

The biology and ecology of cotton (*Gossypium hirsutum*) in Australia

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PREAMBLE

This document addresses the biology and ecology of the species *Gossypium hirsutum*. Included is the origin of *G. hirsutum* as a crop plant (referred to as ‘cotton’), general descriptions of its growth and agronomy, its reproductive biology, toxicity and allergenicity and its general ecology. This document also addresses the potential for cotton to outcross via pollen transfer and seed movement. Special emphasis has been given to the potential hybridisation between cotton and its close native relatives.

1 BIOLOGY OF COTTON

1.1 ORIGIN OF CULTIVATED COTTON

The word ‘cotton’ refers to four species in the genus *Gossypium* (Malvaceae) — *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L.— that were domesticated independently as source of textile fibre (Brubaker et al. 1999a). Globally, the *Gossypium* genus comprises about 50 species (Brubaker et al. 1999a). The place of origin of the genus is not known, however the primary centres of diversity for the genus are west-central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). DNA sequence data from the existing *Gossypium* species suggests that the genus arose about 10 – 20 million years ago (Wendel & Albert 1992; Seelanan et al. 1997).

Cotton lint was spun and woven into cloth even before 3000 B. C. (Gulati and Turner, 1928 cited in (McGregor 1976). Most commercially cultivated cotton is derived from two species, *G. hirsutum* (Upland cotton, 90% of world plantings) and *G. barbadense* (Pima, or Long-staple cotton). *G. hirsutum* is the most widely planted species in Australia but a small amount of *G. barbadense* is also cultivated. Two other species, *G. arboreum* and *G. herbaceum*, are cultivated in Asia, but are not grown commercially in Australia.

Brubaker et al. (Brubaker et al. 1999b) suggest that both *G. hirsutum* and *G. barbadense* were introduced to Australia as a source of textile fibre from Mexico where they are native, and where they were domesticated originally. Commercial cotton cultivation began in Queensland and New South Wales in the 1860s when the American Civil War caused shortages in world cotton supplies. Subsequently, cultivation was attempted in the Northern Territory (1882) and the Kimberley’s, Western Australia (1947), although in these northern regions, the prevalence and impact of insect pests limited the commercial viability of continued plantings (Williams 2002). It was not until the 1960s that a stable Australian cotton industry was established, primarily in northern New South Wales and southern Queensland (Hearn & Fitt 1992).

G. hirsutum also may have arrived in northern Australia naturally, via ocean currents from Central America (Fryxell 1966; Fryxell 1979a). When this may have occurred is unknown, and it has not been substantiated. The primary evidence for this supposition is the presence along coastal river and beach strands in northern Australia of ‘naturalised’ populations of agronomically primitive cotton with morphological

features that suggest they are not derived directly from modern, elite *G. hirsutum* cultivars. They may be descendants of long-distance transoceanic immigrants as proposed by Fryxell, or alternatively, feral derivatives of primitive varieties introduced for cultivation before 1900.

1.2 PHYLOGENY & TAXONOMY

Global radiation of the genus *Gossypium* was accompanied by substantial evolution of chromosome size and structure. The *Gossypium* genus comprises about 45 diploid species with 26 chromosomes and 5 allotetraploid species (tetraploids derived following hybridisation of two diploids) with 52 chromosomes (Brubaker et al. 1999a). *Gossypium* species commonly are grouped into eight diploid genomic groups, designated A - G and K, and one tetraploid genomic group, based on chromosomal similarities (Edwards & Mirza 1979; Endrizzi et al. 1985; Stewart 1995). Each genome represents a group of morphologically similar species that can only rarely form hybrids with species from other genomic groups.

G. hirsutum and *G. barbadense*, the two species cultivated in Australia, are in the AD allotetraploid genomic group. 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 et al. 1989). The identity of the progenitor diploid species, and when these progenitors may have come into physical contact sufficient to enable hybridisation, is unknown. At present, A and D diploid species exist in different hemispheres (Endrizzi et al. 1985).

The taxonomy and distribution of native Australian *Gossypium* species are discussed in detail in Section 1.6.2.

1.3 USES OF COTTON AND ITS BY-PRODUCTS

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

Cotton is primarily grown as fibre crop. It is harvested as 'seed cotton' which is then 'ginned' to separate the seed and lint. The long 'lint' fibres are further processed by spinning to produce yarn that is knitted or woven into fabrics.

The ginned seed is covered in short, fuzzy fibres, known as 'linters'. These must be removed before the seed can be used for planting or crushed for oil, and are used in a variety of products including foods. 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).

The delinted cotton seed can be processed to produce oil, meal and hulls. Cotton seed oil has been in common use since the middle of the nineteenth century and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958 (ANZFA 2002). It is used in a variety of products including edible vegetable oils and margarine, soap, and plastics.

Cotton seed, or meal, flour or hulls derived from it, is also 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 1.8.1).

1.4 GROWTH AND DISTRIBUTION OF CULTIVATED COTTON IN AUSTRALIA

1.4.1 General information on growth and agronomy

In nature, *G. hirsutum* is a perennial shrub that grows to about 1.5 metres in height. Commercially, however, *G. hirsutum* is cultivated as an annual, with destruction of plants after harvesting the fruit for seed and fibre.

In Australia, the bulk of the cotton industry is concentrated in northern New South Wales and southern Queensland. Cotton is grown commercially from Hillston in southern New South Wales to Emerald in central Queensland, as far west as Bourke and Lake Tandou in New South Wales. The total area planted to cotton was about 500 000 hectares in 2000. Cotton is also being grown on a trial basis around Richmond in northern Queensland and in Western Australia and the Northern Territory.

Cotton is grown either as a dryland crop, relying on rainfall, or as an irrigated crop where a reliable water supply is available.

The timing of cotton cultivation varies slightly throughout Australia, depending on climate. Cotton is planted when the soil temperature reaches 14°C at a depth of 10 cm for at least 3 days. In northern New South Wales, the appropriate soil temperature is reached typically in late September or early October, whereas in central Queensland, it is likely to occur four weeks earlier (Cotton Australia 2002b). 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, picking 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 2002a).

Agronomically, the growth of cotton can be divided into three key developmental phases: (1) germination and seedling establishment, (2) leaf area and canopy development and (3) reproduction and dispersal. Total developmental time, from germination to maturation of the first fruit, is usually about 15-17 weeks, although this may be affected by temperature and other environmental variables.

1.4.2 Germination and seedling establishment

Seed dormancy

It is widely accepted that dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture. Additionally, some forms of cotton may produce 'hard seeds' that, upon drying, become impermeable to water and suffer delayed germination (Christiansen & Moore 1959b). This 'induced dormancy' closely resembles the hard-seeded trait of many legumes. In cotton, it can be overcome in a number of ways including by treatment with hot water, which softens the chalazal plug (Christiansen & Moore 1959b), allowing the tissues of the seed and embryo to take up moisture.

Agronomically, hard seeds are undesirable and the trait has been largely eliminated from modern commercial cultivars through breeding and selection (Mauney 1986; Hopper & McDaniel 1999). The existence of a soil seed bank does not appear to have been investigated specifically, although it seems unlikely because dispersed seeds that do not germinate are rapidly weathered, leading to significant decreases in their viability (Hallowin 1975; Woodstock et al. 1985).

In addition to induced dormancy, cotton seeds collected immediately following fruit maturation can display 'innate dormancy' (Taylor & Lankford 1972a) – 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 from no dormancy in certain varieties (Hsi & Reeder 1953) to several months in others (Christidis 1955). Taylor and Lankford (Taylor & Lankford 1972b) demonstrated that the germinability of 1-year old cotton seeds kept under storage was about 8 – 24 % lower than seeds from the same seed lot in subsequent years. They also observed that the positive effect of seed age on germinability could reduce the negative impact of factors that may induce dormancy, such as cold temperature.

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

Germination

'Fuzzy' cotton seed, produced by ginning, is generally delinted by treatment with acid before being used for planting. The delinted seed is also known as 'black' seed. Providing that soil moisture, temperature and oxygen are favourable, a majority (>80%) of seeds germinate after sowing. Germination begins with the entry of

moisture into the seed and embryo via the chalazal aperture, at the seeds' apex (Christiansen & Moore 1959a). The seed/embryo then begins to swell as it absorbs moisture. Under favourable conditions, the radicle (root tip) emerges within 2-3 days from the seed and newly germinated seedlings emerge above the soil 5-10 days after emergence of the radicle (Oosterhuis & Jernstedt 1999).

Other variables affecting cotton seed germination have been studied in large scale field trials in northern Australia (Monsanto, unpublished data). Field experiments explored the germination of black seed, fuzzy seed and seed cotton (just as picked, embedded in the lint) in a variety of habitats into which cotton may be dispersed (native bush, roadsides, cattle feed yards and the edge of waterways) following the manipulation of variables that may affect germination such as the density and burial of sown seeds.

Many of the 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 germinations are likely to have occurred using this technique, than if seeds were dispersed naturally and allowed to germinate with rainwater. The level of germination is, therefore, likely to be comparatively high and reflects potential 'worst case scenarios' for cotton volunteerism. There were highly significant ($P < 0.001$) 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.

Seed form also consistently affected germination ($P < 0.001$). Although dispersal of black seed within the environment is expected to be very limited (see 1.4.4), it germinates more readily than other seed forms. Conversely, seed cotton has the potential to be dispersed widely (along transport routes) but germinates comparatively poorly, even when germination conditions are optimised. Since burying seed increased the likelihood of germination ($P < 0.001$) irrespective of seed form, it may be that lint attached to the seed coat of cotton seed limits contact with the soil, thereby affecting its ability to imbibe soil moisture and germinate (Kerby et al. 1996). The density at which seeds were sown also affected germination at a majority of trial sites ($P < 0.05$). Generally, seeds sown at low density germinated poorly and with greater variability than those sown at high density.

Seedling survival

The survival of seedlings that germinated in Monsanto's field germination experiments was monitored for two years. After one year, there was at least one surviving plant in 16 of the 20 trial sites. After 2 years, there was at least one surviving plant in 8 of the 13 sites at which monitoring was continued for the second year. Typically, the total number of surviving seedlings was low (irrespective of the number of seeds that were sown), precluding robust statistical analyses, and highly variable, ranging from zero at some sites, to about 50 plants at other sites. At sites where the number of surviving seedlings was sufficiently high to warrant statistical analysis, there was no indication that the genetic modifications significantly enhanced survival. However, there were clear trends indicating that the habitat into which seeds were sown affected survival.

Survival at sites located near cattleyards or adjacent to water bodies was consistently high, probably because of high soil nutrients and/or soil moisture. Both factors are clearly critical for survival of cotton seedlings (and probably other life history stages), but the relative importance of each is unknown. The result highlights field observations that the occurrence of naturalised and volunteer cotton appears to be limited by the availability of adequate soil moisture. Significantly, the nutrient-enhanced experimental sites were the only habitats in which a second generation of seedlings was recruited from the original cohort of seeds that was sown.

In northern Australia, the survival of cotton plants is likely to be affected by insect herbivory and, indeed, Monsanto provides data suggesting that damage by leaf-feeding insects on both cotton volunteers and feral cotton can be high. However, factors such as water availability, soil nutrients, grazing by vertebrates, fire and plant competition are likely to affect seedling survival more so than insect pressure. The relative impact of these factors has not been tested specifically.

Among herbivorous insects, Monsanto's observations indicate that grasshoppers appeared to be the most common and destructive insect herbivores at each study site. Indeed, grasshoppers (Orthoptera) are considered to be the most important grazing insects in tropical savanna ecosystems (Andersen & Lonsdale 1991). Grasshoppers are unaffected by the Cry toxins produced by Bollgard II[®] cotton.

Preliminary results from an insect exclusion experiment, conducted at a feral cotton population near the Adelaide River in the Northern Territory, support observations that insect pressure has a relatively small impact on the regulation of cotton populations. The experiment is not yet finalised but the data gathered to date suggest that the survival of naturally occurring seedlings that are protected from potential insect herbivores by insect-proof cages is not significantly higher than the survival of comparable uncaged plants, which are exposed to insect herbivores. As with the observations reported above, grasshoppers appeared to be the most abundant and destructive insect herbivores.

1.4.3 Leaf and canopy development

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 3-5 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 about 5-6 weeks after planting the total area of leaves born on fruiting branches exceeds that of the main stem and vegetative branches, constituting about 60% of the total leaf area at maturity (Oosterhuis & Jernstedt 1999).

Associated with the production and growth of leaves, the plants' canopy begins to 'close'. Early canopy closure may be desirable agronomically, because it can help to limit weed growth and decrease the evaporation of soil moisture.

1.4.4 Reproduction and dispersal

Reproductive maturity is reached about 4-5 weeks after planting, with the formation of floral buds ('squares'). Typically, about 25 days elapse between the initial appearance of a square and anthesis (flower opening) (Oosterhuis & Jernstedt 1999). Under normal crop conditions, about 60% of squares and immature fruit are abscised prematurely. Mature flowers are not usually shed before pollination (Oosterhuis & Jernstedt 1999).

Cotton flowers anthesise at or near dawn and remain open for only one day. At anthesis, the petals of *G. hirsutum* are creamy white. They turn pink-red within about one day of pollination, after which they abscise. Flowers of *G. barbadense* are yellow at anthesis but also turn pink.

Pollen & Pollination

Soon after anthesis, the anthers of cotton flowers dehisce, discharging their pollen. Cotton pollen is relatively large and heavy, and not easily dispersed by wind (Jenkins 1992). Cotton is a facultative self-pollinator, and an opportunistic out-crosser when insect pollinators are present (Oosterhuis & Jernstedt 1999). Cotton pollen remains viable for about 12 hours (Govila & Rao 1969). Fertilisation of ovules occurs about 12-30 hours after pollination.

Out-crossing rates

Insect prevalence strongly influences out-crossing rates for cotton (Elfawal et al. 1976; Moresco et al. 1999), and varies with location and time (Moffett et al. 1975; Elfawal et al. 1976; Moffett et al. 1976). Insect visitation rates, however, 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 & Bridge 1973; Gridley 1974; Theron & van Staden 1975; Elfawal et al. 1976; Umbeck et al. 1991a; Llewellyn & Fitt 1996). Higher estimates (16.5% to 25%) have been reported in a few cases (Smith 1976; Moresco et al. 1999). Indeed, under certain conditions, out-crossing rates may reach 80% (Richmond 1951; Oosterhuis & Jernstedt 1999).

The level of out-crossing observed in Australia is in the order of 1 to 2% between plants in adjacent rows (Thomson 1966a; Mungomery & Glassop 1969a; Llewellyn & 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 & Fitt 1996). Honeybees were implicated as the chief pollinating agent in a Queensland study (Mungomery & Glassop 1969a)). Since honeybees were not present for a similar study in the Ord River valley (Thomson 1966a) it was suggested that native bees might be responsible for the cross-pollination in this region. In cotton out-crossing experiments conducted near Narrabri in New South Wales, no bees were detected, and although small numbers of wasps and flies were recorded, it was suggested that hibiscus beetles were likely to be the major cross-pollinators in these trials (Llewellyn & Fitt 1996).

Pollen dispersal distances

Cotton pollen dispersal studies consistently demonstrate that when out-crossing occurs, it is localised around the pollen source and decreases significantly with distance (Thomson 1966a; Galal et al. 1972; Theron & van Staden 1975; Elfawal et al. 1976; Chauhan et al. 1983; Umbeck et al. 1991a; Llewellyn & Fitt 1996). This presumably represents the effective foraging range of insect pollinators.

The separation distance of 4 metres required in Australia for certified commercial seed production reflects the relatively short distances observed for cotton pollen dispersal in Australian studies. In one CSIRO study involving two cotton seasons carried out in New South Wales, 200 transgenic cotton plants were embedded in an eight-hectare plot of non-transgenic cotton (Llewellyn & Fitt 1996). Seeds from the non-transgenic cotton were collected and assayed for a marker protein in the first season. Of the 37 000 seeds assayed, only six were derived from out-crossing of the transgenic pollen and all of these came from within three metres of the transgenic plot.

In a second study by Llewellyn and Fitt (1996) at the same location, dispersal of pollen from a block of 3 000 transgenic cotton plants was monitored and 60 000 seeds were assayed. Forty-nine cross-pollinated seeds were detected, with the highest level of out-crossing (0.9%) occurring in the first buffer row. Beyond 10 metres, out-crossing events were generally rare, with 0.01% out-crossing detected at distances of 11, 14 and 16 metres, and no out-crossing detected between 16 and 20 metres.

Similar findings have been obtained by cotton breeders in previous studies under Australian conditions. For example, Thomson (Thomson 1966b) looked at out-crossing from a red leafed (partly dominant) variety of cotton planted within a field of green leafed cotton. This study was carried out in the Ord River valley over two growing seasons. Cross-pollination between adjacent plants, measured as the proportion of red leafed progeny, was in the range of 0 to 5 %, with mean values of 1.63 % and 1.02 %, in the first and second seasons respectively. Very little cross-pollination was detected at a distance of more than 3 metres (average less than 0.01%) and none was detected at distances between 3 and 8 metres.

Mungomery and Glassop (Mungomery & Glassop 1969b) used a similar experimental design to look at out-crossing during two seasons in Biloela, Queensland. Cross-pollination between adjacent rows of cotton was around 1.7 % in both years, falling to less than 1 % in rows beyond this. No crossing was observed in rows to the north or south of the red leafed cotton, at 32 or 53 metres (the last two distances tested), with the exception of 0.3% out-crossing detected on the northern side at 53 metres, in one of the two growing seasons.

Umbeck *et al.* (Umbeck et al. 1991b) also investigated pollen dispersal from transgenic cotton embedded in a field of conventional cotton in the United States. 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 transgenic block. The level of out-crossing was generally below 1% at 7 metres, but a low level of sporadic out-crossing was seen at distances of up to 25 metres. Out-crossing at distances greater than 25 metres was not measured.

The studies cited above measured pollen dispersal through buffer rows of cotton. The out-crossing rate in the absence of buffer rows, between cotton plants separated by bare ground, might be expected to be higher. For instance, Green and Jones (Green & Jones 1953) demonstrated that out-crossing through buffer rows decreased from 19.5% at 1.1 metres to 2.6% at 9.6 metres and 1.0% at 10.7 metres. By comparison, out-crossing at a distance of 10 metres, in the absence of a buffer, was 4.7%. Nevertheless, out-crossing in the absence of a buffer did decline with distance, from 6.0% at 5.0 metres, to 4.7% at 10.0 metres, and 0.6% at 25.1 metres. An Egyptian study measured out-crossing from *Gossypium barbadense* and also demonstrated a rapid decline with distance over fallow ground (Galal et al. 1972). In this experiment, the average level of out-crossing varied from 7.8% at 1.1 metres to 0.16% at 35.2 metres.

Fruit development

The growth and development of cotton fruit, known as ‘bolls’, begins immediately following fertilisation although the most rapid period of growth occurs after about 7-18 days (Oosterhuis & Jernstedt 1999). During development, the bolls are spherical to ovoid and pale green. Maximal boll size is achieved about 25 days after fertilisation, with full maturity achieved approximately 20 days later. 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.

Monsanto also provided data from their field experiments in northern Australia on the number of surviving seedlings that reached reproductive maturity. Surviving plants produced floral buds (‘squares’) at a majority of sites, but frequently the squares aborted before maturation of the fruit (‘bolls’), a phenomenon that is common in cultivated cotton. Successful maturation of fruit to produce at least one open boll occurred at 8 of the 20 study sites, although the total number of bolls produced was highly variable, ranging from 1 to >300. Six of these sites were particularly productive, producing a total of ≥ 20 bolls, with estimates suggesting that individual plants produced comparable numbers of bolls to those produced by cultivated cotton plants. Significantly, these were disturbed sites (drain and cattleyard habitats), with high levels of soil nutrients and/or moisture.

Seed morphology

Cotton is grown primarily for its fibres, which are produced by epidermal cells of the seed coat. Prior to ginning and delinting, the seed coat bears two types of fibres – long lint fibres valued by the textile industry and short, fuzzy fibres, known as linters used in various products including foods (see section 2.5.1). After ginning, the cotton seed is still covered in linters and is known as ‘fuzzy seed’. After acid treatment to remove the linters, the cotton seeds are ovoid in shape, slightly pointed, about 10 mm long x 4 mm wide, and dark brown in colour (called ‘black seed’). Each boll produces about 20 - 25 seeds.

Seed dispersal

As cotton does not generally reproduce vegetatively (Serdy et al. 1995), spread within the environment occurs by seed dispersal. Dispersal of cotton seeds is a physical process. Observations of dispersed seeds and the occurrence of volunteer plants in the

northern Australian trials (Monsanto, unpublished data) indicate that delinted black seed has the lowest risk of unintentional spread within the environment. When dispersal of black seed occurs, it is associated with spillage at sowing in cotton production areas.

Fuzzy seed is commonly used as stockfeed and therefore has a high potential for dispersal to non-cotton production habitats, introduced as stockfeed, with spillage from troughs at feeding.

Unprocessed 'seed cotton', that retains all of the fibres attached to the seedcoat, also has a high potential for dispersal within the environment. Monsanto's data suggest that volunteers from dispersed seed cotton were relatively common in irrigation channels and drains, and along roadsides. Roadside volunteers most likely established following seed cotton spillage during transport of cotton modules from the paddock to the gin.

A separate study performed by researchers at the Australian Cotton Research Institute also suggested that seed cotton may be dispersed along transportation routes following spillage from cotton modules. Resultant volunteers were most common close to the studied cotton production areas near Emerald, Queensland, and at the sites to which the seed was being transported (Atherton, north Queensland). In between these extremes, on average at least one roadside volunteer was detected every four kilometres of road in areas south of latitude 22° South; the rate was marginally higher in areas north of latitude 22° South. Volunteers that had reached reproductive maturity and which produced open bolls were detected in all habitats. However, roadside vegetation management practices such as slashing reduced the proportion of flowering volunteers in these habitats relative to the proportion occurring in stockyards.

Post-dispersal, seeds that do not germinate are likely to be removed by seed predators or rot, rather than become incorporated into a persistent soil seed bank, which is in any case unlikely for reasons outlined above.

1.5 PESTS AND DISEASES OF COTTON IN AUSTRALIA

More than 1326 species of insects have been reported in commercial cotton fields worldwide but only a small proportion are pests (Matthews & Tunstall 1994). Of the 30 pests of cultivated *G. hirsutum*, the most important are the caterpillars of *Helicoverpa armigera* and *Helicoverpa punctigera*, and the spider mite *Tetranychus urticae* (Shaw 2000; Pyke & Brown 2000).

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

In cotton, the adult moth lays its eggs on young terminal branches, and the eggs hatch into larvae (caterpillars) within 2 to 3 days. 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 – 20 days and *H. armigera* cotton bollworm may go through four to five generations during the cotton-growing season. The last generation goes into a period of suspended development or ‘diapause’ over winter, burrowing into the soil around the base of the plants. The over-wintering pupae emerge from the soil in the following spring.

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. This strategy, known as “pupae busting”, can kill over 90% of the pupae in the soil. This is an effective mechanism for reducing the number of moths that emerge in the spring and for delaying development of insects with resistance to insecticides used on cotton.

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. In spring, weather conditions cause deterioration of the host plants and this is followed by the large-scale migration of many of the moth species, over distances of 500 to 1500 km, in some cases reaching the cotton growing regions of southeastern Australia. Although some *H. armigera* migrate, *H. punctigera* is more commonly found in these migrations and often arrives in the cotton areas early in the season, before the emergence of *H. armigera*. However, numbers of *H. punctigera* are usually low in late summer and early autumn and winter diapause is not common. The constant influx of *H. punctigera* immigrants to the cotton growing areas is thought to be responsible for the lack of development of resistance to chemical pesticides in this species.

Spider mites are also a significant cotton pest in Australia. The two-spotted spider mite (*Tetranychus uticae*) is the most common but the bean spider mite (*T. ludeni*) and strawberry spider mite (*T. lambi*) are also found. They live and feed on the under side of leaves, causing bronzing, reddening and eventually desiccation of the leaf. Predation is a key factor in reducing early season survival of mites. Predators include thrips (which can also be pests in their own right), ladybeetles, big-eyed bugs, damsel bugs and lacewings. The use of broad-spectrum pesticides to control other pests can result in destruction of beneficial predators and exacerbation of spider mite infestations.

Minor pests of cotton include green mirid (*Creontiades dilutes*), also a pest of other summer crops. The insect feeds on and destroys seedling terminals and small flowerbuds. Cotton aphid (*Aphis gossypii*) is the main aphid pest of cotton. Honeydew produced by the aphid can contaminate cotton lint, reducing its value. However, this is not a major problem for Australian cotton.

The cotton whitefly (*Bemisia tabaci*) is a serious pest of fibre, horticultural and ornamental crops worldwide. It can cause extensive damage through direct feeding, honeydew production and as a viral vector. The first widespread outbreak of this pest in Australian cotton occurred only very recently, in central Queensland in the 2001/2002 cotton growing season. The cotton industry is actively researching pest

and resistance management strategies for use against cotton whitefly (Cotton CRC 2002).

1.5.1 Diseases in cotton

Diseases in cotton may affect the quality of the fibre and seed, as well as the yield and cost of production of the cotton crop (Bell 1999; Cotton Australia 2002a). The main diseases affecting cotton in Australia include:

- Seedling diseases;
- Fungal wilt diseases (Fusarium wilt or verticillium wilt); and
- Leaf spots.

Seedling diseases can be caused by several fungi, commonly *Pythium* and *Rhizoctonia*. The diseases can cause seed rot and damping-off, and are most likely to occur when cool, wet weather occurs soon after planting. Black root rot (*Thielaviopsis basicola*) is another fungus that affects seedlings.

Verticillium wilt and Fusarium wilt are fungal diseases caused by *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *vasinfectum*, respectively. The fungi infect the plant root tips, enter the xylem vessels and proliferate throughout the xylem vessels of the plant. This plugs the vessels and plants develop the wilt symptoms. Verticillium wilt is widespread in most cotton growing areas, and has a wide host range, including many common weeds. Fusarium wilt is relatively new to Australia (first reported in 1993) but has spread rapidly to most cotton growing regions of New South Wales and Queensland. Cotton cultivars with some resistance to these diseases are available.

Leaf spots can be caused by fungi (Alternaria leaf spot, caused by *Alternaria macrospora* or *A. alternata*) or bacteria (Bacterial blight caused by *Xanthomonas campestris*). Most commercial Australian cultivars are resistant to bacterial blight and some also have some level of resistance to *Alternaria*.

1.6 DISTRIBUTION OF FERAL AND NATIVE COTTON POPULATIONS IN AUSTRALIA

1.6.1 Feral (naturalised) populations of cultivated cotton

Small naturalised populations of both *G. hirsutum* and *G. barbadense* occur in parts of northern Australia, particularly in areas associated with a prolonged supply of fresh water (Hnatiuk 1990)(data from Australian State herbaria), but these ‘feral’ populations do not appear to be derived from modern cotton cultivars (see Section 1.7). Data provided by the applicant indicate that under appropriate environmental circumstances, namely where plants have an adequate supply of fresh water and are protected from fire, cotton can persist in northern Australia for decades, and may spread widely without active human intervention.

Although the Queensland herbarium also has specimens of *G. hirsutum* collected from plants that naturalised in Queensland, the majority of feral *G. hirsutum* populations occur in the Northern Territory and northern Western Australia. Notes associated with herbarium specimens suggest that they are restricted to coastal and sub-coastal habitats, or to other environments in which there may be a prolonged supply of fresh water.

Naturalised *G. barbadense* is restricted to Queensland and records from the Queensland herbarium confirm that a total of 28 specimens of this species have been collected. These specimens were collected from most of the eastern botanical regions of Queensland, from Cape York to Moreton Bay. 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.

1.6.2 Taxonomy and distribution of native Australian cotton species

The Australian flora contains 17 native *Gossypium* species that are all members of a distinct group found exclusively in Australia — *Gossypium* subgenus *Sturtia*. They are distant relatives of the cultivated cottons that originated in the Americas (Fryxell 1979b; Fryxell 1992; Seelanan et al. 1999) (Brubaker et al. 1999a; Brubaker et al. 1999b). The Australian *Gossypium* species are all diploid ($2n = 26$) and fall within the three taxonomic sections of the subgenus *Sturtia*, as summarised in Table 1: Section *Sturtia* (C-genome; two species including Sturt’s desert rose, *G. sturtianum*, the floral emblem of the Northern Territory); Section *Hibiscoidea* (G-genome; three species) and Section *Grandicalyx* (K-genome; 12 species).

The centre of *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Including *G. robinsonii*, which is indigenous to the Port Headland area of Western Australia, and *G. rotundifolium*, which occurs in the Broome region, 13 of Australia’s 17 *Gossypium* species occur in this northern region. Of the remaining four species, *G. sturtianum* is the most widely distributed, occurring from Port Headland in Western Australia, through central Australia to the commercial

cotton fields of eastern Australia. *Gossypium sturtianum* also occurs in southern parts of South Australia. 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 Northern Territory to Katherine, in the north of the Northern Territory. Finally, *G. bickii* occurs largely within central Northern Territory, while *G. nelsonii* is distributed in a band from central Northern Territory to central Queensland.

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

1.7 WEEDINESS OF COTTON

Cotton has been grown for centuries throughout the world without any reports that it is a serious weed pest. 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).

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

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

As discussed in Section 1.4, cotton volunteers occur in all Australian cotton growing areas and are relatively common where cotton seed is used as livestock feed. There is no indication, however, that these volunteers sponsor self-perpetuating feral populations. Typically, however, such volunteers are killed by roadside management practices and/or grazed by livestock, thereby limiting their potential to reproduce and become weedy. Also, the relatively low soil moisture of uncultivated habitats probably limits the germination and growth of volunteers.

In northern Australia, cotton volunteers have been observed in areas that have not been cultivated for cotton in many years (Williams, 2001). 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.

1.8 TOXICITY, ALLERGENICITY AND PATHOGENICITY OF COTTON

Cotton is not a pathogen and not capable of causing disease in humans, animals or plants. Cotton pollen is not allergenic. Because it is relatively large and heavy, and is not easily dispersed by wind, the potential for cotton pollen to act as an air born allergen is particularly low. However, inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition.

1.8.1 Seeds

Cotton tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the presence of anti-nutritional and toxic factors including gossypol and cyclopropanoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). Cotton seed is processed into four major products: oil, meal, hulls and linters. After extensive processing to remove toxicants, especially gossypol and its derivatives, the oil and linters are used as premium vegetable oils and as cellulose dietary additives for human consumption, respectively.

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. Human consumption of cotton seed meal is reported mainly in central American countries and India where it is used as a low cost, high quality protein ingredient (Franck 1989; Ensminger et al. 1990).

The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits its use as a protein supplement in animal feed, except for cattle which are unaffected by these components because they are detoxified by digestion in the rumen. Its use as stockfeed is limited, however, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects. Inactivation or removal of gossypol and cyclopropenoid fatty acids during processing enables the use of some cotton seed meal for catfish, poultry and swine.

In the field, the large amounts of fibre present on cotton seed coats deters potential avian seed feeders. Mammals avoid feeding on cotton plants because of both the gossypol content and the morphology of the plant.

1.8.2 Fibre

Cotton lint contains no detectable nitrogen, and hence no DNA or protein (Leffler & Tubertini 1976). The refining and processing of cotton lint (and of cotton seed oil and cotton linters), both chemically and thermally, destroys or removes proteins and nucleic acids to below detectable levels (Sims et al. 1996; Sims & Berberich 1996a; Sims & Berberich 1996b). Processed cotton fibre contains 99.8% cellulose (AgraFood Biotech 2000) and is widely used in pharmaceutical and medical applications because of its very low allergenicity.

2 POTENTIAL FOR GENE TRANSFER FROM COTTON TO OTHER ORGANISMS

The possibility of genes transferring from *G. hirsutum* to other organisms is addressed below. Potentially, genes could be transferred to: (1) cultivated cotton species, including feral populations, (2) native Australian *Gossypium* species, (3) other plant genera, and (4) other organisms. With particular regard to the possibility of gene transfer to other plants (including other cotton plants), each of two potential barriers 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).

2.1 GENE TRANSFER TO CULTIVATED AND FERAL COTTON

Cross-pollination of one *G. hirsutum* plant to another mediated via an insect pollen vector is the most likely means by which cotton genes could be dispersed in the environment. In Australia, gene transfer between adjacent *G. hirsutum* individuals occurs, albeit at relatively low frequencies. For example, as noted in section 2.3.3.1,

Llewellyn and Fitt (Llewellyn & Fitt 1996) estimated that cross-pollination between cotton plants in adjacent rows accounted for only 1 to 2% of seeds.

Fertile progeny are also produced when *G. hirsutum* is cross-pollinated with *G. barbadense* (Brubaker et al. 1999a), thereby potentially providing another ready means by which *G. hirsutum* genes may be spread in the environment. The geographic isolation of naturalised *G. barbadense* from cultivated *G. hirsutum* poses a significant barrier to gene flow between these species in Australia.

Gene flow from cultivated *G. hirsutum* to 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. 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 Northern Territory, particularly potential 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 outcrossing to feral cotton populations. If cotton volunteers establish in areas adjacent to existing feral populations, such as may occur along certain transportation routes, the potential for spread of the transgenes to these feral populations could increase.

2.2 GENE TRANSFER TO AUSTRALIAN *GOSSYPIUM* SPECIES

Of the Australian *Gossypium* species, only four are likely to occur in the existing or potential cotton growing regions and, therefore, are likely to be exposed to *G. hirsutum* pollen. *G. sturtianum* is likely to occur in all commercial cotton growing regions of eastern Australia. *Gossypium rotundifolium* and *G. australe* are the only species whose distribution overlaps potential cotton growing areas in north-western Australia and the Northern Territory, whereas *G. australe* and *G. nelsonii* are the only natives likely to occur in the potential cotton growing area of Richmond, Queensland.

Despite potential co-occurrence of Australian *Gossypium* species and *G. hirsutum*, the native species are found rarely on the heavy clay soils of the major cotton growing regions, preferring well-drained sandy loams. However, at least one population of *G. australe* has been observed within 50 m of cotton plantations near Richmond, Queensland. Also, at Broome, where *G. rotundifolium* is known to occur, cotton may be grown on the same soil type preferred by native *Gossypium*.

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 be pollinated by roadside *G. hirsutum* volunteers. Clearly, however, such potential cross-pollination would depend on chance spillages in areas where native populations occur, and on the possibility of the spilt seed germinating, surviving to reproductive maturity, flowering

synchronously with the native species, and competing for pollination with the predominately self-pollinating native cotton.

Even if these conditions were met, the likelihood of gene transfer from one species to the other is extremely low due to genetic incompatibility, since cultivated cotton is tetraploid (AD-genome) and the Australian *Gossypium* species are diploids (C, G or K genomes) (see 2.2.1 and 2.2.2 below). The likelihood of fertile hybrids occurring, surviving to reproductive maturity and back-crossing to the parental native is, therefore, effectively zero.

2.2.1 Cross-pollination with G- and K-genome natives

Several publications discuss extensive experimental efforts to hybridise *G. hirsutum* with the Australian *Gossypium* species (Brown et al. 1997; Zhang & Stewart 1997; Brubaker et al. 1999b; Brubaker & 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 1. 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 1), 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.

2.2.2 Cross-pollination with C-genome natives

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

Table 1. Summary of attempts to generate hybrid seeds between cultivated cotton (*G. hirsutum*) and native Australian species of *Gossypium*, following hand-pollination. Pollinations representing the greatest potential environmental risk, namely with *G. hirsutum* as the pollen donor, are presented in bold, with the reciprocal pollination presented immediately following.

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

<i>G. hirsutum</i> *	<i>G. exiguum</i>	4 (11)	8 (61)
<i>G. londonderriense</i>	<i>G. hirsutum</i>	ND	ND
<i>G. hirsutum</i> *	<i>G. londonderriense</i>	11 (25)	1 (26)
<i>G. marchantii</i>	<i>G. hirsutum</i>	ND	ND
<i>G. hirsutum</i> *	<i>G. marchantii</i>	17 (23)	0 (72)
<i>G. nobile</i> †	<i>G. hirsutum</i>	0 (14)	0
<i>G. hirsutum</i> *	<i>G. nobile</i>	24 (36)	15 (86)
<i>G. pilosum</i> †	<i>G. hirsutum</i>	0 (6)	0
<i>G. hirsutum</i>	<i>G. pilosum</i>	17 (24)	35 (88)
<i>G. populifolium</i>	<i>G. hirsutum</i>	ND	ND
<i>G. hirsutum</i> *	<i>G. populifolium</i>	14 (40)	18 (65)
<i>G. pulchellum</i>	<i>G. hirsutum</i>	ND	ND
<i>G. hirsutum</i> *	<i>G. pulchellum</i>	7 (16)	1 (15)
<i>G. rotundifolium</i> *	<i>G. hirsutum</i>	0 (57)	0
<i>G. hirsutum</i> *	<i>G. rotundifolium</i>	11 (15)	12 (52)

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

Recently, Brubaker (pers. comm.) observed three individual plants produced following hybridisation of *G. sturtianum* and *G. hirsutum* in the field. These hybrids were produced ‘naturally’, without the application of plant hormones. Genetic analysis confirmed that the hybrids were sterile triploids, probably produced with *G. sturtianum* as the pollen donor, and with a primitive *G. hirsutum* cultivar as the pollen recipient. Although the hybrids were produced without human intervention, it should be noted that both parent species were planted horticulturally, within close proximity of each other, and that *G. hirsutum* is not normally cultivated in the area, either horticulturally or commercially. As with the glasshouse-generated *G. sturtianum* x *G. hirsutum* hybrids, each of these field hybrids were functionally sterile, aborting their flowers before fruit set, thereby eliminating the potential for gene flow.

2.3 GENE TRANSFER TO OTHER PLANTS

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.

2.4 GENE TRANSFER TO OTHER ORGANISMS

Horizontal gene transfer from plants to animals (including humans) or microorganisms is extremely unlikely:

2.4.1 Transfer of genes to humans or other animals

No evidence has been identified for any mechanism by which cotton genes could be transferred to humans or animals, nor any evidence such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. The likelihood of cotton genes transferring to humans and other animals is, therefore, effectively zero.

2.4.2 Transfer of genes to microorganisms

Gene transfer from cotton, or any other plant, to microorganisms is extremely unlikely. Horizontal gene transfer from plants to bacteria has not been demonstrated experimentally under natural conditions (Nielsen et al. 1997; Nielsen et al. 1998; Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (see for example (Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences under conditions of selective pressure (Mercer et al. 1999), and even then only at a very low frequency. Phylogenetic comparison of the sequences of plant and bacterial genes suggests that horizontal gene transfer from plants to bacteria during evolutionary history has been extremely rare, if occurring at all (Nielsen et al. 1998; Doolittle 1999).

The transfer of a gene from a plant to bacteria in the human gut would require a series of steps, each of which has a very low probability (Pittard 1997). An intact copy of the gene would need to:

- survive degradation during processing of food in the gut, and by acid and nucleases in the stomach and intestines;
- be taken up by a bacterium;
- survive efficient bacterial defence mechanisms for degrading foreign DNA; and
- become stably integrated into the bacterial genome or on a plasmid, in precise alignment with a bacterial promoter (if this were not co-transferred, intact, from the plant).

Finally, there would need to be selective pressure for bacteria expressing the gene to persist and multiply in the gut or the environment.

There is also a theoretical possibility of recombination between sequences that have been introduced into the genome of genetically modified cotton and the genome of viruses that might infect the cotton plants (Ho et al. 2000; Hodgson 2000a; Hodgson 2000b). However, recombination between viral sequences and plant transgenes has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, e.g. regeneration of infectious virus by complementation of a defective virus, containing a deletion mutation in its coat protein, by sequences transcribed from a viral coat gene introduced into a transgenic plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999).

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