The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia

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PREAMBLE

This document addresses the biology and ecology of *Carica papaya* L. with particular reference to its growth and cultivation in Australia. Included is the origin of *C. papaya* (referred to as papaya, or paw paw), general descriptions of its growth and agronomy, its reproductive biology, toxicity and allergenicity and its general ecology. This document also addresses the potential for papaya to outcross via pollen transfer.

1. BIOLOGY OF PAPAYA

SECTION 1.1 TAXONOMY, ORIGIN AND DISTRIBUTION

1. Cultivated papaya, *Carica papaya* L., sometimes known as paw paw (or papaw), is a fast-growing tree-like herbaceous plant in the family Caricaceae. In Australia, red and pink-fleshed cultivars are often known as 'papaya' to distinguish them from the yellow-fleshed fruits, known as 'paw paw', but both of these common names refer to the same plant species. Irrespective of its flesh colour, *C. papaya* is generally known as 'papaya' in other countries. In some areas, an unrelated plant, *Asiminia triloba* (Annonaceae), native to north America, is also called paw paw.

2. Until recently, the Caricaceae was thought to comprise 31 species in three genera (namely *Carica*, *Jacaratia* and *Jarilla*) from tropical America and one genus, *Cylicomorpha*, from equatorial Africa (Nakasone & Paull 1998). However, a recent taxonomic revision proposed that some species formerly assigned to *Carica* were more appropriately classified in the genus *Vasconcella* (Badillo 2002). Accordingly, the family's classification has been revised to comprise *Cylicomorpha* and five South and Central American genera (*Carica*, *Jacaratia*, *Jarilla, Horovitzia* and *Vasconcella*) (Badillo 1971), with *Carica papaya* the only species within the genus *Carica* (Badillo 2002).

3. Although opinions differ on the origin of *C. papaya* in tropical America (see Garrett 1995), it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama (Nakasone & Paull 1998). Its seeds were distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa (Villegas 1997).

4. Papaya is now grown in all tropical countries and many sub-tropical regions of the world. It was deliberately introduced to Australia more than a century ago as a horticultural crop for fruit production (Garrett 1995)

SECTION 1.2 USES OF PAPAYA

1. Economically, *Carica papaya* is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams candies and as dried and crystallised fruit (Villegas 1997). Green fruit and the leaves and flowers may also be used as a cooked vegetable (Watson 1997). Nutritionally, papaya is a good source of calcium and an excellent source of vitamins A and C (Nakasone & Paull 1998). The vitamin A and C content of one medium papaya approaches or exceeds USDA minimum daily requirements for adults (see OECD 2003). The fruit of some species of *Vasconcella* may be used as a food source, particularly in some regions of South and central America, but such usage is relatively limited.

2. Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications (El Moussaoui et al. 2001). Of these, however, papain, is a particularly important proteolytic enzyme that is produced in the milky latex of green, unripe papaya fruits (note that ripe papaya fruit contain no latex or papain). The latex is harvested by scarifying the green skin to induce latex flow, which is allowed to dry before collection for processing (Nakasone & Paull 1998). Evolutionarily, papain may be associated with protection from frugivorous predators and herbivores (El Moussaoui et al. 2001). Commercially, however, papain has varied industrial uses in the beverage, food and pharmaceutical industries including in the production of chewing gums, in chill-proofing beer, tenderising meat, drug preparations for various digestive ailments and the treatment of gangrenous wounds. Papain has also been used in the textiles industry, for degumming silk and for softening wool (Villegas 1997) and in the cosmetics industry, in soaps and shampoo.

SECTION 1.3 MORPHOLOGY OF *C. PAPAYA*

3. *Carica papaya* is a soft-wooded perennial plant that lives for about 5-10 years, although commercial plantations are usually replanted sooner (Chay-Prove et al. 2000). Papayas normally grow as single-stemmed trees with a crown of large palmate leaves emerging from the apex of the trunk, but trees may become multi-stemmed when damaged (Villegas 1997). The soft, hollow, cylindrical trunk ranges from 30 cm diameter at the base to about 5 cm diameter at the crown. Under optimal conditions, trees can reach 8-10 metres in height but in cultivation, they are usually destroyed when they reach heights that make harvesting of fruit difficult (Villegas 1997). Cultivated trees in Australia are usually replaced before exceeding 4 m in height.

4. Papaya flowers are born on inflorescences which appear in the axils of the leaves. Female flowers are held close against the stem as single flowers or in clusters of 2-3 (Chay-Prove et al. 2000). Male flowers are smaller and more numerous and are born on 60-90 cm long pendulous inflorescences (Nakasone & Paull 1998). Bisexual flowers are intermediate between the two unisexual forms (Nakasone & Paull 1998). The functional gender of flowers can be altered or reversed, depending on environmental conditions, particularly temperature (see Section 1.5.2).

5. Fruit are ready to harvest five to six months after flowering, which occurs five to eight months after seed germination (Chay-Prove et al. 2000). The fruits range in size from 7-30 cm long and vary in mass from about 250 to 3000g (OECD 2003). Fruit from female trees are spherical whereas the shape of fruit from bisexual trees is affected by environmental factors, particularly temperature, that modify floral morphology during early development of the inflorescence (Nakasone & Paull 1998)(see Section 1.5.2, below).

6. Ripe papaya fruit have smooth, thin yellow-orange coloured skin. Depending on the cultivar, flesh thickness varies from 1.5 to 4 cm (Nakasone & Paull 1998) and flesh colour may be pale yellowish to red (Villegas 1997; Nakasone & Paull 1998). Mature fruits contain numerous grey-black spherical seeds 5 mm in diameter (Villegas 1997).

SECTION 1.4 CULTIVATION

1. Commercial Australian plantings of *C. papaya* occur throughout coastal tropical and sub-tropical regions of New South Wales, Queensland and Western Australia, with Queensland accounting for >95% of production. In Queensland, major production areas include Innisfail in the north, Yarwun and Yeppoon in central Queensland and Gympie and the Sunshine Coast in south Queensland (Garrett 1995).

2. In the absence of irrigation, fruit production is optimal in areas with a minimum monthly rainfall of about 100 mm, minimum relative humidity of 66% (Nakasone & Paull 1998) and where temperatures range between 21 and 33º C (Villegas 1997; Nakasone & Paull 1998; OECD 2003). Temperatures below 12-14º C strongly retard fruit maturation and adversely affect fruit production (Nakasone & Paull 1998). Papaya is extremely sensitive to frost, which can kill the plant.

3. Broadly, there are two distinct types of *C. papaya* plants: dioecious and gynodioecious. Dioecious papayas have male and female flowers on separate trees. Gynodioecious papayas bare female flowers on some trees and bisexual (hermaphrodite) flowers on others. Occasionally, papaya variety Solo may be functionally andromonoecious (male and hermaphrodite flowers on the same plant), with a terminal bisexual flower subtended by a few staminate axillary flowers (OECD 2003). Such changes in floral biology are likely to occur in response to hot and dry conditions, which are known to affect functional gender in papayas (see Section 1.5.2).

4. Both dioecious and gynodioecious varieties of papaya are grown commercially in Australia, but dioecious varieties are generally recommended because they have high fruit yields and relatively predictable fruit shape (Drew et al. 1998; Chay-Prove et al. 2000). In gynodioecious varieties, fruit production on hermaphrodite trees may be affected by air temperatures (see Section 1.5.2) leading to decreased yields and/or poorly shaped fruit (OECD 2003). Accordingly, these lines are only recommended for coastal north Queensland and the Atherton Tableland, where the relatively predictable tropical climate does not affect fruit shape unduly.

5. Australian papaya plantations are established by sowing seeds which may be pre-germinated, or by transplanting established (150 – 200 mm tall) seedlings to the plantation site. As the sex of plants can be difficult to determine before they start flowering (but see Section 1.5.2), 3-4 plants are established in each planting site within the plantation to ensure that the optimum ratio of sex types is achieved. When the sex can be determined, plants are thinned to achieve the desired sex ratio and to reduce competition between plants, which would affect fruit production. For dioecious varieties, a ratio of one male to 8-10 female plants is recommended to maximise yield (Nakasone & Paull 1998; Chay-Prove et al. 2000) whereas for bisexual varieties, the aim of thinning is to have one bisexual plant in each planting position.

SECTION 1.5 REPRODUCTION

1. Fruit production in papaya plants may occur following either cross-pollination (out-crossing), self-pollination or parthenocarpy (a form of asexual reproduction in which fruits may be produced without fertilisation), depending on whether dioecious or gynodioecious lines are planted and the particular cultivar that is grown (Rodriquez-Pastor et al. 1990; Nakasone & Paull 1998; Louw 2000; OECD 2003). Rodriguez-Pastor et al. (1990) demonstrated that when out-crossing is limited experimentally by bagging flowers to prevent pollen flow, 90% and 94.7% of fruit from hermaphrodite flowers of ‘Sunrise Solo’ and ‘Kapoho Solo’, respectively, may be produced following either self-pollination or parthenocarpy. Of these, potentially 35% of the Sunrise Solo fruit were produced parthenocarpically and *ca* 65% were produced following self-pollination. Kapoho Solo produced no parthenocarpic fruit (Rodriquez-Pastor et al. 1990). Parthenocarpic fruit were reported to be of adequate commercial size and quality.

2. Garrett (1995) also investigated the potential for alternative papaya lines to produce fruit parthenocarpically. She demonstrated that such fruit may be produced at much lower frequencies and are of poor size and quality, compared to sexually derived fruit. Garrett (1995) also indicated that low numbers of seeds are produced by parthenocarpic fruit and that = *ca* 4% of such seeds may be viable, depending on the variety of papaya. It is not clear whether these seeds were produced sexually (perhaps by inadvertent contamination with pollen) or asexually. Nevertheless, many researchers (eg. Gillaspy et al. 1993; Vivian-Smith et al. 2001) contend that, by definition, parthenocarpic fruit contain no viable seeds.

1.5.1 Pollination

Pollen production and stigmatic receptivity

1. In Australia, seasonally variable papaya fruit production (in terms of both quantity and quality) have been attributed to several factors including low pollen viability and an absence of suitable pollinators.

2. Garrett (1995) observed that pollen production by papaya trees varies seasonally and according to the variety of plant that is cultivated, but a general trend is for decreased quantities of pollen during winter/early spring (Garrett 1995). The viability of pollen that is produced also varies seasonally and according to variety (Magdalita et al. 1998). Garrett (1995) determined that, on average, 90% of freshly dispersed pollen grains were viable but that in winter, viability dropped to about 45% in some lines and as low as 4.5% in others. No information was provided on the duration of pollen viability, but Allan (1963) indicates that extremes of humidity reduce the storage life of papaya pollen which, under ideal (artificial) storage conditions, potentially remains viable for about 5-6 years.

3. Garrett's (1995) observations of seasonal variation in pollen viability are broadly consistent with similar studies conducted in South Africa (Allan 1963) and Israel (Cohen et al. 1989), where it was concluded that minimum temperatures below 10º C significantly affect pollen viability, possibly as a consequence of degenerated pollen mother cells (Allan 1963).

4. In contrast to pollen production and viability, the receptivity of papaya stigmas remains high throughout the year. If pollinated with viable pollen, both female and hermaphrodite flowers can successfully produce fruit, even in winter (Garrett 1995).

5. Garrett (1995) provides no data regarding the numbers of flowers that are produced in each season (either female, male or hermaphrodite). It is difficult, therefore, to determine the relative importance of variable pollen viability and likely seasonal variations in the number of actual flowers, particularly female flowers, in affecting total fruit production.

Pollinator identity and behaviour

1. Pollinator efficiency or abundance may also affect fruit production. Despite floral morphology suggesting insect pollination, several authors have indicated that wind pollination may also be important (Nakasone & Paull 1998; OECD 2003).

2. In a detailed series of experiments, Garrett (1995) demonstrated that pollination by native and European honeybees, or by wind, is rare. Rather, Garrett (1995) determined that hawkmoths (Lepidoptera: Sphingidae: Macroglossinae), many in the genus *Hyles,* are the primary pollinators of papaya in central Queensland. Further, it was observed that seven hawkmoth species pollinate papaya flowers and that four additional species are likely to be pollinators (Garrett 1995). Of these eleven species, eight were active at dusk (18:00 – 19:00); the remaining three were active during the day. Usually, moths spent about three seconds at each flower before moving to another. Foraging focussed on groups of up to three trees, with each tree in the group being visited in turn, before flying to another part of the orchard. This behaviour did not appear to be affected by wind direction (Garrett 1995). Total foraging time and the distance between groups of trees visited by pollinating moths were not recorded.

1.5.2 Plant gender

3. Papaya flowers can be grouped into three basic forms that reflect whole plant gender: female, male or bisexual (hermaphrodite). With controlled cross-pollinations between flowers of each gender, the ratio of female, hermaphrodite and male offspring are predictable, as summarised in Table 1.

Table 1: Summary of gender ratios following pollinations between male (M), female (F) and bisexual (B) *C. papaya* gender forms*

Pollination	No. of resulting Females	No. of resulting Males	No. of resulting Bisexuals
F x M	1	1	-
F x B	1	-	1
M x M†	1	2	-
1. B x B	1	-	2
B x M	1	1	1
M x B	1	1	1

* summarised from Storey (1976), cited in Garrett (1995);

† note that because of gender reversals or alterations, 'males' may bare functional carpels and set fruit.

1. The genetic or chromosomal basis for the sex ratio of papayas is poorly understood (Villegas 1997; OECD 2003). However, as summarised by Somsri et al. (1998), a common hypothesis is that sex is controlled by a single locus with three alleles — M1 (male), M2 (hermaphrodite) and m (female). Male (M1m) and hermaphrodite plants (M2m) are heterozygous whereas female plants (mm) are homozygous recessive. Combinations of dominants, namely M1M1, M1M2, or M2M2 are lethal, leading to post-zygotic abortion of such ovules. Accordingly, this hypothesis predicts that viable males can only be M1m and viable hermaphrodites cane only be M2m.

2. Considerable effort has been invested recently in developing molecular tests to determine the sex of papaya seedlings (Somsri et al. 1998). These would be advantageous commercially, as plant sex could be determined before reproductive maturity. Male-specific (Parasnis et al. 2000) and hermaphrodite-specific (Lemos et al. 2002) tests have been developed using a variety of molecular techniques. Uraski et al (2002) have also developed a molecular test to distinguish male and hermaphrodite plants from females.

3. Efforts to identify molecular markers for sex are complicated by environmental variables including temperature, humidity, soil nutrients that may modify the functional gender of plants (OECD 2003). In hot (>35 ºC) and dry conditions, for example, bisexual flowers may become functionally male, with poorly developed and non-functional female parts (Watson 1997; Nakasone & Paull 1998). At low (<20º C) temperatures, by contrast, bisexual flowers may become functionally female because of carpelloidy, a condition in which the stamens resemble carpels but remain associated with the developed fruit, leading to distorted fruit shape (OECD 2003). Bisexual flowers of variety 'Solo' may produce 100% carpellodic flowers when minimum temperatures are less than 17º C (Nakasone & Paull 1998). Such changes in functional gender can be either temporary or permanent.

4. Changes in functional gender in response to environmental variables have been used advantageously in papaya breeding programs and to help select the most appropriate varieties for commercial cultivation in particular regions (Chay-Prove et al. 2000; OECD 2003).

SECTION 1.6 PESTS AND DISEASES

1. Singh (1990) reports that about four insect and mite species are major pests of papaya, although 35 other arthropods may infest the plants. In Australia, fruit-spotting bugs (*Amblypelta lutescens* and *A. nitida*), oriental scale (*Aonidiella orientalis*) and yellow peach moth *(Conogethes punctiferalis*) are among the most serious insect pests, each with potential to reduce plant growth and productivity and to damage ripening fruit (Chay-Prove et al. 2000). Among mites, two-spotted mite (*Tetranychus urticae*) and, in particular, broad mite (*Polyphagotarsonemus latus*) are the most serious pests (Chay-Prove et al. 2000). Flying foxes (*Poliocephalus* spp.) are the chief mammalian predator of papaya fruit in Australia.

2. More important than mite and insect pests are pathogens that reduce plant vigour and affect fruit quality (OECD 2003). In Australia, the major fungal pathogens of papaya include phytophthora root and fruit rot (*Phytophthora palmivora*), black spot (*Asperisporium caricae*), brown spot (*Corynespora cassiicola*), anthracnose (*Colletotrichum* spp.) and powdery mildew (*Sphaerotheca* spp.).

3. Yellow crinkle and dieback are both diseases of papaya caused by phytoplasma (OECD 2003). Padovan and Gibb (2001) indicated that these diseases are caused by two different groups of phytoplasma strains.

4. Papaya ring-spot virus (PRSV; Potyviridae) has become the limiting factor for commercial papaya production in many areas of the world (Nakasone & Paull 1998). Internationally, PRSV has significantly reduced crop productivity in Hawaii, the Carribean, Brazil, south-east Asia and other papaya growing areas (OECD 2003). Early symptoms include yellowing and vein clearing in young leaves and sometimes severe blistering and leaf distortion. Dark concentric rings and spots or "C"-shaped markings develop on the fruit which may turn tan-brown as the fruit ripens.

5. Papaya ringspot virus was first identified in Australia in 1991 in suburban Brisbane and has subsequently been recorded in Bundaberg and Beaudesert (Chay-Prove et al. 2000). PRSV is transmitted by aphids, mechanical transmission of sap and the movement of infected plants (Chay-Prove et al. 2000). Its spread is being managed by a quarantine zone, which limits movement of papaya and cucurbits (eg. cucumber, pumpkin and watermelon), which may also host PRSV, from south-east Queensland to other papaya growing districts (Chay-Prove et al. 2000).

6. PRSV remains restricted in distribution in Queensland. Several outbreaks have occurred in south-east Queensland, but these were contained by removing and destroying infected plants. The virus is relatively common in backyards of some northern suburbs of Brisbane, but the rate of spread appears to be low (Persley 2003).

7. Genetically modified PRSV-resistant papaya was developed in response to the devastating impacts of the disease, particularly in Hawaii and south-east Asia. Papaya plants that resist the virus by expressing PRSV coat protein were successfully developed in Hawaii (Ferreira et al. 2002) and their production rapidly reversed the declining Hawaiian papaya industry (Manshardt 1999). GM PRSV-resistant papaya have been available commercially in the USA since 1997 and in Canada since January 10, 2003 (see http://www.agbios.com/main.php).

8. PRSV-resistant GM papayas are not available in Australia.

2. TOXICITY AND ALLERGENICITY OF PAPAYA

1. *Carica papaya* belongs to a group of plant species known as laticiferous plants. These plants contain specialised cells (laticifers), dispersed throughout most plant tissues, that secrete a substance known as ‘latex’. Latex is a complex mixture of chemical compounds with diverse chemical activities. Collectively, these compounds are thought to be involved in defence of the plant against a wide range of pests and herbivores (El Moussaoui et al. 2001).

2. The latex of papaya plants is rich in enzymes known cysteine proteinases, which are used widely for protein digestion functions in the food and pharmaceutical industries. Commercially, papaya latex is harvested from fully grown but unripe fruit, the skin of which contains numerous laticifers. Ripe papaya fruit contains no latex (Villegas 1997), possibly because the latex-producing cells cease functioning or breakdown with age.

3. Cysteine proteinases may constitute as much as 80% of the enzyme fraction in papaya latex (El Moussaoui et al. 2001). The most well studied proteinases from papaya are papain, chymopapain, caricain and glycyl endopeptidase. Other enzymes known from papaya latex include glycosyl hydrolases such as β-1,3-glucanases, chitinases and lysozymes, protease inhibitors such as cystatin and glutaminyl cyclotransferases and lipases (El Moussaoui et al. 2001). Potential roles in defence against pathogenic microorganisms or against herbivory are likely for most of the enzymes isolated from papaya latex to date.

4. Unripe papaya fruit, papaya seeds and latex extracts have been implicated in numerous toxic and allergenic responses in mammals, including humans, as discussed in sections 2.1 and 2.2, below.

SECTION 2.1 INHERENT TOXICITY OF PAPAYA

1. The use of papaya leaf, fruit and root extracts as traditional medicines (Akah et al. 1997; Eno et al. 2000) and the complex, largely uncharacterised, chemical composition of papaya latex, suggests the potential for uncharacterised effects on the health of humans or other organisms. A compound present in crushed papaya seed that is believed to have activity against helminthic intestinal parasites, benzyl isothiocyanate (BITC), has been shown to have an effect on vascular contraction using a canine carotid artery in vitro model (Wilson et al. 2002). Other studies have suggested possible purgative effects of root extracts (Akah et al. 1997) and antihypertensive activity of fruit extracts (Eno et al. 2000). The presence of cyanogenic compounds in papaya has also been reported (Seigler et al. 2002).

2.1.1 Impacts on mammalian reproduction

Impacts on men and other male mammals

2. The antifertility properties of papaya, particularly of the seeds, have been the subject of significant evaluation using animal models, especially in India where there is interest in the development of a safe and effective oral male contraceptive (Lohiya et al. 1999). A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds (Lohiya et al. 1999; Pathak et al. 2000; Lohiya et al. 2002), suggesting that ingestion of papaya seeds may adversely affect the fertility of human males or other male mammals.

Impacts on women and other female mammals

3. In India and parts of south-east Asia and Indonesia, consumption of papaya fruit is widely believed to be harmful during pregnancy, since papaya is believed to have abortifacient properties (induces miscarriage during pregnancy) or teratogenic properties (causes malformations of the foetus) (Adebiyi et al. 2002). For example, Adebiyi et al. (2002) suggest that unripe papaya fruit may induce miscarriage in susceptible pregnant human females. Conversely, a papaya fruit extract is used for prevention of miscarriage by traditional African healers (Eno et al. 2000). A number of early studies, largely conducted in India, suggested that unripe papaya fruit, latex extracts or papaya seeds have deleterious effects on pregnancy in laboratory animals (Schmidt 1995). However, more recent analysis suggests that ripe papaya fruit or purified papain do not cause malformations of rat foetuses.

4. Ingestion of unprocessed ripe papaya fruit has no impact on the number of viable foetuses or foetal weight in rats (Adebiyi et al. 2002). Likewise, purified papain derived from latex of unripe papaya did not impact adversely on prenatal development when administered orally to pregnant rats (Schmidt 1995). However, in vitro, crude latex derived from unripe papaya fruit stimulates contractions in non-pregnant rat uterus (Adebiyi et al. 2002).

SECTION 2.2 INHERENT ALLERGENICITY OF PAPAYA

1. Papain, a product of papaya latex, is widely used in both the food manufacturing and pharmaceutical industries. Sensitisation to papain among workers in these industries is well known (Baur et al. 1988; Iliev & Elsner 1997). Immunoglobin E (IgE) antibodies against all four of the major papaya cysteine proteinases in latex (papain, chymopapain, caricain and glycyl endopeptidase) have been identified in people who show an allergic response to a pharmaceutical product derived from papaya latex (Dando et al. 1995). The presence of these antibodies demonstrates that all four cysteine proteinases are allergenic.

2. Papaya may also have allergenic properties when ingested. For example, an allergic reaction, manifesting as a skin rash, has been reported following use of throat lozenges containing papaya, which appeared to be due to the papaya extract contained in the lozenges (Iliev & Elsner 1997). An extreme allergic reaction to skin contact with unprocessed papaya fruit has also been reported (Ezeoke 1985).

3. WEEDINESS OF PAPAYA

3. There have been numerous attempts to characterise the ‘typical’ traits of weeds (Baker 1965; Bazzaz 1986; Noble 1989; Williamson & Fitter 1996; Roy 2002), but the most successful predictors of weediness remain taxonomic affinity to other weedy species and the history of a given species’ weediness elsewhere in the world (Panetta 1993; Pheloung et al. 1999).

SECTION 3.1 WEEDINESS IN AUSTRALIA

4. In Australia, *Carica papaya* is not considered to be a problematic weed of either agriculture (Groves et al. 2002) or of the natural environment (Groves et al. 2000). However, naturalised (feral) populations of *C. papaya* have been identified from nine locations in coastal Queensland (Australia’s Virtual Herbarium 2003), ranging from the State’s far north-east to south-east. Herbarium records and associated specimen notes indicate that in Queensland, *C. papaya* may form small, low-density self-perpetuating populations (data provided by the Queensland Herbarium).

5. No other papaya (*Vasconcella*) species have naturalised in Australia (Groves et al. 2000; Groves et al. 2002).

SECTION 3.2 WEEDINESS IN OTHER REGIONS

6. Generally, papaya is not considered to be a problematic weed of any region of the world (OECD 2003). Nevertheless, *C. papaya* has naturalised in many tropical and sub-tropical countries (Randall 2002). Space et al. (2000) have suggested that in Rota, of the Mariana Islands, papaya may be invasive in highly disturbed habitats. Similarly, Kwit et al. (2000) observed the establishment of exotic *C. papaya* seedlings following disturbance of forest canopies by a hurricane.

7. Internationally, the small shrub, *Vasconcella pubescens* (formerly *Carica pubescens*; 'mountain papaya'), is the only relative of papaya that has been recorded as a weed (Randall 2002). Mountain papaya has naturalised in parts of New Zealand (Allan Herbarium 2000) and is considered to be 'moderately invasive' in some tropical areas (Bingelli et al. 1999; Randall 2002).

8. *Vasconcella pubescens* does not occur in Australia (Groves et al. 2000; Groves et al. 2002; Australia's Virtual Herbarium 2003; Australian Plant Name Index 2003).

4. POTENTIAL FOR GENE TRANSFER FROM PAPAYA TO OTHER ORGANISMS

1. The possibility of genes transferring from *C. papaya* to other organisms is addressed below. *Potentially*, genes could be transferred to: (1) commercially and domestically cultivated papaya and naturalised papaya populations, (2) wild papaya (*Vasconcella*) species, (3) other plant genera, and (4) other organisms. With particular regard to the possibility of gene transfer to other plants (including other papaya 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.

SECTION 4.1 GENE TRANSFER TO CULTIVATED AND NATURALISED *C. PAPAYA*

2. Cross-pollination of one *C. papaya* plant to another mediated via an insect pollen vector is the most likely means by which papaya genes could be transferred from papaya to other organisms, including cultivated and naturalised *C. papaya*. In Australia, hawkmoths (Lepidoptera: Sphingidae) are the most likely pollen vectors (see Section 1.5.1)

3. Viable seeds and potentially fertile progeny would be produced when pollen is transferred between papaya plants, irrespective of whether the transfer occurred to cultivated or naturalised papayas. As naturalised papayas occur throughout the range of papaya cultivation (Australia's Virtual Herbarium 2003), gene transfer between naturalised and cultivated papayas is likely.

SECTION 4.2 GENE TRANSFER TO WILD PAPAYAS (*VASCONCELLA* SPP.)

4. The closest relatives of *C. papaya* and, therefore, the species with which *C. papaya* is most likely to hybridise and exchange genes are species of *Vasconcella* (formerly, *Carica*)(Badillo 2002). None of these species occurs in Australia, eliminating the likelihood of genes transferring naturally to wild papaya species.

5. Nevertheless, wild papayas (*Vasconcella* spp.) naturally posses a number of desirable traits including resistance to pathogens, cold tolerance and higher sugar content of fruit (Drew et al. 1998), that breeders have sought to introduce into *C. papaya* using traditional plant breeding techniques. Difficulties in producing hybrids between *C. papaya* and *Vasconcella* spp. underscore the negligible risk of gene transfer from *C. papaya* to wild papayas in Australia. For instance, several investigations (Manshardt & Wenslaff 1989a; Manshardt & Wenslaff 1989b; Drew et al. 1998) have indicated that following pollination of *C. papaya* with pollen from wild papayas (and vice versa), pollen grains germinate on the stigma successfully and pollen tubes grow through the style and penetrate the ovules, thereby facilitating fertilisation. Subsequently, however, abortion of these ovules, or endosperm failure, prevents further development of hybrid embryos or production of viable mature seed. These post-zygotic barriers to successful hybrid formation have limited the success of traditional breeding programs (Manshardt & Drew 1998) and indicate that the generation of inter-specific hybrids is only possible with significant human intervention, including techniques such as embryo rescue (Manshardt & Drew 1998). When hybrids are generated using these techniques, they appear as morphological intermediates between the two parental species (Magdalita et al. 1997).

6. In Australia, embryo rescue was used successfully to produce hybrids between *C. papaya* and wild papaya (*Vasconcella*) species including *V. quercifolia*, *V. pubescens*, *V. goudotiana* and *V. parfiflora*. However, many hybrids were of low vigour and did not survive when transplanted to the field (Drew et al. 1998). Although other hybrids survived to reproductive maturity and a few produced viable pollen (Drew et al. 1998), the backcross progeny (derived from re-crossing to the original *C. papaya* parent) were infertile, probably because of large changes to the chromosomes (OECD 2003).

SECTION 4.3 GENE TRANSFER TO OTHER PLANTS

1. The inability of papaya to hybridise naturally with its closest relatives and the infertility of such hybrids when they are formed artificially, illustrates the reproductive isolation of *C. papaya* from other plant groups (Manshardt & Wenslaff 1989a) and indicates that the likelihood of gene transfer between *C. papaya* and other plant species is negligible.

2. 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 papaya to other plant taxa has been identified.

SECTION 4.4 GENE TRANSFER TO OTHER ORGANISMS (MICROORGANISMS AND ANIMALS, INCLDUING HUMANS)

3. The most likely means by which genes could be transferred from papaya to non-plant organisms is by horizontal gene transfer. This is extremely unlikely and has not been demonstrated under natural conditions (Nielsen et al. 1998; Nielsen et al. 1997; Syvanen 1999). Moreover, deliberate attempts to induce such transfers have so far failed (eg. Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory conditions (Gebhard & Smalla 1998; Mercer et al. 1999; Nielsen et al. 1998), but 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).

4. Another example of plant genetic material coming into contact with microorganisms is where a plant pathogen, such as a viral disease, invades a GM plant and the pathogen proliferates inside the GM plant cells. There is a theoretical possibility of recombination of the introduced genes into the genome of the viral pathogen. This has only previously been observed at very low levels between homologous sequences under conditions of selective pressure, eg. 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).

5. A more detailed review of horizontal gene transfer from plants to other organisms is provided in the risk assessment and risk management plan that was prepared in relation to DIR application 026/2002, to release GM papayas into the Australian environment.

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