This is a hypothetical case study and is provided for illustrative purposes. It should not be cited as evidence in an application. Although the data and discussions are representative, this example may not include all considerations needed when assessing risks from a proposed GM plant field trial.

Example answers

Part 4: Suitability of the Applicant

4.1 What measures are proposed to ensure ongoing access and control of areas where dealings with GM plant(s) would occur?

We will use locations belonging to Farm Corporation YYY in Bourke, Central Darling, Coonamble and Gunnedah (NSW). We have contracted this corporation previously both for non-GM cotton trials and GM cotton trials authorised under DIR xxx, xxy and xxz. We will establish signed contracts that allow us access to and control of the land on which trials are intended to be conducted. The contracts will provide for ongoing use of land until licence obligations are satisfied. In our experience, communication with YYY was always satisfactory and we never had any problems maintaining contracts until site sign off by the Regulator.

We also propose other sites for which land owners and contractors have not yet been identified. However, contracts would provide for ongoing use of land, and we have processes in place that alert any land owners and contractors working with us of the requirements of the licence (see Part 4.8 – informing persons covered by the licence of their obligations).

4.2 Informing persons covered by the licence of their obligations

a. Should the Regulator decide to issue a licence, how are you proposing to inform persons covered by the licence of the conditions that apply to them?

For informing people covered by the licence of the relevant licence conditions, we will put a training package together with each relevant licence condition and a plain English explanation of the condition. We will allocate time for a training session and encourage questions to clarify any uncertainties about the meaning of the conditions.

We would prepare a statement for each person covered by the licence that specifies what conditions would apply to them and that they have understood what each condition is about. We would ask each person to sign it after they have been trained appropriately in how to comply with the relevant licence conditions and before they start undertaking any authorised dealings for us.

Should a licence be varied or surrendered, we will ensure to inform and train persons covered by the licence of any changes relevant to them, and get updated signed statements from each person in relation to variations.

b. Should the Regulator decide to issue a licence, how are you proposing to demonstrate that you have informed all persons covered by the licence as required?

A record of the signed statements above (4.8a) would be kept for all persons dealing with GMOs under the licence. Signed statements would be provided to the Regulator upon request.

Part 7: Summary Information

Provide a brief summary of the proposed dealings with the GM plants intended for release.

We are proposing to release GM cotton lines which are herbicide tolerant (HT), insect resistant (IR) or both (HT IR). The IR GM cottons contain a gene derived from a common soil bacterium. Expression of this gene produces an insecticidal substance and confers resistance to the major caterpillar pests of cotton in Australia. The HT GM cottons contain a gene from a common soil bacterium conferring tolerance to the herbicide glyphosate. The HT IR GM cottons contain both these genes.

The main aim of this release is to conduct field trials to measure the agronomic performance of the GM cottons in all current cotton growing areas of Australia south of 22°South under limited and controlled conditions.

We propose to release the GM plants on up to 10 field sites per year on an area of up to 1 ha per site per year, for a total of up to 10 ha per year. The field trials would be run over 5 growing seasons, from 2014 to 2019.

We are proposing a number of control measures to restrict the spread and persistence of the GMOs and their introduced genetic material. These include the use of a pollen trap, post-harvest monitoring of the trial site and destruction of any cotton volunteers, destruction of any seed not required for analysis or future planting, cleaning of equipment prior to use for other purposes, and not allowing plant material from the GM cottons be used in human food or animal feed.

None of the GM cottons have been released into the environment in Australia, but some were approved for field trials in the United States. There have been no reports of harm to human health and safety or the environment resulting from glasshouse or field trials.

Due to their production of insecticidal substances, the IR and HT IR GM cottons are also subject to regulation by the Australian Pesticide and Veterinary Medicines Authority (APVMA). The APVMA is currently assessing a permit application from us. We are also seeking approval from APVMA for glyphosate use on the HT and HT IR GM cottons.

Part 8: Parent Plant(s)

8.1 What is the common name of the parent plant(s)?

Cotton

8.2 What is the scientific name of the parent plant(s)? If the GM plant(s) is the result of crossing between more than one species, please specify both parents.

Gossypium hirsutum L.

Part 9: Description of the GM Plant(s) and Details of the Genetic Modification

9.1 What GM plants are proposed for release?

Three categories of GM cottons are proposed for release:

Category 1. Lepidopteran-resistant (IR) GM cotton – cotton variety Coker 312 was/will be transformed with plasmid pMock808 (see below) – up to 20 lines would be released.

Category 2. Glyphosate-tolerant (HT) GM cotton – cotton variety Coker 312 was transformed with plasmid pMock100 (see below) – up to 50 lines would be released.

Category 3. HT IR GM cotton – up to 100 lines were or will be generated through crossing between Category 1 and 2 GM cottons.

The marker genes *aad* and *nptII* will be present in all GM cottons.

9.2 What genetic material was/will be introduced, deleted or modified compared to the parent plant(s)?

Table: Identity, function and origins of the introduced genetic material

Plasmid pMock100 (used for HT and HT IR GM cottons)

Genetic element	Function in the GM plant	Source organism	Gene accession number	Reference
e-35S	Promoter with duplicated enhancer region.	Cauliflower mosaic virus		(Kay et al., 1987;Odell et

Table: Identity, function and origins of the introduced genetic material

Plasmid pMock100 (used for HT and HT IR GM cottons)

Genetic element	Function in the GM plant	Source organism	Gene accession number	Reference
				al., 1985)
cp4 epsps	Herbicide tolerance gene and selectable marker.	*Agrobacterium sp.* strain CP4	AF464188	(Barry et al., 1992)
nos 3'	3' non-translated region of the nopaline synthase gene; terminator and polyadenylation signal.	*Agrobacterium tumefaciens*		(Bevan et al., 1983;Depicker et al., 1982).
aad	Antibiotic resistance marker gene. This particular genetic element contains its own regulatory sequences, ie promoter and termination sequences.	*Escherichia coli*	X04555	(Fling et al., 1985)
e-35S	Promoter (see above).	Cauliflower mosaic virus		As above.
nptII	Antibiotic resistance marker gene.	*Escherichia coli*	M61152	(Beck et al., 1982)
nos 3'	Terminator and polyadenylation signal (see above).	*Agrobacterium tumefaciens*		As above.

Table: Identity, function and origins of the introduced genetic material

Plasmid pMock808 (used for IR and HT IR GM cottons)

Genetic element	Function in the GM plant	Source organism	Gene accession number	Reference
e-35S	Promoter (see above).	Cauliflower mosaic virus		As above.
cry1X1	Insect resistance gene.	*Bacillus thuringiensis*	MOCK123	(Adams 2001)
nos 3'	Terminator and polyadenylation signal (see above).	*Agrobacterium tumefaciens*		As above.
aad	Antibiotic resistance marker gene (see above).	*Escherichia coli*	As above.	As above.
e-35S	Promoter (see above).	Cauliflower mosaic virus		As above.
nptII	Antibiotic resistance marker gene (see above).	*Escherichia coli*	As above.	As above.
nos 3'	Terminator and polyadenylation signal (see above).	*Agrobacterium tumefaciens*		As above.

9.3 Are any of the source organisms for the introduced genetic material:

a. present in the Australian environment?

Cauliflower mosaic virus and *Agrobacterium tumefaciens* are both present in Australia and overseas.

Escherichia coli is a common gut bacterium which is widespread in human and animal digestive systems world-wide (Beloin et al., 2008;as indicated in Murinda et al., 2004;Sartor, 2008).

Bacillus thuringiensis is a common soil bacterium world-wide (reviewed in the RARMP for DIR 091).

b. known to be allergenic to people, or toxic or pathogenic to people or other organisms?

Cauliflower mosaic virus and *Agrobacterium tumefaciens* are well known plant pathogens, the former has a host range mostly confined to cruciferous plants (reviewed in Schoelz et al., 1986), while the latter is a common soil bacterium with a large host range of plant species (reviewed in Escobar and Dandekar, 2003).

Escherichia coli is a facultative pathogen that may cause urinary tract infections (reviewed by Marrs et al., 2005).

Bacillus thuringiensis produces toxins specific to certain insects (recently reviewed in the RARMP for DIR 091). It has been and still is in use as a biopesticide in (organic) agriculture. See the APVMA website.

9.4 What methods were used to genetically modify the parent species?

IR GM cottons were/will be produced via *Agrobacterium*-mediated transformation. Antibiotic and other bacteriostatic agents were/will be used to minimise or eliminate *Agrobacterium* during in vitro selection of the transformed cotton plants. The GM plants have been propagated by seed and *Agrobacterium* is not normally transmitted from one generation to the next via seed. This will be tested for each plant by PCR using primers specific to regions outside of the T-DNA.

HT GM cottons were produced using biolistics with plasmid pMock100.

HT IR GM cottons were/will be generated through crossing of the IR and HT GM cottons.

9.5 What traits of the parent species were intentionally altered by the genetic modification?

The cry1X1 gene (in IR and HT IR GM cottons)

All GM cottons containing the *cry1X1* gene are expected to show resistance to the affected lepidopteran insects. Cry (crystalline) proteins (also called Bt proteins or Bt toxins), including Cry1X1, belong to a diverse family of insecticidal proteins, each with specific toxicity to certain insect groups. Cry proteins are produced by various subspecies of *B. thuringiensis*. The *cry1X1* gene encodes a Bt toxin which is highly specific to a subset of lepidopteran insects (moths and butterflies), including *H. armigera* and *H. punctigera*, which are major pests of cultivated cotton in Australia (Dankocsik et al., 1990;Macintosh et al., 1990;Widner and Whiteley, 1990).

Cry proteins diffuse through the midgut membrane of feeding lepidopteran insects and bind to specific receptors on the midgut epithelium surface (Hofmann et al., 1988;Karim et al., 2000;Van Rie et al., 1989;Van Rie et al., 1990). Non-target insects, mammals, birds and fish do not possess these receptors and therefore are not susceptible to the toxic effects of these insecticidal proteins.

The toxic effect of Cry proteins requires alkaline conditions (as provided in the larval insect gut) to dissolve the crystals, partial digestion by specific proteases to release the active core toxin, and binding to specific receptors found on the insect midgut epithelium surface. Binding leads to formation of pores in the cell membrane which leads to leakage of intracellular contents into the gut lumen and water into the cell, resulting in cell death, gut paralysis and starvation. It is these steps that provide the high degree of target specificity of each Cry protein (English and Slatin, 1992;Hofmann et al., 1988;Knowles and Dow, 1993;Van Rie et al., 1989).

The *cp4 epsps* gene (in HT and HT IR GM cottons)

The *cp4 epsps* gene confers tolerance to glyphosate (N-phosphonomethyl glycine), the active ingredient of a number of herbicides. It encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996).

In plants, the native *epsps* gene encodes an enzyme (EPSPS) critical for the biosynthesis of aromatic amino acids (tryptophan, tyrosine and phenylalanine), which are essential building blocks for cellular proteins. The EPSPS enzyme catalyses the addition of the enolpyruvyl moiety of

phosphoenolpyruvate to shikimate-3-phosphate. EPSPS performs this function in plants, bacteria, algae and fungi but is absent from mammals, which are not able to synthesise these aromatic amino acids (Bentley, 1990;Padgette et al., 1993).

Glyphosate herbicide inhibits the activity of the naturally occurring EPSPS enzyme in plants, thus blocking the biosynthesis of aromatic amino acids and eventually leading to cell death (Steinrucken and Amrhein, 1980). The *cp4 epsps* gene from *Agrobacterium* is naturally insensitive to the effects of glyphosate (Padgette et al., 1993), as are a number of other microbial EPSPS enzymes (Eschenburg et al., 2002;Schulz et al., 1985). Consequently, in GM plant cells expressing the *Agrobacterium cp4 epsps* gene, biosynthesis of aromatic amino acids is not inhibited in the presence of glyphosate. The resulting plants are expected to be glyphosate tolerant.

The antibiotic selectable marker genes (*nptII* and *aad*) (in all GM cottons proposed for release)

The *nptII* gene was isolated from the *E. coli* Tn5 transposon (Beck et al., 1982). It encodes the enzyme neomycin phosphotransferase type II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. NPTII uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII-producing cell. The *nptII* gene functioned as a selectable marker, which allowed modified cotton plant cells to grow in the presence of the kanamycin or neomycin, and therefore be selected, while inhibiting the growth of non-modified cells.

The *aad* gene was isolated from the *E. coli* Tn7 transposon and encodes the enzyme aminoglycoside adenyltransferase (AAD), which confers resistance to the antibiotics streptomycin and spectinomycin. The *aad* gene is not expressed in the GM cottons because the bacterial regulatory sequence that controls its expression is not active in plants. This gene was used in the laboratory prior to the genetic modification of cotton plant cells to select for bacteria containing the modified DNA.

9.6 What unintended changes due to the genetic modification may be predicted?

Unintended changes in phenotype are not predicted because none of the introduced genetic elements are known to affect other metabolic pathways within the cotton plant. Observation of GM plants grown in the glasshouse does not indicate any unexpected phenotype.

Part 10: Proposed Dealings with the GM Plant(s), including Limits and Controls

10.1 Details of proposed dealings (activities) with the GM plant(s)

Are you proposing to:

a. conduct experiments with the GMO(s)?

Aim: To assess agronomic characteristics of the GM cotton grown under field conditions.

Field experiments: Assessment of agronomic characteristics would include measurement of height; number of flowers, buds, bolls, seeds per boll, number of *Helicoverpa* caterpillars; yield; etc.

Experimentation in a laboratory: Some plant material would be collected from field sites and packaged and transported to our PC2 laboratory in Anytown, NSW under NLRD 1234567 for further assessment such as oil composition. Alternatively, experimentation on lint to assess fibre characteristics would be done under this DIR in Anothertown, NSW. The facility we are proposing to use in Anothertown is the Research Laboratory on the Cotton Research Area. The facility would be locked when not in use and only authorised and trained staff would be permitted entry. We may be able to use other facilities later on and would provide relevant details of these to you as soon as possible, including any information relevant to risk management.

c. breed the GMO(s)?

IR GM cottons will be crossed with HT GM cottons to breed IR HT GM cottons.

Controlled crossing between the GM cotton lines and elite non-GM cotton lines may be done at some of the field trial sites. Glycine bags will be used to cover the flowers on some plants prior to pollen shed and to collect pollen from other flowers to facilitate controlled crossing.

d. propagate the GMO(s)?

The GM cotton will be allowed to set seed at the field trial sites. Harvested seed may be used to plant further trials.

f. grow, raise or culture the GMO(s)?

The GM cotton would be grown at field trial sites, either as an irrigated or dryland crop. Seed would be planted in rows with 1 m spacing, although as a dryland crop it may be planted in skip-rows (every third row would be skipped). Small areas would be hand-planted or planted with a small plot cone-seeder; larger areas would be planted with commercial equipment.

Channel or drip irrigation would be used where necessary.

The crops would be maintained in a similar fashion to commercial cotton crops; some GM plants would be treated differently with respect to weed management within the crop (as some are glyphosate tolerant) and pesticide application (as some are not expected to need as many pesticide applications as non-GM cotton). All application of chemicals would occur in accordance with APVMA requirements.

Harvesting of cotton bolls would occur either by hand (for small plantings) or with commercial equipment.

After leaving the location fallow during the off-season, it may be re-planted to the GM cottons in the following growing season.

g. import the GMO(s)?

Seed for planting would be shipped to Sydney from the US. We have obtained an import permit (Department of Agriculture Biosecurity Division permit number 12345).

10.2 Proposed limits for the DIR

Are you proposing to limit:

a. the scope of the dealings with the GMO(s)?

The range of dealings proposed to be conducted is limited as indicated in the answer to question 10.1. Additionally, the GM plants and products will not be used for animal feed or human food.

b. the scale of the dealings with the GMO(s)?

We propose to release the GM plants on up to 10 field sites per year on an area of up to 1 ha per site per year, for a total of up to 10 ha per year.

c. the locations of the dealings with the GMO(s)?

We are proposing to select only sites that would be in NSW and QLD areas **south of latitude 22° South** where cotton is currently grown as a crop. The LGAs where the release may occur are: Balonne, Banana, Central Highlands, Dalby, Goondiwindi, Isaac, Lockyer Valley, Paroo, Roma, South Burnett, Somerset and Toowoomba in Queensland; and the New South Wales LGAs of Balranald, Bogan, Bourke, Carrathool, Central Darling, Coonamble, Gunnedah, Gwydir, Hay, Inverell, Liverpool Plains, Lachlan, Moree Plains, Narrabri, Narromine, Warrumbungle, Walgett and Warren.

We are proposing to select sites that are not / have not been sites under any other field trial licence. However, GM cotton grown under commercial licences has been and is being grown in these areas.

d. the duration of the dealings with the GMO(s)

All proposed dealings, including breeding, propagating and growing of the GM cottons and experimentation in the field on the GM cottons, are proposed to occur in the cotton growing seasons between September 2014 and May 2019.

After May 2019 we are proposing to continue with transport, storage, experimentation and disposal of the GMOs, eg during post-harvest monitoring of field sites and other relevant areas.

Experimentation on plant material in the laboratory and storage would occur under NLRD 1234567.

e. the persons who are to be permitted to conduct the dealings with the GMO(s)

The project supervisor was identified in Part 2 of this application. Other persons conducting the dealings will include authorised employees or contractors trained and/or experienced in conducting the proposed dealings with GM cotton.

10.3 Proposed controls for the DIR

a. Are you proposing controls to restrict gene flow via pollen dispersal from sexually reproducing GM plants while the GM plants are growing?

The GM cotton would be grown at field trial sites in current cotton growing areas. The GM cotton and non-GM cotton lines used for comparative purposes or breeding would be planted and grown in the location.

Each location would be surrounded by a pollen trap consisting of either non-GM cotton lines or GM cotton lines already approved for commercial release. The plants within the pollen trap would be maintained to flower at the same time as the GMOs. Each pollen trap would have an access road of up to 3 m in width to allow movement of vehicles and equipment to and from the location.

The purpose of the pollen trap is to minimise the dispersal of pollen from the GM cotton to GM or non-GM cotton growing in the surrounding environment. Outcrossing is rare beyond 20 m (Llewellyn et al., 2007), and thus the pollen trap will restrict gene transfer to sexually compatible plants such as commercial cotton crops. In accordance with those findings, for irrigated cotton we propose a 20 m pollen trap where every row would be planted to form a continuous barrier of plants (with the exception of the access path mentioned above).

For dryland cotton, we propose a larger pollen trap: on either side, we would plant a 30 m pollen trap, skipping every third row (as mentioned in Part 10.1.vi). On the head and tail end of each location, we would plant a 32 m pollen trap, whereby the outermost 2 m would be planted every single row and the remainder in a staggered skip row configuration. The latter would create a continuous barrier of plants while avoiding corridors that would facilitate pollinator movement into the trial site (with the exception of the access path mentioned above). There are currently no data available regarding the efficiency of this particular pollen trap configuration.

Some species of native cottons may be in the geographic region of the proposed trials. However, hybridisation between *G. hirsutum* and native cottons is not known to occur naturally. For further details, please see the OGTR document: The Biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton) in Australia (2008).

b. Are you proposing controls to restrict the spread of seeds or asexual propagules from the site while the GM plants are growing?

Site selection: Currently, we have no field sites selected. When selecting field sites, we will select sites so that each location would be a minimum of 100 m from a natural waterway, but may be in closer proximity to irrigation channels or holding ponds. These channels and holding ponds would not flow into natural waterways. In addition, we would only select sites that are not prone to flooding. We would only select well-managed sites, eg where there are no or only few weeds.

All equipment, including harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), tools, shoes and other clothing would be inspected for GM seeds and cleaned before using it for any other purpose.

All non-GM cotton or commercially authorised GM cotton planted at the location and/or in the pollen trap would be treated as if it were the GM cotton proposed for release in this application.

c. Are you proposing to control access to the GM plants or site(s) by people or animals?

The field trials would occur on private land in rural areas where it is not expected that persons other than those conducting dealings on our behalf would enter the field sites. Specific measures such as fences with lockable gates are not proposed.

In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants, and this is likely because they find the seed unpalatable due to its high gossypol content. They are therefore unlikely to carry bolls any great distance from the cotton fields. Similarly, there is no evidence of avian species transporting cotton seed (see OGTR 2008). Thus, fencing to restrict access by large animals, netting to restrict access by birds and baiting/trapping to reduce rodent numbers is not proposed.

d. Are you proposing controls to restrict persistence (and spread) of the GM plant(s) at the site post-harvest?

Non-GM cotton and commercially authorised GM cotton present at the release sites would be used for comparative analysis or destroyed after harvest.

Post-harvest each location would be cultivated when conditions are conducive to germination of volunteers, ie as soon as the weather warms up sufficiently. Cultivation would be shallow to avoid burial of seed and the area would be irrigated if the moisture in the soil is not sufficient for germination.

Post-harvest monitoring would also be conducted. The location, pollen trap, and any areas used to clean equipment or to bury seed would be monitored for cotton volunteers at least every two months for at least 12 months and until the last 6 months are free of volunteers. Any volunteers found would be destroyed before flowering. Inspections would be carried out by the field manager who is trained and experienced at recognising cotton volunteers. The proposed post-harvest measures should restrict the persistence of the GM cottons at the release site.

A location may be re-planted to the GM cottons in the following growing season. In these circumstances, post-harvest monitoring would be conducted at least every two months between harvest at a location and subsequent replanting of that location and would then begin again after the final harvest of the location. During post-harvest monitoring, any cotton plant found would be destroyed before flowering.

Any seed harvested from the field trials, which is not kept for evaluation or seed increase, would be destroyed.

e. Are you proposing controls to restrict dispersal of the GMOs during transport?

All transport of GM plant material would be via the shortest practicable route.

All transport would be in accordance with the Regulator's *Guidelines for the Storage, Transport and Disposal of GMOs* as applicable to transport of GM plants between PC2 facilities: the plant material would be double contained, eg the plant material would be sealed in a bag which would be locked into a sturdy container. The outside of the container would be clearly labelled with the name of the GMO and appropriate contact details.

After importing GM plant seed via airplane into Sydney, the material would be transported either to our PC2 facility in Anytown or to a field site. Transport would also be between the PC2 laboratory and field sites and may be between different field sites. For this, transport would be by car, the driver

being either the project supervisor or his staff. Anyone handling or driving the GM plant material would be trained in the relevant licence conditions and sign a statement to this effect.

It may be necessary to ship seed via courier. In these cases, each package would contain no more than 5 kg seed and the inner container (eg a plastic bag) would also be labelled with the name of the GM plant and appropriate contact details. The outer container would – in addition to the details above – display instructions on how to handle the package and what to do in case the contents are spilt. We will obtain signed statements after training individuals handling the GM plant seed in this case.

f. Are you proposing controls to restrict dispersal of the GMOs during storage?

Storage of harvested seed or plant material would occur at our PC2 facility at Anytown, NSW, under NLRD 1234567. Storage would occur in PC2 glasshouse facilities or in other facilities (subject to approval by the Regulator) and according to the Regulator's *Guidelines for the Storage, Transport and Disposal of GMOs* as applicable to GM plants, ie the material would be double contained and labelled. Storage would only occur in a lockable facility. The facility would remain locked unless accessed by appropriately trained staff or contractors. After removal of seeds and other plant material from the storage area, the area would be cleaned. As all storage areas would have concrete floors, monitoring of storage areas would not be necessary.

g. Are you proposing controls to restrict dispersal of the GMOs during disposal?

Plant material and seed would be subject to destructive analysis or destroyed, although some seed may be stored for future planting. GM plants, other cotton within each field trial location and pollen trap plants would be disposed of either by one or a combination of the following methods:

destructive analysis (eg plant material would be ground up and protein/oil extracted)

non-glyphosate herbicide application

root cutting and mulching

hand weeding

autoclaving or

burial of seed or other plant material under 1m of soil. Burial of seed would take place in an area immediately adjacent to the trial site. Seed in bolls or ginned seed may be buried.

Areas used to bury seed and areas used to clean equipment would be monitored bimonthly for at least 12 months and until the area was free of volunteers for 6 months.

10.4 Are you required to obtain approval for the use of the GM plant(s), or products from the GM plant(s), from other Australian regulatory schemes during the course of the proposed limited and controlled release?

b. Is use as an agricultural chemical intended?

Category 1 (IR) and 3 (HT IR) GM cottons produce an insecticidal substance (Bt toxin) which is expected to control certain insect pests.

c. Would agricultural chemicals be used on the GM plant(s)?

Category 2 (HT) and 3 (IR HT) GM cottons are modified for tolerance to the herbicide glyphosate and we intend to spray with glyphosate during the trial.

Part 11: Assessments and Approvals by Regulatory Authorities

11.1 Provide details of previous approvals for release into the Australian environment of the GM plant(s).

The GM cottons proposed for release have not been released into the Australian environment.

GM cotton containing the same gene for herbicide tolerance and/or a similar *cry* gene for insect resistance have been approved for field trials and commercial release, eg for unrestricted commercial release throughout Australia under DIR 066/2006. We are not aware that the company holding licence DIR 066/2006 has reported any adverse effects from the release, nor are we aware of any adverse or unintended consequences from the release of other similar GM cotton lines.

11.2 Provide details of any previous, future and/or current assessments of the GM plant(s), or products derived from it, by any other regulatory authority in Australia.

APVMA – permit applications have been made: no ABC11111 and ABC11112 for cultivation of GM cotton producing an insecticidal substance and for the application of glyphosate during the proposed trial.

Department of Agriculture Biosecurity Division – permit no 12345 has been obtained to import the GM cotton lines into Australia.

As yet no activity has been undertaken under these permits, as this activity depends on a decision on this GM licence application.

11.3 Provide details on approvals for human food and/or animal feed use or environmental release of the same GM plant(s) in other countries.

Some GM cotton lines proposed for release were approved for field trials in the USA by the USDA in 2009 under permit 09-999-03x. There were no reports of adverse consequences as a result of this release. There have been no approvals for use of the GM cottons for food in other countries.

Part 12: Spread and Persistence of the GM Plant(s) in the Environment

12.1 Compared to the parent species, is the genetic modification expected to alter persistence of the GM plant at the release site?

The introduced genetic material is not known to affect seed numbers or play a role in seed dormancy.

Plant characteristics relating to persistence of the GM cottons in category 1 (IR) at the release sites are not expected to be altered.

GM plants in categories 2 (HT) and 3 (HT IR) are tolerant to the herbicide glyphosate and may survive better in the agricultural environment than their non-GM counterpart in cases where a glyphosate-based herbicide is used to control volunteer cotton. However, control measures including post-harvest monitoring are proposed and this is specifically aimed at controlling GM volunteers by destroying them prior to flowering. Therefore, it is unlikely that any GM plants or GM hybrids would persist at the field sites.

12.2 Provide details on the likelihood of spread and persistence of the GM plant outside of the proposed release site.

a. Is the GM plant more likely to be spread outside the release site than the parent species?

Cotton seed is known to be dispersed deliberately by humans for cultivation and accidentally by humans via transport on vehicles, or possibly on clothing. Cotton seed may also be spread by domestic animals (ie on feet), in stock feed or by wind. The introduced genes are not known to confer any phenotypic changes that would affect any of the mechanisms by which cotton is normally spread. Spread of many GM cotton seeds by these mechanisms is unlikely to occur because of the proposed limits and controls (see Part 10).

b. Compared to the parent species, is the GM plant's ability to persist amongst existing plants expected to increase?

The introduced genes specifically confer herbicide tolerance and insect resistance, and are not known to confer any other phenotypic changes that would affect the ability of the GM cottons to persist amongst existing plants.

In intensive land use areas such as roadsides where application of the herbicide glyphosate is the only approach used to control vegetation, the HT GM cottons in categories 2 (HT) and 3 (HT IR) may have an enhanced ability to establish, survive and reproduce.

GM cottons in categories 1 (IR) and 3 (HT IR) are resistant to certain insect pests of cotton. However, these insect pests do not limit the persistence of cotton in the Australian environment, so this trait is not expected to increase the persistence of the GM cottons among existing plants.

c. Will environmental factors which naturally limit the spread and persistence of the parent species also limit the spread and persistence of the GM plant?

In the proposed release area, south of latitude 22°South, cotton is known to be limited by water availability and frosts. This is not expected to be changed as a result of the genetic modification. The introduced genes specifically confer herbicide tolerance and insect resistance, and are not known to confer any other phenotypic changes that would mitigate the environmental factors which normally limit the spread and persistence of cotton in the areas proposed for release. Experience with very similar commercially approved GM cottons (see Part 11) demonstrates that they are susceptible to water availability and frosts.

12.3 If the GM plant(s) are able to reproduce sexually, which sexually compatible plants may be present in the receiving environment?

Non-GM *G. hirsutum* and *G. barbadense,* and GM cotton already approved for commercial release may be present at or near the proposed release sites. For example, non-GM cotton would be planted in the locations to allow for comparison of agricultural characteristics with the GM cottons and commercial cotton crops are expected to be grown near the proposed release sites.

12.4 Are any characteristics expected to be altered in the GM plant(s) compared to the parent plant that affect the efficiency of gene transfer and introgression into any sexually compatible species?

The introduced genes specifically confer herbicide tolerance and insect resistance, and are not known to confer any changes that would affect either the mechanism of pollen transfer or the efficiency of gene transfer and introgression into the sexually compatible species.

While cotton is primarily self-pollinating, insect pollinators (especially honey bees) are responsible for a low level of cross-pollination. Typically, Bt toxin-producing GM cotton crops are sprayed with pesticides much less frequently than non-GM cotton crops. As insecticide sprays generally reduce numbers of all insect species, Bt cotton crops may have greater in-field pollinator abundance, potentially increasing the rate of cross-pollination relative to that in non-GM cotton crops. The majority of the Australian cotton crop is GM (comprising about 95% of the commercial crop in the 2008/09 growing season, see Chapter 2, Section 2.3 in the RARMP for DIR 091), most of which is modified to produce Bt toxins targeting lepidopteran pests. Therefore, even if pollinator abundance and outcrossing is higher in Bt cotton crops than non-Bt cotton crops, the efficiency of gene transfer from the IR and HT IR GM cottons (categories 1 and 3) proposed for release is expected to be the same as for current commercial GM Bt cotton varieties.

12.5 If the introduced genetic modification were transferred to a different sexually compatible species (not the same species as the GMO), would the presence of the genetic modification enhance the ability of the resultant GMO to spread and persist compared to the non-GM sexually compatible species?

The introduced genes specifically confer herbicide tolerance and insect resistance. Crosses between the GM cotton and non-GM cottons (either *G. hirsutum* or *G. barbadense*) may have enhanced persistence in areas where glyphosate is the only approach used to control weeds. However, the vast

majority of commercial cotton grown in Australia is GM and already contains the same trait for herbicide tolerance and/or Bt genes which target lepidopteran pests of cotton. Hybrids would still be susceptible to the limiting factors of cotton (frost and water availability) as well as other herbicides and/or mechanical control.

Part 13: Potential Harms of the GM Plant(s)

13.1 Is the GM plant expected to be more harmful to people than the parent plant?

The introduced genes specifically confer herbicide tolerance, insect resistance and antibiotic resistance.

Staff handling the GM cottons in the glasshouse have not reported any adverse effects.

Very similar GM plants have been released in Australia and overseas with no known ill-effect (see the answers to Part 11).

The source organisms for the introduced genes are *E. coli*, *A. tumefaciens* and *B. thuringiensis*. They are present in Australia and not known to be toxic (eg TOXNET database) or allergenic (see FARRP database for references).

Other small introduced genetic elements are originally derived from thale cress (*Arabidopsis thaliana*), garden pea (*Pisum sativum*), figwort mosaic virus and cauliflower mosaic virus. All are present in Australia and not toxic or pathogenic in humans. Peas may cause allergies whereby the proteins vicelin and convicilin are implicated (see FARRP database for references). No entries are listed for the other source species for regulatory elements.

E. coli is widespread in human and animal digestive systems as well as in the environment. It may cause urinary tract infections and endocarditis in humans (Branger et al., 2005;Marrs et al., 2005). *A. tumefaciens* and *B. thuringiensis* are not pathogenic in humans.

A number of genetically modified food crops containing the *nptII* gene have been approved for commercial release both in Australia (DIRs 012/2002, 021/2002 and 022/2002) and overseas. No adverse effects on humans, animals or the environment have been reported from these releases (EFB, 2001;Flavell et al., 1992;US FDA, 1998).

While there have been reports in the USA claiming allergic reactions to Bt microbial products in topical insecticidal sprays, these are not due to the Cry1Ac or Cry2Aa proteins present in the Bt sprays. A survey conducted among farm workers who picked vegetables treated with Bt microbial products indicated that exposure to Bt products may lead to allergic skin sensitisation, however there was no clinical allergic disease in any of the workers. Most reactions in these workers were shown to be due to other constituents of the Bt sprays, and there was no evidence of antibodies specific to the Cry proteins of the Bt sprays (Bernstein et al., 1999). The US EPA have also determined that reports of reactions to Bt microbial products have been due to non-Cry proteins produced during fermentation or to other ingredients added to the insecticidal formulations (EPA, 2001).

13.2 Is the GM plant expected to be more harmful to organisms other than people when compared to the parent plant?

The genetic modification regarding insect resistance is aimed at killing lepidopteran pests in cultivated cotton crops. The Cry1X1 protein is known to be specifically toxic to the two major lepidopteran pests of cultivated cotton in Australia, namely *Helicoverpa armigera* and *Helicoverpa punctigera* and some other minor lepidopteran pests such as the pink bollworm (P. gossypiella) and armyworms (*Spodoptera exigua* and *Spodoptera frugiperda*) (Jones & Smith 2005; Fritz et al 1999; Wilks 2003).

The invertebrate fauna of cultivated cotton fields consists of a wide range of species including a number of beneficial species that parasitise or prey on various cotton pests, including Lepidoptera. None of the known predators which attack Lepidoptera in cotton are specialists and can consequently feed on a range of other species (Fitt and Wilson, 2002).

A considerable number of non-target insect species from a range of Orders, which occur on cultivated cotton, have been tested and shown to be insensitive to the Cry1X1 protein. These studies were performed using direct ingestion of the Cry proteins and/or indirect ingestion by being fed susceptible host/prey reared on cotton containing the Cry protein(s). Many of these studies were performed on key beneficial species such as:

the parasitic wasps, *Microplitis mediator* and *Nasonia vitripennis* (order Hymenoptera), which parasitise the caterpillars of *H. armigera* (Authors A and B 2002)

the ladybird beetle, *Hippodamia convergens* (order Coleoptera), a beneficial predatory insect which feeds on aphids and other plant insects commonly found on cotton (Author B et al 2007)

green lacewings, *Chrysoperla carnea* (order Neuroptera), a beneficial predatory insect whose larvae feed on *Helicoverpa* eggs, aphids and other soft-bodied insects commonly found on cotton (Author C 2009).

The above studies indicate that the Cry1X1 protein was toxic only to insects of the Order Lepidoptera. For experience with very similar GM plants see the answers to Part 11.

13.3 Is the phenotype of the GM plant altered such that it could harm the environment more than the parent plant?

The introduced genes specifically confer herbicide tolerance and insect resistance. The introduced genetic material (or its products) is not expected to alter the plant phenotype in any way that would cause more harm to the environment. The GM cotton plants that have been grown in the glasshouse have normal cotton plant phenotype.

Part 14: Additional Information about the Parent Plant(s)

Note that although an OGTR biology document for cotton exists, this part has been completed to provide you with examples of adequate information for these questions.

14.1 Production and use(s) of the parent species

a. Is the parent species grown in Australia?

The current best practice for cotton cropping in Australia is laid out in the Australian Cotton Production Manual published by the Cotton Catchment Communities CRC. Further information on cotton in Australia can be found on the Cotton Catchment Communities CRC website.

b. Is the parent species or products derived from it used in Australia?

Cotton is primarily a fibre crop; lint from the seed is spun into yarn which is woven into fabric.

Cotton seed oil for human consumption and cotton seed (whole or meal) for animal feed have been used in Australia for more than a hundred years. Due to the occurrence of gossypol and anti-nutrients, the use of whole cottonseed is limited even for ruminants.

Linters (shorter fibres than lint) must be removed before the seed can be used for planting or crushed for oil. The linters are produced as first-cut or second-cut linters. The first-cut linters have a longer fibre length and are used in the production of mattresses, furniture upholstery and mops. The second-cut linters have a much shorter fibre length and are a major source of cellulose for both chemical and food uses. They are used as a cellulose base in products such as high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste. In the chemical industry the second-cut linters are used with other compounds to produce cellulose derivatives such as cellulose acetate, nitrocellulose and a wide range of other compounds (Gregory et al., 1999).

14.2 Distribution of the parent species in Australia

a. Is the parent species present in conservation or natural environments?

In conservation areas, eg national parks, where weeds may be defined as any naturalised alien/non-native plant, cotton (*G. hirsutum* and *G. barbadense*) in the form of isolated populations may be considered as a weed (reviewed in Eastick, 2002). *G. hirsutum* is, eg listed under the category 'moderate to minor weed usually in small infestations' in Kakadu National Park (Cowie and Werner, 1987;Storrs, 1996). However, when grown in a glasshouse, seeds from these populations tend to produce plants with poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seed, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2005).

Tufted seeded *G. hirsutum* plants were originally used when hand delinting was required, before the advent of mechanical saw gins in the late 1700s. Tufted seeded *G. hirsutum* plants were subsequently replaced by fuzzy seeded varieties with better lint characteristics and disease resistance. It seems likely, therefore, that many naturalised *G. hirsutum* populations result from attempts in the early 1800s to establish cotton industries in northern Qld and the NT (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2005) and there is no evidence that these isolated *G. hirsutum* populations are invasive or have become problematic weeds.

c. Is the parent species present in areas used for agricultural or plantation production (either dryland or irrigated land use)?

Cotton is mainly grown as an irrigated crop in northern NSW and in QLD but can also be grown as a dryland crop.

d. Is the parent species present in intensive use areas?

Cotton grows as a volunteer along transport routes, eg along roadsides. A survey of the transport routes between Emerald (in the *G. hirsutum* growing region in central QLD) and the Atherton Tablelands QLD, conducted in 2002, indicated that *G. hirsutum* plants established in the roadside environment only infrequently, despite 12 years of use of these routes for transporting ginned seed for stockfeed (Farrell and Roberts, 2002). The study concluded that *G. hirsutum* volunteers tend to establish in highly and regularly disturbed environments and appear to have negligible ability to invade non-disturbed habitats, eg native bush. The following factors that limit survival of *G. hirsutum* volunteers in the roadside environment were identified: competition from already established vegetation, low quantity of seed escapes, high disturbance in areas requiring frequent maintenance and high rate of seed desiccation. Similarly, follow up surveys carried out in 2004 and 2005 found that transient feral *G. hirsutum* populations may occur along cotton transportation routes but weed competition and roadside slashing prevent the establishment of stable populations in areas with otherwise suitable climates (Addison et al., 2007).

Cotton volunteers occur in all Australian cotton growing areas and are relatively common where cotton seed is used as livestock feed (Eastick and Hearnden, 2006). However, there is no indication that these volunteers sponsor self-perpetuating feral populations. Typically, such volunteers are killed by roadside management practices and/or grazed by livestock, thereby limiting their potential to reproduce and become weedy (Addison et al., 2007;Eastick and Hearnden, 2006). Also, the relatively low soil moisture of uncultivated habitats probably limits the germination and growth of volunteers.

Farrell and Roberts (2002) found *G. hirsutum* volunteers at seven of nine dairy farms surveyed (Atherton Tablelands, March 2002) which regularly feed stock with cotton seed. GM *G. hirsutum* (Roundup Ready®, Roundup Ready®/INGARD® or INGARD®) was identified on four of these. Volunteers were all close to dairy infrastructure, suggesting that their ability to invade is negligible. Such volunteers generally do not complete an entire reproductive cycle to produce new seedlings, due to physical damage (eg trampling and grazing), disease and competition, and therefore do not spread into other areas of the farms or natural environment or lead to the development of self-sustaining populations.

14.3 How does the parent species reproduce?

The species reproduces by seed.

Agricultural areas: Although cotton is a perennial in frost-free environments, when used as an agricultural crop it is grown as an annual and produces seed within a year. Based on an average of 10 plants m^{-2} (Cotton Australia, 2002), approximately 29–40 seeds per boll (Eastick, 2002;Yasuor et al., 2007) and 10–12 bolls per plant (Eastick, 2002;Roche and Bange, 2006) cotton yields about 3850 seeds m^{-2} under standard crop production practices. However, seed loss during harvest is minimal, as the picking and transport for ginning of the bolls is an integral part of the isolation of cotton fibre; cotton volunteers are poor competitors and management of volunteer plants is targeted (estimated less than 1000 seeds m^{-2} for these volunteers).

In **intensive use areas**, such as along transport routes and in feedlots, the density of volunteer cotton plants would be much lower than in a cropping system. Control of volunteers in these areas through human intervention, grazing by livestock, and biotic or abiotic factors (Addison et al., 2007;Eastick and Hearnden, 2006;Farrell and Roberts, 2002) would limit seed production to numbers far below those found in a cropping system. Cotton has negligible ability to establish in non-disturbed habitats such as relatively natural environments or conservation/natural environments, and if plants were to establish in these areas, seed production would again be limited by abiotic and biotic factors (estimated less than 1000 seeds m^{-2}).

In **nature conservation areas** the number of volunteer cotton plants is expected to be very low and would suggest low seed production.

Cotton seeds can display innate dormancy of up to 5 months (Christidis, 1955). Innate dormancy prevents the seed from germinating, even under appropriate environmental conditions. Dormancy can also be induced by certain environmental condition, eg low soil temperature or low soil moisture (Taylor and Lankford, 1972). Seeds left in the field will usually not survive until the following season.

14.4 For sexually reproducing species, what are the pollen dispersal mechanisms?

Cotton is primarily self-pollinating with pollen that is large, sticky and heavy, and not easily dispersed by wind (McGregor, 1976;Moffett, 1983). The flowers are large and conspicuous and are attractive to insects (Green and Jones, 1953), thus it is an opportunistic out-crosser when insect pollinators are present (Oosterhuis and Jernstedt, 1999).

In Australia, honeybees are thought to be the most likely insects responsible for any cross-pollination in cotton (Mungomery and Glassop, 1969;Thomson, 1966). *Helicoverpa armigera* has been proposed as an insect which could transport pollen over long distances (Richards et al., 2005). However, a study on the fate of pollen on *H. armigera* showed the quality and quantity of *G. hirsutum* pollen decreased rapidly in contact with *H. armigera* proboscis and therefore this is unlikely to promote wide pollen dispersal (Richards et al., 2005).

Honeybees were implicated as the chief pollinating agent in a QLD study (Mungomery and Glassop, 1969). However, since honeybees were not seen in a similar study in the Ord River valley, WA (Thomson, 1966) it was suggested that native bees might be responsible for the cross-pollination. In cotton out-crossing experiments conducted near Narrabri in NSW, no bees were detected, and although small numbers of wasps and flies were recorded, it was suggested that hibiscus or pollen beetles (*Carpophilus* sp.) were likely to be the major cross-pollinators in these trials (Llewellyn and Fitt, 1996). However, further observations of these insects suggests that they do not move frequently between flowers, and where they have been observed their appearance has been too late in the season and the observed out-crossing rate was low (Llewellyn et al., 2007). In the USA, bumblebees (*Bombus* sp) may also contribute to cotton pollination. These are very effective pollinators as, because of their large size, they cannot enter a flower without depositing and collecting pollen (McGregor, 1976).

Honey bees visit cotton flowers primarily to collect nectar. Honeybees rarely collect cotton pollen, but pollen grains do accidentally adhere to the hairs on their bodies and this effects pollination (Moffett et al., 1975). The reason why honey bees do not collect cotton pollen has not been determined. A

current theory is that the spines affect packing (Vaissière and Vinson, 1994). The larger spines of *G. barbadense* would exacerbate the physical interference of the spines with the pollen aggregation process used by the bees in the packing of their pollen pellets. However, the inability of bees to collect cotton pollen for transport to the hives is not directly related to their ability to cross-pollinate cotton flowers as the pollen collected in pollen baskets is not available for pollination.

Cotton pollen viability decreases dramatically after 8 hours (Govila and Rao, 1969;Richards et al., 2005). Although cotton is mainly self-pollinating, out-crossing can be observed. One study recorded an out-crossing rate of 0.0035% at a distance of 20 m (Llewellyn et al., 2007), another study reported an out-crossing rate of 0.3% at 53 m (Mungomery and Glassop, 1969).

14.5 For sexually reproducing species, what sexually compatible relatives are present in Australia and what is their efficiency of hybridisation with the parent species?

The Australian flora contains 17 native *Gossypium* species that are all members of a distinct group found exclusively in Australia — *Gossypium* subgenus Sturtia. They are distant relatives of the cultivated cottons that originated in the Americas (Brubaker et al., 1999a;Brubaker et al., 1999b;Fryxell, 1979;Fryxell, 1992;Seelanan et al., 1999). The Australian *Gossypium* species are all diploid (2n = 26) and fall within the three taxonomic sections of the subgenus Sturtia, C, G or K: Section Sturtia (C-genome) contains two species including Sturt's desert rose, (*G. sturtianum*, the floral emblem of the Northern Territory (NT)); Section Hibiscoidea (G-genome) contains three species and Section Grandicalyx (K-genome) contains 12 species (Wendel and Cronn, 2003).

In contrast cultivated cotton in Australia (*G. hirsutum* and *G. barbadense*) are in the AD allotetraploid genomic group, subgenus Karpas Rafinesque (Seelanan et al., 1999) and contain one genome similar to those of the A-genome diploids, and one similar to those of the D-genome diploids (Endrizzi et al., 1985;Wendel, 1989;Wendel et al., 1989).

The likelihood of gene transfer from *G. hirsutum* or *G. barbadense* to any of the native Australian species is extremely low due to genetic incompatibility, since cultivated cotton is tetraploid (AD-genome) and the Australian *Gossypium* species are diploids (C, G or K genomes). The likelihood of fertile hybrids occurring, surviving to reproductive maturity and back-crossing to the parental native is, therefore, effectively zero. Indeed, no natural hybrids between Australian *Gossypium* spp. and cotton have been found despite extensive cotton planting over many years (Brown et al., 1997).

The crop species *G. barbadense* is sexually compatible with cotton *G. hirsutum* under natural conditions (Brubaker et al., 1999b). In Australia these hybrids tend to have characteristics intermediate to the parent species and typically have a lower capacity to produce cotton bolls. The hybrids do not form stable populations and instead tend to segregate toward either parental species over a number of generations (Warwick Stiller & Greg Constable, CSIRO, 2002, pers. comm).

14.6 What harms does the parent species cause?

a. Does the parent species have an adverse effect on the health of people and/or animals?

Cotton (*G. hirsutum* and *G. barbadense*) tissue, particularly the seeds, can be toxic to mammals, including people, if ingested in excessive quantities because of the presence of anti-nutritional and toxic factors including gossypol and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids).

Humans only consume cotton linters and highly refined cotton seed oil. Neither of these contain detectable levels of toxins, anti-nutrients, DNA or protein.

The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits its use as a protein supplement in animal feed. Ruminants are less affected by these components because they are detoxified by digestion in the rumen (Kandylis et al., 1998). However, its use as stockfeed is limited in intensive land use areas such as feed lots, to a relatively small proportion of the diet and it must be introduced gradually to avoid potential toxic effects (Blasi and Drouillard, 2002).

The density of cotton volunteers is expected to be low in all relevant land use areas, so exposure to people and animals is expected to be negligible. Thus, the potential of cotton to negatively affect the health of animals and/or people is low.

b. Does the parent species cause a reduction in the establishment or yield of desired plants?

Cotton is not considered a major weed in Australia, and is not considered to threaten agricultural productivity or native biodiversity. The density of cotton volunteers is likely to be low in all relevant land uses and hence there would be a low reduction of yield of other plants.

Cotton is a cultivated plant that may establish where land has been disturbed, most particularly in dryland and irrigated cropping areas. However, the ability of cotton to establish in the relevant land use areas is low. These areas are subject to standard weed management practices that would minimise the impact of any volunteers on the establishment of desired crop plants.

In intensive use areas, such as along roadsides, desired species may range from native flora to introduced trees, bushes and shrubs. Such areas are often managed, for either aesthetic or practical reasons (eg maintaining driver visibility) by the removal of larger trees and invasive weeds. Cotton would be treated as a weed and managed accordingly.

In nature conservation areas, the ability of cotton to establish is so rare that it is unlikely to affect the establishment of native plants.

c. Does the parent species cause a reduction in the quality of products, diversity or services obtained from a relevant land use?

In agriculture, cotton volunteers are managed as part of normal farming practices and therefore do not usually reduce the quality of crops grown in rotation with cotton. If unmanaged or managed poorly, it is possible that cotton seed harvested with the rotation crop could reduce the quality of the crop due to toxins and anti-nutrient components naturally found in cotton seed.

On roadsides, cotton is managed as part of roadside vegetation management and thus is unlikely to cause problems, such as reduced visibility for road users.

Cotton is likely to have a negligible effect on the establishment and yield of desired species in relatively natural or conservation/natural environments and thus is unlikely have any effect on the quality of tourism and nature conservation in these land uses. However, establishment of cotton in these areas could lead to a small reduction in aesthetics.

d. Does the parent species cause a restriction in the physical movement of people, animals, vehicles, machinery and/or water?

In agriculture, cotton volunteers are managed as part of normal farming practices, but if left unmanaged, could lead to a small restriction in the physical movement of people, vehicles and machinery.

On roadsides, feedlots and cattle yards, unmanaged cotton volunteers could lead to a small restriction in the physical movement of people, vehicles and machinery.

Although there are a few reports of cotton establishing in relatively natural or conservation/natural environments (see previous Parts), there are no reports of these plants restricting the physical movement of people, animal, vehicles, machinery and/or water.

e. Does the parent species provide food and/or shelter to pests, pathogens and/or diseases?

Cotton is susceptible to a number of pests and diseases and thus could provide food and/or shelter to pests, pathogens and/or diseases which affect cotton and/or other plants species.

Cotton is susceptible to a range of pathogens, such as Black Root Rot, *Verticillium* wilt, and *Fusarium* Wilt, and insect pests such as the *Heliothis* caterpillar, aphids, thrips, mirids and whitefly. Infected cotton volunteers in dryland or irrigated cropping use areas may act as a reservoir for these pathogens and pests that can infect crops in subsequent years. The magnitude of this effect is difficult to predict, but in some years may constitute a major negative effect. Although in crop rotation regimes, cotton can provide a disease break for other crops and this would constitute a major positive effect, it is unlikely that cotton volunteers would have a major positive effect because volunteer densities are expected to be low.

In intensive or nature conservation use areas the density of cotton volunteers is expected to be low and thus may have only minor or no effect.

14.7 What is the ability of the parent species to establish in competition amongst existing plants in each relevant land use?

Cotton is a domesticated crop that grows best under agricultural conditions. It prefers soils with high fertility and responds well to irrigation.

G. hirsutum volunteers tend to establish in highly and regularly disturbed environments and appear to have negligible ability to invade non-disturbed habitats, eg native bush (Farrell and Roberts, 2002). Seed losses leading to volunteers in dryland and irrigated cropping areas can occur during harvesting and in intensive use areas involved during transport (from field to gin), storage (feedlots) and processing (around the facilities where ginning is conducted).

Two studies identified competition with established vegetation as one of the factors that limit survival of *G. hirsutum* volunteers (Addison et al., 2007; Farrell and Roberts, 2002). Additionally, *G. hirsutum* seed germination was highest in disturbed habitats (Eastick and Hearnden, 2006).

14.8 What factors normally contribute to the long distance (>100 metre) spread of the parent species in the environment?

a. Is the parent species spread by flying animals?

There is no evidence of flying animals transporting cotton seeds in Australia. Glandless cotton seed, which does not contain significant levels of gossypol, is highly susceptible to insect pests and also consumed by rabbits, field mice, crickets and deer, thus suggesting that gossypol normally deters potential predators (Smith, 1995). Additionally, the cotton seeds are large, covered with thick fibres and enclosed in a tough boll that retains most of the seeds on the plant (Llewellyn and Fitt, 1996), which may physically deter consumption by flying animals.

b. Is the parent species spread by wild animals other than flying animals?

Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (eg hooks or spines). Mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll that retains most of the seeds on the plant (Llewellyn and Fitt, 1996).

There are no reports of mammals, including rodents, feeding on mature cotton bolls or carrying seed cotton any great distance from the cotton fields. Glandless cotton seed, which does not contain significant levels of gossypol, is highly susceptible to insect pests and also consumed by rabbits, field mice, crickets and deer, thus suggesting that gossypol normally deters potential predators (Smith, 1995). Dispersal in the hooves of animals is possible, but due to the smooth nature of hooves and the large size of the seed is not expected to be frequent.

c. Is the parent species spread over long distances via water?

Long distance dispersal of viable seed by water is possible as the seeds are enclosed in bolls containing fibres that can float in salt water for up to 3 weeks (Guppy 1906 as cited in Stephens, 1958). Dispersal from cotton fields may occur through flooding or irrigation run-off, however no data is available. Although cotton fields are typically levelled for irrigation purposes which is likely to limit dispersal distances should flooding occur, volunteers can be found along irrigation ditches and water storages in cotton production areas (CDS, 2012), suggesting possible distribution by water. The

impermeability of the seed coat is common in wild cottons, but is largely absent in cultivated varieties (Halloin, 1982). Hence, seed viability of cultivated cottons in water is expected to be low.

Delinted and acid-treated *G. hirsutum* seeds sink in salt water (Guppy 1906 as cited in Stephens, 1958).

d. Is the parent species spread over long distances via wind?

Cotton seeds can be wind dispersed (as reviewed in OECD, 2008). Selection of cultivated cotton varieties which retain their bolls on the plant as they mature has occurred during the domestication of cotton. However, if left too long on the plant, the bolls may fall to the ground and get wind dispersed. Similarly, harvested cotton can be dispersed by wind. The lint present in cotton bolls will easily catch in surrounding vegetation and so the seeds may not be dispersed over long distances. Should mature bolls fall from the plants in severe wind storms, the seeds may be dispersed over greater distances.

e. Is the parent species deliberately spread by people?

As a cultivated crop, viable plant material (seed) is deliberately spread in agricultural areas by people for planting, and accidentally in intensive use areas during transport for ginning, oil extraction and for use as stock feed.

Cotton is a crop species that is purposely cultivated for the production of the fibre, seeds, oil extracted from seeds and for use as animal feed. Thus, it is deliberately transported for cultivation in dryland and irrigated cropping areas and to intensive land use areas for processing and use in feed lots and dairy farms.

Deliberate dispersal of cotton seed in/into nature conservation land use areas by people is not expected.

f. Is the parent species accidentally spread by people?

For agricultural and intensive use areas

In dryland and irrigated cropping areas as well as intensive use areas, cotton seed may be accidently dispersed by people, machinery and vehicles. After picking, cotton bolls are pressed into modules or bales and transported by humans to gins where the fibres are separated from the seeds. In this process, seed could be spread along roadsides and railway lines, as well as near storage and processing facilities. Seed can remain on machinery after harvesting. A survey of the transport routes between Emerald (in the cotton growing region in central QLD) and the Atherton Tablelands (north of latitude 22ºS in QLD), conducted in 2002, indicated that seed cotton was only observed on roadsides in the cotton producing areas between Emerald and Belyando Crossing (Addison et al., 2007). This is likely to have originated during transport from farms to the gin.

For nature conservation areas

No data is available for nature conservation areas. However, human activity in these areas is relatively low and given the reports of isolated pockets of cotton plants in these areas, dispersal of cotton seed in/from these areas is considered unlikely.

g. Is the parent species spread via domestic/farm animals?

Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (eg hooks or spines). Whole cotton seed, meal and hulls are used in stockfeed. A small percentage of cotton seed consumed by stock can pass through the digestive system intact and is able to germinate (Eastick, 2002). It has been estimated that 5.2% of *G. hirsutum* cotton seed fed to cattle is excreted whole (Sullivan et al., 1993), although other studies have indicated that as much as 347 g/day/cow of whole unlinted seed can be excreted (Coppock et al., 1985). Whole seed may be defecated in a cattle yard, or in a field where animals graze after being fed, under conditions which may be suitable for germination. Of the seed that may be excreted whole, there are no reports indicating the portion capable of germinating.

Additionally, due to toxicants and anti-nutritional compounds, cotton seed composes only a small portion of animal feed. Dispersal in the hooves of animals is possible, but due to the smooth nature of hooves and the large size of the seed is not expected to be frequent. A survey of dairy farms which regularly feed stock with cotton seed found that cotton volunteers were all close to dairy infrastructure (Farrell & Roberts 2002), suggesting that spread to other areas of the farms was unlikely. Thus, seed may occasionally be spread from intensive land use areas such as feed lots or cropping areas if domestic or farm animals had access to the cotton crop.

Spread by domestic or farm animals would be highly unlikely in nature conservation areas as they are typically not found in these areas.

h. Is the parent species spread via contaminated produce?

For agricultural areas

Cotton farming in dryland and irrigated cropping areas is often characterised by rotation with other crops, such as wheat or the legumes faba bean (*Vicia faba*) or vetch (*Vicia villosa*). The amount of cotton seed left in the field prior to the planting of a rotation crop would depend upon the efficiency of the harvesting of the bolls, cleaning of machinery, and general weed management procedures. Growth of cotton volunteers within a rotation crop would depend upon the weed management procedures of the latter crop, while the spread of cotton seed with the rotation crop would depend upon the processing of the harvested plant material from the rotation crop.

As a rotation crop, cotton volunteers are targeted for management on-farm by mechanical methods involving mulching, root cutting and cultivation (using cultivators, graders, excavators or chippers), application of herbicides (if in the seedling stage) or burning (Australian Cotton Cooperative Research Centre, 2002;Charles et al., 2002;Roberts et al., 2002). Thus, in agricultural production areas, cotton is unlikely to be a contaminant in any follow on crops.

For intensive use areas

Cotton may be accidentally spread during transport and may appear along transport routes. If plant material along these transport routes were harvested for animal feed (or some other purpose) then contamination with viable cotton seed is possible. Long distance dispersal via contaminated hay and forage may also occur in or from intensive use areas. This could occur from areas purposely producing hay/forage or if roadside vegetation were cut for this purpose. However, considering cotton seed loss in these areas is likely to be low and volunteer plants establishing only rarely, spread via contaminated produce from intensive use areas is unlikely.

For nature conservation areas

Spread from nature conservation areas via produce is considered highly unlikely as there are few and small populations of cotton in these areas and where they occur produce is not transported off to other areas.

14.9 What environmental factors (abiotic and biotic) naturally limit the spread and persistence of the parent species in the environment?

Limiting abiotic and biotic factors that determine whether cotton will persist in the environment include frost, short summer seasons, soil type, fire, competition from other plants, herbivory (insects and other animals) and physical destruction such as trampling (Eastick, 2002;Farrell and Roberts, 2002). The relative impact of each of these factors is dependent on whether the cotton plants are in coastal or inland areas, as well as whether they are in northern or southern areas of Australia. For example, frost is a major limiting factor in southern areas of Australia, whereas the reliable availability of water is a limiting factor in most areas of Australia.

Using the inferential modelling software package CLIMEX, a model has been developed to predict the potential distribution of *G. hirsutum* in Australia (Rogers et al., 2007). Parameter values for temperature, moisture, cold stress, heat stress, dry stress and wet stress were estimated from the literature on cotton physiology and growth, and adopted from known values of perennial *G. hirsutum*

races native to Central America, the Caribbean and the US gulf coast. The final model was substantiated by comparing the potential cotton distribution predicted by CLIMEX for West Africa with the known distribution of naturalised cotton populations in West Africa.

The model indicates that dry stress is the major limiting factor for potential distribution of *G. hirsutum* in northern Australia. It also indicated that, in the absence of supplementary water, the coastal and sub-coastal areas of the east coast from Cape York to just south of the QLD/NSW border, but excluding the dry tropics, were the only climatically suitable areas for long-term survival of cotton populations. When overall soil fertility was considered in addition to climatic data, the area suitable for cotton was further restricted, ie even more closely limited to coastal areas. However, the majority of these most favourable areas for cotton either carry forests or are already used for some form of managed agricultural system and it is therefore not expected that cotton plants would be able to establish in these areas. Weed competition and fire were also identified to further reduce the probability of permanent cotton populations establishing in the identified areas.

14.10 What weed management practices are typically used to restrict the spread and persistence of the parent species in each relevant land use?

Agricultural or plantation production areas

Cotton volunteers are actively managed on-farm by mechanical methods involving mulching, root cutting and cultivation (using cultivators, graders, excavators or chippers), application of herbicides (if in the seedling stage) or burning (Australian Cotton Cooperative Research Centre, 2002;Charles et al., 2002;Roberts et al., 2002). A range of herbicides may be used to control cotton volunteers (at the seedling stage) that emerge after harvest. Herbicides containing carfentrazone-ethyl or paraquat and diquat as active constituents are currently registered by the APVMA for control of volunteer cotton, including Roundup Ready® *G. hirsutum* volunteers (see the APVMA Pubcris database).

Intensive use areas

Feedlots, cattle yards, paddocks, dairy farms or similar: Cotton volunteers are actively managed by the same methods as described above, ie mechanical methods involving mulching, root cutting and cultivation; application of herbicides (if at the seedling stage) or burning. On-farm management practices may vary from farm to farm.

Roadsides: roadside vegetation appears to be managed for two main reasons, the removal of noxious or invasive weeds and to remove obstructions to line of sight around corners and signs (Dignam, 2001). Thus roadside management may focus on safety and removal of specific plants, rather than protection of desired plants. Slashing or mowing and herbicide application are common methods of roadside vegetation management and these would control cotton volunteers.

Conservation or natural environments

The available literature does not indicate that cotton is managed in natural environments. In Kakadu National Park where a few isolated populations exist, cotton is not among the top weeds prioritised for specific control (See the Environment website). Similarly, cotton is not a weed of concern in NSW national parks (DEC NSW, 2006). Both references indicate that standard weed management within national parks incorporates an integrated approach consisting of a variety of techniques including: herbicides, physical removal and, where effective agents exist, biological controls.

14.11 What is the parent species' tolerance to typical weed management practices?

Agricultural or plantation production and intensively used areas

As cotton is used as a crop plant and as feed for ruminants, management is generally targeted to ensure any volunteer plants are controlled (see answer to question 14.10). Cotton volunteers in intensive use areas are not known to sponsor self-perpetuating feral populations.

Conservation or natural environments

In these environments weed management is not targeted specifically to cultivated cotton.

14.12 Provide details of any State or Commonwealth restrictions on the movement of material from the parent species within and between producing regions.

There may be restrictions on bringing cotton seeds into NSW, WA and NT. An on-farm 'come clean, go clean' strategy for on- and between farm hygiene is promoted by the cotton industry due to the presence of fungal cotton pathogens in the soil, including *Thielaviopsis basicola*, *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *vasinfectum* (Cotton Catchment communities CRC, 2002).

14.13 What are the standard practices to restrict the transfer of genetic material from the parent plant to other plants by sexual reproduction (if applicable)?

The OECD recommends a separation distance of 600 m for production of basic seed of *G. hirsutum* (OECD, 2007) and this standard has been adopted by some seed companies in Australia (Cotton Seed Distributors, 2007). In the USA, only minimal (5 m) separation is required between different varieties unless there is obvious differences in morphology, such as flower colour or leaf shape, when 536 m between varieties is required (Jenkins, 1993). Pollen-proof bags (or other physical barriers) are used to cover flowers to enable controlled self-pollination or crossing between different cotton cultivars (Lee, 1980).

Part 15: References Cited in the Application

Addison, S. J., T. Farrell, G. N. Roberts, and D. J. Rogers, 2007, Roadside surveys support predictions of negligible naturalisation potential for cotton (*Gossypium hirsutum*) in north-east Australia: Weed Research, v. 47, no. 3, p. 192-201.

Australian Cotton Cooperative Research Centre, 2002, WEEDpak - A guide for integrated management of weeds in cotton Australian Cotton Research & Development Corporation, Canberra.

Barry, G. et al. 1992, Inhibitors of amino acid biosynthesis: Strategies for imparting glyphosate tolerance to crop plants, in BK Singh, HE Flores, and JC Shannon eds., Biosynthesis and molecular regulation of amino acids in plants: Rockville, USA, American Society of Plant Physiologists, p. 139-145.

Beck, E., G. Ludwig, E. A. Auerswald, B. Reiss, and H. Schaller, 1982, Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5: Gene, v. 19, no. 3, p. 327-336.

Beloin, C., A. Roux, and J. M. Ghigo, 2008, *Escherichia coli* biofilms: Current Topics in Microbiology and Immunology, v. 322, p. 249-289.

Bentley, R., 1990, The shikimate pathway - a metabolic tree with many branches: Critical Reviews in Biochemistry and Molecular Biology, v. 25, no. 5, p. 307-384.

Bernstein, I. L., J. A. Bernstein, M. Miller, S. Tierzieva, D. I. Bernstein, Z. Lummus, M. K. Selgrade, D. L. Doerfler, and V. L. Seligy, 1999, Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides.: Environmental Health Perspectives, v. 107, no. 7, p. 575-582.

Bevan, M., W. M. Barnes, and M. D. Chilton, 1983, Structure and transcription of the nopaline synthase gene region of T-DNA: Nucleic Acids Research, v. 11, no. 2, p. 369-385.

Blasi,D, J Drouillard. Cottonseed feed products for beef cattle, composition and feeding value. 02-426-E. 2002. Kansas State University Agricultural Experiment Station and Co-operative Extension Service.

Ref Type: Report

Branger, S., J. P. Casalta, G. Habib, F. Collard, and D. Raoult, 2005, *Escherichia coli* endocarditis: seven new cases in adults and review of the literature: Eur.J.Clin.Microbiol.Infect.Dis., v. 24, no. 8, p. 537-541.

Brown, A. H. D., C. L. Brubaker, and M. J. Kilby, 1997, Assessing the risk of cotton transgene escape into wild Australian *Gossypium* species, in GD McLean, PM Waterhouse, G Evans, and MJ Gibbs eds., Commercialisation of transgenic crops: risk, benefit and trade considerations: Canberra, Bureau of Resource Sciences, p. 83-94.

Brubaker, C. L., F. M. Bourland, and J. E. Wendel, 1999a, The origin and domestication of cotton, in CW Smith and JT Cothren eds., Cotton: Origin, History, Technology, and Production: New York, John Wiley and Sons, Inc., Wiley Series in Crop Science, p. 3-31.

Brubaker, C. L., A. H. D. Brown, J. M. Stewart, M. J. Kilby, and J. P. Grace, 1999b, Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement.: Euphytica, v. 108, no. 3, p. 199-213.

CDS. Volunteer Cotton Management: 17 Aug 2012. 2012. Cotton Seed Distributors.

Ref Type: Pamphlet

Charles, G., A. Sullivan, I. Christiansen, and G. Roberts, 2002, Managing weeds on roads, channels and water storages, WEEDpak: Australian Cotton Research & Development Corporation, Canberra..

Christidis, B. G., 1955, Dormancy in cotton seed: Agronomy Journal, v. 47, p. 400-403.

Coppock, C. E., J. R. Moya, J. W. West, D. H. Nave, J. M. Labore, and C. E. Gates, 1985, Effect of lint on whole cottonseed passage and digestibility and diet choice on intake of whole cottonseed by Holstein cows: J Dairy Sci, v. 68, no. 5, p. 1198-1206.

Cotton Australia, 2002, How to grow cotton.

Cotton Catchment communities CRC. Integrated Disease Management. Allen, S. J., Nehl, D. B., and Moore, N. 1-42. 2002. Australian Cotton Cooperative Research Centre, Cotton Catchment communities CRC.

Ref Type: Report

Cotton Seed Distributors, 2007, 2007 Variety Guide,

Cowie,ID, P A Werner. Weeds in Kakadu National Park: A Survey of Alien Plants, unpub. report to Australian National Parks and Wildlife Service. 1987.

Ref Type: Report

Dankocsik, C., W. P. Donovan, and C. S. Jany, 1990, Activation of a cryptic crystal protein gene of *Bacillus thuringiensis* subspecies *kurstaki* by gene fusion and determination of the crystal protein insecticidal specificity.: Molecular Microbiology, v. 4, no. 12, p. 2087-2094.

DEC NSW. Protecting our national parks from pests and weeds. 1-44. 2006. Sydney, Department of Enviroment and Conservtion NSW.

Ref Type: Report

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski, and H. M. Goodman, 1982, Nopaline synthase: transcript mapping and DNA sequence: Journal of Molecular and Applied Genetics, v. 1, no. 6, p. 561-573.

Dignam,M. Bush, parks, road and rail weed management survey. Re: CMD.274, 1-24. 2001. Monsanto Australia Ltd.

Ref Type: Report

Eastick,R. Evaluation of the potential weediness of transgenic cotton in northern Australia, 1-177. 2002. Technical Bulletin no. 305Australia, , Technical Bulletin no. 305, Northern Territory Government, CSIRO and Australian Cotton Cooperative Research Centre.

Eastick, R., and M. Hearnden, 2006, Potential for Weediness of *Bt* Cotton (*Gossypium hirsutum*) in Northern Australia.: Weed Science, v. 54, no. 6, p. 1142-1151.

EFB. Antibiotic resistance markers in genetically modified (GM) crops. Briefing Paper no. 10, 1-4. 2001. Barcelona, Spain, European Federation of Biotechnology.

Ref Type: Report

Endrizzi, J. E., E. L. Turcotte, and R. Kohel, 1985, Genetics, cytology and evolution of *Gossypium*: Advances in Genetics, v. 23, p. 271-375.

English, L., and S. L. Slatin, 1992, Mode of action of delta-endotoxins from *Bacillus thuringiensis*: a comparison with other bacterial toxins: Insect Biochemistry and Molecular Biology, v. 22, no. 1, p. 1-7.

EPA. Biopesticides registration action document: *Bacillus thuringiensis* plant-incorporated protectants. IIA1-IIC109. 2001. US EPA.

Ref Type: Report

Eschenburg, S., M. L. Healy, M. A. Priestman, G. H. Lushington, and E. Schonbrunn, 2002, How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from *Escherichia coli*: Planta, v. 216, no. 1, p. 129-135.

Escobar, M. A., and A. M. Dandekar, 2003, *Agrobacterium tumefaciens* as an agent of disease: Trends in Plant Science, v. 8, no. 8, p. 380-386.

Farrell,T, G Roberts. Survey of cotton volunteers north of latitude 22º south. 2002. Report, Australian Cotton CRC and CSIRO Plant Industry.

Ref Type: Report

Fitt, G. P., and L. J. Wilson, 2002, Non-target effects of Bt-cotton: a case study from Australia, in RJ Akhurst, CE Beard, and PA Hughes eds., Biotechnology of *Bacillus thuringiensis* and its environmental impact: Canberra, CSIRO Entomology.

Flavell, R. B., E. Dart, R. L. Fuchs, and R. T. Fraley, 1992, Selectable marker genes: safe for plants?: Biotechnology (N.Y.), v. 10, no. 2, p. 141-144.

Fling, M. E., J. Kopf, and C. Richards, 1985, Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3(9)-O-nucleotidyltransferase: Nucleic Acids Research, v. 13, no. 19, p. 7095-7106.

Fryxell, P. A., 1979, The origin and spread of the tribe, The natural history of the cotton tribe (Malvaceae, tribe Gossypieae): College Station, USA, Texas A & M University Press, p. 131-218.

Fryxell, P. A., 1992, A revised taxonomic interpretation of *Gossypium* L. (Malvaceae): Rheedea, v. 2, no. 2, p. 108-165.

Govila, O. P., and C. H. Rao, 1969, Studies on the *in vitro* germination and storage of cotton pollen: Journal of Palynology, v. 5, p. 37-41.

Green, J. M., and M. D. Jones, 1953, Isolation of cotton for seed increase: Agronomy Journal, v. 45, p. 366-368.

Gregory, S. R., E. Hernandez, and B. R. Savoy, 1999, Cottonseed processing, in CW Smith and JT Cothren eds., Cotton: Origin, History, Technology and Production: New York, John Wiley & Sons, Wiley Series in Crop Science, p. 793-819.

Halloin, J. M., 1982, Localization and changes in catechin and tannins during development and ripening of cottonseed: New Phytologist, v. 90, p. 651-657.

Hofmann, C., H. Vanderbruggen, H. Hofte, J. Van Rie, S. Jansens, and H. Van Mellaert, 1988, Specificity of *Bacillus thuringiensis* δ-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts: Proceedings of the National Academy of Sciences of the United States of America, v. 85, no. 21, p. 7844-7848.

Jenkins, J. N., 1993, Cotton, Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology: Organisation for Economic Cooperation and Development (OECD), p. 61-70.

Kandylis, K., P. N. Nikokyris, and K. Deligiannis, 1998, Performance of growing-fattening lambs fed whole cotton seed: Journal of the Science of Food and Agriculture, v. 78, p. 281-239.

Karim, S., S. Riazuddin, F. Gould, and D. H. Dean, 2000, Determination of receptor binding properties of *Bacillus thuringiensis* delta-endotoxins to cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*) midgut brush border membrane vesicles: Pesticide Biochemistry and Physiology, v. 67, no. 3, p. 198-216.

Kay, R., A. Chan, M. Daly, and J. McPherson, 1987, Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes: Science, v. 236, p. 1299-1302.

Knowles, B. H., and J. A. T. Dow, 1993, The crystal delta-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut: Bioassays, v. 15, p. 469-476.

Lee, J. A., 1980, Cotton, in WR Fehr and HH Hadley eds., Hybridization of crop plants: Madison, Wisconsin, USA, American Society of Agronomy and Crop Science Society of America, p. 313-325.

Llewellyn, D., and G. Fitt, 1996, Pollen dispersal from two field trials of transgenic cotton in the Namoi valley, Australia: Molecular Breeding, v. 2, no. 2, p. 157-166.

Llewellyn, D. J., C. Tyson, G. A. Constable, B. Duggan, S. Beale, and P. Steel, 2007, Containment of regulated genetically modified cotton in the field: Agriculture, Ecosystems & Environment, v. 121, no. 4, p. 419-429.

Macintosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff, and R. L. Fuchs, 1990, Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects: Journal of Invertebrate Pathology, v. 56, p. 258-266.

Marrs, C. F., L. Zhang, and B. Foxman, 2005, *Escherichia coli* mediated urinary tract infections: are there distinct uropathogenic *E. coli* (UPEC) pathotypes?: FEMS Microbiology Letters, v. 252, no. 2, p. 183-190.

McGregor, S. E., 1976, Crop Plants and Exotic Plants - Cotton, Insect pollination of cultivated crop plants: Washington, D. C., USDA, Agricultural Research Service, Agricultural hand book No. 496, p. 171-190 .

Moffett, J. O., 1983, Pollination of entomophilous hybrid seed parents - hybrid cotton, in CE Jones and RJ Little eds., Handbook of experimental pollination biology: New York, Van Nostrand Reinhold, p. 508-514.

Moffett, J. O., L. S. Stith, C. C. Burkhart, and C. W. Shipman, 1975, Honey bee visits to cotton flowers: Environmental Entomology, v. 4, no. 2, p. 203-206.

Mungomery, V. E., and A. J. Glassop, 1969, Natural cross-pollination of cotton in central Queensland: Queensland Journal of Agricultural and Animal Sciences, v. 26, p. 69-74.

Murinda, S. E., L. T. Nguyen, T. L. Landers, F. A. Draughon, A. G. Mathew, J. S. Hogan, K. L. Smith, D. D. Hancock, and S. P. Oliver, 2004, Comparison of *Escherichia coli* Isolates from humans, food, and farm and companion animals for presence of Shiga toxin-producing *E. coli* virulence markers: Foodborne.Pathog.Dis., v. 1, no. 3, p. 178-184.

Odell, J. T., F. Nagy, and N. H. Chua, 1985, Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter: Nature, v. 313, no. 6005, p. 810-812.

OECD. OECD Seed Schemes April 2007. C(2000)146/FINAL incl. 2003, 2004, 2005, 2006 & 2007 amendments, 67-111. 2007. Organisation for Economic Co-operation and Development.

Ref Type: Report

OECD. Consensus Document on the Biology of Cotton. ENV/JM/MONO(2008)33. 2008. Series on Harmonisation of Regulatory Oversight in Biotechnology No. 45, Organisation for Economic Co-operation and Development, available online at.

Oosterhuis, D. M., and J. Jernstedt, 1999, Morphology and anatomy of the cotton plant, in CW Smith and JT Cothren eds., Cotton: Origin, History, Technology and Production: New York, John Wiley & Sons, Wiley Series on Crop Science, p. 175-206.

Padgette,SR, G F Barry, D B Re, D A Eichholtz, M Weldon, K Kolacz, G M Kishore. Purification, cloning and characterisation of a highly glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp.strain CP4. Unpublished. Monsanto Report No. MSL:12738, 1-66. 1993. Monsanto Company.

Ref Type: Unpublished Work

Padgette, S. R., D. B. Re, G. F. Barry, D. E. Eichholtz, X. Delannay, R. L. Fuchs, G. M. Kishore, and R. T. Fraley, 1996, New weed control opportunities: development of soybeans with a Roundup Ready gene, in SO Duke ed., Herbicide-resistant crops: agricultural, environmental, economic, regulatory and technical aspects: Boca Raton, CRC Press, p. 53-84.

Richards, J. S., J. N. Stanley, and P. C. Gregg, 2005, Viability of cotton and canola pollen on the proboscis of *Helicoverpa armigera*: implications for spread of transgenes and pollination ecology: Ecological Entomology, v. 30, no. 3, p. 327-333.

Roberts, G., S. Kerlin, and M. Hickman, 2002, Controlling volunteer cotton, WEEDpak: Australian Cotton Research & Development Corporation, Canberra..

Roche,R, M Bange. Do ultra-narrow row cotton systems offer any benefit to Australian farmers? Turner, N. C., Acuna, T., and Johnson, R. C. 2006. The Regional Institute Ltd. "Ground-breaking stuff". Proceedings of the 13th Australian Agronomy Conference, 10-14 September 2006, Perth, Western Australia. Australian Society of Agronomy. 10-9-2006.

Ref Type: Conference Proceeding

Rogers, D. J., R. E. Reid, J. J. Rogers, and S. J. Addison, 2007, Prediction of the naturalisation potential and weediness risk of transgenic cotton in Australia: Agriculture, Ecosystems & Environment, v. 119, no. 1-2, p. 177-189.

Sartor, R. B., 2008, Microbial influences in inflammatory bowel diseases: Gastroenterology, v. 134, no. 2, p. 577-594.

Schoelz, J., R. J. Shepherd, and S. Daubert, 1986, Region VI of cauliflower mosaic virus encodes a host range determinant: Mol.Cell Biol., v. 6, no. 7, p. 2632-2637.

Schulz, A., A. Kruper, and N. Amrhein, 1985, Differential sensitivity of bacterial 5-enolpyruvyl-shikimate-3-phosphate synthases to the herbicide glyphosate: FEMS Microbiology Letters, v. 28, no. 3, p. 297-301.

Seelanan, T., C. L. Brubaker, J. M. Stewart, L. A. Craven, and J. F. Wendel, 1999, Molecular systematics of Australian *Gossypium* section *Grandicalyx* (Malvaceae): Systematic Botany, v. 24, no. 2, p. 183-208.

Smith, C. W., 1995, Cotton (*Gossypium hirsutum L.*), Crop Production: Evolution, History, and Technology: New York, John Wiley and Sons, Inc., p. 287-349.

Steinrucken, H. C., and N. Amrhein, 1980, The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase: Biochemical and Biophysical Research Communications, v. 94, no. 4, p. 1207-1212.

Stephens, S. G., 1958, Salt water tolerance of seeds of *Gossypium* species as a possible factor in seed dispersal: The American Naturalist, v. 92, no. 863, p. 83-92.

Storrs,M. A Weed Management Strategy for Kakadu National Park 1996 - 2001, unpublished report to Australian Conservation Agency, Kakadu National Park. 1996.

Ref Type: Report

Sullivan, J. L., J. T. Huber, and J. M. Harper, 1993, Performance of dairy cows fed short staple, Pima, and cracked Pima cottonseed and feed characteristics: Journal of Dairy Science, v. 76, no. 11, p. 3555-3561.

Taylor, R. M., and M. K. Lankford, 1972, Secondary dormancy in cotton: Crop Science, v. 12, p. 195-196.

Thomson, N. J., 1966, Cotton variety trials in the Ord valley, North Western Australia: 4. Natural crossing of cotton: Empire Cotton Growing Review, v. 43, p. 18-21.

US FDA, 1998, Guidance for industry: use of antibiotic resistance marker genes in transgenic plants.

Vaissière, B. E., and S. B. Vinson, 1994, Pollen morphology and its effect on pollen collection by honey bees, *Apis mellifera* L. (Hymenoptera: Aphidae), with special reference to upland cotton, *Gossypium hirsutum* L. (Malvaceae): Grana, v. 33, p. 128-138.

Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. Van Mellaert, 1989, Specificity of *Bacillus thuringiensis* delta -endotoxins: importance of specific receptors on the brush border membrane of the mid-gut of target insects.: European Journal of Biochemistry, v. 186, no. 1-2, p. 239-247.

Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. V. Van Mellaert, 1990, Receptors on the brush border membrane of the insect mid-gut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins: Applied and Environmental Microbiology, v. 56, p. 1378-1385.

Wendel, J. F., 1989, New World tetraploid cottons contain Old World cytoplasm: Proceedings of the National Academy of Sciences of the United States of America, v. 86, no. 1, p. 4132-4136.

Wendel, J. F., and R. C. Cronn, 2003, Polyploidy and the evolutionary history of cotton.: Advances in Agronomy, v. 78, p. 139-186.

Wendel, J. F., P. D. Olson, and J. M. Stewart, 1989, Genetic diversity, introgression and independent domestication of Old World cultivated cottons: American Journal of Botany, v. 76, p. 795-1806.

Widner, W. R., and H. R. Whiteley, 1990, Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from *Bacillus thuringiensis*: Journal of Bacteriology, v. 172, no. 6, p. 2826-2832.

Yasuor, H., J. Riov, and B. Rubin, 2007, Glyphosate-induced male sterility in glyphosate-resistant cotton (*Gossypium hirsutum* L.) is associated with inhibition of anther dehiscence and reduced pollen viability: Crop Protection, v. 26, no. 3, p. 363-369.