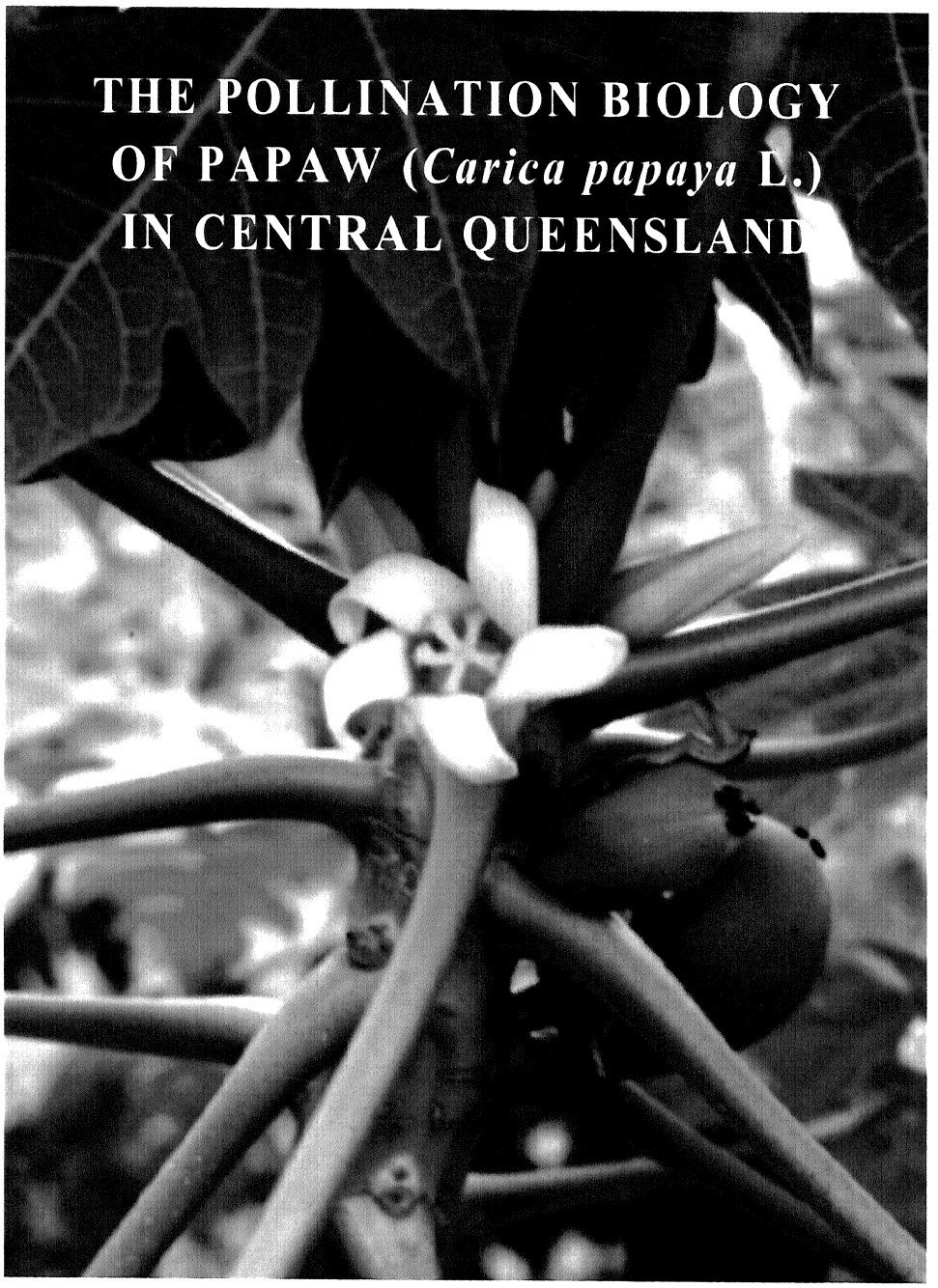
THE POLLINATION BIOLOGY OF PAPAW (*Carica papaya* L.) IN CENTRAL QUEENSLAND



by

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I, the undersigned author, declare that this thesis is my own work and has not been submitted in any other form for any other award at any institution of tertiary education. Information from the published or unpublished works of all other persons has been acknowledged in full in the text, and a complete list of references is provided.

Signature Redacted

Anne Garrett

ABSTRACT

Papaws are one of the most significant fruits in central Queensland, yet there are specific problems associated with consistent fruit production in this subtropical region. This is the first time that a comprehensive analysis into the pollination mechanism of papaw has been conducted, in a country other than that of its origin. Additionally, research into factors influencing fruit set and seed set under subtropical climates has been performed.

Earlier studies on the pollination biology of *C. papaya* have suggested that pollen transfer is wind and/or insect mediated. Whilst papaws grown in Australia are believed to be wind-pollinated, a diverse number of insect species have been proposed to fulfil pollinator function in other areas of cultivation, which reflects that the pollination mechanism in papaw is unknown.

Contrary to all previously published research in the area, pollination of *C. papaya* is carried out by hawkmoths (Lepidoptera: Sphingidae). Seven pollinator species and a further four suspected pollinator species have been identified for the central Queensland region. All species belong to the same subfamily, the Macroglossinae. Contrary to anecdotes from growers, neither native or European bees, nor wind was of significance in the pollination of dioecious papaw cultivars and incidences of apomictic and parthenocarpic fruit set occurred though were rare. Sphingid pollinators showed marked patterns of seasonal occurrence. Weekly observations and light trap results conducted over two years in the vicinity of Rockhampton, indicated that adult sphingids were absent from the central Queensland region during winter from the middle of June until the middle of August. Their absence correlated with relatively low average weekly minimum temperatures of 10.4 °C and below. Overwintering of sphingids in the pupa stage in order to overcome adverse climatic effects, was observed for two species.

Seasonally occurring fruit set and periods of low seed set of *C. papaya*, under the subtropical central Queensland climate were due to three key factors - the availability and viability of pollen and the absence of sphingid pollinators. The overall stagnation of tree growth including a decrease in open flower numbers during winter also played a role. Individual papaw lines (particularly Hybrid 29) showed better adaptations to heat and cold stress in respect to pollen quantity and viability. Seed set, irrespective of viability status, increased fruit size by an average of 0.89 g/seed.

The pollination mechanism by which nectarless pistillate flowers attract pollinator visitation has been identified and involves a multitude of stimuli, including those of olfaction, gustation, tactility and vision. Petals of both flower types show the same visual properties, of absorbing wavelengths of the UV-spectrum (< 405 nm), selectively reflecting only those of longer wavelengths. Additionally, pattern forming trichomes attached to scent glands are present on both pistillate and staminate *C. papaya* flowers. Mechanosensory receptors on the sphingids probosces exactly match trichome patterns and the presence of contact chemoreceptors suggests their involvement for foodsource recognition.

The results of this study provide an information base which can now be integrated into new horticultural practices with regard to pollination requirements of papaw grown under subtropical climates. In particular seasonal trends of pollinator abundance, pollen quantity and pollen viability could translate directly into strategies for improving fruit yield. Plantings of larval host plants in vicinity to papaw orchards could provide the first step towards the management of hawkmoth pollinators. The existence of apomictic seed formation in papaw establishes a basis for further studies concerning sex determination prior to anthesis (as it is only possible at this point of time), making use of the effects of heterosis yet circumventing the constant breeding programmes providing the F_1-crosses. Further investigations into varietal differences of pollen quality and quantity during the subtropical growing season as well as the influence of seedlike structures on fruit set and flesh thickness may also prove useful in the selection of more suitable papaw cultivars grown under subtropical climates. Apart from objectives focusing on the fruit production of papaw, results obtained on the mechanisms of pollinator constancy, in particular the sensory recognition of floral parts by hawkmoth pollinators, offer a far more universal approach into plant-pollinator relationships, which presently are known to function by 'deceit'.

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INTRODUCTION

Carica papaya (Caricaceae) is known as papaw in Australia and the Pacific or papaya in the Americas. The fruit was introduced to Australia from South America more than a century ago. Commercial papaw production is based entirely in Queensland with major production areas situated along the eastern seaboard; Innisfail in north Queensland, Yarwun and Yeppoon in central Queensland and Gympie and the Sunshine Coast in south Queensland. The current industry grossed fourteen million Australian dollars in 1994, placing it amongst the major income earning fruit industries in Queensland (Macleod personal communication).

The husbandry, genetics, and pests of papaw have been intensively researched over the previous decade. Improved seed stock has been made available to orchardists. Nonetheless the market supply of papaw remains inconsistent. Major problem areas are identified in unresolved diseases, inconsistent fruit set and fruit quality (Agnew 1968; O'Hare 1993). Variable fruit set and fruit size, poor shelf life and undesirable fruit flavour are associated with low rates of pollen transfer (Prest 1955; Agnew 1968; Allan 1976; Glennie and Chapman 1976). Growing *C. papaya* under subtropical conditions exacerbates these problems (Agnew 1968).

Identification of pollination vectors and their mode of activity is therefore of high priority if the crop is to be managed optimally and returns are to be maximized. Earlier studies on the pollination biology of *C. papaya* have suggested that it is wind (anemophilic) and/or insect (entomophilic) mediated (Storey 1969b; Free 1970). According to Baker (1976), the pollination agents of *C. papaya* in Central America were probably sphingid moths but the exact genus was not clear. Research into the pollination biology of a related species within the Caricaceae also suggested sphingid involvement in its endemic habitat, although again, pollinator identifications were not made (Bawa 1980b; Bullock and Bawa 1981; Bawa *et al.* 1985b). The involvement of honey bees (*Apis mellifera*) in pollen transfer in papaw was suggested from a study conducted in South Africa (Allan 1963c). Anecdotal accounts propose the involvement of Australian native bees (Goebel 1986; Cheers 1990). Another study undertaken in Jamaica concluded that pollination was not essential to initiate fruit set (Free 1970). It has been suggested that papaws are wind-pollinated in Australia (Prest 1955; Agnew 1968). This is a view widely held by growers. In essence, the diversity of views expressed in the relevant literature reflects the fact that the mechanism of pollination in papaw is

unknown. This lack of consistency in the literature is detailed further in the following sections.

The aim of the research presented here is an evaluation of the pollination biology of *C. papaya* in regards to anemophily, entomophily and agamospermy. The variation in seasonal fruit set typical of subtropical climates such as in central Queensland is investigated. Taken together, the results point the way for improvements in crop management strategies, in particular the continuity, quantity and quality of fruit set.

CHAPTER I

THE BIOLOGY OF *Carica papaya* L.

1.1 *Carica papaya* L.

1.1.1 Origin, Taxonomy and Distribution

The origin, taxonomy and geographical history of *C. papaya* have been reviewed by Storey (1969b), Purseglove (1974) and Giacometti (1987a). The first monograph on the Caricaceae was published by Engler and Prantl (1925) with an update given by Badillo (1971).

The taxonomy of the papaw has been reviewed by Storey (1976), and Sturrock (1980) who both employed classical taxonomical methodology. A bio-molecular approach to analysis of the taxonomy of the Caricaceae was published by Spencer and Seigler (1984). The family consists of 31 known species assigned to four genera (*Cylicomorpha, Jacaratia, Jarilla* and *Carica*), of which 22 species belong to the genus *Carica*. With the exception of the two most primitive species of the genus *Cylicomorpha*, which are indigenous to equatorial Africa, the remainder are indigenous to central tropical America between approximately the 0° and 18° northern latitude (Badillo 1971, as referenced in Giacometti 1987a).

Opinions differ on the origin of *C. papaya* in tropical America. It is generally agreed amongst botanists that the papaw has never been found in the wild (Storey 1969b, 1976; Purseglove 1974; Giacometti 1987a). Purseglove (1974) and Cobley, revised by Steele (1976), state that *C. papaya* arose as an interspecific hybrid of unknown parentage. However, cytological data (in particular the failure of interspecific hybridization of *C. papaya* with other *Carica* species) does not support this view (Mekako and Nakasone 1975; Storey 1976; Chen *et al.* 1991). Papaws share some common characteristics with *C. peltata*, as described by Oviedo y Valdes (1959). In particular, the similarity in distributional range, dioecious breeding and fruit characteristics led Badillo (1971) to conclude that *C. peltata* was synonymous with papaw. Candolle (1908) and Storey (1976) determined that *C. papaya* probably originates from the lowlands of eastern Central America, reaching from Mexico to Panama. Storey (1976) further mentions that

the high diversity of plant types results from variations introduced during cultivation by early civilizations. The opinion is generally held that *C. papaya* is a variation of *C. peltata.* There are no records of Caricaceae native to Australia.

Caricaceae grow in altitudes as high as 5000 feet near the equator. Optimum growing conditions for *C. papaya* occur between altitudes of 0 – 1000 m above sea level with average temperatures of 22 – 25°C and 1500 – 2000 mm rainfall (Kuethe and Spoerhase 1974). Presently, *C. papaya* is cultivated world wide in tropical and subtropical regions between the latitudes 32° North and South of the equator (Glennie and Chapman 1976). The main commercial production centres are Brazil, Australia, South Africa, Taiwan, Hawaii, Thailand, India, Philippines and a few other minor tropical areas (Storey 1969b; Giacometti 1987b).

1.1.2 Sexual System and Genetics

The genetics and sexual system of *C. papaya* has been extensively studied and is reasonably well documented. The somatic chromosome number of *C. papaya* is 18; n = 9 (Foster 1943; Storey 1953; Singh 1960; Datta 1971) a figure which is uniform throughout the genus; according to Kumar and Abraham (1942). Polyploidy has been artificially induced in *C. papaya* by treating seedling stock with colchicine, but this did not prove to be useful for fruit production or breeding purposes (Storey 1976). The natural occurrence of polyploidy within the Caricaceae has never been documented.

The Caricaceae are dioecious with three exceptions, all within the genus *Carica.* The monoecious *C. monoica* and the two hybrids, *C. pentagona* (babaco) and *C. candamarcensis* bear pistillate flowers followed by parthenocarpic fruit set (Baker 1976; Storey 1976; Subramanyam and Iyer 1986; Giacometti 1987a). Propagation in these species occurs vegetatively, and only from cuttings. On the other hand propagation of *C. papaya* is almost entirely by seed. *C. papaya* is dioecious, although cultivated bisexual plants permanently bearing hermaphrodite flowers do exist. This sexual system has been attributed to human selection (Storey 1976; Giacometti 1987a).

The female plant is genetically recessive homozygous for all genes, due to a zygotic lethal factor which eliminates all dominant homozygotes, and phenotypically stable. By contrast male and bisexual trees are genetically heterozygous and phenotypically either stable or ambivalent (Storey 1937, 1953; Hofmeyr 1938). Shorter day lengths and cool night temperatures induce sex reversal from staminate to

hermaphrodite flower set in male trees of subtropical South Africa (Allan *et al.* 1987) and Australia (Agnew 1941, 1954, 1968). In contrast, Nakasone (1967, 1986) regarded the effect of photoperiod as negligible and identified temperature and moisture stress as the main factors influencing sex reversal (see also Awada 1958). A later study also demonstrated the role of nitrogen nutrition in influencing the expression of either female or male functional flowers (Arkle and Nakasone 1984). While studies by Awada (1958) and Arkle and Nakasone (1984) were conducted on bisexual 'Solo' cultivars in Hawaii, studies conducted in South Africa and Australia were based on dioecious papaws. Whilst this difference may explain the discrepancy in obtained results, it is clear that sex determination is not a simple process. Sexual ambivalence of male and hermaphrodite trees is apparently partially determined by genetics. Similar suggestions have also been made by Prest (1955) and Nakasone (1986) to explain the rare incidences of sex reversal of female plants.

Sex reversal in a small number of inbred female trees was observed by Hofmeyr (1941). Storey (1976) comments on the unusual nature of such occurrences. Similar observations were made by Prest (1955) in subtropical Queensland, but unfortunately the author did not elaborate on seasonal or climatic influences. Prest suggests that sexual ambivalence is an inherent genetic characteristic assigned to individual papaw strains, as the lack of stability of flower types increased in successive progenies carrying such strain defects.

Sexual ambivalence is expressed as a higher frequency of distorted flowers of either sex form. The obvious signs are misshapen ovaries and anthers, which either are reduced in numbers and/or in size, or transformed into structures resembling carpels (Hofmeyr 1941; Storey 1958, 1969a).

It is only possible to determine the sex of papaws at the time of anthesis. Attempts to correlate characteristics of vegetative trees or of seeds to the sex of seedlings have so far failed (Singh and Sirohi 1977; Rojas *et al.* 1985; Sao Jose and Cunha 1988). Therefore, it is standard practice to plant four to eight times as many seedlings of dioecious lines per tree position as are required (Macleod and Perrett 1988; O'Hare 1993). Bisexual cultivars also require a greater seedling number at planting, as the following generation exhibits a sex ratio of 1 female : 2 bisexual flowering trees due to selfing. Female plants are disregarded commercially in Hawaii, for no other reason than their round fruit shape compared to oblong fruits of hermaphrodite flowers (Ito 1976; Storey 1976; Nakasone 1986).

The sex segregation of the progeny of matings between the three basic sex forms is listed in Table 1.1, (adapted from Storey 1938, 1976).

Table 1.1: Sex segregations from cross and self pollinations between the three basic *C. papaya* sex forms.

Crosses	Pistillate (P)	Staminate (S)	Bisexual (B)
P x S	1	1	-
P x B	1	-	1
S x S	1	2	-
B x B	1	-	2
B x S	1	1	1
S x B	1	1	1

1.1.3 Tree Habit and Flower Morphology

The dicotyledonous *C. papaya* is in strictly botanical terms a fast growing herb, as it does not produce woody tissue (Sturrock 1980). Plants grow in between two to ten metres in height. The large, lobed leaves radiate from the normally single trunked plant. Senescence of leaves is relatively fast, giving the plant a superficial resemblence to a palm.

Successive flower buds are inserted in the leaf axils at the apex of the plant. There appears to be little variation in flower morphology under normal conditions. Staminate flowers are borne on pendant like axillary inflorescences, whereas the pistillate and hermaphrodite flowers, mostly singular, are borne on short flower stalks. Subsidiary flowers can also occur (Figure 1.1).

Staminate flowers are small, 1 – 3 cm long, sessile, trumpet-shaped and of creamy or white colour. The calyx is cup-shaped and formed by fusion of five small, toothed or lobed green sepals.

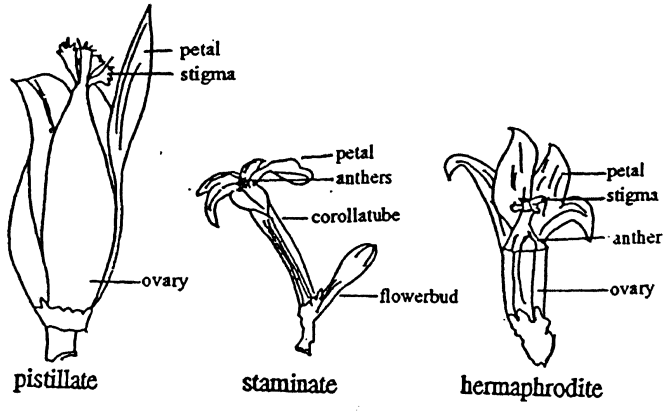


Figure 1.1: The floral structure of *C. papaya* flowers.

Petals are connate and form an elongate, slender corolla tube 2.5 cm long with five spreading lobes of approximately one-third the length of the tube. The ten stamens are inserted at the corolla throat in two alternating whorls. Filaments and anthers are woolly in appearance. Anthers are yellow, oblong, tetra-sporangiate and dithecal with pollen sacs opening by longitudinal slits. A rudimentary pistil exists at the base of the corolla tube (Purseglove 1974; Cronquist 1981).

Pistillate flowers are typically 3.5 – 5 cm long. The calyx is cup shaped, with 3 – 4 mm long teeth. The corolla is formed out of five almost completely free petals. Petals are lanceolate, fleshy and of creamy or white colour. The ovary is large; 2 – 3 cm long and consists of a central cavity with more or less deeply intruded parietal placentas. The stigma is sessile and deeply five-cleft. Ovules are numerous; usually several hundred up to two thousand seeds per fruit (Allan 1963a, 1969; Purseglove 1974; Cronquist 1981).

Fruits (berries) ripen over a period of five to eight months, depending on the climate (Allan *et al.* 1987; O'Hare 1993). Marketable fruit weigh from 0.5 kg – 2 kg and are 10 – 20 cm long, depending on the cultivar (Samson 1986). Plant life expectancy is about 25 years (Storey 1953; Purseglove 1974; Baker 1976). However, commercial plants are usually grown for two seasons and rarely more than three productive seasons as with the increase of plant height harvesting becomes uneconomical (Storey 1953; Agnew 1968; Samson 1986).

1.1.4 Scent

C. papaya flowers of all sex forms are fragrant, all emitting a similar scent. Various authors have tried to describe the scent; from "fragrant" (Purseglove 1974; Theakston 1976), "very sweet and powerful" (Jamieson and Reynolds 1967), "strong, sweet scent" (Baker 1976), to a "lily-of-the-valley" smell (de Wit 1966).

Components of the scent have not been identified (Bullock and Bawa 1981) and the scent glands have not been previously described. The onset of scent release coincides with flower opening at dusk (Sharma and Bajpai 1969; Purseglove 1974; Mekako and Nakasone 1975; Baker 1976; Subramanyam and Iyer 1986).

Although no specific details of either the scent components or the structure of scent glands of the Caricaceae are known, examples from other plant species demonstrate the importance of scents in biotic-mediated pollination systems. Some detailed research has been conducted particularly amongst the Orchidaceae, where 50% of

all species are estimated to employ some form of olfactory and/or visual 'deceit' mechanisms to attract pollinators to the non-reward offering plants (Wiens 1978). Remarkable examples of such 'deceit'-based pollination systems have been discovered among species of the European and North-African *Ophrys* orchids, which primarily attract their male hymenopteran pollinators with pheromone-mimicking substances. Upon attraction, secondary visual lures, which closely resemble the female gender of the approaching insect, stimulate pseudocopulatory behaviour and subsequently result in pollination (Kullenberg and Bergstrom 1975).

Despite the evidence that floral scents play a major role in biotic-mediated pollen transfer, generalizations on suspected plant-pollinator relationships solely based on scent components is not warranted, for reasons such as the by far more sophisticated olfaction sense of insects in comparison to humans (Kevan and Baker 1983) and the concentration of case studies in only a small number of plant families (Wiens 1978). However, the general concensus amongst pollination ecologists, is that scent signals emitted by plants and recognized by animals can be useful in understanding the pollination mechanisms of plants (Proctor and Yeo 1973; Faegri and van der Pijl 1979; Barth 1985).

1.1.5 Nectar

The Caricaceae have nectar-producing staminate and nectarless pistillate flowers according to Baker (1976), although the reverse is claimed by Crane and Walker (1984) and Caron (1985). However, neither of the latter authors support their statements. Nectaries are located distally between the rudimentary ovary and petal insertions (Baker 1976). Pistillate flowers lack nectariferous tissue and this correlates with the trend towards unisexuality; for details see Storey (1967, 1969a). Hermaphrodite flowers produce nectar (Free 1975; Crane and Walker 1984).

C. papaya and the related species *Jacaratia dolichaula* are the only species within the Caricaceae where nectar composition has been examined. Sucrose is the only sugar component in *C. papaya* nectar, whilst the nectar of *J. dolichaula* is a mixture of sucrose, glucose and fructose in the ratio of 55:32:12 (Table 1.2). The total nectar sugar concentrations range between 24 – 34% in *C. papaya* (Allan 1963c) and 23 – 30% in *J. dolichaula* (Bawa 1980b; Bullock and Bawa 1981).

The nectar quantity in *C. papaya* was described by Allan (1963c) as a "drop of nectar at the base of the corolla tube" and quantitative data from *J. dolichaula* indicates a range of 10 – 12 µL of nectar per flower (Bawa 1980b; Bullock and

Bawa 1981). Allan (1963c) states that environmental factors such as low temperature adversely affect nectar secretion. Other influencing factors on nectar secretion such as for instance humidity and soil moisture are discussed by Huber (1956).

Table 1.2: Analyses of the nectar of *J. dolichaula* and *C. papaya* and the stigma sap.

	Jacaratia dolichaula	*Carica papaya*	
	Nectar	Nectar	Stigma sap
Sugars			
Sucrose	54.4%	100%	—
Glucose	32.2%	—	—
Fructose	13.4%	—	—
Amino Acids			
Alanine	15.0%	7.7%	11.7%
Arginine	14.5%	21.2%	11.4%
Aspartic	—	—	1.1%
Cysteine, etc.	0.4%	4.5%	2.7%
Glutamic	8.8%	—	7.3%
Glycine	—	35.6%	15.7%
Isoleucine	0.3%	0.1%	—
Leucine	0.3%	—	—
Serine	49.0%	7.1%	2.6%
Threonine	9.1%	23.7%	40.1%
Valine	0.2%	0.1%	0.1%
β-alanine	—	0.2%	7.3%
γ-amino-butyric	2.5%	—	—

as in Baker (1976)

1.1.6 Amino Acids

While pollen constitutes the main source of amino acids for foraging pollinators, the nectar of some plants also contains amino acids. A study conducted by Baker and Baker (1975) on the amounts and kinds of amino acids present in nectars of a variety of plant species showed that flowers of herbaceous plants (for example *C. papaya* and *J. dolichaula*) contained higher amino acid levels in comparison to woody plant species. Other floral features, such as the form of nectar concealment at the bases of corolla tubes was also positively correlated with the amounts of amino acids present in their nectars. High levels of amino acids in nectars may constitute the only nitrogen source available in nectar feeding animals (such as Lepidoptera) and possibly could offer some clues to the nature of the pollination system of plants (Baker and Baker 1983). However, there is no convincing evidence to support a relationship between the levels of amino acids present in nectars of plants and their pollination system (Inouye and Waller 1984).

The nectar of *C. papaya* and *J. dolichaula* differs in the total number of the detected amino acids as well as in their individual amino acid ratios (Table 1.2). The significance and extent of such differences will only become evident with further study.

1.1.7 Stigmatic Exudates

The stigma surface of the genus *Carica* at the receptive stage has been described by Heslop-Harrison and Shivanna (1977) as "dry" meaning "little or no surface secretion" as well as possessing a unicellular papilla. Stigmas of similar age were described by Sharma and Bajpai (1969) as "moist and shiny" when observed under a handheld lens. Using fluorescence microscopy, Rodriguez Pastor *et al.* (1990), reported that the papilla is coated with a layer of cutin. There is no mention of surface secretion by the authors. Dry stigma types, according to Heslop-Harrison and Shivanna (1977), are generally associated with trinucleated pollen; an observation which is inconsistent with the binucleate pollen of *C. papaya* (see below).

A secretion of stigma sap from undamaged flowers of *C. papaya* was noted by Baker (1976) who locates its origin as a central, longitudinal, stigmatic canal. Constituents are a range of amino acids but sugars are absent (Table 1.2). Various functions of stigmatic exudates have been postulated, including being a part of a pollinators diet (Baker *et al.* 1973; Baker 1976) and/or to assist in pollen germination (Baker *et al.* 1973; Baker 1976; Bawa 1980b). Uniformity of stigma type at genus and at family level were documented by Heslop-Harrison and Shivanna (1977), and the presence of stigmatic exudates secreted in a central stigmatic canal was also noted in *J. dolichaula* (Bawa 1980b).

1.1.8 Pollen

C. papaya pollen grains are smooth, nearly spherical in shape and exhibit a median furrow (Sharma and Bajpai 1969). The size of a single grain varies in between 32 x 34 µm and 42 x 46 µm (width x length) depending on climatic conditions (Allan 1963a). Descriptions of the viscosity of the massed pollen range from the "sticky mass" (Allan 1963c) to "powdery" (Sharma and Bajpai 1969). In both instances, descriptions were based on visual observations alone.

Anthers dehisce about six hours prior to flower opening (Sharma and Bajpai 1969). Pollen is released towards the inside of the flower when anthers split

longitudinally from the apex downwards (Jamieson and Reynolds 1967). Each anther contains a maximum of 14000 pollen grains (Allan 1963a). The number of pollen grains is not correlated with the size of anthers (Allan 1963a). Pollen grains are binucleate when released (Cronquist 1981).

Pollen formation is initiated when flower buds reach a length of 70 – 85 mm. A South African study found that pollen develops over a period of 1 – 2 months depending on climatic conditions (Allan 1963a, 1963b). Climatic influences such as low or excessively high temperatures (5°C or 40°C) and low humidities adversely affect pollen quantity, viability and size (Prest 1955; Allan 1963a; Sharma and Bajpai 1969; Allan *et al.* 1987; Cohen *et al.* 1989). Allan (1963a) mentioned that abundant, well developed pollen was formed in the frost-free coastal areas of South Africa all year around, whilst under cooler subtropical conditions, pollen formation was inhibited at certain times of the year, especially in spring. Lower temperature regimes led to: a) decrease in anther size, b) discolouration of anthers from dark yellow to an off white to brown colour, c) breakdown in pollen formation and d) increased variation in pollen size (Allan 1963a). Similar results in a comparable climate in subtropical Queensland were recorded by Agnew (1948) and Prest (1955).

Adverse effects on pollen viability during the cold period under subtropical climatic conditions can be compensated for by applying pollen collected during the summer and early autumn period via hand pollination (Cohen *et al.* 1989). Pollen germination was satisfactory after storage of two months at 5°C, or six months at -18°C (Cohen *et al.* 1989). Other time intervals are recorded in literature but should be interpreted with caution since the viability test methods are not similar (Sharma and Bajpai 1969; Subramanyam and Iyer 1986; Cohen *et al.* 1989).

1.1.9 Receptivity of the Stigma

The stigma is receptive for one day prior to and three days after flower opening. Stigma receptivity is assumed to be associated with the secretion of stigmatic fluid promoting the germination of pollen grains (Baker 1976; Bawa 1980b). Receptivity is highest on the actual day of flower opening (Sharma and Bajpai 1969; Subramanyam and Iyer 1986). Tissue discolouration indicates the onset of senescence.

During low temperature regimes receptive and initiated ovules bear fruit with high seed numbers when pollinated with viable pollen; an indication of continuous

fertility and cold tolerance. Allan *et al.* (1987) and Cohen *et al.* (1989) base their comparable results on hand pollination studies carried out on orchard trees under Mediterranean and South African winter conditions of similar temperatures, averaging 20°C during the day, 10°C and 12°C during the night. In addition, Allan *et al.* (1987) support their findings with cytological data concerning the development of the embryo sac. All ovules collected post-anthesis apparently indicated normal development of the megagametophyte throughout the year.

Continuous female receptivity under the subtropical Australian climate of south and central Queensland was documented by Prest (1955). Unfortunately, the author does not specify relevant conditions at the time of observation. By contrast, a study undertaken on bisexually flowering papaws in Hawaii showed that warm temperatures, low rainfall and insufficient nitrogen nutrition can lead to female sterility due to the abortion of ovaries. The condition could be overcome by irrigation and fertilizer application (Arkle and Nakasone 1984).

1.1.10 Fertilization

Each seed is the successful outcome of one individually fertilized ovule (Agnew 1941; Free 1975). With possible seed numbers exceeding one thousand seeds per fruit, a comparable amount of pollen has to be deposited on the stigma surface (Agnew 1941; Allan 1963c). *C. papaya* has a seed capacity of appproximately 1800 ovules per fully fertilized fruit, but fruit on average contains about half that amount (Allan 1963a).

The germination and growth of the pollen tube is dependent upon temperature and humidity. *In vitro* results show that pollen tubes reached the base of the style after 10 – 12 hours at 20°C and 70 – 80 percent humidity (Cohen *et al.* 1989). However, ovule fertilization commences after a lapse of 10 days (Rodriguez Pastor *et al.* 1990) and is completed 13 – 15 days after pollination (Foster 1943). Low humidities (30 – 40%), high (40°C) and low (5°C) temperatures either prolong pollen tube growth or in the latter instance, lead to the complete inhibition of pollen germination (Cohen *et al.* 1989).

1.2 REPRODUCTION STRATEGIES

1.2.1 Apomixis

Reproduction in plants either occurs sexually or asexually. Sexual reproduction follows a definite sequential progression of cell formation and differentiation, cell selection and maturation, involving meiosis, mitosis and double fertilization essential for the production of viable seeds. The circumvention of one of these steps in sexually reproducing plants prevents the formation of seed. However, a number of flowering plants maintain the production of viable seed, despite bypassing one or more of the above key events, such as meiosis during megasporogenesis and consequently the fusion of female and male gametes. This process of producing asexual yet viable seed is known as apomixis. A detailed review on the subject has recently been published by Koltunow (1993).

The classical definition of apomixis includes all degrees of asexual reproduction in the angiosperms, either vegetatively or by means of seed (agamospermy; Asker 1979). The recently published literature (e.g. Asker and Jerling 1992; Koltunow 1993) indicates that the definition has become more specific and now includes only those asexual reproductive processes that occur in the ovules of flowering plants themselves giving rise to fertile seeds. Apomixis will be defined here as an asexual reproductive mode by means of seed, synonymous with agamospermy.

Depending on the tissue type from which the embryo is derived, apomictic reproduction can further be described into either sporophytic apomixis, synonymous with adventitious or nucellar embryony or gametophytic apomixis. In adventitious embryony the embryo is formed parthenogenetically from a somatic cell in the ovule outside of the embryo sac and in addition to the regular embryo. In gametophytic apomixis the embryo develops parthenogenetically from a cell (normally an egg cell, of an unreduced diploid embryo sac) following a defect in meiosis of the embryo sac mother cell. Depending on the origin of the embryo sac mother cell, gametophytic apomixis can be further divided into diplospory, where the embryo develops from an unreduced embryo sac, and apospory, where the embryo develops from a somatic cell (Asker 1979; Koltunow 1993). In apospory and adventitious embryony, sexual and asexual reproduction can coexist within the same ovule, which by definition is not possible with diplospory. Further, endosperm development, in apospory and adventitious embryony still depends on fertilization of the polar nuclei, of which diplosporous apomicts are independent (Koltunow 1993).

Apomictic reproduction is present in over 35 plant families encompassing an estimated 300 species over a wide range of taxa (Marshall and Brown 1981; Hanna and Bashaw 1987). It occurs most frequently within the Graminae, Asteraceae and Rosaceae (de Wet and Stalker 1974). Almost all apomicts are classed as facultative apomicts with coexisting sexual and asexual reproduction. Although morphological deviations between sexual and apomictic plants generally exist, quantification is virtually impossible (Gadella 1983). Facultative apomixis has been demonstated in geographically separated clones of the same species (Gadella 1987), within a plant species in the same location (de Wet and Harlan 1970), and where several embryos share the same ovary (de Wet and Stalker 1974). It also has been speculated that dual reproductive modes, such as the coexistence of apomixis and dioecy, possibly occur amongst rainforest species, although individual plant species have not yet been identified (Kaur *et al.* 1977; Bawa *et al.* 1985a). Obligate apomixis (continual asexual seed set) is however infrequent and is only known from 20 genera of the angiosperms. It is common in *Citrus*, *Opuntia* (Nygren 1967) and orchids (Asker 1979).

The chromosome number varies with generation cycles, usually incorporating diploid sexual and polyploid asexual phases (Asker 1966; Wagner 1970). Within the asexual cycle, aposporic and diplosporic plants are most commonly polyploids while adventitious embryony is normally associated with diploidy (Asker and Jerling 1992).

Apomictic behaviour is generally controlled by individual or groups of genes which induce the development of unreduced embryo sacs and the capacity for parthenogenesis (Grant 1971; Asker 1979). It is a frequent phenomenon amongst interspecific hybrids (Stebbins 1950; Grant 1971; de Wet and Stalker 1974). Environmental influences such as temperature and light regimes or interactions of both have also been considered important (de Wet and Stalker 1974; Asker 1980; Marshall and Brown 1981; Matzel 1982; Nogler 1984).

Apomictic reproduction offers a number of advantages, especially for agricultural crops. As apomictic embryos derive from maternal tissues only, the genetic information of the progeny and the parent is identical, except for incidences involving mutation (Maynard-Smith 1978; Hartmann and Kester 1983; Koltunow 1993). Apomictic reproduction also results in complete femaleness of all progeny; an occurrence otherwise only rarely achieved, for example when the embryo arises directly from the haploid egg nucleus (Hartmann and Kester 1983). At present, incidences of agamospermy of agriculturally important plants are uncommon,

except for *Citrus*, *Malus* and several species of Graminae where it occurs naturally (Hanna and Bashaw 1987). Genes linked to apomictic reproduction have not yet been isolated (Koltunow 1993).

Apomictic seed development in *C. papaya* remains a possibility, based on the report by Badillo and Micheleti de Zerpa (cited in Badillo 1971) "who claim to have demonstrated 'parthenogenesis' in a pistillate tree of *C. papaya* in Venezuela" (Baker 1976). Tests for apomixis undertaken on *J. dolichaula* show one successful fruit set with seeds derived from sixteen flowers bagged prior to anthesis (Bullock and Bawa 1981). However, the authors, did not comment on whether or not an embryo was present. Seed set was also reported from an isolated pistillate tree of the related species *J. spinosa* in its native rainforest environment in Costa Rica (Baker 1976). However, all seeds lacked embryos. This strongly indicates existence of parthenocarpic development of fruit in *C. papaya.*

Parthenocarpy is defined as fruit development without the formation of seeds resulting either from a lack of pollinators, failure of fertilization or failure of the embryo to develop. Parthenocarpic fruit set in *C. papaya* has often been reported from various geographical locations of tropical and subtropical distribution such as South Africa (Hofmeyr 1938; Allan *et al.* 1987), the United States of America (Traub *et al.* 1942, Mc Gregor 1976), Israel (Cohen *et al.* 1989), the Philippines (Ordonyo 1959) and Australia (Prest 1955).

Seedlessness generally results in underdeveloped fruit which in most instances reach walnut size, turn yellow, shrivel and abscise (Traub *et al.* 1942; Prest 1955). Parthenocarpic fruit ripens rarely (Agnew 1954; Ordonyo 1959) and the fruit is of small size, thinly fleshed and has an oblong pointed appearance (Foster 1943; Anon. 1987). Seedlessness has also been associated with poor flavour (Anon. 1987). Pollination with non-viable pollen during winter can initiate parthenocarpic fruit set, which indicates that pollination has a stimulating effect on fruit set without the necessity for fertilization (Cohen *et al.* 1989).

1.2.2 Anemophily

It has been stated by various authors that *C. papaya* relies solely on wind pollination (Agnew 1941, 1968; Prest 1955). Prest reached his conclusion after observations in a South Queensland papaw plantation where he described that on "a

fine summers day the air...is laden with pollen grains", enough to ensure successful pollination. Later, Agnew (1968) proposed planting male trees on the eastern side of plantations to guarantee pollen drift with the prevailing wind direction. There are however a number of reasons for questioning these conclusions.

It is generally considered that 10 percent of male trees in a plantation is sufficient to provide a pollen source and this is still standard orchard practice (Agnew 1941; Prest 1955; Jamieson and Reynolds 1967; Free 1970; Anon. 1981). Variations from the male to female ratio do exist, ranging from a reduced ratio of one male to eight female trees (Baxter and Tankard 1990) to one male and 20 or 25 female trees (Samson 1986). Not one of all the proposed ratios has been scientifically evaluated. Insufficient pollination and fruit abortion have been associated with stands of isolated females only (Nakasone 1986).

Anemophilous species generally show similar characteristics of geographical distribution, floral morphology, timing and mechanism of pollen release, dispersal and capture, and a seasonal flowering pattern (Whitehead 1969, 1983; Bawa and Crisp 1980; Niklas 1985). Self compatibility, monoecy and the clumping habit of conspecifics have also been associated with wind pollinators (Niklas 1985). The significance of these factors is developed further below.

The frequency of wind-pollinated plant species increases with higher latitude and altitude. Other parameters include reduced species diversity, deciduous habit, closeness of compatible species and distinct photoperiod and temperature regimes which initiate synchronous flowering (usually before leafing), (Whitehead 1969, 1983; Proctor and Yeo 1973; Faegri and van der Pijl 1979; Harrington 1979; Regal 1982; Niklas 1985). Wind pollination is successful, when either the species number is small (savannas, gymnosperm forests) or the wind velocity is high (islands, montane environments); conditions which generally decrease towards the equator. Ashton (1969), for instance, mentions that only a single species of 760 surveyed tree species is wind-pollinated within the tropical rainforest of Brunei. This particular species is distributed on elevated ridges only. Similar low ratios of 1 – 2 % anemophily have been estimated amongst the approximately 400 tropical wet lowland rainforest species in Costa Rica (Bawa and Crisp 1980; Bawa *et al.* 1985a, 1985b) with a slightly increasing ratio of 4 % towards semi deciduous forest belts (Daubenmire 1972). The latter increase of wind-pollinated species is associated with lower species diversity, deciduous habit and synchronized flowering of conspecifics in response to the dry season.

Records of anemophily in rainforests and in particular in the understorey are scarce (Janzen 1975; Bawa and Crisp 1980; Bawa 1990). Environmental factors such as high levels of humidity, rainfall, low wind velocities within the subcanopy and understorey levels and the scattered distribution of conspecifics hinder the transfer of pollen by either pollen aggregation or depletion due to washouts (Whitehead 1969, 1983; Harrington 1979; Hubbell 1979; Regal 1982). Despite such unsuitable conditions, rare cases of wind-pollinated species have been discovered, for example *Trophis involucrata* (Moraceae), a dioecious understorey tree of the South American tropical rainforest (Bawa and Crisp 1980). The reproductive success of this species relies on an aggregated distribution pattern and the release of pollen for a short interval of time during the lowest annual rainfall period (Bawa and Crisp 1980). Although a similar clumping distributional pattern has also been recorded for *J. dolichaula* and *C. cauliflora* (both Caricaceae) in their native environment in Costa Rica, the characteristics of pollen and floral morphology are atypical of an anemophilic pollination system (Baker 1976; Bawa 1980b).

Adaptations in flower morphology, including the position of insertion of sexual organs, have been recognized amongst anemophilous plant species since these features differ from animal-pollinated plant species. Floral parts of wind-pollinated flowers are reduced in size and number, and do not interfere with pollen release and capture. Flowers also lack scent and nectar and are often of inconspicuous colour, for example, the Graminae (Proctor and Yeo 1973; Faegri and van der Pijl 1979). In anemophilous plants, reproductive organs are generally situated near the apex on the plant axis (Whitehead 1983). Sexually related differences of higher inserted staminate and lower pistillate reproductive organs facilitating pollen capture have been noted from some gymnosperm species (Fowells 1965; Niklas 1985). A study by Allan *et al.* (1987) from South Africa indicates that papaw also shows a sex linked variation in tree height. Papaw plants were tested under various environmental conditions, for example "cool" (20°C day; 12°C night), or "very hot" (30°C day; 20°C night) temperature regimes, and in "glasshouse" and "shadehouse" environments. In all instances male trees grew taller than females. By contrast, Bullock and Bawa (1981) report no significant difference between the heights of female and male *J. dolichaula* trees in their natural environment in Costa Rica. Further, an observation made by Free (1975) indicates that within a papaw plantation, fruit set did not appear to be increased on female trees adjacent to male trees. Gravity, as a mechanism of pollen transfer, has been considered and then disregarded by Allan (1963c), who furthermore excludes anemophilic pollen transfer.

The pollen grains of wind-pollinated species show certain similarities in morphology and in size. Extremes of pollen grain diameters range from 3 µm in the anemophilic "forget-me-not" to 250 µm in the entomophilic pumpkin (Barth 1985). However, grains of wind-pollinated species are normally of small size, ranging between 20 – 60 µm in diameter (Whitehead 1969, 1983; Niklas 1985). Furthermore, the exine (or outermost surface layer of the pollen grain wall) of wind-pollinated species is generally smooth, thin and free of any attachments. Depending on the plant species the exine often incorporates air sacs beneficial to longrange pollen dispersal (Whitehead 1983; Niklas 1985). By contrast, entomophilically distributed pollen is characterized by spiny and/or viscous exines which assist in the attachment to the pollinating vector. Pollen is generally of larger size (Barth 1985). The pollen grains of *C. papaya* are approximately 25 µm in diameter and the exine is smooth, thin and free of any attachments, but is however viscous (Allan 1963a). The existence of pollenkit suggests *a priori* that papaw is not wind-pollinated, but some of the other features are less clear-cut.

Generally the concentration of airborne pollen in anemophilic species is proportional to pollen quantity and inversely proportional to wind turbulence, wind velocity, and the distance between pollen source and capture site (Whitehead 1969). Wind turbulence keeps pollen grains airborne but at the same time lowers their concentration. The release of pollen normally coincides with higher temperatures and/or lower humidities, which generally coincide with conditions of moderate turbulence and adequate wind (Whitehead 1969; Niklas 1985).

Overall, pollen transport in anemophilic plants depends on the interactions between two key variables; the wind velocity and terminal velocity of the pollen grain. The terminal velocity is a function of the diameter and density of the pollen grain and published results indicate that the settling rate of grains is normally between 2 – 6 cm/sec (Whitehead 1969, 1983). The release of pollen normally occurs during the daytime and coincides with rising temperatures, decreasing humidity and increasing wind velocities (Bawa and Crisp 1980; Whitehead 1983).

The capture of pollen depends on many variables but overall the efficacy of pollen collection is proportional to the diameter of the collecting object (Whitehead 1969, 1983; Cruden and Miller-Ward 1981; Niklas 1985). Stigma surfaces enlarged by multiple substructures, such as the feathery stigmas of the Graminae, increase the rate of pollen capture in comparison with a simpler but less complex surface area of similar magnitude (Whitehead 1969). The surrounding vegetational structure also

influences the rate of successful pollen capture, as pollen losses escalate with increasing plant density (Whitehead 1983). The ovaries of wind-pollinated species bear a single or reduced number of ovules, presumably ensuring successful fertilization (Whitehead 1969; Niklas 1985).

The reproductive success of a relatively passive pollination process based on abiotic pollen transfer by wind currents involves the production of abundant amounts of pollen to counteract for undirectionality in pollen capture. The simultaneous release of pollen in so-called 'pollen clouds' increases the chance of a successful encounter of female receptive parts with male microspores (Whitehead 1969; Bawa and Crisp 1980). By contrast, pollination systems which depend on biotic-mediated pollen transfer are generally associated with a lower reproductive investment of the male sex, indicated by a narrower pollen : ovule ratio (Bawa 1980b). Overall, *C. papaya* does not match well with the characteristics typically associated with anemophilous plants.

1.2.3 Entomophily

Although pollination is often essential for reproduction of the plant, it is merely a coincidental event for the animal involved. Generally, animals visit flowers for pollen, nectar and other food sources and in so doing effect pollination. Barth (1985) defines this as "mutualism" in the sense that both species benefit from the interaction (but for quite different reasons).

Pollination by animals is predominantely a phenomenon of the tropics (Janzen 1975; Hubbell 1979; Regal 1982). Bawa (1990) estimates that up to 99% of all flowering plant species in the tropical lowland rainforests of Costa Rica are animal pollinated. Insects are by far the largest group of pollinators (Faegri and van der Pijl 1979; Barth 1985) and constitute the predominant pollinator group of plants breeding by dioecy (Bawa 1980a).

Floral features such as petal colour, flower size and odour are important in flower recognition by pollinators and such features can be used in predicting the pollination mechanism in a plant taxon. The classical example was the prediction made by Darwin (1859) that the Madagascan orchid *Anagraceum sesquipetale* with a spur length of about 30 cm would be pollinated by an insect of comparable proboscis length. Forty years later *Xanotophan morgani*, a hawkmoth, which feeds on the nectar of this species and thereby pollinates it, was discovered; a remarkable example of coevolution and scientific prediction (Barth 1985).

Complex flowers demand pollinators such as bees, hawkmoths and hummingbirds which have sufficient capacity for learning to extract the hidden rewards (Leppik 1957; Kevan 1984). In some plant families the pollination system relies on 'deceit'; for example the relationship between orchids and their predominantly hymenopteran pollinators (Dodson 1975; Wiens 1978). Pollination achieved by 'deceit' based on for instance floral features, or by 'mistake' of the insect, are not uncommon but are limited to a relatively small number of plant species (Dodson 1975; Baker 1976).

While Prest (1955) suggested that *C. papaya* is wind-pollinated, various other observations suggest (at least circumstantially) the involvement of insects. Scattered observations of floral characteristics and studies of potential pollinators have been recorded (Table 1.3), however results remain inconclusive. The floral characteristics of papaw circumstantially suggest entomophilic pollen transfer for the following reasons: the timing of anthesis, the emission of scent, the production of nectar of staminate flowers and the presence of brightly coloured petals (Baker 1976). Records of pollinating insects are diverse but remain inconclusive, except for that of Baker (1976). The possibility of insect-mediated pollination has been suggested by various authors based on: a) observed insect activity on solely staminate flowers, b) insect presence within the orchards and c) floral characteristics. The following examples indicate the nature of some of these studies:

> "Insects were rarely seen visiting female flowers, but nevertheless one must still conclude that they affect pollination" (Allan 1963c);
>
> "I observed honeybees visiting staminate flowers", recommending these insects as pollination vectors (Caron 1985);
>
> "observations at dusk confirmed that...each male tree was being visited by about three Skipper butterflies, *Perichares philetes philetes* (Gmelin). These butterflies also visited hermaphrodite flowers. Their numbers and behaviour indicate that they would be effective pollinators if the need arose" (Free 1975);

and for the related species *J. dolichaula* :

> "But even though moths (Sphingidae) were not observed on female flowers...these are probably the pollinators of the species" (Bawa 1980b);

Traub *et al.* (1942) as cited by Baker (1976) based their conclusions of insect visits exclusively on observations of staminate flowers. In general there is a lack of rigour in these studies.

Table 1.3: Insect visitors and potential insect pollinators of *C. papaya.*

Species	Author	Country of Observation	Animals observed contacting staminate and pistillate C. papaya flowers or staminate flowers only		
Carica papaya	Allan 1963c	South Africa	Hymenoptera	Apidae	*Aphis mellifera* *
			Hymenoptera	Xylocopidae	*Xylocopa* sp.
			Lepidoptera	Sphingidae and others	
			Lepidoptera		Butterflies
	Baker 1976	Ghana			Sunbirds
			Hymenoptera		Bees
			Lepidoptera		Butterflies
			Lepidoptera	Sphingidae	
		Mexico			Hummingbirds
			Diptera	Culicidae	Toxorhynchites
			Diptera	Ceratopogonidae	Midges
		Costa Rica	Hymenoptera	Apidae	*Trigona* sp.
			Hymenoptera	Apidae	*Aphis mellifera*
			Diptera	Syrphidae	
			Lepidoptera	Nymphalidae	*Anartia fatima* (Fabr)
			Lepidoptera	Pieridae	*Phoebis sennae* (L.)
			Lepidoptera	Geometridae	
			Lepidoptera	Sphingidae	*Hyles* sp.*
	Caron 1985	Puerto Rico	Hymenoptera	Apidae	*Aphis mellifera* *
	Free 1975	Jamaica	Lepidoptera	Hesperiidae	*Perichares philetes philetes* *
	Hofmeyr 1938	South Africa	Thysanoptera	Thripidae *	
	Marin Acosta 1969	Venezuela	Thysanoptera	Thripidae	*Frankliniella* sp.
			Lepidoptera	Hesperiidae	
			Lepidoptera	Pieridae	*Phoebis sennae* (L.)
			Coleoptera	Anthicidae	*Acanthinus aequinoctialis* (L.F.)
			Coleoptera	Curculionidae	
			Coleoptera	Elateridae	
			Diptera	Syrphidae	*Mesograpta watsoni* (Curran)
			Hymenoptera	Apidae	*Trigona jaty* Smith
			Hymenoptera	Apidae	*Trigona (Nannotrigona) testaceicornis* var. *punctata* F. Smith
			Hymenoptera	Apidae	*Trigona trinidadensis* Provancher
			Hymenoptera	Apidae	*Trigona* (Plebeia) sp.
			Hymenoptera	Formicidae	*Brachymyrmex* sp.
			Hymenoptera	Formicidae	*Monomorium floricola* (Jerdon)
			Hymenoptera	Formicidae	*Pheidole* sp.
			Hymenoptera	Formicidae	*Solenopsis* (Diplorhoptrum) sp.
			Hymenoptera	Xylopidae	*Xylocopa* sp.
	Traub *et al.* 1942	South East U.S.A.	Lepidoptera	Sphingidae *	
	Stambaugh 1960	South East U.S.A.	Lepidoptera	Sphingidae *	
Jacaratia dolichaula	Bawa 1980b	Costa Rica	Lepidoptera	Sphingidae *	
	Bullock and Bawa 1981	Costa Rica	Lepidoptera	Sphingidae *	

* potential pollinating agents or proposed pollinators of *C. papaya*

Overall, observations of the activity of insects concentrate on staminate flowers and only on rare occasions have insects been observed visiting pistillate flowers (Allan 1963c; Baker 1976; Caron 1985). Baker (1976) noted the presence of mosquitoes and midges on the stigmas of pistillate flowers. The mosquitoes (Culicidae: Toxorhynchites) were also found inside the corolla tube of staminate flowers, although the frequency of this observation was not recorded. The same author documented the involvement of hawkmoths (Sphingidae) in the transfer of papaw pollen, suggesting a species of '*Hyles*' visiting male and female flowers at dusk. This observation was made in Costa Rica within the area of origin of *C. papaya*. Moths were present over a half hour period at dusk, and collected nectar whilst in hovering flight, visiting staminate flowers and making short 'mistake' visits to the pistillate papaw flowers. Baker observed ten such 'mistake' visits over a 25 minute period but did not indicate the number of sphingids involved.

Sphingids also have been mentioned as the pollinating vectors of *C. papaya* in the south east of the United States of America (Traub *et al.* 1942). However, the authors based their observations only on staminate flowers. Based on Traub's *et al.* (1942) conclusion, Stambaugh (1960) later issued the generalization that sphingids are the sole pollinating agents of papaya. Allan (1963c) examined the pollination of papaws in South Africa and concluded that the size of the potential pollinators had to be larger than 16 mm square mesh, since this prevented female trees from setting fruit. He suggested that honey bees where involved, despite the fact that bees were only observed foraging on staminate flowers.

So called 'mistake' pollination or pollination by 'deceit' has often been resorted to in attempts to explain papaw pollination (Baker 1976). A number of such 'mistake' plant-pollinator interactions have been documented, including various bee species (Bawa 1977) and sphingid moths (Baker 1976) amongst others. Insects, in these cases, are supposedly misled into visiting pistillate flowers, either by similar scent emission and petal colouring or misconception of intersexual flower morphologies. No obvious reward is offered to the pollinator (Bawa 1977, 1980b; Bawa and Bullock 1981). Bawa (1980b), for instance, suggests from studies undertaken on *J. dolichaula*, that the petal outline of nectar-producing staminate flowers resembles the shape and size of the stigma outline of nectarless pistillate flowers. Apparently pollinators are unable to discriminate between the resembling floral parts of male and female *J. dolichaula* flowers. According to Bawa (1980b), a pollination mechanism based on mimicry of floral parts of non-reward offering

flowers with reward offering flowers may be widespread among monoecious and dioecious plants such as *Carica* and *Jacaratia* species.

Generally, plants have pollinators belonging to more than one species and sometimes more than one order, and the effectiveness of pollination varies accordingly (Bertin 1989). However, in the moist tropics, where *C. papaya* originates, the large number of animal species is coupled with greater specialization in plant-pollinator relationships (Heinrich and Raven 1972). Generally insects visit a more diverse range of plants than plants receive pollinators (Heinrich and Raven 1972). In summary the literature documents a confusing and conflicting record of insect pollination in papaw.

1.3 FRUIT SET IN TROPICAL AND SUBTROPICAL CLIMATES

C. papaya is a tropical plant species in its native habitat but nowadays is grown commercially in areas well outside the tropics. A number of authors note that fruit set of either dioecious or bisexual papaws is reduced during the colder season in subtropical climates, to the extent that in some instances it does not occur at all. Data are available from Israel (Cohen *et al.* 1989), South Africa (Malan 1964; Allan 1976; Allan *et al.* 1987), the United States of America (Stambaugh 1960) and Australia (Prest 1955; Agnew 1968). Dioecious papaws are more tolerant to subtropical conditions than bisexual clones, which are of purely tropical distribution.

The first established papaw plantations in Queensland were in the south and central coastal districts. Glennie and Chapman (1976) state that 90% of the whole Australian production in 1971/72 was of subtropical distribution. The closeness to the Brisbane markets was advantageous for a fruit of soft texture and short shelf-life. More recently, cultivation has shifted towards the northern tropical climates around Innisfail and Cairns.

Low temperature regimes are associated with inadequate fruit set in *C. papaya* due to lack of pollination and/or fertilization and this is thought to influence the following:

1. time for fruit to reach maturity
2. fruit size and shape
3. fruit taste
4. intensity of flesh colour
5. flesh thickness

Poor fruit set is argued by some authors to be mainly caused by inadequate pollination (Prest 1955; Agnew 1968). Disrupted pollination is considered one of the major problems in cultivation of dioecious papaw cultivars in the central and southern coastal papaw growing districts in Queensland (Agnew 1941; 1954). Although papaws of both sexes flower continously, pollen quantity and pollen viability are impaired during certain times of the year either when average monthly temperatures fall below 14°C – 17°C (Allan 1976) or when temperature amplitudes are wide (Prest 1955). Such climatic conditions occur in the southern and central regions of Queensland annually during the spring and autumn (Prest 1955). Pollen subsequently remains either undeveloped or non-viable (Agnew 1941; Allan 1976; Cohen *et al.* 1989). Similar climatic influences disrupting the events of pollen formation were found in the subtropical conditions of South Africa (Allan 1963a; Allan *et al.* 1987) and Israel (Cohen *et al.* 1989). Although pistillate flowers are less climatically affected and remained fertile when average minimum temperatures decreased below 10°C, fruit set nevertheless declined, due to unsuccessful fertilization (Prest 1955; Allan 1976; Allan *et al.* 1987; Cohen *et al.* 1989). Conditions of fruitlessness in papaw can also result from inadequate nutrition and from soil moisture stress which in most instances leads to premature flower abortion (Agnew 1968; Ong and Kwok 1983). In some instances it may be an inherent characteristic of the plant (Prest 1955).

Fruit maturation can take from 4 – 9 months depending on the time of flower opening. Fruits of flowers that set in early summer ripen quicker than those which set during the late summer and autumn periods. Commercially there are two distinct harvesting peaks for central and southern Queensland conditions; fruit that ripens during May/June is produced by flowers which opened during November/December, whereas the August/September crop is produced by late summer flowers (Agnew 1954; O'Hare 1993). By contrast, papaws grown under tropical northern Queensland climate crop almost continuously (Agnew 1968).

The size, shape, taste, colour and thickness of flesh in fruit are indirectly influenced by colder climate. The number of smaller and misshapen fruits with less or no seed set increases during these conditions, whereas the larger fruit are harvested from fruits flowering during early summer (Allan 1976). Although selected clones of improved taste and deeper flesh colouration do exist, these quality parameters are improving with increased seedset. This also positively affects the flesh thickness (Agnew 1954; Malan 1964).

Although not all authors support the concept of insect pollination, those who do note that climatic conditions such as an increase in cloud cover or in wind velocity, high and low temperatures, adversely affect insect activity and, as such, pollination. Allan (1963c), who suggested honey bees may be pollinators, mentions that their activity naturally ceases with the onset of winter in South Africa. Stambaugh (1960) who proposed hawkmoths as pollinating vectors, notes the different fruit size in papaya grown in Florida but does not indicate the relationship to seasons. Supporters of wind pollination suggest that inadequate fertilization for that particular period is due to the intervention of wet weather (Prest 1955) and higher levels of humidity (Malan 1964). Experimental data show that relatively small rain drops can deplete airborne pollen grains in a relatively short time span (Harrington 1979), and increased humidity levels lead to the rupture of pollen grains due to excessive moisture uptake (Percival 1965).

1.4 SUMMARY

Information outlined in the previous pages concerning the pollination of *C. papaya* indicates the vast array of pollination mechanisms that have been proposed over the last half century. All of these studies are either inconclusive or inadequately supported by comprehensive field observations or experiments.

Sexual reproduction, in which the transfer of pollen is apparently achieved by either abiotic means of anemophily (Agnew 1968) or by biotic means of entomophily (Allan 1963c; Baker 1976) have been proposed alongside asexual/apomictic reproductive mechanisms (Free 1975). Separation of sex (dioecy) which normally is encountered amongst anemophilic plant species, combined with floral features characteristic of insect pollination, such as scent and nectar production, have left observers puzzled. Authors generally agree that insects rarely visit the pistillate flowers of papaw; either in its endemic habitat or in countries where it is grown as an introduced species. Of all available literature searched, the one account where papaw flowers of both sexes were visited by insects, is that of Baker (1976). He proposed that sphingid moths, apparently a species of '*Hyles*' were the natural pollinators of *C. papaya* in Costa Rica. Allan (1963c) suggested that honey bees were pollen transfer agents in South Africa, while in Australia anemophily is seen as the primary mode of pollen transfer; a view generally unchanged and unchallenged since the late 1960's.

The lack of scientific rigour evident in virtually all published results, the multitude of pollination mechanisms and vectors suggested, and the limited and outdated information available, especially for the Australian industry, all suggest the urgent need for a thorough scientific investigation. This study investigates the areas of specification of pollination mode and its influence on variations in fruit set during the flowering season in subtropical central Queensland.

CHAPTER II

STUDY SITE DESCRIPTION

2.1 CLIMATE

The central Queensland region is located between the latitudes 20° and 25° South and the longitudes 146° and 151° East, with Rockhampton as the regional centre on the Tropic of Capricorn. The rainfall and temperature data were obtained from the Bureau of Meteorology in Rockhampton.

2.1.1 Rainfall

The regional climate can be described as subtropical and subhumid. Rockhampton receives on average 843 mm of rain annually (Table 2.1). The frequency of rainfall is highest during the warmer months of summer (December – March) with January/February usually being the wettest months (Figure 2.1). Thunderstorm activity during spring (September – December) provides up to 80% of the spring average rainfall; although its unpredictability causes high variability between years. The same pattern exists for the summer monsoons, which cause rainfall variation during the summer period.

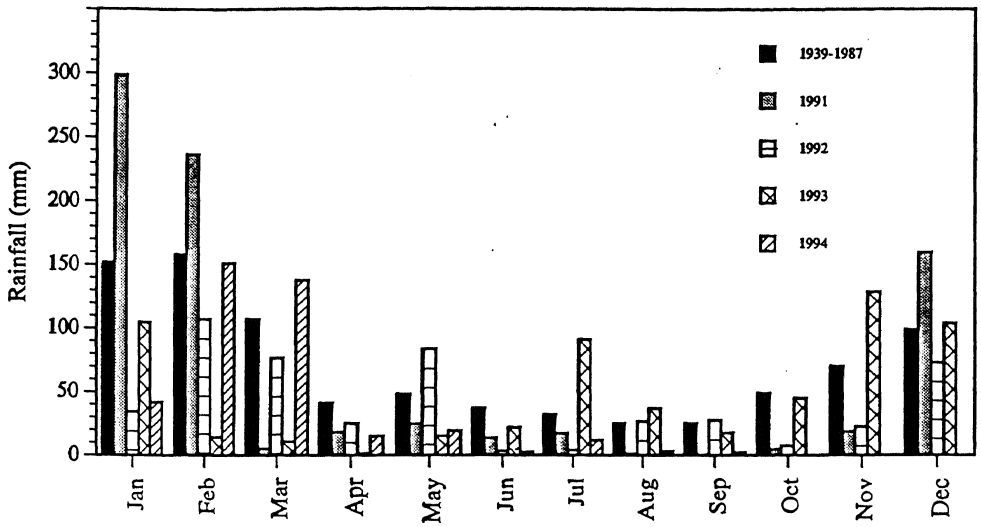
Table 2.1: The total annual rainfall (1991 – 1994) and the longterm annual rainfall mean (1939 – 1987) in Rockhampton.

Year	Rainfall (mm)
1939 - 1987	843.0*
1991	797.4
1992	490.2
1993	589.6
1994	519.2

* 48 year average

Since the flooding of the Fitzroy River in December 1991 and January 1992, rainfall has been below average from March 1992 onwards until December 1994 (Figure 2.1). The rainfall figures show that monsoonal activity during the summers of 1992 and 1993 was low. Although summer rainfall was received late

in 1994 during February and March, the drought since then has continued, making this overall the driest 45 month period on record over the last 95 years until December 1994.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall Mean (mm) 1937 - 1987	152	158	107	41	48	37	32	25	25	49	77	99

Figure 2.1: The total monthly rainfall in 1991, 1992, 1993 and 1994 in comparison to the longterm monthly rainfall means in Rockhampton from 1937 until 1987.

2.1.2 Temperature

The 47 year average maximum temperature in summer in Rockhampton is 31.5°C which decreases to 23.6°C in winter (June – September), while the average minimum temperature during summer is 21.6°C and during winter is 9.9°C. Incidences of frost do occur at the rate of 2 to 3 nights during each of the winter months.

Maximum temperatures during the years of 1991 until 1994 have either met or have been above the long term average; see especially temperatures during the winter periods (Figure 2.2). While 1993 experienced a mild autumn and winter, which is reflected in an average temperature increase of 3°C during the winter season, average winter temperatures during 1992 and 1994 have been closer to the longterm means.

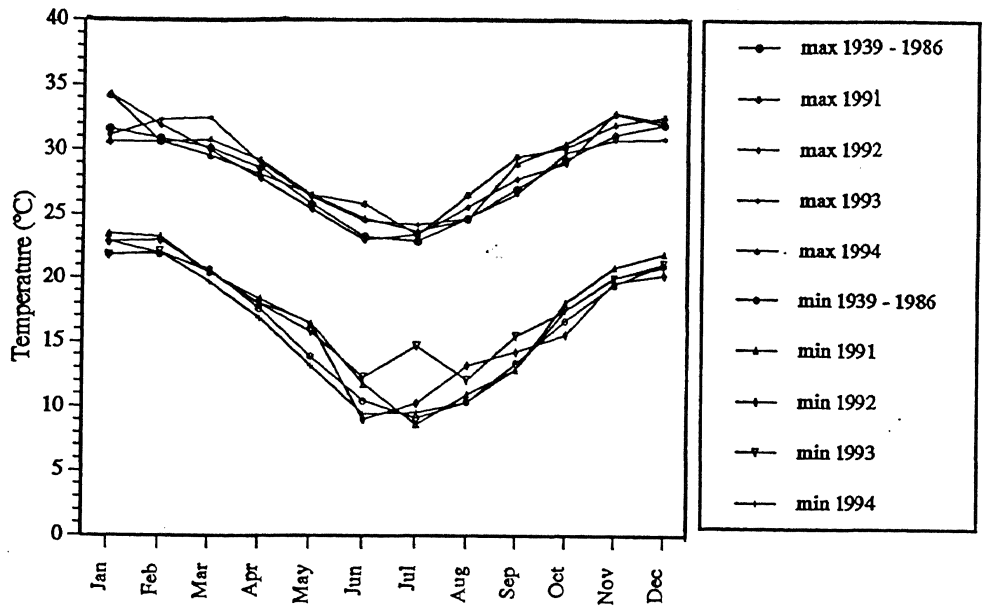


Figure 2.2: Means of minimum and maximum monthly temperatures in Rockhampton in 1991, 1992, 1993 and 1994 in comparison to longterm monthly temperature means in this region from 1939 until 1986.

2.2 DESCRIPTION OF ESTABLISHED ORCHARD SITES

The following paragraphs provide an overview of the experimental design, cultivation practices and location of orchard sites established during the study.

2.2.1 Parkhurst Orchard

Papaws of three different lines (Hybrid 29, Hybrid 1D and siblings of an open pollinated line) most commonly used in central Queensland papaw production were planted as four week old seedlings in Parkhurst (Figure 2.3) on the 2 October 1991. Each line consisted of 30 plants. Seedlings were planted in six rows (plots). Each row consisted of three blocks of five plants per line. Blocks were randomly assigned to rows.

Papaws were planted into top soil, which was mounded into 30 cm high beds. Plants were positioned 1.5 m apart and a trickle irrigation system was installed. Beds were mulched with a 5 cm layer of straw. The nutrition management consisted of a complete fertilizer ('Complete Blau + TE') which was applied in accordance with Queensland Department of Primary Industries (Q.D.P.I.) recommendations (Macleod and Perrett 1988). The orchard remained unsprayed. Plants started flowering after five months, beginning at the end of January 1992.

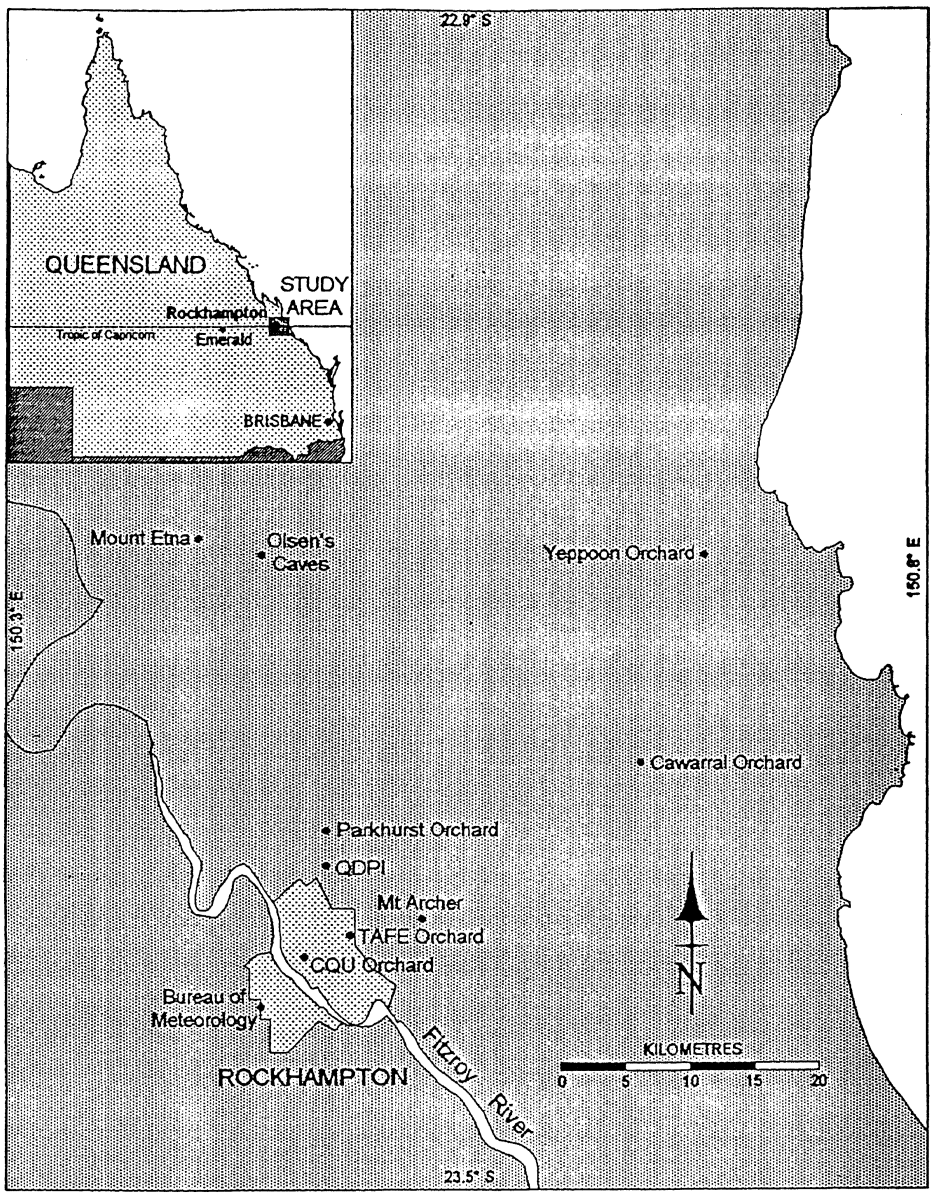


Figure 2.3: Location of study sites in the Rockhampton area. (The map indicates the location of all orchards where sampling was conducted and locations used for hawkmoth observation and collection)

2.2.2 Central Queensland University (C.Q.U.) Orchard

Papaws of the same three lines (Hybrid 29, Hybrid 1D and siblings of an open pollinated line) were planted as four week old seedlings in the orchard of Central Queensland University on the 15 September 1992 (Figure 2.3). Plant material was derived from the same seed collection as previously used at Parkhurst. Each line again consisted of 30 plants. Soil quality, planting procedures and layout, irrigation and nutrition management were identical to the Parkhurst site. Plants started flowering at the end of December 1992.

2.2.3 T.A.F.E. Orchard

In September 1993 papaw seedlings were planted in the Horticulture campus at T.A.F.E., Rockhampton (Figure 2.3). Seedlings consisted of ten trees of the following lines: ER62, JDM362, JDM411, JE1, RO2, TVL7, WH1 and Sunrise Solo, except for the latter where only two trees were available. Seedlings were planted in a completely randomized design. Planting procedures, irrigation and nutrition management were the same as described for the previous sites. Papaws started flowering in January 1994.

CHAPTER III

ANEMOPHILY

3.1 INTRODUCTION

That wind may be involved in the transfer of *C. papaya* pollen was originally suggested by Prest (1955) and Agnew (1968), both of whom worked with papaws grown under subtropical climates in Australia. Prest (1955) described visible pollen clouds during the summer period in a southern Queensland orchard. In addition, most papaw growers appear to believe that pollination is the result of wind dispersal and manage their crops accordingly. However, quantification of anemophilic pollen transfer in papaw and confirmation on its actual occurrence has never been carried out scientifically. A series of experiments was undertaken to determine whether or not *C. papaya* pollen grains are airborne and whether anemophily should be considered as a mode of pollen transfer. Investigations were carried out at various periods throughout the year. Seasonal fluctuations of pollen quantity (Section 4.3.3.2) were considered in the interpretation of results.

In all the following experiments the investigation of anemophilic *C. papaya* pollen transfer was based on the capture of grains on standard glass slides (HD Scientific Supplies, Sydney) greased with vaseline. Slides (75 mm x 25 mm) were examined microscopically for the presence of pollen grains. Papaw pollen grains were easily identified because of their distinctive size, shape and colour.

3.2. MATERIALS AND METHODS

3.2.1 Fixed Slides

During two weeks in spring 1991 a series of standard (i.e. all had the same chance of catching pollen) collection slides coated with vaseline were positioned on the trunks of female trees in an established commercial orchard at Cawarral (Figure 2.3). The orchard consisted of a range of different hybrid lines and open pollinated siblings grown on approximately three acres (2000 plants).

Slides were placed with their surfaces in both horizontal and vertical positions adjacent to a receptive pistillate flower on five randomly chosen trees during two sampling intervals; from the 30 September – 5 October 1991 and from the 5 October – 10 October 1991. The extent of natural pollination was assessed on five newly opened flowers during each sampling interval. Slides and stigmas were collected after five days exposure. The number of deposited *C. papaya* pollen grains per slide was determined using light microscopy.
The data were analysed by analysis of variance using Systat 5.02 software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

3.2.2 Spore Trap

A volumetric spore trap (manufactured by Burkhard, Herefordshire, Great Britain) was used for sampling windborne particles, including pollen (Burkhard Manufacturing 1975; Banik and Chanda 1992). Windborne particles were drawn through a 2 mm x 10 mm opening at a height of 600 mm onto a clockwork driven sampling slide. A constant pressure of 1 bar below atmospheric pressure was maintained throughout the test period by an electrical suction pump. The spore trap was positioned 1 m from the closest male trees.

The spore trap was operated on two different sites during summer and early autumn in 1992; from the 14 January – 8 February 1992 at the orchard of the Queensland Department of Primary Industries (Q.D.P.I.) and from the 19 February – 21 March 1992 at an orchard in Parkhurst; both sites located in Rockhampton (Figure 2.3). Plant material consisted of a selection of parent lines at the Q.D.P.I. site and a mixed planting of hybrid lines (Hybrid 29 and Hybrid 1D) and siblings of an open pollinated papaw (Open) at the Parkhurst orchard. The Q.D.P.I. site consisted of approximately 120 trees. Plants were flowering at trial commencement (14 January 1992).

3.2.3 Swivel Device

Vaseline coated glass slides were fixed to a swivel device able to turn and face the slide into the wind direction. Slides were positioned at 1 m height in 0.5 m range from the lowest inflorescence on the closest male tree. The experiment was carried out during autumn and winter in 1993 and 1994 at the Central Queensland University orchard (see Section 2.2.2 for details). Sampling resumed between 8 March – 8 April 1993 and 20 July – 17 August 1993 and 28 March – 2 May 1994

and 11 July – 15 August 1994. A total of 90 samples were taken; 41 samples during 1993 and the remainder in 1994. Each sampling period lasted 24 hours, except where otherwise stated.

3.3 Results

3.3.1 Fixed Slides

Data collected during both sampling intervals from each of the three treatments (horizontally and vertically positioned slides and stigma surfaces) were combined and analysed for treatment effects, as the sampling time had no effect on the treatments ($P > 0.411$; Table 3.1).

Table 3.1: The quantity of pollen captured on slides fixed in vertical and horizontal positions in the vicinity of receptive pistillate flowers and on the stigma surface over a 120 hour sampling period.

	Average Pollen Number ±SE		
	Vertical Slide	Horizontal Slide	Stigma Surface
Period 1	2 ±2 (n=5) a	12 ± 4 (n=5) b	558 ±129 (n=5) c
Period 2	0 (n=5) a	24 ±15 (n=5) b	398 ±122 (n=5) c
Period 1 & 2	1 ±0 (n=10) d	18±8 (n=10) d	478 ± 88 (n=10) e

values sharing the same subscript do not differ significantly ($P > 0.05$).

The quantity of captured *C. papaya* pollen grains in each treatment was significantly different. Pollen counts on stigma surfaces were significantly higher ($P < 0.001$) in comparison to slides of both vertical and horizontal orientation, averaging 478± 88SE pollen grains per stigma surface (n = 10). On the other hand, no significant differences were apparent between the number of pollen grains trapped on either horizontally or vertically positioned slides adjacent to pistillate flowers ($P > 0.816$). The number of trapped pollen grains was low, averaging 1± 0SE pollen grains on vertically and 18± 8SE pollen grains on horizontally placed slides (n = 10 each).

On the whole, *C. papaya* pollen grains appeared in clusters, irrespective of the nature of the collecting surface. The largest amount of pollen trapped on vertically orientated slides was eight grains per slide after a 120 hour period; of which four grains formed one cluster. In comparison, the highest number of *C. papaya* pollen deposited on horizontally orientated slides was 84 grains, of which 90% or 76 grains formed one cluster. A small number of insect macrotrichia were also found

on these slides. Similarly, the majority of pollen grains on the stigma surface appeared in numerous groups of varying cluster size.

3.3.2 Spore Trap

The Spore Trap procedure recorded less than 5 grains per 24 hour period on most days, and the total amount of intercepted pollen never exceeded 26 grains per 24 hour sampling period (Table 3.2). Pollen grains were mostly distributed singly, although groups of two to four attached grains were occasionally found.

Table 3.2: Pollen grains intercepted by a volumetric spore trap per 24 hours on 30 days during summer and autumn 1992.

Number of Pollen Grains	Number of Recordings (n = 30)
0	9
1 - 10	15
11 - 26	6

3.3.3 Swivel Device

The free standing swivel, placed in close range to a male inflorescence, captured the largest amount of pollen grains. On five of a total of 90 sampling slides, an excess of 50 grains *C. papaya* pollen were captured (Table 3.3). The largest amount (780 grains) was recorded over a 72 hour period, followed by a count of 201 grains over a 24 hour period both during March 1994. However, pollen capture in excess of 50 grains per slide accounted for only 4% and 16% of all samples taken during the March/April sampling period in 1993 and in 1994, respectively (Table 3.3).

Table 3.3: Pollen grains intercepted by a swivel device per 24 hours during autumn (March/April) and winter (July/August), both in 1993 and 1994.

Number of Pollen Grains	Mar/Apr 1993 n = 23	Jul/Aug 1993 n = 18	Mar/Apr 1994 n = 25	Jul/Aug 1994 n = 24
0	9 (3%)	11 (61%)	13 (52%)	22 (92%)
1 - 10	10 (44%)	6 (33%)	2 (8%)	2 (8%)
11 - 50	3 (13%)	1 (6%)	6 (24%)	
≥ 51	1 (4%)		4 (16%)	

Most sample slides captured ≤10 grains per slide per 24 hour period. Pollen capture during both winter periods never exceeded more than 17 grains per slide per 24 hour period. The few individual results, where large numbers of pollen

grains were present, were always associated with extensive pollen clustering, sometimes as high as 170 grains per cluster (Table 3.4). Clustering (i.e. any aggregation of two or more grains) was also observed when numbers of *C. papaya* pollen grains were as low as seven grains per sampling interval (Table 3.4). However, pollen grains formed no ordered arrangement within clusters.

Table 3.4: Aggregation of *C. papaya* pollen grains captured on slides using a swivel device during autumn (March/April) and winter (July/August) both in 1993 and 1994.

Number of Pollen Grains	Sampling Time (hrs)	Largest Number of Pollen Grains per Cluster	Total Amount and % of Clustered Pollen
780	72	122	330 (42%)
201	24	170	198 (99%)
66	24	38	41 (62%)
56	24	40	48 (86%)
52	24	17	32 (62%)
50	72	50	50 (100%)
50	24	32	49 (98%)
48	72	24	48 (100%)
47	24	18	34 (72%)
43	24	17	28 (65%)
24	24	22	22 (92%)
22	72	5	7 (32%)
18	24	6	10 (56%)
11	24	6	11 (100%)
7	24	3	3 (43%)

3.4 DISCUSSION

Of all three wind-orientated experiments, the fixed glass slide experiment probably provided the best assessment of *C. papaya* pollen transfer by wind in an orchard environment, as slides were positioned adjacent to the pistillate flowers. This method provided a net measurement and accounted for the interception of pollen by natural obstacles such as leaves in proximity of receptive flowers. Further, the longer sampling interval (120 hours) better reflected the time period of stigma receptivity, typically estimated as five days (Sharma and Bajpai 1969).

Results from the fixed glass slide experiment indicates that pollen transfer is not wind-mediated but rather insect related. Pollen abundance on the stigma was significantly higher than on the slides even though the stigma surface area is only approximately 5% that of glass slides. If wind pollination was involved then it could be assumed that pollen abundance on vertical slides and vertically exposed stigma surfaces would be significantly greater than that found on horizontally mounted slides. Since significant differences were not recorded between vertical

and horizontal slide orientations, the hypothesis of wind pollination is further weakened.

The results indicate that a directed and selective pollen transfer mechanism is at work, for example insects. This suggestion is supported by the detection of insect macrotrichia on one of the horizontally positioned slides beneath a receptive pistillate flower. Furthermore, the purity of *C. papaya* pollen grains and their clustered appearance on stigma surfaces favour the hypothesis for directed pollen transfer by biotic agents instead of pollination by anemophily or by chance. Large standard errors of pollen counts from the stigma surface may also be indicative of variation in the number of pollinator visits to the same flower. Although clustering of pollen was also observed on vertically positioned slides the pollen aggregations never exceeded four grains per slide.

Capture of *C. papaya* pollen grains remained low even when a volumetric spore trap was used in trapping windborne particles. Considering the volume of air sampled, the close proximity of the installed device to the male inflorescences, and the fact that the space was clear of any interference by leaves, remarkably low amounts of *C. papaya* pollen grains were captured each day. Summing pollen grain numbers over a period of five days, which corresponds to the length of stigma receptivity, led to a maximum estimate of 46 grains per 120 hour period. However, records show that the average number of open flowers on male trees exceeded 50 flowers per tree, producing in excess of 40000 pollen grains each, during a similar time interval in 1993 and 1994 (Sections 4.1.3.3 and 4.3.3.2). These data highlight the inadequacy of pollen transport by wind currents. Although most pollen, when trapped, was distributed as individual grains, small aggregations of up to four grains were indicative of the presence of pollenkit which in turn is associated with biotically mediated pollen transfer.

A similar volumetric spore trap (Model: Burkhard, Herfordshire, Great Britain) was used in a pollen survey conducted in Calcutta, India (Banik and Chanda 1992) to determine the diversity and quantity of airborne pollen causing allergies to humans. Although papaw plants were commonly distributed throughout the sampling area no evidence of anemophilic pollen transport was recorded.

The results from the swivel device also fail to support the hypothesis that papaws are wind-pollinated. When the distance between sampling device (swivel) and pollen source was reduced to approximately 50 cm, the amount of trapped *C. papaya* pollen increased. However, pollen appeared mostly in clustered

aggregations on the sampling slides. These results suggest that pollen was either dislodged from the anthers during a visit by an insect or that gravity may at least be partially involved in the transfer of pollen in the immediate proximity of male and female papaw plants. Observations that male papaw trees are taller than female papaw trees would support the latter assumption (Allan *et al.* 1987). However, sexually related height differences are not universal and did not occur in the related species *J. dolichaula* in its natural environment in Costa Rica (Bullock and Bawa 1981). Therefore, abiotic dispersal of *C. papaya* pollen by means of gravity seems to be of incidental occurrence.

After the existence of an insect pollinator was inferred, then demonstrated, (Section 7.4.1), sampling during the July/August period was conducted to establish whether wind would account for pollen transfer during a period of pollinator absence (Section 7.4.2). In central Queensland, this period often coincides with periods of windy weather. However, the low pollen capture rate in the immediate vicinity of inflorescences suggests that anemophilic pollen transport remains relatively rare even during winter. Nevertheless, some short range pollen dispersal, presumably by means of gravity (see above), cannot be excluded, as some grains were trapped. Limitations of pollen transport in papaw due to pollinator absence and the occasional incidence of anemophily is consistent with the observation that fruit set initiated during winter contains only low numbers of seed (Prest 1955). Other data detailed elsewhere demonstrate that stigma receptivity (Allan *et al.* 1987; Cohen *et al.* 1989) as well as pollen quantity and pollen viability do not limit successful fertilization during the early subtropical winter period. These aspects are discussed in detail in Section 4.3.4.

In summary, three different sets of measurements in a range of orchards with a range of papaw lines and conducted at several different seasons of the year, failed to support the hypothesis for anemophily.

CHAPTER IV

PLANT PARAMETERS

This chapter focuses on plant parameters in relation to pollination biology and seasonal fruit set. These topics are divided into four major areas: a) plant characteristics (growth, flowering onset and average monthly flowering rates), b) floral elements (anthesis, dehiscence and rates of nectar production), c) reproductive elements (stigma receptivity, pollen quantity and pollen viability) and d) the stigma type of *C. papaya* flowers. Results are discussed at the end of each of the four sections.

4.1 SECTION A: PLANT DEVELOPMENT AND FLOWERING

4.1.1 INTRODUCTION

The apical growth rate of *C. papaya* is of special concern in pollination since new flowers and inflorescences are borne in the emerging leaf axils on the normally single stemmed plant. Allan *et al.* (1987) reported sex-related height differences in trees kept under various climatic conditions in South Africa. As stated by several authors (e.g. Agnew 1968 and Allan 1976; Allan *et al.* 1987) subtropical climates, especially those with cold phases during winter and spring, have an adverse effect on the growth of the tree and the amount of flowers produced. Variation of growth rates between female and male papaw trees may also have an influence on means of pollen transfer (Section 3.4).

Only a few published records of the influence of sex related differences on the onset of flowering of *C. papaya* are available and in some instances those records contradict each other when the same species has been considered (i.e. Bawa 1980b and Allen *et al.* 1987). In the current study the time of flower commencement of female and of male trees was monitored in order to evaluate sex related differences: a factor which is normally associated with insect pollination.

Average monthly flowering rates of male and female plants were monitored in order to assess the overall seasonal availability of pollen (in combination with pollen counts of individual flowers; Section 4.3.2.2) under subtropical Queensland conditions. The number of open pistillate flowers provided an estimate of potential fruit set throughout the year. Although flowers are present throughout the year, fruit set and fruit size are markedly seasonal. Published data on open flower numbers are currently unavailable for papaws grown in Australia. An assessment of longterm flower production and longevity of papaw trees was not the aim of this trial as commercially productive trees are kept for no longer than two reproductive seasons.

4.1.2 MATERIALS AND METHODS

4.1.2.1 Growth

The height of five female and five male papaw trees each of Hybrid 29, Hybrid 1D and the open pollinated line was studied at the Parkhurst orchard. Measurements were taken at the end of each month, from the date of flower commencement in February 1992 until February 1993. Using these measurements, the daily linear growth rates were calculated and analysed by a repeated measure analysis of variance using Genstat 5 (release 2.2) software (Payne *et al.* 1988) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.1.2.2 Onset of Flowering

The flowering of young papaw trees was studied at the Parkhurst orchard. The sex of all 30 trees of each of Hybrid 29, Hybrid 1D and the open pollinated line was determined and the stage of flower opening was recorded on four dates over a ten week sampling period. Flowering began at 20 January 1992 (first sampling date) and all trees had flowered by the 30 March 1992 (final sampling date).

Data in the form of the percentage of flowering trees were analysed as a contingency table model using Genstat 5 (release 2.2) software (Payne *et al.* 1988).

4.1.2.3 Average Monthly Rate of Flowering

Five male trees and five female trees of Hybrid 29, Hybrid 1D and the open pollinated line were sampled at the beginning of each month, between March 1992 until February 1993 at Parkhurst. On each occasion the number of open flowers

was recorded. Trees were initially randomly chosen and from then onwards sampling continued on the same trees. Sampling was conducted during the early morning before the onset of flower dehiscence. The number of open flowers on female trees was recorded as the total number of pistillate flowers that opened each month. The number of open staminate flowers was recorded as the open flower number on the day of sampling.
The data were analysed by analysis of variance using Systat 5.02 software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.1.3 RESULTS

4.1.3.1 Growth

Both line and gender were associated with significant differences in the growth rate of papaw plants ($P < 0.001$; Figure 4.1 and Figure 4.2). Male trees grew significantly faster than female trees during February and March and during September and October 1992. The first period of increased growth rates coincided with the beginning of flowering. On average males grew 8.5 mm/day during the first period and and 3.1 mm/day during the second period. In comparison female trees grew 5.5 mm/day and 1.7 mm/day, respectively.

The growth rate showed further significant papaw line effects. During the February/March period, Hybrid 29 grew significantly slower (5.6 mm/day) compared to Hybrid 1D (8.2 mm/day) and the open pollinated line (7.3 mm/day). During the September/October period the open pollinated line exhibited the fastest growth rate with 3.5 mm/day compared to 2.0 mm/day and 1.7 mm/day of Hybrid 29 and Hybrid 1D, respectively. The growth rate of all three papaw lines decreased to below 1 mm/day during June and July.

The overall height of trees is documented as a cumulative average of the growth of five female and five male plants of each papaw line for the length of the experiment, (Figure 4.3). Of all three papaw lines the height of trees of the open pollinated line increased fastest from the beginning of the new growing season (September) onwards. The height of trees of Hybrid 29 advanced slowest. At the end of the trial, on the 26 February 1993, 390 days after flower commencement, the average height of trees of the open pollinated line was 177.7± 7.9 cm, of Hybrid 1D was 170.7± 2.9 cm and of Hybrid 29 was 157.1± 6.1 cm.

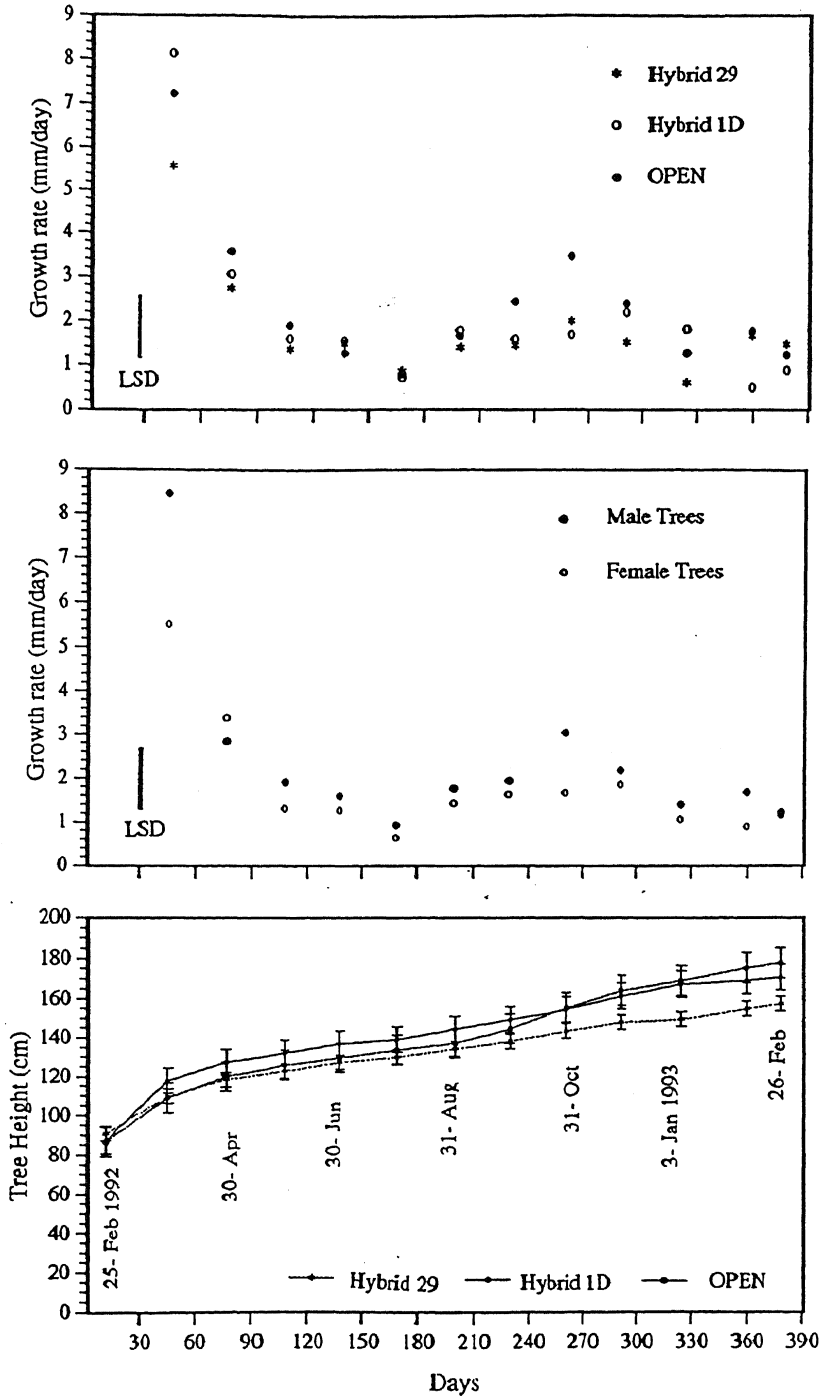


Figure 4.1: The daily growth rate of Hybrid 29, Hybrid 1D and an open pollinated line from anthesis onwards. Each line is represented by n = 30 trees.

Figure 4.2: The daily growth rate of female (n = 39) and male (n = 51) trees, independent of line effects, from anthesis onwards.

Figure 4.3: The average cumulative height increase (± SE) of trees of Hybrid 29, Hybrid 1D and an open pollinated line from anthesis onwards. Each line is represented by n = 30 trees.

4.1.3.2 Onset of Flowering

The timing of initial flowering was significantly different in both different papaw lines and different genders ($P < 0.001$; Figure 4.4 and Figure 4.5). Of the three papaw lines, Hybrid 1D was the last to flower as it had no open flowers on the 20. January 1992. In comparison, on the same date 40% of Hybrid 29 and 17% of the open pollinated strain had commenced flowering. All trees, independent of their line, flowered within 69 days of when the initial open flowers were recorded.

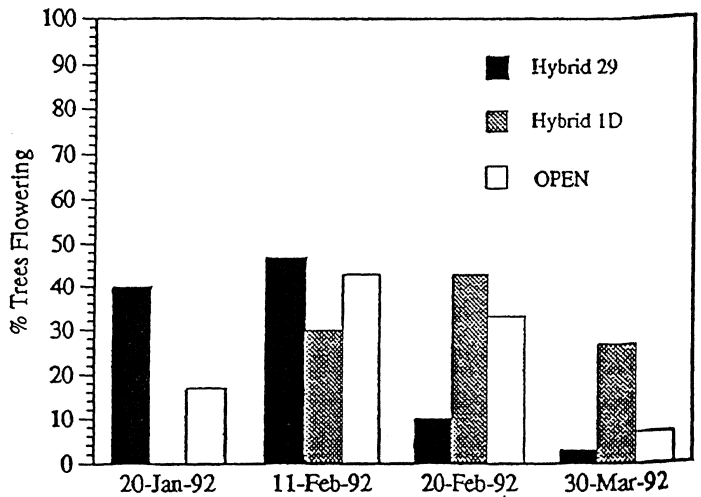


Figure 4.4: The percentage of flowering trees of Hybrid 29, Hybrid 1D and an open pollinated papaw line since the day of the previous inspection at Parkhurst in summer 1992. Each line is represented by n = 30 trees.

Note: The time between sampling intervals is not constant.

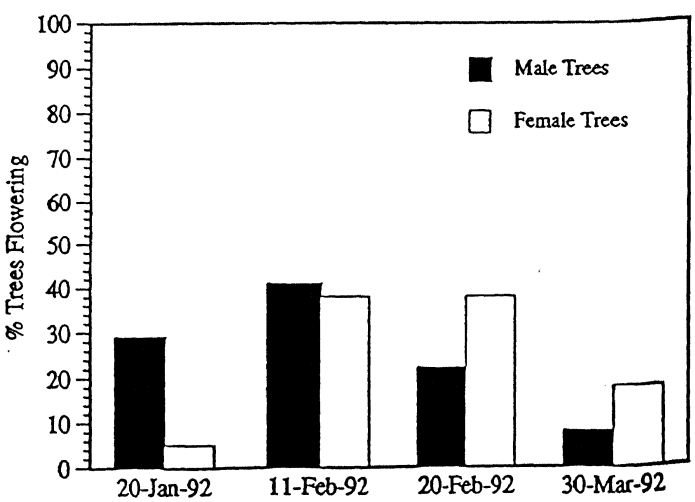


Figure 4.5: The percentage of female (n = 39) and male (n = 51) papaw trees that commenced flowering since the day of the previous inspection at Parkhurst in summer 1992.

Note: The time between sampling intervals is not constant.

The commencement of flowering was different in female and male, irrespective of papaw line. The majority of male trees preceded female trees in the onset of flowering by approximately three weeks (Figure 4.5). Significantly more male trees (29%) had flowered by the 20. January 1992 compared to female trees (5%; $P < 0.001$). The majority of female plants (76%) flowered by the 20. February 1992. On the 30. March 1992, the percentage of female trees starting to flower was more than double (18%) that of male trees (8%). Similar patterns were noted from newly established papaw plants at the University orchard in the following year.

4.1.3.3 Average Monthly Rate of Flowering

Papaws, irrespective of sex, flowered continously throughout the year, although the number of flowers in anthesis varied between seasons (Figure 4.6). Significantly lower numbers of open flowers were observed during the winter months, irrespective of papaw line. While the number of open pistillate flowers was significantly reduced during the months of June and July ($P < 0.001$), the number of open staminate flowers remained significantly reduced during July, August and September ($P < 0.05$).

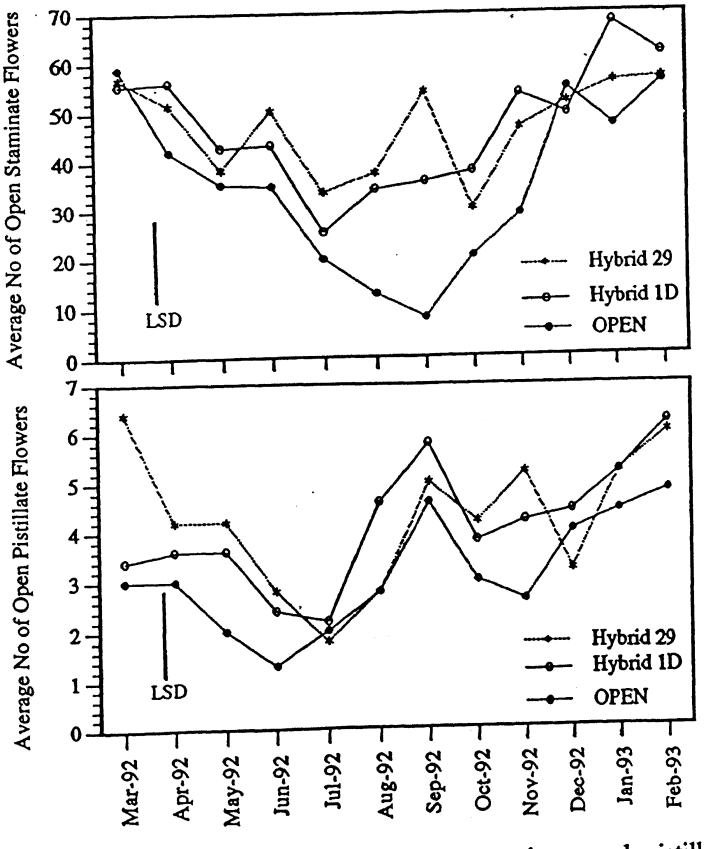


Figure 4.6: The average number of open staminate and pistillate flowers of Hybrid 29, Hybrid 1D and an open pollinated papaw line (March 1992 – February 1993).

Comparisons of the number of open flowers in different lines showed that both pistillate and staminate flowers of the open pollinated papaw line were significantly less ($P < 0.001$) than those recorded for Hybrid 29 and Hybrid 1D. The number of open pistillate ($P = 0.449$) and open staminate ($P = 0.984$) flowers numbers of both hybrid lines did not differ.

4.1.4 DISCUSSION

Male *C. papaya* trees commence flowering earlier than female trees and have significantly greater numbers of open flowers. This resembles the flowering pattern of *Jacaratia dolichaula* in its native rainforest habitat (Bawa 1980b). Bawa suggested that the earlier flowering of males would be an adaptive advantage in accustoming pollinators to foraging sites of plants such as *J. dolichaula* which flower over a prolonged season. It is argued that insects, once accustomed to the location by suitable flowering plant specimens, would forage repeatedly on the same established flight path (Linhart and Mendenhall 1977; Janzen 1984). This is the basis for the term 'traplining'. This specific 'strategy' for attracting pollinators can also be suspected of *C. papaya*, since the same pattern of earlier flowering male trees in comparison to that of female trees was found. Such a flowering strategy most likely ensures the reproductive success of female plants from the commencement of flowering onwards, as pollinators would be accustomed to the location of a food source prior to the initial anthesis of pistillate flowers. Thus earlier flowering of male trees accompanied with 'traplining', increases the reproductive success of the major physiological investment of female plants – the formation and development of ovaries.

C. papaya is a tropical species in its original distribution and probably grows uniformly under those conditions throughout the year. This study demonstrates that under subtropical climates there are two distinct growth spurts; one in January just prior to flowering and a second one in September during spring. During winter, growth rates independent of papaw line effects and gender effects remained low, indicating an overall climatic effect on the tropical plant species. This is demonstrated by an overall small increase of 0.8 mm/day for female plants and 1mm/day for male plants of all three papaw lines during July. These results are in line with observations of tree growth in other subtropical climates such as Israel and South Africa, where the stagnation of tree growth during the winter period has been reported (Allan 1976; Allan *et al.* 1987; Cohen *et al.* 1989). Explicit field records however, are unavailable. An indication of the relationship between

growth rate and various temperature regimes was documented from controlled greenhouse experiments conducted by Allan *et al.* (1987) in South Africa. For example, cool temperature regimes of 20°C day/12°C night, which are similar to subtropical winters of central Queensland, reduced stem growth of male trees to 10 mm/week and stem growth of female trees to 5.6 mm/week, and are of similar magnitude to the results obtained from this field trial. Controlled high temperature regimes (36°C day/28°C night), which are similar to subtropical summer temperatures, were associated however with an increase in stem growth of 37.6 mm/week of male plants and 31.4 mm/week for female plants. Similar growth rates were recorded during the current field trial only at the beginning of the reproductive phase in February (summer 1992). Lower growth rates of field grown plants can probably be explained by the continuing larger variation of field climatical influences in contrast to stable glasshouse environments maintained for almost one year. Physiological obligations towards greater reproductive investments of field grown plants, especially that of female plants, may also play a role (Lloyd 1980).

An overall seasonal effect of climate on the number of open staminate and pistillate flowers was also observed. The most critical period was during winter and early spring; from June until September. Pistillate flowers exhibited a shorter response interval (June/July) to lower temperature regimes in comparison to open staminate flowers. In the latter, significantly lower numbers of open flowers were recorded over a three month interval lasting into September. The decrease in numbers of staminate flowers is possibly due to the significantly higher growth rates of male trees during September (see above) in comparison to growth rates of female trees. Significantly lower numbers of open staminate and pistillate flowers in the open pollinated line in comparison with both hybrid lines demonstrates the superiority of hybrid plant material in terms of potential fruit production.

Seasonal spurts of growth are gender related, with male trees exhibiting a significantly faster growth rate during the onset of the reproductive phase and again during the following spring (September) period, in comparison with growth rates of female trees. The growth of male trees during September most likely accounts for the decrease in the number of open staminate flowers during the same time interval. The reverse (lower growth rate; increase in open flower number) was noted in female trees during September.

4.2 SECTION B: ANTHESIS, DEHISCENCE AND PARAMETERS OF NECTAR

4.2.1 INTRODUCTION

A number of flower-related parameters were considered since reports in the published literature are contradictory. These parameters concerned the timing of floral anthesis and the availability of nectar in *C. papaya* flowers. In addition, the seasonal availability of nectar and its sugar content were investigated. These parameters have not been considered in the published literature.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Anthesis and Dehiscence of *C. papaya* Flowers

Floral anthesis of staminate and of pistillate flowers and the dehiscence of staminate flowers was assessed on five randomly selected male and female trees between 5 March 1992 until the 9 March 1992 at the Parkhurst orchard. Open flowers were removed from male trees and the timing of subsequent flower opening was recorded in hourly intervals between 1700 hours and 2200 hours and at 0800 hours on the following morning. The corolla tubes of opened flowers were marked with a pen and flower dehiscence recorded at midday during the following days.

Anthesis of staminate and pistillate flowers and dehiscence of staminate flowers were analysed by analysis of variance using Systat 5.02 software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.2.2.2 Seasonal Nectar Availability

The availability of nectar of staminate flowers was studied in five male trees of each of the three papaw lines at the orchard at Parkhurst over the twelve month period between March 1992 and February 1993. The flowers were sampled at monthly intervals. Five freshly opened flowers, selected on the basis of their straight, untwisted petal appearance, were collected from each tree at dusk and immediately tested for the amount of available nectar using fine glass microcapillary tubes with a maximum volume of 10 µL (Hirschmann Laborgeraete, Germany). Flowers were cut lengthwise to avoid the blocking of the microcapillary tube mouth and also to

avoid contamination of nectar with pollen grains. These samples were also analysed for their sugar content as described below.
Data were analysed by analysis of variance using Systat 5.02 software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.2.2.3 Concentration of Sugars in Nectar

The sugar concentration of the above nectar samples was examined using a handheld refractometer (Atago ATC, 0 – 32% Brix) with automatic temperature compensation. Measured values were expressed as an estimate of the percentage of sucrose present in the nectar (following the method for nectar analysis established by Baker (1976; Table 1.2). Brix is a standard of measuring sugar concentration as g solute per 100 g solution and is widely used in the sugar industry (Bolten *et al.* 1979).
Data were analysed by analysis of variance using Systat 5.02 software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.2.2.4 Availability of Nectar of Netted and Unnetted Trees

A test on the availability of nectar in freshly opened staminate *C. papaya* flowers was conducted during the dusk and early evening hours in summer and winter to test the feasability of foraging by nocturnally active hawkmoths. The quantity of nectar of staminate flowers of five netted versus five unnetted trees of one papaw line (Hybrid 1E) was tested at the C.Q.U. orchard on two occasions during 1994. The netting material consisted of shade cloth of 2 mm mesh size. Five freshly opened flowers from each tree were collected at two different times (1800 hours and at 2100 hours) on two dates; the 28 March 1994 (summer) and on the 14 July 1994 (winter). Sampling procedures followed the same method as described previously. On both occasions flowers of the same ten trees were collected.
Data were analysed using Student's t- tests.

4.2.3 RESULTS

4.2.3.1 Anthesis and Dehiscence of *C. papaya* Flowers

Significantly greater numbers of staminate (89%) and pistillate (48%) flowers opened between 1800 until 2000 hours (just on dusk) compared to earlier and later

sampling times ($P < 0.05$). Petals of staminate flowers separated completely during the initial two hours and dehisced within the following two days. The receptive phase of pistillate flowers ceased after four days of anthesis when stigmatic and petal tissues had visibly deteriorated.

4.2.3.2 Seasonal Nectar Availability

Nectars of staminate flowers contained a mean nectar volume of 7.1± 0.1SE µL during the year (Figure 4.7). The availability of nectar varied significantly between both, months and between papaw lines ($P < 0.001$). The lowest nectar volumes were recorded during August (≤ 5.5± 0.4SE µL) and December (≤ 5.8± 0.4SE µL). Also, the availability of nectar varied significantly between papaw lines within the same month ($P < 0.001$). Generally, the open pollinated line had less nectar per flower. On six of the twelve sampling dates, nectar volumes per flower were significantly different from either one or both hybrid lines ($P < 0.001$).

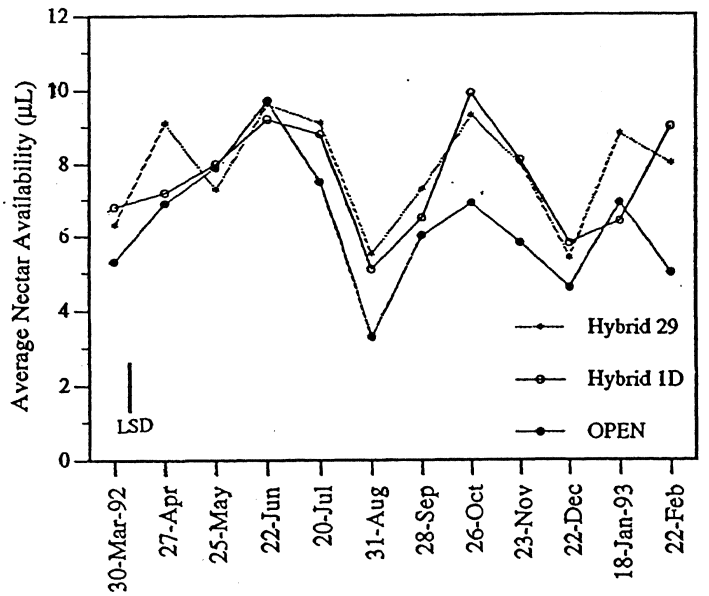


Figure 4.7: The average availability of nectar in staminate flowers of Hybrid 29, Hybrid 1D and an open pollinated papaw line (March 1992 – February 1993).

4.2.3.3 Concentration of Sugars in Nectar

Throughout the year, the mean sugar concentration of nectar was 27.0± 0.1SE % Brix (Figure 4.8). Significant differences ($P < 0.021$) in nectar sugar content were observed between papaw lines and between months. Nectar had the lowest sugar content during September, when sugar concentrations were below 23.2± 0.5SE %

Brix (open pollinated line). The nectar sugar concentrations of all three papaw lines within each sampling month were not significantly different (P > 0.199).

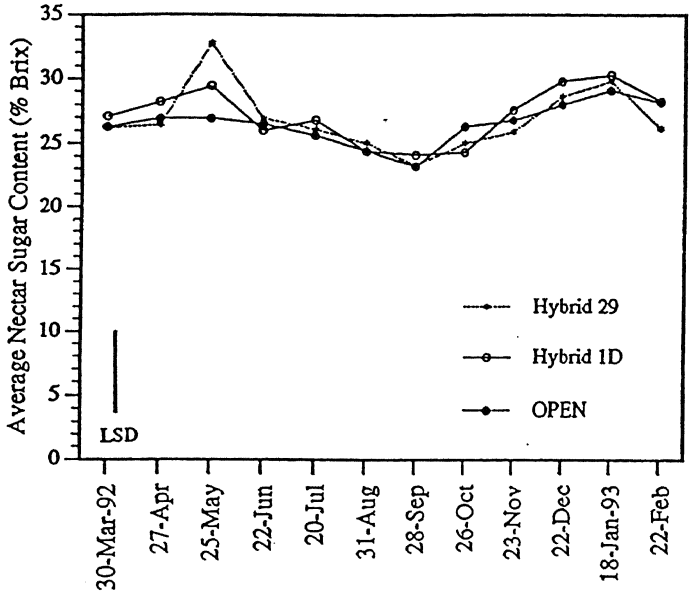


Figure 4.8: The average concentration of sugar (% Brix) in nectar of staminate flowers of Hybrid 29, Hybrid 1D and an open pollinated papaw line (March 1992 – February 1993).

4.2.3.4 Availability of Nectar of Netted and Unnetted Trees

The availability of nectar differed significantly according whether trees were netted or unnetted, at both sampling times (1800 and 2100 hours) during summer 1994 (P < 0.008). The average nectar volumes and standard errors of netted flowers at 1800 and 2100 hours were 7.4± 0.2SE µL and 9.0± 0.2SE µL in comparison to 5.6± 0.5SE µL and 2.8± 0.6SE µL of nectar of flowers from unnetted trees (n = 25 per treatment at both sampling times).

Nectar volumes in netted as opposed to unnetted trees remained similar at treatment application during winter, in July 1994 (P > 0.173). The mean availability of nectar was 6.6± 0.1SE µL per staminate flower (n = 100).

4.2.4 DISCUSSION

The nocturnal anthesis of flowers coincides with the timing of maximum scent release and nectar availability, and this is consistent with a biotic-mediated pollen

transfer in papaw. The results suggest that night-active insects are operative, given that anthesis is nocturnal and a large amount of nectar is available and is concealed at the base of the corolla tube. The sugar concentration of 27% Brix further suggests predominantly that within the potential pollinator groups, the most likely insect would be a lepidopteran species. Bats and nectar eating birds predominantly forage on nectar with sugar concentrations about 20%, bees generally select nectar with sugar concentrations of above 40% (Baker 1978), whereas butterflies and moths forage on nectar with sugar concentrations in the range of 22% – 28% (Pyke and Waser 1981). Since the papaw is an introduced species in Australia, other orders such as the Hymenoptera and Thysanoptera should not be overlooked as possible pollinators.

Nectar volumes of unnetted and netted flowers were similar during winter (July 1994), but differed significantly during summer (March 1994) at both sampling times (18:00 and 21:00 hours). The loss of nectar from unnetted trees in summer from the time of anthesis onwards indicates seasonal foraging activity coinciding with the nocturnal anthesis of *C. papaya.* Therefore, the suggestion that moths (Lepidoptera: Sphingidae) are involved in the pollination of papaw is highly likely, not only in its country of origin (Baker 1976), but also in Australia (Section 7.4.1).

In summary, anthesis in both staminate and pistillate *C. papaya* flowers is nocturnal, as is the onset of nectar release, and evidence is presented that nectar foraging is also nocturnal. The results are consistent with pollination of both staminate and pistillate flowers by nocturnally active insects. Despite continuous nectar production of staminate flowers throughout the year and nectar remaining available during the winter period, it appears that moths do not visit flowers in winter. This indicates seasonality in insect behaviour (Section 7.4.2). Therefore, seasonality of lower rates of fruit set and seed set as experienced during the June – October period (Sections 6.3.1 and 6.3.2) may, at least partially, be incurred by the absence of pollinators.

4.3 SECTION C: RECEPTIVITY OF THE STIGMA AND THE VIABILITY AND QUANTITY OF POLLEN

4.3.1 INTRODUCTION

The focus of this section is primarily on the reproductive 'efforts' of male and female *C. papaya* plants, namely pollen viability and quantity and the receptivity of the stigma. The variability of seasonal fruit set (the primary subject of this research) could have been due to variation in any or all of these parameters. While some information concerning the viability of pollen is available from South Africa (Allan *et al.* 1987) and Israel (Cohen *et al.* 1989), there are no comprehensive reports for Australian papaws.

The quantity and viability of pollen were examined throughout the year, to establish if these parameters are seasonally influenced, and in order to document differences between individual papaw lines should they exist. Such records are not available for papaws grown in Australian climates. Tests on the quantity and germination of pollen derived from bisexual flowers were carried out to establish whether there were differences between these and purely staminate flowers. The development of bisexual flowers on male trees is a recurrent event during spring in tropical and subtropical climates. It seems that individual lines vary in their tendency to set bisexual flowers (Anne Garrett unpublished observation). Insofar as the occurrence of bisexual flower set could influence pollen quantity and pollen viability it was considered relevant to record these parameters.

A sudden decline in pollen germination observed during autumn 1993 coincided with the outbreak of the disease of 'Dieback'. The disease constitutes a major problem to the commercial papaw industry, and tree losses of as high as 90% have been experienced in some years (e.g. Yarwun, 1991). 'Dieback' occurs in all production areas of Queensland, but, is more prevalent in south-east and central Queensland. Outbreaks occur simultaneously for a short recurring period during early summer and autumn. Trees die suddenly within days. The cause of 'Dieback' is unknown but investigations by Liu *et al.* (in press) suggest mycoplasma-like organisms are the disease-causing agent.

A subsequent expansion of the project led to the testing of pollen viability of 'Dieback' affected plants, since the disease may have been indirectly related to the

overall objective of the research, which was to examine reasons for low fruit set in some regions.

Poor fruit set during the summer months in north and south Queensland has also been associated with the rupturing of *C. papaya* pollen grains due to the excess of moisture during periods of wet weather (Agnew 1968; Anon. 1987). However, published data either supporting or refuting this suggestion are not available. The adaptive significance of pollen rupture is somewhat hard to comprehend, especially given the tropical origin of *C. papaya.* Accordingly, a test was conducted to verify or disprove the Agnew's suggestion.

4.3.2 MATERIALS AND METHODS

4.3.2.1 Receptivity of Pistillate Flowers

Five randomly selected pistillate *C. papaya* flowers were hand pollinated during each month from June 1992 until September 1992 on trees at the orchard at Parkhurst. Each pistillate flower received a mixture of pollen from two flowers from each of the three papaw lines; Hybrid 29, 1D and the open pollinated line. Pollen donor trees within each line were randomly selected. The receptivity of pistillate flowers during the months of winter and early spring was assessed as the rate of induced seeded fruit set at trial closure on 24. February 1993.
Data were analysed by analysis of variance using Systat 5.02 release software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.3.2.2 Quantity of Pollen

Three flowers close to anthesis were sampled from each of five male trees of all three papaw lines at the beginning of each month. From February 1992 until February 1993 (Trial period 1) the experiment was carried out on the property in Parkhurst and continued on newly established trees at the C.Q.U. site from March 1993 until October 1994 (Trial period 2).

The amount of pollen was determined using the method of Ramsey and Vaughton (1991). Pollen was teased out from the anthers with a fine needle and submerged in a 10% detergent solution ('Sunlight') of known volume. From this suspension four aliquots, per flower, were quantified using a Neubauer haemocytometer. The mean number of pollen grains per flower was calculated for each month.

Pollen quantity results were separately analysed for the Parkhurst (Trial period 1) and C.Q.U. site (Trial period 2). Data were log transformed followed by analysis of variance using Genstat 5 release 2.2 software (Payne *et al.* 1988). Statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.3.2.3 Quantity of Pollen from Bisexual and Staminate Flowers

The quantity of pollen of staminate and bisexual flowers of male trees was examined at the beginning of October 1994. This date coincided with the annual occurrence of bisexual flower set and flower opening on male trees. Each of the five male sampling trees of Hybrid 29, 1D and the open pollinated line at the C.Q.U. orchard and a further ten randomly chosen male trees of various lines at the orchard at T.A.F.E., were examined between 3 October 1994 and 5 October 1994. Three bisexual and three staminate flowers were collected from each sampled tree. Pollen numbers were quantified by following the same test procedures as previously described.

Data were analysed using paired t–tests. Results for both sites were treated individually.

4.3.2.4 Viability of Pollen

The viability of pollen was determined using the same five male sampling trees of each of the three papaw lines (Hybrid 29, Hybrid 1D and the open pollinated strain) from February 1992 until October 1994. Between February 1992 and February 1993 the experiment was carried out on the property in Parkhurst (Trial period 1) and continued on newly established trees at the C.Q.U. site from March 1993 until October 1994 (Trial period 2). The rate of germination of pollen was tested at bi–weekly intervals until November 1993, and from then onwards sampling continued at intervals of four weeks, commencing at the start of each month.

Five flowers, either in or close to anthesis, were collected from each tree. Their pollen was combined and tested for viability, using the presence of pollen tube growth as an indicator. Initially, three methods were used to obtain results on pollen viability. However, neither staining of pollen with Tetrazolium salts (British Drug House, Poole, England) nor fluorochromatic detection using fluorescein diacetate (Heslop-Harrison *et al.* 1970, Heslop-Harrison *et al.* 1984; ICN Biochemicals, Cleveland, Ohio) produced conclusive results. Neither methods

provided clear differentiation of stain uptake and pollen scoring as viable or unviable was too subjective.

Germination of pollen on modified Brewbaker medium (Brewbaker 1957; Cohen *et al.* 1989) was the most reliable method for obtaining results on pollen viability. Pollen was submerged in liquid modified Brewbaker medium which in turn was distributed on solid modified Brewbaker medium in Petri dishes and incubated at 28°C for 150 minutes following the procedures used by Cohen *et al.* 1989. After incubation, five replicate samples each consisting of 100 grains were examined per plate and pollen grains were considered to have germinated when the pollen tube was longer than the pollen grain.

Data obtained from trees at both sites were analysed separately using analysis of variance performed on Genstat 5 release 2.2 software (Payne *et al.* 1988) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.3.2.5 Viability of Pollen from Bisexual and Staminate Flowers

A comparison between pollen viabilities of staminate and bisexual flowers was carried out on 4 October 1994. Flowers of the same male trees previously tested for pollen quantity (Section 4.3.2.3) were sampled for pollen viability of bisexual and staminate flowers. Three bisexual and three staminate flowers were collected from each tree. Pollen viability was tested using the same procedures as described above (Section 4.3.2.4).

Data were analysed using paired t–tests. Results from different sites were treated individually.

4.3.2.6 Viability of Pollen from 'Dieback' Affected Plants

On three occasions, on the 4 May 1993, 18 October 1993 and 24 October 1994, the viability of pollen in 'Dieback' affected plants was compared to viabilities of pollen sampled from healthy papaw trees. A total of 44 trees were examined; 16 trees each on the sampling dates during May and October 1993 and 12 trees in October 1994. Tests were conducted when the first symptoms of the disease were visible. Sampling procedures followed the same protocol as described above (Section 4.3.2.4).

Data were pooled for each of the sampling dates and analysed using paired t–tests.

4.3.2.7 Performance of *C. papaya* Pollen in Simulated Heavy Rain Environments

Ten flowers in or close to anthesis were collected from five randomly selected male papaw trees at the C.Q.U. orchard during summer in February 1995 for an *in vitro* experiment. Pollen of flowers from each tree was combined and submerged in a Petri dish filled with tap water and kept at room temperature. The control sample consisted of one plate with pollen to which no water was added. Five replicate samples, each consisting of 100 grains, were examined per plate after one hour, three hours, six hours, nine hours, twelve hours and 31 hours. Pollen grains were examined for rupturing and germination.

Data were analysed by analysis of variance using Systat 5.02 release software (Systat Incorporated, U.S.A.) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

4.3.3 RESULTS

4.3.3.1 Receptivity of Pistillate Flowers

Flowers are known from commercial practice to be receptive in summer, and the results in these trials demonstrate that pistillate *C. papaya* flowers remained receptive throughout the year. All pistillate flowers successfully set fruit during the winter/early spring period of June to September when flowers were hand pollinated. Fruit set and seed viabilities in each month from June until September 1992 were not significantly different ($P < 0.954$). Almost all seeds were viable (98%). Seed set varied between months, with fruit setting significantly more seed during June and July than during August and September ($P < 0.001$). Fruits averaged 862± 3.2SE seeds ($n = 10$) during the June/July 1992 interval while seed set reduced to 558± 3.2SE seeds ($n = 10$) during the August/September 1992 interval.

4.3.3.2 Quantity of Pollen

The average number of pollen grains was about 60000 grains per staminate flower during most of the year, irrespective of papaw line and site. However, during short periods of the year, the quantity of pollen significantly varied according to papaw line and month ($P < 0.01$; Figure 4.9). Individual papaw lines responded with varying intensity to the change in climatic conditions, although, in all instances papaw lines indicated a similar trend in their response.

Trial period 1

Figure 4.9 shows the annual minimum and maximum temperatures and the average quantity of pollen per flower of all three papaw lines at the Parkhurst and T.A.F.E. orchard in Rockhampton. With respect to pollen availability two distinct periods were observed. During most of the year (November – July) pollen availability was high (approximately 60000 grains per flower), whereas during winter/early spring (August – October) pollen availability decreased significantly ($P < 0.01$) to below one third (< 20000 grains) of the amount of pollen which was available during most of the year. Although all lines showed a significant reduction in pollen availability during the winter/early spring period, the time of the decrease was shorter for both hybrid lines (two months low; August and September), in comparison to the open pollinated strain (three months low; August – October; Figure 4.9). Line effects were also apparent during August and September when the number of pollen grains in Hybrid 29 (15437± 76SE) was significantly greater ($P < 0.01$) than those of Hybrid 1D (5645± 563SE) and the open pollinated line (2259± 229SE). During periods of low pollen availability, some flowers showed signs of interrupted anther development, indicated by a withered appearance and partial browning of the anthers. Average minimum temperatures during winter in June and July reached 9°C and 10.2°C, respectively.

A significant decrease in pollen availability was also noted during summer (December 1992/January 1993). The quantity of pollen grains of flowers of the open pollinated line (21985± 1723SE) was significantly less ($P < 0.01$) compared to both Hybrid 29 (57156± 1652SE) and Hybrid 1D (53245± 1193SE; Figure 4.9).

During Trial period 1 (from June 1992 until February 1993) low pollen availability was observed during the late winter/early spring period and to a lesser extent during the summer period (January 1993). However, the magnitude of the decrease was dependent on papaw line, and the performance of Hybrid 29 was superior to both other lines during the end of winter/spring period (August – October 1992).

Trial period 2

The results from the C.Q.U. orchard were similar to the results on the quantity of pollen obtained from trees at the Parkhurst site, with average pollen grain numbers significantly affected by sampling month and papaw line ($P < 0.01$). Again, a significantly lower ($P < 0.01$) availability of pollen was recorded during the winter/early spring period (August and September 1994), when pollen quantity

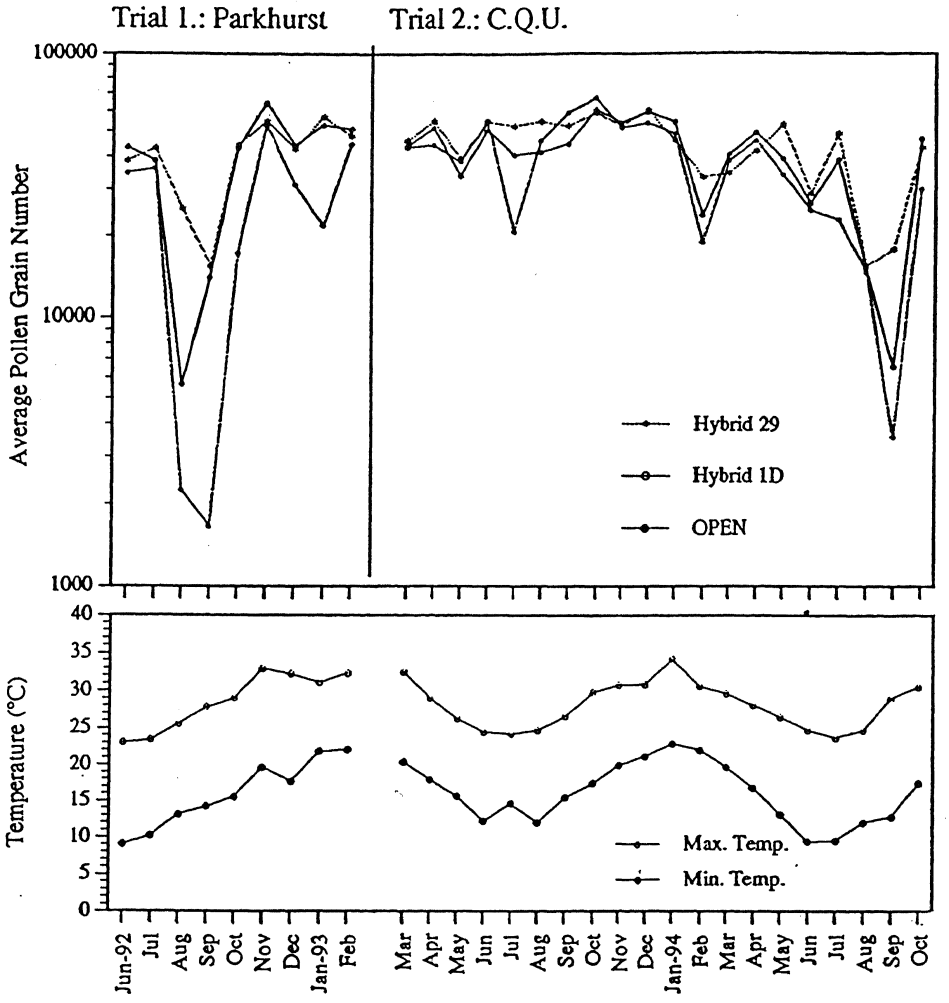


Figure 4.9: The average minimum and maximum temperatures and number of pollen grains per flower of Hybrid 29, Hybrid 1D and an open pollinated papaw line at Parkhurst (June 1992 – February 1993) and at the C.Q.U. orchard (March 1993 – October 1994). (Least Significant Difference in Parkhurst: 0.25; Least Significant Difference for C.Q.U.: 0.23)

decreased to below one third (20000 grains) of its annual average, irrespective of line (Figure 4.9). The quantity of pollen of Hybrid 29 (17941± 1720SE) was again significantly greater ($P < 0.01$) in comparison to Hybrid 1D and the open pollinated lines where pollen quantity decreased to 6655± 511SE and 3606± 495SE respectively. By contrast, pollen availability remained largely unaffected during the winter/spring period of 1993, except for a single occasion in the open pollinated line where a significant reduction ($P < 0.01$) of pollen grains was evident during July 1993 (20800± 1312SE). Hybrid 29 and Hybrid 1D, however, continued producing pollen grain numbers of 40875± 1379SE and above during the entire winter period. Average minimum temperatures during June and July 1993 were above those of the previous year, being 12.2°C and 14.6°C, respectively.

The quantity of pollen in all three papaw lines was also significantly affected during the summer in February 1994. The availability of pollen in Hybrid 1D (24178± 1720SE) and the open pollinated line (19008± 1161SE) decreased significantly (< 0.01), in comparison to Hybrid 29 (33749± 1639SE).

Irrespective of line, papaws showed a similar seasonal trend of lower pollen quantities during the late winter and early spring period in 1994 as previously documented for the winter 1992 period. By contrast, pollen availability did not significantly decrease in any papaw line during the same months in 1993. However, the open pollinated line had significantly less pollen during the month of July 1993 in comparison with both hybrid lines. A significant decrease of pollen availability of Hybrid 1D and the open pollinated line was also noted during summer (February 1994).

4.3.3.3 Quantity of Pollen from Bisexual and Staminate Flowers

Pollen quantities in bisexual flowers were not significantly different from pollen quantities in staminate flowers, neither from trees at the C.Q.U. orchard ($P > 0.25$) nor from trees at the T.A.F.E. orchard ($P > 0.11$; Table 4.1).

Table 4.1: Quantity of pollen from bisexual and staminate flowers.

	Average Pollen Quantity ± SE	
	Bisexual Flower	Staminate Flower
C.Q.U. Orchard	39908± 923*	42975± 1377*
T.A.F.E. Orchard	27660± 4676†	36215± 3442†

* n = 39 † n = 30

4.3.3.4 Viability of Pollen

The average viability of pollen remained about 90%, irrespective of site or line (Figure 4.10). However, during brief parts of the year pollen germination was significantly affected. Individual papaw lines responded to the change in climatic conditions to varying degrees, but responses of lines were similar in all instances.

Trial Period 1

Figure 4.10 shows the average annual minimum and maximum temperatures and the average rate of pollen germination of three papaw lines at the Parkhurst and T.A.F.E. orchard in Rockhampton. With respect to pollen germination two distinct

germination peaks were observed. During most of the year (October – July) the germination rate of pollen was high (70 – 95%), whereas during winter/early spring (August/September) germination was significantly ($P < 0.01$) reduced in each of the three papaw lines. Additionally, significant line effects were recorded. Pollen germination in Hybrid 29 was depressed for one of the three sampling intervals during the August/September period, while pollen germination of Hybrid 1D and the open pollinated line were depressed for all three sampling intervals during the same period. Even at its lowest level (at the end of August), Hybrid 29 (45.7± 2.9SE %) had significantly greater germination than Hybrid 1D (6.8± 1.4SE %) and the open pollinated line (4.5± 1.1SE %), ($P < 0.01$). The rate of pollen germination of Hybrid 1D and the open pollinated line were not significantly different.

A significant decrease in pollen viability was also noted during autumn (May/June 1992) and midsummer (November/December 1992), where the level of germination fell to between 70 – 80%. All three papaw lines had significantly lower germination rates during both intervals ($P < 0.01$). However, the rate of germination in Hybrid 29 (80.1± 2.0SE %) was significantly greater compared to both, Hybrid 1D (68.9± 3.8SE %) and the open pollinated line (70.0± 2.8SE %) during December 1992 ($P < 0.01$).

In summary, during Trial period 1 (from June 1992 until February 1993) the viability of pollen in all papaw lines significantly declined during the winter/early spring period (August/September). There was also a significant decrease observed during midsummer (November/December 1992) and midautumn (May/June). However, the magnitude in the decrease (similarily to pollen quantities) was papaw line dependent and the performance of Hybrid 29 was superior during both, winter and summer 1992.

Trial Period 2

During winter and early spring 1993 pollen viability continued to be high, averaging 90% for most of the season (Figure 4.10). An exception was Hybrid 29 where the rate of pollen germination decreased to 65± 3.5SE % for one bi-weekly sampling interval at the end of August. This was significantly lower ($P < 0.01$) than that of Hybrid 1D (91± 1.2SE %) and siblings of the open pollinated line (84± 2.2SE %). Pollen germination during the following winter and early spring in 1994, decreased significantly from July onwards until October, irrespective of papaw line ($P < 0.01$). The magnitude of the decrease was similar to that experienced during the winter/early spring period of 1992. Pollen germination of

Hybrid 29 (≥ 83.9± 0.7SE %) was significantly greater in comparison to both other lines, where lowest viability levels were 8.0± 0.7SE % (Hybrid 1D) and 16.7± 1.9SE % (open pollinated line) during September 1994 ($P < 0.01$).

Average minimum temperatures in June 1994 (9.4°C) and July 1994 (9.5°C) were similar to those experienced during June (9.0°C) and July (10.2°C) 1992. By contrast, minimum temperatures during the same months in winter 1993 averaged 12.2°C and 14.6°C, respectively. No significant decreases in pollen germination were recorded for the winter period in 1993.

Significantly lower pollen germination rates were also recorded during autumn 1993 (end of April/May), where the rate of germination decreased to below 50% at the end of April 1993 for all three papaw lines (Figure 4.10). The germination rate in Hybrid 29 again remained significantly greater (49.9± 4.8SE %), in comparison to both Hybrid 1D (36.4± 2.8SE %) and the open pollinated line (28.5± 2.1SE %), ($P < 0.01$).

Overall, during Trial period 2 (from February 1993 until October 1994) pollen viability significantly decreased during autumn 1993 (April/May) and winter/early spring 1994 (August/September). Again, the magnitude of decrease was papaw line dependent. The viability of pollen of all papaw lines remained significantly higher during the 1993 winter compared to the winters of 1992 and 1994. Despite averaging the lowest pollen viability during winter 1993 (with 63%), the viability of pollen in Hybrid 29 was superior to Hybrid 1D and the open pollinated line.

4.3.3.5 Viability of Pollen from Bisexual and Staminate Flowers

Germination of pollen from staminate and bisexual flowers of male trees was not significantly different for trees from either site, C.Q.U. or T.A.F.E. orchard, ($P > 0.21$). The mean viability of pollen was above 92%, irrespective of the flower type (Table 4.2).

Table 4.2: Viability of pollen from bisexual and staminate flowers.

	% Viability of Pollen ± SE	
	Bisexual Flower	Staminate Flower
C.Q.U. Orchard	94.7± 0.4*	95.9± 0.3*
T.A.F.E. Orchard	92.9± 0.5†	93.6± 0.5†

* n = 39 † n = 30

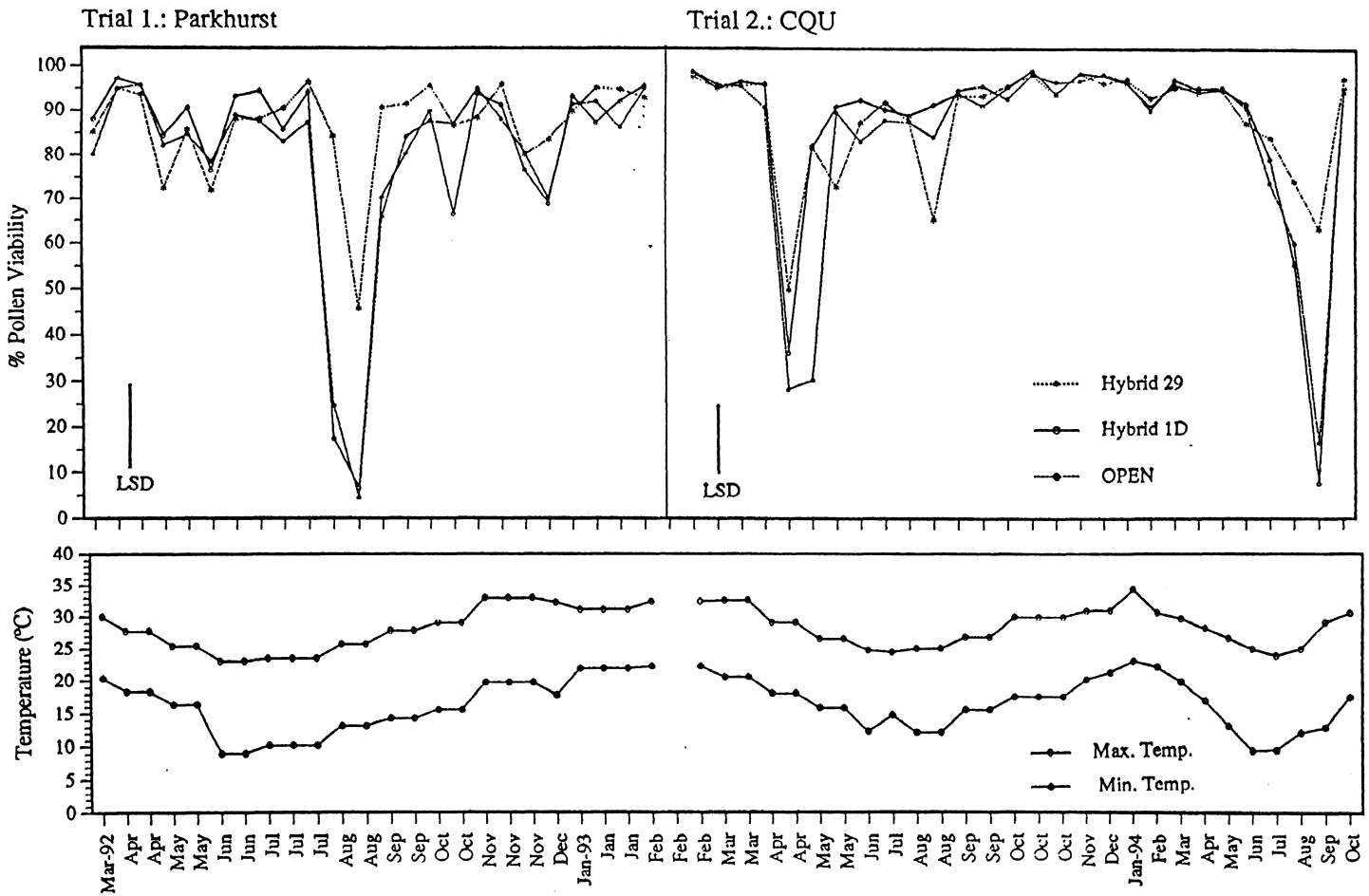


Figure 4.10: Percentage pollen viability of Hybrid 29, Hybrid 1D and an open pollinated line at Parkhurst (March 1992 – February 1993) and at the C.Q.U. orchard (March 1993 – October 1994).

4.3.3.6 Viability of Pollen from 'Dieback' Affected Plants

The viability of pollen from 'Dieback' affected plants was not significantly different ($P > 0.12$) in trees that remained without visible signs of the infection on all three sampling dates (Table 4.3). All affected trees subsequently died of the infection, while all control trees remained visually unaffected.

Table 4.3: Viability of pollen from 'healthy' and 'Dieback' affected plants.

Sampling Date	% Viability of Pollen ± SE	
	'Dieback' Infected Plants	Healthy Plants
4. 5. 1993	66.8± 2.6*	63.8± 3.6*
18. 10. 1993	95.4± 0.5*	98.7± 0.3*
24. 10. 1994	88.1± 1.0 †	86.3± 1.3†

* n = 8 † n = 6

4.3.3.7 Performance of *C. papaya* Pollen in Simulated Heavy Rain Environments

Papaw pollen submerged in tap water germinated. Pollen tubes were visible after three hours of submergence. The increase in the amount of germinated pollen after three, six, nine and twelve hours of submergence was significant ($P < 0.001$), whereas the rate tapered off after twelve and thirty-one hours ($P = 0.265$). Initially, 100% of pollen grains were intact, while 89.4± 1.2SE % and 90.4± 0.8SE % were intact after twelve and thirty-one hours, respectively. Pollen loss due to bursting was negligible ($P > 0.05$). Pollen that did not undergo treatment (control) remained entire.

4.3.4 DISCUSSION

Pistillate flowers remained receptive during the winter period and successfully set fruit when hand pollinated. Therefore, the decline in commercial production during the winter and early spring period can to a degree be counteracted by hand pollination. However, despite hand pollination, fruits may not reach the size of fruit that set during the summer and autumn period, due to a lower rate of cell division and cell enlargement during winter (Kuhne and Allan 1970).

Results from this study show that seasonal variation in fruit and seed set is to a certain extent a consequence of pollen availability and pollen viability. Although pollen quantities were consistent at 60000 grains/flower, and pollen viability

averaged above 90% for most of the year, during certain periods the above parameters appeared to be affected by weather. Cold-affected flowers characteristically showed signs of abnormal anther development and interrupted pollen development. The latter is probably a consequence of degenerated pollen mother cells (Allan 1963a).

The continuing receptivity of pistillate flowers together with the high number of aborted fruitlets, parthenocarpic fruit set or fruit containing low seed numbers (Section 4.3.3.1 and Section 6.3) during the central Queensland winter period suggests that the lack of, or the low number of pollinating insects within the orchard at this time of the year (Section 7.4.2) is the primary causative agent. Fruit set during September may also be impaired due to lower pollen viability in individual papaw lines (for example, the open pollinated line, refer to Section 4.3.3.4) but this is a secondary effect.

Observations of low pollen numbers or complete absence of pollen were previously made by Agnew (1941, 1954, 1968) and Prest (1955) for papaws grown in areas of subtropical Queensland during early spring. Allan (1963a), who conducted pollen studies in an area of subtropical climate in South Africa found similar results. Allan (1963a) found that pollen production remained high during summer until early winter, averaging 100000 grains per flower. At the end of winter and during spring (September and October) he found that the number of pollen grains per flower sharply declined and pollen was unavailable from flowers opening during early spring (September). This present study showed a similar trend of fluctuating pollen quantities throughout the season and a similar decline of pollen availability in September (early spring). However, the duration and magnitude of the decrease was papaw line dependent, as hybrid lines (in particular Hybrid 29) were producing significantly more pollen in comparison to an open pollinated line during spring. The overall lower availability of pollen in the open pollinated line resembled in magnitude, and partially also in duration, the results obtained from both studies conducted under a subtropical climate in South Africa and Israel using non-hybrid lines (Allan 1963a; Cohen *et al.* 1989). Both the South African and Israeli studies indicated 10°C as the critical minimum temperature for normal pollen development. The interval during which minimum temperatures remained below 10°C was shorter in central Queensland than in Israel and South Africa and differed by two months and four months, respectively (Allan 1963a; Cohen *et al.* 1989). Consequently, buds matured faster under central Queensland conditions and periods of low pollen availability were less than those found in overseas studies.

The average number of pollen grains per flower during the main growing season in central Queensland was 60000 grains, compared to an average of 100000 grains per flower during the same growing season in South Africa (Allan 1963a). The reason for the difference is unclear.

The viability of pollen followed a similar seasonal trend to that of the quantity of pollen. The pollen germination rate under the subtropical climate in central Queensland however was greater than those reported from subtropical areas of Israel (Cohen *et al.* 1989) and South Africa (Allan 1963b). During the main growing season germination rates remained above 90% in central Queensland in comparison to approximately 70% in Israel and 60% in South Africa. This indicates a direct effect of temperature or latitude similar to that affecting pollen availability. Again, the duration and magnitude of the decrease was papaw line dependent, as hybrid lines (in particular Hybrid 29) exhibited superior pollen germination in comparison to an open pollinated line during the end of winter/spring period. The overall lower viability of pollen in the open pollinated line resembled in magnitude, and partially also in duration, the results obtained from studies conducted under the subtropical climate in South Africa and Israel using non-hybrid lines (Allan 1963b; Cohen *et al.* 1989). An exception to these results occurred in winter 1993 when pollen production was largely unaffected. Mean minimum temperatures remained 3.1°C above the 10°C critical minimum, in comparison to the mean minimum temperatures of 10.8°C and 9.7°C (three monthly means) in the winters of 1992 and 1994.

High temperatures also led to a decrease in pollen viability, however effects were not as pronounced as at low temperatures. Reasons are most likely found in the faster development of flowers during periods of high temperature (Allan 1963a). Therefore, the immediate effects of high temperature regimes probably remained disguised due to the wide spacings (fortnightly and monthly) between sampling intervals. Nevertheless, results demonstrate that high temperature influences pollen viability, and these results are consistent with studies conducted in South Africa and Israel (Allan 1963a and b; Allan *et al.* 1987; Cohen *et al.* 1989). Differences in performance between papaw lines were noted, and Hybrid 29 was the least affected by a change in climatic conditions. The concept that poor rates of pollination during periods of the northern and southern Queensland summer are due to the rupturing/bursting of pollen grains during wet weather (Agnew 1968; Anon. 1987) was unsubstantiated by the present study, as most of the pollen grains (> 80%) remained intact after thirty-one hours of submergence in water. Most of the 'rupturing' of pollen grains was due to germination.

The performance of bisexual flowers with respect to pollen viability and pollen quantity was not significantly different from 'normal' staminate flowers. Therefore, this annually recurring interlude in spring (May) does not affect the seasonal occurrence of lower fruit set (Section 6.3). Similarily, the results of pollen tested from 'Dieback' affected plants indicate that decreases in pollen viability are primarily climatically related responses.

Therefore, in general, the variable seasonal fruit set of papaw is only secondarily related to the plant parameters such as pollen availability and pollen viability. The major source of variation is attributable to variations in the pollination vector.

4.4 SECTION D: THE STIGMA TYPE OF PISTILLATE PAPAW FLOWERS

4.4.1 INTRODUCTION

The stigmatic surface of pistillate *C. papaya* flowers was described as "dry" meaning "little or no surface secretion" (Heslop-Harrison and Shivanna 1977). Contrary to Heslop-Harrison and Shivanna's (1977) observation, stigmas of *C. papaya* were described as "moist and shiny" by Sharma and Bajpai (1969) when observed under a handheld lens. Secretions of stigmatic exudates from a central stigmatic canal were also reported to occur in papaw flowers (Baker 1976) and in flowers of the related species *J. dolichaula* (Bawa 1980b). This section constitutes an investigation designed to resolve whether or not stigma exudates are present or absent from the receptive surface of pistillate *C. papaya* flowers.

4.4.2 MATERIALS AND METHODS

The stigmatic surface of ten randomly selected newly opened pistillate *C. papaya* flowers was observed using a Nikon Optiphot 2 microscope (Nikon, Tokyo, Japan) set up with darkfield optics and scanning electron microscopy, using a JEOL S.E.M. (5300LV, Tokyo, Japan). Pistillate flowers were freshly collected in the morning during their first day of anthesis. Stigmatic exudates were visible with a handlens. To achieve higher resolution of the stigmatic surface, stigmas were initially gold coated (3 nm) in a sputter coater (Polaron, 510 series, Fissons

Instruments, Sussex, U.K.) but it was found that the hairs collapsed. Therefore, an alternative procedure was adopted by which the untreated stigmas were mounted on aluminium 25 mm stubs and immediately viewed under low vacuum pressure (10^{-1} Torr) S.E.M., without further preparation of the plant material. This method proved to be superior in that no visible collapse or damage to the papillae occurred and stigmatic exudates were easily visible. Photographs were taken using Kodak black and white slide film rated at 200 ISO. Tests were carried out in April 1994 on flowers collected at the C.Q.U. orchard. Observations were made on a precipitation-free day.

4.4.3 RESULTS

Stigmatic papillae are unicellular, smooth and approximately 70 µm in length (Figure 4.11A). Externally, the receptive surfaces showed the presence of stigmatic exudates (Figures 4.11B and 4.11C). Secretion occurred over the entire stigma surface and was not restricted to the area around the central stigmatic canal. The fluid was film-like and made visible by its 'bridging' in the interstices between individual papillae (Figures 4.11B and 4.11C). The quantity of stigmatic exudates was relatively low compared to the wet stigma types such as Rosaceae. Stigmas showed no easily visible differences between the amount of stigmatic exudates.

4.4.4 DISCUSSION

Stigmatic secretions were present on the receptive surfaces of *C. papaya* flowers. Contrary to Bawa's (1980b) and Baker's (1976) observations, stigmatic fluids were not restricted to the area around the central stigmatic canal but occurred instead over the entire stigma surface. Despite the detection of only small amounts of stigmatic exudates, fluids nevertheless were invariably present. Baker (1976) for instance was able to conduct an analysis of the constituents of stigmatic sap of *C. papaya* and Bawa (1980b) found evidence of stigmatic saps on the stigmas of the related species *J. dolichaula.* Additionally, Brewbaker (1957, 1967) reported that binucleated pollen, such as that of *C. papaya*, germinates more easily on liquid or semi-liquid media in *in vitro* germination trials, in comparison to trinucleated pollen which either fails to germinate or bursts prematurely. Thus binucleated pollen grains are usually indicative of 'wet' stigma types as they are adapted to germinate in a liquid medium (Heslop-Harrison and Shivanna 1977). Results of this study confirm that submerging *C. papaya* pollen in water resulted in the germination of

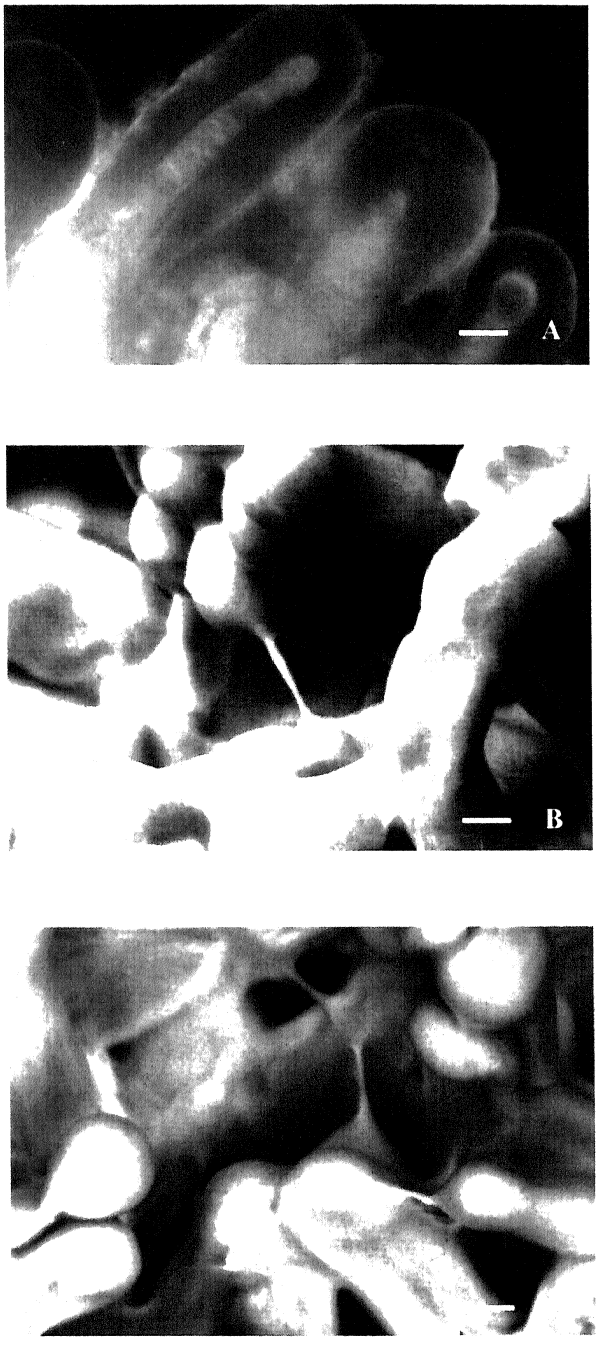


Figure 4.11: Stigmatic papillae of *C. papaya*. (A) Darkfield photograph of papillae, (B) and (C) scanning electron micrographs of exudates on the stigmatic surface.

A, B: x 1000, bar = 10 μm; C: x 750, bar = 10 μm.

grains (Section 4.3.3.7), which is in accordance with their binucleated condition and therefore support the call for reclassification from a 'dry' to a 'wet' stigma type in *C. papaya*. Heslop-Harrison and Shivanna (1977) noted themselves that 'wet' stigma types are sometimes hard to detect as exudates may be restricted to the interstices or that the layer of stigmatic fluid is sometimes so thin that it can only be detected after staining. However, the authors did not indicate if staining procedures were conducted on stigmas of *C. papaya* flowers. Also, Heslop-Harrison and Shivanna were unable to draw from representative data from related species within the Caricaceae as only the receptive surfaces of *C. papaya* flowers were subject to this investigation. Sample size may have also influenced results and remains unstated by the authors. Therefore, it is obvious from the data of this investigation, supported by the studies of Baker (1976), Bawa (1980b) and Brewbaker (1957, 1967) that the receptive surface of *C. papaya* belongs to the 'wet' stigma type following the classification of Heslop-Harrison and Shivanna (1977). Subsequently the characterization of the 'dry' stigma type in association with *C. papaya* should no longer be used.

CHAPTER V

AGAMOSPERMY AND THE EFFECTS OF NON-POLLINATION ON FRUIT PARAMETERS

5.1 INTRODUCTION

This chapter focuses on the occurrence of agamospermy (seed development without fertilization) in papaw. If demonstrated, agamospermy would constitute an alternative reproductive pathway circumventing the necessity for pollen transfer *per se*, and therefore would be of potential value to the papaw industry (Section 1.2.1).

Section A addresses the influences of non-pollination and open pollination on parameters such as fruit set, seed set, and seed viability, while Section B focuses on the consequences of seed numbers and state of seed viability on flesh thickness, fruit weight and seed : fruit weight ratios. These parameters are of particular commercial significance. These experiments also investigate the differences between a number of different commercial varieties and lines of papaw. Results are discussed at the end of each of the two sections.

5.2 SECTION A: FRUIT SET AND SEED SET, SEED NUMBER AND SEED VIABILITY

5.2.1 MATERIALS AND METHODS

Tests were conducted on the ten papaw lines which were WH1, JDM362, RO2, TVL7, JDM411, ER62, JE1, Sunrise Solo, Hybrid 29 and Hybrid 1D. Each line was represented by three initially randomly selected trees, except for S.Solo for which only two fruit-bearing trees were available. Trees of eight lines were planted in a randomized design at the orchard at T.A.F.E. in Rockhampton, while trees of Hybrid 29 and Hybrid 1D were randomly chosen from trees planted in a

randomized block design at the orchard at the University. Site descriptions are given in Section 2.2.

The layout of this trial was based on a split plot design, where each individual tree was considered as a single experimental unit. Consecutively opening pistillate flowers were assigned to blocks of two flowers. One flower was enclosed in a 10 cm wide by 12 cm long white paper bag which was secured tightly at the base with a peg. The other flower was left to open pollination (control). Flowers that opened between the end of January 1994 until the end of April 1994 were considered in this trial and this resulted in four to nine pairwise comparisons (or blocks) for each tree.

The data were split into two groups for statistical analyses; bagged and unbagged flowers, since a distinct biological response of flowers towards bagging was already evident. Analyses of variance were performed on Genstat 5 release 2.2 software (Payne *et al.* 1988) and with Genstat release 5.2, and the significance of differences was assessed using Fisher's Least Significant Differences (Montgomery 1991), except where stated otherwise. One-way analysis of variance was performed for all the parameters. Data concerning fruit set and seed set of bagged flowers was analysed by fitting a generalized linear model to the binary data (Mc Cullagh and Nelder 1983). In so doing, the analyses were based on the proportion of successful fruit set and seed set, taking into account the different numbers of flowers and fruit involved. Fruit related parameters were evaluated at maturity.

The viability of seed derived from fruit that was open pollinated was assessed for embryo presence by sampling seeds to an upper limit of 50 seeds per fruit. The seed viability of seed derived from bagged fruit was determined using germination tests. A small number of seeds from bagged fruit were microscopically evaluated for the presence of embryos, whilst the remainder of seeds were tested for their capacity to germinate. Seeds were cleaned (removal of the sarcotesta) and planted in a 50:50 sand and peatmoss mix and kept under glasshouse conditions at 25°C/60% humidity. The germination rate of 50 seeds of each sampling tree, taken from flowers that remained unbagged, served as a control. Germination was assessed after 28 days.

5.2.2 RESULTS

5.2.2.1 Open Pollinated (Unbagged) Flowers

All flowers which were exposed to open pollination from February until April 1994 successfully set fruit. All fruits which were harvested at maturity contained viable seeds (Table 5.1).

The seed numbers of fruits from different papaw lines were significantly different ($P = 0.009$). Hybrid 1D, JDM411, and ER62 averaged the largest amounts of seeds per fruit (greater than 762± 127SE seeds, n = 16, ER62), significantly higher than TVL7, JE1 and Sunrise Solo (less than 268± 113SE seeds, n = 20, TVL7); (Table 5.1).

The viability of seeds in different papaw lines were also significantly different ($P = 0.015$). Seed viability of S.Solo (48.7± 9.3SE %, n = 10) was significantly less than that of any other line ($P < 0.01$). Also, the seed viability of JE1 (79.5± 6.6SE %, n = 20) was significantly less ($P = 0.05$) than that of JDM362, TVL7, ER62, Hybrid 29 and Hybrid 1D, where viabilities were greater than 96.6 ± 6.6SE % (JDM362; Table 5.2).

5.2.2.2 Non-Pollinated (Bagged) Flowers

Significant varietal effects ($P < 0.01$) in the proportion of flowers which produced fruit and in the proportion of fruit producing some seeds were evident when flowers were bagged (Table 5.3). All flowers of S.Solo resulted in fruit set. A high proportion of fruit set was also evident in TVL7 and JDM411 with 85± 7SE % and 83± 9SE %, respectively. The proportion of fruit set was lowest in Hybrid 29 and Hybrid 1D, with 33± 12SE % and 27± 11SE %, respectively (Table 5.3).

Proportionally more harvested fruit of S.Solo (80± 13SE %), JDM411 (60± 13SE %) and RO2 (43± 13SE %) contained seeds than the remaining papaw lines (Table 5.3). Parthenocarpic fruit set was however observed in all lines (Table 5.4). Seed set from fruit where flowers remained bagged in the receptive stage was observed in seven of the ten papaw lines. Seeds of RO2, JDM411, JE1, Hybrid 29, Hybrid 1D and S.Solo were tested for germinability. While none of the JDM411, JE1 and hybrid line seeds germinated, four of 877 potted seeds of RO2 and thirteen of 308 potted seeds of S.Solo germinated (Table 5.4).

Table 5.1: Average seed number (± SE) of open pollinated fruit of ten papaw lines which fruit set between February 1994 and April 1994. (Each line was replicated in n = 3 trees).

Hybrid 1D	JDM411	ER62	RO2	Hybrid 29	JDM362	WH1	TVL7	S.Solo*	JE1
831± 131	829±120	762± 127	665± 111	621± 131	608± 113	465± 136	268± 113	252± 160	233± 113
n = 15	n = 18	n = 16	n = 21	n = 15	n = 20	n = 14	n = 20	n = 10	n = 20
a	a	a	a	a	a	a			
			b	b	b	b	b		
				c	c	c	c	c	
						d	d	d	d

* n = 2 female trees

means followed by the same letter are not significantly different P<0.01.

Table 5.2: Average percentage of seed viability (± SE) of open pollinated fruit of ten papaw lines which set fruit between February 1994 and April 1994. (Seed viability was assessed on n = 50 seeds per fruit).

TVL7	Hybrid 1D	Hybrid 29	JDM362	ER62	JDM411	RO2	WH1	JE1	S.Solo*
99.5± 6.6	99.2± 7.6	98.5± 7.6	96.6± 6.6	96.5± 7.4	93.9± 7.0	87.2± 6.4	87.1± 7.9	79.5± 6.6	48.7± 9.3
n = 20	n = 15	n = 15	n = 20	n = 16	n = 18	n = 21	n = 14	n = 20	n = 10
a	a	a	a	a	a	a	a		
						b	b	b	
									c

* n = 2 female trees

means followed by the same letter are not significantly different P<0.05.

Table 5.3: Proportion of fruit set and seed set in bagged flowers (February 1994 – April 1994).

Line Name	Total No of Bagged Flowers	Proportion of Fruit Set ± SE	Proportion of Harvested Fruit with Seed ± SE
WH1	14	35± 13	0
JDM362	20	45± 11	0
RO2	21	67± 10	43± 13
TVL7	20	85± 8	0
JDM411	18	83± 9	60± 13
ER62	16	38± 12	17± 15
JE1	20	50± 11	40± 15
Sunrise Solo *	10	100	80± 13
Hybrid 29	15	33± 12	40± 22
Hybrid 1D	15	27± 11	50± 25

* n = 2 female trees

Table 5.4: Fruit set, seed set and seed viability in bagged flowers (February 1994 – April 1994).

Line Name	Total No of Bagged Flowers	No of Harvested Fruit	No of Harvested Fruit with Seeds	No of Seeds of Harvested Fruit	No of Seeds Potted	No. of Seeds Germinated
WH1	14	5	0	0		
JDM362	20	9	0	0		
RO2	21	14	6	1045	877	4
TVL7	20	17	0	0		
JDM411	18	15	9	57556	5394	0
ER62	16	6	1	1	0	
JE1	20	10	4	342	250	0
Sunrise Solo *	10	10	8	422	308	13
Hybrid 29	15	4	1	2	0	
Hybrid 1D	15	4	3	4	0	

* n = 2 female trees

In both instances, viable RO2 and S.Solo seeds were obtained from two different fruit of the same sampling tree. Germination of seeds from open pollinated flowers was greater than 95% for each of the papaw lines.

5.2.3 DISCUSSION

Bagging of flowers, that is the interception of pollen transfer, obviously led to a decrease in successful fruit set, in fruits that produced seeds, and in overall seed number and seed viability. Despite the production of a small number of viable parthenogenetic seeds it can be concluded that in general the reproduction of papaws is primarily a sexual process.

Nevertheless, parthenogenetic seed set did occur, but examples were infrequent (only two of the 169 bagged flowers of 29 sampling trees contained seeds that germinated). As only two trees exhibited a tendency towards agamospermy, I hypothesize that a genetic basis (predisposition) is responsible. The origin of genetic defects possibly results from the multiple inbreeding these plants have undergone in order to 'fix' line characteristics. Generally the establishment of a line requires selfing over at least six generations (Aquilizan 1987). For instance 'Solo' had been selfed for more than 25 generations by 1969 (Storey 1969b). It is generally believed that inbreeding of papaws has no ill-effects with regard to plant vigour and reproductive efforts (Hamilton 1954; Storey 1969b). However, it should be kept in mind that sex reversals of phenotypically stable female papaw trees were only induced after multiple inbreeding efforts (Prest 1955; Nakasone 1986).

Sexual ambivalence in papaws is usually only associated with heterozygosity of both male and hermaphroditic plants and is supposedly triggered by climatic and nutritional influences (Arkle and Nakasone 1984; Nakasone 1967, 1986). Other factors, such as daylength and/or high temperature regimes could also be influential, since in this trial agamospermic seed set occurred on both trees in fruit for which flowers reached anthesis between the 15 February 1994 and 22 February 1994. At present however, the hypothesis remains speculative.

Greater quantities of fruit set in some of the papaw lines (i.e. TVL7 and JDM411, with over 80% of fruit reaching maturity) where flowers remained unpollinated,

was not always associated with seed set. For instance, all bagged fruit of TVL7 developed parthenocarpically. This result supports Agnew's (1954) suggestion that parthenocarpy is an inherited characteristic, as it has reappeared in the progeny of a plant selected for this character. Similar observations on naturally occurring parthenocarpy have also been reported from hermaphroditic cultivars (Rodriguez Pastor *et al.* 1990). In this instance the trait was cultivar dependent. Fruits, according to the authors, were of marketable size.

Overall, seed development of either form, fertile or infertile, significantly stimulated fruit retention on the tree as all aborted fruit was seedless. Fruit set of both hybrid lines was most noticeably affected by non-pollination as less than a third of bagged flowers produced fruit, and almost all fruit developed parthenocarpically. In contrast the proportion of successful fruit set of S.Solo when flowers remained unpollinated was 100%, of which some 80% exhibited some seed development. Parthenocarpic fruit development in S.Solo was observed in two fruits out of six that were bagged on the same sample trees. These results emphasize that the event of agamospermy is most likely linked to inbreeding effects of parent lines and diminishes with the first steps of cross-breeding.

Free (1975) studied pollination of papaw in Jamaica using the method of bagging pistillate flowers. Bagging resulted in 65% successful fruit set three weeks after the initial bagging procedure. The high percentage of asexual fruit set could be interpreted as evidence of varietal influences or could merely have resulted from the conclusions being drawn three weeks after the initial bagging procedure; a period too short to yield biologically significant results. In the present study, the abortion of unfertilized fruit still occurred until twelve weeks after anthesis in central Queensland (personal observation). Furthermore, Free (1975) did not comment on the presence of seed in bagged fruits, and as such the origin of fruit development, either parthenocarpically or asexually, remains unresolved.

Natural pollination led to variable numbers of seed set per fruit and variable numbers when lines were compared. Whilst the former is explicable in terms of variation in pollinator visitation rates towards each flower, the latter is more difficult to explain. Dense leaf canopy and possibly flower and petal size could influence pollinator foraging behaviour on either a visual or an olfactory basis, depending on the light reflection of petals and the amount of scent released from glands located on the petals themselves.

Overall, natural pollination led to 100% successful fruit set during the February to April period in 1994. Results were comparable to those obtained during a similar period in 1993 despite a tenfold increase in rainfall received during each of the three months in 1994 compared to 1993 (Section 2.1). Poor fruit set during the summer months is reportedly common in north Queensland (Anon. 1987) and in south Queensland (Agnew 1968). The explanations proposed by these sources are that fruit loss is due to the loss of pollen washed from anthers or from the air to the ground, or from pollen rupturing due to wet weather conditions. These explanations might be consistent with a wind-pollinated species but the results of the present study cast serious doubts over these explanations (Section 4.3.3.7).

The viabilities of seeds in open pollinated flowers also showed significant differences between lines. Lower viabilities of S.Solo (48.8%) possibly result from the higher incidence of infertile seed development, of which this line seems capable, whilst reasons for lower seed fertility of JE1 (79%) remain unclear.

5.3 SECTION B: EFFECTS OF SEED NUMBER AND VIABILITY ON FRUIT PARAMETERS

5.3.1 MATERIALS AND METHODS

Data concerning fruit weight combining both bagged and open pollinated treatments were analysed using the REML (residual maximum likelihood) technique of Patterson and Thompson (1971) as implemented in the Genstat package. This method accounts for unbalanced data, which in this experiment, derived from the fact that only harvested fruit was included in the analysis.

Varietal differences in fruit weight at maturity were determined using analysis of variance, for both bagged and open pollinated treatments. Analysis of variance was performed on Genstat 5 release 2.2 software (Payne *et al.* 1988) and statistical significance was determined using Fisher's Least Significant Differences (Montgomery 1991).

The influence of seed viability on the flesh thickness of the fruit was analysed from fruit which displayed areas of viable and non-viable seed set, and seedless areas, all

within the same fruit cavity. The flesh thickness was measured as the shortest distance between fruit surface and fruit cavity. Only fruit from the open pollinated treatment were included. Data were analysed using analysis of variance and Fisher's Least Significant Differences for comparisons.

The relationship between fruit weight and seed numbers was analysed by using a general multiple regression model. Analysis of variance was based on the most parsimonious model which only considered factors that were of significant ($P < 0.001$) influence. These terms were the two factors of bagged flowers per tree and the variable seed number. There also was some indication that bagging might influence the relationship between weight and seed numbers ($P = 0.085$), though this was not significant at the 5% level.

5.3.2 RESULTS

5.3.2.1 Comparisons of Fruit Weights of Open Pollinated and Non-Pollinated Flowers between Lines

Fruit weights of the open pollinated treatment showed significant differences ($P < 0.01$) between lines. WH1, JE1 and S.Solo averaged lower fruit weights at harvest (less than or equal to 666± 122g; WH1) than the remainder of lines (greater than 977± 118g; Hybrid 29); (Table 5.5). Of the remaining lines, open pollinated RO2, ER62 and Hybrid 1D fruit weighed significantly more than fruit of WH1, averaging above 1078± 100g (RO2); (Table 5.5). The fruit weights between lines of the bagged treatment were not significantly different ($P = 0.499$).

Table 5.5: Average weight (g ± SE) of open pollinated fruit at maturity in ten papaw lines which flowered between February 1994 and April 1994.

Hybrid 1D	ER62	RO2	JDM411	TVL7	JDM362	Hybrid 29	WH1	JE1	S.Solo*
1166± 118	829± 120	1078± 100	1072± 108	1044± 102	1004± 102	977± 118	666± 122	545± 102	450± 145
n = 15	n = 16	n = 21	n =18	n = 20	n = 102	n =15	n = 14	n =20	n = 10
a	a	a	a	a	a	a			
			b	b	b	b	b		
							c	c	c

* n= 2 female trees

means followed by the same letter are not significantly different P<0.01.

5.3.2.2 Comparison of Fruit Weights of Open Pollinated and Non-Pollinated Flowers within Lines

Harvested fruit of bagged and open pollinated treatments for each of the papaw lines showed a significant ($P < 0.01$) interaction between papaw line and the bagging treatment. Bagging significantly ($P = 0.01$) affected the fruit weight in eight of the ten tested papaw lines (Figure 5.1). The two exceptions were S.Solo and JE1 where non-pollination did not lead to a significant decrease of fruit weights ($P > 0.05$).

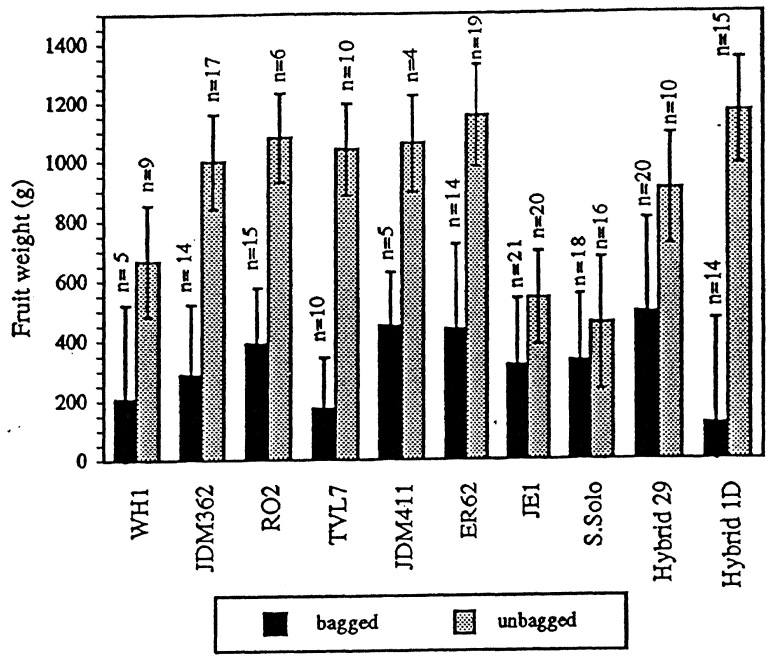


Figure 5.1: Average fruit weights at maturity of bagged and open pollinated fruit of ten papaw lines (February 1994 – April 1994). Confidence limits = 95 percent.

5.3.2.3 Relationship between Fruit Weight and Seed Number

When the influence of seed number, bagging and tree effect on fruit weight was considered, the increase of fruit weight per seed was 0.89g (independent of seed viability) with an estimated difference between bagged and open pollinated fruit of 107g.

Results are described by the regression equation:

Fruit weight = T - B + 0.89 (Seed No)

where	B	=	0	for open pollinated and
		=	107± 48.7	for bagged
and	T	=		the intercept for each tree in Table 5.6

Table 5.6: Estimated y intercept for each tree of open pollinated fruit.

Line	Tree	T	S.E.
WH1	1	447	113
	2	159	116
	3	189	94
JDM362	1	675	114
	2	407	91
	3	383	87
RO2	1	339	117
	2	582	65
	3	405	72
TVL7	1	848	100
	2	681	86
	3	888	82
JDM411	1	245	83
	2	255	76
	3	547	113

Line	Tree	T	S.E.
ER62	1	724	109
	2	295	106
	3	423	99
JE1	1	429	60
	2	211	113
	3	298	77
S.Solo	1	293	70
	2	337	85
Hybrid 29	1	510	103
	2	397	106
	3	375	106
Hybrid 1D	1	589	110
	2	389	108
	3	310	107

5.3.2.4 Flesh Thickness

Flesh thickness was significantly ($P < 0.001$) affected by the presence of seeds and their state of viability. Fertile seed set led to a significant ($P < 0.004$) increase in flesh thickness compared to areas of no seed set and infertile seed set. The flesh thickness of areas with infertile seed set were also significantly greater ($P = 0.049$) compared to areas of no seed set. The average flesh thickness in the presence of fertile seeds was 3.0± 0.1 cm compared to 2.4± 0.1 cm when seeds were infertile and 2.1± 0.1 cm in the absence of seeds (n = 11 fruit).

5.3.3 DISCUSSION

As with much of the literature relating to papaw production, the effects of pollination on fruit development is inconclusive, incomplete or conflicting. The bagging treatment simulated the effects of non-pollination. Growing papaws under subtropical conditions leads to poor pollination during a period in winter and spring, partially due to pollinator absence (Section 7.4), and partially due to insufficient pollen production and viability (Section 4.3). The capacity for unfertilized seed formation is found in some trees more readily than in others, and would be beneficial not only in initializing fruit set at all times, but also is positively influencing the size of winter fruit compared with parthenocarpic fruit. As fruit size also depends on the rate and number of cell divisions during the initial phase of fruit development (two to three months after anthesis), gains in size of fruit that set during winter will be limited by climatic factors (Kuhne and Allan 1970) and intra-tree competition depending on the overall number of fruit set (Potter 1927). However, unfertilized seed development should be of advantage during the spring period (September and October) when pollen supply and viability constitute the limiting resource towards successful fruit set. Potentially apomictic seed set not only prolongs the cropping cycle but also positively increases the weight of fruits.

In the present study, non-pollination affected the weight of all fruit that reached maturity. Non-pollinated fruit averaged half the weight of pollinated fruit. However, considering individual lines, bagging did not significantly influence the fruit weights of JE1 and S.Solo. The similarity in fruit weights between both bagged and unbagged treatments of S.Solo were most likely the consequence of proportionally high (mostly non-viable) seed numbers of fruit that remained non-pollinated. In the instance of JE1, the weight of open pollinated fruit remained relatively low (233± 113g) and possibly concealed differences in fruit weights between the bagged and unbagged treatment. Additionally, some of the bagged JE1 fruits contained some non-viable seed set which would have positively influenced the weight of fruit of the bagged treatment.

Overall, the increase in fruit weight was determined by the number of seeds produced, irrespective of seed viability. These results would support Kobel's (1954) controversial hypothesis that the ultimate size of fruits is correlated with the total number of seeds or seedlike structures that form in fruits, regardless of their viability status. Seeds, according to Kobel (1954) have their greatest influence

(due to the production of hormones) on fruit weight during the early stages of their development. Stimulating influences of underdeveloped seeds on the fruit growth have also been reported from *C. papaya* (Storey 1969c). Storey (1969c) suggested on the basis of partial integument and endosperm development of non-viable seeds, that hormones leading to the development of the former also positively affect fruit growth, possibly by increasing the length of fruit development. In this study endosperm and embryo development of seeds from flowers where flowers were bagged was assessed by subsampling methods only. Partial integument and endosperm development of non-viable seeds in the later development stage cannot therefore be ruled out. However, an instant stimulus from seedlike formations inducing fruit growth might be sufficient not only in triggering fruit growth but also in retaining fruit set. Abbott (1959) for instance found, in a study conducted on apples, that fruit growth was unaffected by the removal or the destruction of seeds after a certain development stage of the fruit.

Non-pollinated fruit that indicated signs of seed development demonstrated an increase in fruit weight, which on the whole was slightly less (107g) compared to open pollinated fruit. Nevertheless, the weight increase of 0.89g per seed (viable or non-viable) remained the same. Results concerning flesh thickness showed that this particular trait was influenced by seed number as well as seed viability. Overall, seed set positively affected flesh thickness, as areas of viable and non-viable seed set were significantly thicker compared to seedless areas. However, a difference was also observed between flesh thicknesses depending on the viability status of seed, supporting the hypothesis that the seed to fruit weight ratio of bagged fruit could indeed be different to a similar ratio of open pollinated fruit.

Instances of increased fruit weight when flowers were not pollinated were due to the performances of individual trees which were able to produce some form of seed set; for example Tree 1. and Tree 2. of JDM411 and Tree 3. of RO2. Seed development without pollination, though, was not continuous, as the same trees also showed signs of fruit abortion and parthenocarpy, although of minor proportion, indicating the importance of environmental influences.

CHAPTER VI

INSECT EXCLUSION TRIALS

6.1 INTRODUCTION

Historically a wide diversity of insect species have been reported in papaw orchards. The list of proposed pollinators is diverse, ranging from minute *Thrips* sp. and midges to larger species of butterflies and moths (Table 1.3). Commonly, bees, either *Apis mellifera* or native *Trigona* species are believed by many authors to act as pollinators of papaw; a view also held and expressed by a majority of the farming community. In order to determine if fruit set is affected by the exclusion of insects, individual female trees were enclosed in wire cages and fruit set was monitored. Two different mesh sizes were trialed in order to evaluate the size of possible insect pollinators.

6.2 MATERIALS AND METHODS

6.2.1 2 mm Mesh

From the 15 February 1992 until the 18 January 1993, four randomly selected female trees (one each of Hybrid 29 and Hybrid 1D and two single trees of the open pollinated line) were enclosed by metal frame cages (0.9 m in length x 0.9 m in width x 1.8 m in height) at the planting at Parkhurst. These frames were covered with light grey flyscreen material of 2 mm mesh size. Enclosures were positioned over trees when they commenced flowering and removed when plants reached cage height. The base of the frame was secured at ground level. Enclosures were free of insects at the commencement of the trial. Trees remained healthy and flowered continously for the length of the trial. In all instances reproductive male trees were adjacent to caged females and within a 1.5 m range.

Trees kept in enclosures were sampled for the number of fruit set, seed set and the embryo presence in seeds. The data were compared with that from five female trees of each line in the adjacent open orchard. Control trees were initially randomly selected, and from then onwards continuously monitored for the above

parameters. The date of flower opening was marked on the stem closest to developing fruits on all sampling trees at the end of each month. At the conclusion of the experiment (when trees outgrew the height of cages) fruit set from trees kept in enclosures was harvested and evaluated using the above seed parameters. Fruits of control trees were sampled at the time of ripening. Seed viability of fruit from control trees was assessed by subsampling 50 seeds for the presence of embryos, while all seeds of fruit harvested from caged trees were evaluated.

6.2.2 2 cm Mesh

From the 9 September 1993 until the 26 April 1994 the same metal frames with 2cm mesh were used to enclose three randomly chosen single female trees (one tree each of Hybrid 29, Hybrid 1D and one sibling of the open pollinated line) at the orchard at the Central Queensland University. The fourth cage included a female and male tree of Hybrid 1D. Controls of three female trees of each of the three papaw lines existed within the adjacent orchard. Plant spacings, sampling parameters and procedures were identical to those described in Experiment 1. All enclosed female plants bore fruit prior to caging.

Both caging experiments were conducted as observational trials only. A large scale screening operation was beyond the scope and budget of this project. Therefore, limited statistical analyses were conducted and data were presented as means, including the standard errors and standard deviations.

6.3 RESULTS

6.3.1 2 mm Mesh

The quantity of fruit set and seed set by trees kept in enclosures, independent of their line, was significantly less than fruit and seed set in unscreened control trees in the surrounding orchard (Tables 6.1 and 6.2). Over the entire observation period, caged female trees produced a total of 75 flowers of which 23 developed into fruit and produced a total of 56 seeds of which only eight seeds showed signs of embryo formation. The highest seed number of a single fruit harvested from enclosures was 34 seeds, of which six were viable. In comparison, flowers of control trees averaged over 60% fruit set and fruit contained seed numbers averaging tenfold the number in fruit from enclosed trees for most of the trial period (Tables 6.1 and 6.2). Most seeds from control trees contained embryos for the length of the sampling interval.

Table 6.1: The average number of open flowers and percentage fruit set of uncaged and caged female trees screened with 2 mm mesh (March 1992 – December 1992).

MONTH	HYBRID 29				HYBRID 1D			
	uncaged*		caged†		uncaged*		caged†	
	AVG Flower No	AVG Fruit set	Flower No	Fruit set	AVG Flower No	AVG Fruit set	Flower No	Fruit set
Mar-92	6.4	6.4	3	0	3.4	3.4	-	-
Apr-92	4.2	3.8	3	0	3.6	3.2	4	4
May-92	4.2	3.6	3	0	3.6	3.4	4	4
Jun-92	2.8	1.8	2	0	2.6	1.8	2	1
Jul-92	2.0	0.6	1	0	2.2	0.6	3	3
Aug-92	2.2	2.0	1	0	4.6	4.4	2	1
Sep-92	5.0	4.0	2	2	5.8	5.4	3	2
Oct-92	4.2	3.4	2	2	3.8	2.8	1	0
Nov-92	5.2	5.2	5	5	4.2	4.2	5	0
Dec-92	-	-	-	-	4.4	4.4	-	-
	(pooled STD= 1.0)				(pooled STD= 0.9)			

MONTH	OPEN						% Fruit set uncaged n = 15 trees	% Fruit set caged n = 4 trees
	uncaged*		Cage 1†		Cage 2†			
	AVG Flower No	AVG Fruit set	Flower No	Fruit set	Flower No	Fruit set		
Mar-92	3.0	3.0	2	0	1	0	100	0
Apr-92	3.0	3.0	2	0	3	1	93	42
May-92	2.0	2.0	3	0	2	0	92	33
Jun-92	0.8	0.2	2	0	1	0	61	14
Jul-92	2.0	0.8	1	0	1	0	32	50
Aug-92	2.2	1.6	2	0	2	0	89	14
Sep-92	4.8	4.4	2	0	2	2	88	67
Oct-92	3.0	2.4	2	1	1	1	78	67
Nov-92	2.6	2.4	-	-	1	1	98	86
Dec-92	4.0	3.8	-	-	2	1	98	50

* n = 5 trees (pooled STD= 1.0)

† n = 1 tree

Table 6.2: The average number of viable seeds formed in fruits of uncaged and caged female trees screened with 2 mm mesh (March 1992 – December 1992).

MONTH	HYBRID 29			HYBRID 1D			OPEN			
	Uncaged* ±SE	n fruit	Caged†±SE	Uncaged* ±SE	n fruit	Caged† ±SE	Uncaged* ±SE	n fruit	Cage1† ±SE	Cage2† ±SE
Mar-92	924±60	32	0	934±42	17	-	599± 70	15	0	0
Apr-92	897±86	21	0	739±82	18	2±2	537± 45	15	0	4±4
May-92	419±58	21	0	555±54	18	1±1	603± 60	10	0	0
Jun-92	24± 9	14	0	89±28	13	0	46± 46	4	0	0
Jul-92	27±17	10	0	55±42	11	0	52± 28	10	0	0
Aug-92	167±64	11	0	183±47	23	0	61± 25	11	0	0
Sep-92	445±79	25	0	250±37	29	0	151± 34	24	0	0
Oct-92	559±81	21	0	332±69	19	0	518±100	15	0	0
Nov-92	672±42	26	0	710±40	21	0	661± 85	13	-	0
Dec-92	937±56	16	-	923±39	22	-	642± 63	20	-	0

* n = 5 trees

† n = 1 tree

All female trees which were kept in enclosures produced seeded fruit after cages were removed. Caged female trees of different lines showed differences in the rate of aborted fruitlets. While the female trees of Hybrid 29 and the open pollinated line showed continuous fruit abortion except for the most recent fruitlets set before trial closure, Hybrid 1D by contrast showed a higher incidence of fruit retention, setting 10 fruit out of the total of 23 fruit set by caged trees. The four oldest fruit (from March until May 1992) of the enclosed Hybrid 1D tree ripened with an average fruit weight of 170± 40g. One fruit contained a total of two seeds. The remainder were seedless. In comparison the average weight of ripe fruit obtained from control trees of Hybrid 1D which set during April 1992 was 1033± 57g (n = 16). Fruit matured at the same rate on caged and uncaged trees. Aborted fruitlets weighed generally less than 10g, with pointed shape, and in most instances skin discolouration had occurred. Fruitlets lacked developing seeds (Tables 6.1 and 6.2). Abscission usually occurred within four weeks of flowering. In some instances, eight to ten week old fruitlets were still aborted, especially those formed during winter. Their fruit weight generally increased but never exceeded 40g.

6.3.2 2 cm Mesh

The rates of fruit set and seed set of trees kept in enclosures surrounded by 2 cm mesh size was lower than that of control trees (Tables 6.3 and 6.4). Control trees exhibited almost 100% of fruit set (except during September) and averaged seed numbers of 436± 70 throughout the observation period, but the three single caged female trees on average set half the amount of fruit (Tables 6.3 and 6.4). Over the entire observation period the three single caged female trees produced a total of 45 flowers of which 22 developed into fruit and produced a total of 77 seeds. Fifteen seeds showed signs of embryo formation. Control seeds exhibited viabilities above 98% over the trial period. The highest total seed number of a single fruit obtained from enclosed trees was 175 seeds (Hybrid 29), but the seeds did not contain embryos. The maximum viable number of seeds per fruit in singly caged females was five.

In the one instance where a female and male tree were enclosed together (Hybrid 1D: Cage 2) all of the 13 opened flowers set fruit. A total of 483 seeds were formed of which 43 seeds exhibited embryo formation. The highest number of seeds per fruit was 217 of which one seed was viable (January 1992). In contrast, the highest count of viable seeds per fruit was 35 of 155 seeds that set (February 1992).

Table 6.3: The average number of open flowers and percentage fruit set of uncaged and caged female trees screened with 2 cm mesh (September 1993 – April 1994).

MONTH	HYBRID 29				OPEN			
	uncaged*		caged†		uncaged*		caged†	
	AVG Flower No	AVG Fruit set	Flower No	Fruit set	AVG Flower No	AVG Fruit set	Flower No	Fruit set
Sep-93	2.7	2.0	2	0	3.0	2.3	1	0
Oct-93	2.8	2.8	3	1	2.3	2.3	2	0
Nov-93	2.0	2.0	2	2	1.3	1.3	1	0
Dec-93	2.3	2.3	2	2	2.7	2.7	1	0
Jan-94	3.0	3.0	2	2	2.3	2.3	2	0
Feb-94	2.3	2.3	2	2	2.3	2.3	2	1
Mar-94	2.3	2.3	2	2	2.0	2.0	2	2
Apr-94	1.7	1.7	3	3	2.0	2.0	3	3
	(pooled STD= 1.0)				(pooled STD= 1.1)			

MONTH	HYBRID 1D						% Fruit set	% Fruit set
	uncaged*		Cage 1†		Cage 2‡		uncaged	caged*
	AVG Flower No	AVG Fruit set	Flower No	Fruit set	Flower No	Fruit set	n = 9 trees	n = 3 trees
Sep-93	3.0	2.3	2	0	-	-	77	0
Oct-93	1.7	1.7	2	0	2	2	100	14
Nov-93	2.0	2.0	2	0	2	2	100	40
Dec-93	1.3	1.3	1	0	2	2	100	50
Jan-94	2.3	2.3	2	0	3	3	100	33
Feb-94	2.0	2.0	3	1.0	3	3	100	57
Mar-94	2.0	2.0	-	-	-	-	100	100
Apr-94	1.3	1.3	2	2	1	1	100	100

* n = 3 trees (pooled STD= 1.2)

† n = 1 tree

‡ 1 female and 1 male tree

Table 6.4: The average number of viable seeds formed in fruits of uncaged and caged female trees screened with 2 cm mesh (September 1993 – April 1994).

MONTH	HYBRID 29			HYBRID 1D				OPEN		
	Uncaged* ±SE	n fruit	Caged† ±SE	Uncaged* ±SE	n fruit	Cage1† ±SE	Cage2‡ ±SE	Uncaged* ±SE	n fruit	Caged† ±SE
Sep-93	569±136	5	0	436± 70	7	0	-	439± 60	7	0
Oct-93	648±115	8	1±1	760±122	5	0	3±2	636± 95	7	0
Nov-93	774±125	6	0	591± 94	6	0	1±1	684±211	4	0
Dec-93	727±124	7	0	548±140	4	0	0	640±101	8	0
Jan-94	526±113	9	0.5±0.5	650± 95	7	0	0.3±0.3	666±117	7	0
Feb-94	927±106	7	0.5±0.5	712± 90	6	5±5	11.7±11.7	594± 94	7	0
Mar-94	876±126	7	0	811± 68	6	-	-	753± 98	6	0
Apr-94	549±120	5	0	588±112	4	0	3±3	642± 86	6	0

* n = 3 trees

† n = 1 tree

‡1 female and 1 male tree

Caged female trees showed differences in the rate of aborting fruitlets. For instance, both single caged female trees of Hybrid 1D and the open pollinated sibling aborted developing fruitlets continuously. At the conclusion of the trial, in April 1994, both females were bearing fruitlets less than eight weeks old. By contrast, the singly enclosed tree of Hybrid 29 exhibited almost complete fruit retention as only two of the 18 fruit in total were aborted.

6.4 DISCUSSION

Enclosing female trees in cages covered with screening material of 2 mm and 2 cm mesh size did not prevent all trees from setting fruit. However, most of the fruit that developed on enclosed females was either seedless or produced a low number of seeds in contrast to unscreened control trees. Also, seeds of fruit set in enclosures lacked embryos in almost all instances. As this experiment was conducted as an observation trial only, standard forms of statistical analyses were restricted due to the limited sample size of enclosed trees. However, the differences observed in fruit set, seed set and seed viability between screened (either using 2 mm or 2 cm mesh material) and unscreened papaw trees warrants the comment that significant biological differences are evident. The reduction of fruit weights of fruit that ripened in enclosures (170± 40g; n = 4) compared to 743± 48g of seed containing fruit of the same line (Hybrid 1D) in the adjacent orchard supports such a claim.

These results indicate the need for pollination to induce consistent fruit set of marketable size (i.e. 500g or above) and for viable seed formation. Similar results were obtained by Allan (1963c) who enclosed an unspecified number of papaw branches with 16 mesh gauze in South Africa. He also found that fruit set and seed numbers were impaired when flowers were enclosed by gauze and the fruits that did set were of no commercial value which has obvious implications for the industry.

Enclosing trees of both sexes within the same cage using 2 cm mesh did not lead to an increase in seed numbers per fruit. However, viable seed set was higher in fruit of the mixed sex set-up compared to singly caged females, i.e. 35 seeds compared to five seeds. These results suggest that viable seed development was likely to be induced by either entomophilic pollen transfer of insects smaller than 2 cm mesh, pollen dispersal on the basis of gravity or apomictically induced seed formation. It is impossible to determine the relative importance of these mechanisms without

extensive experimentation. However, the small number of successfully formed seeds suggests that none of the aforementioned modes adequately provide for the necessary pollen transfer.

The depression of fruit set, seed set and seed viability in all screened trees suggests that both 2 mm and 2 cm sized mesh limited the naturally occurring pollination process. With regard to entomophily, the caging of trees showed that pollination would be most likely carried out by insects larger than 2 cm in body size. Insects, hindered either by their body size or by their means of foraging from passing through 2 cm mesh are likely to act as pollination agents in papaw (refer to Section 7.4.1). Since insects such as *Apis mellifera* and *Trigona* sp. would readily pass through 2 cm mesh when bee attractant plants such as flowering *Ocimum basilicum* (basil) were placed into enclosures within the same orchard, it appears that insects smaller than 2 cm, including bees, are not specifically attracted to female papaw flowers. Almost all fruit set in enclosures developed parthenocarpically. The suggestion that bees act as pollination agents in papaw (Allan 1963c; Caron 1985) is therefore questionable.

In summary, the caging experiments suggest that a large insect is involved in pollination of papaw and that bees are not the primary pollinating agents.

CHAPTER VII

INSECT POLLINATORS OF *Carica papaya* L.

7.1 INTRODUCTION

Numerous insect species have been associated with papaw orchards and have been described as either foragers or as pollinators. The designation of pollinator status, however, relies in most instances on circumstantial evidence as insects were only observed coming into contact with staminate *C. papaya* flowers (e.g. Allan 1963c; Free 1975). Baker (1976) studied the pollination of *C. papaya* in its indigenous environment in Costa Rica and observed sphingid moths coming into contact with pistillate flowers. A study on the related species *Jacaratia dolichaula* undertaken in the same location by Bawa (1980b) suggested the same pollinator group but neither study was definitive either in terms of the species involved or in proof of their involvement as pollinators.

In countries outside the papaw's indigenous distribution, honey bees (*Apis mellifera*) are widely believed to function as pollinators since Allan (1963c) proposed their possible involvement in the pollination of *C. papaya* in South Africa. Although bee mediated pollen transfer in papaw was not clearly established, Allan described the presence of *Apis mellifera* in *C. papaya* orchards as beneficial to pollination. Allan's suggestion spread through the literature (i.e. Free 1970; Caron 1985) and is still widely believed within the farming community. In order to clarify the possible involvement of bees as pollinators in papaw, observations involving three bee species were carried out during two different seasons of the year. Coincidental with these observations, sphingid moths were observed visiting papaw flowers. Subsequent studies confirmed their role in pollination.

An overview of sphingid biology is given below followed by discussion of the involvement of Sphingidae in the pollination of papaws in central Queensland. The latter material is organized around three key areas: species identification and their seasonal occurrence in the Rockhampton area, foraging behaviour, and the capacity to function as a pollinator. Bee mediated transfer of papaw pollen is also evaluated with respect to both native and European species.

7.2 OVERVIEW OF THE FAMILY SPHINGIDAE

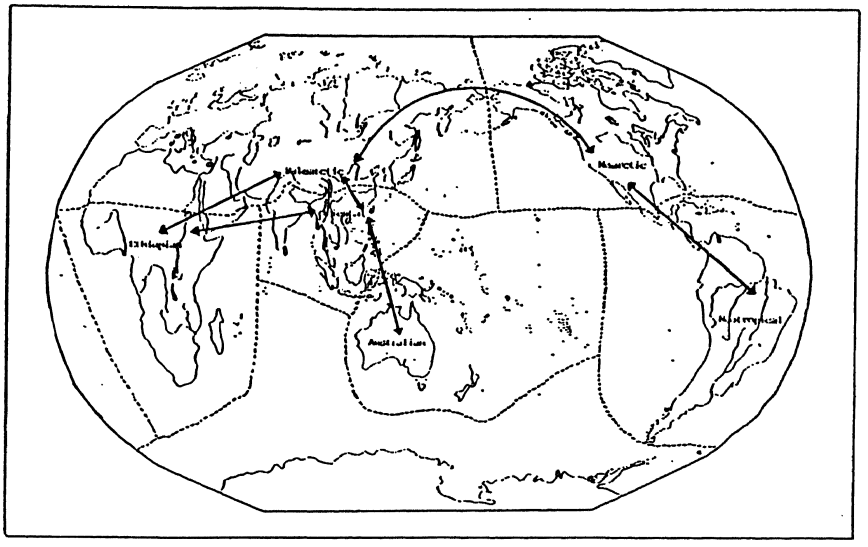
7.2.1 The Sphingidae

The taxonomy, distribution and biology of sphingid moths has been documented by a number of authors. The most comprehensive and recent works were published by Common (1990), D'Abrera (1986) and Munroe (1982). Sphingid moths are foremost of tropical distribution (Rothschild and Jordan 1903). Hypotheses on the origin and routes of their geographical dispersion were made by Darlington (1982) and Lin (1990). However, knowledge of the Australian sphingid fauna is fragmentary and little scientific data has been published (Moulds 1985).

The Family Sphingidae (hawkmoths, sphingids, sphinxes) consists of two subfamilies, the Sphinginae and the Macroglossinae, and is the only family assigned to the superfamily Sphingoidea (Common 1990). Sphingids are estimated to include 1050 species worldwide (D'Abrera 1986), of which 59 species in 25 genera are distributed in Australia. The majority of the Australian species belong to the Macroglossinae, with 50 species in 17 genera (Common 1990).

Australian sphingids are far less diverse and the number of endemic genera proportionally high when compared with all other geographical zones (Lin 1990). The closest phylogenetic link exists with the Oriental fauna (Mackey 1977; Lin 1990). Mackey (1977) for instance, estimated that 70% of the sphingid genera found in Australia and New Guinea are of Oriental origin. There are virtually no close phylogenetic links with the African fauna. Sphingids are most diverse in the wet tropics including the Neotropical, Oriental and African regions. Diversity gradually decreases with an increase in latitude towards the Nearctic and Paleartic regions (Rothschild and Jordan 1903; Common 1990; Lin 1990), and there are only two species which overlap between the Old and New World (Lin 1990). Figure 7.1 shows the map of zoogeographical distribution of the world's Sphingidae, adapted from Lin (1990).

Adult sphingids are medium to large streamlined moths with strong narrow wings and fusiform body shape. Wing spans measure between 36 mm and 190 mm (Common 1990). Males of most species are smaller and in some species darker than females (Burton 1968). At the time of eclosion, most females are double the weight of their male counterparts. The sexually related weight differences diminish with ongoing oviposition (Janzen 1984).



as in Lin (1990)

Figure 7.1: The zoographical distribution of the world's Sphingidae.

Data concerning the fecundity of hawkmoths are absent, but Janzen (1984) presumes that each female will produce 100 eggs if her nutritional requirements are met. Oviposition may occur over weeks to months (Janzen 1984) and according to Haber and Frankie (1989) over the moths whole adult life span. Sphingids are particularly longlived. Janzen (1981) mentioned that adult sphingids were able to complete two reproductive seasons under the tropical climate in Costa Rica, but most female sphingids lay their eggs within the first three to six weeks of the rainy season, depositing a few eggs per night (Janzen 1987a).

The shape of sphingid eggs is almost spherical and slightly flattened at the base. The eggs are commonly various shades of pale green. Females deposit eggs singly on the underside of leaves while hovering (Burton 1968; Janzen 1981; Common 1990). Juveniles hatch within 4 – 8 days. Larval development generally continues for two to five weeks, depending on climate and species (Janzen 1984).

Virtually all hawkmoth larvae show the unique appearance of a dorsal 'horn' on the last abdominal segment. Caterpillars are smooth, hairless and nearly all have conspicuous lateral stripes on thorax and abdomen. Eye spots predominantly on thorax and first abdominal segments, in combination with various forms of larval colouration (green, brown and black) in a single species, function as a cryptic defense mechanism (Janzen 1984). During their early life stages the larvae develop

on the underside of leaves, whilst older, larger larvae feed openly in between host plant foliage and rely on their cryptic colouration for protection (Janzen 1981; Common 1990).

The pupa of hawkmoths is distinctive, in that it is stout and fusiform. The dorsal 'horn' of the larval stage is represented as a dorsal scar in the pupal stage. Caterpillars leave their host plants for pupation and crawl for considerable distances. Larvae spin a cell out of silk, debris and leaf litter in the surface layer of the soil or deeper into the soil profile. There are some exceptions; for instance *Hippotion velox*, a species distributed in the Austral-Oriental region, pupates well above ground in a leaf case spun on the food plant or on vegetation below the food plant (Dodd 1902b; Janzen 1988; Common 1990).

7.2.2 Feeding and Foraging

Adult sphingids feed on nectar. It has been estimated by Baker and Baker (1973) that large, active moths such as sphingids require 1 mL of nectar per day. Sugars may be converted to and stored as fat, which in some species can be directly metabolized to provide energy (Baker and Baker 1973). Sugar occurs predominantly as sucrose in nectar of hawkmoth pollinated flowers (Baker and Baker 1983; Haber and Frankie 1989). Moths also forage to a certain extent on flowers pollinated by bats. Such flowers are characteristically rich in hexose, as bats will not forage on nectars rich in sucrose (Baker and Baker 1983).

Hawkmoths forage almost invariably at dusk or during the night. Exceptions include the diurnally active species of *Cephanodes* (Common 1990), *Macroglossum* (Knoll 1925, 1927; Herrera 1992) and *Hyles* (Cruden *et al.* 1976). Herrera (1992) noticed however that the foraging activity of diurnally active *Macroglossum* species intensified at dusk. The time interval spent on foraging is rather distinct. Eisikowitch and Galil (1971) documented that the group of sphingids pollinating *Pancratium maritimum* L. (Amaryllidaceae) were exclusively foraging for one hour at dusk only. In another study undertaken by Cruden *et al.* (1976) forage times of 3 – 6 hours and 1.5 – 2 hours were recorded and the different time spans interpreted as a direct response to change in elevation and temperature regimes. Shorter foraging periods were observed with increases in altitude.

A number of authors (Haber 1984; Haber and Frankie 1989; Bawa 1990) hypothesize that the moths also search for mates and larval host plants during

feeding flights. Sphingids use their olfactory sense in locating longrange food sources by following odour plumes against the wind to their source (Boeckh 1984; Miller and Strickler 1984; Murlis *et al.* 1992). Such search flights characteristically employ a zigzagging flight pattern (Miller and Strickler 1984; Murlis *et al.* 1992). In close vicinity to the odour source hawkmoths are guided by a combination of scent stimuli and visual orientation (Baker 1970; Brantjes 1973, 1978; Schwemer and Paulsen 1973; Brantjes and Bos 1980; Zacharuk 1980; Finch 1986; Herrera 1993; White *et al.* 1994). Hawkmoths have been shown to possess ultraviolet receptors in their eyes (Schwemer and Paulsen 1973), enabling them to discriminate between UV-reflecting flowers and the remaining vegetation. Despite the ability of hawkmoths to recognize scents and possibly visual cues in their search for food and host plants, moths showed no signs of lasting "learning" capabilities in laboratory experiments (Brantjes 1973). Overall, the consensus amongst pollination ecologists is that sphingids respond to an array of stimuli in both food and host plant location.

Whilst foraging, the initial approach of hawkmoths towards a flower is almost always with their heads turned away from the light source (Brantjes and Bos 1980; Inoue 1986). Virtually all sphingids remain hovering in a stationary position in front of the opened flower whilst imbibing nectar and remove their probosces in a swift, upwardly orientated flight action at the end of the feeding sequence (Brantjes and Bos 1980; Common 1990).

It has been suggested by Janzen (1984) that sphingids achieve their efficiency in flight and foraging by 'traplining', a foraging behaviour where established flight paths in between food plant aggregations are revisited on a daily or nightly basis. Linhart and Mendenhall (1977) observed such foraging behaviour in a pollen dispersal study of the shrub *Lindenia rivalis* Benth. (Rubiaceae) in Belize. By contrast, Haber and Frankie (1989) found no evidence supporting the existence of 'traplining' of hawkmoths. In their experiments, the recapture rate of caught and marked hawkmoths was virtually zero and 'home range' foraging of the type observed in hummingbirds and bees, did not occur. The authors however did agree on the likelihood of 'traplining' in specialized sphingophilous plant-pollinator relationships involving sphingids with longer tongue lengths.

Adult hawkmoths forage for nectar over a wide taxonomic range of plant families but as noted above, the flowers in most instances resemble each other in floral morphology (Eisikowitch and Galil 1971; Faegri and van der Pjil 1979; Janzen 1984; Haber and Frankie 1989; Herrera 1992). However, Silberbauer-Gottsberger

and Gottsberger (1975) and Haber and Frankie (1989) found, that hawkmoth foraging is not restricted to sphingophilous flower types only, and moths search for nectar over a much wider range of flowers which normally are pollinated by bats, hummingbirds, bees or other smaller moths. These authors also found little evidence that hawkmoths choose flowers strictly in accordance to their proboscis length and body size, although they agree on the close correlation between body size and tongue length.

In a study on *Platanthera metabifolia* (Orchidaceae), large fragrant floral displays of white petal colouration attracted large numbers of hawkmoths, irrespective of study site, spike density, flowering time and spur length (Inoue 1986). Brantjes (1973) demonstrated that hawkmoths are attracted by fragrances based on aromatic esters. Such esters are known to occur for instance in a variety of species in the Orchidaceae (Nilsson 1983). Records of scent components of other sphingophilous flowers are sparse and the fragrances described by most authors are based on subjective individual perceptions. Flower density also appears to influence the number of hawkmoth foragers per site (Inoue 1986). In a mixed stand of flowering plant species, hawkmoths preferred multiple flower heads such as spikes and racemes. Moths were also able to dicriminate between various stages of flower opening and selectively foraged on flowers in early anthesis (Nilsson 1983).

7.2.3 Foraging and Climate

Most authors agree on the fact that sphingid abundance and activity patterns are influenced by weather conditions at the time of foraging and by host plant availability and predator density (Frankie 1975; Linhart and Mendenhall 1977; Powell and Brown 1990). The influence of temperature on foraging and flight requirements has been documented under laboratory and field conditions (Heath and Adams 1967; Heinrich and Bartholomew 1971; Bartholomew and Heinrich 1973; Casey 1976; Cruden *et al.* 1976; Bartholomew and Casey 1978). Air temperatures of approximately 14.0°C completely immobilized the flight action of the hawkmoth *Hyles lineata* (Fab.). According to Casey (1976) temperatures had to reach a minimum of 14.6°C, before feeding progressed. Similar results were obtained by Cruden *et al.* (1976) who looked at the distribution of hawkmoth pollinated plants at various altitudes. Hawkmoth foraging, and consequently pollination, was directly related to the ambient temperature. Flight activity ceased at 15°C, as a direct result of physical failure of flight muscle warm-up. An insignificant number of moths were still active at 12 – 13°C, while moths were

absent in the field at 10°C (Cruden *et al.* 1976). The moths foraging ability may be limited by low temperature, but high temperatures have little effect. Herrera (1992) reported bustling forage activity at temperatures reaching 36°C for the diurnally active sphingid *Macroglossum stellatarum.* Moths were not suffering from heat stress due to their efficient thermo-regulating ability.

Wind speed is believed to be another major influence on foraging activity. In the pollination study on the Mediterranean sea shore plant *Pancratium maritimum* L. (Amaryllidaceae), Eisikowitch and Galil (1971) demonstrated a linear relationship between hawkmoth foraging activity and wind speeds but only for wind speeds below 2 – 2.5 m/sec. The medium sized sphingids *Hippotion celerio* and *Macroglossum stellatarum* frequently visited and pollinated *P. maritimum* when wind speeds were below 2 m/sec. However, when speeds exceeded 3 m/sec, their foraging activity and therefore pollination ceased.

Climate has an indirect effect on hawkmoth activity through its influence on the availability of food. Generally, the most concentrated population of sphingid moths is in the tropical and subtropical rainforests, where the climate sustains a continuous supply of food sources (Lin 1990). Within Australia, species richness and diversity is associated with the tropical north-eastern region of Queensland (Moulds 1985). Dry periods, on the other hand, lead to a rapid decrease of sphingids, caused by food shortage, and associated migration (Janzen 1987a). Migration for instance, has been associated with *Hyles lineata* and *Hippotion celerio*; both of which are widely distributed and occur in Australia (Common 1990).

7.2.4 Sphingids and Pollination

The role of sphingids as pollinators has been recognized (Janzen 1987a; Haber and Frankie 1989; Common 1990), although detailed observations of hawkmoth pollination activities in the tropics are rare (Nilsson *et al.* 1985; Bawa 1990). Sphingids are major pollinators of trees, shrubs, vines, epiphytes and some herbs in both wet and dry rainforests of the New World (Janzen 1987a; Haber and Frankie 1989). Their pollinator function is also recognized for tropical trees and shrubs of the Australian flora (Common 1990).

Pollination by hawkmoths does occur over a broad spectrum of plant families. The specificity of sphingophilous plant-pollinator relationships varies according to the lengths of both probosces and corolla tubes of flowers on which they forage.

Examples of specific plant-pollinator relationships have been discovered mainly amongst sphingid species of longer tongue lengths (Nilsson and Rabaconandrianina 1988; Haber and Frankie 1989). Tongue lengths vary between species from extremely long (> 20 cm) to rudimentary and functionless appendages (Barth 1985; Common 1990). Hawkmoths also commonly visit flowers of other pollinator groups such as those of bats, hummingbirds, bees and small moths and carry a variety of different pollen grains on their appendages (Grant 1983; Haber and Frankie 1989). Pollen is primarily attached to the proboscis, which is usually strong and without scales (Kislev *et al.* 1972; Common 1990).

7.2.5 Migration

Migratory movements have been reported for a number of hawkmoth species in the tropics and subtropics, as well as in temperate zones (Seitz 1934; Eisikowitch and Galil 1971; Healy and Smithers 1983; Janzen 1984, 1987a; Nilsson and Rabaconandrianina 1988; Lin 1990; Powell and Brown 1990). Migration is a response to either (a) decrease in temperature (Burton 1968), (b) shortage of food supply for adult moths, (c) unavailability of suitable reproduction sites (Janzen 1987b) or (d) carnivore threat or a combination of these factors (Janzen 1987a). Various sphingid species were observed and captured crossing Central American mountain ranges twice during the year (Powell and Brown 1990). Janzen (1984, 1987b) earlier proposed the hypothesis of directed cyclic migration of sphingids between tropical dry and wet lowland rainforests in Costa Rica triggered by the forces mentioned above. This behaviour is in accord with the reputed longevity of sphingids.

Sphingids reach flight speeds exceeding 25 km/hr and are able to maintain such accelerations for long periods (Healy and Smithers 1983). Easterbrook (1985) mentioned speeds averaging 25 – 38 km/hr and occasionally speeds up to 80 km/hr, and regarded hawkmoths as one of the fastest moving insects.

Widely distributed plant populations benefit from the long-distance foraging and reproductive strategy of hawkmoths. The capacity of moths to carry large pollen loads on their probosces benefits the gene flow of outcrossing plant species (Kislev *et al.* 1972; Haber and Frankie 1989). However, the latter authors cautioned that the outcrossing efficiency of hawkmoths might be limited to their frequently encountered opportunistic feeding behaviour. A mixed pollen load reduces not only the number of potential matings between conspecific plant species but can also

disrupt or block stigmatic surfaces and in some instances can lead to the formation of seed of low viability (Waser 1983).

7.2.6 Characteristics of Sphingophilous Flowers

Hawkmoth pollinated (sphingophilous) flowers are typically white or cream, although minor visitation also occurs to flowers in shades of pale pink, pale yellow, purple and blue (Baker 1961; Eisikowitch and Galil 1971; Silberbauer-Gottsberger and Gottsberger 1975; Grant 1983; Inoue 1986; Janzen 1987b). Characteristically flowers are funnel-shaped and deep corolla tubes conceal the basal nectar source. The number of plant species displaying typical sphingophilous flower syndromes in the tropics is huge, and they are widespread in the plant families of the Rubiaceae, Onagraceae and Apocynaceae (Nilsson *et al.* 1985). In addition, brush type flowers with absent corolla tubes are also frequently visited (Cruden 1970; Faegri and van der Pjil 1979; Inoue 1986; Haber and Frankie 1989; Bawa 1990). Although a large number of plant species display the sphingophilous flower syndrome, observations on hawkmoth pollination are rarely documented.

According to Haber and Frankie (1989) the volume of nectar in sphingophilous flowers is highly variable within species, within individual plants and within flowers on a single plant. In numerous sphingophilous species the onset of nectar production commences generally 2 – 4 hours before anthesis (generally at dusk) and ceases at dawn (Heinrich and Raven 1972; Cruden and Hermann 1983).

Anthesis of sphingophilous flowers occurs almost exclusively at dusk (Eisikowitch and Galil 1971; Grant 1983), and this coincides with the onset of floral scent release (Cruden 1970). Scents (generally based on volatile aromatic esters) have been described as being similar to those of gardenia and jasmine; their strengths vary according to plant species and in some instances flowers are not fragrant to the human nose (Brantjes 1973; Silberbauer-Gottsberger and Gottsberger 1975; Nilsson 1983; Haber and Frankie 1989; Herrera 1993).

Although adult hawkmoths forage for nectar over a wide taxonomic range of plant families, most sphingophilous flowers have similar floral morphology (Faegri and van der Pjil 1979). This suggests that floral morphology is coupled to physical characteristics of the pollinator. However, Haber and Frankie (1989) found that hawkmoths also forage over a range of flowers normally pollinated by bats, hummingbirds, bees and smaller moths. These authors agree that body size and tongue length are correlated, but found little evidence to indicate that hawkmoths

chose flowers in accordance with the length of proboscis and body size. Although there is a large literature on sphingid biology, it is incomplete and at times contradictory. In this chapter an attempt will be made to resolve some of the contradictions by investigating aspects of the unsolved problem "Do hawkmoths pollinate papaw flowers".

7.3 MATERIALS AND METHODS

Diurnal observations were carried out on four properties within a 50 km radius of Rockhampton during the summer of 1991/1992. The properties were adjacent to different plant communities. During initial observations it became apparent that the hawkmoth group (Sphingidae) were the only insects to come into contact with papaw flowers of both sexes. From then on the studies focused on this insect group. The predominant foraging period of the observed pollinators was at dusk and the identification of insects was hindered considerably with the onset of darkness. Initial observations at night were carried out with a red-light torch, which was less disturbing to moth behaviour than ordinary torch light. Even so, red light was suspected of interfering with moth behaviour. Later in the study (from January 1992 onwards) more systematic observations were conducted using photography, light trapping and thermal imaging techniques.

7.3.1 Insect Observation and Capture

From January 1992 until the end of June 1994 visual and written observations were made at the hour of dusk (approximately 1800 hours E.S.T.) on a weekly basis over a period of 123 weeks. The observation period lasted approximately 45 minutes. Behavioural studies on foraging strategies of sphingid moths were restricted to the time interval when it was possible to visually identify moth species using natural light. Insects which came into contact with the pistillate flowers during observations were caught with a net. The rate of successful capture was low, due to the high mobility and non-alighting forage behaviour of sphingid moths. Captured moths were killed using standard killing jars (Common 1990), pinned and identified to species.

Insect species were declared pollinators in accordance with the pollination postulates under field condition proposed by Cox and Knox (1988). Essential requirements were the identification of pollen carrying appendages coming into contact with the stigma of the female flower and the successful deposition of pollen

grains and/or seed set. Pollinator function was detected either by visual observations during the early dusk period and by night photography at the onset of darkness.

7.3.2 Insect Trap

After the initial phase of establishing pollinator species, a standard Robinson insect light-trap fitted with a 240 W ultra violet emitting mercury light bulb was used to sample nocturnally active insects (Cantelo 1990). Sampling was conducted on a weekly basis from the beginning of August 1992 until the end of June 1994, totalling 100 consecutive trap nights. From August 1992 until February 1993 traps were run in the orchard at Parkhurst then sampling continued at the University site until June 1994. Sampling commenced at dusk and continued until collection 30 minutes prior to daybreak.

7.3.3 Photography

A 35 mm camera (Pentax Corporation, Tokyo, Japan) fitted with a flash and 100 mm macro lens was mounted on a tripod 1.2 m from pistillate and staminate *C. papaya* flowers. Photographs were taken using coloured Kodak print film rated at 100 ISO. Pistillate flowers at various stages of opening (from petals just beginning to reflex until the late receptive phase where petals are completely separated and reflexed) were observed and the insect activity photographed. The insect forage pattern was disturbed if the camera was placed closer to flowers. Infra red beam-triggering devices for taking photographs of the moths proved unsuccessful, because of the greater speed of the moths relative to the response time of the camera.

7.3.4 Night Observations

Night observations were conducted during different seasons in 1992 and 1993 between 18:00 hours (dusk) and 06:00 hours (dawn). For ten minutes after every full hour the orchard was searched for insects using a red-light torch. Observations were carried out on three dates each during 1992 (18 March, 29 July and 15 October) at the Parkhurst orchard and 1993 (23 February, 22 June and 14 November) at the University site.

In summer 1994 (3 – 8 February), observational night studies were conducted using a Thermal Imager (AN/TAS-6A) in collaboration with the 1st Divisional

Intelligence Company Surveillance Platoon, Brisbane. The instrument was able to detect temperature changes up to 0.5°C identifying targets in the approximate range of 5 km. The search for hawkmoth activity was carried out at two locations; the University orchard and at a private papaw orchard 10 km from the coast near Yeppoon (Figure 2.3). Observations were divided into six intervals of six consecutive hours of surveillance time covering the time from dusk to midnight and from midnight to dawn (Table 7.1). The instrument was positioned at the end of rows to allow for deeper screening penetration down the rows. The equipment itself was set up in a stationary position but was capable of traversing 360°.

Table 7.1: Sampling periods and locations of observations on hawkmoth activity using a Thermal Imager during summer 1994.

Date	Yeppoon Orchard	C.Q.U. Orchard
3-Feb		18:00 – 24:00 hrs
4-Feb	18:00 – 24:00 hrs	
5-Feb		18:00 – 24:00 hrs
6-Feb	24:00 – 06:00 hrs	
7-Feb		24:00 – 06:00 hrs
8-Feb	24:00 – 06:00 hrs	

7.3.5 Transfer and Deposition of *C. papaya* Pollen Grains

The probosces of 79 field collected sphingid moths of the pollinator and suspected pollinator species which were determined from previous experiments, were measured. The number of attached *C. papaya* pollen grains of field collected sphingids was estimated using a score system consisting of five step classifications: 0, 1 – 100, 101 – 500, 501 – 5000 and > 5000. The lengths of probosces of successfully eclosed adults from field collected larvae of *Hippotion celerio, H. velox, Macroglossum hirundo errans, Theretra oldenlandiae firmata* and *Cephanodes janus janus* (Section 9.4.1) were also recorded.

On three occasions (9 – 12 March 1992, 26 – 29 October 1992 and 16 – 19 March 1993), a total of thirty pistillate flowers (i.e. 10 flowers per sampling period) remained bagged during the day and were unbagged from dusk (18:00 hours) until dawn (06:00 hours) during four consecutive days. The four day period marked the time when the pistillate flowers remain receptive, while the choice of dates related to seasons when pollinator activity was to be expected. Insect flower visitation was observed during the dusk periods. After four days the stigma of each flower was removed and the number of deposited pollen grains microscopically scored using

four step classifications: 0, 1 – 100, 101 – 500 and > 500 grains present per stigma surface.

7.3.6 Scanning Electron Microscopy

Probosces and antennae carrying pollen of sphingid moths were examined using scanning electron microscopy (S.E.M.). Appendages were mounted on 9 mm aluminium stubs, gold coated (3 nm) in a Sputter Coater (Polaron, 510 Series, Fisons Instruments, Sussex, U.K.) and viewed using a JEOL scanning electron microscope. (5300 LV Series, JEOL, Tokyo, Japan). Exposures were taken on Kodak black and white slide film rated at 100 ISO.

7.3.7 Bee Observations

The foraging and pollinator activity of *Apis mellifera* and *Trigona carbonaria* were observed weekly at the T.A.F.E. orchard site (Rockhampton) during two six week intervals in summer and in winter 1994. *Trigona carbonaria* hives were positioned 3 m distant and seven *Apis mellifera* hives at 20 m distance from the first row of flowering papaws. Observations were conducted between 12 January – 16 February 1994 and from 24 May – 5 July 1994, covering a total of 20 experimental hours. Bee visits to *C. papaya* flowers were counted for 10 minutes past every full hour in between 0800 hours and 1700 hours. Observations were carried out on six adjacent papaw trees (three of either sex).

7.3.8 Meteorological Data

Meteorological data of minimum and maximum daily temperatures and rainfall in the study site areas were obtained from the Rockhampton Meteorological Station (Section 2.1).

7.4 RESULTS

7.4.1 Identification of Pollinator Species

The following hawkmoth species were recorded over the period from January 1992 until June 1994 as the pollinators of *C. papaya* in central Queensland: *Macroglossum hirundo errans* Walk., *Macroglossum micaceum* Walk., *Nephele subvaria* Walk., *Hippotion celerio* L., *Hippotion velox* Fab., *Theretra oldenlandiae firmata* Walk. and *Theretra silhetensis intersecta* Butl., (Figures 7.2a and 7.2b).

Several other species may be involved in the pollination of papaw, although the results were inconclusive for one of the following reasons: (a) no evidence that moth species came into direct contact with receptive stigma, although observed foraging on staminate flowers (b) moth species observed on pistillate flower, but pollen deposition inconclusive or (c) inability to capture moth specimens after contact with stigma surface.

The following species were hand netted and carried *C. papaya* pollen on their probosces but failed one of the Cox and Knox postulates: *Hippotion scrofa* L., *Hyles lineata livornicoides* Fab., *Cephanodes hylas cunnighami* Boisd. and *Cephanodes janus janus* Miskin, (Figure 7.2c). All seven pollinator and four suspected pollinator species belong to the same subfamily, namely the Macroglossinae.

7.4.2 Abundance and Seasonal Occurrence of Pollinators

The number of moths in the trial orchards was monitored from January 1992 until June 1994 using both traps and written observations. A total of 96 hawkmoths of various species were trapped. Hand netting of hawkmoths during observational studies resulted in the capture of 34 sphingid moths which all belonged to the species group identified as pollinators or suspected pollinators. Light traps attracted a total of 62 sphingid moths, of which 47 specimens were pollinators or suspected pollinators (Table 7.2).

Table 7.2: The number of sphingid moths caught with a light trap and hand net at the Parkhurst and C.Q.U. papaw orchards in Rockhampton (weekly analyses; January – June 1994).

Moth Species	Light Trap	Hand Net	Total
Hippotion celerio	7	9	16
Hippotion velox	0	3	3
Macroglossum hirundo errans	10	2	12
Macroglossum micaceum	1	2	3
Nephele subvaria	4	1	5
Theretra silhetensis intersecta	12	7	19
Theretra oldenlandiae firmata	4	2	6
Cephanodes hylas cunninghami *	1	2	3
Cephanodes janus janus *	0	4	4
Hippotion scrofa *	6	1	7
Hyles lineata livornicoides *	2	1	3
Agrius godarti †	1	0	1
Coenotes eremophilae †	11	0	11
Gnathothlibus erotus eras †	3	0	3
Total	62	34	96

* suspected pollinator species
† non-pollinator species

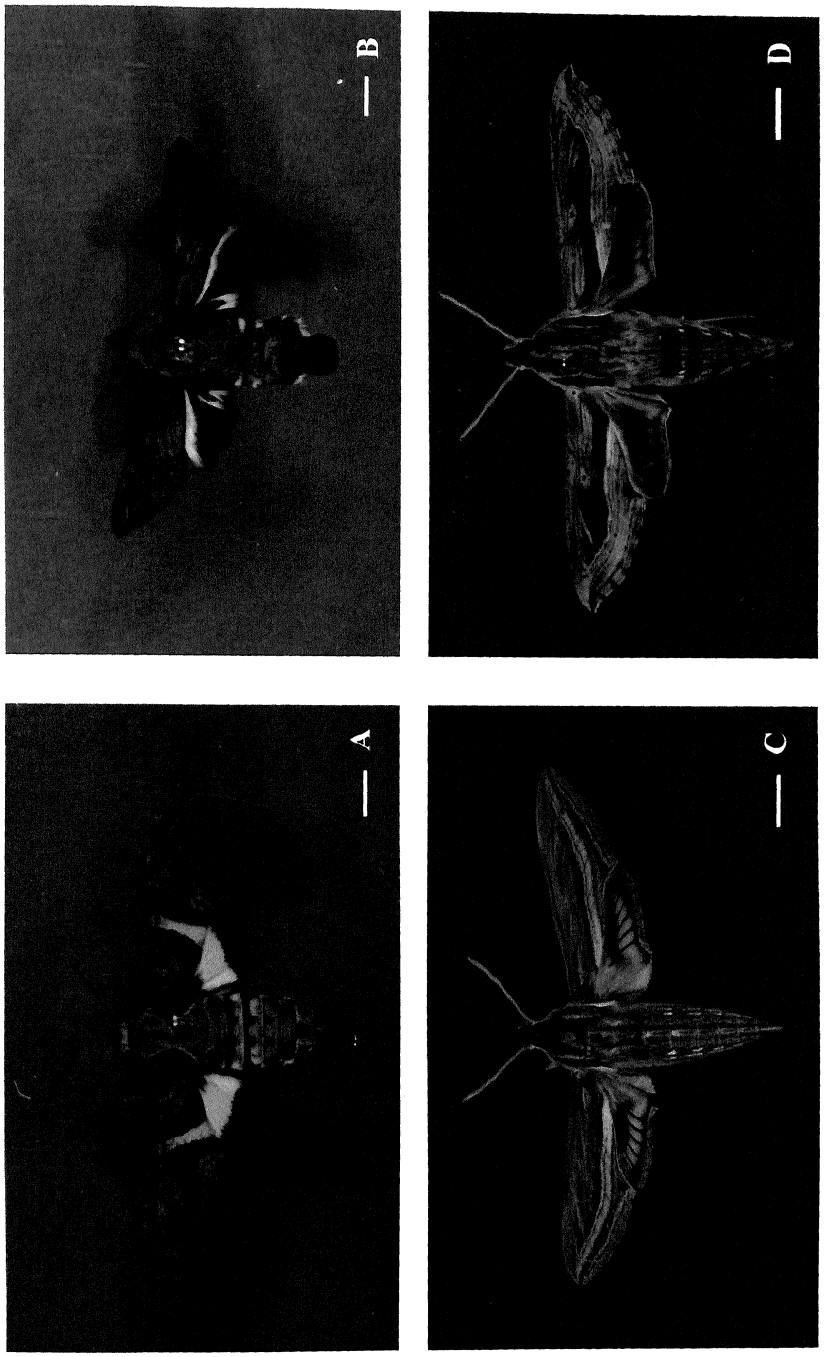


Figure 7.2a: Lepidopteran species identified as pollinators of *C. papaya* in central Queensland. (A) *Macroglossum hirundo errans* Walk., (B) *Macroglossum micaceum* Walk., (C) *Hippotion celerio* L., (D) *Hippotion velox* Fab. Bar = 1 cm

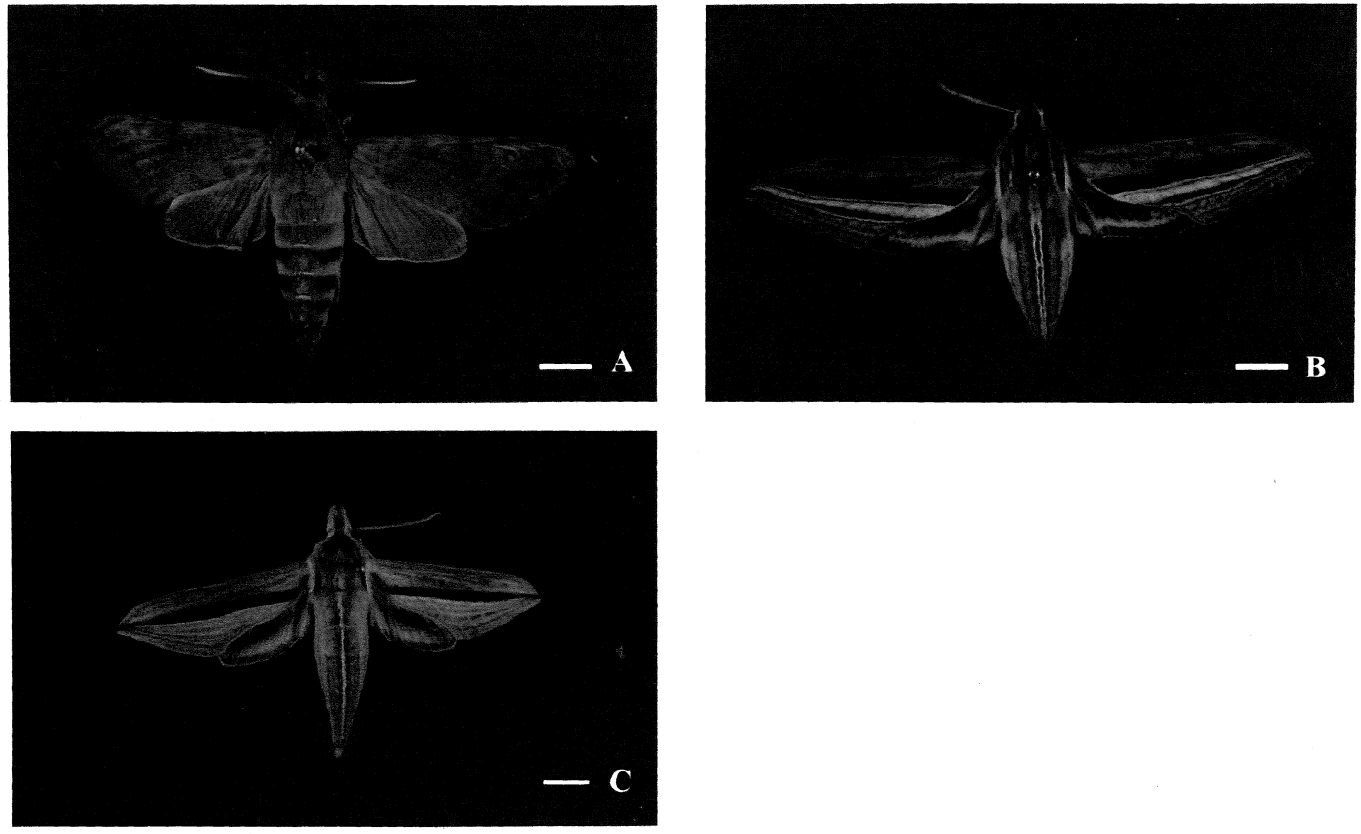


Figure 7.2b: Lepidopteran species identified as pollinators of *C. papaya*. (A) *Nephele subvaria* Walk., (B) *Theretra oldenlandiae firmata* Walk., (C) *Theretra silhethensis intersecta* Butl.. Bar = 1 cm.

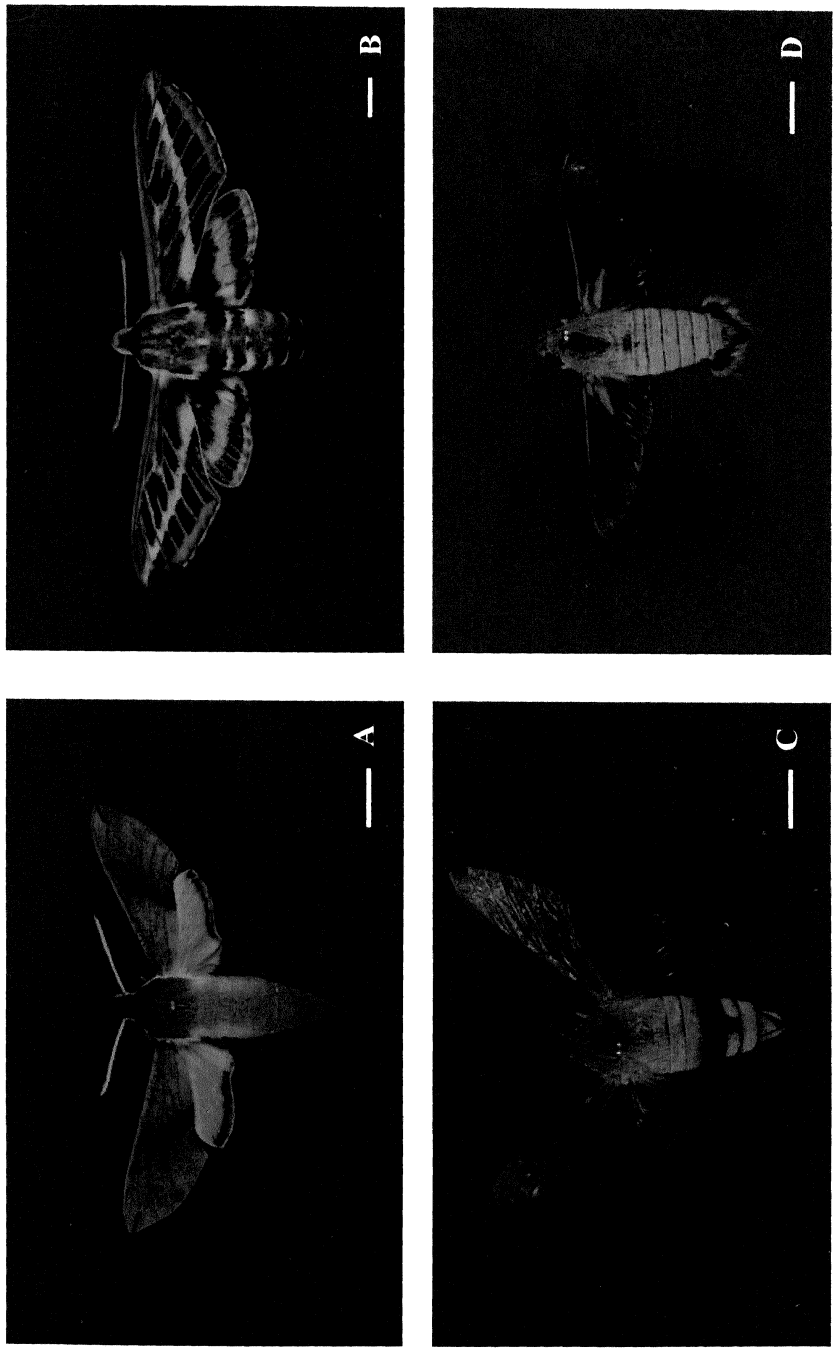


Figure 7.2c: Lepidopteran species suspected of being pollinators of *C. papaya* in central Queensland. (A) *Hippotion scrofa* L., (B) *Hyles lineata livornicoides* Fab., (C) *Cephanodes hylas cunninghami* Boisd., (D) *Cephanodes janus janus* Miskin. Bar = 1 cm

Sphingid species not associated with papaw pollination made up a total of 15 specimens belonging to three species of the same subfamily, the Sphinginae (Table 7.2). These species were caught in light traps but were not observed foraging on papaw flowers. By contrast, all pollinator and suspected pollinator species belonged to the subfamily of Macroglossinae.

The diversity of species varied little between the years of the study. Five of the 11 species were caught or observed in all three years, whilst the remaining six species were trapped during two years of observation. Of the 11 definite and potential papaw pollinating sphingids, *M. hirundo errans, T. silhetensis intersecta* and *H. celerio* were consistently caught and observed foraging in papaw orchards in all years.

Sphingid moths were present for most of the year, except during the winter period from mid June until mid August (Figures 7.3a-c). Hawkmoths were declared absent when neither visual observation nor light trapping demonstrated their presence. Light trap results should be seen as an underestimate of sphingid numbers present, while observational sphingid counts are most likely an overestimate, since individual specimens might have been counted twice. However, when hawkmoths were abundant, the two methods of counting gave comparable results.

There appears to be a relationship between the number of moths, temperature and rainfall. For example distinct peaks in moth numbers were observed during February and March 1992 and again during May 1992. Other peaks occurred during March 1993 and the November/December period of 1993 and during February and March 1994. Although the influence of rainfall has been recognized, the results are difficult to analyse statistically because the years 1992 until 1994 were unusually dry and therefore not representative of the typical seasonal conditions (Section 2.1).

The influence of temperature on the occurrence of hawkmoths was observed during winter. Moths were clearly absent each year from the beginning/middle of June until the middle of August for a period of approximately eight weeks. The disappearance of moths corresponded to minimum weekly temperatures of 10.4°C and below in all three years. The first hawkmoths appeared when minimum weekly temperatures were equal to or above 9.9°C in the following season (Figures 7.3a–c). The maximum weekly temperatures in the time interval during which hawkmoths disappeared and reappeared was between 24°C and 25.5°C.

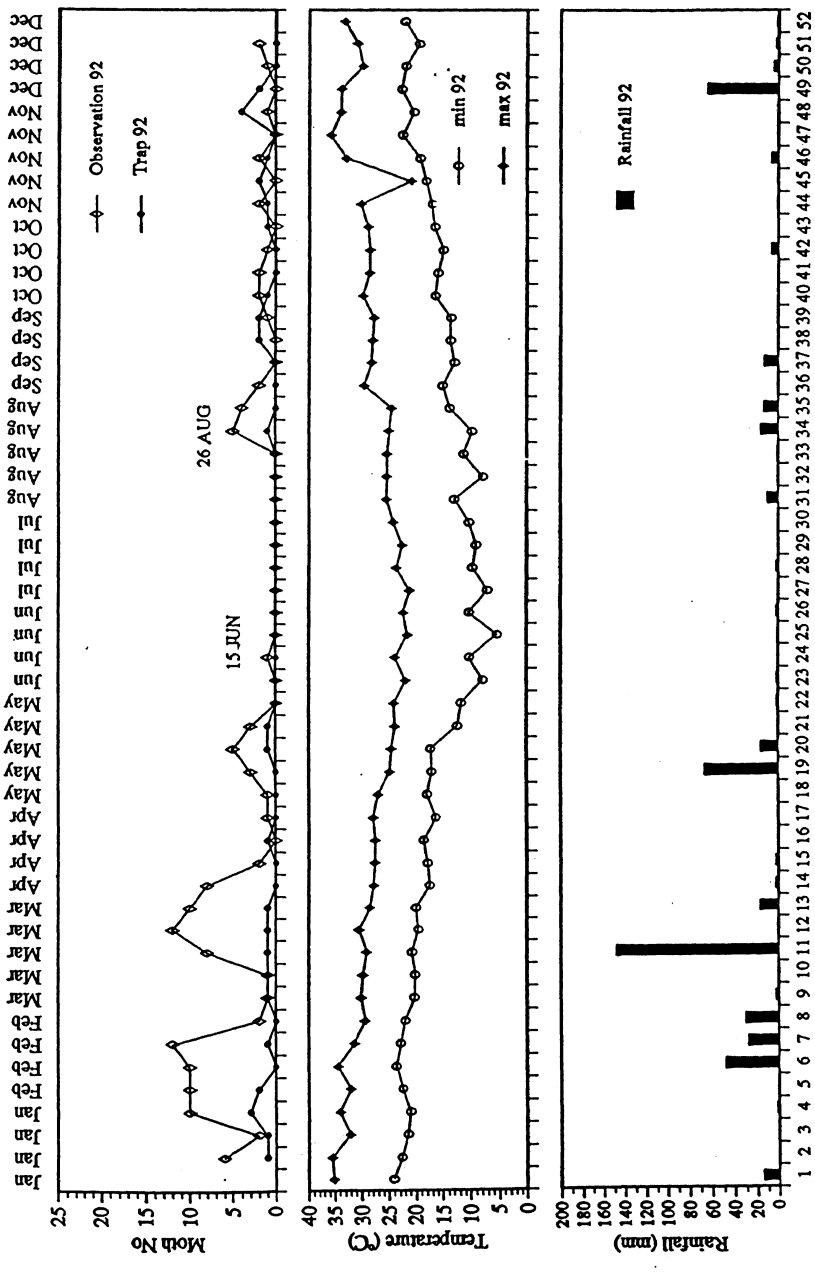


Figure 7.3a: The number of sphingid moths trapped with a standard Robinson light trap or observed foraging in *C. papaya* orchards in Rockhampton during weekly observations from January until December 1992 (Figure includes average weekly minimum and maximum temperatures and rainfall).

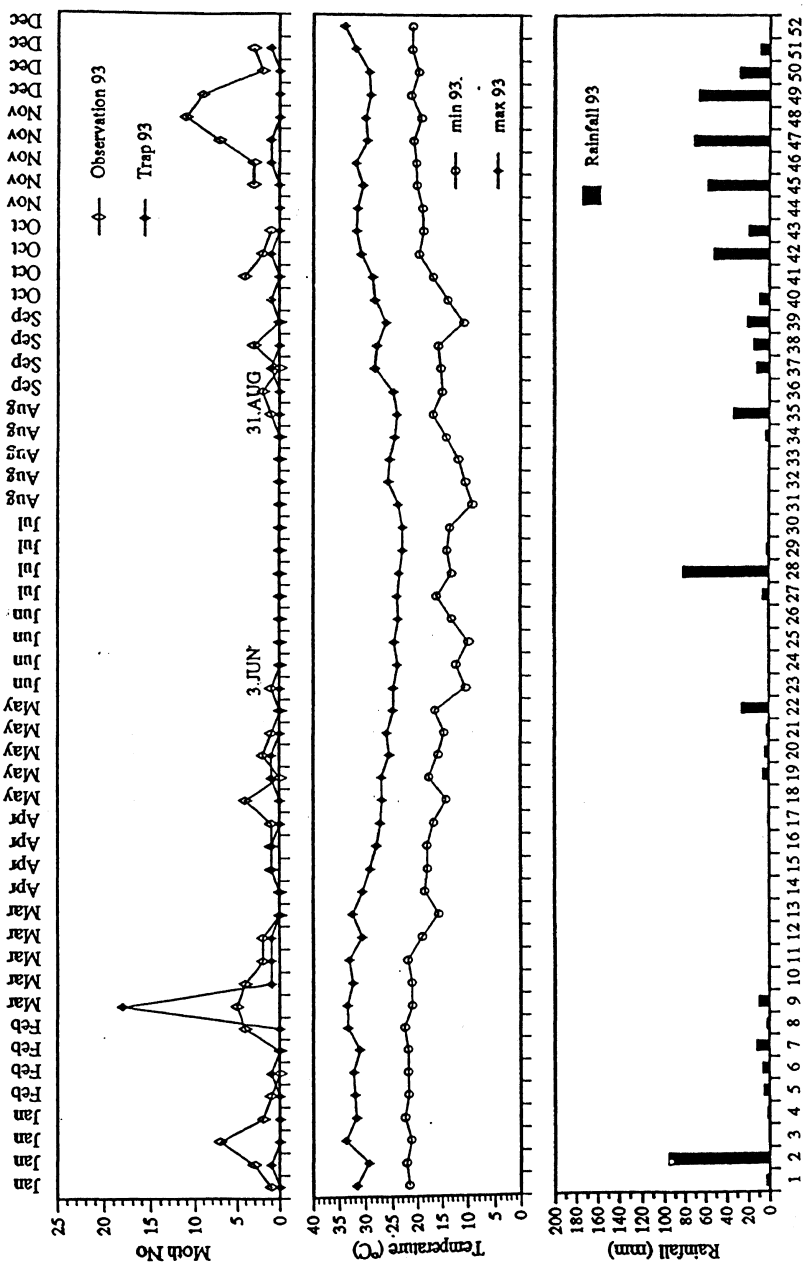


Figure 7.3b: The number of sphingid moths trapped with a standard Robinson light trap or observed foraging in *C. papaya* orchards in Rockhampton during weekly observations from January until December 1993. (Figure includes average weekly minimum and maximum temperatures and rainfall).

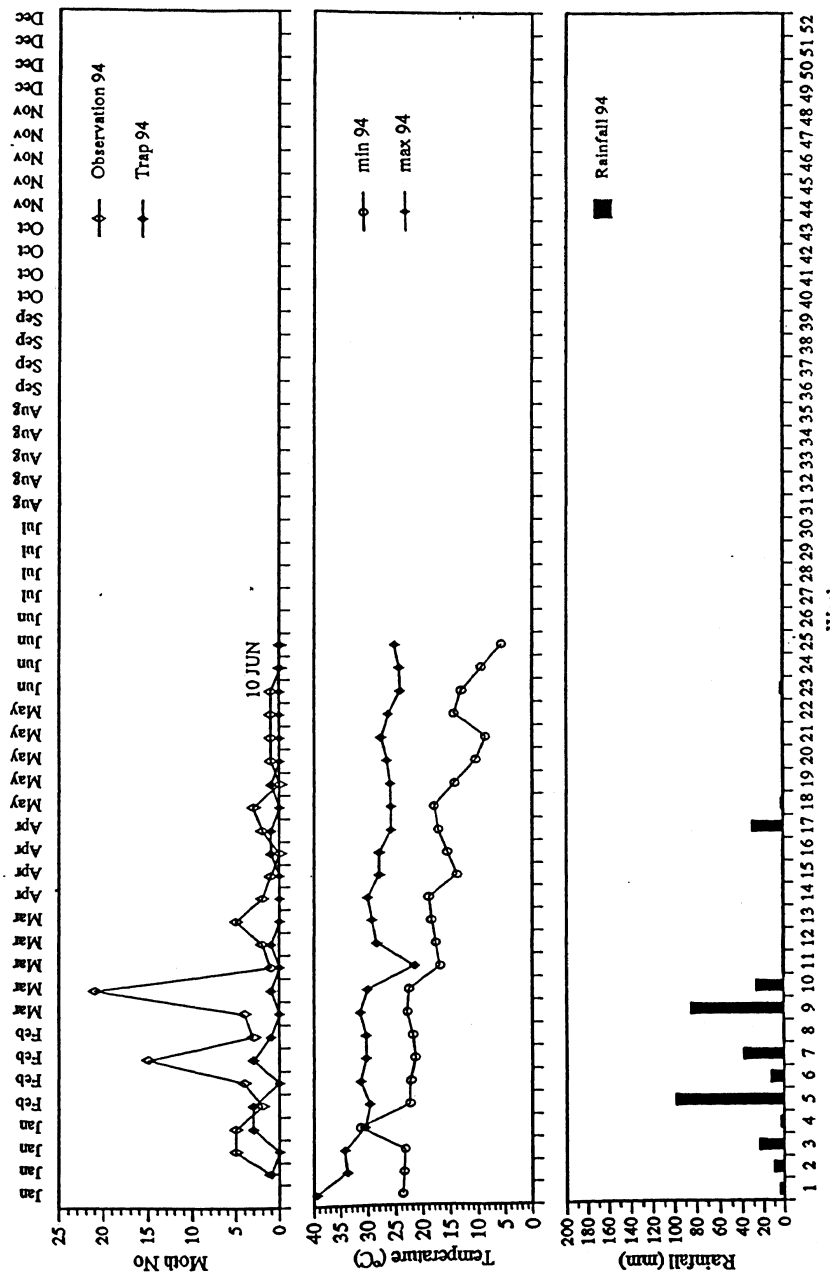


Figure 7.3c: The number of sphingid moths trapped with a standard Robinson light trap or observed foraging in *C. papaya* orchards in Rockhampton during weekly observations from January until June 1994. (Figure includes average weekly minimum and maximum temperatures and rainfall).

7.4.3 Observations of Foraging Behaviour

Of the eleven pollinating and potential pollinating sphingid species, three species were diurnal foragers while the majority foraged nocturnally at the period of dusk (18:00 – 19:00 hours). The foraging behaviour of hawkmoths on *C. papaya* flowers was observed at dusk since most moths were nocturnal. A total of 262 foraging contacts of sphingid moths on staminate *C. papaya* flowers were observed during the 123 weeks of observational studies (Table 7.3). This number represents discrete foraging episodes. When species identification was inconclusive, insect contacts were listed under the category 'unidentified species'.

Table 7.3: The total number of observed contacts of sphingid species with staminate and pistillate *C. papaya* flowers (January 1992 – June 1994).

Moth Species	Number of Visits to Staminate Flowers	Number of Visits to Pistillate Flowers
*Cephanodes hylas cunninghami**	3	0
*Cephanodes janus janus**	1	0
Macroglossum hirundo errans	56	9
Macroglossum micaceum	11	2
Nephele subvaria	23	3
Hippotion celerio	46	5
Hippotion velox	15	2
*Hippotion scrofa**	4	0
*Hyles lineata livornicoides**	0	0
Theretra oldenlandiae firmata	10	2
Theretra silhetensis intersecta	8	3
unidentified sphingid species	85	16

* proposed pollinator status

Sphingids initially approached the papaw field from the nearby scrub, irrespective of wind direction. Moths initially moved in a zigzag pattern between the rows of papaw plants before starting to feed. A general pattern was followed by moths feeding on the nectar-secreting staminate *C. papaya* flowers. Sphingids concentrated their feeding activity on individual trees. Foraging on the tree commenced with the imbibition of nectar from flowers of lower inserted inflorescences. Moths then turned to flowers of an adjacent inflorescence, gradually moving around the axis of the tree. Their probosces remained extended during intra-tree foraging. Foraging moths concentrated on newly opened flowers, avoiding the curled and yellow petals of day-old flowers. Moths manoeuvred their bodies with ease in order to insert their probosces into adjacent staminate flowers. Mostly flowers open horizontally or are slightly pendant, but moths were not deterred in their foraging efforts by perpendicular flower orientations, either upright or downward. Sphingids remained in constant flight and were never observed

landing on flowers. Moths spent on average three seconds per flower to imbibe nectar before approaching the next target and visited 15± 7 SE flowers per minute (n = 71 observations), irrespective of pollinator species. On rare occasions moths spent up to 8 seconds feeding on staminate flowers. Such extended visits were observed with six different moths, namely *T. oldenlandiae firmata*, *T. silhetensis intersecta*, *H. velox*, *H. celerio*, *M. hirundo errans* and *N. subvaria*. Zigzagging flights were common between episodes of nectar imbibition. Normally moths foraged on three trees or less before flying to another part of the orchard, where foraging continued. During periods of high sphingid abundance (i.e. January until March 1992, March 1993) numerous hawkmoths were observed foraging on the same tree, in some instances on the same inflorescence. As many as five sphingids were observed foraging at the same time on the same tree which in some instances led to multiple foraging visits to the same flowers. Sphingids spent less time on flowers devoid of nectar and normally terminated foraging altogether on flowers of the same inflorescence. On a number of occasions (n = 31), especially when moth numbers were small, sphingids were observed foraging over a period of only 10 to 20 minutes.

7.4.4 Sphingid Moths as Pollinators

Sphingid moths were observed visiting pistillate *C. papaya* flowers on a total of 42 occasions during the length of the study period. The moths made highly irregular contact with pistillate flowers. Methodological difficulties included restricted visibility (by either foliage or dimness of light) and the ease with which moths were disturbed. The photographic record provided details of species, illustration of the probosces contacting stigma surfaces, the stage of floral anthesis and details of the means by which moths approached the flowers (Figure 7.4). Sphingid moths visited flowers from the very early stage of anthesis, when petals have just begun to separate and the stigma is still concealed, to the later stages of fully reflexed petals when the entire stigma surface is completely exposed (Figure 7.4). Moth probosces remained extended between the last contact with staminate flowers and succeeding visits to pistillate flowers (n = 9 observations). Probosces either touched the stigma surface and/or were directed between the five stigma lobes towards the base of the flower. Hawkmoth visits to pistillate flowers lasted less than an average of three seconds (n = 23).

The small number of sphingids observed coming into contact with pistillate flowers hindered the compilation of the moth visitation ratio between staminate and pistillate flowers. However, an indication of this ratio was derived from three observations

Figure 7.4: A selection of *C. papaya* pollinating sphingid species contacting pistillate flowers at dusk. (A) *Nephele subvaria* Walk., (B) *Theretra oldenlandiae firmata* Walk., (C) *Hippotion velox* Fab., (D) *Macroglossum hirundo errans* Walk..

in February and March 1992, during a time when moth numbers were high. On one occasion, one pistillate flower received five sphingid visits from three different hawkmoth species within one minute. Species were identified as *Macroglossum hirundo errans*, *Macroglossum micaceum* and *Theretra* sp.. Nectar probing of 42 successive staminate flowers prior to the contact with the pistillate flower was observed from one individual of *M. hirundo errans*. The male and female papaw trees were adjacent to each other. On another occasion *Theretra* sp. was observed foraging on 65 staminate flowers on two adjacent trees before contacting a pistillate flower five tree positions (approximately 9 m distance) away from the last nectar source. On a third occasion *H. celerio* was observed foraging on 38 staminate flowers before inserting its proboscis into a pistillate flower on the adjacent tree. Visits to pistillate flowers were predominantly followed by a zigzagging flight pattern before moths continued foraging within another area of the orchard.

Moths were trialed in flight cage experiments in order to establish staminate to pistillate flower visitation rates and the number of visits individual pistillate flowers would receive from moths within a given time. Experiments were carried out on two occasions; during November 1993 and April 1994 using two differently sized flight cages, with dimensions of 2 m x 1 m x 2 m, and 4 m x 9 m x 20 m (height x width x length) respectively. The smaller cage was stocked with a single flowering male and female papaw plant, while the larger enclosure was planted with 30 papaw trees of mixed sex. A total of twelve sphingid moths (*H. celerio* (n = 6), *H. velox* (n = 4) and *T. oldenlandiae firmata* (n = 2)) were released singly into the enclosures. All sphingids were observed resting in stationary position throughout the day. With the approach of dusk sphingids began to fly and orientated themselves towards the setting sun. Moths vigorously attempted to escape the enclosures and never foraged inside the cages during the entire observation period. Experiments where other flowering food plants of sphingid moths were positioned inside the enclosures were similarly unsuccessful in that moths did not forage. As a result the flight cage trials were abandoned. Similar results of failing to forage when held in captivity were obtained by Murcia (1990).

7.4.5 Night Observations

Observations were carried out at night in all seasons, but sphingids were observed foraging only during the summer and autumn period (18 March 1992, 15 October 1992, 23 February 1993, 14 November 1993). Hawkmoths were not present during winter (29 July 1992 and 22 June 1993). A total of six sphingid moths (three *H. celerio*, two *M. hirundo*, one each of *T. silhetensis silhetensis* and *C.*

hylas cunninghami) were hand netted during the summer/autumn observation periods. All species were caught during the dusk period with the exception of *C. hylas cunninghami* which was observed and caught foraging on staminate papaw flowers at dawn (05:30 hours). Observations were made hourly, but sphingids were scarcely present between 22:00 and 05:00 hours.

Observations conducted in February 1994 using the Thermal Imager led on two occasions to the sighting of hawkmoths within the orchard. Both incidents occurred between 18:00 and 24:00 hours. At the University site two foraging specimens were observed at dusk and a further specimen sighted at 23:15 hours. In the Yeppoon orchard one sphingid was recorded foraging just on dusk (18:00 hours) and a further specimen at 23:25 hours. Hawkmoths, as a group, were identified by their characteristic flight patterns, but the identification of individual species was not possible using the Thermal Imager.

7.4.6 Pollen-Carrying Appendages and Quantity of Attached Pollen

All eleven hawkmoth species for which pollinator behaviour was observed, carried *C. papaya* pollen on their probosces. Although 70 of the 81 captured sphingids had *C. papaya* pollen present on their probosces, numbers of pollen grains varied considerably (Table 7.4). The majority of sphingids carried more than 100 pollen grains on their probosces, while 16 specimens carried in excess of 5000 pollen grains. Both pollinator and suspected pollinator species bore similarly high loads of pollen. Grains were found in all areas on the proboscis. In a number of instances pollen grains were completely immersed in nectar which covered the entire surface of the proboscis. By contrast, none of the specimens of the three Sphinginae species which were caught in light traps (Section 7.4.2) carried papaw pollen on any of their appendages.

Table 7.4: The number of *C. papaya* pollen grains attached to the probosces and the antennae of papaw-pollinating and suspected papaw-pollinating sphingid species caught in light traps and hand nets in the Rockhampton area (January 1992 – June 1994).

Number of Pollen Grains	0	1–100	101–500	501–5000	>5000
Probosces (n = 81)	11	17	12	25	16
Antennae (n = 9)		7	2		

Hand-netted specimens had larger quantities of pollen attached to their probosces than individuals caught in light traps and the eleven species which did not carry pollen were all caught in light traps. All pollen grains that were attached to the probosces and antennae of sphingid species were identified as grains of *C. papaya* which were easily identified by their characteristic shape and size (Figure 7.5A). Pollen grains carried on the probosces were distributed in clumps (Figure 7.5B). Some individuals had also small amounts of pollen attached to their antennae, and these grains were either clumped or singularly distributed (Figure 7.5C).

7.4.7 Length of Proboscis in Pollinator and Suspected Pollinator Species

The length of the proboscis in all pollinating hawkmoth species varied between 2.5± 0.13 cm and 3.6± 0.14 cm (Table 7.5). This is similar to the corolla tube length of staminate *C. papaya* flowers collected from 10 different lines (2.1± 0.3 cm; n = 100 flowers).

Table 7.5: The average lengths of the probosces of pollinator and suspected pollinator species of *C. papaya.*

Moth Species	n	Average Proboscis Length ± STD (cm)
Hippotion celerio	25	3.36± 0.23
Hippotion velox	5	3.58± 0.13
Macroglossum hirundo errans	10	2.47± 0.13
Macroglossum micaceum	2	2.45± 0.21
Nephele subvaria	3	3.49± 0.23
Theretra silhetensis intersecta	14	2.75± 0.12
Theretra oldenlandiae firmata	2	3.23± 0.14
Cephanodes hylas cunninghami *	2	2.53± 0.14
Cephanodes janus janus *	10	2.45± 0.12
Hippotion scrofa *	3	2.88± 0.38
Hyles lineata livornicoides *	3	2.97± 0.21
Agrius godarti †	1	4.2
Coenotes eremophilae †	11	6.45± 0.27
Gnathothlibus erotus eras †	3	6.62± 0.21

* suspected pollinator species
† non-pollinator species

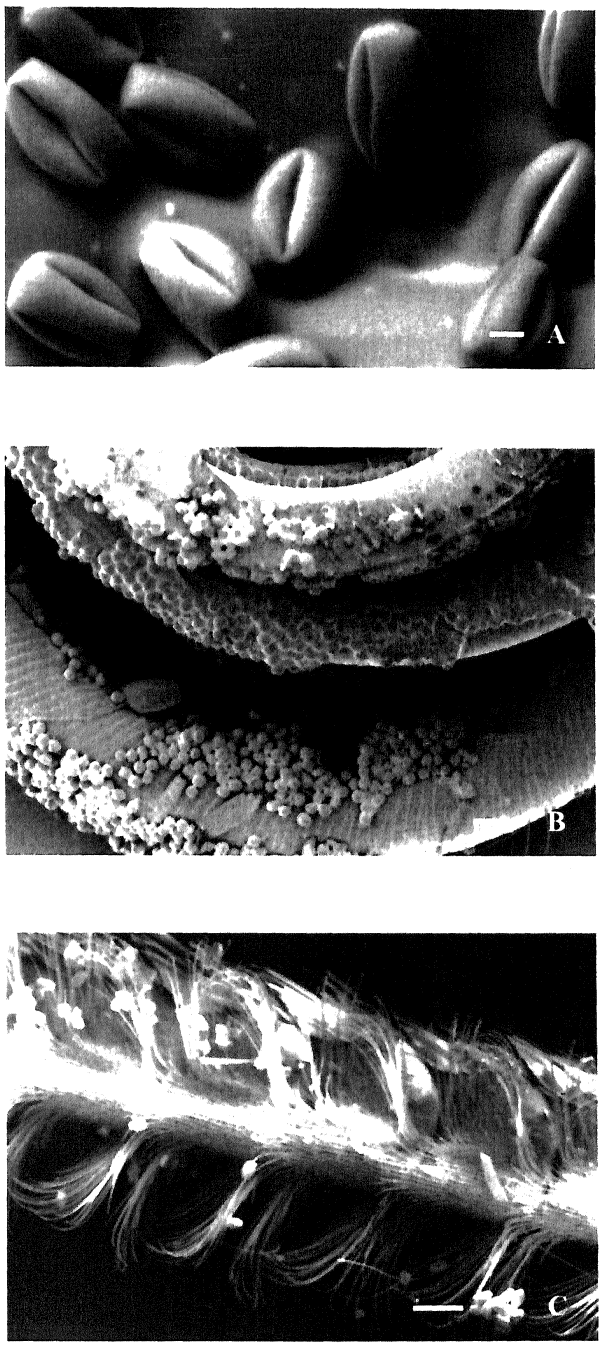


Figure 7.5: Scanning electron micrographs of (A) *C. papaya* pollen grains and their attachment to (B) the proboscis of *H. celerio* and (C) the antenna of *N. subvaria*.

A: x 750, bar = 10 μm; B, C: x 100, bar = 100 μm.

7.4.8 Deposition of Pollen on Stigmas of Unbagged Flowers from Dusk until Dawn

All thirty pistillate flowers which remained bagged during the day and were uncovered from dusk until dawn had *C. papaya* pollen deposited on their stigmas. The quantity of deposited pollen grains varied (Table 7.6), although the majority of stigmas were coated with more than 100 grains after exposure over four consecutive nights. On no occasion was pollen other than of *C. papaya* found on the examined stigmas. The majority of the pollen on the stigma surface was aggregated into clumps.

Table 7.6: The number of deposited *C. papaya* pollen grains on stigma surfaces of pistillate papaw flowers exposed to night flying insects over four consecutive nights.

Number of Pollen Grains	0	1-100	101-500	>500
Number of Stigmas (n = 30)	1*	4	8	17

* shrivelled

7.4.9 Observations on Bees in *C. papaya* Orchards

Both bee species *Apis mellifera* and *Trigona carbonaria* foraged during the summer and winter sampling period. The number of foraging visits to papaw flowers was insignificant, and indeed, only nine *Apis mellifera* visits and 38 *Trigona carbonaria* visits to staminate *C. papaya* flowers were observed during a total of 20 hours of observation. Honey bees never made contact with receptive pistillate flowers, while only one such visit was observed from a specimen of *Trigona carbonaria*. Both bee species were commonly foraging on flowering native (i.e. *Eucalyptus* sp., *Melaleuca* sp., *Grevillea* sp.) and introduced plant species (i.e. *Tecoma stans*, *Tabernaemontana coronaria*, *Senecio confusus*) adjacent to the orchard during the same periods.

7.5 DISCUSSION

The level of pollinator species diversity found in central Queensland greatly expands upon Baker's observation that a single sphingid species pollinated *C. papaya* in Costa Rica (Baker 1976). Baker observed a species of *Hyles* in the geographical region where the papaw originates. Moths belonging to the same genus (*Hyles*) are also suspected pollinators of papaw in Australia, and the

subspecies *H. lineata livornicoides*, indigenous to the Australasian-Asian Pacific area, is similar in size to *Hyles lineata*. The latter organism is globally distributed throughout the tropics and subtropics (Common 1990). Identification of the same genus pollinating papaws in Australia supports Baker's original proposition (Baker 1976). However, the discovery that a large number of moth species pollinate papaws differs from the single-species model proposed for pollination of papaw by Baker (1976). Also, the number of specimens of *H. lineata livornicoides* caught and observed in the Rockhampton area suggests that this species is not the dominant pollinator species. The data indicate that *T. silhetensis intersecta, H. celerio* and *M. hirundo errans* are the most important pollinators.

The majority of the eleven pollinator and suspected pollinator species are distributed throughout the Asian-Pacific region; two species, *H. celerio* and *H. lineata* are of even wider distribution. When taking the subspecies level into account, eight of the eleven pollinator species are of Australian origin (including nearby islands) of which *T. silhetensis intersecta* and *C. janus janus* are indigenous to the Rockhampton area only. These findings suggest that a number of indigenous sphingid species have independently adapted to using papaw as a new food source and also achieve successful pollination. *C. papaya* introduced to dry forest environments in Costa Rica, where it nowadays grows wild, is an important nectar source to hawkmoths (Haber and Frankie 1989). The overall acceptance of papaw as a nectar food source by small to medium sized sphingids suggests that sphingids will be major pollinators. The actual mechanism of foodsource finding and recognition by hawkmoth pollinators including previously formulated hypotheses will be discussed in detail in Chapter VIII.

The observation that all species involved in pollen transfer of papaw in the Rockhampton area belong to the same subfamily, the Macroglossinae, is possibly due to the relatively small species number of the other subfamily, the Sphinginae, as both subfamilies share similar areas of distribution (both locally and Australia-wide). Macroglossinae outnumber the Sphinginae on an identified species level in a ratio of 50 : 9 (Common 1990). Additionally, species belonging to the Australian distributed Sphinginae are overall larger in size (including the length of the proboscis) than the Macroglossinae for which papaw pollinator function has been established. All the identified sphingid species which are involved in the transfer of papaw pollen had similar proboscis lengths (in between 2.5± 0.13 cm and 3.6± 0.14 cm) contrary to non pollinator species which had proboscis lengths of 4.2 cm and above. Whilst the proboscis lengths of the pollinating Macroglossinae species matched the size of the lengths of corollas of staminate *C. papaya* flowers, the

proboscis lengths of non pollinator species were greater than double the length of corolla tubes of staminate *C. papaya* flowers. Therefore, these results demonstrate that hawkmoth foraging is linked to an optimum length of the proboscis of insects that pollinate *C. papaya*. Whilst the selection of food plants is limited on one hand to the physical length of the hawkmoth proboscis with which it has to reach the concealed nectar source, probosces which on the other hand are too long, seem also to be disadvantageous. The net energy return from small flowers with proportionally small amounts of nectar in comparison with larger flowers containing proportionally larger quantities of nectar (Baker 1978) is expected to influence the foraging strategy of hawkmoths. For instance, a flower of *Bombax malabaricum* (Bombacaceae) that produces approximately 250 µL of nectar each day is attractive to larger pollinating bird and bat species. By contrast, small individual flowers as those of *Cordia* sp. (Borginaceae) which produce only a fraction of one microliter of nectar per day are usually pollinated by small species of Diptera or Coleoptera (Opler *et al.* 1975). Furthermore, hawkmoths have also been shown to preferentially forage on flowers which are either large or numerous (Inoue 1986).

Pollen deposition on stigmas of flowers which remained covered during the day and were uncovered from dusk until dawn shows that hawkmoths or specific species of hawkmoths not only forage at dusk but also during later hours of the night, as sphingids were not observed coming into contact with the stigma during the dusk period. Janzen (1984) stated that some hawkmoth species, and in particular those newly eclosed, continue searching for food and mates during all hours of the night. Silberbauer-Gottsberger and Gottsberger (1975) also observed continuing forage activity of individual sphingid species in Brazil until the hour of midnight. The two sightings of unidentified hawkmoths foraging on staminate papaw flowers demonstrates that such "out-of-hours" foraging activity does occur. However, the main period for pollination is clearly the one hour period at dusk.

The demonstrated involvement of sphingid moths in the pollination of *C. papaya* is a major finding of this project. The finding is consistent with the hypothesis that plant species which remain almost constantly in flower are pollinated by only one major pollinator group (Augspurger 1980). The generality of this finding seems likely but now needs confirmation in other continents. As such, the length of the proboscis is the major characteristic linking hawkmoths which pollinate *C. papaya*.

Weekly observations and insect trapping conducted from January 1992 until June 1994 showed that sphingid moths are present in papaw orchards for most of the

year, with the exception of winter. Their presence is greatly influenced by climatic conditions, in particular temperature and rainfall. The overall low hawkmoth abundance during most of the trial period seems to be a reflection of the persistent drought conditions especially during 1993/1994 period. Peaks of moth numbers which occurred during the seasons of spring, summer and autumn indicate that given, a minimum temperature of above 10°C, moth numbers suddenly increased within the same or following week after rainfall. Such sudden increases repeatedly occurred during summer (March 1992, January 1993, February and March 1994) and were to a lesser degree also noticed during spring (November 1993) and autumn (May 1992). These results correspond with observations made by Haber and Frankie (1989) and Janzen (1987a) on the seasonal occurrence of hawkmoths in dry tropical rainforest habitats in Costa Rica, where mass population increases of sphingid moths coincided with rainfall (Janzen 1987a). Besides rainfall, moth numbers were also influenced by temperatures, in particular by minimum temperatures. Moth numbers decreased to zero from the middle of June onwards when minimum temperatures decreased to 9.9°C and below. This trend was consistent throughout the three years of moth trapping and observational studies. Even incidences of high winter rainfall as experienced during July 1993, did not trigger an increase in sphingid numbers and moths continued to be absent from the area (Figure 7.2a). In a field study conducted in a high elevation system in Mexico Cruden *et al.* (1976) showed that sphingids were absent from the area when temperatures decreased to 10°C. According to the authors, temperatures of approximately 15°C immobilized the flight muscles of *Hyles lineata*, and only small numbers of hawkmoths were able to forage when temperatures were below 15°C. Similar minimum temperatures led to a decrease of hawkmoth activity in the present study, which is indicative of a fundamental requirement of the physiology of the Sphingidae. With decreasing temperatures moths not only need to provide more energy for the initial warm-up of flight muscles (Heath and Adams 1967) but also need an increased supply of energy during the event of flight and foraging. Reasons are seen in the maintenance of body temperature and in extracting nectar of higher viscosity, as the viscosity of nectar increases with a decrease in temperature (Cruden *et al.* 1976).

The majority of sphingid moths captured in the vicinity of papaw orchards had *C. papaya* pollen attached almost solely to their probosces. The average number of attached pollen grains per proboscis exceeded 500 grains. In fact 20% of all examined specimens had more than 5000 pollen grains attached to their probosces. Hawkmoth species of similar size to *C. papaya* pollinating species have been reported to carry pollen loads in excess of 15000 grains on their probosces (Kislev

et al. 1972). Pollen grains of their investigated plant species were comparable in size and shape to that of papaw pollen; demonstrating the enormous capacity of sphingid moths as pollen transfer agents.

C. papaya pollen grains were visible in all areas on the proboscis surface of sphingid moths and in some cases almost completely covered the entire surface of the proboscis. Results on the distribution of pollen grains on hawkmoth probosces in this study differ from observations made by Kislev *et al.* (1972). According to Kislev *et al.* pollen grains were predominantly attached to the median furrow of the sphingid proboscis. The discrepancies between observations are probably due to differences associated with varying degrees of pollen viscosity, exine configuration and position of anthers.

Pollen that was identified from probosces of sphingids captured in papaw orchards was that of *C. papaya*. Pollen quantity and purity and its aggregation to the proboscis suggests that hawkmoths are efficient pollinators and therefore emphasizes their pollinator quality especially useful for outbreeding plant species (Levin *et al.* 1971; Kislev *et al.* 1972; Augspurger 1980) such as papaw. Data on the exclusive nature of pollen carried on captured sphingid probosces strongly suggest that hawkmoths most probably forage by 'traplining', supporting the view held by Linhart and Mendenhall (1977) and Janzen (1984). Opportunistic foraging behaviour of sphingids, as it was recorded by Kislev *et al.* (1972) and Haber and Frankie (1989), on the other hand is very likely to be due to the intermingled distribution of flowering plant species of low individual nectar volumes, which necessitate the diverse local food intake for the obvious reason of survival. Despite the finding of opportunistic feeding behaviour of sphingid moths, Kislev *et al.* (1972) stated also that the degree of pollen uniformity in almost half of their investigated pollinating hawkmoth species exceeded 90% for the predominant plant species. Those observations would lend support to the results on pollen purity found in this study as well as to the most probable foraging strategy of 'traplining'. The absence of pollen on probosces of some sphingids caught in light traps indicates their direct attraction to the light source without prior foraging activity conducted in the orchard.

Bees rarely contacted the stigma of pistillate *C. papaya* flowers. Their function as pollinators in *C. papaya* is minor and secondary. Although both bee species were observed foraging on staminate *C. papaya* flowers, the majority of *Trigona carbonaria* and *Apis mellifera* bees preferred foraging on flowers other than papaw. Both bee species were observed foraging preferentially on flowering native plant

species such as *Eucalyptus* sp. and *Melaleuca* sp.. Generally, either *Apis mellifera* or *Trigona* sp. bees avoided visiting pistillate *C. papaya* flowers, most probably due to their capacity for learning and communication, leading them to avoid visiting non-reward offering flowers.

In summary, the suggested involvement of bees (e.g. *Apis mellifera* or *Trigona carbonaria*) in the pollination of *C. papaya* can be altogether eliminated as no evidence was found in support of Allan's hypothesis (Allan 1963c), proposing that papaws are pollinated by bees.

CHAPTER VIII

FOODSOURCE RECOGNITION BY HAWKMOTH POLLINATORS

8.1 INTRODUCTION

Petals comprise an important part of the floral structure and their role is generally associated with the attraction of pollinators. Flowers most commonly produce nectar as a floral reward in close vicinity to the reproductive parts, and advertise its source by so called 'nectar guides' (Simpson and Neff 1983; Barth 1985). Most of the available literature has focused on the visual properties of 'nectar guides' (patterns in which ultraviolet and longer wavelengths are contrasted), but fewer studies have documented the importance of petal microstructures in locating intrafloral food resources (e.g. Kevan and Lane 1985). Floral scents are also important, as insects can detect and discriminate between scents and mixtures of scents attributable to various parts of the flower (Barth 1985; Bertin 1989).

Little is known about the mechanisms by which sphingids locate and recognize their food sources (Altner and Altner 1986; Bawa 1990). Recently, White *et al.* (1994) demonstrated that sphingids possess photoreceptors in their eyes, enabling them to discriminate between ultraviolet and longer wavelengths. In this study, hawkmoths were shown to rely solely on visual stimuli when foraging, selecting flowers where the petals reflected wavelengths over 400 nm, and absorbed ultraviolet wavelengths. Related studies concerning the oviposition behaviour of hawkmoths indicate however, that an array of stimuli, including visual, tactile and olfactory cues, have to be present before egg laying occurs (Ramaswamy 1988).

The following section reports on the mechanisms by which hawkmoth pollinators recognize *C. papaya* flowers, including assessments of visual (wavelength properties of *C. papaya* flowers), tactile and olfactory stimuli associated with both staminate and pistillate flowers. These features are discussed in relation to intrasexual pollinator attraction of outbreeding plant species, and location of intrafloral food resources by nocturnally active sphingid moths. The results demonstrate for the first time, that nocturnally foraging hawkmoths respond to a

combination of contact chemosensory (olfactory and gustatory) and mechanosensory (tactile) stimuli when locating intrafloral food resources.

8.2 MATERIALS AND METHODS

8.2.1 Plant Material

The dorsal and ventral petal surfaces of 30 pistillate and 30 staminate *C. papaya* flowers were examined using a JEOL scanning electron microscope (JEOL, 5300LV series, Tokyo, Japan). Freshly opened flowers were collected at random from trees grown at the T.A.F.E. orchard on a precipitation-free day during February 1995. Two petals of each flower were mounted on 25 mm aluminium stubs, one of each exposing the dorsal and the other the ventral petal surface. Preparations were immediately viewed under low vacuum pressure (10^{-1} Torr) S.E.M., without further preparation of the plant material. Photographs were taken using Kodak black and white slide film rated at 200 ISO. Exposures were enhanced using computer image intensifying techniques (Adobe Photoshop 3.0, Adobe Systems Incorporation, California, U.S.A., and Quark XPress 3.31, Quark Incorporation, U.S.A. Software and Cadonics Printer, NP 1600 series, Cadonics Incorporation, Ohio, U.S.A.).

8.2.2 Proboscis Material

Probosces of sphingid moths (n = 5) were examined using scanning electron microscopy following the same procedures as outlined in Section 7.3.2. The moth species were *H. celerio*, *H. scrofa*, *M. hirundo errans*, *T. oldenlandiae firmata* and *T. silhethensis intersecta*. Photographs were taken using Kodak black and white slide film rated at 200 ISO. Exposures were enhanced using computer image intensifying techniques (Section 8.2.1).

8.2.3 Staining of Glandular Plant Tissue

A further petal of all the above flowers (Section 8.2.1) was stained with 1% aqueous Neutral Red (Molecular Probes, from British Drug House, Poole, England) solution following the procedure of Vogel (1962). Neutral Red is a vital stain which accumulates in the vacuoles of plant tissues (Vogel 1962) and is an excellent method by which to highlight glandular plant tissues. Petals were examined after staining using a Nikon, Optiphot 2 microscope (Nikon, Tokyo,

Japan) set up for both bright and darkfield microscopy and photographed on Kodak colour slide film rated at 200 ISO.

8.2.4 Spectrophotometry

Freshly collected petals of pistillate and staminate flowers were cut into small pieces and immersed in methanol for 24 hours to extract pigments (Thompson *et al.* 1972). Preparations consisted of 40 g of petals (from either staminate or pistillate flowers) to which 200 mL of 98% methanol was added. Samples were stirred and covered to prevent evaporation. Prior to analysis, samples were filtered. The relative absorption was assessed using a spectrophotometer (Perkins-Elmer, Lambda 3B, Connecticut, U.S.A.). Spectral absorption was measured between the wavelengths of 260 nm and 700 nm.

8.3 RESULTS

8.3.1 Plant Material

The trichomes of both staminate and pistillate *C. papaya* flowers were not randomly arranged but fell into definite patterns. Patterns of longitudinally aligned trichomes were found in the basal half of flowers. In pistillate flowers this region corresponds to the area between the receptacle until petals begin to fully reflex at the height of the stigma (Figure 8.1). In staminate flowers, trichomes lined the inside of corolla tubes and were situated between the mouth of the corolla tube and the meniscus of the nectar at the base of the tube (Figure 8.1).

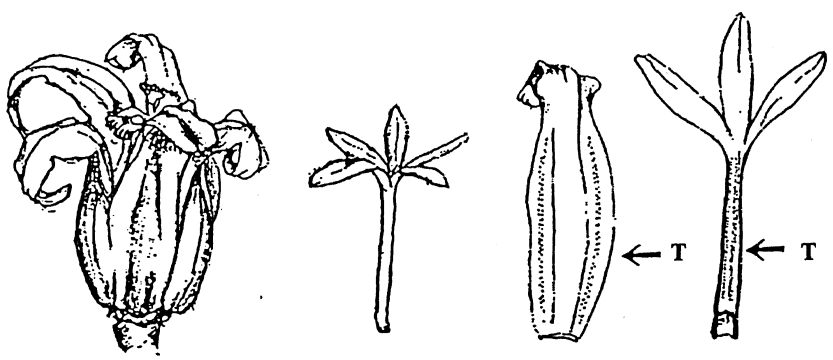


Figure 8.1: Distribution of trichomes on pistillate and staminate flowers.

Scanning electron micrographs of the adaxial petal surfaces of pistillate flowers showed patterns of aligned trichomes in the ligule of all the examined petals. Trichomes were distributed in narrow strips following the longitudinal petal axis, situated median between the midrib and the petal edge (Figure 8.2A; compare with Figure 8.5B). These microtextures occurred equally to both sides of the midrib and continued for approximately half the length of all the examined petals. Structurally these hairs were simple, unicellular, approximately 100 µm long and of fingerlike appearance tapering towards their distal end (Figure 8.2B). Trichomes were attached to scent glands at their base. These trichomes differed from longer hairs (250 – 500 µm) which occasionally appeared in the distal area of the adaxial petal surface (e.g. Figure 8.5C) and were also attached to scent glands. With the exception of these few trichomes, the distal adaxial petal surface was smooth and free of trichomes (Figure 8.2C).

Trichomes were also found lining the inside of corolla tubes of staminate flowers, situated between the mouth of the corolla tube and the meniscus of nectar at the base of the tube. Their alignment in narrow strips was of ribbonlike appearance (Figure 8.2D; compare with 8.5A). Despite closer interspacings between the rows of trichomes within corolla tubes, their longitudinal alignment was almost identical to that found in the ligule of pistillate flowers. A distinct line of transition occurred within the corolla tube (Figure 8.2E), between the hairlined region of the corolla tube wall and the hairless nectar-containing area at the base of the tube (Figure 8.2F). On the whole, the patterned nature of trichome distribution was associated with both pistillate and staminate *C. papaya* flowers. Trichomes always appeared in the basal area of the adaxial petal surface, which relates to the corolla tube of staminate flowers, and the area of the petal below the stigma of pistillate flowers. In all instances trichomes were attached to scent glands, irrespective of the gender of papaw flowers.

8.3.2 Proboscis Material

Scanning electron micrographs of the probosces of sphingid moths showed that sensory organs were present along the length of the proboscis (Figure 8.3A). These sensory organs differed in shape, size and position. Hairlike sensillae which measured between 50 – 150 µm (Figures 8.3B and 8.3C) were concentrated in the median and distal areas of the proboscis, whilst the tip was hairless. Hairs possessed an extremely sharp tip and no terminal opening. Other sensillae which were of distinctive styloconic appearance, consisted of a basal stylus (part of the epidermis) and an apical blunt-tipped peg (sensilla), and measured between 10 – 40

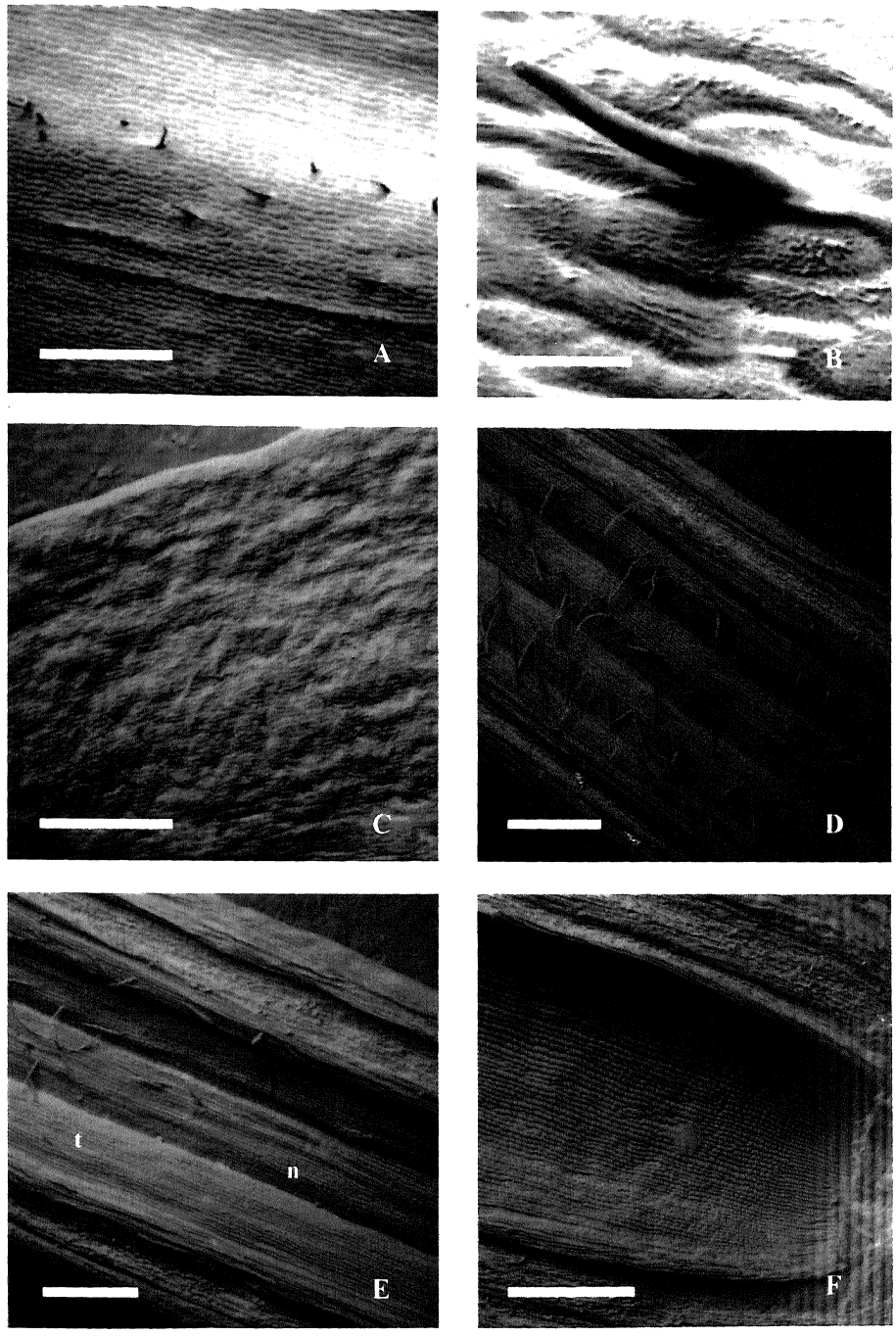


Figure 8.2: Scanning electron micrographs of trichomes on pistillate and staminate *C. papaya* flowers. (A) aligned trichomes in the basal area of a pistillate flower, (B) single trichome, (C) abaxial petal surface of pistillate flower, (D) aligned trichomes in the corolla tube of a staminate flower, (E) transition between distal end of aligned trichomes (t) and hairless nectary (n) at the base of the corolla tube, (F) hairless nectary.

D, E: x 35, bar = 500 μm; A, C, F: x 50, bar = 500 μm; B: x 500, bar = 50 μm.

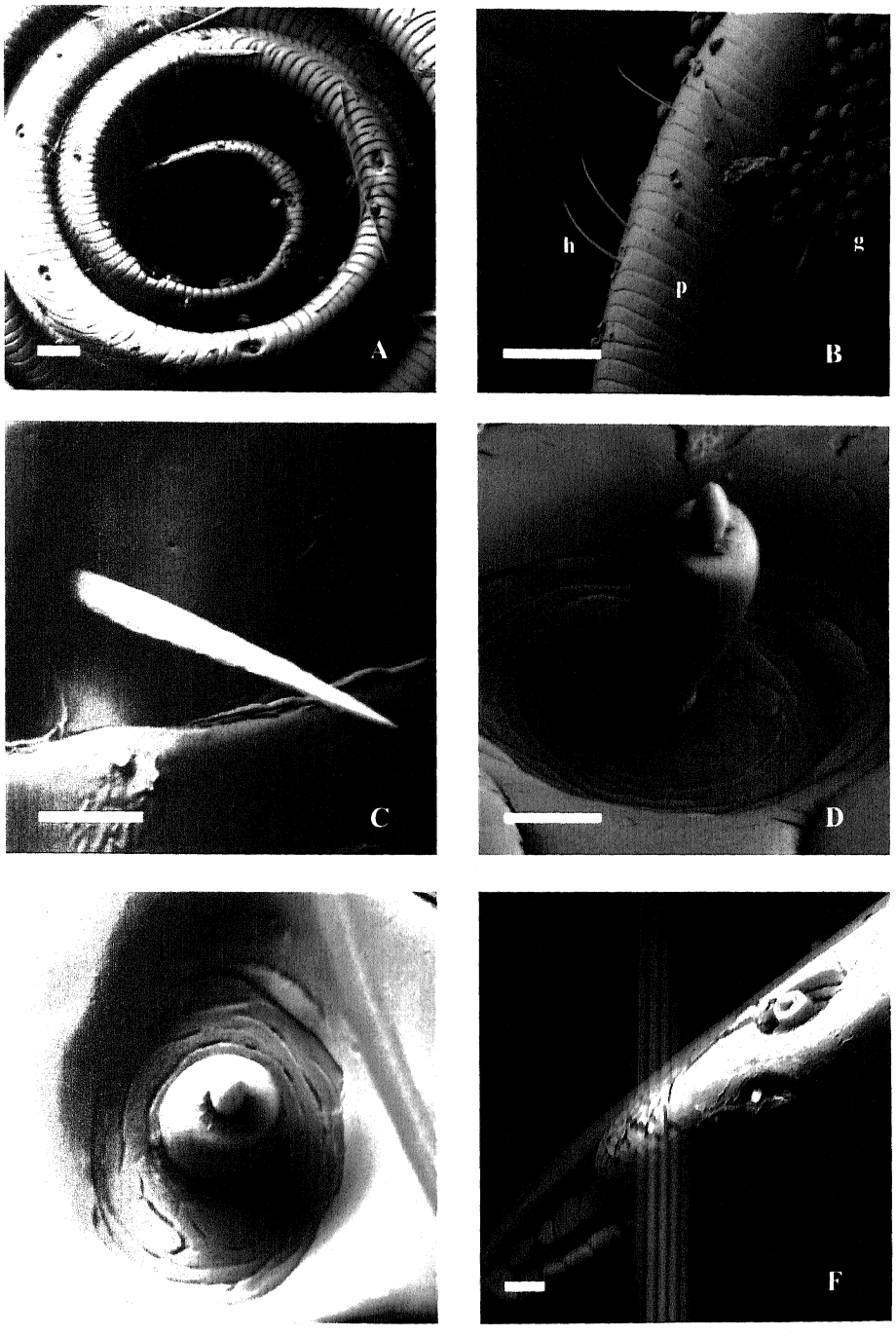


Figure 8.3: Scanning electron micrographs of the probosces of hawkmoths (Lepidoptera: Sphingidae). (A) sensory organs on the proboscis, (B) hairs (h) attached to the proboscis (p); note also *C. papaya* pollen grains (g), (C) hairlike sensilla, (D) and (E) styloconic sensillae; note their sunken position into grooves of the proboscis, (F) styloconic sensillae at the tip of the proboscis.

A: x 75, bar = 100 µm; B: x 200, bar = 100 µm; C, D, E: x 2000, bar = 10 µm: F: x 750, bar = 10 µm.

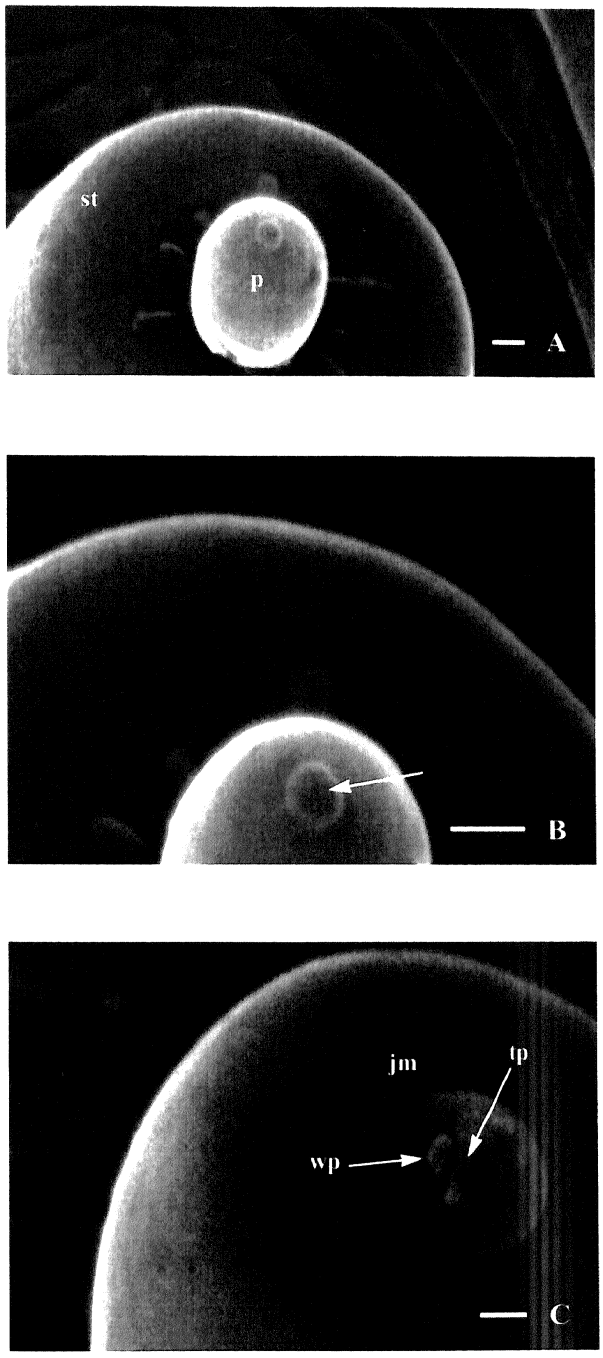


Figure 8.4: Terminal pores of styloconic sensillae showing (A) stylus (st) and the sensory peg (p); (B) the terminal opening and (C) the basal joint membrane (jm), terminal pore (tp) and wall pores (wp).

A: x 7500; B: x 15 000; C: x 10 000;
bars = 1 μm.

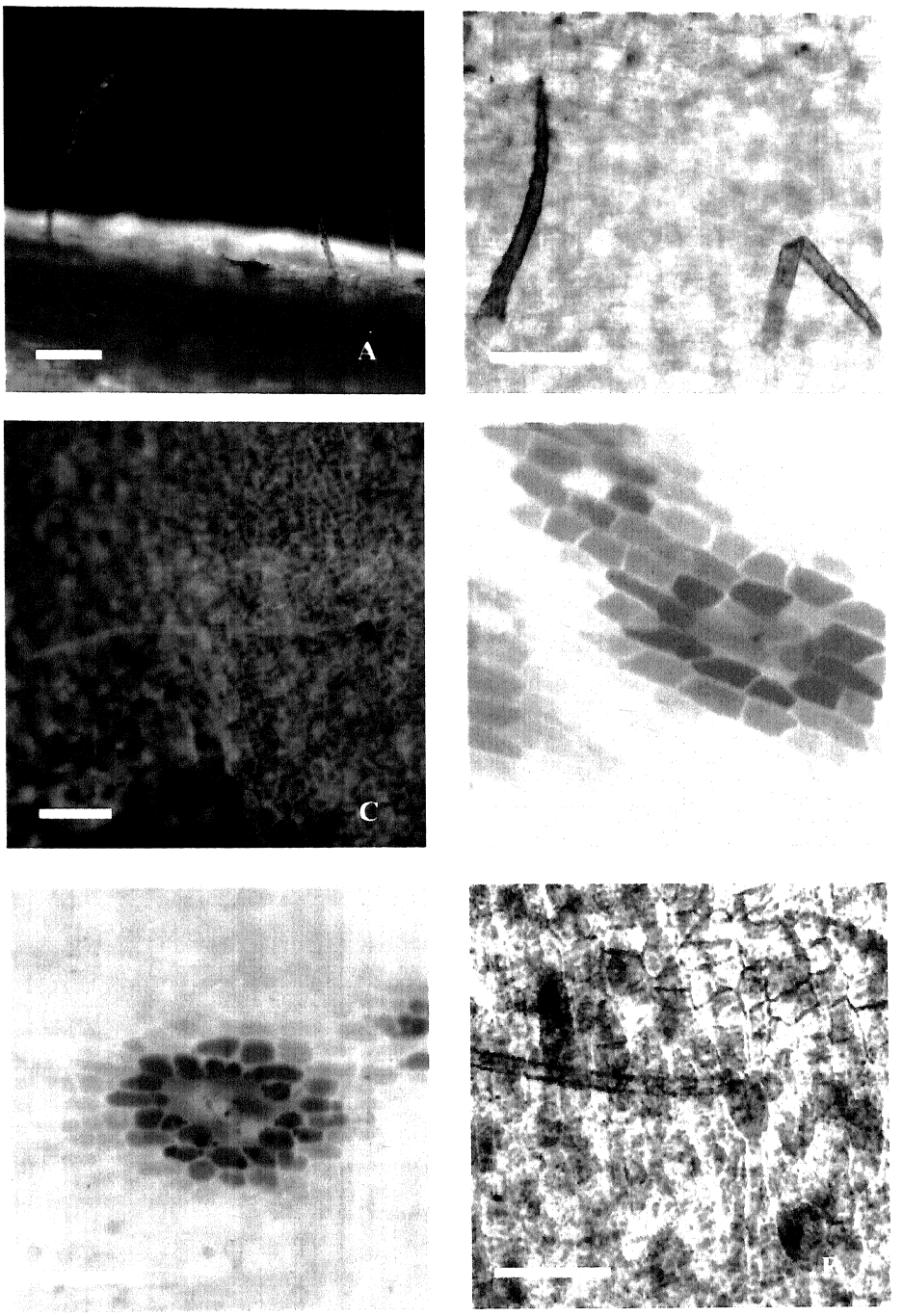


Figure 8.5: The staining of pistillate and staminate petal tissues with Neutral Red. (A) trichomes of staminate 'nectar guide', (B) trichomes of pistillate 'nectar guide', (C) trichome, (D) glandular tissue of *C. papaya* petals, (E) scent gland, (F) basal area of a trichome attached to a scent gland.

A: x 200, bar = 100 μm; B, D, F: x 550, bar = 50 μm; C: x 350, bar = 100 μm; E: x 450, bar = 50 μm. A, C : Darkfield photographs

μm in length (Figures 8.3D and 8.3E). They were attached in lateral positions on the surface of the proboscis, equidistant along two thirds of the proboscis towards its tip (Figure 8.3F). Styloconic sensillae varied in size due to the variation in the length of the stylus. Pegs were approximately 5 – 8 μm long and possessed a single terminal pore (Figure 8.4A and Figure 8.4B), surrounded by four wall pores (Figure 8.4C). Overall the cuticle of hairlike and styloconic sensillae was smooth. Types of sensory organs on the hawkmoths probosces were similar, irrespective of the species of hawkmoth.

8.3.3 Staining of Glandular Plant Tissue

Staining *C. papaya* petals with Neutral Red clearly distinguished the secretory structures of glandular hairs (Figure 8.5A – C) and tissue (Figure 8.5D – F) in the epidermal layer of the petals. All flowers contained large areas of scent glands or osmophores (Vogel 1962) on both the abaxial and adaxial petal surface, irrespective of the gender of flowers (Figure 8.5D). Osmophores were distributed in longitudinal strips median between midrib and petal edge, covering almost the entire length of the petals. However, the stain was preferentially taken up by cells in the basal region of the petal surface, irrespective of the gender of flowers.

Staining also highlighted the cell configuration of individual scent glands. The secretory cells of the epidermal surface form a ring pattern of 8 – 12 cells surrounding a central gland cell. Neutral Red stain accumulated in the cell vacuoles (Figure 8.5E) as previously reported by Vogel (1962). When trichomes were present, these hairs were always attached to the central cell (Figure 8.5F). The intensity of Neutral Red staining varied with the length of staining period and photographic exposure time, hence colouration of photographs is not identical.

8.3.4 Spectrophotometry

The relative wavelength absorption of pistillate and staminate *C. papaya* petals extracted with methanol was identical. Whilst wavelengths below 405 nm were absorbed, those above were reflected (Figure 8.6).

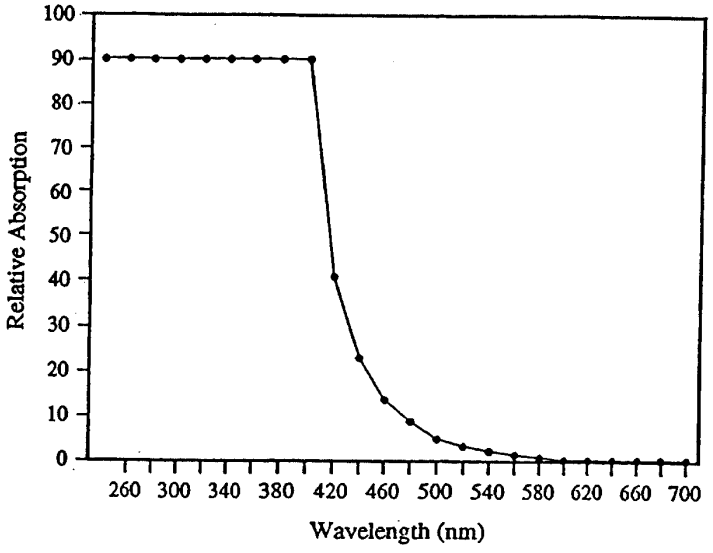


Figure 8.6: The relative absorption of methanol extracts of petals of *C. papaya.*

8.4 DISCUSSION

Trichomes attached to scent glands were found on both pistillate and staminate *C. papaya* flowers, whilst bisexual flowers were almost free of trichomes. The presence of specialized scent hairs in close proximity to the nectar source of staminate *C. papaya* flowers, and the reproductive organ of pistillate *C. papaya* flowers, would suggest their involvement in the 'deceit' mechanism by which nectarless pistillate flowers attract pollinator visits. Trichomes most likely provide a combination of olfactory and tactile stimuli by which pollinators (in this instance sphingid moths) recognize the location of an intrafloral nectar source. Brantjes (1973) mentioned that hawkmoths touch 'invisible scent sources' with their probosces whilst foraging for nectar, however did not provide the relevant evidence. The presence of pattern-forming trichomes attached to scent glands in specific areas of both, pistillate and staminate *C. papaya* flowers demonstrates the existence of such a combined stimulus incorporating the olfactory and tactile senses of hawkmoths. Therefore, these specialized scent hairs indicate the necessity for both chemoreception as well as mechanoreception in hawkmoths to induce feeding behaviour, similarly to the typical requirements to induce oviposition behaviour (Ramaswamy 1988). Visual stimuli also play a role in the shortrange recognition of suitable food plants, and the wavelength properties of petals of *C. papaya* flowers were shown to conform with spectral properties of flowers typically pollinated by hawkmoths (White *et al.* 1994), see below.

Scanning electron micrographs from the proboscis surface of hawkmoths indicate the existence of mechanosensory and contact chemosensory receptors. Mechanosensory receptors usually are hairlike and possess an extremely sharp tip with no distal opening (Slifer 1970), whilst chemosensory sensillae normally appear in pits sunken into the surface of the proboscis. The presence of a distal, central pore at the apex of chemosensory sensillae has been associated with receptors for gustation, whilst wall pores, which surround the terminal pore, have been associated with olfactory receptors (Altner and Altner 1986). However, a clear distinction between receptor types cannot be made with certainty as they demand further electrophysiological analysis (Altner and Altner 1986). However, the descriptions of either mechanosensory and chemosensory sensillae clearly match the types of sensory organs found on the surface of hawkmoth probosces.

The moths receptors exactly match the trichome patterns found attached to the scent glands of staminate and pistillate *C. papaya* flowers. These results indicate that the tactile sensory organs of hawkmoths are sensitive enough to provide the insect with a mechanosensory as well as with contact chemosensory (olfactory and gustatory) recognition mechanism of its food source.

Olfactory stimuli play a major role in the plant-pollinator relationship of hawkmoths, and all hawkmoth pollinated flowers are highly scented (Baker 1961; Eisiowitch and Galil 1971; Silberbauer-Gottsberger and Gottsberger 1975; Grant 1983; Janzen 1987b). Scent signals not only guide pollinators from afar to the immediate location of an intrafloral food resource, but also act as specific stimuli, which trigger the instinctive extension of the proboscis. According to Brantjes (1973) and Brantjes and Bos (1980) the extension of the proboscis in response to a scent stimulus constitutes an innate release mechanism which seems to be universal in hawkmoths. However, their theory was recently challenged by White *et al.* (1994) who claimed that the extension of the proboscis of hawkmoths was elicited solely by visual stimuli. The eyes of hawkmoths contain photoreceptors, which enable moths to discriminate between wavelengths. Sphingids were found to unroll their probosces while approaching artificial flowers that absorbed wavelengths of the UV spectrum and reflected those above 400 nm. Moths selectively foraged on flowers which provided those characteristics. However, vision seems to play only a partial role in the recognition of food sources by hawkmoths, as only 15 – 20% of all moths which came into contact with the appropriate flowers, engaged in actual feeding. As White *et al.* (1994) also used a large number of moths (15 – 30 specimens) in their flight cage experiments, and experimental sessions were carried out over a two hour period, the possibilty of a

small number of moths repeatedly feeding on the provided artifical flowers does exist. According to Brantjes (1973), hawkmoths would solely forage by visual stimuli, as long as moths were rewarded for their visits. However, their preference for a sole visual stimulus was not lasting, as it was automatically replaced by the innate preference for specific scent stimuli, if they were provided (Brantjes 1973). This indicates the necessity for or the coexistence of other floral stimuli to induce feeding behaviour and does strengthen the concept that both olfactory and tactile stimuli are involved in the recognition of intrafloral food resources. For instance Brantjes and Bos (1980) observed that hawkmoths hover nearer to flowers as soon as the proboscis tip presumably receives tactile stimuli located at the 'entrance' of the flower, which emphasizes the importance of tactile cues in intrafloral food source recognition.

In papaws the initial attraction of pollinators to plants depends on scent and visual stimuli. As staminate flowers outnumber pistillate papaw flowers (Section 4.1.3.3), chances of pollinators encountering nectar-offering staminate flowers first are by far greater. Upon the initial flower contact with nectar-offering staminate flowers, pollinators would additionally receive tactile stimuli with which the insect could associate a nectar reward. In order for moths to get to the concealed nectar source at the base of the corolla tube, their probosces would have to pass, and inevitably contact, the longitudinally aligned trichomes between the corolla tube mouth and the level of nectar at its base. The function of trichomes serving as nectar guides is supported by the observation that hairs were not present past the level where a full complement of nectar was to be expected. Also, the concealed position of trichomes within the narrow corolla tubes would create a scent gradient in comparison with the remainder of the exposed scented petal tissue.

Neutral Red accumulated more rapidly in the glandular tissue, including entire trichomes, which indicates that the water repellent cuticle is thin or absent above these cells. Therefore, greater emittence of volatile substances should be suspected of scent glands to which trichomes are attached as opposed to glands without attached hair, or the remaining petal surface. Pollinators, or better, their specific feeding appendages, would also be guided to the location of the intrafloral food resource by following a scent gradient. Chemosensory sensillae at the tip of the proboscis support the hypothesis that such a mechanism exists. Staminate flowers, therefore, may fulfil the role of accustoming pollinators to specific scent and tactile stimuli by which they recognize a food reward, and possibly also provide pollinators with the triggering scent stimulus upon which they automatically extend their probosces (Brantjes 1973).

Trichomes were also present on the petals of pistillate *C. papaya* flowers. Their presence, form of alignment, location, and close association with the scent producing tissue, suggests that they too perform an analogous function in attracting pollinators. Despite morphological differences between staminate and pistillate flowers, such as the lack of a corolla tube of the latter, floral parts of pistillate flowers are arranged in a form that resembles the corolla tubes of staminate flowers. The slightly concave petals which closely follow the contours of the ovary below the stigma, and the alternate arrangement of petals with the stigmatic lobes, creates a multiplicity of 'funnel-like' structures which are similar in length to the corolla tubes of staminate flowers (Figure 8.1). As trichomes were only found in the basal part of the adaxial petal surface, which corresponds to the 'funnel'-shaped area of the petal below the stigma, they most likely present the same olfactory and tactile stimuli associated by hawkmoths with the nectar reward offered by staminate flowers. By providing pollinators with both olfactory and tactile stimuli in the lower area of the adaxial petal surface, parts of the proboscis would inevitably come into contact with the deeply segmented stigmatic lobes during foraging for nectar. Observations of hawkmoths coming into contact with pistillate *C. papaya* flowers confirm that moths inserted their probosces in between the stigmatic lobes (Section 7.4.4). Their behaviour is indicative of their searching for a nectar resource, guided by the tactile and olfactory stimuli of the nectar guide. As the alignment of trichomes in pistillate flowers continues from the height of the stigma to the base of petals, pistillate flowers may lure pollinators into inserting their probosces even deeper than they normally need to do before reaching the nectar source of staminate flowers. By doing so, more surface area of the pollen carrying proboscis would come into contact with the stigma and therefore not only increase the likelihood of pollination *per se,* but also increase the probability of a greater load of pollen grains being deposited.

In all 42 observed contacts of hawkmoths with pistillate papaw flowers, moths had their probosces extended before coming into contact with the stigma (Section 7.4.4 and Figure 7.4D), irrespective of hawkmoth species. These observations not only strengthen the hypothesis of an innate extension mechanism of the proboscis in response to an accustomed scent stimulus but also its claim on universality amongst hawkmoths. Therefore, the nectarless pistillate *C. papaya* flowers, which emit a similar scent and wavelength spectrum to that of staminate flowers, would depend on the innate feeding mechanism of hawkmoths in response to an accustomed combination of visual, olfactory and tactile stimuli. As both pistillate and staminate flowers provide each of the three stimuli, repetitive visits of hawkmoths to the nectarless pistillate flowers most likely continue on the basis that moths are unable

to discriminate between flowers that are constantly nectarless, or nectarless due to prior nectar foraging activity (Section 7.4.4).

Notwithstanding the significance of olfactory and tactile stimuli, Brantjes (1973) recognized earlier that foraging of hawkmoths in close proximity to flowers can be compensated for or improved by visual orientation which would provide the animal with greater foraging efficiency. This is in accordance with observations made by Inoue (1986) who reported that light reflections by pale coloured flowers, which were enhanced by larger petal size or increased flower density, markedly increased the number of sphingid pollinators. Visual cues may become the only means by which hawkmoths continue to locate their food sources and may also initially be sufficient for identification of appropriate hawkmoth flowers (refer to White *et al.* 1994). However, the provision of floral scents and tactile cues would enable hawkmoth pollinators to discriminate between visually equivalent flowers, and therefore would constitute a useful tool by which plant species ensure pollinator constancy. Pollinator constancy is complementary to the 'traplining' behaviour suspected of hawkmoth pollinators (Janzen 1984). Requirements for visual and olfactory cues in order to locate food plants may also vary between hawkmoth species (Brantjes 1973).

The pollination mechanism of papaw was previously described by Baker (1976), who classified it as 'mistake' pollination (from the insects point of view). Bawa (1980b) later described the process as pollination by 'deceit' (from the plants point of view) when he studied the pollination mechanism in the related species *J. dolichaula.* Since Badillo (1971) showed earlier that the floral structure within all species in the Caricaceae is remarkedly constant, the 'deceit' based mechanism may equally apply to *C. papaya* and therefore will also be discussed with respect to *C. papaya.*

'Mistake' pollination, according to Baker (1976), implies the transfer of pollen by insects with low discriminatory powers. These insects are misled into visiting both staminate and pistillate *C. papaya* flowers due to the similarity of scent emission from both. Poor light at dusk and fast flight of the sphingid pollinator was assumed to aide the event of pollination. By contrast, Bawa (1980b) proposed a 'deceit' based pollination system that primarily focused on interfloral mimicry, by which the reproductive parts of pistillate flowers resemble the petal outline of staminate *C. papaya* flowers. Principally, the terminology of 'mistake' or 'deceit' based pollination system is acceptable but for quite different reasons to those proposed by either author.

Recent knowledge on the visual abilities of hawkmoths refute all parts of former hypotheses that are based on low discriminatory visual powers. Hawkmoths have been shown to possess visual senses enabling them to discriminate and successfully operate between typical hawkmoth pollinated flowers which shared similarities of UV wavelength absorbance and longer wavelength reflectance (Brantjes 1973; White *et al.* 1994). Entire flowers seem to appear white against the dark background (White *et al.* 1994). As papaw flowers complied with the above wavelength pattern, the entire petal outline of both pistillate and staminate flowers would be visible to hawkmoths. The smoothness of the cutin covered stigmatic papillae (Rodriguez Pastor *et al.* 1990) in association with a thin film of stigmatic fluids (Section 4.4.3) would contribute to the reflection of wavelengths by entire flowers rather than a highlighting of the stigmatic outlines as proposed by Bawa (1980b). Observations on the foraging activity of hawkmoths on pistillate *C. papaya* flowers also showed that moths inserted their probosces into flowers which were at various stages of anthesis (Section 7.4.4). Similar observations of hawkmoths visiting nocturnally opening flowers in their early stages of anthesis were also reported by Silberbauer-Gottsberger and Gottsberger (1975). These findings indicate that hawkmoths are able to associate a particular scent with a food source. Therefore, a pollination system that relies on interfloral mimicry as proposed by Bawa (1980b) is highly unlikely, particularly since the stigmatic outlines are still concealed by the petals in the process of flower opening. Consequently, floral scent signals are as important (possibly even more so) as visual signals for the initial contact of moths with flowers. Thus hawkmoth pollination by no means constitutes a pollination system based on mere coincidence. Rather, the results open a new perspective into the level of sophistication plants employ to promote themselves to pollinators by using an array of signals, including those that stimulate the olfactory, gustatory, tactile and visual senses of their pollinators.

CHAPTER IX

LARVAL FOOD PLANT ASSOCIATIONS OF SPHINGID MOTHS

9.1 INTRODUCTION

After establishing that hawkmoths are the pollinators of *C. papaya* the focus was on their larval ecology including the identification of host plants in the vicinity of papaw orchards. Also, known larval host plants were introduced to the papaw orchard to determine whether pollinator species would accept them as breeding sites, which if found, would constitute a useful tool towards the management of hawkmoths as pollinators of papaw.

Furthermore, the literature concerning larval ecology in Australian sphingid species is rather scarce. Larval food plant associations are completely unknown for some species (e.g. *Macroglossum micaceum*) and are incomplete for other species (e.g. *Hyles lineata* or *Hippotion celerio;* Common 1990). Similarily, species-specific data concerning the time interval for larval development is not available and records pertaining to pupal dormancy in sphingids distributed throughout Australia cannot be found. In that respect every record contributes a further insight into hawkmoth ecology.

A brief overview of the reproductive behaviour of adult sphingids and the feeding requirements of the immature stages will be given, prior to the presentation and discussion of results.

9.2 OVERVIEW ON THE REPRODUCTIVE REQUIREMENTS OF THE SPHINGIDAE AND THEIR LARVAL ECOLOGY

The reproductive behaviour of sphingid moths is scarcely described in the literature. An early report mentioned that forage flights usually occur during the early evening hours and are followed by mating flights (Lederer 1923, as cited in Eisikowitch and Galil 1971). Haber and Frankie (1989) briefly mention repetitive

mating and continuous oviposition during adult sphingid life, and this in turn presumes a continuous search for mates.

The nature of the close search range for recognition of larval host plants of female sphingids has recently been reviewed by Jackson (1990). It has been hypothesized that olfactory and visual cues are used for the orientation of hawkmoths towards suitable host plants and contact chemoreception and mechanoreception are usually essential before oviposition is initiated (see also Brantjes 1973; Bernays 1982; Miller and Strickler 1984; Ramaswamy 1988).

A number of hawkmoth species respond to the depletion of adult and larval food sources, and possibly increased predation, by migrating (Janzen 1987a, 1987b; Powell and Brown 1990). Janzen (1984, 1987a) hypothesized that such events are of a cyclic nature and constitute a normal part of the seasonal life cycle of a number of sphingid species in Costa Rica. Adult sphingids recur in a habitat they had previously left after cross-country migration. Huge population increases of adult and larval stages of sphingid moths have been associated with wet season rains. Similar observations have been made in tropical areas in Sierra Leone (Cross and Owen 1970), Costa Rica (Janzen 1987a, 1987b, 1988; Haber and Frankie 1989) and Australia (Moulds 1981). Haber and Frankie (1989) specified the timing of such sudden explosions of species numbers to approximately two weeks after the onset of rain in the dry rainforest environment of Santa Rosa National Park, Costa Rica. Janzen (1987a) remarked that within the same habitat the number of adult moths attracted to a light increased into the thousands on the same night that the first rainfall of the season was recorded.

Sphingid larvae feed on more recently evolved plant families (post-Cretaceous; Lin 1990) and most of the species feed on host plants which are related at a family or genus level (Janzen 1981, 1984, 1988). Host plants typically belong to the families Rubiaceae, Apocynaceae, Euphorbiaceae, Solanaceae, Bignoniaceae, Asclepiadaceae, Moraceae, Sapodaceae and Lauraceae and range in form from annual herbs to vines or saplings and large trees (Merz 1959; Janzen 1981; Janzen and Waterman 1984; Bernays and Janzen 1988). The majority of the sphingid species exhibit monophagous, and to a certain extent, oligophagous feeding behaviour. Janzen (1981) estimated that sphingids feed on less than 2% of the approximately 600 broad-leaf plant species in the Santa Rosa National Park in Costa Rica reserve. This was interpreted by him to indicate an explicit close relationship between larvae and host plants. Polyphagous sphingid species are the exception, although they do occur. For example, *Hyles lineata* feeds on more than

20 plant species in South America (Janzen 1988). A similar diversity of host plant choices was recorded from five hawkmoth species in Australia (Moulds 1981). Moulds reported that these species foraged on native as well as on introduced plant species belonging to more than five plant families. All five sphingids were of widespread distribution, covering at least the Indo-Australian region.

The occurrence of sphingid caterpillars is generally linked to the rainy season. Larval numbers suddenly explode over a period of three to six weeks in which most caterpillars complete their larval development. Hawkmoth larvae typically feed on soft, new foliage but with ongoing development incorporate older leaf material into their diets (Bernays and Janzen 1988). Their gregarious feeding habits can cause heavy defoliation in some years, depending on larval abundance, which may fluctuate enormously within seasons and between years (Janzen 1981). Although larval food plants of Australian sphingids are poorly documented, Moulds (1981, 1984) identified 21 species of hawkmoths which can be classified as pests of commercial and ornamental species. Of the commercial crops, grape vines (*Vitis vinifera*, Vitaceae) and sweet potatoes (*Ipomea batatas*, Convolvulaceae) in particular are attacked by numerous hawkmoth species, almost all of which belong to the subfamily Macroglossinae. The list of garden ornamentals attacked by sphingids at pest proportions is extensive but contains as many native as introduced plant species (Moulds 1981). Almost all hawkmoth larvae pupate in leaf litter or in pupation chambers in the soil surface layer (Janzen 1987a; Common 1990).

9.3 MATERIALS AND METHODS

9.3.1 Larval Ecology

Scrub and open woodland adjacent to papaw plantations at the Parkhurst and C.Q.U. orchard were searched for known and suspected larval food plants and inspected for eggs and larval stages. The survey was based on the known host plants published by Common (1990) and Moulds (1981, 1984).

Five fortnightly observations were conducted over an eight week period commencing in the second week of January continuing until the second week of March 1993. Survey areas also included relatively undisturbed dry rainforest scrubs around Mt. Etna and Mt. Archer (Figure 2.3). Larval host plant associations were recorded and larvae were collected and photographed.

Collected larvae were reared on the host plant species on which they had been feeding. They were identified and the time taken between the commencement of the pupa stage until eclosion was recorded. Specimens were kept in 400 mL glass containers covered with gauze and kept outside in the shade. Minimum temperatures were 10°C or above. Pupae were misted occasionally with water, preventing the pupae from drying.

A collection of successfully pupated adults was produced for identification. Specimens overwintering in the pupa stage were kept in containers in a shelter from 2 June 1993 until 10 September 1993.

9.3.2 Acceptance of Larval Host Plants by Sphingid Pollinators in the Vicinity of Papaw Orchards

In order to investigate the effects of artificial enhancement of host plant numbers a total of 55 plants were potted into 12 inch buckets and distributed between rows of the papaw orchard at the C.Q.U. orchard between February 1993 until June 1994. Thirtyeight plants belonging to 12 recognized host plant species within seven plant families were used (Table 9.1). Selected plants were known hosts to six *C. papaya* pollinating and proposed pollinating sphingid species. The remainder of plant specimens were closely related species of known larval host plants. Additionally, sweet potato (*Ipomea batatas*, Convolvulaceae) a known food plant of hawkmoth larvae, was planted underneath the papaw trees. Weekly observations were carried out to record eggs and larval stages present.

In 1993 an experimental papaw orchard was established 300 km inland at Emerald (Figure 2.3), where 200 grape vines were planted adjacent to the papaw orchard of approximately 500 plants. In Australia, grape vines (Vitaceae) are attacked by the larvae of six hawkmoth species (Moulds 1981) including *H. celerio*, *T. oldenlandiae firmata* and *Hyles lineata livornicoides* which have confirmed or suspected pollinator status in *C. papaya*. Therefore this large scale host plant trial would provide insight in the acceptance of host plants by sphingid pollinators in direct vicinity to the crop they pollinate. Grape vines were selected because of their dual function as a commercial crop plant and their proven attractiveness as larval hosts to a number of papaw-pollinating and suspected papaw-pollinating sphingid species.

During the last week of March 1994 sphingid larvae were collected from newly established grape vines (*Vitis vinifera*) and photographed for identification. Hawkmoth larvae were reared on *V. vinifera* leaves following the procedures listed

Table 9.1: List of plant species of larval host plant trial.

Plant Species	Family	Origin	Sphingid Species
Alocasia macrorrhizos	Araceae	N	*H. celerio / T. oldenlandiae firmata*
Carissa ovata	Apocynaceae	N	*N. subvaria*
Impatiens balsamina	Balsaminaceae	I	*H. celerio / H. scrofa / T. oldenlandiae firmata*
Ipomea batatas	Convolvulaceae	I	*H. celerio / H. scrofa / H. velox*
Ipomea indica	Convolvulaceae	I	*H. celerio*
Hibbertia scandens	Dilleniaceae	N	*H. celerio / T. oldenlandiae firmata*
Cissus oblonga	Rubiaceae	N	*H. scrofa / T. oldenlandiae firmata*
Morinda jasminoides	Rubiaceae	N	*M. hirundo*
Pavetta australis	Rubiaceae	N	*M. hirundo*
Pentas lanceolata	Rubiaceae	I	*H. celerio / H. scrofa / T. oldenlandiae firmata*
Psychotria loniceroides	Rubiaceae	N	*M. hirundo*
Vitis vinifera	Vitaceae	I	*H. celerio / H. lineata livornicoides / T. oldenlandiae firmata*
Canthium gracillipes	Rubiaceae	I T	
Cayratia acris	Rubiaceae	N T	
Cissus repens	Rubiaceae	N T	
Cissus rhombifolia	Rubiaceae	N T	
Psychotria daphnoides	Rubiaceae	N T	
Randia benthamiana	Rubiaceae	N T	
Randia fitzalanii	Rubiaceae	N T	
Timonius timon	Rubiaceae	N T	
Cissus antarctica	Vitaceae	N T	

Introduced species (I), Native species (N), species on trial (T) - close relatives to kown sphingid host plant species -.

in Section 9.3.1. The interval of time between commencement of pupation until eclosion was recorded.

9.4 RESULTS

9.4.1 Larval Ecology

The survey of larval stages of sphingid moths resulted in successful identification of eight hawkmoth species of which five contribute towards the pollination of papaw, all within the same subfamily, the Macroglossinae (Table 9.2 and Figure 9.1).

Table 9.2: Identification of sphingid species and their larval host plants in the vicinity of Rockhampton (January/February 1993).

Moth Species	Collection Site	Larval Host Species	Plant Family
Cephanodes hylas cunninghami	Mt. Etna	*Canthium odoratum*	Rubiaceae
Cephanodes janus janus	Mt. Etna	*Canthium odoratum*	Rubiaceae
	Olsen's Caves	*Aidia racemosa*	Rubiaceae
Hippotion celerio	Olsen's Caves	*Boerhavia diffusa*	Nyctaginaceae
	C.Q.U. orchard	*Ipomea batatas*	Convolvulaceae
Hippotion velox	Olsen's Caves	*Pisonia aculeata*	Nyctaginaceae
Macroglossum hirundo errans	Mt.Archer	*Coelospermum reticulatum*	Rubiaceae
	Olsen's Caves	*Psychotria loniceroides*	Rubiaceae
Gnathothlibus erotus eras *	Rockhampton	*Cissus repens*	Vitaceae
Theretra clotho celata *	Olsen's Caves	*Cissus repens*	Vitaceae
Theretra queenslandi *	Olsen's Caves	*Dendrochnides photinophylla*	Urticaceae

* non papaw pollinating species

Larvae of pollinating and suspected pollinating hawkmoth species were collected at Mt. Archer, Mt. Etna and at Olsen's Caves, which constitute areas of more or less undisturbed vine thicket habitat and partially open woodland. Hawkmoth larvae were not detected on endemic larval host plants (i.e. *Canthium odoratum, Canthium oleifolium, Coelospermum reticulatum, Carissa ovata* and *Planchonia carreya)* in the vicinity of disturbed dry scrub adjacent to papaw orchards at the C.Q.U. and Parkhurst orchards.

A total of 35 hawkmoth specimens were recorded at Mt. Archer, Mt. Etna and at Olsen's Caves, of which 27 (two *H. celerio*, seven *H. velox*, six *M. hirundo errans*, one *C. hylas cunninghami* and eleven *C. janus janus*) had either pollinator or suspected pollinator status in papaw. The remainder belonged to the species of *G.erotus eras*, *T. queenslandi* (one each) and *T. clotho celata* (six specimens).

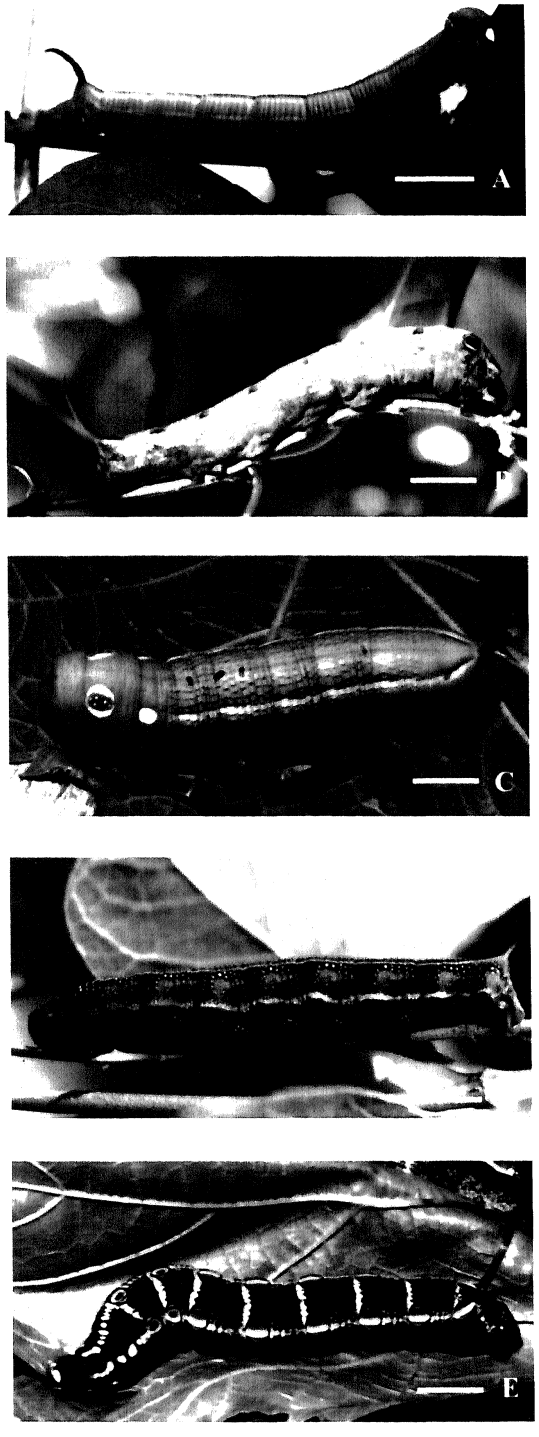


Figure 9.1: The larvae of a number of identified and suspected *C. papaya* pollinating sphingid species. (A) *Cephanodes janus janus* Miskin, (B) *Hippotion velox* Fab., (C) *Hippotion celerio* L., (D) *Macroglossum hirundo errans* Walk., (E) *Theretra oldenlandiae firmata* Walk.. Bar = 1 cm

Moth larvae were collected during two weeks; the last week of January 1993 and the first week of February 1993. Sphingids eclosed during a period of persistent low rainfall totalling 4.4 mm of rain over 15 days (14– 28 February 1993).

In almost all instances, sphingid larvae were collected from plants already identified as host species with the exception of *Aidia racemosa*, which is an addition to the larval food plant list of *C. janus janus*. Furthermore, the collection of *H. celerio* on *Boerhavia diffusa* supports its status as a suspected larval food plant (refer to Moulds 1981).

The feeding habits of hawkmoth larvae that were relevant to the study were observed in the field and during rearing. For example, the larvae of *H. velox* consumed complete young leaves of *P. aculeata,* whereas *M. hirundo* was observed eating whole young flower buds of *C. reticulatum.* As larvae usually consumed entire leaves and fed on the sheltered underside of leaves, their presence in the field was difficult to detect when leaf damage alone was used as an indicator. Also, the different larval colourations (i.e. green and brown forms) which were noted for *C. hylas cunninghami, M. hirundo* and *H. velox* camouflaged the caterpillars. Larvae of *H. velox* and *M. hirundo errans* changed larval colourations (i.e. from green to brown forms) during their last instar development. In many instances, presence of larvae was inferred from large amounts of characteristic droppings below their food plants rather than from obvious plant damage.

Sphingid larvae which were collected during summer 1993, remained on average two to three weeks in the pupa stage (Table 9.3). The shortest pupation interval was noted for *M. hirundo errans* with 14± 0.8 days and the longest interval was recorded for *C. hylas cunninghami*, with 21 days.

Table 9.3: The average pupation time of hawkmoth species collected in the vicinity of Rockhampton (January/February 1993).

Moth Species	Moth No	Pupation Time Days± STD
Cephanodes hylas cunninghami	1	21
Cephanodes janus janus	11	19± 2.9
Hippotion celerio	2	19± 1.4
Hippotion velox	7	18± 1.3
Macroglossum hirundo errans	6	14± 0.8
*Gnathothlibus erotus eras**	1	18
*Theretra clotho celata**	6	16± 1.5
*Theretra queenslandi**	1	†

*non papaw pollinating species

†specimen died

9.4.2 Acceptance of Larval Host Plants by Sphingid Pollinators in the Vicinity of Papaw Orchards

During the 72 week experimental period of the host plant trial at the C.Q.U. orchard a total of two hawkmoth larvae were recorded feeding on introduced garden and commercial plant specimens Larvae were identified as those of *T. oldenlandiae firmata* on *Impatiens balsamina* and *H. celerio* on *Ipomea batatas.* Sightings of both larvae coincided with periods of wet weather during the beginning (37.2 mm rainfall) and end (70.8 mm rainfall) of November 1993.

A total of 30 *H. celerio* and 9 *T. oldenlandiae firmata* larvae were collected from grape vines in Emerald in March 1994. These were the only species present on grape vines within the orchard from November 1993 until June 1994. Between one to four larvae of either *H. celerio* or *T. oldenlandiae firmata* or both were collected from individual vines. All larvae were of similar size and had entered their last (fourth) developmental stage. Green and brown coloured forms of *H. celerio* larvae were found and in some instances collected from the same vine. Sightings of hawkmoth larvae coincided with a period of wet weather.

Larvae collected in Emerald were reared following the same procedures as in the previous year. Larvae of both species entered the pupa stage within the week following collection (27 March 1994 – 1 April 1994). Eclosure of *H. celerio* larvae was recorded on three separate occasions. Whilst four of the pupae eclosed after 19± 1.4 days, the majority of pupae overwintered until the first hatching occurred during the middle of October, 207± 1.2 days after entering the pupa stage. The remainder entered the adult stage in November, 240± 2.0 days after entering the pupa stage (Table 9.4).

Table 9.4: The average pupation time of *H. celerio* and *T. oldenlandiae firmata* collected on *V. vinifera* in the Emerald district (March 1994).

Moth Species	Moth No	Pupation Time Days± STD
Hippotion celerio	4	22± 1.4
	4	207± 1.2
Theretra oldenlandiae firmata	8	240± 2.0
	8	197± 1.1

All nine *T. oldenlandiae firmata* specimens overwintered in the pupa stage and eclosed after 197± 1.1 days during the second week of October 1994. One pupa was mummified. All eclosure times of *H. celerio* and of *T. oldenlandiae firmata*

coincided with the occurrence of rain within five days of eclosure. The amount of rainfall varied between registered traces below 1 mm and 70.8 mm per day.

Parasitism was noted of six of the 30 *H. celerio* pupae which were parasitized by flies (Tachinidae: *Palexorista* sp.). Cantrell (1986) listed the names of five sphingid species (including those of *H. celerio*) parasitized by trachiniid flies belonging to four different genera. Identification of flies to species level in the genus *Palexorista* has not yet been revised (Elson-Harris personal communication). In this study, fly species hatched 14.3± 1.2 days after larvae entered the pupa stage. In three instances the sphingid pupae were parasitized by two individual *Palexorista* sp., while a single fly hatched from the remaining two pupae, totalling eight tachinid parasites. Feeding and pupation of parasitized larvae seemed unaffected, as no visual signs of defects were observed on either larval or pupal stages.

9.5 DISCUSSION

Most of the larval host plants were associated with open woodland and dense vine thicket habitats in the Rockhampton area. Such vegetation communities are diminishing in the Rockhampton area with ongoing development spreading into and around the ranges. Formerly, gully and creek vegetation would have provided much of the breeding grounds for hawkmoth species. Previously papaw orchards would have been in the immediate vicinity of such plant communities, benefiting from the close interaction between breeding and feeding grounds of hawkmoths. As no long term data of hawkmoth numbers and species composition are available for any region within Australia, the effects of habitat destruction on the distribution and density of sphingid species are undocumented. Possibly the non-detection of hawkmoth caterpillars on larval host plants in the immediate vicinity of the Parkhurst and University orchards indicates the consequences of habitat destruction since caterpillars were collected during the same time interval from less disturbed dry rainforest habitats. Endemic species such as for instance *C. janus janus*, the distributional range of which is restricted to the Rockhampton area, would be most vulnerable, as host plant specificity of sphingid moths is generally correlated with their distributional range. Relatively few *C. janus janus* host plant species are known. Moth species which are widely distributed are most likely to be polyphagous (e. g. *Hyles lineata*); (Janzen 1988). Although some sphingid species are endemic to certain regions, the majority of sphingid species, including all other papaw-pollinating and suspected pollinating species, are of wider distributional

range. Hawkmoths cover a large area of habitat for foraging and feeding and as such their numbers might not necessarily decrease in the short term in areas where clearing or urbanisation has occurred. However, studies addressing the range covered by female hawkmoths between foraging sites and reproductive sites are not available. There is an obvious need to investigate further the biodiversity of hawkmoths and threats to their longer term survival. The results from the present study indicate the commercial importance of conserving the biodiversity of hawkmoths.

In the present study, the occurrence of immature sphingids as well as the timing of eclosion of adult moths appears to be linked to the wet season or at least isolated occurrences of rain. These observations are consistent with those made by Moulds (1981) who recorded a huge population increase of sphingid larvae (reaching pest status) on the Atherton Tablelands in tropical Australia. According to Moulds, such rapid breeding potential is only associated with wet season rains. Janzen (1984, 1987a) reported similar seasonal breeding behaviour of sphingid moths from dry rainforest habitats in Costa Rica. He observed that several hawkmoth species which have a normal distributional range within Santa Rosa National Park (Costa Rica) are known to migrate in search of more favourable breeding habitats across the Andes during dry seasons. Some species were also suspected of crossing the ranges twice; timing their recurrence in Santa Rosa National Park with the beginning of the rainy season (Janzen 1987b; Powell and Brown 1990). Large scale migration is not documented in Australian sphingids, although suspected from a small number of species (Common 1990).

Pupation of sphingid species required at least two weeks but could be delayed to as long as 34 weeks after larvae entered the pupa stage. These observations were made on larvae collected through the subtropical summer period in Rockhampton and Emerald in central Queensland. Larvae that were collected during the January/February period (in 1993) all eclosed within three weeks following pupation, however most larvae that were collected at the end of March (in 1994) remained in the pupal stage until the following spring. Although this overwintering process of sphingids was only observed from two species (*H. celerio* and *T. oldenlandiae firmata*), it is possibly common amongst a much larger species group. Janzen (1987a) reported that sphingids overcame unfavourable survival conditions such as dry spells in Santa Rosa National Park, Costa Rica by remaining in the pupal stage. He hypothesized that pupal dormancy would last for four to six months and that the first flux of adult sphingid numbers during the new seasons rains was partially the result of mass eclosure of dormant pupae from the previous

season. Pupal dormancy has been associated with eight to twelve species within Santa Rosa National Park (Janzen 1987a). The above results concerning pupal dormancy lasting, in case of *H. celerio,* for over 34 weeks, are proof of such a survival mechanism. The consistent small standard deviation in the timing of each flux of eclosure suggests a precise mechanism of control, with the pupae potentially sensitive to barometric pressure, day length or soil temperature.

The provision of larval hosts in the papaw orchard did not encourage sphingid reproduction in the locality itself. The overall low host plant acceptance level at the University site is difficult to explain but may be related to the fact that it was a disturbed urban site. However, observations from the Emerald orchard indicate that supplementary host plants such as *V. vinifera* placed adjacent to an orchard would be accepted by hawkmoth species such as *H. celerio* and *T. oldenlandiae firmata.* Such plants induce moth breeding activity in the immediate vicinity of papaw orchards. These preliminary results suggest that alternative hosts may be better exploited if planted adjacent to papaws rather than underneath the crop.

Parasitism of hawkmoth larvae and pupae occurred with some specimens of *H. celerio.* The likelihood of parasites including flies (Trachinidae) and wasps (Brachonidae and Ichneumonidae), predating the larval and pupal stages of sphingid moths was previously mentioned by Janzen (1988). Janzen reported that at least two species of *Belvosia* (Diptera: Tachinidae) are known to parasitize both small and large hawkmoth species in Santa Rosa National Park, Costa Rica. Parasitism of hawkmoth species within the Australasian region was mentioned by Cantrell (1986) in his updated host catalogue of the Australian Trachinidae. Ramifications of such parasitism on the population dynamics of sphingids is unknown, however this is another area which requires further investigation if the hawkmoth populations are to be managed to maximize papaw pollination.

The uncontrolled removal of native vegetation will certainly have an impact on pollination of papaw. At this stage it should be remembered that native pollinators provide the option of a cost effective pollination system, as management costs for habitat enhancement are relatively low (Corbet 1991). Preservation of local scrub and vine thicket sites would be considered a long-term investment in sustaining crop pollination dependent on native pollinators, as there is usually no replacement for specific plant-pollinator relationships. Results from the present study suggest also that it may be possible to optimize conditions for pollinators by manipulating the local plant community (i.e. the planting of larval host species). Dempster (1983) for instance reported that besides predation the most significant factor

determining fluctuations in moth and butterfly abundance is the failure to oviposit a full complement of eggs. Factors such as host plant density and distribution are of importance as hawkmoths generally lay their eggs singly on individual host plant species. The papaws sexual reproductive system will function as long as certain hawkmoths and their larval food plants survive in these areas.

CONCLUSIONS

The mechanism by which the commercially significant exotic plant species *Carica papaya* (papaw) is pollinated remained obscure for many years. This study conclusively demonstrates that the previously unknown pollination mechanism of papaw is neither anemophilic nor agamospermic as formerly hypothesized, but is entirely entomophilic. In contrast with another prior hypothesis that pollination is bee mediated, several species of hawkmoths (Lepidoptera: Sphingidae), some of which are endemic to the central Queensland area, are identified as the pollinators of *C. papaya.*

The mechanism by which non-nectar offering pistillate flowers receive nocturnal pollinator visits is demonstrated for the first time. In contrast with former concepts of 'deceit' based plant-pollinator relationships a specific anatomic relationship exists between moths and flowers. Pollen transfer in papaws involves the recognition of both sexes of *C. papaya* flowers by their hawkmoth pollinators by their similar scent emission and UV reflecting properties of petals. The nectar-producing staminate as well as the nectarless pistillate *C. papaya* flowers both have nectar guides (the former in their corolla tubes, the latter along the base of their petals), which provide nocturnally foraging sphingid moths with tactile and contact chemosensory stimuli when locating intrafloral food resources. Mechanosensory sensillae on the probosces of sphingid pollinators matched the length of trichomes found on pistillate and staminate papaw flowers. Contact chemosensory sensillae on the proboscis were identified as receptors for olfaction and gustation, which presence shows the multiplicity by which hawkmoth pollinators recognize their food source.

The identity of the pollinator was clearly established for the first time. There was no evidence to support common beliefs of the involvement of other insect species such as *Apis mellifera* and *Trigona* sp. as pollinators of papaw. Pollination of dioecious papaws under natural conditions was demonstrated to be carried out by hawkmoths (Lepidoptera: Sphingidae). Seven pollinator species, namely *Macroglossum hirundo errans, Macroglossum micaceum, Nephele subvaria, Hippotion celerio, Hippotion velox, Theretra oldenlandiae firmata* and *Theretra silhetensis intersecta* have so far been identified in the central Queensland region and the potential of an additional four species, namely *Hippotion scrofa, Hyles lineata livornicoides, Cephanodes hylas cunnighami* and *Cephanodes janus janus*

has been recognized. It was shown that pollinator and suspected pollinator species had in common a similar length of the proboscis (2.5 cm – 3.6 cm). Additionally all pollinator species belong to the same subfamily, the Macroglossinae. With the exception of three diurnally active species the remaining moths are nocturnal foragers and their activity coincides with the dusk floral anthesis of *C. papaya* flowers.

The identification of hawkmoths as the primary pollinators of *C. papaya* inspired investigations into the nature of the larval host plants. Species such as *Vitis vinifera* increased hawkmoth reproduction adjacent to papaw orchards, a finding which immediately suggests methods for maximizing fruit production. Furthermore, seasonal fruit set under the subtropical climate was influenced by a combination of factors, relating to the seasonal absence of pollinators and the seasonal variability of pollen quantity and viability. Whilst low pollen viabilities and pollen availability correlated with the decrease in fruit set and seed numbers during the end of the August and September period, pollinator absence was the primary cause of reduced fruit and seed set during winter, from approximately the middle of June until the middle of August. Hand pollination should be considered as an option to ensure fruit set during the period of pollinator absence as pollen viability and pollen quantity of hybrid lines (especially that of Hybrid 29) and stigma receptivity overall were found to be adequate during the early winter period to permit fruit set.

Overall, this study has documented a unique plant-pollinator relationship involving an at present horticulturally unmanaged insect group, the Sphingidae. In studying the interactions between crop plants, their pollinator and its ecological requirement, a major step has been accomplished in understanding seasonal papaw production under subtropical climates. These results will be integrated into existing crop management practices. Future studies should seek to optimize the relationship between *C. papaya* and its sphingid pollinators and the potential significance of incidences of infrequently occurring agamospermy. This would make papaws breeding by dioecy independent of the present need for pollen transfer.

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