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Annual report to ERMA on the GM onion field trial

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Environmental Risk Management Authority and the Māori Consultative Group

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1 Executive summary

This report describes progress to the end of February 2006 on the field trial approved by ERMA of genetically transformed onion seedlings.

1. The 2005/06 field trial planned to test eight lines with a total of 2680 transplants of selfed and backcrossed material. Most lines were repeats from the 2004/05 season. This meant that the field trial was to operate at around 98% of capacity. Unfortunately, poor germination of some seed reduced the number of transplants available for assessment, and meant that this figure had to be amended. Only 1958 transplants were actually planted out: this is 75% of capacity.

2. Due to a delay while awaiting the outcome of a 67A minor amendment to the trial application, onions were transplanted later than optimal for normal growth and development. As a consequence, yield was compromised and many lines, especially those containing short-day germplasm, only produced small bulbs.

3. Weed establishment varied from plot to plot. This made accurate assessment of weed control efficacy between treatments difficult. Generally weed growth was diverse and dense, suggesting that there was a large weed seed bank in the soil.

4. Glyphosate herbicide was applied 3 weeks after transplantation. By 3 weeks after application all weeds were dead except for a few clovers that were severely stunted. At 5 to 6 weeks before harvest a second application of glyphosate was used to kill the second flush of weeds. This left the plots clean through to harvest. One transgenic line responded poorly to the glyphosate, although the others showed no readily discernable effect. The late planting and early maturing of the short-day material compromised our study of the material with respect to normal phenotypic development.

5. A major thrips infestation occurred on the site. This was not treated in time and severe thrips damage resulted, which affected the onions' storage ability. This problem may have been triggered by the use of oats as a buffer. This will be reviewed for next year.

6. Harvested material was returned to the PC2 glasshouse, cured and stored. Some of this material will be used for initial equivalency analysis. The remainder will be inspected by plant breeders.

7. Security measures were refined during the trial to prevent false alarms from being triggered.

8. Onions continue to represent a relatively safe initial starting crop for testing GM systems in New Zealand and for developing methodologies that will reassure industry, regulatory bodies and other interested groups that the testing is efficient and effective.

9. All ERMA controls were complied with. However, it is important to recognise that the controls, their implementation, regulation and interpretation, are an evolving process between ERMA, MAF and the operator. It is vital that effective communication channels are maintained and discretion is shown.

2 Trial activities for the preceding year

2.1 Trial design

The field trial site was situated at least 2 km from any commercially grown onions and no flowers were allowed to develop on the test onions within the field site. The planting of a field site is shown below (Figure 1) in relation to the 2004/05 site.

In total, material representing eight original transformation events was planted. The material was derived from transgenic seed produced in hybrid short-day germplasm, open pollinated Longkeeper-type germplasm, and an inbred Longkeeper germplasm. The resulting seed was germinated in the PC2 glasshouse, sprayed with glyphosate, and surviving seedlings were used for transplants. Owing to poor germination, some blocks had reduced numbers and only 1958 transgenic plants were actually planted (2206 including controls).

Due to a delay in initiating the trial, whilst awaiting the result of a 67A amendment application, transplants were not transferred until late in November. Transplantation was achieved in 1 day and this did contribute to a more uniform trial than in the 2004/05 season (Figure 2). The late planting severely compromised the growing season and allowed little time for the short-day germplasm to grow before forming bulbs. As a consequence, little data could be gathered on growth, phenotype and yield, etc.

In addition to testing the lines for field tolerance to glyphosate, we also initiated some preliminary studies towards developing synergistic activities between biotech crop lines and other agronomic practices, such as the use of biocontrol agents. At this preliminary stage, this testing involved planting buckwheat as border rows at each end of the blocks in an attempt to observe improved thrips control (buckwheat is reported to attract predatory insects). The second measure was to include additional replicate onion plots with the biocontrol agent trichoderma (Onionmate™, Agrimm Technologies, Christchurch, New Zealand) added to these according to the manufacturer's instructions, as a control agent for onion white rot.

The severe thrips infestation this season indicated that buckwheat has an insignificant effect on thrips numbers under these conditions. No definitive conclusions could be made from the trichoderma versus non-trichoderma

plots because of the small number of replicates. However, we believe that synergistic associations could be developed between the biocontrol agent and the herbicide tolerance trait. We intend to investigate this further in future seasons.

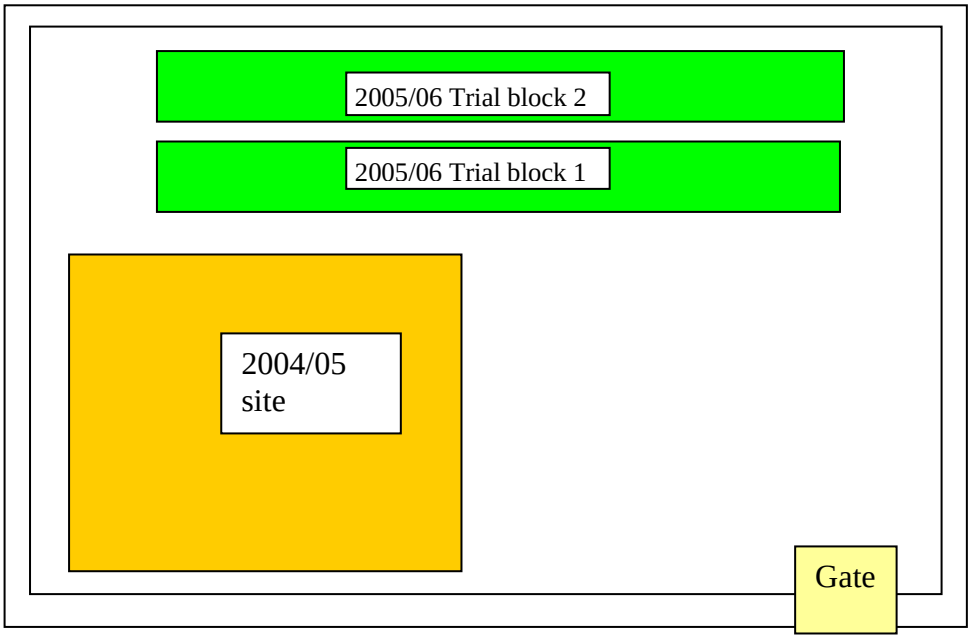

Figure 1: Field site planting plan.

Figure 2: 2005/06 trial site with the oat and buckwheat buffer rows. Block 1 is the two rows on the left, block 2 are the rows on the right of the picture.

2.2 Onion growth and development

All onion lines grew as well as could be expected considering the late planting date. The segregating seed from the short-day hybrid material and the backcrossed material gave rise to a range of short-day phenotypes that produced small to large bulbs. The selfed, open pollinated material was generally like the parental, open pollinated population, but some detrimental characteristics, such as multiple centres, occasionally arose within these plots. This was almost certainly due to inbreeding rather than to the transgenic event and was witnessed in the 2004/05 season within the same line. The hybrid material produced uniform hybrid bulbs throughout all replicate plots. From the lines in the trial, six were sampled by taking leaf (young and old) and bulb tissue. This material has been frozen and will be used to assess the activity of the transgene and to quantify the amount of transgenic protein present in the various tissues in order to compare the transgenic onions with other transgenic crops.

2.3 Onion plants displaying pipes

No onion plants displayed pipes.

2.4 Weed diversity

Weed diversity studies were not undertaken this year due to resource allocations. However, the weed profile was similar to that observed in the 2004/05 season.

2.5 Weed control

Glyphosate herbicide was applied 3 weeks after transplantation. Ten days after this, all weeds had stopped growing and most had started to die off. By 3 weeks after spraying, all weeds were dead except for a few clovers that were severely stunted. The transgenic onions were not affected by the treatment, except for one line that was showing poor transplant survival anyway. Control non-transgenic plots received three applications of Totril and Tribunal, each at 400 mL/Ha and 500 g/Ha respectivlely, throughout the growing season. This gave reasonable control of weeds in the non-transgenic plots.

At between 5 and 6 weeks before harvest, a second application of glyphosate was used to control a second flush of weeds.

The amount of glyphosate used was double the effective required dose for broad-spectrum weed control. This dose was used in order to check that the onions could cope on the margins of the spray boom where they might be subject to a double dose of spray. Under field conditions, it would be expected that the amount of glyphosate active ingredient would be half that used in this trial.

2.6 Harvest

All material that survived transplantation and spray was harvested and returned to the PC2 glasshouse, cured and stored. Some of this material will be used for initial equivalency analysis. The remainder will be inspected by plant breeders, and elite bulbs selected for crossing and seed production later this year. Unfortunately, as a result of thrips damage in the field, much of the material succumbed to disease during storage and has had to be destroyed.

3 Unanticipated events and interference

Essentially there were no major problems with the trial. However, minor technical and physical problems arose that were not foreseen when the trial was planned.

1. Security – There was still some trouble with false alarms this season but this appeared much reduced from the 2004/05 trial. No deliberate breaches of the site were noted.
2. Resources – These were much improved upon the 2004/05 season and allowed most activities to proceed as required.
3. Transplants – Due to problems associated with transplants noted in the 2004/05 season, Crop & Food Research applied for a 67A amendment to the field trial so that seed could be planted for the 2005/06 trial. This approval was not granted as applied for and, as a consequence transplants had to be used in the 2005/06 trial. This has compromised our ability to gather meaningful data about yield, growth, performance and the development of optimal management strategies for the crop. We intend to rectify this for the 2007/08 season.
4. Interference – there was no observed interference with the site.

4 Compliance issues

Controls 1-1.12: controls concerning the containment facility were all adhered to correctly.

Amended control 6.4: this made keeping registers of plant material much easier during the transfer from field to glasshouse and throughout the trial.

Sections 1.16-1.18: specification of the plants to be field-tested. These controls were adhered to.

Sections 2 and 3: controls governing the exclusion of unauthorised people and other organisms were adhered to.

Section 4: the amendment to this control was in reality a cosmetic change, as the definition of “traces of onion material or soil potentially harbouring onion material” could be interpreted as referring to many different degrees of cleanliness. In reality it did remove the requirement to clean footwear if they were only percieved to be dusty.

Section 5: controls governing accidental release or escape were adhered to.

Section 6: controls addressing monitoring requirements were adhered to.

Section 7: all additional controls were adhered to. The amendment allowing for alternative buffer row species considerably eased the ability of the operator to account for all the onion material on the site.

5 *Continuing viability of the project and proposed activities for next year*

The project has been essentially on hold in this second season of plantings, due to our inability to get approval to plant seed as we requested under a 67A amendment. However, we do have a commitment for the 2006/07 season from our collaborators and have designed some new activities to test that we can fit within the current approval system. We intend to carry these out next year and then focus efforts on a new application that will enable more relevant evaluations to be carried out.

We believe that the continued growth in biotech crops means that it is inevitable that in the future New Zealand will need the capability to grow such crops. Onions can easily be prevented from flowering and do not disperse clonal material in the ground. They represent a relatively safe initial starting point for testing ERMA and MAF systems and for developing methodologies that will reassure industry, regulatory bodies, and other interested groups that the testing is efficient and effective. This will set a basis for the control of other potentially more difficult crops (i.e. ones that flower easily or leave clonal material widespread) in New Zealand.