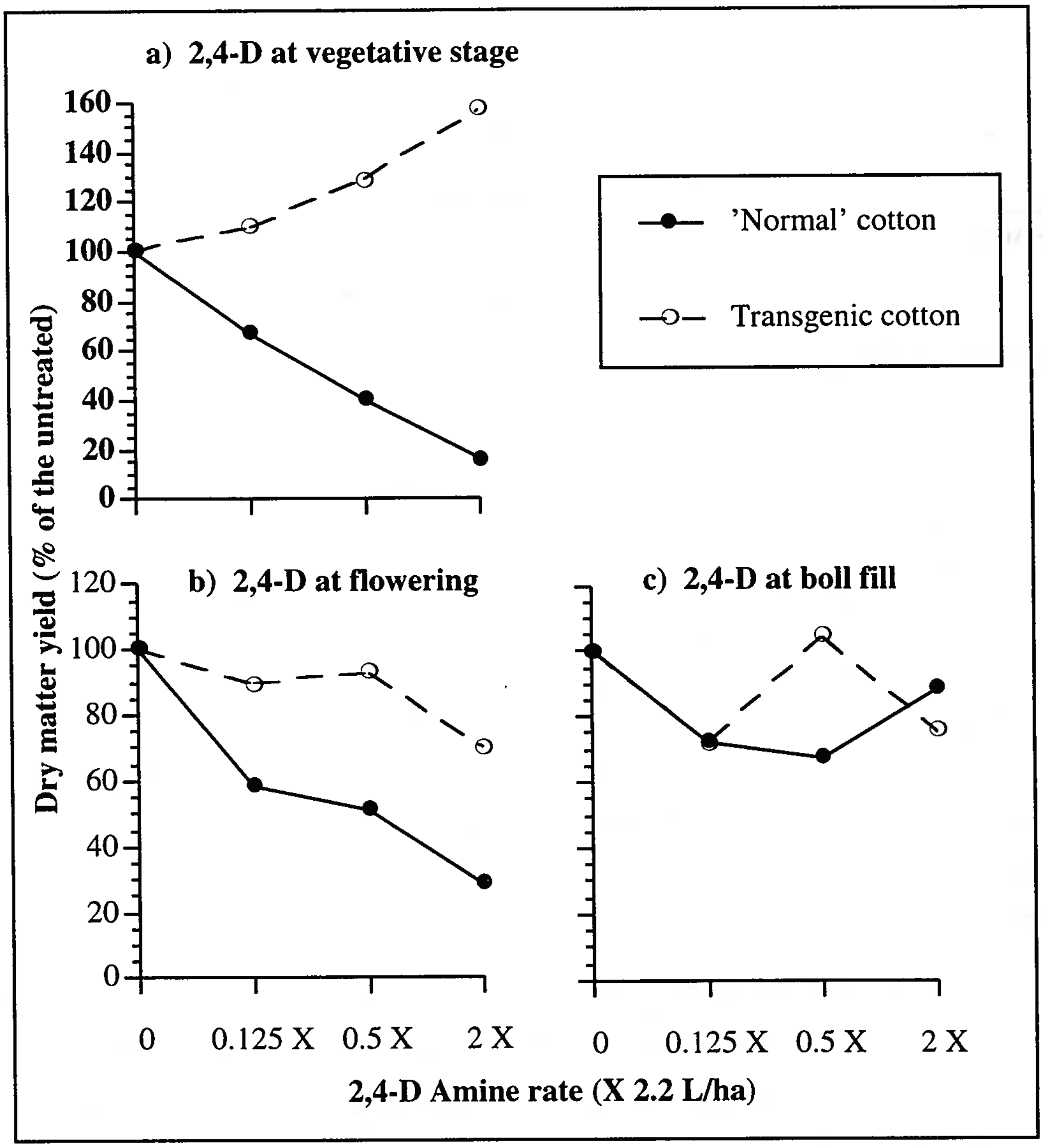

Figure 2. Cotton dry-matter production in 96/97. 2,4-D was applied over-the-top of cotton at the growth stages and rates indicated. The increase in yield of the transgenic cotton in figure a) is an artefact caused by the experimental design.

Examination of the full data set (Figure 2) expressed as a percentage of the unsprayed plots, showed an advantage to the transgenic material from herbicide applied during the vegetative stage (Figure 2a), but this is an artefact of the split-plot design, where the transgenic plants had the advantage of no competing plants in the adjoining rows. There is also indication of a depression in growth of the transgenic material from 2,4-D applied at high rates during flowering (Figure 2b), suggesting that the tolerance gene is expressed less well later in development.

Nevertheless, the transgenic material clearly performed well when compared to normal cotton, exhibiting high levels of tolerance to extremely high levels of 2,4-D. The levels of 2,4-D used in the experiment were far higher than would be experienced by cotton under herbicide drift conditions.

The 1997/98 season

The experiment was repeated last season with basically the same design, but with the inclusion of MCPA and an additional application time. Also the herbicide rates used were lower, with 2,4-D amine applied at 2.2, 0.45, 0.09 & 0.02 L/ha, corresponding to 1 X, 0.2 X, 0.04 X and 0.008 X (or 0.8%) of normal field rates, and MCPA applied at 4.0, 0.8 & 0.16 L/ha, corresponding to 1 X, 0.2 X and 0.04 X of normal field rates.

The cotton was planted on November 10 and establishment was again variable. The field was irrigated twice and had 2 insecticides applied. Herbicides were applied at vegetative, squaring, flowering and boll-fill stages (7 January, 30 January, 27 February and 5 March, respectively), and the cotton was picked on 11 June with a single-row, plot picker. The lint yields are shown in Figure 3.

Cotton lint yield following 2,4-D application was lower on the normal than the transgenic cotton at all times except for the lightest application when applied at boll-fill (Figure 3a). Generally, the level of lint yield reduction declined with later applications and declined as the 2,4-D rate was reduced.

Cotton lint yield following MCPA application was lowest at the vegetative and boll-fill stages (Figure 3b). There was relatively little impact of MCPA at flowering.

Examination of the full data set shows that the transgenic cotton was not adversely affected by 2,4-D applied at the vegetative stage (Figure 4a), but that there was a 30% yield penalty from applying the full rate of MCPA.

2,4-D applied at squaring (Figure 4b) did reduce the yield even of the transgenic material, with a 45% yield penalty at the highest rate, although this was still far better than the result for the non-transgenic cotton which was severely affected (95% yield penalty).

MCPA at the 2 higher rates also resulted in a yield penalty on the transgenic cotton, consistent with lower tolerance to MCPA observed in glasshouse trials (Llewellyn, unpublished data).

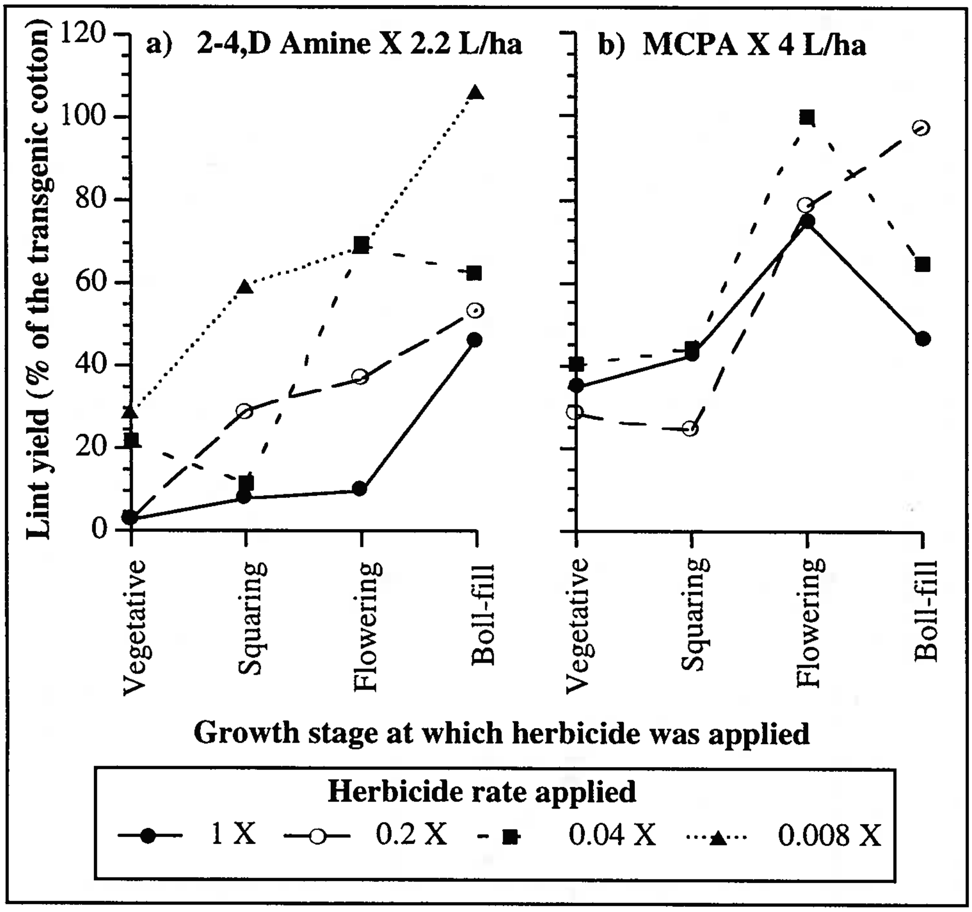

Figure 3. Cotton lint yield of normal cotton relative to herbicide tolerant cotton in 97/98. Herbicides was applied over-the-top of cotton at the growth stages and rates indicated.

2,4-D applied at flowering (Figure 4c) had little, if any adverse effect on the yield of the transgenic material, but MCPA at the 2 higher rates again resulted in a large yield penalty on the transgenic cotton of around 55%.

The results for 2,4-D applied at boll-fill (Figure 4d) are inconsistent, but indicate there was no adverse effect on the yield of the transgenic material, and there was also probably no effect of MCPA on the transgenic cotton at this stage.

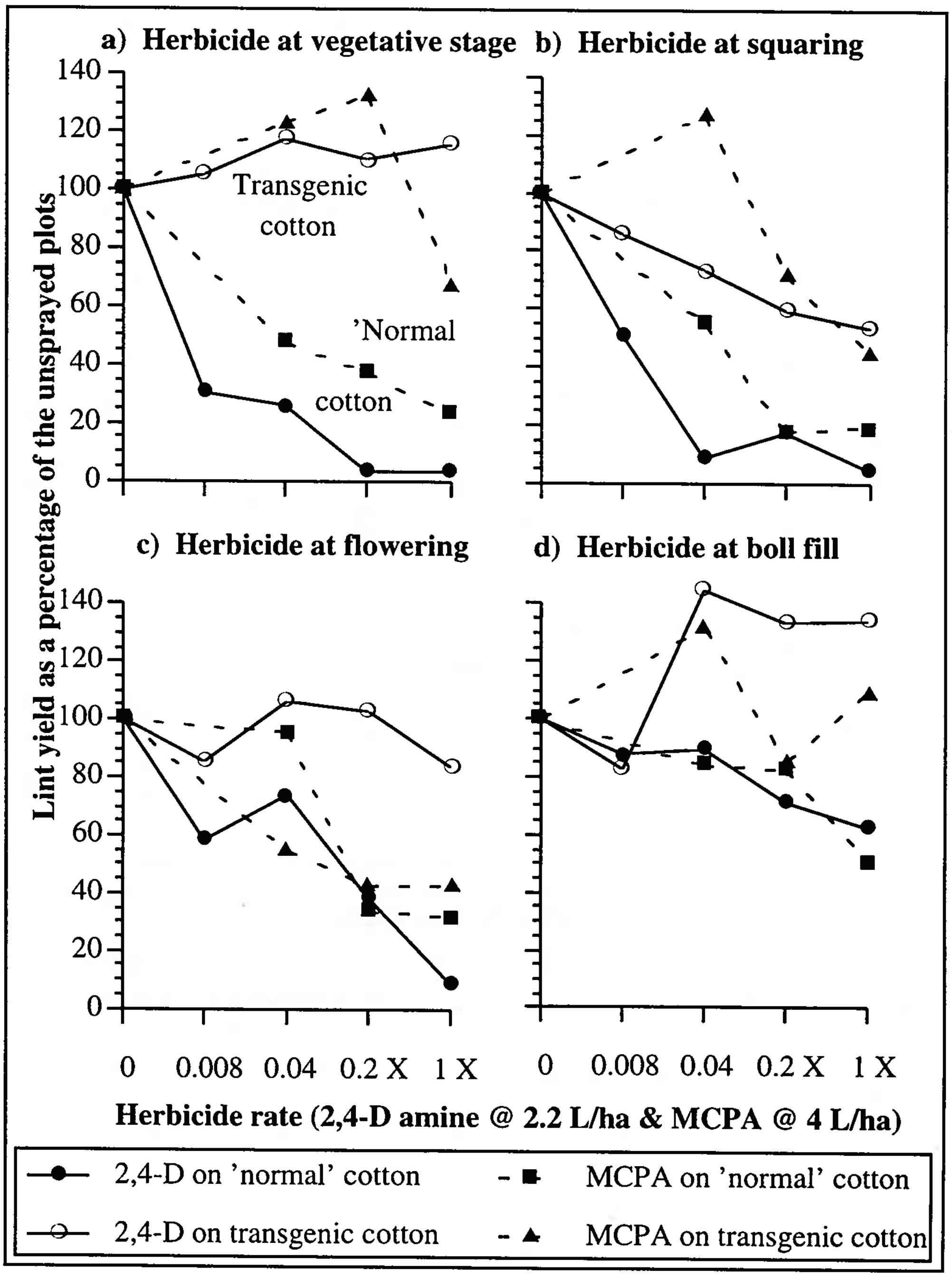

Figure 4. Cotton lint yield in 97/98. Herbicides was applied over-the-top of cotton at the growth stages and rates indicated.

Conclusion

The transgenic, 2,4-D tolerant cotton has shown very good field tolerance to 2,4-D at all tested rates over two seasons. However, there does seem to be some decline in tolerance during the squaring phase of plant development, although tolerance increases again upon flowering. The cotton also has significant tolerance to MCPA during the early vegetative growth stage, but less tolerance during later growth stages. The enzyme being expressed in the plants is known to have less but significantly less activity in detoxifying MCPA than 2,4-D, and this is reflected in the lower overall tolerance to MCPA in the field.

This level of tolerance to 2,4-D should be of great value to the cotton industry and if incorporated into elite commercial lines, should greatly reduce the occurrence of 2,4-D damage from drift. The field tolerance to MCPA is marginal and is likely to have little practical impact on the cotton industry.

Acknowledgments

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References

Lyon, B. R., Cousins, Y. L., Llewellyn, D. J., Dennis, E. S. (1993) Cotton plants transformed with a bacterial degradation gene are protected from accidental spray drift damage by the herbicide 2,4-dichlorophenoxyacetic acid. *Transgenic Res.* **2**: 162-169.