



Australian Government

Department of Health and Aged Care
Office of the Gene Technology Regulator

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 commercial release.

Example answers

Part 7: Summary Information

We are proposing to release three lines of GM cotton (*Gossypium hirsutum*) which are herbicide tolerant (HT), insect resistant (IR) or both (HT IR). The lines have been assigned unique OECD identifiers MK-AB808-7, MKAB2230-7 and MK-AB808-7 x MK-AB2230-7, respectively. The IR GM cotton contains 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 cotton contains a gene from a common soil bacterium conferring tolerance to the herbicide glyphosate. The HT IR GM cotton was generated through crossing between these two lines and contains both these genes.

The aim of the release is for the commercial production of these GM cotton lines throughout Australia. There are no limits or controls proposed on the release. The cotton would be grown and utilised in the same way that commercial cotton is currently grown and utilised, including as feed for animals, cotton seed oil for humans and for fibre production (eg. denim or other cotton products).

The GM cottons have been released into the environment in Australia for field trials (DIR XXX) from 2009 to 2014 and have been approved for commercial release in the USA (2010) and Brazil (2011). There have been no reports of harm to human health and safety or the environment resulting from the field trials in Australia or the overseas commercial releases.

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 for use of herbicide XXX on the HT GM cotton lines, and Food Standards Australia (FSANZ) is assessing the use in food of cotton seed oil derived from the GM cotton lines.

Part 9: Description of the GM Plants and Details of the Genetic Modification

9.1. What GM plants are proposed for release?

Three separate lines of GM cottons are proposed for release:

Line 1. Insect-resistant (IR) GM cotton – cotton variety Coker 312 was transformed with plasmid pMock808 (see below) to provide resistance to specific lepidopteron insect pests. The OECD identifier for this line is MK-AB808-7.

Line 2. Herbicide-tolerant (HT) GM cotton – cotton variety Coker 312 was transformed with plasmid pMock100 to provide tolerance to the herbicide glyphosate. The OECD identifier for this line is MK-AB2230-7.

Line 3. HT IR GM cotton which was generated through crossing between the IR and HT GM cottons. The OECD identifier for this line is MK-AB808-7 x MK-AB2230-7.

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

9.2. What genetic modification was introduced, deleted or modified compared to the parent species?

Table X below describes the genetic components for pMock808. [Note that you must provide the details for all components for each construct used].

Table X. Identity, function, origin, accession number, reference and location of the introduced genetic material for plasmid pMock808

Genetic element	Function of the DNA region	Source organism	Gene Accession number	Reference
Right Border	DNA region containing right border sequence used for transfer of the T-DNA	<i>Agrobacterium tumefaciens</i>		Depicker et al., 1982
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>nos 3'</i>	Terminator and polyadenylation signal (see above).	<i>Agrobacterium tumefaciens</i>		Bevan et al. 1983, Depicker et al. 1982
<i>cry1X1</i>	Insect resistance gene.	<i>Bacillus thuringiensis</i>	MOCK123	(Adams 2001)
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>e-35S</i>	Promoter with duplicated enhancer region	Cauliflower mosaic virus		(Kay et al. 1987; Odell et al. 1985)
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>aad</i>	Antibiotic resistance marker gene containing its own regulatory sequences, ie promoter and termination sequences.	<i>Escherichia coli</i>	X04555	(Fling et al. 1985)
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>nos 3'</i>	Terminator and polyadenylation signal (see above).	<i>Agrobacterium tumefaciens</i>		As above.
<i>nptII</i>	Antibiotic resistance marker gene.	<i>Escherichia coli</i>	M61152	(Beck et al. 1982)
<i>e-35S</i>	Promoter (see above).	Cauliflower mosaic virus		As above.
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
Left Border	DNA region containing left border sequence used for transfer of the T-DNA	<i>Agrobacterium tumefaciens</i>		Depicker et al., 1982
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>ori-V</i>	Origin of replication for maintenance of plasmid in <i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>		Stalker et al. 1981
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		
<i>ori-322</i>	Origin of replication for maintenance of plasmid in <i>E. coli</i>	<i>Escherichia coli</i>		Sutcliffe 1979
Intervening sequence	Sequence used in DNA cloning	<i>Escherichia coli</i>		

9.3. Are any of the source organisms for the introduced genetic modification:

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 found in soil and plant communities worldwide and strains have been isolated from habitats including soil, insects, stored-product dust and deciduous and coniferous leaves (Schnepf et al. 1998).

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 & Dandekar 2003).

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

Bacillus thuringiensis (Bt) produces toxins specific to certain insects including Lepidoptera (butterflies and moths), Coleoptera (beetles and weevils), Hymenoptera (wasps and bees) and Diptera (flies and mosquitoes), or to nematodes (reviewed in Bravo et al. 2007). Bt does not have a history of causing allergenicity in humans. There have been rare reports of occupational allergies associated with the use of Bt insecticidal products containing Bt. It has been and still is in use as a [biopesticide in \(organic\) agriculture](#).

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

IR GM cottons were produced via *Agrobacterium*-mediated transformation using plasmid pMock808. The intent was to integrate the T-DNA region of the plasmid (ie between the left and right borders) in the cotton genome. The *nptII* gene provides tolerance to the antibiotic kanamycin and this trait was used to select genetically modified plants. Antibiotic and other bacteriostatic agents were 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. Presence/absence of *Agrobacterium* was tested for each plant by PCR using primers specific to regions outside of the T-DNA. The transformation method is based on the work by Karl et al. (2002).

HT GM cottons were produced using biolistics with plasmid pMock100. The plasmid was linearized by digestion with restriction enzyme Eco R1 (see plasmid in Part 9.2), purified and used to coat gold particles for bombardment of cotton cells. Further detail on the transformation methodology is available in the article by Simpson et al (1999). The intention was to integrate the plasmid DNA into the cotton genome. As above, the *nptII* gene was used as the selectable marker to identify genetically modified cotton plants.

HT IR GM cottons were generated through conventional 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 & 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 & Slatin 1992; Hofmann et al. 1988; Knowles & 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 (Padgett 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; Padgett 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 & Amrhein 1980). The *cp4 epsps* gene from *Agrobacterium* is naturally insensitive to the effects of glyphosate (Padgett 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 adenosine triphosphate (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 adenylyltransferase (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.

9.11. Unintended changes in the GM plants

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

Unintended changes to the phenotype are not predicted because none of the introduced genetic elements are known to affect other metabolic pathways within the cotton plant. Observation of the GM plants grown in glasshouse, in field trials in Australia or overseas commercial releases did not indicate any unexpected phenotype.

e. Have you tested the GM plants for any predicted potential changes identified in (d) above?

Unintended changes were not predicted (see above) thus specific testing was not conducted.

Part 10: Proposed Dealings with the GM Plants, including any Limits and Controls on the Dealings

10.1. Details of proposed dealings (activities) with the GM plants

c. breed the GMOs?

Controlled crossing between the GM cotton lines and elite non-GM cotton lines will occur to introduce the GM traits into modern advanced cotton lines. Crossing between IR and HT GM lines will occur to produce HT IR GM cotton lines. Under this application, we do not intend to intentionally cross these GM cotton lines with other GM cottons already approved in Australia, nor do we intend to cross the GM cottons with Pima cotton (*G. barbadense*).

g. import the GMOs?

Seed for planting would be shipped to Sydney from the USA as authorised by the GTR under NLRD 321. We have obtained an import permit from the Department of Agriculture and Water Resources. The permit number is 12345.

Part 11: Assessments and Approvals by Regulatory Authorities

11.1. Provide details of previous approvals for release into the Australian environment of the GM plants.

The GM cotton lines were trialled under limited and controlled conditions (field trials) in Australia from 2007 to 2011 under DIR XXX. There were no adverse effects resulting from the field trials. Seed of the three GM cotton lines will be imported under NLRD 321.

11.2. Provide details of any previous and/or current assessments of the GM plants, or products derived from them, by any other regulatory authority in Australia.

APVMA – issued permits: no ABC11111 and ABC11112 for cultivation of GM cotton producing an insecticidal substance and for the application of glyphosate during field trials of these GM cotton lines.

We have applied to APVMA for approval of the cultivation of IR and HT IR GM cotton lines on a commercial scale as they produce an insecticidal substance, and for registration of glyphosate containing herbicides for use on the HT and HT IR GM cotton lines.

Department of Agriculture Biosecurity Division – permit no 12345 has been obtained to import the GM cotton lines into Australia, and FSANZ is assessing the use in food of cotton seed oil derived from the GM cotton lines.

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

The GM cotton lines proposed for release were approved for field trials in the USA by the USDA in 2009 under permit 09-999-03x. The lines were later approved for environmental release and use in food and feed in the USA (2009) and Brazil (2011). These approvals are still current. There have been no reports of adverse consequences as a result of these releases.

Part 12: Spread and Persistence of the GM Plants in the Environment

12.1. Provide details on the likelihood of spread and persistence of the GM plants in the environment.

a. Are the GM plants more likely to be spread in the environment 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 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. There were no observed phenotypic changes which would change dispersal of the cotton seed.

b. Are the GM plants more likely to persist amongst existing plants compared to the parent species?

Studies conducted on the Australian field trials found no significant differences between the GM cotton lines proposed for release and commercially grown non-GM cotton lines for a number of agronomic traits. There was no significant difference for seed numbers, seed size, time to germination, germination frequency, days to maturity, or any of the fibre characteristics among the GM and non-GM lines tested (refer to report 1234). These results suggest the genetic modification has not altered the GM plant's ability to persist compared to the parent species. The introduced genetic material is not known to play a role in seed dormancy. The germination frequency and time to emergence data indicated above would suggest that the dormancy of the GM cotton seed is not different from other GM cottons or the non-GM parental species.

Cotton is not known to be highly competitive amongst other plants. The IR and HT IR GM cottons lines are resistant to certain insect pests of cotton. However, these insect pests are not known to limit the persistence of cotton in the Australian environment, so this trait is not expected to increase the persistence of the GM cottons in the environment. The IR trait is unlikely to increase the competitive advantage of the GM cotton amongst existing plants.

The HT and HT IR GM cotton lines 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. 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 cotton lines may have an enhanced ability to establish, survive and reproduce.

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

In Australia cotton is known to be limited by a number of environmental factors, with water availability (northern Australia) and frosts (southern Australia) being the main limiting factors. Other limiting factors include nutrient availability, temperature and soil type. The factors naturally limiting spread and persistence of cotton are 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 Australia. Experience with cotton demonstrates that

cotton is susceptible to water availability and frosts (see Part 14 / the OGTR Biology document for cotton).

12.2. If the GM plants are able to reproduce sexually, which sexually compatible plants may be present in the receiving environment?

The proposed release is for throughout Australia, thus non-GM *G. hirsutum* and *G. barbadense*, and any GM cotton (*G. hirsutum* and *G. barbadense*) already approved for commercial release or for field trials may be present in the receiving environment. Native Australian cotton species are not sexually compatible with *G. hirsutum*.

12.3. Are any characteristics expected to be altered in the GM plants compared to the parent species 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, most of which is modified to produce Bt toxins targeting lepidopteran pests. Therefore, although pollinator abundance and outcrossing may be higher in Bt cotton crops than non-Bt cotton crops, the efficiency of gene transfer from the IR and HT IR GM cottons proposed for release is expected to be the same as for current commercial GM Bt cotton varieties.

12.4. 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 14: Additional Information about the Parent Species

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 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, e.g. national parks, where weeds may be defined as any naturalised alien/non-native plant, cotton (*G. hirsutum* and *G. barbadense*) is found in isolated populations and may be considered as a weed (reviewed in Eastick 2002). *G. hirsutum* is, e.g. listed under the category 'moderate to minor weed usually in small infestations' in Kakadu National Park (Cowie & 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 New South Wales and in Queensland 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, e.g. along roadsides. A survey of the transport routes between Emerald (in the *G. hirsutum* growing region in central Queensland) and the Atherton Tablelands in Queensland, 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 & 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, e.g. 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 & 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 & Hearnden 2006). Also, the relatively low soil moisture of uncultivated habitats may limit 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 (e.g. 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 & Bange 2006), cotton yields about 3850 seeds per square metre under standard crop production practices. However, seed loss during harvest is minimal because the picking and transport for ginning of the bolls is an integral part of the isolation of cotton fibre and the seed is attached to the fibre. If seed were lost during harvest, the emerging cotton volunteers are poor competitors and management of volunteer plants would be targeted in follow on crops. It is estimated that due to low density and poor competitive ability, these volunteers would produce considerably less seed square metre than when grown as a crop plant.

In **intensively used 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 & Hearnden 2006; Farrell & Roberts 2002) would limit seed production to numbers far below those found in a cropping system. Cotton has little 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.

In **nature conservation areas** the number of volunteer cotton plants is expected to be very low and this 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 conditions, e.g. low soil temperature or low soil moisture (Taylor & 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 & Jones 1953), thus it is an opportunistic out-crosser when insect pollinators are present (Oosterhuis & Jernstedt 1999).

In Australia, honeybees are thought to be the most likely insects responsible for any cross-pollination in cotton (Mungomery & 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). Nonetheless, this does not rule out pollen transfer via other body parts.

Honeybees were implicated as the chief pollinating agent in a QLD study (Mungomery & 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 this region. 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 & 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).

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 of cotton affect the pollen aggregation process used by the bees in the packing of their pollen pellets (Vaissière & Vinson 1994). 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 & 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 & 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); Section *Hibiscoidea* (G-genome) contains three species and Section *Grandicalyx* (K-genome) contains 12 species (Wendel & 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 in Australia?

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

Cotton (*G. hirsutum*) 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 (e.g. 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 & 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 (e.g. 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 to 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 change 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, e.g. native bush (Farrell & 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 & Roberts 2002). Additionally, *G. hirsutum* seed germination was highest in disturbed habitats (Eastick & 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 & 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 (e.g. 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 & 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 (Halooin 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, thus limiting their ability to be transported (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.

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 known to occur.

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 accidentally 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 22°S 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 or farm animals?

Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g. 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 delinted 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 water availability, 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 & 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, i.e. 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. For example, herbicides containing carfentrazone-ethyl as an active constituent are currently registered by the APVMA for control of volunteer cotton, including Roundup Ready® *G. hirsutum* volunteers (see [APVMA Pubcris database](#) accessed on 15 April 2016).

Intensively used areas

Feedlots, cattle yards, paddocks, dairy farms or similar: cotton volunteers are actively managed by the same methods as described above, i.e. 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](#) ((accessed on 15 April 2016). Similarly, cotton is not a weed of concern in New South Wales 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 control.

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.

For interstate movement of cotton seed, the relevant quarantine department must be contacted. 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 species to other organisms 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).

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