



**Australian Government**

**Department of Health and Ageing  
Office of the Gene Technology Regulator**

# **The Biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton)**



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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

Version 2 (February 2008) has been updated to include an appendix containing a weed risk assessment of cotton based on the National Post-Border Weed Risk Management Protocol. References to the new appendix have been added in the text. Minor updates to the sections on seed dispersal, out-crossing rates and commercial production of GM cotton have also been made.

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## PREAMBLE

This document describes the biology of *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (pima cotton), with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *G. hirsutum* and *G. barbadense*, general descriptions of their morphology, reproductive biology, development, biochemistry, biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism in risk assessments of genetically modified *G. hirsutum* or *G. barbadense* that may be released into the Australian environment.

In this document, the word “cotton” is used to refer to information relevant to both *G. hirsutum* and *G. barbadense*, where the information only relates to one species it will be stated as *G. hirsutum* or *G. barbadense*.

In nature, *G. hirsutum* and *G. barbadense* are perennial shrubs. However, in the agricultural system both species are cultivated as annuals, with destruction of plants after harvesting the fruit for seed and fibre. The plants are mainly grown for their fibre, cotton lint, which is used in textiles and clothing. Neither species is native to Australia, but grown as a mostly irrigated crop in northern New South Wales (NSW) and Queensland (QLD).

## SECTION 1 TAXONOMY

The genus *Gossypium* was named by Linneaus in the middle of the 18th century. It is in the Family *Malvaceae*, Order *Malvales* and Tribe *Gossypieae*. (Smith 1995). *Gossypium hirsutum* L. was named due to its hairiness (hirsute), although it has also been referred to as *Gossypium hirsutum* ssp. *latifolium*, *Gossypium hirsutum* var. *punctatum*, *Gossypium jamaicense*, *Gossypium mexicanum*, *Gossypium morrillii*, *Gossypium punctatum*, *Gossypium purpurascens*, *Gossypium religiosum*, *Gossypium schottii*, *Gossypium taitense* and *Gossypium tridens*. It is commonly known as upland cotton, American cotton or Mexican cotton.

*G. barbadense* L. was named after its assumed habitat of Barbados. It has been known by alternative scientific names as *Gossypium evertum*, *Gossypium peruvianum*, *Gossypium vitifolium* and *Gossypium brasiliense* (USDA 2006). It is commonly known as Creole cotton, Egyptian cotton, extra long-staple or ELS cotton, Indian cotton, Sea Island cotton or pima cotton.

The common name cotton comes from the Arabic ‘quṭn’ and generally refers to species that produce spinnable fibres (lint) on their seed coat (Lee 1984). The oldest known words for cotton are ‘karparsa-i’, in the language Sanskrit, and ‘Karapas’ used in early Bible manuscripts (Smith 1995).

The taxonomy of *Gossypium* is still a subject for debate. Smith (Smith 1995) described the genus *Gossypium* as containing 43 species consisting of 37 diploid species ( $2n = 2x = 26$ ) and six tetraploid ( $2n = 4x = 52$ ) species. This is in contrast to Fryxell (Fryxell 1992) who lists 50 species in total or other authors (Brubaker et al. 2002; Percival et al. 1999) who list 49 species in total but include only five tetraploids. The different number of tetraploids relates to discussion on the status of *G. lanceolatum* Todaro and the evidence presented that it is actually a locally developed, domesticated form of *G. hirsutum* and should be classified as *G. hirsutum* race ‘palmeri’ not a separate species (Brubaker & Wendel 1993). There has also been debate about the status of *G. nandewarensense* and whether it should be classified as separate species (Brown et al. 1997; Fryxell 1992) or as a variety of

*G. sturtianum* (Fryxell 1965). The *Gossypium* genus is commonly grouped into eight diploid genomic groups, designated A–G and K, and one tetraploid genomic group, based on chromosomal similarities (Edwards & Mirza 1979; Endrizzi et al. 1985; Stewart 1995). Each genome represents a group of morphologically similar species that can only rarely form hybrids with species from other genomic groups (Table 1).

**Table 1 Taxonomy of *Gossypium* Species<sup>a</sup>**

Species	Genomic Group	Distribution
<b>Diploid species</b>		
<i>G. herbaceum</i> L.	A <sub>1</sub>	Old World cultigen, Africa, Asia Minor
<i>G. arboreum</i> L. (syn. <i>G. aboreum</i> L.)	A <sub>2</sub>	Old World cultigen, Asia Minor, SE Asia, China, Africa
<i>G. anomalum</i> Wawr. and Peyr.	B <sub>1</sub>	Africa
<i>G. triphyllum</i> (Harv. And Sand.) Hochr	B <sub>2</sub>	Africa
<i>G. captis-viridis</i> Mauer	B <sub>3</sub>	Cape Verde Islands
<i>G. trifurcatum</i> Vollesen <sup>b</sup>	B?	Somalia
<i>G. sturtianum</i> J.H. Willis	C <sub>1</sub>	Australia
<i>G. robinsonii</i> F. Muell.	C <sub>2</sub>	WA, Australia
<i>G. nandewareense</i> Derera <sup>c</sup>	C	Australia
<i>G. thurberi</i> Tod.	D <sub>1</sub>	Mexico, Arizona
<i>G. amourianum</i> Keam.	D <sub>2-1</sub>	Mexico
<i>G. harknessii</i> Brandg.	D <sub>2-2</sub>	Mexico
<i>G. davidsonii</i> Kell.	D <sub>3-d</sub>	Mexico
<i>G. klotzschianum</i> Anderss.	D <sub>3-k</sub>	Galapagos Islands
<i>G. aridum</i> (Rose & Standl.) Skov	D <sub>4</sub>	Mexico
<i>G. raimondii</i> Ulbr	D <sub>5</sub>	Peru
<i>G. gossypoides</i> (Ulbr.) Standl.	D <sub>6</sub>	Mexico
<i>G. lobatum</i> Gentry	D <sub>7</sub>	Mexico
<i>G. laxum</i> Phillips	D <sub>8</sub>	Mexico
<i>G. trilobum</i> (DC.) Skov.	D <sub>9</sub>	Mexico
<i>G. tumeri</i> Fryx.	D <sub>10</sub>	Mexico
<i>G. schwendimanii</i> Fryxell & S. Koch	D <sub>11</sub>	Mexico
<i>G. stocksii</i> Mast.ex. Hook.	E <sub>1</sub>	Arabia
<i>G. somalense</i> (Gürke) Hutch.	E <sub>2</sub>	Arabia
<i>G. areysianum</i> (Defl.) Hutch.	E <sub>3</sub>	Arabia
<i>G. incanum</i> (Schwartz) Hille.	E <sub>4</sub>	Arabia
<i>G. benadireense</i> Mattei	E	Somalia, Kenya, Ethiopia
<i>G. bricchettii</i> (Ulbrich) Vollesen	E	Somalia
<i>G. vollesenii</i> Fryxell	E	Somalia
<i>G. longicalyx</i> Hutch. and Lee	F <sub>1</sub>	Africa
<i>G. bickii</i> Prokh	G <sub>1</sub>	Central Australia
<i>G. nelsonii</i> Fryx.	G	Australia

Species	Genomic Group	Distribution
<i>G. australe</i> F. Muell.	G	Australia
<i>G. anapoides</i> Stewart, Wendel and Craven	K	Australia
<i>G. costulatum</i> Tod.	K	Australia
<i>G. cunninghamii</i> Tod.	K	Northern NT, Australia
<i>G. enthyle</i> Fryxell, Craven & J.M. Stewart	K	WA, Australia
<i>G. exgiuum</i> Fryxell, Craven & J.M. Stewart	K	WA, Australia
<i>G. londonderriense</i> Fryxell, Craven & J.M. Stewart	K	Australia
<i>G. marchantii</i> Fryxell, Craven & J.M. Stewart	K	Australia
<i>G. nobile</i> Fryxell, Craven & J.M. Stewart	K	WA, Australia
<i>G. pilosum</i> Fryx.	K	WA, Australia
<i>G. populifolium</i> (Benth.)Tod.	K	WA, Australia
<i>G. pulchellum</i> (C.A. Gardn.) Fryx.	K	WA, Australia
<i>G. rotundifolium</i> Fryxell, Craven & J.M. Stewart	K	WA, Australia
<b>Allotetraploid species</b>		
<i>G. hirsutum</i> L.	(AD) <sub>1</sub>	Cultivars, Central America
<i>G. barbadense</i> L.	(AD) <sub>2</sub>	Cultivars, South America
<i>G. tomentosum</i> Nutt. ex Seem.	(AD) <sub>3</sub>	Hawaiian Islands
<i>G. mustelinum</i> Miers ex Watt	(AD) <sub>4</sub>	Brazil
<i>G. darwinii</i> Watt	(AD) <sub>5</sub>	Galapagos Islands
? <i>G. lanceolatum</i> Tod <sup>d</sup>	(AD)	Mexico

<sup>a</sup> Modified from (Endrizzi et al. 1985; Percival et al. 1999; Seelanan et al. 1999; Stewart 1995)

<sup>b</sup> Retained in *Gossypium* genus as (Rapp et al. 2005).

<sup>c</sup> May be classified as a subspecies of *G. sturtianum* (Fryxell 1965).

<sup>d</sup> May be classified as a subspecies of *G. hirsutum* (Brubaker & Wendel 1993).

*G. hirsutum* and *G. barbadense*, the two species cultivated in Australia, are in the AD allotetraploid genomic group, subgenus *Karpas* Rafinesque (Seelanan et al. 1999). Like the other AD-genome species, *G. hirsutum* and *G. barbadense* 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 identity of the progenitor diploid species, and when these progenitors may have come into physical contact sufficient to enable hybridisation is unknown as, at present, A and D diploid species exist in different hemispheres (Endrizzi et al. 1985).

### 1.1 Taxonomy and distribution of native Australian cotton 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 1979b; 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 & Cronn 2003).

The centre of *Gossypium* diversity in Australia is in northern Western Australia (WA) and NT. Including *G. robinsonii*, which is indigenous to the Port Hedland area of WA, and *G. rotundifolium*, which occurs in the Broome region, 13 of Australia's 17 *Gossypium* species occur in this northern region. Of the remaining four species, *G. sturtianum* is the most widely distributed. It is a shrubby species, occurring as small isolated populations, widely scattered across the sub-tropical to warm temperate arid zones of Australia, in QLD, NSW, South Australia (SA) and WA (Seelanan et al. 1999). Like *G. sturtianum*, *G. australe* has a broad east coast – west coast distribution, but its indigenous range is north of that of *G. sturtianum*, extending from southern areas of the NT to Katherine, in the north of the NT. Finally, *G. bickii* occurs largely within central NT, while *G. nelsonii* is distributed in a band from central NT to central QLD.

## SECTION 2 ORIGIN AND CULTIVATION

### 2.1 Centre of diversity and domestication

The word 'cotton' is used in this document to refer to *G. hirsutum* and *G. barbadense*, however, generally 'cotton' refers to four species in the genus *Gossypium* (Malvaceae) - *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L. - that were domesticated independently as source of textile fibre (Brubaker et al. 1999a). Today, *G. hirsutum* and *G. barbadense* are the major cultivated cotton species, with *G. hirsutum* accounting for 90% of world production (Jenkins 2003). *G. barbadense* represents approximately 5% of world fibre production (Wu et al. 2005) and is cultivated primarily in Egypt, Peru, Sudan, USA and parts of the former Soviet Union. *G. arboreum* is grown mainly in India and *G. herbaceum* is grown in the drier regions of Africa and Asia (Jenkins 2003). Only *G. hirsutum* and *G. barbadense* are grown commercially in Australia with *G. hirsutum* comprising 99% of plantings in 2006/2007 (Information supplied by Monsanto).

The place of origin of the *Gossypium* genus is not known, however the primary centres of diversity for the genus are west-central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). The genus *Gossypium* is thought to have separated from *Kokia* and *Gossypioides*, the most closely related genera in the *Gossypieae*, approximately 12.5 million years ago in the Miocene period (Seelanan et al. 1997; Wendel & Albert 1992) or slightly more recently in the Pliocene (Cronn et al. 2002). There is still debate regarding when the allelotetraploids originated (reviewed in Wendel & Cronn 2003). Some authors have suggested an ancient origin (60–100 million years ago - Cretaceous or early Tertiary period), so that hybridisation of the A and D genomes took place prior to separation of the South American and African continents. Alternatively, human transfer of African or Asian A genome plants may have occurred followed by accidental or deliberate hybridisation with a D genome species. This would have occurred much more recently, approximately 6000 years ago. However, neither of these theories is supported by molecular evidence such as DNA sequence data which estimates and supports a mid-Pleistocene origin (1-2 million years ago) (Wendel 1989). This period was characterised by fluctuating sea levels due to glaciation, and the coastal distribution of the allelotetraploids may have enabled them to exploit the disturbed littoral areas (Fryxell 1979b).

Archaeological records indicate that *Gossypium* fibre has been used since 6000 BC. A *Gossypium* thread, used to string copper beads, from Mehrgarh in Pakistan has been dated

at 6<sup>th</sup> millennium BC (Moulherat et al. 2002). It is unknown whether this is from a domesticated cotton species, but it suggests that cotton fibre was known and used at this time. Cotton was probably used as wadding, packing or for dressing wounds prior to being used for spinning into yarn (Smith 1995). *Gossypium* remains in the form of cloth, string, assorted bits of fibre and boll fragments were found in different layers of deposits in caves in Tehuacan Valley in Mexico (Smith, Jr. & MacNeish 1964). These have been identified as being from tetraploid *Gossypium*, with the earliest bolls dating from approximately 5800 BC. Archaeological remains of scraps of fabrics and cords, unprocessed fibres formed into plugs and cotton boll segments from a site in Peru are thought to be the earliest forms of domesticated *G. barbadense*. The finds show a continuum of increasing seed size and fibre diameter from the earlier (2500 BC) to later (1000 BC) levels (Stephens & Moseley 1973).

The geographic centre of origin for *G. hirsutum* is North and Central America and Mexico, and for *G. barbadense* is South America (Jenkins 2003). *G. hirsutum* was probably first domesticated by pre-Columbian people of the Yucatan peninsula (Brubaker & Wendel 1994). These early semi-domesticated forms dispersed into the rest of Mesoamerica as well as northern South America and into the Caribbean (Iqbal et al. 2001). Selection then occurred for reduced seed dormancy, annualised growth habit and photoperiod independent flowering creating genotypes more similar to modern cultivars. Interestingly, modern North American *G. hirsutum* has a very limited genetic diversity, thought to be due to a genetic bottleneck resulting from the selection pressure of domestication (Iqbal et al. 2001). This is hypothesised to partly result from the Kekchi Indians of Guatemala intercropping cotton with capsicums and harvesting the cotton as soon as the first bolls developed to prevent competition with the capsicums, thus rigorously selecting for early maturity along with reduced seed dormancy and annual growth.

The maritime subsistence for the Andean civilisations, depending in part on cotton fishing nets, has led to the perception that the domestication of *G. barbadense* took place along the coastline (Westengen et al. 2005). Cotton seed, fibres, fabric and fishing nets have been found at Huaca Prieta on the north coast of Peru, dating from 1500–2400 BC. From this centre *G. barbadense* dispersed into South America, West Indies and the Galapagos. This may have been carried by humans or naturally by ocean currents (Smith 1995).

Cotton remains from archaeological excavation sites from northern and central coastal Peru show a continuum to a strongly reduced fuzz layer (tufted seed) with a kidney shaped seed which was more easily ginned by hand, with no hard seeds and no delayed germination. Later domestication introduced higher percentage lint, longer and stronger lint and different colour fibres (Westengen et al. 2005).

It is believed that *G. hirsutum* was cultivated by the Pueblo Indians in the south west USA as early as the first century AD (Fryxell 1979a). Most wild cottons have a short day photoperiod response for flowering so during domestication cotton has been selected to be insensitive to photoperiod (Lee 1984). Annuals are unknown amongst the wild species of *Gossypium* (Fryxell 1979a). Annual growth habit and the concomitant day-neutral flowering response is a major evolutionary step which occurred due to human selection and enabled growth of these plants outside of the tropics. Wild species of cotton have a fairly high percentage of 'hard' or dormant seed which can persist in a seed bank prior to germination (Jenkins 2003). This trait has been bred out of modern cotton cultivars as it is advantageous for all the seed planted to germinate immediately after sowing. Similarly, modern annual cultivars have seed aggregated in compact locks which remain in bolls to aid harvesting whereas the wild species have seeds that drop individually and scatter freely (Stephens 1965; Stephens 1970). Data suggests that a doubling of seed size has led

to a 3-fold increase in lint index (g lint/100 seed) and an 80% increase in mean fibre length during domestication (Stephens 1965). This increased fibre length has been achieved by a prolonging of the fibre elongation period and greater growth rate early in fibre development in modern cultivars compared to wild *G. hirsutum* (Applequist et al. 2001).

Today, indigenous *G. hirsutum* is widely distributed in Central and South America, the Caribbean and some Pacific Islands. *G. barbadense* has a more southerly indigenous range centred on the northern third of South America but with a large region of overlap with *G. hirsutum* in the Caribbean (Wendel and Cronn 2003). However, both species are cultivated commercially in many countries.

### **2.2.1 Origin in Australia**

Cotton was introduced to Australia as a source of textile fibre. Although sporadic attempts were made to produce cotton in the years following European settlement in 1788, commercial cotton cultivation began in QLD and NSW in the 1860s when the American Civil War caused shortages in world cotton supplies (Constable et al. 2001). Subsequently, cultivation was attempted in the NT (1882) and the Kimberley's, Western Australia (1947), although in these northern regions, the prevalence and impact of insect pests limited the commercial viability of continued plantings (Wood & Hearn 1985). It was not until the 1960s that the modern intensive Australian cotton industry was established, primarily in northern NSW and southern QLD (Hearn & Fitt 1992).

*G. hirsutum* also may have arrived in northern Australia naturally, via ocean currents from Central America (Fryxell 1966; Fryxell 1979b). When this may have occurred is unknown, and it has not been substantiated. The primary evidence for this supposition is the presence along coastal river and beach strands in northern Australia of 'naturalised' populations of agronomically primitive cotton with morphological features that suggest they are not derived directly from modern, elite *G. hirsutum* cultivars. They may be descendants of long-distance transoceanic immigrants as proposed by Fryxell, or alternatively, feral derivatives of primitive varieties introduced for cultivation before 1900.

## **2.2 Commercial uses**

Cotton is currently the leading plant fibre crop worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries (Smith 1999). It is estimated that cotton is cultivated on approximately 2.4% of the World's arable land (Blaise 2006). Specific areas of production include countries such as USA, India, China, America, the Middle East and Australia, where climatic conditions suit the natural growth requirements of cotton, including periods of hot and dry weather and where adequate moisture is available, often obtained through irrigation.

Average world cotton production was at 24.88 Mt (mega tons) in 2005–06 and is forecast to rise to 28.04 Mt by 2011–12 (Wood et al. 2007). Ninety-five percent of Australian cotton production is exported (<http://www.daff.gov.au/agriculture-food/hort-crops-wine/crops/cotton/industry>). Australia exported 650 kt (kilo tons) of raw cotton in 2005–06 worth \$1137 million (ABARE 2007). In 2005–06 Australian exports comprise only approximately 7% of the world cotton export market with most cotton being exported from USA (3821 kt), sub-Saharan Africa (1422 kt) and Uzbekistan (1045 kt) (ABARE 2006). The major markets for Australian cotton are (in descending order) China, Indonesia, Thailand, Republic of Korea and Japan (ABARE 2006). No cotton was imported into Australia in 2005–06, although a small amount (0.1–0.4 kt) was imported in the previous five seasons.

*G. barbadense* is grown for its fibre quality as it has longer staple length (44–46 staple length) and higher fibre strength than *G. hirsutum* (Smith 1999). *G. barbadense* fibre had a price premium in the USA of approximately 80% more than *G. hirsutum* fibre in 2004 (ICAC 2004). The world production for extra fine cotton was estimated to be 774,000 t (metric tons) for 2004. This included 224,000 t of *G. hirsutum* cotton grown in Egypt which has fibre long enough to be classified as extra fine cotton. The main producers of *G. barbadense* cotton in 2004 were estimated to be the USA (157,000 t), China (98,000 t), India (90,000 t) and Egypt (68,000 t). Australia, Israel and Peru were estimated to produce 24,000 t in total in 2004. (ICAC 2004).

Cotton is primarily grown as a fibre crop. It is harvested as ‘seed cotton’ which is then ‘ginned’ to separate the seed and lint. The long ‘lint’ fibres are further processed by spinning to produce yarn that is knitted or woven into fabrics. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from cotton lint.

The ginned *G. hirsutum* seed is covered in short, fuzzy fibres, known as ‘linters’. These 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). *G. hirsutum* ginned seed comprises 17% crude oil, 45% meal, 10% linters and 28% hulls (Smith 1995). It should be noted that *G. barbadense* cotton seed does not produce linters and therefore is only processed into oil, meal and hulls.

De-linted cotton seed (ie. seed with no lint or linters) is processed into oil, meal and hulls (Cherry & Leffler 1984). The processing of cotton seed oil involves a series of steps including heating, addition of sodium hydroxide, bleaching with clay, filtering and treating with steam under vacuum (OECD 2004). Cotton seed oil has been in common use since the middle of the nineteenth century and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958 (ANZFA 2002). It is used in a variety of products including edible vegetable oils and margarine, soap, and plastics (Frank 1987).

Cotton seed meal is the product remaining once the oil has been removed by crushing and can contain up to 41% protein (Smith 1995). Cotton seed, or meal, flour or hulls derived from it, is used in food products and for animal feed, but this is limited by the presence of natural toxicants in the seeds (gossypol and cyclopropenoid fatty acids; see Section 5). Although cotton seed meal is not used for human consumption in Australia or New Zealand, it has been approved for use in human food in the USA and other countries, when derived from gossypol-free varieties of cotton or after processing to remove the gossypol. The FAO and WHO permit up to 0.6 µg/mg (600 ppm) free gossypol in edible cotton seed products, whereas the FDA has a lower limit of 450 ppm (Lusas & Jividen 1987). Human consumption of cotton seed meal is reported mainly in central American countries and India where it is used as a low cost, high quality protein ingredient (Frank 1987).

Cotton trash can be used as a bulking agent to improve the efficacy of animal manure composting (Brampton 2001). In the USA, cotton trash has been investigated as a fuel. The cotton stalks have a similar specific energy (17.1–18.1 MJ/kg) to wood (Coates 2000) which has led to the proposal that the trash could be used as an industrial fuel for a power

plant (Gomes et al. 1997) or combined with pecan shells to produce BBQ briquettes (Coates 2000). There has also been some interest in using cotton waste to ferment to produce ethanol (Jeoh & Agblevor 2001).

Extracts from cotton plants, which would be primarily gossypol, have been used as a medicine. In traditional medicine *G. barbadense* leaves have been used as a treatment for nausea during pregnancy or for ‘proud flesh’ (swollen tissue around a wound) (Sawyer 1955). *G. barbadense* extracts are still sold for treatment of hypertension, fungal infection and menstrual stimulant (Tropilab Inc 2007) (See Section 5.4 for more information).

## 2.3 Cultivation in Australia

### 2.3.1 Commercial propagation

Cotton is generally propagated by seed. In Australia, seed can be ordered with various seed treatments such as fungicides, systemic insecticides or a plant activator, thought to provide increased plant resistance against diseases (Cotton Seed Distributors 2007).

Seed for planting is generally delinted. This can be achieved using a mechanical, flame or acid delinting process (Gregory et al. 1999). Sulphuric acid delinting is used most commonly and is a commercial process carried out in Australia at plants in WeeWaa and Narromine. Acid delinting heats up the seed and slightly scarifies the seed coat which can help break dormancy and improve germination rates (Gregory et al. 1999).

The isolation distances for production of certified seed of *G. hirsutum* and *G. barbadense* in Australia are different. 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 2003). The OECD recommends separation distances of 800 m for certified commercial seed production of *G. barbadense* and 600 m for *G. hirsutum* (OECD 2007) and this standard has been adopted by some seed companies in Australia (Cotton Seed Distributors 2007). QSEED specify 600 m for *G. barbadense* and 200 m for *G. hirsutum* (QSEED 2004). This difference is thought to reflect the higher value of *G. barbadense* cotton lint and the low tolerance to the presence of *G. hirsutum* genes (Brett Ross 2007 pers. comm.) rather than a greater likelihood of out-crossing by *G. barbadense*.

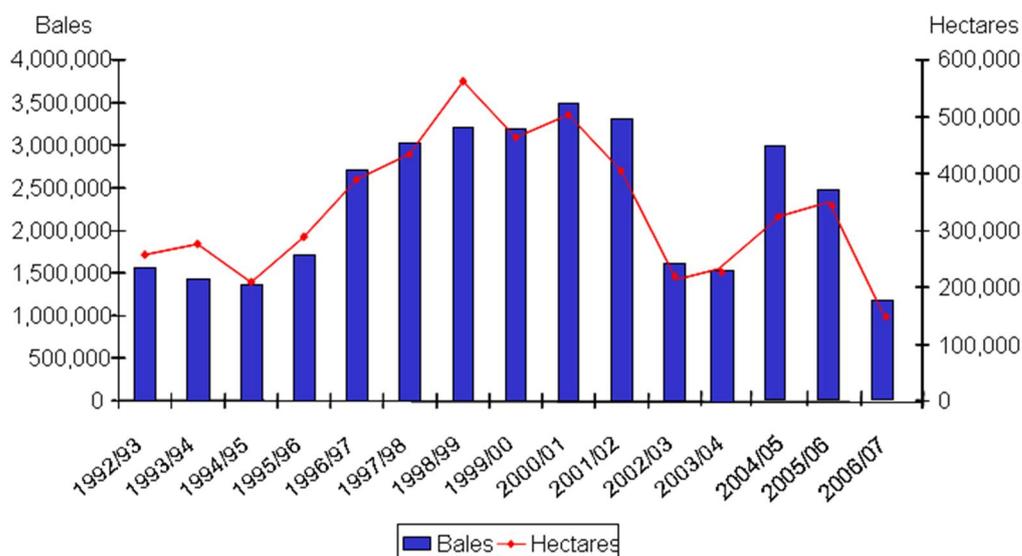
Hybrid cotton, consisting of either intraspecific or interspecific hybrids between *G. hirsutum* and *G. barbadense* is widely grown in some countries including India and China. It was estimated in 2006 that 50% of the cotton acreage in these countries is planted to hybrid cotton (Blaise 2006). In India seeds of hybrid cotton are commercially produced by hand emasculation and pollination, or hand pollination of male sterile lines. However in Australia and other countries where labour costs are high, this process is considered economically unfeasible. Research into insect pollination of male sterile lines in Arizona, USA (Moffett et al. 1975) indicated that insect pollination rates were probably not high enough for hybrid cotton production.

### 2.3.2 Scale of cultivation

The total area planted to cotton varies from season to season with 150 000 ha planted in 2006–07 compared with over 500 000 ha planted in 2000–01 as can be seen in Figure 1. The size of cotton farms in Australia range from 300–4400 ha, with an average size of 800 ha (Hearn & Fitt 1992). In 2004–05, Australia yielded a world record of 2,038 kg/ha (9.2 cotton bales/ha). This figure was three times the world average (732 kg/ha). The next highest yielding countries were Syria (1,571 kg/ha), Mexico (1,312 kg/ha) and Turkey (1,289 kg/ha) (Cotton Australia 2006b).

In Australia, the bulk of the cotton industry is concentrated in northern NSW and southern QLD. *G. hirsutum* is grown commercially from Hillston in southern NSW to Emerald in central QLD, as far west as Bourke and Lake Tandou in NSW. *G. barbadense* is cultivated around Bourke, Tandou and Hillston in NSW.

**Figure 1 Seasonal cotton crop in Australia<sup>1</sup>**



<sup>1</sup> Compiled from data from Cotton Australia and International Cotton Advisory Committee (ICAC)

The major cotton growing regions in Australia are listed in Table 2. Cotton Australia produce an annual report with detailed information about cotton production and the individual valleys where cotton is grown commercially each season (Cotton Australia 2005). A map showing the local government areas in which cotton is grown is available on the OGTR website <<http://www.ogtr.gov.au/pdf/public/cotmaplga.pdf>>.

**Table 2 Major cotton growing regions as of 2003**

State	Cotton growing region	LGAs	Towns
QLD	Central Highlands	Emerald, Peak Downs	Emerald
QLD	Dawson - Callide	Banana	Theodore, Biloela, Moura
QLD	St George - Dirranbandi	Balonne	St George, Dirranbandi
QLD	Darling Downs	Wambo, Dalby, Jondaryan, Chinchilla, Pittsworth, Milmerran	Dalby, Chinchilla, Oakey, Pittsworth, Milmerran, Toowoomba
QLD/NSW	Macintyre Valley	Waggamba (QLD), Moree Plains (NSW)	Goondiwindi, Mungindi, Bogabilla
NSW	Gwydir Valley	Moree Plains, Walgett	Moree, Collarenebri
NSW	Upper Namoi	Gunnedah	Gunnedah, Boggabri, Curlewis
NSW	Lower Namoi	Narrabri, Warren	Narrabri, Wee Waa, Walgett
NSW	Macquarie Valley	Narromine, Warren	Narromine, Warren, Trangie, Dubbo
NSW	Bourke	Bourke	Bourke
NSW	Lachlan - Murrumbidgee	Carrathool, Lachlan	Hillston, Lake Cargellico, Griffith

Source: modified from (Reeve et al. 2003)

Climates with long, warm summers are typical for summer *G. hirsutum* growing regions in Australia. *G. barbadense* has similar requirements, although, due to its requirement for a longer growing season, little or no rainfall after March is essential for fibre maturation. Climatic data for some of these areas are given in Table 3.

**Table 3 Climatic data for some of the current cotton growing regions in Australia**

Representative site (within area)	Av. daily max/min temperature °C (summer)	Av. daily max/min temperature °C (winter)	Av. monthly rainfall mm (summer)	Av. monthly rainfall mm (winter)
Bourke NSW (Bourke)	35.6/20.3	19.0/5.6	38.8	23.6
Hillston Airport NSW (Carrathool)	32.4/17.6	15.8/4.6	28.7	32.1
Emerald QLD Post Office (Emerald)	34.2/20.3	23.3/7.8	84.4	27.8
Hay NSW (Hay)	32.2/15.9	16.0/4.2	27.3	32.9
Menindee NSW (Lake Tandou)	33.5/17.7	17.8/4.7	21.9	19.2
Moree NSW (Moree Plains)	34.4/18.7	19.5/4.3	64.1	73.2
Narrabri West Post Office NSW (Narrabri)	32.3/17.3	18.9/4.5	72.5	45.7
Cunnamulla QLD (Paroo)	35.3/21.5	19.8/6.5	45.3	22.1
Warren NSW (Warren)	33.0/17.9	17.0/3.4	56.8	30.3

Source: <<http://www.bom.gov.au>>.

#### POSSIBLE AREAS FOR EXPANSION OF THE COTTON INDUSTRY

Opportunities for further expansion of the *G. hirsutum* industry in southern Australia are limited mainly by the length of growing season in VIC and southern NSW, or availability of irrigation water in NSW, SA, and WA (See Section 2.3.2).

Opportunities for further expansion of the *G. barbadense* industry in southern Australia are limited mainly by the humidity and rainfall during crop maturation and the length of growing season (G. Constable; CSIRO; pers. comm. 2007).

A study by the Australian Cotton Cooperative Research Centre (ACCRC) (Australian Cotton Cooperative Research Centre 2001), based on average temperatures during the growing season, timing of rainfall, and the suitability of the soil for cotton cultivation, indicates considerable potential for expansion into northern Australia in particular areas of WA, the NT and QLD. The ACCRC study examined potential regions for cotton growing in northern Australia and suggested at least 200,000 ha of potential irrigation areas that could be developed over the next ten years.

There is potential for growing cotton crops in both summer and winter in different locations, particularly in north QLD. However, the wet season (approximately November through to March) in more northern areas of Australia would impact greatly on cotton fibre quality (Eastick 2002; Farrell & Roberts 2002) and the ability to access and operate in the cotton fields. Dry season cropping may be necessary in certain areas to avoid periods of highest insect abundance (Australian Cotton Cooperative Research Centre 2001). Data on the climate and the most suitable growing season for cotton in each of the five regions in northern Australia, as suggested in the ACCRC study, is provided in Table 4.

**Table 4 Climatic data for sites where the Australian Cotton CRC is currently involved in northern Australia**

	Broome Post Office (northern WA)	Kununurra ORIA (northern WA)	Katherine Council (northern NT)	Richmond Post Office (northern QLD)	Lower Burdekin Ayr DPI RS (northern QLD)
Average daily max/min temperature (summer <sup>a</sup> )	33.6°C/26.1°C	36.7°C/25.2°C	35.3°C/24.0°C	36.9°C/22.6°C	31.7°C/22.5°C
Average daily max/min temperature (winter <sup>b</sup> )	28.6°C/14.9°C	31.4°C/16.1°C	30.9°C/14.3°C	26.8°C/9.4°C	25.6°C/12.3°C
Average monthly rainfall (summer)	126.1 mm	171.6 mm	216.4 mm	98.7 mm	182.4 mm
Average monthly rainfall (winter)	10.1 mm	1.8 mm	0.9 mm	9.3 mm	18.2 mm
Growing season	May– November	April– October	March– October	December– July	March– November
Arable soil type	Sandy loam	Cracking clay	Clay loam and sandy clay loam	Cracking clay, some inherent salinity	Cracking clay
Irrigation system	Sub surface drip	Furrow	Sub surface drip/ overhead	Furrow	Furrow
Development status	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.	New area under development or evaluation	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.

<sup>a</sup> December, January, February

<sup>b</sup> June, July, August

ORIA: Ord River Irrigation Area

DPI RS: Department of Primary Industries Research Station

Sources: <http://www.bom.gov.au> and ACCRC (2001)

Previous attempts at commercial cotton cultivation in northern Australia over the past 100 years have ended in failure. This has been attributed to a combination of factors including cultivation during the wet season (inconsistent rainfall, season too short), low plant populations, poor choice of soils, unsustainable insect pest management practices, lack of effective irrigation techniques, use of unsuitable cotton varieties, and pathogens (Australian Cotton Cooperative Research Centre 2001). General overall problems were geographic distance, ignorance of the physical environment, and an aversion to learning from experience (Bauer 1985). Attempts at growing other large scale commercial agricultural crops in northern Australia in the past have also failed due to similar reasons (Australian Cotton Cooperative Research Centre 2001).

#### COMMERCIAL GM COTTON IN AUSTRALIA

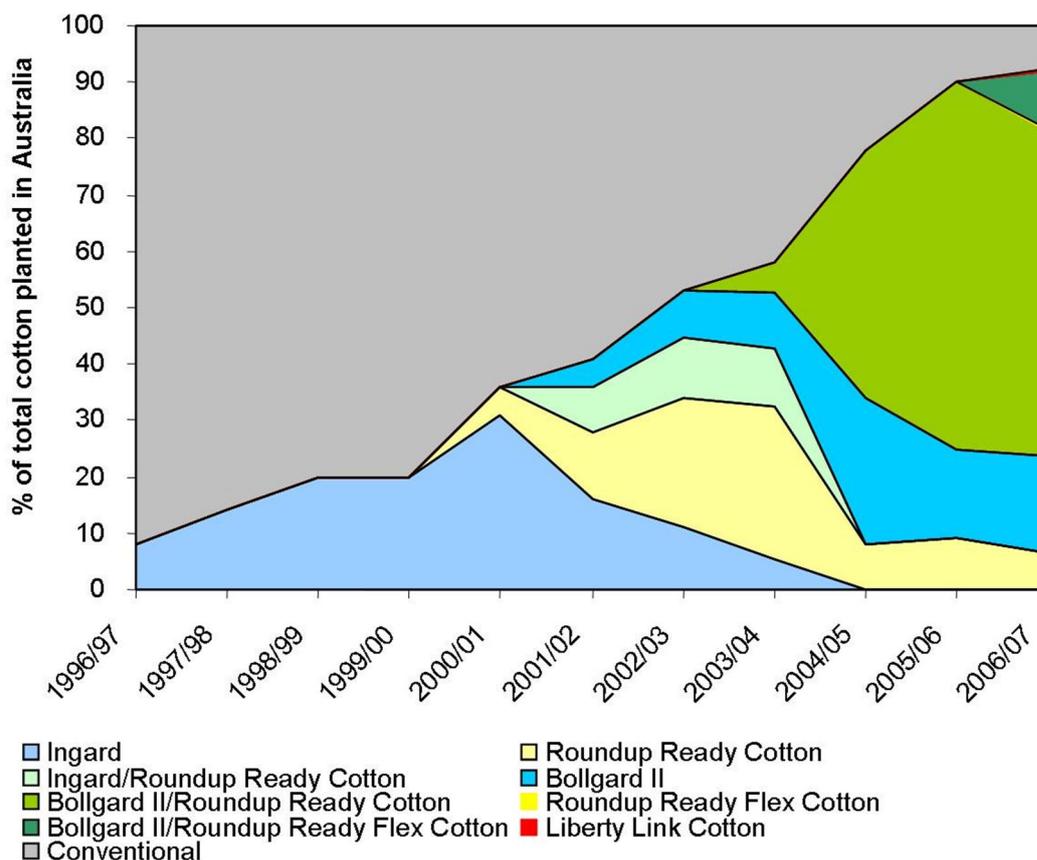
The following GM *G. hirsutum* lines have been approved for commercial release in Australia:

- insect resistant INGARD<sup>®</sup> *G. hirsutum* (DIR 022/2002; withdrawn from the market in 2004 in favour of Bollgard II<sup>®</sup> *G. hirsutum*)
- glyphosate tolerant Roundup Ready<sup>®</sup> *G. hirsutum* (DIR 023/2002 and DIR 066/2006)

- glyphosate tolerant/insect resistant Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> *G. hirsutum* (DIR 023/2002; withdrawn from the market in favour of Bollgard II<sup>®</sup>/Roundup Ready<sup>®</sup> *G. hirsutum*)
- insect resistant Bollgard II<sup>®</sup> *G. hirsutum* (DIR 012/2002 and DIR 066/2006)
- insect resistant/glyphosate tolerant Bollgard II<sup>®</sup>/Roundup Ready<sup>®</sup> *G. hirsutum* (DIR 012/2002 and DIR 066/2006)
- glyphosate tolerant Roundup Ready Flex<sup>®</sup> *G. hirsutum* (DIR 059/2005 and DIR 066/2006)
- glyphosate tolerant/insect resistant Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> *G. hirsutum* (DIR 059/2005 and DIR 066/2006)
- glufosinate ammonium tolerant LibertyLink<sup>®</sup> *G. hirsutum* (DIR 062/2005).
- glufosinate ammonium tolerant/insect resistant LibertyLink<sup>®</sup>/Bollgard II<sup>®</sup> *G. hirsutum* (DIR 062/2005)
- insect resistant Widestrike<sup>™</sup> *G. hirsutum* (DIR 091).

Various of these lines have been approved internationally in countries including Argentina, Canada, China, European Union, India, Japan, Korea, Mexico, Phillipines, South Africa and United States.

There is almost 99.5% uptake of GM cotton varieties by cotton growers in Australia. In the 2010/11 season, 597,000 hectares of GM cotton varieties was planted and 95% of those varieties contained stacked traits for insect resistance and herbicide tolerance (<http://grains.agric.wa.gov.au/node/genetically-modified-crops>). A second source indicates that Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cottons currently occupy about 97% of the total cotton area, most of it stacked with the Bollgard<sup>®</sup> II trait (CSIRO 2010). For the 2010/11 season, LibertyLink<sup>®</sup> comprised about 0.5% of the hectares sown to cotton, while no Widestrike<sup>™</sup> cotton was planted commercially (Bayer CropScience and Dow AgroScience 2011 Annual Reports to OGTR, respectively). For the adoption of GM cotton in Australia upto 2007, see Figure 2.

**Figure 2 Australian GM cotton adoption by product (1997–2007)<sup>1</sup>**

<sup>1</sup> Compiled from data from Cotton Australia and Monsanto Australia 2007

### 2.3.3 Cultivation practices

Temperature is the dominant environmental factor affecting *G. hirsutum* development and yield (Australian Cotton Cooperative Research Centre 2002c; Constable & Shaw 1988). Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least three successive days. Cotton seedlings may be killed by frost and a minimum of 180–200 frost-free days of uniformly high temperatures (averaging 21–22°C) is required after planting for *G. hirsutum* (Duke 1983) and 200–250 days for *G. barbadense* (Unruh & Silvertooth 1997). Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields (Constable & Shaw 1988).

The timing of cotton cultivation varies slightly throughout Australia, depending on climate. In northern NSW, the appropriate soil temperature is reached typically in late September or early October, whereas in central QLD, it is likely to occur four weeks earlier (Cotton Australia 2002). Cotton farming activities include soil preparation during August–September, planting in September–October, managing weeds, pests and watering during the growing season in November–February. Defoliation, harvesting and transportation for processing are done during March–May. Cotton growers may also plant other crops during the off-season period from May–August (Cotton Australia 2002). *G. barbadense* may be planted earlier and harvested later than *G. hirsutum*.

Cotton rotation systems traditionally involve two years of cotton followed by a year of wheat and occasionally may include a legume crop in the rotation (Anthony 2003). The cereal rotation was used primarily to break cotton disease cycles, however the inclusion of legumes such as faba bean (*Vicia faba*) is becoming more common in NSW (Rochester et al. 1998). Recent research has indicated that the inclusion of a forage legume crop such as

vetch (*Vicia villosa*) in the rotation can increase the yield of the following cotton crop by 13% (Rochester & Peoples 2005). In Australia, cotton is normally grown as a sole crop, although research in Pakistan has shown that intercropping with sesame, sorghum and soybean can reduce weeds (Iqbal et al. 2007).

Cotton is generally planted 4 cm deep into the soil with 16 seeds sown per metre. This is to achieve 10 plants per metre at picking time. Dry land cotton may be sown as full rows, or single or double skip row configuration to maximise utilisation of soil moisture (Cotton Australia 2002).

The timing of planting for Bollgard II® *G. hirsutum* (and its stacks with other genes) is prescribed by the Resistance Management Plan (RMP) as approved by the cotton industry's Transgenic and Insect Management Strategy (TIMS) Committee. The RMP requires various resistance mitigation measures by each grower to ensure resistance to the endotoxins can be effectively managed. These measures include requiring the grower to plant refuge crops of minimum sizes, types and distances from the Bollgard II® crop, fixed planting windows, post harvest crop destruction, control of volunteer and ratoon cotton, pupae destruction and trap cropping (APVMA 2003).

Egyptian studies have found factors affecting transpiration rates in *G. barbadense* have a limiting effect on yield even when adequate soil moisture is available (Sawan et al. 2002; Sawan et al. 2004; Sawan et al. 2005). *G. barbadense* requires low humidity conditions during growth to limit conditions favourable to diseases such as *Alternaria* and boll rots (and to Bacterial Blight if the variety is not resistant). Dry conditions are also especially required during fibre development and crop ripening as the fibre is susceptible to weathering resulting in price discounts which totally remove any of the normal *G. barbadense* premiums (G. Constable; CSIRO; pers. comm. 2007).

Crop yields may be lower in southern growing regions as a result of the shorter summer season. The minimum day degrees required from planting of cotton to 60% boll opening is 2050 (information from the Australian Cotton CRC; available at <<http://www.cotton.crc.org.au>>). For example, cotton planted on 1 October near Warren (Macquarie Valley, NSW) could be expected to reach 60% boll opening by 31 March the following year. Day degrees, or heat units, are calculated progressively during the season from the number of days with a temperature over 12°C using the formula:

Day degrees = [(daily max. temp - 12) + (daily min. temp - 12)]/2

The majority of Australia's cotton crop is grown in the Murray-Darling Basin under irrigation (Anthony 2003). In the 2005–06 season in Australia, 84% of cotton was grown as a furrow irrigated crop (Cotton Australia 2006a) and fields are commonly irrigated five or six times during the growing season between flowering and peak boll development (McLeod et al. 1998). In NSW, cotton production occurs mainly on cracking grey clay soils (vertisols) of the Namoi and Gwydir River Valleys which have inherently low drainage rates (Hodgson & Chan 1982) so waterlogging may occur.

Alternatively, cotton may be grown as an unirrigated crop known as dryland cotton. In some years up to 20% of the total cotton production area consists of dryland cotton although this has accounted for less than 10% of total production (Australian Cotton Cooperative Research Centre 2002a). When cotton is grown as an unirrigated crop the biggest climatic factor influencing cotton yield is rainfall (Ford 2002). In Australia, the majority of dryland production occurs in areas that have a moderate to high variability in rainfall during January to March, the crucial period of the growing season determining yield quantity and quality (Ford 2002; Gibb & Constable 1995). In this period cotton has a daily water use of up to 8–10 mm (Gibb & Constable 1995).

The indeterminate nature of cotton means some varieties have a tendency to excessive vegetative growth at the expense of reproductive growth. The vegetative growth of the cotton crop can be managed using the application of plant growth regulators such as mepiquat chloride (1,1-dimethyl piperidinium chloride) which reduces gibberellic acid formation (Jost et al. 2006). *G. hirsutum* plants treated with either mepiquat chloride or PGR-IV (indolebutyric acid and gibberellic acid) showed increased yield and boll numbers (Biles & Cothren 2001). In *G. barbadense* the application of mepiquat chloride significantly increased seed cotton and lint yields due to increased boll retention and larger bolls (Sawan 2006). Chemical defoliant are also often used in cotton prior to harvest to facilitate mechanical picking and prevent lint contamination with leaves (Shaw 2002). These can also be used to enhance crop maturity and improve uniformity. The use of defoliant is widespread in Australia and Israel, but less than 50% of the cotton in the USA is treated, with most applications occurring in the western states (Chaudhry 1996). Due to the greater sensitivity of *G. barbadense* to nitrogen availability the crop may have denser foliage than *G. hirsutum* and so greater rates of defoliant are often needed (Cotton Seed Distributors Extension and Development Team 2005).

Ratoon cotton is cotton that has regrown from left over root stock, either from volunteer cotton slashed earlier in the same season or from cotton grown in a previous season. Control of ratoon cotton is important as it is capable of acting as a host reservoir for diseases or insect pests of cotton. Herbicides are generally ineffective on ratoon cotton. However, the cultivation and soil disturbance practices used to destroy over-wintering *Helicoverpa* pupae (as discussed in Section 7.2.1) are an effective control measure for ratoon cotton (Roberts et al. 2002).

High levels of farm hygiene are commonly maintained on cotton farms (for example all equipment is cleaned on entry and exit to a field/farm to prevent the transfer of disease or the spread of weeds) and this is discussed further in Sections 7.1.1 and 7.12. Weeds and cotton volunteers on roads and irrigation structures are controlled by mechanical removal or herbicides (Charles et al. 2002) and this is discussed further in Section 8.5. Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. Transport of ginned cotton seed is conducted in covered vehicle to minimise loss of seed.

## 2.4 Crop Improvement

### 2.4.1 Breeding

Cotton is primarily self-pollinating, although out-crossing can occur. The first *G. hirsutum* cotton lines grown in Australia were from the USA. Generally in the USA breeding of *G. hirsutum* has focused on maximum yield and broad adaptation, whereas breeding in *G. barbadense* has emphasised fibre quality (Chee et al. 2005a). A survey of USA breeders in 2000 concluded that most *G. hirsutum* work involved crossing closely related parents followed by backcrossing or reselecting from existing crosses, with less than 3% of the breeding material coming from non-*G. hirsutum* sources (Bowman 2000). In Australia the American lines have now been superseded by locally bred lines which are adapted to Australian conditions. Currently, most breeding work performed is with locally bred parental lines, although USA cultivars may still be used and even Russian cotton varieties have been involved in breeding programmes (Constable et al. 2001). Over 80% of the Australian cotton crop consists of CSIRO developed varieties (CSIRO Plant Industry 2007). Current work at CSIRO includes breeding for resistance to Fusarium and Cotton Bunchy Top and for improved cotton fibre (CSIRO Plant Industry 2007) and the

production of cultivars for dryland production systems that have high yield potential and enhanced water use efficiency (Stiller et al. 2005). It is estimated that breeding has contributed 45% to the improvements in yield seen since 1983 (Constable et al. 2001).

Modern *G. barbadense* cultivars are highly introgressed with *G. hirsutum* (Percival et al. 1999). Introgressed traits between *G. hirsutum* and *G. barbadense* such as day length neutral flowering, disease resistance and heat tolerance have been maintained through selection (Brubaker et al. 1999a; Wang et al. 1995). This has led to most commercial cultivars of *G. barbadense* having an average of 8–12% introgressed *G. hirsutum* DNA (Wang et al. 1995).

*G. hirsutum* and *G. barbadense* share the AD tetraploid genomes, are not separated by any large-scale chromosomal rearrangements (Gerstel & Sarvella 1956), and can be hybridised to produce fertile F<sub>1</sub> progeny. However, F<sub>2</sub> progeny show evidence of lethal gene combinations in succeeding generations (Gerstel 1954; Stephens & Phillips 1972). The two species have different ribosomal DNA sequences (Wendel et al. 1995) and chloroplast genomes (Wendel & Albert 1992), although sequencing of the chloroplast genomes has revealed many similarities (Ibrahim et al. 2006; Lee et al. 2006). Genetic and physical isolating mechanisms have evolved to keep the two species distinct; these include incompatibility at the ‘corky’ locus (Stephens 1946; Stephens 1950a; Stephens 1950b; Stephens & Phillips 1972), differences in the timing of pollen shedding (Stephens & Phillips 1972), and selective fertilisation (Brubaker et al. 1999a; Kearney & Harrison 1932). However, these can be overcome with directed breeding. Recent research has involved crossing *G. barbadense* and *G. hirsutum* followed by back crossing into *G. hirsutum* to create mapping families for QTL (quantitative trait loci) analysis of fibre elongation (Chee et al. 2005a), fibre fineness (Draye et al. 2005), fibre length (Chee et al. 2005b) as well as improved fibre and agronomic traits (Saha et al. 2006).

Wild relatives of the cultivated tetraploid cottons are being investigated as sources of novel genes. For example, *G. sturtianum* accessions have been identified which are resistant to Fusarium wilt (McFadden et al. 2004). Hybrids formed between these and *G. hirsutum* also show enhanced wilt resistance, suggesting that *G. sturtianum* may possess a useful source of resistance which could be introgressed into commercial cultivars (Becerra Lopez-Lavalle et al. 2007), however many backcross generations are needed to produce a commercial quality phenotype. *G. raimondii* shows resistance to jassids and this species has been used in an attempt to transfer this resistance to *G. hirsutum*. The *G. raimondii* x *G. hirsutum* hybrids produced showed jassid resistance and after colchicine treatment to restore fertility these are being backcrossed to the *G. hirsutum* parent (Saravanan et al. 2007).

#### 2.4.2 Genetic modification

The first report of regeneration of cotton from tissue culture was in 1983 (Davidonis & Hamilton 1983). Since then, transformation of cotton has been achieved, but mainly using the readily regenerable *G. hirsutum* Coker varieties of cotton, followed by backcrossing to commercial varieties. Although many varieties will form callus and differentiate into somatic embryos they do not successfully regenerate into mature plants (Sakhanokho et al. 2004). Protocols have now been developed for regeneration of commercial varieties of *G. hirsutum* and *G. barbadense* (Gould et al. 1991; Sakhanokho et al. 2004; Sakhanokho et al. 2001), including the Australian cultivar Siokra 1-3 (Cousins et al. 1991).

Initial transformation experiments used *Agrobacterium tumefaciens* to insert foreign DNA into *G. hirsutum* hypocotyls or cotyledons (Firoozabady et al. 1987), which were then cultured to promote embryogenesis and regenerate plants (Umbeck et al. 1987), a process

taking 6–12 months. This has remained the most popular method despite reports of transformation of embryonic suspension cultures via particle bombardment (Finer & McMullen 1990; McCabe & Martinell 1993; Rajasekaran et al. 2000). To overcome the widespread problem of regeneration from somatic embryos seen in commercial cotton varieties, protocols have been developed in which transformation is achieved via particle bombardment of meristems (McCabe & Martinell 1993). More recently, chloroplast transformation using particle bombardment has been reported (Kumar et al. 2004). Transformation of *G. barbadense* has also been achieved using polybrene-spermidine treatment to facilitate the uptake of plasmid DNA (Sawahel 2001).

The major focus in the production of GM plants has been on resistance to insects and herbicides. Resistance to lepidopteran insect pests has been achieved by using genes from *Bacillus thuringiensis* (*cry1Ac* in INGARD<sup>®</sup> and *cry1Ac* and *cry2Ab* in Bollgard II<sup>®</sup>). Resistance to the herbicide glyphosate has been achieved using a single gene from *Agrobacterium* sp. CP4 (*cp4 epsps*) to produce Roundup Ready<sup>®</sup> *G. hirsutum*, or two copies of the gene to produce Roundup Ready Flex<sup>®</sup> *G. hirsutum*. Resistance to the herbicide glufosinate ammonium has been achieved with the *bar* gene from *Streptomyces hygroscopicus* to produce the Liberty Link<sup>®</sup> *G. hirsutum*. These are approved for commercial release and for use in human food and animal feed in Australia (as discussed in Section 2.3.2) and overseas.

Later stage research is still focussed on different agronomic properties. Field trials have been approved in Australia for GM *G. hirsutum* with increased tolerance to waterlogging, increased water use efficiency, resistance to insects (*vip*), altered oleic acid and improved fungal resistance. In the USA, similar trials are being conducted and also include traits such as improved cold, heat and salinity tolerances. There are also trials of GM cotton plants with altered oleic acid content of the seed and improved fibre quality (USDA 2007). Although progress in genetic engineering of improved cotton fibre yield and quality is not yet at commercial stage there are reports of GM plants with increased yield, fibre length and fibre strength (pers comm. in (Wilkins et al. 2000), and improved thermal properties (Chowdhury & John 1998). As cotton is one of the world's largest oil seed crops and cotton seed meal is a highly nutritious food source (Wilkins et al. 2000), there has been interest in altering seed gossypol levels to make it suitable as a human food (reviewed in Lusas & Jividen 1987). Recent research has produced GM *G. hirsutum* plants with significantly reduced gossypol levels in the seed, with no reduction in the levels in foliage, floral organs and roots (Sunilkumar et al. 2006).

## SECTION 3 MORPHOLOGY

### 3.1 Plant morphology

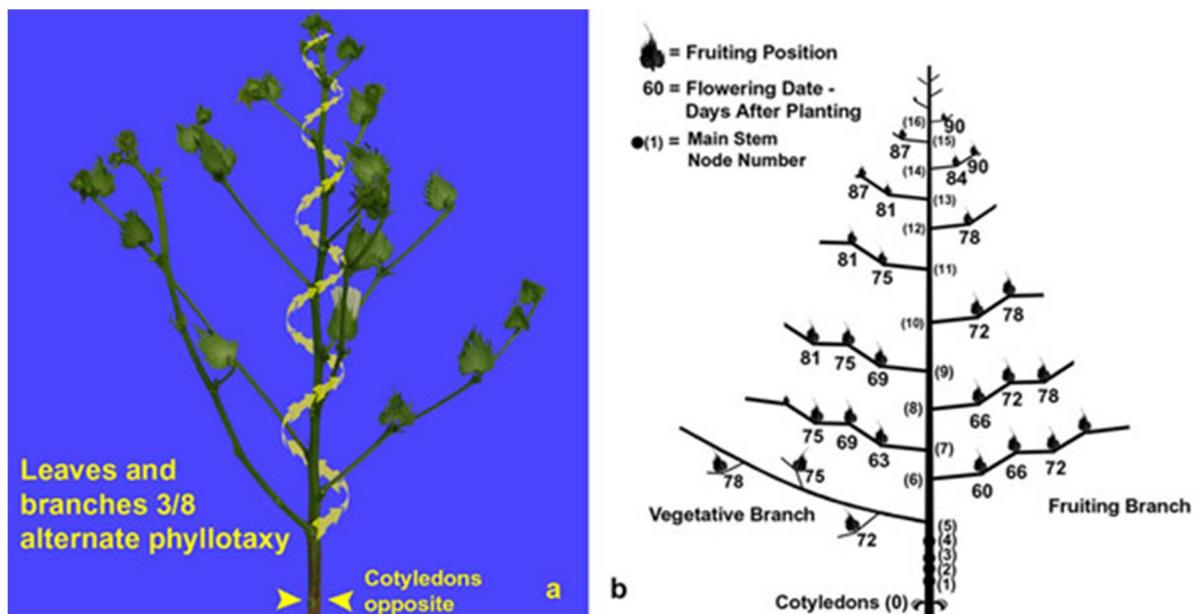
In nature, *G. hirsutum* is a perennial shrub that grows to approximately 1.5–2 m in height, while *G. barbadense* grows to approximately 3 m in height. Commercially, however, both species are cultivated as approximately 1–1.5 m tall annuals, with destruction of plants after harvesting the fruit for seed and fibre.

Cotton plants have an indeterminate growth habit, meaning the plant can develop leaves, stems, flowers, fruit (bolls) and seed all at the same time. The branches on the cotton plant can be classified as either vegetative or fruiting branches. Vegetative branches have only one meristem and so grow long and straight, whereas fruiting branches have multiple meristems, each starting after the previous fruiting bud and as such exhibit a zig-zag growth habit. The first five main stem nodes support primarily vegetative growth and fruiting branches commence thereafter, with branches showing a  $\frac{3}{8}$  alternate phyllotaxy as shown in Figure 3 (Oosterhuis & Jernstedt 1999; Ritchie et al. 2007).

**Figure 3 Cotton plant morphology**

(a) A defoliated cotton plant shows the  $\frac{3}{8}$  alternate phyllotaxy of branches. Each branch is  $\frac{3}{8}$  of a turn around the stem from the branch below it. The branches form from the axils of main stem leaves.

(b) A diagram of the general timing of flower emergence from buds on the fruiting branches by fruiting position. (used with permission from Ritchie et al. 2007)



*G. hirsutum* is heliotropic, its leaves are generally flat and track the sun to maximise light adsorption throughout the day, whereas *G. barbadense* leaves are stationary and are cupped to maximise capture morning and afternoon sunlight, but provide shading in the middle of the day to reduce photobleaching and transpiration (Sassenrath-Cole 1995; Wise et al. 2000). *G. barbadense* also have more stomata than *G. hirsutum*, but these stomata are smaller so the stomatal area per leaf is less than *G. hirsutum* (Lu et al. 1997; Wise et al. 2000). Generally leaves on the main stem axis have seven lobes in *G. barbadense* or five for *G. hirsutum*, whereas those on the fruiting branches have three lobes in either species (Gore 1935). Further comparisons between the vegetative morphology of *G. hirsutum* and *G. barbadense* are outlined in Table 5.

**Table 5 Comparative cotton plant morphology (Fryxell 1984)**

	<i>G. hirsutum</i>	<i>G. barbadense</i>
Habit	Shrubs 1–2 m (or more) tall, usually widely branching, more or less stellate-pubescent, gland-dotted throughout	Shrubs 1–3 m tall, sometimes arborescent, the stems sparsely stellate-pubescent to glabrate, prominently gland-dotted
Leaves	long-petiolate, cordate, weakly 3–5-lobed, the lobes broadly triangular to ovate, acute to acuminate	petiolate, cordate, 3–7-lobed, palmately 7–9-nerved, glabrate, the lobes ovate, entire, acuminate, with 1–5 foliar nectaries beneath
Stipules	subulate, 5–15 (rarely to 20) mm long	subulate to falcate, 10–50 mm long, often prominent
Distribution	Indigenous to Middle America and the Antilles and in certain Pacific Islands (Socorro, the Marquesas, Samoa, etc.); now virtually cosmopolitan in cultivation.	From South America and parts of Central America and the Antilles, now cosmopolitan in cultivation.

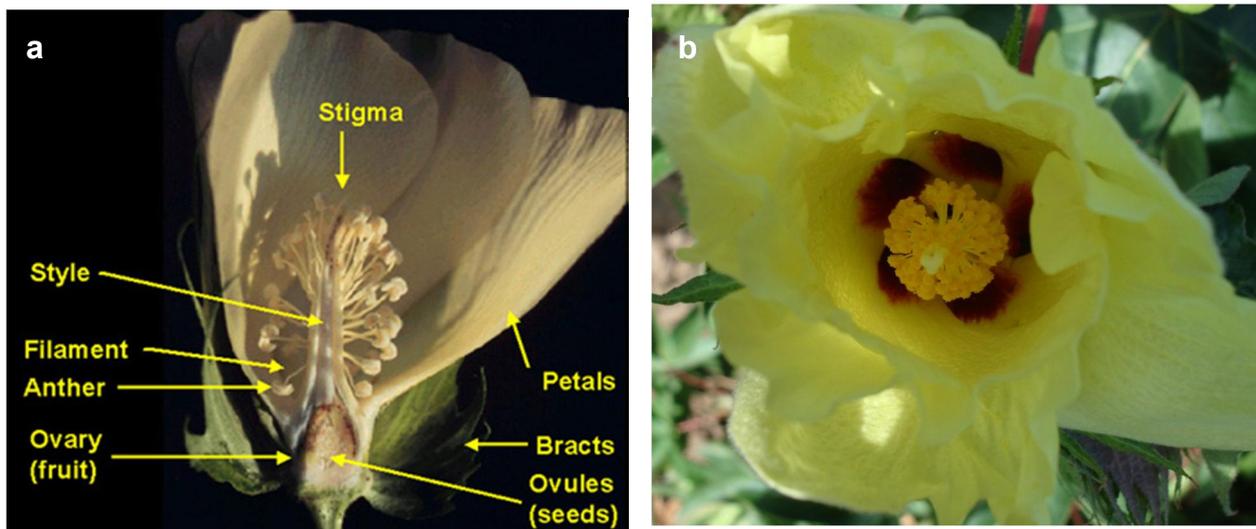
### 3.2 Reproductive morphology

Cotton flowers are large (5–9 cm), perfect (that is contain both male and female structures) and pentamerous (parts arranged in fives). They have both floral and extra-floral nectaries (Moffett 1983). The style is 2–5 cm long and terminates in the 0.5-1 cm-long stigma. The ovary contains 5–10 ovules in each of 3–5 sections, or locules. The stamina sheath, which encloses most of the style, bears numerous stamens 0.5–1 cm long, each terminating in an anther that normally produces an abundance of viable self-fertile pollen (McGregor 1976). There are approximately 20,000 pollen grains per flower (Ter Avanesian 1978).

The flowers of *G. hirsutum* and *G. barbadense* differ in appearance and in their presentation of pollinator foraging cues (see Figure 4). *G. hirsutum* flowers are cream in colour, with cream pollen and secrete a low volume of nectar, whereas *G. barbadense* flowers are yellow, with a maroon nectar guide, orange pollen and produce more nectar with a lower sugar concentration than *G. hirsutum* (McGregor 1976; Moffett 1983). Furthermore, the *G. barbadense* stigma extends well above the anthers, unlike *G. hirsutum* (McGregor 1976), and this may affect the likelihood of cross pollination occurring. It has not been determined whether or not these differences make *G. barbadense* flowers more attractive to native Australian insect pollinators than *G. hirsutum*. Further comparisons between the reproductive morphology of *G. hirsutum* and *G. barbadense* are outlined in Table 6.

#### Figure 4 Cotton flowers

- (a) Annotated *G. hirsutum* flower (used with permission from Ritchie et al. 2007)  
 (b) *G. barbadense* flower (photo OGTR 2007).



**Table 6 Comparative cotton reproductive morphology (Fryxell 1984)**

	<i>G. hirsutum</i>	<i>G. barbadense</i>
Flowers	flowers usually in sympodial inflorescences, the pedicels 20–40 mm long, surmounted by three involucellar nectaries	flowers solitary or in sympodial inflorescences, the pedicels 10–40 mm long, gland-dotted, usually glabrate, surmounted by three involucellar nectaries
Bracts	bracts of the involucrel inserted above each nectary, foliaceous (enclosing the bud), ovate, three to 19-lacinate	bracts of the involucrel three, inserted above the nectaries, ovate, up to 60 mm long, 45 mm broad, seven to 19-lacinate
Calyx	truncate or five-toothed, 5–6 mm long (excluding teeth)	6–10 mm long, undulate or truncate, prominently gland-dotted, ciliate on margin, otherwise glabrous, a trio of nectaries often present at juncture of calyx and involucrel, alternate with bracts
Petals	up to 50 mm long, cream-colored or pale yellow, with or without a dark spot at base; androecium included	up to 80 mm long, usually yellow with dark-red spot at base, minutely gland-dotted; staminal column ca. 25 mm long, pallid, glabrous, gland dotted, the filaments 2–4 mm long
Style	single with decurrent stigmatic lobes, more or less enclosed by androecium or somewhat exceeding androecium	exceeding the androecium, gland-dotted
Capsule	three to five-celled, glabrous, smooth, broadly ovoid or subglobose	three-celled, glabrous, prominently pitted, usually narrowly elongate (35–60 mm long) and beaked
Seeds	several per locule, lanate, the seed fibres white, tan, or red-brown	several per cell, free or fused together, lanate, the fibres usually white

## SECTION 4 DEVELOPMENT

Agronomically, the growth of cotton can be divided into three key developmental phases: (1) reproduction and dispersal, (2) germination and seedling establishment and (3) leaf area and canopy development. Total developmental time for *G. hirsutum*, from germination to maturation of the first fruit, is usually approximately 15–17 weeks, although this may be affected by temperature and other environmental variables (Oosterhuis & Jernstedt 1999; Ritchie et al. 2007).

### 4.1 Reproduction

Cotton plants generally reproduce sexually, although there have been reports of cuttings rooting as discussed below in Section 4.1.1.

#### 4.1.1 Asexual reproduction

In a natural situation cotton does not reproduce vegetatively, however there has been rooting under experimental conditions. Cuttings of *G. barbadense* (referred to as *G. vitifolium*) can be propagated (Khafaga 1983a; Khafaga 1983b) under laboratory conditions, where significant rooting only occurs where the cuttings are several internodes long and the parent plants are between six to ten weeks old (Khafaga 1983a; Khafaga 1983b). Other work with *G. barbadense* cuttings indicated that few roots formed without application of naphthaleneacetic acid (NAA) or tannic acid (Fadl & El-Ghandour 1975). In *G. hirsutum* and a *G. hirsutum* x *G. barbadense* hybrid, rooting of semi-hardwood cuttings was observed under experimental conditions, but only when hormones (indole butyric acid and NAA) were applied (Sheelavantar et al. 1975). *G. hirsutum* has also been successfully grafted onto a different root stock, thus achieving asexual reproduction (Rea 1931; Rea

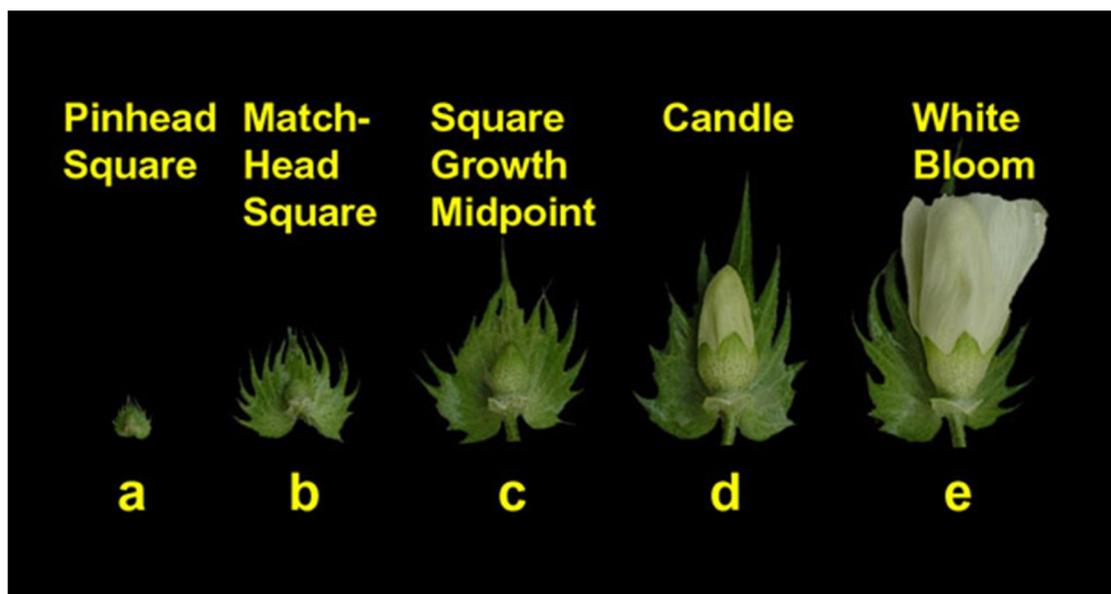
1933). To be successful the grafts had to be completed less than one hour after the pieces were cut and the cambial layers carefully aligned before sealing the graft with paraffin.

#### 4.1.2 Sexual reproduction

Reproductive maturity is reached approximately four to five weeks after planting, with the formation of floral buds ('squares'). The floral buds first appear as small pyramidal structures which are composed of three large green bracts which completely enclose the developing flower (Figure 5). Typically, approximately 25 days elapse between the initial appearance of a square and anthesis (flower opening) (Oosterhuis & Jernstedt 1999; Ritchie et al. 2007).

#### Figure 5 Cotton flower development

Development of the bud from match head square (a) to flower (e) involves both a size increase and petal development. Two bracts have been removed from each square, candle and bloom to show this development. (used with permission from Ritchie et al. 2007)



*G. hirsutum* generally begins to flower 775 day degrees (see Section 2.3.3 for description) after planting (Bange et al. 2002), and *G. barbadense* requires at least 100 day degrees more than *G. hirsutum* to reach full maturity (Cotton Seed Distributors Extension and Development Team 2005).

Generally *G. hirsutum* is planted in NSW in October-early November and flowering will occur approximately 80 days later, with peak flowering occurring at the end of January to early February (Bange et al. 2002). *G. barbadense* is generally planted earlier in the season rather than later and in most regions of Australia *G. barbadense* planting should be finished by mid October to ensure adequate season length (Cotton Seed Distributors Extension and Development Team 2005). The flowering of modern cotton varieties is not sensitive to daylength but may still show a preference for fruiting under cool nights and mild water stress by increasing fruit set under these conditions (Hearn 1981).

Under normal crop conditions, approximately 60% of squares and immature fruit are abscised prematurely. Mature flowers are not usually shed before pollination (Oosterhuis & Jernstedt 1999). The flowers open in a predictable sequence, as illustrated in 0b, with the first flower opening low on the plant and closest to the stem. Approximately three days later the next flower will open in the same relative position on the next highest branch, and

three days after that the next flower will open on the lowest branch. Thus the flowering progresses in an upwards and outwards spiral pattern (Oosterhuis & Jernstedt 1999).

Cotton flowers anthesise at or near dawn and remain open for only one day. Approximately 90% of the flowers opening on a single day do so within a single hour (Beasley 1975). *G. barbadense* flowers begin opening slightly earlier in the day than *G. hirsutum* flowers (Brubaker et al. 1999a). At anthesis, the petals of *G. hirsutum* are creamy white. They turn pink-red within one day of pollination, after which they abscise. Flowers of *G. barbadense* are yellow at anthesis but also turn pink (Oosterhuis & Jernstedt 1999). Cotton has an indeterminate flowering pattern and thus flowers are initiated over a period of several weeks (Cherry & Leffler 1984). At the peak of flowering there are usually four flowers open on each cotton plant (McGregor 1976).

## 4.2 Pollination and pollen dispersal

### 4.2.1 Pollen

Soon after anthesis, the anthers of cotton flowers dehisce, discharging their pollen. In *G. barbadense* the pollen is released just prior to anthesis and is therefore available as soon as the corolla has expanded enough to permit entry for insects. The *G. hirsutum* pollen is shed later, after the corolla aperture is large enough for pollinators to gain access (Brubaker et al. 1993). The stigmas are receptive soon after this, so generally the flowers are self-pollinated as no self-incompatibility mechanisms exist. Cotton pollen is relatively large with long spines. There is some confusion over which species has the larger pollen grains (El Nagger 2004), but most authors have stated that *G. barbadense* pollen is larger than *G. hirsutum* (Kakani et al. 1999; Kearney & Harrison 1932; Saad 1960) (Table 7).

**Table 7 Pollen size and spine length of *G. hirsutum* and *G. barbadense***

Species	Size ( $\mu\text{m}$ )	Spines ( $\mu\text{m}$ )	Spine density	Reference
<i>G. hirsutum</i>	85–88	7.5	-	(El Nagger 2004)
	100.9	12.1	$8.3 \times 10^{-3}$ spines/ $\mu\text{m}^2$	(Kakani et al. 1999)
	$103 \pm 6.2$	-	-	(Saad 1960)
<i>G. barbadense</i>	66–73	11	-	(El Nagger 2004)
	117.9	15.4	$4.9 \times 10^{-3}$ spines/ $\mu\text{m}^2$	(Kakani et al. 1999)
	$115 \pm 9.0$	-	-	(Saad 1960)

The viability of *G. hirsutum* pollen decreases rapidly after 8 hours (Govila & Rao 1969; Richards et al. 2005). High temperatures found in *G. hirsutum* flowers which are exposed to full sun has been shown to lead to reduced pollen grain germination *in vitro* (Burke et al. 2004; McGregor 1976). A study of the cardinal temperatures (lowest, highest and optimum for survival) of 12 cultivars of cotton gave averages for pollen germination and growth of 14°C (minimum), 31°C (optimum) and 43°C (maximum) (Kakani et al. 2005). Pollen grains germinate within 30 min after deposition on the stigma then fertilisation of ovules occurs within 24–48 after pollination (Pundir 1972). For full fertilisation leading to a full complement of seed approximately 50 ovules must be fertilised therefore at least 50 viable pollen grains must contact the stigma (McGregor 1976). A greater number of pollen grains on the stigma has been shown to lead to faster pollen tube growth in *G. hirsutum* (Ter Avanesian 1978).

As the pollen tube grows down the style, its nucleus moves a few microns ahead of the sperm. The sperm and contents are discharged into the germ sac of the ovule after

approximately 15 hours in *G. hirsutum* (Gore 1932). Fertilisation is completed from 24–30 hours after opening of the flower (Gore 1932).

#### 4.2.2 *Pollination*

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).

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 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). 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. Cotton has been considered a major honey plant, with *G. barbadense* producing more nectar than *G. hirsutum* (Vansell 1944). The larger volume of nectar and the larger number of flowers in *G. barbadense* led Vansell to conclude that one acre of *G. barbadense* is equivalent to 30 acres of *G. hirsutum* for honey production. 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 that honey bees do not collect cotton pollen has not been determined. It was thought to be slightly repellent to bees (Moffett et al. 1975) due to the gossypol concentration (Moffett 1983), however, neither *G. barbadense* nor *G. hirsutum* pollen contains gossypol (Loper 1986). The relatively large size of cotton pollen and absence of pollenkitt (sticky material) on the surface of the pollen of *G. hirsutum* have also been discounted, in favour of the theory that the spines affect packing (Vaissière & 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.

#### 4.2.3 *Out-crossing rates*

Insect prevalence strongly influences out-crossing rates for cotton (Elfawal et al. 1976; Llewellyn et al. 2007; Pheloung 2001), and varies with location and time (Elfawal et al.

1976; Moffett et al. 1976; Moffett et al. 1975). Insect visitation rates, however, may over estimate cross-pollination rates because many potential pollinators preferentially target nectaries rather than the pollen (Moffett et al. 1975; Rao et al. 1996). Many field-based assessments, in Australia and overseas, estimate out-crossing at 10% or less (Elfawal et al. 1976; Gridley 1974; Llewellyn & Fitt 1996; Meredith & Bridge 1973; Umbeck et al. 1991). Higher estimates have been reported in a few cases (Smith 1976).

The level of out-crossing observed in Australia is in the order of 1 to 2% between plants in adjacent rows (Llewellyn & Fitt 1996; Mungomery & Glassop 1969; Thomson 1966). This is relatively low compared to that seen in some other countries. Differences in pollinator species may be responsible for the lower rate, in particular the absence of bumble bees, which are known to be very effective pollinators (Llewellyn & Fitt 1996).

Cotton pollen dispersal studies consistently demonstrate that when out-crossing occurs, it is localised around the pollen source and decreases significantly with distance (Chauhan et al. 1983; Elfawal et al. 1976; Galal et al. 1972; Llewellyn & Fitt 1996; Thomson 1966; Umbeck et al. 1991). This presumably represents the effective foraging range of insect pollinators.

In Australia, studies using plots of GM *G. hirsutum* surrounded by buffer rows of non-GM *G. hirsutum* have observed pollen flow into the non-GM cotton (Llewellyn & Fitt 1996). The levels of out-crossing varied between seasons and with wind direction. The highest level of out-crossing (0.9%) occurred in the first buffer row. Beyond 10 m, out-crossing events were generally rare, with 0.01% out-crossing detected at up to 16 m, and no out-crossing detected between 16 and 20 m. Further experiments have indicated that out-crossing is rare beyond 20m, averaging 0.0035% of seed tested (Llewellyn et al. 2007).

Similar findings have been obtained by cotton breeders in previous studies under Australian conditions. For example, Mungomery and Glassop (Mungomery & Glassop 1969) looked at out-crossing from a red leafed (partly dominant) variety of *G. hirsutum* planted within a field of green leafed *G. hirsutum* during two seasons in Biloela (QLD). Cross-pollination between adjacent rows of *G. hirsutum* was around 1.7% in both years, falling to less than 1% in rows beyond this. In one of the two growing seasons, 0.3% outcrossing was detected on the northern side at 53 m.

The above experiments were all performed in southern cotton growing areas of Australia. The possible expansion of cotton into tropical northern regions (see Section 2.3.2), has prompted investigations into out-crossing in these areas with higher insect numbers and different environmental conditions (Llewellyn et al. 2007). In Kununurra, WA, outcrossing rates were higher than seen in southern Australia, with 7.9% at 1 m, falling to 0.79% at 50 m. A similar, earlier experiment had recorded much higher outcrossing rates of 30% at 1 m then down to 0.76% at 50 m. These higher rates were thought to be due to large numbers of pollinators due to beehives in an adjacent field (Llewellyn et al. 2007). A previous experiment looked at out-crossing from a red leafed (partly dominant) variety of *G. hirsutum* planted within a field of green leafed *G. hirsutum* (Thomson 1966) in the Ord River valley, WA over two growing seasons. Cross-pollination between adjacent plants, measured as the proportion of red leafed progeny, was in the range of 0 to 5%, with mean values of 1.6% and 1.0%, in the first and second seasons, respectively. Very little cross-pollination was detected at a distance of more than 3 m (average less than 0.01%) and none was detected at distances between 3 and 8 m. However, insecticides were applied at least weekly to control insect pests as without the sprays it was not possible to obtain seed, which would have affected the abundance of insect pollinators.

In Mississippi in the USA, Umbeck *et al.* (1991) also investigated pollen dispersal from GM *G. hirsutum* embedded in a field of non-GM cotton. They found higher out-crossing

rates (up to 5.7% in the first buffer row), but as with the Australian studies, the rate of out-crossing fell rapidly with distance from the GM block. The level of out-crossing was generally below 1% at 7 m, but a low level of sporadic out-crossing was seen at distances of up to 25 m. Out-crossing at distances greater than 25 m was not measured. A later study in California, USA found higher outcrossing rates in a field where honey bees were present (7.6% at 0.3 m) compared to a field in an area with fewer bees (4.9% at 0.3 m) (Van Deynze et al. 2005). In a field in which bees were present 0.32% outcrossing was still detected at 30 m. In Greece, a study of outcrossing using phenotypic traits showed 2.2% outcrossing at 1 m, dropping to zero at 10 m, whereas a second experiment had a slightly higher rate of 3.8% at 1 m, dropping to zero beyond at 20 m (Van Deynze et al. 2005; Xanthopoulos & Kechagia 2000).

There have also been reports of out-crossing occurring over longer distances for *G. hirsutum*. Van Deynze et al (2005) measured pollen-mediated gene flow in California, between a herbicide resistant pollen source field and commercial cotton fields. The fields were separated by open space and sampling occurred in each of three years, at distances of 200, 400, 800 and 1625 m away from the GM pollen source field. From this study, pollen mediated gene flow was found to vary over the three years, ranging from 0.01 to 0.1% at distances between 200 and 1625 m; gene flow was on average less than 0.1% at 400 m and an average of 0.04% was detected at 1625m on the basis of samples taken at three different sites over three years.

More recently, Heuberger et al (2010) developed an empirical model for gene flow patterns for cotton in the commercial agricultural landscape which simultaneously accounted for the effects of pollinator abundance, the area of relevant surrounding fields and seed mediated gene flow over an initial range of 3 km. These authors found that pollen mediated gene flow rates were low (especially as compared with seed-mediated gene flow) and concluded that GM cotton fields at distances more than 750 m from the edge of monitored non-GM fields did not appear to contribute to outcrossing.

Under Australian conditions no out-crossing was detected 1800m from the pollen source (Llewellyn et al. 2007). The higher out-crossing rates seen in the USA compared to Australia is thought to be due to the presence of bumblebees (*Bombus* sp) (Llewellyn & Fitt 1996).

Studies of pollen movement by bees has shown that *G. barbadense* pollen is transported a similar distance to *G. hirsutum* pollen despite its larger size and longer spines (Galal et al. 1972; Llewellyn & Fitt 1996; Reddy et al. 1992b), with around 8% cross pollination occurring within the first 2 m, falling to less than 2% at 8 m and negligible cross pollination detectable at a distance of 20 m.

The studies cited above measured out-crossing through buffer rows of cotton. The out-crossing rate in the absence of buffer rows, between cotton plants separated by bare ground, might be expected to be higher. For instance, Green and Jones (Green & Jones 1953) demonstrated in Oklahoma, USA that out-crossing through buffer rows of *G. hirsutum* decreased from 19.5% at 1.1 m to 2.6% at 9.6 m and 1.0% at 10.7 m. By comparison, out-crossing in the absence of a buffer decreased from 6.0% at 5.0 m, to 4.7% at 10.0 m, and 0.6% at 25.1 m. An Egyptian study measured out-crossing from *Gossypium barbadense* and also demonstrated a rapid decline with distance over fallow ground from an average level of 7.8% at 1.1 m to 0.16% at 35.2 m (Galal et al. 1972). In an Australian study, out-crossing occurred over 50 m of bare ground to give an average level of 1.9% in the first row of the cotton plants (Llewellyn et al. 2007). The out-crossing level dropped to 0.19% at 5 m into the cotton field, suggesting that pollinators did not carry viable pollen far into the field to effect pollination but remained at the edges. In northern Australia, the

out-crossing rate over 50 m of bare ground was 0.3% (Llewellyn et al. 2007), lower than in Southern regions.

As bees are sensitive to insecticides, it should be noted that extensive use of insecticides for control of insect pests will essentially limit the extent of cross-pollination (Jenkins 2003) due to repellence as well as bee mortality (Rhodes 2002).

### 4.3 Fruit/seed development and seed dispersal

#### 4.3.1 *Fruit development*

Approximately five to seven days after a flower appears it usually dries and falls from the plants exposing the developing cotton fruit or boll (Ritchie et al. 2007).

The growth and development of the boll begins immediately following fertilisation although the most rapid period of growth occurs after approximately 7–18 days (Oosterhuis & Jernstedt 1999). During development, the bolls are spherical to ovoid and pale green. Boll development can be separated into three phases. Initially the cotton fibres elongate and the maximum volume of the boll and seeds are attained. After three weeks, the filling phase begins in which cellulose is deposited inside the hollow cotton fibre. After approximately six weeks the boll maturation phase begins and the boll dries out (Ritchie et al. 2007). Each mature boll is divided into three, four or five locks, each lock contains several seed surrounded by their long staple or fibres (Berardi & Goldblat 1980) producing in total 29–34 seed per boll (Yasuor et al. 2007). Mature bolls are thick and leathery, and dry rapidly to become brittle and brown. Such fruit often split open, revealing the seeds and associated fibres. Since seed cotton is usually harvested only once or twice, many open bolls remain in the field for a considerable time before harvest (Cherry & Leffler 1984). Once the bolls open and the fibre covered seed are exposed to the weather, seed quality deteriorates producing loss of vigour and reduced germination potential (Hopper & McDaniel 1999).

Cotton fibres are unique amongst vegetable fibres as they are derived from single epidermal cells (Smith 1995). The initiation of lint development does not depend on pollination or fertilisation as it begins as soon as the flower opens (Gore 1932). Approximately 20% of the epidermal cells per seed begin to elongate immediately after anthesis and will grow long enough to be spun into fibre. Other epidermal cells begin to elongate approximately six days after anthesis and form the short thick fibres called linters. During the elongation phase the fibre consists of a primary and secondary wall, a layer of protoplasm and the lumen (central vacuole). In the filling phase cellulose microfibrils are deposited on the inside of the lumen and can be observed under a microscope as daily growth rings. During the final maturation phase the fibre dries and the lumen collapses, producing the twisted ribbon-like appearance (Smith 1995).

The mature cotton seed is a pointed oval shape, approximately 8–12 mm in length, consisting principally of a hull and kernel, with a thin membrane separating the hull from the kernel. The gossypol pigment glands are visible as 100–400 µm long oval shaped specks throughout the kernel tissues (Berardi & Goldblat 1980).

Under Australian conditions a *G. hirsutum* plant produces approximately 29–40 seeds per boll (Eastick 2002; Yasuor et al. 2007) with 10–12 bolls per plant (Eastick 2002; Roche & Bange 2006). *G. hirsutum* and *G. hirsutum* x *G. barbadense* interspecific hybrids grown in Turkey produced a slightly higher number of bolls per plant (13–21) (Basbag & Gencer 2007), yet data from the former USSR suggested that *G. hirsutum* C-15 cultivar produced up to 33 bolls (Ter Avanesian 1978). Data on *G. barbadense* from Sudan indicated that

approximately 10 bolls per plant were produced (Siddig 1967), although this data is not from modern cultivars.

#### 4.3.2 *Seed dispersal*

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). At maturity the bolls split open, and under natural conditions the fibres can catch the wind and facilitate seed dispersal (Calhoun & Bowman 1999).

In commercial cotton farming, some cotton seed may be lost from the plants into the fields during harvesting. Some dispersal of cotton seed may also occur in areas where cotton seed is stored. Seed is stored on farms in various ways (for example in sheds) that maintain its quality and protect it from animals and weathering thereby limiting dispersal. Wider dispersal of cotton seed may occur during transport, stockfeeding, adverse weather conditions and animals and these are discussed below.

##### TRANSPORT

The amount of cotton seed being transported and the distances transported depends on the amount of the cotton grown each year and its end use. This can be highly variable, for example, cotton seed is used as a supplementary food for cattle in drought, so transport to these areas would increase (Knights 2007; NSW-DPI 2007).

There are three sources of transported seed that may be distributed onto roadsides (Addison et al. 2007). These are:

- seed cotton (as harvested from the plant) escaping during transport from the field to the gin
- seed which had been ginned escaping during transport away from the gin to oil crushing facilities or for stock feed. In the case of *G. hirsutum* this is commonly called 'fuzzy seed' as it is still coated with linters
- planting seed escaping during transport to cotton farms for planting. For *G. hirsutum* this seed is delinted and is often called black seed.

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.

##### DISPERSAL VIA USE AS STOCKFEED

As discussed in Section 2.2, cotton seed is fed to both sheep and cattle as a protein supplement, although the amount of *G. barbadense* seed available is much lower than that of *G. hirsutum* reflecting the smaller quantity of *G. barbadense* grown. The quantity of cotton seed used is generally limited to a relatively small proportion of the diet, and must be introduced gradually to avoid potential toxic effects due to the presence of anti-nutrients (that is gossypol and cyclopropenoid fatty acids) in cotton seed (see Section 5.1).

Farrell and Roberts (Farrell & Roberts 2002) surveyed nine dairy farms which used cotton seed to feed cattle and observed instances of spilled cotton seed. These seed were observed in seed storage areas, along paths in feed lots and grazing paddocks.

In addition to seed dispersal during feeding, a small percentage of cotton seed consumed by stock can pass through the digestive system intact and is able to germinate (Eastick 2002). *G. barbadense* seed is not digested as thoroughly as *G. hirsutum* and so more

whole seed is likely to pass through into the faeces (Solomon et al. 2005; Sullivan et al. 1993a; Sullivan et al. 1993b; Zinn 1995). It has been estimated that 11% of fed *G. barbadense* cotton seed are excreted whole compared to 5.2% of the *G. hirsutum* cotton seed that is fed to cattle (Sullivan et al. 1993a), although other studies have indicated that as much as 347 g/day/cow of whole (Sullivan et al. 1993b) 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.

#### DISPERSAL VIA WIND

The fibres on cotton seeds may facilitate dispersed by wind (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 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.

#### DISPERSAL VIA FLOODING OR OTHER EXTREME ENVIRONMENTAL CONDITIONS

Some seed from cotton plants may be dispersed from areas where the cotton is grown or harvested, or from areas used for stockfeed and storage of GM cotton seed, during flooding or other extreme environmental conditions such as cyclones. Seed may also be washed into drains, creeks, rivers and sinkholes close by.

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, eg through flooding or irrigation run-off, but 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. 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.

If seed were dispersed, it is not expected to survive as seeds of modern cotton varieties have been bred to be soft-seeded (Hopper & McDaniel 1999; Mauncy 1986). The viability of *G. hirsutum* cotton seed is affected by moisture (Halooin 1975) and extended soaking of both *G. barbadense* and *G. hirsutum* seed in water generally reduces cotton seedling emergence and results in smaller seedlings (Buxton et al. 1977). Areas that get flooded regularly may not be favourable for commercial production, as cotton plants are poorly adapted to waterlogging (Hodgson & Chan 1982). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice would reduce the dispersal of seed.

In the event of cotton seed reaching the sea, experiments using seawater showed that the viability of modern cultivated cottons with thin seed coats decreased markedly after one week, probably due to the thin seed coat enabling rapid water uptake (Stephens 1958). Delinted and acid-treated *G. hirsutum* seeds sink in salt water (Guppy 1906 as cited in Stephens 1958), thus they unlikely to be dispersed and survive.

### DISPERSAL BY ANIMALS

Mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll that retain most of the seeds on the plant (Llewellyn & Fitt 1996). In Australia, there are no reports of mammals, including rodents, feeding on mature cotton bolls or carrying seed cotton any great distance from the cotton fields. Similarly there is no evidence of avian species transporting cotton seeds. 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).

## **4.4 Seed dormancy and germination**

### **4.4.1 Seed dormancy**

Primitive cotton accessions of cotton generally have a high percentage of 'hard seed'. On drying these become impermeable to water and suffer delayed germination (Christiansen & Moore 1959). This is a positive survivability mechanism in wild cotton. Agronomically, hard seeds are undesirable and the trait has been largely eliminated from modern commercial cultivars through breeding and selection (Hopper & McDaniel 1999; Mauncy 1986). Cotton seed in commercial trade must be handled properly to preserve germination quality. In humid environments, seed left in the field will not usually survive until the next season (Jenkins 2003). The existence of a soil seed bank seems unlikely because dispersed seeds that do not germinate are rapidly weathered, leading to significant decreases in their viability (Hallowin 1975; Woodstock et al. 1985).

It is widely accepted that dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture. This 'induced dormancy' can be overcome in a number of ways including by treatment with hot water, which softens the chalazal plug (Christiansen & Moore 1959), allowing the tissues of the seed and embryo to take up moisture.

In addition to induced dormancy, cotton seeds collected immediately following fruit maturation can display 'innate dormancy' (Taylor & Lankford 1972) – an inherent condition of the mature seed/embryo that prevents the seed from germinating, even when exposed to appropriate environmental conditions. The duration of innate dormancy varies between varieties and timing of maturity (Christidis 1955; Hsi & Reeder 1953). Experiments with *G. barbadense* have shown no significant dormancy (Hsi & Reeder 1953). In *G. hirsutum* it can depend on when in the season the boll opened, with those maturing early in the season requiring 25 days for satisfactory germination, whereas those which mature last needing up to five months (Christidis 1955). A longer experiment determined that *G. hirsutum* seed stored for two years showed higher germination than seed stored for one year, or seed planted the season following harvest (Taylor & Lankford 1972). They also observed that the positive effect of seed age on germination ability could reduce the negative impact of factors that may induce dormancy, such as cold temperature or salinity.

Hopper and McDaniel (Hopper & McDaniel 1999) observed that the 'vigour' of *G. hirsutum* seed – those properties of the seed that determine its potential for rapid, uniform emergence – may vary between seed lots. Seed vigour may indicate varying degrees of innate dormancy. Several researchers have attempted to improve seed vigour by incorporating its selection into *G. hirsutum* breeding programs (see, for example, Bourland 1996).

#### 4.4.2 Germination

The cotton seed imbibes moisture predominately through the chalazal cap which initiates germination. Water uptake is rapid during the first 12 hours for initial wetting, and then continues at a lower rate (Smith 1995). Seedling emergence occurs in five to seven days under appropriate air and soil temperatures. Cold temperatures have a significant effect on cotton germination and can lead to decreased yield, shorter plants and delayed flowering (Table 8). However, fatty acid treatment of *G. barbadense* seeds can overcome the inhibitory effect of cold temperatures on germination (Bartkowski et al. 1978).

**Table 8 Effect of cold stress on *G. hirsutum* seed following planting (Smith 1995)**

Days of chill	Days delayed flowering	Fibre maturity	Percent 1 <sup>st</sup> harvest	Final plant height (cm)
0	0	3.9	60	165
2	3	3.8	59	155
4	6	3.6	54	150
6	10	3.4	46	137

Once the cotyledons have emerged, it may be seven to ten days before the first true leaf appears. This will then be followed by a new leaf every 2.5 to 3 days. (Smith 1995).

*G. hirsutum* is routinely planted when the soil temperature reaches 14°C at a depth of 10 cm for at least three days, and lint yield is adversely affected if planted too early (due to cold temperatures) or too late (due to shortened growing season) (Kittock et al. 1987). However *G. barbadense* is more tolerant of early planting and can show increased yield due to the longer growing season (Kittock et al. 1987; Kittock et al. 1985). Germination field tests have shown some cultivars of *G. barbadense* can have up to 60% germination even when minimum temperatures are as low as 7°C (Bartkowski et al. 1977) whereas the germination of *G. hirsutum* falls to 56% at 10°C (Constable & Shaw 1988).

The type of cotton seed has a large impact on the likelihood of germination (Eastick & Hearnden 2006). Experiments in northern Australia have shown that for *G. hirsutum* black seed, which has been ginned and acid delinted and is used for planting, has the highest germination rate. Seed cotton, directly harvested from the plant, has a low germination rate which is attributed to mechanical impedance of cotyledon emergence through the lint cover (Eastick & Hearnden 2006). Fuzzy *G. hirsutum* seed had an intermediate germination rate between seed cotton and black seed. It is unknown whether the absence of linters for *G. barbadense* impacts on germination potential.

The type of habitat that the cotton seed is dispersed into has also been shown to affect germination for *G. hirsutum*. A study on the spread and persistence of *G. hirsutum* cotton seed showed germination was highest in disturbed habitats, especially if the seed was buried (Eastick & Hearnden 2006). There were highly significant differences between alternative habitats, with germination much less likely to occur in undisturbed bush and roadside sites, than in disturbed sites such as stockyards and the edges of waterways. However, these experiments aimed to maximise the germination and establishment of seedlings, by sowing seed into cleared ground, lightly burying the seeds and then hand-watering. More germination is likely to have occurred using this technique, than if seeds were dispersed naturally and allowed to germinate with rainwater. The density at which seeds were sown also affected germination at a majority of trial sites. Generally, seeds sown at low density germinated poorly and with greater variability than those sown at high density (Eastick & Hearnden 2006).

#### 4.4.3 *Seedling survival*

The survival of seedlings has also been shown to relate to density, with those germinating at highest density showing highest survival rates (Eastick & Hearnden 2006). This study also showed that survival of plants for two years was low, with only eight out of the original 20 sites having at least one surviving plant, although the total number of surviving seedlings was low, and highly variable, ranging from zero at some sites, to approximately 50 plants at other sites. However, there were clear trends indicating that the habitat into which seeds were sown affected survival. Survival at sites located near cattleyards or adjacent to water bodies was consistently high, probably because of high soil nutrients and/or soil moisture. The result is in agreement with field observations that the occurrence of naturalised and volunteer cotton appears to be limited by the availability of adequate soil moisture (Addison et al. 2007).

Grazing and trampling may also limit seedling survival. In the study of *G. hirsutum* in northern Australia grasshoppers appeared to be the most common and destructive insect herbivores. Grazing and trampling by cattle were also factors which prevented seedling survival and growth (Eastick 2002; Eastick & Hearnden 2006).

#### 4.5 *Vegetative growth*

Following germination, plant growth continues with the development of a central, main stem that bears the first true leaves spirally, along its axis. Leaves are typically 10–15 cm wide, palmately-lobed, with 3–7 lobes on each leaf.

Branching of the main stem occurs initially from axillary buds of the main stem leaves. Either vegetative (monopodial) or fruiting (sympodial) branches are produced. Both branch types bear true leaves, but approximately 5–6 weeks after planting the total area of leaves born on fruiting branches exceeds that of the main stem and vegetative branches, constituting approximately 60% of the total leaf area at maturity (Oosterhuis & Jernstedt 1999).

### SECTION 5 *BIOCHEMISTRY*

Cotton is not a pathogen and not capable of causing disease in humans, animals or plants. However, it does contain a number of compounds which have adverse effects on human and animal health. The most studied of these is gossypol [1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-(2,2-binaphthalene)-8,8'-dicarboxaldehyde]. This is a yellow polyphenolic compound found primarily in the pigment glands of the cotton plants on the seed, leaves and roots (Coutinho 2002; Smith 1961) and is generally removed before cotton seed can be eaten. However, gossypol has also been investigated as a human medicine, as a male contraceptive, anti-cancer drug and anti-hypertensive agent (Blackstaffe et al. 1997; Coutinho 2002; Hasrat et al. 2004). Cotton plants also contain cyclopropenoid fatty acids (CPFA) in the seed and tannins in the leaves (Lane & Schuster 1981; Mansour et al. 1997) and flower buds (Chan et al. 1978) which are both thought to act as deterrents to insect herbivory and may affect utilisation as animal feed.

#### 5.1 *Toxins*

Cotton (*G. hirsutum* and *G. barbadense*) tissue, particularly the seeds, can be toxic 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).

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, to a relatively small proportion of the diet and it must be introduced gradually to avoid potential toxic effects (Blasi & Drouillard 2002).

### 5.1.1 *Gossypol*

Gossypol intake from cotton seed feeding of lactating dairy cows has been shown to cause increased plasma gossypol concentrations and erythrocyte fragility (Mena et al. 2001). In red deer, consumption of 1.7% of body weight of cotton seed led to reduced antler growth (Burns & Randel 2003; Sullivan et al. 1993b). However, no effect of cotton seed consumption was seen on reproductive development in brahman bulls (Chase, Jr. et al. 1994) and overseas studies report that feeding cotton seed meal up to 30% of diet shows no evidence of gossypol toxicity to sheep (Kandylis et al. 1998). Inactivation or removal of gossypol and cyclopropenoid fatty acids during processing enables the use of some cotton seed meal for catfish, poultry and swine.

Generally the fatty acid composition of *G. barbadense* and *G. hirsutum* seed (Khalifa et al. 1999; Khattab et al. 1977) and oil (Pandey & Thejappa 1981) are similar. However, *G. barbadense* cotton seed does not possess linters and has been shown to be digested differently in cattle compared to *G. hirsutum*, possibly due to the naked seed. It is believed that the unlinted cotton seed sinks in the rumen so is less masticated and therefore less digested than linted cotton seed (Coppock et al. 1985). This leads to a higher proportion of the *G. barbadense* seed appearing undigested in the faeces (Solomon et al. 2005; Sullivan et al. 1993a; Sullivan et al. 1993b; Zinn 1995). To improve the digestibility of the *G. barbadense* seed it is often cracked prior to feeding to cattle but this increases the animals' exposure to gossypol. Cotton seed is used extensively throughout QLD as a feed supplement for sheep, however it is recommended that care should be taken when feeding *G. barbadense* seed as, due the absence of lint it can be consumed faster and therefore intakes can be higher (Knights & Dunlop 2007).

Gossypol in cotton seed exists in both the free and bound forms. In intact whole seed the gossypol is found in the biologically active, free form, however heat or moisture occurrence during processing causes the gossypol to bind to proteins creating the less toxic bound form. In ruminants, with a well-developed rumen microflora, free gossypol can be converted to bound gossypol, thus preventing it entering the bloodstream (Santos et al. 2002).

Gossypol content, form and enantiomer differ between the two cotton species. The gossypol content of *G. barbadense* cotton is generally higher than that of *G. hirsutum* (Table 9) with more of the gossypol in the more biologically active, free (unbound) form. The difference in composition alters the amount of cotton seed of *G. barbadense* and *G. hirsutum* cotton that is recommended for cattle feed. It has been suggested that adult cattle should have less than 0.1–0.2% free gossypol in the total ration, which amounts to 1.8–2.7 kg of *G. hirsutum* seed per day or 2.7–3.6 kg of unprocessed *G. barbadense* seeds per day (Kirk & Higginbotham 1999). The higher free gossypol levels in cracked *G. barbadense* seed resulted in higher plasma gossypol concentrations in dairy cows, but this did not significantly affect milk yield (Prieto et al. 2003; Santos et al. 2002). However, the cows which consumed cracked *G. barbadense* seed at approx. 7.5% of their diet had reduced fertility as seen by decreased conception rates and increased incidence of abortions (Santos et al. 2003).

**Table 9 Gossypol concentration and composition in cotton seed**

	<i>G. barbadense</i>	<i>G. hirsutum</i>
Total gossypol (% DM)	0.60–1.15	0.51–0.77
Free gossypol (% DM)	0.93–1.08	0.47–0.70
(-) – isomer (% total gossypol)	51.2–54.1	35.4–43.4
(+) – isomer (% total gossypol)	45.9–48.8	56.6–64.6

Data compiled from values presented in (Arana et al. 2001; Prieto et al. 2003; Santos et al. 2002; Sullivan et al. 1993a).

Gossypol also exists as two different enantiomers (mirror image forms of the same compound) as it has chiral rotation about the binaphthyl bond. These two enantiomers have different toxicity levels and are present in different relative proportions in *G. barbadense* and *G. hirsutum* cotton (Stipanovic et al. 2005), with *G. barbadense* cotton containing more of the (–)- gossypol (Sullivan et al. 1993a). This form has been shown to have greater biological activity (Wang et al. 1987). Studies have shown that toxicity of the gossypol enantiomers varies between different animals.

Generally the (–)- gossypol isomer is thought to be more toxic from studies on rats (Wang et al. 1987) and appears to be more detrimental to fertility of male hamsters (Lindberg et al. 1987; Matlin et al. 1985) and rats (Wang et al. 1987). Similarly, broiler chickens showed reduced weight gain when fed cotton seed containing a higher proportion of (–)-gossypol isomer (Bailey et al. 2000; Lordelo et al. 2005). However, a study of laying hens fed the two different isomers provided evidence that the (+)- gossypol is more toxic, showing increased tissue accumulation of gossypol, increased egg discolouration and reduced egg weight compared to those fed the (–)- gossypol enantiomer (Lordelo et al. 2007).

Studies investigating the toxic effects of the two gossypol enantiomers have also been conducted on the plant pathogen *Rhizoctonia solani* (Puckhaber et al. 2002) and the insect pest *Helicoverpa zea* (Stipanovic et al. 2006). Both the (+) and (–)-gossypol enantiomers, or a mixture were equally effective at inhibiting the growth of *R. solani* and *H. zea*.

As discussed in Section 2.2, cotton seed meal or flour has been sold for use in human food. Various studies (summarised in (Berardi & Goldblat 1980) have observed no deleterious effects when moderate amounts of cotton seed products containing low levels of gossypol have been consumed. It is also stated that there are no reports of gossypol toxicity in humans who have consumed gossypol-containing products.

### 5.1.2 Cyclopropenoid Fatty Acids

Cotton also contains cyclopropenoid fatty acids (CPFA) such as malvalic, sterculic and dihydrosterculic acids, which constitute approximately 0.5–1.0% of the total lipid content of the seed (Schneider et al. 1968). The level of CPFAs is generally higher in *G. hirsutum* than in *G. barbadense* (Frank 1987). The CPFAs are destroyed by the processing of cotton seed oil for use in margarine or salad oil for human food (Hendricks et al. 1980), but can produce undesirable effects when used in less processed animal feed. For example, rainbow trout (*Salmo gairdnerii*) fed glandless cotton seeds, showed reduced weight gain and liver carcinomas (Hendricks et al. 1980). Glandless cotton seed do not produce gossypol so the resulting effects have been attributed to the CPFA. Similarly, cockerels

fed cotton seed oil (estimated to contain 0.5–0.7% CPFA (Obert et al. 2007) or the equivalent concentration of CPFAs from *Sterculia foetida* caused increased plasma cholesterol and aortic atherosclerosis (Goodnight, Jr. & Kemmerer 1967). Hens fed cotton seed meal show pink coloration of the white of the eggs following storage, which has been attributed to CPFAs (Phelps et al. 1964).

## 5.2 Allergens

Cotton pollen is not allergenic. It is relatively large, sticky and heavy, and not easily dispersed by wind (McGregor 1976; Moffett 1983), so the potential for cotton pollen to act as an airborne allergen is particularly low.

Inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. In the 1970's the incidence of this disease was estimated at 20–50% in cardroom workers and 5–10% in spinners (Nicholls 1992). Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition, and there is some evidence that the condition may be due to fungal contamination of the cotton dust (Salvaggio et al. 1986).

*G. hirsutum* linters are a major component of house dust, a known allergen, although some individuals are actually sensitive to the house dust mite rather than the dust itself (Nicholls 1992). *G. barbadense* cotton seed does not possess linters and therefore does not contribute to this dust.

No allergic reactions to fats (including cotton seed oil) have been reported in people. The processing of cotton seed oil involves a series of steps including heating, addition of sodium hydroxide, bleaching with clay, filtering and treating with steam under vacuum (OECD 2004). These processes are expected to remove all traces of protein from the oil (ANZFA 2001).

Processed cotton fibre contains over 99% cellulose (Wakelyn et al. 2007) and is widely used in pharmaceutical and medical applications because of its low capacity to cause irritation (AgraFood Biotech 2000). The refining and processing of cotton lint (and *G. hirsutum* linters), both chemically and thermally, destroys or removes proteins and nucleic acids to below detectable levels (Sims et al. 1996; USDA 2004).

## 5.3 Beneficial phytochemicals

### 5.3.1 Medicines

Leaf extracts from *G. barbadense* have been used in traditional medicine in Inagua (Bahamas, USA) to cure 'proud flesh' (swollen tissue around a wound), and for nausea during pregnancy (Sawyer 1955). Currently, *G. barbadense* extracts are sold for use in alternative medicine for treatment of hypertension, fungal infections, and as an abortifacient or emmenagogue (menstruation stimulant) (Tropilab Inc 2007). Extracts from *G. barbadense* have been shown to have anti-hypotensive effects in rats (Hasrat et al. 2004) and to increase smooth muscle contraction in guinea pigs (Mans et al. 2004).

Gossypol has also been studied for its use as a treatment for cancer. Human melanoma cells show cytotoxicity to gossypol, with a 5-fold greater cytotoxic sensitivity to the (–)-gossypol enantiomer than the (+)-enantiomer (Blackstaffe et al. 1997), suggesting that the (–)-gossypol enantiomer may have some potential therapeutic benefits in melanoma patients. Gossypol has also been investigated as a human contraceptive, and shown to be highly effective, although it has irreversible effects in approximately 20% of men (Coutinho 2002). It has also been investigated as an antiparasitic agent. *In vitro* experiments showed that gossypol reduced the growth of both *Trypanosoma cruzi*, the causal agent of Chagas

disease, (Montamat et al. 1982) and *Entamoeba histolytica*, which causes amoebiasis (Gonzalez-Garza et al. 1989).

### 5.3.2 *Stock feed*

Cotton seed is a valuable foodstuff for cattle as it combine high energy, high fibre and high protein (Ensminger et al. 1990b). It is generally difficult to maintain both high fibre content for milk fat percentage and high energy density for maximum milk production (Palmquist & Jenkins 1980). In *G. hirsutum* seed, the fibre is composed of linters (approximately 10% by weight of the seed) which is nearly pure cellulose and highly digestible. The seed also contains oil, which gives it a high energy value (Coppock et al. 1985). Cattle and sheep may also be fed cottonseed hulls, which are an important source of roughage. Gin trash is also fed to ruminants, and is thought to have approximately 90% of the food value of cottonseed hulls. (Ensminger et al. 1990a).

## SECTION 6 ABIOTIC INTERACTIONS

### 6.1 Nutrient requirements

Nitrogen and phosphorous are both important nutrients for cotton growth in Australia. Nitrogen levels have a large impact on the yield and quality of lint produced and can also affect the seed yield of cotton plants. Nitrogen deficiency can lead to reduced growth and yield; whereas excess nitrogen can lead to excessive vegetative growth and reduced reproductive growth (Fritschi et al. 2003; Fritschi et al. 2004; Hutmacher et al. 2004; Reddy et al. 2004). Excessive vegetative growth may also lead to increased pest and disease susceptibility (Cisneros & Godfrey 2003) and complicate cotton defoliation. However, the use of growth hormones such as mepiquat chloride can prevent excessive vegetative growth and reduce the effect of excess nitrogen (Fritschi et al. 2003; Sawan 2006; Sawan 2007; Sawan et al. 1998). This has lead to an increase in the amount of nitrogen added to cotton crops in America from 120 kg/ha to around 200 kg/ha (Fritschi et al. 2003).

*G. barbadense* is more sensitive to nitrogen than *G. hirsutum* with excess available nitrogen leading to excessive vegetative growth and reduced yield (Fritschi et al. 2003; Silvertooth 2001; Unruh & Silvertooth 1996a; Unruh & Silvertooth 1996b). When nitrogen is not in excess, increasing nitrogen levels leads to an increase in dry weight and yield, although the response is not as great as that seen in *G. hirsutum* (Fritschi et al. 2003; Fritschi et al. 2004; Reddy et al. 1996). *G. barbadense* plants deprived of nitrogen between flowering and harvest produce 10% less dry weight than nitrogen sufficient plants, compared to 15% less dry weight for nitrogen deficient *G. hirsutum* (Bettmann et al. 2006).

As can be seen in Table 10, Australian soils are rich in many of the required nutrients as symptoms of deficiency are not seen. The process of crop rotation to aid in the control of Black root rot and Verticillium wilt (see Sections 2.3.3 and 7.2.2) may also aid in the maintenance of soil nutrient levels.

**Table 10 Nutrient requirements of commercial cotton grown in Australia<sup>a</sup>**

Nutrient	Uptake per hectare <sup>b</sup>	Removal per hectare <sup>c</sup>	Fertiliser	Deficiency	Toxicity
Nitrogen (N)	180 kg	120 kg	urea ammonium carbonate	small pale yellow leaves, stunted growth, autumn coloured leaves	rank growth, shedding, reduced lint quality, increased susceptibility to insects and disease
Phosphorus (P)	25–30 kg	20–25 kg	Mono-ammonium phosphate (NPK 9:22:0)	stunted growth, dark green or purple foliage, delayed fruiting	
Potassium (K)	200 kg	41–48 kg	potassium chloride potassium sulphate potassium nitrate	Premature senescence, increased susceptibility to insects and disease, yellowish white mottling of leaves, leading to rusty bronze colour, necrotic spots and then shrivelling of leaves. – <b>Not common in Australia</b>	
Zinc (Zn)	100–150 g	100 g	zinc oxide zinc sulphate heptahydrate	interveinal chlorosis <sup>d</sup> , cupped, bronzed leaves, stunted growth, reduced yield and fibre quality	
Iron (Fe)	600 g	80 g	iron chelate	interveinal chlorosis, eventual white leaves <b>Linked to waterlogging</b>	
Copper (Cu)	50 g	20 g	copper chelate copper oxide	chlorosis of lower leaves, dieback of terminal bud in severe cases – <b>not observed in Australia</b>	
Boron (B)	400 g	100 g	borax boric acid	young leaves light green at base, older leaves twisted, flowers deformed, boll shedding.	leaf cupping, chlorosis, necrotic spots.
Calcium (Ca)	220 kg	10 kg	calcium carbonate calcium sulphate	collapsing petioles. <b>Not seen in Australia</b>	
Magnesium (Mg)	24–40 kg	12 kg	dolomite lime magnesium sulphate	purple/red leaves with green vein, premature senescence of mature leaves. <b>Not seen in Australia</b>	high soil Mg ratios with Ca and K affect soil structure
Sulphur (S)	30–50 kg	10 kg	usually provided as part of other fertilizers	yellowing of young leaves, spindly plants, short slender stems. Reduced boll size	
Manganese (Mn)	450 g	60 g	manganese sulphate	leaf cupping, interveinal chlorosis starting with younger leaves, upper leaves may have necrotic spots. <b>Rarely seen in Australia</b>	linked to acid soils. Leaves crinkled, mottles and chlorotic. Can induces iron and zinc deficiency. <b>Linked to waterlogging.</b>

Nutrient	Uptake per hectare <sup>b</sup>	Removal per hectare <sup>c</sup>	Fertiliser	Deficiency	Toxicity
Molybdenum (Mo)	10 g	2–5 g	ammonium molybdate, molybdenum trioxide	interveinal chlorosis, greasy leaf surface with interveinal thickening, leaf cupping and eventual white or grey necrotic spots on the leaf margin. <b>Not seen in Australia</b>	can cause copper imbalance

<sup>a</sup> Compiled from NUTRIpak (Australian Cotton Cooperative Research Centre 2002c)

<sup>b</sup> Amount of nutrient removed from soil during growth

<sup>c</sup> Amount of nutrient removed from field as seed cotton (the remaining nutrients taken up by the plants during growth consist of leaf litter and other plant waste and are usually reincorporated into the soil)

<sup>d</sup> Chlorosis is a yellowing of leaf tissue due to a lack of chlorophyll

## 6.2 Temperature requirements and tolerances

Cotton originated in hot, dry regions and requires consistently hot temperatures for best yield, while dry conditions during boll maturation contribute to fibre quality.

*G. hirsutum* has a base temperature of 12°C, below which all plant development ceases. *G. hirsutum* seedlings can suffer from cold shock when minimum daily temperatures fall below 11°C. However, unless the exposure is prolonged little or no damage will occur and plant development will be delayed, but continue once temperatures rise (Bange & Milroy 2004; McDowell et al. 2007). *G. hirsutum* seedlings can also be killed by frost (Constable & Shaw 1988). As discussed in Section 4.4.2, *G. barbadense* is more tolerant of cool temperatures and early planting than *G. hirsutum*.

*G. barbadense* seedling development in the first two weeks is generally insensitive to temperatures between 15°C and 40°C, although once the seedling has established the height, yield and rate of development can all be affected by temperature (Reddy et al. 1992a; Reddy et al. 1992b). The optimum daytime temperature range for *G. hirsutum* is 30–35°C, with rapid fruit loss above 35°C, and a 50% yield reduction at 25°C (Reddy et al. 1992b), whereas the optimum range for *G. barbadense* is between 25–30°C with only 30% yield at 35°C (Reddy et al. 1992a). A long term study in the USA indicated that the yield differential between advanced cultivars of *G. hirsutum* and *G. barbadense* cotton nearly doubled when mean July temperature increased from 31–35°C (Lu et al. 1997). However, *G. barbadense* cultivars with heat tolerance approaching that of *G. hirsutum* have been developed, mainly through changes in *G. barbadense* stomatal conductance (Cornish et al. 1991; Radin et al. 1994; Srivastava et al. 1995).

## 6.3 Water use

To meet the water demand of cotton (approximately 7 ML/ha of irrigated water utilised per crop) for good economic returns the majority of Australia's crop is grown under irrigation, mostly in the Murray-Darling Basin (Anthony 2003). Alternatively, cotton may be grown as an unirrigated crop known as dryland cotton. In some years up to 20% of the total cotton production area consists of dryland cotton although this has accounted for less than 10% of total production (Australian Cotton Cooperative Research Centre 2002a). When cotton is grown as an unirrigated crop the biggest climatic factor influencing cotton yield is rainfall during January to March (Ford 2002; Gibb & Constable 1995). In this period cotton has a daily water use of up to 8 to 10 mm (Gibb & Constable 1995).

Water use efficiency in cotton can be simply defined as the measure of total yield (lint) produced per unit of water supplied to the crop (Gibb & Constable 1995). Because of the reliance on rainfall that is highly variable, dryland production has the biggest potential to

benefit through improved water use efficiency of cotton plants (Australian Cotton Cooperative Research Centre 2002a; Cooperative Research Centre for Sustainable Cotton Production 1995). However, irrigated cotton may also benefit from improved water use efficiency particularly in drought years where less water is available for irrigation (Cooperative Research Centre for Sustainable Cotton Production 1995), if water allocations were to be reduced because of environmental demands or the cost of water were to rise.

In Australia, waterlogging in cotton is estimated to cause annual yield losses of approximately 1 bale/ha or 11% (Dennis et al. 2000). Waterlogging occurs mainly when heavy rain follows a scheduled irrigation, especially when combined with poorly draining soils and inadequate field slope. In the 2005–06 season in Australia, 84% of cotton was grown as a furrow irrigated crop (Cotton Australia 2006a) and fields are commonly irrigated five or six times during the growing season between flowering and peak boll development (McLeod et al. 1998). In NSW, cotton production occurs mainly on cracking grey clay soils (vertisols) of the Namoi and Gwydir River Valleys which have inherently low drainage rates (Hodgson & Chan 1982).

Research in the early 1980's showed that a 32 hour waterlogging treatment of cotton could lead to yield losses of 42% (Hodgson 1982; Hodgson & Chan 1982), although another study showed a recovery of plants following waterlogging stresses leading to no reduction in yield (Hocking et al. 1987). A more recent experiment following a similar protocol to the Hodgson study recorded approximately 40% yield loss but only when more severe waterlogging conditions were imposed (Bange et al. 2004b). The reduced yield loss due to waterlogging seems to be partly related to improvements in field design and soil structure. An increased awareness of soil management programs by cotton farmers has led to a reduction in soil compaction and there have been improvements in the furrow irrigation fields with more even water flow due to the use of laser guided levelling systems. The more even slope and hill heights have meant that water does not collect in low areas.

Waterlogging damages plants due to low oxygen concentrations (hypoxia) around the roots. This is caused because water displaces the oxygen in the soil, and cannot be replaced by diffusion of atmospheric oxygen. The low oxygen conditions inhibit energy production in the plant roots and other oxygen-dependent pathways, including those involving cytochromes, oxidases and desaturases.

The visual symptoms of waterlogging are initially wilting (Hocking et al. 1985; Reicosky et al. 1985) then leaf chlorosis, premature senescence and reduced boll number, leading to lint yield loss (Hodgson & Chan 1982). Damage to crop yields has already occurred once leaf yellowing is observed (Constable 1995). The impact of waterlogging early in crop growth has a far greater influence on yield than waterlogging at mid-flowering or later (Bange et al. 2004a), although yield loss due to waterlogging can be sustained at all stages of crop growth (Hodgson & Chan 1982).

Uptake of potassium, phosphorus (Hocking et al. 1987) and nitrogen (Constable 1995; Hocking et al. 1985) is impaired in waterlogged cotton, especially in young plants just before flowering and can result in the plants becoming temporarily deficient in these nutrients. During the first three to four days of waterlogging most of the yield loss is due to less nitrogen being absorbed from the soil (Constable 1995).

#### **6.4 Other tolerances**

Cotton is classified as a salt tolerant plant. The most common effect of salinity stress is the general stunting of growth (Cothren 1999). However, salinity also has adverse effects on germination and emergence of cotton (Ashraf 2002). Variation in salt tolerance exists

between *G. barbadense* and *G. hirsutum* with *G. barbadense* being more salt tolerant (Ashour & Abd-El'Hamid 1970).

## SECTION 7 BIOTIC INTERACTIONS

### 7.1 Weeds

Although the weed spectrum varies between fields, there are commonly 60–70 weed species found in cotton fields (Australian Cotton Cooperative Research Centre 2002d). A list of the most important weeds in cotton in Australia can be found in Appendix A. Weeds may impact on the crop in a number of ways, primarily in competition for water and nutrients (Charles 1991). Cotton is particularly susceptible to competition from weeds, which may be a consequence of its ancestral arid environment where it may have been a primary coloniser (Hearn & Fitt 1992). Weeds may also indirectly impact on the cotton crop. They may act as hosts for pests and diseases, adversely affect cotton harvesting or lint quality (Charles 2002), and interfere with water flow through irrigation channels (Charles 1991).

The types of weeds present in fields vary from those such as *Xanthium occidentale* (Noogoora burr), *X. spinosum* (Bathurst burr) and *Datura* spp. (thornapples) which are large plants that compete with cotton, obstruct harvest and contaminate lint (Charles 1991). Thornapples may also host Heliothis, mites and Verticillium wilt (Charles 2002). These are hard seeded plants which represent a long term problem.

Other important weeds may include *Ipomoea lonchophylla* (cow vine) and *Tribulus micrococcus* (yellow vine or spine less caltrop) which can tangle in the picker heads at harvest time, thus requiring frequent head cleaning. Grass weeds such as *Cyperus rotundus* (nut grass) can contaminate the lint and the grass seeds are difficult to remove (Charles 2002). The *Cyperus* spp. produce rhizomes and are resistant to cultivation. One of the most problematic weeds in *G. barbadense* is volunteer *G. hirsutum* which is difficult to recognise but reduces overall lint quality (Cotton Seed Distributors Extension and Development Team 2005).

The weed spectrum varies in different cotton regions with *Sesbania cannabina* (sesbania pea) the main weed in dryland cotton fields in QLD, *Hibiscus trionum* (bladder ketmia) and *Tribulus micrococcus* (caltrop) in southern QLD, and grasses, especially *Urochloa panicoides* (liverseed) and *Echinochloa colona* (barnyard grass), in northern NSW (Taylor & Walker 2006).

The introduction of Roundup Ready<sup>®</sup> GM *G. hirsutum* beginning in 2001–02 season has not significantly affected the weed spectrum reported by growers. A survey of cotton growers in 2003 indicated that the only weed which has become more problematic is *Cyperus rotundus* which is the fourth most common weed in GM glyphosate tolerant fields compared to the seventh in non GM cotton fields (Werth et al. 2006). However, this may be due to farmers preferentially selecting fields with problem weeds such as *C. rotundus* to grow glyphosate-resistant cultivars.

#### 7.1.1 Weed Control

The control of weeds, although expensive, is necessary but may adversely affect growth of the cotton crop itself by herbicide damage or root disturbance due to chipping. In the 1988–89 season control of weeds was estimated to cost \$187/ha of cotton grown in NSW for irrigated cotton (Charles 1991). The highest cost was herbicides followed by chipping costs. A 2001 survey of dryland cotton growers produced a slightly higher weed control estimate of \$220/ha (Walker et al. 2006).

Weeds are commonly managed with a combination of herbicides and hand chipping (Charles 1991). The cotton CRC has developed an Integrated Weed Management guide for cotton which advocates reducing reliance on single herbicide groups and incorporating chipping and cultivation (Roberts & Charles 2002). This should also involve crop rotations, farm hygiene to prevent weed seed spreading and may involve the use of herbicide resistant varieties (Charles 2002).

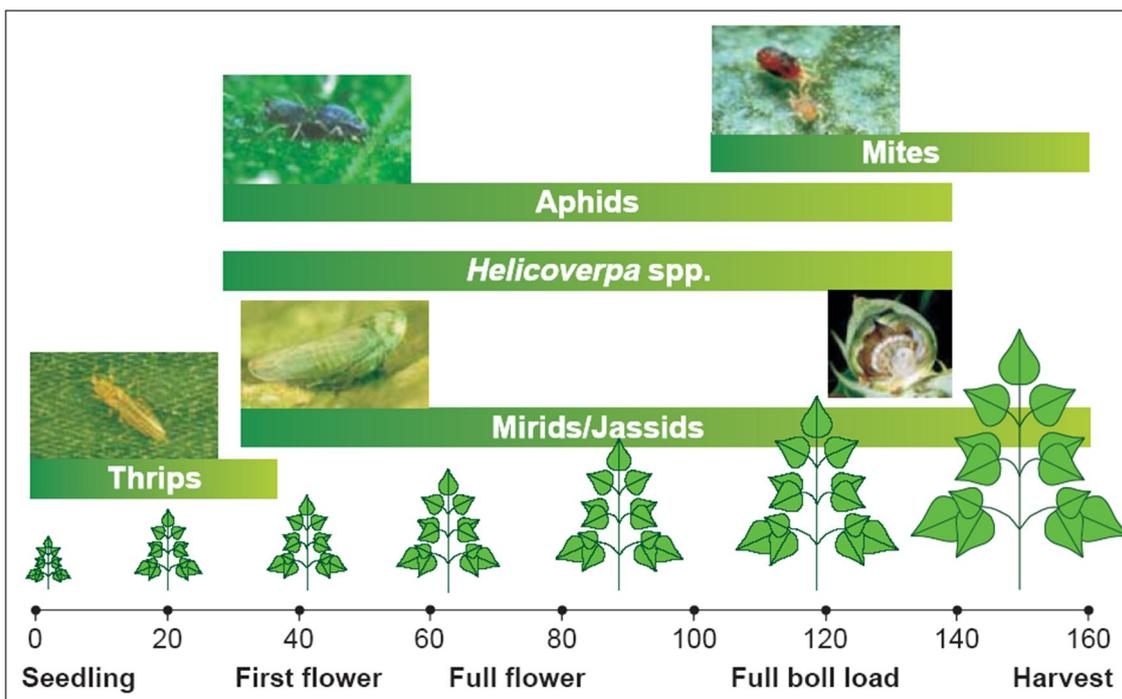
The introduction of Roundup Ready<sup>®</sup> GM *G. hirsutum* has altered the herbicides which are used in cotton fields. A survey of cotton growers in 2003 indicated that glyphosate usage had increased more than four-fold whereas application of other herbicides eg. Group C and D had decreased slightly (Werth et al. 2006). Group C herbicides used on cotton include prometryn and fluometuron and group D include trifluralin and pendimethalin (Charles 2002). A crop management plan for the use of glyphosate tolerant cotton varieties, which specifies an Integrated Weed Management Strategy and a weed management audit endorsed by the TIMS committee, is in place to minimise the potential for development of glyphosate-resistant weeds. Compliance with the crop management plan is implemented through a Technology User Agreement between the grower and Monsanto. There are currently no reports of glyphosate resistant weeds in GM cotton fields in Australia (Cerdeira & Duke 2006).

## 7.2 Pests and pathogens

### 7.2.1 Pests

More than 1326 species of insects have been reported in commercial cotton fields worldwide but only a small proportion are pests (Matthews & Tunstall 1994) with the type and number of pests differing from season to season and between different regions. Of the 30 pests of cultivated *G. hirsutum*, the most important in southern Australia are the caterpillars of *Helicoverpa armigera* and *Helicoverpa punctigera*, and the two-spotted spider mite *Tetranychus urticae* (Pyke & Brown 2000; Shaw 2000). Other pests include cotton aphid (*Aphis gossypii*), green mirid (*Creontiades dilutus*), silverleaf whitefly (*Bemisia tabaci* b-biotype), thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*) and the green vegetable bug (*Nezara viridula*) (Farrell & Johnson 2005). Beneficial predatory insects can include ladybeetles (*Coccinella* spp., *Adalia* spp.), blue beetles (*Dicranolais* spp.), damsel bugs (*Nabis* spp.), big eyed bugs (*Geocoris* spp.), shield bugs (*Cermatulus* spp., *Ochelia* spp.), pirate bugs (*Coranus* spp.), lacewings (*Chrysopa* spp., *Micromus* spp.) and spiders (*Lycosa* spp., *Oxyopes* spp., *Salticidae*, *Araneus* spp.) (Mensah 1999).

Insect herbivory can occur at all stages in the plant lifecycle with different insects preferring different stages (Figure 6). Experience from growing cotton previously in northern regions of Australia suggests that insect pressure is higher in tropical areas during the wet season compared to the current southern cotton growing regions. The four key lepidopteran pests of cotton in northern Australia are cotton bollworm (*H. armigera*), native budworm (*H. punctigera*), cluster caterpillar (*Spodoptera litura*) and pink bollworm (*Pectinophora gossypiella*) (Cotton Catchment communities CRC 2006; Strickland et al. 2003; Strickland et al. 2000)

**Figure 6 Insect pests of cotton in Australia<sup>a</sup>**

<sup>a</sup> Taken from (Holloway 2005) illustration reproduced with permission from Bayer CropScience

*Helicoverpa armigera*, also known as the cotton bollworm, is a noctuid moth that occurs throughout the Australasia-Pacific region, in Africa and in Western Europe. It has a wide host range and its caterpillars attack many field and horticultural crops (Common 1953; Fitt 1989; Zalucki et al. 1986). Over the past thirty years it has been largely controlled by synthetic pesticides, leading to widespread evolution of resistance to many of these chemicals (King 1994). For example, typically 80 to 90% of the insects are now resistant to synthetic pyrethroids.

In cotton, the adult moth lays its eggs on young terminal branches, and the eggs hatch into larvae (caterpillars) within 2 to 3 days (King 1994; Zalucki et al. 1986). The caterpillars attack young leaves and flower buds (squares) and can burrow into the developing fruit, consuming developing seeds and fibres.

The caterpillar stage lasts for 15–24 days and *H. armigera* cotton bollworm may go through four to five generations during the cotton-growing season (Scott et al. 2003). The last generation goes into a period of suspended development or ‘diapause’ over winter, burrowing into the soil around the base of the plants. The over-wintering pupae emerge from the soil in the following spring (Duffield & Steer 2006; Fitt 1989; King 1994; Zalucki et al. 1986).

Mechanical cultivation of the soil at the end of the cotton-growing season disturbs the exit tunnels made by the larvae when they burrow into the soil (Duffield & Dillon 2005). This strategy, known as “pupae busting”, can kill over 90% of the pupae in the soil. This is an effective mechanism for reducing the number of moths that emerge in the spring and for delaying development of insects with resistance to insecticides used on cotton. However, the proportion of the population in diapause varies greatly between years, ranging from less than 10% to as much as 90% and so mechanical cultivation would only target a fraction of the winter population in any given year (Sequeira & Playford 2001).

*Helicoverpa punctigera*, or native budworm, is morphologically similar to *H. armigera* but is endemic to Australia. Large populations of both *Helicoverpa* species and other

noctuid moths can develop in the semi-arid areas of inland Australia in response to rainfall and abundant growth of native host plants (Zalucki et al. 1994). In spring, weather conditions cause deterioration of the host plants and this is followed by the large-scale migration of many of the moth species, over distances of 500 to 1500 km, in some cases reaching the cotton growing regions of southeastern Australia (Farrow & Daly 1987; Oertel et al. 1999). Although some *H. armigera* migrate, *H. punctigera* is more commonly found in these migrations and often arrives in the cotton areas early in the season, before the emergence of *H. armigera*. However, numbers of *H. punctigera* are usually low in late summer and early autumn and winter diapause is not common (Duffield & Steer 2006). The constant influx of *H. punctigera* immigrants to the cotton growing areas is thought to be responsible for the lack of development of resistance to chemical pesticides in this species (Scott et al. 2003).

Spider mites are also a significant cotton pest in Australia. The two-spotted spider mite (*Tetranychus uticae*) is the most common but the bean spider mite (*T. ludeni*) and strawberry spider mite (*T. lambi*) are also found. They live and feed on the under side of leaves, causing bronzing, reddening and eventually desiccation of the leaf (Gutierrez 1994). Predation is a key factor in reducing early season survival of mites. Predators include thrips (Wilson et al. 1996) (which can also be pests in their own right), ladybeetles (*Hippodamia convergens*), big-eyed bugs (*Geocoris spp.*), damsel bugs (*Nabis spp.*) and lacewings (*Chrysopa, Micromus spp.*). The use of broad-spectrum pesticides to control other pests can result in destruction of beneficial predators and exacerbation of spider mite infestations (Wilson et al. 1991). *G. barbadense* is less susceptible to mites than *G. hirsutum* (Cotton Seed Distributors Extension and Development Team 2005; Trichilo & Leigh 1985).

Minor pests of cotton include green mirid (*Creontiades dilutes*), which is also a pest of other summer crops. The insect feeds on and destroys seedling terminals and small flowerbuds. Cotton aphid (*Aphis gossypii*) is the main aphid pest of cotton. Honeydew produced by the aphid can contaminate cotton lint (Slosser et al. 2002), reducing its value. Aphids are not a major problem for Australian *G. hirsutum*. However, the long growing season of *G. barbadense* may lead to aphids migrating from *G. hirsutum* and so honeydew contamination of *G. barbadense* bolls can occur (Cotton Seed Distributors Extension and Development Team 2005).

The silverleaf whitefly (*Bemisia tabaci*) is a serious pest of fibre, horticultural and ornamental crops worldwide. It can cause extensive damage through direct feeding, honeydew production and as a viral vector. It was first identified in Australia in 1994 (Gunning et al. 1995). The first widespread outbreak of this pest in Australian cotton occurred in central QLD in the 2001/2002 cotton growing season and has moved south into NSW, where its spread has been limited by cold winter temperatures (Cotton Catchment communities CRC 2007b). The cotton industry is actively researching pest and resistance management strategies for use against cotton whitefly (Australian Cotton Cooperative Research Centre 2002b).

The major pests of *G. barbadense* are similar to those of *G. hirsutum*. However, *G. barbadense* shows some resistance to *Earias spp* (Reed 1994), jassids (Hemiptera: Cicadellidae) (Matthews 1994) and spider mites which is possibly due to the higher gossypol content of *G. barbadense* plants (Gannaway 1994; Matthews & Tunstall 1994; Sengonca et al. 1986). Modern *G. barbadense* cultivars have moderately hairy leaves which are more attractive to silverleaf whitefly than the smooth leaves of *G. hirsutum*. Also, *G. barbadense* has a longer growing season than *G. hirsutum* and this may expose the plants to a wider range of insect pest predators or to different stages in the insect life

cycles. This has the potential to increase the impact of insect predation, or conversely, to allow the plant extra time to recover from early season insect damage.

Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they do not seem to be a major limiting factor in naturalised *G. hirsutum* populations in northern Australia. Monitoring of seven naturalised *G. hirsutum* populations in the NT revealed abundant seed production, suggesting that these *G. hirsutum* plants were not significantly affected by lepidopteran pests (Eastick 2002). The major insect herbivores observed, particularly over the wet season, were grasshoppers (Orthoptera: Caelifera). Grasshoppers are considered to be the most important insect herbivores in tropical savannah ecosystems (Andersen & Lonsdale 1990).

When insects were sampled from three naturalised *G. hirsutum* populations in the NT, only 16% were from the order Lepidoptera (Eastick 2002). The dominant insect order found was Hemiptera (28% of total insects) suggesting that sucking insects possibly influenced naturalised cotton populations more than lepidopteran insects. A number of non-lepidopteran pests, including sucking insects, also attack cultivated cotton and require pest management via insecticides (Farrell & Johnson 2005).

In Northern Australia the abundance of pests such as *H. armigera*, *S. litura*, and *Pectinophora gossypiella* partly caused the switch to dry season cropping (Cotton Catchment communities CRC 2007c). *P. gossypiella* is a major pest in the USA. The larvae feed early in the season in cotton squares and later on the green bolls as they develop, causing lint yield loss (George & Wilson 1983).

*S. litura* larvae feed on leaves, flowers and bolls in cotton crops and are generally a problem in northern, but not eastern Australia. Heavy infestations of larvae can destroy large areas (Cotton Catchment communities CRC 2007a). They are pests of various crops including strawberries, tobacco, tomato, apple, cabbages and cauliflowers.

### 7.2.2 Pathogens

Cotton is infected by a range of diseases which can affect the quality of the fibre and seed, as well as the yield and cost of production of the cotton crop (Bell 1999; Cotton Australia 2002). The type and severity of infection differs from season to season and between different regions. The most significant diseases of cotton in Australia include: black root rot (*Thielaviopsis basicola*), Verticillium wilt (*Verticillium dahliae*), Fusarium wilt (*Fusarium oxysporum* var. *vasinfectum*), alternaria leaf spot (*Alternaria macrospora* and *A. alternata*), and boll rot (*Phytophthora nicotianae* var. *parasitica*) (Farrell & Johnson 2005). There are also over 30 species of fungi that can cause cotton seedling death, but this is predominantly caused by *Rhizoctonia solani*, *Pythium* spp. or *Fusarium* spp. (not Fusarium wilt) (Farrell & Johnson 2005)

Black root rot, caused by the fungal pathogen *Thielaviopsis basicola*, is widespread in all cotton growing areas of NSW (Nehl et al. 2004), and QLD (Australian Cotton CRC <<http://cotton.crc.org.au>>). Disease surveys show a steady rise in the number of farms with the disease since it was first detected in 1989.

Symptoms of Black root rot include stunted, slowing seedlings with black roots and lateral root death (Nehl & Allen 2004). As Black root rot cannot be controlled using fungicides, the management of the disease relies on farm management practices that slow down or prevent pathogen infection, for example planting after cold weather has passed, planting varieties that are able to 'catch up' later in the season, pre-irrigation in preference to 'watering up', planting of non-host crops such as cereals, sunflower, brassicas and onions for more than one season between cotton crops (Jhorar 2003) and adapting a 'come clean, go clean' strategy (Cotton Catchment communities CRC 2002). All cotton varieties and

many legumes are hosts for *T. basicola*. Therefore, legumes should be avoided as rotation crops in cotton growing regions infested with *T. basicola* (Allen et al. 2003).

Verticillium wilt is caused by the fungal pathogen *Verticillium dahliae*. Its incidence has increased in recent years, mainly due to the increasing use of susceptible varieties (Johnson & Nehl 2004). Symptoms include yellow leaf mottle, brown discoloration in the stem, stunted growth and some defoliation which is more severe in cold weather or under waterlogging (Cotton Catchment communities CRC 2002; Johnson & Nehl 2004; Nehl & Allen 2004). Control strategies for Verticillium wilt include planting of resistant cotton varieties, planting after cold weather has passed, avoiding waterlogging, crop rotation with non host crops such as sorghum and cereals, and adapting a 'come clean, go clean' strategy. *V. dahliae* has a wide host range including the crop plants sunflower, soybean, potato, tomatoes and olives as well as weeds such as saffron thistle (*Carthamus lanatus*) and pigweed (*Portulaca oleracea*) and many others and so control of these weeds is essential (Allen et al. 2003).

Fusarium wilt was first detected in Australia in 1993 (Kochman 1995) and by 2005 it was widespread on the Darling Downs in southern QLD, St George and from the McIntyre Valley into northern NSW. However, Emerald QLD, Hillston and Tandou NSW were still free of the disease at that time (Swan & Salmond 2005). The disease is caused by the fungal pathogen *Fusarium oxysporum* f.sp. *vasinfectum* (Fov), which can be maintained in spore form in the soil for over 10 years and cannot be controlled by the use of fungicides. Genetic analysis of Australian Fov samples indicate that it has arisen indigenously from Fusarium associated with native *Gossypium* spp. (Wang et al. 2006; Wang et al. 2007). Symptoms include wilting, tissue necrosis and death, and production of a characteristic browning of the vascular tissue (Nehl & Allen 2004). The severity of Fusarium wilt is strongly influenced by environmental conditions and farm management (plant stress) and may be affected by plant gossypol levels (Turco et al. 2004). The control strategies for Fusarium wilt recommended by the Cotton Catchment communities CRC include planting resistant cotton varieties (all cotton seed sold in Australia now come with a Fov resistance rating), planting of surface-treated seeds, avoiding waterlogging and adapting a 'come clean, go clean' strategy (Cotton Catchment communities CRC 2002; Swan & Salmond 2005). The type and timing of nitrogen fertilizer application may also affect the level of Fov in the soil (Wang et al. 1999). Cotton and also some weeds, for example bladder ketmia (*Hibiscus trionum*), sesbania pea (*Sesbania cannabina*) and dwarf amaranth (*Amaranthus macrocarpus*), are hosts for *F. oxysporum* f.sp. *vasinfectum* (Allen et al. 2003) and the possibility of management through crop rotation is being investigated (Swan & Salmond 2005). The possibility of introducing Fov resistance traits from *G. sturtianum* is also being investigated (Becerra Lopez-Lavalle et al. 2007; McFadden et al. 2004).

Alternaria leaf spot is caused by *Alternaria macrospora* (primarily *G. barbadense*) or *A. alternata* (primarily *G. hirsutum*) or a combination of both (Bashan et al. 1991). Symptoms include brown, grey or tan lesions predominantly on lower leaves, rapid defoliation and dry circular bolls lesions (Cotton Catchment communities CRC 2002; Nehl & Allen 2004), and is more severe with potassium deficiency (Blachinski et al. 1996; Hillocks & Chinodya 1989) or in humid conditions. Most commercial varieties of *G. hirsutum* are relatively resistant however *G. barbadense* is very susceptible and yield reductions of up to 40% have been reported overseas (Shtienberg 1993). Control measures include planting only resistant varieties in infected fields, incorporating crop residues into soil as soon after harvest as possible, appropriate potassium fertilisation, fungicide applications (Bhuiyan et al. 2007), and control of volunteer cotton plants and host weed species (Cotton Catchment communities CRC 2002). Cotton and some malvaceous weeds

such as bladder ketmia (*Hibiscus trionum*), sida (*Sida spp.*) and anoda weed (*Anoda cristate*) are also hosts for *Alternaria macrospora*.

Bacterial blight, caused by *Xanthomonas campestris*, is a major disease of *G. barbadense*. Symptoms include angular, dark green, water soaked lesions on the leaves, bracts and bolls (Cotton Seed Distributors Extension and Development Team 2005). Most *G. barbadense* cultivars are highly susceptible to bacterial blight (Brinkerhoff 1970; Delannoy et al. 2005) with reports of losses up to 80% in Australia although new resistant cultivars are being developed (Cotton Catchment communities CRC 2002). Control measures include foliar copper sprays, avoiding excessive vegetative growth and incorporating crop residues into soil as soon after harvest as possible (Cotton Catchment communities CRC 2002; Cotton Seed Distributors Extension and Development Team 2005).

There are also a number of viral diseases which can infect cotton. The most economically important of these is Cotton leaf curl virus (CLCuV) which caused substantial yield loss to cotton crops in Pakistan in the 1990's (Briddon & Markham 2000). This virus is transmitted by *Bemisia tabaci* (whitefly) and causes leaf curl, foliar discoloration, vein thickening and stunting. It was originally classed as a begomovirus in the family *Geminiviridae* (Briddon & Markham 2000), although further research has shown that the begomovirus acts in a complex with a nanovirus component and a single stranded satellite-like molecule (Briddon et al. 2001). Another related virus has been isolated more recently from *G. barbadense* and named *Cotton leaf curl Bangalore virus* (Chowda Reddy et al. 2005). Neither of these viruses are currently present in Australia (Plant Health Australia 2007).

Cotton bunched top has been observed in Australia since 1998 (Reddall et al. 2004). It is thought to be transmitted by the cotton aphids (*Aphis gossypii*) and causes pale patterns on leaf margins, leathery leaves and short petioles and internodes which leads ultimately to reduced lint yields. The causal agent for this disease has not yet been identified, although it is thought to be viral (Ali et al. 2007).

### 7.3 Other interactions

Successful cotton growth in most soils depends on the interaction with mycorrhizal fungi (Australian Cotton Cooperative Research Centre 2002c; Cotton Catchment communities CRC 2002; Nehl & Allen 2004; Youssef & Mankarios 1974). The fungal species interacting with cotton roots, for example *Glomus mosseae*, grow intercellularly in the root cortex. They form arbuscules, highly branched, tree-like structures in intimate contact with the plant's plasma membrane within the cortex cells of the plant. The arbuscules are characteristic of this type of endophytic symbiosis called vesicular arbuscular mycorrhizae (VAM) and are the sites of mineral exchange from the fungus to the plant and carbohydrate exchange from the plant to the fungus. For the plant, improvement of phosphate uptake is the main advantage in engaging in VAM (reviewed in Strack et al. 2003). VAM fungi are widespread in the environment.

The VAM fungal species *Glomus mosseae*, as many other VAM fungi, is capable of colonising a variety of plant species. For example, Giovannetti et al. (2004) demonstrated that an isolate of *G. mosseae* is able to colonise cotton (*G. hirsutum*), eggplant (*Solanum melongena*), carrot (*Daucus carota*), lettuce (*Lactuca sativa*) and leek (*Allium porrum*).

VAM fungi can influence the severity of plant diseases on cotton. Liu (1995) reported mutual inhibition of infection of cotton after simultaneous inoculation with VAM fungi and *V. dahliae* as well as reduced disease incidence and disease indices of plants sequentially inoculated with AM fungi and *V. dahliae*. In another report, Zhengjia and

Xiangdong (1991) showed reduced severity of Fusarium wilt in *G. hirsutum* plants inoculated with *G. mosseae*.

## SECTION 8 WEEDINESS

### 8.1 Weediness status on a global scale

An important indicator of potential weediness of a particular plant is its history of weediness in any part of the world and its taxonomic relationship to declared weeds (Panetta 1993; Pheloung 2001). Cotton has been grown for centuries throughout the world without any reports that it is a serious weed. Worldwide, there are approximately 50 species of *Gossypium* (Craven et al. 1994; Fryxell 1992), none of which is listed as a serious weed (Groves et al. 2003; Holm et al. 1997; Holm et al. 1979; Randall 2002).

Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds, such as seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance dispersal of seeds (Keeler 1985; Keeler 1989).

### 8.2 Weediness status in Australia

Cotton is not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2003). No *Gossypium* species are recognised as problematic weeds in Australia, either agriculturally or environmentally (Lazarides et al. 1997; Tothill et al. 1982). Cotton has no relatives that are problematic weeds (Keeler et al. 1996), although locally *G. sturtianum* can be weedy (Lazarides et al. 1997).

In conservation areas, for example 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 for example 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 have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (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.

A small number of other *G. hirsutum* plants appear to be of more recent origin, but none seem to have originated from the current commercial types of *G. hirsutum* that have been cultivated since the 1970s (for example Eastick 2002). These naturalised *G. hirsutum* plants are confined to areas of disturbed land with at least a seasonal water supply; typical locations are above the high tide mark on beaches and near river banks in northern Australia.

Even though *G. hirsutum* has been grown previously in a number of places in northern Australia, only isolated *G. hirsutum* populations have been able to naturalise. For

example, *G. hirsutum* has not persisted in the environment in the Ord River Irrigation Area following the abandonment of *G. hirsutum* farms, with actively growing *G. hirsutum* plants in the fields, in the 1960s and 70s (Eastick 2002).

Naturalised *G. barbadense* has been found in QLD and NT and data from the Australian Virtual Herbarium confirm that these specimens were collected primarily from the eastern coastal regions of QLD and northern areas of NT (Australia's Virtual Herbarium 2007). Unfortunately, few ecological data accompany the herbarium records. It is difficult, therefore, to assess the abundance or 'weediness' of *G. barbadense* in Australia, although specimen notes suggest that several of the collections were of 'escaped' or 'naturalised' plants growing in habitats such as roadsides and drainage lines. As *G. barbadense* is not regarded as a problematic weed, it is probable that the herbarium specimens highlight the existence of occasional individuals, and/or small ephemeral populations, rather than a significant weed problem.

### 8.3 Weediness in agricultural ecosystems

*G. hirsutum* and *G. barbadense* may occur as escapes from agriculture and/or as small populations of naturalised exotic species (Lazarides et al. 1997; Sindel 1997). Where such populations have established, they are not considered to threaten agricultural productivity or native biodiversity.

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 probably limits the germination and growth of volunteers.

In northern Australia, cotton volunteers have been observed in areas that have not been cultivated for cotton in many years (Williams 2002). Many of these volunteers appear to benefit from water and nutrients that may run off other areas that are tended regularly and which occur within metres of the volunteer plants.

### 8.4 Weediness in natural ecosystems

There are abiotic and biotic factors that determine whether *G. hirsutum* will persist in the environment including short summer seasons, soil type, fire, competition from other plants, herbivory (insects and other animals), and physical destruction such as trampling (Eastick & Hearnden 2006; Farrell & Roberts 2002). The relative impact of each of these factors is dependent on whether the *G. hirsutum* 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.

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 had established in the roadside environment only infrequently, despite 12 years of use of these routes for transporting ginned seed (including GM *G. hirsutum* varieties since their respective commercial releases) 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 (for example 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).

The above results were supported by the Eastick study (Eastick & Hearnden 2006), where *G. hirsutum* seed germination was highest in disturbed habitats especially when the seed was buried rather than remaining exposed on the soil surface. Persistence of *G. hirsutum* plants for more than 1–2 years was only seen in habitats with increased water availability or nutrition such as cattle yards. Eastick also found that although *G. hirsutum* growing in cattle yards may reach reproductive maturity, persistence and seed dispersal from these areas is limited by trampling and grazing. No *G. hirsutum* volunteers were found in the undisturbed bush habitats surrounding these areas (Eastick 2002; Eastick & Hearnden 2006). Similarly, monitoring of Bt cotton volunteers in Kununurra (WA) showed considerable damage by leaf-eating insects during the wet season (Eastick 2002).

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<sup>®</sup>, Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> or INGARD<sup>®</sup>) 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 (for example 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.

Climex<sup>®</sup> models to predict the areas that are climatically suitable for long-term survival of *G. hirsutum* (Rogers et al. 2007) and *G. barbadense* (Rogers 2007) in Australia have been developed. Both models indicate that dry stress is the major limiting factor for potential distribution of cotton in northern Australia. The modelling program predicted similar naturalisation potentials for *G. barbadense* and *G. hirsutum* in Australia, with matching climates confined to the eastern coast of QLD consistent with the majority, but not all, of the reports of naturalised populations in Australia (Australia's Virtual Herbarium 2007). The modelling program also predicted that the winter temperatures in all of the current cotton growing areas of Australia were too cold to support the establishment of permanent populations of *G. hirsutum* and *G. barbadense*.

When overall soil fertility was considered in addition to climatic data, the area suitable for cotton is further restricted (that is even more closely limited to coastal areas). However, the majority of these most favourable areas for cotton either carry forests (with >50% canopy closure) 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 (Rogers et al. 2007).

## 8.5 Control measures

The control of cotton volunteers is important both in cotton fields and outside the fields such as roadsides and drains. There are three types of cotton volunteers that need to be controlled: seedling cotton, established cotton, and regrowth or 'ratoon' cotton.

Herbicides can be used to control seedling cotton volunteers. Glyphosate has been the most common herbicide used to control these volunteers but, with the uptake of Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> GM *G. hirsutum*, alternative herbicides are being used, including glufosinate ammonium. However, the use of glufosinate ammonium is limited

on *G. hirsutum* volunteers as its effectiveness on *G. hirsutum* seedlings at the 4 and 8 leaf stage offers incomplete control. Also the commercial release of LibertyLink<sup>®</sup> *G. hirsutum* in 2006 means that glufosinate ammonium tolerant *G. hirsutum* is now available. Other herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective (Roberts et al. 2002). Cultivation is also a very effective method to control seedling cotton volunteers (Australian Cotton Cooperative Research Centre 2002d).

Established or ratoon cotton plants, whether GM or non-GM, are difficult to control by herbicides alone. Instead, established or ratoon cotton plants are most effectively controlled by mechanical methods involving mulching, root cutting and cultivation (Roberts et al. 2002).

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 2002d; 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<sup>®</sup> *G. hirsutum* volunteers (APVMA Pubcris database available at <<http://services.apvma.gov.au/PubcrisWebClient>>).

Integrated weed management strategies stress the need to avoid relying on one control method (Roberts & Charles 2002). To avoid development of glyphosate resistant weeds for example, it is recommended that the application of glyphosate alone should not be used as the sole management strategy.

## 8.6 Weed risk assessment of cotton

The weed risk potential of cotton has been assessed (Appendix B) using methodology based on the Australia/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness (Virtue et al. 2008). These properties relate to invasiveness, impacts and potential distribution. The distribution of cotton is driven by economics, as well as factors such as climate and soil suitability.

In summary, as a volunteer (rather than as a crop), cotton is considered to:

- have a low ability to establish amongst existing plants
- have a low tolerance to average weed management practices in cropping and intensive land uses, but a high tolerance in nature conservation areas
- have a short time to seeding (less than one year)
- have a low annual seed production in dryland and irrigated cropping areas, and a low ability for volunteers to establish in any land use
- not reproduce by vegetative means
- unlikely to undergo long distance spread by natural means
- be commonly spread long distance by people from dryland and irrigated cropping areas, as well as from intensive land uses, but unlikely from nature conservation areas
- have a limited ability to reduce the establishment or yield of desired plants
- have a low ability to reduce the quality of products or services obtained from all land use areas

- have a low potential to restrict the physical movement of people, animals, vehicles, machinery and/or water
- have a low potential to negatively affect the health of animals and/or people
- can act as a reservoir for a range of pests and pathogens
- have a low effect upon soil nutrients, salinity, stability or the water table

This is consistent with previous assessments of cotton in Australia described in Section 8.2, and provides a baseline for the assessment of GM cotton.

## SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

The possibility of genes transferring from *G. hirsutum* to other cultivated cotton species, including feral populations, and native Australian *Gossypium* species organisms is addressed below. There are two potential barriers which must be overcome before gene flow can occur successfully. Pre-zygotic barriers include geographic separation, differences in floral phenology, different pollen vectors and different mating systems such as stigmatic or stylar incompatibility systems. Post-zygotic barriers include genetic incompatibility at meiosis, selective abortion, lack of hybrid fitness and sterile or unfit backcross progeny (Brown et al. 1997).

### 9.1 Intraspecific crossing

Cotton is generally self-pollinating, however cross-pollination can occur (see Section 4). In Australia, cross-pollination between adjacent individuals occurs, albeit at relatively low frequencies. For example, as noted in Section 4.2, Llewellyn and Fitt (Llewellyn & Fitt 1996) estimated that cross-pollination between *G. hirsutum* plants in adjacent rows accounted for only 1 to 2% of seeds.

Crossing between cultivated cotton and feral cotton populations is also possible and viable seeds would be generated if it occurred. The likelihood of this occurring is remote, however, given the geographic separation of feral cotton populations from existing cotton plantations (see Section 8). Geographic distances between these feral populations and most cotton growing regions exceeds conceivable pollinator foraging ranges and therefore serves as an effective natural barrier to cross-pollination. However, certain potential cotton growing areas in the NT, particularly areas in the Roper and Sturt Plateau regions, may occur in relatively close proximity to some feral cotton populations. In these areas, there is an increased probability of out-crossing to feral cotton populations.

### 9.2 Natural interspecific and intergeneric crossing

#### 9.2.1 Crosses between *G. barbadense* and *G. hirsutum*

Hybridisation can occur naturally between *G. barbadense* and *G. hirsutum* (Brubaker et al. 1999b). In older studies hybrid vigour or heterosis has been observed in *G. barbadense* x *G. hirsutum* hybrids (McGregor 1976; Moffett 1983) and hybrid cotton is widely cultivated in India and China. A study in Turkey of *G. hirsutum* x *G. barbadense* hybrids showed high yields and good fibre characteristics (Basbag & Gencer 2007). However, observations in Australia suggest that hybrid progeny exhibit characteristics intermediate to the parents but typically with a lower capacity to produce cotton bolls (Warwick Stiller & Greg Constable, CSIRO, 2002, pers. comm.). Hybrids between the two species do not form stable populations and instead tend to segregate towards either parental phenotype over a number of generations.

*G. barbadense* and *G. hirsutum* share the AD tetraploid genomes, are not separated by any large-scale chromosomal rearrangements (Gerstel & Sarvella 1956), and can be hybridised to produce fertile F<sub>1</sub> progeny. However, F<sub>2</sub> progeny show evidence of lethal gene combinations in succeeding generations (Gerstel 1954; Stephens & Phillips 1972). The two species have different ribosomal DNA sequences (Wendel et al. 1995) and chloroplast genomes (Wendel & Albert 1992). Genetic and physical isolating mechanisms have evolved to keep the two species distinct.

Genetic isolation mechanisms include incompatibility at the 'corky' locus (Stephens 1946; Stephens 1950a; Stephens 1950b; Stephens & Phillips 1972) and selective fertilisation (Brubaker et al. 1999a; Kearney & Harrison 1932). When equal mixtures of pollen from *G. hirsutum* or *G. barbadense* are simultaneously placed on the stigma of either species, only approximately 25% of the mature seed arise from interspecific fertilisations, compared to the expected 50% (Kearney & Harrison 1932). This selective fertilisation was determined to result from a reaction in the stigma by 'like pollen' that inhibits the growth of 'unlike pollen'.

In addition, a physical isolating mechanism is also present which prevents *G. hirsutum* pollinating *G. barbadense*. It is thought that early flowering of *G. barbadense* compared with *G. hirsutum* allows *G. barbadense* to be preferentially pollinated early in the day when *G. hirsutum* pollen is unavailable, whereas *G. hirsutum* can be pollinated by the still abundant *G. barbadense* pollen later in the day (Stephens & Phillips 1972).

Interspecific introgression between the two species has been extensively studied, with gene flow primarily occurring from *G. barbadense* into *G. hirsutum* where natural populations overlap. However, commercial cultivars primarily show gene flow in the opposite direction due to targeted breeding and, as noted previously, most commercial cultivars of *G. barbadense* now contain an average of 8–12% introgressed *G. hirsutum* chromatin (Wang et al. 1995).

In Australia, *G. hirsutum* generally comprises 99% of the commercial cotton crop in any given year and there is overlap between the growing areas of *G. hirsutum* and *G. barbadense*. Therefore, it is possible that crossing between *G. barbadense* and *G. hirsutum* could occur in agricultural fields. Geographical separation of feral cotton populations of *G. hirsutum* and *G. barbadense* from existing cotton plantations would generally prevent crossing between feral and cultivated cotton.

### **9.2.2 Crosses with native *Gossypium* spp**

Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distances from cultivated cotton fields. Generally, the Australian species do not have the properties of invasive agricultural or environmental weeds, although *G. sturtianum* has the potential to form localised weedy populations (Lazarides et al. 1997). *Gossypium australe*, and to a much lesser extent *G. nelsonii* and *G. bickii*, may form roadside populations in some areas of some states but typically the Australian cottons are found only in native vegetation, not in human-modified environments including agricultural areas (Groves et al. 2000).

Of the Australian *Gossypium* species, only three are likely to occur in the existing cotton growing regions and, therefore, are likely to be exposed to *G. barbadense* or *G. hirsutum* pollen. *G. sturtianum* and *G. nandewarensis* are likely to occur in all commercial cotton growing regions of eastern Australia and *G. australe* may be at the edge of its distribution (Brown et al. 1997). In the Theodore district in QLD, *G. sturtianaum* populations were found within 2 km of land used for growing cotton (Brown et al. 1994).

*Gossypium rotundifolium* and *G. australe* are the only species whose distribution overlaps potential cotton growing areas in north-western Australia and the NT, whereas *G. australe* and *G. nelsonii* are the only natives likely to occur in the potential cotton growing area of Richmond, QLD (Australia's Virtual Herbarium 2007).

Despite potential co-occurrence of Australian *Gossypium* species and cotton, the native species are found rarely on the heavy clay soils of the major cotton growing regions, preferring well-drained sandy loams. However, at Broome, where *G. rotundifolium* is known to occur, cotton may be grown on the same soil type preferred by native *Gossypium* (Australian Cotton Cooperative Research Centre 2001).

During transportation of cotton modules, seed cotton can be spilled and may germinate, giving rise to ephemeral roadside populations of *G. hirsutum*. Such populations may be associated spatially with several Australian *Gossypium* species, thereby placing these species, which ordinarily would be isolated geographically from cultivated cotton, within pollinator distance of *G. hirsutum*. Herbarium records indicate that all of the Australian C- and G-genome species, and one K-genome species (*G. rotundifolium*), have populations that are intersected by major transportation routes. Potentially, each of these species could receive pollen from roadside *G. hirsutum* volunteers. Clearly, however, such potential cross-pollination would depend on chance spillages in areas where native populations occur, and on the possibility of the spilt seed germinating, surviving to reproductive maturity, flowering synchronously with the native species, and competing for pollination with the predominately self-pollinating native cotton.

Even if these conditions were met, the likelihood of gene transfer from one species to the other 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) (see Section 9.3). 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).

### 9.3 Crossing under experimental conditions

Crossing of cotton with *Gossypium* species other than the A or D genomes involves the production of hybrids through tetraploid (trispecific) or hexaploid (bispecific) bridging populations followed by successive backcrossing (Brubaker et al. 1999b; Stewart 1995). Tetraploid bridging involves generating a tetraploid between the wild species and an A or D genome bridging species. The chromosome number is doubled using colchicine then this is crossed to the cultivated tetraploid and backcrossed. Hexaploid bridging is simpler, involving direct hybridisation of the wild species with the tetraploid cotton, doubling of the chromosomes and then backcrossing to the tetraploid parent (Brubaker et al. 1999b), but autopolyploidisation (pairing of the homologous chromosomes from the same parent during meiosis in polyploids) reduces the recombination of homoeologous chromosomes (Becerra Lopez-Lavalle et al. 2007).

Experiments with artificially created *G. hirsutum* hybrids suggest that interspecific crosses among *Gossypium* species are more likely to be successful when the plant with the highest chromosome number is the pollen recipient (Brubaker & Brown 2001), therefore successful gene transfer is more likely from wild *Gossypium* species to cultivated cottons than *vice versa* (Refer to Table 11).

#### 9.3.1 Cross-pollination with G- and K-genome natives

Several publications discuss extensive experimental efforts to hybridise *G. hirsutum* with the Australian *Gossypium* species (Brown et al. 1997; Brubaker & Brown 2001; Brubaker

et al. 2002; Brubaker et al. 1999b; Zhang & Stewart 1997). Although some hybrid seeds have been produced by crossing *G. hirsutum* (as a pollen donor; ♂) with *G. australe* (as pollen recipient; ♀), none of the seeds were viable. Numerous attempts to hybridise *G. hirsutum* (♂) with the remaining Australian G- and K-genome species (♀) generated no viable seeds (Brown et al. 1997; Brubaker et al. 1999b), as summarised in Table 11. The reciprocal pollinations, in which pollen from the Australian species (♂) is used to pollinate *G. hirsutum* (♀), have produced viable seed for several of the inter-specific crosses (Table 11), but only under ideal glasshouse conditions and with significant human intervention including, for example, the application of plant hormone (gibberellic acid) to retain fruit that otherwise would be aborted. Even so, the resultant seedlings were not robust, were difficult to maintain under glasshouse conditions and would not be expected to persist in the field.

Backcrosses between the *G. hirsutum* x K-genome species (ADK) hybrids and *G. hirsutum* (AD) results in the production of pentaploid progeny (AADDK). These successful backcrosses were possible due to the production of unreduced gametes in the hybrid (Brubaker & Brown 2001). The pollen from these pentaploid plants was functionally sterile which would limit the possibility of further introgression into the native K-genome species. The ADK hybrids themselves would not be maintained in the populations because the pentaploid hybrids would contain a single set of K-genome chromosomes, which cannot pair up during meiosis. Thus, in subsequent backcrosses to *G. hirsutum* or the native K-genome species the K-genome or AD genomes chromosomes would be lost respectively, unless they recombined. Transfer of introduced genes by recombination between chromosomes of different genomic origin is thought to be extremely rare, as demonstrated by studies in hexaploid wheat (Hedge & Waines 2004). This is likely due to the spatial separation of chromosomes from different genomes during the cell cycle as observed in hexaploid wheat which contains three genomes (Avivi et al. 1982) and the F1 hybrid generated by crossing barley and wild rye (Leitch et al. 1991). There has been some research into the hybridisation potential of *G. barbadense* with native Australian *Gossypium* spp. Attempts to pollinate the K genome species *G. anapoides* with *G. barbadense* pollen did not result in seed set (Zhang & Stewart 1997).

### 9.3.2 Cross-pollination with C-genome natives

The native species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. This species is the only native for which hybrid seedlings have been produced with the native parent as the recipient of cultivated cotton pollen and then, only with human intervention. Hybrids between *G. sturtianum* and cultivated cotton are sterile, however, regardless of which species serve as the pollen recipient. This effectively eliminates any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations (Brown et al. 1997; Brubaker et al. 1999b).

Artificial hybrids between *G. barbadense* and the C-genome species *G. sturtianum* have been produced in a glasshouse without application of plant hormones (Skovsted 1937; Webber 1935; Webber 1939). However, these hybrids were sterile, again effectively eliminating any potential for introgression of *G. barbadense* genes into *G. sturtianum* populations.

The similarity between the AD tetraploid genomes of *G. barbadense* and *G. hirsutum* and their genetic distance from the diploid C, G and K genomes of the native Australian *Gossypium* spp. indicates that *G. barbadense* will have the same barriers to hybridisation

as *G. hirsutum*. Therefore, the likelihood of fertile hybrids occurring, surviving to reproductive maturity and back-crossing to the parental native is effectively zero.

**Table 11 Summary of attempts to generate hybrid seeds between cultivated cotton and native Australian species of *Gossypium*, following hand-pollination<sup>1</sup>**

Genome of native	Female (♀) parent (pollen recipient)	Male (♂) parent (pollen donor)	No. fruit with seed (no. pollinations attempted)	No. plants established (no. seed sown)
C	<b><i>G. sturtianum</i></b> *	<b><i>G. hirsutum</i></b>	25 (122)	5 (149)
	<i>G. hirsutum</i> *	<i>G. sturtianum</i>	25 (39)	134 (193)
	<b><i>G. robinsonii</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. robinsonii</i>	8 (9)	54 (89)
G	<b><i>G. australe</i></b> *	<i>G. hirsutum</i>	38 (122)	0 (151)
	<i>G. hirsutum</i> *	<i>G. australe</i>	0 (16)	0
	<b><i>G. bickii</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. bickii</i>	0 (13)	0
	<b><i>G. nelsonii</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. nelsonii</i>	2 (14)	0 (2)
K	<b><i>G. anapoides</i></b> †	<b><i>G. barbadense</i></b>	0 (4)	0
	<i>G. hirsutum</i> *	<i>G. anapoides</i>	7 (15)	12 (26)
	<b><i>G. costulatum</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. costulatum</i>	2 (4)	4 (13)
	<b><i>G. cunninghamii</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. cunninghamii</i>	1 (15)	0 (1)
	<b><i>G. enthyle</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. enthyle</i>	10 (18)	9 (48)
	<b><i>G. exiguum</i></b> †	<b><i>G. hirsutum</i></b>	0 (7)	0
	<i>G. hirsutum</i> *	<i>G. exiguum</i>	4 (11)	8 (61)
	<b><i>G. londonderriense</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. londonderriense</i>	11 (25)	1 (26)
	<b><i>G. marchantii</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. marchantii</i>	17 (23)	0 (72)
	<b><i>G. nobile</i></b> †	<b><i>G. hirsutum</i></b>	0 (14)	0
	<i>G. hirsutum</i> *	<i>G. nobile</i>	24 (36)	15 (86)
	<b><i>G. pilosum</i></b> †	<b><i>G. hirsutum</i></b>	0 (6)	0
	<i>G. hirsutum</i>	<i>G. pilosum</i>	17 (24)	35 (88)
	<b><i>G. populifolium</i></b>	<b><i>G. hirsutum</i></b>	ND	ND
	<i>G. hirsutum</i> *	<i>G. populifolium</i>	14 (40)	18 (65)
<b><i>G. pulchellum</i></b>	<b><i>G. hirsutum</i></b>	ND	ND	
<i>G. hirsutum</i> *	<i>G. pulchellum</i>	7 (16)	1 (15)	

Genome of native	Female (♀) parent (pollen recipient)	Male (♂) parent (pollen donor)	No. fruit with seed (no. pollinations attempted)	No. plants established (no. seed sown)
	<b><i>G. rotundifolium</i></b> *	<b><i>G. hirsutum</i></b>	0 (57)	0
	<i>G. hirsutum</i> *	<i>G. rotundifolium</i>	11 (15)	12 (52)

<sup>1</sup> Pollinations representing the greatest potential environmental risk, namely with *G. hirsutum* or *G. barbadense* as the pollen donor, are presented in bold, with the reciprocal pollination presented immediately following.

\* = data from Brown et. al. (Brown et al. 1997); † = data from Zhang and Stewart (Zhang & Stewart 1997); ND = no data available

### 9.3.3 *Cross-pollination with other plant taxa*

Gene transfer to unrelated plant species is highly improbable because of pre- and post-zygotic genetic incompatibility barriers that are well documented for distantly related plant groups. No evidence for horizontal gene transfer from cotton to other plant taxa has been identified.

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**APPENDIX A WEEDS OF COTTON****Major weeds of cotton crops in Australia<sup>b</sup>**

<b>Scientific name</b>	<b>Common name</b>
<b>Grasses</b>	
<i>Cyperus rotundus</i>	Nutgrass
<i>Echinochloa colona</i>	Awnless barnyard grass
<i>Urochloa panicoides</i>	Liverseed grass
<b>Broadleaf weeds</b>	
<i>Amaranth spp.</i>	Amaranths
<i>Chamaesyce drummondii</i>	Caustic weed
<i>Citrullus lanatus var. lanatus</i>	Wild melon
<i>Convolvulus erubescens</i>	Australian bind weed
<i>Cullen tenax</i>	Emu foot
<i>Datura ferox</i>	Thornapple
<i>Hibiscus trionum</i>	Bladder ketmia
<i>Ibicella lutea</i>	Devils claw
<i>Ipomoea lonchophylla</i>	Cowvine
<i>Medicago polymorpha</i>	Burr medic
<i>Physalis minima</i>	Wild gooseberry
<i>Polymeria pusilla</i>	Polymeria
<i>Portulaca oleracea</i>	Pigweed
<i>Sesbania cannabina</i>	Sesbania pea
<i>Sonchus oleraceus</i>	Common sowthistle
<i>Tribulus micrococcus</i>	Yellow vine or spineless caltrop
<i>Xanthium italicum</i>	Italian cocklebur
<i>Xanthium occidentale</i>	Noogoora burr
<i>Xanthium spinosum</i>	Bathurst burr

<sup>b</sup> Data compiled from (Australian Cotton Cooperative Research Centre 2002d; Charles et al. 2004; Taylor & Walker 2006; Walker et al. 2006)

## APPENDIX B WEED RISK ASSESSMENT OF COTTON

**Species:** *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton)

### Relevant land uses:

1. Intensive<sup>b</sup> uses (ALUM<sup>c</sup> classification 5),
2. Production from dryland agriculture (ALUM classification 3.3.6 Cotton)
3. Production from irrigated agriculture (ALUM classification 4.3.6 Irrigated Cotton)
4. Nature conservation<sup>d</sup> (ALUM classification 1.1)

**Background:** The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The questions and ratings (see table) used in this assessment are based on the South Australian Weed Risk Management Guide (Virtue 2004). The terminology is modified to encompass all plants, including crop plants.

Weeds are usually characterised by one or more of a number of traits, these including rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they cause one or more harms to human health, safety and/or the environment. Cotton has been grown globally for centuries, without any reports that it is been become a serious weed. In Australia, cotton is grown mainly in New South Wales and Queensland. Unless cited, information in this weed assessment is taken from the document *The Biology of Gossypium hirsutum* L. and *Gossypium barbadense* L. (*Cotton*) (OGTR 2008). This WRA is for non-GM cotton volunteers in the land use areas identified above. Reference is made to cotton as a cultivated crop only to inform its assessment as a volunteer.

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<sup>b</sup> *Intensive use* includes areas of intensive horticulture or animal production, areas of manufacture or industry, residential areas, service areas (eg shops, sportsgrounds), utilities (eg. facilities that generate electricity, electrical substations, along powerlines) areas of transportation and communication (eg along roads, railways, ports, radar stations), mine sites and areas used for waste treatment and disposal.

<sup>c</sup> ALUM refers to the Australian Land Use and Management classification system version 7 published May 2010.

<sup>d</sup> *Nature conservation* refers to land use areas that have relatively low level of human intervention, with nature conservation the prime use. This class of land use includes nature reserves, wilderness areas, national parks and other protected or conserved areas.

Invasiveness questions	Cotton
<p><b>1. What is cotton's ability to establish amongst existing plants?</b></p>	<p><b>Rating: Low in all relevant land uses</b></p> <p>Cotton is a domesticated crop that grows best under agricultural conditions. It prefers soils with high fertility and responds well to irrigation. Volunteers tend to establish in highly and regularly disturbed environments, and have a poor ability to compete with established vegetation (Farrell &amp; Roberts 2002). Seed losses leading to volunteers in <i>dryland and irrigated cropping areas</i> can occur during harvesting, and in <i>intensive use areas</i> during transport (from field to gin), storage (feedlots) and processing (around the facilities where ginning is conducted). Naturalised populations of both <i>G. hirsutum</i> and <i>G. barbadense</i> have been found in few relatively natural areas in the north of Australia, indicating that it is possible for these species to establish outside agricultural cultivation. However, cotton seems to have a limited ability to invade and establish in undisturbed nature conservation areas.</p>
<p><b>2. What is cotton's tolerance to average weed management practices in the land use?</b></p>	<p><b>Rating: Low in cropping and intensive land uses High in nature conservation land uses</b></p> <p>Weed management practices (preventive, cultural and chemical) aim at reducing the loss in yields due to weeds.</p> <p>In <i>dryland and irrigated cropping areas</i>, cotton volunteers in subsequent crops or along field margins are typically controlled by mechanical methods such as mulching and root cutting, as well as the application of appropriate herbicides.</p> <p>Cotton volunteers in <i>intensive use areas</i> are not known to sponsor self-perpetuating feral populations. Typically, such volunteers are killed by roadside management practices (eg herbicide treatment or slashing/mowing) and/or grazed by livestock, thereby limiting their potential to reproduce (Addison et al. 2007; Eastick &amp; Hearnden 2006).</p> <p>Cotton is not known to be specifically targeted in <i>nature conservation areas</i> and, in some areas where small cotton populations occur, no weed management is conducted. Both these reasons give rise to the high tolerance rating for this land use area.</p>

Invasiveness questions	Cotton
<b>3. Reproductive ability of cotton in the land use:</b>	
<b>3a. What is the time to seeding in the land uses?</b>	<p><b>Rating: &lt; 1 year in all relevant land uses</b></p> <p>Cotton is a perennial that has been adapted and bred to act as an annual crop. Under standard agricultural conditions, it generally takes four months to complete a lifecycle from germination to the maturation of the first seeds. However, in <i>nature conservation areas</i> of northern Australia, feral cotton does exist as a perennial, with annual seed production.</p>
<b>3b. What is the annual seed production in the land use per square metre?</b>	<p><b>Rating: Low in all relevant land use areas (from volunteers)</b></p> <p>When grown as a crop in <i>dryland and irrigated cropping areas</i>, cotton seed production would be considered high (&gt;1000 viable seed per m<sup>2</sup>)<sup>e</sup>. However, volunteers will generally not occur at a high density, as seed loss during crop harvest is minimal, cotton volunteers are poor competitors, and management of volunteer plants is targeted. Similarly, in <i>intensive use areas</i>, conditions for establishment and survival of cotton volunteers would not be ideal, and weed management practices in these areas would severely limit volunteer numbers and seed production. Therefore, the number of seeds produced by volunteers in these land uses is expected to be low (&lt;1000 viable seed per m<sup>2</sup>).</p> <p>In <i>nature conservation areas</i> the number of volunteer cotton plants is expected to be very low and would suggest low seed production.</p>
<b>3c. Can cotton reproduce vegetatively?</b>	Under natural conditions, cotton <b>cannot</b> reproduce by vegetative propagation.

<sup>e</sup> When grown as a crop, *G. hirsutum* usually produces 29-40 seeds per boll, and 10-12 bolls per plant. In Australia, cotton is typically planted in rows that are 1 m apart, corresponding to 12 plants per m<sup>2</sup>. However, row spacing can be 38 cm, or even as narrow as 25 cm, enabling 24 or more plants per m<sup>2</sup> (Brodrick & Bange 2010; Roche et al. 2006). Assuming a range of 12-24 plants per m<sup>2</sup>, the number of seeds per m<sup>2</sup> could range from approximately 3,500 to over 10,000 per m<sup>2</sup>. Based on the above, seed production would be considered high (>1000 viable seed per m<sup>2</sup>).

Invasiveness questions	Cotton
<b>4. Long distance seed dispersal (more than 100m) by natural means in land uses</b>	
<b>4a. Are viable plant parts dispersed by flying animals (birds and bats)?</b>	<p><b>Rating: Unlikely in all relevant land uses</b></p> <p>There is no evidence that flying animals play a role in the dispersal of cotton seeds. Mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll that retain most of the seeds on the plant (Llewellyn &amp; Fitt 1996), so dispersal from cotton volunteers is highly unlikely.</p>
<b>4b. Are viable plant parts dispersed by wild land based animals?</b>	<p><b>Rating: Unlikely to Occasional in all relevant land uses</b></p> <p>Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (<i>eg</i> hooks or spines). Whole cotton seed, meal and hulls are used in stockfeed. Dispersal of viable seed by ingestion and then later excretion has been reported for livestock, but only a small percentage of seed that passes through the digestive system remains intact and viable. 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. Mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll that retain most of the seeds on the plant (Llewellyn &amp; Fitt 1996), so dispersal from cotton volunteers is unlikely.</p>
<b>4c. Are viable plant parts dispersed by water?</b>	<p><b>Rating: Occasional in all relevant land uses</b></p> <p>Dispersal of viable seed by water is possible, for example through flooding or irrigation run-off, but no data is available. Cotton 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.</p>
<b>4d. Are viable parts dispersed by wind?</b>	<p><b>Rating: Unlikely in all relevant land uses</b></p> <p>The fibres attached to cotton seeds may catch the wind and facilitate seed dispersal, however this is not expected to approach a distance of 100m, except perhaps during severe wind storms.</p>

Invasiveness questions	Cotton
<b>5. Long distance seed dispersal (more than 100m) by human means in land uses:</b>	
<b>5a. How likely is deliberate spread via people?</b>	<p><b>Rating: Common in/dryland and irrigated cropping and intensive land uses</b>  <b>Highly unlikely in nature conservation land use</b></p> <p>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 <i>dryland and irrigated cropping areas</i> and to <i>intensive land use</i> areas for processing and use in feed lots and dairy farms.</p> <p>Cotton seed is not deliberately dispersed within/into <i>nature conservation</i> land use areas.</p>
<b>5b. How likely is accidental spread via people, machinery and vehicles?</b>	<p><b>Rating: Common in dryland and irrigated cropping areas and intensive land uses</b>  <b>Unlikely in nature conservation land use</b></p> <p>In <i>dryland and irrigated cropping areas</i> as well as <i>intensive use areas</i>, 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.</p> <p>No data is available for <i>nature conservation</i> areas. However, human activity in these areas is relatively low and given the reports of isolated pockets cotton plants in these areas, dispersal of cotton seed in/from these areas is considered unlikely.</p>
<b>5c. How likely is spread via contaminated produce?</b>	<p><b>Rating: Unlikely in/from all relevant land use areas</b></p> <p>Cotton farming in <i>dryland and irrigated cropping areas</i> is often characterised by rotation with other crops, such as wheat or the legumes faba bean (<i>Vicia faba</i>) or vetch (<i>Vicia villosa</i>). 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</p>

Invasiveness questions	Cotton
	<p>rotation crop would depend upon the processing of the harvested plant material from the rotation crop.</p> <p>Long distance dispersal via contaminated hay and forage may also occur in or from <i>intensive use areas</i>. 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 <i>intensive use areas</i> is unlikely.</p>
<p><b>5d. How likely is spread via domestic/farm animals?</b></p>	<p><b>Rating: Unlikely in nature conservation areas</b>  <b>Occasional in all other relevant land uses</b></p> <p>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. Dispersal of viable seed by ingestion and then later excretion has been reported for livestock, but only a small percentage of seed that passes through the digestive system remains intact and viable. 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 &amp; Roberts 2002), suggesting that spread to other areas of the farms was unlikely. Thus, seed may occasionally be spread from <i>intensive land use areas</i> such as feed lots or <i>cropping areas</i> if domestic or farm animals had access to the cotton crop.</p> <p>Spread by domestic or farm animals would be highly unlikely in <i>nature conservation areas</i> as they typically not found in these areas.</p>

Impact questions	Cotton
<p><b>6. Does cotton reduce the establishment of desired plants?</b></p>	<p><b>Rating: Reduces establishment by &lt; 10% in all relevant land uses</b></p> <p>Cotton is a cultivated plant that may establish where land has been disturbed, most particularly in <i>dryland and irrigated cropping areas</i>. However, as noted in 1, 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 <i>intensive use areas</i>, 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 (<i>eg</i> maintaining driver visibility) by the removal of larger trees and invasive weeds. Cotton would be treated as a weed and managed accordingly. In <i>nature conservation areas</i>, the ability of cotton to establish is so rare that it is unlikely to affect the establishment of native plants.</p>
<p><b>7. Does cotton reduce the yield or amount of desired plants?</b></p>	<p><b>Rating: Reduces yield/amount by &lt; 10% in all relevant land uses</b></p> <p>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.</p>
<p><b>8. Does cotton reduce the quality of products or services obtained from the land use?</b></p>	<p><b>Rating: Low in all relevant land uses</b></p> <p>As discussed in questions 6 and 7 above, cotton has a low impact on both the establishment and yield/amount of desired species and thus there is no expectation that cotton would reduce the quality or characteristics of products, diversity or services available from the relevant land use areas.</p>
<p><b>9. What is the potential of cotton to restrict the physical movement of people, animals, vehicles, machinery and/or water?</b></p>	<p><b>Rating: Low in all relevant land uses</b></p> <p>Cotton is unlikely to establish in <i>nature conservation areas</i> and although it may establish in <i>dryland and irrigated cropping areas</i> or <i>intensive use areas</i>, standard management practices as well as environmental conditions would keep the density of the cotton volunteers very low. Thus, the potential for cotton to restrict the physical movement of people, animals or water would be low.</p>

Impact questions	Cotton
<p><b>10. What is the potential of cotton to negatively affect the health of animals and/or people?</b></p>	<p><b>Rating: Low in all relevant land uses</b></p> <p>Cotton contains compounds, specifically gossypol and the cyclopropenoid fatty acids, that are toxic if ingested in excessive quantities. The presence of these compounds 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 (Kandyliis et al. 1998). However, in <i>intensive use</i> areas, such as feedlots, its use as stockfeed is limited to a relatively small proportion of the diet and it must be introduced gradually to avoid potential toxic effects (Blasi &amp; Drouillard 2002). Although people use cotton seed oil for cooking, they generally do not consume cotton plants or seed.</p> <p>The density of cotton volunteers is expected to be low in the 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.</p>
<p><b>11. Major positive and negative effects of cotton on environmental health in the land use</b></p>	
<p><b>11a. Does cotton provide food and/or shelter for pathogens, pests and/or diseases in the land use?</b></p>	<p><b>Rating: Major negative effects in dryland or irrigated cropping use</b>  <b>Minor or no effect in all other relevant land use areas</b></p> <p>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 <i>dryland or irrigated cropping use</i> areas may act as a reservoir of these pathogens and pests that can infect crops in subsequent years. 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 due to standard weed management practices. The magnitude of this effect is difficult to predict (eg. under sub-standard weed management), thus in some years may constitute a major negative effect.</p> <p>In <i>intensive or nature conservation use</i> areas the density of cotton volunteers is expected to be low and thus may have only minor or no effect.</p>

Impact questions	Cotton
<b>11b. Does cotton change the fire regime in the land use?</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to affect fire regimes.
<b>11c. Does cotton change the nutrient levels in the land use?</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to affect nutrient levels.
<b>11d. Does the species affect the degree of soil salinity in the land use?</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil salinity.
<b>11e. Does the species affect the soil stability in the land use?</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil stability.
<b>11f. Does the species affect the soil water table in the land use</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to affect the soil water table.
<b>11g. Does the species alter the structure of nature conservation by adding a new strata level?</b>	<b>Rating: Minor or no effect in all relevant land uses</b> The number and density of cotton volunteers is expected to be low for all relevant land uses, and would not be expected to add a new strata level.