A bromoxynil tolerant transgenic subterranean clover

GA Sandral[1], BS Dear[1], TJ Higgins[2] and D Spencer[2]

[1]NSW Agriculture, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW 2650
[2]CSIRO, Division of Plant Industry, Black Mountain, Canberra, ACT 2600.

Abstract

The effect of inserting the Oxy-gene into subterranean clover on its tolerance to the herbicide bromoxynil was examined in 3 transgenic lines of the cultivar Gosse under glasshouse and field conditions. The Oxy gene greatly enhanced the tolerance of the 3 transgenic lines at both the cotyledon and 3-4 leaf stage. One of the transgenic lines had elevated levels of the isoflavone genistein but the levels of the oestrogens Biochanin A and formononetin remained low in all 3 transgenics. The inclusion of the Oxy-gene offers the potential to greatly improve broadleaf weed control in subterranean clover pastures and the opportunity to apply bromoxynil earlier and at lower rates.

Key words

Herbicide tolerance, oxy gene, genetic engineering.

Subterranean clover cultivars are known to be very sensitive to most broadleaved herbicides (1) suffer-ing large reductions in herbage yield following herbicide application to control broadleaf weeds in newly establishing pastures. Although subterranean clover becomes more tolerant to herbicides with age, weeds also increase their tolerance thus reducing the efficacy of herbicides when applied later in the season. Bromoxynil is one of the more selective herbicides on subterranean clover but still reduces the herbage yield by up to 34% 30 days after herbicide application (DAHA) (1), with damage becom-ing greater if applied at temperatures above 20oC (5). This greatly restricts the period in which the herbicide can be applied and consequently its effectiveness. In an attempt to improve the tolerance of subterranean clover to bromoxynil, the Oxy gene, isolated from the soil bacterium Klebsiella ozaenae, was inserted using a dis-armed Agrobacterium into the cultivar Gosse. (3).

The effectiveness of this gene in enhancing bromoxy-nil tolerance in 3 transgenic Gosse constructs (Oxy 5, 7 and 10) was evaluated in separate field and glasshouse experiments.

Materials and methods

Glasshouse experiment

Seeds of the same 3 transgenic lines and Gosse were sown into 15 cm diameter pots containing a red earth soil in the glasshouse and thinned to 5 plants per pot at the cotyledon stage. Treatments consisted of 3 herb-icides (bromoxynil @ 1.5 L/ha, Jaguar? @ 1.5 L/ha and Brominil M? @ 0.6 L/ha), applied to the 4 lines/cultivars at two leaf stages (cotyledon and 3-4 true leaves) with an unsprayed control. The pots were arranged in a completely randomised block design with 2 replications. Only the results of the bromoxynil treatment will be reported here. Herbicide phytotoxicity was determined by scoring seedlings 10 DAHA using the EWRC scale and plant dry weight by harvesting all plants 30 DAHA.

Field experiment

Seeds of the 3 transgenic Oxy lines and Gosse were sown into small peat pots and grown in the glasshouse until they reached the 1-2 true leaf stage. They were then transplanted into the experimental area as spaced plants in a randomised plot experiment with 15 plants/plot and 3 replications. The experiment was located at the Agricultural Institute at Wagga Wagga. The trial area was fully fenced and covered with covered with weed matting and bird netting in accordance with the requirements of the Genetic Manipulation and Advisory Committee (GMAC).

The herbicide bromoxynil was applied at 1.5 L/ha at the 4-5 leaf stage to half the plots using a hand held boom spray. Plants were scored for herbicide damage 30 DAHA using the EWRC log rating scale (1=no damage, 5= 20% reduction, 9=all plants dead) (6) and 3 plants per plot harvested at ground level 80 DAHA to determine herbage yield responses.

Results

Glasshouse

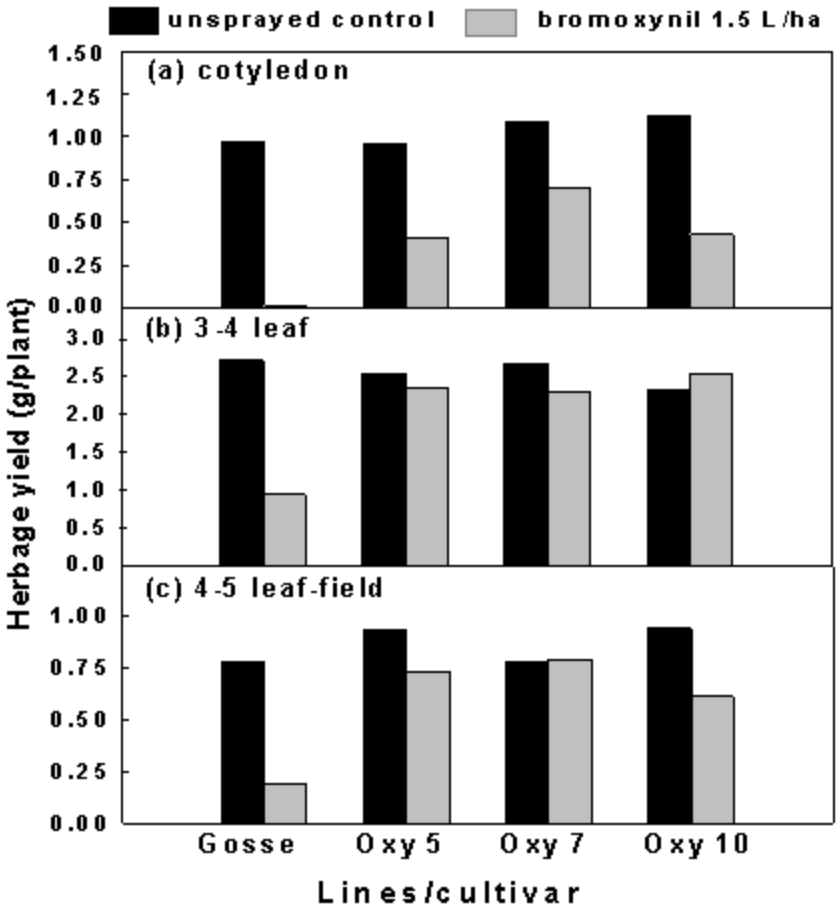
The application of 1.5 L/ha of bromoxynil at the cotyledon stage caused seedling death in Gosse but only retarded the growth of the 3 transgenic lines (Fig. 1a). When applied later at the 3 - 4 leaf stage it caused severe marginal burning on the leaves of Gosse compared with no significant burning in the 3 oxy lines (Fig. 1b).

Field

When bromoxynil was applied to subterranean clover seedlings in the field, the EWRC scores indicated that Gosse exhibited marked leaf burn and substantial stunt-ing (Table 1). In contrast, the 3 transgenic lines showed only a mild reduction in yield. Subsequent herbage yield measurements after the plants had an opportunity to recover showed little or no yield reduction in the 3 sprayed transgenic lines compared to the unsprayed controls (Fig. 1c). All 3 transgenics had very low for- mononetin levels similar to Gosse. Biochanin A and genistein levels were also low in Oxy 5 and 10, but Oxy 7 exhibited significantly higher genistein concentrations (Table 1). The application of bromoxynil did not change the oestrogen levels in any of the lines or Gosse.

Discussion

The inclusion of the Oxy gene substantially increased the tolerance of the 3 transgenic lines compared to Gosse in both glasshouse and field experiments. This effect was most pronounced when the herbicide was applied at the cotyledon stage. Of the 3 transgenics, Oxy 7 appeared to have a slightly greater tolerance at the cotyledon stage but all lines had a high and similar level of tolerance when sprayed at the 3-4 leaf stage.



The insertion of the Oxy gene did not appear to change the levels of formononetin, genistein or bio-chanin A in Oxy 5 or Oxy 10, however there was a significant increase in biochanin A and genistein in Oxy 7. As these two oestrogens are not associated with sheep infertility these raised values should not be of concern. It is important however to recognise the possible effects of gene insertion. For example Moses et al (4) found genetically transformed potatoes which had increased chlorsulfuron tolerance, yielded lower than their untransformed parents. However, the studies reported here indicate that herbage yield was not altered by the insertion of the oxy gene, although other measures of plant success such as seed yield are yet to be examined.

One of the issues raised with inserting new genes into cultivated plants, and particularly herbicide tolerance genes, is the risk they may escape into wild species. This aspect has been reviewed in detail and the risk rated as low (2). However, the need to use this technology carefully and in conjunction with an integrated weed management program is emphasised to ensure the full potential of the technology is captured.

Conclusion

The experiments have clearly shown the ability of the Oxy gene to raise the tolerance of subterranean clover to the herbicide bromoxynil. The marked increase in tolerance at the cotyledon stage provides the opportunity to bring forward the time of herbicide application and thus the ability to control weeds when they are more susceptible. The increased tolerance should enable herb-icide application at higher temperatures in autumn and thereby increase the phytotoxicity of the herbicide on weed species. The

combination of earlier application and higher temperatures is likely to lead to a reduction in herbicide rates.

Further work is currently under way to examine the seed setting ability of the transgenic lines as well as the effect the Oxy gene may have on herbicide mixtures containing bromoxynil.

Acknowledgments

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