ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

FORM NO-04

Application for approval to

FIELD TEST
IN CONTAINMENT ANY GENETICALLY MODIFIED ORGANISM

under section 40 of the Hazardous Substances and New Organisms Act 1996

Application Title: Field test of genetically modified vegetable and forage brassicas

Applicant Organisation: NZ institute for Crop & Food Research Ltd

	IBSC	ERMA NZ
Considered by:		X

ERMA Office use only

Application Code: ☐☐☐☐☐☐☐☐

Formally received:____/____/____

ERMA NZ Contact: _____

Initial Fee Paid: $

Application Status:

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IMPORTANT

1. An associated User Guide is available for this form on the ERMA New Zealand website. If you need additional guidance in completing this form please contact ERMA New Zealand.
2. This application form covers field testing in containment of any genetically modified organism and may be used to seek approvals for field testing in containment of more than one genetically modified organism where the organisms are of a similar nature.
3. If the application does not involve a defined organism and involves ongoing genetic manipulation this is not the correct form. Instead such applications covering **development of genetically modified organisms outside a containment structure** as defined within the Act 1996 should be made on **Form NO3O,** instead of this form (Form NO-04).
4. This version of the application form is the most recently approved by the Chief Executive of ERMA New Zealand and replaces all other versions. Older versions will not be accepted. You should check with ERMA New Zealand or on the ERMA New Zealand web site for the current version of this form.
5. You should talk to an Applications Advisor at ERMA New Zealand who can help you scope and prepare your application. We need all relevant information early on in the application process. Quality information up front will speed up the process.
6. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included as appendices to the application form.
7. Commercially sensitive information must be collated in a separate Appendix. You need to justify why you consider the material commercially sensitive, and make sure it is clearly labelled as such.
8. Applicants must sign the form and enclose the correct application fee (plus GST). The initial application fee can be found in our published Schedule of Fees and Charges. Please check with ERMA New Zealand staff or the ERMA New Zealand website for the latest schedule of fees. We are unable to process applications that do not contain the correct initial application fee.
9. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed.
10. Please provide an electronic version of the completed application form, as well as sending a signed hard copy. Until we receive the signed hard copy we will not be able to process your application.
11. **Note: A**pplications to field-test genetically modified organisms shall be publicly notified by the Authority (s 53(1)(d) of the HSNO Act) and may go to a hearing (s 60 of the HSNO Act).
12. This application form was approved by the Chief Executive of ERMA New Zealand on 28th June 2005.

You can get more information by contacting us. One of our staff members will be able to help you.

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Section One – Applicant Details

1.1 Name and postal address in New Zealand of the organisation or individual making the application:

Name > Crop & Food Research

Postal Address >
Crop & Food Research
Private Bag 4704
Christchurch 8140

Physical Address >
Canterbury Agriculture and Science Centre,
Gerald St
Lincoln

Phone > 03- 325 9400
Fax > 03 325 2074
E-mail > brassicatrial@crop.cri.nz

1.2 If application is made by an organisation, provide the name and contact details of a key contact person at that organisation

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name > Mary Christey

Position > Research Leader

Address > Crop & Food Research
Private Bag 4704
Christchurch 8140

Phone > 03- 325 9400
Fax > 03 325 2074
E-mail > brassicatrial@crop.cri.nz

1.3 If the applicant is an organisation or individual situated overseas, provide the name and contact details of the agent in New Zealand authorised to transact the applicant's affairs in relation to the application

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name >Not applicable

Section Two – Purpose of the Application

This form is to be used for an application to **field test** in containment any genetically modified organism. For applications for **development of genetically modified organisms outside of a containment structure** as defined within the HSNO Act 1996, use **Form NO3O.** For applications for development of genetically modified organisms meeting the requirements of the *HSNO (Low-Risk Genetic Modifications) Regulations 2003,* the **rapid assessment** application form (**NO3P**) should be used.

2.1 Give a short summary statement of the purpose of this application to be used on ERMA New Zealand's public register - *Maximum of 255 characters including spaces and punctuation.*

Briefly describe the organism(s) to be field tested, and the purpose(s) for which you wish to field test the organism(s).

To assess agronomic performance, in the Lincoln region, over 10 years of vegetable and forage brassicas, specifically cabbage, broccoli, cauliflower and kale, modified for resistance to caterpillar pests like cabbage white butterfly and diamond-back moth.

2.2 Provide a description of the background and aims of the project suitable for lay readers

Describe **in less than one page** the rationale for the overall project these organisms are to be used in so that people not directly connected with the research and with a limited knowledge of science can understand why these organisms are being field tested in containment. This explanation is particularly important if the field test involves DNA from human genes or native and valued introduced flora and fauna. In addition, discuss whether expression of the foreign genetic material is anticipated, and whether any unusual procedures will be involved in the field-testing. Detail should also be included on the methods to be used, the general location, associated facilities and duration of the field test.

Diamondback moth (DBM) (*Plutella xylostella*) and cabbage white butterfly (*Pieris rapae*) are serious pests of cruciferous crops such as broccoli, forage kale and cabbage not only in New Zealand but also worldwide. While there are numerous chemical control methods available for these pests, there are problems particularly in DBM with the development of resistance to these chemicals. In addition there are concerns about the presence of chemical residues in the environment. As a result there is growing interest in the development of alternative control methods. The introduction of insecticidal transgenes into plants offers such an alternative.

The soil bacterium *Bacillus thuringiensis* (*Bt*) has been used for biological control of insects for over 30 years. When the bacterium sporulates it produces large crystalline (*cry*) proteins that have insecticidal activity. When susceptible insects ingest these crystalline proteins, they are converted into active toxins that kill the insect by disruption of the midgut cells. In NZ commercial products containing this bacterium include DIPEL® ES, AGREE® WDG and FORAY® 48 B. These products are used for caterpillar control in orchards and on vegetables and are also used by organic growers. There are many different strains of *B. thuringiensis,* each producing different insecticidal proteins that target different insect groups. For example, cry1A and cry9A are insecticidal against lepidoptera (caterpillars). The genes for these insecticidal proteins are being introduced into plants by genetic modification techniques to confer insect resistance. Since the first overseas commercial release

of a genetically modified crop in 1994, the technology has been widely adopted with 90 M ha planted world wide in 2005 (James 2005) ─equivalent to about three times the total land area of NZ. Approx. 30% of this area includes *Bt*-containing crops, especially maize and cotton.

We have used *Agrobacterium tumefaciens*-mediated transformation to produce broccoli, kale, cabbage and cauliflower transgenic for various *Bt* genes and demonstrated excellent control of DBM, soybean looper (*Thysanoplusia orichalcea*) and cabbage white butterfly larvae in laboratory and greenhouse assays. *Bt*-containing plants cause mortality of neonate larvae within 48h with no visible damage to the plant. However, field-testing of these plants is required to confirm the extent of insect control under agronomic situations. This aim of this field test is to assess the agronomic performance of these plants under field conditions and in particular their response to cabbage white butterfly and diamond-back moth caterpillars. In addition we will monitor the insecticide input on the plants. We expect that the *Bt*-containing plants will require no insecticide applications to control these pests compared with non-*Bt* containing plants. The agronomic performance of these plants will be assessed weekly by measurements on plant phenotype including height, leaf number, survival, days to head initiation and diameter of heads compared with non-*Bt* containing plants of the same cultivar. In addition the field test will be used to study potential environmental concerns about the field testing of GM plants. Impacts of *Bt*-containing plants on soil microflora, horizontal gene transfer and non-target beneficial invertebrates will be compared with non-*Bt* containing plants in the same trial. Experiments will also be conducted to study the persistence of transgenic DNA in soil over time.

This field test does not involve DNA from human genes or native and valued introduced flora and fauna. The sequence of the DNA to be used was originally derived from the bacterium *Bacillus thuringiensis* but the sequence has been modified to allow optimal expression in plant cells. This DNA was originally manufactured in a DNA synthesiser. A selectable marker gene and/or reporter gene is also inserted into these plants and is expressed to enable selection of the genetically modified cells and plants. These genes; *cry*, selectable marker, and reporter genes; also contain bacterial, viral, fungal or plant-derived promoter and terminator regions to ensure expression in plant cells. The plant material to be used, vegetable and forage Brassicas, all belong to the Brassicaceae or Crucifer family. They are not native to NZ and are not closely related to other native members of this family occurring in a different taxonomic tribe. Therefore inter-crossing with native Brassicaceae is not possible.

Expression of the introduced insecticidal gene is expected. Prior to transfer to the field all plants will be checked with molecular techniques to confirm gene presence. Some plants from each line will also be checked by either a caterpillar assay or RT-PCR to ensure the insecticidal gene is still active. Not every plant of each line will be checked for expression as the plants are either progeny or clonally propagated and therefore expression should be consistent within a line.

These plants will be grown in a secure contained field location near Lincoln. The field test will be small, occupying an area of up to 500 sq.m (0.05ha) for the first year and possibly increasing to up to 0.4 hectares in subsequent years. Each plant in the field test is expected to be in the ground for up to 5 months. The plants will be transplanted into the field as 6-12 week old seedlings or tissue-culture derived plantlets or cuttings with 4-10 leaves and their growth and development monitored until the formation of marketable heads-up to 5 months depending on plant type. The plants will then be removed from the field and either destroyed by autoclaving or replanted in a contained PC2 greenhouse for flowering and seed collection. The field test site will be subjected to the normal agronomic practises associated with growing vegetable and forage brassicas including control of insects, weeds and diseases by chemical application as required. Fertiliser applications will also be made as required. The trial will be monitored carefully at least every 3-4 days to ensure that no caterpillars are surviving on transgenic plants and that no open flower buds are visible on the plants. There is a visibly significant change in the plants before they bolt to produce open flowers and plants will be removed from the field test site as they bolt

and placed back into the laboratory greenhouse well before flowers open to ensure no dispersal of genetically modified pollen. At the end of the field test, plants will be removed from the field and either destroyed by autoclaving or replanted into a contained PC2 greenhouse for flowering and seed collection.

The field test will be conducted over a 10 year period to enable the evaluation of new combinations of *Bt* gene constructs and also for the evaluation of new plant lines or the re-evaluation of existing lines. In the first year, most plants to be tested will contain single *Bt* genes. This will enable us to confirm our laboratory and greenhouse experiments that the individual genes are effective. Some plants may also contain two or more *Bt* genes- introduced via transformation or via conventional crossing of 2 transgenic lines. In subsequent years we will test further plants containing single *Bt* genes but concentrate on plants with one or more different *Bt* gene constructs. A 10 year period also ensures we have sufficient time to adequately evaluate these plants as environmental conditions may prevent sufficient insect infestation in some years to adequately assess the effectiveness of the genes being used.
The field test site will be monitored monthly for one year after the completion of the trial to ensure no volunteer GM plants remain. The personnel associated with this trial are experienced with conducting field trials of genetically modified plants and are trained in the procedures associated with such trials.

Section Three – Information on the organism(s) to be field tested

If more than one type of organism is to be field tested this section must be completed separately for each organism. If there are commercial reasons for not providing full information here alternative approaches must be discussed with and agreed to by ERMA New Zealand.

3.1 Provide unique name(s) for the new organism(s) to be tested

These name(s) will be on the public register and should clearly identify each new organism. This must describe both the host organism as well as how it has been modified.

Brassica oleracea L. vegetables and forages including cabbage, cauliflower, broccoli and forage kale each modified by *Agrobacterium tumefaciens* mediated transformation for the introduction of either one or more *cry* genes to confer resistance to DBM and CWB caterpillars, and selectable marker and/or reporter genes e.g. NPTII or Green Fluorescent Protein (GFP).

3.2 Give the unequivocal identification of the host organism(s)

Please include details on the following:

Latin binomial, including full taxonomic authority: (e.g. ----- Linnaeus 1753) **class, order and family:**

The plants to be field tested are *Brassica oleracea* vegetable and forage types including broccoli, cauliflower, cabbage and forage kale. They all belong to the Brassicaceae or Crucifer family and are closely related to turnip, Chinese cabbage (both *Brassica rapa* syn. *Brassica campestris*), forage rape and swede (both *Brassica napus*), Broccoflower and Brussels sprouts (also *Brassica oleracea).* The genetic relationships between these *Brassica oleracea* diploid and amphidiploid species are shown in Appendix 1 (Figure 1).

Taxonomy: (from Williams and Hill, 1986; Fast Plants 1987)
Class: Angiospermae (flowering plants)
Subclass: Dicotyledonae (Dicotyledons, Dicots)
Superorder: Dilleniidae
Order: Capparales
Family: Brassicaceae (syn. Cruciferae) or Crucifer family.
Tribe: Brassiceae

Diploid with Chromosome number n=9

GENUS AND SPECIES	**COMMON NAME**
Brassica oleracea var. *italica*	broccoli
Brassica oleracea var. *botrytis*	cauliflower
Brassica oleracea var. *capitata*	cabbage
Brassica oleracea var. *acephala*	forage kale

Taxonomic authority: Linnaeus 1753

Common name(s), if any:

broccoli, cabbage, cauliflower and forage kale

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Type of organism (e.g. bacterium, virus, fungus, plant, animal, animal cell):

plant

Strain(s), breed(s) **and genotype(s***)*, if relevant:

A range of broccoli, cabbage, cauliflower and forage kale commercial cultivars and breeding lines under development will be tested. In addition, crosses will be conducted between the same plant types to produce plants containing a combination of *Bt* genes and a combination of marker and reporter genes.

Other information, including presence of any inseparable or associated organisms; whether a prohibited organism is involved; and information on consideration of the organism(s) by other states, countries or organisations:

There are no likely inseparable or associated organisms. A prohibited organism is not involved. There is to our knowledge no information on consideration of the organism(s) by other states, countries or organisations.

3.3 Provide details on the genetic modifications and processes involved in the development of each of the organism(s) to be tested

Experimental data and information obtained from the development phase of each genetically modified organism should be attached to this application as an Appendix to assist in risk evaluation.

The transgenic plants to be field tested contain one or more crystalline protein genes (*cry* genes) which are derived from the bacteria *Bacillus thurigiensis*, and confer resistance to certain caterpillars.
The *cry* genes are under the control of regulatory elements such as promoters and terminators, which permit expression in a plant system. In addition, these plants will also contain selectable marker genes and/or reporter genes to enable selection of transgenic plants. Each genetically modified line has been characterised by a series of molecular and other techniques including PCR, RT-PCR, Elisa, flow cytometry and caterpillar feeding assays. See Appendix 2 for full details of these results.

Identify the category or categories of the genetic modification(s) involved in the development as described in the HSNO Low-Risk Genetic Modification Regulations 2003.

The genetically modified *Brassica oleracea* plants to be field-tested were developed as Category B genetic modifications of Category 2 host organisms.

The production of the transgenic material described in this application was conducted under ERMA application GMD01086 with approval codes GMD000814 for *Brassica oleracea*, GMD000808 for *Agrobacterium tumefaciens* and GMD000817 for *E. coli*. *Agrobacterium tumefaciens* and *E. coli* are the intermediary hosts used in the development of the transgenic plants and are not part of this field trial application.

Vector system(s) used in development of the genetically modified organisms.

Agrobacterium tumefaciens disarmed strains such as EHA105 (Hood *et al*., 1993) or LBA4404 (Hoekema *et al*., 1983) were used to transfer standard plant binary vectors containing a selectable marker gene eg NPTII or a reporter gene e.g. GFP and insecticidal *cry* gene(s) into the *Brassica oleracea* plants described in this application. Alternatively, conventional crossing methods will be used to obtain plants containing 2 or more different *cry* genes per plant line. PCR analysis will be used to ensure that only material inside of the Left and Right borders of the binary vectors has been transferred to the modified plants.
See Appendix 2 for examples of the constructs.

Type and source of additional genetic material.

The genetic material incorporated into the *Brassica oleracea* plants may consist some or all of the following:

- One or more crystalline protein genes (*cry* genes) encoding insect resistance derived from *Bacillus thurigiensis*. These genes may be plant preferred versions of insecticidal *cry* genes from *Bacillus*

thurigiensis. Different *cry* genes may be used as they target different binding sites in the insect gut. These genes may also contain bacterial, viral or plant derived promoter and terminator regions to ensure constitutive or tissue specific expression in plants.
- Gene regulatory elements including promoters and terminators may be derived from plants, including *Brassica* species, *Arabidopsis thaliana*, tobacco and other crop species.

The genetic material may also contain any of a number of other standard and commercially available regulatory elements derived from vertebrates, invertebrates, plants, fungi, bacteria and viruses with established use in plant transformation including:

- the CaMV35S promoter and the CaMV35S polyadenlylation region, sourced from cauliflower mosaic virus; the Octopine synthase (OCS) and Nopaline synthase (NOS) promoters and terminators derived from *Agrobacterium tumefaciens*;
- tissue specific promoters may be used to target *cry* gene expression eg the chlorophyll AB binding protein
- Antibiotic resistance markers or selectable markers such as hygromycin phosphotransferase II (*hptII*), neomycin phosphotransferase (*nptIII*) and phosphinotricin acetyl transferase (*bar*) or other antibiotic and selectable markers commonly used in plant transformation (i.e. available from research groups or companies on request to researchers);
- Other markers and reporters such as the *uidA* gene (GUS) and fluorescent proteins including Green Fluorescent Protein (GFP) and DsRed commonly used in plant transformation.
- The use of genetic material from native flora and fauna is explicitly excluded.

The foreign DNA will be contained in the T-DNA regions within the binary vectors. This region between the Left and Right border is transferred into the plant genome by disarmed *Agrobacterium tumefaciens* strains (Grant et al., 1991). Examples of some of the constructs used in this development are given in Appendix 2.

Use of special genetic material: please complete this table by marking the correct box

	Yes	**No**
Does this application use native flora or fauna as **host organism(s)**?		**X**
Does this application use **genetic material** from native or valued introduced flora and fauna?		**X**
If native flora and fauna are involved, are the species concerned endemic to New Zealand?		**X**
Was human DNA or cell lines used that are of known Māori origin?		**X**
Does this application use genetic material obtained **directly** from human beings? *If Yes, provide additional details below.*		**X**

If native flora and fauna are involved, from where in New Zealand or elsewhere has this material been obtained? What consultation with Māori has taken place and specifically with whom? What were the outcomes of this consultation e.g. what were the key issues raised, were options discussed to address/mitigate concerns?
Be as specific as possible as this information may be needed to decide whether Māori have been appropriately involved.

These developments do not involve the use of native flora and fauna.

If the genetic modification involves DNA of human origin, provide details of where the material was obtained (including provenance and/or informed consent), and whether approval was obtained from an Ethics Committee, and what consultation with Māori has taken place. Consultation with Māori will be required when human DNA or cell lines are used. In recording consultation, ensure you append any relevant information to the application including consultation feedback, minutes of meetings or other correspondence.

These genetic modifications do not involve the use of DNA of human origin.

Other relevant details (such as what techniques or experimental procedures were used, whether any unusual manipulations were carried out, and how the foreign genetic material is expressed)**.**

Appendix 2 contains full details of the methods used in the production of these transgenic plants. Briefly, plasmids containing the DNA regions of interest were manipulated using *E. coli*. The final vector was introduced into *Agrobacterium tumefaciens* strains using the freeze thaw method. Standard *Agrobacterium tumefaciens* mediated transformation experiments involving the immersing of leaf explants in an *Agrobacterium tumefaciens* culture followed by culture on hormone-containing shoot regeneration medium were conducted. Explants were subsequently transferred to antibiotic-containing medium to eliminate the *Agrobacterium* and to enable selection of transgenic cells and subsequently plants. Plants were confirmed as containing and expressing the DNA of interest by a mixture of PCR analysis, ELISA analysis and insect feeding assays. The material for the field test will be either clonally propagated in tissue culture or the greenhouse or seedlings grown from seed collected on the original transgenic plants in a PC2 greenhouse

3.4 Characteristics of the organism(s) to be field tested

Provide information on the main features or essential characteristics of the organism(s) to be field tested. Specify in what way the organism to be tested differs phenotypically from the unmodified organism from which it is derived. You should note characteristics of the host organism as well as any new characteristics introduced by the genetic modifications. For example, note pathogenicity, production of spores/seeds/pollen, conditions for growth and reproduction. This information should be relevant to the identification of the risks of the organism (section 5.2).

The plants to be field tested were developed by *Agrobacterium tumefaciens* mediated transformation with non-tumourigenic strains such as EHA105 or strain LBA4404. Genes such as reporter genes, selectable marker genes and the *cry* genes(s), were introduced into these plants during the transformation process. These genes were introduced into the nuclear genome and will be expressed in all plant cells if they under the control of constitutive promoters. The use of tissue-specific promoters will target gene expression to specific tissues such as leaves. The marker or reporter genes enable the selection of transgenic plants. For example NPTII, confers resistance to kanamycin and its presence enables the selection of transgenic cells and plants. In addition, the plants contain one or more *cry* genes to confer resistance to specific caterpillar pests including caterpillars of cabbage white butterfly, diamond back moth and soybean looper. In the field it is expected that these plants will be phenotypically indistinguishable from unmodified control plants except if these caterpillars are present. These plants will differ from the unmodified plant from which it is derived only in terms of its response to feeding damage from these specific caterpillar pests. Soybean looper is a caterpillar pest of Brassicas in the north of the North Island but is not known to be a pest in the South Island (G. Walker, Crop & Food, pers.com.). If caterpillars are present we would expect the modified plants to show no visible signs of feeding. In contrast, unsprayed non-transgenic control plants will have clear feeding damage with holes or damaged regions visible on leaves (See Fig. 18 in Appendix 8). Under severe caterpillar infestations whole leaves will be removed. The modified plants are not expected to have improved reproductive fitness. They may have improved biological fitness due to the expected reduction in caterpillar damage. This may allow the plants to produce larger marketable heads compared with controls that have not been sprayed for caterpillar control. They should have similar biological fitness to sprayed control plants.

Seedlings or plants will be transplanted into the field as small plants with approx. 4-10 leaves each. During the course of the following weeks vegetative growth will increase on the plants resulting in the formation of further leaves and increased plant height. In broccoli and cauliflower, vegetative growth will then cease followed by the initiation of heads followed by an increase in size of these heads. In cabbage, the inner leaves develop as a ball-like structure of overlapping leaves resulting in the formation of a firm round head. Appendix 3 describes and illustrates in more detail the developmental stages for broccoli, cabbage, cauliflower and forage kale. Depending on environmental conditions, DBM and CWB are likely to lay eggs on plants in the field immediately after planting. Therefore the first caterpillars are likely to be present within a week of planting. It is expected that DBM and CWB could continue to lay eggs on plants through-out the duration of the field trial. This means that after the first few weeks that a variety of caterpillar developmental stages could be apparent. We expect eggs to hatch on *Bt*-containing plants but for no further development of the caterpillars with death within 2-3 days. Appendix 8 illustrates the different stages in the DBM and CWB life cycles. The time taken to progress through each stage is dependent on environmental conditions.

Broccoli, cabbage, cauliflower and forage kale are annual crops whereas forage kale is biennial, requiring cold to induce flowering. Under normal field conditions if left to mature these plants would produce open fertile flowers within 6 months with the potential for pollen dispersal and seed set. However, for the course of this field test the plants will be monitored at least every 3-4 days, to ensure that bolting plants are removed so that flowers do not open and there is no potential for pollen or seed dispersal. There is a visibly significant

change in the plants before they flower. In **broccoli**, bolting, or peduncle elongation, is easily noted by the elongation of the stem the head sits on followed subsequently by the loosening of the head and then the appearance of open flowers. In **cabbage**, the head alters shape from the normally round to slightly pointed. Over a few weeks the head becomes more pointed and eventually the central region of the head splits open as the floral meristem pushes through. Small closed flower buds are still not visible at this stage but become apparent as the meristem elongates. In **cauliflower**, the first noticeable change is the loosening of the head and the slight elongation of the stems composing the head. The stems continue to elongate with tightly compressed flower buds on their ends. During the next stage elongation of the stem is accompanied by enlargement and maturation of the flower buds. These closed flower buds subsequently open. The **forage kale** cultivars used in this research only flower after a period of cold induction (or vernalisation). Typically at least 4 weeks of exposure to temperatures below 8°C is required (S. Gowers, Crop & Food, pers. comm.). As with the vegetables the transition from the vegetative to reproductive meristem is obvious. The first visible sign is the thickening of the main meristem at the top of the plant followed by a small (0.5cm) compact cluster of several flower buds at the apex. As the stem continues to elongate the flower buds enlarge, and separate along the stem before opening.

Appendix 3 describes and illustrates in more detail the sequence of events clearly visible prior to any flowers opening in broccoli, cabbage, cauliflower and forage kale. This visually clear sequence of events and the use of trained staff to monitor the plants every 3-4 days will ensure that plants are removed from the field as they bolt, well before any flower would open.

The majority of *Brassica* species are native to the Mediterranean region (Tsunoda, 1980). They are dicotyledonous, herbaceous plants grown either as annuals or biennials, depending on the part of the plant harvested. Broccoli, cabbage and cauliflower are cultivated worldwide for human consumption. Brassicas survive through seed propagation. Pollen transfer to the same or closely related species could occur. However, the strict regime we have in place for monitoring and removal of plants when they bolt will ensure that these plants do not produce open flowers outside a contained greenhouse. Appendix 4 lists the potential of *B. oleracea* for crossing with other brassicas.

Plants containing two or more different *Bt* genes will also be field tested. Some overseas studies have suggested that the use of multiple *Bt* genes targeting different sites in insects could delay the development of insect resistance in insects feeding on plants modified to produce multiple *cry* proteins. The use of more than 1 site of action means that the insect will not be able to rapidly develop resistance to *Bt* proteins by a single mechanism thereby delaying the development of insect resistance (Zhao *et al*., 2003).

Section Four – Outline of the proposed field test, containment and control system and its effectiveness

4.1 Describe the field test design

Include details of the location and layout of the field test and associated facilities, the number of sites involved, the number of organisms to be field-tested and the duration of the field test.

The field test will be located at Lincoln, Canterbury (exact location is Confidential and attached as Confidential Appendix C1). It will be at one location and will occupy an area of up to 0.4 ha. Plots within the trial site will contain transgenic plants for up to 20 weeks per year. The field test will run for 10 years to allow the evaluation of an extensive array of transgenic plants.

The field test consists of growing replicate blocks or rows of each transgenic and control line of broccoli, cauliflower, cabbage and forage kale. Six to twelve week old seedlings, or tissue-culture derived plantlets or greenhouse-derived cuttings with 4 -10 leaves will be hand planted (see Appendix 11). Several independent transgenic lines will be tested in any one season. Each block or row will contain up to 10 plants of the appropriate line spaced 40cm apart, with 50cm between blocks or rows. Plants are spaced so as to prevent migration of caterpillars from *Bt*-containing plants to non-*Bt* plants. For each independent transgenic line up to 10 rows of transgenic and up to 5 rows of non-transgenic progeny as a control will be planted. Segregating non-transgenic progeny will be used as the controls. As these plants are derived originally from transgenic plants they are still treated as GM. They are regarded as the best comparison with *Bt*-containing plants as they represent the same generation. Some of the non-transgenic progeny control rows will include rows that are sprayed as required for caterpillar control. However, some rows will be left un-sprayed to replicate the reduced insecticide treatment given to the *Bt*-containing plants. In addition, the entire plot will be surrounded by one to two border rows of a non-GM plant to prevent edge effects such as from wind or other environmental factors.

The trial will be harvested i.e. removal of entire plants, once marketable heads (approx. 10-15cm diameter) are produced and prior to the opening of any flower buds. The exact harvest date will vary between the different crop types relating to their different maturity dates. Broccoli is likely to be harvested first, followed by cauliflower, with cabbage and kale remaining in the field for the longest. For the first few years the trial will be smaller and occupy an area of up to 500 sq. m . Larger trials will only be conducted once it is established that lines perform well under field conditions. Further details on the proposed field site and plan are shown in Confidential Appendix C1.

Natural populations of CWB and DBM will be used to infect the field trial. It is likely that cabbage white butterflies in particular will commence laying eggs on the crop within days of planting. If the weather conditions have been unsuitable for survival of CWB and DBM butterflies then both will be deliberately released at the trial site. CWB and DBM will be obtained from either laboratory reared populations or from other field sites in NZ.

This field test site has been chosen as it is close to the laboratory allowing easy access for close monitoring, as well as being relatively inaccessible to the general public. The surrounding environment will consist of experimental trials of a range of crops including potatoes, cereals, and brassicas. Full research facilities for conducting the various aspects of the field trial will be available including cultivation, spraying and irrigation equipment associated with normal maintenance of field trials on an Agricultural Experiment Station. The field trial will also be supported by well-equipped tissue culture and molecular biology laboratories.

4.2 Describe the proposed containment system (physical and operational)

A full account is required of how the organisms will be contained securely. Describe the physical characteristics of the containment system, including security features. Outline procedures followed in the operation and management of the containment system, and the supervision, training and qualifications of staff. Refer to relevant containment or other manuals as appropriate. Identify if the facility is currently registered by MAF as a containment facility. If so provide registration details.

The field test will be planted and supervised by experienced staff from the Plant Biotechnology laboratory of the New Zealand Institute for Crop & Food Research Limited. Staff and researchers authorised to work with PC2 material will only be allowed to work on the field test. All staff associated with the planting, data collection, monitoring and disposal of this trial are fully trained in plant tissue culture and genetic engineering to PC2 standards. They are all authorised users of restricted organisms and authorised by our Biological Safety Officer to use the Lincoln containment facility GMO laboratories and GMO plant house. In addition the two main staff associated with the planting, data collection, monitoring and disposal of this field trial were involved with these aspects with the previous three GM *Brassica* field trials conducted at Lincoln. It will be the responsibility of the authorised staff to ensure that new staff or temporary staff are trained in documenting visits, handling and transporting material, and recording changes made to plant numbers etc. New staff involved in the field test will be trained by their immediate supervisor who will always be a registered PC2 user and fully experienced in either GMO's or field tests involving GMO's. On-going training will be conducted as required. All new staff will receive routine training as outlined in the containment manual. Subcontractors will also have to be authorised and new staff trained appropriately. Once material is back within a PC2 contained facility the procedures outlined in the containment manual for this facility (Confidential Appendix C2) will apply. An internal audit by a biological safety officer, appointed by the local IBSC will be performed on 6 monthly intervals or as required by ERMA to ensure the field test is being carried out as required.

The field test will be a standard *Brassica* trial involving the hand planting of seedlings or cuttings produced in the containment greenhouses or laboratories at Lincoln. This planting material will be produced in contained laboratories or greenhouses following protocols previously described (Christey and Braun 2004). Selfed seed collected from the original transgenic plants or tissue-culture or greenhouse derived clonal cuttings of the original transgenic plants will be used. The exact number of seedlings, plantlets or cuttings required for the trials will be transported to the trial site in secure double contained containers. Material to be transported to and from the field test site will be placed into double wrapped containers (i.e. a seedling tray or pot within another closed container or bag within a closed bag). Containers will be clearly labelled. Prior to transfer to the field test site the number of plants and containers/bags will be recorded on a datasheet. On arrival at the field test site these details will be checked to ensure all plant material has been transferred to the field test site. After completion of planting numbers will again be checked to ensure all plants are accounted for.

Should records demonstrate that material has been lost from the field test site or during transport then an investigation by the project senior scientist will be undertaken to determine whether actual loss of

material has occurred or whether record-keeping abnormalities have occurred. If loss of material has occurred then the route, the field test site, and the containment structure will be searched by personnel on the project in order to find the missing plant(s). If material cannot be found then post-monitoring of the field test site will be undertaken to check for missed material. If any *Bt Brassica* material is found beyond the field test site then this will be assessed for the presence of the transgenes used in this field test. If present then these *Bt* brassicas will be removed and destroyed by autoclaving and an area 5m around the site monitored for a period of 1 year to ensure that no further *Bt Brassica* material grows.

This proposed field test will be completely surrounded by one to two buffer rows of non-transgenic wild type plants. This is essentially the same for any agronomic trial to prevent "edge effects" from wind or other environmental factors. These buffer rows will be either be planted with a commercially obtained line of non-transgenic wild type red broccoli or red cauliflower or with a non-*Brassica* plant like *Phacelia* or buckwheat. These lines have been selected to ensure that the buffer rows contain a genotype phenotypically distinct from that of the transgenic *Brassica* lines. The inclusion of *Phacelia* or buckwheat is to encourage the presence of beneficial predators and parasitoids. The buffer row(s) will be removed and composted at the end of the field test.

Because plants will only be grown to heading and not to reproductive maturity (see section 4.1) there will be no pollen or seed to escape from the trial. Any plants that bolt will be removed to ensure that plants do not flower in the field. An isolation distance of 700m is used in NZ for production of certified *Brassica* kale seed (Christey and Woodfield, 2001). However, as there are risk management strategies in place (plants checked every 3-4 days) to ensure that no open flowers will be produced on the trial site an exclusion zone of brassicas surrounding the field test site is not required. The field test will be monitored at least every 3-4 days to ensure that plants that bolt are removed prior to flowers opening. The surrounding land will contain trials of other crop plants including *Brassicas* (see confidential Appendix C1 for details).

Each plant in the field test has a unique row/number identifier which will ensure all plants are easily accounted for. Seedlings on transplant are large enough that accidental removal e.g. on footwear, is not possible. The field test will be monitored at least every 3-4 days to ensure that all plants are accounted for.

Physical barriers are considered necessary to prevent the unintentional dispersal of these genetically modified brassicas by birds and animals. The trial will be securely fenced and will have weed cloth to prevent the entry of rabbits, hares and pukeko. Bird scarers will be used to decrease the number of birds attracted to the site. In addition the crop will be sprayed with bird repellent. Commercially available mammal and bird repellents eg Pindone and X-Pel will also be applied around the trial site. Several studies have shown no adverse effects of *Bt* strains on a range of birds (EPA, 2001) and also rodents and livestock (EPA, 2001).

At harvest, the buffer rows of non-transgenic plants surrounding the field test will be harvested via hand picking and composted. The plants in the experimental plots will be individually hand lifted and picked. As each plant is removed the details will be recorded to ensure that all plants are accounted for. Any plants in the experimental plots with bolting heads will be completely removed before flowering and autoclaved or the whole plant transferred to the containment glasshouse in a secure bag for repotting for seed collection. After plants have been lifted, they will all be transported to the PC2 containment greenhouse facility (Crop & Food Research Lincoln

containment facility registered to MAF Biosecurity Authority Standard 155.04.09 under ERMA application GMD01086 with approval code GMD000814) in secured autoclave bags for counting and weighing of heads and plants. Post harvest assays and extractions will be performed within the containment greenhouse facility or containment biotechnology laboratories (which operate at category PC2). Plants will be autoclaved at the completion of these assays unless they are being kept for seed production. Approval GMD000814 allows for the production of seed off these plants in the PC2 containment greenhouse facility.

Within a week following harvest, the site will be thoroughly checked to ensure that no plants have been left in the soil. The field test site will be left fallow for the remainder of the year to enable the detection of volunteers. In the following year the site will be sown with a cover crop such as grass or cereal as is standard agronomic practice to improve soil structure and fertility and to help prevent the build-up of plant pathogens in the soil. The site will be monitored visually monthly for at least one year following removal of the last GM plant from the field trial site for the appearance of escape plants.

Human involvement would be required to accidentally move transgenic plants off the site. To prevent this, all harvesting and cultivation equipment will be thoroughly checked and cleaned and maintained. All autoclave bags containing harvested plants will be securely tied up before transport from the trial site.

Appropriate measures to prevent sabotage and detect human disturbance on the site have been arranged and are provided along with further details of the site and the containment facilities, their structure, operation, management, supervision, staff and training in confidential Appendices C2 and C3.

4.3 Inspection of the site *before* field testing commences

Clause 6A(b) of Part 1 of the Third Schedule of the HSNO Act *may* require inspection of a site *before* field testing commences. Please provide reasons as to whether you think this clause 6A(b) should apply to your field test and describe how you would achieve this.

Prior to every planting the field trial site will be ploughed and sprayed to ensure removal of any other plants. MAF staff will be notified prior to the planting of each years trial to enable inspection of the site.

4.4 Inspection and monitoring of containment facilities *during* the field test

Clause 6A(a)(i) of Part 1 of the Third Schedule of the HSNO Act requires inspection and monitoring of containment facilities *during* the field test. Describe how this would be done.

As described in Appendix 3, the trial will be monitored every 3-4 days to ensure that plants that bolt are detected and removed so that no open flowers are present. In addition, weekly measurements will be taken on plant agronomic features including leaf number, plant height, head initiation and head diameter. Plants will also be counted weekly to ensure that all plants are present. During the course of the field test, plants will also be monitored for the presence of insect pests e.g. aphids and for fungal and bacterial diseases. Plants will be sprayed as required to ensure the control of these pests and diseases.

To ensure that the *Bt*-containing transgenic plants in this field trial do not contribute towards the development of *Bt* resistant insects only plants that have adequate expression levels by causing 100% mortality in laboratory assays will be field tested. In addition *Bt*-containing plants in the field test will be monitored visually every 3-4 days for visible signs of caterpillar damage to ensure no survival of caterpillars to maturity on *Bt* plants. The rows in the field test will be spaced 50cm apart to ensure that plants do not touch to ensure that if any caterpillars survive they cannot move from a *Bt* to a non-*Bt* containing plant. Any *Bt*-containing plant showing signs of caterpillar damage will be immediately removed from the field test and the plant and caterpillars destroyed by autoclaving.

MAF and ERMA staff are welcome to inspect the trial site during the field test as they wish with prior notification.

4.5 Clean up controls and *post* field test monitoring and inspection

Section 45A(2)(a) and (b) of the HSNO Act requires that at the completion of a field test the organism and any heritable material from the organism (along with some or all of the remaining genetic elements) be removed or destroyed. Clause 6A(a)(ii) of Part 1 of the Third Schedule of the same Act also requires inspection and monitoring to ensure that this has taken place. Describe how you would achieve these objectives.

During the trial the plants will be removed as they commence bolting (see section 4.1). Any remaining non-bolting plants will be removed at the end of the field test. This will involve digging the entire plant from the soil. Some plants may be transferred to the PC2 GMO facility and repotted for future seed collection. The remaining plants will be placed in a secured autoclave bag for transfer to the GMO facility for disposal by autoclaving. The removal of each plant will be recorded to ensure no plant is left behind. However, visual assessment will detect any remaining plants. The field test site will be cleared of any remaining plant material by hand harvesting at the end of the trial and, if not required for assessment, this will be disposed of by autoclaving. At the completion of the trial, all field test plants will be dug out of the ground and accounted for and then the field test site ploughed to cut up all other non-*Brassica* plant material e.g. weeds to prevent regrowth. It is highly unlikely that any viable plant material from the GMO plants will remain, but as a precaution the site will be monitored visually monthly for at least one year following removal of the last GM plant from the field trial site for the appearance of escape plants. Any plants that appear to be vegetable or forage brassicas of the type field-tested will be removed by digging the entire plant from the soil and PCR analysis conducted to determine if the plant is GMO. Appropriate positive controls will be included to ensure PCR analysis accurately detects any escapes. Any GMO plant will be then destroyed by autoclaving. Section 4.2 describes the crop rotation scheme to be followed.

The method described above will ensure all heritable material is removed. However, a small amount of plant material will remain on the site as removing the plant from the soil is likely to leave behind a small amount of root material. It is extremely unlikely that plants could regenerate from the root material left behind as such regeneration requires specialised tissue culture conditions. Any such regenerates would be detected during the routine monitoring for escapes. In addition as the plants grow senescing leaves will be dropped from the plants onto the soil. Non-heritable, genetic elements contained in this plant material will be left to decompose on the trial site. Leaving genetic elements to undergo natural degradation is appropriate for this trial for the following reasons:

1. Similar genetic elements from introduced transgenes are being left as genetic elements in ~45 million hectares of soil worldwide last year alone and have been left in commercial acreages since 1996 without any evidence of adverse effects (Nap *et al.* 2003, Conner *et al.* 2003).
2. There is a negligible likelihood of the genetic elements from the introduced transgenes being transferred horizontally, and, due to the ubiquitous nature of similar 'natural' genetic elements in plants and microbes, the consequences of such an event would most likely be harmless (see section 5 and Appendix 4).
3. Previous field trial studies at Crop & Food research on the persistence of the NPTII gene in soil have shown that the NPTII gene was only detectable in soil when the potato plants were growing. After harvest no DNA was detectable in the soil (S. Keenan, Crop & Food, pers. comm.).

4.6 Discuss the ability of the organism(s) to escape from the proposed containment system

Describe the biological features of the organism(s) that would affect its ability to escape from the containment system. For example, "Plants will be grown to reproductive stage, but inflorescences will be bagged to prevent escape of pollen or seeds." "The double fencing system will be high and wide enough to prevent escape of the animals by jumping.

The GM plants to be field tested in this application were developed through *Agrobacterium tumefaciens*-mediated transformation. There have been concerns raised about the possibility of GM *A. tumefaciens* from the co-cultivation step remaining as a contaminant of GM plants and being transferred to the environment on field testing. During the tissue culture phase of this research plants were grown on Timentin-containing medium which has been shown to be effective in eliminating *Agrobacterium.* In addition, PCR analysis has detected no contaminating *Agrobacterium* in the plants to be field-tested (see Appendix 2 for more details).

Brassicas survive through seed propagation. They do not form tubers or bulbs or propagate vegetatively through stolons or runners. Plantlets with 4-10 leaves will be transplanted to the trial site. These plantlets are approx. 15cm tall and therefore the plants are easily visualised and easily counted. The lack of tuber or bulb formation and the lack of vegetative propagation mean all plants will be easily monitored and accounted for with no possibility of further vegetative propagation or of tubers/bulbs remaining in the field.

Plants will be grown to harvest maturity i.e. at the stage when heads of cauliflower, cabbage and broccoli would normally be removed for market. Plants will not be grown to reproductive maturity therefore there will be no opportunity for escape of pollen or seed. In all cases the difference in morphology of these plants prior to bolting and flowering is easily noted enabling sufficient time to remove the plants from the field prior to any pollen release (see Figures 13-15 in Appendix 3). From the initiation of heads to peduncle elongation followed by opening of flowers takes over 4 weeks in broccoli, cauliflower, cabbage and kale. Therefore the

monitoring every 3-4 days from the start of the trial, with removal of plants when they begin bolting, will ensure that plants are removed well before flowers open.

Forage kale and cabbage require a period of cold to induce the initiation and formation of reproductive structures. In cabbage the change to inflorescence production is easily noted from the cracking of the head as the inflorescence structure breaks through. In forage kale bolting of the main stem occurs well before flower buds are open. Cauliflower and broccoli will bolt to flowering after the heads have matured. However, elongation of the peduncles that occurs well before the flower buds open is easily noted allowing removal of plants well before flowers open. Appendix 3 illustrates and describes in more detail the sequence of events involved in the transition to fully flowering plants in the material to be field tested.

All plants will be monitored every 3-4 days for any evidence of bolting and any plants bolting will be removed and either transferred to the GMO containment greenhouse for seed production or disposed of by autoclaving.

As discussed in section 4.2, the presence of shade cloth, bird scarers and repellents will be used to prevent and minimise the escape of plant material by birds, rodents, hares and rabbits feeding on and dispersing the plant material. Indirect dispersal is still possible for example via ladybirds feeding on aphids that have fed on the *Bt*-containing plants. However, these plants will either be sprayed to contain aphid infestation to minimise this effect or the leaves removed to the laboratory to study the effect of this feeding on non-target invertebrates.

There are several potential pathways by which the GM Brassicas may escape from containment. These include human and animal mediated pathways as well as escape due to natural events such as flooding. The trial location, security and monitoring procedures in place will ensure that the likelihood of these events leading to plant removal is highly improbable and missing plants would be quickly identified.

Section Five – Identification and Assessment of adverse and beneficial effects (risks, costs, and benefits)

This section must include information on the adverse and beneficial effects referred to in the HSNO Act. Adverse effects include risks and costs, and beneficial effects are described as benefits. All effects should be described in terms of the magnitude of the effect if it should occur and the likelihood of occurrence. Monetary and non-monetary effects should be considered, and a comment should be included on the distribution of the adverse and beneficial effects across affected parties. When identifying and assessing the risks of the genetically modified organism it is important to consider the increase in risk that it poses when compared to the unmodified parent organism from which it has been derived. Provide a brief account of where the information in the application has been sourced from, e.g. from in-house research, independent research, technical literature, community or other consultation. Comment on the significance of uncertainty in the information and thus in the assessment of effects.

5.1 Ability of organism(s) to establish a self-sustaining population

Discuss the ability of the organism to establish a self-sustaining population outside of containment, taking into account the ease of its eradication. You should consider the ability of the organism to survive and reproduce if it did escape from containment.

As discussed in section 4.6 Brassicas survive through seed propagation and do not propagate via tubers, bulbs, runners or stolons. Monitoring the field test every 3-4 days for any bolters and subsequent removal of bolting plants from the field site before the flowers open will ensure the prevention of pollen or seed spread from the trial site.
Seedlings or plantlets planted out onto the site will be large enough to prevent their accidental removal by physical elements, such as the wind, or biological elements, such as birds. If any plants are uprooted or dug up they are likely to be left close to the initial planting site and the accurate data recording will ensure any missing plants are immediately identified. Accurate record keeping of plant numbers being taken to the site or removed from the site will prevent loss in transit. Security measures (confidential Appendix C1) will prevent deliberate escape. Monitoring post experiment will ensure no escape from any material that could have accidentally been left in the ground. At the completion of the field test the site will be monitored visually monthly for at least one year following removal of the last GM plant from the field trial site for the appearance of escape plants. Any plants will be removed by digging the entire plant from the soil and then destroyed by autoclaving.

Appendix 4 discusses the potential for transfer of the introduced genes to non-GM *B. oleracea* but the use of management methods to ensure plants are removed before flowers open and the frequency of pollination means this is a rare event. Monitoring the field test every 3-4 days for any bolters and subsequent removal of bolting plants from the field site before the flowers open will ensure the prevention of pollen spread from the field test site. Additional management measures such as removal of any weedy flowering Brassicaceae from the field test site and immediately adjacent to the field test will further reduce the likelihood of gene transfer by out-crossing occurring. If the unlikely event that any plant flowered there is the potential for pollen dispersal by bees or wind and therefore the extremely remote possibility of pollen transfer to another *Brassica* plant. If any plant flowered they are unlikely to form any viable selfed seed as the lines used are mainly self-incompatible and therefore cross pollination with a different compatibility background is required. Self-pollination is not possible as hand bud pollination would be required. In addition, some lines may be tetraploid. This means that this plant can produce fertile flowers but on crossing with any diploid *Brassica* only sterile triploid plants would form. In the extremely unlikely event that viable plants or seed remain in the field, such plants would be a volunteer in

the following crop and thus only of nuisance value. These plants would be easily visible during the post-trial monitoring and could be removed by spraying with an appropriate herbicide, e.g. Roundup or Glean, or by roguing and autoclaving them.

5.2 Identify all potential adverse effects of the organism(s) (risks and costs)

Identify potential adverse effects associated with the organism(s). Consider effects on the environment (for example ecosystems), human health and safety, and any ethical and cultural effects. Remember to consider adverse effects of the organism(s) both within and out of containment should the organism escape. Identification is primarily concerned with setting out all the effects that might occur, and deciding which of them warrant more detailed assessment. The detail of assessment should be in section 5.3. Adverse effects should be identified for the following categories:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. **adverse** effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health).

The potential adverse effects these plants pose are thought to be negligible. The genes (*cry* and NPTII) introduced are similar to ones in the soil already. The *Bt* endotoxin can enter the soil from both root exudates and from post harvest decomposition of plant material. However, there are no reports of any detrimental effects on the soil ecosystem from the use of *Bt*-containing crops. Soils are already exposed to *Bt* as *Bacillus thurigiensis* is a naturally occurring soil organism. Therefore *Bt* is already constantly available for ingestion by all soil invertebrates. In NZ, Chilcott and Wigley (1993) found that between 60-100% of soils sampled contained *Bt*. Of these isolates, 37-88% were toxic against Lepidopteran larvae. In addition; commercially available *Bt*-containing insecticides are already applied to crops in NZ to control Lepidopteran larvae. However, the presence of a *Bt*-containing crop is likely to expose the soil to higher levels than that derived from these sources. In addition, *Bt*-containing crops express the activated toxin whereas commercial *Bt* products consist of a mixture of the bacterium and spores. NPTII resistance is already quite ubiquitous in nature; species such as monocots are already highly tolerant to kanamycin (Nap *et al.*, 1992). In NZ and overseas numerous field trials have been conducted of plants containing the other reporter and marker genes proposed in this research. For example in Australia GMOs containing various combinations of these genes (*bar*, *hph*, *nptII*, *uidA* and *gfp*) have been field-tested with no reports of adverse effects on human health or the environment resulting from these releases (http://www.ogtr.gov.au/rtf/ir/dir052finalrarmp.rtf).

There are concerns about the impact of *Bt*-containing plants on the soil ecosystem. However, *Bt* plants appear to have little impact on non-target soil biota such as earthworms, collembolans, and general soil microflora (reviewed in O'Callaghan *et al*., 2005). Studies in NZ on GM plants have shown little effect on soil microflora. GM potatoes expressing antimicrobial proteins have shown only very minimal impacts on soil microbes associated with the GM plants. The soil biota in the field and during decomposition was studied. These minor differences may represent only transient changes or reflect the natural variation found between individual plants in natural populations (Conner and O'Callaghan, 2005). Another NZ study analysed rhizosphere bacterial and fungal communities of two transgenic lines of pine trees (*Pinus radiata*) in comparison with microbial communities associated

with the non-transgenic parental line. The results to date suggest that the transgenic pine trees have no significant effects on the dominant bacterial and fungal communities, including ectomycorrhizal fungi (Lottmann *et al.*, 2006).

These plants will be contained to a small field test site (see section 4.1) with no possibility for dispersal. The modified plants are not expected to influence the characteristics or abundance of any other species. There is no information to suggest that the field testing of these genetically modified brassicas will have any consequences (positive or negative) on tangata whenua, human and animal health, agricultural production, target or non-target organisms, the general ecology, environmental quality or pollution in the area.

There are no native Brassicaceae in the same tribe as *Brassica oleracea* (Bourdot *et al*., 1999); therefore no effect on native flora is expected. In NZ, the Brassicaceae family is represented by 101 species, classified into 40 genera, which are further grouped into 10 tribes (Bourdot *et al*., 1999). Some of these species are represented by up to three sub-species or varieties. Of the 108 entities listed by Bourdot *et al*. (1999) as members of the Brassicaceae family present in the flora of NZ, 25 are listed as endemic (i.e. confined to the flora of NZ). These 25 endemic members are found in the *Cardamine*, *Cheesemania*, *Rorippa* (all Arabideae tribe), *Ischnocarpus*, *Iti*, *Pachycladon* (all Sisymbrieae tribe), *Lepidium* and *Notothlaspi* (both Lepidieae tribe) genera and most are alpine plants. The plants to be field-tested all belong to the *Brassica* genus and the Brassiceae tribe and therefore no effect on native flora is expected due to the taxonomic distances involved. In addition, as the endemic plants are mostly alpine plants they are not likely to come in close contact with cultivated brassicas.

Intertribal sexual hybridisation in the Brassicaceae has not been reported but intertribal somatic hybridisation has been accomplished (reviewed in Christey 2004). Since the production of the first intertribal somatic hybrids between *B. campestris* and *A. thaliana* by Gleba and Hoffmann over 20 years ago, intertribal somatic hybrids have been produced between *Brassica* species and four other Brassicaceae tribes including genera from the Arabideae (*Armoracia, Barbarea*), Sisymbrieae (*Arabidopsis*, *Camelina*), Lepidieae (*Capsella*, *Thlapsi*), and Drabeae (*Lesquerella*) tribes (reviewed in Christey 2004). When *Pachycladon exilis* was pollinated by *B. rapa*, no hybrids formed (R. Bicknell, Crop & Food Research, pers. comm.). In addition, no increase in silique elongation was noted following these crosses, indicating that *P. exilis* failed to respond at all to the presence of *B. rapa* pollen.

Of the seven Brassicaceae listed as being used traditionally by Māori for food or medicinal purposes (Appendix 5), four are naturalised and three are indigenous to NZ and none are endemic. Two of the naturalised plants are in the same tribe and genus as the plants to be field tested in this application. The remainder are in different tribes to *Brassica oleracea*.

The potential for cross pollination is discussed in more detail in Appendix 4. As discussed in section 5.1 and Appendix 4 the potential for pollen transfer is remote due to the strict monitoring in place to ensure plants do not flower and the production of a viable plant from pollen transfer is extremely remote.

The potential effects on the biodiversity of non-target organisms such as aphids, parasitoids, predators and pollinators is not likely to be adverse and may be beneficial due to the reduction in non-specific insecticide use. These effects are discussed in more detail below.

Effect on non-target pests, parasitoids and predators

Another concern is the potential impact of *Bt*-containing plants on non-target organisms, including pests and natural enemies, feeding on and/or visiting *Bt* plants. In particular it is important to determine if the *Bt* toxins have any detrimental effect on biological control agents such as parasitic wasps that are

important bio-control agents for biological control of CWB, DBM and aphids. These beneficial non-target organisms may come in contact with the *Bt* toxin from the transgenic plants by feeding directly on the plant themselves or indirectly by feeding on target or non-target herbivorous insects.

Effect on parasitoids There are several parasitoids that are important natural enemies of DBM and CWB larvae in NZ (Table 1). These wasps have slightly different modes of action but essentially lay an egg inside the larvae. The resulting parasitoid larvae feeds on the caterpillar and emerges as a cocoon or wasp, usually after killing the caterpillar before it causes serious damage (Berry, 2000). Direct effects of the toxin can be expected only if the toxin is ingested and the parasitoid is susceptible. It is not possible to study the effects directly on CWB or DBM parasitoids as the plants kill the larvae. Schuler *et al.* (2003, 2004) studied the effect of *cry*1Ac oilseed rape plants on the DBM parasitoid *Cotesia plutellae*. Although unable to survive in *Bt*-susceptible DBM larvae on highly resistant *Bt* oilseed rape plants due to premature host mortality, *C. plutellae* is able to complete its larval development in *Bt*-resistant DBM larvae. The parasitoid was as effective in controlling *Bt*-resistant DBM larvae on *Bt* plants as on wild type plants. Equal or higher numbers of parasitoid adults emerged per transgenic as per wild type plants.

Table 1: Parasitoids of DBM and CWB in NZ[1]

Host	**Parasitoid (stage attacked)**
DBM	*Diadegma semiclausum* (larvae)
	Diadromus collaris (pupa)
CWB	*Pteromalus puparum* (pupa)
	Cotesia glomerata (larvae)
	Cotesia rubecula (larvae)

[1] from Berry (2000)

Effect on non-target pests It is important to consider the effect of the *Bt*-containing plants on the natural enemies of other pests which are not controlled by the *Bt* gene but are potentially exposed to the *Bt* toxin when feeding on hosts. For example, aphids are a major pest of *Brassica* crops and are not controlled by *Bt* genes therefore it is important to ensure no detrimental effect of the *Bt*-expressing plants on the ability of the aphid parasitoid to control aphid populations. Schuler *et al.* (2001) conducted a detailed study on the effect of *cry*1Ac expressing oilseed rape on the peach potato aphid (*Myzus persicae*) and its hymenopteran endoparasitoid *Diaeretiella rapae*. They found no detrimental effects of the transgenic oilseed rape on the ability of the parasitoid to control aphid populations. Adult parasitoid emergence and sex ratio were also not consistently altered on the transgenic oilseed rape lines compared with the wild-type lines.

In NZ, Walker *et al.* (2006) have conducted several studies on the effect of *Bt* toxins on non-target Lepidopteran pests and their parasitoids. Laboratory bioassays with *Helicoverpa armigera* (tomato fruitworm, heliothis) and *Spodoptera litura* (tropical armyworm) were conducted with the *Bt* foliar insecticide Dipel 2X and also with the Cry1Ac toxin. *S. litura* and *H. armigera* are potential non-target pests on *Bt* brassicas. They are both controlled by several larval parasitoids and it is therefore important to examine the impact of *Bt*-crops on them. *H. armigera* larvae parasitised with *Cotesia kazak* and *S. litura* larvae parasitised with *Cotesia kazak* and *Meteorus pulchricornis* were assayed. The results showed reduced weights of host larvae, pupae and moths with delayed development. But this had little effect on parasitoid viability. Larval development of the parasitoids was delayed only when there was a significant effect on the host Lepidoptera. Walker *et al.* (2006) concluded that their studies confirm

previous observations that there are no direct effects of *Bt* toxins on developing parasitoid larvae; the effects are indirect and mediated through the health of the host. The *Bt*-containing plants developed in this research are currently being used in similar non-target impacts assays in containment with *S. litura* and *H. armigera* and their caterpillar parasitoids *Meteorus pulchricornis* and *Cotesia kazak* by Crop & Food Research.

Effect on non-target predators In addition, it is important to determine if the *Bt* toxins affect predators or natural enemies of non-target pests such as aphids or caterpillars. For example brown lacewing or ladybird beetles feeding on aphids or hoverfly feeding on aphids and caterpillars. Zhang *et al.* (2006) have shown in laboratory feeding experiments that trace amounts of *Bt* toxins were detected in the cotton aphid *Aphis gossypii* feeding on *Bt* cotton. *Bt* toxin was also detected in ladybirds feeding on *Bt*-fed aphids. The presence of the *Bt* toxin was not detrimental as the ladybird beetles preying on *Bt*-reared aphids matured faster and mated more frequently than those fed on *Bt*-free aphids. Kalushkov and Nedved (2005) have shown in laboratory experiments that *Bt* potatoes had no effect on the aphid *Myzus persicae* and that aphids fed on *Bt* potatoes had no effect on the larval development and mortality of the aphidophagous coccinellid *Coccinula septempunctata*. Kalushkov and Nedved (2005) also showed that six different aphid species cultured on *Bt* potato were suitable food for the aphidophagus coccinellid *Propylea qualuordecimpunctata* compared with those cultured in non-*Bt* potato. The *Bt*-containing plants developed in this research are currently being used in similar non-target predator impact assays in containment with aphids and lacewings by Crop & Food Research.

A detailed recent review by Romeis *et al.* (2006) summarised the published data from laboratory, greenhouse and field trials on the effect of *Bt* crops on arthropod biological control agents (parasitoids and predators). These studies mainly relate to maize, cotton, rice and potato but field studies have confirmed that the abundance and activity of parasitoids and predators are similar in *Bt* and non-*Bt* crops. In contrast, applications of conventional insecticides have usually resulted in negative impacts on biological control organisms.

Effect on pollinators

The main pollinators of *Brassica* crops are honey bees with bumble bees also visiting. The plants to be field tested will not be allowed to flower therefore no effect on pollinators is expected. In the unlikely event of any plant flowering and being pollinated the current research indicates that the effects will be negligible. Nectar contains no DNA or RNA and is also free of protein, being composed principally of sugars (Malone, 2002). In addition, *cry* gene expression in the plants to be field tested is controlled by the CaMV 35S promoter. However, this promoter does not work efficiently in the pollen of the plant and therefore protein expression is very low (Malone 2002).

Research in NZ and overseas has investigated the potential impact of *Bt* transgenic products on honeybees and shown negligible effects of *Bt* genes on honey bees. Pre-release honey bee biosafety tests are conducted for each *Bt* crop registered in the USA and in each case no effects were observed (reviewed in O'Callaghan *et al.*, 2005). In a NZ study, Malone *et al.* (2001) fed newly emerged adult honey bees (*Apis mellifera*) 625 ug/g activated Cry1Ba toxin and found no effect on several parameters including timing of their first flight, the period during which flights took place or in estimated longevity. The rate used approximates expression in pollen of 0.25% (of total soluble protein). This confirms pervious studies showing that bees were unaffected when fed on purified *Bt* gene products (Malone *et*

al., 1999). In a subsequent NZ study, Malone *et al.* (2004) investigated the effect of *Bt* on hypopharyngeal gland development. The hypopharyngeal gland of adult bees secretes jelly for feeding to bee larvae. Therefore compounds interfering with gland development might affect the survival of larvae in the colony, even if adult survival is not affected. They fed honey bees pollen-food containing 0.3% Cry1Ba toxin and found no effect on bee survival, or the mean diameters of gland acini, gland mass or protein content. There was no evidence of Cry1Ba in the glands of bees fed this protein. The Cry1Ba1 protein used is the same source as used in the production of transgenic plants in this study. Hanlkey *et al.* (2003 as cited in Malone *et al*., 2004) also found no significant impacts attributable to transgenic pollen in experiments where honeybee larvae were fed pollen from cry1Ab expressing corn.

B. Potential adverse effects on human health and safety (including occupational exposure).

There is no evidence to suggest that handling these plants will have adverse effects on human health and safety. *Bt* toxins have a long history of safe use as insecticidal sprays with applications to crops for over 30 years with no reports of human or mammalian toxicity (Kough 2003). Several *Bt* containing insecticides are currently registered for use in NZ including Foray, Dipel, Delfin and Agree. These products are used on a variety of crops including fresh produce such as lettuce and tomatoes with no reports of human or mammalian toxicity. The nucleotide sequence modifications made to alter the *Bt* gene sequence to enable more efficient expression in plants did not alter the amino acid sequence of the final protein (Strizhov *et al*. 1996). Therefore no novel allergen effects are expected. Plants containing similar genes have been field-tested in NZ and overseas for several years with no reports on adverse effects on human health and safety. In 2005 over 30M ha of Bt-containing crops were grown commercially (James 2005). In addition plants containing similar genes have been consumed. *Bt*-containing foods are approved for human consumption in several countries. GM ingredients can only be sold in New Zealand if Food Standards Australia and New Zealand (FSANZ) has assessed them for safety and they have been approved by the FSANZ. Currently several different GM foods have been approved by FSANZ including GM derived soybean, canola, corn, potato, sugarbeet and cotton. Fourteen of these include *Bt* genes and six include NPTII in combination with *Bt* genes. In addition, FSANZ has approved the use of products derived from GMOs expressing the PAT, HPH, NPTII and GUS proteins for food http://www.foodstandards.govt.nz/foodmatters/gmfoods/gmcurrentapplication1030.cfm). *Bt*-containing potato, tomato, cotton and maize have been approved for human consumption in the USA (http://www.cfsan.fda.gov/~lrd/biocon.html; http://www.agbios.com). In some cases approvals were granted 10 years ago. *Bt*-maize has been approved for human consumption in 9 countries including Argentina, Canada, EU, Japan, Netherlands, Philippines, Switzerland, Taiwan and the UK. *Bt*-tomato has been approved for human consumption in Canada; *Bt*-potato in Canada, Japan, Philippines and *Bt*-cotton in Australia, Canada, Japan, Philippines, and the USA (http://www.agbios.com.).

A detailed report by Read (2000) on the use of antibiotic resistance marker genes in GMOs concluded that the potential impact of the use of the NPTII antibiotic resistance gene in GMOs on the prevalence of antibiotic resistance is far less significant than the impact of the current use of antibiotics in humans and animals in NZ (See Appendix 4 for more details).

C. Potential effects resulting from the transfer of any genetic elements to other organisms in or around the site of the field test

(**"Genetic element",** in relation to a new organism, means- (a) heritable material; and (b) any genes, nucleic acids, or other molecules from the organism that can, without human intervention, replicate in a biological system and transfer a character or trait to another organism or to subsequent generations of the organism).

As discussed in section 4 the proposed monitoring of this field trial will ensure that no heritable material from pollen or seed will be available for transfer to another organism.

There is the potential for DNA to become incorporated into the soil through the natural senescence of plant material especially leaves but also roots. As discussed in section 4.5 this DNA is likely to be undetectable a year after the trial is completed.

The presence of DNA in the soil from the GM plants raises the issue of the possibility for horizontal gene transfer (HGT). HGT is defined as the transfer of genetic material from one organism to another outside the context of parent to offspring reproduction. It is a common event in microbial evolution and there are many non-GM examples of HGT (Nielsen *et al.*, 1998). The three different mechanisms whereby horizontal transfer of genetic material may occur include conjugation, transduction and transformation. Conjugation involves the transfer of DNA between bacteria and involves the temporary connection of the cells. It is unlikely to be a mechanism for transfer of DNA from GM brassicas to other organisms as the transgenic DNA is not present on a plasmid but is integrated into the *Brassica* genome. Transduction involves the movement of genetic material via viruses. However, viruses that function in both plants and bacteria and thereby possibly facilitate HGT from plants to bacteria have not yet been identified (Nielsen *et al*., 1998). Transformation involves the uptake of free DNA from the environment. Some competent bacteria can take up naked DNA independent of its sequence (Nielsen *et al.*, 1998). As discussed by Nielsen *et al* (1998) there are several sequential steps required for genetic material to cause an effect via HGT. The recipient organism must be exposed to the foreign DNA, followed by uptake, integration and expression of the foreign DNA in the organism. There is potential for organisms to be exposed to GM DNA but studies have shown that DNA from senescing cells in soil is rapidly degraded. As discussed in detail by Nielsen *et al* (1998), HGT is theoretically possible but based on previous studies in the laboratory and in the field detection of such an event is improbable.

The likelihood of HGT is extremely remote but more importantly the consequences if HGT did occur are minimal. The *cry* genes used in this research have been extensively modified for optimal plant codon use and are therefore unlikely to function in a bacterial or invertebrate system. Various Lepidopteran active *Bacillus thuringiensis* strains already naturally occur in soil in New Zealand (Chilcott and Wigley, 1993) and therefore various *cry* genes are already available for any potential horizontal gene transfer from this bacterium to other soil species. Therefore *Bt* crops are not adding anything new to the already existing flow of *cry* genes among the soil micro-organisms. In NZ research of horizontal gene transfer is being conducted to evaluate the potential for HGT under NZ conditions (Watson and Carter, 2003).

Nap *et al.* (1992) discussed the potential and consequence for horizontal gene transfer of the NPTII gene. As they discussed kanamycin resistant micro-organisms already exist naturally in soil, therefore the contribution of the GM derived NPTII gene to the natural kanamycin resistance is minimal. In addition, the transfer of the NPTII gene to a soil micro-organism will not immediately result in kanamycin resistance because the gene carries regulatory sequences that will not generally work in micro-organisms (Appendix 4; Nap *et al.*, 1992). Upon transfer, recombination is required to make the gene functional, the micro-organism should be present in a natural environment in which selective advantage for kanamycin resistance occurs and should be able to out-compete the kanamycin resistant organism already present. Nap *et al.* (1992) concluded that the likelihood for a subsequent occurrence for all these events is negligible and that therefore horizontal transfer of the NPTII gene DNA will not alter or disturb a soil ecosystem.

As discussed above the potential for horizontal gene transfer of this genetic material to other organisms is negligible. In addition, preliminary experiments using one line of these *Bt*-containing plants in a laboratory study by ESR on HGT with *Acinetobacter*, a bacterium highly competent for horizontal gene transfer has detected no evidence of HGT under experimental conditions (S. Watson, ESR, pers. comm.).

D. Potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued introduced flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi).

Consultation

We were advised by ERMA New Zealand that this application to field trial genetically modified vegetable and forage brassicas triggered the requirement to consult nationally with Māori. In addition we were advised to consult with local iwi groups, namely Te Rūnanga ō Ngāi Tahu and Te Taumutu Rūnanga, early in the development of our proposal. In following this advice we have provided both Te Rūnanga ō Ngāi Tahu and Te Taumutu Rūnanga with an 8 page summary of the proposed field trial for feedback. We also met with the HSNO committee of Te Rūnanga ō Ngāi Tahu on 26 July 2006 to present our proposal and discuss issues raised. A copy of their response is included in this application (Appendix C3). We have not yet received a response from Te Taumutu Rūnanga.

National Maori consultation has also been initiated with the distribution of application information and Consultation Response forms to more than 80 iwi/Māori organisations. Further information about the Maori consultation undertaken is attached (Appendix 9).

Potential adverse effects of interest to Māori

This field trial proposal is not expected to have an adverse effect on Māori traditional resources. No material from native or valued introduced species is used and no material sourced directly from human beings is involved. Cabbage, broccoli, cauliflower and kale are all introduced plants and the genetically modified plants represent lines developed in New Zealand.

We note however that Māori continue to raise concerns about genetic modification. With regard to this proposal, Te Rūnanga ō Ngāi Tahu noted a range of concerns particularly in regard to the possibility of cross pollination or hybridisation, and the safety and security of the containment area. We have provided information about the Brassicaceae family to establish that there are no native members of the *Brassica* genus and therefore no effect on native flora is expected due to the taxonomic distances involved. In addition, as the endemic plants (all from other genera) are mostly alpine plants they are not likely to come in close contact with cultivated brassicas. We also consider that the containment regime and field trial plan (see section 4) proposed in this application satisfactorily addresses the other concerns raised reducing the risk of potential adverse effect to negligible levels.

Although noting the concerns mentioned above, Te Rūnanga ō Ngāi Tahu concluded in their response to consultation that (see their response letter – appendix C3):

'Following our assessment of the information you have provided, we do not oppose the proposed field tests of genetically modified Brassica oleracea vegetable and forage brassicas. While the tribal policy on genetic modification remains relevant, three points contribute to our position:

- *Knowledge and experience gained through the recent Crop and Food Research onion trial process*
- *The trials do not involve a native or taonga species*
- *There is no intention to release any GMO material into the environment.'*

We will also forward any feedback we receive from our national consultation to ERMA New Zealand for consideration as part of our application.

E. Other potential adverse effects (such as effects on society and community, effects on the market economy and effects on New Zealand's international obligations).

The local community is well aware of the previous GM research being conducted at Crop & Food Lincoln. All our previous field trial applications covering potatoes, Brassicas, peas and onions have been publicly notified in the local newspaper. Since 1989, Crop & Food have conducted over 25 separate GM field trials at Lincoln. The researchers associated with the potato and onion trials have given numerous talks to the local community including addressing school groups and community groups such as rotary and the Te Taumutu runanga. The local community are also aware of the GM *Brassica* research as Dr Christey has previously conducted 3 separate GM Brassica trials at Lincoln. In addition she has addressed the local high school on several occasions on her group's research as well as shown local school, university and industry groups her research facilities. Articles on her group's research have appeared in local newspapers and in local industry newsletters. Through her research contacts, local seed companies, CRIs and universities are aware of her research and plans for field testing. Neighbours directly adjacent to the field trial site have also been contacted (See Appendix C3). A Crop & Food information sheet and letter summarising the research has been circulated to industry groups (See Appendix C3).

Response from the local community, including both the public and scientific community, has been mixed with both positive responses and also concerns raised. Positive feedback is obtained about the reduction in insecticide use these plants may confer. Concerns generally relate to the containment of the plants, particularly pollen containment, and also to the possibility for insect resistance to *Bt* developing. These concerns are being addressed by ensuring that the strict monitoring in place will ensure that these plants do not flower and that any caterpillars surviving on GM plants are detected and removed.

These plants are being grown in a contained field trial and will not be marketed. There is no possibility that these plants or their products would be accidentally marketed due to the strict monitoring we have in place to ensure that all plants are accounted for.

5.3 Provide an assessment of the potentially significant adverse effects identified in Section 5.2.

The assessment should include the nature, likelihood or probability of occurrence and magnitude (in monetary or non-monetary terms) of each adverse effect. The uncertainty bounds of the information contained in the assessment should also be discussed. The assessment should consider options and proposals for managing identified adverse effects and consider whether they can be adequately managed by the proposed containment system. Adverse effects should be assessed in relationship to:

A. Potentially significant adverse effects on the environment, in particular ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health).

As discussed in section 5.2A there are no potentially significant adverse effects on the environment identified from this research to date. The operating procedures and containment system in place for this field test will ensure that the potential for any such events is minimal. If any such effects were

subsequently identified the consequences and impact would be minimal as the plants could easily be destroyed.

B. Potentially significant adverse effects on human health and safety (including occupational exposure).

As discussed in section 5.2B there are no potentially significant adverse effects on human health and safety identified from this research to date. If any such effects were subsequently identified the consequences and impact would be minimal as the plants could easily be destroyed.

C. Potentially significant effects resulting from the transfer of any genetic elements to other organisms in or around the site of the development or field test.

As discussed in section 5.2C there are no potentially significant adverse effects resulting from the transfer of genetic elements to other organisms identified from this research to date. If any such effects were subsequently identified the consequences and impact would be minimal as the plants could easily be destroyed.

D. Potentially significant adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued introduced flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi). If consultation with Māori has been undertaken you should provide details of the outcome, including options and proposals for managing risks identified.

Consultation with Ngai Tahu has already occurred and the response to their concerns outlined in Section 5.2D. Consultation with Te Taumutu runanga and National Consultation has been initiated as outlined in Appendix 9.

E. Other potentially significant adverse effects (such as effects on society and community, effects on the economy and effects on New Zealand's international obligations).

As discussed in section 5.2E there are concerns raised about the testing of GM plants but the strict monitoring of this trial will ensure that there are no adverse effects on the market economy or society and the community from this field test.

5.4 Identify all potential beneficial effects of the organism(s) (benefits)

Identify monetary and non-monetary benefits associated with the organism(s) being field tested. Focus on immediate as well as long-term benefits and specify whether these are likely to be direct or indirect benefits. Substantiate claims by reference to sources of information. Benefits should be identified for the following categories:

A. Potential beneficial effects on the environment, in particular on ecosystems and their constituent parts (e.g. beneficial effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health).

This field test will also provide an opportunity to conduct impacts research to assess the environmental effect of such trial. Resources from our current FRST-funded impacts programme will be used to conduct monitoring of this field test. As part of the proposed impacts research associated with this field test the effect of the *Bt*-containing plants on the soil microflora and biota will be assessed. In addition, research will be conducted on the effect on non-target parasitoids, invertebrates and predators and on the potential for HGT. As some of the field test will be treated with conventional spray regimes including *Bt* sprays this field test will provide an ideal opportunity to compare and contrast the effect these different regimes have on the soil ecosystem and non-target organisms.

The potential for future reduced environmental exposure to pesticides is a benefit of this field test. In NZ vegetable Brassica growers routinely use several insecticide applications to control cabbage white butterflies and diamond back moths. As outlined in Appendix 6 in 2004 vegetable brassicas in NZ were sprayed with 2.81 kg a.i./ha of insecticides ranking these vegetables 4th highest in terms of insecticide use. In addition, overuse has resulted in resistance of DBM to several of these chemicals. The chemicals used are toxic and reduced environmental use is a potential benefit. For example, Orthene is a broad-spectrum systemic organophosphate insecticide used to control caterpillar pests and aphids. It is toxic to bees and birds with a residual activity of 10-15 days in plants and a half-life in the soil of 7-10 days. There is a withholding period of 7 days and human health effects are possible (NZ Agrichemical Manual 2005). Decis Forte is a non-systemic quick acting synthetic pyrethroid. It is toxic to bees and humans and highly toxic to aquatic organisms.

In addition, the reduced use of insecticides may lead to increased biodiversity of non-target organisms such as beneficial invertebrates that are normally harmed by insecticide use and enhance biological control methods on other insect pests such as aphids.

B. Potential beneficial effects on human health and safety.

This field test will not immediately result in beneficial effects on human health and safety as it is designed to evaluate the agronomic performance of insect resistant plants. However, the future adoption of these insect resistant plants will have beneficial effects on human health and safety through the reduced pesticide usage these plants will require. This reduced exposure to pesticides is beneficial to human health and safety. The insecticides currently used to control cabbage white butterfly and diamond back moth have proven human health effects. In the US the average number of insecticide applications for cotton has decreased from 4.6 to 0.8 largely owing to the introduction of *Bt* cotton (Romeis *et al*., 2006). In China, *Bt* cotton plants have provided a 60-80% decreased in the use of foliar insecticides (Romeis *et al.*, 2006).

C. Potential beneficial effects specific to Māori.

There are no potential beneficial effects of this trial specific to Māori; however Māori could benefit from this field test in the future if these plants were released commercially through reduced insecticide applications on an important food crop and through the associated benefits of reduced exposure of humans and the

environment to toxic chemicals. This could potentially reduce pesticide residues in food, soil and ground water in the future if the outcomes of this research are successful. This may lead to the improved long term health and well-being of the land and its people. In addition, through the interaction, consultation and information exchange between the applicant and the local iwi and runanga it is hoped that this field test will increase the knowledge and understanding of cultural issues associated with genetic modification.

D. Other potential beneficial effects ((such as effects on society and community, effects on the market economy and effects on New Zealand's international obligations).

This field test will hopefully show that use of GM crops can lead to reduced pesticide input on vegetable and forage brassicas. It will enhance our knowledge and understanding of the use of GM crops for reduced insecticide use and possibly as part of an IPM regime. In addition, this field test will provide more information to the public about the potential beneficial effects of transgenic brassicas and enable more informed debate about the use of transgenic crop plants. This field test will also provide an opportunity to conduct impacts research to assess the environmental effect of such a field test. The increased scientific knowledge in these areas will be made available to the wider scientific community through published papers and conference presentations. This field test will enhance the international reputation of NZ agricultural research and demonstrate to the international science community that NZ are at the fore front of GM research. This will enhance our ability to attract and retain scientists and to attract research funds, both from NZ and possibly overseas.

5.5 Provide an *assessment* of the potentially significant beneficial effects identified in Section 5.4.

Estimate the likelihood that the benefits will be realised, the magnitude of benefits associated with the new organism(s) and any uncertainties associated with this assessment. Be as specific as possible, and, where appropriate, provide documentation to support your assessment. Discuss the likelihood of any of these benefits being realised by any of the alternative methods identified in section 6.1. You should also indicate who would receive the benefits, and the time-course of delivery of the benefits. Beneficial effects should be assessed in relationship to:

A. Potentially significant beneficial effects on the environment, in particular on ecosystems and their constituent parts (e.g. beneficial effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health).

As discussed in section 5.4A the potential for future reduced environmental exposure to pesticides is a benefit of this field test. This field test will also provide an opportunity to conduct impacts research to assess the environmental effect of such trial. These benefits will be immediately realised by the researchers involved who will be able to publish this research. This will benefit the wider scientific community both in NZ and internationally as the results will be of use for other researchers contemplating similar research.

B. Potentially significant beneficial effects on human health and safety.

As discussed in section 5.4B this field test will not immediately result in beneficial effects on human health and safety as it is designed to evaluate the agronomic performance of insect resistant plants. However, the future adoption of these insect resistant plants will have beneficial effects on human health and safety through the reduced pesticide usage these plants will require. However, for these benefits to be fully realised the public acceptance of GM products in NZ will have to increase.

C. Potentially significant beneficial effects specific to Māori.

As discussed in section 5.4C there are no potential beneficial effects of this field test specific to Māori; however Māori could benefit from this field test in the future if these plants were released commercially through reduced insecticide applications on an important food crop and through the associated benefits of reduced exposure of humans and the environment to toxic chemicals.

D. Other potentially significant beneficial effects (such as effects on society and community, effects on the market economy and effects on New Zealand's international obligations).

As discussed in section 5.4D this field test will enhance our knowledge and understanding of the use of GM crops for reduced insecticide use and possibly as part of an IPM regime. In addition, this field test will provide more information to the public about the potential beneficial effects of transgenic brassicas and enable more informed debate about the use of transgenic crop plants. This field test will also provide an opportunity to conduct impacts research to assess the environmental effect of such a field test. These beneficial effects of the field test will benefit both researchers and also in the longer term the environment, the local community, farmers and consumers due to the production of vegetable and forage brassicas with reduced use of toxic insecticides. In addition, another potentially significant benefit of this field test will be to address questions from the Royal Commission on Genetic Modification about the impact of GM plants on the NZ environment.

5.6 *Overall evaluation* of adverse and beneficial effects (risks, costs, and benefits)

This overall evaluation is the main task of the Authority. The Authority has to decide whether the beneficial effects of the field trial outweigh the adverse effects. The Authority must also be satisfied that the organisms can be safely contained. You may wish to express a view on the relative importance of the different adverse and beneficial effects and how they should be brought together in making a decision.

From the research and reasons presented in section 5 and Appendix 4 we conclude that the overall adverse effects of this field test are negligible. The containment and monitoring systems in place will minimise the risk of any inadvertent transfer of genetic material to the environment. The beneficial effects of this field test in terms of the potential for reduced exposure of humans, beneficial organisms and the environment to toxic insecticides outweigh any potential adverse effects. In addition, this field test will address important questions regarding the impact of GM plants on the environment.

Section Six – Other requirements

6.1 Identify any alternative method(s) of achieving the research objective(s).

Assess each alternative method and compare the adverse effects on the environment and human health and safety with this field test. Indicate how practical the alternative method is.

To fully assess the insect resistance, environmental impact and phenotype of these plants field testing is required. There is no alternative method for achieving these research objectives as no genes are known for durable resistance to these caterpillars in the Brassicaceae family, therefore production of these plants via conventional breeding techniques is not yet possible.
To undertake this research entirely within a contained greenhouse is not feasible as it would be impossible to replicate natural environmental conditions, in particular natural caterpillar infestation. In addition, subtle phenotypic variations may only be visible under field situations as shown with previous potato trials.

6.2 Monitoring of effects

Field tests may often provide an opportunity to collect information related to the occurrence of adverse effects. The Authority wishes to encourage applicants to take full advantage of the field test to conduct monitoring which will provide an assurance that risks are being effectively managed and/or provide information which will assist in the consideration of any future release application. Describe any such monitoring you propose to put in place.

As part of this field test several areas of monitoring are being conducted relating to plant phenotype, chemical input, effect on non-target organisms and soil ecology. Detailed phenotypic observations will be made on all plants to assess the effect of the introduced genes on plant phenotype and phenology e.g. plant height, leaf number, head diameter and days to head initiation etc will be measured throughout the trial. In addition, the chemical input required for caterpillar control on transgenic and non-transgenic plants will be documented. Experiments will be conducted to measure the fate of DNA in the soil during the field test and after the plants have been removed. In addition, assessment of the effect of the transgenic plants on the soil biota will be conducted. The effect of the *Bt*-containing plants on non-target invertebrates such as aphids and ladybirds will also be studied (see section 5.2). The information to be obtained is based on studying areas that address the perceived risks of this field test and will be of value for any future field tests. In addition, the Royal Commission on Genetic Modification recommended that more research be conducted on the environmental impacts of GM organisms.

Section Seven – Additional Information

7.1 Do any of the organism(s) need approvals under any other New Zealand legislation or are affected by international obligations?

For example, the field test may require an approval under the Animal Welfare Act 1999; or if genetic material from species listed by CITES is used, then approval is required from both the importing and exporting countries.

The organisms to be field tested do not require approvals from any other NZ legislation.

7.2 Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere?

For example, has the organism(s) been considered for import under the Plants Act? Has the organism been developed as a result of a genetically modified development approval from either ERMA New Zealand or a delegated IBSC? Have any other countries' regulatory bodies assessed the organism for approval?

The plants described in this application were developed under ERMA application GMD01086 with approval code GMD000814 for *Brassica oleracea*. No other countries' regulatory bodies have assessed these organisms for approval.

Previously we have conducted several field trials of vegetable and forage brassicas developed via *Agrobacterium tumefaciens* and *A. rhizogenes* mediated transformation (Appendix 7). The majority of the plants were transgenic for NPTII and in addition some plants contained a non-modified *Bt* gene. Other genes include reduced ethylene production, Basta resistance or GUS. Potato plants containing the genes used in this research have been developed and greenhouse assays showed good control of potato tuber moth larvae (Meiyalaghan *et al*., 2004). Transgenic potato plants containing the *cry*1Ca5 gene gave 100% mortality and lines containing the *cry*1Ba1 gene 54-93% mortality (Meiyalaghan *et al*., 2004).

In New Zealand several field tests of *Bt*-containing transgenic plants have already been conducted. Potato plants transgenic for either *cry*9Aa2 (Conner *et al.*, 2002) or *cry*1Ac9 (Davidson *et al*., 2004) have been field tested. Davidson *et al.* (2004) compared the resistance of potatoes transgenic for a *cry*1Ac9 gene to potato tuber moth (*Phthorimaea operculella*) over three field seasons. The transgenic potato lines exhibited stable resistance to larvae across field seasons, between affected plant organs and between plant organs of different ages. In addition to field trials, several groups in NZ are developing *Bt* containing plants e.g. Pine containing *cry*1Ac (Grace *et al*., 2005).

World wide *Bt*-containing maize and cotton crops were grown commercially on over 26M ha in 2005 (James 2006), approximately three times the land area of NZ. Food products from these crops have been approved for human consumption in several countries including NZ.

7.3 Is there any additional information that you consider relevant to this application that has not already been included?

There is no other information to add.

7.4 Provide a glossary of scientific and technical terms used in the application.

See Appendix 10.

7.5 List of appendices. Give the names of any Appendices included with this application. Appendices should contain any information that is commercially sensitive, or additional material included with the application (such as details of consultations, vector diagrams, referenced articles). The main application should refer to the relevant Appendices but be able to be read as a stand-alone document.

- Appendix 1: Triangle of U
- Appendix 2: Gene constructs and Transformation methods
- Appendix 3: Prevention of flowering under field conditions
- Appendix 4: Summary of potential impacts considered
- Appendix 5: Maori traditional uses of Brassicaceae
- Appendix 6: Current control methods for CWB and DBM in NZ
- Appendix 7: Summary of previous field trials
- Appendix 8: CWB and DBM life cycles
- Appendix 9: Maori consultation
- Appendix 10: Glossary of scientific terms
- Appendix 11: Operational Manual for *Bt Brassica* field test
- Appendix 12: References Cited

Confidential Information

Confidential Appendix C1: Field trial location, design and security
Confidential Appendix C2: Containment manual for the Crop & Food Research Lincoln Containment facility
Confidential Appendix C3: Correspondence

7.6 References. Please include a list of the references cited in and supplied with this application form. Originals of the references must be supplied in full. Where the reference supplied is an extract from a book only the specific pages quoted must be supplied, along with the cover page of the book.

See Appendix 11.

Section Eight – Application Summary

Summarise the application in clear, simple language that can be understood by the general public. Include a description of the organism(s), the purpose of the field test, how the field test will be conducted to achieve its objectives, the proposed containment system, and any risks and benefits associated with the field test. This summary will be used to provide information for those people and agencies who will be notified of the application (e.g. Ministry of Agriculture and Forestry, Ministry for the Environment, Department of Conservation, Regional Councils, etc) and for members of the public who request information. Do not include any commercially sensitive information in this summary.

Vegetables brassicas are widely grown in NZ for both the domestic and export market. Insect pests are a large problem on these crops with control requiring the use of 2.81 kg of active ingredient of insecticide per hectare. The most serious caterpillar pests are cabbage white butterfly and diamond back moth (DBM). While there are numerous chemical control methods available for these pests, there are problems particularly in DBM with the development of resistance to these chemicals. In addition there are concerns about the presence of chemical residues in the environment. As a result there is growing interest in the development of alternative control methods. The soil bacterium *Bacillus thuringiensis* (*Bt*) has been used for biological control of insects for over 30 years. In NZ commercial products containing this bacterium are available. As there are no *Brassica* cultivars or close relatives with caterpillar resistance the introduction of insecticidal genes from *Bt* into plants offers an alternative method for caterpillar control. In 2005 maize and cotton crops containing *Bt* genes were grown commercially on over 26M ha world wide, approximately three times the land area of NZ. Food products from these crops have been approved for human consumption in several countries including NZ.

We have used *Agrobacterium tumefaciens*-mediated transformation to produce broccoli, kale, cabbage and cauliflower transgenic for a *Bt* gene and demonstrated excellent control of DBM, soybean looper and cabbage white butterfly larvae in laboratory and greenhouse assays. *Bt*-containing plants cause mortality of freshly hatched larvae within 48h with no visible damage to the plant. However, field-testing of these plants is required to confirm the extent of insect control under agronomic situations and to assess any unexpected environmental effects of these plants. We expect that the *Bt*-containing plants will require fewer insecticide applications compared with non-*Bt* containing plants. In addition, we expect no detrimental effects on non-target organisms.

This field test does not involve DNA from human genes or native and valued introduced flora and fauna. The sequence of the DNA to be used was originally derived from the bacterium *Bacillus thuringiensis* but the sequence has been modified to allow optimal expression in plant cells. This DNA was manufactured in a DNA synthesiser. The plant material to be used, vegetable and forage Brassicas, all belong to the Brassicaceae or Crucifer family. They are not native to NZ and are not closely related to other native members of this family occurring in a different taxonomic tribe. Therefore inter-crossing is not possible.

These plants will be grown in a secure contained field location near Lincoln. The field test will be small, occupying an area of up to 0.4 ha and is it expected that each plant will be in the ground for up to 5 months. The plants will be transplanted into the field as small seedlings or plantlets and their growth and development monitored until the formation of marketable heads. The plants will then be removed from the field and either destroyed by autoclaving or replanted in a contained PC2 greenhouse for flowering and seed collection. The field test site will be subjected to the normal agronomic practises associated with growing vegetable brassicas including control of insects, weeds and diseases by chemical application as required. The field test will be monitored carefully every 3-4 days to ensure that no caterpillars are surviving on transgenic plants and that no open flower buds are visible on the plants. Plants will be removed when they initiate bolting and therefore well before flowers open to ensure no dispersal of genetically modified pollen. The field test site will be monitored monthly for volunteer plants for one year after the completion of the trial. The personnel associated with this field test are

experienced with conducting field tests of genetically modified plants and are trained in the procedures associated with such field tests.

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Checklist

Please check and complete the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes
Confidential information identified and enclosed separately	Yes
Copies of additional references attached	Yes
Cheque for initial fee enclosed (incl. GST)†	No
If "yes", state amount:	$…….
Fee direct credited to ERMA bank account:	Yes
If 'yes" give date of DC 26…/10…/06… and amount:	$39,375 …….
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

*NA – not applicable

† The cost of processing the application will be charged to you in accordance with our pricing policy. A fees and charges schedule, including the initial fee required with the application can be found on our web site under new organism applications.

Signed: **Date:**

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