Australian Government
Department of Health and Aged Care
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 the risk analysis of genetically modified forms of the species that may be released into the Australian environment.

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ABBREVIATIONS

ACT	Australian Capital Territory
ACSA	Australian Cotton Shippers Association
AFLP	Amplified Fragment Length Polymorphism
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bt	*Bacillus thuringiensis*
CBTV	*Cotton bunchy top virus*
CLCuV	*Cotton leaf curl virus*
CPFA	Cyclopropenoid fatty acids
CRC	Cooperative research Centre
CRDC	Cotton Research and Development Corporation
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industry
ELS	Extra-long staple cotton
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
Ha	Hectare
ML	megalitre
N	Haploid number of chromosomes
NSW	New South Wales
NT	Northern Territory
OECD	Organisation for Economic Co-operation and Development
Qld	Queensland
QTL	Quantitative Trait Locus
RFLP	Restriction Fragment Length Polymorphisms
SA	South Australia
spp.	Species
Tas	Tasmania
VAM	Vesicular arbuscular mycorrhizae
Vic	Victoria
WA	Western Australia

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 (GM) *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. Since the early 2010s, only *G. hirsutum* has been commercially grown in Australia, mostly as an irrigated crop in northern New South Wales (NSW) and Queensland (Qld). In recent years, the cotton industry has expanded into northern Victoria (Vic), Western Australia (WA) and the Northern Territory (NT).

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.

Gossypium barbadense L. was named after its assumed habitat of Barbados. It has been known by alternative scientific names as *Gossypium peruvianum, Gossypium vitifolium* and *Gossypium brasiliense*. It is commonly known as Creole cotton, Egyptian cotton, extra-long staple or ELS cotton, Indian cotton, Sea Island cotton or Pima cotton (Dube, 2017).

The common name cotton comes from the Arabic 'quotn' 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 centre of origin for the *Gossypium* genus is most likely Africa where the most diverse group of species exists. Great diversity of wild cotton species is also found in Australia (especially in the NT and the north-east of WA). The taxonomy of *Gossypium* and phylogenetic relationship of species within the genus have been clarified using DNA sequencing followed by phylogenetic analysis (Grover et al. 2016; Wendel & Grover 2015).

The genus *Gossypium* contains around 50 species; some of which have been discovered or resurrected and new species are likely to be discovered. For example, *G. nandewarense* has been downgraded to subspecies of *G. sturtianum* after rigorous genetic studies (Wajahatullah et al., 1997). In 2017, a new species was identified in the islets of Wake Atoll (located in the Western Pacific), using nuclear and chloroplast genome sequences. It was named *G. stephensii* formerly thought to belong to *G. hirsutum.* It is suggested that this species originated from Mexico's west coast following ocean dispersal (Gallagher et al., 2017). Figure 1 combines phylogenetic data, genome size and distribution for known Gossypium species.

Based on chromosomal similarities, the known Gossypium species are classified into 8 diploid and one tetraploid genomic groups (Edwards and Mirza, 1979; Endrizzi et al., 1985; Stewart, 1995). The diploid groups are designated A, B, C, D, E, F, G and K. The tetraploid group designated as AD because it contains both A and D genomes. Each group represents morphologically similar species that can only rarely form hybrids with species from other genomic groups (Table 1). There are 44 diploid species ($2n = 2x = 26$) and 7 allotetraploid ($2n = 4x = 52$) species known (Wendel et al., 2009; Gallagher et al., 2017). Despite sharing the same number of chromosomes, diploid species exhibit more than a 3-fold variation in DNA content per genome due to the extensive chromosomal evolution experienced by *Gossypium* species (Wendel et al., 2009).

The 2 species cultivated in Australia, *G. hirsutum* and *G. barbadense*, 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).

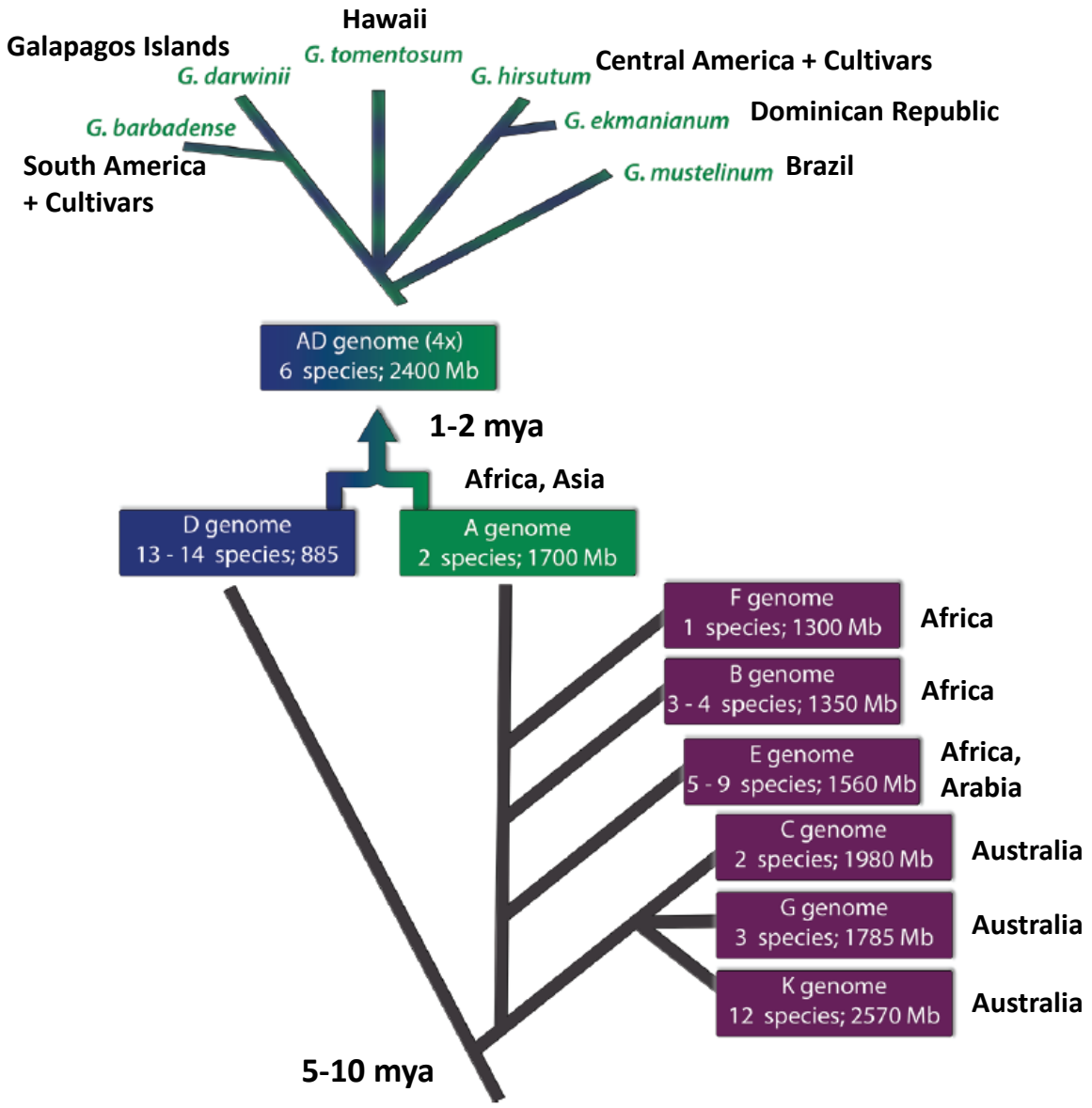

Figure 1. Phylogenetic tree of *Gossypium* species (modified from Wendel and Grover, 2015). The phylogenetic tree at the bottom represents the 8 diploid groups and contains information about the size of the genome in million bases and number of species in each group. The tree at the top represents the polyploid species and their phylogenetic relationships. Note this phylogenetic tree was representative when produced in 2015 and there have been additions and restructuring since, these are identified in Section 1.

Table 1. Taxonomy of *Gossypium* Species

Genomic Group	Species	Distribution
A	*G. herbaceum* L., *G. arboretum* L. (syn. *G. aboreum* L.)	Africa, Asia Minor Africa, Asia Minor, China
B	*G. anomalum* Wawr. and Peyr., *G. triphyllum* (Harv. And Sand.) Hochr, *G. captis-viridis* Mauer, *G. trifurcatum* Vollesen	Africa, Cape Verde Islands, Somalia
C	*G. sturtianum* J.H. Willis, *G. robinsonii* F. Muell.	Australia
D	*G. klotzschianum* Anderss., *G. raimondii* Ulbr, *G. thurberi* Tod., *G. armourianum* Kearn., *G. harknessii* Brandg., *G. davidsonii* Kell., *G. aridum* (Rose & Standl.) Skov, *G. gossypioides* (Ulbr.) Standl., *G. lobatum* Gentry, *G. laxum* Phillips, *G. trilobum* (DC.) Skov., *G. turneri* Fryx., *G. schwendimanii* Fryxell & S. Koch	Galapagos islands Peru Mexico, Arizona Mexico
E	*G. stocksii* Mast.ex. Hook., *G. somalense* (Gürke) Hutch., *G. areysianum* (Defl.) Hutch., *G. incanum* (Schwartz) Hille., *G. benadirense* Mattei, *G. bricchettii* (Ulbrich) Vollesen, *G. vollesenii* Fryxell	Arabian Peninsula Somalia, Kenya, Ethiopia & southwest Asia Somalia & southwest Asia
F	*G. longicalyx* Hutch. and Lee	East Africa
G	*G. bickii* Prokh, *G. nelsonii* Fryx., *G. australe* F. Muell.	Australia
K	*G. costulatum* Tod., *G. cunninghamii* Tod., *G. enthyle* Fryxell, Craven & J.M. Stewart, *G. exiguum* Fryxell, Craven & J.M. Stewart, *G. londonderriense* Fryxell, Craven & J.M. Stewart, *G. marchantii* Fryxell, Craven & J.M. Stewart, *G. nobile* Fryxell, Craven & J.M. Stewart, *G. pilosum* Fryx., *G. populifolium* (Benth.) Tod., *G. pulchellum* (C.A. Gardn.) Fryx., *G. rotundifolium* Fryxell, Craven & J.M. Stewart, *G. anapoides* J.M. Stewart, Craven, Brubaker and Wendel	northwest Australia, Cobourg Peninsula, NT
AD	*G. hirsutum* L. *G. barbadense* L. *G. tomentosum* Nutt. ex Seem. *G. mustelinum* Miers ex Watt *G. darwinii* Watt *G. lanceolatum ekmanianum* Tod *G. stephensii*	Cultivars, Central America Cultivars, South America Hawaiian Islands Brazil Galapagos Islands Dominican Republic Wake Atoll (West Pacific)

Sources: Endrizzi et al. (1985); Stewart (1995); Percival et al. (1999); Seelanan et al. (1999); Rapp et al. (2005); Krapovickas and Seijo (2008); Wendel et al. (2009); Grover et al. (2015); Stewart et al. (2015); Wendel and Grover (2015); Gallagher et al. (2017)

1.1 Taxonomy and distribution of native Australian cotton species

The Australian flora contains 17 native *Gossypium* species (Appendix A) 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 (Fryxell, 1979b, 1992; Brubaker et al., 1999a;

Brubaker et al., 1999b; Seelanan et al., 1999). The Australian *Gossypium* species are all diploid (2*n* = 26) and fall within the 3 taxonomic sections of the subgenus *Sturtia*, C, G or K. Section *Sturtia* (C-genome) contains 2 species, including Sturt's desert rose (*G. sturtianum*, the floral emblem of the NT); Section *Hibiscoidea* (G-genome) contains 3 species and Section *Grandicalyx* (K-genome) contains 12 species (Wendel and Cronn, 2003).

The centre of *Gossypium* diversity in Australia is in northern WA and the NT. Interestingly, 12 out of 17 Australia's *Gossypium* species are found in the relatively small coastal area in northern WA. Of the remaining 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 (Atlas of Living Australia, accessed September 2023). 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. Figure 2 shows the distribution of all native Australian *Gossypium* species combined.

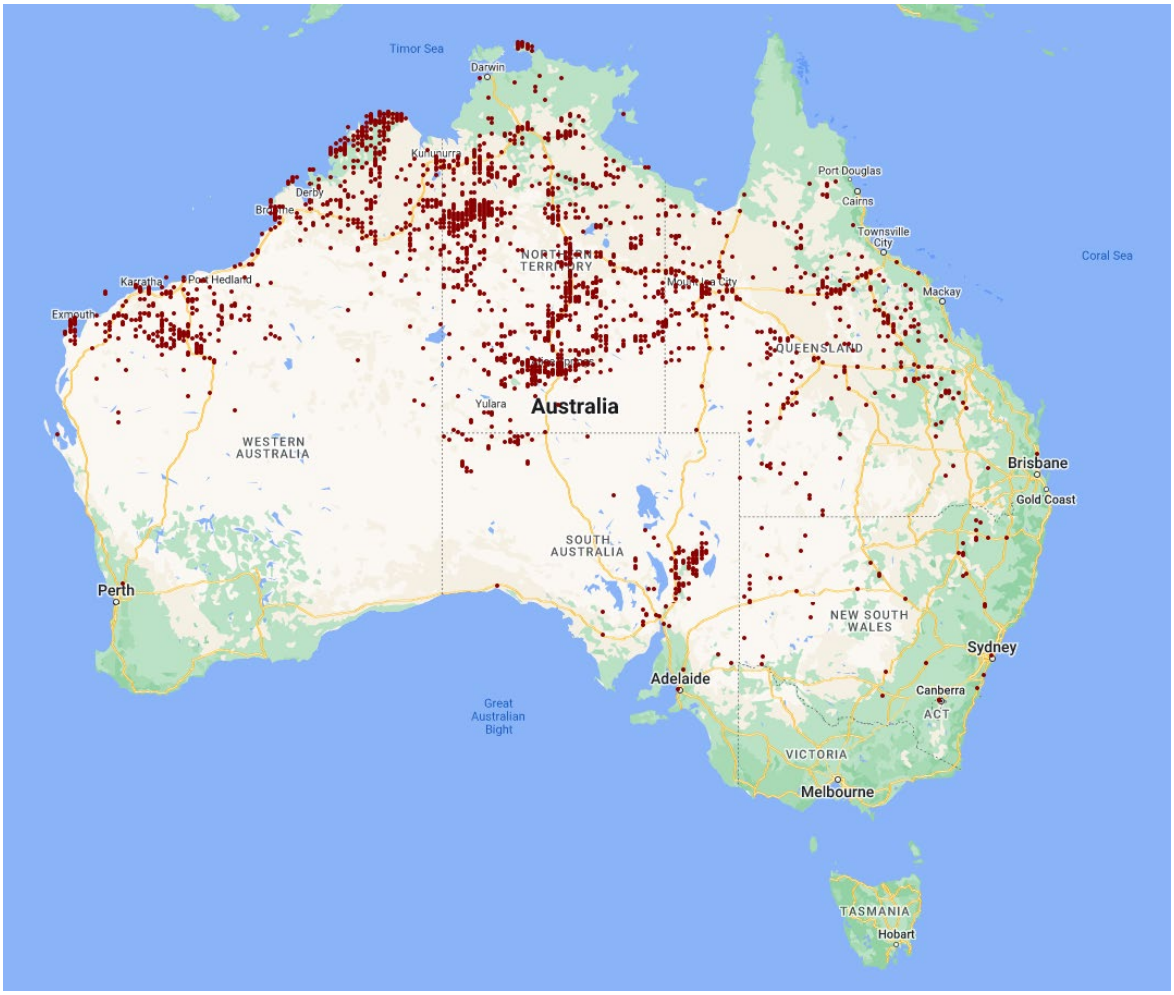

Figure 2. Distribution of native *Gossypium* species in Australia, as at September 2023. The map has been created using the "Atlas of Living Australia" interactive map service by including the species listed in Appendix A.

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 4 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; Li et al., 2020). *G. barbadense* represents approximately 5-8% of world fibre production (Wu et al., 2005; Li et al., 2020) and is cultivated primarily in Egypt, Peru, Sudan, USA and parts of the former Soviet Union. *G. arboreum* and *G. herbaceum* represent less than 1 % of the world cotton and are grown main in Bangladesh, India, Myanmar and Pakistan (ICAC, 2023a).

In the past, *G. barbadense* represented about 2% of commercial cotton cultivated in Australia. However, it has not been grown commercially since 2010. Currently, the Australian cotton industry is based on *G. hirsutum* (Stiller and Wilson, 2014; CRDC and CottonInfo, 2023b). In Australia, CSIRO has released at least 113 varieties of cotton, such as Sicot 746B3F, that have increased yield by 2% per annum and extended the growing seasons since 1984 (Ward, 2013; CSIRO, 2021).

As mentioned in Section 1, the place of origin of the *Gossypium* genus is thought to be Africa, based on genetic diversity of African/Arabian species. *Gossypium* species have been demonstrated to constitute a single natural lineage, despite their vast global distribution and apparent morphological differences (Wendel et al., 2009).The primary centres of diversity for the genus are West-central and Southern Mexico (18 species), North-east Africa and Arabia (14 species) and the Kimberley region of 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 (Wendel and Albert, 1992; Seelanan et al., 1997) or slightly more recently in the Pliocene (Cronn et al., 2002). Based on molecular evidence such as DNA sequence data, allotetraploid cotton species (AD genomes) are the result of hybridisation between A-genome and D-genome species in mid-Pleistocene, 1-2 million years ago (Figure 1). This event occurred when Old World's A-genome species crossed the Atlantic and hybridised with American D-genome species followed by genome duplication (Wendel and Grover, 2015). This period was characterised by fluctuating sea levels due to glaciation, and the coastal distribution of the allotetraploids may have enabled them to exploit the disturbed littoral areas (Fryxell, 1979b).

Archaeological records indicate that *Gossypium* fibre has been used since 6000 BCE. A *Gossypium* thread, used to string copper beads, from Mehrgarh in Pakistan has been dated at 6th millennium BCE (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 that 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 Tehuacán Valley in Mexico (Smith and MacNeish, 1964). These have been identified as being from tetraploid *Gossypium,* with the earliest bolls dating from approximately 5800 BCE. 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 BCE) to later (1000 BCE) levels (Stephens and 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 and Wendel, 1994). It is believed that *G. hirsutum* was cultivated by the Pueblo Indians in the South-west USA as early as the first century CE (Fryxell, 1979a). 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 Kekchí 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.

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).

Most wild cottons have a short day photoperiod response for flowering, however, 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 that 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, 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 (Coppens d'Eeckenbrugge and Lacape, 2014).

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 seeds, fibres, fabric and fishing nets have been found at Huaca Prieta on the north coast of Peru, dating from 1500-2400 BCE. From this centre *G. barbadense* dispersed into South America, the West Indies and the Galapagos. This may have been carried by humans or naturally by ocean currents (Smith, 1995).

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.

2.1.1 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 in 1882 and the Kimberley, WA in 1947, although in these northern regions the rainfall, high UV and prevalence and impact of insect pests limited the commercial viability of continued plantings (Wood and 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 and Fitt, 1992).

G. hirsutum may also have arrived in northern Australia naturally, via ocean currents from Central America (Fryxell, 1966, 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 (1966, 1979b), or alternatively, feral derivatives of primitive varieties introduced for cultivation before 1900.

2.2 Commercial uses

Cotton is currently the world's leading plant fibre crop and is grown commercially in the temperate and tropical regions of more than 75 countries (Jabran and Singh, 2020). Specific areas of production include the USA, India, China, America, the Middle East and Australia. In these regions, climatic conditions suit the natural growth requirements of cotton, including periods of hot and dry weather, with adequate moisture available, often through irrigation. The top 6 producers in 2021/2022 (metric tonnes - t) were China, India, USA, Brazil, Pakistan and Australia, that produce approximately 80% of the world's cotton (USDA, 2022). In 2022, cotton ranked as the third most valuable agricultural export in Australia (DAFF, 2023). Depending on the season, Australia's export volume equates to approximately 3-9% of the world cotton export (Ward, 2013; USDA, 2022).

The forecast global cotton production for 2023-2024 is 24.8 million t (i.e. 114.1 million US bales; each US bale weights approximately 480 lb or 218 kg), a decrease of 3.5 % compared to 2022-2023 (USDA, 2023). In Australia, the annual production for 2023-2024 is forecast at 1.17 million t, about 5.18 million Australian bales (Australian bales weigh approximately 500 lb or 227 kg) (USDA, 2023). The highest annual production in Australia was observed in the 2011-2012 and 2021-2022 seasons at approximately 1.15-1.22 million t (5.3 – 5.6 million Australian bales) and the lowest in 2019-2020 at approximately 133,930 t (590,000 Australian bales) (Cotton Australia, 2023a).

Over 99% of Australian cotton production is exported. Despite an increase in cotton production, Australian cotton exports fell approximately 51% in value to AU$510 million for 2020 as new crop supplies were only made available for purchase in April 2021, still recovering from the effects of the droughts of previous years (NSW DPI, 2021). Strains on the export market were exacerbated by tariffs and global market conditions, as well as the impacts of COVID-19, which affected all stages of the cotton production process (Voora et al., 2020). In 2021, Australian cotton exports surged to AU$2.2 billion, an increase of approximately 250% in value compared to the previous year (NSW DPI, 2022b). A portion of the 2021 crop was sold in the following season due to a delay in harvesting, and the 2022 season was estimated at AU$4.6 billion, a new record in export value. In 2019-2020, the Chinese market comprised 62% of the Australian cotton exports. Due to changes in market conditions, Australia diversified its export destinations. Currently, the major markets for Australian cotton are Vietnam (41%), Indonesia (20%), Turkey (7%), Thailand (6%) and India (6%) (DAFF, 2022b). The Australian cotton export value for the period 2017 to 2022 is shown in Table 2.

G. barbadense is known for its fibre quality as it has longer staple length between 32 and 40 mm compared to *G. hirsutum*, which has staple length between 22 and 33 mm (Cotton Incorporated, 2018). It is also referred to as long staple (LS) or Extra-long staple (ELS) cotton. Fine fibre produced from *G. barbadense* demonstrates higher strength than fibre from *G. hirsutum* (Smith, 1999). In the period 2012-2022, the global LS and ELS cotton production varied between approximately 375,000 and 496,000 t, with the primary producers being the USA, China, India and Egypt (Reinhart, 2018accessed September 2022; ICAC, 2022, 2023b). In 2021/2022, *G. barbadense* accounted for just 1.2% of the world's total cotton production, with a volume of 308,000 t. Projections for 2022/2023 anticipates a production increase to around 430,000 t (ICAC, 2023b).

Table 2. Australian cotton export value for the period 2017 to 2022 (Trend Economy 2023)

Year	Value ($US)
2017	$1,332,026,736
2018	$1,836,953,486
2019	$1,111,232,821
2020	$314,934,213
2021	$1,448,102,346
2022	$3,031,258,406

Cotton is primarily grown as a fibre crop. It is harvested as seed cotton, which is then ginned to separate the seed and lint. Cotton fibre can undergo a secondary mechanical processing step, after which chemical processing involving heat, pressure and a sodium hydroxide solution is used to saponify the natural wax coat and remove non-cellulosic material as well as pectins. To whiten the fibres, an oxidising agent such as hydrogen peroxide is applied followed by a fibre finish and a drying process. The length and degree of processing applied by each step is dependent on the type and quality of cotton required by the consumer. 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 (Barnhardt Natural Fibres, 2023).

After the ginning process, some short, fuzzy fibres, known as 'linters' are still attached to the *G. hirsutum* seed. 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 (i.e. seed with no lint or linters) is processed into oil, meal and hulls (Cherry and 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 19th 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 seeds contain around 23% of crude protein (Bertrand et al. 2005). Cotton seed meal is the product remaining once the oil has been removed by crushing and can contain up to 41% of 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. In 2019, a GM ultra-low gossypol cottonseed was approved for food and feed in the USA (Rathore et al., 2020).

While some international bodies have not established a safe daily intake of gossypol (European Food Safety Authority, 2009), the United States Food and Drug Administration (FDA) permits up to 0.45 µg/mg (450 ppm) of free gossypol in edible cotton seed products for human consumption (FDA, 2023). Cotton seed meal is mainly consumed in central American countries and India where it is used as a low cost, high quality protein ingredient (Frank, 1987).

Cotton trash, a bioproduct generated during the ginning process, can be used as a bulking agent or as one of the components in the production of compost (Steiner et al., 2011; Qurat ul et al., 2021). 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 fermenting cotton waste to produce ethanol (Jeoh and 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 sold for treatment of hypertension, fungal infection and menstrual stimulant (Tropilab Inc., 2007) (See Section 5.3 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 (CRDC and CottonInfo, 2023b).

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 (CSD, 2020). 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).

Isolation distances for production of certified seed of *G. hirsutum* and *G. barbadense* may vary. To promote international standardisation, the OECD Seed Schemes recommends separation distances to produce certified agricultural seeds. A country may choose to participate in one or more of the crop groups listed in the scheme. Recommendations for certified cotton seeds are based on the category of seed being produced, the cotton species, whether it is the product of a hybrid, and if the hybrid was produced using cytoplasmic male sterility (CMS) lines (OECD, 2023), as summarised in Table 3. The production of cotton seeds in Australia adheres to OECD Seed Scheme standards and other international protocols (CSD, 2020). A full list of countries participating in the OECD Seed Scheme can be found in the OECD website (accessed November 2023).

Table 3. Isolation distances recommended by the OECD for the production of cotton seed

	G. barbadense	*G. hirsutum*	*G. hirsutum* × *G. barbadense*
Basic Seed	200 m	100 m	200 m
Certified Seed - Non-hybrid varieties	150 m	30 m	150 m
F_1 hybrids - produced without CMS	150 m	30 m	150 m
F_1 hybrids - produced using CMS	800 m	800 m	800 m

Source: OECD (2023)

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 was 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 (Santhy et al., 2008). Due to high labour costs, these processes are considered economically unfeasible in many countries. Research into insect pollination of male sterile lines in Arizona, USA indicated that insect pollination rates were probably not high enough for hybrid cotton production (Moffett et al., 1975).

2.3.2 Scale of cultivation

In Australia, the total area planted to cotton varies from season to season with ~60,000 ha planted in the 2019-2020 season compared to over ~560,000 ha planted in the 2021–2022 season. (Cotton Australia, 2022). An overview of the total crop volume and area planted from 2000 to 2022 is shown in Figure 3.

Cotton farms in Australia typically range from 300 to 4400 ha (Hearn and Fitt, 1992). In the 2021-2022 season, the average size of cotton farms was 1,056 ha (CRDC, 2022). Yield also varies depending on the season, with the lowest recorded at ~6.7 bales/ha in 2011 and the highest at ~11.1 bales/ha in 2015 (Figure 3) (Cotton Australia, 2022). The 2021/2022 season saw an average yield of ~9.67 bales/ha across irrigated and dryland cotton (CRDC, 2022). The global cotton lint yield in major cotton producing countries is shown in Figure 4. Since 2000, Australian cotton yield has varied from 1.5 to 3.5 times the word's average cotton production. The main limiting factor for cotton production in Australia is availability of water. In 2021/2022 season, 78% of Australian cotton planted area was irrigated (CRDC, 2022).

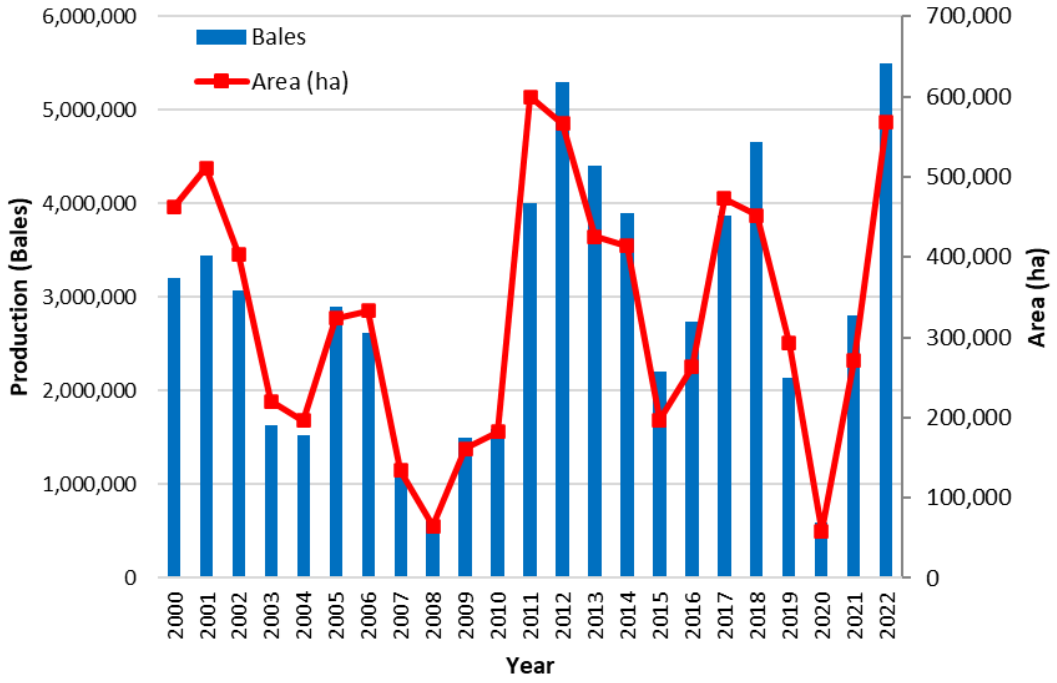

Figure 3. Seasonal cotton crop in Australia. Data sourced from Cotton Australia (2022) and the Cotton Research Development Corporation (CRDC, 2022). The year listed is when the season finished.

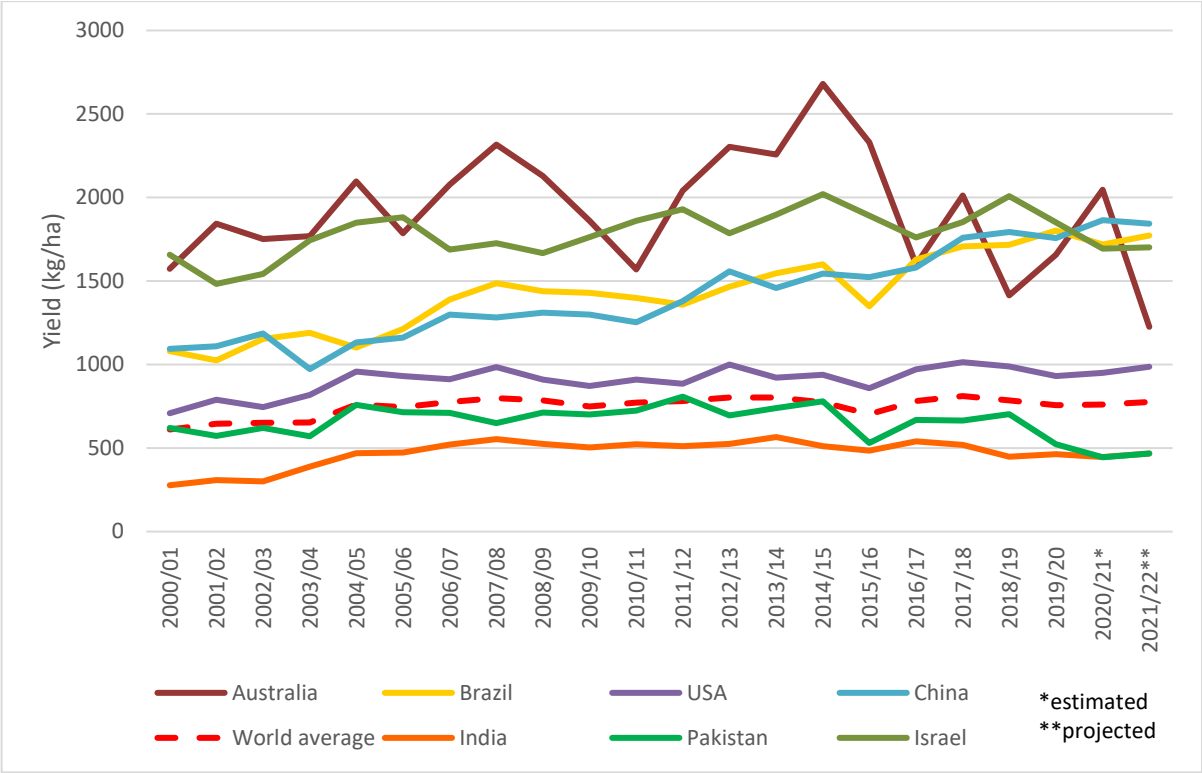

Figure 4. Dynamics of cotton lint yield in major cotton producing countries. Data sourced from International Cotton Advisory Committee website, accessed September 2023. Although not a major producer, Israel is included as a high-yielding cotton producing country.

In Australia, the bulk of the cotton industry is concentrated in northern NSW and southern Qld around river valleys (Figure 5). In NSW, cotton is grown along the Barwon and Darling rivers in the west and the Lachlan and Murrumbidgee rivers in the south. It is also cultivated south from the Macintyre River on the Qld border and covers the Gwydir, Namoi and Macquarie valleys. In Qld, cotton is grown in the Darling Downs, St George, Dirranbandi and Macintyre Valley regions and also near Emerald, Theodore and Biloela in Central Qld (DAFF, 2019).

Previously, *G. barbadense* (Pima) was cultivated around Bourke, Tandou and Hillston in western NSW and accounted for approximately 1-2% of the total cotton production (CRDC, 2013a). However, more recently no varieties of Pima cotton are currently commercially available in Australia (CRDC, 2013a; CRDC and CottonInfo, 2023b). The long drought from 2010 and the eventual loss of infrastructure to support Pima cotton processing, may have contributed to its commercial downfall in Australia (Stiller and Wilson, 2014).

The major cotton growing regions in Australia are listed in Table 4 and illustrated on the map (Figure 5). A detailed map for the current growing season is available at SataCrop.

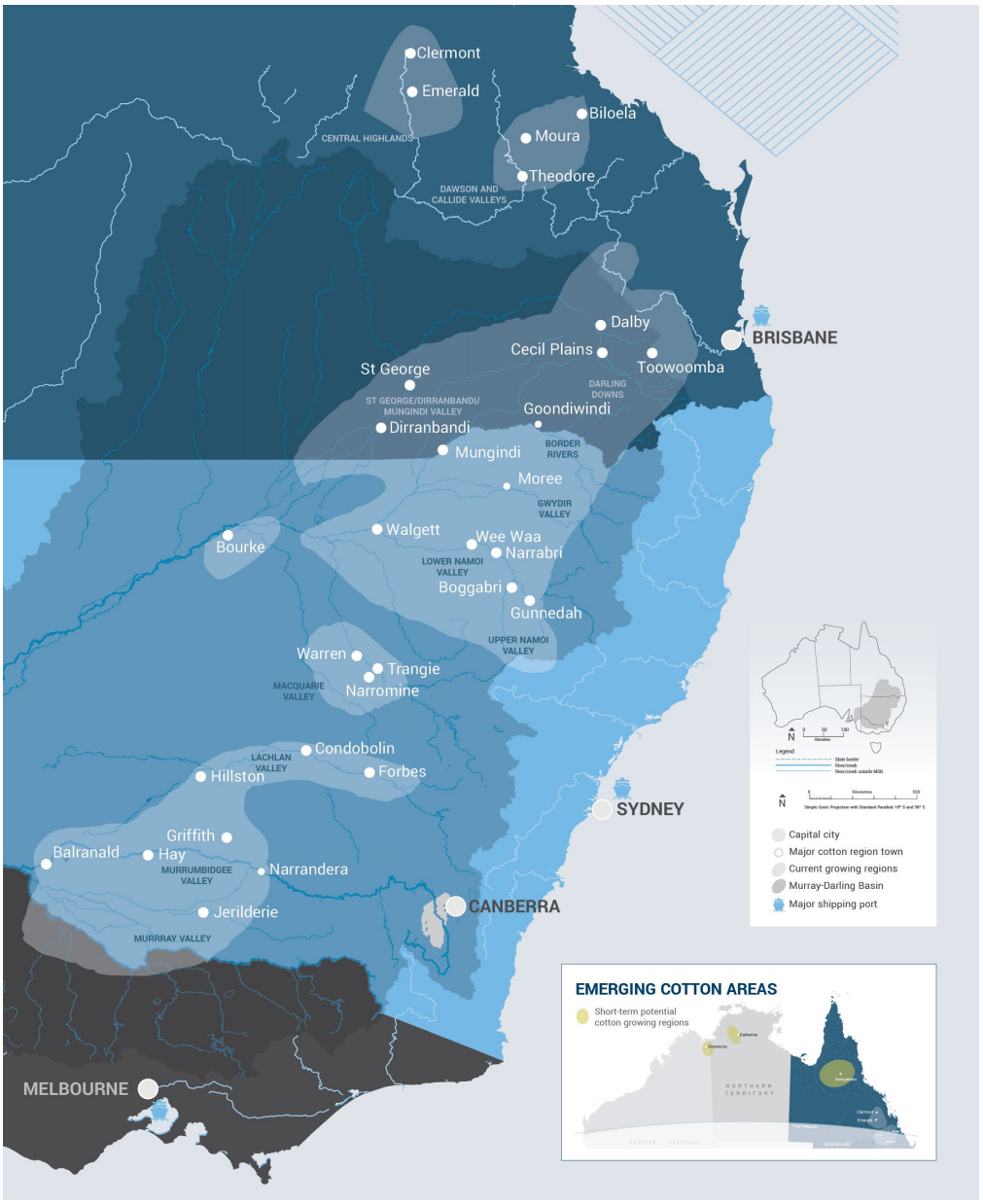

Figure 5. Cotton growing regions in Australia. Image as produced by Cotton Australia, accessed September 2023.

Table 4. Major cotton growing regions in Australia

State	Cotton growing region	LGAs	Towns
Qld	Central Highlands	Isaac, Central Highlands	Clermont, Emerald
Qld	Dawson and Callide Valleys	Banana	Theodore, Biloela, Moura
Qld	St George/Dirranbandi Valley	Balonne	St George, Dirranbandi
Qld	Darling Downs	Western Downs, Millmerran, Toowoomba, Goondiwindi	Dalby, Cecil Plains Toowoomba, Goondiwindi

State	Cotton growing region	LGAs	Towns
Qld/NSW	Macintyre Valley	Moree Plains (NSW), Balonne (QLD), Goondiwindi (QLD)	Mungindi
NSW	Gwydir Valley	Moree Plains	Moree
NSW	Upper Namoi	Gunnedah, Narrabri	Gunnedah, Boggabri,
NSW	Lower Namoi	Narrabri, Walgett	Narrabri, Wee Waa, Walgett
NSW	Macquarie Valley	Narromine, Warren	Narromine, Warren, Trangie,
NSW	Bourke	Bourke	Bourke
NSW	Lachlan - Murrumbidgee	Lachlan, Forbes, Carrathool, Griffith, Hay, Narrandera, Murrumbidgee, Balranald	Condobolin, Forbes, Hillston Griffith, Hay, Narrandera, Jerilderie, Balranald

Source: Major cotton growing regions and the corresponding local government areas (LGAs) as presented in Figure 5.

Climates with long, warm summers are typical for *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 cotton growing areas are shown in Table 5.

Table 5. Climatic data for select cotton growing region towns in Australia, as identified in Figure 5

Representative site (weather station)	Mean daily max/min temperature °C (summer)	Mean daily max/min temperature °C (winter)	Mean monthly rainfall mm (summer)	Mean monthly rainfall mm (winter)	Reporting Period
Emerald QLD (Emerald Airport)	34.3/21.9	24.2/9.9	82.8	22.5	1992-2023
Biloela QLD (Thangool Airport)	33.4/19.7	24.0/6.4	93.7	27.4	1992-2023
Toowoomba QLD (Toowoomba Airport)	27.9/17.3	17.5/7.3	99.8	31.0	1996-2023
St George QLD (St George Airport)	34.4/21.3	20.6/5.9	57.6	24.7	1997-2023
Moree NSW (Moree Aero)	33.5/19.5	19.3/5.3	70.8	31.7	1995-2023
Bourke NSW (Bourke Airport)	36.2/21.7	19.7/5.1	32.0	18.6	1998-2023
Walgett NSW (Walgett Airport)	34.9/19.7	19.6/4.3	50.1	26.3	1993-2023
Narrabri NSW (Narrabri Airport)	34.0/19.4	19.0/4.8	64.3	36.3	2001-2023
Trangie NSW (Trangie Research Station)	32.6/17.9	16.4/3.9	48.5	34.1	1991-2023

Representative site (weather station)	Mean daily max/min temperature °C (summer)	Mean daily max/min temperature °C (winter)	Mean monthly rainfall mm (summer)	Mean monthly rainfall mm (winter)	Reporting Period
Hillston NSW (Hillston Airport)	33.3/18.0	16.6/4.2	34.4	32.1	1991-2020
Griffith NSW (Griffith Airport)	32.6/17.3	15.9/4.1	31.8	31.9	1991-2020
Balranald NSW (Balranald RSL)	32.3/16.4	16.7/4.4	29.7	27.0	1991-2020

Source: Bureau of Meteorology, accessed November 2023.
Summer months include December, January and February. Winter months include June, July and August, seasons as defined by the Bureau of Meteorology.

2.3.2.1 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).

Cultivation of cotton in North Qld and NT historically proved difficult due to abundance of pests. Growing of cotton during summer is also affected by high humidity at the harvesting period and impact greatly on cotton fibre quality (Eastick, 2002; Farrell and Roberts, 2002). Since the introduction of pest-resistant GM cotton varieties, this problem has become less acute. Cotton production is emerging in NT, with a total of 800 ha dedicated to cotton planting between 2019-2020, spread across 6 farms (Northern Territory Government, 2023). Besides climate suitability and pest control, the main limiting factor for industry expansion is the absence of infrastructure for processing and high transportation cost. For example, cotton produced in WA is currently transported for processing in Queensland with a cost of approximately AU$ 100-200 per bale. To address this issue, a cotton gin is planned to be built in Kununurra (northern WA), with completion expected by mid-2025 (Government of Western Australia, 2023a, b).

2.3.2.2 Commercial GM cotton in Australia

In Australia, over 99% of currently grown cotton is genetically modified (GM) and 95% of varieties grown contain stacked traits for insect resistance and herbicide tolerance (Cotton Australia, 2015). The list of GM cotton lines approved for commercial release in Australia can be found at the OGTR website.

2.3.3 Cultivation practices

Temperature is the dominant environmental factor affecting *G. hirsutum* development and yield (Constable and Shaw, 1988; Australian Cotton Cooperative Research Centre, 2018). Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least 3 successive days (CottonInfo, 2016a; CRDC and CottonInfo, 2023b). 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, 1998) and 200-250 days for *G. barbadense* (Unruh and 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 and Shaw, 1988). Ideal soil temperatures for cotton establishment are between 16 and 28°C (CottonInfo, 2016a).

The timing of cotton cultivation varies slightly throughout Australia, depending on climate and therefore when the appropriate soil temperature is reached. 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, 2016b). To achieve the optimal growth as described above, *G. barbadense* may be planted earlier and harvested later than *G. hirsutum.*

Rotation and fallow can enhance soil properties, nutrients and can break cotton disease and weed cycles, leading to increased crop productivity (CRDC and CottonInfo, 2023b; Lv et al., 2023). Intercropping, when 2 crops are planted in the same season, enhances overall land productivity, however, yields of intercropped cotton are generally lower compared to monocropping (Lv et al., 2023). In Australia, cotton is normally grown as a sole crop and fields may rotate into grains or legumes crops (i.e. wheat, maize, chickpeas, faba beans), fallowed or used for short period of sustainable livestock grazing (Cotton Australia, 2023d; CRDC and CottonInfo, 2023b).

Cotton is generally planted between 2.5 and 4.5 cm deep into moist soil or 2.5 cm deep into dry soil, with the aim of establishing 8-12 plants per metre for irrigated cotton or 6-8 per metre for dryland cotton. Row configurations may include skip row configurations, particularly for dryland cotton or in situations where irrigation water is scarce (CRDC and CottonInfo, 2023b).

The timing of planting for GM insect resistant *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 insect resistance proteins can be effectively managed. These measures include requiring the grower to plant refuge crops of minimum sizes, types and distances from the GM crop, fixed planting windows, post-harvest crop destruction, control of volunteer and ratoon cotton, pupae destruction and trap cropping (APVMA, 2003).

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, 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 80% boll opening is 2050 (Acres of Opportunity, 2021). For example, cotton planted on 1 October near Warren (Macquarie Valley, NSW) could be expected to reach 80% 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°C) + (daily min. temp. - 12°C)]/2

When the minimum temperatures are less than 12°C, the day degree formula becomes:

Day degrees = (daily max. temp. - 12°C)/2

Water availability is another major factor influencing yield, with the majority of Australia's cotton production occurring under irrigation (CRDC, 2022). Furrow irrigation, when water is channelled into trenches dug between the rows of plants, is the most common system. Other irrigation systems used in Australia include Centre Pivot Lateral Move (CPLM), subsurface Drip (SSD) and Siphonless/Bankless channels (CottonInfo, 2023b).

Cotton crops require in average 6-7 megalitres of irrigation water per hectare (ML/ha) (Cotton Australia, 2023b). It is estimated that fields are irrigated 5 or 6 times during the growing season between flowering and peak boll development (McLeod et al., 1998). Changes in irrigation practices

(such as soil moisture monitoring, laser-assisted soil levelling and use of GPS-guided tractors) have improved the water usage efficiency by about 48% since 1992 (Cotton Australia, 2023b).

When cotton is grown as an unirrigated crop the biggest climatic factor influencing cotton yield is rainfall (Ford and Forrester, 2002). In Australia, the majority of dryland production occurs in areas that have a moderate to high variability in rainfall during December to March, the crucial period of the growing season determining yield quantity and quality (CRDC and CottonInfo, 2023b) .

The area dedicated to dryland cotton varies each season. In 2021/2022, about 20% of the cotton planted area was dryland cotton, up from 8% in 2016/2017 (Cotton Australia, 2015, 2016a, 2018). The main driver for this reduction is the lower yield for unirrigated cotton, which depending on the season can vary between 2.3-7.5 times lower compared to irrigated cotton (Cotton Australia, 2015, 2016a, 2018). On average, irrigated cotton crop yields over 10 bales/ha, while cotton grown under dryland conditions yields 1.5-3 bales/ha (Cotton Australia, 2023a).

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 and 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 defoliants 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 defoliants 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 defoliants 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 7.2.2) 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 7.3. 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. 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 all Australian cotton is planted to CSIRO varieties, some

of which are also used internationally, including the USA, Brazil, Turkey and Greece (CSIRO, 2016). Plant breeding has been focused on crop traits including high yield, improved fibre characteristics, disease resistance, regional adaptation and suitability for dryland growing conditions (Conaty et al., 2022). In 2001 it was estimated that breeding has contributed 45% to the improvements in yield seen since 1983 (Constable et al., 2001) and that CSIRO varieties have improved water efficiency and reduced pesticide and herbicide use (CSIRO, 2016).

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 (Wang et al., 1995; Brubaker et al., 1999a). 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 and Sarvella, 1956), and can be hybridised to produce fertile F_1 progeny. However, F_2 progeny show evidence of lethal gene combinations in succeeding generations (Gerstel, 1954; Stephens and Phillips, 1972). The 2 species have different ribosomal DNA sequences (Wendel et al., 1995) and chloroplast genomes (Wendel and 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 2 species distinct; these include incompatibility at the 'corky' locus (Stephens, 1946, 1950a, b; Stephens and Phillips, 1972), differences in the timing of pollen shedding (Stephens and Phillips, 1972), and selective fertilisation (Kearney and Harrison, 1932; Brubaker et al., 1999a). However, these can be overcome with directed breeding. 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 jassid insect pests and this species has been used in an attempt to transfer this resistance to *G. hirsutum*. The *G. raimondii* × *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 and 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 were developed for regeneration of commercial varieties of *G. hirsutum* and *G. barbadense* (Gould et al., 1991; Sakhanokho et al., 2001; Sakhanokho et al., 2004), 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). These were then cultured to promote embryogenesis and regenerate plants (Umbeck et al., 1987). This process takes 6-12 months to complete. Agrobacterium-mediated transformation has remained the most popular method despite reports of transformation of embryonic suspension cultures via particle bombardment (Finer and McMullen, 1990; McCabe and Martinell, 1993; Rajasekaran et al., 2000;

Srivastava et al., 2023). 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 and Martinell, 1993). Chloroplast transformation using particle bombardment has also 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). CRISPR/Cas editing system delivered via Agrobacterium-mediated transformation has been used to achieve cotton gene editing (Gao et al., 2017; Long et al., 2018; Zhu et al., 2018; Khan et al., 2023)

In 2018, GM cotton occupied 76% of the global cotton area, mostly involving insect-resistant varieties (ISAAA, 2018). The major focus in the production of GM plants has been on resistance to insects and herbicides. The trend has been for usage of cotton varieties with several genes for insect resistance stacked together with 2 or t2 different types of herbicide resistance. It ensures that pests and weeds are not developing resistance (CSIRO, 2016).

Later stage research is still focussed on different agronomic properties. Field trials have been approved in Australia for *G. hirsutum* with 1) improved fibre properties, 2) increased yield, and 3) altered lipid composition in seeds. The list of GM cotton lines approved for field trial in Australia can be found at the OGTR website.

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 (Lusas and Jividen, 1987). Research has produced GM *G. hirsutum* plants with significantly reduced gossypol concentration in the seed, but normal gossypol level 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 5 main stem nodes support primarily vegetative growth and fruiting branches commence thereafter, with branches showing a ⅜ alternate phyllotaxy as shown in Figure 6 (Oosterhuis and Jernstedt, 1999; Ritchie et al., 2007).

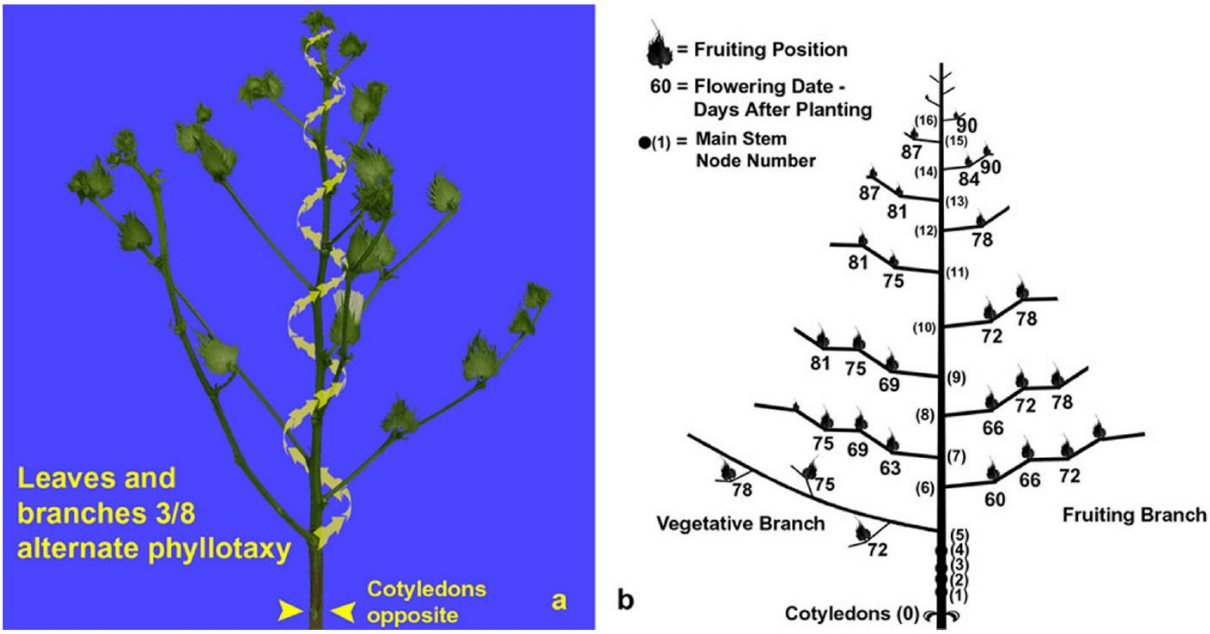

Figure 6. Cotton plant morphology. (a) A defoliated cotton plant shows the 3/8 alternate phyllotaxy of branches. Each branch is 3/8 of a turn around the stem from the branch below it. The branches form from the axils of main stem leaves. (b) A diagram of the general timing of flower emergence from buds 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 7 lobes in *G. barbadense* or 5 for *G. hirsutum*, whereas those on the fruiting branches have 3 lobes in either species (Gore, 1935). Further comparisons between the vegetative morphology of *G. hirsutum* and *G. barbadense* are outlined in Table 6.

Table 6. Comparative cotton plant morphology

	G. hirsutum	***G. barbadense***
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.

Source: Fryxell (1984)

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 5). 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 7). *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 7.

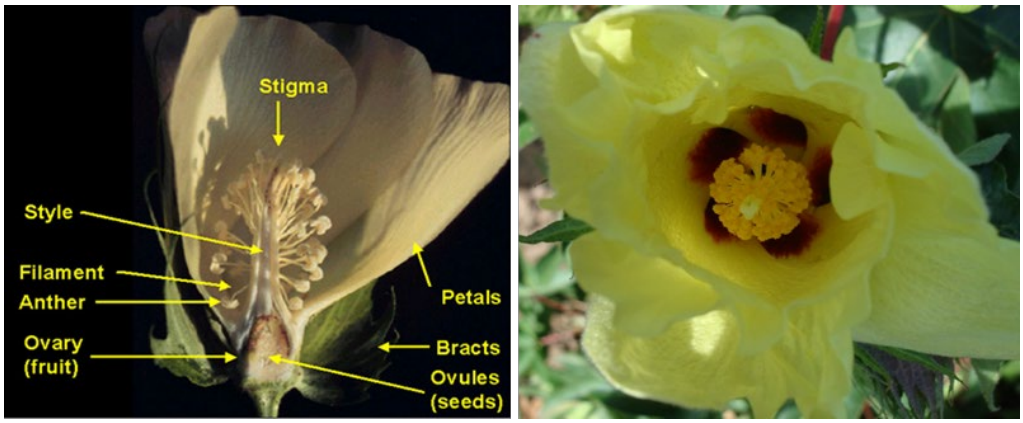

Figure 7. Cotton flowers. (a) Annotated *G. hirsutum* flower (used with permission from Ritchie et al., 2007); (b) *G. barbadense* flower (photo OGTR 2007).

Table 7. Comparative cotton reproductive morphology

	G. hirsutum	***G. barbadense***
Flowers	flowers usually in sympodial inflorescences, the pedicels 20-40 mm long, surmounted by 3 involucellar nectaries	flowers solitary or in sympodial inflorescences, the pedicels 10-40 mm long, gland-dotted, usually glabrate, surmounted by 3 involucellar nectaries
Bracts	bracts of the involucel inserted above each nectary, foliaceous (enclosing the bud), ovate, 3 to 19-laciniate	bracts of the involucel 3, inserted above the nectaries, ovate, up to 60 mm long, 45 mm broad, 7 to 19-laciniate
Calyx	truncate or 5-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 involucel, 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 androedium	exceeding the androecium, gland-dotted
Capsule	3 to 5-celled, glabrous, smooth, broadly ovoid or subglobose	3-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

Source: Fryxell (1984)

SECTION 4 DEVELOPMENT

Agronomically, the growth of cotton can be divided into 3 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 approximately 15-17 weeks, although this may be affected by temperature and other environmental variables (Oosterhuis and 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 rooting has been observed under experimental conditions. Cuttings of *G. barbadense* (referred to as *G. vitifolium*) can be propagated under laboratory conditions, where significant rooting only occurs where the cuttings are several internodes long and the parent plants are 6-10 weeks old (Khafaga, 1983a, b). Other work with *G. barbadense* cuttings indicated that few roots formed without application of napthaleneacetic acid (NAA) or tannic acid (Fadl and El-Ghandour, 1975). In *G. hirsutum* and a *G. hirsutum* × *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, 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 4-5 weeks after planting, with the formation of floral buds ('squares'). The floral buds first appear as small pyramidal structures, which are composed of 3 large green bracts that completely enclose the developing flower (Figure 8). Typically, approximately 25 days elapse between the initial appearance of a square and anthesis (flower opening) (Oosterhuis and Jernstedt, 1999; Ritchie et al., 2007).

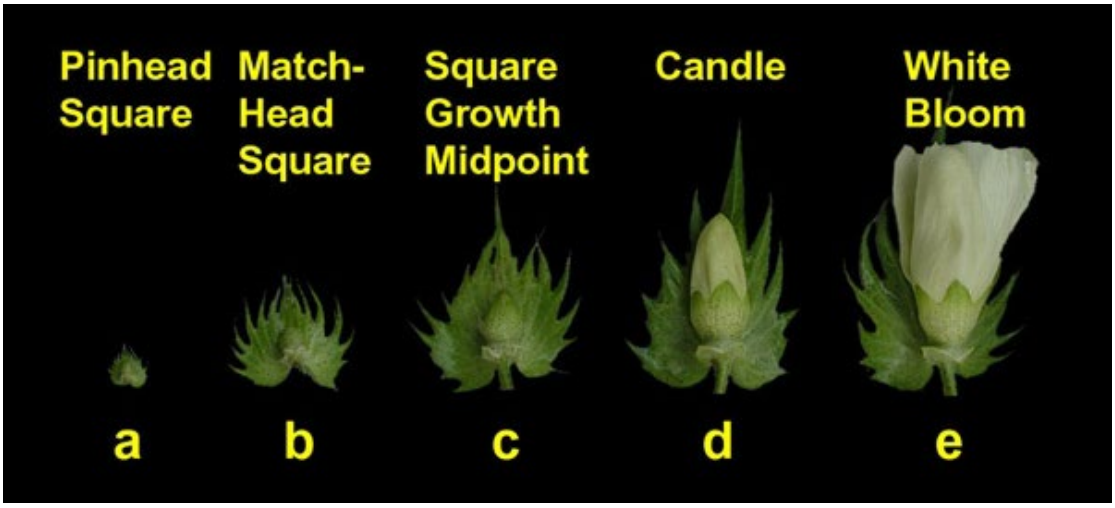

Figure 8. Cotton flower development. Development of the bud from pinhead square (a) to flower (e) involves both a size increase and petal development. Two bracts have been removed from each square, candle and bloom to show this development. Used with permission from Ritchie et al. (2007).

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 day length 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 fruits are abscised prematurely. Mature flowers are not usually shed before pollination (Oosterhuis and Jernstedt, 1999). The flowers open in a predictable sequence, as illustrated in Figure 8b, with the first flower opening low on the plant and closest to the stem. Approximately 3 days later the next flower will open in the same relative position on the next highest branch, and 3 days after that the next flower will open on the lowest branch. Thus the flowering progresses in an upwards and outwards spiral pattern (Oosterhuis and Jernstedt, 1999).

Cotton flowers anthese 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 and Jernstedt, 1999). Cotton has an indeterminate flowering pattern and thus flowers are initiated over a period of several weeks (Cherry and Leffler, 1984). At the peak of flowering there are usually 4 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. *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* (Kearney and Harrison, 1932; Saad, 1960; Kakani et al., 1999) (Table 8).

Table 8. Pollen size and spine length of *G. hirsutum* and *G. barbadense*

Species	Size (µm)	Spines (µm)	Spine density	Reference
G. hirsutum	85-88	7.5	-	(El Nagger, 2004)
	100.9	12.1	$8.3x10^{-3}$ spines/μm^2	(Kakani et al., 1999)
	103 ± 6.2	-	-	(Saad, 1960)
G. barbadense	66-73	11	-	(El Nagger, 2004)
	117.9	15.4	$4.9x10^{-3}$ spines/μm^2	(Kakani et al., 1999)
	115 ± 9.0	-	-	(Saad, 1960)

The viability of *G. hirsutum* pollen decreases rapidly after 8 hours (Govila and 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* (McGregor, 1976; Burke et al., 2004). 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 of 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 and Jones, 1953), thus it is an opportunistic out-crosser when insect pollinators are present (Oosterhuis and Jernstedt, 1999).

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

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

Honeybees 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 (1944) 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 for honeybees not collecting 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 and Vinson, 1994). The larger spines of *G. barbadense* would exacerbate the physical interference of the spines with the pollen aggregation

process used by the bees in the packing of their pollen pellets. However, the inability of bees to collect cotton pollen for transport to the hives is not directly related to their ability to cross-pollinate cotton flowers as the pollen collected in pollen baskets is not available for pollination.

4.2.3 Out-crossing rates

Insect prevalence strongly influences out-crossing rates for cotton (Elfawal et al., 1976; Pheloung, 2001; Llewellyn et al., 2007), and varies with location and time (Moffett et al., 1975; Elfawal et al., 1976; Moffett et al., 1976). Cotton pollen dispersal studies consistently demonstrate that when out-crossing occurs, it is localised around the pollen source and decreases significantly with distance (Thomson, 1966; Galal et al., 1972; Elfawal et al., 1976; Chauhan et al., 1983; Umbeck et al., 1991; Llewellyn and Fitt, 1996). This presumably represents the effective foraging range of insect pollinators. However, insect visitation rates, may overestimate 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 estimate out-crossing at 10% or less (Meredith and Bridge, 1973; Gridley, 1974; Elfawal et al., 1976; Umbeck et al., 1991; Llewellyn and Fitt, 1996). Higher estimates have been reported in a few cases (Smith, 1976).

Studies conducted in Australia, and discussed below, have shown out-crossing levels at 1 to 2% between plants in adjacent rows (Thomson, 1966; Mungomery and Glassop, 1969; Llewellyn and Fitt, 1996). 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 and Fitt, 1996).

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 and 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 20 m, 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 (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 2 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 as evidenced by 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* in the Ord River valley, WA over 2 growing seasons (Thomson, 1966). 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 (on 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 which would have affected the abundance of insect pollinators as without sprays it was not possible to obtain seeds (Thomson, 1966).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 honeybees 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 (Xanthopoulos and Kechagia, 2000; Van Deynze et al., 2005).

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 3 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 3 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 1625 m on the basis of samples taken at 3 different sites over 3 years.

In another study, 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 1800 m 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 and 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; Reddy et al., 1992b; Llewellyn and Fitt, 1996), 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 (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 (Green and Jones, 1953). An Egyptian study measured out-crossing from *G. 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, extensive use of insecticides for control of insect pests may limit the extent of cross-pollination (Jenkins, 2003) due to repellence as well as bee mortality (Rhodes, 2002). It should be noted that the introduction of GM insect resistant cotton varieties and optimised pest control methods have reduced the insecticide usage in Australia (see Section 7.3).

4.3 Fruit/seed development and seed dispersal

4.3.1 Fruit development

Approximately 5 to 7 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 and Jernstedt, 1999). During development, the bolls are spherical to ovoid and pale green. Boll development can be separated into 3 phases. Initially the cotton fibres elongate and the maximum volume of the boll and seeds are attained. After 3 weeks, the filling phase begins in which cellulose is deposited inside the hollow cotton fibre. After approximately 6 weeks the boll maturation phase begins and the boll dries out (Ritchie et al., 2007). Each mature boll is divided into 3, 4, or 5 locks and each lock contains several seeds surrounded by their long staple or fibres (Berardi and Goldblat, 1980) producing in total 29-34 seeds 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 and 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 and 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 6 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 and 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 and Bange, 2006). *G. hirsutum* and *G. hirsutum* × *G. barbadense* interspecific hybrids grown in Turkey produced a higher number of bolls per plant (13-21) (Basbag and 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

Cotton seeds are large, covered with thick fibres and enclosed in a tough boll that retains most of the seeds on the plant (Llewellyn and Fitt, 1996). At maturity the bolls split open, and under natural conditions the fibres can catch the wind and facilitate seed dispersal (Calhoun and 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, stock feeding, adverse weather conditions and animals and these are discussed below.

4.3.2.1 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 (NSW DPI, 2016).

There are 3 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 stockfeed. 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.

4.3.2.2 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 0).

Farrell and Roberts (2002) surveyed 9 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 (Sullivan et al., 1993a; Sullivan et al., 1993b; Zinn, 1995; Solomon et al., 2005). It has been estimated that 11% of cattle-fed *G. barbadense* cotton seed are excreted whole compared to 5.2% of the *G. hirsutum* cotton seed (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.

4.3.2.3 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 windstorms, the seeds may be dispersed over greater distances.

4.3.2.4 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, e.g. 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 (Bayer, 2021), suggesting possible distribution by water. 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.

If seed were dispersed, it is not expected to survive as seeds of modern cotton varieties have been bred to be soft-seeded (Mauncy, 1986; Hopper and McDaniel, 1999). The viability of *G. hirsutum* cotton seed is affected by moisture (Halloin, 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 are flooded regularly may not be favourable for commercial production, as cotton plants are poorly adapted to waterlogging (Hodgson and Chan, 1982). As part of Good Management Practice (GMP) of the cotton industry, most farms growing cotton under irrigation conditions are designed to retain irrigation water run-off. This aims to optimise water consumption and minimise the entry of pesticide residues into natural waterways (CRDC and CottonInfo, 2023b). 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 are unlikely to be dispersed and survive.

4.3.2.5 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 and 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 generally have a high percentage of ‘hard seed’. On drying, these become impermeable to water and suffer delayed germination (Christiansen and Moore, 1959). This is a positive survival mechanism in wild cotton. Agronomically, hard seeds are undesirable and the trait has been largely eliminated from modern commercial cultivars through breeding and selection (Mauncy, 1986; Hopper and McDaniel, 1999). 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 decreases in their viability (Halloin, 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 and Moore, 1959), allowing the tissues of the seed and embryo to take up moisture.

In addition to induced dormancy, *G. hirsutum* seeds collected immediately following fruit maturation can display 'innate dormancy' (Taylor and 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 (Hsi and Reeder, 1953; Christidis, 1955). Experiments with *G. barbadense* have shown no significant dormancy (Hsi and 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 5 months (Christidis, 1955). A longer experiment determined that *G. hirsutum* seed stored for 2 years showed higher germination than seed stored for one year, or seed planted the season following harvest (Taylor and 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 (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. Differences in 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 5 to 7 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 9). However, fatty acid treatment of *G. barbadense* seeds can overcome the inhibitory effect of cold temperatures on germination (Bartkowski et al., 1978).

Table 9. Effect of cold stress on *G. hirsutum* seed following planting

Days of chill	Days delayed flowering	Fibre maturity	Percent 1st 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

Source: Smith (1995)

Once the cotyledons have emerged, it may be 7 to 10 days before the first true leaf appears. This will then be followed by a new leaf every 2.5 to 3 days. (Smith, 1995).

As described in Section 2.3.3, *G. hirsutum* is routinely planted when the soil temperature reaches 14°C at a depth of 10 cm for at least 3 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., 1985; Kittock et al., 1987). 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 and Shaw, 1988).

The type of cotton seed has a large impact on the likelihood of germination (Eastick and Hearnden, 2006). Experiments in northern Australia have shown that *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 and 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 into which cotton seed is dispersed 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 and 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 and 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 and Hearnden, 2006). This study also showed that survival of plants for 2 years was low, with only 8 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 cattle yards 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 and 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 to 6 weeks after planting the total area of leaves borne on fruiting branches exceeds that of the main stem and vegetative branches, constituting approximately 60% of the total leaf area at maturity (Oosterhuis and Jernstedt, 1999).

SECTION 5 BIOCHEMISTRY

5.1 Toxins

Cotton is not a pathogen and not capable of causing disease in humans, animals or plants. However, it does contain several compounds which have adverse effects on human and animal health.

5.1.1 Gossypol

Extensive research has focused on the toxin gossypol (1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-(2,2-binaphthalene)-8,8'-dicarboxaldehyde), which exists as a yellow polyphenolic compound found primarily in the pigment glands of the cotton plants on leaves, stems and roots and at its highest concentration in the seeds (Smith, 1961; Coutinho, 2002). It is produced by cotton plants as a defence mechanism to deter insect and vertebrate herbivory (Wani and Nazir, 2022).

Gossypol in cotton seed exists in both the free and bound forms. In intact whole seed the gossypol is found in the active, free form, however heat or moisture occurrence during processing causes the gossypol to bind to proteins creating the less toxic bound form (Santos et al., 2002). The total concentration of gossypol depends on the cotton variety with an average of ~1% of dry weight in cotton seeds (Harrison et al., 2013). Biotic and abiotic stress can also impact gossypol production, for example, high temperatures reduce gossypol production, while induced damage to cotton leaves can increase gossypol levels in the plant (Harrison et al., 2013; Chappuis et al., 2023). The gossypol content, form and structure (isomers or enantiomers) differ between the 2 cotton species. The gossypol content of *G. barbadense* cotton is generally higher than that of *G. hirsutum* (Table 10), with more of the gossypol in the toxic, free form (Arana et al. 2001; Prieto et al. 2003; Santos et al. 2002; Sullivan et al. 1993a).

Table 10. Gossypol concentration and composition in cotton seed

	G. barbadense	***G. hirsutum***
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

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

Gossypol can exist as 2 different isomers (mirror image forms of the same compound) as a result of chiral rotation about the binaphthyl bond. These 2 isomers have different toxicity and are present in different relative proportions in *G. barbadense* and *G. hirsutum* (Stipanovic et al., 2005), with *G. barbadense* containing more of the (-)-gossypol Table 10 (Sullivan et al., 1993a). Studies have shown that toxicity of the gossypol isomers varies between different animals. Generally the (-)-gossypol isomer is more toxic based on studies on rats (Wang et al., 1987) and appears to be more detrimental to fertility of male hamsters (Matlin et al., 1985; Lindberg et al., 1987) 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 2 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 isomer (Lordelo et al., 2007).

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). Gossypol consumption was shown to reduce sperm mobility of domestic

boars (Baker, 2019). However, no effect of cotton seed consumption was seen on reproductive development in brahman bulls (Chase 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 (see Section 5.1.2) during processing enables the use of some cotton seed meal for catfish, poultry and swine.

Studies investigating the toxic effects of the 2 gossypol isomers 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 isomers, or a mixture, were equally effective at inhibiting the growth of *R. solani* and *H. zea*. However, against the growth of *A. flavus*, (-)-gossypol exhibited inhibitory activity 4 times more effective than (+)-gossypol (Mellon et al., 2011).

As discussed in Section 2.2, cotton seed meal or flour has been sold for use in human food. Various studies (summarised in Berardi and Goldblat, 1980) have observed no deleterious effects when moderate amounts of cotton seed products containing low levels of gossypol have been consumed.

5.1.2 Cyclopropenoid fatty acids

Cotton plants also contain cyclopropenoid fatty acids (CPFA) in seeds and tannins in the leaves (Lane and 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. CPFA's such as malvalic, sterculic and dihydrosterculic acids constitute approximately 0.5-1.0% of the total lipid content of the seed (Schneider et al., 1968; Harrison et al., 2013).

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 increased number of 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 and 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 1970s 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).

Cotton lint contains no detectable nitrogen, and hence no DNA or proteins after refining processing (Narayanan et al., 2011; Barnhardt Natural Fibres, 2020)Processed cotton fibre contains over 99% cellulose (Wakelyn et al., 2007), is hypoallergenic, does not cause irritation and is used in medical, hygiene and cosmetic products (Australian Cotton, 2022)

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 (Renner et al., 2022). Human melanoma cells show cytotoxicity to gossypol, with a 5-fold greater cytotoxic sensitivity to the (-)-gossypol isomer than the (+)isomer(Blackstaffe et al., 1997), suggesting that the (-)-gossypol isomer 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 Stockfeed

Cotton seed is a valuable foodstuff for cattle due to its combination of 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 and Jenkins, 1980). 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).

Generally, the fatty acid composition of *G. barbadense* and *G. hirsutum* seed (Khattab et al., 1977; Khalifa et al., 1982) and oil (Pandey and Thejappa, 1981) are similar. However, *G. barbadense* 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, possibly due to it not being thoroughly chewed 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 (Sullivan et al., 1993a; Sullivan et al., 1993b; Zinn, 1995; Solomon et al., 2005). To improve the digestibility of the *G. barbadense* seed it is often cracked prior to feeding to cattle, but this increases the animal's exposure to gossypol. Cotton seed is used extensively throughout Qld as a feed supplement for sheep. However, feeding cotton seed to sheep under 5 months of age is not recommended as their rumen is not developed enough to handle gossypol (Business QLD, 2016). 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).

Differences in composition influence the amount of *G. barbadense* and *G. hirsutum* cotton seed that is recommended for cattle feed. For adult cattle, a maximum of 24 g of free gossypol per day should not be exceeded when feeding to lactating cows (Cotton Incorporated, 2023), which amounts to 2.3 to 3.6 kg of cotton seed per day. 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

(Santos et al., 2002; Prieto et al., 2003). However, the cows consuming cracked *G. barbadense* seed at a level of approximately 7.5% of their diet had reduced fertility as indicated by decreased conception rates and increased incidence of abortions (Santos et al., 2003).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Nutrient requirements

Nitrogen and phosphorous are key nutrients for cotton growth. Nitrogen levels have a large impact on the yield and quality of lint produced and can also affect the seed yield. 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, 2004; Hutmacher et al., 2004; Reddy et al., 2004). Excessive vegetative growth may also lead to increased pest and disease susceptibility (Cisneros and 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 (Sawan et al., 1998; Fritschi et al., 2003; Sawan, 2006, 2007). This has led 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 (Unruh and Silvertooth, 1996a, b; Silvertooth, 2001; Fritschi et al., 2003). 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* (Reddy et al., 1996; Fritschi et al., 2003, 2004). *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 11, Australian soils have sufficient levels of many of the required nutrients, as symptoms of deficiency are not seen. The use of crop rotation to aid in the control of black root rot and *Verticillium* wilt (see Sections 2.3.3 and 7.4) may also aid in the maintenance of soil nutrient levels.

Table 11. Nutrient requirements of commercial cotton grown in Australia[a]

Nutrient	Uptake per hectare [b]	Removal per hectare [c]	Fertiliser	Deficiency	Toxicity
Nitrogen (N)	64–403 kg	39–168 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)	18–43 kg	14–28 kg	Mono-ammonium phosphate (MAP - NPK 10:22:0)	Stunted growth, dark green or purple foliage, delayed fruiting.	
Potassium (K)	43–264 kg	17–88 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. **Not common in Australia.**	
Zinc (Zn)	66–214 g	59–109 g	Zinc oxide Zinc sulphate heptahydrate	Interveinal chlorosis[d], cupped, bronzed leaves, stunted growth, reduced yield and fibre quality.	
Iron (Fe)	350–2022 g	102–161 g	Iron chelate	Interveinal chlorosis, eventual white leaves **Linked to waterlogging.**	
Copper (Cu)	26–89 g	14–28 g	Copper chelate Copper oxide	Chlorosis of lower leaves, dieback of terminal bud in severe cases – **not observed in Australia.**	
Boron (B)	168–682 g	26–65 g	Borax Boric acid	Young leaves light green at base, older leaves twisted, flowers deformed, boll shedding.	Leaf cupping, chlorosis, necrotic spots.
Calcium (Ca)	71–266 kg	2.7–6.5 kg	Calcium carbonate Calcium sulphate	Collapsing petioles. **Not seen in Australia.**	

Nutrient	Uptake per hectare [b]	Removal per hectare [c]	Fertiliser	Deficiency	Toxicity
Magnesium (Mg)	13.9–73.3 kg	8.7–17.9 kg	Dolomite lime Magnesium sulphate	Purple/red leaves with green vein, premature senescence of mature leaves. **Not seen in Australia.**	High soil mg ratios with ca and k affect soil structure.
Sulphur (S)	24–66 kg	5.8–11.8 kg	Usually provided as part of other fertilizers	Yellowing of young leaves, spindly plants, short slender stems. Reduced boll size.	
Manganese (Mn)	127–729 g	6–22 g	Manganese sulphate	Leaf cupping, interveinal chlorosis starting with younger leaves, upper leaves may have necrotic spots. **Rarely seen in Australia.**	Linked to acid soils. Leaves crinkled, mottles and chlorotic. Can induces iron and zinc deficiency. **Linked to waterlogging.**
Molybdenum (Mo)	3-5 g	1-2 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. **Not seen in Australia.**	Can cause copper imbalance.

[a] Compiled from NUTRIpak (Australian Cotton Cooperative Research Centre, 2018)
[b] Amount of nutrient removed from soil during growth
[c] 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)
[d] 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 will continue once temperatures rise (Bange and Milroy, 2004; McDowell et al., 2007). *G. hirsutum* seedlings can also be killed by frost (Constable and 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 2 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* nearly doubled when mean July temperature increased from 31 to 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

As discussed in Section 2.3.3, cotton is usually grown as an irrigated crop but can also be grown as an unirrigated crop, also known as dryland or rainfed cotton. Water availability is a limiting factor in cotton production (Cotton Australia, 2023b, a).

Water use efficiency in cotton is defined as the measure of bales of cotton produced per unit of megalitre of water supplied to the crop. It can also describe the efficiency of water delivery, application and use (Cotton Australia, 2023b). Over the past decades, researchers have focused on developing cotton varieties appropriate for the Australian climate, improving both dryland and irrigated cotton production (Gibb. D and Cooperative Research Centre for Sustainable Cotton Production, 1995; Cotton Australia, 2023b). Combined with better water delivery systems, these improvements led to a 52% reduction in water used to grow one bale of cotton in 2021, compared to 1997 (Cotton Australia, 2023a).

Excess water, called waterlogging, has a negative impact on cotton plants and leads to yield loss. Waterlogging damages plants due to low oxygen concentrations (hypoxia) around the roots. It occurs when excess water inhibits the diffusion of atmospheric oxygen in the soil. The low oxygen conditions in turn inhibit energy production in the plant roots and other oxygen-dependent pathways, including those involving cytochromes, oxidases and desaturases (Bange et al., 2004b).

The first visual symptom of waterlogging is wilting, followed by leaf chlorosis (yellowing), premature senescence and reduced boll number, leading to lint yield loss (Hodgson and Chan, 1982; Hocking et al., 1985; Reicosky et al., 1985). Damage to crop yields has already occurred once leaf yellowing is observed (Constable, 1995). 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 occur at all stages of crop growth (Hodgson and Chan, 1982).

Uptake of potassium, phosphorus and nitrogen 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 3 to 4 days of waterlogging, most of the yield loss is due to less nitrogen being absorbed from the soil (Constable, 1995).

In Australia, waterlogging in cotton has been 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. The majority of the Australian crop is grown under furrow irrigation on cracking clay soils (Silburn et al., 2013). For example, cotton production in NSW occurs mainly on cracking grey clay soils (vertisols) of the Namoi and Gwydir River Valleys, which have inherently low drainage rates (Hodgson and Chan, 1982).

Research in the early 1980s showed that a 32 hour waterlogging treatment of cotton could lead to yield losses of 42% (Hodgson, 1982; Hodgson and Chan, 1982), although another study showed a recovery of plants following waterlogging stresses leading to no reduction in yield (Hocking et al., 1987). An experiment following a similar protocol to the Hodgson study recorded approximately 40% yield loss, but only when more severe waterlogging conditions (up to 72h) 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 of fields, with more even water flow due to the use of laser guided levelling systems. More even slope and hill heights have meant that water does not collect in low areas (Bange et al., 2004b).

6.4 Other tolerances

Cotton is classified as a salt tolerant plant. The most common effect of salinity stress in cotton 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 and Abd-El'Hamid, 1970).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Although the weed spectrum varies between localities, there are commonly 60-70 weed species found in cotton fields (CRDC, 2013b). A list of the most important weeds in cotton in Australia can be found in Appendix B. Competition from weeds reduces a cotton crop's access to nutrients, water, light and area, having the greatest impact in the period from germination to 2 months of growth when the plants are most vulnerable (Pala and Mennan, 2021). Late season, evenly established crops of cotton with vigorous growth can compete with weeds. However, initial crops are particularly susceptible to weed competition (Hearn and Fitt, 1992; CRDC and CottonInfo, 2022). Weeds may also indirectly impact on the cotton crop. Pests and diseases that affect cotton can establish themselves on weed populations or cotton volunteers before moving onto cotton plants (CottonInfo, 2016b), adversely affecting cotton harvesting or lint quality (Charles, 2002), and interfering with water flow through irrigation channels (Charles, 1991). The presence of weeds can also reduce the product quality as they can be included in the material collected during harvest (Pala and Mennan, 2021). The distribution and spectrum of weed species varies between each state depending on the environmental conditions they experience.

Notable weeds include *Ipomoea lonchophylla* (cow vine) and *Tribulus micrococcus* (yellow vine or spineless caltrop), which can tangle in the picker heads at harvest, 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). Long-term problems can arise from larger weeds such as *Xanthium occidentale* (Noogoora burr), *X. spinosum* (Bathurst burr) and *Datura* spp. (thornapples), which have the ability to host *Heliothis*, mites and *Verticillium* wilt (Charles, 2002). In Australia, some of the most difficult weeds to control are liverseed grass (*Urochloa panacoides*), flaxleaf fleabane (*Conyza bonariensis*), feathertop Rhodes grass (*Chloris virgata*), awnless barnyard grass (*Echinochloa colona*) and sowthistle (*Sonchus oleraceus*) (CRDC and CottonInfo, 2022).

7.1.1 Weed control

While weed control is expensive and can adversely affect growth of the cotton crop itself by herbicide damage or root disturbance due to chipping, it is imperative to the growth of a healthy crop. A 2018 survey of dryland cotton growers estimated that the cost of crop and pasture chemical costs was $744/ha, with an average of 20% allocated to those used for weed control (estimated at $150/ha) (McLeod, 2018). This is a reduction from a 2001 study that found weed control expenditure to be $220/ha (Walker et al., 2006). The study also found that the additional non-chemical expenditure for weed control in 2018 was $15/ha on average. Additionally, further yield loss due to weed competition was estimated between 4.5% to 7.5% depending on the region (McLeod, 2018).

To control weeds, it is advised that a specialised and unique strategy is developed so that the most appropriate response can be employed. To do this, farmers should keep an accurate record of weed species present, the crop rotations employed, herbicide types used as well as any alternate weed control methods used. It is important to correctly identify the type of weed/s that are present (identification publications and weed experts are useful resources) and if any problem patches are occurring. A personalised control plan can be developed using a Herbicide Resistance Management Strategy (HRMS) (CRDC and CottonInfo, 2022).

Intensive herbicide use was triggered by the cultivation of glyphosate-resistant Roundup Ready® GM *G. hirsutum* in the 2001-02 season, which has led to an ever increasing level of herbicide use, leading to a change in the spectrum of weeds observed (Werth et al., 2010). For example, flaxleaf fleabane (*C. bonariensis*) is poorly controlled by glyphosate and only emerges from the top 0.5 cm of soil. Heavy reliance on glyphosate and reduced tillage combined with flaxleaf fleabane's naturally high

glyphosate resistance have favoured the spread of this weed, making it the second most important weed in cotton in 2010 compared to its 14th position in 2005 (CRDC and CottonInfo, 2022).

As of 2023, there were 523 unique cases of herbicide resistant weeds across the globe, with weeds developing resistance to 21 of 31 herbicide sites of action. Glyphosate is under intense scrutiny due a strong dependency by the farming industry, leading to an increased amount of weed resistance. In Australia, 70% of all herbicides used on cotton crops contain glyphosate and an industry wide HRMS has been implemented, which indicates the most effective weed control method combination to help prevent resistance developing and to attack the weed seed bank. Paired with an HRMS, Integrated Weed Management (IWM) plans aid in the control of weeds and they advise that to avoid herbicide reliance, alternative tactics should be employed, including introduction of rotation crops, removal of weed and cotton volunteers/ratoon reservoirs, maintenance of non-crop areas (i.e. channels and fence lines), soil maintenance pre-cotton sowing, manual chipping and spot spraying. Compliance with the crop management plan is implemented through a Technology User Agreement between the grower and Monsanto (CRDC and CottonInfo, 2022).

7.2 Pests

7.2.1 Overview

More than 1326 species of insects have been reported in commercial cotton fields worldwide but only a small proportion are pests (Matthews and Tunstall, 1994) with the type and number of pests differing from season to season and between different regions, influenced by rainfall during autumn and winter. This allows for the growth of other plants in the cropping area that can host pests throughout the cold season. These pest then move onto cotton crops in the spring (Wilson et al., 2018).

Of the 30 pests of cultivated *G. hirsutum*, the most important in Australia are the caterpillars of *Helicoverpa armigera* (cotton bollworm) and *H. punctigera* (native budworm)*,* the two-spotted spider mite *(Tetranychus urticae),* the green mirid (*Creontiades dilutus*), the cotton aphid (*Aphis gossypii*) and the silverleaf whitefly (*Bemisia tabaci* b-biotype)(Pyke and Brown, 2000; Shaw, 2000; Cotton Australia, 2023c). Other pests include thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*), the green vegetable bug (*Nezara viridula*), other armyworms (*Spodoptera litura* (cluster caterpillar), *S. exigua* (lesser armyworm) and *S. frugiperda* (fall armyworm)), brown and yellow mirids (*Campylomma pacificus* and *C. liebknechti*), other mite species (*T. ludeni* (bean spider mite), *T. lambi* (Strawberry spider mite) and *Polyphagotarsonemus latus* (broad mite)), the greenhouse whitefly (*Trialeurodes vaporiorum*) and others (Farrell and Johnson, 2005; CRDC and CottonInfo, 2023a). Beneficial predatory insects are used as an alternative mode of pest control (reducing reliance on insecticides) where insects including ladybeetles (*Coccinella* spp., *Adalia* spp.), blue beetles (*Dicranolauis* 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.) are utilised as pest predators (Mensah, 1999; CottonInfo, 2016b). Insect herbivory can occur at all stages in the plant lifecycle with different insects preferring different stages (Figure 9). 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 most important cotton insect pests are discussed in the following sections.

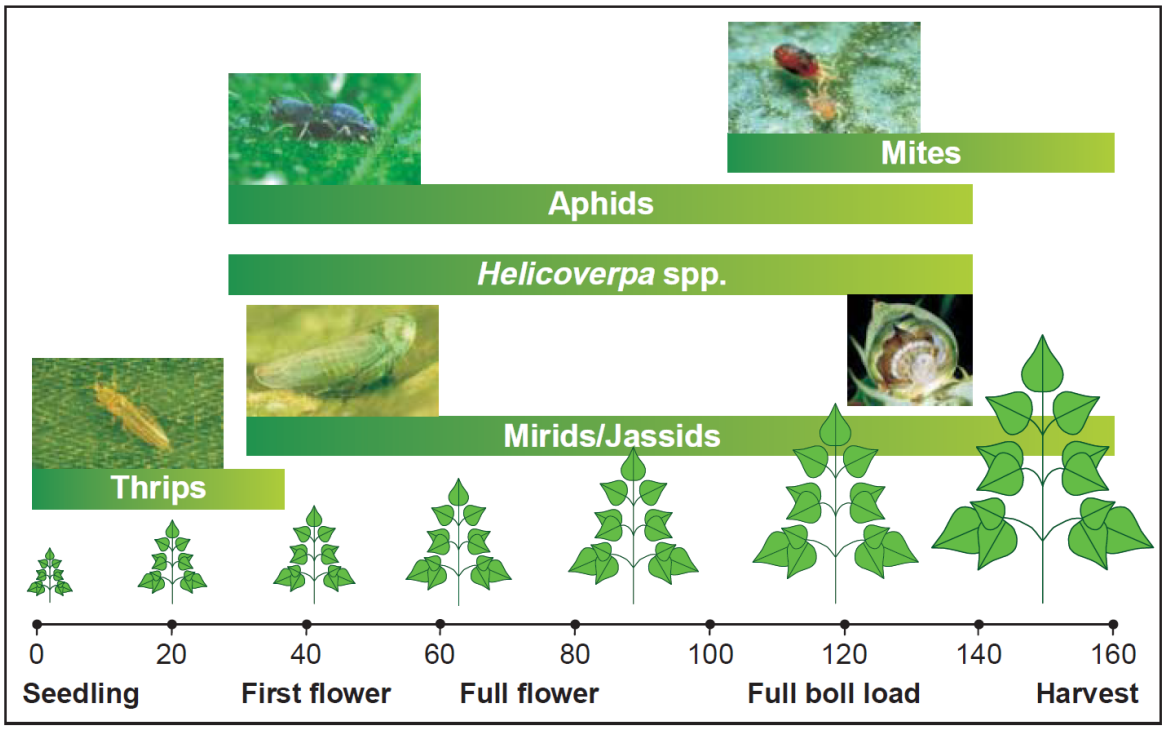

Figure. 9. Insect pests of cotton in Australia.

Figure sourced from (Holloway, 2005) and illustration reproduced with permission from Bayer CropScience.

7.2.2 Cotton bollworm

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 attacks many field and horticultural crops (Common, 1953; Zalucki et al., 1986; Fitt, 1989). Cotton bollworm is able attack cotton plants at all life stages, however, the reproductive tissue of the plant is preferred. Greatest plant consumption occurs when the bollworm is at its largest, (approximately 24 mm long) (CottonInfo, 2016b). Prior to the 1970s in the Ord River irrigation area, WA, *H. armigera* was almost of no consequence in cotton. From the 1970s, several non-selective insecticides were beginning to be used against various pests and resistance to these insecticides was developing by *H. armigera*. Eventually, *H. armigera* could not be controlled and cotton cultivation was abandoned in the Ord in 1974. Similarly, in the eastern states of Australia, resistance by *H. armigera* was increasing and by the late 1980s and 1990s cotton required application of pesticides at a rate of 14-20 times a season (Walsh et al., 2022).

In cotton plants, the adult moth lays its eggs on young terminal branches, and after 3-4 days the eggs hatch into larvae (caterpillars), followed by 5 to 6 growth stages, ending in the pupal stage where the larva has moved into the soil for metamorphosis (Zalucki et al., 1986; King, 1994). 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* may complete 4 to 5 generations during the cotton-growing season (Scott et al., 2003). During summer, pupal development lasts approximately 16 days but as the temperature begins to drop and the days shorten, the last generation goes into a period of suspended development or 'diapause' over winter, remaining in the soil around the base of the plants in pupal form. The over-wintering pupae emerge from the soil in the following spring (Zalucki et al., 1986; Fitt, 1989; King, 1994; Duffield and Steer, 2006).

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 and 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 insect 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%, so mechanical cultivation may only target a fraction of the winter population in any given year (Sequeira and Playford, 2001).

7.2.3 Native budworm

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 (usually flowering plants from inland Australia) and this is followed by the large-scale migration of many moth species, over distances of 500 to 1500 km, in some cases reaching the cotton growing regions of southeastern Australia, aided by the warm winds preceding cold fronts (Farrow and Daly, 1987; Oertel et al., 1999; QLD DPI&F, 2005). Although some *H. armigera* migrate, *H. punctigera* are more commonly found in these migrations and often arrive 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 and Steer, 2006). The constant influx of *H. punctigera* immigrants to 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). Both *Helicoverpa* species share a majority of the same predators, namely spiders, lacewings, ants and beetles (CottonInfo, 2016b).

7.2.4 Spider mites

Spider mites are also a significant cotton pest in Australia. The two-spotted spider mite (*Tetranychus uticae*) is more common than the bean spider mite (*T. ludeni*) and strawberry spider mite (*T. lambi*). They live and feed on the underside of leaves using piercing mouthparts, causing bronzing, reddening and eventually desiccation of the leaf, mainly in younger leaves (Gutierrez, 1994). Predation is a key factor in reducing early season survival of mites. Predators include Western flower thrips (*Frankliniella occidentalis*; 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*.). *G. barbadense* is less susceptible to mites than *G. hirsutum* (Trichilo and Leigh, 1985; Cotton Seed Distributors Extension and Development Team, 2005).

Other mite pests include; blue oat mites (*Penthaleus major*), redlegged earth mite (*Halotydeus destructor*), brown wheat mite (*Petrobia lateens*) and broad mite (*Polyphagotarsonemus latus*) (CottonInfo, 2016b).

7.2.5 Silverleaf whitefly

The silverleaf whitefly (*Bemisia tabaci*) is a serious pest of fibre, horticultural and ornamental crops worldwide. It can cause extensive damage through direct feeding, and honeydew production, which contaminates cotton fibre and inhibits photosynthesis (CottonInfo, 2016b). It was first identified in Australia in 1994 (Gunning et al., 1995), however, an outbreak did not occur until 2001 in Qld (Wilson et al., 2018). An integrated approach has been developed to monitor and control the population of silverleaf whitefly in cotton (CottonInfo, 2015b).

7.2.6 Fall armyworm

The Fall armyworm (FAW; *Spodoptera frugiperda*) was first identified in Australia in 2020. It is believed they have migrated from the tropics of the Americas. It is currently present in Australia and has been found in Qld, NT, WA and NSW (NSW DPI, 2022a; CRDC and CottonInfo, 2023a). FAW eggs are laid in groups of 100-200, protected by a layer of scales to produce a felt-like appearance. When larvae feed on plants, they leave a characteristic opaque ‘window’ of leaf cuticle behind that is highly

indicative of larval infestation (Plant Biosecurity and Product Integrity, 2020). FAW can fly hundreds of kilometres and while their impact on cotton plants is not well documented, the rate at which clusters of FAW can eliminate crops (notably sweet corn, maize and sorghum) through defoliation indicates a serious threat to the cotton industry (Business QLD, 2021).

7.2.7 Other pests

Other insect 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 flower buds, and can cause damage to lint in developing bolls. 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 quality and value. Aphids feed on the underside of leaves, in the terminals, young stems and on developing fruit (CRDC and CottonInfo, 2023a). Large cluster caterpillar (*S. litura*) larvae feed on leaves, flowers and bolls in cotton crops while young larvae skeletonise leaves. It has been a serious pest in northern Australian cotton growing areas since 1950s (CottonInfo, 2016b; CRDC and CottonInfo, 2023a). *S. litura* are pests of various crops including strawberries, tobacco, tomato, apple, cabbages and cauliflowers (EFSA Panel on Plant Health et al., 2019).

Reniform nematode (*Rotylenchulus reniformis*) is a significant threat especially in the areas where cotton is not rotated with other crops ('back-to-back' cotton). Reniform nematodes are capable of attacking a wide range of plant species, causing stunting and poor plant growth when feeding on the root system. In turn, this reduces yield and delays maturity of the crops. Reniform nematodes exhibit extreme resilience, are able to reproduce parthogenetically and are also able to enter an ametabolic state during periods of water scarcity (anhydrobiosis) (Wang, 2001). Rotation with sorghum or corn is an efficient measure for controlling nematodes (Smith et al., 2015) as these are non-host crops that do not support nematode growth, while also restoring the soil structure and quality (Marshall et al., 2012).

7.2.8 Gossypium barbadense and Gossypium hirsutum pest commonalities

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 (Sengonca et al., 1986; Gannaway, 1994; Matthews and Tunstall, 1994). 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 7 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 and Lonsdale, 1990).

When insects were sampled from 3 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 and 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 (Davies et al., 2007). *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 and Wilson, 1983).

7.3 Pest control methods

Heavy use of insecticides during the late 1990s resulted in resistant cotton bollworm, which almost destroyed the Australian cotton industry. As a result, the industry has turned to alternative methods of pest control, developing an integrated pest management (IPM) system that includes use of insecticides in conjunction with other measures such as crop rotations, timing adjustments, barrier/trap additions, rigorous quarantine and biosecurity measures, as well as the use of GM cotton varieties (Schellhorn et al., 2013; NSW EPA, 2021).

Introduction of GM cotton has changed cultivation practices, reducing the use of insecticides and changing the distribution and abundance of pests (CottonInfo, 2015a). First grown in Australia in 1996, the GM cotton known as 'Bt cotton', incorporated the *Cry1Ac* gene from *B. thuringiensis* (Bt). This Bt cotton plant produced a insecticidal protein lethal to wide variety of pests, particularly lepidopteran species (DAFF, 2018). In 2004, a second variety of Bt cotton was released incorporating the *Cry1Ac* and *Cry2Ab* genes and aimed to improve insecticidal activity and reduce the potential for target insects to develop resistance. (CSIRO, 2023). Despite researchers' efforts, insect resistance to Bt cotton has been observed in the USA, India and Pakistan (Tabashnik et al., 2023)

In Australia, specific limitations and requirements are imposed on farms that grow Bt cotton to ensure that insect control mechanisms retain long-term effectiveness and that the development of resistance is minimised (CRDC and CottonInfo, 2022). Insecticide Resistance Management Strategies (IRMS) aim to strengthen pest management by identifying appropriate insecticides, rates and timings to ensure effective control of target pests, delay resistance, and conserve naturally occurring biological control for enhanced sustainability of ecosystems. IRMS also involve the destruction of crops at the end of the season with pupae busting techniques, maintenance of farm hygiene and inclusion of crop rotations. It has been adapted for the different growing seasons of northern and central/southern Australia (CottonInfo, 2023a).

Use of insect resistant GM cotton combined with improved IRMS has reduced insecticide usage in Australia by about 95% (Cotton Australia, 2023a). When considering insecticide inclusion in pest control strategies, the grower must consider the benefits and harms it can cause. Opting for species-specific insecticides instead of broad spectrum sprays favours some natural enemies of cotton pests, while the overuse of insecticides may result in target pests developing insecticide resistance (Kranthi and Russell, 2009; Naranjo, 2010; CRDC and CottonInfo, 2022). For instance, the use of broad-spectrum pesticides may destroy beneficial predators and worsen spider mite infestations (Wilson et al., 1991).

7.4 Pathogens

Cotton is susceptible to a range of diseases, which can act individually or in combination to affect the quality of the fibre and seed, as well as the yield and cost of production of the cotton crop (Bell, 1999; CottonInfo, 2016a). 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 and 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 and Johnson, 2005).

7.4.1 Fungal pathogens

Black root rot (BRR), caused by the fungal pathogen *Berkeleyomyces rouxiae* (formerly *Thielaviopsis basicola*), is widespread in all cotton growing areas of NSW and Qld, dominant in regions that

experience cooler planting temperatures (Nehl et al., 2004; O'Keeffe et al., 2021). Disease surveys show a steady rise in the number of farms with the disease since it was first detected in 1989. BRR is transmitted via residual infected plant tissue, contaminated soil, mite/arthropod activity and can persist over 5 to 6 cotton crops even when crop rotation is utilised (Nel et al., 2019).

Symptoms of BRR include stunted and slow seedling development with black roots and lateral root death (Nehl and Allen, 2004). As BRR cannot be controlled using fungicides, the management of the disease relies on farm management practices that slow down or prevent pathogen infection. Examples include planting after cold weather has passed, planting varieties that are able to 'catch up' later in the season, intentional field flooding, biofumigation, pre-irrigation in preference to 'watering up' (irrigation after planting), planting of non-host crops such as cereals, sunflower, brassicas and onions for more than one season between cotton crops (Jhorar, 2003; O'Keeffe et al., 2021), and adopting a 'come clean, go clean' strategy (Holman, 2016). All cotton varieties and many legumes are hosts for *B. rouxiae.* Therefore, legumes should be avoided as rotation crops in cotton growing regions infested with *B. rouxiae* (Allen et al. 2003).

Verticillium wilt is caused by the fungal pathogen *Verticillium dahliae*. Its incidence across Australia has increased over time and it is established in all states, mainly due to the increasing use of susceptible varieties of cotton (Johnson and Nehl, 2004; Subbarao, 2020). It is transmitted through contaminated soil, where *V. dahliae* can persist without a host for many years as microsclerotia, on infected roots and even in infected fruit (Michigan State University, 2008). Symptoms include yellow leaf mottle, brown discolouration in the stem when cut, stunted growth, wilting and some defoliation, which is more severe in cold weather or under waterlogging (Ayele et al., 2020; CRDC and CottonInfo, 2022). 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 adopting 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*), pigweed (*Portulaca oleracea*) and many others, therefore, control of these weeds is essential (Allen et al. 2003). The effectiveness of different control strategies have been shown to be highly variable across different localities due to the presence of varying soil microflora, weed host presence and differing strains of the pathogen (Subbarao, 2020).

Fusarium wilt was first detected in Australia in 1993 (Kochman, 1995) and since then it has become widespread across Australia, mainly in Qld and eastern Australia, preferring cool, wet, spring conditions (Allen, 2007). 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 with fungicides. It is transmitted through soil or water contaminated with spores and infected plant material (Plant Health Australia, 2013). 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 and 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 include planting resistant cotton varieties (all cotton seed sold in Australia has a Fov resistance rating), using a plant activator to treat seeds prior to planting, avoiding waterlogging and adopting a 'come clean, go clean' strategy (CRDC and CottonInfo, 2022). The type and timing of nitrogen fertiliser 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 Fov (Allen et al. 2003). Management of these species can include crop rotation using plants such as white oat, and taking care not to use crops that can act as hosts or reservoirs e.g. corn or tomato (Leoni, 2013).

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). It is transmitted via air-borne spores released by air movement, water splashing, or moving of infected plant material (Michigan State University, 2015). Symptoms include brown, grey or tan lesions predominantly on lower leaves, rapid defoliation and dry circular bolls lesions (Nehl and Allen, 2004; CottonInfo, 2017), and is more severe with potassium deficiency (Hillocks and Chinodya, 1989; Blachinski et al., 1996) 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 (CRDC and CottonInfo, 2022). Cotton and some malvaceous weeds such as bladder ketmia (*Hibiscus trionum*), sida (*Sida* spp.) and anoda weed (*Anoda cristate*) are also hosts for *A. macrospora.*

7.4.2 Bacterial pathogens

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 (Allen et al., 2003). 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 (Allen et al., 2003). 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). In Australia, most varieties of *G. hirsutum* are completely resistant to the *Xanthomonas* strains present in Australia (Allen et al., 2003; CRDC and CottonInfo, 2022).

7.4.3 Viral pathogens

There are 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 and 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 and 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 from *G. barbadense* grown in Bangalore, India and named *Cotton leaf curl Bangalore virus* (Chowda Reddy et al., 2005). Cotton leaf curl disease is not present in Australia and is considered a threat to Australia's cotton industry (DAFF, 2022a; Industries, 2023).

Cotton bunchy top (CBT) is a viral disease caused by cotton bunchy top virus (CBTV) which has been observed in Australia since 1998 (Reddall et al., 2004). It is thought to be transmitted by cotton aphids (*Aphis gossypii*) and causes pale patterns on leaf margins, leathery leaves and short petioles and internodes that leads to reduced lint yield (CRDC and CottonInfo, 2023a).

7.5 Other interactions

Successful cotton growth in most soils depends on the interaction with mycorrhizal fungi (Youssef and Mankarios, 1974; Nehl and Allen, 2004; CRDC and CottonInfo, 2018, 2022). 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 *G. mosseae*, like many other VAM fungi, can colonise a variety of plant species. For example, Giovannetti et al. (2004) demonstrated the ability of an isolate of *G. mosseae* to colonise the roots of 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* (Fryxell, 1992; Craven et al., 1994), none of which is listed as a serious weed (Holm et al., 1979; Holm et al., 1997; Groves et al., 2003; Randall, 2017).

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, 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 (Tothill et al., 1982; Lazarides et al., 1997). 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 and 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 riverbanks in northern Australia.

Even though *G. hirsutum* has been grown previously in several 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 (Atlas of Living Australia). 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 and Hearnden, 2006). However, there is no indication, that these volunteers sponsor self-perpetuating feral populations. Typically, such volunteers are killed by roadside management practices and/or grazed by livestock, thereby limiting their potential to reproduce and become weedy (Eastick and Hearnden, 2006; Addison et al., 2007). Additionally, 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 (Farrell and Roberts, 2002; Eastick and Hearnden, 2006). The relative impact of each of these factors is dependent on whether the *G. hirsutum* plants are in coastal or inlands areas, as well as whether they are in northern or southern 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 and Roberts, 2002). The study concluded that *G. hirsutum* volunteers tend to establish in highly and regularly disturbed environments and appear to have negligible ability to invade non-disturbed habitats (for example native bush). 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).

These results were supported by another study, where *G. hirsutum* seed germination was highest in disturbed habitats, especially when the seed was buried rather than remaining exposed on the soil surface (Eastick and Hearnden, 2006). 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 (2002) 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 and 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 7 of 9 dairy farms surveyed (Atherton Tablelands, March 2002) which regularly feed stock with cotton seed. GM *G. hirsutum* (Roundup Ready®, Roundup Ready®/INGARD® or INGARD®) was identified on 4 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® 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 were developed. Both models indicated 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 (Atlas of Living Australia, accessed September 2023). 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 in areas such as roadsides and drains. There are 3 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® and Roundup Ready Flex® 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. Additionally, the commercial release of LibertyLink® *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 (CRDC, 2013b).

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 (using cultivators, graders, excavators or chippers) (Roberts et al., 2002).

Cotton volunteers are actively managed on-farm by mechanical methods described above, application of herbicides (if in the seedling stage) or burning (Charles et al., 2002; Roberts et al., 2002; CRDC, 2013b). A range of herbicides may be used to control cotton volunteers (at the seedling stage) that emerge after harvest. Herbicides containing carfentrazone-ethyl or paraquat and diquat

as active constituents are currently registered by the APVMA for control of volunteer cotton, including Roundup Ready® *G. hirsutum* volunteers(APVMA, 2021, 2022).

Integrated weed management strategies stress the need to avoid relying on one or 2 control method (Charles, 2013). 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 (CRDC and CottonInfo, 2023a).

8.6 Weed risk assessment

The weed risk potential of cotton has been assessed (Appendix C) 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 experience with cotton in Australia described in Section 8.2, and provides a baseline for the evaluation of activities with 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 is addressed below. There are 2 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 (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* × *G. hirsutum* hybrids (McGregor, 1976; Moffett, 1983) and hybrid cotton is widely cultivated in India and China. A study in Turkey of *G. hirsutum* × *G. barbadense* hybrids showed high yields and good fibre characteristics (Basbag and 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 and Greg Constable, CSIRO, 2002, pers. comm.). Hybrids between the 2 species do not form stable populations and instead tend to segregate towards either parental phenotype over a number of generations.

As described in Section 2.4.1, *G. barbadense* and *G. hirsutum* share the AD tetraploid genomes, are not separated by any large-scale chromosomal rearrangements (Gerstel and Sarvella, 1956), and can be hybridised to produce fertile F_1 progeny. However, F_2 progeny show evidence of lethal gene combinations in succeeding generations (Gerstel, 1954; Stephens and Phillips, 1972). The 2 species have different ribosomal DNA sequences (Wendel et al., 1995) and chloroplast genomes (Wendel and Albert, 1992). Genetic and physical isolating mechanisms have evolved to keep the 2 species distinct.

Genetic isolation mechanisms include incompatibility at the ‘corky’ locus (Stephens, 1946, 1950a, b; Stephens and Phillips, 1972) and selective fertilisation (Kearney and Harrison, 1932; Brubaker et al., 1999a). 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 and 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’.

The extended stigma of *G. barbadense* (see Section 3.2) may affect the likelihood of cross pollination with *G. hirsutum*. In addition, *G. barbadense* early flowering compared to *G. hirsutum* is thought to enable it to be preferentially pollinated early in the day when *G. hirsutum* pollen is unavailable. Whereas *G. hirsutum* can be pollinated later in the day by the still-abundant *G. barbadense* pollen (Stephens and Phillips, 1972).

Interspecific introgression between the 2 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 in Section 2.4.1, most commercial cultivars of *G. barbadense* now contain an average of 8-12% introgressed *G. hirsutum* chromatin (Wang et al., 1995; Van Deynze et al., 2011). Gene flow can be reduced by increasing the distance between cotton planted areas (Van Deynze et al., 2011)

In Australia, the primary cotton crop is *G. hirsutum*, while *G. barbadense* has not been grown commercially since 2010 (see Section 2.1). This eliminates the potential crossing between *G. barbadense* and *G. hirsutum* in agricultural fields, provided that *G. barbadense* continues not to be planted. 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 species

Most of the Australian *Gossypium* species have limited distribution 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 3 are likely to occur in the existing cotton growing regions and, therefore, are likely to be exposed to *G. hirsutum* pollen. *G. sturtianum* and *G. nandewarense* 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 (Atlas of Living Australia, accessed September 2023).

Despite potential co-occurrence of Australian native *Gossypium* species and cultivated 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 cultivated on the same soil type (sandy loam) preferred by native *Gossypium* (Yeates, 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. 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 *Gossypium* 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 (Stewart, 1995; Brubaker et al., 1999b). 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 autosyndesis (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 and Brown, 2001), therefore successful gene transfer is more likely from wild *Gossypium* species to cultivated cottons than *vice versa* (refer to Table 12).

9.3.1 Cross-pollination with G- and K-genome *Gossypium* natives

Several publications discuss extensive experimental efforts to hybridise *G. hirsutum* with the Australian *Gossypium* species (Brown et al., 1997; Zhang and Stewart, 1997; Brubaker et al., 1999b; Brubaker and Brown, 2001; Brubaker et al., 2002). 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 12. 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 12), 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* × 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 and 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 (Hegde and 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 3 genomes (Avivi et al., 1982) and the F_1 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 and Stewart, 1997).

9.3.2 Cross-pollination with *Gossypium* 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, regardless of which species serves 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 (Webber, 1935; Skovsted, 1937; 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 12. Summary of attempts to generate hybrid seeds between cultivated cotton and native Australian species of *Gossypium*, following hand-pollination[a]

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	***G. sturtianum*** [b]	***G. hirsutum***	25 (122)	5 (149)
	G. hirsutum [b]	*G. sturtianum*	25 (39)	134 (193)
	G. robinsonii	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. robinsonii*	8 (9)	54 (89)
G	***G. australe*** [b]	*G. hirsutum*	38 (122)	0 (151)
	G. hirsutum [b]	*G. australe*	0 (16)	0
	G. bickii	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. bickii*	0 (13)	0
	G. nelsonii	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. nelsonii*	2 (14)	0 (2)
K	***G. anapoides*** [c]	***G. barbadense***	0 (4)	0
	G. hirsutum [b]	*G. anapoides*	7 (15)	12 (26)
	G. costulatum	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. costulatum*	2 (4)	4 (13)
	G. cunninghamii	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. cunninghamii*	1 (15)	0 (1)
	G. enthyle	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. enthyle*	10 (18)	9 (48)

Genome of native	**Female (♀) parent** (pollen recipient)	**Male (♂) parent** (pollen donor)	**No. fruit with seed** (no. pollinations attempted)	**No. plants established** (no. seed sown)
	G. exiguum [c]	***G. hirsutum***	0 (7)	0
	G. hirsutum [b]	*G. exiguum*	4 (11)	8 (61)
	G. londonderriense	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. londonderriense*	11 (25)	1 (26)
	G. marchantii	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. marchantii*	17 (23)	0 (72)
	G. nobile [c]	***G. hirsutum***	0 (14)	0
	G. hirsutum [b]	*G. nobile*	24 (36)	15 (86)
	G. pilosum [c]	***G. hirsutum***	0 (6)	0
	G. hirsutum	*G. pilosum*	17 (24)	35 (88)
	G. populifolium	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. populifolium*	14 (40)	18 (65)
	G. pulchellum	***G. hirsutum***	ND	ND
	G. hirsutum [b]	*G. pulchellum*	7 (16)	1 (15)
	G. rotundifolium [b]	***G. hirsutum***	0 (57)	0
	G. hirsutum [b]	*G. rotundifolium*	11 (15)	12 (52)

[a] Pollinations representing the greatest environmental risk, namely with *G. hirsutum* or *G. barbadense* as the pollen donor, are presented in bold, with the reciprocal pollination presented immediately following.
[b] data from Brown et al. (1997); [c] data from Zhang and 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 AUSTRALIAN NATIVE GOSSYPIUM SPECIES

The following are Australian native *Gossypium* spp. as listed on the Atlas of Living Australia, accessed September 2023:

Gossypium anapoides

Gossypium australe

Gossypium bickii

Gossypium costulatum

Gossypium cunninghamii

Gossypium enthyle

Gossypium exiguum

Gossypium londonderriense

Gossypium marchantii

Gossypium nelsonii

Gossypium nobile

Gossypium pilosum

Gossypium populifolium

Gossypium pulchellum

Gossypium robinsonii

Gossypium rotundifolium

Gossypium sturtianum

APPENDIX B WEEDS OF COTTON

Table B1. Major weeds of cotton crops in Australia

Scientific name	Common name
Grasses	
Chloris truncata	Windmill grass
Chloris virgata	Feathertop Rhodes grass
Cyperus rotundus	Nutgrass
Echinochloa colona	Awnless barnyard grass
Lolium rigidum	Annual ryegrass
Urochloa panicoides	Liverseed grass
Broadleaf weeds	
Amaranth spp.	Amaranths
Chamaesyce drummondii	Caustic weed
Citrullus lanatus var. *lanatus*	Wild melon
Convolvulus erubescens	Australian bind weed
Conyza bonariensis	Flaxleaf fleabane
Cullen tenax	Emu foot
Datura ferox	Thornapple
Hisbiscus trionum	Bladder ketmia
Ibicella lutea	Yellow-flowered devils claw
Ipomoea lonchophylla	Cowvine
Ipomoea plebeia	Bellvine
Medicago polymorpha	Burr medic
Phyla nodiflora	Lippia
Physalis minima	Wild gooseberry
Polymeria pusilla	Polymeria
Portulaca oleracea	Pigweed
Salvia reflexa	Mintweed
Sesbania cannabina	Sesbania pea
Sonchus oleraceus	Common sowthistle
Tribulus micrococcus	Yellow vine or spineless caltrop
Xanthium italicum	Italian cockleburr
Xanthium occidentale	Noogoora burr
Xanthium spinosum	Bathurst burr

Sources: Data compiled from (Charles et al., 2004; Taylor and Walker, 2006; Walker et al., 2006; CRDC, 2013b)

APPENDIX C WEED RISK ASSESSMENT OF COTTON

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

Relevant land uses:

1. Intensive[1] uses (ALUM[2] 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[3] (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 sourced from this document "*The Biology of Gossypium hirsutum* L. and *Gossypium barbadense* L. (*Cotton*) (OGTR, 2024)". 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.

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

[2] ALUM refers to the Australian Land Use and Management classification system version 8 (ABARES, 2016).

[3] *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**
1. What is cotton's ability to establish amongst existing plants?	**Rating: Low in all relevant land uses** 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 and Roberts, 2002). Seed losses leading to volunteers in *dryland and irrigated cropping areas* can occur during harvesting, and in *intensive use areas* during transport (from field to gin), storage (feedlots) and processing (around the facilities where ginning is conducted). Naturalised populations of both *G. hirsutum* and *G. barbadense* 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.
2. What is cotton's tolerance to average weed management practices in the land use?	**Rating: Low in cropping and intensive land uses** **High in nature conservation land uses** Weed management practices (preventive, cultural and chemical) aim at reducing the loss in yields due to weeds. In *dryland and irrigated cropping areas*, 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. Cotton volunteers in *intensive use areas* are not known to sponsor self-perpetuating feral populations. Typically, such volunteers are killed by roadside management practices (e.g. herbicide treatment or slashing/mowing) and/or grazed by livestock, thereby limiting their potential to reproduce (Eastick and Hearnden, 2006; Addison et al., 2007). Cotton is not known to be specifically targeted in *nature conservation areas* 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.

Invasiveness questions	**Cotton**
3. Reproductive ability of cotton in the land use:	
3a. What is the time to seeding in the land uses?	**Rating: < 1 year in all relevant land uses** Cotton is a perennial that has been adapted and bred to act as an annual crop. Under standard agricultural conditions, it generally takes 4 months to complete a lifecycle from germination to the maturation of the first seeds. However, in *nature conservations areas* of northern Australia, feral cotton does exist as a perennial, with annual seed production.
3b. What is the annual seed production in the land use per square metre?	**Rating: Low in all relevant land use areas (from volunteers)** When grown as a crop in *dryland and irrigated cropping areas*, cotton seed production would be considered high (> 1000 viable seed per m^2)[4]. 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 *intensive use areas,* 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 (< 1000 viable seed per m^2). In *nature conservation areas* the number of volunteer cotton plants is expected to be very low and would suggest low seed production.
3c. Can cotton reproduce vegetatively?	Under natural conditions, cotton **cannot** reproduce by vegetative propagation.

[4] 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^2. However, row spacing can be 38 cm, or even as narrow as 25 cm, enabling 24 or more plants per m^2 (Roche et al., 2006; Brodrick and Bange, 2010). Assuming a range of 12-24 plants per m^2, the number of seeds per m^2 could range from approximately 3,500 to over 10,000 per m^2. Based on the above, seed production would be considered high (> 1000 viable seed per m^2).

Invasiveness questions	Cotton
4. Long distance seed dispersal (more than 100 m) by natural means in land uses	
4a. Are viable plant parts dispersed by flying animals (birds and bats)?	**Rating: Unlikely in all relevant land uses** 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 and Fitt, 1996), so dispersal from cotton volunteers is highly unlikely.
4b. Are viable plant parts dispersed by wild land-based animals?	**Rating: Unlikely to Occasional in all relevant land uses** Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g. hooks or spines). Whole cotton seed, meal and hulls are used in stockfeed. 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 and Fitt, 1996), so dispersal from cotton volunteers is unlikely.
4c. Are viable plant parts dispersed by water?	**Rating: Occasional in all relevant land uses** 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 (Bayer, 2021), 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.
4d. Are viable parts dispersed by wind?	**Rating: Unlikely in all relevant land uses** The fibres attached to cotton seeds may catch the wind and facilitate seed dispersal, however this is not expected to approach a distance of 100 m, except perhaps during severe windstorms.

Invasiveness questions	**Cotton**
5. Long distance seed dispersal (more than 100 m) by human means in land uses:	
5a. How likely is deliberate spread via people?	**Rating: Common in/from dryland and irrigated cropping and intensive land uses** **Highly unlikely in nature conservation land use** Cotton is a crop species that is purposely cultivated for the production of the fibre, seeds, oil extracted from seeds and for use as animal feed. Thus, it is deliberately transported for cultivation in *dryland and irrigated cropping areas* and to *intensive land use* areas for processing and use in feed lots and dairy farms. Cotton seed is not deliberately dispersed within/into *nature conservation* land use areas.
5b. How likely is accidental spread via people, machinery and vehicles?	**Rating: Common in dryland and irrigated cropping areas and intensive land uses** **Unlikely in nature conservation land use** In *dryland and irrigated cropping areas* as well as *intensive use areas*, cotton seed may be accidently dispersed by people, machinery and vehicles. After picking, cotton bolls are pressed into modules or bales and transported by humans to gins where the fibres are separated from the seeds. In this process, seed could be spread along roadsides and railway lines, as well as near storage and processing facilities. Seed can remain on machinery after harvesting. No data is available for *nature conservation* 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.

Invasiveness questions	Cotton
5c. How likely is spread via contaminated produce?	**Rating: Unlikely in/from all relevant land use areas** Cotton farming in *dryland and irrigated cropping areas* is often characterised by rotation with other crops, such as wheat or the legumes faba bean (*Vicia faba*) or vetch (*Vicia villosa*). The amount of cotton seed left in the field prior to the planting of a rotation crop would depend upon the efficiency of the harvesting of the bolls, cleaning of machinery, and general weed management procedures. Growth of cotton volunteers within a rotation crop would depend upon the weed management procedures of the latter crop, while the spread of cotton seed with the rotation crop would depend upon the processing of the harvested plant material from the rotation crop. Long distance dispersal via contaminated hay and forage may also occur in or from *intensive use areas*. This could occur from areas purposely producing hay/forage or if roadside vegetation were cut for this purpose. However, considering cotton seed loss in these areas is likely to be low and volunteer plants establishing only rarely, spread via contaminated produce from *intensive use areas* is unlikely.
5d. How likely is spread via domestic/farm animals?	**Rating: Unlikely in nature conservation areas** **Occasional in all other relevant land uses** Cotton seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g. hooks or spines). Whole cotton seed, meal and hulls are used in stockfeed. 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 and Roberts, 2002), suggesting that spread to other areas of the farms was unlikely. Thus, seed may occasionally be spread from *intensive land use areas* such as feed lots or *cropping areas* if domestic or farm animals had access to the cotton crop. Spread by domestic or farm animals would be highly unlikely in *nature conservation areas* as they are typically not found in these areas.

Impact questions	Cotton
6. Does cotton reduce the establishment of desired plants?	**Rating: Reduces establishment by < 10% in all relevant land uses** Cotton is a cultivated plant that may establish where land has been disturbed, most particularly in *dryland and irrigated cropping areas*. However, as noted in Impact question 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 *intensive use areas*, such as along roadsides, desired species may range from native flora to introduced trees, bushes and shrubs. Such areas are often managed, for either aesthetic or practical reasons (e.g. maintaining driver visibility) by the removal of larger trees and invasive weeds. Cotton would be treated as a weed and managed accordingly. In *nature conservation areas*, the ability of cotton to establish is so rare that it is unlikely to affect the establishment of native plants.
7. Does cotton reduce the yield or amount of desired plants?	**Rating: Reduces yield/amount by < 10% in all relevant land uses** 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.
8. Does cotton reduce the quality of products or services obtained from the land use?	**Rating: Low in all relevant land uses** As discussed in Impact 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.
9. What is the potential of cotton to restrict the physical movement of people, animals, vehicles, machinery and/or water?	**Rating: Low in all relevant land uses** Cotton is unlikely to establish in *nature conservation areas* and although it may establish in *dryland and irrigated cropping areas* or *intensive use areas*, 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.

Impact questions	**Cotton**
10. What is the potential of cotton to negatively affect the health of animals and/or people?	**Rating: Low in all relevant land uses** 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 (Kandylis et al., 1998). However, in *intensive use* 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 & Drouillard 2002). Although people use cotton seed oil for cooking, they generally do not consume cotton plants or seed. 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.
11. Major positive and negative effects of cotton on environmental health in the land use	
11a. Does cotton provide food and/or shelter for pathogens, pests and/or diseases in the land use?	**Rating: Major negative effects in dryland or irrigated cropping use** **Minor or no effect in all other relevant land use areas** Cotton is susceptible to a range of pathogens, such as Black Root Rot, Verticillium wilt, and Fusarium wilt, and insect pests such as the Heliothis caterpillar, aphids, thrips, mirids and whitefly. Infected cotton volunteers in *dryland or irrigated cropping use* areas may act as a reservoir 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 (e.g. under sub-standard weed management), thus in some years may constitute a major negative effect. In *intensive* or *nature conservation use* areas the density of cotton volunteers is expected to be low and thus may have only minor or no effect.

Impact questions	Cotton
11b. Does cotton change the fire regime in the land use?	**Rating: Minor or no effect in all relevant land uses** 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.
11c. Does cotton change the nutrient levels in the land use?	**Rating: Minor or no effect in all relevant land uses** 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.
11d. Does the species affect the degree of soil salinity in the land use?	**Rating: Minor or no effect in all relevant land uses** 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.
11e. Does the species affect the soil stability in the land use?	**Rating: Minor or no effect in all relevant land uses** 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.
11f. Does the species affect the soil water table in the land use	**Rating: Minor or no effect in all relevant land uses** 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.
11g. Does the species alter the structure of nature conservation by adding a new strata level?	**Rating: Minor or no effect in all relevant land uses** 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.