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Report on GMF06001 GM *Bt Brassica* Field Test

M C Christey

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A report prepared for
ERMA

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New Zealand Institute for Crop and Food Research Limited
Private Bag 4704, Christchurch 8140, New Zealand

Contents

1 Executive summary

This report describes progress to 31 July 2008 on the GM *Brassica* field trial approved by ERMA (GMF06001). During this period a total of 551 kale, broccoli, cauliflower and cabbage seedlings were transplanted to the field trial site. The entire trial was surrounded by one buffer row of 136 plants containing mixed brassicas phenotypically different to those in the trial for example, radish, ornamental kale, red kale, red cabbage and kohlrabi. Plants tested in the field trial contained either a *cry*1Ba1 or *cry1Ca5* gene under the control of the 35S promoter. In addition, some cauliflower plants had the *cry1Ca5* gene under the control of the potato chlorophyll AB binding protein promoter. All *Bt*-containing lines also contained an *NPT*II (*nos*-*NPT*II-*nos*) gene used for selection of transgenic plants.

One of the primary aims of this field trial was to determine the extent of insect control under natural infestation. Overall low caterpillar infestation was noted and thus the full potential of these plants was not seen. However, clear differences were noted between control and *Bt*-containing plants with damage noted on control plants and not on *Bt*-containing plants.

All plants were removed prior to flowers opening thus preventing the release of heritable material from the trial site. No evidence of bolting or floral initiation was noted on any of the cabbage or the majority of forage kale plants grown in the trial. This is expected as a sufficient period of cold inductance (vernalisation) had not yet been experienced.

During the course of this trial the opportunity was taken to initiate several areas of impacts research. DNA persistence in the soil, CRY protein levels and the composition of the rhizosphere community were analysed.

2 Field test activities for the previous year

2.1 Trial design

During the course of this trial a total of 112 blocks of up to 5 plants were transplanted to the field trial site (Table 1). The majority of plants used in the trial were obtained from selfed seed collected from transgenic plants planted in pots in the GMO greenhouse facility. Molecular analysis was conducted on the segregating progeny to identify plants with/without the genes of interest. In the case of cabbage and some cauliflower lines plants for the trial were obtained from in vitro tissue culture derived plants as per control 7.3. These plants were transplanted to the GMO greenhouse prior to transplanting into the field.

Table 1: Summary of material field tested

Plant type	gene	no. of plants	Survival (%)
Cabbage	*cry*1Ba1	14	100
	None	27	100
Kale	*cry1C*	113	94
	*cry*1Ba1	50	100
	none	69	95
Cauliflower	*cry1C*	12	100
	cab- cry1C	16	100
	*cry*1Ba1	43	100
	none	10	80
Broccoli	*cry1C*	67	100
	*cry*1Ba1	65	95
	none	65	92
TOTAL		551	
buffer rows	none	136	
TOTAL		687	

The initial planting of the trial occurred late November 2007 when just over 340 plants (not including buffers) of broccoli, cauliflower and forage kale were transplanted. In mid December 2007 a further 72 plants were planted including buffer row plants, cabbage and cauliflower plants. In late January 2008 a further 156 plants were planted. The entire trial was surrounded by one row of mixed brassicas phenotypically different to those in the trial for example, radish, ornamental kale, red kale, red cabbage and kohlrabi, as per control 7.4. Plants tested in the field trial contained either a *cry*1Ba1 or *cry1Ca5* gene under the control of the 35S promoter. In addition, some cauliflower plants had the *cry1Ca5* gene under the control of the potato chlorophyll AB binding protein promoter. All *Bt*-containing lines also contained an *NPT*II (*nos-NPT*II-*nos*) gene used for selection of transgenic plants. Prior to field testing all lines were checked to ensure no backbone T-DNA was present and no contaminating *Agrobacterium* was detectable.

2.2 Plant growth and development

Excellent survival of all lines was obtained in the field with only 20 plants not surviving to maturity, an overall survival rate of 96% (Table 1). Some plants did not survive transplanting and some kale plants became diseased resulting in their death prior to maturity. A mixture of transgenic and non-transgenic kale plants were diseased, all from the same cultivar suggesting a cultivar effect.

During the course of the trial observations and data were collected on agronomic characteristics such as head diameter, leaf number and plant height and at the conclusion fresh weight and dry weight were determined for forage kale.

2.3 Insect infestation

One of the primary aims of this field trial was to determine the extent of insect control under natural infestation. Overall low caterpillar infestation was noted and thus the full potential of these plants was not seen. However, clear differences were noted between control and *Bt*-containing plants with damage noted on control plants and not on *Bt*-containing plants (Figures 1 and 2). Other non-GM *Brassica* trials in the region also did not have significant caterpillar damage but other areas of Canterbury were more affected. However, we did obtain good biological control in the trial. Often caterpillar damage was noted (on control plants) with no evidence of the presence of a caterpillar suggesting that the total absence of chemical control enabled predators to increase. Harvestmen and parasitic wasps were often noted on the plants. In future years we will use a better nectar source as the buffer row to encourage more cabbage white butterfly (CWB) and diamond back moth (DBM) visits.

Figure 1: Detached leaves of *Bt*-containing (top) and control (bottom) cauliflower leaves from the field trial showing the extent of caterpillar damage on control plants.

Figure 2: *cry*1Ba1 expressing cabbage (top) and control cabbage (bottom) 8.5 weeks after planting

2.4 Surveillance for bolting and flowering

Head initiation and commencement of bolting was noted on broccoli and cauliflower plants in the field. All plants were removed prior to flowers opening thus preventing the release of heritable material from the trial site. Plants were removed at varying times from the trial depending on maturity. Cabbages were removed 5 months after planting, cauliflower 4 months after planting, and kale removal started 6 months after planting. At removal cabbage and kale were all still vegetative with no sign of flower bud initiation. No evidence of bolting or floral initiation was noted on any of the cabbage plants grown in the trial. This is expected as a sufficient period of cold inductance (vernalisation) had not yet been experienced. Some of the kale plants still remaining in the field are now starting to show flower bud initiation but only on close inspection of the apex.

In contrast broccoli were removed due to signs of the commencement of bolting starting January 2008, approximately 2 months after transplanting. The majority of plants were removed directly to an autoclave bag on site and then transported to the GMO greenhouse facility for autoclaving. The remainder and some plants of each type were transported back to the GMO greenhouse where they have been repotted to enable flowering for seed collection. Currently, a total of 11 cabbages, 16 cauliflowers and 31 broccoli have been kept for this purpose. Some forage kale plants will also be kept and repotted in the GMO greenhouse to enable flowering for seed collection.

2.5 Weed diversity

Hand weeding of the trial was conducted as necessary. A range of weeds were noted including fathen, shepherd's purse, nightshade, ryegrass, clover and pansy. A few turnips (*Brassica rapa*) were also noted but no *Brassica oleracea* weeds.

2.6 Invertebrate biodiversity

During the course of this trial the opportunity was undertaken to document the range of invertebrate species found within the trial both on the plants themselves and also in the soil. While this is not yet completed a wide community were found to be present including caterpillars (CWB, DBM, woolly bear), butterflies (CWB, DBM,) aphids, parasitic wasps, harvestmen, millipedes, earthworms, flatworms, ladybirds and whitefly.

2.7 Harvest

As of 31 July 2008 107 forage kale remain in the field site.

3 Any unanticipated events

3.1 Interference

During the trial extra bird scarers were put in place to prevent birds entering the trial. No evidence of rabbit or hares were noted in the field trial site though they were seen in the vicinity and pellets noted outside the trial site. This indicates the wind break barrier was sufficient to prevent their access.

3.2 Unanticipated events

There were no unanticipated events associated with the trial except for weather effects. However, even though the trial was subjected to extreme weather conditions on occasion, such as high rain, hail, snow and strong winds, the only adverse effect noted were that the kale plants grew at a slight angle. Planting deeper on transplanting would have prevented this.

3.3 Security

There were no security issues with the trial site and there was no observed interference with the trial.

4 Any issues with controls

All controls concerning the containment facility as outlined in the Guidance Document were adhered to correctly as required. There were no issues with any of the controls. During the course of the trial clarification was sought on one issue involving flowering of buffer row plants.

In addition, during the harvest of the kale plants it was realised that determination of dry weight would require access to a large drying oven outside the designated PC2 area. Approval was sought and obtained to use this facility as no transfer of heritable material was involved.

5 Proposed activities for next year

Postharvest monitoring for the appearance of volunteers will continue monthly. Details of next year's activities will be provided once confirmed.

6 Educational and public awareness activities

During the time period of this trial two public seminars to Men's Probus groups have been given as well as one talk to Canterbury university Biotech students, one talk to Cashmere High teachers and one talk to Lincoln High School students. The Probus talks specifically covered the *Brassica* trial and the other two talks covered general biotechnology research as well. In all cases, high interest was shown with positive feedback about the research. There has also been media coverage in newspapers and radio mainly in relation to the High Court Appeal. In addition, an interview with Straight Furrow was published in April 2008.

Ngāi Tahu has been kept informed of the field test activities through a meeting held earlier in the year to discuss our overall GM research and also via email. Ngāi Tahu have not approached us for any other further information. In addition, I have completed and passed a 12 week Stage 1 Te Reo Māori course run by Culture Flow. Crop & Food research have just awarded us a Māori summer scholarship which will enable us to have a student to help us in identifying cultural and ecological risk concerns of Māori in relation to this research.

7 Impacts research

During the course of this trial the opportunity was taken to initiate several areas of impacts research. DNA persistence in the soil has been studied by using qPCR analysis to detect the presence of the NPTII and *cry* transgenes in soil from the field trial. Soil samples were collected prior to planting and at 8-10 weeks after planting from immediately around the plants and also midway between adjacent plants. In addition, samples were collected at harvest and after harvest. The transgenic DNA was only detected within the immediate growing region of the plants during the active growing stage of the plant at 8–10 weeks after planting. We did not detect it midway between adjacent plants or when plants were no longer present. It is possible that transgenic plant DNA was only detectable when viable plant tissue was collected along with the soil sample.

During the trial CRY protein levels of *cry*1c expressing plants have been assessed using ELISA analysis. In addition, CRY protein levels in senescing material both on plants and on the ground has been determined. These results show that low levels of CRY protein are detectable in senescing leaves.

As mentioned above a survey of invertebrate species within the trial including soil was conducted.

The introduction of transgenic plants into agricultural ecosystems has raised the question of the ecological impact of these plants on non-target organisms, such as beneficial predators and parasitoids, soil bacteria and fungi. As part of this research comparisons were made on the type and number of non-target organisms present on *Bt* and non-*Bt* plants and in the soil zone. As the zone around roots (rhizosphere), is considered to be the most likely place where effects can be expected a cultivation-independent approach was chosen to characterise microbial communities associated with *Bt* and non-*Bt* plants. Rhizosphere samples were taken from transgenic broccoli plants from independent transformation events and untransformed control plants. General bacterial, fungal and taxon-specific primers (*Alpha*- and *Betaproteobacteria*, *Actinobacteria,* and *Pseudomonas*) were applied to PCR-amplify 16S/18S rRNA gene fragments from rhizosphere DNA followed by denaturing gradient gel electrophoresis (DGGE). Even minor shifts in the composition of the microbial community can be detected via the DGGE approach. The results showed that the soil bacterial community associated with transgenic *Bt* broccoli plants did not differ from the non-*Bt* control plants. The only influence on the bacterial communities was observed between the two cultivars.

8 Scientific publications

Poster abstracts relating to this research have been accepted for two international conferences being held later this year.

Christey MC, Braun R, Keenan S, Gerard E, Lottman J 2008. Agronomic Performance and Nontarget Impacts of Bt-containing Forage and Vegetable Brassicas under Field Conditions. Poster abstract for Brassica 2008 Norway. http://www.brassica2008.no/

Christey MC, Braun R, Niu K, Gerard E, Lottman J 2008. Evaluation of Non-target Impacts of Bt-containing Forage and Vegetable Brassicas under Field Conditions. Poster abstract for ISBGMO conference in Wellington 2008. http://www.isbgmo.info/

Keenan S, Bulman SR, Christey MC, Conner T 2008. Persistence of transgenic plant DNA in soil from New Zealand field trials. Poster abstract for ISBGMO conference in Wellington 2008. http://www.isbgmo.info/