The Biology & Ecology of Pineapple (*Ananas comosus* var. *comosus*) in Australia

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Office of the
Gene Technology Regulator

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1 EXECUTIVE SUMMARY

Cultivated pineapple (*Ananas comosus* (L.) Merrill, which is now called *Ananas comosus* var *comosus*) belongs to the family *Bromeliaceae.* It is grown mainly for fresh and canned fruit and juice and is the only source of bromelain, an enzyme used in pharmaceuticals and as meat-tenderising agent.

Pineapple originated in South America, was discovered by Europeans in 1493 and is now grown in various parts of the world, including Australia. In Australia, it is mainly grown in the narrow coastal strip along the eastern seaboard from Cairns in north to Brisbane in south. Small commercial fields are in the Northern territory and northern New South Wales. In Australia, pineapple is only found under cultivation and occurs as a managed cultigen.

Pineapple is a perennial monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit. Adult pineapple plants are up to 1 m high and 0.5 m wide while adult 'Smooth Cayenne' plants are up to 1.5 m high and 1 m wide. The inflorescence consists of 50-200 individual hermaphrodite flowers with tubular corolla. Flowers are normally self-sterile and fruit development is parthenocarpic.

Wind pollination is not known to occur. While there are no humming birds in Australia, honeybees, native bees (*Trigona* spp.) and ants have occasionally been observed visiting pineapple flowers, probably for the nectar and may play a secondary role in cross-pollination. However, sexual reproduction is rare in nature.

Vegetative propagation is the dominant form of reproduction by the use of vegetative shoots including the crown, slips and suckers. Commercial propagation of pineapple is not through seeds but by vegetative propagation. Except for *A. macrodontes, Ananas* sp. does not produce underground stolons. Dispersal of vegetative structures or seeds occurs only through flooding or human or animal intervention.

Pineapple seeds lack dormancy, have a very tough seed coat and a hard, flinty endosperm. Without treatment germination is slow and very irregular. Commercially, seeds are desired only in breeding programs and are usually the result of hand pollination. Although pineapple can be grown from seed, fertility in commercially grown cultivars of pineapple is very low and consequently seed production is very rare. Seeds retain germination capacity for about six months at tropical ambient temperature.

Although all *Ananas* species are found in Australia, they have a very limited distribution, with only *A. comosus* var *comosus* cultivar 'Smooth Cayenne' used mainly in commercial production. In addition, *Ananas* species are not naturalised in Australia and are not recognised as weeds in Australia.

Cultivars of pineapple are only compatible with each other if they are not closely related. However, all *Ananas* species are sexually compatible with pineapple. Although there are reports of hybrids obtained by hand-pollination, sexual reproduction is rare in nature. The frequency of natural cross-pollination depends on how closely compatible plants are planted, synchronicity of flowering and the presence of pollinators. The grower controls all of these factors to a large extent. In the unlikely event of successful pollination, the seed is contained in the fruit as commercial pineapples have no seed releasing mechanisms. In addition, the seed is not used for commercial planting.

There are a few reports of intergeneric hand-pollinated crosses between *Ananas* and some members of family *Bromeliaceae*, although there are no scientific publications or reports of these crosses occurring in nature. There have been no other reports of hybridisation between *Ananas* and other plants. Gene transfer to unrelated plant species is highly improbable as no evidence for horizontal gene transfer from pineapple to other plant taxa has been identified.

Horizontal gene transfer from plants to animals (including humans) or microorganisms is extremely unlikely. No evidence has been identified for any mechanism by which pineapple genes could be transferred to humans or animals. Horizontal gene transfer from plants to bacteria has not been demonstrated experimentally under natural conditions.

Pineapple is not a pathogen and is not capable of causing disease in humans, animals or plants. Pineapple pollen is relatively sticky and not easily dispersed by wind, and therefore the potential for pineapple pollen to act as an aerially born allergen is particularly low. Pineapple is not known to be allergenic but can be toxic to workers who cut pineapples. When unripe, pineapple is inedible and poisonous, and can irritate the throat, cause burning sensation on lips and mouth and act as a drastic purgative.

Common pests infesting vegetative propagules are mealybugs, scale and pineapple red mites. In addition to these pests, the diseases termed heart rot, root rot, fruit rot and butt rot may be major problems when handling, storing or planting fresh materials. Two types of plant viruses – a closterovirus and a bacilliform also infect pineapples.

2 PREAMBLE

2.1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

Australia's first national regulatory system for gene technology was established on 21 June 2001, when the *Gene Technology Act 2000* (the Act) took effect. The regulatory system is designed to protect the health and safety of people and the environment, by identifying risks posed by, or as a result of, gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Commonwealth regulatory body located within the Health and Ageing portfolio.

Section 50 of the Act requires the Regulator to prepare a risk assessment and risk management plan in relation to any proposed intentional release of a GMO into the environment. The Regulator must consider a number of specific issues in preparing the risk assessment and risk management plan including:

- the properties of the parent organism;
- the effect of the genetic modification;
- the potential for dissemination or persistence of the GMO or its genetic material in the environment; and
- any likely impacts of the proposed dealings on the health and safety of people.

This document addresses the biology and ecology of *Ananas comosus* var. *comosus* with particular reference to its growth and cultivation in Australia. Included is the origin of pineapple, general descriptions of its growth and agronomy, its reproductive biology, toxicity and allergenicity and its general ecology. This document also addresses the potential for pineapple to outcross to other plants.

2.2 THE PURPOSE OF THIS DOCUMENT

This document is designed to be a regularly revised resource document for dealings involving the intentional release (DIR) into the Australian environment of genetically modified (GM) pineapples either commercially or for the purpose of field trials. This document has been prepared to summarise current information on conventional (non-genetically modified) pineapple (*A. comosus* var. *comosus*) that may be relevant to assessing any risks to the health and safety of people and the environment posed by various GM pineapple varieties. It will also support the detailed risk assessment and risk management plan prepared for each DIR application involving GM pineapples. Particular genetic modifications, and how the Regulator would assess their associated risks would be detailed in the risk assessment and management plan prepared for each application.

3 BIOLOGY OF PINEAPPLE

3.1 INTRODUCTION

The parent organism is cultivated pineapple (*Ananas comosus* (L.) Merrill, which is now called *Ananas comosus* var *comosus*) and belongs to the family *Bromeliaceae*. Any reference to pineapple in this document refers to *Ananas comosus* var *comosus* (formerly *Ananas comosus*). The pineapple is the leading edible member of *Bromeliaceae* which embraces about 2,000 species, mostly epiphytic and many strikingly ornamental.

The pineapple shares the distinction accorded to all major food plants of the world of having been selected, developed, and domesticated by peoples of prehistoric times and passed on to us through earlier civilisations.

3.1.1 Origin and Evolution

The pineapple, as we know it now, originated in South America. Pineapple was domesticated by the Tupi-Guarani Indians from *A. comosus* var. *ananassoides* and accompanied them in their northward migrations to the Antilles, northern Andes and central America (Bertoni, 1919).

It seems that *A. comosus* var. *comosus* and *A. comosus* var. *erectifolius* were developed from *A. comosus* var. *ananassoides* and/or *A. comosus* var. *paraguazensis*. Selection of *A. comosus* var. *comosus* appears to be based on large fruit size, through an increase in the fruitlet number and size, high fruit quality (lower acidity) and reduced seediness. Selection of *A. comosus* var. *erectifolius* appears to be based on selection for long, fibrous and smooth leaves for fibre production. Other traits were necessarily modified in the course of domestication and selection. The excellence of selections obtained by the Amerindians, their knowledge of the crop and wide pre-Columbian distribution of the pineapple, all indicate an ancient domestication, dating back several thousand years (Coppens d'Eeckenbrugge and Leal, 2003).

3.1.2 History

Cristobal Colo`n (Christopher Columbus) and his sailors discovered pineapple in 1493, when they landed on an island in the Lesser Antilles of the West Indies (Collins, 1960).

In 1557, a clergyman in Brazil first used the name 'ananas' for pineapples, derived from the Tupi Indian name *nana*. The english name 'pineapple' came from the comparison of the fruit with the exotic pine cone as the outermost modified scales of the fruit make it appear like a pine cone (Collins, 1960), the fruit has acquired few vernacular names. It is widely called *pina* by Spanish-speaking people, *abacaxi* in the Portuguese tongue, *ananas* by the Dutch, Germans and French and the people of former French and Dutch colonies; *nanas* in southern Asia and the East Indes (Morton, 1987).

Following the discovery of pineapple, it was soon to be found in various foreign countries either by accident or by intent to introduce the species to a new land. Spaniards introduced the pineapple into the Philippines and may have taken it to Hawaii and Guam early in the 16th Century. The first sizeable plantation 5 acres (2 ha)—was established in Oahu in 1885. Portuguese traders are said to have taken seeds to India from the Moluccas in 1548, and they also introduced the pineapple to the east and west coasts of Africa. The plant was growing in China in 1594 and in South Africa about 1655. It reached Europe in 1650 and fruits were being produced in Holland in 1686 but trials in England were not successful until 1712. Greenhouse culture flourished in England and France in the late 1700's. Captain Cook planted pineapples on the Society Islands, Friendly Islands and elsewhere in the South Pacific in 1777. Lutheran missionaries in Brisbane, Australia, imported plants from India in 1838. A commercial industry took form in 1924 and a modern canning plant was erected about 1946 (Morton, 1987).

3.1.3 Uses of Pineapple and By-products

Pineapple is cultivated predominantly for its fruit that is consumed fresh or as canned fruit and juice. Pineapple is the only source of bromelain, a complex proteolytic enzyme used in the pharmaceutical market and as a meat-tenderising agent. The stems and leaves of pineapple plant are also a source of fibre that is white, creamy and lustrous as silk. Pineapple fibre has been processed into paper with remarkable qualities of thinness, smoothness and pliability (Collins, 1960; Montinola, 1991). Parts of the plant are used for silage and hay for cattle feed. Processing wastes in the form of shell, core materials and centrifuged solids from juice production are also used as animal feed. Alcoholic beverages can also be made from juice.

3.1.4 Production and Trade

Pineapple is now the third most important commercial tropical fruit crop in the world. The processing of pineapple has made the fruit well known even in the temperate parts of the world.

Early commercial trade was limited to relatively short transportation routes due to the short shelf life of fresh pineapple. Jams and sweets were the first commercial products made from pineapples (The`vet, 1557; Acosta, 1590; Loudon, 1822). In the early 19th century, fresh pineapples were sent from the West Indies to Europe attached to the entire plant (Loudon, 1822). Commercialisation in the mid-19th century developed based on the shortest trade routes.

A retired sailor first canned pineapples in Malaya in 1888. Export from Singapore soon followed with shipments reaching half a million cases by 1900. In Hawaii, a machine that could peel and core pineapples automatically was invented and refined between 1911 and 1919 that allowed large-scale economically viable canning industry in Hawaii. Similar canning operations started in Taiwan, Philippines, Australia, South Africa, the Caribbean and Kenya. The industry grew and flourished. However, World War II ruined the South-East Asian industry and destroyed international trade in the region for three and a half years during World War II. Hawaii gained supremacy but other competitors soon followed. The Malaysian Pineapple Industry Board was established in 1959 with steady progress thereafter (Morton, 1987).

After World War II, refrigerated sea transport developed that reduced the need for proximity to the market and as a result the fresh-fruit market started expanding. Today the canned-product market remains very important but the value of the international fresh-fruit market is rapidly increasing.

Pineapple is currently grown commercially over a wide range of latitudes from approximately 30^{o}N in the northern hemisphere (30^{o}45'N in India (Hayes, 1960) and 28^{o}30' in the Canary Islands (Galan Sauco *et al*, 1988)) to 33^{o}58'S in South Africa (Bartholomew and Kadzimin, 1977).

Based on the statistics (Anon., 2002) collected by Food and Agriculture Organisation (FAO) of United Nations, mean pineapple production for 1999-2001 was 13,527,149 metric tonnes (t) and was approximately constant for the 3 years. World production has more than tripled during the past 30 years (3,833,137 t in 1961 to 13,738,735 t in 2001). The leading pineapple producing countries in 2001 are Thailand (2,300,000 t), Philippines (1,571,904 t), Brazil (1,442,300 t), China (1,284,000 t), India (1,100,000) and Nigeria (881,000 t). Australia produced 140,000 t of pineapples in 2001. Australia markets canned and fresh fruit almost exclusively within the country (Rohrbach *et al*, 2003).

World trade in pineapple mainly consists of processed products. World exports of canned pineapple doubled between 1983 and 1992 passing 1 million tonnes (t) and representing a value of more than US$600 million (Rohrbach *et al*, 2003). The leading countries are Thailand (315,000 t), the Philippines (209,000 t), Indonesia (95,000 t), Kenya (84,000 t) and Malaysia (44,000 t).

The international fresh-pineapple market (about 670,000 t) is dominated by Costa Rica, the Philippines and the Cote d`Ivoire. The chilled fresh-cut fruit pineapple packed as spears or chunks in sealed plastic bags for retail sale is a relatively new product. Commercialisation of this process will depend on the costs versus benefits of high-pressure processing equipment (Rohrbach *et al*, 2003).

The market for concentrated pineapple juice and frozen concentrate has also increased from an estimated 40,000 t in 1983 to 167,000 t in 1993 and is expected to increase further. The supply is dominated by Thailand and the Philippines (Rohrbach *et al*, 2003).

In international trade, the numerous pineapple cultivars are grouped in four main classes: 'Smooth Cayenne', 'Red Spanish', 'Queen', and 'Pernambuco (Abacaxi)' despite much variation in the types within each class (Morton, 1987). The fifth group or class comprising of 'Motilona' or 'Perolera' is commercially very important in South America (Sanewski, 2003). However, most of the world production (about 70%), and most of the canned pineapple (about 95%), comes from the cultivar 'Smooth Cayenne'. Since 'Smooth Cayenne' does not provide the best quality fresh fruit all year round, there is pressure on distributors/growers to switch to cultivars with superior quality fresh fruit than 'Smooth Cayenne' (Sanewski and Scott, 2000). Taiwan, Hawaii, Malaysia, Australia, Cuba, Brazil and France are trying to develop cultivars for the fresh-fruit markets.

3.2 TAXONOMY

Pineapple belongs to the order *Bromeliales,* family *Bromeliaceae,* subfamily *Bromelioideae.* The *Bromeliaceae* have adapted to a very wide range of habitats. They are monocots but are set apart from other monocots by several unique characters (Gilmartin and Brown, 1987). The *Bromeliaceae* are divided into three subfamilies, the *Pitcarnioideae,* the *Tillandsioideae* and the *Bromelioideae*. Pineapple is the most important economic plant in the *Bromeliaceae*.

From the first observation of the pineapple by European explorers to the present time, pineapple taxonomy has varied considerably. The first botanical description of cultivated pineapple was by Charles Plumier at the end of the 17th century when he created the genus *Bromelia* for the plants called *karatas*, in honour of the Swedish physician Olaf Bromel and also described *Ananas* as *Ananas aculeatus fructu ovato, carne albida*. Linnaeus in 1753 in his *Species Plantarum* designated the pineapple as *Bromelia ananas* and *Bromelia comosa*. However, Miller (1754, 1768) maintained the name *Ananas*, with all six cultivated varieties.

In 18th and 19th century, pineapple classification resulted in a number of different names (Leal *et al*, 1998). To simplify classification, Mez (1892) recognised in the *Flora Brasiliensis* only one species, *Ananas sativus*, with five botanical varieties. In 1917, Merrill established the binomial *Ananas comosus*. In 1919, Hassler divided the genus *Ananas* into two sections *Euananas* and *Pseudananas* and *Pseudananas* was raised to genus by Harms in 1930.

From 1934 onwards, pineapple taxonomy was dominated by the views of L. B. Smith and F. Camargo who divided the genus *Ananas* and renamed and multiplied species. Ultimately this resulted in 2 genera and nine species recognised in 1979 (Coppens d'Eeckenbrugge and Leal, 2003). This classification has been criticised on the basis of practicality and inconsistency with available data on reproductive behaviour and morphological, biochemical and molecular diversity (Leal, 1990; Loison-Cabot, 1992; Leal and Coppens d'Eeckenbrugge, 1996; Coppens d'Eeckenbrugge *et al*, 1997; Leal *et al*, 1998) and therefore a much simpler and consistent classification has been prepared taking the above information into consideration (Leal and Coppens d'Eeckenbrugge, 1996; Leal *et al*, 1998).

The present classification is as follows (Coppens d'Eeckenbrugge and Leal, 2003):

- *Ananas comosus* var. *ananassoides* (formerly two species: *A. ananassoides* and *A. nanus*);
- *Ananas comosus* var. *bracteatus* (formerly two species: *A. bracteatus* and *A. fritzmuelleri*)
- *Ananas comosus* var. *comosus* (formerly *A. comosus*)
- *Ananas comosus* var. *erectifolius* (formerly *A. lucidus*.(formerly *A. erectifolius*))
- *Ananas comosus* var. *parguazensis* (formerly *A. parguazensis*)
- *Ananas macrodontes* (formerly *Pseudananas sagenarius*)

Ananas monstrous has been invalidated by Leal (1990) because the crownless fruit characteristic is not stable.

3.3 GROWTH AND DEVELOPMENT OF PINEAPPLE

Pineapple is a perennial monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit. It continues to grow after fruiting by means of one or more axillary buds growing into vegetative branches with a new apical meristem. These branches come to maturity and produce fruit while still attached to the old plant, through which they obtain most of their nourishment.

Adult pineapple plants are about 1 m tall and 0.5 m wide while adult 'Smooth Cayenne' plants are usually up to 1-1.5 m high and 1 m wide. The main morphological structures of the plant are the stem, the leaves, the peduncle (stem which bears fruit), the multiple fruit or syncarp or sorosis (fusion of many fleshy fruitlets), the crown, the shoots and the adventitious roots (Coppens d'Eeckenbrugge and Leal, 2003). The peduncle and inflorescence develop from the apical meristem (Kerns *et al*, 1936).

The stage of inflorescence emergence is called 'red heart' because of the 5-7 reddish peduncle bracts at its base. The inflorescence consists of 50-200 individual flowers, capped by a crown composed of numerous short leaves (up to 150) on a short stem. The flowers of the pineapple open in the sequence of their origin, starting with the whorl of flowers at the base of the inflorescence. About 5-10 flowers open daily, and the flowering lasts for 10-15 days. Flowers are hermaphrodite (a plant having stamens and pistils in the same flower) with tubular corolla. The flowers open and the anthers dehisce in the late morning, begin to whither in late afternoon, and are closed at sundown.

A. comosus produces functional germ cells but it is self-sterile through gametophytic incompatibility (Brewbaker and Gorrez, 1967). This incompatibility is generally stronger in *A. comosus* var. *comosus* than in other botanical varieties. Gametes from other plants of the same cultivar are also incompatible. However, some cultivars exhibit pseudo-self-incompatibility, expressed in the variable production of self-seeds, although the resulting self-fertility is always lower than cross fertility (Coppens d'Eeckenbrugge and Leal, 2003). *A. comosus* var. *comosus* is naturally compatible with other cultivars of *A. comosus* var. *comosus* and other *A. comosus* subspecies. Self-incompatibility occurs due to inhibition of pollen tube growth in the upper third of the style (Kerns, 1932; Majumdar *et al*, 1964).

A. comosus var. *comosus* flowers are normally self-sterile and fruit development is parthenocarpic (does not require fertilisation) (Py *et al*, 1987).

Wind pollination is not known to occur in pineapples (Kerns e*t al*, 1968) due to sticky nature of pollen. The flowers are elongated and tubular in shape and pollen is sticky (OGTR planned release PR-137). Humming birds are considered to be principal pollinating agents in the USA (Purseglove, 1972). Honeybees (*Apis mellifera*) and pineapple beetles (*Nitidulid* spp.) also occasionally affect cross-pollination between different compatible cultivars if they are grown near each other (Wee and Rao, 1974). Although nectar is secreted inside the blossom cup, it seeps out into the sepal and bract surfaces (Okimoto, 1949). Usually honeybees seen visiting pineapple flowers feed on the nectar outside the flower (Purseglove, 1972).

While there are no humming birds in Australia, honeybees, native bees (*Trigona* spp.) and ants have occasionally been observed visiting pineapple flowers, probably for the nectar (Sanewski, 2003) and may play a secondary role in cross-pollination. However, pollination is unlikely because compatible cultivars in adjacent rows (in Hawaii) do not intercross (Collins, 1960). Sexual reproduction is rare in nature, probably because of the slow germination and fragility of young seedlings. Vegetative propagation is the dominant form of reproduction because of the vegetative nature of the plant and desiccation resistance in the various kinds of vegetative propagules (Janick and Moore, 1996).

Commercial propagation of pineapple is not through seeds but by the use of vegetative shoots including the crown, slips, suckers and occasionally shoots induced from the dissected stem of mature plant and less frequently by tissue culture of meristems.

3.3.1 Vegetative propagating structures

Crowns (or "tops"), slips, suckers and stem sections have all been commonly utilised for vegetative multiplication of the pineapple. The definition of vegetatively propagable parts and their locations (see Fig 1 below) are as follows:

- slips / hapa (leafy branches attached below the fruit, on the peduncle, grouped near the base of the fruit, sometimes produced from basal eye of the fruit (collar of slips); commonly preferred; may produce fruit within 14-16 months after planting);
- ground (ratoon) suckers (shoots produced at or below ground from the stem and, when used, will produce fruit in 12-14 months after planting);
- side shoots or suckers/stem shoots/ (shoots produced from the above ground portion of the stem, when used, will produce fruit in 18-20 months after planting); and

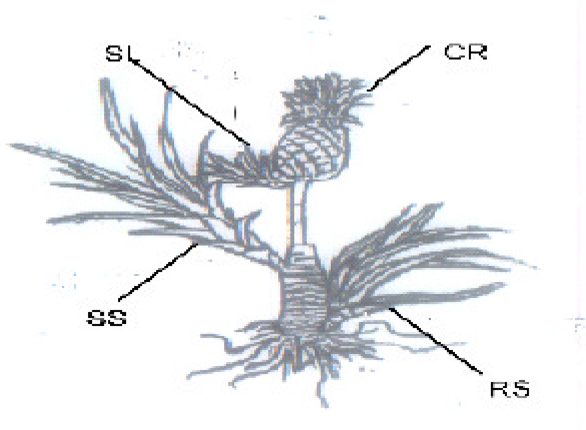

Fig. 1
Plant shoots used for planting material
CR = crown; SL = slip; SS = side shoots or suckers; RS = ratoon suckers

- crown (the short stem and leaves growing from the apex of the fruit; not commonly used; may take up to 24 months after planting to produce fruit) (Pineapple Technical Group, 1999).

Some plants may lack a crown or may produce multiple crowns. Crownlets can also grow at the base of the main crown or from some of the upper fruitlets (Coppens d'Eeckenbrugge and Leal, 2003).

The crown, slips and suckers are capable of forming a new plant. All these structures can survive detached from the parent plant for up to 6 months depending on the prevailing conditions. However, all vegetative shoots (crown, slips, suckers) are attached to the parent plant and not easily removed. Underground shoots or stolons are only produced by *A. macrodontes* (not grown commercially). Dispersal of vegetative structures or seeds would occur only through floods or human or animal intervention.

Vegetative propagation does not ensure exact copies/clones due to the possibility of mutations that may lead to occurrence of distinct clones from the parent cultivar.

3.3.2 Seeds

Pineapple seeds have a very tough seed coat and a hard, flinty endosperm. Treatment with sulphuric acid will often improve the uniformity of germination but is not essential. Commercially, seeds are desired only in breeding programs and are usually the result of hand pollination. Seed is usually treated with a fungicide before sowing under intermittent mist and, where there is no inbreeding, a germination rate of 80-90% should be achieved. The seedlings are planted in the field when 15-18 months old and require 16-30 months to reach the mature fruiting stage. Vegetatively propagated plants fruit in 15-22 months (Morton 1987).

Although pineapple can be grown from seed, fertility in commercially grown cultivars of pineapple is very low and consequently seed production is very rare (Coppens d'Eeckenbrugge *et al*, 1993). In the unlikely event of successful pollination, the seed is contained in the fruit as commercial pineapples have no seed releasing mechanisms. In addition, the seed is not used commercially for propagating further plants. Only clonally propagated plants are used for replanting.

Seed viability is not long. Longevity of hybrid seeds has been estimated to be less than six months in storage at Cote d'Ivoire (Loison-Cabot, 1990). In addition there does not appear to be any seed dormancy in pineapple seeds (Loison-Cabot, 1988).

3.3.3 Maturation and ripening

More than 3 months are necessary from flowering to fruit maturity (Janick and Moore, 1996) but in south-east Queensland it usually takes at least 5 months (Sanewski, PC, 2003). The entire pineapple blossom develops parthenocarpically (development of fruit without fertilisation) into a berry-like fruitlet. The edible part of the fruit consists mainly of the ovaries, of the bases of sepals and bracts and of the cortex of the axis. The fruit shell is mainly composed of sepal and bract tissues and the apices of the ovaries (Okimoto, 1948).

Pineapple flowering is uneven and it is highly desirable to attain uniform maturity and to control the time of harvest in order to avoid overproduction in the peak periods. Several different chemicals are available to achieve uniformity and control over flowering.

It is difficult to judge when the pineapple is ready to be harvested. Size and colour changes are not fully reliable indicators. In general, for the fresh fruit market, the summer crop is harvested when the eye shows a light pale green colour. The winter crop is slower to mature and the fruits are picked when there is a slight yellowing around the base. The winter fruit tends to be more acidic and have a lower sugar level than summer fruit. Fruits for canning are acceptable at a more advanced stage. Overripe fruits are deficient in flavour and highly perishable. Harvesting of fruits can be manual or semi-mechanised.

After maturation of the first fruit, the plant continues to grow by means of one or more axillary buds that grow into vegetative branches with a new apical meristem. These branches at maturity produce fruit while still attached to the old plant. The original plant is called the 'plant crop' and while the fruit developed from the lateral, axillary branch attached to the axis of the plant crop is called 'first ratoon'. The first ratoon may then produce the 'second ratoon' although this is rarely utilised in commercial situations.

In most commercial plantings, the plants are not allowed to produce more than 2-3 crops due to reduction in fruit size and uniformity (Coppens d'Eeckenbrugge and Leal, 2003). Then a new plantation must be established through regular procedures using vegetative propagules.

3.4 PINEAPPLE CULTIVATION IN AUSTRALIA

Pineapple is exotic to Australia and is grown as an agricultural crop almost exclusively in Queensland. They were first introduced into Australia from India by Lutheran missionaries in the southern coastal district of Queensland in about 1838 (Collins, 1960).

The growing area in Queensland is confined to the narrow coastal strip along the eastern seaboard ranging from Cairns in the north to Brisbane in the south. Centres for supplying pineapples for canning include Yeppoon, Bundaberg, Mary Valley, Gympie, Nambour, the Glasshouse Mountains area and Brisbane. The most northerly plantations are at Mossman in Far North Queensland and most southerly are at Dayboro (just north-west of Brisbane). The majority of plantations are in Wamuran and Caboolture, 100 km north of Brisbane. Small commercial fields are in the Northern Territory and northern New South Wales.

Land for pineapple farming is chosen on the basis of four major factors - elevation, aspect, soil and drainage. In the subtropical areas of southern Queensland, elevation and aspect are of particular importance as the pineapple is sensitive to the cold and most are planted on hillsides to escape the frost. Pineapples thrive when planted on a north-east aspect where they receive the maximum amount of sunlight and warmth.

Although all *Ananas* species are found in Australia as per the latest taxonomic classification (Coppens d'Eeckenbrugge and Leal, 2003), distribution of *Ananas* species is limited except for *A. comosus* var. *comosus* (cultivated pineapple) (Sanewski, 2003):

- *A. ananassoides* (*A. comosus* var. *ananassoides*), *A. parguazensis* (*A. comosus* var. *parguazensis*), *A. fritzmuelleri* (*A. comosus* var. *bracteatus*) and *Pseudananas sagenarius* (*Ananas macrodontes*) are located only at Maroochy Research Station, Queensland; and
- *A. nanus* (*A. comosus* var. *ananassoides*), and *A. bracteatus* (*A. comosus* var. *bracteatus*) are sometimes found in nurseries or home gardens as ornamental plants mainly in Queensland. (A cultigen of *A. comosus var erectifolius* is being assessed by a few Queensland pineapple growers for the production of cut flowers).

3.4.1 Wild relatives of pineapple in Australia

Pineapple is not found in nature but only found under cultivation (Collins, 1960) and currently within Australia it occurs almost exclusively as a managed cultigen.

In countries like Brazil, Paraguay, North Argentina and Venezuela, *A. comosus* var. *ananassoides* (*A. ananassoides*, *A. nanus*) and *A. comosus* var. *parguazensis* (*A. parguazensis*) are the only species found in the wild while *A. comosus* var. *comosus* (*A. comosus*), *A. comosus* var. *erectifolius* (*A. lucidus* formerly known as *A. erectifolius*), *A. comosus* var. *bracteatus* (*A. bracteatus* and *A. fritzmuelleri*) are always found under cultivation or as escapes (Janick and Moore, 1996).

None of the pineapple species have become naturalised in southeast Queensland (Stanley and Ross, 1989). Except for cultivated pineapple, all the other pineapple species have very limited distribution in Australia (Sanewski, 2003).

4 WEEDINESS OF PINEAPPLE AND POTENTIAL FOR OUTCROSSING

Currently within Australia, pineapple occurs almost exclusively as a managed cultigen. It is not recognised as a weed in Australia. However it can become an agricultural weed by re-shooting from large pieces of stem that are left intact and buried within a commercially cultivated field of pineapples if the plants from the previous crop are not completely destroyed. These volunteers can be easily destroyed by thorough cultivation and herbicide application.

None of the pineapple species are known to occur as weeds in Australia. Although it is considered that they could theoretically survive in some environments within Australia, they are unlikely to become weeds, as they are slow growing, susceptible to common fungi like *Phytophthora cinnamomi* and sensitive to common herbicides. None have become naturalised in south-east Queensland (Stanley and Ross, 1989) or in any other part of Australia (Sanewski, 2003).

Although pineapple can be grown from seed, fertility in commercially grown cultivars of pineapple is very low and consequently seed production is very rare (Coppens d'Eeckenbrugge *et al*, 1993). In the unlikely event of successful pollination, the seed would stay contained in the fruit as commercial pineapples have no seed releasing mechanisms and commercial propagation of pineapple is not through seeds but by the use of vegetative shoots. Vegetative propagules can only be distributed through flooding or human or animal intervention. Viability of seed is substantially reduced after six months and vegetative propagules can stay viable in the field for a maximum of six months (University of Queensland, 2002). All these factors ensure that pineapple does not become a weed in nature.

Pineapple hybridises with other subspecies of *Ananas comosus* to produce fertile offsprings. Hand-pollinated crosses between *A. comosus* var. *comosus* (diploid, 2n=50) and *Ananas macrodontes* (tetraploid, 4n=100)) produced 5-10% fertile seeds, most of which were tetraploid and grew to be fully fertile (Collins, 1960).

5 TOXICITY, ALLERGENICITY AND PATHOGENICITY

Pineapple is not a pathogen and is not capable of causing disease in humans, animals or plants. Pineapple pollen is not a known allergen. Because it is relatively sticky, it is not dispersed by wind, and therefore the potential for pineapple pollen to act as an aerially born allergen is particularly low.

Pineapples are not known to be allergenic or pathogenic, although they can be toxic (poisonous) to workers who cut pineapples. Almost complete obliteration of the fingerprints by removal of the stratum corneum in workers employed in cutting up pineapples has been ascribed to the effects of pressure together with the keratolytic effect of bromelain, a protease found in the plant sap. The nails were unaffected, in contrast to the nail damage that occurs in workers exposed to proteases of animal origin. Moniliasis of the finger webs has been occasionally observed in pineapple cutters (Polunin, 1951).

In addition, the raw fruit, when eaten in large quantity, produces a burning sensation of the lips and mouth (Watt & Breyer-Brandwijk, 1962) and can also produce angular stomatitis (Fasal, 1945). When unripe, the pineapple is inedible and poisonous, irritating the throat and acting as a drastic purgative (Morton, 1987). Excessive consumption of pineapple cores can cause the formation of fibre balls (bezoars) in the digestive tract (Morton, 1987). Pineapple fruits contain ethyl acrylate. Ethyl acrylate (4% in petrolatum) produced sensitisation in 10 of 24 subjects in a skin sensitisation test (Opdyke, 1975).

6 POTENTIAL FOR GENE TRANSFER FROM PINEAPPLE TO OTHER ORGANISMS

The possibility of genes transferring from *A. comosus* to other organisms is addressed below. Potentially, genes could be transferred to: (1) other pineapple plants, including other *A. comosus* subspecies, and other *Ananas* species, (2) other plant genera, and (3) other organisms.

6.1 GENE TRANSFER TO OTHER PINEAPPLE PLANTS

6.1.1 To other *A. comosus* var *comosus* plants

About 70% commercial cultivation of pineapple in the world (Loeillet, 1996) and about 94% in Australia (Sanewski, 2003) comprises of the clones of the cultivar known as 'Smooth Cayenne'. Since plants of *A. comosus* var. *comosus* are self incompatible, the clones of a cultivar (eg. clones of cultivar 'Smooth Cayenne') are also incompatible with each other because they are very closely related. Therefore, cross-pollination and consequent gene transfer does not occur in 'Smooth Cayenne' plantations.

Cultivars of *A. comosus* var. *comosus* are compatible with each other provided they are not too closely related. The frequency of natural cross-pollination would depend on how closely they are planted, synchronicity of flowering and the presence of pollinators. All of these factors are usually controlled by the grower although some natural flower initiation can occur. Considering the factors required for natural cross-pollination, it is unlikely that there would be much, if any, natural cross-pollination of different cultivars as observed in farms that have small areas of 4-5 different cultivars growing with no seed observed (Sanewski, 2003).

Cross-pollination of an *A. comosus* var. *comosus* plant to another cultivar of this variety via an insect pollen vector is the most likely but not the ususal means by which pineapple genes could be dispersed in the environment. This is because humming birds which are the main pollinators, are not found in Australia but honeybees (*Apis mellifera*), native bees (*Trigona* spp.) and ants have occasionally been observed visiting pineapple flowers, probably for the nectar (Sanewski, 2003) and may play a secondary role in cross-pollination. However, Collins (1960) observed (in Hawaii) that even when compatible cultivars were planted in adjacent rows, pollination did not occur.

Reduced longevity of the seeds, relatively long, delicate germination, vulnerable seedlings and a long sexual cycle all appear to be factors which limit production by seed (Loison-Cabot, 1992). In the unlikely event of successful pollination, the seed would stay contained in the fruit as commercial pineapples have no seed releasing mechanisms and propagation of commercial pineapple is not through seeds but by vegetative shoots. Fertility in commercially grown cultivars of pineapple is very low and consequently seed production is very rare (Coppens d'Eeckenbrugge *et al*,1993). In addition, viability of seed is substantially reduced after six months.

6.1.2 To other *A. comosus* subspecies

A. comosus var *comosus* can hybridise with other subspecies of *A. comosus*. Hand-pollinated hybrids have been obtained from crosses between *A. comosus* var *comosus* and *A. comosus* var. *ananassoides*, *A. comosus* var *bracteatus*, and *A. comosus* var *erectifolius* to produce viable seeds and fertile offsprings (Collins, 1960). However, researchers/breeders rarely find seed in fruit of the different subspecies probably due to lack of synchrony in flowering and minimal pollinator activity unless they are hand-pollinated (Sanewski, 2003).

6.1.3 To other *Ananas* species

A. comosus can hybridise with the only other *Ananas* species – *A. macrodontes* (also known as *Pseudananas sagenarius*). Hand-pollinated crosses between *A. comosus* var. *comosus* (diploid, 2n=50) and *A. macrodontes* (tetraploid, 4n=100)) produced 5-10% fertile seeds (the remainder having mostly flat empty seed coats), most of which were tetraploid and grew to be fully fertile (Collins, 1960). Sanewski (2003) also hybridised *A. macrodontes* with *A. comosus* var. *erectifolius* and *A. comosus* var. *bracteatus*.

6.2 GENE TRANSFER TO NATURALISED PINEAPPLE SPECIES

Neither *A. comosus* var. *comosus* nor any subspecies of *A. comosus* nor any species of *Ananas* are naturalised in Australia (Sanewski, 2003).

6.3 GENE TRANSFER TO OTHER PLANTS

There are a few reports of intergeneric hand-pollinated crosses between *Ananas* and some members of family *Bromeliaceae* although there are no scientific publications or reports of these crosses occurring in nature. Some websites like http://fcbs.org/articles/Bigenerics.htm, http://fcbs.org/, and http://www.geocities.com/RainForest/Canopy/5376/bromeliad-lst.txt refer to hand-pollinated crosses between *Ananas comosus* and *Neoreglia*, between *Aechmea serrata* and *Ananas comosus*, between *Cryptanthus* and *Ananas* (species not given) and between *Ananas bracteatus* and *Aechmea* sp. There is also a scientific report of *Tillandsia fasciculata* X *A. comosus* var. *comosus* (Valds *et al*, 1998).

In the wild, Bromeliad bigenerics (crosses between plants from two different genera, i.e. genus x genus) do not apparently occur because of the fertility barriers, different blooming times and geographical range that prevents most species in different genera from cross-breeding by specialised pollinators. The biggest obstacle appears to be genetic incompatibility between potential parents (http://fcbs.org/articles/Bigenerics.htm). Sanewski (2003) was unsuccessful in obtaining hybrids from *Aechmea* sp X *A. comosus* and

Aechmea sp X *A. macrodontes* as it is not easy to obtain the intergeneric hybrids even with controlled hand pollination.

There have been no other reports of hybridisation between *Ananas* and other plants. Gene transfer to unrelated plant species is highly improbable as no evidence for horizontal gene transfer from pineapple to other plant taxa has been identified.

6.4 GENE TRANSFER TO OTHER ORGANISMS

It is considered that there is negligible risk of transferring pineapple genes to other sexually incompatible organisms such as humans, other animals, fungi, bacteria, other microorganisms and viruses. A complex series of events would be necessary for successful horizontal gene transfer to occur.

In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence and Ochman, 1998). 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 (Doolittle, 1999; Nielsen *et al,* 1998).

Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman *et al*, 2000). On some occasions viruses have transferred genes to their hosts (Harper *et al,* 1999). Most gene transfers have been identified through analyses of gene sequences (Ochman *et al*, 2000; Worobey and Holmes, 1999).

In contrast, evidence from gene sequences indicate that transfers of genes between plants and other organisms such as animals, bacteria, fungi or viruses is exceedingly rare (Mayo and Jolly, 1991 and Aoki and Syono, 1999). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Nielsen *et al,* 2000; Schoelz and Wintermantel, 1993 and Greene and Allison, 1994). However, in all cases this was achieved only with the presence of related gene sequences (homologous recombination), using highly sensitive or powerful selection methods to detect rare gene transfer events. Horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen, 1999; Nielsen *et al*, 1997; Nielsen *et al*, 1998) and deliberate attempts to induce such transfers have so far failed (e.g. Schlüter *et al,* 1995; Coghlan, 2000).

7 PESTS AND DISEASES OF PINEAPPLE IN AUSTRALIA

The pineapple plant is most productive under xerophytic (dry) environments where low rainfall is supplemented by irrigation in well-drained soils. Once the root system (adventitious roots) is damaged or destroyed, it does not regenerate significantly. Several characteristics of the pineapple plant and commercial pineapple production systems have contributed to the severity of several pest and disease problems (Rohrbach and Johnson, 2003).

Common pests infesting vegetative propagules (crowns, slips, suckers) are mealybugs, scale and pineapple red mites. In addition to these pests, the diseases termed heart rot, root rot, fruit rot and butt rot may be major problems when handling, storing or planting fresh materials. Two types of plant viruses – a closterovirus and a bacilliform also infect pineapples.

The most serious and widespread disease of pineapples in Queensland, *ie* heart rot, root rot and green fruit rot is caused by the fungus *Phytophthora cinnamomi* (mainly in Mary Valley and the areas between Mary Valley and Mackay), while *Phytophthora nicotianae* var. *parasitica* (mainly in Mackay and the areas between Mary Valley and Mackay) causes heart (top) rot only (Pegg, 1993).

Other important disease causing fungi are *Thielaviopsis paradoxa* (which causes butt or base rot, white leaf spot and water blister in fruit) and *Penicillium funiculosum* (which causes fruitlet core rot or green eye, leathery pocket and interfruitlet corking). The yeast, *Saccharomyces* spp., causes yeastly rot in overripe or damaged fruit, mainly during spring (Pegg, 1993).

Bacteria *Erwinia ananas*, *Gluconobacter oxydans* or *Acetobacter aceti* normally cause pink disease of fruit, but in Queensland the disease causing bacteria are undetermined. Since pink disease causes discolouration of fruit, it is considered a problem during processing. *E. ananas* also causes marbling of fruit (Pegg, 1993).

Tomato spotted wilt virus causes yellow spot on leaves and young crowns. Mealybug wilt is possibly caused by pineapple closterovirus transmitted by mealybug (*Dysmicoccus brevipes*). Mealybugs feed on an infected plant and then move to other plants with help of ants or wind and transmit the wilt to other plants while feeding on them (Pegg, 1993).

Root-knot nematode (*Meloidogyne javanica*) and root-lesion nematode (*Pratylenchus brachyurus*) are widespread in all pineapple growing districts. Root-knot nematode is the most damaging of all nematodes in Queensland pineapple fields, causing stunting, yellowing and dieback of plants. Reniform nematode (*Rotylenchulus reniformis*) is largely restricted to north Queensland where it can cause major losses. It has been recorded only occasionally in southeast Queensland pineapple fields. Spiral nematode (*Helicotylenchus dihystera*) and ring nematode (*Criconemella ornata*) are also common in pineapple fields although they do not cause any economic damage in Queensland (Stirling, 1993).

Symphyla or symphylids (*Hanseniella* spp.) are wingless, soil-inhabiting arthropods and are distantly related to insects (Py *et al*, 1987). They occur throughout pineapple growing areas of Queensland and in most countries where pineapples are grown (Waite, 1993). Symphylids that are important as pineapple pests feed on plant root tips and hairs (Carter, 1963; Py *et al*, 1987) resulting in many short, branching roots giving the ‘witches broom’ effect that can lead to dramatic yield decreases in severe cases (Lacoeuilhe, 1977; Kehe, 1979), particularly when soil moisture is a limiting factor. Symphylids usually feed on the younger roots of the meristematic region. Symphylid-inflicted injury to the roots may also provide entrance to ‘wound’ pathogens which can destroy roots (Sakimura, 1966).

Pineapple mealybug (*Dysmicoccus brevipes*) is found in all pineapple growing areas and can infest roots, butt, stem, leaves, flowers and fruit (Waite, 1993). Mealybugs feed on plant sap in the phloem of their host plants. They produce honeydew (a sweet,sticky liquid) as a by-product of their feeding on plant sap. The honeydew when accumulated in large quantities supports the growth of sooty mould (*Capnodium* sp) which may be found on the pineapple, but does not cause significant damage (Rohrbach and Johnson, 2003). Mealybug feeding does not normally cause a problem unless they transmit closterovirus that causes mealybug wilt.

Pineapple scale (*Diaspis bromeliae*) occurs in all pineapple production areas of the world and varies in its impact on pineapple. Frequently, it builds up on the crown of the developing fruit and at harvest the vegetative propagules may be heavily infested, desiccating the planting material, thereby making it unusable. In Australia for example, it does not typically reduce fruit yield directly but affects fruit appearance so that the value is reduced. If ratoon planting is shaded, fruits and suckers may be greatly damaged (Waite, 1993). When fruits are highly infested, cracks may develop in fruitlets (Py *et al*, 1987).

The white grubs (i.e. larval stage) of several beetle species in the family *Scarabaeidae* (*Antitrogus mussoni*, *Anoplognathus porosus*, *Rhopaea magnicornis*, *Lepidiota squamulata L. grata*, *L. noxia*, and *L. gibbifrons*) are reported in Australia as feeding on pineapple roots (Waite, 1993). They may injure plants by feeding on the roots, which interferes with nutrient and water uptake and transport (Carter, 1967; Petty, 1978; Waite, 1993); weakening or destroying the roots that anchor the plants in the soil (Waite, 1993); and wounding plant tissues, thus enabling secondary plant pathogens to enter the plant (Carter, 1967). Crop loss may occur due to serious infestation, particularly the ratoon crop (Waite, 1993). Plants may become stunted, wilted and chlorotic (Petty, 1978). Fungal pathogens like *Pythium* and root-knot nematode may infect the injured plant (Carter, 1967).

The pineapple red mite (also known as red spider or false spider mite), *Dolichotetranychus floridanus*, is the largest mite found on pineapple (Rohrbach and Johnson, 2003). It is a minor pest in most production areas, but more serious in the Yeppoon district particularly when dry climatic conditions prevail. The mites feed primarily on the white tissue at the base of the leaves causing lesions and allowing rot organisms to invade the dead tissue. Under dry conditions mites can become a problem on the stored vegetative propagules (Waite, 1993).

Other invertebrate pests that infest pineapples in Australia include grasshoppers, cane weevil borer (*Rhabdoscellus obscurus*), African black beetle (*Heteronychus arator*), pineapple mite (*Steneotarsonemus ananas*), common armyworm (*Mythimna convecta*), rutherglen bug (*Nysius vinitor*), grey cluster bug (*N. clevelandensis*) and moth larvae (possibly belonging to the family *Pyralidae*) (Waite, 1993).

The Australian crow (*Corvus orru*) is a pest in all pineapple growing districts in Queensland and usually attacks the largest and ripest fruit to feed on fruit flesh. Too much crow feeding could cause severe crop damage. Eastern swamphen (*Porphyrio porphyrio*) is mainly found in swamps and other water storage areas and pulls out young plants from fields close to their habitats and partially eats them. Native rodents (eg. *Rattus sordidus*, *Melomys cervinipes*, *M. burtoni*) feed on fruit and small leaves of crowns and thereby damage the fruit and the crown. Feral pigs (*Sus scrofa*) feed on pineapple fruit after removing the fruit from plants. To eat the fruit, pigs carry it to unploughed land at the end of furrows. In addition to eating fruit, pigs can injure a large number of plants by eating the heart of young plants (Broadley, 1993).

8 DEFICIENCIES IN AVAILABLE DATA

In attempting to summarise the available data on biology and ecology of pineapple in Australia, several gaps in current knowledge have been highlighted. In particular, these include:

1. Pollinator identity and efficiency;
2. Pollen viability and dispersal;
3. Detailed studies on natural cross-pollination between various pineapple cultivars, subspecies and species in Australian environment, consequent seed setting and hybrid fertility; and
4. Interaction of pineapple plant with soil organisms (eg, microorganisms, invertebartes).

Data in these areas would be needed before significantly large scale, less restricted or commercial release of genetically modified pineapple could be approved.

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