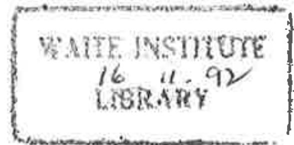
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The Floral Biology of Cashew (*Anacardium occidentale* L.) in relation to Pollination and Fruit set

By

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The floral biology of cashew (Anacardium occidentale L.) in relation to pollination and fruit set

Summary

This study of the floral biology of cashew (*Anacardium occidentale* L.), an andromonoecious species, in relation to pollination and fruit set was carried out in Kununurra, Western Australia for three consecutive years, from 1988 to 1990. The reproductive phase occurred in the dry season between June and November, and flowering started after vegetative flushing. There were more male than hermaphrodite flowers in a panicle, and the two flower types had different opening patterns. The hermaphrodite flowers dominated in the first two weeks but were replaced by male flowers from week three onward. Diurnal opening patterns of hermaphrodite and male flowers were variable but peak opening was generally in the morning. The number of open hermaphrodite flowers was correlated with initial and final fruit set. Premature fruit drop peaked in weeks five and six after flower opening. Means of hermaphrodite and male flowers, initial and final fruit set and fruit shed per panicle were 95, 578, 29, 6, and 22 respectively.

The stigma had a thick continuous cuticle with little exudate. The stigmatic papilla cells were mainly unicellular but occasionally bicellular or tricellular, and the stigmatic exudate contained lipid, protein and carbohydrate. The style consisted of three layers of epidermis, parenchymatous tissue with three vascular bundles in a triangular arrangement, and solid transmitting tissue in the centre. The cashew pistil has a dry stigma and a solid style.

Both hermaphrodite and male flowers have large and small stamens, all of which produce pollen grains. The four pollen types were similar in morphological structure being tricolpate of about 44.0 x 26.3 micron dimensions. There were similar numbers of total pollen grains produced by hermaphrodite and male flowers,

but the pollen grains differed in viability and chemical composition. The pollen grains produced by the hermaphrodite flowers had a reduced capacity for *in vivo* pollen germination and ovule penetration, but higher contents of both sugars and amino acids than the pollen grains from the male flowers. It is suggested that the hermaphrodite flower pollen grains are adapted to provide a food source to attract insects to visit the flowers, while the male flower pollen grains are adapted for pollination.

Nectaries were located in both hermaphrodite and male flowers, at the junctions of the panicle branches, on the leaf petiole and on the young developing fruits. Floral and panicle nectar secretion was synchronised with pollen presentation. The hermaphrodite flower secreted a higher volume of nectar than the male, but the male floral nectar had the highest concentration of sucrose and amino acids. This suggests that male flowers are adapted to encourage insects to visit large numbers of flowers to collect sufficient pollen for effective pollen transfer. It is suggested that the function of the extrafloral nectar produced from panicle branches, leaves and young fruit is to attract ants to forage and protect the cashew trees from leaf, flower and fruit eating fauna.

The cashew pistil had a relatively short period of receptivity to pollen. For optimum fertility the hermaphrodite cashew flower had to be pollinated within the first three hours of anthesis. Pollen germination and pollen tube growth was rapid in the pistil with the majority of the pollen tubes reaching the base of the style by three hours after pollination. Pollen tube growth was slower in the ovary prior to penetration of the ovule. Ovule penetration had occurred twenty four hours after pollination.

There was a reduction in yield following selfing as compared with crossing, but this was not due to pollen-pistil incompatibility. It appears that postzygotic factors were probably the cause of premature fruit drop and yield reduction

following self-pollination. Diallel analysis showed that the arrangement of compatible genotypes in the plantation is an important yield determinant. There was significant general combining ability (GCA) in all three experiments conducted but in only one experiment was specific combining ability (SCA) significant. The characters of high GCA values as male and female parents should be selected for, along with other flowering and fruiting criteria such as synchronised flowering, the ratio of hermaphrodite to male flowers, the number of initial and final fruits set per panicle and kernel characteristics.

Declaration

I hereby declare that this research has been carried out by myself and contains no material which has been accepted as part of the requirements for any degree or diploma in any university, or any material previously published or written unless due reference to this material is made. I am willing to make this thesis available for photocopy and loan if it is accepted for the award of the degree.

W. Wunnachit

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1 Introduction

Cashew cutivation is distributed over many countries which have a tropical climate, but the majority of cashew nut production comes from the new growing areas of Asian and African countries and from Brazil, its country of origin (Ohler, 1979; IBPGR, 1986). A number of products can be harvested from the cashew tree (Figure 1.1). The edible kernel is the most important product, but the swollen fruit pedicel, the cashew apple, is also edible (Figure 1.2). The shell of the cashew nut contains a valuable industrial oil, the cashew nut shell liquid, and the young leaves are edible and are consumed as a vegetable in some countries. The increasing demand for the cashew nut has stimulated many cashew growing countries to develop their production for both domestic consumption and for export. In Australia, trial plantings of cashew have been established mainly by the three state governments of the Northern Territory, Queensland, and Western Australia, along with pioneer private agencies (Gunn and Cocks, 1971; Millington, 1983; Wait and Jamieson, 1986). The Kununurra, area which is located in the tropics of Western Australia, has high potential for commercial cashew production (Dept. Nat. Res., 1976; Ord River Project Co-ordination Committee, 1983), and cashew seeds from different origins have been introduced for evaluation in the area since 1972. The cashew trees in this area show excellent vegetative and reproductive growth with wide variation of flowering and fruiting habits. The characteristic of poor final fruit set with excessive premature drop is common among these seedling trees, as reported overseas (Damodaran *et al.*, 1965; Free and Williams, 1976; Wunnachit *et al.* 1986). Superior genotypes have been selected using broad vegetative and reproductive criteria, and budwood of the selected genotypes are vegetatively propagated to prepare planting material for commercial orchard establishment. Because inadequate pollination and sexual incompatibility are significant factors contributing to poor bearing and low yield in many tree crops (de Nettancourt, 1977; Faegri and van der Pijl, 1979; Heslop-Harrison, 1975; Knox, 1984), so it

Figure 1.1 Five year old cashew tree at Kununurra

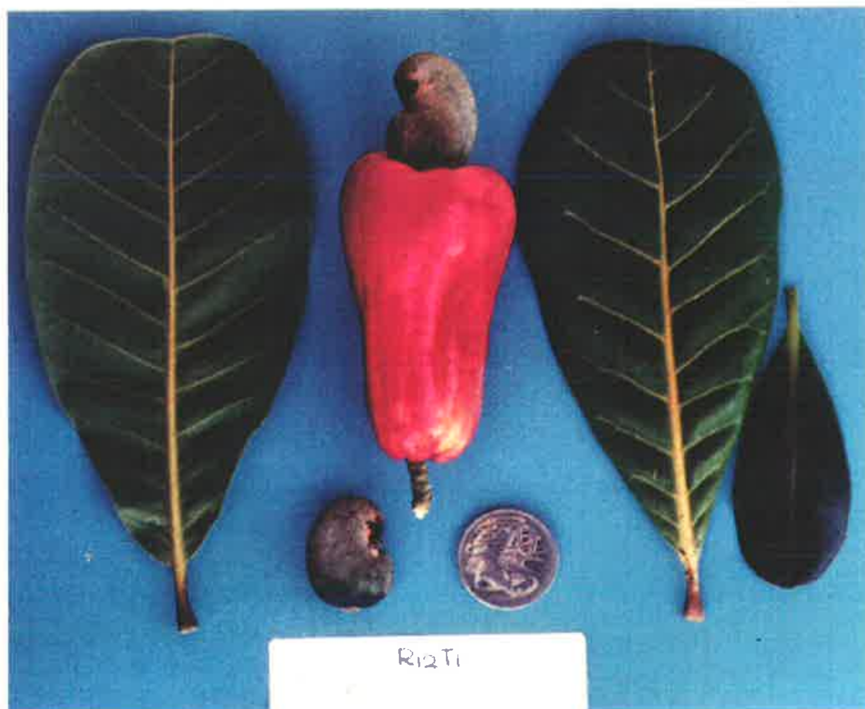

Figure 1.2 Leaf and fruit of cashew showing cashew apple and kernel in shell

is important to know the pollination and compatibility requirements of the clonal cashew genotypes before planting them together in the same orchard. The optimum orchard layout using compatible genotypes coupled with ensuring adequate pollination can minimize the problem of poor fruit set and improve yield.

This project has emphasised three main aspects of research; pollination, sexual compatibility, and genotypic selection criteria based on pollination and compatibility requirements. This is achieved via the following steps;

1. Study of the flowering and fruiting phenology of cashew in north-western Australia.

2. Examination of the structure and histochemistry of the cashew pistil.

3. Investigation of the characteristics of cashew pollen grains and the quantity and quality of pollen production.

4. Investigation of floral and extrafloral cashew nectaries and the quantity and quality of nectar production

5. Study of the pollen-pistil interaction and breeding system of the cashew

2. Literature review

2.1 Introduction

Pollination is a natural process in the reproductive phase of flowering plants, leading to fertilisation and fruit set. It is one of the limiting factors in the production of many tropical crops, including cashew (Free, 1970). There have been reports that cashew trees produce many flowers but that mature fruit set is very low and variable with excessive premature fruit drop. As vegetative propagation of superior cultivars becomes more widespread, it is important to understand the pollination and compatibility requirements of the cultivars. Many crops require pollinator cultivars for optimum fruit production. This is often related to sexual incompatibility which results in the inhibition of pollen grain germination or inhibition of pollen tube growth in the style, ovary, or ovule. It may thus reduce fruit set and has been reported in many tropical crops (Singh *et al.*, 1962; de Wet and Robbertse, 1986). Inadequate pollination and fertilisation and sexual incompatibility are probably the most significant factors contributing to poor bearing and these are the main areas of discussion in this review.

2.2. The cashew tree

Cashew belongs to the family Anacardiaceae. It is native to the South American tropics but now is widely grown in the tropical areas of many countries in Asia, Africa and Australia (Johnson, 1973; Ohler, 1979; Wait and Jamieson, 1986). The tree is fast growing and produces branches near the base giving a wide spreading canopy (Figure 1.1). With the large tap root system and an extensive network of lateral roots, the tree is hardy and drought resistant (Morton, 1961). Cashew producing areas are mainly in coastal regions (Rao, 1958; Ascenso, 1970; Nambiar, 1979; Ohler, 1979). This may be because of the good drainage, sandy soil and good aeration (Nambiar, 1979; Ohler, 1979). Trees growing under favourable

conditions can produce their first flowers at the age of 2-3 years (Johnson, 1973; Ohler, 1979). The first crop is small but it increases each year up to ten years when the tree reaches maturity.

2.2.1 Flowering habit

The flowering habit of cashew is controlled by both internal and external factors (Nambiar, 1979). In areas of evenly distributed rainfall, cashew flowering may occur throughout the year. In climates with two wet periods flowering may take place twice a year. Usually, flowering occurs after the growth flush at the end of the rainy season. The inflorescences are produced terminally on newly-formed shoots. Argles (1969) reported that in the bearing cashew tree two or three peak periods of growth were usually observed, even though under favourable conditions of soil moisture and other environmental factors, some shoot growth may occur in almost every month. Thus rainfall appears to be an important controlling factor in the flowering of cashew. The flowering period varies amongst the production areas from two to four months, with the peak opening period between four to six weeks after commencement (Bigger, 1960; Ohler, 1979).

Nambiar (1979) reported that the variation in flowering seasons of cashew in different areas is also controlled by latitude and temperature. There is a gradual delay in the flowering time as one moves north or south from the equator. Increasing altitude and increasing distance from the equator both result in lower minimum temperatures. This is probably a major limiting factor to flowering of cashew through the effect on shoot growth.

2.2.2 Floral characteristics

Cashew flowers occur in large inflorescences termed panicles, in common with some other tropical crops such as mango, avocado, and rambutan (Purseglove, 1968). The panicles are produced terminally on newly developed shoots at the

surface of the canopy. Studies in different areas show that the panicles vary in shape, size, and numbers of branches and flowers (Rao and Hassan, 1957; Northwood, 1966; Ascenso and Mota, 1972; Wunnachit, 1979). The panicle shape may be conical, pyramidal, or irregular, but most are conical in shape and between 14 and 21 cm long. The panicle comprises one main rachis and 3-11 secondary branches each with several tertiary branches. Cashew is andromonoecious (Purseglove, 1968), with hermaphrodite and male flowers in the same panicle. The arrangement of the flowers is a typical monochasial cyme with the terminal flower of each cyme a hermaphrodite, with two male flowers occupying lateral positions (Copeland, 1961). The number of flowers on each panicle varies depending on the vigour of the tree (Morada, 1941), the age of the tree (Wunnachit *et al.*, 1986), and the environmental conditions. The cashew tree produces more male than hermaphrodite flowers and the total number of flowers and their sex ratio are variable between both trees and locations.

The male flower is smaller than the hermaphrodite flower but both have the same numbers of sepals (5), petals (5), large stamens (1), and small stamens (6-14) (Copeland, 1961; Purseglove, 1968; Ascenso and Mota, 1972; Kumaran *et al.*, 1976; Ohler, 1979). The five sepals are green and protect the flower when it is closed. They open once only and then fall at about seven days after anthesis (Wunnachit, 1979). The five petals arise within the tube of sepals. They are white on first opening, but later turn purple-red. The petal is a protective organ, and in many flowers also serves as the organ of attraction. They tend to be large in flowers pollinated by animals (Faegri and van der Pijl, 1979) and are present as reduced organs in many wind pollinated flowers (Whitehead, 1969). Colour is an important floral attribute (Harbourne, 1977), as it functions as a visual signal to insect pollinators. The array of floral colours visible to insects allows them to distinguish species of flowers at a distance. Honeybees, which are the most important insect pollinators of many economic crops, exhibit their colour preference by visiting

blue, yellow, and white coloured flowers (Harbourne, 1977; Faegri and van der Pijl, 1979). Flower colour may develop or change during anthesis, as in the cashew flower, in many species such as *Fuchsia spp.* (Yazaki, 1976) and *Murtensia spp.* (Weevers, 1952). There have been many attempts to determine the significance of this phenomenon in relation to the pollination system. The generally accepted interpretation is that the colour change indicates to the insect that floral rewards are no longer available.

The hermaphrodite and male flowers normally have the same complement of one large and 6-14 small stamens. Flowers with 0, 3, or 4 large stamens do occur but are very rare (Ascenso and Mota, 1972). The stamens of cashew are arranged in an ellipse with the large stamen located at one of the narrow ends (Copeland, 1961). Moncur and Wait (1986) reported that the large stamen of the male flower is longer than that of the hermaphrodite flower and is usually the same length as the style. The significance of the different length of the stamens is considered to be an adaptation to accommodate various types and sizes of potential insect pollinators. In mango, which belongs to the same family as the cashew (Anacardiaceae), there is also one large fertile stamen which is longer than the four staminodes and is nearly equal to the length of the style (Singh, 1954). Each stamen of cashew has a slender filament on which the anther is borne. The anther is bilobed and dehisces along the slit between the two pollen sacs of each lobe. The anther is rounded and pink in colour, turning grey at the time of dehiscence, then brown following pollen shed (Ohler, 1979). Studies on anther dehiscence in the large and small stamens of cashew flowers show different results. Northwood (1966) reported that the large and most of the small stamens dehisce normally and produce pollen, whereas according to Rao and Hassan (1957) and Damodaran *et al.* (1965), the large one was the only true stamen, the others being sterile staminodes. The significance of the different stamen size requires further clarification as it may have an effect on the pollination system of cashew.

The pistil of the cashew flower has three main parts; the stigma, the style, and the ovary. The simple style arises from the distal margin of the ovary. It is long and slender, usually longer than the large stamen, tapering toward the apex then expanding slightly to form the stigma. It is occasionally shorter than the major stamen, or of equal length (Northwood, 1966). The ovary is laterally compressed, containing a single locule and a single anatropous ovule, the funiculus arising from the base of the locule in the narrow plane of the ovary (Copeland, 1961). It is a superior ovary borne on a receptacle which is fused with the pedicel. When fruit set occurs the ovary develops into the cashew fruit (true fruit) and the receptacle develops into the cashew apple (pseudo or false fruit) (Figure 1.2).

2.2.3 Flower opening pattern

The hermaphrodite and male flowers of cashew have different opening patterns. In many growing areas, the male flower starts to open first in the early morning and is followed by the hermaphrodite flower one to several hours later. Male flower opening commences between 6 a.m. and 2 p.m. in India (Damodaran *et al.*, 1965), 6 a.m. and 6 p.m. in Tanzania (Northwood, 1966), and 5 a.m. and midday in Thailand (Wunnachit, 1979) with the peaks about two to four hours after the start of opening. The hermaphrodite flower opens four hours after the male with the peak about two hours after the start of opening (Wunnachit, 1979).

Flower opening and anther dehiscence are influenced by climate (Nambiar, 1979; Ohler, 1979), with reports from different growing areas showing slight differences. Even at the same locality the opening times may differ from day to day according to the location of the flowers in the canopy. In general, flower opening and anther dehiscence start and finish earlier on the sunny than on the shady side of the tree (Damodaran *et al.*, 1965; Northwood, 1966). The opening of the hermaphrodite and male flowers is different not only in diurnal pattern, but reports also show different phases of flowering. Pavithran and Ravindranathan (1974) reported three

distinct sex phases; the first was a male phase, the next a mixed phase of male and hermaphrodite flowers, and the third a male phase at the end of flowering period. Kumaran *et al.* (1976) also reported that the flowers occurred in phases but they found an extra bisexual phase at the start of the flowering period. It appears that genotype and environment have a combined effect on the flowering of cashew. The sequence and time of flower opening are important for efficient fruit set and yield. Only the hermaphrodite flowers will set, but ample pollen must be available for efficient pollen transfer. The timing of flower opening is also important with respect to the scheduling of honeybee introduction into the orchard.

2.2.4 Pollination of cashew

Cashew nut production depends on pollination, and is reported to have a mixed system of both selfing and crossing (Free, 1970), although cross pollination is more likely to occur (Northwood, 1966; Free and Williams, 1976). Bigger (1960) reported that the high percentage of male flowers suggested that wind was the pollinating agent, but this was contested by Northwood (1966) and Elsy *et al.* (1986) who considered that wind pollination is not important in the cashew pollination system.

It has been reported that cashew flowers are self fertile, but that hand pollination improves fruit set (Northwood, 1966; Free, 1970; Free and Williams, 1976). However, the extent of self pollination in cashew has not been intensively studied. Most of the research on cashew pollination is on cross pollination, particularly by insects, and it is accepted that insects are the major vectors for cashew pollen (Rao and Hassan, 1957; Northwood, 1966; McGregor, 1976). Cashew flowers have characteristics which are well adapted for cross and insect pollination such as the stigma at a similar level to the anthers, the strongly scented coloured flowers and floral nectar production. However, it is generally found that there is poor fruit set under open pollination conditions (Rao and Hassan, 1957;

Damodaran *et al.*, 1965; Free and Williams 1976; Wunnachit *et al.*, 1986). Nambiar (1979) commented that the failure of pollination was one of the reasons for poor final fruit set of cashew in India. On the other hand Northwood (1966) observed that in Tanzania, flower production, pollination, and fruit set were efficient, and these factors did not limit the yield. McGregor (1976) suggested that the evidence strongly indicated that the concentration of honeybee colonies in cashew plantations during flowering would at least alleviate the problem of poor setting of fruit.

2.3. The pollen-pistil interaction

Pollen contains the male gametes. It is produced in the pollen sac of the anther and has a complex structure and composition. The pistil contains the female gametes and normally consists of three main parts; the stigma, style and ovary. These are also complex multicellular organs. The structure, composition, and function of the pollen and pistil control pollination and fertilisation in flowering plants.

2.3.1. Pollen

The development of the pollen grain takes place in the pollen sac in the anther (Esau, 1977). During pollen formation, development is coordinated with the inner wall of the anther, the tapetum (Echlin, 1971). The tapetum supplies nutritive materials to the developing pollen (Echlin, 1971; Dickinson and Lewis, 1973; Christensen and Horner, 1974). In particular, it contributes to the formation of the outer wall of the pollen grain, the exine. The tapetum is also the origin of proteins present in the cavities of the exine which are believed to play a role in cell recognition on the arrival of the pollen grains at the stigma surface (van den Ende, 1967; Knox, 1984). The exine is either a uniform sheath or subdivided into the outer sexine and the inner nexine (Heslop-Harrison,1975). The sexine is the sculptured part. It is attached to the nexine by means of rods, the baculae, which

may be united into a tectum above, or remain free. The inner layer of the pollen wall, the intine, is made up of cellulose and pectin, resembling the primary wall of a vegetative cell. It contains several enzymes which are involved in pollen metabolism. These are synthesized by the spore and inserted during pollen development. Mattsson *et al.* (1974) reported that the intine protein was gametophytic, synthesized by the pollen cytoplasm and the exine protein sporophytic, synthesized by the parental cytoplasm. These enzymic wall proteins seem to be concerned with germination, penetration of the stigma surface and the early growth of the pollen tube. Other functions include involvement in the recognition responses which control breeding behaviour, in both inter and intra-specific incompatibility systems.

The pollen grain varies in size and shape depending on the species. It generally has apertures, thin wall areas in the exine, through which the pollen tube emerges during germination. These also allow the grain to change in volume during changes in humidity (Walker, 1974). Eames (1961) reported that pollen of the majority of dicotyledons have three apertures (tricolpate pollen). In higher plants, two types of mature pollen grain are found; bicellular and tricellular (Knox, 1984). In the bicellular type, the pollen grain comprises two cells, a vegetative cell which is concerned with tube growth and metabolism, and a generative cell which divides after pollen germination within the pollen tube to produce two sperm cells. In tricellular pollen, the generative nucleus divides in the ungerminated grain, giving rise to a pair of sperm cells within the pollen grain. Dumas *et al.* (1983) reported differences between the two pollen types when the pollen germinates. In the tricellular type, the metabolism of the grain is geared to rapid pollen tube growth so that fertilisation may occur within minutes or hours of pollen deposition on the stigma. In contrast, in the bicellular type pollen tube growth is slow and fertilisation occurs several hours or days after pollination.

At the time of dispersal, the pollen grain is in a stage of temporary dormancy (van den Ende, 1967). In mature pollen the water content is about 10% in most species and respiration is extremely low. Upon hydration, respiration and other metabolic processes increase rapidly.

For successful fertilisation the pollen grain must be viable. There are a number of different methods to test pollen viability. Knox (1984) summarised *in vitro* culture methods using an agar medium or liquid culture with carbon sources such as sucrose, and essential minerals including boron and calcium. It is possible to assess such parameters as germination percentage, pollen tube length and growth rate. However, the test is most effective with bicellular pollen as tricellular pollen germinates poorly *in vitro*. Heslop-Harrison and Heslop-Harrison (1970) developed the fluorochromatic reaction (FCR) method which is assessed by fluorescence microscopy. It is a rapid test in which fluorescein diacetate is hydrolysed to fluorescein in the cytoplasm and accumulates intracellularly, making it readily detectable by its fluorescence. The stain has been used successfully in determining the pollen viability of many species (Ockendon and Gates, 1976; Shivanna and Heslop-Harrison, 1981; Widrlechner *et al.*, 1983). Dumas *et al.* (1983) developed the use of nuclear magnetic resonance spectroscopy (NMR) to test pollen viability. The technique is based on the changes in the water status of the pollen as it becomes non viable.

2.3.1.1 Pollen tube growth

Following pollen hydration, the pollen tube emerges from one of the previously differentiated germinal apertures in the wall of aperturate grains. Cresti *et al.* (1977) studied morphological changes during pollen germination in *Lycopersicum peruvianum* and found that the grains have three semi-spherical apertures which are extruded at hydration. The developing tube wall is bilayered comprising an outer pectocellulose wall continuous with the intine, and an inner

layer of callose. As the tube grows, the vegetative nucleus and generative nucleus enter the tube. Frankel and Galun (1977) reported that in plants with a hollow style the pollen germinates on the surface of the stigma papillae and the tube grows in the mucilage-filled canal in the centre of the style. This canal has a characteristic transfer-type wall in the transmitting tissue. Substances which may have a nutritional role are transfered via this wall to the pollen tube during the growth of the tube. In plants with solid styles, the pollen tube penetrates the intercellular space of the pistil tissue. The tube penetrates the cuticle of the papilla cells and then grows in an exudate between the cuticle and cell wall. On reaching the style, the tube finds its way to the transmitting tissue and from there grows toward the ovary.

2.3.2. Pistil

The pistil consists of three main parts, the stigma, the style and the ovary. The stigma is the organ providing the cells for pollen reception and germination. It is connected with the style through which the pollen tube grows to fertilise the ovule within the ovary. The ovary has one or more ovules each of which contains the target cells for the pollen tube, the egg and the central cell. Their nuclei participate in the double fertilisation process, characteristic of the angiosperms (Esau, 1977). The pistil is precisely adapted for its interaction with the male gametophyte. During development, the style and stigma secrete components which provide a medium for pollen germination and tube growth (Knox, 1984).

2.3.2.1 The stigma

The stigma is the expanded tip of the style. It may be looked upon as a gland. At maturity the stigma can be classified as either wet; when the stigma surface is covered with a copious liquid exudate, or as dry; with only an adhesive coating or pellicle on the stigmatic surface (Heslop-Harrison and Shivanna, 1977). The epidermal cells of stigmas are commonly elongated into papillae which may be short or long and may also be branched. They are either unicellular or multicellular

units. Non-papillate types also occur especially amongst aquatic monocots (Knox, 1884). Sedgley and Buttrose (1978) reported that the structure of the stigma/style of the avocado was asymmetrical with a groove lined with transmitting tissue, extending the whole length. Stigma papillae fringed the groove for about a third of its length. The stigmatic fluid was secreted between the papillae of the stigmatic surface and between the cells of the transmitting tissue. Stosser and Anvari (1982) reported that the stigmatic surface of sweet cherry was of the wet type consisting of papillae which were turgid at anthesis. The cell wall was very thin and the cells shrivelled within one to two days after anthesis and at four to five days were collapsed completely. The stigma turned brown and no longer produced secretion, but at that stage they were still receptive to pollen germination and tube growth. de Wet and Robbertse (1986) reported that the mango cultivars Haden and Sensation had a dry stigma. The papillae had a thin cuticle with an external pellicle.

There have been reports that the sub-cellular characteristics of angiosperm stigmas may vary in many aspects. Some stigma cells contain a prevalence of endoplasmic reticulum, such as the stigma of *Acacia* (Kenrick and Knox, 1981) and avocado (Sedgley, 1979). Others contain Golgi bodies and vesicles, such as the stigma of satsuma mandarin (Shiraishi *et al.*, 1976) and petunia (Konar and Linskens, 1966). It is suggested that there may be differences in the nature of the secreted products and also in the modes of secretion between the two types.

The cell wall structure of mature stigma cells prior to pollination shows various adaptations to their role in the interaction with the pollen. Sedgley (1981) found wall ingrowths of transfer cell type in the *Citrullus* stigma and concluded that the wall ingrowths may facilitate secretion. Clarke *et al.* (1980), studying *Gladiolus* stigma walls revealed the presence of tubules radiating from the plasma membrane to the surface cuticle, which is perforated, presumably permitting secretion of the surface exudate. Knox (1984) concluded that stigmatic secretion can be classified into two types.

1. Stigmas with lipophilic and hydrophobic surface exudate as the continuous phase, involving holocrine secretion such as of *Petunia hybrida* (Konar and Linskens, 1966).

2. Stigmas with mucilaginous secretion of carbohydrates and proteins as the continuous phase, involving merocrine secretion mechanisms.

A similar classification for the wet stigma type was suggested by Dumas *et al.* (1984), the hydrophilic type with the granulocrine pathway of secretion and the lipophilic type with holocrine secretion. Dry stigmas have a pellicle that is defined as the outermost extracellular coating of the stigma surface. Mattsson *et al.* (1974) reported that the coating is a hydrophilic layer containing proteins and lipids. The pellicle is separated from the cuticle by an intervening layer which is mucilaginous.

2.3.2.2 The style

The style is the pathway for pollen tube growth to the ovary. Knox (1984) classified styles based on structural and morphological features into three types.

1. Open style in which the route for pollen tube growth to the ovary is through the mucilage-filled stylar canal. It is characteristic of many monocots.

2. Closed style in which the pollen tube passes through solid transmitting tissue to the ovary. Many dicotyledonous plants have this type of style. The transmitting tissue of the closed style has been classified on structural and cytoplasmic features into two zones. Firstly, the glandular stigmatic zone or neck encountered by pollen tubes on passing from the stigma to the stylar transmitting tissue (Dumas *et al.*, 1978; Herrero and Dickinson, 1979). The stigmatic zone may differ from the transmitting tissue in both cell shape and in the content of cytoplasmic organelles. Secondly, the transmitting zone in which the cells are usually elongate and fusiform in the axial direction and have relatively thick

pectocellulosic cell walls. Both tissues secrete an intercellular matrix into the intercellular spaces.

3. Semiclosed style which exhibits intermediate features.

The stylar secretory cells have a similar glandular role to the stigma cells in secreting exudate, even though there may be chemical differences between the intercellular matrix and the surface exudate. The files of cells within closed styles are usually directly connected via plasmodesmata on their transverse walls, although their longitudinal walls are separated by intercellular mucilage at maturity. The transmitting tissue cells are connected directly to the papillae of the receptive surface by plasmodesmata, and the tract terminates in the ovarian cavity. The transmitting tissue not only provides a nutritional guide tissue for the pollen tubes, but possibly also a means for the passage of electrical and other signals from the stigma surface to the ovarian cavity.

2.3.2.3 The ovary

The ovary contains one or more ovules. An embryo sac, the female gametophyte occurs inside each of the ovules (Esau, (1977). The ovule commonly consists of following parts; the nucellus - the central tissue with vegetative cells enclosing the sporogenous tissue; the integument - one or two layers enclosing the nucellus and forming the micropyle; and the funiculus - the stalk connecting the ovule with the placenta. The region where the nucellus, the integument and the funiculus merge is called the chalaza. The vascular system, connected to that of the placenta through the funiculus, extends to the chalaza usually as a single strand. The sporogenous cell, or megaspore mother cell undergoes meiosis resulting in a row of four cells. Three of these cells generally degenerate leaving one functional megaspore which develops into the embryo sac. Three mitotic divisions occur in the functional megaspore so that an eight-nucleate embryo sac is formed. The three cells at the micropylar end constitute the egg apparatus which is comprised of the egg and

two synergids. At the opposite end of the embryo sac there are three antipodal cells. Between the two groups of cells is the large central cell containing two polar nuclei.

2.3.3. Self incompatibility

Cross pollination makes it possible for genes to be exchanged between different individuals of a species so that they have a higher chance of survival if the surrounding environment changes. There are various floral mechanisms that ensure outcrossing rather than selfing;

dioecy; the individual plant is either male or female,

monoecy; male and female flowers occur on the same plant but the sexes are separated into different flowers,

dichogamy; the stamen and stigma of the same flower mature at different times,

male and female sterility; the failure to produce viable pollen grains or egg cells.

Self incompatibility is another important outcrossing mechanism. It is a genetically controlled mechanism which reduces the prevalence of inbreeding depression in a population (Heslop-Harrison, 1975). Fertile pollen will not produce seed set when placed on the stigma of the same flower or another flower on the same plant. The self incompatibility mechanism resulting in an inhibition of pollen germination or arrest of pollen tube growth can occur in the stigma, style, ovary or ovule, and according to the stage of arrest it is classified into pre and postzygotic incompatibility.

2.3.3.1 Prezygotic self incompatibility

Prezygotic self incompatibility is particularly well developed in the angiosperms. It is determined by the action of genes (Lewis, 1960; Pandey, 1967; Heslop-Harrison, 1975), through a multi-allelic system such that the possession of the same allele in both male and female results in incompatibility (Frankel and Galun, 1977; Knox, 1984). External factors such as temperature also influence incompatibility (Lewis, 1942; Ascher and Peloquin, 1966; Sedgley, 1977; Raff and Knox, 1982). Self incompatibility has been distinguished by the site of action into two types, sporophytic and gametophytic (van den Ende, 1967; Heslop-Harrison, 1975; Knox, 1984). Sporophytic incompatibility is controlled by the genotype of the diploid sporophyte. Pollen grains display the parental genotype rather than expressing their own haploid genotype. The enzymic wall proteins from the exine cavities of the pollen control recognition, pollen germination, penetration of the stigma surface and the early growth of the pollen tubes (Mattsson *et al.*, 1974). In the sporophytic system, in the Cruciferae and Compositae, inhibition of pollen germination occurs on the stigma. The surface of the stigma papillae bear a pellicle, an external protein coating overlying the cutinised outer layer of the wall. It is functionally important in the capture and hydration of pollen grains and is also the site of the recognition reactions involved in incompatibility responses. Knox (1973) and Heslop-Harrison *et al.* (1974) found that callose (beta-1,3-glucan) is deposited in the stigma cells adjacent to incompatible pollen grains, and may inhibit the penetration of the pollen tube. Roggen (1972) reported that in *Brassica spp.* the pollen grains stick to the wax layer which is present on the stigma papillae. It is pierced, apparently by enzyme hydrolysis, and the pollen exine contacts the cuticle whereupon the papillae collapse. The wax layer seems to be the incompatibility barrier because after incompatible pollination no sticking reaction and consequently no germination takes place and the papillae stay turgid. The sporophytic self

incompatibility system operates in some tropical tree crops including the carambola (Knight, 1982).

In gametophytic incompatibility, the expression of the S gene is determined by the genotype of the individual pollen grain. This means that in a plant heterozygous for the S gene, the pollen grains will segregate equally within the anther for the S alleles, each grain then acting according to its own S genotype (Knox, 1984). Pollen tube inhibition can occur at a variety of sites, from the stigma to the embryo sac (Lewis, 1956; Brewbaker, 1957). Self pollen inhibition may occur at the stigma e.g. in the grasses, *Oenothera organensis* (Emerson, 1940) and *Tradescantia pallida* (Herd and Beadle, 1980) but generally pollen tube arrest occurs within the transmitting tissue of the style, as in *Nicotiana alata* and *Lycopersicum peruvianum* (de Nettancourt *et al.*, 1973). In a few cases, inhibition may take place in the ovary or in the embryo sac such as in many monocotyledons (Arasu, 1968) and in *Borago officinalis* (Crowe, 1971). Pacini (1983) reported that *Lycopersicum peruvianum*, *Petunia hybrida*, and *Prunus avium* did not show cytological differences between compatible and incompatible pollen during germination and early pollen tube growth. Differences became evident after a short period of pollen tube growth in the style. The incompatible pollen tubes had thick walls, cytoplasmic necrosis, formation of small spherical vesicles with fibrillar contents and their generative cell did not divide. The incompatible pollen tube tips burst at various distances from the stigma. Raff and Knox (1982) found that in sweet cherry (*Prunus avium*) the initial growth of incompatible tubes was at least as rapid as that of the compatible. Self incompatible pollen tubes ceased growth after penetrating the style. There were differences between the cultivars in the length of self pollen tube penetration into the style before inhibition.

2.3.3.2 Postzygotic incompatibility

Postzygotic self incompatibility is particularly well developed in the gymnosperms, but it is also reported in angiosperms in which it generally occurs in the very early stages of embryo development. In mango Sharma and Singh (1970) found that the zygote underwent a few cell divisions prior to degeneration. Sedgley (1980) found that premature fruit shed is common in the avocado. Large numbers of fruits were shed within a month of anthesis when the embryo was at the globular stage but had not started cotyledon differentiation. Degani *et al.* (1989) added that premature fruit drop in the avocado was due to genetic selection during the period of early fruit development. Postzygotic self incompatibility mechanisms are also suspected to occur in pecan (Romberg and Smith, 1946) and *Eucalyptus regnans* (Griffin *et al.*, 1987).

2.4. Floral rewards

Animal-pollinated flowers have to attract vectors, and so they are usually large, coloured and scented (Faegri and van der Pijl, 1979). This is true of flowers specialised for pollination by birds, bees, butterflies or a range of other insects or mammals. Most animals visit flowers for food, the pollen and nectar, which are thus important floral rewards.

2.4.1. Nectar

Nectar is the most important reward of animal-pollinated flowers (Fahn, 1979). Nectaries are classified into floral and extrafloral, the first occurring in the flower and the second on non-floral tissue such as the petiole. Nearly all parts of the flower have been recorded as bearing nectaries in various species. The nectary is made up of nectariferous tissue (Fahn, 1979), with an epidermis subtended by specialized parenchymatous tissue. The parenchymatous tissue is generally composed of small cells with thin walls, relatively large nuclei, dense granular

cytoplasm and small vacuoles. Dukee *et al.* (1981) reported that the floral nectary of *Passiflora* spp. consists of a ring of tissue which is located on the inner surface of the calyx tube and encircles the base of the gynophore. At maturity three general areas can be distinguished, the epidermis, the secretory tissue and the ground parenchyma. The parenchyma contains vascular bundles that branch and extend into the secretory tissue terminating near the epidermis. Elias and Gelband (1976b) reported that the extrafloral nectaries of *Campsis* (Bignoniaceae), found on the petiole, calyx, corolla, and fruit were one to two layered secretory cups, with well defined basal cells beneath the secretory layer. The secretory cells were arranged in a narrow column, vertically within the nectary, except in the rim where there was a transition from vertical to horizontal alignment. The cells had conspicuous nuclei and dark staining cytoplasm, and the secretory layer was covered with a thin cuticle.

Nectar is secreted either via epidermal cells e.g. in *Passiflora spp.* (Dukee *et al.*, 1981) and *Ipomoea pandura* L. (Convolvulaceae) (Beckmann and Stuckey, 1981) or via trichomes e.g. *Lonicera japonica* (Fahn and Rachmillevitz, 1970) and *Vicia faba* (Figier, 1971). When secretion occurs via epidermal cells which are covered by a cuticle the exudation is assisted by the rupture of the cuticle, by the occurrence of pores in the cuticle, or by the cuticle being permeable. Nectar is mostly a derivative of phloem sap, and secretion is a complex physiological process (Ziegler, 1956; Dukee *et al.*, 1981). Ziegler (1956) reported that the sugar of the phloem sap was transported to the location where the sugar concentration was lowest, such as an actively secreting nectary. Maurizio (1962) noted that the sugar spectrum of phloem sap was by no means standard, it varied between species and within species and also showed seasonal changes. Nectar is a complex mixture of chemicals, and sugars are the major constituents (Wykes, 1952; Percival, 1961; Baker and Baker, 1983). Other components include amino acids, proteins, lipids, antioxidants, alkaloids, vitamins, allantonin and allantonic acids, dextrins and inorganic materials such as minerals. All may have some significance in pollination.

2.4.1.1 Sugars and amino acids in nectar

A large amount of work has been done on sugar analysis of nectar. Percival (1961) studied nectar of 889 species of angiosperms and found that the three main sugars are sucrose, glucose and fructose. Baker and Baker (1983) examined 765 angiosperm nectars and divided them into four groups based on monosaccharide (glucose + fructose) to disaccharide (sucrose) ratios. Wykes (1951) tested the nectar of 60 angiosperm species and showed that glucose, fructose and sucrose occurred in nectar from every species tested except *Fritillaria imperialis* L. in which sucrose was absent. The amount of nectar sugar presented to a flower visitor is an important factor in determining the behaviour of that visitor, and so has important consequences for the breeding system of the plant (Heindrich and Raven, 1972). Different flowers contribute differently in this matter of energy supply. The volume of nectar produced is roughly proportional to the size of the flowers, but a compact inflorescence of small flowers, as in many in umbelliferous plants and composites, can equal the output of a large single flower. Corbet (1978) investigated the nectar of *Echium vulgare* and showed that the nectar fluctuated in volume and in sugar concentration from hour to hour and from day to day. The fluctuation is due to the different rates of secretion by the plant, removal by bees, and evaporation and concentration. Diurnal patterns of visits by social bees are related to changes in the quantity and quality of nectar in the flowers. Corbet *et al.* (1979) reported that changes in the nectar of *Crataegus*, *Tilia* and *Echium* were related to microclimate and insect visits. They found that nectar concentration is highly correlated with ambient relative humidity, and the microclimate inside the flower may influence the rate of equilibrium of nectar with the humidity of the air. The quantity of sugar per flower depends on the relative rates of secretion and reabsorption. Morning and evening are the two peak periods of nectar secretion and the pattern of visits by bees to the flowers is related to the changing concentration of sugar in the nectar. Vansell and Watkins (1942) studied nectar secretion and sugar

concentration in orange and found that nectar collected during a low humidity period contained 40% sugar but the average for all the samples was about 34%. During a foggy, humid season, the average concentration was about 20% and then the bees were practically absent from the blossoms. When the concentration approached or exceded 30%, bees were actively collecting nectar and bees prefered to visit the older orange blossoms which contained richer nectar. Waller *et al.* (1972) proposed that honeybees prefered nectar containing sucrose to that with either glucose or fructose. Sugar solutions containing 30-50% sucrose were more attractive than solutions containing higher or lower sugar concentrations. In contrast Wykes (1951) reported that flowers that were attractive to honeybees were characterised by approximately equal amounts of sucrose, glucose and fructose. Haydak (1970) suggested that not all nectar sugars had the same nutritive value, and several were even toxic to some insects. For example galactose, lactose and raffinose were toxic to honeybees (Baker and Lehner, 1976) but not to other insects such as Dipterans. Kevan and Baker (1983) concluded that the role of individual sugars in insect metabolism were complex and differed between the insect groups. However, energy was the main outcome of sugar ingestion and was used in flight or other activities. The sugar may be converted to fat, as in migratory Lepidoptera, and may be stored for long distance flight or for egg production.

Amino acids and proteins are always present in nectar, although the amounts are small by comparison with sugars (Baker, 1976). Baker and Baker (1975) analysed the amino acid complements of over 300 species and found that amino acids occurred in amounts that may be significant nutritionally to the flower visitors either in protein metabolism or as gustatory stimuli. They drew a relationship between amino acid concentration in floral nectar and the insect pollinators. The nectar of flowers visited by moths and wasps were richer in amino acids than the nectar of those pollinated by bees and unspecialized flies that also taken pollen. Not all amino acids were equally available. Overall, alanine, arginine, serine, proline,

and glycine were most frequently available and were reported in the floral nectar of all species examined (Baker and Baker, 1983). At the other extreme, tryptophan, histidine, and methionine were scarcer essential amino acids in nectar.

2.4.1.2 Lipids, oils and antioxidants in nectar

Lipids and oils are also present in the nectar of many plants (Baker and Baker, 1975). The nutritional role of nectar lipid is unclear, but it must be regarded as a minor energy source and it may function in ovarian maturation (Downer, 1978). Corbet *et al.* (1979) speculated that lipids may act as a water proofing monolayer and thus retard evaporation of nectar.

Antioxidants also occur in nectar (Baker and Baker, 1975). Ascorbic acid is required by some plant-feeding insects to maintain normal growth, moulting and fertility (Chippendale, 1978). However, the importance of ascorbic acid and other antioxidants in nectar is unknown. Baker and Baker (1975) reported that the antioxidants and lipids tended to be found together in both the nectar and in the stigmatic exudate. They suggested that antioxidants may prevent oxidation (rancidity) of lipoidal compounds.

2.4.2 Pollen

Pollen is also an important floral reward in many plants. It contains many kinds of nutrients such as amino acids, proteins, sugars, fats, vitamins and minerals (Stanley and Linskens, 1974). Pollen also contains starch that can be used as an energy source (Kevan and Baker, 1983). Some flowers such as tulip and poppy do not secrete nectar but produce a great deal of pollen (Holm, 1979). Numerous insects are lured to these flowers by the pollen. Some flowers have special food stamens alongside the normal ones, and the pollen of these serves as nutrition for insects. Baker (1976) reported that there were short stamens for insect food and long stamens providing pollen for pollination in *Tibouchina spp.* (Melastomataceae).

Cassia spp. and *Swartzia* spp. (Fabaceae) (Dulberger, 1981). Pollen is a vital food for many insects, especially the Apidae, beetles, flies, thrips, and butterflies (Kevan and Baker, 1983). Protein and free amino acid contents of pollen vary with species but are regularly high (Stanley and Linskens, 1974), up to 6% peptide and 13% protein. Stace (1988) proposed that bees ideally needed a crude protein level of 20% or more. Degroot (1953) investigated the amino acids required by bees, and showed that the minimum amounts of each that were needed (g. amino acid/100 g. protein) were threonine 3.0, valine 4.0, methionine 1.5, leucine 4.5, isoleucine 4.0, phenylalanine 2.5, lysine 3.0, histidine 1.5, arginine 3.0, and tryptophan 1.0. He commented that if one of the amino acids was in short supply, then when it was exhausted, the rest of the amino acids were not used efficiently. Kleinschmidt (1984) and Stace (1988) examined the pollen quality of *Eucalyptus albens*, white box, and showed that its crude protein content ranged from 18 to 24%. Isoleucine was low, and at some stages it was only two thirds the amount required by bees. Other amino acids like valine and methionine were sometimes in short supply, but not to the same degree as isoleucine. They suggested that the available amino acids were not sufficient for the bees to thrive, and that the bees tended to become weak, so that diseases such as nosema and European Brood Disease caused problems.

Free sugars are less important in pollen than in nectar. Starch may or may not be present in mature pollen, and starch and lipids may be alternative reserves of energy that are utilized in pollen germination and pollen tube growth (Kevan and Baker, 1983). Lipid rich pollen grains are found particularly in species where pollen is the only floral reward offered (Stanley and Linskens, 1974). Baker and Baker (1979) reported that there was a correlation between pollen size and the nature of its reserve substances. Small pollen grains always store lipid, whereas very large grains may store starch. Oils on the outside of pollen grains may act as attractants and also cause the grains to adhere to each other and to the pollinators.

2.5 Environment and pollination

There are many environmental factors that can affect either the breeding system of the flower or pollinator behaviour. Climatic effects include temperature, irradiation, wind, rain and humidity. It is well known that bees will not leave the hive in cool overcast conditions, but equally important is the effect of climate on the pollen-pistil interaction of the pollination process.

The effect of temperature on sex expression and the pollen-pistil interaction has been reported for a number of important crop plants. Frankel and Galun (1977) reported that in some dioecious annual species, a shift from higher to lower temperature usually caused not only an increase in the ratio of female to male plants but also the appearance of female flowers on the male plants. The change in sex regulation can take place at various stages in the floral ontogeny of the plant. Sedgley (1977) reported that temperature affected floral behaviour and pollen tube growth of the Fuerte avocado. At 33/28 C and 25/20 C (day/night temperature) the flowers opened in the afternoon in the female stage and again the following morning in the male stage with some overlap of male and female stages at 25/20 C. At 17/12 C very few flowers had a female stage, the majority opening once only in the male stage. The duration of the flowering period decreased with a rise in temperature, as did the total number of flowers opening. The rate of pollen tube growth increased with a rise in temperature, but abnormal growth was frequently observed at 33/28 C and tubes failed to reach the ovary at 17/12 C. There have also been reports that high temperatures have an adverse effect on pollination by shortening the period of stigma receptivity, and reducing pollen viability and pollen tube growth (Kendall and Taylor, 1969; Herrero and Dickinson, 1980; Sedgley and Annells, 1981).

3. Experimental material

3.1. Experimental orchards

Two cashew orchards located in Kununurra, Western Australia, were used in this study. The first orchard belonged to the Western Australian Department of Agriculture. It was planted in 1984 as a trial planting with bulk seed from the best trees which had been established in Broome, Western Australia in 1979, from seeds introduced from Brazil and from Kamerunga, Queensland, Australia. The orchard was situated in the Cockatoo sand or Pindan, a sandy-surfaced soil with clay increasing with depth. The orchard consisted of 15 rows with 15 trees in each row, at a spacing of 8.0 m. between rows and 4.0 m. between trees within rows. The trees were drip irrigated and received nitrogen, phosphorus, potassium (11:12:10) and zinc at planting and twice annually. They grew quickly in the early years, and were approaching maturity in 1988 when the experiments started.

The second orchard was in a commercial plantation belonging to Voyager Enterprises Pty. Ltd. on Block 240 of the Ord River Irrigation Project. It was about 4 km from the first orchard, and located in the black cracking Cununurra clay. The plantation was divided into different sections, and G block was selected. It was planted in 1987 using large grafted plants with budwood from the best Broome trees as well as from the superior genotypes in the first orchard. The orchard design was 7.5 x 4.0 m. spacing between rows and between trees respectively, with furrow irrigation. Fertilizer was applied as for the first orchard with the addition of sulphur, boron, molybdenum, magnesium, copper and iron. Because of the large planting material and the fast growth rate, most of the trees flowered in 1988. Both orchards were provided with honeybee hives during flowering period.

In this study the first orchard is called the sandy soil block and the second orchard the black soil block.

3.2. Experimental trees

Fourteen cashew genotypes from the first orchard and their clonal progeny in the second orchard were selected for use in the experiments in this study. Most of the genotypes had been selected by the Western Australian Department of Agriculture and Voyager Enterprises as superior. They all produced high numbers of hermaphrodite flowers per panicle, high final fruit set and acceptable nut size. The origin of the selected genotypes is shown in Table 3.1.

3.3 Climate data

The Ord River Irrigation area is located at latitude 15 39 S, longitude 128 43 E, in the far north of Western Australia. The climatic conditions are described as semi-arid monsoonal (Petheram and Kok, 1983) or semi-arid monsoonal with a hot rainy season (Dept. Nat. Res., 1976). A summary of meteorological data collected at Kimberley Research Station, in Kununurra is shown in Table 3.2.

Table 3.1 Tree numbers and origin of the cashew genotypes used in the study.

Kununurra tree (row/tree) no.	Origin
1.2	Broome
1.3	unknown
1.11	unknown
1.12	Kamerunga AB 9/86
4.3	Kamerunga MS 90/81
6.6	Brazil CP 7
7.1	Broome
9.8	Brazil CP 65
10.9	Brazil CP 7
11.7	Brazil CP 65
11.9	Brazil CP 75
12.1	Weipa red
12.5	unknown
15.15	unknown

Table 3.2 Climate data for the Ord River Irrigation area, Western Australia. Data recorded at Kimberley Research Station, Kununurra (Delane, 1987).

Month	Mean temperature (°C)	Rainfall (mm)	Relative humidity (%)	Total Radiation (w/sqm/d)	Daylength (hr)
Jan	30.2	189	65	23.8	12.8
Feb	29.6	189	69	22.7	12.5
Mar	29.5	131	61	22.9	12.1
Apr	27.9	31	44	22.4	11.6
May	25.4	8	36	19.9	11.3
Jun	23.0	4	36	19.0	11.1
Jul	22.3	3	32	20.0	11.2
Aug	24.4	1	33	21.5	11.4
Sep	27.6	2	35	23.8	11.8
Oct	30.6	23	39	25.5	12.7
Nov	31.7	70	48	25.5	12.7
Dec	31.4	127	56	25.4	12.9

4. Flowering and fruiting phenology of cashew in north-western Australia

4.1 Introduction

This chapter reports the study of the flowering and fruiting phenology of cashew in the Kununurra area of north-western Australia. Cashew has been introduced to Kununurra recently and as a new crop plant in the area there is no information on phenology. The flowering habit, flower opening pattern, fruit set, fruit drop, and final fruit set are observed and recorded. The results provide basic information for further studies of floral biology and will also be useful in genotypic selection and in orchard design for future cashew growing in the area.

4.2 Materials and methods

Details of the plants and growing conditions are presented in chapter 3. Nine cashew trees in the sandy soil block were selected. The tree numbers were 1.2, 1.3, 1.11, 1.12, 6.6, 7.1, 9.8, 11.7, and 11.9. Four new panicles with nearly open flowers were chosen and tagged on each tree. The panicles were of similar size and were located randomly around the canopy at about 1-1.5 m above the ground. When the flowers commenced opening, the hermaphrodite and male flowers were counted and recorded on each panicle every Monday, Wednesday, and Friday in the afternoon. To avoid double recording, the counted flowers were marked by removing half of one petal with fine forceps. The numbers of newly set fruit, shed fruit, and ripe fruit were recorded at every counting date. The fruit set criteria were a swollen ovary about 0.5 cm in length visible from the top of the flower with the petals and style dying. Shed fruit had a brown shrunken nut with a green or brown coloured apple. They remained attached to the panicle for a few days prior to shed and were categorized as small (0.5-1 cm long) and large (more than 1 cm long). Ripe fruit was recorded when the nut and apple colour changed from green to yellow or red at

about two months after setting. The study was started at the peak of flowering and continued until fruit maturity for three consecutive years; 1988, 1989, and 1990. Differences between panicles on one tree, between weeks, and between years in the flowering period and in number of hermaphrodite and male flowers, percentage hermaphrodite flowers, number of fruit set, percentage fruit set, and in the number of shed fruit and ripe fruit were analysed by analysis of variance (ANOVA) using Genstat. Some of the data were transformed into log or arcsine before analysis.

The diurnal opening patterns of hermaphrodite and male flowers were also studied on the same nine trees. Four new panicles were chosen and tagged on each tree. The observations were started when the flowers opened in the early morning and continued until they finished in the late afternoon. Numbers of hermaphrodite and male flowers opening were recorded and the counted flowers were removed at each hourly observation. The study was done each Monday and Tuesday for 4 weeks in the 1989 flowering season. The pooled data were used to derive the mean opening pattern.

4.3. Results

4.3.1 Flowering and fruiting habit

For the three years of observations, the nine selected cashew trees in Kununurra consistently flowered from June to October after the hot rainy season. This coincided with a decline in temperature, rainfall, relative humidity, daylength, and radiation (Table 3.2, Figure 4.1). The inflorescence buds appeared two weeks after the leaf flush. Many trees had a small flush and flowering at the beginning of the season, in May, but the main flushing and flowering started in June when the temperature and relative humidity were low, and daylength and radiation reached their lowest levels. Flowers started opening two weeks after visible floral buds appeared and the panicles consisted of hermaphrodite and male flowers. In a single panicle the two types of flower opened from the first week but the hermaphrodite

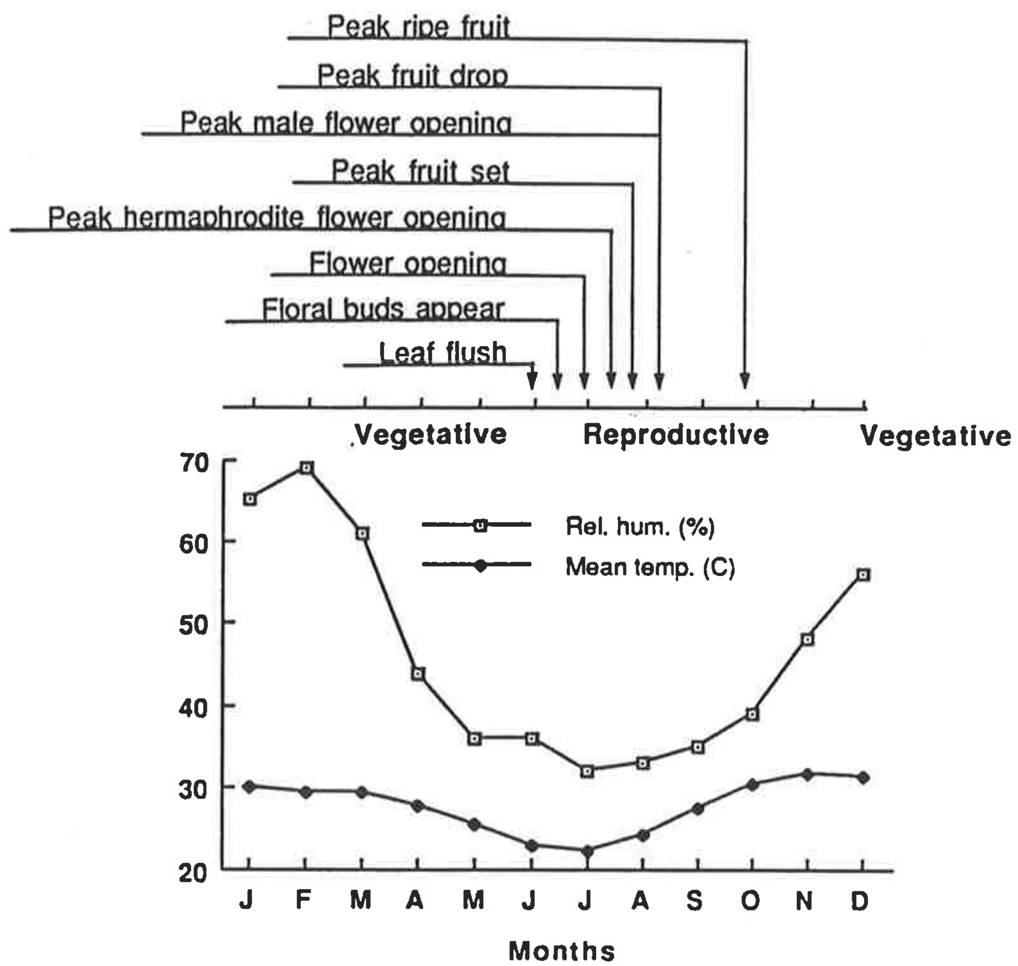

Figure 4.1 The timing of flowering and fruit development of the cashew in Kununurra in relation to climate.

Table 4.1 Mean number per panicle of hermaphrodite and male flowers, percentage hermaphrodite flowers, and flowering period of hermaphrodite and male flowers of nine cashew trees from 1988 to 1990.

Tree No.	Mean no. of hermaph. flowers per panicle	Mean no. of male flowers per panicle	Percentage hermaph. flowers per panicle	Mean period of hermaph. flowering (weeks)	Mean period of male flowering (weeks)
1.2	89.6	580.0	13.4	10.4	12.1
1.3	44.8	715.5	5.9	6.3	8.7
1.11	69.8	645.7	9.8	5.01	8.6
1.12	78.2	576.8	11.9	5.6	8.6
6.6	160.3	691.3	18.8	5.7	8.8
7.1	95.6	442.9	17.8	6.0	8.7
9.8	101.3	454.0	18.2	5.0	7.4
11.7	57.4	545.3	9.5	4.67	9.1
11.9	157.0	553.3	22.1	7.2	9.3
Average	94.9	578.3	14.2	6.2	9.0

Table 4.2 Mean number per panicle of fruit set, shed fruit, and ripe fruit of 9 cashew trees from 1988 to 1990.

Tree No.	Mean fruit set per panicle	Mean small fruit drop per panicle	Mean large fruit drop per panicle	Mean ripe fruit per panicle
1.2	40.0	27.9	8.2	1.7
1.3	10.4	3.4	1.3	5.8
1.11	23.4	9.9	2.1	8.8
1.12	21.5	12.1	3.1	6.9
6.6	41.7	19.8	6.5	11.0
7.1	28.0	10.6	7.1	8.7
9.8	40.9	22.2	12.3	4.8
11.7	17.5	8.8	5.0	1.5
11.9	42.4	31.0	3.8	7.8
Average	29.2	16.2	5.5	6.4

flowering ended before the male. The hermaphrodite flowering reached a peak in early August resulting in maximum fruit set two weeks later. Fruit drop and male flowering shared the same peak period at the end of August. After setting, fruit growth took two months to ripening. The peak of ripe fruit and harvesting was mid October.

There were differences between trees in the number of flowers and fruits (Tables 4.1, 4.2). Tree 6.6 had high numbers per panicle of hermaphrodite flowers and ripe fruit, while tree 11.7 had low numbers per panicle of hermaphrodite flowers, fruit set, and ripe fruit. Trees 9.8 and 11.9 had good fruit set but high fruit drop lowered the number of ripe fruit. The average mean flowering period of male flowers was longer than that of hermaphrodite flowers. Within each year, the nine selected trees were significantly different in flowering period, flower numbers, number of fruit set, fruit drop, and ripe fruit. However there was no significant difference either between years or between panicles within trees in any of the above parameters (Tables 4.3, 4.4). The analysis also showed a significant correlation ($p<0.0001$) between the number of hermaphrodite flowers and fruit set and ripe fruit.

4.3.2 Weekly opening pattern of hermaphrodite and male flowers and sex ratio

There was variation in the number of hermaphrodite and male flowers opening per week amongst the 9 trees. Most of the hermaphrodite flowers opened early in the flowering period and reached a peak in week 2 (Figure 4.2). More than 90% of the hermaphrodite flowers opened between week 1 and week 3 and the number opening in weeks 1 and 2 was higher than the number of male flowers. The number dropped gradually after week 3 to the end of the flowering period. Very few male flowers opened in the first week but the number increased after week 2 and reached a peak in week 5, followed by a gradual reduction to the end of the flowering

Table 4.3 Analysis of flowering period and flower number data for hermaphrodite and male cashew flowers over three years.

Source of variation	Degrees of freedom	Mean square	
		Hermaphrodite	Male
Flowering period			
between years	2	1.1ns	13.4 ns
between trees	24	15.1***	8.2 ***
within tree (error)	78	1.5	0.8
total	104		
Flower number			
between years	2	2.4 ns	5.7 ns
between trees	24	6.2 ***	3.0 ***
within tree	78	1.3 ns	1.0 ns
within panicle (error)	545, 839	1.1	2.8
total	649, 943		
% hermaphrodite flowering			
between years	2	1482.8 ns	
between trees	24	1288.4 ***	
within tree	78	240.4 ns	
within panicle (error)	839	869.3	
total	943		

ns not significant, *** highly significant (p=0.001)

Table 4.4 Analysis of number of fruit set, shed fruit, and ripe fruit of cashew over three years.

Source of variation	Degrees of freedom	Mean square
Fruit set		
between years	1	3.9 ns
between trees	16	2.4 ***
within tree	53	0.4 ns
within panicle (error)	363	1.2
total	438	
Fruit drop Large/Small		
between years	2	2.0 ns/0.8 ns
between trees	24	2.7 ***/0.8 ***
within tree	78	0.3 ns/0.3 ns
within panicle (error)	587	0.8/0.4
total	691	
Ripe fruit		
between years	2	6.5 ns
between trees	24	57.7 ***
within tree (error)	78	15.5
total	104	

ns not significant, *** highly significant (p=0.001)

period. Over the whole flowering period, there were many more male than hermaphrodite flowers and the sex ratio changed from week to week. In weeks 1 and 2, the ratio of hermaphrodite to male flowers was approximately 5:1 and 2:1 respectively. After week 2 number of male flowers increased sharply while the hermaphrodite flower number decreased thus lowering the sex ratio through the rest of the flowering period.

4.3.3 Weekly fruit set and fruit drop

Fruit set started in week 2 after anthesis (Figure 4.3) and fruit drop from week 3 (Figures 4.3, 4.4). There was high variation in the numbers of fruit set and fruit shed per week among the 9 trees, but their patterns were consistent between years. Fruit set reached a peak in week 3 and more than 82% of fruit set occurred between weeks 3 and 5. The maximum fruit drop was in week 5, two weeks after the fruit set peak. More than 77% of the fruit dropped between weeks 4 and 6 and the number slowly decreased after week 6. Large and small fruit drop showed similar patterns but differed in numbers. Large fruit drop started a week after the small fruit drop, but they shared a peak in week 5 (Figure 4.4). The majority of the hermaphrodite flowers did not set fruit ranging from a minimum of 60% failure between week three and seven up to almost 100% failure in week 11 (Figure 4.5).

4.3.4 Diurnal opening pattern of male and hermaphrodite flowers

In most panicles there were hermaphrodite and male flowers open on the same day. Flowers started opening from early morning at 6 am until the afternoon at about 4 pm (Figure 4.6). Very few flowers opened after late afternoon. There tended to be two peaks of opening of both types of flower per day, one in the early morning and the other toward midday.

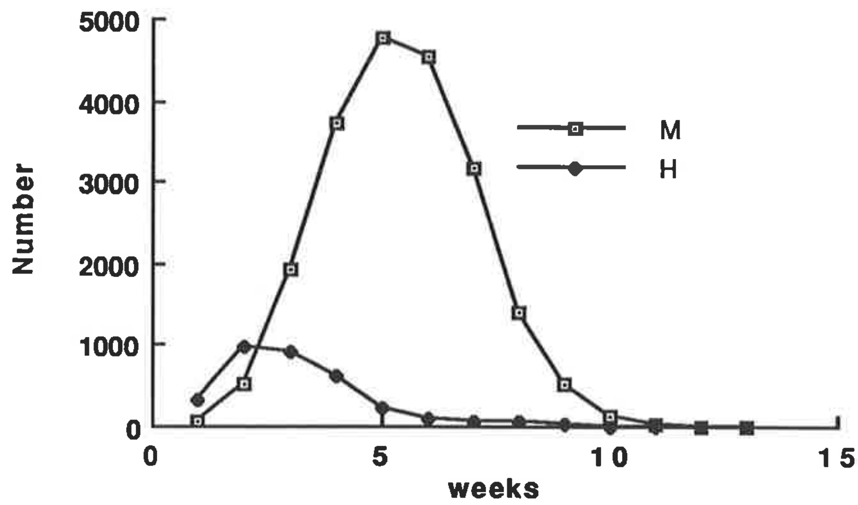

Figure 4.2 Average weekly hermaphrodite (H) and male (M) flower opening of nine cashew trees.

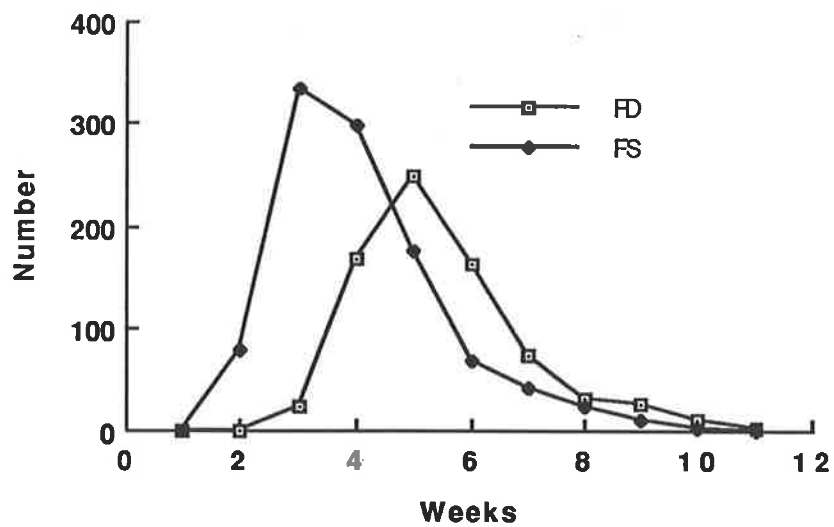

Figure 4.3 Average weekly fruit set (FS) and fruit drop (FD) of nine cashew trees.

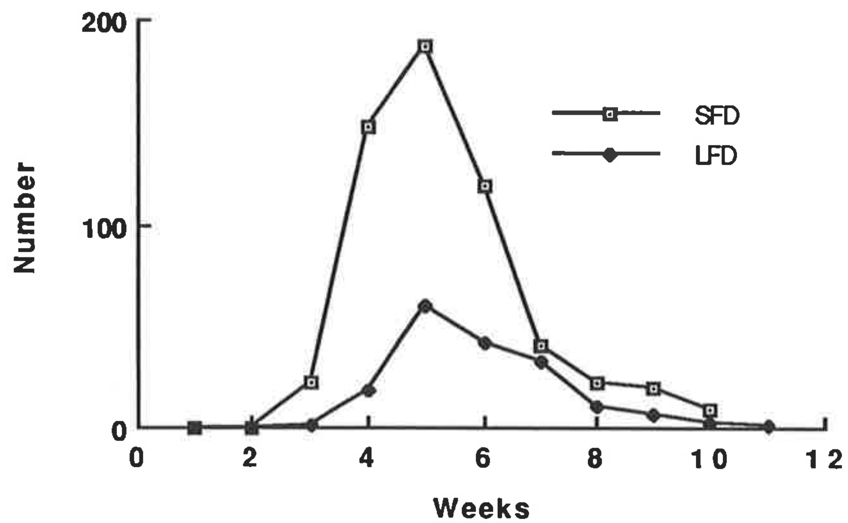

Figure 4.4 Average weekly small fruit drop (SFD) and large fruit drop (LFD) of nine cashew trees

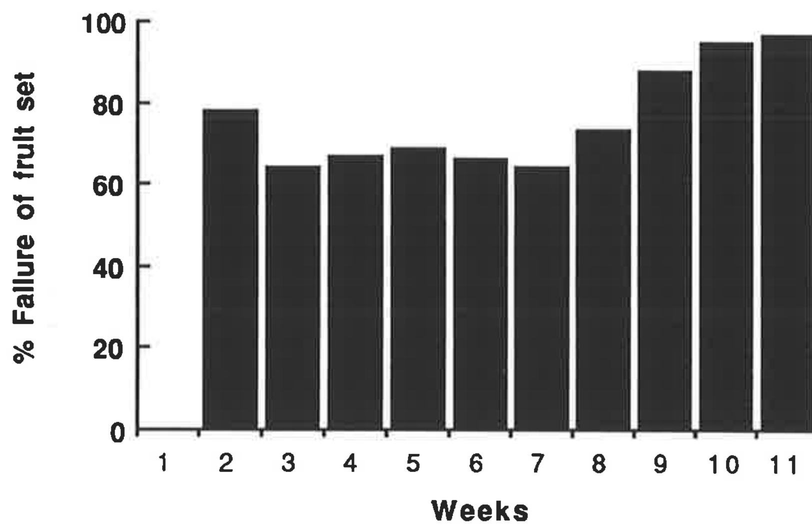

Figure 4.5 Weekly failure of fruit set of hermaphrodite flowers.

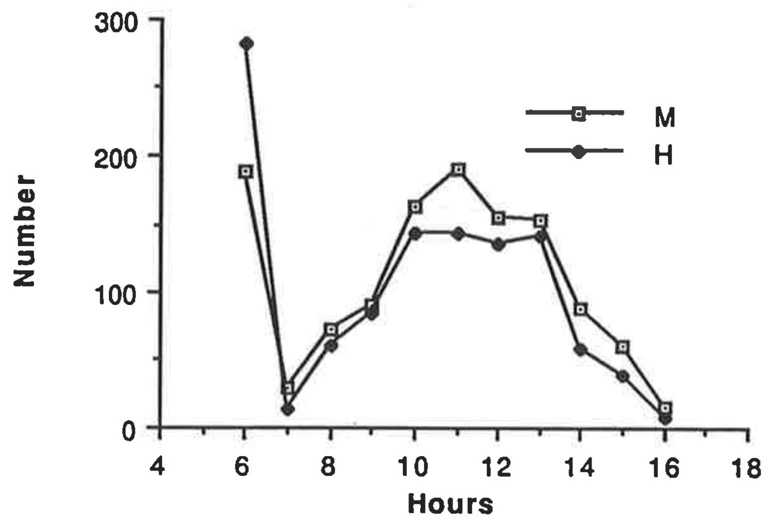

Figure 4.6 Diurnal hermaphrodite (H) and male (M) flower opening of nine cashew trees over a four week period.

4.4 Discussion

Cashew flowering varies with the climate. Under conditions of well distributed rainfall the cashew may flower more than once a year, but in areas of distinct wet and dry seasons its flowering occurs only once a year after the wet season (Medway, 1972; Nambiar, 1979; Ohler, 1979; Wait and Jamieson, 1986). The cashew trees in Kununurra flower only once a year after the hot rainy season in June, when the temperature, humidity, daylength, and radiation are lowest. Latitude also influences the cashew flowering season, but Randhawa (1957) stated that the relationship between latitude and flowering probably reflects the effects of latitude on temperature. In the main growing areas of the southern hemisphere (up to 25 S), cashew flowering occurs between June and November (Northwood, 1966; Ohler, 1979; Sturtz, 1981). This is true of the cashew trees in Kununurra which flower between June and late October. Cashews in other areas of northern Australia located in similar latitude and climate conditions to Kununurra, have been reported to flower at nearly the same time i.e. July in northern Queensland (Wait and Jamieson, 1986) and early June in the Northern Territory (Sturtz, 1981; Heard *et al.*, 1990).

Flowering starts after the growth flush in terminal branches on the surface of the canopy. Nearly all the terminal branches flush and flower. This is different from the flowering habit of mango, in which flowers appear directly from the mature dormant terminal branches after the wet season (Scholefield, 1982; Chacko, 1984). Thus in cashew, leaf development precedes flowering, whereas in the mango the flowers and leaves appear contemporaneously. In addition the mango shows alternate flowering. Over the three year observation period there was no off year in any of the 9 cashew trees observed.

Flowering and fruiting of the cashew trees in Kununurra occurs in the dry season. This has the advantage of low pest and disease levels, thus avoiding major

damage to the flowers and fruits (Ellis, 1976; Holliday, 1980; Krisaneepaiboon *et al.*, 1988). Sturtz (1981) indicated, however, that both too much rain, and humidity below 50% at flowering and fruit setting are undesirable, as the latter shortens stigma receptivity and pollen viability. The very high fruit drop, particularly of small fruit, might be in part an effect of the dryness.

The numbers of open hermaphrodite and male flowers and the sex ratios throughout the panicle life are important in terms of effective pollination. Weeks 1 and 2 were the most effective pollination periods, as numbers of open hermaphrodite flowers were high. Both the hermaphrodite and male flowers open from 6 am and the peak of opening is early in the day. Reports from overseas (Damodaran *et al.*, 1965; Northwood, 1966; Wunnachit, 1979) also report the peak of flowering in the morning.

In this study there was a high rate of failure of fruit set and high fruit drop in the early weeks of the cashew flowering period, indicating that pollination was limiting. This is in contrast to the report by Heard *et al.* (1990) that pollination was not limiting in studies in the Northern Territory. However, high initial fruit set, high fruit drop, and low ripe fruit numbers in cashew have been reported by Rao and Hassan (1957), Murthy *et al.* (1975) and Wunnachit *et al.* (1986). The heavy fruit drop may be the result of unsuccessful pollination of hermaphrodite flowers due to pollen limitation, failed pollen-pistil interaction, or pollen vector limitation. The nutritional status of the tree, and environmental conditions could also play a role.

The number of hermaphrodite flowers varies significantly between trees and is highly correlated with fruit set and ripe fruit numbers. These results indicate that the number of hermaphrodite flowers can be used as a criterion for cashew selection.

5. Structure and histochemistry of the cashew pistil

5.1 Introduction

To improve the pollination and fruit set of cashew it is necessary to understand the structure of the pistil. The stigma is the specialized structure of the pistil to which pollen grains adhere and then germinate to grow through the style to reach the embryo sac for fertilization. Pistil receptivity is the period during which effective pollination can occur and has its basis in temporal changes that occur in the receptive cells. These changes in the structure and histochemistry of the stigma and style can influence the germination of pollen grains and the growth of the pollen tubes. This chapter investigates the structure and histochemistry of the cashew stigma and style using light and scanning electron microscopy.

5.2. Materials and methods

Twenty newly opened hermaphrodite flowers from tree 11.7 in the sandy soil block were collected and fixed for 24 h in 3% glutaraldehyde in 0.025 M phosphate buffer pH 7.0 containing 0.5% caffeine for suppression of phenolics (Mueller and Greenwood, 1978).

5.2.1 Scanning electron microscopy (SEM)

Five whole flowers and excised stigmas were hydrated via 25, 50, 75, and 100% ethanol each for a minimum of 15 minutes, and then critical point dried. The samples were mounted on aluminium stubs and sputter coated with gold. They were observed with a Phillips 505 scanning electron microscope at 20 kV.

5.2.2. Light microscopy

Five flowers were dissected into pieces of stigma, upper style, mid style, and lower style. The samples were dehydrated via 25, 50, 75, and 100% ethanol, each

for a minimum of 15 minutes and then embedded in glycol methacrylate (GMA) (Feder and O'Brien, 1968). Alternatively tissue was postfixed in 1% osmium tetroxide in 0.025 M phosphate buffer until the samples turned black. They were dehydrated via 25, 50, 75, and 100% ethanol followed by two changes in propylene oxide, each for ten minutes. The samples were embedded in low viscosity Spurr embedding medium overnight at 70 C. Serial longitudinal (LS) and transverse (TS) sections were cut at 1.5, 3, and 6 micron thickness using a Reichert-Jung 2050 microtome with glass knives. The sections were collected with fine forceps and floated onto a drop of distilled water on a microscope slide. The sections were straightened using dissecting needles and dried overnight at 60 C before staining.

5.2.2.1 Staining

Five dyes were used for staining as follows;

Periodic acid-Schiff's reagent (PAS) for insoluble polysaccharides (McGuckin and McKensie, 1958; Jensen, 1962)

Toluidine blue O (TBO) for phenolics and nucleic acids (Feder and O'Brien, 1968)

Periodic acid-Schiff's reagent followed by toluidine blue O (PAS/TBO) for general structure (Feder and O'Brien, 1968)

Aniline blue black (ABB) for proteins (Fisher, 1968)

Auramine O (AO) for lipid and cuticle (Heslop-Harrison, 1977)

Sudan black B (SBB) for lipids (Jensen, 1962; Bronner, 1975)

Periodic acid-Schiff's reagent

Slides of GMA sections were placed in a saturated solution of 2,4-dinitrophenyl hydrazine (0.5 g. in 100 ml. 15% acetic acid) for 30 minutes, then

rinsed in running water for one hour before placing in 0.1% periodic acid for 30 minutes. The slides were rinsed in running water for 5 minutes and then stained with Schiff's reagent for one hour. They were transfered to 10% sodium metabisulphite in 1 N hydrochloric acid for 2 minutes with 2 repeats in fresh solution. The slides were then rinsed in distilled water to remove excess dye.

Toluidine blue O

Toluidine blue O was used individually and as a counter stain to periodic acid-Schiff staining by placing the slides in 0.05% toluidine blue O in benzoate buffer pH 4.5 for 5 minutes. The slides were rinsed in water to remove excess dye. Sections were mounted in Dammar in xylene and were examined by bright field light microscopy.

Aniline blue black

Slides of GMA sections were placed in a solution of 1% aniline blue black in 7% acetic acid at 60 C for 10 minutes. They were removed and briefly dipped in 7% acetic acid to remove excess dye and then air dried. The sections were mounted in 5% acetic acid and examined by bright field light microscopy.

Auramine O

A solution of 0.01% auramine O in 0.01 M tris hydrochloric acid buffer pH 7.2 was used to mount GMA sections prior to observation using fluorescence microscopy.

Sudan black B

A fresh saturated solution of sudan black B in 70% ethanol was prepared and filtered. The solution was warmed to 60 C for 30 minutes before staining. The slides of Spurr-embedded sections were dipped into 70% ethanol for 1 minute before transfer to the sudan black B solution at 60 C. After 15-20 minutes, they were

removed and rinsed in 70% ethanol followed by distilled water. The slides were mounted in 60% glycerol in water and observed by bright field light microscopy.

5.3 Results

The hermaphrodite flower consisted of 5 sepals, 5 petals, one large stamen, 6-14 small stamens, and a single pistil with a long tapering style (Figure 5.1). Each stamen comprised an anther and a short filament (Figure 5.1, 5.2). The stamens were arranged in an elliptical whorl surrounding the ovary, with the large stamen located at one end of the ellipse. The large stamen was nearly twice the length of the small stamens.

The stylar tip was slightly bent in the bud but straightened following anthesis. The stigmatic surface was papillate with a central depression and a small amount of secretion (Figure 5.3, 5.4). A thick continuous cuticle, detected by auramine O staining, covered the stigmatic and stylar surfaces (Figure 5.4).

The stigma papillae were mainly unicellular but occasionally bicellular or tricellular (Figure 5.5). Radiating files of cells connected the papillae with the transmitting tissue in the upper style region. The papillae cells were highly vacuolated with peripheral cytoplasm. The cytoplasm stained intensely with SBB (Figure 5.6), ABB (Figure 5.7), and TBO indicating the presence of lipids, protein and phenolics. The cell walls stained with PAS, and PAS-positive starch grains were present in the papillae cells.

The style consisted of three layers. The outermost epidermis comprised a single layer of regular cells with stomata (Figure 5.5). The tissue located adjacent to the epidermis consisted of parenchymatous cells (Figures 5.8, 5.9) which were highly vacuolated, elongated longitudinally and loosely arranged. They contained starch grains (Figure 5.8). There were three vascular bundles which extended to the base of the papillae.

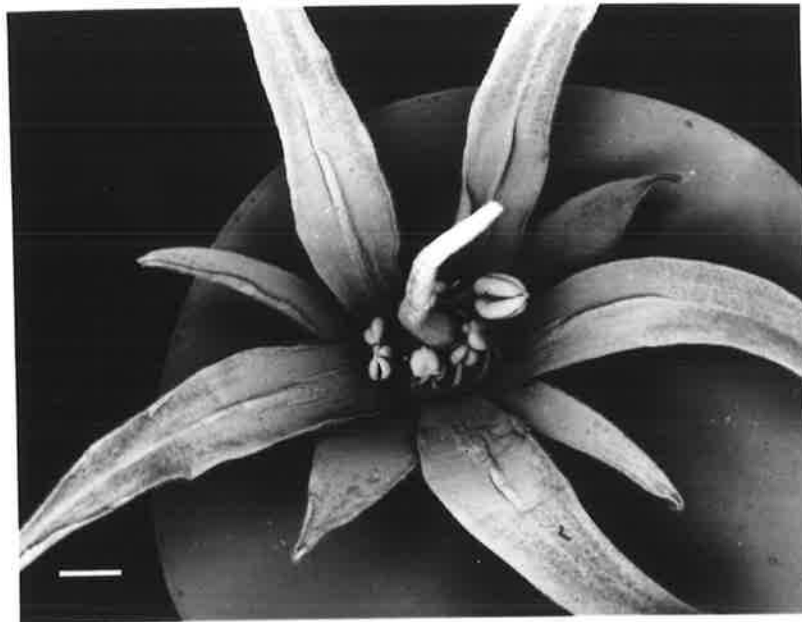

Figure 5.1 Scanning electron micrograph of hermaphrodite cashew flower. Bar represents 1 mm.

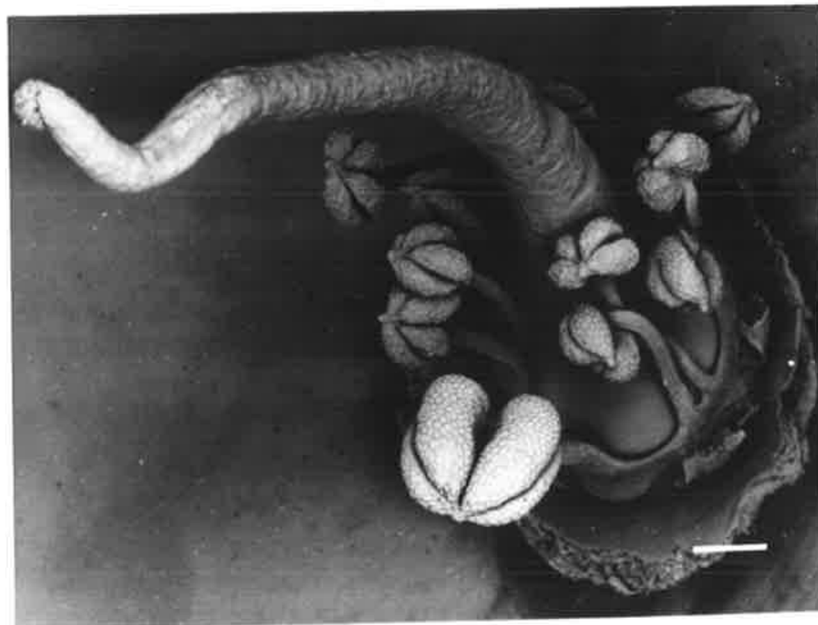

Figure 5.2 Scanning electron micrograph of hermaphrodite cashew flower with sepals and petals removed showing the single pistil and one large and ten small stamens. Bar represents 400 µm.

Figure 5.3 Scanning electron micrograph of stigma showing papillate surface with a central depression and a small amount of secretion. Bar represents 100 µm.

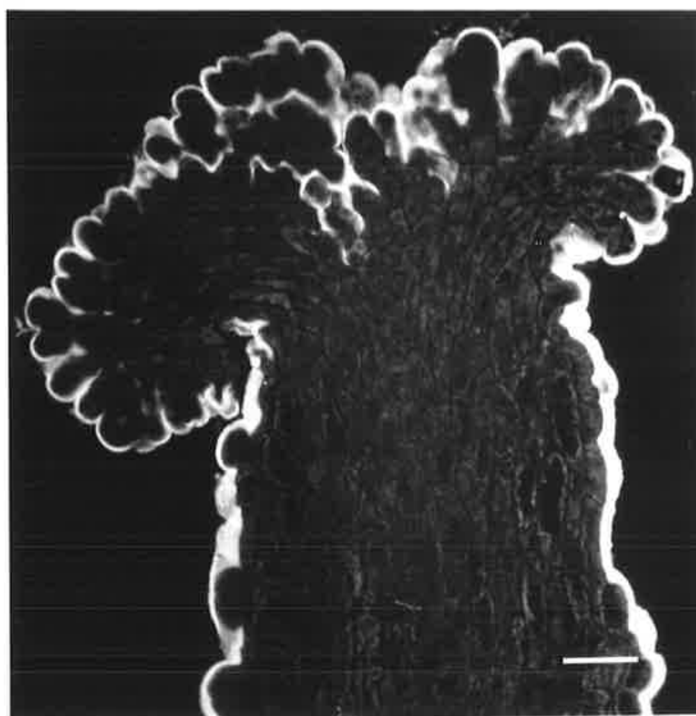

Figure 5.4 Fluorescence micrograph of GMA section stained with AO showing continuous cuticle. Bar represents 100 µm.

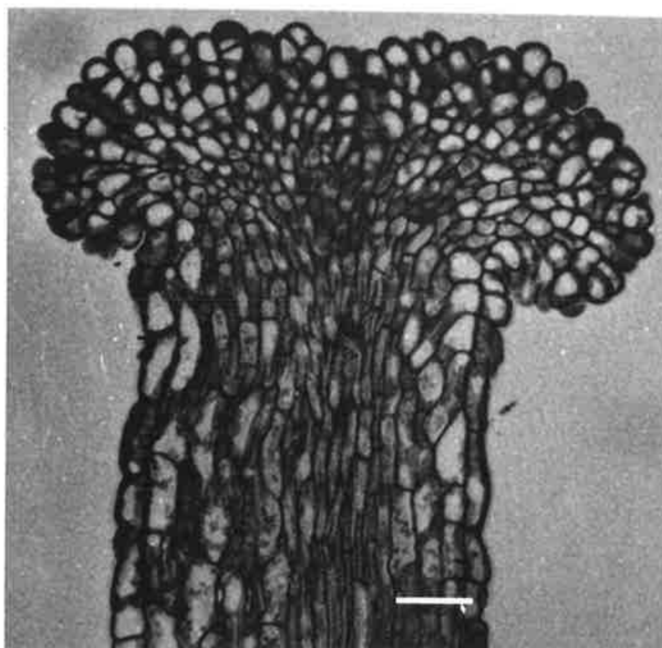

Figure 5.5 Bright field micrograph of longitudinal GMA section of mature cashew stigma stained with PAS and TBO showing short papilla cells. Bar represents 50 μm.

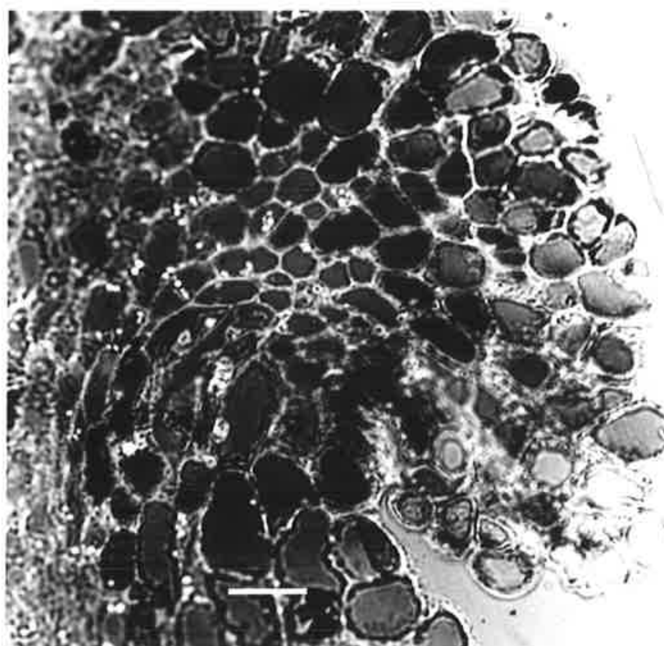

Figure 5.6 Bright field micrograph of longitudinal section of Spurr embedded material showing stigma papillae stained with SBB with lipid droplets in the cells. Bar represents 75 μm.

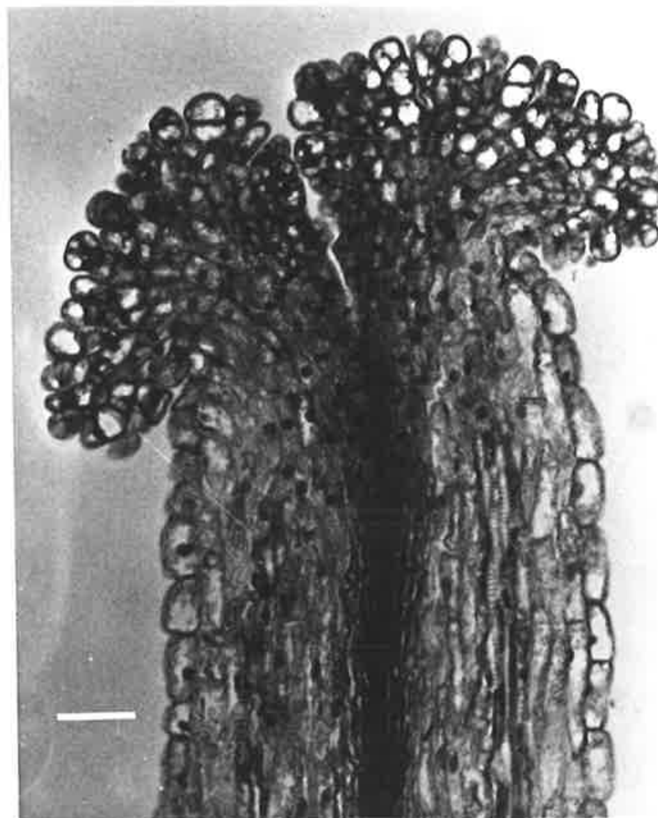

Figure 5.7 Bright field micrograph of longitudinal GMA section stained with ABB showing prominent nuclei of solid transmitting tissue. Bar represents 40 µm.

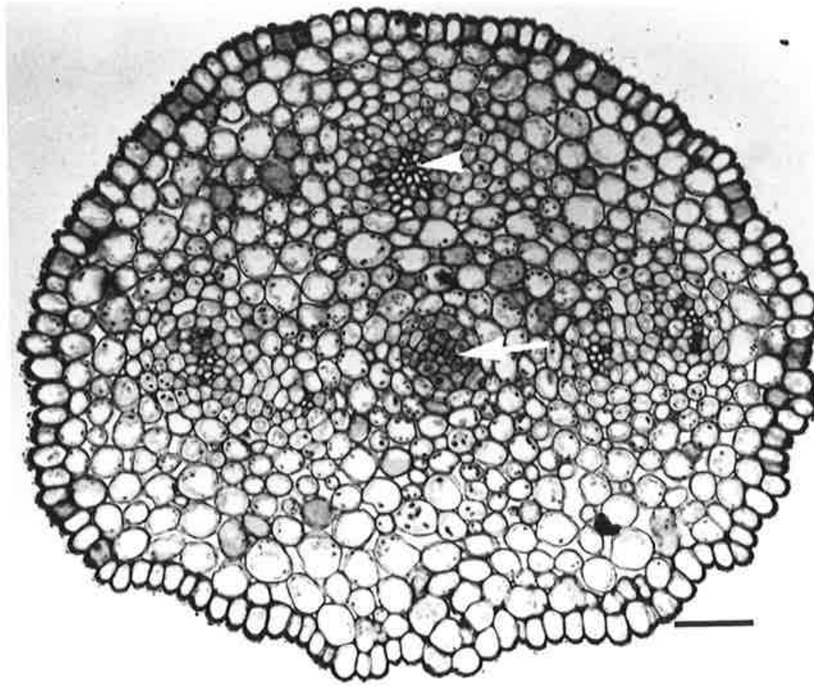

Figure 5.8 Bright field micrograph of GMA transverse section of mid style stained with PAS and TBO showing epidermal layer, ground tissue, vascular bundles (arrowhead) and transmitting tissue (arrow). Bar represents 50 µm.

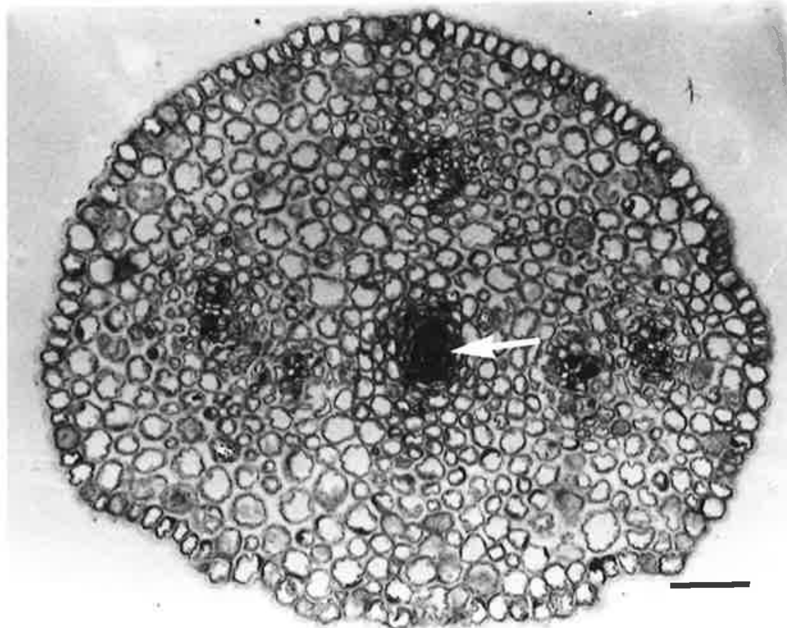

Figure 5.9 Bright field micrograph of GMA transverse section of mid style stained with ABB showing transmitting tissue (arrow) without intercellular spaces. Bar represents 50 µm.

The centre of the style consisted of a solid core of transmitting tissue (Figures 5.7-5.9). In the upper style the transmitting tissue was wider than at the base and radiated out to join the papillae. Toward the base of the style the transmitting tissue consisted of only 8-9 cells in TS, while the parenchymatous ground tissue increased in cell layers. Thus the overall diameter of the style increased toward to the base.

The transmitting tissue comprised closely-packed cells (Figure 5.8, 5.9) with small intercellular spaces. The cells had dense cytoplasm and the large nuclei occupied the median zone of the cells. With ABB and TBO staining, the nuclei were particularly prominent (Figure 5.7). In longitudinal section the cells were narrow, elongated, and fusiform. The transmitting tissue cytoplasm was intensely stained with ABB, TBO, and PAS but not stained with SBB. Thus the cells contained proteins, phenolics, and starch grains.

5.4 Discussion

The cashew stigma at anthesis has only a small amount of visible exudate, but has a continuous cuticle and can be classified as a dry type papillate stigma (Heslop-Harrison and Shivanna, 1977). The pistil shares this feature, and that of the solid style with the mango (Scholefield, 1982; de Wet and Robbertse, 1986; John *et al.*, 1987). Pistils with dry stigmas and solid styles have been reported in many other species also (Jensen and Fisher, 1966; Gosh and Shivanna, 1984; Shivanna *et al.*, 1989). Stigma papillae are modified epidermal cells (Heslop-Harrison and Shivanna,1977; Knox, 1984), and may have thin or thick walls, with or without cuticle, depending on the species. Jensen and Fisher (1966) found that the papillae of cotton had relatively thin walls covered by a distinct cuticle. The stigma of *Grevillea banksii* was not cuticularized, but had thin walls (Herscovitch and Martin,1989). Mackenzie *et al.* (1990) reported that the stigma of *Solanum tuberosum* had both unicellular and occasionally bicellular papillae with a

continuous cuticle and thick cell walls. The cuticle in dry stigmas has been reported to play a role in protection against desiccation and pathogen attack. John *et al.* (1987) reported that in mango (*Mangifera indica*) the cuticular deposition is massive at the corners of the compactly arranged papilla cells and comparatively thin at their convex surface. Flowering in mango occurs during the dry season, and the wavy and unevenly thick cuticle that covers the stigma surface may be an adaptation to prevent desiccation. Cashew flowering also occurs in the dry season, and the thick and continuous cuticle may have a similar function. There have been reports in some other species that the stigma cuticle has an important role in protection, for example in sweet potato (Martin and Ortiz, 1967) and petunia (Konar and Linskens, 1966). The papilla cells and extracellular secretions of the cashew contained protein, phenolics, starch and lipid, and the transmitting tissue cells contained protein, phenolics and starch. Lipid, protein, and carbohydrate are common in papillae and in stigmatic secretion of many species (Vasil and Johri, 1964; Konar and Linskens, 1966; Martin and Brewbaker, 1971; Sedgley and Buttrose, 1978; Schou, 1984; Kandasamy and Kristen, 1987; Herscovitch and Martin, 1989; MacKenzie *et al.*, 1990). They are proposed to have an important role in the pollen-pistil interaction and in the support of pollen tube growth.

Similarly the transmitting tissue may provide a source of nutrient for pollen tube growth (Kroh and Munting, 1967; Vasil, 1974; Bell and Hicks, 1976). The transmitting tissue is wide at the top of the style and can accommodate many pollen tubes. In the lower style region, however, the narrow transmitting tissue provides a smaller area for pollen tube growth. This may be an adaptation to select only the most vigorous pollen tube to reach the single ovule for fertilization.

6. Cashew pollen characteristics

6.1 Introduction

The cashew tree bears hermaphrodite and male flowers in the same panicle. Both types of flower have both large and small stamens which produce pollen grains. There have been no previous studies into the relative characteristics of the four pollen types. In this study the morphology and structure of the four pollen types are examined by light and scanning electron microscopy. Pollen viability is tested by fluorescein diacetate staining and by *in vivo* testing, and the sugar and amino acid contents of the pollen are analysed using high performance liquid chromatography.

6.2. Materials and methods

6.2.1 Experimental plants

Genotypes 7.1, 11.7, 12.1 and 12.5 in both orchards were used. Details of the trees are presented in chapter 3.

6.2.2 Anther dimensions and pollen grain numbers

Anther dimensions and number of pollen grains were measured from the large and small stamens of hermaphrodite and male flowers. Prior to anther dehiscence, five male and five hermaphrodite flowers from each tree were removed and fixed in FPA 50 (40% formaldehyde : propionic acid : 50% ethanol; 5:5:90). Anthers of individual flowers were measured, then dissected and collected into four separate groups; large hermaphrodite (LH), small hermaphrodite (SH), large male (LM), and small male (SM). Each anther was gently forced to open with needles and fine forceps under a binocular microscope and the pollen grains per anther were counted. The data of pollen grain numbers were analysed by analysis of variance (ANOVA).

6.2.3 Morphology and structure of the pollen grains

Fresh pollen from dehiscing large and small anthers of three hermaphrodite and three male flowers from each of trees 7.1 and 12.5 in the sandy soil block were dried at room temperature for 60 minutes and then placed on adhesive tape attached to aluminium stubs. Duplicate samples were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer pH 7.0, dehydrated via an ethanol series and critical point dried. The dry samples were attached to aluminium stubs using adhesive glue. All samples were sputter coated with gold and paladium alloy (60:40) to 200 A thickness and observed with a Philips 505 SEM at 20 kV. Morphological characters and the surface exine of each pollen type were examined and photographs taken. Pollen dimensions were measured from the photomicrographs.

Anthers just prior to dehiscence were removed from three hermaphrodite and three male flowers of trees 7.1 and 12.5 in the sandy soil block, and fixed in 3% glutaraldehyde in 0.025 M phosphate buffer pH 7. The samples were dehydrated via 25, 50, 75 and 100% ethanol, each for 15 minutes and then embedded in glycol methacrylate (GMA) (Feder and O'Brien, 1968). Series of longitudinal sections were cut at 1.5 and 3 microns using a Reichart-Jung 2050 microtome with glass knives. The sections were collected onto microscope slides and dried at 70 C overnight. The dry slides were stained with periodic acid-Schiff's reagent followed by toluidine blue O (PAS/TBO) for general structure (Jensen, 1962), sudan black B (SBB) for lipids (Bronner, 1975), and auramine O (AO) for cuticle (Heslop-Harrison, 1977) as described in section 5.2.2.1. The samples were examined and photographed using a Zeiss Axiophot photomicroscope.

6.2.4. Viability of pollen

6.2.4.1 Fluorescein diacetate test

Each of the four pollen types was pooled from 10 flowers of the four experimental trees from the sandy soil block. Pollen samples were stored at room temperature (20 C) for 0, 12, 24, and 48 hours after anther dehiscence and were then tested for viability with fluorescein diacetate. Pollen samples were placed on microscope slides. A solution of fluorescein diacetate was prepared, using the procedure of Heslop-Harrison and Heslop-Harrison (1970). Stock solutions of 2 mg fluorescein diacetate in 100 ml acetone, and 10% sucrose in distilled water were prepared. Fluorescein diacetate was added dropwise to the sucrose solution until milkiness appeared. The fresh solution was applied to the pollen samples, covered with a cover slip, and allowed to incubate for 5-10 minutes before examination by fluorescence microscopy. Each sample was separated into 3 replicates. The data of percent fluorescing pollen grains were analysed by analysis of variance (ANOVA).

6.2.4.2 In vivo test

Hand pollination of flowers followed by observation of pollen tube growth was performed to assess pollen viability. It was carried out in the sandy soil block. Trees 7.1 and 11.7 were used as female parents and trees 12.1 and 12.5 were used as pollen sources. Ten panicles on each tree were chosen and all open flowers were removed. The panicles were bagged with paper bags. Next morning the bags were removed from the panicles, all open male flowers were removed, and the newly opened hermaphrodite flowers were emasculated. Fresh dehiscing large and small anthers of hermaphrodite and male flowers from the male parents were collected. Each pollen type was directly applied to twenty hermaphrodite flowers by gently attaching the dehiscing anthers to the stigmas. The pollinated flowers were rebagged and left on the panicles for 48 hours before removal. The flowers with sepals and

petals removed were fixed in Carnoy's fixative (ethanol : chloroform : acetic acid; 6:3:1).

The fixed pistils were dissected and the ovules removed. The pistils and ovules were hydrated via 70 and 30% ethanol followed by two changes of distilled water each for 10 minutes, then softened in 0.8 N sodium hydroxide at 60 C for one hour. They were stained in 0.1% aniline blue in tri potassium phosphate, and an individual pistil with its own ovule was mounted in 80% glycerol. The preparations were observed using a Zeiss Axiophot fluorescence microscope. Pollen grains were counted on the stigma, and pollen tubes were counted in the upper style, lower style and ovary. The number of penetrated ovules was also counted.

The number of flowers (of 20) which had pollen grains on the stigma and with styles, ovaries, and ovules penetrated by at least one pollen tube were analysed for the stigma, upper style, lower style, ovary and ovule. A binomial model was fitted to each data set and the three way interaction between female parent, male parent, and pollen type factors was tested for significance.

6.2.5. Pollen sugars and amino acids

6.2.5.1 Pollen collection and preparation of extracts

Pollen was collected from dehiscing large and small anthers of 2850 hermaphrodite and 2850 male flowers from the four experimental trees from the black soil block between July and August 1990. The four pollen types were weighed and stored in small glass vials at -20 C. The samples were finely ground in a small glass mortar with a drop of 90% ethanol and a pinch of acid washed sand. Two ml of 90% ethanol was added to each sample prior to sonication and centrifugation at 2500 rpm for 20 minutes. The supernatant was removed and divided equally into two lots, one for sugar and one for amino acid analysis.

6.2.5.2 Sugar analysis using high performance liquid chromatography

The samples were passed through a mixed resin column (Aberlite CG-4B : Aberlite CG-120, 2:1; Mallinckrodt, U.S.A.). The eluate was vacuum dried with a Savant speed vac concentrator. The dry samples were dissolved with 1 ml HPLC water (filtered Milli Q water which was degassed in an ultrasonic bath), heated at 80 C for 4 minutes, shaken, and sonicated in an ultrasonic bath for 20 seconds. Four hundred μl of each sample was passed through a syringe tip filter (0.45 μm HV) into HPLC glass vials. Sugars were identified and their relative masses quantified by Waters high performance liquid chromatography. A Waters Associates liquid chromatograph with Model 510 pump, SugarPak column (30 cm x 6.5 mm), and Waters 410 refractive index detector was used together with a WISP 710 B automatic injector. The mobile phase solvent was 0.1 mMolar Ca^{2+} EDTA in HPLC water maintained at about 75 C. Standard solutions of fructose, glucose, and sucrose were prepared from dry AR grade reagents and 20 μg was injected. Sample injection volume was 100 μl with a running time of 31 minutes at a constant temperature of 90 C. For calibration, regressions based on a response peak area of standard sugar solutions were used. Quantities of each sugar were determined in comparison to the standards using regression equations and then were expressed as relative percentage mass.

6.2.5.3 Amino acid analysis using high performance liquid chromatograpy

The ethanolic extracts were vacuum dried with a Savant speed vac concentrator. The processes of sample preparation followed the recommendations of Waters for the PICO TAG amino acid analysis system. The dry samples were taken up in 10 μl of methanol : 1 M sodium acetate : triethylamine (2:1:1). They were vacuum dried and derivatized with 20 μl of methanol : triethylamine : phenylisothiocyanate : water (7:1:1:1), for 10 minutes at room temperature. The

vacuum dried samples were reconstituted in 100 μl of 4 mM phosphate buffer pH 7.4 with 5% acetronitrile. They were passed through a syringe tip filter (0.45 μm HV) into HPLC glass vials for injection.

Waters Associates HPLC components for PICO TAG amino acid analysis consisted of two Model 510 pumps, a PICO TAG 30 cm column, a Lambda Max Model 481 UV spectrophotometer detector, and a data and chromatography control system with Digital Professional 350 computer. They were used with a Model 710B WISP TM auto injector and column heater.

Mobile phases A and B were prepared as recommended. A was a mixture of 19.0 g sodium acetate trihydrate and 0.5 ml triethylamine in 1 litre of water. The solution was titrated to pH 6.4 with glacial acetic acid. 940 ml of the resulting solution was mixed with 60 ml acetronitrile. B was a mixture of 600 ml acetronitrile and 400 ml water. The mobile phases were degassed by sonicating in an ultrasonic bath for 20 seconds.

Standard amino acid solutions which contained 2.5 μmol. of amino acid per ml were used. 5 μl of standard was derivatized with 20 μl of derivatizing solution for 10 minutes at room temperature, and then vacuum dried. It was reconstituted in 400 μl of diluent and syringe tip filtered for injection. Sample injection volume was 25 μl with a running time of 73 minutes at 38 C. Separation of amino acids occurred over a 60 minute period.

Amino acids were identified by comparing retention times in the samples and in the standards and quantified by comparing the peak areas. Values were expressed in n mol/mg fresh weight of sample.

6.3. Results

6.3.1 Anther dimensions and pollen grain numbers

The hermaphrodite and male flowers both had one large and between 6 and 9 small stamens. The average dimensions of the large and small anthers of the hermaphrodite flower stamens were 1.01 x 0.58 mm and 0.54 x 0.33 mm, and of the male flower stamens were 1.03 x 0.56 mm and 0.56 x 0.32 mm. Within a flower, the numbers of pollen grains produced by the large and small anthers were significantly different (Table 6.1). Large anthers produced between 752 and 1047 pollen grains and small anthers produced between 116 and 201 pollen grains. The total numbers of pollen grains produced by hermaphrodite and male flowers were 2238 and 2247 respectively. There were no significant differences in the numbers of pollen grains produced by the four trees, or between hermaphrodite and male flowers within trees.

6.3.2 Morphology and structure of pollen grains

All pollen grain types were similar in morphology. They were tricolpate with three germinal furrows extending the length of the grain (Figure 6.1). The exine had a ridged pattern. Upon hydration of the grain, the germination aperture was visible in the centre of the furrow (Figure 6.2). Dehydrated pollen grains were subspherical and trilobate in polar view and broadly elliptical with slightly flattened ends in equatorial view. There was no significant variation in pollen size between the four types. The average dimensions of LH, SH, LM and SM pollen grains were 43.9 x 25.4, 44.6 x 26.2, 44.6 x 25.4, 43.1 x 28.5 µm respectively.

The pollen exine was thick and intensely stained with AO (Figure 6.3), SBB (Figure 6.4) and PAS/TBO (Figure 6.5). With ABB the cytoplasm was stained and the two nuclei were prominent (Figure 6.6). SBB also stained the cytoplasm,

Table 6.1a Number of pollen grains produced from large and small anthers of hermaphrodite and male flowers of four cashew trees.

Tree	Hermaphrodite		Male	
	Large	Small Average/total	Large	Small Average/total
7.1	1019.3	131.1/1048.8	797.3	161.7/1347.5
11.7	792.3	126.0/1008.0	751.7	115.8/1080.8
12.1	1046.7	201.3/1677.5	1044.0	186.6/1617.2
12.5	885.3	191.9/1471.2	846.0	188.0/1504.0
Mean	937.5	162.6/1031.4	859.8	163.0/1387.4

Table 6.1b Analysis of variance.

Source of variation	Degrees	Mean squares	Significance
Between trees	3	102426	ns
Between flowers	4	3221	ns
Between anther	8	1473499	p<0.001
Within anther (Error)	207	2434	
Total	222		

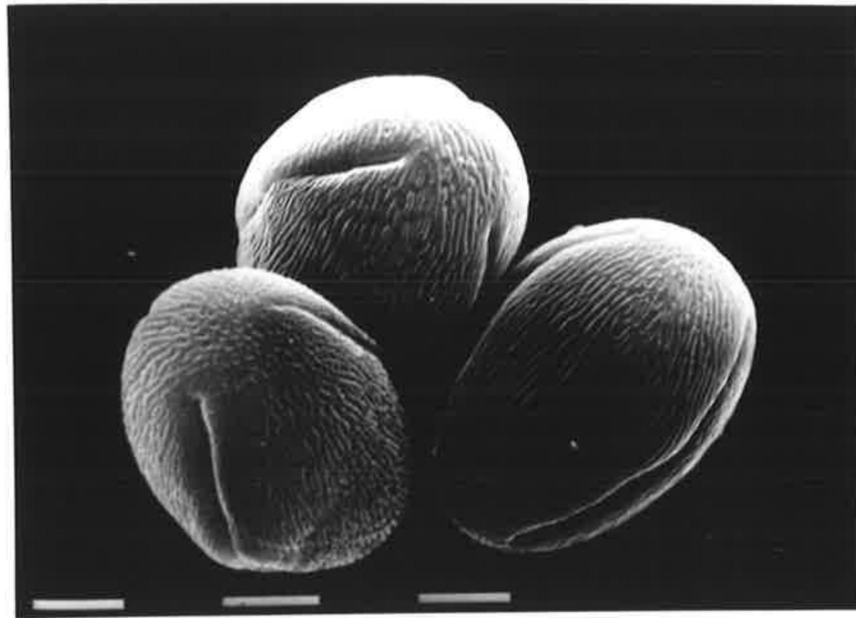

Figure 6.1 Scanning electron micrograph of dehydrated LM cashew pollen grains showing germination furrows and ridged exine. Bar represents 10 µm.

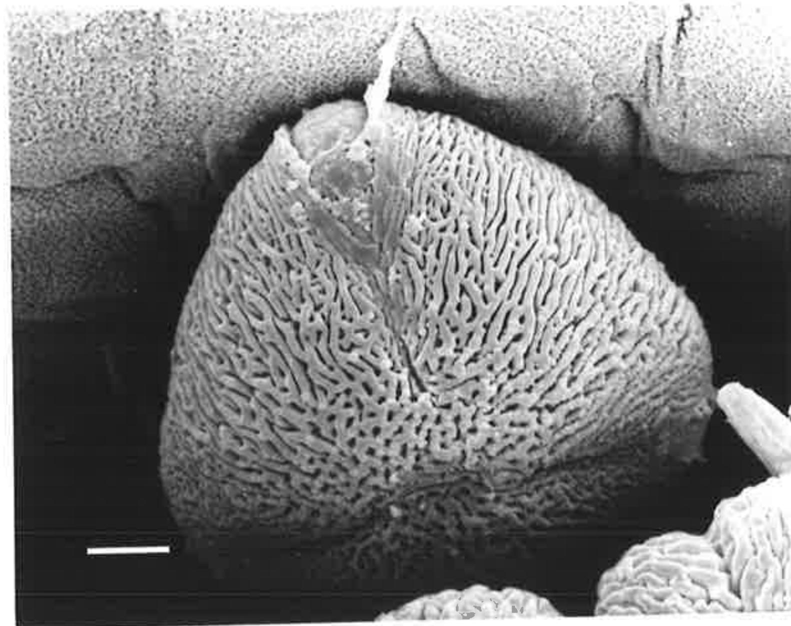

Figure 6.2 Scanning electron micrograph of hydrated LM cashew pollen grain showing germination aperture. Bar represents 4 µm.

particularly at the site of the germinal pore (Figure 6.4). There was no difference in the staining characteristics of the four pollen types.

6.3.3 Fluorescein diacetate test

Pollen stained with fluorescein diacetate was fluorescent yellow indicating enzyme activity or dull brown indicating dead pollen (Figure 6.7). All pollen types showed high fluorescence at 0 hours which declined with increased storage time (Table 6.2). There was a significant difference between the pollen types. LH pollen showed the highest mean percent fluorescence followed by LM, SM, and SH pollen. There were also significant differences in pollen fluorescence at different times after storage. There was no interaction between pollen type and storage time.

6.3.4 In vivo test

There were major differences between the pollen types in ability to germinate on the stigma and penetrate the single ovule (Table 6.3). When the four pollen types were compared there was a highly significant effect at all levels of the pistil ($p<0.001$) with LM pollen the most fertile followed by SM, LH and SH respectively. When pollen from hermaphrodite anthers was compared with pollen from male anthers there was a significant interaction of female parent and pollen type at all levels of the pistil (Table 6.4). There was also a significant interaction of female parent and male parent in the stigma, upper style and lower style.

6.3.5 Sugar analysis using high performance liquid chromatography

Glucose, fructose and sucrose were identified in the four pollen types but the concentration of sucrose was very low (Figure 6.8). Glucose and fructose were found at three times concentration in the hermaphrodite flower pollen than in the male flower pollen (Table 6.5). The glucose : fructose ratios in the four pollen types were consistent at about 1:1. The total concentration of the two sugars was highest in SH pollen followed by LH, LM, and SM pollen respectively.

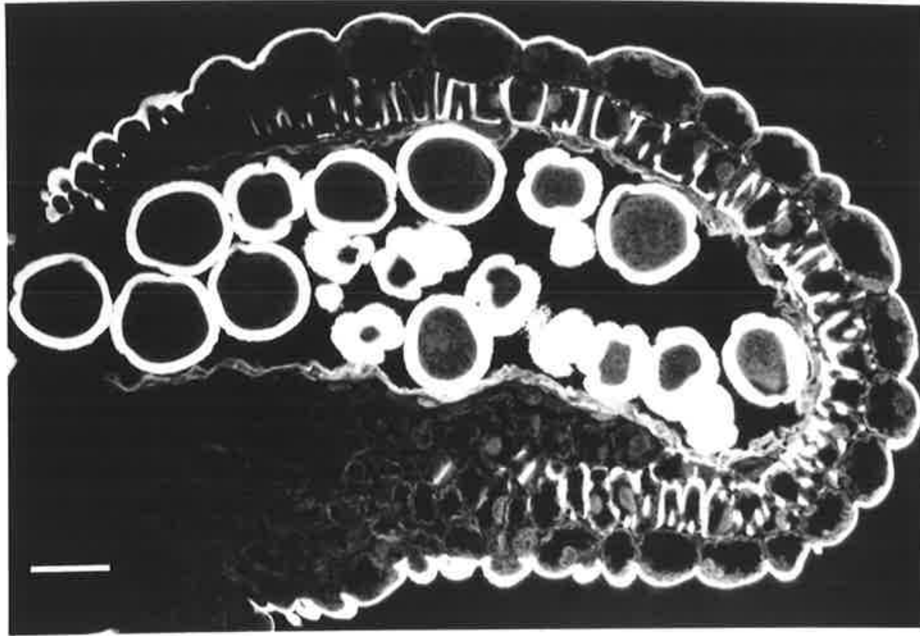

Figure 6.3 Fluorescence micrograph of LM cashew pollen grains showing prominent lipoidal wall. Bar represents 30 µm.

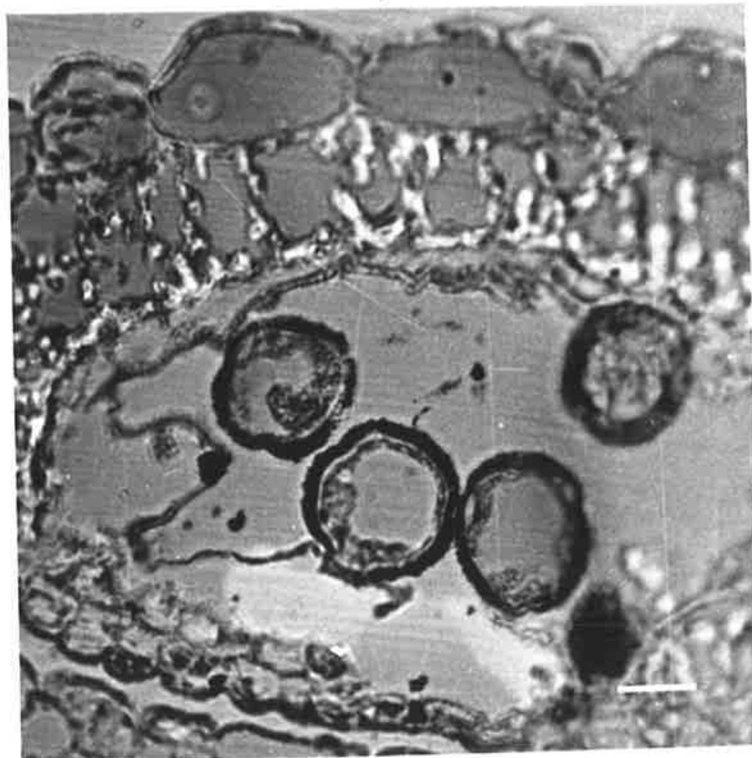

Figure 6.4 Cashew pollen grains from the small stamen of the male flower stained with SBB showing lipid in the wall and in the cytoplasm. Bar represents 20 µm.

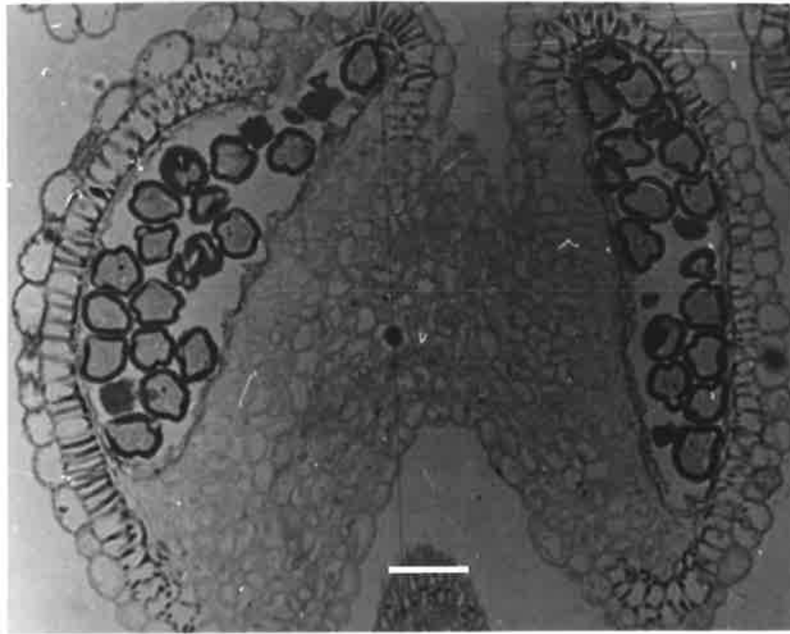

Figure 6.5 Large anther of the male flower stained with PAS and TBO showing bilobed anther and pollen grains. Bar represents 95 μm.

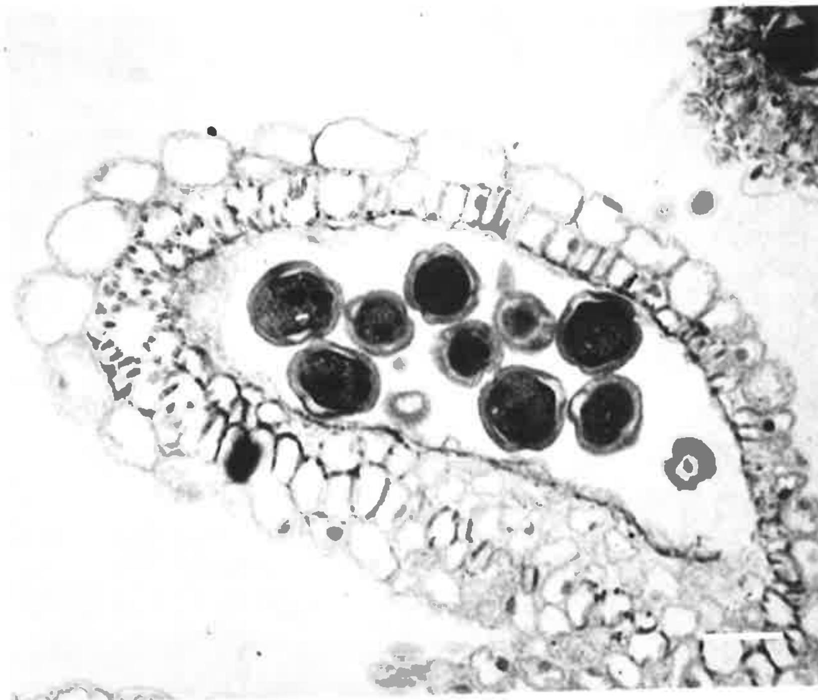

Figure 6.6 Cashew pollen grains from the large stamen of the male flower stained with ABB showing two prominent nuclei. Bar represents 40 μm.

Figure 6.7 Cashew pollen grains from the large stamen of the hermaphrodite flower stained with fluorescein diacetate showing fluorescing and non fluorescing pollen. Bar represents 50 μm.

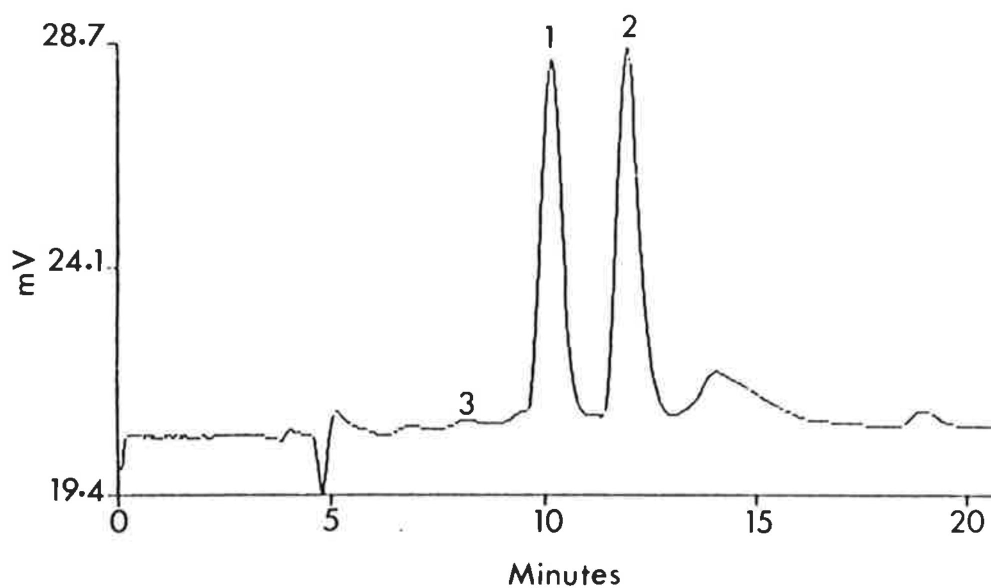

Figure 6.8 HPLC chromatogram of sugar from the pollen grains of the large stamen of the male cashew flower; (1) glucose; (2) fructose; (3) sucrose

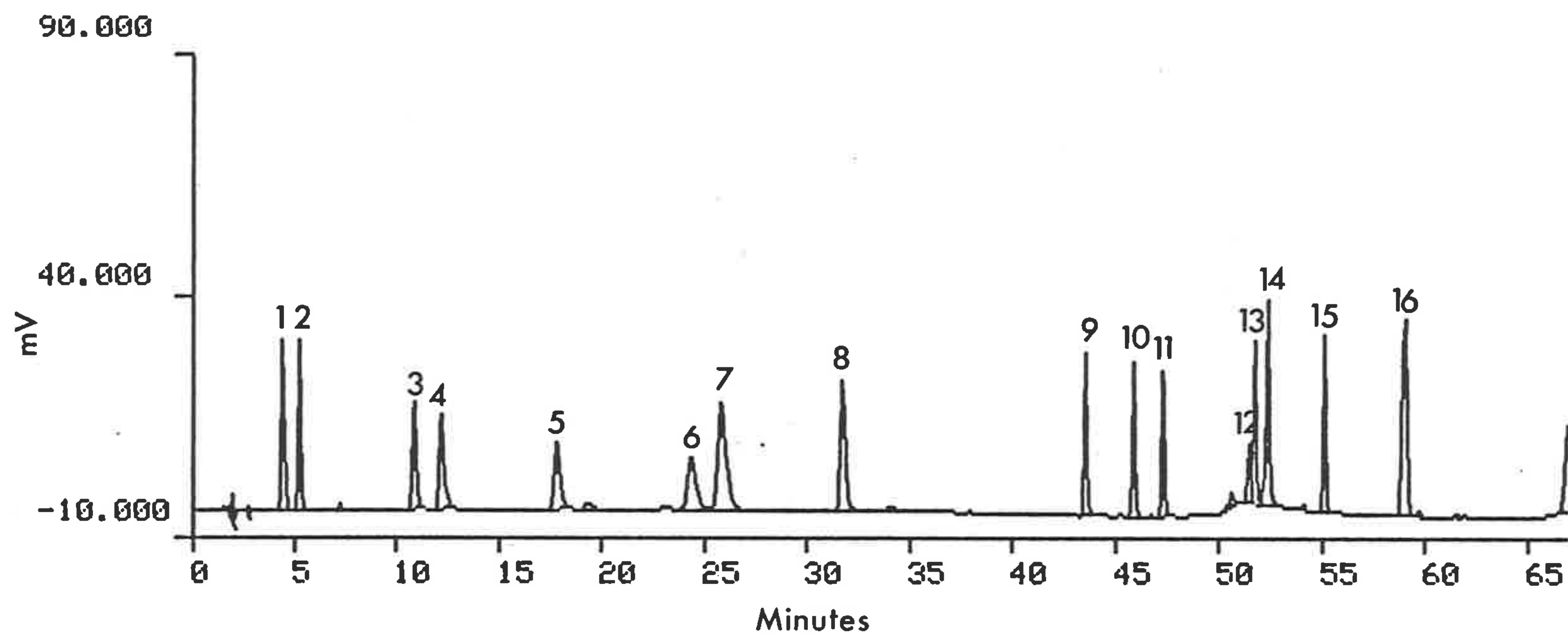

Figure 6.9 HPLC chromatogram of amino acid standards: (1) aspartic acid, (2) glutamic acid, (3) serine, (4) glycine, (5) histidine, (6) threonine, (7) alanine+arginine, (8) proline, (9) tyrosine, (10) valine, (11) methionine, (12) cysteine, (13) isoleucine, (14) leucine, (15) phenylalanine, (16) lysine. Asparagine and glutamine are not included.

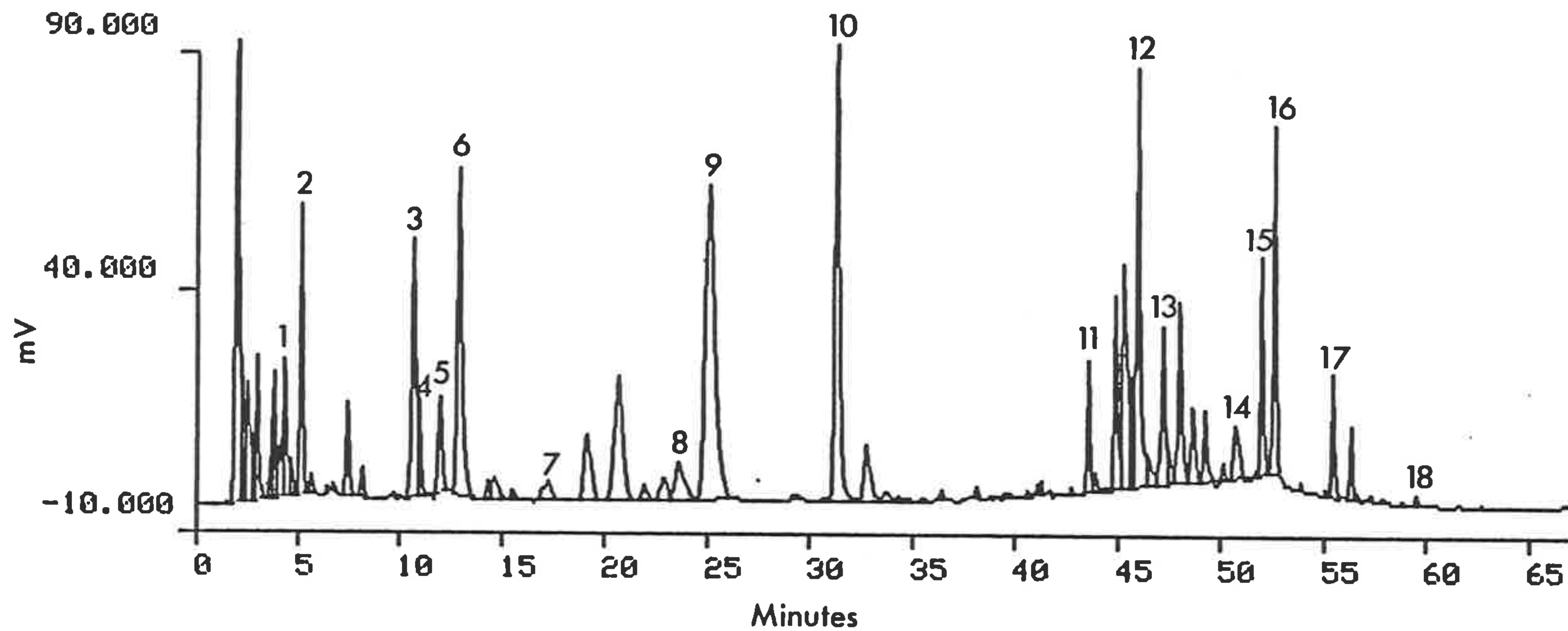

Figure 6.10 HPLC chromatogram of amino acids in pollen from the small stamen of the male cashew flower: (1) aspartic acid, (2) glutamic acid, (3) asparagine, (4) serine, (5) glycine, (6) glutamine, (7) histidine, (8) threonine, (9) alanine+arginine, (10) proline, (11) tyrosine, (12) valine, (13) methionine, (14) cysteine, (15) isoleucine, (16) leucine, (17) phenylalanine, (18) lysine.

Table 6.2a Percent fluorescing grains of four cashew pollen types stained with fluorescein diacetate at different times after storage at room temperature.

Pollen	0h	6h	24h	48h	Mean
Large male	100.0	75.3	39.1	12.8	56.7
Small male	95.5	61.2	40.2	12.3	52.3
Large hermaphrodite	100.0	77.4	80.0	39.9	73.1
Small hermaphrodite	90.8	42.7	44.1	30.1	51.9
Mean	96.6	64.1	50.8	22.4	

Table 6.2b Analysis of variance.

Sources of variation	Degrees of freedom	Mean square	Significance
Pollen type	3	1190.9	$p = 0.002$
Time	3	11378.6	$p < 0.001$
Pollen.time	9	400.3	ns
Error	32	191.0	
Total	47		

Table 6.3 Number of pistils (of 20) with pollen grains or pollen tubes following cross pollination by four cashew pollen types.

Pollination	LM	SM	LH	SH
Stigma				
7.1x12.1	15	17	8	0
7.1x12.5	17	11	3	1
11.7x12.1	14	6	6	6
11.7x12.5	18	12	12	2
Mean	16.0	11.5	7.3	2.3
Upper style				
7.1x12.1	15	17	8	0
7.1x12.5	17	11	2	1
11.7x12.1	14	5	5	6
11.7x12.5	17	12	11	1
Mean	15.8	11.3	6.5	2.0
Lower style				
7.1x12.1	14	15	6	0
7.1x12.5	16	9	1	1
11.7x12.1	14	4	3	6
11.7x12.5	15	10	8	0
Mean	14.8	9.5	4.5	1.8
Ovary				
7.1x12.1	13	15	6	0
7.1x12.5	16	9	1	1
11.7x12.1	14	4	3	6
11.7x12.5	15	9	8	0
Mean	14.5	9.3	4.5	1.8
Ovule				
7.1x12.1	8	13	4	0
7.1x12.5	15	8	0	1
11.7x12.1	14	4	2	5
11.7x12.5	12	8	8	0
Mean	12.3	8.3	3.5	1.5

Table 6.4 Analysis of deviance using binomial distribution comparing pollen from hermaphrodite and male flowers.

Source of variation	Degrees of freedom	Deviance				
		Stigma	Upper style	Lower style	Ovary	Ovule
Female parent	1	0.201	0.000	0.000	0.053	0.230
Male parent	1	0.201	0.051	0.000	0.053	0.058
Pollen type	1	67.750	72.263	67.500	68.591	58.378
Female.male	1	6.361*	6.513*	4.241*	2.417	0.275
Female.pollen	1	9.501**	9.791**	8.937**	7.226**	6.547*
Female.male.pollen	1	0.304	0.102	0.022	0.010	1.443

* $p<0.05$, ** $p<0.01$

Table 6.5 Glucose and fructose content of four cashew pollen types.

Pollen	Sugar content µg/mg fresh weight of sample			
	Glucose	Fructose	Total	G:F
LM pollen	24.6	26.8	51.4	1:1.1
SM pollen	18.2	19.4	37.6	1:1.1
LH pollen	75.8	82.6	158.4	1:1.1
SH pollen	83.4	94.4	177.8	1:1.1
Mean	50.5	55.8	106.3	1:1.1

Table 6.6 Composition of free amino acids in four pollen types of cashew (nmol/mg fresh weight).

Amino acids	LH	SH	LM	SM
Aspartic acid	6.00	8.81	1.57	1.38
Glutamic acid	10.26	12.24	2.52	2.52
Serine	5.86	4.79	1.34	1.19
Asparagine	10.89	9.95	2.40	2.23
Glycine	7.03	7.09	1.45	1.30
Glutamine	24.03	16.92	6.88	3.40
Histidine	1.96	2.46	0.38	0.57
Threonine	4.40	3.70	0.61	1.09
Alanine+arginine	27.33	17.37	7.29	4.74
Proline	118.14	19.76	27.42	5.64
Tyrosine	3.71	3.66	0.90	1.27
Valine	22.40	17.91	9.24	5.37
Methionine	15.88	6.11	5.04	1.73
Cysteine	5.87	11.97	1.31	1.62
Isoleucine	7.62	7.66	2.14	1.73
Leucine	13.19	14.98	4.77	2.88
Phenylalanine	5.70	3.46	0.63	1.19
Lysine	0.57	1.19	0.08	0.10
Total	290.85	170.04	75.97	39.97

6.3.6 Amino acid analysis using high performance liquid chromatography

Nineteen free amino acids were detected in the four pollen types (Table 6.6, Figures 6.9, 6.10). Pollen from hermaphrodite flowers had 4 times the free amino acid content of pollen from male flowers. Within flower types, pollen from the large stamen had a higher total free amino acid content than pollen from the small stamens. Proline was the major amino acid detected in all the four pollen types, and the amount of proline in LH pollen was more than one third of the amount of the rest of the amino acids combined.

6.4 Discussion

Different numbers of pollen grains are produced by flowers of different species (Iwanami *et al.*, 1988; Vear *et al.*, 1990). The number of pollen grains produced per anther is under both genetic and physiological control and has been used in classification of pollination types (Faegri and van der Pijl, 1979). Anemophilous flowers, such as *Juglans* produce many thousands of pollen grains per anther in contrast to entomophilous flowers, such as some species of *Acacia*, which produce only 64 pollen grains per anther (Sedgley, 1989). Cashew flowers have been observed to be pollinated by insects (Northwood, 1966; Free and Williams, 1976; Khoo *et al.*, 1982; Heard *et al.*, 1990). The observation of relatively low numbers of pollen grains produced by a cashew flower in this study supports these reports.

The exine pattern of the pollen wall is genetically stable for different varieties and species, and morphological and utrastructural characters of pollen have been used for cultivar identification in a number of crops (Fogle, 1977; Maas, 1977; Westwood, 1978; Martens and Fretz, 1980; Ahmedullah, 1983; Marcucci *et al.*, 1984; Martens *et al.*, 1989; Mulas *et al.*, 1989). The morphological characters of the four types of cashew pollen were similar with no significant variation in pollen size. The exine of cashew pollen consists of lipid and phenolics and is

histochemically similar to that of other angiosperm species (Heslop-Harrison, 1979; Knox, 1984). The pollen is binuclear at maturity, as is reported in two-thirds of flowering plant families (Knox, 1984).

The fluorescein diacetate test relies on the presence of active enzymes in the pollen to reduce a non-fluorescent substrate to a fluorescent product. It is regularly used for assessing pollen viability (Heslop-Harrison and Heslop-Harrison, 1970). The technique together with *in vitro* or *in vivo* germination tests has been widely used in many species and the results are often highly correlated (Ockenden and Gates, 1976; Shivanna and Heslop-Harrison, 1981; Widrlechner *et al.*, 1983). In this study however the results of the two tests were not correlated. For example, the pollen from the short anther of the hermaphrodite flower showed low viability in the *in vivo* test but had a high percentage of fluorescing grains with fluorescein diacetate. Thus a fluorescing pollen grain is not necessarily capable of germinating on the pistil and effecting fertilisation. The *in vivo* pollination test is more accurate and this showed that LM pollen has the highest viability. For this reason it was used as a pollen source in all further experiments. The fluorescein diacetate test indicated that within 6 hours after dehiscence the pollen retained reasonable viability, but this finding should be checked by *in vivo* tests.

Glucose, fructose and sucrose are the three main sugars found in pollen of most plant species (Stanley and Linskens, 1974; Schmidt *et al.*, 1989) but only glucose and fructose are significant in cashew pollen. The glucose : fructose ratios of about 1:1 of the four pollen types are consistent with those reported in species such as *Lilium lanciofolium* and *Lilium auratum* (Motomura *et al.*, 1962), *Ranunculus spp.* (McLellan, 1977), and *Typha latifolia* (Schmidt *et al.*, 1989). As pollen is a floral reward to insect visitors the difference in sugar content of the four pollen types may have significance in pollination (Stanley and Linskens, 1974; Baker 1976). In terms of sugar reward, pollen from hermaphrodite flowers provides more fructose and glucose to potential pollinators than the pollen from male flowers.

Stanley and Linskens (1974) found that the concentration of pollen amino acids was considerably higher in the bound than the free fraction but that their distribution in both fractions tended to follow the same pattern. In the analysis of the four cashew pollen types, only free amino acids were analysed and higher levels would be expected if the bound amino acids were also analysed. Baker (1976) stated that pollen provides a flower visitor with food that can be used in its own nutrition or that of its brood. Proline has been reported to be the major amino acid in pollen of many species (Bathurst 1954; Pfahler and Linskens, 1970; Rayner and Langridge, 1985; Moezel *et al.*, 1987; Grunfeld *et al.*, 1989) and this is the case in the four pollen types of cashew. There is no correlation between free proline content and pollination system (Britikov *et al.*, 1964), and Lue and Dixon (1967) reported that proline is not essential in the honeybee diet because bees can synthesis proline from glucose. Thus the role, if any, of proline in pollination is still unclear. It may, however, have a function in stress tolerance, as pollen is a dehydrated structure potentially separated from the plant for long periods (Zhang and Croes, 1983), and proline has been identified as accumulating in some plants in response to stress (Paleg *et al.*, 1981). Degroot (1953) quantified the essential amino acids for bee nutrition but the amounts in the four pollen types of cashew is low, particularly methionine, lysine, leucine, and phenylalanine. In general the pollen of the hermaphrodite flower was a richer source of amino acids than the pollen of the male flower. The honeybee can discriminate between food sources of different nutritive value and will convey this information to others in the hive (Gould and Gould, 1983).

Thus the hermaphrodite cashew flower produces pollen that is more nutritious than that of the male flower but has a reduced capacity to effect fertilisation. This suggests that the function of the pollen of the hermaphrodite cashew flower is primarily to attract insect pollinators to the flower, whereas that of the male flower is to provide pollen for pollination. The ideal situation would

appear to be the production of pollen with both high fertility and high nutritive value. It is possible, therefore, that these two factors may be mutually exclusive in the cashew. A distinction can also be made between the large and the small stamen within each flower type. The large anthers produce more pollen grains on a per anther basis with higher fertility than the small anthers. The large anther is also more prominent than the small anthers and so is most readily investigated by insect visitors to the flower. This suggests that the large anther is the major source of pollen for pollination purposes. Distinctions between anther types and function have also been reported in *Cassia* (Dulberger, 1981).

7. Cashew nectar production

7.1 Introduction

Both hermaphrodite and male flowers of cashew produce nectar which attracts pollinating insects (Free and Williams, 1976). This study investigates the location of the nectaries, the volume and concentration of the nectar, and the nectar sugar and amino acid composition using high performance liquid chromatography. The structure and histochemistry of nectary tissues are also investigated by light and scanning electron microscopy. As nectar is a reward to insect pollinators an understanding of cashew nectar production will contribute to knowledge of the pollination system.

7.2. Materials and methods

7.2.1 Experimental plants

This study was carried out on trees in the sandy soil block. The details of the cashew trees are presented in chapter 3.

7.2.2 Location, structure, and histochemistry of cashew floral and extrafloral nectary tissues

Cashew trees were observed for evidence of nectar secretion. Drops of exudate were produced at the junction of the corolla and androecium of both hermaphrodite and male flowers (Figure 7.1), at the angles of the branches of the panicle (Figure 7.2), on the adaxial surface at the proximal end of the midrib (Figure 7.3), and on the young developing fruit at the site of abscission of the style (Figure 7.4). Detailed structural and histochemical studies were conducted on the floral nectaries of the hermaphrodite and male flowers and on the extrafloral nectaries of the panicles.

Figure 7.1 Nectar secretion at the base of the floral tube of hermaphrodite (a) and male (b) cashew flowers

Figure 7.2 Nectar secretion at the angles of cashew panicle branches

Figure 7.3 Nectar secretion on the adaxial surface of the proximal end of the midrib of the cashew leaf

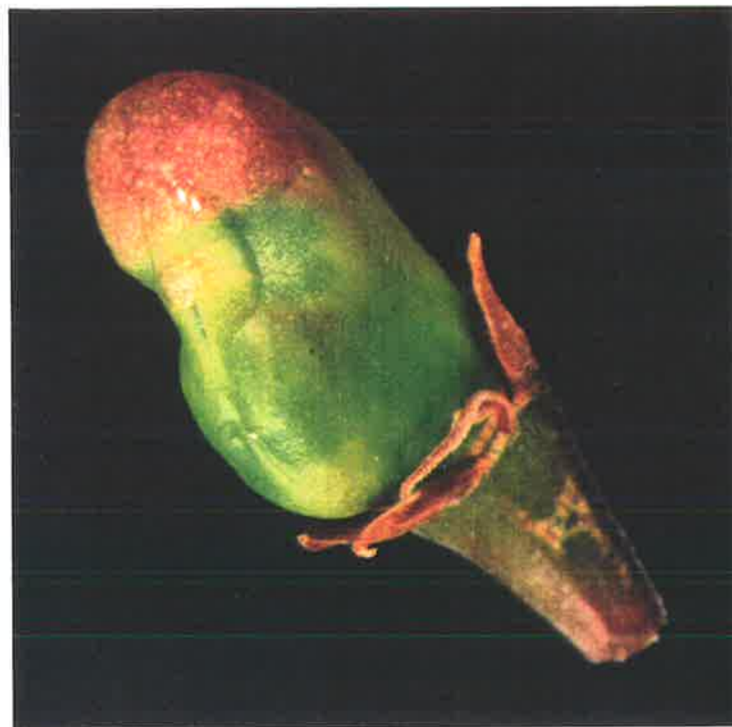

Figure 7.4 Nectar secretion by the young cashew fruit at the site of abscission of the style

Three young panicles of tree 12.5 were bagged with fine mesh cotton bags. The bags were left on the panicles until drops of nectar appeared at the angles of the panicle branches. The bags were then removed and the secretory tissues were dissected from the panicle branches and from newly opened hermaphrodite and male flowers. Samples were fixed in 3% glutaraldehyde in 0.025M phosphate buffer pH 7.0, dehydrated via an ethanol series, and embedded in glycol methacrylate (Feder and O'Brien, 1968). Some samples were post fixed in 1% osmium tetroxide in 0.025M phosphate buffer pH 7.0 at room temperature followed by dehydration via an ethanol/propylene oxide series before embedding in Spurr's resin (chapter 5.2.2.). Serial longitudinal sections were cut at 1.5 and 3 microns using a Reichart-Jung 2050 microtome with glass knives. The sections were stained with periodic acid Schiff's reagent and toluidine blue O (PAS/TBO) for general structure (Jensen, 1962), PAS for carbohydrates, aniline blue black (ABB) and coomassie brilliant blue (CBB) for proteins (Fisher, 1968), and sudan black B (SBB) for lipids (Bronner, 1975) (chapter 5.2.2.1).

Fixed samples were also processed for scanning electron microscopy. They were dehydrated via an ethanol series and critical point dried using carbon dioxide. The samples were attached to aluminium stubs using adhesive glue and sputter-coated with gold and palladium alloy (60:40). They were examined using a Philips 505 scanning electron microscope at 20 kV.

7.2.3 Volume, concentration, and sugar and amino acid composition of floral and extrafloral nectar

Ten panicles on each of trees 2.6, 7.1, 11.7, 12.1, 12.5 were chosen. The panicles were located randomly around the canopy at about 1.5 m above the ground. All open flowers were removed and the panicles were bagged with fine mesh cotton bags to protect them from insect visitors. To avoid damage to the panicle a wire support was used to prevent rubbing of the bag against the panicle. The bags were

removed the following day and nectar was withdrawn from open hermaphrodite and male flowers with a one microlitre capillary tube (Drummond, U.S.A). The tube was gently inserted into the base of the floral tube between the petals and the stamens and the nectar was sucked into the tube by capillary force. Collection of one microlitre required up to 50 flowers. Extrafloral nectar was examined from four bagged panicles at peak anthesis of trees 2.6, 7.1, 11.7, and 12.5. All nectar from all of the nectaries (mean of six) present on each panicle was withdrawn once only using a two microlitre capillary tube. Nectar volume was read directly from the tube and its concentration was measured using a refractometer (Erma, Tokyo). The samples were stored in 80% ethanol for sugar and amino acid analysis using high performance liquid chromatography as described in chapter 6, except that a PICO TAG 15 column was used for amino acid analysis.

7.3. Results

7.3.1 Structure and histochemistry of floral and extrafloral nectaries of cashew

Nectariferous tissues of hermaphrodite and male floral and of extrafloral nectaries were similar in structure and histochemistry. The nectar was secreted via multicellular trichomes (Figure 7.5) which in the extrafloral nectary were interspersed with unicellular non-secretory hairs (Figures 7.6, 7.7). Cuticle covered the outer surface of the trichomes (Figure 7.8) and became distended by the secretion of nectar (Figure 7.9). Lipid was present at the surface of the trichomes and in the epidermal cells below the trichomes (Figure 7.10), but protein was not detected. Starch was present in the cells of the trichomes (Figure 7.9), and vascular tissue terminated in the parenchyma below the nectariferous tissue.

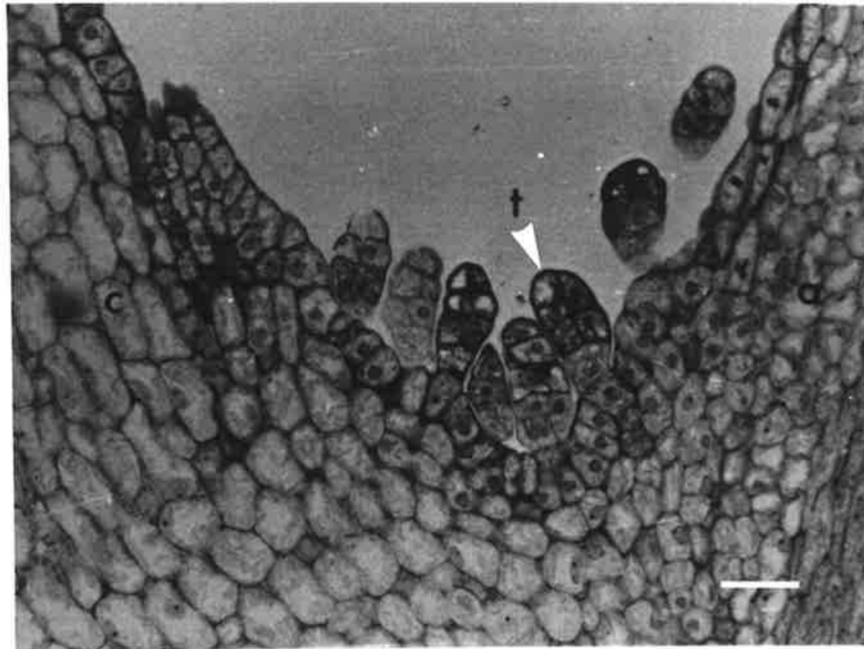

Figure 7.5 Floral nectary tissue at the junction of the corolla (c) and androecium (a) of the male cashew flower stained with PAS and TBO showing nectary trichomes (t). Bar represents 30μm.

Figure 7.6 Scanning electron micrograph of secretory trichomes (arrow) and non secretory hairs (arrowhead) in the extrafloral panicle nectary of cashew. Bar represents 100μm.

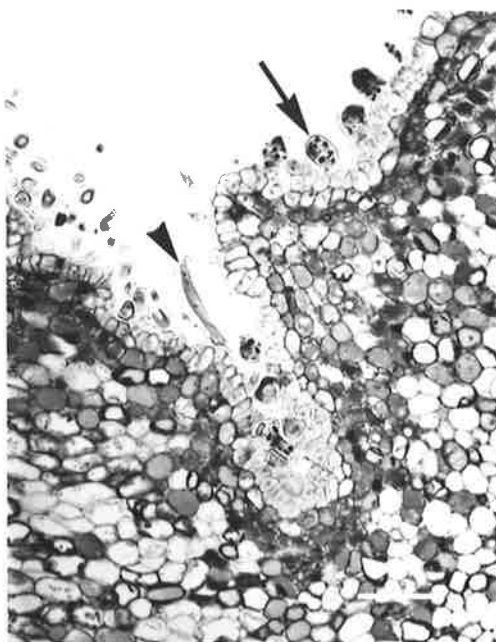

Figure 7.7 Extrafloral nectary tissue from the cashew panicle branch stained with PAS and TBO showing nectary trichomes (arrow) and non secretory unicellular hairs (arrowhead). Bar represents 100 μm.

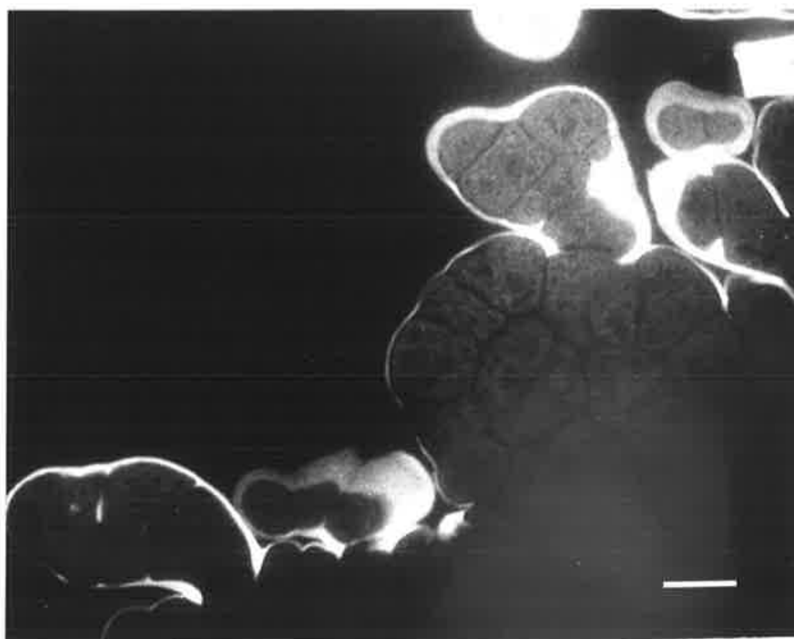

Figure 7.8 Floral nectary tissue of hermaphrodite cashew flower stained with AO showing cuticle over the trichomes. Bar represents 20 μm.

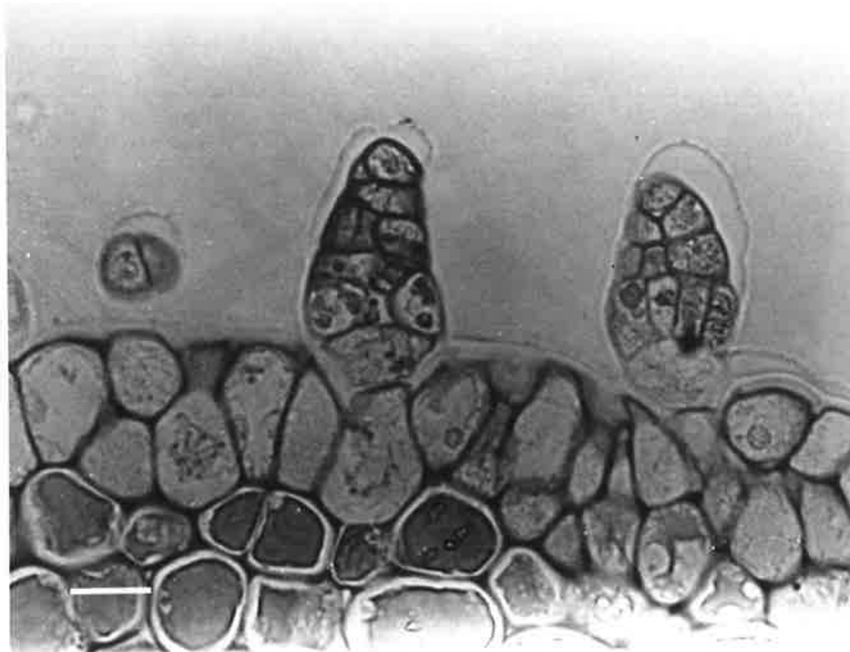

Figure 7.9 Extrafloral nectary of the cashew panicle branch stained with PAS showing starch grains in the secretory cells, and distended cuticle. Bar represents 20 μm.

Figure 7.10 Floral nectary of hermaphrodite cashew flower stained with SBB showing lipid at the surface of the trichomes and in the cells beneath the trichomes. Bar represents 20 μm.

Table 7.1 Volume (μl) per flower and per panicle and concentration (% brix) of floral and extrafloral nectar.

Tree	Hermaphrodite		Male		Panicle	
	Volume	Conc.	Volume	Conc.	Volume	Conc.
2.6	0.07	73.4	0.05	69.5	12.7	79.9
7.1	0.07	66.1	0.04	69.7	29.0	75.3
11.7	0.07	73.7	0.04	70.1	17.3	75.0
12.1	0.10	74.6	0.04	73.2	–	–
12.5	0.10	71.9	0.04	73.8	31.3	80.6
Mean	0.08	71.9	0.04	71.2	22.6	77.7

-not measured

7.3.2. Volume, concentration, and sugar and amino acid composition of floral and extrafloral nectar

7.3.2.1 Sugars

The volume of hermaphrodite flower nectar was significantly higher ($p<0.001$) than that of the male (Table 7.1), but there was no significant difference between trees in volume of either floral or extrafloral nectar and no significant tree by flower interaction. Hermaphrodite and male floral and extrafloral nectar concentration was high at around 70% in most cases.

Glucose, fructose and sucrose were present in both floral and extrafloral nectar with a predominance of glucose and fructose (Table 7.2). There were significant differences in the contents of the sugars ($p<0.001$) due to the higher amount of sucrose in the floral nectar of the male flower ($p=0.049$). In the extrafloral nectar the amounts of fructose and glucose were slightly higher than in the floral nectar.

7.3.2.2. Amino acids

Fourteen free amino acids were detected in the floral nectar and 15 in the panicle nectar (Table 7.3). Histidine was not present in any floral nectar samples but occurred in all extrafloral nectar with the exception of tree 11.7. Isoleucine and leucine were absent from the hermaphrodite floral nectar of trees 7.1 and 12.1 and from the extrafloral nectar of tree 11.7. Leucine was also absent from the male floral nectar of tree 7.1.

Individual and total amino acid concentrations of both floral and extrafloral nectar varied between trees. Male floral nectar had an amino acid content slightly higher than that of the hermaphrodite nectar and about three times that of the extrafloral nectar. Proline was the dominant amino acid in the floral nectar and leucine and isoleucine were the two lowest components. Serine and glutamic acid

Table 7.2 Glucose (G), fructose (F) and sucrose (S) contents (µg/µl) in floral and extrafloral nectar of cashew.

Tree	Hermaphrodite flower				Male flower				Panicle			
	G	F	S	G:F:S	G	F	S	G:F:S	G	F	S	G:F:S
2.6	345.4	334.7	60.0	5.8:5.6:1	285.5	277.7	140.3	2.0:2.0:1	494.7	469.7	70.2	7.1:6.7:1
7.1	518.5	310.8	41.5	7.7:7.6:1	372.1	361.0	60.7	6.1:6.0:1	487.7	473.0	70.0	7.0:6.8:1
11.7	329.9	321.7	76.0	4.3:4.2:1	345.1	335.8	109.1	3.2:3.1:1	398.8	385.6	58.7	6.8:6.6:1
12.1	358.9	347.2	54.9	6.5:6.3:1	366.3	352.3	72.7	5.0:4.9:1	–	–	–	–
12.5	360.5	341.5	50.2	7.2:6.9:1	416.1	392.8	67.6	6.2:5.8:1	437.6	428.1	43.8	10.0:9.8:1
Mean	342.6	331.2	56.5	6.1:5.9:1	357.0	343.9	90.1	4.0:3.8:1	454.7	439.1	60.7	7.5:7.2:1

- not measured

Table 7.3 Amino acids in nectar of hermaphrodite and male flowers and of panicles of cashew (nmol/μl).

	Amino acid*															Total
Tree	ASP	GLU	SER	GLN	HIS	ARG	THR	ALA	PRO	VAL	MET	LEU	ILE	PHE	LYS	
Hermaphro. flower																
2.6	.15	.21	.11	.07	ND	.05	.04	.01	.26	.05	.03	<.01	<.01	.02	.01	1.09
7.1	.14	.26	.14	.10	ND	.08	.06	.08	.52	.07	.02	ND	ND	.01	.01	1.47
11.7	.14	.17	.15	.13	ND	.10	.07	.08	1.14	.06	.03	<.01	<.01	.01	.01	2.07
12.1	.14	.15	.12	.07	ND	.06	.05	.05	.52	.08	.03	ND	ND	.01	.01	1.31
12.5	.24	.41	.20	.45	ND	.05	.05	.10	.12	.08	.06	.01	.02	.05	.03	1.89
Mean	.16	.24	.14	.16	ND	.07	.05	.08	.51	.07	.03	<.01	.01	.02	.01	1.57
Male flower																
2.6	.29	.37	.38	.20	ND	.07	.08	.19	.51	.09	.07	.01	.02	.03	.03	2.34
7.1	.28	.42	.31	.18	ND	.08	.06	.12	.48	.08	.04	ND	<.01	.03	.02	2.08
11.7	.16	.21	.20	.14	ND	.09	.05	.10	.52	.07	.03	<.01	<.01	.01	.01	1.58
12.1	.18	.18	.20	.13	ND	.10	.08	.09	.80	.08	.04	<.01	<.01	.01	.02	1.90
12.5	.14	.21	.16	.31	ND	.04	.04	.07	.18	.10	.06	.01	.01	.01	.02	1.36
Mean	.21	.28	.25	.19	ND	.08	.06	.11	.50	.08	.05	<.01	.01	.02	.02	1.85
Panicle																
2.6	.07	.05	.26	.28	.04	.04	.05	.08	.07	.07	.02	.02	.02	.02	.02	1.08
7.1	.03	.02	.11	.10	.01	.02	.02	.03	.11	.02	.01	.01	.01	.02	.01	.51
11.7	.02	.02	.04	.04	ND	.02	.01	.02	.03	.05	.02	ND	ND	.01	<.01	.28
12.5	.03	.02	.11	.08	.03	.02	.02	.04	.15	.04	.02	.01	.01	.01	.01	.57
Mean	.04	.03	.13	.13	.02	.03	.02	.04	.09	.05	.02	.01	.01	.01	.01	.61

*ASP aspartic acid; GLU glutamic acid; SER serine; GLN glutamine; HIS histidine; ARG arginine; THR threonine; ALA alanine; PRO proline; VAL valine; MET methionine; LEU leucine; ILE isoleucine; PHE phenylalanine; LYS lysine; ND not detectable

were the highest components of the extrafloral nectar and phenylalanine, lysine, leucine, and isoleucine were present in the lowest amounts.

7.4 Discussion

The nectariferous tissues of the hermaphrodite and male flowers and of the panicle were similar anatomically and consisted of secretory trichomes. This is a common nectary type in the flowers of many species including those of the family Bignoniaceae (Subramanian and Inamdar, 1985; Findlay and Mercer, 1971a, b) and in *Lonicera japonica* (Fahn and Rachmilevitz, 1970). The situation in cashew, however, differs from that in some other plants such as *Kigelia pannata* (Subramanian and Inamdar, 1985) and *Campsis* (Elias and Gelband, 1976) in which floral and extrafloral nectaries differed in anatomical structure. The thick continuous cuticle observed covering the outer surface of the cashew secretory cells has been reported in nectaries of many other species (Findlay and Mercer, 1971a, b; Marginson *et al.*, 1985; Elias and Gilband, 1976; Beardsell *et al.*, 1989). In *Abutilon* the nectar passes through the cuticle via pores (Subhramanian and Inamdar, 1985), but in *Thryptomene calycina* (Beardsell *et al.*, 1989), *Acacia terminalis* (Marginson *et al.*, 1985), and *Campsis* (Elias and Gelband, 1976) there were no pores, as is the case in the cashew. The nectar must either tear the cuticle or pass through it by diffusion. Torn cuticles were not observed in cashew nectaries indicating that the nectar reaches the exterior of the trichome via diffusion.

The histochemical evidence shows that the nectariferous tissues contain starch grains. This is probably related to sugar metabolism and nectar secretion. In *Passiflora* spp. (Dukee *et al.*, 1981) the nectar is composed of sugars which are supplied by the phloem of the vascular bundles terminating in the secretory tissue. Nectariferous tissues of cashew have no direct vascular connection. The deposition of starch grains in the nectariferous and parenchymatous tissues beneath may be

supplied by phloem sugar, and the starch grains may be subsequently broken down to nectar sugar.

Cashew nectar is glucose and fructose dominant as is the nectar of many species (Wykes, 1951; Percival, 1961). Many factors have been reported to influence the ratio of nectar sugars. Walker *et al.* (1974) reported that genetic and environmental factors have a combined effect on the sugar ratio in the nectar of alfalfa (*Medicago sativa*). Frey-Wyssling *et al.* (1954) found that the enzyme invertase, which is secreted by nectary tissue, can cause changes in hexose : sucrose ratios in nectar. The mean nectar volume per panicle from one collection at peak secretion is very high, and an even higher volume is expected over the life of the panicle. The nectar volume of the hermaphrodite flower is twice as high as the male. This is probably due to the difference in floral size, as the hermaphrodite flower is larger than the male. A positive relationship between floral size and nectar volume has been reported in soybean, *Glycine max* (Robacker *et al.*, 1983), alfalfa, *Medicago sativa* (Barnes and Furgala, 1978), and in birdsfoot trefoil, *Lotus corniculatus* (Murrell *et al.*, 1982).

Cashews in Kununurra flower in the dry season which has a relatively high daytime temperature and very low relative humidity. A dry environment increases the concentration of nectar (Corbet, 1978; Corbet *et al.*, 1979) and may explain the high sugar concentrations recorded. The anatomical structure of the nectary can also affect concentration of the nectar. Due to the lack of a direct vascular connection, the nectary tissue may have a limited water supply, and this may result in a high nectar concentration (Beardsell *et al.*, 1989).

Proline is uncommon in nectar (Baker and Baker, 1975; Gottsberger *et al.*, 1989) and its presence in the nectar of cashew may be related to the dry conditions under which the trees flower. Alternatively it is possible that there was some contamination of the floral nectar with pollen, as proline is the dominant amino acid

of cashew pollen (chapter 6) and the flowers sampled were not emasculated. The three nectar types varied slightly in both amino acid composition and content and the variation between floral and extrafloral nectar was greater than between the two floral nectars. In the floral nectar proline, glutamic acid, aspartic acid, glutamine, serine, and alanine were present in the greatest concentrations and with the exception of proline these amino acids are also common in the nectar of other species (Gottsberger *et al.*, 1989; Gottsberger *et al.*, 1990).

The cashew tree produces nectar secretions from a range of locations over most of the year. The function of the panicle and floral nectar is probably the attraction of insects for pollination. The panicle nectaries commence secretion prior to anthesis of the first flower and reach peak secretion at peak anthesis. This nectar has a very high sugar concentration and so is a good food source for foraging insects. Amino acids are particularly important for social insects as nutrition for their larvae, thus indicating that the cashew is adapted to pollination by bees or wasps.

The secretion provided by the developing fruit is clearly not associated with attraction of insects for pollination, as is the case for the leaf secretion for most of the year. The function of these secretions may be to attract ants for protection of the fruits and leaves from predators. Ants were commonly observed on the cashew trees, and similar associations have been described in a number of species (Bentley, 1977). The ants guard the tree, which provides them with year-round food, from predatory leaf or fruit eating insects. It is possible that the panicle nectaries may share the role of attraction of pollinators with the attraction of protective insects to deter flower-consuming predators. The fact that the cashew tree produces secretion from so many areas over such a long period indicates important associations with insect visitors to the tree. This is an area which requires further research, as the ants repel honeybees from the panicles, and may also adversely affect pollination via an inhibitory effect on pollen viability (Beattie *et al.*, 1984).

8. Pollen pistil interaction and breeding system

8.1 Introduction

This chapter investigates three aspects of the breeding system of cashew, including timing of pistil receptivity to pollen, timing of pollen tube growth, and genotypic compatibility in the field situation. The results will improve our knowledge of the breeding system and provide information useful in the selection of superior genotypes and in orchard design.

8.2. Materials and methods

Three experiments were carried out in the sandy soil block, and a repeat of one was done in the black soil block. Details of the trees are presented in chapter 3.

8.2.1 Timing of pistil receptivity to pollen

Four cashew trees; 7.1, 11.7, 12.1, and 12.5 were selected in 1988; two (7.1, 11.7) were used as female parents, and the other two (12.1, 12.5) as male parents. Newly opened panicles were labeled, all open flowers were removed and the panicles were bagged. Next morning the bags were removed from the panicles and the hermaphrodite flowers were emasculated. Fifteen flowers were pollinated with pollen from each male parent, by brushing a freshly-dehisced large anther against the stigma, at each of 0, 3, 6, and 24 hours after first opening of the flower. A further 15 flowers were left unpollinated. The pollinated and unpollinated control flowers were rebagged, harvested 24 hours after pollination and emasculation respectively, and the pistils fixed in Carnoy's fixative. The single ovule was dissected from each pistil, and both ovule and pistil were hydrated, softened, stained with decolourised aniline blue and observed using fluorescence microscopy (Martin, 1959). Pollen grains were counted on the stigma, and pollen tubes were counted in the upper style, the lower style, the ovary, and ovule. The results were analysed

using three way analysis of variance for the number of pollen grains on the stigma, and the number of pollen tubes in the upper and lower style. A three way contingency table was used to analyse the number of ovaries and ovules with pollen tubes.

8.2.2 Timing of pollen tube growth

The cashew trees, pollination technique, microscopy procedure, and statistical analysis were as described above. Flowers were pollinated in 1988 at anthesis, and 15 were harvested at each of 3, 6, 24, and 48 hours after pollination. Unpollinated control flowers were harvested 24 hours after anthesis

8.2.3 Genotype compatibility

Three 5 x 5 diallel pollination experiments were conducted using single tree plots. The pollination technique and microscopy procedure were as described above. In experiment 1, conducted in 1988, fifty flowers from each of trees 2.6, 6.6, 10.9, 12.1 and 15.15 were emasculated and pollinated at anthesis, and 25 of these were harvested and fixed in Carnoy's fixative 24 hours after pollination. The remaining 25 flowers were left to set fruit, and final set was assessed by counting the remaining mature fruit two months after pollination. Experiments 2 and 3 were conducted in 1990 using genotypes 1.2, 1,12, 4.3, 6.6, 10.9, 11.7 and 12.1, and results were assessed by mature fruit set only. For the pollen tube data set, the following binomial model was fitted:

$$y_{ijk} \sim B(n_{ijk}, p_{ij})$$

where $i = 1,2,.....,5$; $j = 1,2,.....,5$; $k = 1,2,.....s_{ij}$; s_{ij} is the number of pistils for female i and male j; y_{ijk} is the number of pollen tubes in the ovule for female i, male j and pistil k; n_{ijk} is the number of pollen tubes in the upper half of the style for female i, male j and pistil k; p_{ij} is the probability that a pollen tube reaches the ovule for female i and male j. The model was fitted using Genstat (Rothamstead

Experimental Station) to find estimates of pij ($\hat{p}_{ij}$) to provide information on which crosses were the best. yijk and nijk were also expressed as a ratio xijk. The data were then analysed as a diallel cross (Sedgley *et al.*, 1990), following removal of the self pollination data which may have introduced bias (Griffing, 1956a,b). Similarly for the fruit set data the following binomial model was used:

$$yijk' \sim B(nijk',pij')$$

where i= 1,2,.....,5; j= 1.2,.....,5; k= 1,2,.....,sij'; sij' is the number of flowers for female i and male j; yijk' is the number of flowers showing fruit set for female i and male j and flower k; nijk' is the number of flowers pollinated for female i and male j and flower k; pij' is the probability that a flower will show final fruit set for female i and male j. Genstat was again used to estimate pij'($\hat{p}_{ij}$') and a diallel analysis of the cross pollination data was carried out on the ratio xijk' where xijk'=yijk'/nijk' (Sedgley *et al.*, 1990).

8.3. Results

Pollen germination and pollen tube growth in the pistil of cashew is shown in figure 8.1.

8.3.1 Timing of pistil receptivity to pollen

There was a trend toward reduced numbers of pollen grains germinating on the stigma, and reduced pollen tube growth in the upper and lower style, when pollination was delayed beyond 3 hours after anthesis (Table 8.1). Moreover, the number of pistils with the ovule penetrated by a pollen tube was significantly reduced when pollination was delayed until 6 or 24 hours after anthesis (Table 8.2).

8.3.2 Timing of pollen tube growth

There was a trend toward increased numbers of pollen grains germinating on the stigma, and increased pollen tube growth in the upper style, at 48 hours after

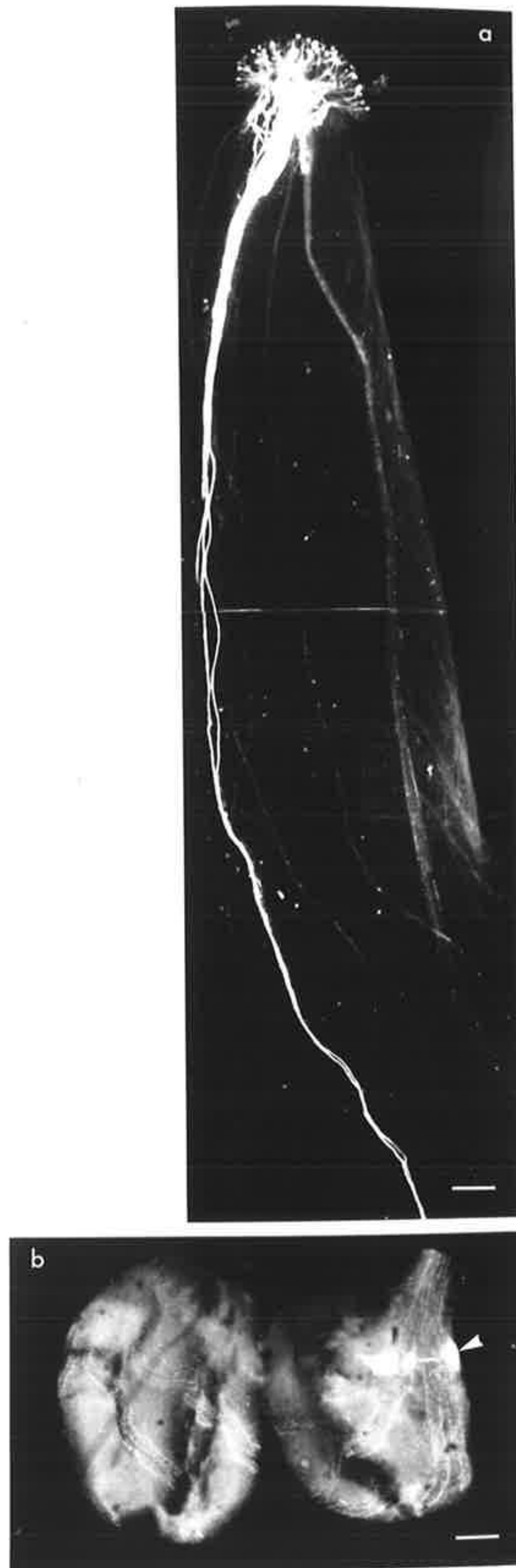

Figure 8.1 Fluorescence micrograph of a) pollen grain germination and pollen tube growth and b) ovule penetration (arrow) in the pistil of cashew at 24 hours after cross pollination. Bar represents 200 µm.

pollination as compared with 3, 6, or 24 hours (Table 8.3) indicating a progressive increase in adhesion, germination and pollen tube growth. In addition, the number of pollen tubes in the lower style, and the number of ovaries and ovules penetrated by a pollen tube, increased with increasing time after pollination (Tables 8.3, 8.4). A few pollen tubes had reached the ovary by three hours after pollination, and penetration of the ovules was observed at 24 hours.

8.3.3 Genotype compatibility

There was significant variation between the pollinations as measured by both pollen tube growth and final fruit set (Tables 8.5, 8.6, 8.7). There was no difference between the fertility of self and cross pollinations as measured by pollen tube growth, but the final fruit set values generally showed lower yields following self than cross pollination (Table 8.5). The analysis of variance (Table 8.6) of the cross pollen tube growth figures in experiment 1 demonstrated significant specific combining ability (SCA) amongst the genotypes, whereas that for the final fruit set results showed significant general combining ability (GCA) in all three experiments, but significant SCA in experiment 3 only. Thus for the pollen tube growth results of experiment 1, and the final fruit set of experiment 3, the actual $\hat{p}_{ij}$ and $\hat{p}_{ij}'$ values should be compared (Table 8.5) whereas for the final fruit set results for experiments 1 and 2 the $\hat{p}_i'$ (probability of fruit set for female i across all male parents), and $\hat{p}_j'$ (probability of fruit set for male j across all female parents) values should be compared (Table 8.8). Genotypes 12.1 and 10.9 generally performed well as either female or male parent when crossed with other trees, whereas genotype 4.3 performed very poorly as a female parent. SCA for final fruit set was significant in experiment 3, with 10.9 x 11.7 and 2.6 x 12.1 the most successful combinations. Selfing of 2.6 and 6.6 was poor, as was the cross between 2.6 and 6.6. When the crosses were ranked according to level of fertility as measured by both methods in experiment 1 and for fruit set in all three experiments, the ranking between pollen tube growth and final fruit set in

Table 8.1 Pollen germination on the stigma and pollen tube growth in the upper and lower style in cashew flowers hand pollinated at varying times after anthesis, and harvested 24h after pollination.

Cross female x male	Mean number of pollen grains on the stigma following pollination at x hours after anthesis				Mean number of pollen tubes in the upper style following pollination at x hours after anthesis				Mean number of pollen tubes in the lower style following pollination at x hours after anthesis			
	x=				x=				x=			
	0	3	6	24	0	3	6	24	0	3	6	24
7.1 x 12.1	23.5	22.8	21.9	10.5	6.4	9.1	6.4	4.0	1.5	2.3	1.0	0.9
7.1 x 12.5	20.6	12.9	14.6	14.8	5.4	3.6	6.8	3.7	2.1	0.6	0.7	0.9
11.7 x 12.1	14.2	20.7	17.7	19.3	5.9	8.0	5.3	6.2	1.9	1.3	1.2	1.7
11.7 x 12.5	23.6	24.5	9.9	18.1	6.9	8.3	4.2	4.1	1.4	2.2	1.0	1.6
Mean	20.5	20.9	16.0	15.7	6.2	7.3	5.7	4.5	1.7	1.6	1.0	1.3

There was a significant interaction of female parent, male parent and time of pollination after anthesis on number of pollen grains on the stigma ($p=0.01$), the number of pollen tubes in the upper style ($p=0.001$) and the number of pollen tubes in the lower style ($p=0.001$).

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Table 8.2 Number of ovaries and ovules penetrated by at least one pollen tube in cashew flowers hand pollinated at varying times after anthesis, and harvested 24 hours after pollination.

Cross female x male	Number of ovaries, of 15 flowers, penetrated by a pollen tube following pollination at x hours after anthesis				Number of ovules, of 15 flowers, penetrated by a pollen tube following pollination at x hours after anthesis			
	x=				x=			
	0	3	6	24	0	3	6	24
7.1 x 12.1	15	15	12	13	14	11	1	1
7.1 x 12.5	15	9	10	11	15	5	1	0
11.7 x 12.1	15	15	15	14	12	9	3	7
11.7 x 12.5	14	15	12	13	11	14	4	1
Mean	14.8	13.5	12.3	12.8	13.0	9.8	2.3	2.3

There were no significant differences due to female parent, male parent or time of pollination after anthesis on the number of ovaries penetrated by a pollen tube, but there was a significant interaction between female parent and time of pollination on the number of ovules penetrated by a pollen tube ($p=0.05$).

Table 8.3 Pollen germination on the stigma and pollen tube growth in the upper and lower style in cashew flowers hand pollinated 0 hours after anthesis and harvested at varying times after pollination.

Cross female x male	Mean number of pollen grains on the stigma at x hours after pollination x=				Mean number of pollen tubes in the upper style x hours after pollination x=				Mean number of pollen tubes in the lower style x hours after pollination x=			
	3	6	24	48	3	6	24	48	3	6	24	48
7.1 x 12.1	15.9	12.4	13.5	24.9	3.2	3.6	2.5	6.8	0.3	0.9	1.3	1.6
7.1 x 12.5	14.5	7.1	16.8	13.3	3.3	3.4	3.7	3.3	0.5	1.1	2.1	1.5
11.7 x 12.1	16.9	23.9	24.0	23.1	4.8	4.9	5.1	4.3	1.0	1.1	2.8	3.1
11.7 x 12.5	23.5	20.3	18.7	28.3	4.5	3.5	5.1	4.9	0.9	1.0	2.7	2.6
Mean	17.7	18.4	18.3	22.4	4.0	3.8	4.1	4.9	0.7	1.0	2.2	2.2

There was a significant interaction of female parent, male parent and time of harvest after pollination on the number of pollen grains on the stigma ($p=0.05$) and the number of pollen tubes in the upper style ($p=0.001$). There was no three way interaction on the number of pollen tubes in the lower style, but there was a significant interaction of female parent and time ($p=0.001$) and of female and male parent ($p=0.05$).

Table 8.4 Number of ovaries and ovules penetrated by at least one pollen tube in cashew flowers hand pollinated 0 hours after anthesis and harvested at varying times after pollination.

Cross female x male	Number of ovaries, of 15 flowers, penetrated by a pollen tube following pollination at 0 hours after anthesis and harvested x hours after pollination				Number of ovules, of 15 flowers, penetrated by a pollen tube following pollination at 0 hours after anthesis and harvested x hours after pollination			
	x=				x=			
	3	6	24	48	3	6	24	48
7.1 x 12.1	0	6	10	15	0	0	8	14
7.1 x 12.5	0	9	14	12	0	0	13	12
11.7 x 12.1	7	3	15	15	0	1	11	13
11.7 x 12.5	1	4	15	15	0	0	11	15
Mean	2.0	5.5	13.5	14.3	0	0.2	10.8	13.5

There was a significant interaction of female parent and time of harvest after pollination on the number of ovaries penetrated by at least one pollen tube (p=0.05). There was a significant difference in the number of ovules penetrated by a pollen tube and time of harvest after pollination (p=0.05). There were no significant differences due to female parent, male parent and time when only 24 and 48 hours were considered.

Table 8.5 Pollen tube growth ($\hat{p}_{ij}$) and final fruit set ($\hat{p}_{ij}'$) following self and cross pollination of cashew genotypes.

Experiment 1
Pollen tube growth

		Male				
		2.6	6.6	10.9	12.1	15.15
	2.6	0.0890	0.1239	0.0901	0.1410	0.0925
	6.6	0.1131	0.1146	0.0861	0.1111	0.1382
Female	10.9	0.0381	0.0610	0.1130	0.1494	0.0960
	12.1	0.0802	0.0763	0.1583	0.1111	0.1145
	15.15	0.1442	0.1314	0.1240	0.0978	0.1810

Experiment 1
Final fruit set

		Male				
		2.6	6.6	10.9	12.1	15.15
	2.6	0.0001	0.0001	0.0001	0.4000	0.0001
	6.6	0.0714	0.0909	0.2381	0.4348	0.4348
Female	10.9	0.1500	0.1364	0.0909	0.2083	0.1429
	12.1	0.1739	0.2273	0.5652	0.0870	0.2800
	15.15	0.2414	0.1111	0.3333	0.2000	0.0714

Experiment 2
Final fruit set

		Male				
		2.6	1.12	4.3	10.9	12.1
	2.6	0.0000	0.1613	0.0303	0.1714	0.1212
	1.12	0.2162	0.1471	0.3056	0.1818	0.3529
Female	4.3	0.0294	0.0263	0.0000	0.0000	0.0000
	10.9	0.0303	0.2000	0.3125	0.0857	0.3226
	12.1	0.1842	0.2903	0.1429	0.4333	0.0645

Experiment 3
Final fruit set

		Male				
		2.6	6.6	10.9	11.7	12.1
	2.6	0.0556	0.0000	0.3125	0.2727	0.5294
	6.6	0.0001	0.0001	0.4872	0.3611	0.3243
Female	10.9	0.3871	0.4857	0.0857	0.5854	0.3226
	11.7	0.5000	0.3030	0.4194	0.0909	0.2857
	12.1	0.3143	0.2286	0.4333	0.1176	0.645

Table 8.6 Analysis of variance of pollen tube growth and final fruit set following cross pollination of cashew genotypes.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio	Significance
Experiment 1					
Pollen tube growth					
Mean	1	8.67	8.67	367.75	p<0.01
GCA	4	0.14	0.04	1.52	NS
SCA	5	0.91	0.18	7.73	p<0.01
Reciprocal	10	0.35	0.04	1.49	NS
Error	424	10.00	0.02		
Total	444	20.08			
Experiment 1					
Final fruit set					
Mean	1	2.80	2.80	73.07	p<0.001
GCA	4	0.67	0.17	4.35	p=0.005
SCA	5	0.05	0.01	0.25	NS
Reciprocal	10	0.77	0.08	2.02	NS
Error	41	1.57	0.04		
Total	61	5.85			
Experiment 2					
Final fruit set					
Mean	1	2.13	2.13	99.53	p<0.001
GCA	4	0.48	0.12	5.65	p=0.008
SCA	10	0.18	0.04	1.72	NS
Reciprocal	10	0.40	0.04	1.88	NS
Error	58	1.03	0.02		
Total	83	4.23			
Experiment 3					
Final fruit set					
Mean	1	10.10	10.10	191.32	p<0.001
GCA	4	1.07	0.27	5.06	p=0.001
SCA	5	1.05	0.21	3.96	p=0.003
Reciprocal	10	0.52	0.05	0.99	NS
Error	77	4.07	0.05		
Total	97	16.81			

Table 8.7 Variance components of pollen tube growth and final fruit set following cross pollination of cashew genotypes.

Source of variation	Value	Standard deviation	% total variance
Experiment 1			
Pollen tube growth			
GCA	0.0011	0.0007	3.86
SCA	0.0036	0.0022	12.55
Reciprocal	0.0003	0.0003	1.16
Error	0.0236	0.0016	82.68
Experiment 1			
Final fruit set			
GCA	0.0088	0.0053	16.30
SCA	0.0054	0.0016	0.00
Reciprocal	0.0069	0.0058	12.73
Error	0.0383	0.0083	70.97
Experiment 2			
Final fruit set			
GCA	0.0041	0.0036	13.49
SCA	0.0022	0.0030	7.22
Reciprocal	0.0028	0.0025	9.27
Error	0.0214	0.0043	70.02
Experiment 3			
Final fruit set			
GCA	0.0018	0.0066	2.57
SCA	0.0164	0.0118	23.09
Reciprocal	0.0000	0.0024	0.00
Error	0.0528	0.0084	74.34

Table 8.8 Final fruit set of cashew genotypes when used as either female ($\hat{p}_{i'}$) or male ($\hat{p}_{j'}$) parents.

Genotype	Female	Male
Experiment 1		
2.6	0.0941	0.1600
6.6	0.2842	0.1170
10.9	0.1609	0.2935
12.1	0.3118	0.3043
15.15	0.2222	0.2222
Experiment 2		
1.2	0.1212	0.1197
1.12	0.2642	0.1630
4.3	0.0139	0.1986
10.9	0.2137	0.1866
12.1	0.2537	0.1940
Experiment 3		
2.6	0.2586	0.2789
6.6	0.2801	0.2381
10.9	0.4564	0.4165
11.7	0.3652	0.3473
12.1	0.2685	0.3543

Table 8.9 Cashew pollinations in order of decreasing fertility.

Experiment 1 Pollen tube growth female x male	Experiment 1 Final fruit set female x male	Experiment 2 Final fruit set female x male	Experiment 3 Final fruit set female x male
15.15 x 15.15	12.1 x 10.9	12.1 x 10.9	10.9 x 11.7
12.1 x 10.9	6.6 x 15.15	1.12 x 12.1	2.6 x 12.1
10.9 x 12.1	6.6 x 12.1	10.9 x 12.1	11.7 x 2.6
15.15 x 2.6	2.6 x 12.1	10.9 x 4.3	6.6 x 10.9
2.6 x 12.1	15.15 x 10.9	1.12 x 4.3	10.9 x 6.6
6.6 x 15.15	12.1 x 15.15	12.1 x 1.12	12.1 x 10.9
15.15 x 6.6	15.15 x 2.6	1.12 x 1.2	11.7 x 10.9
15.15 x 10.9	6.6 x 10.9	10.9 x 1.12	10.9 x 2.6
2.6 x 6.6	12.1 x 6.6	12.1 x 1.2	6.6 x 11.7
6.6 x 6.6	10.9 x 12.1	1.12 x 10.9	6.6 x 12.1
12.1 x 15.15	15.15 x 12.1	1.2 x 10.9	10.9 x 12.1
6.6 x 2.6	12.1 x 2.6	1.2 x 1.12	12.1 x 2.6
10.9 x 10.9	10.9 x 2.6	1.12 x 1.12	2.6 x 10.9
6.6 x 12.1	10.9 x 15.15	12.1 x 4.3	11.7 x 6.6
12.1 x 12.1	10.9 x 6.6	1.2 x 12.1	11.7 x 12.1
15.15 x 12.1	15.15 x 6.6	10.9 x 10.9	2.6 x 11.7
10.9 x 15.15	6.6 x 6.6	12.1 x 12.1	12.1 x 6.6
2.6 x 15.15	10.9 x 10.9	10.9 x 1.2	12.1 x 11.7
2.6 x 10.9	12.1 x 12.1	1.2 x 4.3	11.7 x 11.7
2.6 x 2.6	15.15 x 15.15	4.3 x 1.2	10.9 x 10.9
6.6 x 10.9	6.6 x 2.6	4.3 x 1.12	12.1 x 12.1
12.1 x 2.6	2.6 x 15.15	4.3 x 12.1	2.6 x 2.6
12.1 x 6.6	2.6 x 10.9	4.3 x 10.9	6.6 x 2.6
10.9 x 6.6	2.6 x 6.6	4.3 x 4.3	6.6 x 6.6
10.9 x 2.6	2.6 x 2.6	1.2 x 1.2	2.6 x 6.6

experiment 1 was similar with the exception of the self pollinations, and the fruit set rankings were similar between the three experiments across the common genotypes (Table 8.9).

8.4 Discussion

This work has shown that the cashew pistil has a relatively short period of receptivity to pollen, that pollen tube growth is rapid, and that there is a reduction in yield following self as compared with cross pollination which is not due to pollen-pistil incompatibility. The results have significance with regard to the commercial production of cashew, as the provision of insect pollinators in the orchard must ensure the rapid transfer of pollen between the trees, and the arrangement of the genotypes in the plantation should give the maximum opportunity for cross pollination to occur.

For optimum fertility, the hermaphrodite cashew flower should be pollinated within the first three hours of anthesis. Limited periods of pistil receptivity are not unusual amongst crop plants, and have lead to the concept of the effective pollination period (EPP). This is the length of the period of ovule longevity, minus the time taken for pollen tubes to reach the ovule (Williams, 1969). In effect, it is the period during which pollen transfer must occur in order that fertilisation and fruit set will ensue. Research with apple has shown that supplementary pollination beyond the EPP will not increase fruit set (Williams, 1970). In general, the EPP of a species decreases with increasing temperature (Vasilakakis and Porlingis, 1985), and tropical crops thus tend to have particularly short periods of receptivity. In avocado, a dichogamous subtropical crop, the flower must be pollinated while the flower is in the female stage, or fruit set will not occur (Sedgley, 1977). This female stage lasts for only a few hours, and pollinating insects must be active during this limited period. Similarly in the mango, Spencer and Kennard (1955) have reported that the stigma remains receptive to pollen for only a few hours.

Pollen tube growth is rapid in cashew pistils, with the majority of the pollen tubes reaching the base of the style by three hours after pollination. Despite this initial rapid growth, however, only one ovule was penetrated by a pollen tube at six hours, with significant penetration at 24 hours after pollination. This slowing of pollen tube growth upon reaching the ovary is a common phenomenon in tree crops, and has been reported in avocado (Sedgley,1979), almond (Pimienta *et al.*, 1983), cherry (Anvari and Stosser, 1978), and citrus (Ton and Krezdorn, 1967). In some other crops, this may be due to immaturity of the ovule at anthesis. Full maturity of the pear embryo sac is delayed until five days after anthesis (Herrero, 1983), and the extracellular secretion required for pollen tube growth is not produced by the peach ovary until 12 days after anthesis (Arbeloa and Herrero, 1987).

A particularly interesting finding of this work is that there is no pollen-pistil incompatibility mechanism operating in the cashew pistil, yet final nut yield is reduced following selfing. Self incompatibility mechanisms commonly operate via inhibited or retarded pollen tube growth in the stigma or style (de Nettancourt, 1977). In the case of the cashew, however, there was no difference in pollen germination or pollen tube growth to the ovule between self and cross pollination. Thus, the self sterility mechanism in cashew is operating after ovule penetration. Inhibition of self pollen tube growth occurs in the nucellus in *Acacia retinodes* (Kenrick *et al.* 1986), but this did not appear to be the case in cashew. There is increasing evidence that postzygotic mechanisms may be responsible for self sterility of many tree crops (Sedgley and Griffin, 1989). Abortion of selfed embryos occurs at the four to six celled stage in *Liquidambar* (Schmitt and Perry, 1964), and preferential premature shed of selfed rather than crossed fruits occurs in avocado (Degani *et al.*, 1989).

Although there was SCA amongst the genotype crosses as measured by pollen tube growth, the yield measurement demonstrated GCA with SCA effects in only one of

the three experiments. SCA indicates that some specific genotype combinations are better or worse than most of the others, whereas GCA shows that some female or male parents are better or worse than others across all pollinations. Thus the diallel analysis can be used to identify genotypes of superior fertility and suggests that 12.1, 10.9 and 11.7 would provide the basis of a high yielding cashew orchard, but that on the basis of fertility genotype 4.3 should omitted. Significant reciprocal effects indicate that some genotype combinations are more fertile in one direction than in the other, but this was not detected amongst the cashew genotypes. It must be remembered, however, that considerations other than fertility, such as kernel quatility or size, are significant factors in the assessment of a genotype, and that synchrony of flowering must be also be tested in a new production area. The three experiments showed differences in the results with regard to SCA and GCA effects. This is probably due to the inclusion of different genotypes, different season, and different orchards, and highlights both the large amount of variability between trees and the complex interaction between genotype and environment.

This is the first study involving pollen tube growth and compatibility relationships of cashew genotypes, and the results have important implications for plantation establishment for this crop. It is clear that the arrangement of genotypes in the plantation should minimise the possibility of self pollination, by ensuring effective interplanting of superior types. In addition, adequate provision of active insect pollinators is essential for pollen transfer of compatible pollen to the hermaphrodite flowers as soon as possible after anthesis. Most important of all, however, is the selection of genotypes as this has a major bearing on the potential yield of the orchard.

9 General discussion

The flowering and fruiting phenological data in this study generally agree with the results of studies in other areas. Flowering started after the rainy season, there were more male than hermaphrodite flowers in a panicle, and there were large numbers of initial fruit set but relatively small numbers of fruit maturing (Rao and Hassan, 1957; Northwood, 1966; Wunnachit *et al.*, 1986; Heard *et al.*, 1990). There are two areas of difference however. Firstly in this study hermaphrodite flowers dominated in weeks 1 and 2 of flowering. Pavithran and Ravindranathan (1974) and Paramesawaran *et al.* (1984) found that flower opening in cashew panicles occurred in three phases of male, mixed and male. They suggested that the duration of the mixed phase should be considered while selecting superior cashew trees along with other selection indices related to flowering. In the Northern Territory of Australia, Heard *et al.* (1990) found that three quarters of the breeding lines investigated had more male than hermaphrodite flowers throughout the flowering period. Secondly there is a highly positive correlation between number of hermaphrodite flowers and initial and final fruit set. This does not agree with the study of Heard et al. (1990), in which there was no significant correlation between number of hermaphrodite flowers and fruit set. It must be accepted that both genetics and environment control the flowering and fruiting of the cashew (Nambiar 1979).

There was a high failure of fruit set in the first week after flowering which may be an effect of pollen limitation. This was followed by fruit drop after week three, which was unlikely be the result of pollen limitation because it happened after setting. Northwood (1966) reported that pollination was adequate and that physiological limitations caused abortion of young fruit. Heard *et al.* (1990) considered that post-pollination events and not pollen transfer were limiting yield. In this study the results indicate that both pollen transfer and postzygotic factors are

limiting yield in Kununurra. The problem of inadequate pollen transfer may indicate that the bees were inefficient, and this is an area which requires further research. The post-pollination fruit drop is a more complex problem. There are several hypotheses relating fruit drop to post-zygotic factors such as selective abortion and resource limitation (Stephenson, 1981; Wiens, 1984; Carr, 1991). Stephenson (1980) reported that in *Catalpa speciosa* inflorescences that set 1, 3, and 6 fruits aborted 42, 68, and 81% of subsequent fruits respectively. Experiments on artificial thinning of flowers or juvenile fruits resulted in little or no abortion, and on unthinned inflorescences fruit drop increased in proportion to the number of fruit initiated (Quinlan and Preston, 1968). Postzygotic self-incompatibility has been reported to affect premature fruit drop in many species (Romberg and Smith, 1946; Bradley and Griggs, 1963; Griffin *et al.*, 1987; Sedgley and Griffin, 1989), including mango (Sharma and Singh, 1970). There was synchronised opening of hermaphrodite and male flowers thus facilitating pollen from one flower to pollinate another flower on the same plant. In addition the four types of pollen grains all have a chance to pollinate a hermaphrodite pistil, but all are not equally effective in pollination. So the poor final fruit set of open pollination possibly resulted from selfing, particularly if LH and SH pollen grains of a tree pollinated it's own pistils. The high proportion of male to hermaphrodite flowers over the whole flowering period of the cashew is probably an adaptation to produce high enough numbers of pollen grains to ensure maximum pollination (Faegri and van der Pijl, 1979; Sutherland, 1987).

Most of the trees in Kununurra had a small flush and flowering at the beginning of the season, prior to the main flushing and flowering in June. This split flowering and fruiting habit is costly in terms of management, and it is better for the plantation to have one major peak of flowering. To achieve this selection of superior genotypes which have only one flowering period should be considered.

Flowering and fruiting of the cashew trees in Kununurra occurs in the dry season. This on the one hand has the advantage of decreasing disease levels (Krisaneepaiboon *et al.*, 1988) but on the other hand the very high temperatures of the dry season can have an adverse effect on pollination by shortening stigma receptivity, pollen viability, and pollen tube growth (Kendall and Taylor, 1969; Sedgley, 1977; Herrero and Johnson, 1980; Sedgley and Annells, 1981; Mascarenhas and Altschuler, 1983; Maestro and Alvarez, 1988). The structure of the stigma is well adapted to dry weather by having a thick continuous cuticle over the stigmatic surface. But this thick cuticle may also be a barrier to pollen germination (Roggen, 1972; Kambal *et al.*, 1976; Lord and Heslop-Harrison, 1984), particularly when the dryness decreases pollen viability. There was very little exudate on the stigmatic surface, but the papilla cells and extracellular secretions contained lipid, protein and carbohydrate which are common in stigmatic exudates of many species (Konar and Linskens, 1966; Martin, 1969; Knox *et al.*, 1976; Clarke *et al.*, 1979; Herrero and Dickinson, 1979). The presence of the stigmatic exudate must presumably have an adaptive advantage for those genera possessing it. The exudate may be important in pollen recognition (Knox, 1984), have a protective role (Konar and Linskens,1966), or it may serve as an attraction or reward to pollinating insects which may collect it (Lord and Webster, 1979). For the cashew, the little exudate secreted onto the thick stigmatic cuticle is more likely to have a function in pollen recognition and in protection from desiccation in dry weather. There have also been reports in many species that stigmatic exudate has a role in the pollen-pistil interaction leading to incompatibility (Dickinson and Lewis, 1973; Heslop-Harrison, 1975; Sedgley, 1977; Knox, 1984), but this is unlikely in the cashew. Pollen tube growth showed no signs of arrest and the pollen germinated quickly. Pollen tube growth was delayed after entering the ovary prior to penetration of the ovule, and this is probably because of the immaturity of the ovule as reported in pear (*Pyrus communis*) (Herrero and Gascon, 1987) and in

peach (*Prunus persica*) (Herrero and Arbeloa, 1989). No structural or histochemical investigations of the ovule were conducted in this study and in further research the structural development of the ovule from prior to post anthesis, with and without pollination, is required.

This is the first comparative study of the four pollen types from cashew hermaphrodite and male flowers. Morphological characters and the numbers of pollen grains produced were similar between the two flower types, and this is in agreement with the study by Solomon (1986) on andromonoecious *Solanum carolinense* (Solanaceae), but not with the study of McKone and Webb (1988) in two andromonoecious species of the Apiaceae. The differences in pollen morphology, numbers of pollen grains and pollen composition between the two types of flowers in andromonoecious families might be the result of different steps of evolution from hermaphrodism to unisexuality (Stephenson, 1980; Stephenson, 1981; Solomon, 1986; McKone and Webb, 1988). The four pollen types of the hermaphrodite and male flowers of cashew differed in a number of aspects including number of grains per anther, longevity ', fertility and chemical composition. The pollen grains produced by the hermaphrodite flowers have reduced capacity for *in vivo* pollen germination and ovule penetration, but have higher contents of both sugars and amino acids than pollen grains from the male flowers. It is also possible that the hermaphrodite flowers produce pollen grains which attract insects to visit the flowers, while the male flowers produce pollen grains which effect pollination. The phenomenon of large and small stamens within the same flower has been reported in some other genera including *Mangifera* (Anacardiaceae) (Singh, 1954), *Tibouchina* (Melastomataceae) (Baker, 1976), *Cassia* and *Swartzia* (Fabaceae) (Dulberger, 1981). In the latter cases the short stamens were proposed to be a source of pollen for food while the large stamens produced pollen for pollination. This is also true of cashew to some extent as the pollen from the small stamens of both hermaphrodite and male flowers showed lower viability than that from the corresponding large

stamens. In cashew, however, the difference in viability between the pollen of the two flower types was generally greater than that between the two stamen sizes. This research has shown that selection for high yielding cashew genotypes should not be based solely on trees with a high proportion of hermaphrodite flowers. It is also important to have a proportion of male flowers for effective pollination. Faegri and van der Pijl (1979) stated that in the life of the plant, pollen must have a double function both as microspores and as an attractant. As nectar is presented at a certain period only, and as in insect-pollinated plants nectar is generally the chief attractant, the pollen presentation must be synchronized with nectar presentation.

Nectar is the most important floral reward to insect pollinators (Wykes, 1951; Percival, 1961; Corbet, 1978). There were differences in volume, and in sugar and amino acid contents between hermaphrodite and male floral nectar of the cashew. The volume of nectar produced by hermaphrodite flowers was twice as high as that of the male flowers and this increases the attraction of the hermaphrodite flower to insect pollinators. It is interesting to note that the male floral nectar has the highest sucrose and amino acid contents of all the nectar types sampled. Thus insects with brood, especially honeybees which are used commercially for crop pollination, may be encouraged to visit the male flowers in preference to other sources. The relatively low nectar volume of the male flower may further encourage insects to visit large numbers of male flowers. In doing so they would accumulate large amounts of pollen on their bodies which could then be transfered to the stigmas of hermaphrodite flowers. Thus cashew floral nectar has a direct role in pollination, while the extrafloral nectar may have a further role. The function of extrafloral nectar in many species is to attract ants to protect the plant (Bentley, 1977; Pickett and Clarke, 1979). Ants were observed to forage for nectar on the panicle branches, leaves and young fruits of all the trees in this study. They were aggressive and attacked any intruders approaching the panicles. This indicates that the function of the extrafloral nectar of the cashew may be to attract ants for panicle protection.

However, no studies have been conducted in the native habitat in South America to confirm this.

The investigation of the cashew pollen-pistil interaction by *in vivo* pollen germination and pollen tube growth showed that the pistil has a relatively short period of receptivity to pollen, and that the pollen showed rapid germination and tube growth. The effective period of the stigma receptivity to pollen was within three hours after anthesis, which in the field situation in Kununurra is between mid morning and midday. This is also the time of maximum insect foraging (Heard *et al.*, 1990). The rapid pollen tube growth can be explained in terms of an adaptation to the short stigma receptivity, and may also be due to the high ambient temperature. The slowing of pollen tube growth upon reaching the ovary is not uncommon for tropical tree crops (Sedgley and Griffin, 1989), and is not the result of self incompatibility. This conclusion is supported by the similar rates of pollen tube growth following selfing and crossing, followed by ovule penetration within 48 hours after pollination.

The selected genotypes of cashew were tested for self and cross compatibility as assessed by pollen tube growth and final fruit set. These results showed a particularly important characteristic. There was no pollen pistil incompatibility as measured by pollen tube growth but there was a reduction in final fruit set following selfing as compared with crossing. This reduction in final fruit set following selfing has important implications for genotype combinations in the orchard. A similar investigation of self and cross compatibility was carried out with macadamia cultivars by Sedgley *et al.* (1990). They observed that a small number of crosses in the diallel analysis increases the error component of the total variance. This is also true of the diallel analysis of the cashew in this study. However, the analysis of final yield was repeated twice, and GCA was highly significant in all three experiments, while SCA was significant in one experiment only. There was no reciprocal effect in any experiment. All genotypes showed reduced final fruit set following selfing as

compared with crossing. The fact that the cashew shows a degree of self sterility has not been recognised previously. This is probably because, in the past, cashew orchard were established using seedling trees and thus contained a large number of different genotypes which were cross compatible. With the current trend towards orchard establishment using clonally-propagated superior genotypes, the question of compatibility assumes greater importance. Selection of genotypes with high GCA is essential, and genotypes 12.1, 10.9 and 11.7 were the best for interplanting. However, the phenological data for tree 11.7 showed that this genotype is not superior in other characters such as final fruit set. The characters of quantity and quality of pollen and nectar production may also be important, although hermaphrodite and male flowers produced similar numbers of pollen grains and similar volume, concentration and sugar and amino acid composition of nectar. Thus the diallel analysis has to be used together with other flowering and fruiting criteria for genotypic selection and orchard layout.

This study has significantly advanced knowledge of cashew pollination and breeding biology, and the information can now be applied to improvement of cashew production. The finding that the cashew trees tested show a degree of self-sterility means that genotypes must be interplanted in the orchard for maximum production. Some genotypes are superior in terms of combining ability and thus provide further advantages in terms of potential yield. The cashew is insect pollinated and the introduction of honeybee hives during flowering should be considered as an important management practice. This research has shown that the first few weeks of the flowering season are the most likely to set fruit, so strong, active hives must be prepared in advance of the commencement of bloom.

Selection and breeding of improved cultivars is an important goal for the industry in Australia and overseas. This study has identified some new selection criteria. General combining ability is clearly important for high yield potential. Other less obvious features concern the differential pollen fertility and composition

between the hermaphrodite and male flowers. Past selection has concentrated on maximising the number of hermaphrodite flowers produced per tree. The discovery that the pollen from the hermaphrodite flower shows lower fertility than that from the male indicates that a certain proportion of male flowers is necessary for optimum pollination. The existence of extrafloral nectaries has not been previously recognised in cashew. This may also be a significant selection criterion in terms of pest control in the orchard. Further research is required on the insect-plant relationships in this regard.

In conclusion, the pollination and breeding biology of cashew is fundamental to productivity in three major ways. These are optimisation of yield in the orchard, the development of selection criteria for cultivar development, and finally efficient hybridisation techniques, developed in this study, are now available for future plant breeding.

10. References

Ahmedullah, M. 1983. Pollen morphology of selected *Vitis* cultivars. Journal of the American Society for Horticultural Science, 108; 155-160.

Anvari, S. F. and Stosser, R. 1978. Fluorescence microscopic investigations on pollen tube growth and viability of ovules in sour cherry. Mit Klosterneuberg, 28; 23-30.

Arasu, N. N. 1968. Self incompatibility in angiosperms; a review. Genetica, 39; 1-24.

Arbeloa, A. and Herrero, M. 1987. The significance of the obturator in the control of pollen tube entry into the ovary in the peach (*Prunus persica*). Annals of Botany, 60; 681-685.

Argles, G. K. 1969. *Anacardium occidentale* (cashew), ecology and botany in relation to propagation. FAO Conference on Propropagation of Tropical and Subtropical Fruits, pp. 1-24.

Ascenso, J. C. 1970. Patrimonio fruiteiro de Mocambique. Agron Mocambibana, 4; 221-226.

Ascenso, J. C. and Mota, M. I. 1972. Studies on the flower morphology of cashew. Agron Mocambicana, 6, 107-118.

Ascher, P. D. and Peloquin, S. J. 1966. Effect of floral aging on the growth of compatible and incompatible pollen tubes in *Lolium longiflorum* Thumb. American Journal of Botany, 53; 99-102

Baker, H. G. 1976. Chemical aspects of the pollination biology of woody plants in the tropics. In: Tomlinson, P. B. and Zimmermann, M. H. (eds.). Tropical Trees as Living Systems. pp. 57-82. Cambridge University Press, Cambridge, U. K.

Baker, H. G. and Baker, I. 1975. Studies of nectar constitution and pollinator-plant coevolution. In: Gilbert, L. F. and Raven, P. H. (eds.). Coevolution of Animals and Plants. pp. 100-140. Texas University Press.

Baker, R. J. and Lehner, Y. 1976. Milk sugar poisonous to honeybees. American Bee Journal, 116; 322-332.

Baker, H. G. and Baker, I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. American Journal of Botany, 66; 591-600.

Baker, H. G. and Baker, I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones, C. E. and Little, J. (eds). Handbook of Experimental Pollination. pp. 117-141. Van Nostrand, New York.

Baker, H. G. and Baker, I. 1986. The occurrence and significance of amino acids in floral nectar. Plant Systematics and Evolution, 151;175-186.

Barnes, D. K. and Furgala, B. 1978. Nectar characteristics associated with sources of alfalfa germplasm. Crop Science, 18; 1087-1089.

Bathurst, N. O. 1954. The amino acids of grass pollen. Journal of Experimental Botany, 5; 253-256.

Beardsell, D. V., Williams, E. G. and Knox, R. B. 1989. The structure and histochemistry of the nectary and anther secretory tissue of the flowers of *Thryptomene calycina* (Lindl) Stapf (Myrtaceae). Australian Journal of Botany, 37; 65-80.

Beattie, A., Turnbull, C., Knox, R. B., and Williams, E. G. 1984. Ant inhibition of pollen function: a possible reason why ant pollination is rare. American Journal of Botany, 71; 421-426.

Beckmann, R. L. and Stuckey, J. M. 1981. Extrafloral nectaries and plant guarding in *Ipomea pandurata* L. (Convolvulaceae). American Journal of Botany, 68; 72-79.

Bell, J. and Hicks, G. 1976. Transmitting tissue in the pistil of tobacco; light and electron microscopic observation. Planta, 131; 187-200.

Bentley, B. L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annual Review of Ecology and Systematics, 8; 107-427.

Bigger, M. 1960. *Selenothrips rubrocintus* (Giard) and the floral biology of cashew in Tanganyika. East African Agricultural Journal (Kenya), 25; 229-234.

Bradley, M. V. and Griggs, W. H. 1963. Morphological evidence of incompatibility in *Olea europea* L. Phytomorphology, 13; 141-156.

Brewbaker, J. L. 1957. Pollen cytology and incompatibility systems in plants. Journal of Heredity, 48; 271-277.

Brikitov, E. A., Musatova, N. A., Vladimirtseva, S. U. and Protsenko, M. A. 1964. Proline in the reproduction system of plants. In: Linskens, H. F. (ed.). Pollen Physiology and Fertilization. pp. 77-85. North Holland Publishing Company, Amsterdam.

Bronner, R. 1975. Simultaneous demonstration of lipids and starch in plant tissue. Stain Technology, 50; 1-4.

Carr, D. E. 1991. Sexual dimorphism and fruit production in a dioecious understory tree *Ilex opaca* Ait. Oecologia, 85; 381-388.

Chacko, E. K. 1984. Physiology of vegetative and reproductive growth in mango (*Mangifera indica* L.) trees. Proceedings of the first Australian Mango Research Workshop. pp. 54-70. C.S.I.R.O., Melbourne, Australia

Chipendale, G. M.1978. The function of carbohydrates in insect life processes. In Rockstein, M (ed). Biochemistry of Insects. pp. 1-55. Academic Press, New York.

Christensen, J. E. and Horner, H. T. 1974. Pollen pore development and its spatial orientation during microsporogenesis in the grass; *Sorghum bicolor.* American Journal of Botany, 61; 604-623.

Clarke, A. E., Gleeson, P., Harrison, S. and Knox, R. B. 1979. Pollen-stigma interactions: identification and characterisation of surface compounds with recognition potential. Proceedings of the National Academy of Sciences (U. S. A.) 76; 3358-3362.

Clarke, A. E., Abbot, A., Mandel, T. E. and Pettitt, J. M. 1980. Organisation of the wall layers of the stigmatic papillae of *Gladiolus gandavensis*; a freeze fracture study. Journal of Ultrastructural Research, 79; 269-281.

Copeland, H. F. 1961. Observations on the reproductive structures of *Anacardium occidentale* L. Phytomorphology, 11; 314-325.

Corbet, S. A. 1978. Bee visits and the nectar of *Echium vulgare* L. and *Sinapsis alba* L. Ecological Entomology, 3; 25-37.

Corbet, S. A. 1978. Bees and nectar of *Echium vulgare* L. In: The Pollination of Flowering Plants Richard, A. J. (ed). Linnaean Society Symposium Series, London. pp. 21-30.

Corbet, S. A., Unwin, D. M. and Prys-Jones, O. E. 1979. Humidity, nectar and insect visits to flowers, with special reference to *Crataegus*, *Tilia* and *Echium*. Ecological Entomology, 4; 9-22.

Cresti, M., Pacini, E., Ciampolini, F. and Sarfatti, G. 1977. Germination and early tube development *in vitro* of *Lycopersicum peruvianum* pollen: ultrastructural features. Planta, 136; 239-247.

Crowe, L. K. 1971. The polygenic control of outbreeding in *Borago officinalis*. Heredity, 27; 111-118.

Damodaran, V. K., Abraham, J. and Alexander, K. M. 1965. The morphology and biology of the cashew flower; flowering habit, flowering season, morphology of the flower, and sex ratio. Agricultural Research Journal, Kerala., 3; 23-28.

Degani, C., Goldring, A. and Gazit, S. 1989. Pollen parent effect on outcrossing rate in Hass and Fuerte avocado plots during fruit development. Journal of the American Society for Horticultural Science, 114; 106-111.

Degroot, A. P. 1953. Protein and amino acid reqiurements of honeybees (*Apis mellifera*). Physiologia Comparata et D'Ecologia, 3; 197-285.

Delane, R. J. 1987. Climate of the Ord River Irrigation Area Western Australia; Implication for crop production. Western Australian Department of Agriculture Technical Paper No. 9, 29 pp.

de Nettancourt, D. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin. 230pp.

de Nettancourt, D., Devreux, M., Bozzini, A., Cresti, M., Pacini, E. and Sarfattii, G. 1973. Ultra structural aspects of self-incompatibility mechanism in *Lycopersicum peruvianum* Mill. Journal of Cell Science, 12; 403-419.

Department of Natural Resources. 1976. An Outline of the Ord River Irrigation Project Western Australia. AGPS Canberra, Australia. 50 pp.

de Wet, E. D. and Robbertse, P. J. 1986. Studies on pollination problems in mango cultivars. In: Williams, E. G. and Knox, R. B. (eds.), Pollination '86. pp. 31-41. Plant Cell Biology Research Centre, Melbourne, Australia.

Dickinson, H. G. and Lewis, D. 1973. Cytochemical and ultrastructural differences between intraspecific compatible and incompatible pollinations in *Raphanus*. Proceedings of the Royal Society of London, Series B, 183; 21-38.

Dickinson, H. G. and Lewis, D. 1973. The formation of tryphine coating the pollen grains of *Raphanus*, and its properties relating to the self incompatibility system Proceedings of the Royal Society of London, Series B, 184; 149-165.

Downer, R. G. H. 1978. Functional role of lipids in insects. In Rockstein, M. (ed). Biochemistry of Insects. pp. 57-92. Academic Press New York.

Dukee, L. T., Gaal, D. T. and Reisner, W. H. 1981. The floral and extrafloral nectaries of *Passiflora*. I. the floral nectary. American Journal of Botany, 68; 453-462.

Dulberger, R. 1981. The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpiniaceae). American Journal of Botany, 68; 1350-1360.

Dumas, C., Rougier, M., Zandonella, P., Ciampolini, F., Cresti, M. and Pacini, E. 1978. The secretory stigma in *Lycopersicum peruvianum* Mill., ontogenesis and glandular activity. Protoplasma, 96; 173-187.

Dumas, C. Duplan, J. C. Said, C. and Soulier, J. P. 1983. Nuclear magnetic resonance to correlate water content and pollen viability. In Mulcahy, D. C. Ottaviano, E. (eds). Pollen Biology and Implications for Plant Breeding. pp. 15-20. Elsevier Biomedical, New York.

Dumas, C. Knox, R. B. and Gaude, T. 1984. Pollen-pistil recognition; new concepts from electron microscopy and cytochemistry. International Review of Cytology, 90; 239-272.

Eames, A. J. 1961. Morphology of Angiosperms. McGraw Hill, New York. 518 pp.

Echlin, P. 1971. The role of the tapetum during microsporogenesis of angiosperms. In Heslop-Harrison, J. (ed). Pollen Development and Physiology. pp. 41-61. Butterworths, UK.

Elias, T. and Gelband, H. 1976a. Morphology and anatomy of floral and extrafloral nectaries of *Turera ulmifolia* L. I. the floral nectary. American Journal of Botany, 4; 453-462.

Elias, T. S. and Gelband, H. 1976b. Morphology and anatomy of floral and extrafloral nectaries in *Campsis* (Bignoniaceae). American Journal of Botany, 63; 1349-1353.

Ellis, M. B. 1976. More Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey England. pp. 149.

Elsy, C. R., Namboodiri, K. M. N., Vidyadharan, K. K. and Oommen, A. 1986. Role of pollen and pollinating agents in cashew yield. Cashew Causerie, 8; 3-4.

Emerson, S. H. 1940. Growth of incompatible tubes in *Oenothera organensis*. Botanical Gazette, 101; 890-899.

Esau, K. 1977. Anatomy of Seed Plants. John Wiley and Sons, New York. 550 pp.

Faegri, K. and Pijl, L. van der. 1979. The Principles of Pollination Ecology. Pergamon Press, London. 244 pp.

Fahn, A. 1979. Secretory Tissues in Plants. Academic Press, London. 302 pp.

Fahn, A. and Rachmilevitz, T. 1970. Ultrastructure and nectar secretion in *Lonicera japonica*. In: Robson, N. K. B., Cutler, D. F. and Gregory, M. (eds.). New Research in Plant Anatomy. pp. 51-56. Academic Press, London.

Feder, N. and O'Brien, T. P. 1968. Plant microtechnique; some principles and new methods. American Journal of Botany, 55; 123-142.

Figier, J. 1971. Etude infrastructure de la Stipule de *Vicia faba* L. au niveau du nectaire. Planta, 98; 31-49.

Findlay, N. and Mercer, F. V. 1971. Nectar production in *Abutilon*. I. movement of nectar through the cuticle. Australian Journal of Biological Sciences, 24; 647-656.

Findlay, N. and Mercer, F. V. 1971. Nectar production in *Abutilon*. II. submicroscopic structure of the nectary. Australian Journal of Biological Sciences, 24; 657-664.

Fisher, B. B. 1968. Protein staining of ribboned epon sections for light microscopy. Histochemie, 16; 92-96.

Fogle, H. W. 1977. Identification of clones within four tree fruit species by pollen exine patterns. Journal of the American Society for Horticultural Science, 102; 552-560.

Fogle, H. W. 1977. Identification of tree fruit species by pollen ultrastructure. Journal of the American Society for Horticultural Science, 102; 548-551.

Frankel, R. and Galun, E. 1977. Pollination Mechanisms, Reproduction and Plant Breeding. Springer Verlag. 281 pp.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, London. 544 pp.

Free, F. B. and Williams, I. H. 1976. Insect pollination of *Anacardium occidentale* L., *Mangifera indica* L., *Blighia sapinda* Koeing, and *Persea americana* Mill. Tropical Agriculture, Trinidad, 53; 125-139.

Frey-Wyssling, A. M., Zimmerman, M. and Maurizio, A. 1954. Uber den enzymatischen Zuckerumbau in Nektarien. Experimentia, 10; 490-492.

Gosh, S. and Shivanna, K. R. 1984. Structure and cytochemistry of the stigma and pollen pistil interaction in *Zepharanthes*. Annals of Botany, 53; 91-105.

Gottsberger, G., Arnold, T. and Linskens, H. F. 1989. Intraspecific variation in the amino acid content of floral nectar. Botanica Acta, 102;141-144.

Gottsberger, G., Arnold, T. and Linskens, H. F. 1990. Variation in floral nectar amino acids with aging of flowers, pollen contamination, and flower damage. Israel Journal of Botany, 39;167-176.

Gould, J. L. and Gould, C. G. 1983. The honey bee: programmed learning. In: Real, L. (ed.). Pollination Biology. pp. 185-219. Academic Press, London.

Griffin, A. R., Moran, G. F. and Fripp, Y. J. 1987. Preferential outcrossing in *Eucalyptus regnans* F. Muell. Australian Journal of Botany, 35; 465-475.

Griffing, B. 1956a. A generalised treatment of the use of diallel crosses in quantitative inheritance. Heredity, 10; 31-50.

Griffing, B. 1956b. Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Sciences, 9; 463-493.

Grunfeld, E., Vincent, C. and Bagnara, D. 1989. High performance liquid chromatography analysis of nectar and pollen of strawberry flowers. Journal of Agricultural Food Chemistry, 37; 290-294.

Gunn, R. H. and Cocks, K. D. 1971. Potentialities for cashew in northern Australia. Journal of the Australian Institute of Agricultural Science, 37; 25-31.

Harbourne, J. B. 1977. Introduction to Ecological Biochemistry. Academic Press, London. 243 pp.

Haydak, M. V. 1970. Honeybee nutrition. Annual Review of Entomology, 15; 143-156.

Heard, T. A., Vithanage, V. and Chacko, E. K. 1990. Pollination biology of cashew in the Northern Territory of Australia. Australian Journal of Agricultural Research, 41; 1101-1114.

Heinrich, B. and Raven, P. 1972. Energetics and pollination ecology. Science, 176; 597-602.

Herd, Y. R. and Beadle, D. J. 1980. The site of the self incompatibility mechanism in *Tradescantia pallida*. Annals of Botany, 45; 251-256.

Herrero, M. 1983. Factors effecting fruit set in Agua de Aranjuez pear. Acta Horticulturae, 139; 91-96.

Herrero, M. and Dickinson, H. G. 1979. Pollen-pistil incompatibility in *Petunia hybrida*: Changes in the pistil following compatible and incompatible intraspecific crosses. Journal of Cell Science, 36; 1-18.

Herrero, M. and Dikinson, H. G. 1980. Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific matings. Journal of Cell Science, 47; 365-383.

Herrero, M. P. and Johnson, R. R. 1980. High temperature stress and pollen viability of maize. Crop Science, 20; 796-780.

Herrero, M. and Gascon, M. 1987. Prolongation of embryosac viability in pear (*Pyrus communis*) following pollination or treatment with gibberellic acid. Annals of Botany, 60; 187-293.

Herrero, M. and Arbeloa, A. 1989. Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). American Journal of Botany, 76; 1441-1447.

Herscovitch, J. C. and Martin, A. R. H. 1989. Pollen-pistil interactions in *Grevillea banksii*, the pollen grain, stigma, transmitting tissue, and in vitro pollinations. Grana, 28; 69-84.

Heslop-Harrison, J. 1975. Incompatibility and the pollen-stigma interaction. Annual Review of Plant Physiology, 26; 403-425.

Heslop-Harrison, J. 1975. Physiology of the pollen grain surface. Proceedings of the Royal Society of London, Series B, 190; 275-300.

Heslop-Harrison, Y. 1977. The pollen stigma interaction; pollen tube penetration in *Crocus*. Annals of Botany, 41; 913-922.

Heslop-Harrison, J. 1979. Aspects of the structure, cytochemistry, and germination of the pollen of rye (*Secale cereale* L.). Annals of Botany (suppl), 1; 1-47.

Heslop-Harrison, J. and Heslop-Harrison, Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intercellular hydrolysis of fluorescein diacetate. Stain Technology, 45; 115-120.

Heslop-Harrison, J., Knox, R. B. and Heslop-Harrison, Y. 1974. Pollen wall proteins: exine held fraction associated with the incompatibility response in Cruciferae. Theoretical and Applied Genetics, 44; 133-137.

Heslop-Harrison, Y. and Shivanna, K. R. 1977. The receptive surface of the angiosperm stigma. Annals of Botany, 41; 233-258.

Holliday, P. 1980. Fungus Diseases of Tropical Crops. Cambridge University Press, London. 486 pp.

Holm, E. 1979. The Biology of the Flower. Penguin Books. 140 pp.

International Board of Plant Genetic Resources. 1986. Genetic Resources of Tropical and Sub-tropical Fruits and Nuts (Excluding *Musa*). IBPGR, Rome, Italy. 162 pp.

Iwanami, Y. Sasakuma, T. and Yamada, Y. 1988. Pollen: Illustration and Scanning Electronmicrographs. Kodansha, Tokyo. 198 pp.

Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman and Co. London. 408pp.

Jensen, W. A. and Fisher, D. B. 1966. Cotton embryogenesis; The tissue of the stigma and style and their relation to the pollen tube. Planta, 84; 97-121.

John, P., Raghuram, Y. and Nair, G. M. 1987. Uncommon cuticular ornamentation on the stigma surface of mango (*Mangifera indica* L.). Current Science, 56; 783-784.

Johnson, D. 1973. The botany, origin and spread of the cashew, *Anacardium occidentale* L. Journal of Plantation Crops, 1; 1-7.

Kambal, A. E., Bond, D. A. and Tonynbee-Clarke, G. 1976. A study on the pollination mechanism in field beans (*Vicia faba* L.). Journal of Agricultural Science, 87; 519-526.

Kandasamy, M. K. and Kristen, U. 1987. Developmental aspects of ultrastructure, histochemistry and receptivity of the stigma of *Nicotiana sylvestris*. Annals of Botany, 60; 427-437.

Kendall, W. A. and Talor, N. L. 1969. Effect of temperature on pseudo self-compatibility in *Trifolium pratense* L. Theoretical and Applied Genetics, 39; 123-126.

Kenrick, J and Knox, R. B. 1981. Structure and histochemistry of the stigma and style of some Australian species of *Acacia*. Australian Journal of Botany, 29; 733-745.

Kenrick, J., Kaul, V. and Williams, E. G. 1986. Self-incompatibility in *Acacia retinodes*: site of pollen-tube arrest is the nucellus. Planta, 169; 245-250.

Kevan, P. G. and Baker, H. G. 1983. Insects as flower visitors and pollinators. Annual Review of Entomology, 28; 407-453.

Khoo, K. C., Ibrahim, Y., Maelzer, D. A. and Lim, T. K. 1982. Entomofauna of cashew in West Malaysia. Proceedings of International Conference for Plant Protection in the Tropics. pp. 289-295. Kuala Lumpur, Malaysia.

Knight, R. J. Jr. 1982. Partial loss of self-incompatibility in Golden Star carambola. HortScience, 17, 72.

Knox, R. B. 1973. Pollen wall proteins: cytochemical observations of pollen-stigma interactions in ragweed and *Cosmos* (Compositae). Journal of Cell Science, 12; 421-443.

Knox, R. B. 1984. Pollen pistil interaction. In Linskens, H. F. and Heslop-Harrison, J (eds.). Encyclopedia of Plant Physiology, 17; 508-608. Springer-Verlag, Berlin.

Knox, R. B., Clarke, A. E., Harrison, S., Smith, P. and Marchalonis, J. J. 1976. Cell recognition in plants: determinants of the stigma surface and their pollen interactions. Proceedings of the National Academy of Sciences (U. S. A). 73, 2788-2792.

Konar, R. N. and Linskens, H. F. 1966. Physiology and biochemistry of the stigmatic fluid of *Petunia hybrida*. Planta, 71; 372-387.

Konar, R. N. and Linskens, H. F. 1966. The morphology and anatomy of the stigma of *Petunia hybrida*. Planta, 71; 356-371.

Krisaneephiboon, W., Petcharat, V., Lim, S., and Chuenchitt, S. 1988. Study on disease of cashew (*Anacardium occidentale* L.) in southern regions of Thailand. Songklanakarin Journal of Science and Technology, 10; 263-266.

Kroh, M. and Munting, A. J. 1967. Pollen germination and pollen tube growth in *Diplotaxis tenuifolia* after cross pollination. Acta Botanica Neerlandica, 16; 182-187.

Kumaran, P. M., Vimala, B. and Murthy, K. N. 1976. On the occurrence of pistillate and neutral flowers in cashew. Journal of Plantation Crops, 4; 82-84.

Lewis, D. 1942. The physiology of incompatibility in plants. I the effect of temperature. Proceedings of the Royal Society of London, Series B, 131; 13-26.

Lewis, D. 1956. Incompatibility and plant breeding. Brookhaven Symposia in Biology, 9; 89-100.

Lewis, D. 1960. Genetic control of specifity and activity of the S antigen in plants. Proceedings of the Royal Society of London, Series B, 151; 468-477.

Lord, E M. and Webster, B. D. 1979. The stigmatic exudate of *Phaseolus vulgaris* L. Botanical Gazette, 120; 109-117.

Lord, E. M. and Heslop-Harrison, Y. 1984. Pollen-stigma interaction in the Leguminosae: Stigma organisation and the breeding system in *Vicia faba* L. Annals of Botany, 54; 827-836.

Lue, P. F. and Dixon, S. E. 1967. Studies on the mode of action of royal jelly in honey bee development. Annals of the Entomological Society of America, 45; 595-599.

Mackenzie, C. J., Yoo, B. Y. and Seabrook, J. E. A. 1990. Stigma of *Solanum tuberosum* cv. Shepody; ultrastructure and secretion. American Journal of Botany, 77; 1111-1124.

Maestro, M. C. and Alvarez, J. 1988. The effect of temperature on pollination and pollen tube growth in muskmelon (*Cucumis melo* L.). Scientia Horticulturae, 36; 173-181.

Marcucci, M. C., Sansavini, S., Ciampolini, F. and Cresti, M. 1984. Distinguishing apple clones and cultivars by surface morphology and pollen physiology. Journal of the American Society for Horticultural Science, 109; 10-19.

Marginson, R., Sedgley, M. and Knox, R. B. 1985. Structure and histochemistry of the extrafloral nectary of *Acacia terminalis* (Salb) MacBride (Leguminosae, Mimosoideae). Protoplasma, 127; 21-30.

Martens, J. and Fretz, T. A. 1980. Identification of eight crabapples by pollen surface sculpture. Journal of the American Society for Horticultural Science, 105; 257-263.

Martens, M. H. R., Reisch, B. I. and Mauro, M. C. 1989. Pollen size variability within genotypes of *Vitis*. HortScience, 24; 659-662.

Martin, F. and Ortiz, S. 1967. Anatomy of the stigma and style of sweet potato. New Phytologist, 66; 109-113.

Martin, F. and Brewbaker, J. L. 1971. The nature of the stigmatic exudate and its role in pollen germination. In: Heslop-Harrison, J. (ed.). Pollen Development and Physiology, Buterworths, UK.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology, 34; 125-128.

Martin, F. W. 1969. Compounds from the stigma of ten species. American Journal of Botany, 56; 1023-1027.

Mascarenhas, J. P. and Altschuler, M. 1983. The response of pollen to high temperature and its potential applications. In: Mulcahy, D. L. and Ottaviano, E. (eds). Pollen: biology and implications for plant breeding. pp. 3-8. Elsevier Science Publishing

Mass, J. L. 1977. Pollen ultrastructure of strawberry and other small-fruit crops. Journal of the American Society for Horticultural Science, 102; 560-571.

Mattsson, O., Knox, R. B., Heslop-Harrison, J. and Heslop-Harrison, Y. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature, 247; 298-300.

Maurizio, A. 1962. From raw material to the finished product; honey. Bee World, 43; 66-81.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants. US Department of Agriculture Paper Number 496, Washington. 411 pp.

McGuckin, W. F. and McKenzie, B. F. 1958. An improved periodic acid fuchsin sulfite staining method for evaluation of glycoproteins. Clinical Chemistry, 4; 476-483.

Mckone, M. J. and Webb, C. J. 1988. A difference in pollen size between the male and hermaphrodite flowers of two species of Apiaceae. Australian Journal of Botany, 36; 331-337.

McLellan, A. R. 1977. Minerals, carbohydrates and amino acids of pollen from some woody and herbaceous plants. Annals of Botany, 41; 1225-1232.

Medway, L. 1972. Phenology of tropical rain forest in Malaya. Biological Journal of the Linnean Society, 4; 117-146.

Millington, A. J. 1983. Cashew; Australia's potential for a major tropical crop. In: Noel, D. (ed). Tree Crops: The Third Component Proceedings of the First Australian Conference on Tree and Nut Crops. pp. 43-48. Cornucopia Press, Perth, Western Australia

Moezel, P. G. van der, Delfs, J. C., Pate, J. S., Loneragan, W. A. and Bell, D. T. 1987. Pollen selection by honeybees in shrublands of the northern sandplains of Western Australia. Journal of Agricultural Research, 26; 224-232.

Moncur, M. W. and Wait, A. J. 1986. Floral ontogeny of the cashew, *Anacardium occidentale* L. (Anacardiaceae). Scientia Horticulturae, 30; 203-211.

Morada, E. K. 1941. Cashew culture. Philippines Journal of Agriculture, 12; 89-106.

Morton, J. F. 1961. The cashew's brighter future. Economic Botany, 15; 57-78.

Mueller, W. C. and Greenwood, A. D. 1978. The ultrastructure of phenolic-storing cells fixed with caffeine. Journal of Experimental Botany, 29; 757-764.

Mulas, M., Agabbio, M. and Neiddu, G. 1989. Ultrastructural morphology of Nonpareil almond pollen. HortScience, 24; 816-818.

Murrell, D. C., Shuel, R. W. and Tomes, D. T. 1982. Nectar production and floral characteristics in birdsfoot trefoil (*Lotus corniculata* L.). Canadian Journal of Plant Science, 62; 361-371.

Murthy, R. N., Kumaran, P. M. and Nayer, N. M. 1975. Increasing fruit set in cashew by hormone spraying. Journal of Plantation Crops, Kerala, 3; 81-82.

Motomura, Y., Watanabe, T. and Aso, K. 1962. Studies on honey and pollen. VI. On the sugar composition of several kinds of pollen. Tohoku Journal of Agricultural Research, 13; 237-244.

Nambiar, M. C. 1979. Cashew. In: Alvim, P. T. and Kozlowski, T. T. (eds). Ecophysiology of Tropical Crops. Academic Press, New York.

Northwood, P. J. 1966. Some observations on flowering and fruit setting in the cashew (*Anacardium occidentale* L.). Tropical Agriculture, Trinidad, 1; 35-42.

Ockendon, D. J. and Gates, P. J. 1976. Reduced pollen viability in the onion (*Allium cepa*). New Phytologist, 76; 511-517.

Ohler, J. G. 1979. Cashew. Communication No. 71. Department of Agricultural Research, Royal Tropical Institute, Amsterdam. pp 260.

Ord River Project Co-ordination Committee. 1983. Potential for cashew production in the Kimberleys. Western Australian Department of Agriculture, Perth, Western Australia.

Pacini, E. 1983. Pollen-pistil interactions in plants with gametophytically controlled self incompatibility. Phytomorphology, 31; 175-180.

Paleg, L. G., Douglas, T. J., Van Daal, A. and Keech, D. B. 1981. Proline, betaine and other organic solutes protect enzymes against heat inactivation. Australian Journal of Plant Physiology, 8;107-114.

Pandey, K. 1967. Time and site of S gene action, breeding system and relationship in incompatibility. Euphytica, 19; 364-372.

Parameswaran, N. K., Damodaran, V. K. and Praphakaran, P. B. 1984 Relationship between yield and duration of different phases in flower opening in cashew (*Anacardium occidentale* L.). Indian Cashew Journal., 16; 15-19.

Pavithran, K. and Ravindranathan, P. P. 1974. Studies on the floral biology in cashew (*Anacardium occidentale* L.). Journal of Plantation Crops, Kerala, 2; 32-33.

Percival, M. S. 1961. Types of nectar in angiosperms. New Phytologist, 60; 235-281.

Peteram, R. J. and Kok, B. 1983. Plants of the Kimberley Region of Western Australia. University of Western Australia Press, Perth, Western Australia. 556pp.

Pfahler, P. L. and Linskens, H. F. 1970. Biochemical composition of maize (*Zea mays* L.) pollen I. Effects of the endosperm mutants, waxy (wx), shrunken (sh2), and sugary (su1) on the amino acid content and fatty acid distribution. Theoretical and Applied Genetics, 40; 6-10.

Pickett, C. H. and Clark, W. D. 1979. The function of extrafloral nectaries in *Opuntia acanthocarpa* (Cactaceae). American Journal of Botany, 66; 618-625.

Pimienta, E., Polito, V. S. and Kester, D. E. 1983. Pollen tube growth in cross- and self-pollinated Nonpareil almond. Journal of the American Society for Horticultural Science, 108; 643-647.

Purseglove, J. W. 1968. Tropical Crops 1. Dicotyledons. Longmans, London. 332 pp.

Quinlan, J. D. and Preston, A. P. 1968. Effect of thinning blossom and fruitlets on growth and cropping of Sunset apple. Journal of Horticultural Science, 43; 373-381.

Raff, J. W. and Knox, R. B. 1982. Pollen tube growth in *Prunus avium*. In: Williams, E. G., Knox, R. B., Gilbert, J. W. and Bernhardt, P. (eds). Pollination '82. Plant Cell Biology Research Centre, Melbourne, Australia.

Randhawa, M. S. 1957. Flowering Trees in India. Farm Bulletin No. 8, Indian Council for Agricultural Research, New Delhi.

Rao, V. N. M. 1958. Cashew nut cultivation in India. Farm Bulletin No. 9, Indian Council for Agricultural Research, New Delhi.

Rao, V. N. M. and Hassan, M. V. 1957. Preliminary study on the floral biology of cashew (*Anacardium occidentale* L.). Indian Journal for Agricultural Science, 27; 277-288.

Rayner, C. J. and Langridge, D. F. 1985. Amino acids in bee collected pollen from Australian indigenous and exotic plants. Australian Journal of Experimental Agriculture, 25; 722-726.

Robacker, D. C., Flottum, P. K. and Erickson, E. H. 1983. Climatic and edaphic effects on soybean flowers and subsequent attractiveness of plants to honey bees. Field Crops Research, 6; 267-278.

Roggen, H. P. J. R. 1972. Scanning electron microscopical observation on compatible and incompatible pollen-stigma interactions in *Brassica*. Euphytica, 21; 1-10.

Romberg, L. D. and Smith, C. L. 1946. Effect of cross pollination, self pollination and sib pollination on the dropping, the volume and kernel development of pecan nuts and on the vigour of the seedling. Proceedings of the American Society for Horticultural Science 47, 130-138.

Schmidt, J. O., Buchmann, S. L. and Glaiim, M. 1989. The nutritional value of *Typha latifolia* pollen for bees. Journal of Apicultural Research, 28; 155-165.

Schmitt, D. and Perry, T. O. 1964. Self-sterility in sweetgum. Forest Science, 10; 302-305.

Scholefield, P. B. 1982. A scanning electron microscope study of flowers of avocado, litchi, macadamia, and mango. Scientia Horticulturae, 16; 262-272.

Schou, O. 1984. The dry and wet stigma of *Primula obconica*; ultrastructure and cytochemical dimorphism. Protoplasma, 131; 99-113.

Sedgley, M. 1976. Control by the embryosac over pollen tube growth in the style of the avocado (*Persea americana* Mill.). New Phytologist, 72; 149-152.

Sedgley, M. 1977. Reduced pollen tube growth and presence of callose in the pistil of the male floral stage of the avocado. Scientia Horticulturae, 7; 27-36.

Sedgley, M. 1977. The effect of temperature on floral behaviour, pollen tube growth and fruit set in avocado. Journal of Horticultural Science, 52; 135-141.

Sedgley, M. 1979. Light microscope study of pollen tube growth, fertilisation and early embryo and endosperm development in the avocado varieties Fuerte and Hass. Annals of Botany, 44; 353-359.

Sedgley, M. 1979. Structural changes in the pollinated and unpollinated avocado stigma and style. Journal of Cell Science, 38; 49-60.

Sedgley, M. 1980. Anatomical investigation of abscissed avocado flowers and fruitlets. Annals of Botany, 46; 771-777.

Sedgley, M. 1981. Ultrastructure and histochemistry of the watermelon stigma. Journal of Cell Science, 48; 137-146.

Sedgley, M. 1989. Acacia. In: Halevey, A. H. (ed.). Handbook of Flowering, Volume 6, pp. 1-11. CRC Press, Florida.

Sedgley, M. and Buttrose, B. 1978. Structure of the stigma and style of the avocado. Australian Journal of Botany, 26; 663-682.

Sedgley, M. and Annells, L. M. 1981. Flowering and fruit set response to temperature in avocado cultivar Hass. Scientia Horticulturae, 14; 27-33.

Sedgley, M. and Griffin, A. R. 1989. Sexual Reproduction of Tree Crops. Academic Press, London. 378 pp.

Sedgley, M., Bell, F. D. H., Bell, D., Winks, C. W., Pattison, S. J. and Hancock, T. W. 1990. Self- and cross-compatibility of macadamia cultivars. Journal of Horticultural Science, 65; 205-213.

Sharman, D. K. and Singh, R. N. 1970. Self-incompatibility in mango (*Mangifera indica* L.). Horticultural Research, 10; 108-118.

Shirashi, M., Matsumoto, K. and Shigemasu, A. 1976. Morphological studies on fertilisation and development of citrus fruit. I Ultrastructural examination of papillary cells of the stigma of satsuma madarin (*Citrus unshui* Marc.). Journal of the Japanese Society of Horticultural Science, 45; 231-237.

Shivanna, K. R. and Heslop-Harrison, J. 1981. Membrane state and pollen viability. Annals of Botany, 47; 759-770.

Shivanna, K. R., Ciampolini, F. and Cresti, M. 1989. The structure and cytochemistry of the pistil of *Hypericum calycinum* ; the stigma. Annals of Botany, 63; 613-620.

Singh, R. N. 1954. Studies in floral biology and subsequent development of fruits in the mango (*Mangifera indica* L.) varieties Dashehari and Langra. Indian Journal of Horticulture, 11; 69-88.

Singh, R. N., Majumdar, P. K. and Sharman, D. K. 1962. Self incompatibility in mango (*Mangifera indica* L.) var Dashehari. Current Science, 5; 209-211.

Solomon, B. P. 1986. Sexual allocation and andromonoecy: resource invesment in male hermaphrodite flowers of *Solanum carolinense* (Solanaceae). American Journal of Botany, 73; 1215-1221.

Spencer, J. L. and Kennard, W. C. 1955. Studies on mango (*Mangifera indica* L.) fruit set in Puerto Rico. Tropical Agriculture, Trinidad, 32; 323-330.

Stace, P. 1988. Pollen quality, honey production and strong bees. Australian Bee Journal, 69; 13-15.

Stanley, R. G. and Linskens, H. F. 1974. Pollen: Biology, biochemistry and management. Springer Verlag, Berlin. 307 pp.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction. and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). Ecology, 61; 57-64.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics, 12; 253-279.

Stosser, R. and Anvari, S. F. 1982. Pollen tube growth and fruit set as influenced by senescence of stigma, style and ovules. Acta Horticulture, 139; 13-22.

Sturtz, J. D. 1981. Cashew. In: Australian Tropical Tree Fruits Workshop. Queensland Department of Primary Industries Conference and Workshop Series. pp. 53-59.

Subramanian, R. B. and Inamdar, J. A. 1985. Occurrence, structure, ontogeny and biology of nectaries in *Kigelia pinnata* DC. Botanical Magazine, Tokyo, 98; 67-73.

Sutherland, S. 1987. Why hermaphrodite plants produce many more flowers than fruits: experimental test with *Agave mckelveyana*. Evolution, 41; 750-759.

Ton, L. D. and Krezdorn, A. H. 1967. Growth of pollen tubes in three incompatible varieties of citrus. Proceeding of the American Society for Horticultural Science, 89; 211-215.

van den Ende, H. 1967. Sexual Interaction in Plants; the role of specific substances in sexual reproduction. Academic Press, London. 186 pp.

Vansell, G. H. and Watkins, W. G. 1942. Orange nectar and pollen in relation to bee activity. Journal of Economic Entomology, 35; 321-323.

Vasil, I. K 1974. The histology and physiology of pollen germination and pollen tube growth on the stigma and in the style. In: Linskens, H. F. (ed.), Fertilisation in Higher Plants. pp. 105-118. North-Holland, Amsterdam.

Vasil, I. K. and Johri, M. M. 1964. The style, stigma and pollen tube. Phytomorphology, 14; 352-369.

Vasilakakis, M. D. and Porlingis, I. C. 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. HortScience, 20; 733-735

Vear, F., Pham-Delegue, M., Tourvieille de Labrouhe, D., Marilleau, R., le Loublier, Y., Metayer, M., Dovault, P. and Philippon, J. P. 1990. Genetical studies of nectar and pollen production in sunflower. Agronomie, 10; 219-231.

Wait, A. J. and Jamieson, G. I. 1986. The cashew: its botany and cultivation. Queensland Agricultural Journal, 112; 253-257.

Walker, A. K., Barnes, D. K. and Furgala, B. 1974. Genetic and environmental effects on quantity and quality of alfalfa nectar. Crop Science, 14; 235-238.

Walker, J. 1974. Aperture evolution in the pollen of primitive angiosperms. American Journal of Botany, 61; 1112-1137.

Waller, G. D., Carpenter, E. W. and Ziehl, O. A. 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honeybees. Journal of the American Society for Horticultural Science, 97; 535-539.

Weevers, T. 1952. Flower colours and their frequency. Acta Botanica Neerlandica, 1; 81-92.

Westwood, M. N. 1978. Morphology and surface topography of pollen and anthers of *Pyrus* species. Journal of the American Society for Horticultural Science, 103; 28-37.

Whitehead, D. R. 1969. Wind pollination in the angiosperms; evolutionary and environmental considerations. Evolution, 23; 28-35.

Widrlechner, M. P., Pellett, H. M., Ascher, P. D. and Fuhrman, S. 1983. *In vitro* pollen germination and vital staining in deciduous azaleas. HortScience, 18; 86-88.

Wiens, D. 1984. Ovule survivorship, brood size, life history, breeding system and reproductive success in plants. Oecologia, 64; 47-53.

Williams, R. R. 1969. Factors effecting pollination in fruit trees, In: Williams, R. R. and Cutting, C. V. (Eds). Physiology of Tree Crops. pp. 193-207. Academic Press, London.

Williams, R. R. 1970. The effect of supplementary pollination on yield. In: Williams, R. R. and Wilson, D. (Eds). Toward Regulated Cropping. pp. 7-10. Grower Books, London.

Wunnachit, W. 1979. Studies on floral morphology, biology, and fruit growth of cashew in Thailand. Special Report submitted to Faculty of Agriculture, Kasetsart University, Bangkok.

Wunnachit, W., Paechana, P., and Sirisongkram, P. 1986. Studies on floral biology and fruit setting of cashew in eastern coast of southern Thailand. Sonklanakarin Journal of Science and Technology, 8; 259-265.

Wykes, G. R. 1951. An investigation of the sugars present in the nectar of flowers of various species. New Phytologist, 51; 210-215.

Yazaki, Y. 1976. Co-pigmentation and colour change with age in petals of *Fuschia hybrida*. Botanical Magazine, 89; 45-57.

Zhang, H. Q. and Croes, A. F. 1983. Protection of pollen germination from adverse temperature: a possible role for proline. Plant Cell and Environment, 6; 471-476.

Ziegler, H. 1956. Untersuchungen Uber die Leitung und Sekretion der Assimilate. Planta, 47; 447-500.