# POLLEN TUBE GROWTH AND FRUIT DEVELOPMENT OF *PISTACIA*

by

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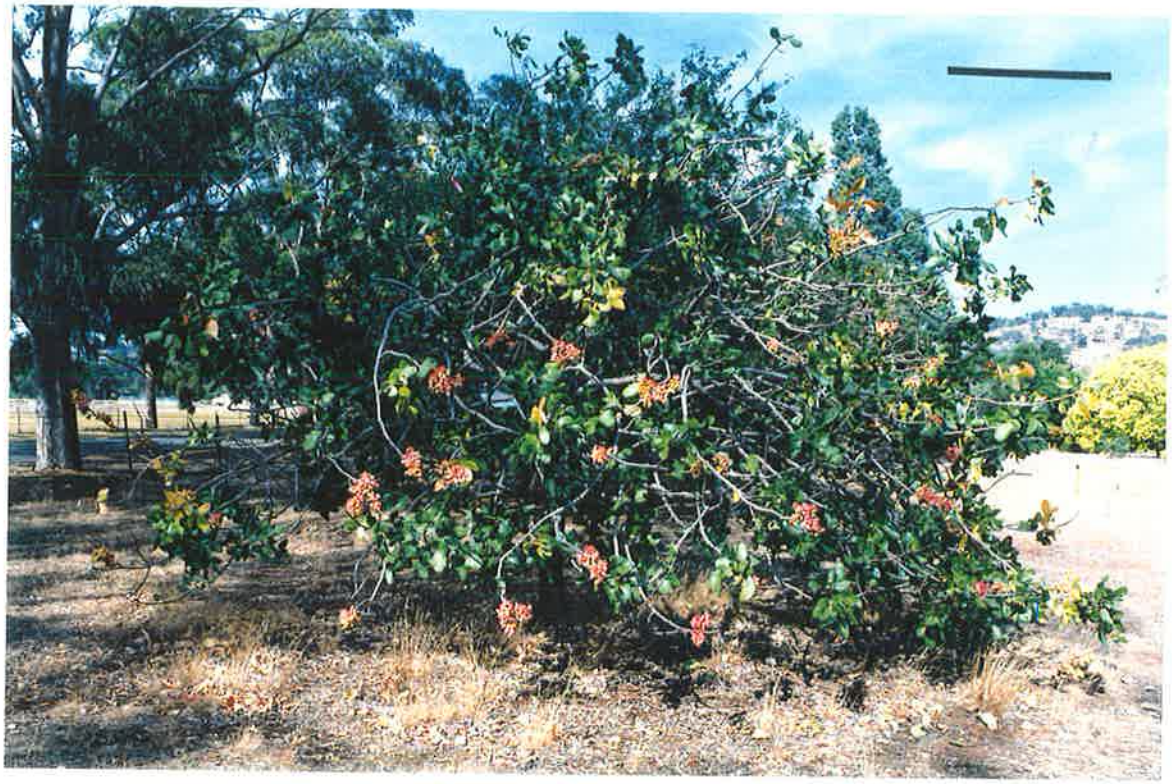

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# Pollen tube growth and fruit development of *Pistacia.*


## Summary

In *Pistacia vera* the majority of fruits did not reach maturity and were dropped up to 8 weeks after pollination. Of those that reached maturity a proportion were abnormal in that they were seedless (blank) or the shell did not split. In this study, pollination and fruit development were investigated in relation to abscission and abnormalities.

Two different media were used to assess pollen germination of *Pistacia vera*, *P. atlantica* and *P. terebinthus* Fresh and 24 hour dried pollen showed high germination *in vitro* as did pollen stored at -15°C for one month. Stored pollen of *P. atlantica* and *P. terebinthus* showed low germination at 6 months in contrast to *P. vera.* It was concluded that all pollen tested could be stored for one month at -15°C, and that pollen of *P. vera* could be stored for at least 6 months.

The stigma surface of *P. atlantica* turned red at anthesis, but that of *P. vera* did not. Female *Pistacia vera* trees were hand pollinated with pollen from three different male genotypes at 0, 1, 2, 3 and 4 days after anthesis. Results were measured by mature fruit set and by fluorescence microscopy of pollen germination and tube growth in the pistil. Maximum pollen tube growth and fruit set of split nuts was achieved following pollination within two days of anthesis. Three day old pistils supported pollen tube growth, but fruit set was low. Four day old pistils supported little pollen tube growth or fruit set. Significant differences were also apparent between pollen parents, with low pollen tube growth and fruit set following inter-specific pollination with *P. atlantica* pollen. All pollen tubes were observed to penetrate chalazogamously, and parthenocarpic production of blank fruits occurred in both unpollinated and pollinated treatments. The results indicate that for optimum fruit production of pistachio, pollen transfer must be achieved within two days of anthesis and inter-specific pollinator genotypes should be avoided.

Pistil anatomy of the *Pistacia vera* cultivars Kerman and Sirora was studied in relation to the pathway of pollen tube growth using fluorescence, bright field and scanning electron microscopy. The pistil comprised a three-lobed stigma, a short style and a unilocular ovary containing a single ovule with a large funicle. The ovule was crassinucellate and anatropous, with the mid-point of the funicle directly below the base of the style. The transmitting tract comprised the stigma, the style, the ovary wall and the funicle, with the ovarian transmitting tissue ensheathing the ovule, and the funicular transmitting tissue forming a central core. Most pollen tubes grew towards the embryo sac via the stigma and the style, and penetrated the funicle via the ovarian cavity at the base of the style. They grew through the distal funicular transmitting tissue and penetrated the nucellus via the chalaza. A minority of pollen tubes grew via the ovary wall transmitting tissue before penetrating the proximal funicular transmitting tissue. Some tubes grew in the space between the inner integument and nucellus, and some stopped growing in the nucellus or integument. Successful tubes entered the embryo sac via the egg apparatus. At anthesis unpollinated embryo sacs were immature and comprised a megaspore mother cell, a tetrad or a one cell embryo sac. Following anthesis unpollinated embryo sacs developed slowly and a maximum of 50% of them reached maturity by four days after anthesis. In contrast all embryo sacs of pollinated pistils were mature by one or two days after pollination. In the cultivar Kerman approximately 25% of embryo sacs were penetrated and fertilised at the second day after pollination compared with 43% fertilisation of Sirora at three days. Up to 60% of pistils had no pollen tube, and up to 50% of unpollinated and 29% of pollinated pistils, had no embryo sac.

Fruit development of the *Pistacia vera* cultivars Kerman and Sirora was investigated using bright field microscopy. Normal fruit development was compared with that of seedless (blank) and small seeded (semi-blank) fruits to identify the stages at which degeneration occurred. In normal fruits pericarp growth took place up to week four after pollination and preceded embryo, endosperm and funicle proliferation between weeks four and 16. Shell and hull development occurred between weeks

eight and 16. Blank and semi-blank fruits were identified by slower growth and lack of hull colouration. Blank fruits resulted from one of five patterns of ovule degeneration caused by funicle degeneration, embryo sac absence, embryo sac degeneration, lack of pollen tube penetration of the embryo sac, or failure of endosperm cellularisation. Funicle degeneration was the most common cause of blanking and was observed either at the site of pollen tube penetration or at the chalazal end of both pollinated and unpollinated ovules. Degeneration of funicular tissue extended towards the vascular bundles. Small seeded or semi-blank fruit resulted from degeneration of the cotyledons during the latter stage of embryo development.

Intra- and inter-specific pollinated fruits of *Pistacia vera* and *P. atlantica* were harvested up to fruit ripening for bright field microscopic studies of embryo development and shell structure. A high proportion of ovules of *P. atlantica* lacked an embryo sac at anthesis. In *P. atlantica* ovules with an embryo sac a ponticulus developed after pollination to make a bridge for pollen tube growth which was present up to eight weeks after pollination. Nomarski and bright field studies of shell structure indicated structural change from eight weeks after pollination. In *P. vera* about 4 - 6 weeks prior to harvest, shells developed a narrow angle at the split zone with a small area of spindle shape cells located at the edge. Up to four restricted splitting zones were seen in the inner layer of shell at the edge of the dorsal and vertical sutures. Intra-specific pollination decreased yield and quality of *Pistacia vera* cultivars Kerman and Sirora. Spontaneous early splitting of hull and shell were observed, particularly in *P. vera* cv. Sirora pollinated with *P. atlantica*.

## DECLARATION

I HEREBY DECLARE that the thesis presented here contains no work which has been accepted for the award of any other degree or diploma in any university, or any material previously published or written, except where due reference has been made in the text.

I am willing to have this copy of my thesis available for loan and photocopying, when deposited in the University Library.

YAHYA DEHGHANI SHURAKI

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# Chapter 1

# 1 Literature review

## 1.1 Introduction

In the flowering plants, pollination is one of the most important factors in reproduction and regeneration. Pollination begins by the transfer of pollen to the stigmatic surface. Normally, the pollen germinates, and a pollen tube enters the stigma tissue and passes through the style via the apoplast, finally reaching the ovary. Usually the pollen tube enters the ovule via the micropyle (porogamy), then enters the embryo sac through one of the synergids. Sometimes the tube enters the ovary through the chalaza (chalazogamy) or other parts of ovule wall (aporogamy). The pollen tube has a vegetative cell at its tip, and two generative nuclei which fuse with the egg and polar nuclei. The result of these fusions is an embryo, and an endosperm which acts as a food store for embryo development, and at the time of seed germination in some species. The developing embryo and endosperm may secrete hormones which stimulate the ovary wall to grow. With regard to pollination, out-crossing is a very important feature. Most out-crossing in plants results from self-incompatibility, dichogamy, dioecy and monoecy, in which physical or physiological factors are involved.

Fruit development takes place after the fusion of sperms with the polar nuclei and egg nucleus. Usually the development of the seed and fruit do not occur at the same rate. After fruit maturity, fruit ripening begins and this is related to ethylene emission and, in some fruits, to abscisic acid. During ripening, the fruit becomes soft, and starch, chlorophyll and fruit acids decrease, as sugar, pigments, flavours and odours increase.

## 1.2 Pollination

### 1.2.1 Pollen

Pollen contains the male gametes of flowering plants. Comprising two or three cells, the pollen grain transfers genetic information from the male parent to the offspring.

In the early stage of pollen development, hypodermal cells in the pre sporangia produce two kinds of cells. The inner cells form the microsporocytes, and the outer cells produce tapetal layers (Iwanami *et al.*, 1988). Microsporocytes or pollen mother cells (PMC) become covered with callose, a b-1, 3-glucan, secreted by the PMC cytoplasm. The PMCs are larger than the tapetal cells and their cytoplasm is denser.

#### 1.2.1.1 Tapetum

The sporogeneous tissue is surrounded by between two and four nutritive tapetal cell layers. These are polynucleate with between two and 16 nuclei. They also possess a large vacuole and are connected to each other and to the pollen mother cells via plasmodesmata. Two kinds of tapetum are recognised: (1) secretary (glandular) where the tapetal cells remain in position, but eventually undergo autolysis; and (2) amoeboid (plasmodial) where the cells migrate into the anther lumen during pollen grain development (Davis, 1961; Heslop-Harrison, 1971). The cytoplasm of the micro-spores becomes progressively denser and contains smooth endoplasmic reticulum, plastids, starch granules, dictyosomes, proteins, micro bodies and ribonucleic acids (Echlin and Godwin, 1968; Horner and Rogers, 1974). The most conspicuous event in the development of the tapetal cells is the formation of sporopollenin plaques in the proximity of the locular plasmalemma. During the tetrad phase, the tapetal cells degenerate and sporopollenin orbicules are deposited outside the plasmalemma. This continues after micro spore release (Horner and Lersten, 1971), and acid phosphatase is associated with the sites of exine and orbicule

formation (Zavada, 1984). Sporopollenin, a polymer unique to the walls of spores and pollen, is highly resistant to environmental factors such as heat. Deposition occurs immediately after micro spore callose dissolution (Skvarla and Larson, 1966; Shoup *et al.*, 1980).

#### 1.2.1.2 Pollen wall development

Pollen wall development can be divided into three phases: the PMC phase, the tetrad phase and the free spore stage. PMC development is initiated in the central part of the anther locule and proceeds centrifugally (Zavada, 1984). Callose is deposited on the PMC wall by Golgi vesicles. Ultimately, meiotic division and tetrad formation occur. After meiosis, the primexine appears between the callose layer and the plasmalemma, and serves as a template for sporopollenin deposition (Rowley and Skvarla, 1975). Early exine patterning is distinguishable within the primexine (Horner and Lersten, 1971). The primexine elements are synthesized by the micro spores (Heslop-Harrison, 1968).

The exine at maturity consists of sexine and nexine, external to the intine, and is an electron dense layer. The intine is electron translucent (Burns-Balogh, 1983), and contains osmiophilic materials such as the polysaccharides starch and cellulose (Knox, 1971).

### 1.2.2 Pollen germination and tube growth

The mature pollen grain is a dehydrated structure. When pollen grains are placed on the stigma, they swell due to water absorption. Pollen components including sugars, amino acids, and enzymes begin to exude from the pollen wall, and at the same time water and other molecules enter. The pollen germinates usually through one of the germination apertures, or at the contact point with the stigma in non-aperturate pollen (Iwanami *et al.*, 1988).

Pollen can grow not only on the stigma, but also on a culture medium. Usually the medium contains sugar, agar and inorganic elements such as potassium, calcium, magnesium and boron. Other important factors are the pH of the medium, the temperature of the environment, and relative humidity (Lee *et al.*, 1985; Kim *et. al.*, 1985; Bednarska, 1989; Dhar, 1989). Boron and calcium are generally essential for germination and tube growth (Xu *et. al.*, 1984; Wet *et al.*, 1990).

#### 1.2.2.1 Chemicals and pollen tube growth

Pollens of various genotypes have different responses to plant growth regulating substances, depending on hormone concentration and environmental conditions. For example, 10 ppm indole acetic acid enhanced germination and pollen tube growth in Jackfruit (Sinha, 1972), whereas pollen germination and tube growth of olive was inhibited by the same concentration. Pollen tube growth was inhibited after the addition of naphthalene acetic acid, gibberellic acid (GA) and indole butyric acid to grape pollen germination medium (Okamoto *et al.*, 1989), whereas GA3 increased pollen germination in magnolia (Kim and Lee, 1989). Coumarin, kinetin, and gibberellins increased pollen germination and tube growth of some cultivars of apple and *Vicia faba* (Popov, 1984; Gupta and Murty, 1985). Agricultural chemicals such as alar-89 and cycocel (CCC) both reduced pollen viability in *Lycopersicon esculentum* (Pastogi and Singh, 1989), and maleic hydrazide and ethylene reduced pollen germination and tube growth in *Impatiens balsamina* (Rana *et al.*, 1989). Many substances applied to crops such as pesticides, paclobutrazol and heavy metals including copper, lead and zinc generally have inhibitory effects on pollen germination and tube growth (Bhandal and Bala, 1989; Gomoa, 1989; Vitagliano and Viti, 1989; Wet *et al.*, 1990; Abbott *et al.*, 1991).

### 1.2.3 Pollen storage

Pollen from different species needs different conditions for storage. The best temperature for long term storage is that of liquid nitrogen (-196°C) (Polito and Luza,

1988b; Yates and Sparks, 1990). In some species such as *Pistacia vera*, pollen can be stored at -15°C for as long a time as in liquid nitrogen (Vithanage and Alexander, 1985). Pollen can be stored at different humidities and temperatures, depending on the pollen type and length of storage. In most species, pollen viability falls dramatically with storage at room temperature.

### 1.2.4 Pollen transfer

Transfer of pollen to the stigma takes place by various methods. The natural agents of pollination are mainly wind and insects, but sometimes it is done artificially in order to increase yields, such as in the date palm.

Air currents are involved in the pollination of many crop plants such as corn, wheat and barley. Insects, especially honeybees, are also very important in pollination and the majority of out-crossing is done by honeybees and wind (Hartmann and Kester, 1990). Following pollen transfer, three types of pollen-pistil reaction have been distinguished: incompatibility, full-compatibility and partial-compatibility. Self-incompatibility is an important cause of out-crossing in plants.

## 1.3 Out-crossing

### 1.3.1 Self-incompatibility

In many plant species, out-crossing is achieved by hereditary factors which impose a physiological barrier to self-fertilization. Such plants produce functional gametes, but genetic factors limit the effective mating combinations in the population.

Many species of flowering plants have evolved genetically controlled mechanisms to prevent inbreeding. Self-incompatibility (SI) systems occur widely in angiosperms and, in the majority of plant species, SI is controlled by a single multiple allelic S-locus (Lewis, 1954). Different plant families vary extensively with respect to the genetic control and cytological manifestation of the phenomenon. However, SI systems have been classified into two main groups. A distinction can be made

between SI systems in which the incompatibility phenotype is associated with a number of morphological differences in the flower (heteromorphic systems) and SI systems with no associated morphological differences (homomorphic systems) (Pandey, 1980). A sub-division can be observed between the homomorphic groups in which the SI phenotype of pollen is determined by the haploid genome of the pollen (gametophytic systems) or by the diploid genome of its parent plant (sporophytic systems). The major difference between the two systems is in the timing of SI gene action. In gametophytic systems, the relevant gene(s) is expressed in the pollen or pollen tube, whereas in the sporophytic systems expression occurs in early anther development, either in the premeiotic phase of the PMC or in the tapetum (Lewis, 1954; Pandey, 1958; Pandey, 1979).

#### 1.3.1.1 Origin of new self-incompatibility specificities

There are three main mechanisms which can lead to the appearance of new incompatibility specificities: (1) mutation and changes in the main locus (Lewis, 1954; Hayman and Richter, 1992); (2) breakdown of activity due to doubling of the genome; (3) hybridization between relatively distant species. Environmental conditions such as temperature, humidity and carbon dioxide ($CO_2$) concentration may have an effect on incompatibility systems, often by influencing modifier gene action (Bubar and Miri, 1965; Nasrallah and Wallace, 1968). O'Neill (1986) reported that 5% $CO_2$ reduced the level of self-incompatibility in rape. It may affect the activity of the enzyme esterase.

#### 1.3.1.2 Heteromorphic self-incompatibility

In this system both the stamens and styles are different lengths, with two types, distyly and tristyly, common in plants. In distylous systems there are two types of flowers, thrum and pin. Thrum flowers have short styles and long stamens with large pollen grains, and pin flowers are the opposite (Lewis, 1954; Ganders, 1974; Ornduff, 1980; Swamy and Bahadur, 1984; Cornish *et al.*, 1988). Distyly is

common in the families Rubiaceae (Ornduff, 1980) and Boraginaceae (Olesen, 1979). Yield in the long x short or short x long style flowers is higher than in self-pollinated or short x short and long x long style flowers (Mulcahy and Comparello, 1970; Ganders, 1974; Mulcahy and Mulcahy, 1983). Distyly is controlled by a single locus with two alleles. In some plants heterostyly is not connected with incompatibility (Whitehouse, 1950; Crowe, 1964). Usually in long style flowers the pollen tubes are arrested in the style, whereas in short styles the tubes are arrested in the stigma (Bawa and Beach, 1983).

In the tristylous system, the style may be one of three lengths, long, medium or short. The stamen length varies according to the stigma position (Lewis, 1954).

#### 1.3.1.3 Homomorphic self-incompatibility

More than half of the flowering plants have specific self-incompatilbility genes (S-genes) which prevent inbreeding. There are two major types of homomorphic multiallelic incompatibility systems, sporophytic and gametophytic.

Pandey (1962) reported differences between gameto- and sporophytic self incompatibility (GSI and SSI) which are correlated with a shift in timing of gene action. Gene action in gametophytic systems takes place in the gametophyte immediately after meiosis and before microspore formation (de Nettancourt, 1977). In sporophytic systems, breakdown of the tapetal layer transfers the molecules of incompatibility substances (glycoproteins) from the parent (sporophyte) into the exine cavities of the pollen (Knox, 1973; Pandey, 1979).

##### 1.3.1.3.1 Gametophytic self-incompatibility

In this group of self-incompatible plants, the pollen-pistil interaction is genetically controlled by the haploid genome of the pollen grain and the diploid genome of the pistil tissue. This system, in many cases, is controlled by a single gene locus with multiple alleles. The gametophytic self-incompatibility (GSI) system is

found in the families Solanaceae, Liliaceae, Poaceae, Commelinaceae, Papaveraceae, Onagraceae, Rosaceae and Rubiaceae (Cornish *et al.*, 1988).

At the time of arrest the tip of the pollen tube thickens, callose deposits occur close to the tip, and the pollen tube may then burst (Kahn and Demason, 1985). Successful fertilization in plants with the GSI system occurs only when the S-alleles in pollen and pistil are different. The GSI in some monocots such as Poaceae (Gramineae) is more complicated than in dicots and is controlled by two independent S and Z loci (Hayman, 1956; Lundqvist, 1975).

#### 1.3.1.3.2 Sporophytic self-incompatibility

In sporophytic self-incompatibility (SSI) pollen behaviour is determined by the genotype of the pollen parent. Inhibition usually occurs on the stigmatic surface. It is often associated with the deposition of callose on the stigma or in the cell walls of the stigmatic surface (Thompson, 1967; Knox, 1982; Bob *et al.*, 1986). In SSI the pollen tube may erode the cuticle, but is arrested within or under the cuticle layer (Clarke, 1982; Bob *et al.*, 1986).

### 1.3.2 Biochemical and molecular basis of self-incompatibility

In the pollen and pistil of self-incompatible plants are substances which may be involved directly or indirectly with incompatibility. Lewis (1954) reported that S-proteins control SI in *Oenothera organensis*, and Nasrallah and Wallace (1968) detected S-allele antigens in the style of *Brassica oleracea* using antisera raised in rabbits. A glycoprotein extracted from the style of *Brassica campestris* has been isolated by Nishio and Hinata (1977) and Nishio *et al.* (1992), which may be a product of the S7-allele or from a gene adjacent to that locus, and Williams *et al.* (1982) reported that a glycoprotein in the style of *Prunus avium* which caused inhibition of pollen tube growth of the $S_3S_4$ genotype probably corresponded to the S3 or S4 alleles. In *Nicotiana alata*, growth of pollen tubes with the S2-allele is inhibited by the S2-glycoprotein (Anderson *et al.*, 1987). Takayama *et al.* (1987)

reported that SI in *B. campestris* correlated with an S-glycoprotein containing a series of peptides at the N-terminal end which are homologous with the same sequence in the C-terminal region of the *B. oleracea* glycoprotein reported by Nasrallah *et al.* (1985). Anderson *et al.* (1986) distinguished a unique 30-base pair oligonucleotide from mature style extract in *Nicotiana alata* which may be involved in SI. Arabinogalactan proteins have a high proportion of galactose and arabinose attached to the proteins (typically to the hydroxyproline amino acid position). These proteins are reported to be a major part of the stylar mucilage in hollow styles such as that of *Gladiolus*, and they are also reported in the transmitting tissue of *Nicotiana alata*. The function of the arabinogalactan proteins within styles is not established, but presumably they are involved in pollen tube growth and nutrition, and may be related to the S-genes. mRNAs and proteins play a role in the regulation of pollen tube growth (Lin *et al.*, 1987; Chen *et al.*, 1992), and self-incompatibility due to ribonuclease activity has been reported in the style of *Nicotiana alata* (Koltunow *et al.*, 1990; McClure *et al.*, 1990), and in *N. tabaccum* (Murfett *et al.*, 1992).

### 1.3.3 The time and site of incompatibility reactions

The tissues involved in the incompatibility reactions are the male gametophyte (pollen and pollen tube) and the pistil tissue of the stigma, style and ovary. Three major sites of pollen tube inhibition are observed: (1) inhibition on the stigma surface such as in the Brassicaceae, Compositae (2) inhibition during pollen tube growth in the transmitting tissue in the style, for example, in the Solanaceae and Rosaceae; and (3) inhibition in the ovary, ovule or embryo sac such as in *Gasteria*, *Theobroma*, and *Lotus* (Heslop-Harrison and Dickinson, 1969; Heslop-Harrison and Heslop-Harrison, 1981; Heslop-Harrison, 1982; Cornish *et al.*, 1988).

#### 1.3.3.1 Prezygotic self-incompatibility

In prefertilization incompatibility, the pollen tubes generally do not grow through the full length of the style. Inhibition can also occur in the ovary, however

(Seavy and Bawa, 1986), and McKay (1942) in *Castanea mollissima*, and Turcotte and Feaster (1967) in *Gossypium barbadense* reported that after pollination and tube penetration the embryo sac the sperms did not fuse with the female nuclei.

### 1.3.3.2 Postzygotic self-incompatibility

Postzygotic SI mainly occurs at the early stage of embryo development. Either the zygote fails to divide, or it undergoes a few divisions and then aborts.

In alfalfa some self pollinated ovules abort very soon after fertilization (Busbice *et al.*, 1975), and Griffin *et al.* (1987) reported that in *Eucalyptus regnans* also, the zygote may abort after fertilization. In mango embryo abortion is due to nucellus and endosperm degeneration and at the time of abortion, endosperm nuclei are abnormally large (Sharma and Singh, 1970). Seedlessness in *Camellia* is related to embryo abortion (Yueh-Jiang *et al.*, 1992), and incompatibility in grasses is sometimes due to zygotic inviability, embryo abortion, or endosperm abnormalities, which occur after fertilization (Heslop-Harrison, 1982).

### 1.3.3.3 The stigma

The stigma is the part of the pistil which is specialized for pollen reception at the time of pollination, and stigmas can be divided into two main groups: dry and wet. Dry stigmas have little or no surface secretion at maturity, whereas wet stigmas have copious exudate (Heslop-Harrison and Shivanna, 1977). In general, stigma inhibition of pollen germination and tube growth is related to SSI and dry stigmas. In *Brassica* the S-glycoproteins are first produced a few days prior to anthesis and the concentration increases dramatically about one day before flower opening (Nishio and Hinata, 1977; Nasrallah, 1985; Moore and Nasrallah, 1990).

### 1.3.3.4 The style

Two kinds of style can be distinguished: the solid style and the hollow style. Hollow styles usually have a canal lined by transmitting tissue, and sometimes the canal is divided into branches. Solid styles have a central core of transmitting tissue.

The hollow style is very common in monocotyledonous plants. Pollen tubes pass through the canal to reach the ovary, and in SI species the pollen tube usually is arrested in the style (Ascher and Drewlow, 1971). In *Trifolium* the hollow style is surrounded by secretory cells, and the canal and stigma exudates are of the same composition (Heslop-Harrison, 1982). In citrus (Kahn and Demason, 1985) and gooseberry (Arasu, 1985) the pollen tube is arrested within the canal. Brewbaker and Gorrez (1967) reported that SI in the ovary may also be correlated with hollow styles.

Solid styles have transmitting tissues with the files of cells separated by intercellular substance. Acid phosphatase, peroxidase and carbohydrates are found in the intercellular material (Bell and Hicks, 1976), and Went and Willemse (1984) observed that the cells of transmitting tissue in *Lycopersicon peruvianum* are vacuolated and connected via plasmadesmata. S-glycoproteins are associated with pollen tube inhibition in the style, and Pandey (1962) observed in *Solanum* that most incompatible pollen tubes in self- and cross-incompatible crosses were arrested about one-third of the length down the style from the stigma. In the neck of the style of *Petunia hybrida* are large spherical cells, in which area the pollen tubes are arrested and the upper part of the style is also the inhibitory site in *Panicum* (Burson and Young, 1983), *Secale* (Heslop-Harrison and Heslop-Harrison, 1981) and *Pyrus serotina* (Yamashita *et al.*, 1990). Sedgley *et al.* (1985) observed that the pollen tubes of macadamia were arrested in the upper style, and Ellis *et al.* (1991) reported that the upper part of the style in *Eucalyptus* was the site of inhibition in interspecific crosses. In *Nicotiana alata* the highest concentration of S-glycoproteins was present in the upper part of the style at flowering time. Anderson *et al.* (1986) reported that using the stigmatic secretion from a compatible mature flower with mature pollen on

immature stigmas (green bud) from the same plant resulted in selfed seed. Thus, incompatibility substances (S-glycoproteins) are not present in immature flowers. Koltunow *et al.* (1990) supported this finding in tobacco.

### 1.3.3.5 The ovary

Ovarian inhibition is divided into two main kinds, prezygotic and post zygotic. Prezygotic inhibition includes micropylar, nucellar, and embryo sac inhibition (Bawa and Beach, 1983). Post zygotic inhibition occurs either immediately after egg fusion or after a few zygotic divisions. For example, ovule abortion of *Gasteria verrucosa* occurs after the first division of the primary endosperm nucleus (Seavey and Bawa, 1986).

Spiss and Paolillo (1969) observed that the pollen tube was arrested at the end of the funiculus or at the micropyle of *Lotus corniculatus*. In *Paspalum* species the highest percentage of pollen tubes was arrested at the micropyle; the ovary and style were the next most important points of arrest (Burson and Young, 1983). Brewbaker and Gorrez (1967) reported that in a *Gasteria* hybrid (*G. verrucosa* x *G. picta*) the pollen tube is arrested adjacent to the micropyle.

### 1.3.3.6 The ovule

In *Acacia retinodes* the pollen tube cannot pass beyond the integuments, and is arrested in the nucellus (Kenrick *et al.*, 1986). Self-incompatibility in *Theobroma cacao* is controlled within the embryo sac. The pollen tube enters the ovule, but male gametes fail to fuse with female gametes. This incompatibility is controlled by two S-loci (Cope, 1962). In *Lotus corniculatus* self pollen tubes grow into the ovary, but fail to fertilize the ovule (Dobrofsky and Grant, 1980 a and b) and pollen tubes in self-pollinated flowers of *Warzewiczia* also failed to fertilize the egg (Bawa and Beach, 1983). In *Rhododendron* the pollen tube released the sperm on entry into a synergid but these failed to fertilize the egg (Williams *et al.*, 1986).

### 1.3.4 Overcoming self-incompatibility

Overcoming SI is very important for producing homozygous pure lines. The methods of overcoming SI are:

(1) Application of hormones to the ovary, for example, in apple (Emsweller and Stuart, 1948);

(2) Temperature treatment. Immersing the stigma of *Oenothera* in hot water (45-50°C) for five minutes removed SI (Matsubara, 1980; Hiratsuka *et al.*, 1989), whereas low temperatures of about 0-2°C increased the self seed set in Golden Star carambola (Knight, 1982);

(3) Mentor pollen application, for example, compatible irradiated pollen of pear improved selfed seed production (Visser and Marcucci, 1986). Yamashita and Tanimoto (1985) used pollen extract and/or stigma exudate in citrus for overcoming SI. They succeeded in producing some seeds from the self-incompatible Hassanaka and Huganatsu cultivars. Dayton (1974) increased fruit set in apple by using a mixture of killed and self-incompatible pollen;

(4) Placental, ovarian, or test tube pollination (Kanta and Maheshwari, 1963);

(5) Mechanical or electrical methods (Roggen and van Dijk, 1972, 1976; Roggen *et al.*, 1972);

(6) Double pollination. For example, in apple and pear double pollination increased self-seed production (Visser and Marcucci, 1983);

(7) Carbon dioxide concentration. $CO_2$ application to self-incompatible *Brassica* species increased self-seed production. $CO_2$ concentration ranging from 3 to 10% in air enabled the self-incompatible pollen tube to penetrate the stigma and overcome the self-incompatibility (Nakanishi and Savano, 1984).

### 1.3.5 Monoecy

Monoecious plants have the sexes separated in different flowers on the same plant. Pollination is by wind or insects. Many plants are monoecious including *Corylus* (filbert), *Castanea* (chestnut), *Alnus* (alder), *Betula* (beech), and *Juglans* (walnut). The andromonoecious cashew, *Anacardium occidentale*, has hermaphrodite and male flowers (Moncur, 1986).

### 1.3.6 Dioecy

In dioecious plants the male and female flowers are on different plants, such as in date palm, asparagus, kiwifruit, ginkgo and pistachio. Armstrong and Tucker (1986) described dioecy in *Myristica fragrans*, with staminate and pistilate flowers produced on axillary flowering structures with different morphology. Dioecious plants may have derived from monoecious or hermaphrodite ancestors (Whitehouse, 1950) via gynodioecy or andromonoecy (Lloyd, 1980). Commercially the ratio of male and female plants for pollination is very important. For example, one male is recommended per nine female plants of kiwifruit (Hartmann and Kester, 1990), and pistachio (Maggs, 1973). The segregation of the sexes makes it simple to produce hybrid seed in breeding programs. In some species sex expression can be correlated with a chromosomal or hormonal basis (Frey, 1983). In dioecious plants, such as *Spinacia oleracea,* the sex determination gene is located on sex chromosomes, such that xx are female and xy are male (Duvick, 1967). A genetic male may very occasionally produce a small percentage of hermaphroditic flowers, such as in pistachio (Crane, 1974).

### 1.3.7 Dichogamy; protandry and protogyny

Dichogamy is the maturity of the stigma and anther of one flower at different times, or of the unisexual flowers of one plant at different times. Dichogamy can be divided into two main groups. Protandry occurs when the mature pollen is shed prior to stigma receptivity, and protogyny is vice versa. Protandrous dichogamy occurs in

*Amyema* (Loranthaceae) where the anther dehisces from 12 hours to over a week before pistil maturity, depending on the taxon (Bernhardt and Knox, 1983). The mechanism is associated with the presence of a dense layer of cuticle over the stigma, containing the enzyme esterase, which is involved with both the site of pollen hydration and tube penetration into the stigmatic surface (Bernhardt and Knox, 1983). Sedgley *et al.* (1985) reported that in the macadamia the pollen is shed before anthesis, and the stigma produces exudate as a pollen germination medium two days later.

Protogyny, in which pistil maturity is attained before pollen shed, occurs in many taxa including members of the families Poaceae, Moraceae, Lauraceae and Rosaceae (Elliott, 1958; Lloyd, 1980; Gleeson, 1982; Shaulp, 1986). In the caprifig the staminate flowers mature a few weeks after the pistillate flowers in the same fig (Condit, 1947). In the family Lauraceae, including the avocado, protogynous flowers have a reciprocal daily cycle (Lloyd, 1980; Gleeson, 1982). The avocado plant has two mating types. In mating type A, the flowers first open in the morning when their stigmas are receptive, but the anthers are not dehisced. The flowers reopen the next afternoon in the male stage. In type B, the female stage flowers open in the afternoon, and then re-open in the male stage the next morning when the stamens are upright and the anthers are dehisced (Bergh, 1975; McGregor, 1976; Sedgley, 1977a).

Heterodichogamy occurs when the mating system exhibits phenotypic dimorphism (protandrous and protogynous). It is common in the family *Juglandaceae* including walnuts, hickories and pecans, and also in the *Betulaceae* in filberts. An individual tree may be either protogynous or protandrous, and in walnut the female flower primordial of protogynous clones are initiated during the year before flowering, whereas those of protandrous clones do not reach this stage until the following year, just prior to anthesis (Polito and Li, 1985). In pecan, heterodichogamy is controlled genetically with protogyny dominant to protandry (Wetzstein and Sparks, 1986).

In many monoecious plants the male and female flowers may not mature simultaneously. This phenomenon can be used for hybridization programs, especially in crop plants such as sweet corn and some species of the Cucurbitaceae (Duvick, 1967).

### 1.3.8 Male sterility

Male sterility includes pollen abortion and sterile pollen production. It is used commercially in the production of hybrid seeds of pepper, onion, carrot, sorghum, and sweet corn (Edwardson, 1970; Horner and Rogers, 1974; Hossain, 1983).

In flowering plants, male gamete formation is a highly regulated developmental process that occurs in the anther. In this organ haploid micro spores are produced after meiotic division of the pollen mother cell. The micro spores develop into pollen grains that carry the generative or sperm cells. One of the most important tissues in the anther is the tapetum. It plays roles in the development and maturity of the pollen, in nutrition of the micro spores, and contributes to the sporopollenin deposition of the exine. The tapetum also produces the enzymes that release the micro spores from the callose wall (Horner and Lersten, 1971; Laser and Lersten, 1972; Mascarenhas, 1990). Tapetal cells have specific genes which control pollen development, which may be in the cytoplasm, the nucleus or both. Mutations in these genes may act as abortive factors in each stage of pollen development (Laser and Lersten, 1972; Warmke and Overman, 1972; Bino, 1985), and may be stimulated by environmental conditions such as temperature, light, and humidity (Nasrallah and Wallace, 1968; Mariani *et al.*, 1991; Hormaza and Herrero, 1992).

Cytoplasmic male sterility, in which the sterility genes are in the mitochondria or plastids, has been reported in many commercial plants. It is controlled by single or multiple genes (Edwardson, 1970; Sage, 1972; Koltunow *et al.*, 1990). Cytoplasmic male sterility can arise as follows:

(1) Intergeneric crosses such as *Aegilops* x *Triticum* (Kihara, 1966), *Lycopersicon* x *Solanum* and *Triticum* x *Secale*, in which pollen abortion is accompanied by reduction in anther size and lengthening of filaments (Kihara, 1966; Edwardson, 1970);

(2) Interspecific crosses, for example, *Begonia schmidtiana* x *B. subvilosa*, and *Capsicum annuum* x *C. peruvianum*, in which male flower buds are abscised early in the $F_1$ generation (Daskaloff, 1968);

(3) Intra-specific crosses such as Australian Brown x Persian White onion, in which male sterility appears in the $F_2$ generation (Peterson and Foskett, 1953). In rice sterility arises in the $F_1$ generation from the cross between Indica female and Japonica male, and appears to be controlled by three pairs of genes (Edwardson, 1970). Male sterility in rice was increased by cooling treatment particularly at an early stage of microsporogenesis (Iwao, 1983);

(4) Spontaneously, as in *Allium* (Jones and Clarke, 1943). In this case segregation appears in the $F_1$ with a ratio of 1:1, and in the $F_2$ and $F_3$ with a ratio of 3:2 and 3:3 male sterile : male fertile plants. This kind of sterility has also been observed in *Beta* and *Capsicum* (Edwardson, 1970; Laser and Lersten, 1972).

Nuclear male sterility occurs when nuclear genes are homozygous. *Sorghum* and *Zea* are examples in which the $Rf_1$ male sterile gene on chromosome 3 controls sterility (Herbert *et al.*, 1955). In nuclear cytoplasmic male sterility the genes are both in the cytoplasmic organelles and in the nucleus, for example, in wheat (Hossain, 1983.

## 1.4 Fertilization, fruit set and development

### 1.4.1 Fertilization

Fertilization is the fusion of male and female nuclei. After pollination the pollen tube enters the stigma and style, and penetrates the ovule via the micropyle. In

a minority of cases it penetrates via the chalaza and/or other parts of the ovule, depending on ovule structure and maturity. The pollen tube generally enters the embryo sac via one of the synergids, either after previous degeneration or accompanied by the destruction of that synergid (Went and Willemse, 1984). A pore develops in the pollen tube tip and the tube contents including sperm cells and vegetative nucleus are released. The pollen tube then degenerates (Russell, 1982; Went and Willemse, 1984).

The phenomenon of nuclear fusion has been described by Jensen (1964) and by Went and Willemse (1984). The plasma membrane of one sperm cell comes into contact and fuses with the plasma membrane of the egg cell, and the plasma membrane of the second sperm fuses with the central cell plasma membrane. The fusion process leads to the formation of bridges between the two gametes, through which the sperm nuclei and possibly sperm cytoplasm can pass. Therefore, there is no pore or rupture in the female gamete membranes to facilitate entrance of the pollen tube contents. One of the sperms fuses with the egg to form the zygote which develops into the embryo, and the other fuses with the polar nuclei to produce the triple fusion nucleus, which develops into the triploid endosperm.

### 1.4.2 Fruit set

Except in some apomictic and parthenocarpic species, the flowers must be pollinated in order to set fruit. Fruit set may occur without fertilization, by pollen tube stimulation (Gustafson, 1942; Okamoto and Imai, 1982; George *et al.*, 1984). Auxins are produced by growing pollen tubes and plant growth substances may be involved in fruit set (Stephenson, 1981), as auxins, gibberellins, and cytokinins have all been implicated in fruit set (Popov, 1984, Coombe; 1989). The percentage of fruit set and ovary development, which usually commences at anthesis or fertilization, may depend on variety, rootstock, nutrition, pollen parent, time of pollination or environmental conditions (Schroeder, 1947; Thompson and Liu, 1973; Bookman, 1983; Coombe, 1989; Degani *et al.*, 1990).

In some plants with two ovules in the ovary, such as *Macadamia* and *Prunus avium*, fertilization normally takes place in only one ovule and the second one usually aborts (Eaton, 1959; Sedgley, 1981a). In almond degeneration of the second ovule may be due to callose accumulation in the chalaza (Pimienta and Polito, 1982). The endosperm plays an important role in embryo development of all species. It may be absorbed during development or persist to seed maturity.

### 1.4.3 Apomixis

Apomixis is asexual reproduction in plants and includes the phenomena of adventive embryony, parthenogenesis, apospory and diplospory. The most common form of vegetative apomixis, apospory, occurs when the embryo develops from the embryo sac without meiosis either following megaspore mother cell (MMC) failure, or from an ovarian somatic cell. In *Amelanchier* aposporous embryo sacs occur close to a mass of degenerated tissue at the centre of the young ovule, and all degenerate except those with eight nuclei at maturity (Green, 1984). Longly (1984) reported that aposporous embryo sac initials in *Cichorium intibus* derived from nucellus and chalazal tissue.

Apospory occurs in many families of plants such as the Ranunculaceae, in *Ranunculus auricomus*, and the Rosaceae, in *Amelanchier* and *Crataegus*, (Nogler, 1984; Dickinson and Phipps, 1985; Campbell *et al.*, 1987). Apomixis can arise via mutation and is controlled by multiple genes (Olien, 1987). Pollination is necessary for seed and fruit production in *Amelanchier laevis* despite aposporous replication (Campbell *et al.*, 1987). When the embryo arises directly from an unreduced MMC the condition is called diplospory and occurs in *Poa alpina* and in *Paspalum* (Nogler, 1984). Some species, such as *Poa pratensis* are classified as facultative apomicts that can produce both sexual and apomictic seeds. Sexual plants can be mated with facultative apomictic plants to produce new hybrids (Tisser *et al.*, 1987; Campbell *et al.*, 1987). In adventitious embryony the embryo develops directly from the somatic

ovarian tissues, nucellus or integument without the formation of an embryo sac, as in nucellar embryos of citrus (Soost and Cameron, 1975).

Viviparous propagation is where the seed germinates before dispersal or harvesting. This characteristic is controlled genetically and by external stimulants, such as certain environmental conditions. In some plants such as mangrove, some varieties of pecan, watermelon, citrus and some perennial forage grasses the embryo starts growing when temperature and other factors are favourable (Bashaw, 1980; Hartmann and Kester, 1990). Vivipary can occur following both sexual and asexual seed formation.

### 1.4.4 Polyembryony

Polyembryony occurs when more than one embryo develops within a single seed. Two main types of polyembryony can be distinguished, which take place in either the sporophytic (integuments and nucelli) or gametophytic (egg and synergids) tissues.

Polyembryony may occur by cleaving of the apical embryogenic mass of cells produced by the zygotes as in maize (Sarkar and Coe, 1966) and *Pterocarya fraxinifolia* (Bouman and Boesewinkle, 1969). In *Araucaria juss* polyembryony occurs from the suspensor without zygote cleavage (Haines and Prakash, 1980). Alternatively polyembryony may occur when accessory embryos are produced from one or more embryo sacs in the same ovule (Soost and Cameron, 1975; Lakshmanan and Ambegaokar, 1984). In *Gossypium barbadense* there is one fertilized egg with a number of parthenocarpic eggs developing in separate embryo sacs, which may be due to abnormal fertilization (Turcotte and Feaster, 1967).

Adventive polyembryony is when the embryos develop from the nucellar or integument cells. This occurs in the family Juglandaceae, in *Juglans regia* (Nast, 1935), Rutaceae, in *Citrus* (Soost and Cameron, 1975) and Anacardiaceae, in *Mangifera* (Philipson, 1978) and pistachio (Grundwag and Fahn, 1969). The number

of embryos in the nucellar tissue varies, and is related to the age of the plant, the nutritional status of the fruit, the pollen parent, and environmental conditions. Polyembryony is used in propagation and breeding, for example, in citrus, virus-free seedlings are produced via nucellar embryos (Lakshmanan and Ambegaokar, 1984).

### 1.4.5 Parthenocarpy

Parthenocarpy is the ability to form fruit without fertilization. It can be divided into two main forms: vegetative and stimulative parthenocarpy. Vegetative parthenocarpy is induced by endogenous or exogenous plant growth regulating substances, and may also be stimulated by environmental factors (Gustafson, 1942; Garcia-Papi and Garcia-Martines, 1984). The plant growth regulators auxins, gibberellins and cytokinins, can be utilized to increase parthenocarpy in some fruits such as fig, grape, apple, and tomato (Crane *et al.*, 1959; Schwabe and Mills, 1981). Stimulative parthenocarpy requires the stimulus of pollination. Parthenocarpy may be inherited by a single gene and can be observed in many taxa, including fig, citrus, grape, banana, tomato, cucumber, date, pear, apple and pineapple (Gustafson, 1942; Crane *et al.*, 1959; Storey, 1975; Lin, S. *et al.*, 1983, 1984).

In cucumber and *Ribes* the top flowers of the branch possess high levels of auxins which can increase the number of parthenocarpic fruits (Nitsch *et al.*, 1952; Takeno and Ise, 1992). Beger and Quebedeaux (1975) reported that an auxin transport inhibitor applied to the peduncle of cucumber may induce vegetative parthenocarpy.

Parthenocarpic seedless fruits may be produced in response to environmental stimuli such as high or low temperature, humidity, light and wind (George *et al.*, 1984; Lin, S. *et al.*, 1984). These factors may act on either the sporophytic or gametophytic tissue of the plants to increase fruit development (Rick, 1978; Levy *et al.*, 1978). Insufficient pollen shed plus rapid growth of the ovary can produce parthenocarpic fruits in tomato (Lin, S. *et al.*, 1983). Mentor and self-incompatible

pollen can stimulate parthenocarpy, for example, in Clemantine mandarin (Garcia-Papi and Garcia-Martines, 1984), and *Citrus grandis* (Li, 1980), and irradiated pollen is successful in pear (Sniezko and Visser, 1987), and in some apples (Zhang and Lespinasse, 1991).

In nut crops parthenocarpy (blanking) is undesirable. In pistachio it has been reported to result from early pollination, or may be attributable to certain rootstocks, pollen genotypes, environmental or nutritional conditions (Whitehouse *et al.*, 1964; Crane, 1973; Bradley and Crane, 1975). In filbert a single gene controls self-incompatibility, which can be a cause of blanking in this crop (Thompson, 1967).

### 1.4.6 Fruit development

Fruit growth in many species is sigmoidal and includes an initial period of slow growth, phase I; a period of exponential growth, phase II; and a period of declining growth, phase III. Phase I is characterised by increase in cell number, and endogenous auxins and gibberellins are responsible for this growth (Coombe, 1965; Bottini *et al.*, 1985). In phase II the cells enlarge rapidly and storage substances such as carbohydrates accumulate in both seed and pericarp cells. In phase III ripening occurs. The fruit flesh softens, storage materials such as starches and oils convert into sugars, organic acids decline and pigments develop (Leopold and Kriedemann, 1975; Kingston, 1992).

Some species have a double sigmoidal fruit growth curve. These include the stone fruits, *Ficus carica*, *Ribes nigrum*, *Rubus* spp, *Vaccinium* spp, *Vitis* spp, and *Olea europea* (Green, 1971; Coombe, 1973; Coombe, 1989). Cell division occurs during the initial period of slow growth and may continue into the first rapid growth phase. In stone fruits, the stone and seed usually reach full size during the first rapid growth phase. In the second period of slow growth, the stone hardens and the embryo enlarges rapidly. In the second rapid growth phase, the fleshy part of the fruit enlarges and finally the fruit ripens. In addition to the two types of growth curve,

species differ in the final size of their fruits (Leopold and Kriedemann, 1975; Hartmann *et al.*, 1988).

### 1.4.7 Fruit abscission

Abscission involves cell separation by dissolution of one or more layers of the cell wall, or of the middle lamella (Kozlowski, 1973). In abscission of many fruits, cell separation occurs in an irregular zone of parenchyma cells localized at the point where the fruit and receptacle tissue are contiguous (Stösser *et al.*, 1969a, b).

When premature fruit abscission occurs in fruit trees, it is usually prior to developmental phase II in species with sigmoidal fruit growth, and prior to phase III in double sigmoidal species. Apples, for instance, have two distinct periods of abortion, 'early drop' just after fertilization and 'June drop' after cell division in the endosperm and before rapid cell division in the embryo (Leopold and Kriedemann, 1975). In stone fruit such as peaches, cherries and apricots, the periods of fruit abortion precede the major developmental changes during the first two phases of the growth curve. Some species appear to have only one major period of abscission (Stephenson, 1980). Many factors are involved with fruit abscission. Factors influencing fruit abscission include soil moisture and aeration, mineral deficiencies and toxicities, salinity and alkalinity and soil diseases. Other influences include late frost and high temperatures in spring, atmospheric pollutants, biotic factors such as insects and pathogenic micro-organisms, and incompatible pollination (Addicot and Lyon, 1973).

Chemicals may influence fruit abscission. Auxins, such as indole acetic acid, naphthalene acetic acid and 2,4 dichlorophenoxy acetic acid, when sprayed on trees generally delay fruit abscission. These auxins are abscission inhibitors and are translocated rapidly from leaf to fruits (Cooper and Henry, 1973). Blanped (1972) reported that the second fruit drop in apple, the June drop, correlated with increased production of ethylene which is emitted from fleshy tissues and acts upon the adjacent

abscission zone. In citrus fruits, cellulase, a cell separation enzyme, is produced after application of ethylene (Jackson and Osborne, 1970; Abeles *et al.*, 1971). Abscisic acid (ABA) and 2,4,5-trichlorophenoxy acetic acid have been shown to stimulate ethylene production in apples, tomatoes, avocados and ber (*Ziziphus mauritima*) Their levels are constant during maturity and increase at ripening (Rhodes, 1980; Bal *et al.*, 1984). The presence of mature leaves may increase fruit maturity and abscission in citrus because of the ABA transferred from the old leaves to the fruits. In contrast, young leaves produce GA3 and reduce fruit abscission (Cooper and Henry, 1973). In *Phaseolus vulgaris* older fruits inhibit younger fruits and cause their abscission by producing ABA (Tomas *et al.*, 1979). Ethylene increases cellulase activity in the separation layer, and gibberellins, auxins and ABA stimulate ethylene production (Abeles *et al.*, 1992).

Some undamaged juvenile fruits of legumes were reported to abscise due to genetic disorders (Martin *et al.*, 1961), and fruit abscission on different rootstocks of *Hachiya persimmon* and of *Zizyphus mauritima* is also controlled by genetic factors (Schroeder, 1947; Singh *et al.*, 1991). Herbivory, defoliation, and leaf shedding are also possible causes for fruit abscission (Martin *et al.*, 1961; Hocking and Pate, 1977). Fruit thinning and removal of the shoot tips of apple trees increase the size of fruits and reduces fruit drop significantly (Quinlan and Preston, 1968).

### 1.4.8 Ripening

Ripening of fruits refers to pigment accumulation and the cell wall composition changes that result in the softening of fruit (Biale, 1964; Coombe, 1976; Bouranis; Niavis, 1992). These changes occur from the latter stage of growth and development to full maturation and may occur either before or after harvesting.

Fleshy fruits can be divided into climacteric fruits, such as avocado and banana, which undergo a large increase in respiration with major changes in fruit composition and texture, and non-climacteric fruits, such as citrus, which show little

or no change in respiration. The increase in respiration appears to be a consequence of an increase in endogenous ethylene, which results in increased ATP levels in the cells, followed by ripening and senescence (McMurchie *et al.*, 1972; Brady, 1987). Ethylene synthesis is normally limited by the supply of 1-aminocyclopropane-1-carboxylic acid (ACC) (Bufler, 1984). High calcium concentration in fruit tissue results in a slower rate of ripening, reduced ethylene production, and slower softening (Ferguson, 1984). Various enzymes are involved with ripening, including cellulase and polygalacturonase (Frenkel *et al.*, 1968; Leopold and Kriedemann 1975; Tucker and Grierson, 1982; Christoffersen *et al.*, 1984; Kingston, 1992). Softening of fruit is normally accompanied by an increase in the concentration of soluble pectin, polysaccharide, and uronic acid residues that are solubilised by polyuronic hydrolyzing enzymes (Huber, 1983a and b). In tomatoes, at the softening period, polygalacturonase increases as pectic acid decreases (Brady *et al.*, 1982; Tucker and Grierson, 1982). Polygalacturonase production in tomato fruit is controlled genetically (Givannoni *et al.*, 1992).

Auxin advances ripening in vegetative parthenocarpic fruits such as banana (Abeles *et al.*, 1971). This is because of its effects on ethylene stimulation in the fruit. Endogenous ABA and ethylene increase during ripening of pear and during colouring of citrus (Green, 1971).

Senescence follows fruit development and ripening and leads to the death of the tissue. Senescence may occur before or after harvesting. In this stage abscisic acid frequently increases, and induces osmotic and salt stress in the tissues. Thus, salt treatment of plants may advance ripening, for example, in tomato (Huber, 1983a). Gibberellins retard senescence and delay colour change at maturation and ripening (Abeles *et al.*, 1971).

## 1. 5 The genus *Pistacia*

### 1.5.1 Introduction

*Pistacia* is a genus of the family Anacardiaceae, which originated in the lower mountains and plateaus of central Asia between latitudes 35° and 45° N (Maggs, 1973). *Pistacia* includes 11 species, of which only *Pistacia vera*, the pistachio, produces edible nuts, and the deciduous tree grows to 5 m high and 10 m across. *Pistacia* species have pinnate leaves with 3-11 ovate leaflets, but only 3-5 in *P. vera*. The vegetative buds are easily distinguished from the generative buds by their narrower shape, and flowering in spring precedes foliage development. Flowering extends for about two weeks (Crane and Takeda, 1979; Ducke, 1989), and the individual flowers are small and clustered together in short spikes. New vegetative shoots grow rapidly and shoot growth is complete by mid-summer, but is then retarded by the commencement of seed growth. Under favourable conditions cropping starts at five years but maximum yield is produced at 20-40 years (Maggs, 1973).

The pistachio is dioecious and wind pollinated, with many commercial female cultivars including Kerman, Sirora, Kalleh Ghoochi and Bronte, and male cultivars including Peters, Chico, Ask and 15-12. The quality and quantity of the pistachio crop may be influenced by the pollen donor (Peebles and Hope, 1937; Whitehouse *et al.*, 1964; Martínez-Pallé and Herrero, 1994). Male cultivars should be established as 8-12% of the pistachio orchard (Maggs, 1973).

Although the plant is very tolerant of high and low temperatures, the best yield is produced in sub-tropical and semi-arid conditions. Pistachio trees grow well where the summer is long, hot and dry, and the winters moderately cold so that about 1000 h of chilling below 7°C is provided to break the winter rest. High humidity and rainfall in summer and autumn are not desirable (Maggs, 1973; Joley, 1981; Ducke, 1989).

Pistachio trees can adapt to many kinds of soils, but *P. vera* as a rootstock grows best in well drained, rich, sandy-loam soils. Other suitable species as a rootstock for *P. vera* are *P. atlantica*, which is highly tolerant of heavy and rocky soils, high salinity and root knot nematode, and *P. terebinthus* with tolerance to *Phytophthora* and both alkaline and acidic soils. Other species of *Pistacia* such as *P. lentiscus*, *P. palaestina* and *P. chinensis* are not suitable as *P. vera* rootstocks (Maggs 1973; Joley, 1981; Ducke,1989). All pistachio rootstocks are produced from seed, and this introduces variability in the orchard.

### 1.5.2 Reproductive biology

Male and female flowers are small and in axillary panicles consisting of several hundred individual flowers. Staminate flowers have 3-5 short stamens with large anthers. Female flowers consist of an ovary with three carpels, of which two abort, and a short thick style (Grundwag and Fahn, 1969). The stigma has three white to pink lobes of which one is longer than the other two. The ovule is basifixed and anatropous and has a bent funiculus. The micropyle faces an outgrowth of the funiculus and the nucellus is thick (crassinucellate). There is a hypostase in the chalaza containing phenolic compounds. The pistachio fruit is a drupe which encloses one dicotyledonous seed within a bony shell surrounded by a hull. Hull colour changes at fruit maturity, the colour depending on the cultivar (Grundwag, 1975; Ducke, 1989).

When the female flowers are receptive the stigmatic surface has been reported to become tinged with red (Maggs, 1973). Mature anthers are bright yellow and, after bursting, release many pollen grains. Male flowers generally open sooner than the females, and commercial orchards must use male and female cultivars which mature synchronously.

After pollination, the nucellus and integuments grow and the funiculus becomes large and spongy. Lignification of the shell occurs by the 10th week. The

ovule then grows to fill the ovary cavity by about 18-20 weeks after fertilization, when the fruit matures (Crane and Nelson, 1971; Crane and Al-Shalan, 1974; Grundwag, 1976).

### 1.5.3 Problems in pistachio production

#### 1.5.3.1 Alternate bearing

Alternate bearing is reduction in fruit load in the season after a heavy crop year, which can be seen in many fruit trees, but to different degrees (Davis, 1957). Alternate bearing in many deciduous fruit trees is a result of the inhibitory effects of fruits on flower bud initiation (Schroeder, 1947; Woodbridge, 1973; Porlingis, 1974). It is prevalent in mango, apple, pear, pecan, prune and pistachio. Alternate bearing in pistachio results from inflorescence bud abscission when a heavy crop is present (Crane and Nelson, 1971). In this case dominance by the developing seeds overcomes the inflorescence buds in competition for metabolites and leads to bud abscission (Davis, 1957; Crane and Nelson, 1971; Addicot and Lyon, 1973; Porlingis, 1974). Crane and Al-Shalan (1974) found that carbohydrate levels in branches are not correlated with flower bud abscission. In *P. vera* early or late pollination, particularly accompanied by high temperature at anthesis has been reported to increase alternate bearing. This may be attributable to lack of pistil receptivity, paternal genotype or to age of the female tree (Whitehouse and Stone, 1941). When defruiting occurs before seed completion, bud retention increases, and this can be influenced by neighbouring branches. Thus, an alternate bearing habit may be established on whole trees or on individual branches (Wolper and Ferguson, 1990).

Growth substances may influence *Pistacia vera* yield, for example, $GA_3$ induces cell division and elongation in the base of buds which can bring about bud abscission (Lin, T. S. *et al.*, 1984 a, b). Abscisic acid (ABA) and auxins have been

detected in fruiting branches of pistachio. They were found in leaves and wood and may control fruit bud abscission (Takeda *et al.*, 1980; Crane and Nelson, 1971).

### 1.5.3.2 Blanking and semi-blanking

Blanking (empty shell) and semi-blanking (partially empty shell) are two important problems in *P. vera* production which are common wherever pistachio is grown. Between 23 and 100% of the pistachio crop, depending on cultivar, can be blank and in *P. vera* cv. Kerman the blnk proportion is about 26% (Crane, 1973).

Variability of seedling rootstocks is one of the possible factors in blanking as this is a major source of variability within an orchard (Crane and Forde, 1976; Crane and Iwakiri, 1981). Nevo *et al.* (1974) suggested that late blooming is a possible cause of semi-blanking and recorded such fruits in *P. vera* cv. Red Aleppo. Crane and Takeda (1979) reported that most late bloom follows insufficient chilling, so a moderate winter may result in high fruit blanking and semi-blanking. Chalazogamy followed by embryo abortion at an early stage of fruit growth may also be a factor. Grundwag and Fahn (1969) reported that the pollen tubes grow through the short style and penetrate the funiculus. In the funiculus the pollen tubes were reported to grow through the vascular bundle to the chalaza, and then into the nucellus via the hypostase. In the nucellus the pollen tubes encircle the embryo sac to reach the micropyle (Copeland, 1955; Crane, 1973; Grundwag, 1976). This unusual pathway of pollen tube growth requires confirmation. It is possible that pistachio may be similar to walnut, in which chalazogamy has also been reported (Luza and Polito, 1991). If walnut flowers are pollinated at an early stage then chalzogamous penetration is increased because of undeveloped integuments (Luza and Polito, 1991). This can cause pre- or post-fertilization degeneration of the ovule.

Unpollinated flowers can produce parthenocarpic fruits (Peebles and Hope, 1937; Gustafson, 1942; George *et al.*, 1984), and in this case the ratio of male and female trees in the orchard could be important. There are some reports showing an

effect of pollen genotype on the number of blank fruits in pistachio. Slow growth of the embryo leading to a delay in seed maturation was observed after inter-specific pollination of *P. vera* (Peebles and Hope, 1937; Whitehouse *et al.*, 1964), and ovule degeneration was reported by Grundwag (1975).

#### 1.5.3.3 Indehiscence

*Pistacia vera* is the only *Pistacia* species in which the shell splits naturally at fruit maturity. A split shell in pistachio is desirable from the consumer point of view. Peebles and Hope (1937) reported that the pollen of a *P. vera* hybrid produced more split fruits than pollen of *P. chinensis* or *P. atlantica.* Peters and Ask, which are two male cultivars of *P. vera* were in turn better than the *P. vera* hybrid and *P. atlantica* (Crane and Iwakiri, 1980). However, Crane and Forde (1976) reported that there was no significant difference between the split rates of Kerman fruits pollinated with male cultivars of *P. vera* and other species, in contrast to the findings of Whitehouse *et al.* (1964). In spite of the fact that there exists a split zone in pistachio fruits, splitting may not occur if the seed is small at fruit maturity. Pollen genotype may influence seed size, as interspecific pollination often results in smaller kernels than intraspecific pollination. This may be a xenia effect due to the fact that other species of *Pistacia* have smaller seeds than *P. vera* (Nevo *et al.*, 1974; Crane and Iwakiri, 1981). Nutrition and irrigation are also important for shell splitting (Maggs, 1973).

## 1.6 Conclusions

The aim of this study is to investigate the reasons for poor fruit set and quality in pistachio. This will be achieved by controlled hand pollination at different stages of pistil maturity and with different pollen parents. In addition, a detailed anatomical study of pollination, fertilization and seed development will be conducted to extend and amplify work conducted previously (Grundwag and Fahn, 1969; Grundwag 1975, 1976).

# Chapter 2

# 2 Flowering, fruiting and *in vitro* pollen germination

## 2.1 Experimental area

The pistachio experiments were conducted at the Waite Agricultural Research Institute, which is located in South Australia (34°58' S, 138°38' E and 122.5 m elevation) with a moderate mediterranean climate. Long term climatic data are shown in Table 2.1.

## 2.2 Experimental trees

Experimental collections and orchards have been established on a deep sandy-loam soil with drip irrigation in the orchards. In the Waite Arboretum, a *Pistacia* species collection of seedling trees was planted, whereas in Alverstoke orchard and the CSIRO collection *Pistacia vera* cultivars were grafted onto *P. atlantica* rootstocks (Table 2.2).

## 2.3 Flowering

Flowering period and time of anthesis of the different species of *Pistacia* varies considerably and this may be related to environmental factors such as air temperature, humidity and solar radiation. In this location, flowering of *Pistacia atlantica* commences first followed by the other species with *P. vera* the last to flower (Tables 2.3, 2.4). Flowering in some cultivars of *P. vera* such as Sirora continues to November, which may be due to abnormal bud burst because of the mild winter temperatures. Meteorological data during the 1992 and 1993 flowering periods are shown in Tables 2.5 and 2.6. Female and male flowers of *P. vera* are shown in Figures 2.1 and 2.2.

**Table 2.1** Meteorological data (mean) for the Waite Agricultural Research Institute from 1925-1989. Waite Agricultural Research Institute Biennial Report 1988-1989.

| Month | Rainfall (mm) | Relative humidity (%) | Bright sunshine (hours/day) | Solar radiation (MegJ/sqm/day) | Air temperature ($^{o}$C) |
|---|---|---|---|---|---|
| Jan | 23.4 | 49.5 | 9.3 | 27.0 | 22.1 |
| Feb | 24.3 | 52.5 | 8.9 | 24.0 | 22.1 |
| Mar | 23.8 | 53.9 | 7.6 | 18.9 | 20.6 |
| Apr | 54.9 | 61.0 | 5.7 | 12.9 | 17.3 |
| May | 80.3 | 69.2 | 4.0 | 8.6 | 14.3 |
| Jun | 74.6 | 74.5 | 3.3 | 7.2 | 11.9 |
| Jul | 87.9 | 75.8 | 3.2 | 7.3 | 11.0 |
| Aug | 74.8 | 71.7 | 4.4 | 10.2 | 11.7 |
| Sep | 61.9 | 64.4 | 5.6 | 14.4 | 13.5 |
| Oct | 52.6 | 58.8 | 6.7 | 19.5 | 15.6 |
| Nov | 36.9 | 54.3 | 7.8 | 23.4 | 18.1 |
| Dec | 29.9 | 51.3 | 8.4 | 25.6 | 20.3 |

**Table 2.2** *Pistacia* species and cultivars in the Waite orchards and Arboretum.

| *Pistacia* species | Sex | Number of trees | Tree code(s) | Origin of species[1] | Year planted |
|---|---|---|---|---|---|
| Arboretum | | | | | |
| *P. atlantica* | Female | 2 | 666B, | Mediterranean to W. Asia | 1964 |
| | | | 670A | | 1966 |
| *P. atlantica* | Male | 1 | 671D | Mediterranean to W. Asia | 1965 |
| *P. terebinthus* | Male | 1 | 673D | Mediterranean | 1964 |
| *P. lentiscus* | Male | 1 | 673A | Mediterranean | 1963 |
| *P. vera* seedlings: | | | | | |
| Red Aleppo | Female | 1 | 665B | Western Asia | 1966 |
| Trabonella | Female | 1 | 664A | Western Asia | 1966 |
| Trabonella | Male | 1 | 664B | Western Asia | 1966 |
| CSIRO collection | | | | | |
| *P. vera* cultivars: | | | | | |
| Kerman | Female | 2 | | Western Asia | 1977 |
| | | | | | 1980 |
| 15-12 | Male | 1 | | Western Asia | 1977 |
| Alverstoke orchard | | | | | |
| *P. vera* cultivar: | | | | | |
| Sirora | Female | 2 | | Western Asia | 1984 |

[1] Zohary (1952)

**Table 2.3** Flowering time of *Pistacia* species and cultivars in 1992.

| *Pistacia* species | Tree code | Sex | Flowering period | Peak flowering[1] |
|---|---|---|---|---|
| Arboretum | | | | |
| *P. atlantica* | 666B | Female | 17.9-14.10 | 23.9-3.10 |
| *P. atlantica* | 670A | Female | 22.9-11.10 | 2-9.10 |
| *P. atlantica* | 671D | Male | 4-23.9 | 16-21.9 |
| *P. terebinthus* | 673D | Male | 21.9-17.10 | 23-29.9 |
| *P. lentiscus* | 673A | Male | 6-18.9 | 11-13.9 |
| *Pistacia vera* seedlings: | | | | |
| Red Aleppo | 665B | Female | 28.9-21.10 | 11-17.10 |
| Trabonella | 664A | Female | 27.9-16.10 | 4-10.10 |
| Trabonella | 664B | Male | 23.9-9.11 | 27.9-19.10 |
| CSIRO collection | | | | |
| *P. vera* cultivars: | | | | |
| Kerman (2 trees) | | Female | 21.10-19.11 | 27.10-14.11 |
| 15-12 | | Male | 16.10-3.11 | 19-25.10 |
| Alverstoke orchard | | | | |
| *P. vera* cultivar: | | | | |
| Sirora[2] (2 trees) | | Female | 29.10-17.11 | 5-13.11 |

[1] Peak flowering was when 30% of male flowers in 5 randomly observed branches were shedding pollen and when 30% of pistils of female trees had all three stigma lobes completely diverged.

[2] 10 out of 103 flower buds did not open until March 1993, and then dropped.

**Table 2.4** Flowering time of *Pistacia* species and cultivars in 1993.

| *Pistacia* species | Tree code | Sex | Flowering period | Peak flowering[1] |
|---|---|---|---|---|
| Arboretum | | | | |
| *P. atlantica* | 666B | Female | 2-19.9 | 9-16.9 |
| *P. atlantica* | 670A | Female | 8-26.9 | 15-23.9 |
| *P. atlantica* | 671D | Male | 21.8-4.9 | 27-31.8 |
| *P. terebinthus* | 673D | Male | 28.8-17.9 | 3-10.9 |
| *P. lentiscus* | 673A | Male | 3-18.9 | 8-17.9 |
| *Pistacia vera* seedlings: | | | | |
| Red Aleppo | 665B | Female | 23.9-19.10 | 27.9-10.10 |
| Trabonella | 664A | Female | 24.9-14.10 | 27.9-10.10 |
| Trabonella | 664B | Male | 20.9-8.11 | 14-26.10 |
| CSIRO collection | | | | |
| *P. vera* cultivars: | | | | |
| Kerman (2 trees) | | Female | 16.10-13.11 | 19-29.10 |
| 15-12 | | Male | 9-30.9 | 14-23.9 |
| Alverstoke orchard | | | | |
| *P. vera* cultivar: | | | | |
| Sirora[2] (2 trees) | | Female | 7.11 | 7.11 |

[1] Peak flowering was when 30% of male flowers in 5 randomly observed branches were shedding pollen and when 30% of pistils of female trees had all three stigma lobes completely diverged.

[2] Only 2 of 72 flower buds burst. All but 13 of the unopened buds had dropped by January 1994.

**Table 2.5** Meteorological data during the 1992 flowering period of the *Pistacia* species and cultivars used in the experiments. Waite Agricultural Research Institute Biennial Report 1992-1993.

| Day of month | August | | | September | | | October | | | November | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *MT | RH | SR | MT | RH | SR | MT | RH | SR | MT | RH | SR |
| 1 | 9.5 | 63 | 6.2 | 12.2 | 86 | 8.7 | 11.4 | 67 | 11.4 | 11.0 | 64 | 13.1 |
| 2 | 9.7 | 65 | 10.3 | 12.2 | 91 | 10.9 | 13.2 | 51 | 8.1 | 11.4 | 54 | 18.5 |
| 3 | 13.1 | 59 | 9.7 | 10.6 | 79 | 12.1 | 17.5 | 49 | 14.2 | 12.6 | 75 | 24.3 |
| 4 | 14.2 | 34 | 6.8 | 11.2 | 72 | 10.1 | 13.5 | 96 | 8.5 | 12.2 | 58 | 22.4 |
| 5 | 14.7 | 58 | 10.5 | 12.1 | 91 | 10.9 | 14.1 | 60 | 16.7 | 13.8 | 56 | 28.1 |
| 6 | 11.7 | 88 | 9.5 | 12.6 | 95 | 10.8 | 15.9 | 35 | 21.5 | 14.1 | 66 | 28.2 |
| 7 | 12.1 | 56 | 4.1 | 11.8 | 90 | 10.4 | 18.9 | 32 | 7.4 | 19.3 | 39 | 23.6 |
| 8 | 9.9 | 84 | 8.8 | 9.4 | 55 | 12.8 | 15.7 | 94 | 26.0 | 23.2 | 24 | 4.2 |
| 9 | 8.9 | 66 | 11.2 | 10.9 | 57 | 16.3 | 11.2 | 67 | 13.3 | 10.2 | 88 | 19.7 |
| 10 | 11.2 | 52 | 22.0 | 13.1 | 62 | 10.8 | 12.7 | 83 | 7.9 | 11.5 | 82 | 14.0 |
| 11 | 9.1 | 69 | 8.3 | 12.0 | 66 | 11.2 | 13.2 | 67 | 11.8 | 13.4 | 73 | 14.6 |
| 12 | 10.9 | 93 | 7.4 | 9.7 | 64 | 11.2 | 14.5 | 49 | 21.3 | 13.7 | 54 | 23.3 |
| 13 | 12.7 | 84 | 7.1 | 11.3 | 53 | 16.4 | 20.4 | 44 | 17.8 | 12.4 | 54 | 28.6 |
| 14 | 13.2 | 96 | 4.4 | 10.0 | 91 | 25.0 | 23.9 | 34 | 18.6 | 17.0 | 41 | 26.2 |
| 15 | 16.3 | 94 | 12.7 | 9.1 | 79 | 9.5 | 15.6 | 80 | 11.1 | 20.9 | 42 | 23.8 |
| 16 | 9.9 | 41 | 11.0 | 11.3 | 71 | 14.1 | 13.1 | 67 | 7.6 | 23.1 | 33 | 21.5 |
| 17 | 8.6 | 76 | 10.3 | 10.4 | 86 | 5.3 | 14.4 | 89 | 9.5 | 20.1 | 52 | 5.9 |
| 18 | 9.9 | 65 | 7.2 | 13.0 | 85 | 14.1 | 11.7 | 71 | 9.7 | 23.2 | 83 | 12.5 |
| 19 | 9.1 | 91 | 6.0 | 9.4 | 62 | 12.4 | 13.4 | 85 | 19.6 | 20.3 | 82 | 15.2 |
| 20 | 13.6 | 61 | 9.0 | 10.3 | 60 | 15.4 | 12.4 | 56 | 25.9 | 23.5 | 65 | 15.9 |
| 21 | 9.9 | 60 | 14.2 | 12.6 | 57 | 22.0 | 11.7 | 51 | 26.2 | 12.5 | 90 | 8.6 |
| 22 | 13.1 | 54 | 11.9 | 13.0 | 98 | 10.3 | 11.8 | 37 | 26.5 | 15.9 | 70 | 19.7 |
| 23 | 8.6 | 72 | 12.0 | 14.0 | 87 | 10.2 | 19.0 | 26 | 26.9 | 17.0 | 91 | 21.6 |
| 24 | 7.7 | 74 | 9.4 | 12.6 | 93 | 4.8 | 20.1 | 30 | 25.1 | 15.1 | 65 | 14.8 |
| 25 | 9.0 | 70 | 10.3 | 11.4 | 64 | 14.2 | 19.0 | 49 | 22.5 | 12.7 | 74 | 22.1 |
| 26 | 12.1 | 59 | 9.9 | 9.5 | 54 | 19.5 | 21.9 | 65 | 22.0 | 13.1 | 56 | 19.1 |
| 27 | 11.8 | 91 | 11.0 | 14.0 | 53 | 17.4 | 18.6 | 82 | 17.7 | 14.0 | 69 | 25.9 |
| 28 | 11.2 | 81 | 14.5 | 13.0 | 93 | 7.8 | 18.6 | 74 | 14.5 | 15.1 | 65 | 25.3 |
| 29 | 10.3 | 95 | 22.0 | 10.1 | 73 | 18.1 | 21.5 | 84 | 9.5 | 15.4 | 65 | 24.3 |
| 30 | 9.7 | 91 | 5.9 | 10.2 | 59 | 15.7 | 17.0 | 85 | 14.8 | 16.0 | 54 | 30.3 |
| 31 | 10.9 | 92 | 4.4 | | | | 13.6 | 92 | 12.3 | | | |

*MT: mean temperature ($^{o}$C)

RH: relative humidity (%)

SR: solar radiation (MegJ/sqm/day)

**Table 2.6** Meteorological data during the 1993 flowering period of the *Pistacia* species and cultivars used in the experiments. Waite Agricultural Research Institute Biennial Report 1992-1993.

| Day of month | August | | | September | | | October | | | November | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *MT | RH | SR | MT | RH | SR | MT | RH | SR | MT | RH | SR |
| 1 | 11.2 | 42 | 5.7 | 12.4 | 90 | 8.9 | 14.9 | 71 | 18.6 | 19.8 | 72 | 20.8 |
| 2 | 10.0 | 73 | 7.4 | 11.8 | 46 | 11.5 | 14.6 | 51 | 15.3 | 20.2 | 74 | 17.9 |
| 3 | 9.5 | 86 | 10.8 | 11.3 | 96 | 9.8 | 16.1 | 82 | 4.3 | 12.7 | 79 | 12.0 |
| 4 | 9.4 | 84 | 12.2 | 11.3 | 52 | 16.5 | 13.5 | 56 | 19.9 | 13.2 | 56 | 13.6 |
| 5 | 11.5 | 54 | 12.5 | 14.6 | 34 | 9.8 | 13.9 | 84 | 12.5 | 12.8 | 62 | 23.5 |
| 6 | 12.9 | 41 | 9.4 | 16.2 | 55 | 15.1 | 16.9 | 77 | 21.8 | 14.9 | 62 | 26.0 |
| 7 | 12.8 | 37 | 3.4 | 18.8 | 43 | 7.2 | 20.4 | 37 | 22.6 | 12.2 | 68 | 12.5 |
| 8 | 14.4 | 99 | 8.9 | 12.9 | 75 | 4.7 | 20.0 | 71 | 19.2 | 16.6 | 57 | 16.4 |
| 9 | 12.8 | 64 | 6.5 | 11.5 | 63 | 11.4 | 13.0 | 69 | 14.3 | 9.3 | 51 | 26.5 |
| 10 | 14.6 | 81 | 10.6 | 10.0 | 63 | 6.1 | 9.8 | 53 | 15.0 | 15.1 | 49 | 25.7 |
| 11 | 11.2 | 78 | 11.3 | 11.9 | 77 | 10.8 | 9.9 | 60 | 11.7 | 20.0 | 18 | 20.7 |
| 12 | 12.8 | 49 | 12.7 | 10.3 | 50 | 16.6 | 14.5 | 33 | 23.3 | 26.0 | 70 | 20.8 |
| 13 | 13.3 | 80 | 23.0 | 9.6 | 65 | 16.4 | 16.2 | 35 | 23.3 | 18.7 | 66 | 24.0 |
| 14 | 12.0 | 95 | 26.0 | 11.3 | 69 | 8.4 | 17.0 | 34 | 21.2 | 17.5 | 55 | 28.0 |
| 15 | 12.1 | 81 | 6.2 | 10.4 | 58 | 10.9 | 13.3 | 43 | 17.5 | 17.7 | 54 | 26.7 |
| 16 | 11.0 | 56 | 11.6 | 14.1 | 54 | 13.0 | 19.5 | 37 | 12.4 | 19.5 | 45 | 19.7 |
| 17 | 11.0 | 88 | 7.6 | 16.6 | 46 | 18.8 | 19.0 | 85 | 11.6 | 25.6 | 34 | 16.2 |
| 18 | 11.6 | 100 | 9.7 | 16.6 | 53 | 9.9 | 11.9 | 89 | 16.0 | 26.4 | 84 | 15.1 |
| 19 | 10.8 | 91 | 10.9 | 13.1 | 80 | 9.1 | 12.6 | 80 | 16.2 | 18.4 | 69 | 7.9 |
| 20 | 11.5 | 84 | 14.6 | 11.4 | 47 | 15.1 | 12.9 | 60 | 21.6 | 15.6 | 55 | 26.9 |
| 21 | 13.8 | 63 | 14.9 | 10.7 | 78 | 16.6 | 12.3 | 52 | 18.3 | 14.1 | 60 | 22.5 |
| 22 | 13.8 | 59 | 15.4 | 11.9 | 78 | 14.4 | 16.8 | 44 | 20.6 | 14.7 | 54 | 21.2 |
| 23 | 14.9 | 60 | 15.6 | 13.1 | 57 | 21.0 | 21.8 | 27 | 17.0 | 16.0 | 46 | 29.6 |
| 24 | 12.7 | 32 | 15.8 | 16.3 | 45 | 20.0 | 16.7 | 70 | 17.3 | 20.2 | 53 | 30.5 |
| 25 | 18.6 | 26 | 15.7 | 16.8 | 42 | 21.6 | 15.1 | 65 | 21.6 | 27.4 | 44 | 30.7 |
| 26 | 18.5 | 27 | 15.7 | 16.4 | 47 | 18.7 | 16.0 | 54 | 21.6 | 17.7 | 18 | 30.6 |
| 27 | 18.2 | 39 | 10.0 | 21.2 | 29 | 19.5 | 20.0 | 31 | 21.3 | 10.6 | 52 | 30.1 |
| 28 | 19.8 | 47 | 12.9 | 22.3 | 43 | 17.6 | 13.2 | 90 | 19.2 | 8.7 | 47 | 30.4 |
| 29 | 19.2 | 42 | 15.3 | 19.3 | 81 | 19.0 | 13.5 | 54 | 24.4 | 9.5 | 36 | 29.4 |
| 30 | 20.7 | 44 | 6.6 | 19.8 | 42 | 10.1 | 14.5 | 63 | 21.2 | 17.7 | 12 | 26.0 |
| 31 | 14.4 | 80 | 5.7 | | | | 19.8 | 36 | 23.6 | | | |

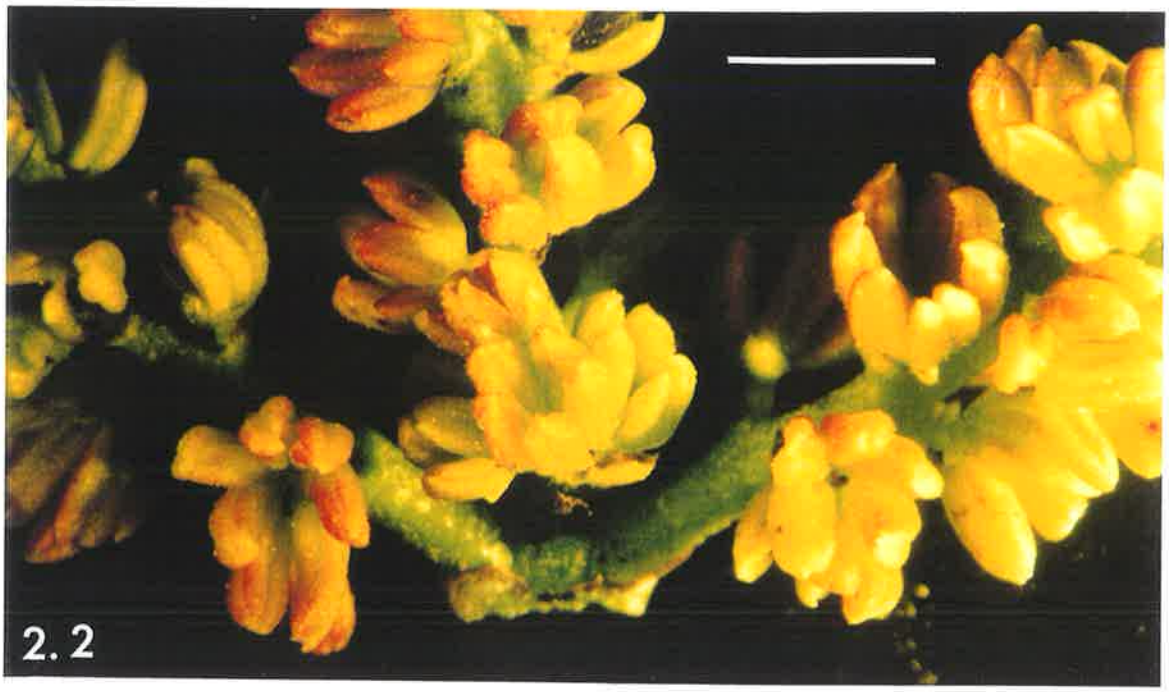
*MT: mean temperature ($^{o}$C)

RH: relative humidity (%)

SR: solar radiation (MegJ/sqm/day)

**Figure 2.1** Female inflorescences of pistachio cv. Kerman from bud to open flower. Bar represents 1 cm.

**Figure 2.2** Male inflorescences of pistachio cv. Trabonella showing mature anthers at anthesis. Bar represents 5 mm.


2. 2

## 2.4 Fruiting

Final fruit set at maturity varied from 12.1 up to 43.3 %, but the latter set occurred during a year of poor bud burst and hence low flower numbers (Table 2.7). A proportion of the mature fruits was always blank or semi-blank in both cultivars (Figure 2.3).

Fruit abscission in pistachio can be a major restriction on production (Table 2.7). Following open pollination, abscised fruitlets ranged from 1.6 to 11.4 mm in diameter. Flower and fruit drop in pistachio occurs over three periods: 1) a gradual period after anthesis up to week four in which aborting pistils, 1.6 - 4.8 mm in diameter, were shed; 2) a period of heavy fruitlet abscission during four to eight weeks after anthesis with most fruitlets dropped by week six. Almost all fruitlets were smaller than normal. Fruitlet development had ceased, followed by browning prior to abscission; 3) a slow rate of fruit abscission after eight weeks of anthesis up to fruit maturity. In this period, the abscission symptoms were hull discolouration from the fruit tip or adjacent to the pedicel which extended rapidly, with increased fruit abortion after rainfall.

**Figure 2.3** Pistachio cv. Trabonella clusters at fruit harvest, and blank fruits (left) and full seeded fruits with a split shell (right). Bar represents 1 cm.


2.3

**Table 2.7** Fruit abscission (mean ± standard error) following open pollination of two trees of each of *P vera* cvs. Kerman and Sirora from anthesis to fruit harvest in 1992 and 1993. One hundred flowers of each cultivar were labelled at anthesis.

| Cultivar/year | Number of fruits abscissed during fruit development up to fruit harvest (mean ± standard error) | | | | | Fruit retained at harvest (%) |
|---|---|---|---|---|---|---|
| | week 2 | week 4 | week 8 | week 16 | week 21 | |
| Kerman/1992 | 8.01 ±5.026 | 6.10 ±0.595 | 65.77 ±7.102 | 3.05 ±0.641 | 0.32 ±0.120 | 16.7 |
| Kerman/1993 | 6.43 ±3.121 | 13.81 ±9.260 | 57.02 ±14.133 | 9.09 ±4.195 | 0.81 ±0.672 | 12.8 |
| Sirora/1992 | 20.67 ±2.284 | 22.26 ±8.744 | 41.66 ±7.651 | 1.66 ±0.771 | 1.68 ±0.848 | 12.1 |
| Sirora/1993 | 4.7 ±2.513 | 7.76 ±4.003 | 40.42 ±15.805 | 2.53 ±0.958 | 1.24 ±0.600 | 43.3 |

## 2.5 Stigma surface colour changes during anthesis

### 2.5.1 Introduction

It is important to identify the duration of the receptive period for pollination. Both the quality and quantity of yield may be dependent on the pollination period, as early or late pollination has been hypothesised to result in problems in date, pistachio (Whitehouse and Stone, 1941) and walnut (Luza and Polito, 1991). Stigma receptivity of plants may be recognised by a change in colour, increase in stigma secretion, or by reflective properties which may differ between genotypes (Lord and Webster, 1979; Heslop-Harrison and Shivanna, 1977). Stigma colour in some plants may be genetically or environmentally controlled. In *Buchloe dactyloides* the stigma colour changes under different environmental conditions, particularly air temperature, and genotype also influences the colour of the stigma surface (Huff, 1991) The aim of this experiment is to record the duration of the different periods of stigma surface colour in *Pistacia vera* and *P. atlantica* flowers.

### 2.5.2 Materials and methods

One female tree of *P. atlantica* and of each of *Pistacia vera* Red Aleppo and Trabonella, two trees of the cultivar Kerman and two trees of the cultivar Sirora were used. All trees were located on the Waite campus.

Six separate branches were selected for each tree. On each branch three buds each with about 300 flowers were isolated one to three days before flowering using glassine bags with two 5 x 8 cm transparent windows. Twenty flowers were labelled from each bud when the stigma lobes first opened. Those on three of the branches of each cultivar were pollinated after 24 h with fresh pollen of Trabonella or 15-12 using a paint brush. The flowers on the other

three branches were left unpollinated. Stigma surface colour was recorded every 12 h up to three weeks.

### 2.5.3 Results

In all *Pistacia vera* trees except seedling Red Aleppo the unpollinated and pollinated stigma was green for one day (Table 2.8). Thereafter, in the trees Kerman, Trabonella and Red Aleppo the unpollinated stigma was white for one, two and three weeks respectively prior to flower shed. The unpollinated stigma of Sirora darkened to a yellow colour from the sixth day after lobe expansion. Unpollinated pistils of *P. atlantica* had a different colour sequence from *P. vera.* After being green for 6 days and white for 5 days a pink colour appeared on the stigma for a further 10 days. This colouration was firstly observed as very light sporadic pinkish points at the tip of the largest lobe and then gradually extended to other parts of the lobe.

Pollinated stigmas of all *P. vera* cultivars had turned dark yellow by at least five days after lobe opening, and all were brown by between 9 and 15 days. Only the stigma of *P. atlantica* developed red colouration. The pollinated flowers were not shed, but the stigma abscised by 22 days.

**Table 2.8** Unpollinated and pollinated stigma colour changes during anthesis in six trees of *Pistacia vera* and one of *P. atlantica*.

| Trees | Stigma colour duration (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Green | White | Dark yellow | Pink | Red | Brown | Flower shed |
| Unpollinated | | | | | | | |
| *P. vera*: | | | | | | | |
| Trabonella | 1 | 2-14 | - | - | - | - | >15 |
| Sirora (2 trees) | 1 | 2-5 | 6-14 | - | - | - | >15 |
| Kerman (2 trees) | 1 | 2-8 | - | - | - | - | >9 |
| Red Aleppo | 0 | 1-21 | - | - | - | - | >22 |
| *P. atlantica* | 6 | 7-11 | - | 12-21 | - | 22-30 | >31 |
| Pollinated | | | | | | | Stigma shed |
| *P. vera*: | | | | | | | |
| Trabonella | 1 | 2-3 | 4-14 | - | - | 15-21 | >22 |
| Sirora (2 trees) | 1 | 2-3 | 4-14 | - | - | 15-21 | >22 |
| Kerman (2 trees) | 1 | 2-4 | 5-8 | - | - | 9-12 | >13 |
| Red Aleppo | 0 | 1-6 | 7-10 | - | - | 11-13 | >14 |
| *P. atlantica* | 1 | 2-3 | 4-6 | - | 7-8 | 9-14 | >15 |

### 2.5.4 Discussion

This work has shown that stigma colour during anthesis varies between genotypes and species and may not be a reliable criterion for identification of the stage of stigma receptivity of pistachio. Unpollinated *P. atlantica* stigmas showed a period of pink colour which was not observed in the *P. vera* genotypes. This is in contrast to results from Iran, where the unpollinated stigma of *P. vera* cultivars turned pink to red and this stage was assumed to indicate receptivity (Shuraki and Sheibany, unpublished). The difference may be due to climatic factors and genotypes (Huff, 1991). In contrast to the unpollinated stigma the surface colour of pollinated *P. vera* pistils changed to dark yellow and then to brown, thereby indicating that pollination had taken place. Extension of the white stage in unpollinated flowers may be due to lack of damage caused by growth of the pollen tubes in the pistil. In further experiments, the time after first opening of the stigma lobes was used to indicate age, rather than stigma colour.

## 2.6 Storage and *in vitro* germination of *Pistacia* pollen

### 2.6.1 Introduction

As part of the research program on pollination and fruit set in pistachio, two methods for evaluating quality and longevity in fresh and stored pollen were investigated, based on work conducted by several researchers (Crane *et al.*, 1974; Vithanage and Alexander, 1985; Golan-Goldhirsh *et al.*, 1991; Martínez-Pallé and Herrero, 1994). Crane *et al.* (1974) found that germination of pistachio pollen decreased significantly when it was stored at ambient temperature for a few days, and after four months storage in a freezer, but pre-incubation hydration had a positive effect on germination of four month -20°C stored pollen of *P. vera* (Polito and Luza, 1988b). However, Stone *et al.* (1943) reported 30% viability of pollen of some *Pistacia* species stored for one year at -1°C and 30-40% relative humidity.

Breeding and improvement of *P. vera* commercial cultivars is an important research aim. However, improvement of yield via assisted pollination may be a possibility, and stored pollen could be used to pollinate late blooming female cultivars (Pontikis, 1977; Vithanage and Alexander, 1985). In some dioecious plants such as *Pistacia* species, there is variation in the coincidence of male and female flowering, which can cause low set despite a high number of male trees per hectare (Maggs, 1973). Although pollen of early flowering cultivars showed some tolerance to early season cool temperatures (Polito and Luza, 1988a), viability of pistachio pollen was reduced in adverse environmental conditions (Crane *et al.*, 1974). Thus selection of male cultivars with long flowering and high fertility along with assisted pollination may reduce the number of males per hectare, and improve pistachio yield and quality.

### 2.6.2 Materials and methods

In this experiment pollen was collected from *Pistacia vera* Trabonella and 15-12, and from *P. atlantica* and *P. terebinthus* all of which were growing on the Waite campus. Inflorescences that had 10% of flowers with dehisced anthers were removed from the trees, brought into the laboratory, spread over white paper and dried at 20°C for 24 h on the laboratory bench or in the oven. Pollen was cleaned by passing the flowers through double layers of tissue paper and stored at -15°C in glass tubes stoppered with plastic lids. Germination was measured of fresh pollen, after drying, and after storage at -15°C for 1, 6 and 12 months. Pollen viability was measured using two different germination media: 1) 10% sucrose and 1% agar (Crane *et al.*, 1974); 2) 15% sucrose and 1% agar plus 0.01% boric acid, 0.03% calcium nitrate, 0.02% magnesium sulphate, and 0.01% potassium nitrate, (Brewbaker and Kwack, 1963). Three replicates of 100 grains were scored per treatment as germinated or ungerminated after 24h incubation at 20°C. A grain was scored as germinated if the length of the tube equalled the diameter of the grain. Analysis of variance of the pollen germination data was carried out.

### 2.6.3 Results

Germination percentages of fresh and dried pollen were uniformly high, and both media appeared satisfactory for assessing germination percentage (Table 2.9). There was a slight reduction in germination of pollen of *P. atlantica* and *P. terebinthus* after storage for one month. After 6 months storage *P. vera* and *P. terebinthus* pollen germination decreased and pollen of *P. atlantica* did not germinate. Only pollen of *P. vera* cv. 15-12 showed significant germination after a year of storage. There was a strong three way interaction between pollen, medium and time of storage ($P < 0.001$) (Table 2.10). Pollen germinated *in vitro* is shown in figures 2.4 - 2.7.

**Table 2.9** Percentage pollen germination on two media after 0, 1, 6 and 12 months of storage at -15°C.

| | *P. vera* | | *P. atlantica* | *P. terebinthus* |
|---|---|---|---|---|
| | Trabonella | 15-12 | | |
| Medium 1 | | | | |
| Fresh pollen | 80.6 | 87.7 | 79.8 | 74.2 |
| Dried 24h at 20°C | - | - | 75.7 | 70.5 |
| Stored 1 month at -15°C | 79.2 | 87.6 | 74.0 | 64.4 |
| Stored 6 months at -15°C | 69.7 | 66.4 | 0 | 44.0 |
| Stored 12 months at -15°C | 12.5 | 34.5 | 0 | 1.4 |
| Medium 2 | | | | |
| Fresh pollen | 83.9 | 83.4 | 88.8 | 80.7 |
| Dried 24h at 20°C | - | - | 88.6 | 83.7 |
| Stored 1 month at -15°C | 81.1 | 87.3 | 62.2 | 64.6 |
| Stored 6 months at -15°C | 61.5 | 63.9 | 0 | 49.7 |
| Stored 12 months at -15°C | 12.1 | 49.7 | 0 | 4.7 |

- Not tested

**Table 2.10** Analysis of variance of pollen germination data for four sources of *Pistacia* pollen

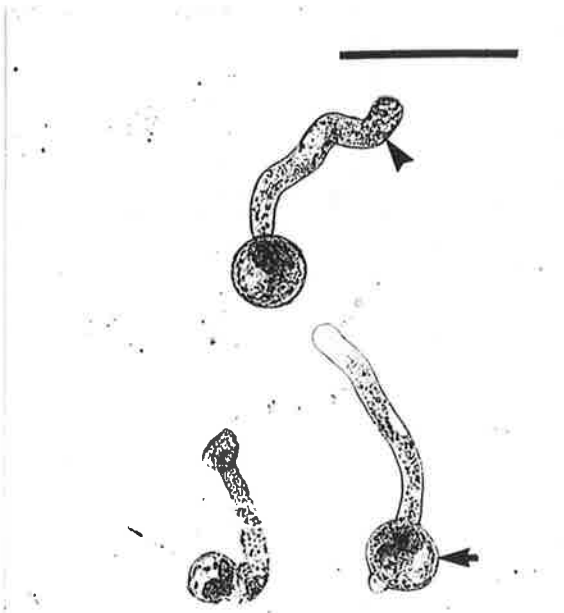
| Source of variation | Degrees of freedom | Sum square | Mean square | Variance ratio | Significance |
|---|---|---|---|---|---|
| Pollen stratum | 3 | 7908.4 | 2636.1 | | |
| Pollen x vial stratum | 104 | 96102.6 | 924.1 | | |
| Total | 107 | 104011.0 | | | |
| | | | | | |
| Pollen | 3 | 7908.4 | 2636.1 | | |
| Medium | 1 | 105.7 | 105.7 | 7.3 | |
| Time | 4 | 81399.7 | 20349.9 | 1405.5 | |
| Medium x Time | 4 | 455.8 | 113.9 | 7.9 | |
| Pollen x Medium | 3 | 65.3 | 21.8 | 1.5 | |
| Pollen x Time | 10 | 12388.2 | 1238.8 | 85.6 | |
| Pollen x Medium x Time | 10 | 645.4 | 64.5 | 4.5 | <0.001*** |
| Residual | 72 | 1042.44 | 14.5 | | |

**Figure 2.4** Germination of fresh pollen of pistachio cv. 15-12 after four hours incubation on the medium with microelements; arrow shows pollen grain and arrow head pollen tube. Bar represents 20 μm.

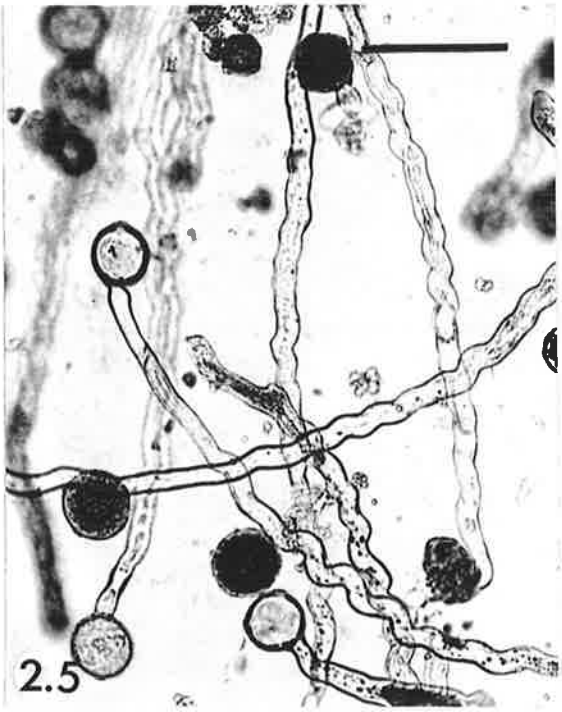
**Figure 2.5** Germination of fresh pollen of pistachio cv. 15-12 after 24 hours incubation on the medium with microelements. Bar represents 20 μm.

**Figure 2.6** Germination of one-year old stored pollen of pistachio cv. Trabonella after four hours incubation on the medium with microelements. Bar represents 20 μm.

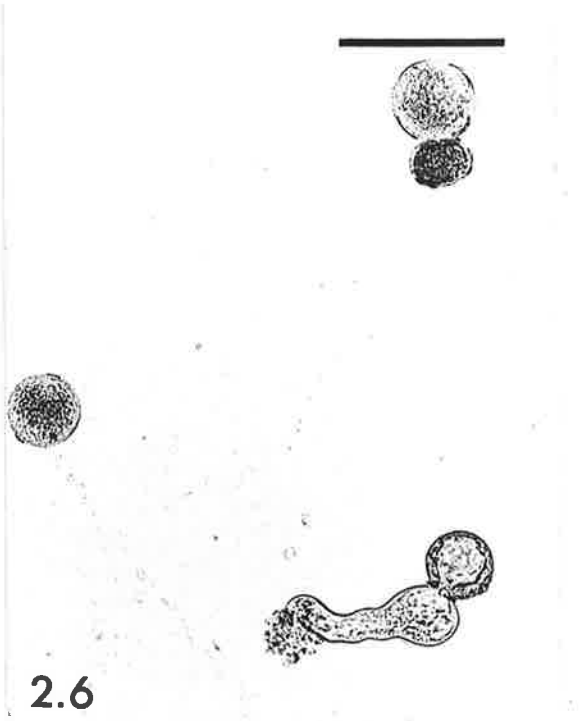
**Figure 2.7** Germination of one-year old stored pollen of pistachio cv. Trabonella after 24 hours incubation on the medium with microelements. Bar represents 20 μm.



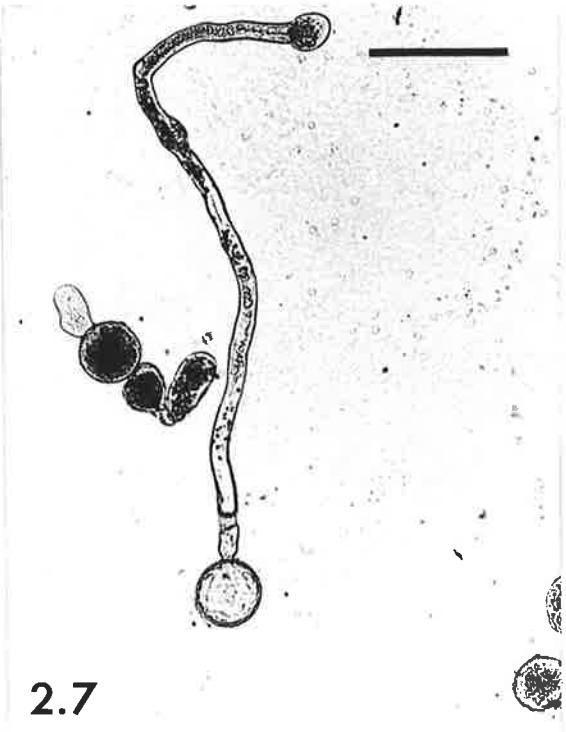
2.4



2.5



2.6



2.7

### 2.6.4 Discussion

Male trees of pistachio often shed pollen before pistil receptivity of female trees, and coincidence is necessary for pollination to occur during a relatively short period of anthesis (Whitehouse *et al.*, 1964; Maggs, 1973). Manipulation of pistachio pollen shed and pollination period have been carried out to improve pistachio yield and quality (Procopiou, 1973; Porlingis and Voyiatzis, 1993). This may be achieved via assisted pollination. For this it is necessary to develop an easy method to store pistachio pollen for periods of time. According to Sedgley (1981b) and Lee *et al.* (1985) pollen of some species can be stored indefinitely at -196°C without affecting viability. However, this is not a simple method for orchardists. On the other hand, pollen viability is highly susceptible to ambient room conditions (Stanley and Linskens, 1974), and Stone *et al.* (1943) reported low germination of *Pistacia* pollen after 2 months storage in the refrigerator.

In this research all pollen except *P. atlantica* germinated after six months in the freezer, and after a year pollen of *P. vera* cv. 15-12 showed up to 50% germinability. In previous research pistachio pollen stored after freeze-drying at -15°C remained viable for 8 months (Vithanage and Alexander, 1985). Thus a freezer can be used for storage of some pistachio pollen for up to a year. Artificial pollination applied for yield improvement is possible in some plants such as chestnut (McKay and Crane, 1938), date palm (Nixon, 1956), hazelnut (Thompson, 1967), pistachio (Peebles and Hope, 1937; Crane and Iwakiri, 1980), pecan (Marquard, 1988) and kiwifruit (King and Ferguson, 1991). In pistachio some marketing factors such as splitting, and fruit weight may also be manipulated by pollen source.

Some authors have reported higher pollen germination on a medium including micro elements, especially boron and calcium (Brewbaker and Kwack, 1963), and Therios *et al.* (1986) reported the highest germination of *P. vera* cultivars on media with both boron and calcium. However Crane *et al.* (1974) and Gilissen (1977) found

that pre-incubation and storage treatments were more important than micro elements. In this experiment the medium containing trace elements gave, on average, slightly higher germination percentages than that lacking elements. It was concluded that pollen of all species and cultivars tested from plants growing under South Australian conditions could be stored for up to one month at -15°C prior to use in pollination experiments. Pollen of *P. vera* could be stored for up to six months.

# Chapter 3

# 3 Effect of pistil age and pollen parent on pollen tube growth and fruit production of pistachio

## 3.1 Introduction

In many tree crops a large number of flowers and fruits is shed prior to fruit maturity and aborted ovaries may be unpollinated, pollinated with incompatible pollen or shed following postzygotic breakdown (Stephenson, 1981). Pollination of the immature pistil can induce fruit abscission in walnut (Luza and Polito, 1991), and in pistachio abortion may be correlated with abnormal zygote or embryo development (Bradley and Crane, 1975). Fruit drop can also be influenced by the timing of pollination, either in relation to flower age or environmental conditions at anthesis such as temperature (Crane and Iwakiri, 1981). In the dioecious, wind-pollinated pistachio, the main problems at harvest are empty (blank), semi-blank and non-split fruits (Crane, 1973; Nevo *et al.*, 1974; Crane and Iwakiri, 1980). It is possible that pollen parent incongruity may lead to fruiting problems. The duration of the effective pollination period is another possibility (Ducke, 1989) as is premature pollination (Niklas and Buchmann, 1988) or pollination of overmature flowers (Egea and Burgos, 1992). These problems may be improved by pollination at the time of peak pistil maturity with a compatible pollen source. The aim of this research was to investigate the effects of pistil maturity and paternal genotype on pollen germination, tube growth and fruit production.

## 3.2 Materials and methods

Controlled pollination experiments were conducted using mature female trees of *Pistacia vera* cv. Kerman grafted onto *P. atlantica* rootstock, plus seedling trees of the *P. vera* cvs. Red Aleppo and Trabonella (Trabonella female). These were

pollinated with pollen from the male *P. vera* genotype cv. 15-12 grafted onto *P. atlantica* rootstock, plus seedling trees of *P. vera* cv. Trabonella (Trabonella male) and of *P. atlantica*. The pistachio plantings were located close to Adelaide, South Australia (34°56' S; 138°35' E). Pollinations were conducted between Kerman and Red Aleppo with all male genotypes in 1992, and in 1993 Kerman was pollinated with 15-12 and *P. atlantica*.

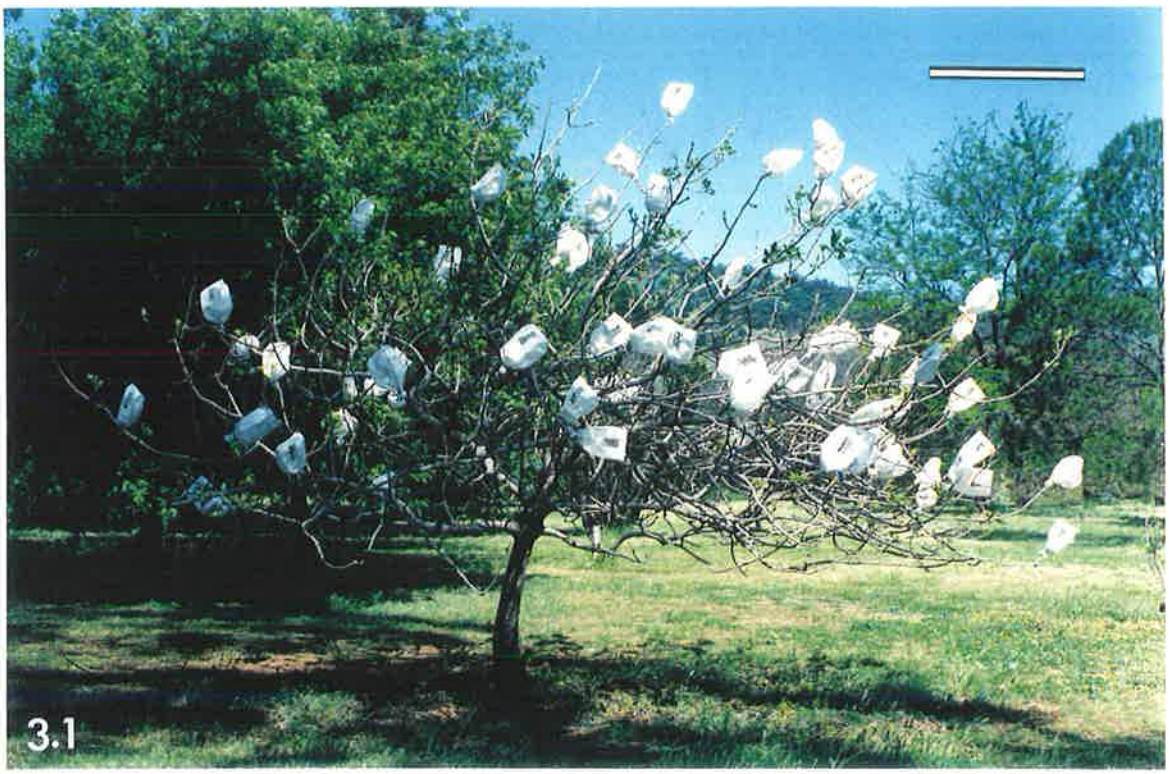
Pollen was collected when about 10% of male flowers had dehisced anthers. Flowers were dried for a day at room temperature (20°C) and the pollen sieved through tissue paper. Pollen germination percentages *in vitro* (Brewbaker and Kwack, 1963) at anthesis were 83.9, 83.4 and 74.0 for Trabonella male, 15-12 and *P. atlantica* respectively in 1992, and 83.5 and 70.4 for 15-12 and *P. atlantica* in 1993.

Between three and six replicates of each female pistachio genotype were selected, each having three clusters of about 300 flowers. Flowers were covered prior to anthesis with a glassine bag to exclude pollen (Figure 3.1). Pollination was conducted at anthesis and at one, two, three, and four days after flower opening. All flowers not at the correct stage were removed, and control treatments were not pollinated. Hand pollination was carried out with fresh pollen of Trabonella male and 15-12 and four week stored pollen (-15°C) of *P. atlantica*, using either a paint brush or a syringe. The *P. atlantica* pollen was stored as peak flowering preceded that of *P. vera* during both 1992 and 1993. The pollinated flowers were covered again after pollination. Ten pistils were harvested at 24 hours and 48 hours (48 hours only in 1993) after pollination from three of the replicates. The rest were left to set fruit.

Pistils were fixed in Carnoy's fluid, hydrated via an ethanol series and softened with 0.8N NaOH at 60°C for 10 - 30 minutes. The ovules were dissected from the flowers and processed separately. Tissues were stained with water soluble aniline blue (Martin, 1959; Sedgley, 1979). Samples were mounted on slides with

**Figure 3.1** Isolation of pistachio inflorescences using a glassine bag before and after hand pollination. Bar represents 1 m.

**Figure 3.2** After fruit set hand pollinated inflorescences were covered by mesh bags to protect the fruit up to harvest. Bar represents 1 m.



3.1

3.2

80% glycerol, covered with a glass cover slide and squashed gently. Preparations were observed using a fluorescence microscope. Pollen grains were counted on the stigma, and pollen tubes were counted in the stigma, the upper and lower style, ovary, ovule and nucellus.

The number of pollen grains on the stigma was analysed by fitting a Poisson model using Genstat 5. Binomial models (McCullagh and Nedler, 1983) were fitted for the number of germinated pollen grains on the stigma, the number of pollen tubes in the stigma, the number of pollen tubes in the upper style and so on. The data were analysed for effects of pollination time, pollen source and time of harvest.

The pistils for fruit set were covered for two weeks after pollination and the glassine bags were then removed and replaced with nylon mesh bags (Figure 3.2). Fruits were counted at maturity, at about 20 weeks after pollination. Mature fruits were categorised as split (normal) or abnormal (Figure 3.3) and the abnormal fruits were divided into non-split, blank, semi-blank and mis-shapen (Figures 3.3, 3.4).

## 3.3 Results

The pistil consisted of a large three-lobed stigma (Figure 3.5), a short style and a large ovary containing a single ovule (Figure 3.4). Pollen germination and tube growth was observed in all pollinations conducted (Tables 3.1 - 3.6, Figures 3.5, 3.6). Pollen germination and tube penetration were highest when pollination was carried out at two and three days after flower opening, and by the 48 hours harvest there was an increase in the number of tubes in pollinated flowers over the 24 hours harvest. At four days after flowering some pistils failed to support pollen grains and tubes, particularly when *P. atlantica* pollen was used and a high percentage of pollen tubes were short and coiled with swollen tips.

Up to eleven pollen tubes were observed to enter the ovule, but only one or two penetrated the nucellus, always via the chalaza (Figure 3.6). Branching of pollen

**Table 3.1** Average number of pollen grains and tubes (mean ± standard error) per pistil of cv. Kerman pollinated with 15-12 at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 or 48 hours.

| Time of pollination (days) | Time of harvest | Number of pollen grains on the stigma | Number of pollen tubes in | | | | | | Mean ± SE of pollen tube number in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 1992 | 24 h | | | | | | | | |
| 0 | | 72.0±9.48 | 45.5±6.57 | 3.66±0.563 | 2.66±0.450 | 1.46±0.302 | 0.50±0.141 | 0 | 1.66±0.291 |
| 1 | | 126.5±14.52 | 110.4±13.12 | 7.87±0.604 | 6.47±0.452 | 4.16±0.462 | 1.86±0.302 | 0.17±0.080 | 4.31±0.380 |
| 2 | | 128.0±10.60 | 116.8±10.47 | 10.6±0.57 | 9.23±0.550 | 5.90±0.405 | 2.20±0.188 | 0.10±0.055 | 5.60±0.354 |
| 3 | | 169.7±13.39 | 149.4±13.15 | 10.5±0.74 | 8.73±0.706 | 4.50±0.370 | 0.23±0.092 | 0 | 4.78±0.382 |
| 4 | | 110.5±15.77 | 73.7±11.14 | 1.50±0.414 | 0.97±0.330 | 0.66±0.260 | 0.10±0.073 | 0 | 0.65±0.215 |
| 1992 | 48 h | | | | | | | | |
| 0 | | 132.5±16.35 | 118.2±15.50 | 4.43±0.459 | 3.66±0.416 | 1.97±0.330 | 1.03±0.200 | 0.43±0.141 | 2.30±0.309 |
| 1 | | 122.7±14.68 | 111.0±13.15 | 8.17±0.659 | 6.80±0.588 | 4.23±0.468 | 2.40±0.242 | 1.20±0.211 | 4.56±0.433 |
| 2 | | 128.5±17.95 | 120.4±17.05 | 8.83±0.864 | 8.33±0.828 | 3.60±0.309 | 2.36±0.242 | 1.33±0.193 | 4.89±0.487 |
| 3 | | 148.6±18.64 | 135.9±17.70 | 8.46±0.837 | 7.53±0.801 | 3.96±0.475 | 2.31±0.318 | 1.30±0.230 | 4.71±0.532 |
| 4 | | 97.6±11.79 | 66.3±9.24 | 1.73±0.479 | 1.02±0.299 | 0.50±0.223 | 0.43±0.218 | 0.30±0.180 | 0.80±0.279 |
| 1993 | 48 h | | | | | | | | |
| 0 | | 13.6±2.17 | 11.8±1.91 | 2.90±0.348 | 2.42±0.208 | 1.72±0.235 | 0.83±0.147 | 0.30±0.089 | 1.63±0.205 |
| 1 | | 94.9±11.32 | 88.2±10.45 | 4.67±0.424 | 4.45±0.424 | 3.33±0.354 | 2.03±0.204 | 0.78±0.161 | 3.05±0.313 |
| 2 | | 107.2±9.82 | 103.8±9.56 | 6.40±0.472 | 6.12±0.497 | 5.42±0.431 | 3.88±0.396 | 1.82±0.230 | 4.73±0.405 |
| 3 | | 108.0±11.70 | 103.8±11.39 | 5.98±0.419 | 5.78±0.392 | 4.98±0.370 | 3.68±0.356 | 1.27±0.177 | 4.30±0.342 |
| 4 | | 54.3±7.20 | 43.1±8.58 | 1.55±0.262 | 1.45±0.260 | 1.00±0.199 | 0.27±0.100 | 0.05±0.039 | 0.86±0.172 |

**Table 3.2** Average number of pollen grains and tubes (mean ± standard error) per pistil of cv. Kerman pollinated with *Pistacia atlantica* at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 or 48 hours.

| Time of pollination (days) | Time of harvest | Number of pollen grains on the stigma | Number of pollen tubes in | | | | | | Mean ± SE of pollen tube number in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 1992 | 24 h | | | | | | | | |
| 0 | | 76.6±9.96 | 58.6±8.82 | 2.86±0.590 | 2.13±0.522 | 1.46±0.422 | 0.43±0.156 | 0 | 1.37±0.338 |
| 1 | | 106.9±16.87 | 98.9±15.89 | 2.43±0.506 | 2.16±0.460 | 0.63±0.222 | 0.46±0.184 | 0 | 1.14±0.274 |
| 2 | | 139.3±16.50 | 130.4±15.67 | 5.86±0.651 | 4.23±0.586 | 1.86±0.306 | 0.46±0.157 | 0 | 2.48±0.340 |
| 3 | | 193.7±17.65 | 176.5±16.09 | 3.50±0.535 | 2.20±0.402 | 0.83±0.230 | 0.16±0.096 | 0 | 1.34±0.252 |
| 4 | | 112.8±12.66 | 67.7±9.15 | 0.10±0.055 | 0 | 0 | 0 | 0 | 0.020±0.011 |
| 1992 | 48 h | | | | | | | | |
| 0 | | 106.2±13.96 | 94.3±13.35 | 4.13±0.639 | 3.70±0.597 | 1.46±0.243 | 0.73±0.191 | 0.23±0.103 | 2.05±0.354 |
| 1 | | 83.9±10.04 | 75.0±9.52 | 6.86±0.725 | 5.96±0.640 | 1.46±0.388 | 0.73±0.191 | 0.36±0.122 | 3.07±0.413 |
| 2 | | 110.2±15.38 | 97.6±14.21 | 6.70±0.819 | 5.93±0.837 | 2.50±0.449 | 1.33±0.216 | 0.67±0.175 | 3.43±0.499 |
| 3 | | 105.8±13.53 | 91.7±12.95 | 4.33±0.563 | 3.76±0.598 | 1.56±0.376 | 0.80±0.181 | 0.40±0.123 | 2.17±0.368 |
| 4 | | 86.7±15.27 | 31.5±7.64 | 0.70±0.220 | 0.36±0.155 | 0 | 0 | 0 | 0.21±0.075 |
| 1993 | 48 h | | | | | | | | |
| 0 | | 71.4±10.48 | 67.2±9.83 | 3.53±0.279 | 3.38±0.266 | 2.20±0.194 | 0.98±0.149 | 0.23±0.082 | 2.06±0.194 |
| 1 | | 14.2±2.66 | 12.3±2.10 | 2.70±0.238 | 2.58±0.243 | 1.92±0.175 | 1.20±0.146 | 0.62±0.117 | 1.90±0.188 |
| 2 | | 86.9±9.56 | 84.0±9.24 | 5.42±0.345 | 3.30±0.345 | 4.22±0.312 | 3.08±0.274 | 0.97±0.168 | 3.39±0.288 |
| 3 | | 126.8±12.04 | 122.7±11.74 | 5.72±0.416 | 5.50±0.382 | 4.30±0.323 | 3.10±0.208 | 0.88±0.146 | 3.90±0.295 |
| 4 | | 40.1±6.10 | 31.3±5.27 | 1.80±0.316 | 1.70±0.319 | 1.20±0.266 | 0.27±0.122 | 0.03±0.033 | 1.03±0.211 |

**Table 3.3** Average number of pollen grains and tubes (mean ± standard error) per pistil of Kerman pollinated in 1992 with Trabonella male at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 and 48 hours.

| Time of pollination (days) | Time of harvest | Pollen grains on the stigma | Pollen tubes in | | | | | | Mean ± SE of pollen tubes in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 0 | 24 h | 66.43 ±10.64 | 43.10 ±8.78 | 3.46 ±0.57 | 2.76 ±0.43 | 0.86 ±0.23 | 0.33 ±0.11 | 0.06 ±0.04 | 1.49 ±0.28 |
| 1 | | 122.43 ±11.85 | 110.73 ±11.85 | 5.50 ±0.38 | 4.46 ±0.38 | 1.46 ±0.25 | 0.93 ±0.18 | 0.16 ±0.08 | 2.50 ±0.25 |
| 2 | | 136.20 ±11.67 | 126.97 ±11.24 | 7.73 ±0.31 | 6.81 ±0.32 | 4.13 ±0.32 | 1.16 ±0.24 | 0.07 ±0.04 | 3.98 ±0.24 |
| 3 | | 134.06 ±14.74 | 121.70 ±13.92 | 7.43 ±0.47 | 6.90 ±0.42 | 3.36 ±0.34 | 1.03 ±0.21 | 0.06 ±0.04 | 3.76 ±0.30 |
| 4 | | 82.90 ±9.76 | 48.34 ±7.30 | 0.93 ±0.23 | 0.76 ±0.19 | 0.41 ±0.14 | 0.07 ±0.04 | 0 | 0.43 ±0.12 |
| Mean ± SE | | 108.40 ±11.73 | 90.17 ±10.63 | 5.01 ±0.39 | 4.34 ±0.35 | 2.04 ±0.26 | 0.70 ±0.15 | 0.07 ±0.04 | 2.43 ±0.24 |
| 0 | 48 h | 93.02 ±12.84 | 82.63 ±12.10 | 3.26 ±0.42 | 2.96 ±0.40 | 1.30 ±0.23 | 0.83 ±0.20 | 0.40 ±0.14 | 1.76 ±0.28 |
| 1 | | 155.01 ±16.37 | 143.80 ±15.80 | 7.36 ±0.55 | 6.16 ±0.42 | 3.76 ±0.33 | 2.07 ±0.32 | 1.01 ±0.24 | 4.07 ±0.38 |
| 2 | | 121.33 ±16.14 | 114.67 ±15.67 | 7.91 ±0.46 | 7.30 ±0.45 | 5.31 ±0.37 | 2.23 ±0.26 | 0.90 ±0.20 | 4.73 ±0.35 |
| 3 | | 124.43 ±13.48 | 112.77 ±13.10 | 6.86 ±0.63 | 6.13 ±0.54 | 2.80 ±0.29 | 1.73 ±0.18 | 0.86 ±0.13 | 3.68 ±0.35 |
| 4 | | 135.33 ±15.56 | 91.33 ±11.04 | 1.13 ±0.30 | 1.03 ±0.27 | 0.60 ±0.18 | 0.32 ±0.12 | 0.23 ±0.10 | 0.66 ±0.19 |
| Mean ± SE | | 125.86 ±14.88 | 109.04 ±13.54 | 5.30 ±0.56 | 4.72 ±0.42 | 2.75 ±0.29 | 1.43 ±0.24 | 0.68 ±0.16 | 2.98 ±0.31 |

**Table 3.4** Average number of pollen grains and tubes (mean ± standard error) per pistil of Red Aleppo pollinated in 1992 with 15-12 at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 and 48 hours.

| Time of pollination (days) | Time of harvest | Pollen grains on the stigma | Pollen tubes in | | | | | | Mean ± SE of pollen tubes in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 0 | | 87.37 ±9.52 | 55.87 ±6.61 | 1.43 ±0.36 | 1.10 ±0.31 | 0.50 ±0.18 | 0.10 ±0.05 | 0 | 0.62 ±0.18 |
| 1 | | 112.70 ±13.71 | 104.50 ±12.95 | 7.73 ±0.68 | 6.67 ±0.57 | 3.93 ±0.44 | 1.36 ±0.18 | 0.10 ±0.46 | 3.96 ±0.47 |
| 2 | 24 h | 131.70 ±16.32 | 125.73 ±15.66 | 11.02 ±0.86 | 9.43 ±0.70 | 3.56 ±0.33 | 1.63 ±0.20 | 0.13 ±0.06 | 5.15 ±0.43 |
| 3 | | 87.07 ±13.51 | 83.97 ±12.00 | 12.33 ±0.93 | 10.60 ±0.82 | 5.36 ±0.47 | 2.67 ±0.32 | 0.06 ±0.04 | 6.20 ±0.52 |
| 4 | | 137.23 ±14.94 | 100.63 ±11.19 | 2.30 ±0.55 | 1.43 ±0.50 | 0.70 ±0.28 | 0.20 ±0.12 | 0 | 0.92 ±0.29 |
| Mean ± SE | | 111.21 ±13.60 | 94.14 ±11.68 | 6.96 ±0.67 | 5.84 ±0.58 | 2.81 ±0.34 | 1.49 ±0.14 | 0.06 ±0.11 | 3.37 ±0.38 |
| 0 | | 140.10 ±17.06 | 124.30 ±16.64 | 5.43 ±0.68 | 4.77 ±0.62 | 2.36 ±0.29 | 1.10 ±0.19 | 0.43 ±0.14 | 2.82 ±0.38 |
| 1 | | 138.80 ±15.26 | 127.33 ±14.63 | 7.83 ±0.83 | 7.10 ±0.72 | 3.33 ±0.46 | 2.10 ±0.27 | 0.93 ±0.18 | 4.26 ±0.49 |
| 2 | 48 h | 138.30 ±15.59 | 129.53 ±15.05 | 10.10 ±0.94 | 9.02 ±0.86 | 4.13 ±0.45 | 2.41 ±0.26 | 1.13 ±0.21 | 5.35 ±0.54 |
| 3 | | 117.03 ±14.97 | 109.23 ±13.84 | 8.53 ±0.89 | 7.63 ±0.83 | 4.13 ±0.40 | 2.10 ±0.19 | 0.83 ±0.12 | 4.64 ±0.48 |
| 4 | | 124.83 ±17.15 | 68.60 ±11.08 | 1.10 ±0.30 | 0.83 ±0.27 | 0.40 ±0.14 | 0.17 ±0.07 | 0.03 ±0.03 | 0.51 ±0.16 |
| Mean ± SE | | 131.80 ±16.02 | 111.39 ±14.25 | 6.60 ±0.73 | 5.87 ±0.66 | 2.87 ±0.34 | 1.57 ±0.19 | 0.67 ±0.13 | 3.52 ±0.41 |

**Table 3.5** Average number of pollen grains and tubes (mean ± standard error) per pistil of Red Aleppo pollinated in 1992 with Trabonella at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 and 48 hours.

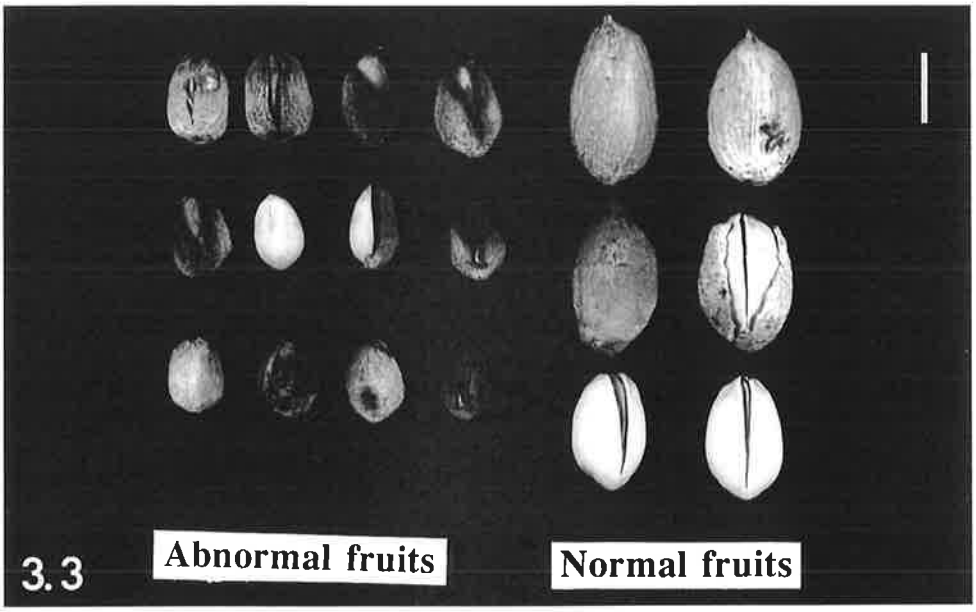
| Time of pollination (days) | Time of harvest | Pollen grains on the stigma | Pollen tubes in | | | | | | Mean ± SE of pollen tubes in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 0 | | 160.86 ±18.02 | 100.50 ±13.96 | 1.06 ±0.32 | 0.90 ±0.28 | 0.53 ±0.20 | 0.23 ±0.10 | 0.03 ±0.03 | 0.55 ±0.18 |
| 1 | | 150.53 ±16.65 | 140.50 ±10.71 | 5.80 ±0.47 | 5.36 ±0.43 | 1.77 ±0.29 | 0.80 ±0.17 | 0.06 ±0.04 | 2.73 ±0.28 |
| 2 | 24 h | 124.37 ±14.65 | 117.73 ±14.18 | 8.13 ±0.41 | 7.43 ±0.32 | 3.81 ±0.28 | 1.86 ±0.23 | 0.10 ±0.05 | 4.26 ±0.26 |
| 3 | | 114.20 ±14.63 | 106.63 ±14.17 | 7.04 ±0.46 | 6.33 ±0.42 | 4.23 ±0.30 | 2.06 ±0.22 | 0.13 ±0.06 | 3.96 ±0.29 |
| 4 | | 120.03 ±10.24 | 76.47 ±7.12 | 1.57 ±0.51 | 0.97 ±0.36 | 0.40 ±0.21 | 0.16 ±0.13 | 0 | 0.62 ±0.24 |
| Mean ± SE | | 134.01 ±13.64 | 108.36 ±11.97 | 4.72 ±0.43 | 4.19 ±0.36 | 2.15 ±0.26 | 1.02 ±0.17 | 0.06 ±0.04 | 2.42 ±0.25 |
| 0 | | 137.60 ±16.36 | 124.27 ±15.74 | 3.83 ±0.47 | 3.53 ±0.44 | 1.63 ±0.29 | 0.73 ±0.17 | 0.20 ±0.10 | 1.98 ±0.29 |
| 1 | | 141.90 ±15.67 | 132.73 ±14.92 | 7.10 ±0.52 | 6.46 ±0.55 | 3.86 ±0.36 | 2.13 ±0.21 | 1.13 ±0.17 | 4.14 ±0.36 |
| 2 | 48 h | 116.23 ±15.36 | 108.47 ±14.67 | 6.67 ±0.60 | 5.83 ±0.52 | 3.50 ±0.33 | 2.36 ±0.24 | 1.36 ±0.21 | 3.94 ±0.38 |
| 3 | | 122.87 ±16.88 | 112.93 ±15.81 | 7.63 ±0.45 | 6.83 ±0.35 | 4.63 ±0.36 | 2.73 ±0.28 | 1.66 ±0.27 | 4.69 ±0.34 |
| 4 | | 180.23 ±15.36 | 113.90 ±11.52 | 1.93 ±0.39 | 1.30 ±0.35 | 0.76 ±0.26 | 0.56 ±0.23 | 0.26 ±0.15 | 0.96 ±0.27 |
| Mean ± SE | | 139.76 ±15.92 | 118.45 ±14.53 | 5.43 ±0.48 | 4.79 ±0.44 | 2.87 ±0.32 | 1.70 ±0.23 | 0.92 ±0.18 | 3.14 ±0.33 |

**Table 3.6** Average number of pollen grains and tubes (mean ± standard error) per pistil of Red Aleppo pollinated in 1992 with *P. atlantica* at 0, 1, 2, 3 and 4 days after flower opening and harvested at 24 and 48 hours.

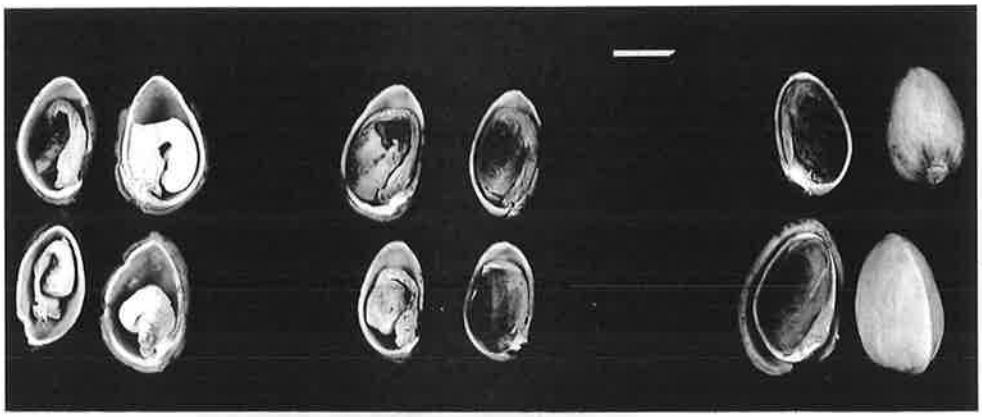
| Time of pollination (days) | Time of harvest | Pollen grains on the stigma | Pollen tubes in | | | | | | Mean ± SE of pollen tubes in style and ovary |
|---|---|---|---|---|---|---|---|---|---|
| | | | stigma | upper style | lower style | ovary | ovule | nucellus | |
| 0 | 24 h | 132.90 ±14.63 | 101.93 ±11.29 | 2.36 ±0.57 | 1.83 ±0.50 | 0.56 ±0.20 | 0.07 ±0.04 | 0 | 0.96 ±0.26 |
| 1 | | 97.43 ±13.96 | 70.87 ±12.56 | 1.60 ±0.51 | 1.36 ±0.47 | 0.67 ±0.28 | 0.23 ±0.10 | 0 | 0.77 ±0.27 |
| 2 | | 116.60 ±13.78 | 99.73 ±13.31 | 3.20 ±0.46 | 2.46 ±0.36 | 1.46 ±0.32 | 0.83 ±0.17 | 0 | 1.59 ±0.26 |
| 3 | | 83.13 ±14.31 | 73.96 ±12.97 | 2.05 ±0.37 | 1.76 ±0.35 | 0.66 ±0.18 | 0.30 ±0.10 | 0 | 0.95 ±0.20 |
| 4 | | 111.50 ±15.61 | 54.57 ±8.03 | 0.07 ±0.05 | 0 | 0 | 0 | 0 | 0.02 ±0.01 |
| Mean ± SE | | 108.31 ±14.43 | 80.21 ±11.63 | 1.85 ±0.39 | 1.48 ±0.34 | 0.67 ±0.19 | 0.29 ±0.08 | 0 | 0.68 ±0.20 |
| 0 | 48 h | 113.83 ±12.59 | 96.50 ±11.91 | 3.60 ±0.59 | 2.96 ±0.55 | 1.03 ±0.23 | 0.57 ±0.16 | 0.27 ±0.09 | 1.68 ±0.32 |
| 1 | | 105.63 ±13.41 | 94.20 ±12.48 | 4.67 ±0.65 | 3.63 ±0.60 | 1.80 ±0.37 | 1.02 ±0.22 | 0.50 ±0.13 | 2.32 ±0.39 |
| 2 | | 111.67 ±16.91 | 101.30 ±15.57 | 3.46 ±0.60 | 2.56 ±0.52 | 1.36 ±0.33 | 0.91 ±0.21 | 0.53 ±0.14 | 1.76 ±0.36 |
| 3 | | 78.90 ±13.22 | 64.53 ±11.89 | 2.06 ±0.40 | 1.56 ±0.39 | 0.93 ±0.26 | 0.40 ±0.12 | 0.20 ±0.07 | 1.03 ±0.25 |
| 4 | | 123.43 ±13.73 | 53.57 ±6.79 | 0.27 ±0.13 | 0.06 ±0.06 | 0 | 0 | 0 | 0.06 ±0.04 |
| Mean ± SE | | 106.69 ±13.97 | 82.02 ±11.73 | 2.81 ±0.46 | 2.15 ±0.42 | 1.02 ±0.24 | 0.57 ±0.14 | 0.30 ±0.08 | 1.37 ±0.27 |

**Figure 3.3** Normal and abnormal fruits of *Pistacia vera*. Normal fruits consist of kernel, shell and hull. The hull is removed during processing and the shell split naturally. Abnormal fruits may be blank, semi-blank, non-split or mis-shapen. Bar represents 1 cm.

**Figure 3.4** Abnormal fruits of *P. vera* including blank, semi-blank and non-split. Bar represents 1 cm.


Abnormal fruits
Normal fruits

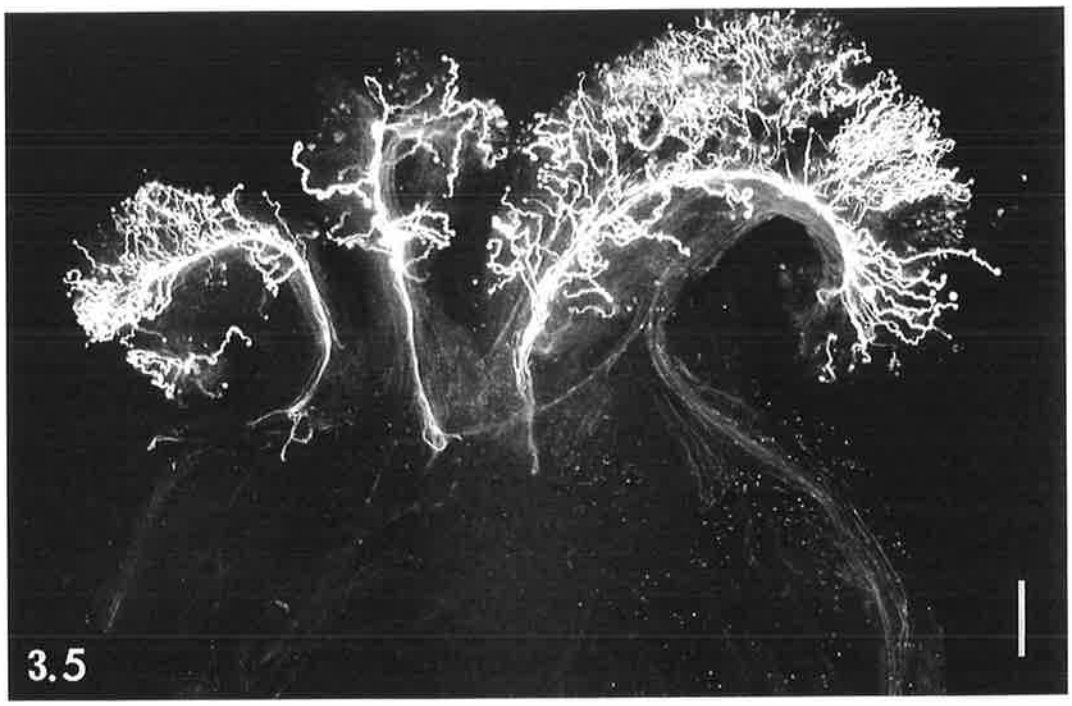
3.3


Blank fruits
Semi-blank fruits
Nonsplit fruits

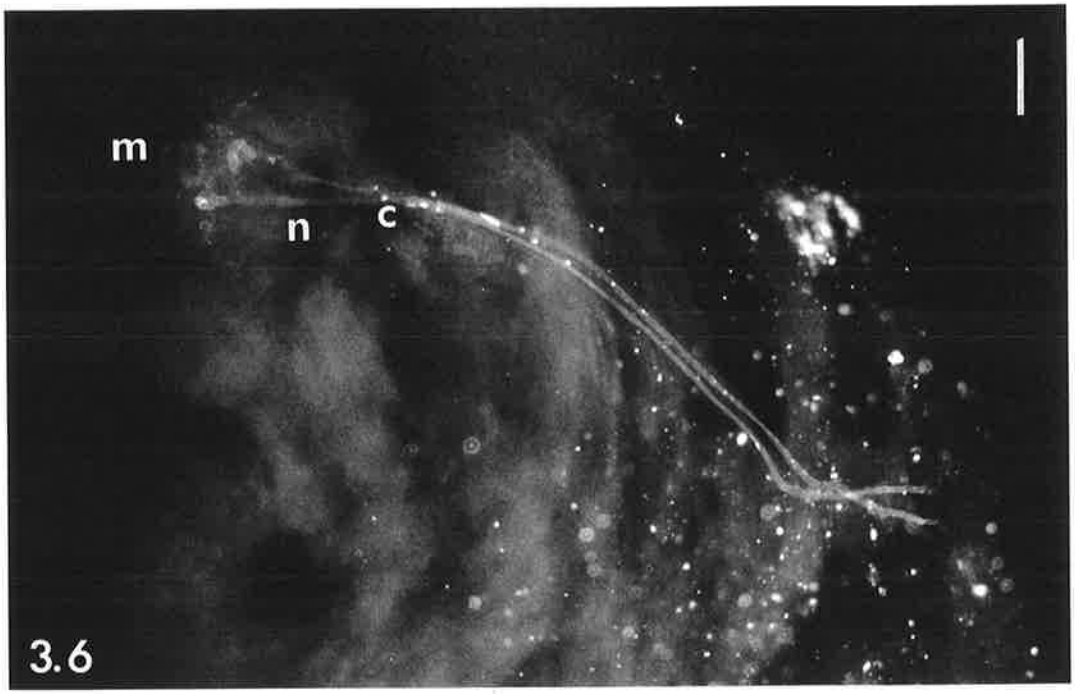
3.4

**Figure 3.5** Fluorescence micrograph of cv. Kerman pollinated with 15-12 pollen three days after anthesis and harvested at 48 hours showing pollen germination on the stigma and tube growth in the stigma and style. Bar represents 500 μ m.

**Figure 3.6** Fluorescence micrograph of cv. Kerman ovule pollinated with 15-12 pollen two days after anthesis and harvested at 48 hours showing chalazogamous penetration of the nucellus (n) via the chalaza (c) rather than the micropyle (m) route by two pollen tubes. Bar represents 100 μ m.



3.5




3.6

tubes was also observed in the nucellus. Analysis of deviance showed that all of the variables of male parent, time of pollination and time of harvest were significant, either singly or as interactions (Table 3.7). Unpollinated pistils had no pollen grains or pollen tubes.

Pistil fertility as measured by total fruit set and production of split fruit was highest up to two days after anthesis (Table 3.8). In inflorescences pollinated with *P. vera* pollen many flowers persisted up to 10 weeks, but relatively few fruits remained to fruit maturity. Three day old pistils produced mainly abnormal mis-shapen fruits which were much smaller than normal fruits (Figure 3.3). These abnormal fruits were usually split but sometimes had an irregular crack on the husk and shell. The majority of pistils pollinated at day 4 after flower opening were shed before maturity.

Most pistils of Kerman and Trabonella female pollinated with *P. atlantica* pollen had dropped by 10 weeks after anthesis, and only one split fruit of Kerman was produced in 1993 (Table 3.9). Some one day old flowers of Kerman pollinated with *P. atlantica* remained up to 10 weeks, and then split and abscised prematurely, in contrast to *P. vera* x *P. vera* fruits which split at 18-20 weeks after anthesis.

Blank fruits developed from unpollinated and four-day old pollinated Trabonella female and Red Aleppo pistils (Figure 3.7), and from pollinated pistils of all crosses except Kerman and Trabonella female by *P. atlantica* in 1992.

**Table 3.7** Analysis of deviance of pollen grain and pollen tube data.

| | Probability | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tubes in | | | | | |
| Source of variation | Grains on stigma | stigma | upper style | lower style | ovary | ovule | nucellus |
| 1992 | | | | | | | |
| 1. Time of pollination | ns | ns | ns | <0.001 | ns | ns | ns |
| 2. Male parent | <0.05 | ns | ns | <0.001 | ns | ns | ns |
| 3. Time of harvest | ns | ns | ns | <0.001 | ns | <0.001 | ns |
| Interaction 1. 2 | ns | <0.001 | <0.05 | ns | <0.01 | ns | ns |
| Interaction 1. 3 | ns | <0.001 | ns | ns | ns | ns | <0.05 |
| Interaction 2. 3 | ns | <0.001 | <0.001 | ns | <0.001 | ns | <0.001 |
| Interaction 1. 2. 3 | ns | ns | ns | ns | ns | ns | ns |
| 1993 | | | | | | | |
| 1. Time of pollination | ns | ns | ns | <0.01 | 0.001 | <0.001 | ns |
| 2. Male parent | ns | ns | ns | <0.05 | <0.05 | ns | <0.05 |
| Interaction 1. 2 | <0.001 | ns | <0.001 | ns | ns | ns | ns |

ns=not significant

**Table 3.8** Fruit set of *Pistacia vera* x *Pistacia vera* following pollination up to four days after anthesis.

| Day pollinated | Number of pistils | Total number of fruits retained | Number of fruits at maturity* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Split | Non-split | Blank | Semi-blank | Mis-shapen |
| 1992 Kerman x 15-12 | | | | | | | |
| 0 | 909 | 42 | 4 | 22 | 9 | 4 | 3 |
| 1 | 903 | 107 | 15 | 26 | 36 | 28 | 2 |
| 2 | 891 | 33 | 10 | 8 | 7 | 6 | 2 |
| 3 | 924 | 18 | 1 | 3 | 5 | 1 | 8 |
| 4 | 861 | 1 | 0 | 0 | 1 | 0 | 0 |
| Unpoll. control | 934 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1992 Red Aleppo x Trabonella | | | | | | | |
| 0 | 935 | 42 | 3 | 23 | 8 | 5 | 3 |
| 1 | 915 | 66 | 10 | 29 | 14 | 12 | 1 |
| 2 | 921 | 124 | 22 | 64 | 26 | 6 | 6 |
| 3 | 918 | 20 | 1 | 2 | 4 | 1 | 12 |
| 4 | 908 | 3 | 0 | 0 | 0 | 0 | 3 |
| Unpoll. control | 932 | 9 | 0 | 0 | 9 | 0 | 0 |
| 1993 Kerman x 15-12 | | | | | | | |
| 0 | 803 | 38 | 4 | 7 | 23 | 2 | 2 |
| 1 | 851 | 129 | 13 | 52 | 47 | 14 | 3 |
| 2 | 761 | 85 | 12 | 23 | 30 | 13 | 7 |
| 3 | 811 | 35 | 0 | 2 | 26 | 2 | 5 |
| 4 | 764 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unpoll. control | 875 | 2 | 0 | 0 | 2 | 0 | 0 |

*Some fruits appear in more than one group

**Table 3.9** Fruit set of *Pistacia vera* x *Pistacia atlantica* following pollination up to four days after anthesis.

| Day pollinated | Number of pistils | Total number of fruits retained | Number of fruits at maturity* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Split | Non-split | Blank | Semi-blank | Mis-shapen |
| 1992 Kerman x *P. atlantica* | | | | | | | |
| 0 | 922 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 983 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 963 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 918 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 881 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unpoll. control | 946 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. vera* Trabonella female x *P. atlantica* | | | | | | | |
| 0 | 963 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 840 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 893 | 4 | 0 | 0 | 0 | 0 | 4 |
| 3 | 936 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 1025 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unpoll. control | 964 | 12 | 0 | 0 | 12 | 0 | 0 |
| 1993 Kerman x *P. atlantica* | | | | | | | |
| 0 | 803 | 26 | 0 | 2 | 21 | 3 | 0 |
| 1 | 851 | 17 | 1 | 0 | 16 | 0 | 3 |
| 2 | 764 | 33 | 0 | 6 | 27 | 0 | 0 |
| 3 | 918 | 12 | 0 | 0 | 12 | 0 | 1 |
| 4 | 764 | 6 | 0 | 0 | 6 | 0 | 0 |
| Unpoll. control | 783 | 0 | 0 | 0 | 0 | 0 | 0 |

*Some fruits appear in more than one group

**Figure 3.7** Four-day pollinated flowers of cv. Trabonella x 15-12 produced blank fruits at harvest. Bar represents 1 cm.

3.7

## 3.4 Discussion

The highest yield of split fruit resulted from pollination up to two days after flower opening. Three day old pistils supported pollen tube growth but this resulted in little fruit set, indicating that embryo sac degeneration may have occurred by this time. There was reduced pollen tube growth and little fruit set in four day old pistils. Lack of pistil receptivity in older flowers has also been reported to reduce fruit set in avocado and rubber (Sedgley, 1977b; Sedgley and Attanayake, 1988).

Pollen of *P.vera* produced higher fruit set than *P. atlantica*. These observations were supported by the pollen tube growth data, as pollen of *P. atlantica* did not grow well in the *P. vera* pistil particularly in 1992. This could be due to inter-specific incompatibility, and may result in ovule or seed abortion, and thus in blanking. *P. atlantica* is used as a rootstock for *P. vera*, and trees, which frequently flower at the same time as *P. vera*, are often grown near to commercial orchards. Synchronous flowering was not observed in either of the years of this study, but *in vitro* tests showed adequate germination of all pollens, although it is possible that the stored *P. atlantica* pollen had lost the ability to effect fertilisation and fruit set. This requires further investigation as some authors have reported fruit set with fresh *P. atlantica* pollen comparable to that with *P. vera* (Whitehouse and Stone, 1941, Whitehouse *et al.*, 1964). However, Crane and Iwakiri (1980) obtained lower fruit set with *P. atlantica* pollen, and Whitehouse *et al.* (1964) reported delayed ripening and small kernel size. Grundwag (1975) observed ovule degeneration following inter-specific pollination within the *Pistacia* genus, although the *P. vera* x *P. atlantica* combination was not studied. The data available suggest that *P. atlantica* should not be allowed to flower in the vicinity of commercial *P. vera* cultivars, as its pollen may compete with *P. vera* pollen during the very short period of pistil receptivity.

All pollen tubes of all genotypes at all pollination times that reached the nucellus were observed to enter via the chalaza. Pollen tube penetration in pistachio

has been reported previously to be chalazogamous (Grundwag and Fahn, 1969), but this is the first study to investigate a range of pollination times. Luza and Polito (1991) reported that only immature ovules of walnut were penetrated chalazogamously, with porogamous penetration of mature ovules. Pistachio ovules up to four days after anthesis were penetrated chalazogamously, indicating that this is the normal route of pollen tube growth in this species.

The fact that blank fruits were reported in both unpollinated and pollinated treatments indicates that vegetative and stimulative parthenocarpy may occur in pistachio. This has major implications with regard to yield and quality, and reinforces the importance of selecting a pollinator cultivar with overlap in time of pollen shed with pistil maturity of the *P. vera* female cultivars.

# Chapter 4

# 4 Chalazogamous pollen tube pathway and stimulation of embryo sac development in *Pistacia vera*

## 4.1 Introduction

Problems in the pollen pistil interaction in pistachio may be responsible for the abortion and abscission of flowers, early developing fruitlets and premature fruits. Irregularities in ovule development have been found in the megagametophyte of a range of species. In *Phytolacca americana*, ovule abortion occurs either during the formation of the female gametophyte or at the globular embryo stage after fertilisation (Mikesell, 1988). In the almond, there is callose deposition in the chalazal area or in the integument layers which may block the flow of nutrients to the ovule (Pimienta and Polito, 1982). Grundwag (1975) has reported pre-anthesis ovule degeneration in pistachio as well as post pollination degeneration of the embryo sac (Grundwag and Fahn, 1969).

This study was undertaken to document pistachio pistil structure, path of pollen tube growth and early development of pollinated and unpollinated pistils. Chalazogamous ovule penetration has been reported in pistachio (Grundwag and Fahn, 1969), and this phenomenon is reported in detail in relation to pollen tube growth.

## 4.2 Materials and methods

Three buds on three branches each with about 300 female flowers on two trees of each of *Pistacia vera* cvs. Kerman and Sirora were bagged during budburst. Ten flowers of each treatment were harvested at each of 0, 1, 2, 3 and 4 days after flowering, and the pistils processed for microscopy. In addition, about 1000 female

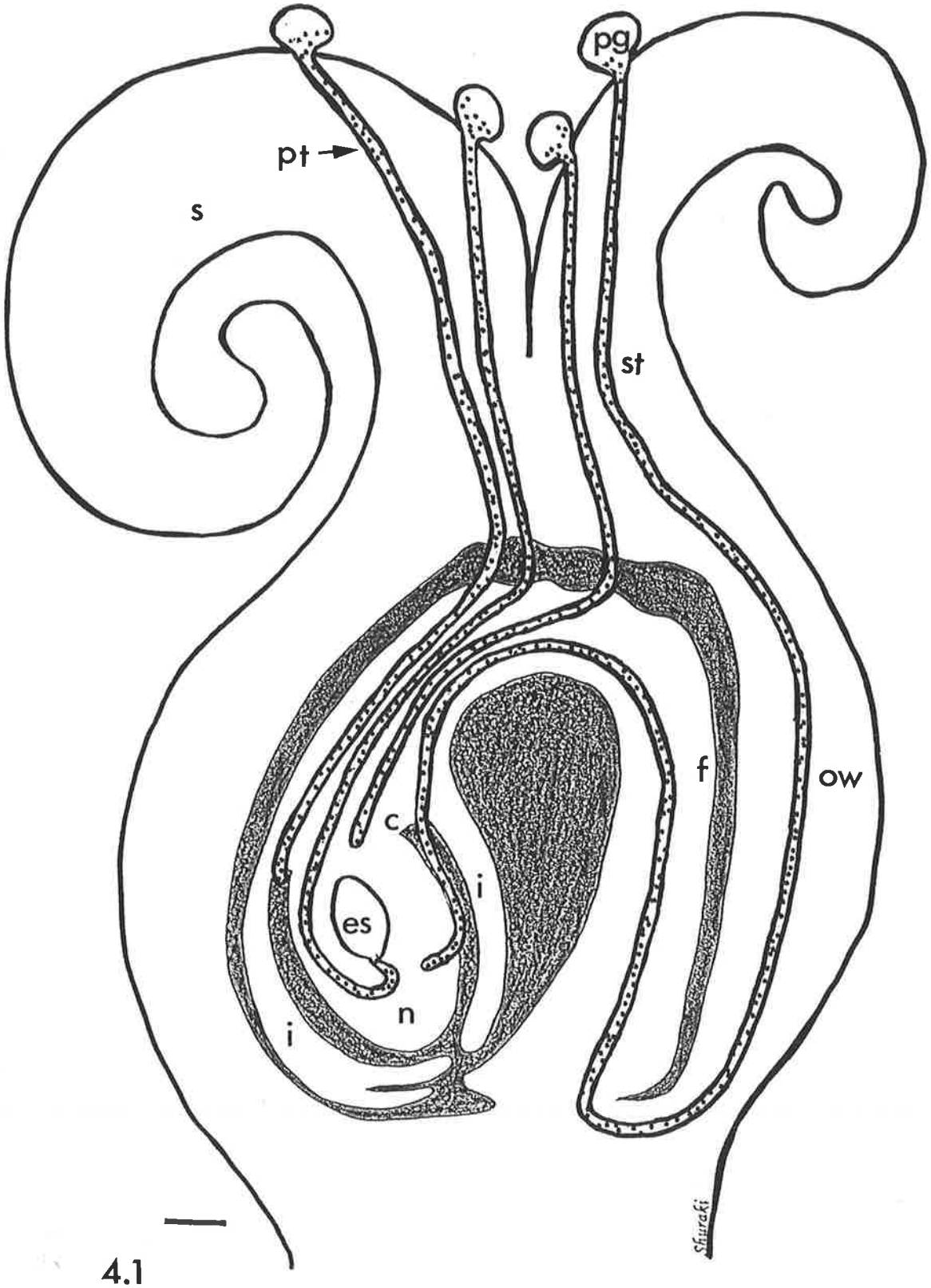
flowers on three branches with three buds on two trees of each of *P. vera* cvs. Kerman and Sirora were pollinated with pollen from *P. vera* cv. 15-12 at 0, 1, 2, 3 and 4 days after flower opening. Ten pistils were harvested at 0, 1, 2, and 3 days after pollination. Specimens were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer (pH 7.0) containing 0.5 % caffeine for suppression of phenolics (Muller and Greenwood, 1978), or in FAA (formalin: acetic acid: 50% ethanol, 5: 5: 90). The pistils were dehydrated via an alcohol series and embedded in glycol methacrylate in gelatine capsules for two days at 60°C (Feder and O'Brien, 1968). Sections 3 or 4 µm thick were stained with periodic acid-Schiff's reagent and toluidine blue O (PAS and TBO) (O'Brien and McCully, 1981) and observed using light microscopy or with decolourised aniline blue (AB) and observed using fluorescence microscopy (Sedgley, 1982). Following fixation and dehydration, flowers for scanning electron microscopy (SEM) were critical point dried and coated with 20 nm gold. Samples were observed with a Cambridge S250 SEM at 20 kV. Fresh samples were observed with an environmental SEM, Electro scan ES3, at 15 kV.

## 4.3 Results

### 4.3.1 Female flower structure

Pistillate inflorescences were panicles with hundreds of small flowers. A large bract covered three to five female flowers up to two of which were small and sterile. The small flowers were not sampled for microscopy. The large bracts reflexed at four days after anthesis and dropped toward the end of week one. Each flower had five to eight small but variably-sized perianth parts located in two spirals surrounding the single large pistil (Figures 4.1, 4.2). The bracts dropped four weeks after pollination. In both cultivars, the three stigmatic lobes diverged to expose the papillate stigma which had a white to pinkish surface at maturity. The curvature of the largest stigma lobe was distinctive for Kerman and Sirora. That of Sirora extended vertically and then reflexed, while that of Kerman extended horizontally before reflexing.

**Figure 4.1** Diagram of longitudinal section (LS) of pistil of pistachio showing pollen tube (pt) pathways. Pollen grains (pg) germinated on the stigma (s) and the majority of pollen tubes penetrated the funicle (f) via the lower stylar (st) transmitting tract, and grew via the chalaza (c) and nucellus (n) to penetrate the embryo sac (es). A minority of tubes penetrated via the ovary wall (ow) transmitting tissue. Some unsuccessful tubes penetrated between the nucellus (n) and integuments (i). Bar represents 135 μm.




4.1

**Figures 4.2-4.7** Figures are arranged such that the nucellus is located at the bottom left in relation to all photographs.

**Figure 4.2** Scanning electron micrograph of the female pistachio flower comprising perianth (pe) and pistil (p) with anatropous ovule (o) exposed. Bar represents 400 μm.

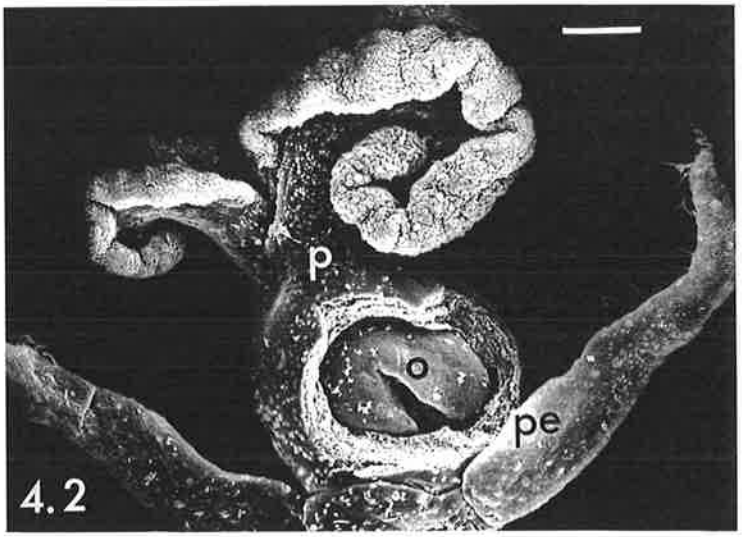
**Figure 4.3** Light micrograph of LS stained with PAS and TBO of the ovule consisting of a large curved funicle (f) with a relatively small nucellus (n) and integuments (i). Bar represents 380 μm.

**Figure 4.4** Light micrograph of transverse section (TS) stained with PAS and TBO of upper style showing three tracts of transmitting tissue (arrow) surrounded by resin ducts (r) and vascular bundles (vb). Bar represents 100 μm.

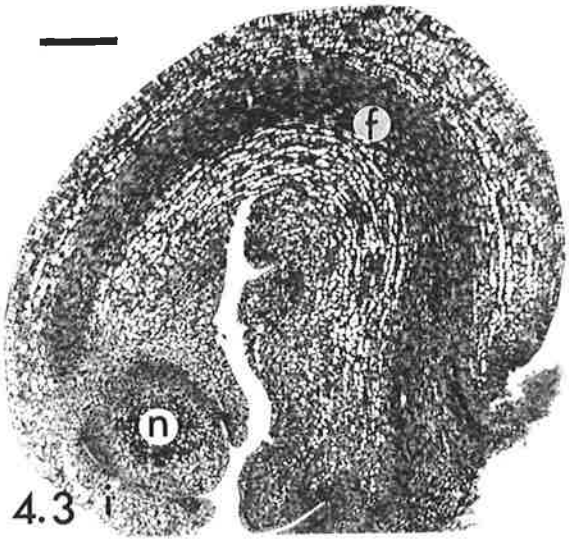
**Figure 4.5** Light micrograph of LS stained with PAS and TBO of the lower style showing the ovarian projection (op) and the funicular protuberance (fp) in the free space between ovary wall (ow) and the funicle (f). Bar represents 170 μm.

**Figure 4.6** Light micrograph of LS stained with PAS and TBO of the funicle showing the funicular protuberance (fp) and loose parenchymatous tissue linking with the transmitting tissue (tt) of the funicle. Bar represents 75 μm.

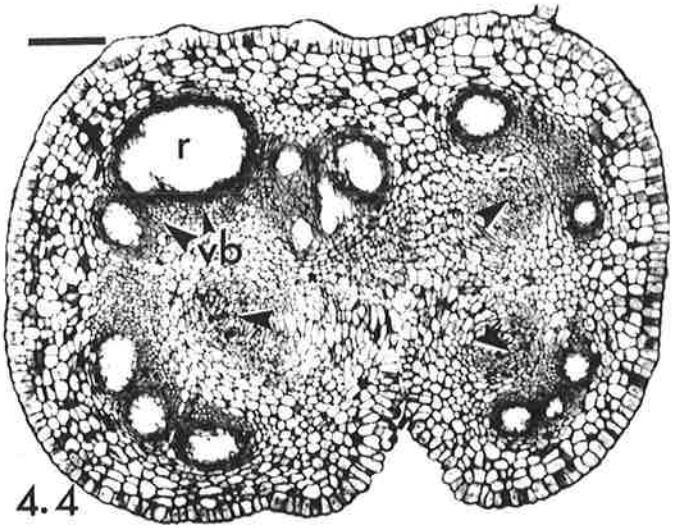
**Figure 4.7** Light micrograph of TS stained with PAS and TBO of the funicle showing three transmitting tissue tracts (tt) in proximal funicle (pf) and one in the distal funicle (df). Bar represents 165 μm.



4.2



4.3



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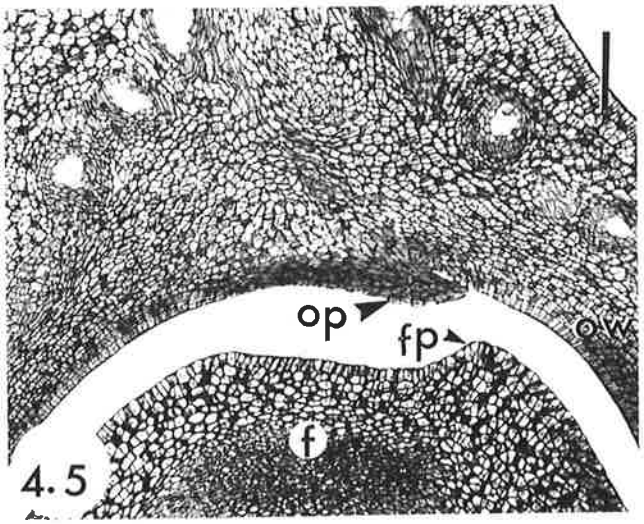

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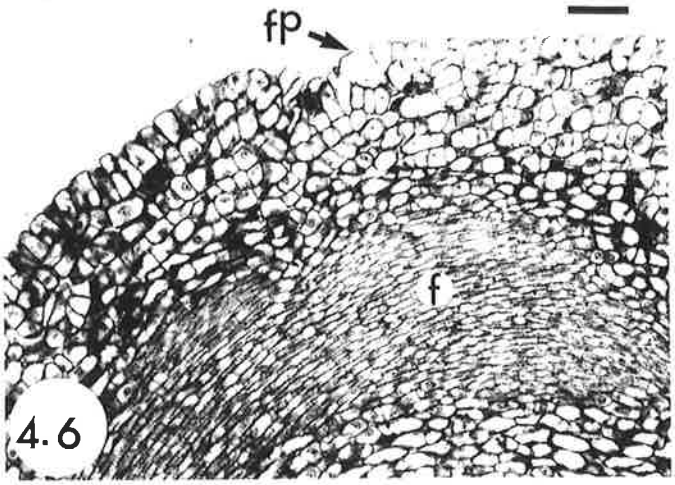
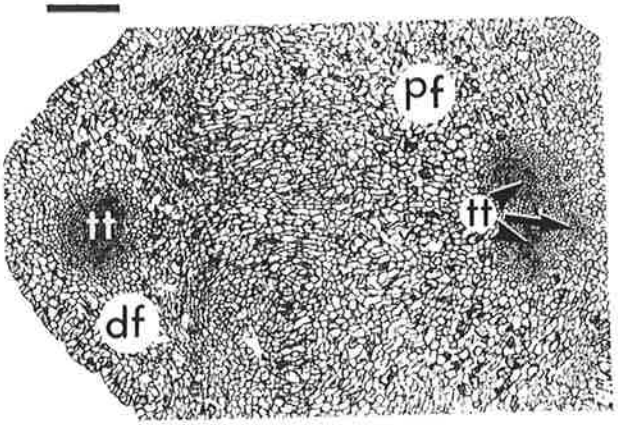


4.6

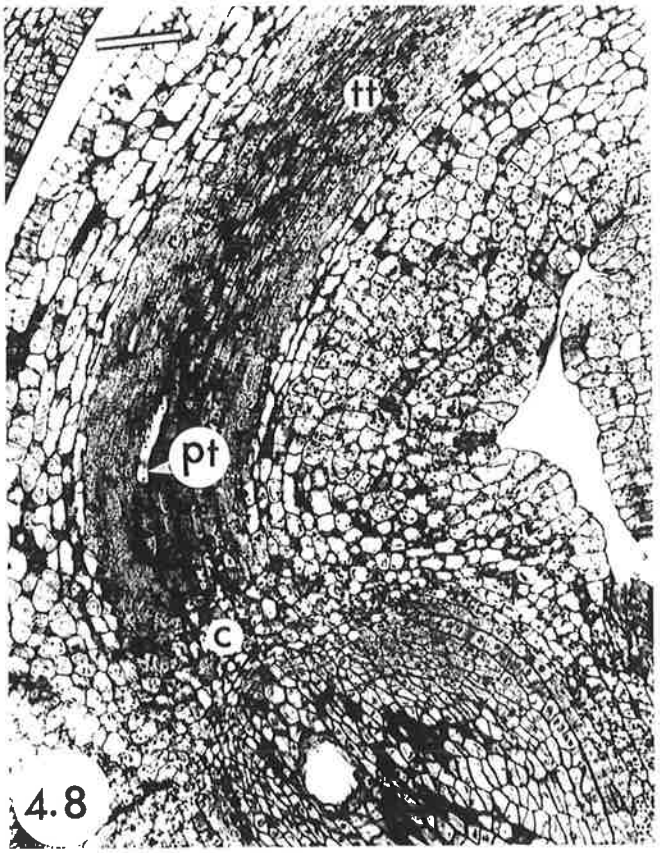


4.7

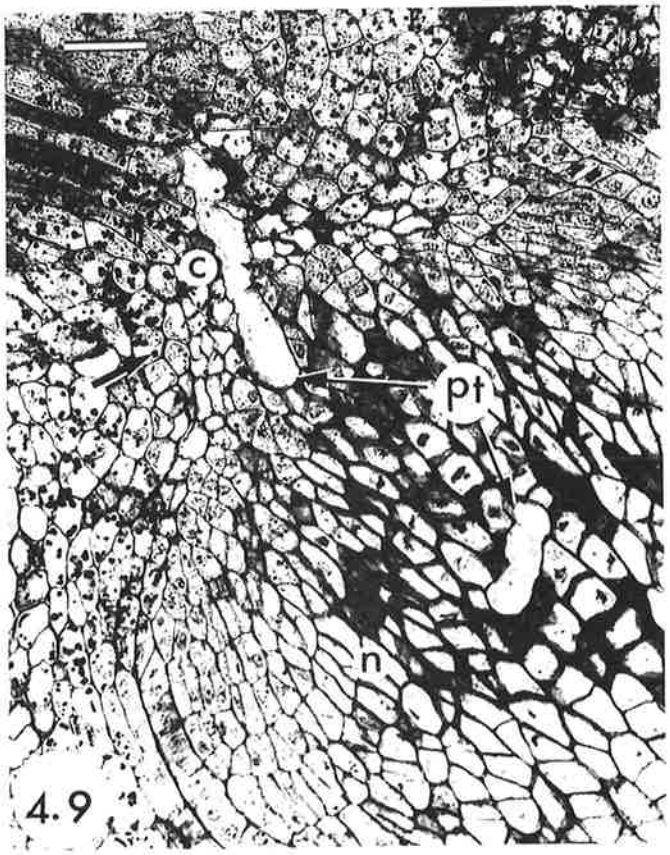
**Figures 4.8 and 4.9** Figures are arranged such that the nucellus is located at the base of the photograph.

**Figure 4.8** Light micrograph of LS stained with PAS and TBO of the funicular transmitting tissue (tt) with a pollen tube (pt) approaching the chalaza (c). Bar represents 50 μm.

**Figure 4.9** Light micrograph of LS stained with PAS and TBO of chalaza (c) and nucellus (n) with a pollen tube (pt). Note the starch grains (arrow) in the chalaza (c). Bar represents 30 μm.




4.8




4.9

**Figures 4.10-4.14** Figures are arranged such that the nucellus is located at the bottom left in relation to all photographs.

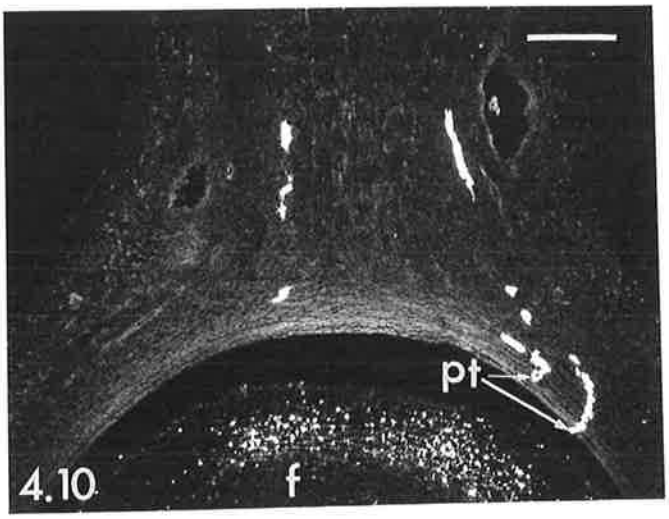
**Figure 4.10** Fluorescence micrograph of LS stained with AB of the lower style showing the main pollen tube pathway. Pollen tubes (pt) cross the ovarian free space to penetrate the funicle (f). Bar represents 100 μm.

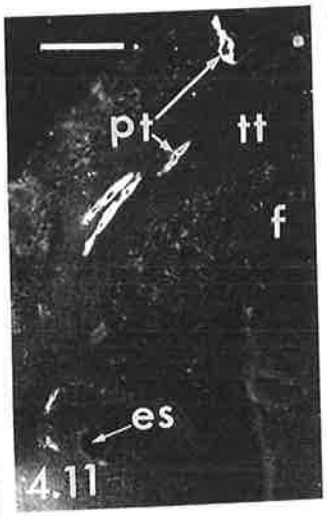
**Figure 4.11** Fluorescence micrograph of LS stained with AB showing the main pollen tube pathway in the distal funicle (f). The pollen tube (pt) passed the parenchymatous tissue then penetrated the central transmitting tissue (tt) to reach the embryo sac (es). Bar represents 100 μm.

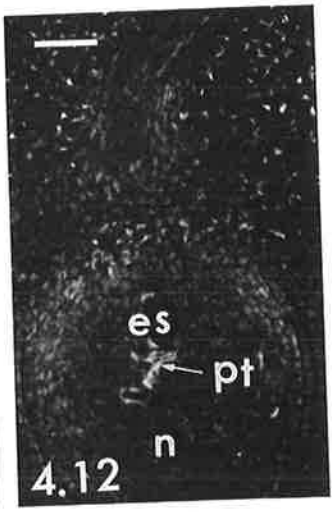
**Figure 4.12** Fluorescence micrograph of LS stained with AB of the nucellus (n) showing a pollen tube (pt) penetrating the embryo sac (es), two days after pollination. Bar represents 60 μm.

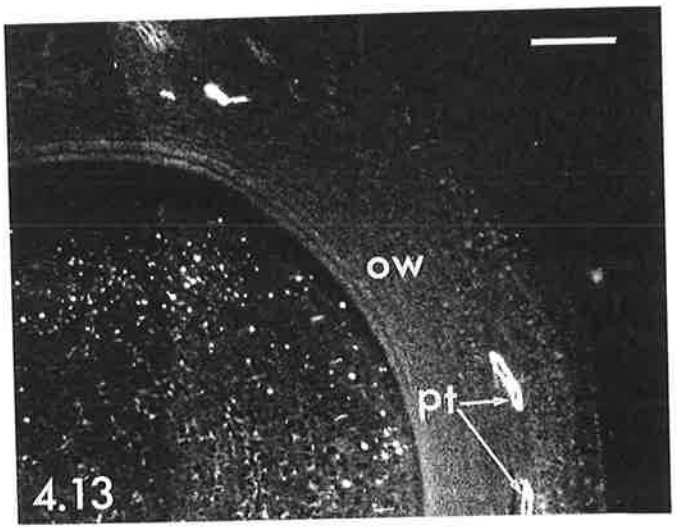
**Figure 4.13** Fluorescence micrograph of LS stained with AB showing a pollen tube (pt) in the ovary wall (ow), a second route of pollen tube growth. Bar represents 80 μm.

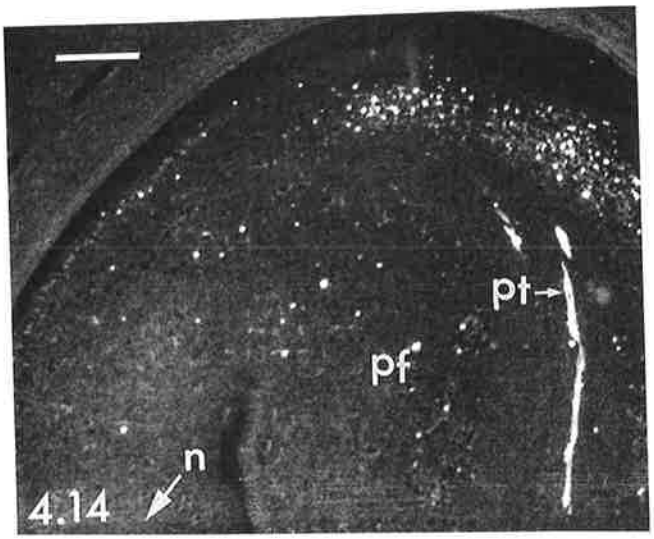
**Figure 4.14** Fluorescence micrograph of LS stained with AB showing a pollen tube (pt) growing toward the nucellus (n) via the proximal funicle (pf). Bar represents 100 μm.


pt
f
4.10


pt
tt
f
es
4.11


es
pt
n
4.12


ow
pt
4.13


pt
pf
n
4.14

The two small lobes of cv. Sirora appeared about 15 hours later than those of cv. Kerman which diverged at flower opening. Flower opening of both cultivars started from the top of the panicle so that a range of developmental stages was present, but after a day of flowering the majority of flowers had the large stigma lobe exposed. The unilocular ovarian chamber contained a single ovule with a large funicle (Figure 4.3). The ovule was crassinucellate and anatropous with a large chalaza. The inner integuments were well developed, but the outer integuments were reduced.

The receptive stigma of pistachio was papillate, with large distal cells and small proximal cells with many intercellular spaces. The large papilla cells were highly vacuolate with peripheral cytoplasm and extracellular secretion. The upper style of both cultivars continued the lobing of the stigma and consisted of epidermis with one to four layers of regular cells, parenchymatous cortex with latex ducts, vascular bundles close to the latex ducts, and the central tissue with three main transmitting tissue tracts (Figure 4.4). In the lower style, the transmitting tissue tracts joined into a single core of tissue, part of which ended at a protuberance of the ovary wall (Figure 4.5). The ovary wall protuberance lay opposite a protuberance of the funicle which comprised loose papillate cells which connected with the funicular transmitting tissue (Figure 4.6). The remainder of the transmitting tract spread to enter the ovary wall and ensheath the ovule. At the base of the ovary the transmitting tissue was continuous with that of the funicle (Figure 4.1). The funicle was largely parenchymatous with three vascular bundles and three tracts of transmitting tissue in the lower part which formed one large tract in the upper funicle (Figures 4.6, 4.7, 4.8). The funicular transmitting tissue ended at the chalaza. The chalaza and integuments accumulated starch (Figure 4.9).

### 4.3.2 Pollen tube pathway

Pollen grains germinated on the stigma after two hours, and after six hours tubes penetrated the stigma tissue between the papilla cells. After six hours of

pollination, when pollen tube length was about twice the pollen grain diameter, stigmatic secretion increased, and after 21 hours copious secretion appeared on the stigma surface, particularly around and in the groove between the lobes. Tubes grew through the transmitting tissue in the style, and the majority of pollen tubes penetrated the ovarian cavity via the central transmitting tissue (Figure 4.10). Most penetrated the funicle by crossing the gap between the inner ovary wall and the funicle (Figures 4.10, 4.11). They grew in the funicular transmitting tissue towards the chalaza (Figure 4.11, Table 4.1), through the nucellus (Figure 4.9), then beyond the embryo sac, finally turning to penetrate at the micropylar end (Figure 4.12). A minority of pollen tubes grew via a different route, penetrating the lower funicular transmitting tissue via the ovary wall transmitting tissue (Figures 4.13, 4.14, Table 4.2). At the chalaza, some tubes stopped growing or grew into the integuments and stopped there (Table 4.2). Some pollen tubes penetrated between the inner integument and the nucellus, but none of them penetrated the embryo sac (Table 4.2). In young flowers more tubes penetrated via the nucellus and grew direct to the embryo sac, with highest penetration of the embryo sac following pollination of 1, 2, and 3 day old flowers (Table 4.2). Pollen tube penetration of the nucellus decreased in old flowers and more pollen tubes stopped at the chalaza (Tables 4.1, 4.2).

### 4.3.3 Embryo sac development

One of the innermost cells of the nucellar tissue close to the chalazal region differentiated as a megaspore mother cell (MMC). It was the largest cell in the nucellus, approximately 3 μm in length and 1 μm in diameter (Figure 4.15). Some small MMCs failed to continue into meiosis but most formed a linear tetrad (Figure 4.16), from which the chalazal megaspore cell grew and the other three micropylar megaspores degenerated.

**Table 4.1** Pollen tube penetration of ovules of *Pistacia vera* cvs Kerman and Sirora pollinated with cv. 15-12.

| Days after anthesis | | Number of samples | Number of ovules with no pollen tube | Number (mean± standard error) of pollen tubes in | | | |
|---|---|---|---|---|---|---|---|
| Pollinated | Harvested | | | funicle | chalaza | nucellus | embryo sac |
| Kerman | | | | | | | |
| 0 | 1 | 3 | 0 | 2.50±0.36 | 1.40±0.70 | 0 | 0 |
| 1 | 2 | 7 | 42.9 | 2.00±0.35 | 1.50±0.25 | 1.25±0.25 | 0.25±0.22 |
| 2 | 3 | 5 | 40.0 | 2.30±0.27 | 0.64±0.17 | 0 | 0 |
| 3 | 4 | 5 | 60.0 | 1.50±0.71 | 0 | 0 | 0 |
| Kerman | | | | | | | |
| 1 | 1 | 10 | 100 | 0 | 0 | 0 | 0 |
| 1 | 2 | 10 | 30.0 | 2.00±0.53 | 1.00±0.31 | 0.70±0.24 | 0.20±0.12 |
| 1 | 3 | 10 | 20.0 | 1.60±0.38 | 1.00±0.24 | 0.80±0.23 | 0.30±0.14 |
| 1 | 4 | 10 | 40.0 | 0.91±0.27 | 0.45±0.15 | 0.36±0.14 | 0.09±0.09 |
| Sirora | | | | | | | |
| 1 | 1 | 10 | 100 | 0 | 0 | 0 | 0 |
| 1 | 2 | 10 | 30.0 | 1.70±0.34 | 0.80±0.30 | 0.20±0.12 | 0.10±0.09 |
| 1 | 3 | 10 | 20.0 | 1.20±0.27 | 0.80±0.23 | 0.40±0.15 | 0.10±0.09 |
| 1 | 4 | 10 | 50.0 | 0.60±2.53 | 0.30±0.14 | 0.21±0.13 | 0.10±0.10 |

**Table 4.2** Route of pollen tube growth in ovules of *Pistacia vera* cv. Kerman pollinated with cv. 15-12 and harvested 48 hours after pollination. Minimum of thirty ovaries per treatment.

| Day pollinated after anthesis | Percent pollen tubes | | | | | | |
|---|---|---|---|---|---|---|---|
| | Entered funicle via style transmitting tissue | Entered funicle via ovarian transmitting tissue | Ceased growth in chalaza or integument | Grew between inner integument and nucellus. Embryo sac: | | Entered nucellus directly Embryo sac: | |
| | | | | Penetrated | Not penetrated | Penetrated | Not penetrated |
| 0 | 100 | 0 | 8.3 | 0 | 0 | 0 | 91.7 |
| 1 | 97.6 | 2.4 | 8.2 | 0 | 10.3 | 10.2 | 71.3 |
| 2 | 93.1 | 6.9 | 8.8 | 0 | 5.3 | 24.6 | 61.3 |
| 3 | 98.2 | 1.8 | 12.3 | 0 | 6.1 | 16.9 | 64.7 |
| 4 | 98.6 | 1.4 | 33.3 | 0 | 11.1 | 0 | 55.6 |

**Table 4.3** Development of unpollinated ovules of *Pistacia vera* cv. Kerman following anthesis, and of ovules pollinated with cv. 15-12 up to three days after anthesis and harvested up to four days after anthesis.

| Days after anthesis | | Number of samples | Length (mean ± standard error) of | | Stage of embryo sac development (%) | | | | | | | Fertilised ovules (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ovule (mm) | embryo sac (mm) | No embryo sac | MMC[a] | Tetrad | Embryo sac nuclei | | | | |
| | | | | | | | | 1 | 2 | 4 | 8 | |
| Unpollinated | Harvested | | | | | | | | | | | |
| 0 | 0 | 12 | 0.82±0.04 | 30.8±3.080 | 16.7 | 41.7 | 25.0 | 16.7 | 0 | 0 | 0 | 0 |
| 1 | 1 | 6 | 0.89±0.09 | 30.4±0.913 | 16.7 | 0 | 33.3 | 50.0 | 0 | 0 | 0 | 0 |
| 2 | 2 | 6 | 1.09±0.06 | 32.0±5.011 | 16.7 | 0 | 33.3 | 16.7 | 16.7 | 16.7 | 0 | 0 |
| 3 | 3 | 6 | 1.11±0.05 | 40.1±2.361 | 16.7 | 0 | 0 | 0 | 66.7 | 16.7 | 0 | 0 |
| 4 | 4 | 6 | 1.22±0.04 | 44.0±1.702 | 16.7 | 0 | 0 | 0 | 16.7 | 16.7 | 50.0 | 0 |
| Pollinated | Harvested | | | | | | | | | | | |
| 0 | 1 | 6 | 1.19±0.03 | 64.2±4.041 | 16.7 | 0 | 0 | 0 | 0 | 33.3 | 50.0 | 0 |
| 1 | 2 | 5 | 1.23±0.03 | 74.3±3.021 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| 2 | 3 | 4 | 1.50±0.07 | 86.1±3.691 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| 3 | 4 | 3 | 2.38±0.03 | 88.3±3.603 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| Pollinated | Harvested | | | | | | | | | | | |
| 1 | 1 | 7 | 0.70±0.18 | 46.0±4.166 | 28.6 | 14.3 | 14.3 | 0 | 28.6 | 14.3 | 0 | 0 |
| 1 | 2 | 7 | 1.21±0.02 | 78.5±3.062 | 14.3 | 0 | 0 | 0 | 0 | 0 | 85.7 | 0 |
| 1 | 3 | 7 | 1.23±0.04 | 80.7±2.411 | 0 | 0 | 0 | 0 | 0 | 0 | 71.4 | 28.6 |
| 1 | 4 | 7 | 1.89±0.22 | 88.2±4.095 | 14.3 | 0 | 0 | 0 | 0 | 0 | 71.4 | 28.6 |

[a]Megaspore mother cell

**Table 4.4** Development of unpollinated ovules of Pistacia vera cv. Sirora following anthesis, and of ovules pollinated with cv. 15-12 at one day after anthesis and harvested up to four days after anthesis.

| Days after anthesis | | Number of samples | Length (mean ± standard error) of | | Stage of embryo sac development (%) | | | | | | | Fertilised ovules (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ovule (mm) | embryo sac (mm) | No embryo sac | MMC[a] | Tetrad | Embryo sac nuclei | | | | |
| | | | | | | | | 1 | 2 | 4 | 8 | |
| Unpollinated | Harvested | | | | | | | | | | | |
| 0 | 0 | 12 | 0.67±0.04 | 28.7±2.072 | 25.0 | 50.0 | 25.0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 6 | 1.13±0.09 | 30.4±2.091 | 33.3 | 0 | 16.7 | 33.3 | 16.7 | 0 | 0 | 0 |
| 2 | 2 | 6 | 1.27±0.05 | 38.7±0.883 | 50.0 | 0 | 0 | 0 | 33.3 | 16.7 | 0 | 0 |
| 3 | 3 | 6 | 1.22±0.06 | 36.2±0.891 | 16.7 | 0 | 0 | 16.7 | 0 | 50.0 | 16.7 | 0 |
| 4 | 4 | 6 | 1.38±0.10 | 37.8±8.611 | 33.3 | 0 | 0 | 0 | 0 | 66.7 | 0 | 0 |
| Pollinated | Harvested | | | | | | | | | | | |
| 1 | 1 | 7 | 0.87±0.08 | 50.3±4.330 | 28.6 | 0 | 0 | 0 | 57.1 | 14.3 | 0 | 0 |
| 1 | 2 | 7 | 1.18±0.05 | 57.1±8.461 | 14.3 | 0 | 0 | 0 | 0 | 14.3 | 71.4 | 0 |
| 1 | 3 | 7 | 1.30±0.06 | 72.5±2.266 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| 1 | 4 | 7 | 1.76±0.18 | 75.3±1.933 | 0 | 0 | 0 | 0 | 0 | 0 | 57.2 | 42.8 |

[a] Megaspore mother cell

**Figure 4.15** Light micrograph of LS stained with PAS and TBO showing the megaspore mother cell (mmc). Bar represents 10 μm.

**Figure 4.16** Light micrograph of LS stained with PAS and TBO showing three cells of a tetrad with the functional chalazal megaspore (m). Bar represents 10 μm.

**Figure 4.17** Light micrograph of LS stained with PAS and TBO showing a uninucleate embryo sac with a large vacuole (v) at the micropylar end and three degenerated megaspores. Bar represents 10 μm.

**Figure 4.18** Light micrograph of LS stained with PAS and TBO of a two nucleate embryo sac showing one of the two nuclei (arrow). Bar represents 10 μm.

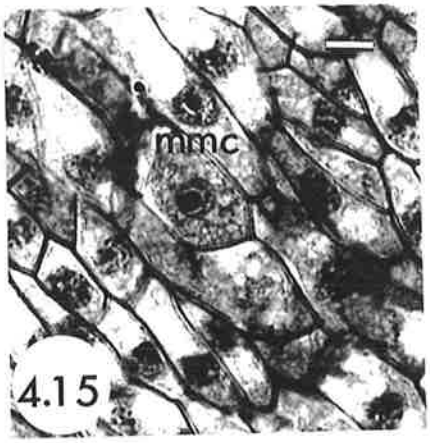
**Figure 4.19** Light micrograph of LS stained with PAS and TBO showing the four nucleate embryo sac with two nuclei (arrows) at each end. Bar represents 10 μm.

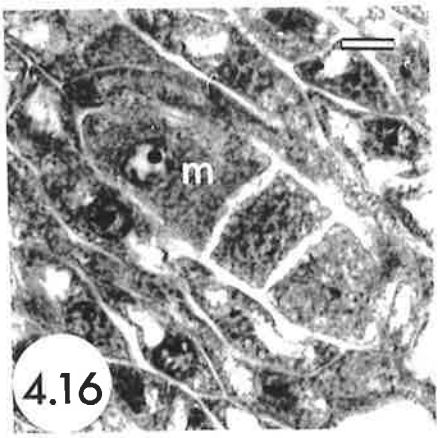
**Figure 4.20** Light micrograph of LS stained with PAS and TBO of the egg apparatus with two synergids (s) and the egg (e). Bar represents 10 μm.

**Figure 4.21** Light micrograph of LS stained with PAS and TBO showing two of the three antipodals (a) and egg cell (e). Bar represents 25 μm.

**Figure 4.22** Light micrograph of LS stained with PAS and TBO showing polar nuclei (pn). Bar represents 25 μm.

**Figure 4.23** Light micrograph of LS stained with PAS and TBO showing zygote (z) and primary endosperm nucleus (en). Bar represents 10 μm.


mmc
4.15


m
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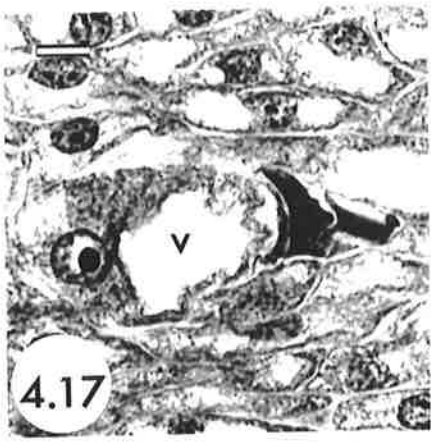
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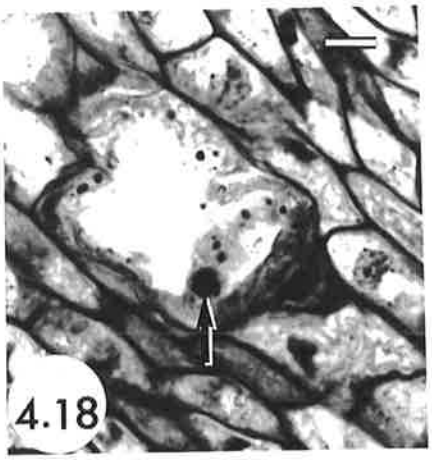
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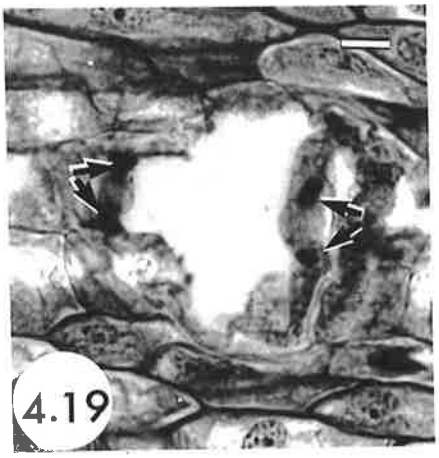
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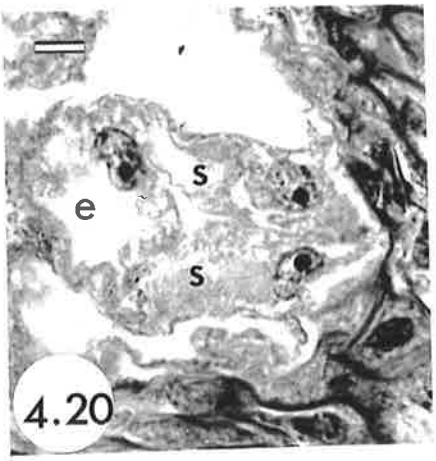
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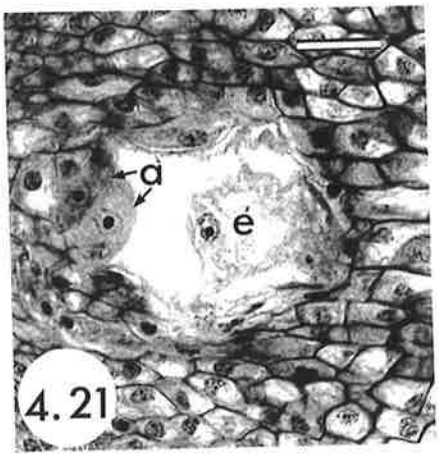
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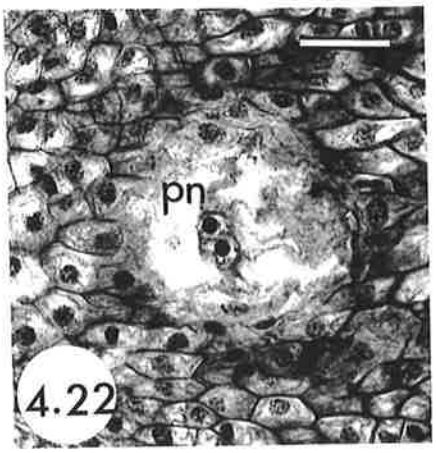
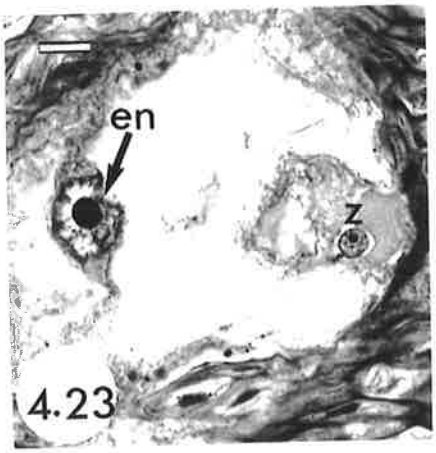

pn
4.22


en
z
4.23

The functional megaspore was approximately 3 μm in length, 1.5 μm in diameter, and had a large nucleus and a large vacuole in the micropylar half (Figure 4.17). Some small functional megaspores stopped growth at this stage of development (Tables 4.3, 4.4). The one nucleate embryo sac underwent mitosis to form the binucleate embryo sac, with a nucleus at each of the micropylar and chalazal ends (Figure 4.18). The four nucleate embryo sac had two nuclei at the chalazal end and two at the micropylar end (Figure 4.19). Some of the nucellar cells surrounding the embryo sac were compressed due to embryo sac growth. The immature eight nucleate embryo sac had two groups each with four nuclei at both the chalazal and micropylar ends and in the mature embryo sac, the two polar nuclei migrated to the mid region.

The embryo sac of both Kerman and Sirora was small and immature at anthesis (Tables 4.3, 4.4). The unpollinated embryo sac developed following anthesis, but maturity was accelerated by pollination. The mature embryo sac consisted of an egg cell and two synergids (Figure 4.20), three antipodals (Figure 4.21) and two polar nuclei (Figure 4.22). Ovule and embryo sac length also increased following anthesis, but increase was greater following pollination. However, some ovules with no embryo sac were observed even three days after pollination, and the first fertilised embryo sacs were observed at two days after pollination in Kerman (Figure 4.23). Both pollinated and unpollinated pistils contained some nucelli without an embryo sac (Tables 4.2, 4.3).

## 4.4 Discussion

The pollen tube pathway in *Pistacia* is unusual in that the funicle comprises an integral part, and ovule entry is via the chalaza. Chalazogamy has been reported in relatively few genera, including *Alnus*, *Mangifera*, *Casuarina*, *Juglans*, *Carya* and *Betula* in addition to *Pistacia* (Sedgely and Griffin, 1989). A chalazal route has been reported in the walnut, but only for immature flowers (Luza and Polito, 1991). In *Pistacia*, the pollen tube pathway has been previously studied by Copeland (1955) and by Grundwag and Fahn (1969), but in this study we have also identified the ovary wall as a route for pollen tube growth. These tubes penetrated the funicular transmitting tissue through the lower funicle. An ovarian route has been reported in *Grevillea banksii*, with branched tubes which did not fertilise the ovule (Martin and Herscovitch, 1989). In the pistachio, pollen tubes entered the ovary wall and proximal funicle only when other tubes had penetrated via the distal funicle.

In pistachio, the majority of pollen tubes crossed a free space between the locular wall and funicular epidermis. In some species the pollen tubes pass this space through a protuberance or bridge. In the mango, pollen tubes travel within a bridge called a ponticulus formed from joined projections of tissue from ovary wall and ovule (Joel and Eisenstein, 1980). This structure is analagous to the projections from the ovary wall and funicle observed in this study in *Pistacia*. In mango, pollination enhanced the development and completion of the ponticulus (de Wet *et al.*, 1986), but we did not observe this in pistachio. In the walnut two wings of integumentary outgrowth tissues functioned as the pollen tube pathway (Luza and Polito, 1991), and *Raphanus* and *Ornithogalum* ovules also have an outgrowth with epidermal transmitting tissue on the surface of which the pollen tubes travel (Tilton and Horner, 1980; Hill and Lord, 1987).

Pollination enhanced the development and maturity of the pistachio embryo sac. At anthesis, some unpollinated ovules had undifferentiated megaspores and only

of half of pistils had complete embryo sacs four days after anthesis. However, all embryo sacs of pollinated pistils had eight nuclei at one day after pollination. A similar positive influence on embryo sac development has been reported in almond (Pimienta and Polito, 1983) and in pear (Herrero and Gascon, 1987). In some pistachio pistils, embryo sac initiation did not occur, and this may be related to maternal genotype. Undeveloped flowers have been reported in other plants, and in the pecan these flowers are dropped (Yates and Sparks, 1994).

Despite abundant pollen tube growth (Shuraki and Sedgley, 1994), relatively few pistachio embryo sacs were penetrated by a pollen tube. Young flowers were more likely to be fertilised, but the numbers were still low. In some species low fertilisation and fruit set have been attributed to slowness of pollen tube growth or to the distance between the stigma and the ovule (Yano *et al.*, 1975; Bassiri *et al.*, 1987). In addition, higher fertilisation and lower fruit abscission has been reported with few pollen grains on the stigma, which may be related to less competition between pollen tubes in the pollen tube pathway (McGranahan *et al.*, 1994; Stanton, 1994). This research has shown that future work should investigate the reasons for the low level of embryo sac penetration in pistachio.

# Chapter 5

# 5 Fruit development of *Pistacia vera* in relation to embryo abortion and abnormalities at maturity

## 5.1 Introduction

Fruit abnormalities and embryo abortion have been studied in a wide variety of angiosperms and the proportion of flowers that fail to develop and reach mature fruits varies from 1 to 100 percent (Sedgley and Griffin, 1989). Fruit abnormality and abortion can be correlated with factors such as temperature (Thompson and Liu, 1973), genotype (Mehlenbacher and Smith, 1991), plant growth regulators and nutrients (Stephenson, 1981; Guitian, 1993).

Embryo abortion in pistachio has been variously attributed to lack of pollination, poor nutrition, rainfall during anthesis and to water deficit during seed development (Maggs, 1973; Grundwag, 1975; Crane *et al.*, 1976; Jordano, 1988). In addition, the time of pollination of the female flower after first opening has been shown to be a significant determinant of yield (Shuraki and Sedgley, 1994), as has the high proportion of ovules which lack an embryo sac (Shuraki and Sedgley, 199$\frac{1}{4}$). Post-fertilisation aberrations in pistachio have been reported to result in low seed set, with a high rate of embryo abortion leading to seedless (blank) or small seeded (semi-blank) fruits (Grundwag and Fahn, 1969). Bradley and Crane (1975) found that early pistachio fruit abortion occurred after degeneration of the funicle at the chalaza, but our understanding of fruit abnormalities and seed abortion of pistachio is still very limited and more information is need to determine the causes of the blanking and semi-blanking problems. In this study two commercially important *P. vera* cultivars Kerman and Sirora were hand pollinated with the male cultivar 15-12, and morphological characteristics of fruit and seed development in relation to abnormalities were investigated to maturity.

## 5.2 Materials and methods

Three branches each with three clusters (200-250 flowers per cluster) on two trees of *Pistacia vera* cvs. Kerman and Sirora were covered with glassine bags with transparent windows prior to flower opening. Flowers were hand pollinated with pollen of *P. vera* cv. 15-12 between one and four days after first flower opening, and pollinated flowers were re-bagged. After one week the glassine bag was replaced with a mesh bag until fruit harvest when normal, blank and semi-blank fruits were counted. Fruits were measured with vernier callipers weekly during development.

Flowers pollinated at one day after anthesis were harvested at each of 0, 1, 2, 3 and 4 days and at 1, 2, 4, 8, 16 and 20 weeks after pollination and processed for bright field microscopy. Specimens were fixed either in 3% glutaraldehyde in 0.025 M phosphate buffer (pH 7.0) containing 0.5 % caffeine for suppression of phenolics (Muller and Greenwood, 1978), or in FPA50 (formalin: propionic acid: 50% ethanol, 5: 5: 90). The pistils were dehydrated via an alcohol series and embedded in glycol methacrylate in gelatine capsules for two days at 60°C (Feder and O'Brien, 1968). Longitudinal sections 3 or 4 µm thick were stained with periodic acid-Schiff's reagent and toluidine blue O (PAS and TBO) (O'Brien and McCully, 1981) and observed using light microscopy. Tissue measurements were made using a micrometer eyepiece.

## 5.3 Results

### 5.3.1 Normal fruit set and development

Fruit set was low and a high proportion of fruits was blank or semi-blank, particularly unpollinated fruit of Kerman in 1993 and 4-day pollinated fruits of Kerman in 1992 and Sirora in 1993 (Table 5.1, Figure 5.2). At fruit ripening, normal, blank and semi-blank fruits were interspersed in the cluster and were

distinguished by hull colouration. None of the blank and semi-blank fruits had a coloured hull, and the hull firmly adhered to the shell.

Fruit growth commenced soon after pollination with rapid growth up to week 4 (Table 5.2). No difference in fruit structure was observed between glutaraldehyde and FPA50 fixation. Shell and hull thickness reached maximum size between eight and 16 weeks after pollination which coincided with the commencement of shell hardening. In the weeks following pollination there was significant development of the funicle, integuments and nucellar tissue (Figure 5.4) of both cultivars such that the funicle was still clearly recognisable at fruit maturity (Table 5.3, Figure 5.1). The greatest size increase of funicle, embryo sac and embryo occurred between weeks 4 and 16 (Tables 5.3, 5.4). At the time of fertilisation the egg was a highly vacuolated cell 1.9 mm in length with a broad chalazal end, and synergids were equal in size, of 1.7 mm in length. Shortly after pollen tube penetration of the embryo sac, the penetrated synergid underwent cytoplasmic changes, including darkened shrunken cytoplasm, and digestion of starch granules. The occurrence of fertilisation, which took place two days after pollination, was confirmed by the presence of one or more sperms or a zygote and polar fusion nucleus. During fertilisation the cell wall of the central cell disappeared and the antipodals persisted up to one week after pollination. Soon after fertilisation the zygote shrank and only one of the synergids was visible. A week following pollination some ovules had free nuclear endosperm and a one cell pro-embryo.

One to four cell pro-embryos were observed in up to 19 % of four-week pollinated pistils (Table 5, Figure 5.3). One-cell embryos were 2.5-3.7 μm in diameter, contained a large vacuole, had a nucleus close to the micropylar end and had a PAS positive cell wall. At this stage less than ten free endosperm nuclei were present in a cytoplasmic ribbon at the embryo sac periphery. After eight weeks of pollination early embryos consisted of 12-20 cells, with about 20-35 free endosperm nuclei.

**Table 5.1** Percent fruit set at maturity and percent of set fruit which were blank or semi-blank of *Pistacia vera* cvs. Kerman and Sirora pollinated with cv. 15-12 up to four days after anthesis.

| Day pollinated | Percent fruit retained | Percent of set fruit which were blank | semi-blank | total blank and semi-blank |
|---|---|---|---|---|
| Kerman x 15-12 | | | | |
| 1992 | | | | |
| 0 | 4.6 | 21.4 | 9.5 | 30.9 |
| 1 | 11.8 | 33.6 | 26.2 | 59.8 |
| 2 | 3.7 | 21.2 | 18.2 | 39.4 |
| 3 | 1.9 | 27.8 | 5.5 | 33.3 |
| 4 | 0.1 | 100 | 0 | 100 |
| Unpollinated | 0 | 0 | 0 | 0 |
| Kerman x 15-12 | | | | |
| 1993 | | | | |
| 0 | 4.5 | 60.5 | 5.3 | 65.8 |
| 1 | 7.2 | 36.4 | 10.9 | 47.3 |
| 2 | 13.5 | 35.3 | 15.3 | 50.6 |
| 3 | 2.2 | 74.3 | 6.7 | 80.0 |
| 4 | 0.3 | 0 | 0 | 0 |
| Unpollinated | 1.0 | 100 | 0 | 100 |
| Sirora x 15-12 | | | | |
| 1993 | | | | |
| 0 | 6.13 | 13.2 | 17.4 | 30.6 |
| 1 | 8.0 | 19.1 | 14.0 | 33.1 |
| 2 | 14.4 | 20.4 | 2.5 | 22.9 |
| 3 | 7.1 | 47.9 | 10.8 | 58.7 |
| 4 | 4.1 | 100 | 0 | 100 |
| Unpollinated | - | - | - | - |

- not measured

**Table 5.2** Normal fruit dimensions (mean ± standard error) of *Pistacia vera* cvs. Kerman and Sirora pollinated with pollen of cv. 15-12 at one day after flower opening and harvested up to maturity. Five samples for each harvest.

| Weeks after pollination | Fruit length (mm) | Fruit height (mm) | Fruit width (mm) | Ovary wall thickness (mm) | Shell thickness (mm) |
|---|---|---|---|---|---|
| Kerman | | | | | |
| 0 | 1.55±0.054 | 1.56±0.043 | 1.30±0.041 | 0.20±0.014 | - |
| 1 | 3.12±0.195 | 2.82±0.125 | 2.30±0.122 | 0.21±0.011 | - |
| 2 | 11.52±1.934 | 7.40±1.094 | 6.44±1.196 | 0.92±0.141 | - |
| 4 | 23.05±0.565 | 13.91±1.086 | 13.17±0.434 | 2.03±0.063 | - |
| 8 | 26.61±0.341 | 15.92±0.254 | 15.09±0.314 | 2.14±0.043 | 1.03±0.070 |
| 16 | 27.50±0.316 | 16.39±0.219 | 15.85±0.191 | 2.49±0.058 | 1.10±0.043 |
| 20 | 27.47±0.332 | 16.19±0.348 | 15.87±0.356 | 2.48±0.058 | 1.13±0.033 |
| Sirora | | | | | |
| 0 | 1.56±0.073 | 1.30±0.097 | 1.19±0.083 | 0.20±0.025 | - |
| 1 | 3.20±0.542 | 2.70±0.449 | 2.31±0.385 | 0.20±0.008 | - |
| 2 | 10.28±1.768 | 6.62±1.021 | 5.91±1.145 | 0.91±0.180 | - |
| 4 | 22.60±0.594 | 14.76±0.304 | 14.75±0.414 | 2.04±0.037 | - |
| 8 | 26.13±0.189 | 15.40±0.172 | 15.15±0.188 | 2.25±0.032 | 1.22±0.039 |
| 16 | 26.57±0.524 | 16.19±0.096 | 16.24±0.242 | 2.45±0.029 | 1.09±0.027 |
| 20 | 27.45±0.375 | 16.27±0.164 | 16.02±0.262 | 2.51±0.062 | 1.08±0.025 |

- tissue not discernable

Embryo and endosperm underwent extensive divisions after eight weeks of pollination (Figure 5.4), and the majority of sixteen week embryo sacs had a fully cellular endosperm (Figure 5.5), some embryos with cotyledons and a testa (Figure 5.6). In 1993, 62.4 % of normal fruits of cv. Kerman and 57.5 % of Sirora had an embryo with cotyledons at 16 weeks after pollination (Figure 5.6). Shell splitting in some fruits started before ripening and progressed during hull softening and colouration.

### 5.3.2 Abnormal fruit set and development

Abnormal fruits were identified by slower growth than that presented in Tables 5.2 - 5.5, and these fruits often showed external signs of degeneration and were shed prematurely (Figure 5.7). Not all were shed, however, and when these retained fruits were observed microscopically, all showed degeneration of one or more tissues. Ovules lacking embryo sacs and with degenerated or unpenetrated embryo sacs were observed at anthesis, and nucellar degeneration commenced one week after pollination (Tables 5.6, 5.7; Figure 5.8).

Degeneration of the funicle occurred during anthesis, with necrosis of the funicular epidermis. Degeneration of the funicle was observed at the site of pollen tube penetration or the epidermis of the chalazal end (Table 5.7, Figure 5.9) and necrosis extended towards the vascular tissue of the funicle. This early funicle degeneration was observed in both pollinated and unpollinated abnormal pistils of both cultivars and was never observed in normal fruits.

There were three major post fertilisation causes of fruit abnormality. Lack of cellularisation of the endosperm, and degeneration of the nucellus (Figure 5.10) both resulted ultimately in death of the pro-embryo at between four and 20 weeks after pollination (Table 5.7). From 16 weeks after pollination, degeneration of the developing cotyledons arrested embryo growth.

**Table 5.3** Normal dimensions (mean ± standard error) of funicle, integuments and nucellus of *Pistacia vera* cvs. Kerman and Sirora pollinated with cv. 15-12 at one day after flower opening and harvested up to fruit maturity. Five samples for each harvest.

| Weeks after pollination | Length of lower funicle (mm) | Length of upper funicle (mm) | Length of total funicle (mm) | Diameter of lower funicle (mm) | Diameter of upper funicle (mm) | Length of outer integument[1] (mm) | Length of inner integument[1] (mm) | Length of nucellus (mm) |
|---|---|---|---|---|---|---|---|---|
| Kerman | | | | | | | | |
| 1 | 2.19±0.179 | 1.67±0.151 | 3.87±0.323 | 1.53±0.165 | 0.57±0.051 | 410.0±25.701 | 446.7±17.641 | 430.0±19.135 |
| 2 | 3.73±0.267 | 3.00±0.368 | 6.72±0.634 | 2.22±0.207 | 0.90±0.114 | 445.7±19.440 | 434.3±12.125 | 402.9±23.140 |
| 4 | 5.07±0.519 | 4.12±0.471 | 9.20±0.827 | 4.10±0.203 | 1.27±0.145 | 542.5±43.330 | 480.0±17.332 | 380.0±10.710 |
| 8 | 7.59±0.454 | 6.87±0.636 | 12.99±0.748 | 4.63±0.588 | 2.04±0.225 | 715.7±108.420 | 681.4±126.60 | 597.1±14.400 |
| 16 | 10.28±0.812 | 8.57±0.415 | 18.19±1.377 | 6.41±0.446 | 2.62±0.169 | - | - | - |
| 20 | 10.77±0.322 | 9.22±0.108 | 19.99±0.426 | 7.60±0.413 | 3.09±0.221 | - | - | - |
| Sirora | | | | | | | | |
| 1 | 2.25±0.131 | 1.75±0.088 | 4.00±0.194 | 1.32±0.087 | 1.02±0.174 | 476.7±33.235 | 466.7±19.101 | 403.3±30.730 |
| 2 | 3.47±0.302 | 2.97±0.373 | 6.45±0.662 | 2.10±0.108 | 1.00±0.955 | 428.6±30.421 | 460.0±20.472 | 397.1±14.210 |
| 4 | 6.20±0.102 | 4.27±0.266 | 10.47±0.248 | 3.45±0.175 | 2.17±0.258 | 504.2±83.044 | 448.6±72.923 | 324.9±54.651 |
| 8 | 6.92±0.366 | 6.97±0.470 | 13.55±0.562 | 4.90±0.348 | 2.17±0.258 | 791.4±70.920 | 608.6±70.901 | 428.6±22.632 |
| 16 | 10.53±0.631 | 9.20±0.381 | 21.17±1.207 | 7.15±0.530 | 3.08±0.414 | - | - | - |
| 20 | 10.89±0.338 | 10.04±0.518 | 20.90±0.837 | 7.17±0.139 | 2.47±0.210 | - | - | - |

[1]both integuments measured from chalazal mid-point

- tissues not discernable

**Table 5.4** Length (mean ± standard error) of normal embryo sac and embryo of *Pistacia vera* cvs. Kerman and Sirora pollinated with cv. 15-12 at one day after flower opening and harvested up to fruit maturity.

| Weeks after pollination | Number of samples | Length of embryo sac | Length of embryo |
|---|---|---|---|
| | | µm | µm |
| Kerman | | | |
| 1 | 10 | 117.6±13.150 | 28.1±1.691 |
| 2 | 8 | 167.5±34.784 | 31.1±0.132 |
| 4 | 6 | 327.9±67.510 | 45.3±7.970 |
| | | mm | mm |
| 8 | 8 | 1.7±0.315 | 0.11±0.043 |
| 16 | 14 | 13.15±0.516 | 12.47±0.533 |
| 20 | 15 | 17.47±0.394 | 16.51±0.368 |
| | | µm | µm |
| Sirora | | | |
| 1 | 10 | 110.2±9.952 | 21.5±1.104 |
| 2 | 6 | 164.6±2.711 | 36.1±6.020 |
| 4 | 7 | 275.3±35.961 | 37.0±2.110 |
| | | mm | mm |
| 8 | 8 | 1.8±0.310 | 0.12±0.035 |
| 16 | 14 | 12.39±1.422 | 11.72±1.428 |
| 20 | 12 | 14.17±1.473 | 13.07±0.588 |

**Table 5.5** Normal embryo development of *Pistacia vera* cvs. Kerman and Sirora pollinated with cv. 15-12 at one day after flower opening and harvested up to fruit maturity.

| Weeks after pollination | Number of samples | Percent | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | embryo sac with fused polar nuclei | 1 cell embryo | 2 cell embryo | 4 cell embryo | <1mm globular embryo | 1-5 mm embryo | 5-15 mm embryo | >15 mm embryo |
| Kerman | | | | | | | | | |
| 1 | 16 | 18.75 | 6.25 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 16 | 31.25 | 12.50 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 14 | 0 | 14.28 | 7.14 | 7.14 | 0 | 0 | 0 | 0 |
| 8 | 16 | 0 | 0 | 0 | 0 | 43.75 | 0 | 0 | 0 |
| 16 | 20 | 0 | 0 | 0 | 0 | 0 | 35.00 | 25.00 | 10.00 |
| 20 | 20 | 0 | 0 | 0 | 0 | 0 | 15.00 | 10.00 | 50.00 |
| Sirora | | | | | | | | | |
| 1 | 17 | 23.56 | 5.89 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 16 | 25.00 | 12.50 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 16 | 0 | 12.50 | 18.75 | 6.25 | 0 | 0 | 0 | 0 |
| 8 | 16 | 0 | 0 | 0 | 0 | 50.00 | 0 | 0 | 0 |
| 16 | 21 | 0 | 0 | 0 | 0 | 0 | 35.00 | 20.00 | 15.00 |
| 20 | 17 | 0 | 0 | 0 | 0 | 0 | 11.76 | 11.76 | 41.17 |

**Table 5.6** Abnormal embryo sac development of *Pistacia vera* cvs. Kerman and Sirora pollinated with cv. 15-12 at one day after flower opening and harvested up to fruit maturity.

| Weeks after pollination | Number of samples | Percent ovules with | | |
|---|---|---|---|---|
| | | no embryo sac | unpenetrated embryo sac | degenerated embryo sac |
| Kerman | | | | |
| 1 | 16 | 18.75 | 18.75 | 6.25 |
| 2 | 16 | 12.50 | 0 | 12.50 |
| 4 | 14 | 14.28 | 0 | 35.70 |
| 8 | 16 | 18.75 | 0 | 0 |
| 16 | 20 | 0 | 0 | 0 |
| 20 | 20 | 0 | 0 | 0 |
| Sirora | | | | |
| 1 | 17 | 17.64 | 23.52 | 23.52 |
| 2 | 16 | 31.25 | 0 | 25.00 |
| 4 | 16 | 18.75 | 0 | 18.75 |
| 8 | 16 | 6.25 | 0 | 0 |
| 16 | 21 | 0 | 0 | 0 |
| 20 | 17 | 0 | 0 | 0 |

**Table 5.7** Degeneration in different parts of the abnormal ovule during fruit development in *Pistacia vera* cvs. Kerman and Sirora pollinated with pollen of cv. 15-12 at one day after flower opening and harvested up to fruit maturity.

| Date of harvest | Number of samples | Percent ovules with degeneration of | | | | | |
|---|---|---|---|---|---|---|---|
| | | site of pollen tube penetration | chalazal epidermis | vascular tissue | nucellus | endosperm | embryo |
| Kerman | | | | | | | |
| Days | | | | | | | |
| 0 | 8 | 50.0 | 50.0 | 0 | 0 | 0 | 0 |
| 1 | 8 | 75.0 | 25.0 | 0 | 0 | 0 | 0 |
| 2 | 5 | 100 | 0 | 0 | 0 | 0 | 0 |
| 3 | 8 | 50.0 | 50.0 | 0 | 0 | 0 | 0 |
| 4 | 8 | 50.0 | 50.0 | 0 | 0 | 0 | 0 |
| Weeks | | | | | | | |
| 1 | 8 | 0 | 25.0 | 0 | 75.0 | 0 | 0 |
| 2 | 8 | 0 | 25.0 | 0 | 75.0 | 0 | 0 |
| 4 | 6 | 0 | 0 | 33.3 | 66.7 | 0 | 0 |
| 8 | 6 | 0 | 0 | 16.7 | 66.7 | 16.7 | 0 |
| 16 | 6 | 0 | 0 | 33.3 | 16.7 | 16.7 | 33.3 |
| 20 | 5 | 0 | 0 | 40.0 | 0 | 20.0 | 40.0 |
| Sirora | | | | | | | |
| Days | | | | | | | |
| 0 | 6 | 66.6 | 33.4 | 0 | 0 | 0 | 0 |
| 1 | 5 | 0 | 100 | 0 | 0 | 0 | 0 |
| 2 | 5 | 0 | 100 | 0 | 0 | 0 | 0 |
| 3 | 5 | 100 | 0 | 0 | 0 | 0 | 0 |
| 4 | 8 | 25.0 | 75.0 | 0 | 0 | 0 | 0 |
| Weeks | | | | | | | |
| 1 | 5 | 0 | 0 | 0 | 100 | 0 | 0 |
| 2 | 5 | 0 | 0 | 0 | 100 | 0 | 0 |
| 4 | 8 | 0 | 0 | 0 | 75.0 | 25.0 | 0 |
| 8 | 8 | 0 | 0 | 25.0 | 75.0 | 0 | 0 |
| 16 | 6 | 0 | 0 | 33.3 | 33.3 | 16.7 | 16.7 |
| 20 | 6 | 0 | 0 | 33.3 | 16.7 | 16.7 | 33.3 |

## 5.4 Discussion

The majority of pollinated pistils of pistachio did not reach maturity and were either shed or resulted in seedless or small-seeded fruits. This is the first study to document in detail the normal development of the pistachio fruit and so to identify the stage which degeneration occurs. In normal pollinated ovules pericarp development took place up to week four, whereas proliferation of the embryo, endosperm and funicle occurred between weeks four and 16, with shell and hull development between weeks eight and 16. Previous authors have reported a longer period to the first division of the pistachio zygote of six weeks (Lin *et al.*, 1984c) and between four and 18 weeks (Grundwag, 1976). Division of the zygote and free nuclear endosperm development occurred after zygote shrinkage, and division of the endosperm nucleus preceded division of the embryo. The endosperm and pro-embryo grew quickly from four to six weeks after pollination when about one-third of the fruit cavity was filled by the large funicle. Endosperm wall formation started from the periphery of the embryo sac, first at the micropylar area, as in walnut (Tadeo *et al.*, 1994) and in pistachio it was observed near the globular embryo at 16 weeks after pollination.

Blank pistachio fruits contain only funicular and degenerated ovule tissue at maturity. The results suggest that the major causes of blanking are the lack of an embryo sac at anthesis (up to 31% of ovules lacked an embryo sac), embryo sac degeneration, failure of pollen tube penetration and funicle degeneration. Failure of endosperm cellularisation was a further, although relatively minor, cause of blanking. Embryo sac degeneration continued to four weeks after pollination and has also been reported in *Persea* (Sedgley, 1980; Tomer *et al.*, 1980), *Juglans* (Catlin and Polito, 1989) *Eucalyptus* (Sedgley and Griffin, 1989) and *Quercus* (Mogensen, 1975). The majority of degeneration in the pistachio, however, appears to be related to funicle degeneration. It commenced at the site of pollen tube penetration or at the chalazal end and spread towards the vascular bundles where it may have blocked sap flow to the

ovule. Penetration of the funicle by a pollen tube is relatively unusual amongst flowering plants (Shuraki and Sedgley, 1995), but degeneration of the chalazal tissue has been reported in *Persea* and *Prunus* in which damaged vascular bundles may have resulted in seed abortion (Pimienta and Polito, 1982; Tomer *et al.*, 1980; Steyn *et al.*, 1993). The reasons for these abnormalities are not clear, but may relate to competition for nutrients between flowers and developing fruits within the inflorescence.

Semi-blank small-seeded fruits in pistachio appear to be related to post-fertilization degeneration. Breakdown of tissue was observed in the growing cotyledon of the pistachio seed, and appeared to start from the vascular tissue or from the epidermis late in development. Similar spontaneous tissue degeneration has been observed in other plant tissues and results indicate that a deficiency or imbalance of nutrient elements such as calcium, potassium, boron or nitrogen are responsible (Goode and Ingram, 1971; Lewis and Martin, 1973; Shear, 1975). Lawson and Dienelt (1991) reported that vascular degeneration of the *Chrysanthemum* leaf was due to manganese toxicity which occurred in a limited range of soil pH. Thus it may be possible to control the semi-blanking problem via soil and water management. In addition spontaneous tissue degeneration in leaf, ovule and vascular bundles in many plants have been reported to be correlated with gene activity and environmental factors such as temperature (Hoisington *et al.*, 1982; Delong *et al.*, 1993; Mittler *et al.*, 1995).

This study has identified the anatomical causes of blank and semi-blank fruit in pistachio. Blank fruits results from funicle degeneration, embryo sac absence, degeneration or lack of penetration by a pollen tube or from endosperm failure. Semi-blank fruit results from cotyledon breakdown during the latter stages of fruit development. Of particular interest in the fact that abnormal pistachio fruits may not be shed, as occurs in other tree species, but may be retained on the tree as abnormal fruits to maturity.

**Figure 5.1** Normal fruit of cv. Kerman at maturity with large funicle (arrowhead) still present . Bar represents 1 cm.

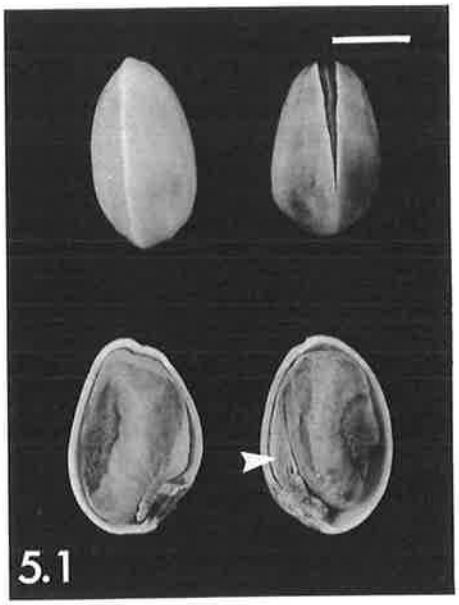
**Figure 5.2** Abnormal fruits of cv. Sirora with (centre) aborted seed (blank), and (left and right) a seed smaller than normal (semi-blank). Bar represents 1 cm.

**Figure 5.3** Normal four-cell pro-embryo (arrowhead) of cv. Kerman at four weeks after pollination with free endosperm nuclei (en). Bar represents 10 μm

**Figure 5.4** Normal eight-week ovule of cv. Kerman with prominent funicle (f), a globular embryo (arrowhead), free endosperm nuclei (en) and proliferation of integuments (i), nucellus (n) and chalaza (c). Bar represents 500 μm.

**Figure 5.5** Normal sixteen-week embryo (arrowhead) of cv. Sirora surrounded by cellular endosperm (en), nucellus (n) and integuments (i). Bar represents 50 μm.

**Figure 5.6** Normal sixteen-week embryo of cv. Sirora with small cotyledons (ct) and remnant endosperm (en) surrounded by a darkened testa (arrowhead). Bar represents 1 mm.



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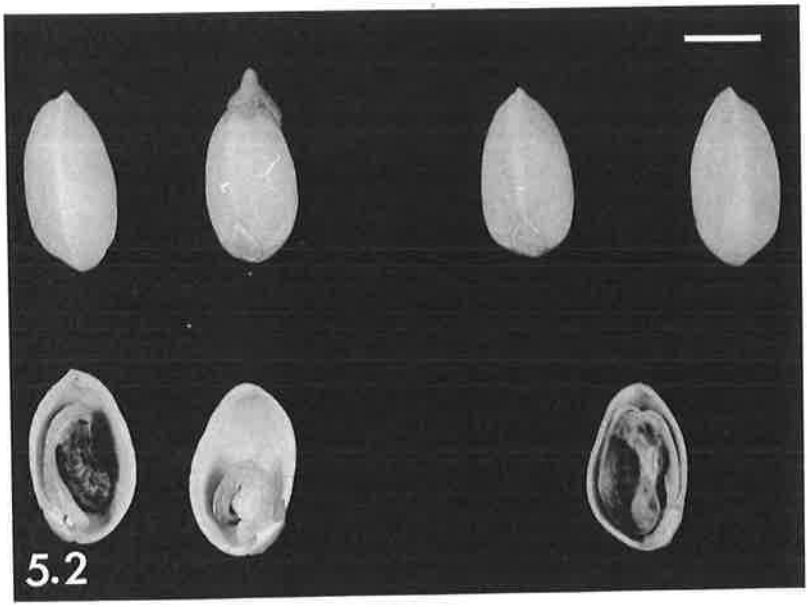

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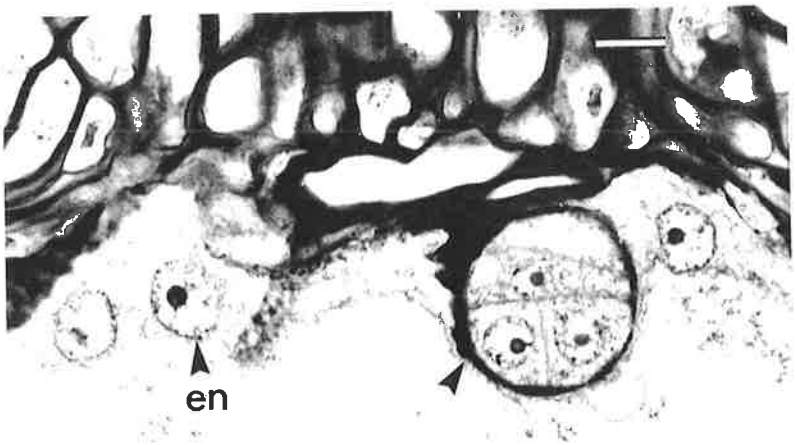


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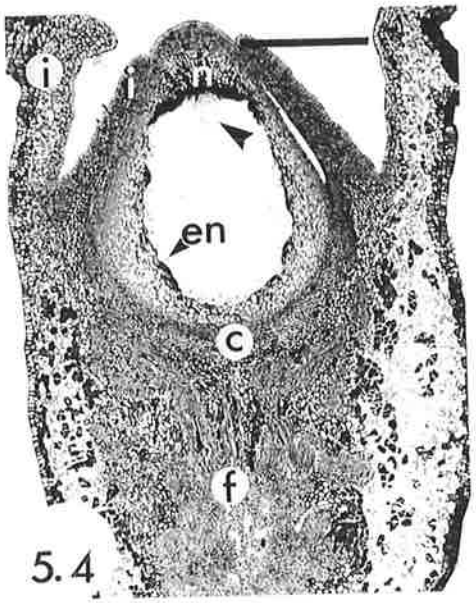


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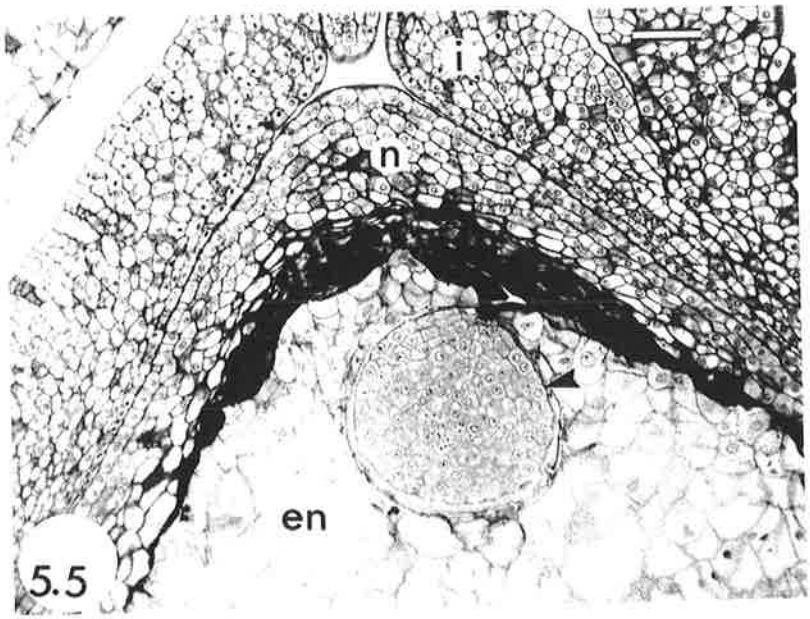


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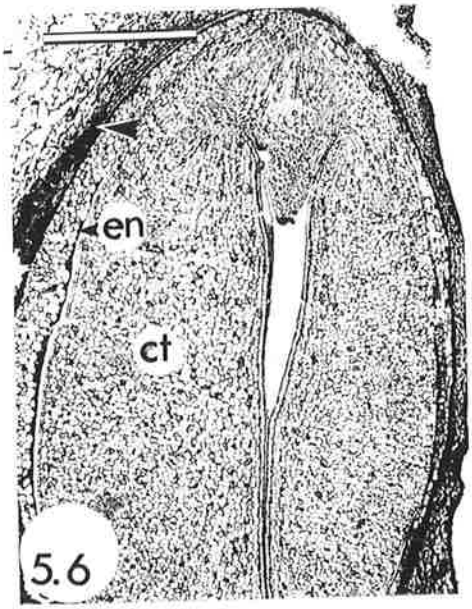



5.6

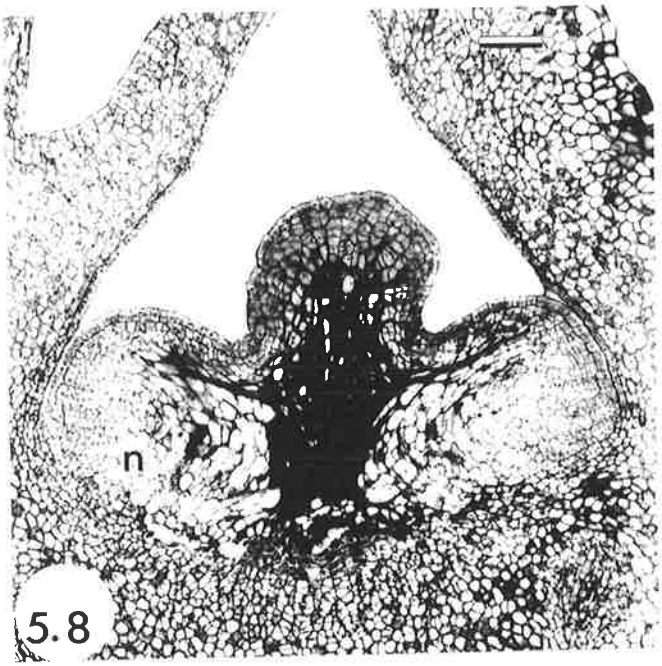
**Figure 5.7** Abnormal fruits of cv. Sirora showing hull degeneration. Bar represents 1 cm.

**Figure 5.8.** Eight-week old abnormal ovary of cv. Sirora showing degeneration of nucellar tissue (n). Bar represents 100 μm.

**Figure 5.9** One-day old abnormal ovary of cv. Sirora showing degeneration of the funicle (f) at the site of pollen tube penetration (d); nucellus (n). Bar represents 100 μm.

**Figure 5.10** Sixteen-week old abnormal ovary of cv. Sirora showing globular embryo (em), cellular endosperm (en) and degenerated nucellus (n) and vascular bundles (dv) at the chalazal (c) area. Bar represents 100 μm.

5.7

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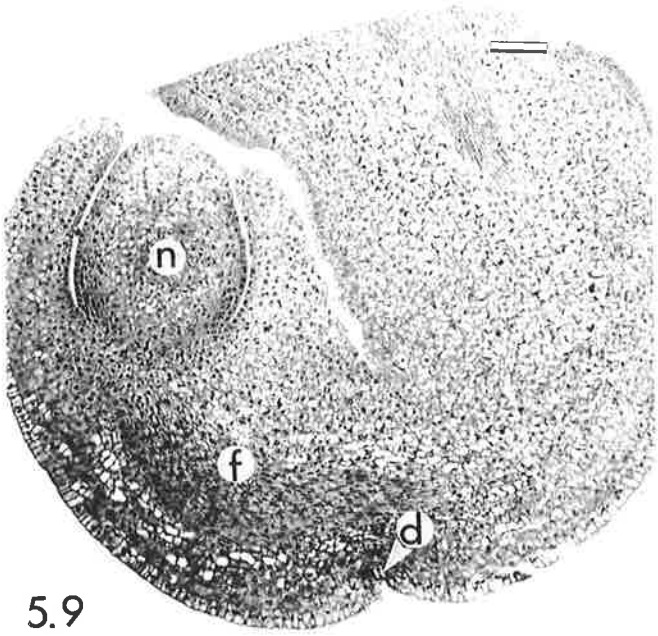


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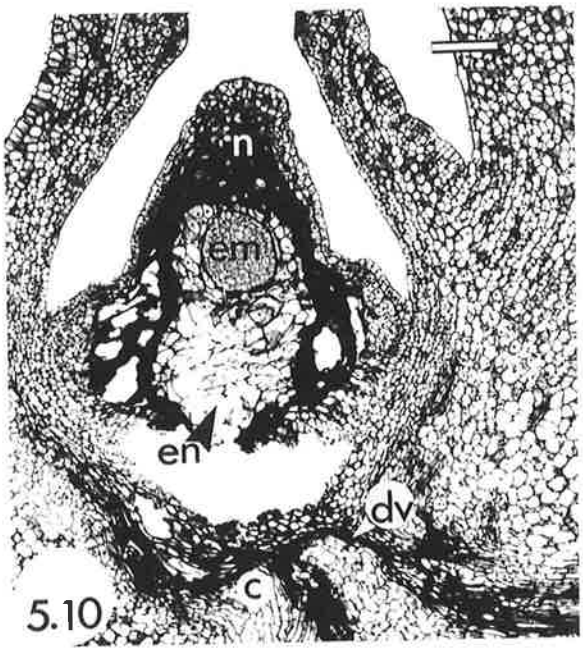



5.10

# Chapter 6

# 6 Embryo development and shell structure of *Pistacia vera* and *P. atlantica* following intra- and inter-specific pollination.

## 6.1 Introduction

The mature *Pistacia* fruit is a bilaterally symmetrical structure consisting of the embryo enclosed in the pericarp which comprises a woody endocarp and fleshy mesocarp and exocarp. The endocarp forms the shell, with a dorsal and ventral suture such that at maturity of the *P. vera* fruit spontaneous splitting occurs along the distal portion of both sutures. Shell splitting is an important economic attribute of *P. vera* (Crane and Iwakiri, 1982), but does not occur in any other species in the genus. Shells of the small seeded *P. atlantica* never split, and a proportion of the *P. vera* crop always fails to split, which is sometimes, but not always, related to the seedless (blank) or partially seeded (semi-blank) condition. Nevo *et al.* (1974) reported that shell indehiscence of *P. vera* was attributable to small seed size, with pressure of the mature full-sized seed causing rupture of the endocarp along the suture. An additional problem is that a small proportion of the crop may have a prematurely split shell and hull, often in areas other than the usual split zone. This detracts from the value of the crop as *Aspergillus* may enter the wound and cause aflatoxin problems (Sommer *et al.*, 1986; Pearson *et al.*, 1994).

There is little information on the structure of the *Pistacia* shell split zone. It has been reported that the dehiscence area in the dorsal and ventral zones has the same structure in both dehiscent and indehiscent fruits (Nevo *et al.*, 1974). Crane (1978) reported that shell splitting started prior to fruit ripening, and this was enhanced by ethylene application (Torabi, 1980). Spontaneous splitting prior to fruit maturity is a serious source of economic loss in many fruits other than pistachio. Sensitivity of

fruits to irregular splitting is related to a smooth cuticle structure which splits because of rapid extension of the hypodermal layer. This is often triggered by environmental factors such as high air temperature and humidity (Skene, 1980; Ohta *et al.*, 1991). This study aims to further our understanding of pistachio fruit maturity in relation to embryo development and shell dehiscence by comparing intra- and inter-specific pollination of *P. vera* and *P. atlantica.*

## 6.2 Materials and methods

### 6.2.1 Hand pollination

Experimental trees were situated in the Arboretum and Alverstoke collections of the Waite Agricultural Research Institute of the University of Adelaide, South Australia. In 1994, eight buds on five branches on each of two trees of *P. vera* cvs. Kerman and Sirora and *P. atlantica* were bagged before budburst. Each bud had about 1000 pistils, which were pollinated either with one year stored pollen from *P. vera* cv. 15-12, or with fresh pollen of *P. atlantica* . Hand pollination was conducted one day after flower opening and flowers not at the correct stage were removed. Stored pollen of cv. 15-12 was used as *P. atlantica* flowers before *P. vera* in Adelaide. Its *in vitro* germinability was 35%, as compared to 81% germinability of the fresh *P. atlantica* pollen.

### 6.2.2 Microscopy

At least five pistils were harvested after 2 and 4 days, and at 1, 2, 4, 8, 16 and 20 weeks (fruit maturity) after pollination. Ovary walls and ovules were fixed in FPA 50 (formaldehyde: propionic acid: 50% ethanol 5: 5: 90) for 24 hours at 4°C. The pistils were dehydrated via an alcohol series and embedded in glycol methacrylate in gelatine capsules for two days at 60°C (Feder and O'Brien, 1968). Longitudinal 4 µm sections of ovules were stained with periodic acid-Schiff's reagent (PAS) and toluidine blue O (TBO). Ovule measurements were made using a micrometer eyepiece. All 16 and 20 week samples were full seeded. Woody shells were soaked

in water at 60°C for 24 hours and then placed in 1M NaOH and stored for one week at 60°C, with the NaOH changed daily. Shells were sampled at four locations, the proximal and distal portions of the dorsal and ventral sutures (Figure 6.1). Ventral and dorsal proximal samples were taken at one quarter of the distance along the ventral or dorsal suture from the insertion of the funicle to the distal join of the ventral and dorsal surfaces. Ventral and dorsal distal samples were taken at three quarters of the distance along the ventral or dorsal suture from the insertion of the funicle to the distal join of the ventral and dorsal surfaces. In all cases the dimension of the tissue sampled was 1 mm along the suture and 1 mm along both surfaces at right angles to the suture. Longitudinal 4 μm sections were taken at right angles to the suture, stained with TBO and observed under bright field or differential interference contrast (Nomarski) optics. Tissue thickness at the suture join at 4, 8, 16 and 20 weeks was measured using a micrometer eyepiece, and the angles of the join at the sutures were measured using computer assisted video images and a protractor. Data were analysed using ANOVA.

## 6.3 Results

### 6.3.1 Fruit set

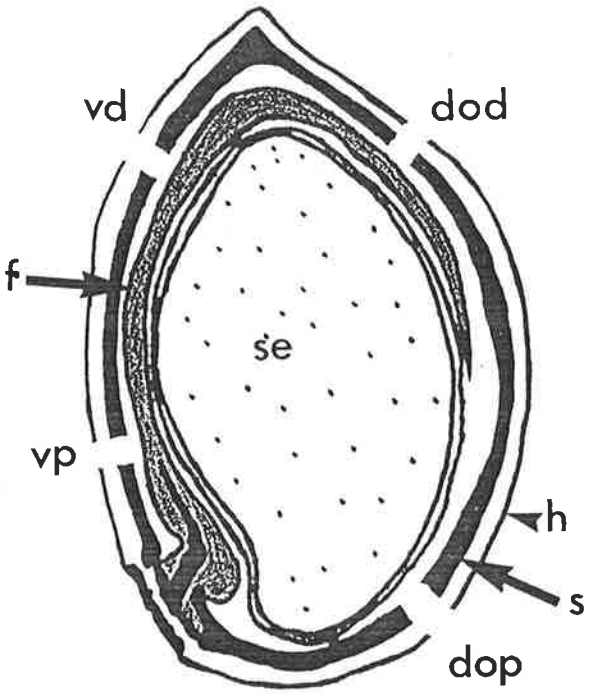
Highest fruit set followed intra-specific pollination (Table 6.1) with partial compatibility between *P. vera* and *P. atlantica* (Table 6.1, Figure 6.2) Over 65% of Sirora and Kerman fruits pollinated with 15-12 had full seeds and split shells, with lower proportions following inter-specific pollination. In *P. vera* failure of shell splitting and premature shell splitting were more common following inter-specific pollination (Figure 6.3). Shells of *P. atlantica* did not split even following inter-specific pollination. Fruit dimensions of Kerman following inter-specific pollination were lower than following intra-specific pollination, but there was no difference for Sirora or *P. atlantica.*

**Table 6.1** Mature fruit set of *Pistacia vera* cvs. Sirora and Kerman and *P. atlantica* following intra- and inter-specific pollination with *P.vera* cv. 15-12 and *P. atlantica*. Different letters within columns indicate a significant difference (P = 0.05).

| Cross | Fruit retained at harvest (%) | Percentage of mature fruits which were* | | | | | | Average fruit length at harvest (mm) | Average fruit diameter at harvest (mm) |
|---|---|---|---|---|---|---|---|---|---|
| | | full seed split shell | full seed premature shell split | full seed non-split shell | small seed non-split shell | no seed non-split shell | total non-split shell | | |
| Sirora x 15-12 | 29.1 | 68.4 | 3.5 | 14.8 | 7.5 | 5.8 | 28.1 | 23.6 [a] | 15.5 [a] |
| Sirora x *P. atlantica* | 14.5 | 4.91 | 25.6 | 33.9 | 25.6 | 30.5 | 90.0 | 20.3 [ab] | 14.5 [a] |
| Kerman x 15-12 | 25.4 | 65.5 | 4.3 | 8.7 | 13.5 | 8.0 | 30.2 | 22.0 [a] | 15.4 [a] |
| Kerman x *P. atlantica* | 3.1 | 23.5 | 5.8 | 5.8 | 17.6 | 64.7 | 88.1 | 19.3 [b] | 13.6 [b] |
| *P. atlantica* x 15-12 | 11.7 | 0 | 0 | 37.5 | 2.8 | 59.7 | 100 | 8.6 [c] | 6.6 [c] |
| *P. atlantica* x *P. atlantica* | 18.2 | 0 | 0 | 24.3 | 1.9 | 73.8 | 100 | 8.2 [c] | 6.3 [c] |

*Some fruits appear in more than one group

**Figure 6.1** Longitudinal diagram of the mature *Pistacia* fruit showing the shell (s), hull (h), seed (se) and funicle (f). Gaps represent the the points of sampling of the dorsal proximal (dop), dorsal distal (dod), ventral proximal (vp) and ventral distal (vd) hull and shell sutures. Bar represents 5 mm.




6.1

**Figure 6.2** Fruit set of *P. vera* cv. Kerman four weeks after pollination with *P. vera* cv. 15-12 (top) and *P. atlantica* (bottom). Bar represents 1 cm.

6.2

**Figure 6.3** Premature shell splitting of *P. vera* cv. Sirora at 16 weeks after pollination with *P. atlantica*. Fruits are smaller than normal and some seeds are infected. Bar represents 1 cm.

6.3

### 6.3.2 Ovule development

Ovule development following intra-specific pollination was similar for the two species, except that ponticulus development differed in *P. atlantica* x *P. atlantica* from the other crosses. At two days after intra-specific pollination, a protuberance had developed from the *P. atlantica* funicle (Figure 6.4). This connected with a corresponding invagination at the base of the style at the junction with the ovary wall (Figure 6.5), to form a bridge or ponticulus along which the pollen tube grew (Figure 6.6). In some samples, up to five protuberances were seen on the funicle at the site of pollen tube penetration, but only one of them joined with the ovary wall. Pollen tubes either passed the free space between the ovary and funicle and penetrated the funicle ponticulus or passed directly through the ponticulus from the ovary wall. The ponticulus of *P. atlantica* continued to grow after intra-specific pollination (Table 6.2, Figure 6.7). Following inter-specific pollination of *P. atlantica*, ponticulus development was similar to that of *P. vera.*

Embryo sac length tended to be greater following intra- than inter-specific pollination of *P. vera* although there was no difference in nucellus length at 8 weeks after pollination (Table 6.2). There was no difference in embryo sac or nucellus length of *P. atlantica* following intra- or inter-specific pollination at 8 weeks after pollination. A higher proportion of *P. atlantica* than *P. vera* ovules lacked an embryo sac at anthesis and more *P. vera* ovules lacked an embryo sac following inter-specific than intra-specific pollination (Table 6.3). Other causes of fruit failure were embryo sac degeneration and degeneration of the embryo and endosperm. The latter phenomenon was particularly high in Kerman pollinated with *P. atlantica.*

### 6.3.3 Ovary wall and shell development

The ovary wall and shell thickness at both the dorsal and ventral sutures of *P. vera* were lower following inter- than intra-specific pollination, but there was no

**Table 6.2** Length of ponticulus, nucellus and embryo sac of *Pistacia vera* cvs.Sirora and Kerman and *P. atlantica* following intra- and inter-specific pollination with *P. vera* cv. 15-12 and *P. atlantica* up to 8 weeks after pollination.

| Cross | Mean length (± standard error) of | | |
|---|---|---|---|
| | funicle ponticulus (µm) | nucellus (µm) | embryo sac (µm) |
| Sirora x 15-12 | 17.8 ± 5.1 | 389.2 ± 41.1 | 273.9 ± 41.8 |
| Sirora x *P. atlantica* | 20.1 ± 1.6 | 440.6 ± 68.1 | 188.4 ± 76.9 |
| Kerman x 15-12 | 19.4 ± 4.7 | 481.4 ± 54.7 | 293.3 ± 45.0 |
| Kerman x *P. atlantica* | 27.1 ± 5.3 | 434.7 ± 40.7 | 187.3 ± 67.0 |
| *P. atlantica* x 15-12 | 25.8 ± 4.9 | 388.4 ± 29.3 | 105.4 ± 30.4 |
| *P. atlantica* x *P. atlantica* | 149.7 ± 70.6 | 386.8 ± 33.1 | 79.7 ± 21.5 |

**Table 6.3** Causes of fruit degeneration of *Pistacia vera* cvs. Sirora and Kerman and *P. atlantica* up to 16 weeks after intra- and inter-specific pollination with *P. vera* cv. 15-12 and *P. atlantica*.

| Cross | Number (%) of ovules: | | | | Total observed |
|---|---|---|---|---|---|
| | normal | no embryo sac | degenerated embryo sac | degenerated embryo and endosperm | |
| Sirora x 15-12 | 27 (79) | 1 (3) | 2 (6) | 4 (12) | 34 |
| Sirora x *P. atlantica* | 25 (73) | 6 (18) | 1 (3) | 2 (6) | 34 |
| Kerman x 15-12 | 26 (76) | 2 (6) | 2 (6) | 4 (12) | 34 |
| Kerman x *P. atlantica* | 18 (43) | 5 (12) | 2 (5) | 17 (40) | 42 |
| *P. atlantica* x 15-12 | 15 (46) | 14 (42) | 2 (6) | 2 (6) | 33 |
| *P. atlantica* x *P. atlantica* | 12 (31) | 16 (41) | 5 (13) | 6 (15) | 39 |

consistent effect of pollination on *P. atlantica* ovary wall and shell thickness (Table 6.4). In *P. vera*, both the cross and time of harvest had significant effects on ovary wall and shell thickness, with few significant interactions (Table 6.5). In *P. atlantica*, only time of harvest was a consistently significant factor in ovary wall and shell thickness.

Endocarp structure of all sutures of all crosses was similar up to 8 weeks after pollination, with irregularly-shaped cells with interlocking projections and thick walls (Figures 6.8 - 6.12). At 8 weeks the ventral distal suture angle of *P. vera* pollinated with *P. vera* was narrow (Figure 6.8) in contrast to the wide angle of *P. atlantica* following intra-specific pollination (Figure 6.9). The inner epidermis of the *P. vera* endocarp had developed thickened walls by 16 weeks, except at the suture join (Figure 6.10). Splitting of the shell commenced from the inner epidermis by separation of the thin walled cells (Figure 6.11) and progressed by separation of the cells of subsequent layers. Splitting commenced at the mid points of the ventral and dorsal distal sutures and extended in both directions, with complete separation of the distal portions but only partial separation of the proximal areas. In some fruits more than one groove was present in areas other than the suture (Figure 6.12) and this was observed to result in irregular splitting.

The angle of the ventral distal suture was significantly higher following inter-specific than following intra-specific pollination of both *P. vera* cultivars Sirora and Kerman (Table 6.6). Conversely, the angle of the ventral distal suture of *P. atlantica* was lower following inter-specific than following intra-specific pollination. Other suture angles were more variable, although inter-specific pollination increased the dorsal proximal angle of Kerman and reduced the ventral proximal angle of *P. atlantica*. Analysis of the data showed a significant effect of the cross on all angles except that of the ventral proximal suture, with time of harvest significant only for the distal shell angles (Table 6.7). Interactions of cross and time of harvest were significant for all suture angles.

**Table 6.4** Ovary wall and shell thickness at the suture of *Pistacia vera* cvs. Kerman and Sirora and *P. atlantica* at maturity following intra- and inter-specific pollination with *P. vera* cv. 15-12 and *P. atlantica*. Differences between proximal and distal portions were not significant so results were pooled. Different letters within columns indicate a significant difference (P = 0.05).

| Weeks after pollination | Ovary wall thickness (mm) | | Shell thickness (mm) | |
|---|---|---|---|---|
| | dorsal suture | ventral suture | dorsal suture | ventral suture |
| Sirora x 15-12 | 1.67[b] | 1.61[b] | 0.94[b] | 0.97[b] |
| Sirora x *P. atlantica* | 1.45[a] | 1.42[a] | 0.66[a] | 0.76[a] |
| Kerman x 15-12 | 1.60[b] | 1.62[b] | 0.92[b] | 0.97[b] |
| Kerman x *P. atlantica* | 1.44[a] | 1.39[a] | 0.71[a] | 0.78[a] |
| *P. atlantica* x 15-12 | 0.72[a] | 0.82[b] | 0.44[b] | 0.42[a] |
| *P. atlantica* x *P. atlantica* | 0.79[b] | 0.74[a] | 0.42[a] | 0.41[a] |

**Table 6.5** Data analysis of ovary wall and shell thickness at the suture of *Pistacia vera* cvs. Kerman and Sirora and *P. atlantica* following intra- and inter-specific pollination with *P. vera* cv. 15-12 and *P. atlantica* during fruit maturation.

| Source of variance | Probability | | | |
|---|---|---|---|---|
| | Ovary wall thickness | | Shell thickness | |
| | dorsal suture | ventral suture | dorsal suture | ventral suture |
| *Pistacia vera* | | | | |
| 1. Cross | <0.001 | <0.001 | <0.001 | ns |
| 2. Time of harvest | <0.001 | <0.001 | <0.05 | <0.001 |
| 3. Interaction 1 . 2 | ns | <0.05 | ns | ns |
| *Pistacia atlantica* | | | | |
| 1. Cross | ns | ns | ns | ns |
| 2. Time of harvest | <0.001 | <0.001 | <0.001 | <0.001 |
| 3. Interaction 1 . 2 | ns | ns | <0.05 | ns |

**Table 6.6** Angles at the proximal and distal portion of the dorsal and ventral sutures of the mature shell after intra- and inter-specific pollination of *P. vera* cvs. Sirora and Kerman and *P. atlantica* with *P. vera* cv. 15-12 and *P. atlantica*. Different letters within columns indicate a significant difference (P = 0.05).

| Cross | Mean angle of shell in mature fruits at: | | | |
|---|---|---|---|---|
| | dorsal suture | | ventral suture | |
| | proximal | distal | proximal | distal |
| Sirora x 15-12 | 107.0$^{ab}$ | 67.7$^{a}$ | 73.2$^{a}$ | 41.1$^{a}$ |
| Sirora x *P. atlantica* | 116.5$^{bc}$ | 69.3$^{ab}$ | 87.3$^{ab}$ | 69.8$^{b}$ |
| Kerman x 15-12 | 99.3$^{a}$ | 67.8$^{a}$ | 68.1$^{a}$ | 39.3$^{a}$ |
| Kerman x *P. atlantica* | 126.1$^{cd}$ | 89.6$^{abc}$ | 92.4$^{ab}$ | 86.3$^{b}$ |
| *P. atlantica* x 15-12 | 111.9$^{abc}$ | 95.4$^{bc}$ | 77.8$^{a}$ | 83.0$^{b}$ |
| *P. atlantica* x *P. atlantica* | 139.8$^{d}$ | 105.6$^{c}$ | 114.1$^{b}$ | 117.6$^{c}$ |

**Table 6.7** Data analysis of angles from the proximal and distal portions of the dorsal and ventral sutures of the shell after intra- and inter-specific pollination of *P. vera* cvs. Sirora and Kerman and *P. atlantica* with *P. vera* cv. 15-12 and *P. atlantica* during fruit maturation.

| Source of variance | Probability | | | |
|---|---|---|---|---|
| | dorsal suture | | ventral suture | |
| | proximal angle | distal angle | proximal angle | distal angle |
| 1. Cross | <0.001 | <0.05 | ns | <0.001 |
| 2. Time of harvest | ns | <0.001 | ns | 0.05 |
| 3. Interaction 1. 2 | <0.05 | <0.001 | <0.05 | <0.001 |

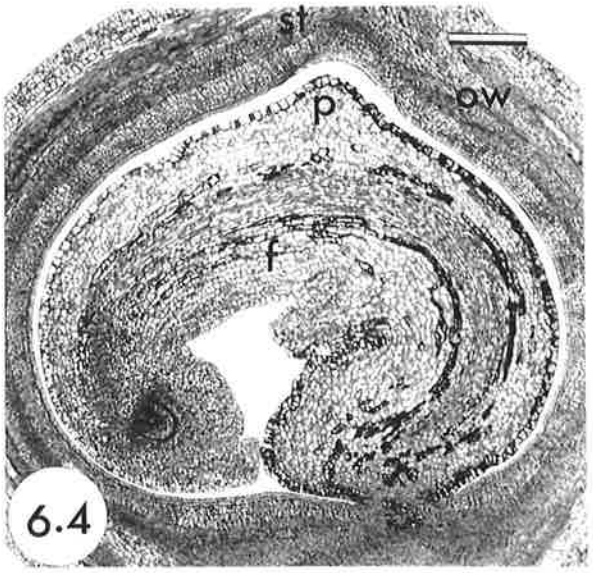
**Figures 6.4 - 6.7** Longitudinal sections of *P. atlantica* ovary pollinated with *P. atlantica* pollen stained with PAS and TBO and photographed using bright field optics.

**Figure 6.4** Ovary 2 days after pollination showing a ponticulus (p) of the funicle (f) and invagination at the base of the style (st) at the junction with the ovary wall (ow). Bar represents 50 μm.

**Figure 6.5** Funicle ponticulus (p) in contact (arrow) with the ovary wall (ow) 2 days after pollination. Bar represents 20 μm.

**Figure 6.6** Pollen tube (pt) penetrating the funicle ponticulus (p) 2 days after pollination. Bar represents 20 μm.

**Figure 6.7** Proliferated ponticulus (p) of the funicle (f) 4 weeks after pollination. Bar represents 20 μm.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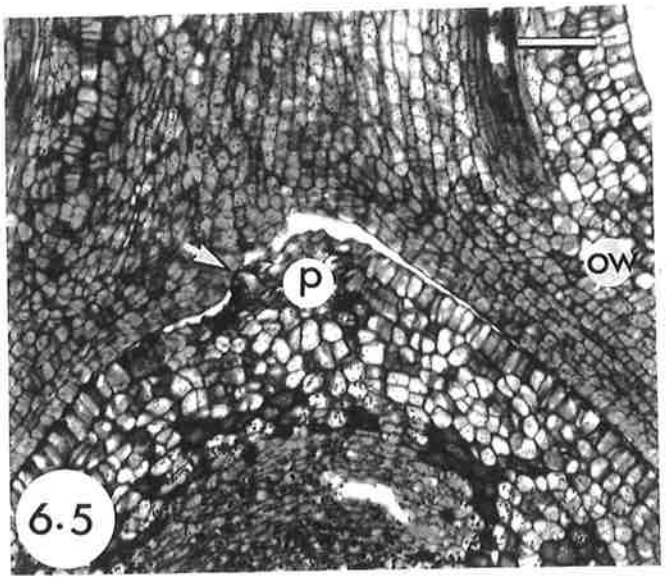
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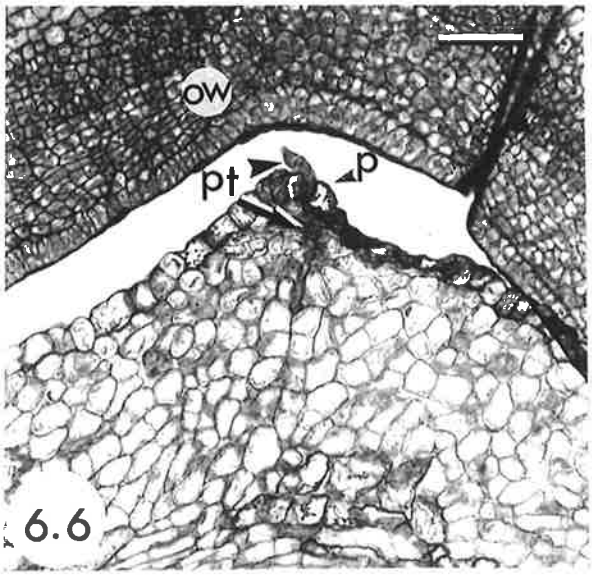
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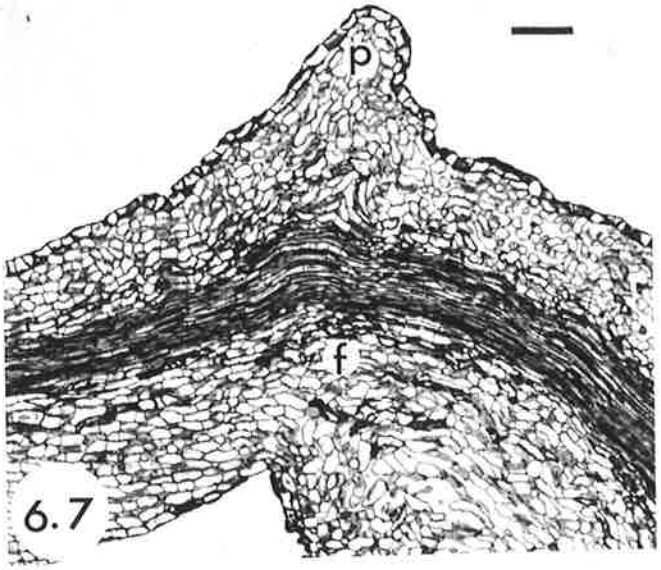
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## 6.4 Discussion

This study has shown that shell thickness and suture angle may be significant factors in the splitting of *Pistacia vera* fruit at maturity. Both ovary wall and shell thickness at the suture were lower following inter-specific pollination, indicating that the thinner suture character of *P. atlantica* was imparted to the *P. vera* ovary wall tissue during fruit development. Similarly, the higher ventral distal shell suture angle of *P. atlantica* appeared to be imposed on the inter-specific pollinated *P. vera* fruit. Previous reports of metaxenia effects in pistachio (Peebles and Hope, 1937; Whitehouse *et al.*, 1964) have been questioned (Crane and Iwakiri, 1980), as it is difficult to perceive how a hybrid seed can impose its genetic character on the maternal tissue of the ovary. Nevertheless, the data reported here suggest that such an effect is occurring, perhaps as proposed previously (Swingle, 1928) via plant growth regulators produced by the hybrid embryo and endosperm.

Nevo *et al.* (1974) concluded that small seed size and not shell characteristics were the cause of indehiscence of *Pistacia vera* fruits. The findings reported here do not contradict this work, as Kerman had a smaller fruit following inter-specific pollination, and the embryo sac of both *P. vera* cultivars also tended to be smaller. This study also supports previous observations on endocarp structure, although Nevo *et al.* (1974) did not find a difference in shell thickness with pollen parent. The lack of clearly-defined dehiscence zone is in contrast to the situation in some other species, such as almond (*Prunus dulcis*) (Weis and Polito, 1990). The narrow shell suture angle of *P. vera* was also observed by Nevo *et al.* (1974), but was not measured.

This study is the first to investigate the anatomical basis of the premature fruit splitting problem in *Pistacia vera.* The hull and shell of fruits close to harvest splits prematurely, often in areas other than the usual split zone. This leaves an open wound which may become infected and downgrade the value of the crop (Sommer *et al.*,

1986). The incidence of the problem varies, but accounted for 3.5% of Sirora and 4.3% of Kerman pollinated with 15-12. Of particular concern is the premature splitting incidence of 23.5% in Sirora and 5.8% in Kerman pollinated with *P. atlantica* pollen. Observations reported here indicate that this phenomenon is related to the development of split zones in areas other than the morphological suture between the two valves of the shell. These other areas do not seal the separation zone and so may allow access of disease causing micro-organisms. The phenomenon is quite different from that of premature splitting of many fleshy fruits which occurs in humid conditions following expansion of hypodermal cell layers with subsequent splitting of the cuticle (Skene, 1980; Ohta *et al.*, 1991). In *Pistacia vera*, splitting commences from the inner epidermis of the endocarp, even in those fruits showing abnormal premature splitting.

The difference in ponticulus structure between *Pistacia vera* and *P. atlantica* may be one cause of the observed reduction in inter-specific as compared with intra-specific fertility. In *P. vera* the ponticulus develops from both the ovary wall and from the funicle (Chapter 4), whereas only the funicle produced the ponticulus in *P. atlantica*. A similar structure has also been described in *Mangifera indica* which also belongs to family Anacardiaceae (Joel and Eisenstein, 1980). The reason for continued proliferation of the *P. atlantica* ponticulus following pollination is not clear, and it is noteworthy that this proliferation did not occur following inter-specific pollination. Intra-specific pollination has been shown to stimulate embryo sac maturation in *P. vera* (Chapter 4), and the diminished stimulation observed here following inter-specific pollination may be another possible cause of reduced inter-specific fertility. Under the conditions of these experiments, *P. atlantica* had both lower fruit set and a higher proportion of blank fruits than *P. vera*, and this may be related to the higher incidence of ovules lacking an embryo sac in *P. atlantica*. Embryo sac degeneration and embryo and endosperm failure are also common causes of fruit abortion in other genera such as *Persea* (Sedgley, 1980) and *Quercus* (Mogensen, 1975).

**Figures 6.8 -6.12** Ventral distal shell sutures of *Pistacia* stained with TBO.

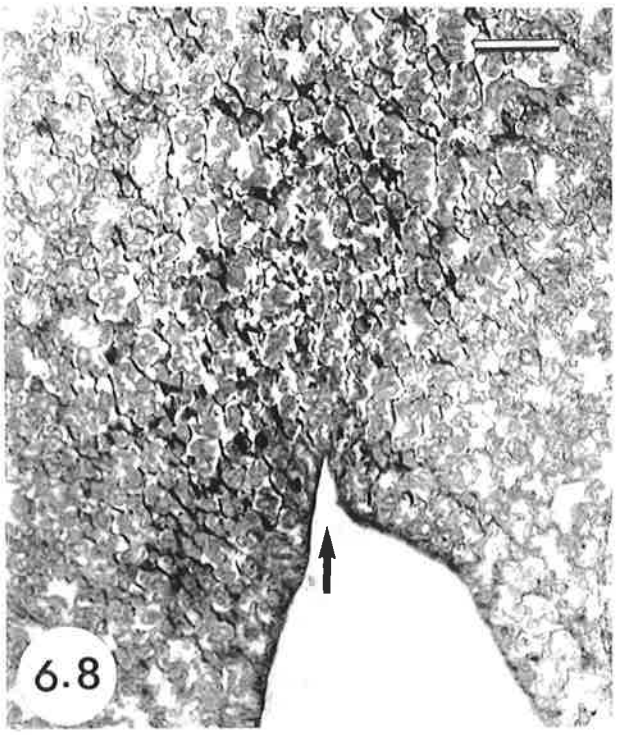
**Figure 6.8** *Pistacia vera* cv. Kerman 8 weeks after pollination with *P. vera* cv. 15-12, photographed using Nomarski optics. Note the narrow suture angle (arrow) and absence of a clearly-defined dehiscence zone. Bar represents 20 μm.

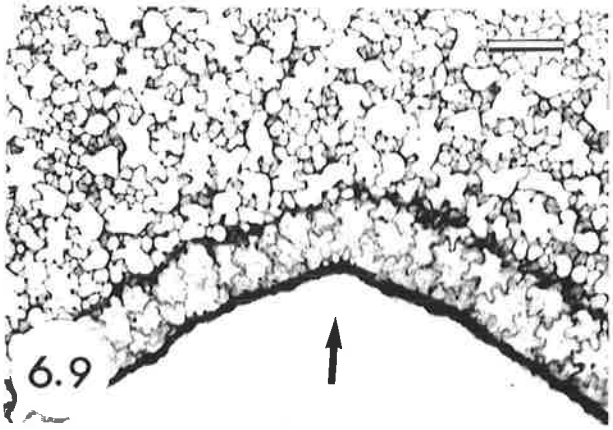
**Figure 6.9** *Pistacia atlantica* 8 weeks after pollination with *P. atlantica* photographed using bright field optics. Note the wide suture angle (arrow). Bar represents 100 μm.

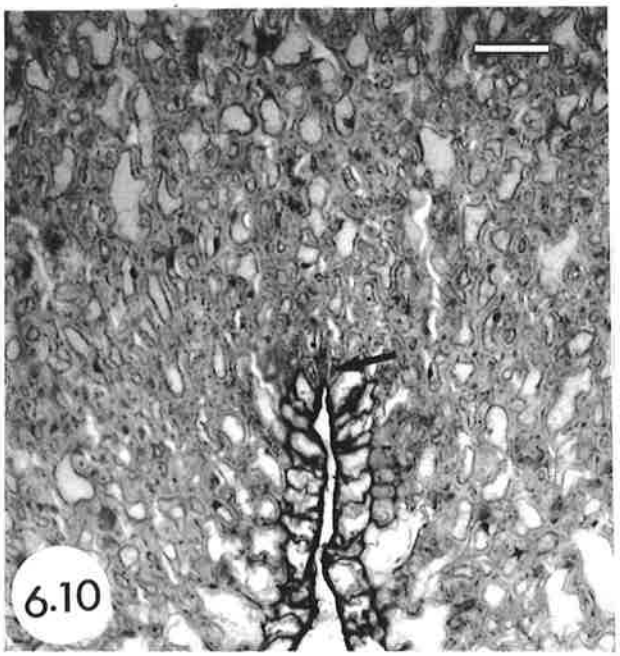
**Figure 6.10** *Pistacia vera* cv. Sirora 16 weeks after pollination with *P. vera* cv. 15-12 photographed using Nomarski optics. Note that the thick walls of the inner epidermis are absent at the point of splitting (arrow). Bar represents 20 μm.

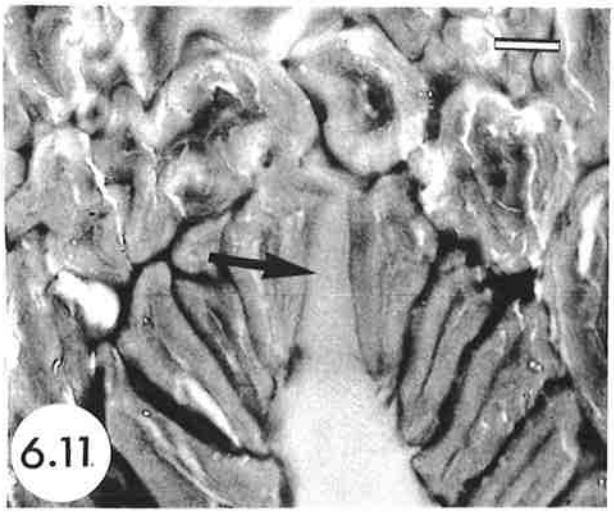
**Figure 6.11** *Pistacia vera* cv. Sirora pollinated with *P. vera* cv. 15-12 at maturity photographed using Nomarski optics. Note that splitting has commenced from the inner surface (arrow). Bar represents 20 μm.

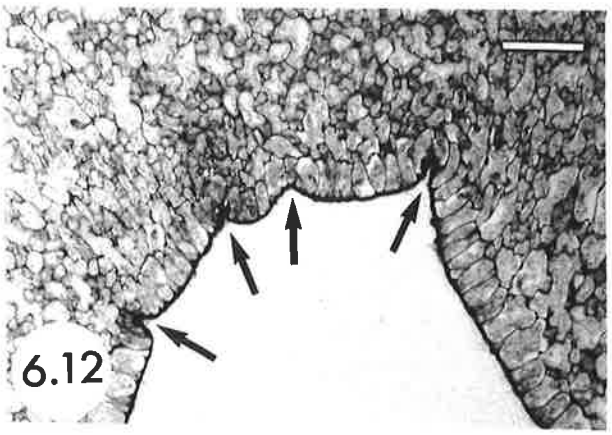
**Figure 6.12** *Pistacia vera* cv. Kerman 16 weeks after pollination with *P. vera* cv. 15-12 photographed using bright field optics. Four grooves are visible (arrows), which may result in premature abnormal splitting. Bar represents 20 μm.


6.8


6.9


6.10


6.11


6.12

It should also be noted that although the stored *P. vera* pollen had an *in vitro* germinability of only 35%, it gave fruit set comparable with that obtained in other experiments using fresh pollen. This indicates that pollen stored for one year can be used with confidence for experimental work and, if required, for pollen supplementation.

This work has shown that the genotype of the male parent can influence important quality characteristics of pistachio, including shell splitting at maturity, premature shell splitting and the production of blank fruits. It raises the question that more attention should be paid to quality aspects of *P. vera* male cultivars. Currently the major considerations are whether or not the male cultivar flowers concurrently with the female and produces large amounts of pollen. In future, further attention should be paid to potential effects of the pollen parent on fruit yield and quality.

# Chapter 7

## 7 General Discussion

Pistachio (*Pistacia vera* L.) production problems include blanking, semi-blanking, non-splitting and premature splitting. This study has shown that the period of stigma receptivity and thus the successful pollination period of pistachio cultivars is extremely short, with maximum economic fruit set from pistils pollinated at one and two days after flower opening (Shuraki and Sedgley, 1994). The majority of three and four day old pistils of pistachio hardly supported pollen germination and tube growth. In old pistils pollen tubes were arrested in the style, or in the chalazal and nucellar tissue and this resulted in less fruit set and more blank, semi-blank or mis-shapen fruits. In addition, lack of pistil receptivity in freshly opened pistils resulted in low fruit set. The pollination period in some other plants such as cherry and kiwifruit is also short (Stösser and Anvari, 1982; Gonzalez *et al.*, 1995), and in plants such as *Persea americana* and *Rubus bellandii*, low fruit production in relation to limited pistil receptivity has been reported (Sedgley, 1977b; Czapik, 1992).

In this study the pollen tube pathway in pistachio was documented precisely. The pollen grain germinated on the stigma surface and penetrated the stigma and style transmitting tissues. Then tubes passed either a gap between the ovary wall and funicle or entered a ponticulus (bridge). In the *P. vera* cultivars Kerman and Sirora the ponticulus formed from projections of tissue from both the ovary wall and funicle whereas in *P. atlantica*, a larger projection from the funicle interlocked with an ovarian invagination to make the ponticulus. This report of a ponticulus in *P. vera* has recently been supported by Martínez-Pallé and Herrero (1995). In mango (*Mangifera indica*: Anacardiaceae), the pollen tubes also travel within a ponticulus (Joel and Eisenstein, 1980), and pollination enhanced its development and completion (de Wet *et al.*, 1986). In *Pistacia vera* this was not seen, but a *P. atlantica* pistil pollinated with

*P. atlantica* pollen produced a larger ponticulus than a pistil pollinated with *P. vera* cv. 15-12. Some tubes passed through the longer route of ovary wall transmitting tissue, but none of them penetrated the embryo sac and all tubes were arrested at the chalazal or nucellar tissue. An ovarian route has also been reported in *Grevillea banksii*, with abnormal tubes which did not fertilise the ovule (Martin and Herscovitch, 1989). Thus it was found that pollen tubes penetrated receptive pistils via an unusual pathway. They failed to penetrate and fertilise old and immature pistils efficiently, and this increased the proportion of abnormal fruits.

Pollination enhanced the development and maturity of the *Pistacia vera* embryo sac. Only 50% of unpollinated ovules had mature embryo sacs at four days after anthesis, whereas all pistils pollinated with *P. vera* pollen had a mature embryo sac, which indicates a pollination effect on embryo sac development. Pollination of *P. vera* with *P. atlantica* pollen did not stimulate embryo sac development to the same extent as intra-specific pollination. A positive influence of compatible pollination on embryo sac development has been reported in almond (Pimienta and Polito 1983) and in pear (Herrero and Gascon 1987). Ovules without embryo sacs were more common in *P. atlantica* than in *P. vera* and may account for the lower fruit set.

Final fruit set in pistachio was generally low, with maximum production of 29.1% set recorded on Sirora pollinated with *P. vera* cv. 15-12 in 1995. Low fruit set can be attributed to both pre-fertilisation and early post-fertilisation degeneration (Stephenson, 1981; Sedgley, 1994). Pollen tube competition in the pollen tube pathway, and the long distance between stigma and embryo sac are possible causes for failure of fertilisation and early embryo development of pistachio, as has been reported in some other plants (Bassiri *et al.*, 1987; McGranahan *et al.*, 1994).

In this study the growth periods of normal and abnormal fruits of pistachio were investigated. In normal ovules pericarp development took place up to week four, and maximum embryo, endosperm and funicle growth occurred up to 16 weeks after pollination. The results suggest that the major causes of blanking are the lack of an

embryo sac at anthesis, degenerated embryo sac, failure of pollen tube penetration and funicle degeneration. Embryo sac degeneration continued to four weeks after pollination and has also been reported in *Persea americana* (Sedgley, 1980; Tomer *et al.*, 1980), in *Juglans regia* (Catlin and Polito, 1989) and in *Quercus gambelii* (Mogensen, 1975).

Semi-blank fruit contained a funicle either with a degenerated ovule or with a small and underdeveloped seed. Failure of endosperm cellularisation, funicle degeneration and seed degeneration are causes of semi-blanking. Funicle degeneration was the major cause of premature embryo degeneration, and started either from the site of pollen tube penetration or from the chalazal tissue and spread towards the vascular bundles where it may have blocked sap flow to the ovule. Bradley and Crane (1975) reported black spots on the funicle which may be sites of degeneration. In this investigation it was found that semi-blanking or small-seededness of pistachio correlated with post-fertilization degeneration in the early embryo or endosperm. Moreover, tissue degeneration in the growing cotyledon of the pistachio seed started from the vascular tissue or from the epidermis late in development and resulted in half-seeded fruits. Similar spontaneous tissue degeneration has been observed in other plant tissues and results indicate that a deficiency or imbalance of microelements can damage tissues leading to embryo degeneration and abortion ( Gubbels and Carolus, 1971; Hoisington *et al.*, 1982; Delong *et al.*, 1993; Mittler *et al.*, 1995).

Inter-specific crossing of *P. vera* cultivars increased blank and mis-shapen fruit production, particularly in the cultivar Kerman. This could be due to inter-specific incompatibility which resulted in ovule and seed abortion, and thus in blanking. In the present study, blank fruits were observed in both pollinated and unpollinated treatments, but increased after inter-specific pollination of *P. vera* cultivars, especially cv. Kerman pollinated with *P. atlantica.* Consequently, pistachio yield and quality can be improved by selection of an appropriate compatible male cultivar for each commercial cultivar. Grundwag (1975) also reported ovule

degeneration following inter-specific pollination within the *Pistacia* genus. None of the intra- or inter-specific crosses of *P. atlantica* produced split shell fruits, and inter-specific pollination decreased the number of split fruit in *P. vera* cultivars. This seems to be a direct effect of pollen source on fruit morphology of pistachio. Shell thickness and suture angle were also important factors. This study also addressed the problem of premature shell splitting, and found that multiple split zones in areas other than the shell suture were responsible.

Further research is needed to clarify the effects of intra-specific incompatibility on blanking, semi-blanking and premature splitting in commercial cultivars of *P. vera* pollinated with *P. vera* male cultivars, and mis-shapen and non-split fruits may also be caused by inappropriate paternal genotype. The interaction with external influences such as air temperature, irrigation and nutrition, particularly during the rapid growth phase of the seed, and at fruit ripening, should also receive further attention.

# 8 References

Abbott, J. D., B. D. Brutonson and C. L. Patterson (1991). Fungicidal inhibition of pollen germination and pollen tube elongation in muskmelon. HortScience 26, 529-530.

Abeles, F. B., P. W. Morgan and M. E. Saltveit (1992). Ethylene in Plant Biology. 2nd ed, Academic Press, New York, USA.

Abeles, F. B., R. G. Leather, L. E. Forrence and L. E. Craker (1971). Abscission, regulation of senescence, protein synthesis and enzyme secretion by ethylene. HortScience 6, 371-376.

Addicott, F. T. and J. L. Lyon (1973). Physiological ecology of abscission. In 'Shedding of Plant Parts', (T. T. Kozlowski, ed.), Academic Press, New York.

Anderson, M. A, P. J. Harris, I. Bonig and A. E. Clarke (1987). Immunogold localization of L-arabinofuranosyl residues in the pollen tube of *Nicotiana alata* Link et Otto. Planta 171, 438-442.

Anderson, M. A., E. C. Cornish, S-L. Mau, E. G. Williams, R. Hogart, A. Atkinson, I. Bonig, G. W. Tegear, J. P. Coghlan. J. Crawford and A. E. Clarke (1986). Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature 321, 38-44.

Arasu, N. T. (1985). Self-incompatibility in *Ribes*. Euphytica 19, 373-378.

Armstrong, J. E. and S. C. Tucker (1986). Floral development in *Myristica* (Myristicaceae). American Journal of Botany 73, 1131-1143.

Ascher, P. D. and L. W. Drewlow (1971). Pollen development and physiology. In 'Pollen', (Heslop-Harrison, J. ed.), Butterworths, London.

Bal, J. S., S. N. Singh, J. S. Randhu and J. S. Javanda (1984). Effects of growth regulators on fruit drop, size and quality of ber (*Zizyphus mauritiana* Lamk.). Indian Journal of Horticulture 41, 182-185.

Bashaw, E. C. (1980). Apomixis and its application in crop improvment. In 'Hybridization of Crop Plants', (W. R. Fehr and H. H. Hadley, eds.), American Society of Agronomy and Crop Science, Madison, WI, USA.

Bassiri, A., F. Ahmad and A. E. Slinkard (1987). Pollen grain germination and pollen tube growth following *in vivo* and *in vitro* self and interspecific pollination in annual *Cicer* species. Euphytica 36: 667-675.

Bawa, K. S. and J. H. Beach (1983). Self -incompatibility systems in the Rubiaceae of a tropical lowland wet forest. American Journal of Botany 70, 1281-1288.

Bednarska, E. (1989). The effect of exogenous $Ca^{2+}$ ions on pollen germination and pollen tube growth. Investigation with $^{45}Ca^{2+}$ ions together with verapamil, $La^{3+}$, and ruthenium red. Sexual Plant Reproduction 2, 53-58.

Beger, E. M. and B. Qubedeaux (1975). Parthenocarpic cucumber: mechanism of action of auxin transport inhibitors. Journal of the American Society for Horticultural Science 99, 385-390.

Bell, J. and G. Hicks (1976). Transmitting tissue in the pistil of tobacco. Light and electron microscopic observations. Planta 131, 187-200.

Bergh, B. O. (1975). Avocado. In 'Advances in Fruit Breeding', (J. Janick and J. N. Moore, eds.), Purdue University Press, West Lafayette, Indiana, USA.

Bernhardt, P. and R. B. Knox (1983). The stigmatic papilla of *Amyema* (Loranthaceae): development, response to protandry and surface adaptation for bird pollination. American Journal of Botany 71, 1313-1319.

Bhandal, I. S. and R. Bala (1989). Heavy metal inhibition of *in vitro* pollen germination and pollen tube growth in *Amaryllis vitata* (Ait). Horticultural Abstracts 61, 10270.

Biale, J. B. (1964). Growth, maturation and senescence in fruits. Science 146, 880-888.

Bino, R. J. (1985). Histological aspects of microsporogenesis in fertile, cytoplasmic male sterile and restored fertile *Petunia hybrida*. Theoretical and Applied Genetics 69, 423-428.

Blanped, G. D. (1972). A study of ethylene in apple, red raspberry, and cherry. Plant Physiology 49, 627-630.

Bob, C. F., B. L. Redmond and D. F. Karnosky (1986). On the nature of intra- and inter-specific incompatibility in *Ulmus*. American Journal of Botany 73, 465-474.

Bookman, S. S. (1983). Effects of pollination timing on fruiting in *Asclepias speciosa* Torr. (Asclepiadaceae). American Journal of Botany 70, 897-905.

Bottini, R., D. D. Bottini, M. Koshioka, R. Pharis and B. G. Coombe (1985). Identification of gibberellins $A_1$, $A_5$, $A_{29}$, and $A_{32}$ from immature seed of apricot (*Prunus armeniaca* L.). Plant Physiology 78, 417-419.

Bouman, F. B. and F. D. Boesewinkle (1969). On a case of polyembryony in *Pterocarya fraxinifolia* (Juglandaceae) and on polyembryony in general. Acta Botanica Neerlandica 18, 50-57.

Bouranis, D. L. and C. A. Niavis (1992). Cell wall metabolism in growing and ripening stone fruits. Plant and Cell Physiology 33, 999-1008.

Bradley, M. V. and J. C. Crane (1975). Abnormality in seed development in *Pistacia vera* L. Journal of the American Society for Horticultural Science 100, 461-464.

Brady, C. J. (1987). Fruit ripening. Annual Review of Plant Physiology 3, 155-178.

Brady, C. J., G. M. Alpine, W. B. McGlasson and Y. Ueda (1982). Polygalacturonase in tomato fruits and the induction of ripening. Australian Journal of Plant Physiology 9, 172-178.

Brewbaker, J. L. and D. D. Gorrez (1967). Genetics of self-incompatibility in the monocot genera *Ananas* (pineapple) and *Gasteria*. American Journal of Botany 54, 611-616.

Brewbaker, J. L. and B. H. Kwack (1963). The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50, 859-865.

Bubar, J. S. and R. K. Miri (1965). Inheritance of self-incompatibility and brown keel tip in *Lotus corniculatus*. Nature 205, 1035-1036.

Bufler, G. (1984). Ethylene enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening apples. Plant Physiology 75, 192-195.

Burns-Balogh, P. (1983). A theory on the evolution of the exine in Orchidaceae. American Journal of Botany 70, 1304-1312.

Burson, B and B. Young (1983). Pollen pistil interactions and interspecific-incompatibility among *Panicum antidotale*, *P. coloratum*, and *P. deustum*. Euphytica 32, 397-405.

Busbice, T. H., R. Y. Gurgis and H. B. Collins (1975). Effects of selection for self-fertility and self-sterility in alfalfa and related characters. Crop Science 15, 471-475.

Campbell, C. S., Greene, C. W., Greene and S. E. Bernquist (1987). Apomixis and sexuality in three species of *Amelanchier* shade bush (Rosaceae: Maloideae). American Journal of Botany 74, 321-326.

Catlin, P. B. and V. S. Polito (1989). Cell and tissue damage associated with pistilate flower abscission of persian walnut. HortScience 24, 1003-1005.

Chen, C. G., E. C. Cornish and A. E. Clarke (1992). Specific expression of an extensine-like gene in the style of *N. alata*. The Plant Cell 114, 1053-1062.

Christoffersen, R. E., M. L.Tucker and G. G. Laties (1984). Cellulase gene expression in ripening avocado fruit: the accumulation of cellulase mRNA and protein as demonstrated by cDNA hybridization and immunodetection. Plant Molecular Biology 3, 385-392.

Clarke, A. E. (1982). The molecular basis of pollination. In, 'Pollination 82', pp. 27-35. Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Australia.

Condit, I. J. (1947). The Fig. Chronica Botanica Company, USA.

Coombe, B. G. (1965). The effect of growth substances and leaf number on fruit set and size of Corinth and Sultana grapes. Journal of Horticultural Science 40, 307-316.

Coombe, B. G. (1973). The regulation of set and development of the grape berry. Acta Horticulturae 34, 261-269.

Coombe, B. G. (1976). Development of fleshy fruits. Annual Review of Plant Physiology 27, 507-528.

Coombe, B. G. (1989). Fruit setting, development, and ripening. Acta Horticulturae 240, 209-215.

Cooper, W. C. and W. H. Henry (1973). Chemical control of fruit abscission. In 'Shedding of Plant Parts', (T. T. Kozlowski ed.), Academic Press, New York, USA.

Cope, F. W. (1962). The mechanism of pollen incompatibility in *Theobroma cacao* L. Journal of Heredity 17, 157-182.

Copeland, H. F. (1955). The reproductive structures of *Pistacia chinensis* (Anacardiaceae). Phytomorphology 5, 440-449.

Cornish, E. C., M. A. Anderson and A. E. Clarke (1988). Molecular aspects of fertilization in flowering plants. Annual Review of Cell Biology 4, 209-228.

Crane, J. C. (1973). Parthenocarpy: a factor contributing to the production of blank pistachio. HortScience 8, 388-390.

Crane, J. C. (1974). Hermaphroditism in pistachio. California Agriculture 28, 1-3.

Crane, J. C. (1978). Quality of pistachio nuts as affected by time of harvest. Journal of the American Society for Horticultural Science 103, 332-333.

Crane, J. C. and I. M. Al-Shalan (1974). Physical and chemical changes associated with growth of the pistachio nut. Journal of the American Society for Horticultural Science 99, 87-89.

Crane, J. C. and H. I. Forde (1976). Effects of four rootstocks on yield and quality of pistachio nuts. Journal of the American Society for Horticultural Science 101, 604-606.

Crane, J. C. and B. T. Iwakiri (1980). Xenia and metaxenia in pistachio. HortScience 15, 184-185.

Crane, J. C. and B. T. Iwakiri (1981). Morphology and reproduction of pistachio. Horticultural Reviews 3, 376-393.

Crane, J. C. and B. T. Iwakiri (1982). Shell dehiscence in pistachio. HortScience 17, 797-798.

Crane, J. C. and M. M. Nelson (1971).The unusual mechanism of alternate bearing in the pistachio. HortScience 6, 489-490.

Crane, J. C. and F. Takeda (1979). The unique response of the pistachio tree to inadequate winter chilling. HortScience 14, 135-137.

Crane, J. C., M. V. Bradley and L. C. Luckwill (1959). Auxin in parthenocarpic and non parthenocarpic figs. Journal of Horticultural Science 34, 142-153.

Crane, J. C., P. B. Catlin and I. Al-Shalan (1976). Carbohydrate levels in the pistachio as related to alternate bearing. Journal of the American Society for Horticultural Science 101, 371-374.

Crane, J. C., H. I. Ford and C. Daniel (1974). Pollen longevity in *Pistacia*. California Agriculture 28, 8-9.

Crowe, L. K. (1964). The evolution of outbreeding in plants. Journal of Heredity 19, 435-457.

Czapik, R. (1992). Development of embryo and endosperm after intra-specific and inter-specific pollination in *Rubus bellardii* weihe. Acta Biologica Cracoviensia Series Botanica 34, 59-69.

Daskaloff, S. (1968). A male sterile pepper mutant. Theoretical and Applied Genetics 38, 370-372.

Davis, G. L. (1961). The life history of *Podolepis jaceoides*, 1. Microsporogenesis and male gametogenesis. Phytomorphology 11, 69-96.

Davis, L. D. (1957). Flowering and alternate bearing. Journal of the American Society for Horticultural Science 70, 545-557.

Dayton, D. F. (1974). Overcoming self-incompatibility with killed compatible pollen. Journal of the American Society for Horticultural Science 99, 190-192.

de Nettancourt, D. (1977). Incompatibility in Angiosperms. Springer-Verlag, Berlin, Germany.

de Wet, E. P., J. Robbertse and L. A. Coetzer (1986). Pollination and ponticulus development of *Mangifera indica* L. cvs. Haden and Sensation. South African Journal of Plant and Soil 3, 76-79.

Degani, C., A. Goldring, I. Adato and R. El-Bastri (1990). Pollen parent effects on outcrossing rate, yield, and fruit characteristics of 'Fuerte' Avocado. HortScience 25, 471-473.

Delong, A., A. Calderon-Urrea and S. L. Dellaporta (1993). Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. Cell 74, 757-768.

Dhar, A. K. (1989). *In-vitro* germination and pollen tube growth of belladona (*Atropa belladona*). Horticultural Abstracts 59, 563.

Dickinson, T. A. and J. B. Phipps (1985). Studies in *Crataegus* (Rosaceae: Maloideae) XIV. The breeding system of *Crataegus crus-galli* sensu lato in Ontario. American Journal of Botany 73, 116-130.

Dobrofsky, S. and W. F. Grant (1980a). An investigation into the mechanism for reduced seed yield in *Lotus corniculatus*. Theoretical and Applied Genetics 57, 157-160.

Dobrofsky, S. and W. F. Grant (1980b). Electrophoretic evidence supporting self-incompatibility in *Lotus corniculatus*. Canadian Journal of Botany 58, 712-716.

Ducke, J. A. (1989). *Pistacia vera* L. (Anacardiaceae). In 'Handbook of Nuts', CRC Press, Boca Raton Florida, pp 240-243, USA.

Duvick, D. N. (1967). Influence of morphology and sterility on breeding methodology. In 'Plant Breeding', (K. J. Frey, ed.), University Press, Iowa, USA.

Eaton, G. W. (1959). Cytological study of the megaspore in *Prunus avium*. Canadian Journal of Plant Science 39, 466-476.

Echlin, P. and H. Godwin (1968). The ultrastructure and ontogeny of pollen in *Heleborus foetidus* L. II. Pollen grain development through the callose special wall stage. Journal of Cell Science 3, 175-186.

Edwardson, J. R. (1970). Cytoplasmic male sterility. Botanical Review 36, 341-420.

Egea, J. and L. Burgos (1992). Effective pollination period as related to stigma receptivity in apricot. Scientia Horticulturae 25, 77-83.

Elliott, C. F. (1958). Plant Breeding and Cytogenetics. McGraw-Hill, USA.

Ellis, M. F., M. Sedgley and J. A. Gardner (1991). Inter-specific pollen-pistil interaction in *Eucalyptus* L'Her. (Myrtaceae): The effect of taxonomic distance. Annals of Botany 68, 185-194.

Emsweller, S. L. and N. W. Stuart (1948). Use of growth regulating substances to overcome incompatibilities in *Lilium*. Proceedings of the American Society for Horticultural Science 51, 581-589.

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

Ferguson, I. B. (1984). Calcium in plant senescence and fruit ripening. Plant, Cell and Environment 7, 477-489.

Frenkel, C., I. Klein and D. R. Dilley (1968). Protein synthesis in relation to ripening of pome fruits. Plant Physiology 43, 1146-1153.

Frey, K.J. (1983). Plant population management and breeding. In 'Crop Breeding', (K. M. Rawal and M. N. Wood, eds.), American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin, USA.

Ganders, F. R. (1974). Disassortative pollination in the distylous plant *Jepsonia heterandra*. Canadian Journal of Botany 52, 2401-2407.

Garcia-Papi, M. A. and G. L. Garcia-Martines (1984). Endogenous plant growth substances content in young fruit of seeded and seedless Clemantine mandarine as related to fruit set and development. Biological Abstracts 78, 22692.

George, J. W. L., J. W. Scott and W. E. Splittstoesser (1984). Parthenocarpy in tomato. Horticultural Reviews 6, 56-84.

Gilissen, L. J. W. (1977). The influence of relative humidity on the swelling of pollen grains *in vitro*. Planta 13, 299-301.

Givannoni, J. J., D. Dellapenna, A. B. Bennett and R. L. Fischer (1992). Polygalacturonase and tomato fruit ripening. Horticultural Reviews 13, 67-105.

Gleeson, S. A. (1982). Heterodichogamy in walnuts: inheritance and stable ratios. Evolution 36, 892-902.

Golan-Goldhirsh, A., U. Schmidhalter, M. Müller and J. J. Oertli (1991). Germination of *Pistacia vera* L. pollen in liquid medium. Sexual Plant Reproduction 4, 182-187.

Gomoa, A. H. (1989). Effect of some chemicals on a variety of pollen grains and growth of the pollen tube. Horticultural Abstracts 62, 2807.

Gonzalez, M. V., M. Coque and M. Herrero (1995). Papillar integrity as an indicator of stigmatic receptivity in kiwifruit (*Actinidia deliciosa*). Journal of Experimental Botany 46, 263-269.

Goode, J. E. and J. Ingram (1971). The effect of irrigation on the growth, cropping and nutrition of Cox's orange pippin apple trees. Journal of Horticultural Science 46, 195-208.

Green, A. (1971). The physiology and nutrition of developing fruits. In 'The Biology of Fruits and their Products', (A. C. Hulme ed.), Academic Press, USA.

Green, G. W. (1984). Sexual apomictic reproduction in *Clamagrostis* (Graminae) from eastern north America. American Journal of Botany 71, 285-293.

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

Grundwag, M. (1975). Seed set in some *Pistacia* L. (Anacardiaceae) species after inter- and intra-specific pollination. Israel Journal of Botany 24, 205-211.

Grundwag, M. (1976). Embryo and fruit development in four species of *Pistacia* L. (Anacardiaceae). Botanical Journal of the Linnean Society (London) 73, 355-370.

Grundwag, M. and A. Fahn (1969). The relation of embryology to the low seed set in *Pistacia vera* (Anacardiaceae). Phytomorphology 19, 225-235.

Gubbels, G. H. and R. L. Carolus (1971). Influence of atmospheric moisture, ion balance, and ion concentration on growth, transpiration and blackheart of celery (*Apium graviolens* L.). Journal of the American Society for Horticultural Science 96, 201-204.

Guitian, J. (1993). Why *Prunus mahaleb* (Rosaceae) produces more flowers than fruits. American Journal of Botany 80, 1305-1309.

Gupta, M. and Y. S. Murty (1985). *In vitro* hormonal regulation of pollen germination and pollen tube growth in *Vicia faba*. Horticultural Abstracts 57, 1939.

Gustafson, F. G. (1942). Parthenocarpy: natural and artificial. Botanical Review 80, 599-654.

Haines, R. J. and N. Prakash (1980). Proembryo development and suspensor elongation in *Araucaria juss*. Australian Journal of Botany 28, 511-522.

Hartmann, H. T. and D. E. Kester (1990). Plant Propagation, Principles and Practices, 4th ed., Prentice-Hall, USA.

Hartmann, H. T., N. M. Kofranek, V. E. Rubatzky and W. J. Flocker (1988). Plant Science, 4th ed, Prentice-Hall, USA.

Hayman, D. L. (1956). The genetical control of incompatibility in *Phalaris coerulescens* Desf. Australian Journal of Biological Science 9, 321-331.

Hayman, D. L. and J. Richter (1992). Mutation affecting self-incompatibility in *Phalaris caerulescens* Desf (Poaceae). Journal of Heredity 63, 495-503.

Herbert, K. H., F. R. Immer and D. C. Smith (1955). Methods of Plant Breeding. 2nd ed, McGraw-Hill, New York, USA.

Herrero, M. and M. Gascon (1987). Prolongation of embryo sac viability in pear (*Pyrus communis*) following pollination or treatment with gibberellic acid. Annals of Botany 60, 287-293.

Heslop-Harrison, J. (1968). The pollen grain wall. Science 161, 230-237.

Heslop-Harrison, J. (1971). Pollen Development and Physiology. Butterworths, London.

Heslop-Harrison, J. (1982). Pollen stigma interaction and cross-incompatibility in the grasses. Science 215, 1358-1364.

Heslop-Harrison, J. and H. G. Dickinson (1969). Time relationship of sporopollenin synthesis associated with tapetum and microspores in *Lilium*. Planta 46, 199-214.

Heslop-Harrison, J. and Y. Heslop-Harrison (1981). The pollen stigma interaction in grasses. II. Pollen tube penetration and the stigma response in *Secale*. Acta Botanica Neerlandica 30, 289-306.

Heslop-Harrison, Y. and K. R. Shivanna (1977). The receptive surface of the angiosperm stigma. Annals of Botany 41, 1233-1258.

Hill, J. P. and E. M. Lord (1987). Dynamics of pollen tube growth in the wild radish *Raphanus raphanistrum* (Brassicaceae). II. morphology, cytochemistry and ultrastructure of transmitting tissue, and path of pollen tube growth. American Journal of Botany 74: 988-997.

Hiratsuka, S., T. Tezuka and Y. Yamamoto (1989). Analyses of self-incompatibility reaction in Easter Lily by using high temperature. Journal of the American Society for Horticultural Science 114, 505- 508.

Hocking, P. J. and J. S. Pate (1977). Mobilization of minerals to developing seeds of legumes. Annals of Botany 41, 1259-1278.

Hoisington, D. A., M. G. Neuffer and V. Walbot (1982). Disease lesion mimics in maize 1. effects of genetic background, temperature, developmental age, and wounding on necrotic spot formation with Les1. Developmental Biology 93, 381-388.

Hormaza, J. I. and M. Herrero (1992). Pollen selection. Theoretical and Applied Genetics 83, 663-672.

Horner, H. T. J and N. R. Lersten (1971). Microsporogenesis in *Citrus limon* (Rutaceae). American Journal of Botany 58, 72-79.

Horner, H. T. J. and M. A. Rogers (1974). A comparative light and electron microscopic study of microsporogenesis in male-fertile and cytoplasmic male sterile pepper (*Capsicum annuum*). Canadian Journal of Botany 25, 435-443.

Hossain, M. A. (1983). Cytoplasmic study involving a nuclear male sterile mutant in wheat. Ph.D thesis, The University of Adelade, Waite Agricultural Research Institute.

Huber, D. J. (1983a). Polyuronid degeneration and hemicellulose modifications in ripening tomato fruit. Journal of the American Society for Horticultural Science 108, 405-409.

Huber, D. J. (1983b). The role of cell wall hydrolases in fruit softening. Horticultural Reviews 5, 169-219.

Huff, D. R. (1991). Sex ratio and inheritance of anther and stigma colour in diploid buffalo grass. Crop Science 31, 328-332.

Iwanami, Y., T. Sasakuma and Y. Yamada (1988). Pollen Illustrations and Scanning Electronmicrographs. Springer-Verlag, Berlin, Germany.

Iwao, N. (1983). Male sterility caused by cooling treatment at the young microspore stage in rice plant. Japanese Journal of Crop Science 52, 307-313.

Jackson, M. B. and D.J. Osborne (1970). Ethylene, the natural regulator of leaf abscission. Nature 225, 1019-1022.

Jensen, W. A. (1964). Observation of the fusion of nuclei in plants. Journal of Cell Biology 23, 669-670.

Joel, D. M. and D. Eisenstein (1980). A bridge between the ovule and ovary wall in *Mangifera indica* L. (Anacardiaceae). Acta Botanica Neerlandica 29, 203-206.

Joley, L. E. (1981). Pistachio. In 'Nut Tree Culture in North America', (R. A. Jaynes, ed.), The Northern Nut Growers Association, USA.

Jones, H. A. and A. E. Clarke (1943). Inheritance of male sterility in the onion and the production of hybrid seed. Proceedings of the American Society for Horticultural Science 43, 189-194.

Jordano, P. (1988). Pollination and variability of seed production in *Pistacia lentiscus* L. (Anacardiaceae). Anales Jardin Botanico de Madrid 45, 213-231.

Kahn, T. L. and D. A. Demason (1985). A quantitative and structural comparision of citrus pollen tube development in cross-compatible and self-incompatible gynoecia. Canadian Journal of Botany 64, 2548-2555.

Kanta, K. and P. Maheshwari (1963). Intra ovarian pollination in some Papaveraceae. Phytomorphology 13, 215-220.

Kenrick, J., V. Kaul and E. G. Williams (1986). Self-incompatibility in *Acacia retinodes*: site of pollen tube arrest is the nucellus. Planta 169, 240- 250.

Kihara, H. (1966). Factors affecting the evolution of common wheat. Indian Journal of Genetics 26, 14-28

Kim, H. S. and S. W. Lee (1989). Studies of pollen germination in magnolia species. Horticultural Abstracts 61, 1352.

Kim, S. K., H. B. Lagersted and L. S. Daley (1985). Germination responses of filbert pollen to pH, temperature, fruit set and sucrose. HortScience 20, 944-946.

King, M. J. and A. M. Ferguson (1991). Collection and use of dry pollen for pollination of kiwi fruit. New Zealand Journal of Crop and Horticultural Science 19, 385-389.

Kingston, C. M. (1992). Maturity indices for apple and pear. Horticultural Reviews 13, 407-432.

Knight, R., J. Jr (1982). Partial self compatibility in "Golden star" *Carambola*. HortScience 17, 72-73.

Knox, R. B. (1971). Pollen wall proteins: localization, enzymatic and antigenic activity during development in *Gladiolus* (Iridaceae). Journal of Cell Science 9, 209-237.

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

Knox, R. B. (1982). Pollination systems. In 'Pollination 82', Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Australia.

Koltunow, A. M., J. Truettner, K. H. Cox, M. Wallorth and R. B. Goldberg (1990). Different temporal and spatial gene expression patterns occur during anther development. The Plant Cell 2, 1201-1224.

Kozlowski, T. T. (1973). Extent and significance of shedding of plant parts. In 'Shedding of Plant Parts', (T. T. Kozlowski, ed.), Academic Press, New York, USA.

Lakshmanan, K. K. and K. B. Ambegaokar (1984). Polyembryony. In 'Embryology of Angiosperms', (B. M. Johri, ed.), Springer-Verlag, Berlin, Germany.

Laser, K. D. and R. N. Lersten (1972). Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Botanical Review 36, 425-454.

Lawson, R. H. and M. M. Dienelt (1991). *Chrysanthemum* foliar necrosis: symptoms, histochemistry, and x-ray analysis of leaf lesion. Phytopathology 81, 1071-1078.

Lee, C. W., J. C. Thomas and S. L. Buchmann (1985). Factors affecting *in vitro* germination and storage of jojoba pollen. Journal of the American Society for Horticultural Science 110, 671-676.

Leopold, C. A. and P. E. Kriedemann (1975). Plant Growth and Development. McGraw-Hill, New York, USA.

Levy, A., H. D. Rabinowitch and N. Kedar (1978). Characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica 27, 211-218.

Lewis, D. (1954). Comparative incompatibility in angiosperms and fungi. Advances in Genetics 6, 235-271.

Lewis, T. L. and D. Martin (1973). Longitudinal distribution of applied calcium, magnesium and potassium, in Merton apple fruits. Australian Journal of Agricultural Research 24, 363-371.

Li, S-J. (1980). Self-incompatibility in "Matou" wentan (*Citrus grandis* (L.) obs.). HortScience 15, 298-300.

Lin, J. J., D. B Dickinson and T. H. D. Ho (1987). Phytic acid metabolism in lily pollen. Plant Physiology 83, 408-413.

Lin, S., S. E. Walter and G. L. William (1983). Factors controlling the expression of parthenocarpy in "Severianin" tomato. Scientia Horticulturae 19, 45-53.

Lin, S., G. L. William and S. E. Walter (1984). Expression and inheritance of parthenocarpy in "Severianin" tomato. Journal of Heredity 75, 62-64.

Lin, T. S., J. C. Crane and K. Ryugo (1984a). Gibberellin-like substances in pistschio as related to inflorescence bud abscission. HortScience 19, 267-268

Lin, T. S., J. C. Crane, K. Ryugo (1984b). Effects of gibberellin on vegetative and inflorescence buds of pistachio. Journal of the American Society for Horticultural Science 109, 39-42.

Lin, T. S., J. C. Crane, K. Ryugo, V. S. Polito and T. M. Dejong (1984c). Comparative study of leaf morphology, photosynthesis, and leaf conductance

in selected *Pistacia* species. Journal of the American Society for Horticultural Science 109, 325-330.

Lloyd, D. G. (1980). The distribution of gender in four angiosperm species illustrating two evolutionary pathway to dioecy. Evolution 34, 123-134.

Longly, B. (1984). Tetrades irregulieres developments apomeiotiques et aposporie dans les ovules de *Cichorium intibus* L. Canadian Journal of Botany 62, 635-646.

Lord, E. M. and B. D. Webster (1979). The stigmatic exudate of *Phaseolus vulgaris* L. Botanical Gazette 140, 266-271.

Lundqvist, A. (1975). Complex self-incompatibility systems in angiosperms. Proceedings of the Royal Society of London, Series B 188, 235-245.

Luza, J. G. and V. S. Polito (1991). Porogamy and chalazogamy in walnut (Juglandaceae). Botanical Gazette 152, 100-106.

Maggs, D. H. (1973). The pistachio as an Australian crop. Journal of the Australian Institute of Agricultural Science 39, 10-17.

Mariani, C., R. B. Goldberg and J. Leemans (1991). Engineered male sterility in plants. In 'Molecular Biology of Plant Development', (G. I. Jenkins, and W. Schouch, eds.), Symposia of the Society for Experimental Biology, Symposium XIV. The Society for Experimental Biology, The University of Cambridge, UK.

Marquard, R. D. (1988). Outcrossing rates in pecan and the potential for increased yields. Journal of the American Society for Horticultural Science 113, 84-88.

Martin, A. R. H. and J. C. Herscovitch (1989). Fertilizing pollen tubes parasitise ovary wall in *Grevillea*. Annals of Botany 64, 163-165.

Martin, D., T. L. Lewis and J. Cerny (1961). Jonathan spot–three factors related to incidence: fruit size, breakdown, and seed numbers. Australian Journal of Agricultural Research 12, 1039-1049.

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

Martínez-Pallé, E. and M. Herrero (1994). Male performance in pistachio (*Pistacia vera*). Journal of Horticultural Science 69, 1117-1122.

Martínez-Pallé, E. and M. Herrero (1995). The ponticulus: a structure bridging pollen tube access to the ovule in *Pistacia vera*. Sexual Plant Reproduction 8, 217-222.

Mascarenhas, J. (1990). Gene activity during pollen development. Annual Review of Plant Physiology and Plant Molecular Biology 41, 317-338.

Matsubara, S. (1980). Overcoming self-incompatibility in *Raphanus sativus* L. with high temperature. Journal of the American Society for Horticultural Science 105, 842-846.

McClure, B. A., J. E. Gray, M. A. Anderson and A. E. Clarke (1990). Self-incompatibility in *Nicotiana alata* involving degradation of pollen rRNA. Nature 347, 757-760.

McCullagh, P. and J. A. Nelder (1983). Generalized linear models. Chapman and Hall, London.

McGranahan, G. H., D. G. Voyiatzis, P. B. Catlin and V. S. Polito (1994). High pollen loads can cause pistillate flower abscission in walnut. Journal of the American Society for Horticultural Science 119, 505-509.

McGregor, S. E. (1976). Insect Pollination of Cultivated Crop Plants. United States Department of Agriculture, Handbook No. 496, USA.

McKay, J. W. (1942). Self-sterility in the chinese chestnut (*Castanea mollissima*). Proceedings of the American Society for Horticultural Science 41, 156-160.

McKay, J. W. and H. L. Crane (1938). The immediate effect of pollen on the fruit of the chestnut. Proceedings of the American Society for Horticultural Science 36, 293-298.

McMurchie, E. J., W. B. McGlasson and I. L. Eaks (1972). Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237, 235-236.

Mehlenbacher, S. A. and D. C. Smith (1991). Partial self-compatibility in 'Tombul' and 'Montebello' hazelnut. Euphytica 56, 231-236.

Mikesell, J. (1988). Comparative development of viable and aborted ovules in *Phytolacca americana* L. (Phytolaccaceae). Botanical Gazette 149, 196-202.

Mittler, R., V. Shulaev and E. Lam (1995). Coordinated activation of programmed cell death and defence mechanisms in transgenic tobacco plants expressing a bacterial proton pump. The Plant Cell 7, 29-42.

Mogensen, H. L. (1975). Ovule abortion in *Quercus* (Fagaceae). American Journal of Botany 62, 160-165.

Moncur, M. M. (1986). Floral ontogeny of the cashew, *Anacardium occidentale* (Anacardiaceae). Scientia Horticulturae 30, 203-211.

Moore, H. M. and Nasrallah, J. B. (1990). A *Brassica* self-incompatibility gene is expressed in the stylar transmitting tissue of transgenic tobacco. The Plant Cell 2, 29-38.

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

Mulcahy, D. L. and D. Camparello (1970). Pollen flow within the tristylous species *Lythrum salicaria*. American Journal of Botany 57, 1027-1030.

Mulcahy, D. L. and G. B. Mulcahy (1983). Gametophytic self-incompatibility reexamined. Science 220, 1247-1251.

Murfett, J. E., E. C. Cornish, P. R. Ebert, I. Bonig, B. A. McClure and A. E. Clarke (1992). Expression of a self-incompatibility glycoprotein ($S_2$-ribonuclease) from *Nicotiana alata* in transgenic *N. tabaccum*. The Plant Cell 4, 1063-1074.

Nakanishi, T. and M. Savano (1984). Pollen tube behavior by carbon dioxide in relation to overcoming self-incompatibility in *Brassica*. In 'Sexual Reproduction In Seed Plants, Ferns and Mosses', (M. T. M. Willemse and J. L. van Went, eds.), Proceedings of the 8th International Symposium on Sexual Reproduction in Seed Plants, Ferns and Mosses. Wageningen, the Netherlands.

Nasrallah, J. B., R. C. Doney and M. E. Nasrallah (1985). Biosynthesis of glycoproteins involved in the pollen-stigma interaction of incompatibility in developing flowers of *Brassica oleracea* L. Planta 165, 100-107.

Nasrallah, M. E. (1985). The molecular basis for sexual incompatibility. In 'Plant Biotechnology: Developmental Regulation of Plant Gene Expression', (D. Grierson, C. Biol and F. I. Biol, eds.), Volume 2, Blackie, Glasgow, UK.

Nasrallah, M. E. and D. H. Wallace (1968). The influence of modifier genes on the intensity and stability of self-incompatibility in cabbage. Euphytica 17, 495-503.

Nast, C. G. (1935). Morphological development of the fruit of *Juglans regia*. Hilgardia 9, 345-362.

Nevo, A., E. Werker and R. Ben-Sassen (1974). The problems of indehiscence of pistachio (*Pistacia vera* L. ) fruit. Israel Journal of Botany 23, 1-13.

Niklas, K. J. and S. L. Buchmann (1988). Aerobiology and pollen capture of orchard-grown *Pistacia vera* (Anacardiaceae). American Journal of Botany 75, 1813-1829.

Nishio, T. and K. Hinata (1977). Analysis of S-specific proteins in stigma of *Brassica oleracea* L. by isoelecteric focusing. Journal of Heredity 38, 391-396.

Nishio, T., K. Toriyama, T. Sato, M. K. Kandasamy, D. J. Paolillo, J. B. Nasrallah and M. E. Nasrallah (1992) Expression of S-locus glycoprotein gene from *Brassica oleracea*. Sexual Plant Reproduction 5, 101-109.

Nitsch, J. K., J. L. Liverman and F. W. Went (1952). The development of sex expression in *Cucurbita* flowers. American Journal of Botany 39, 32-43.

Nixon, R. W. (1956). Effect of metaxenia and fruit thinning on size and checking of Deglet Noor dates. Proceedings of the American Society for Horticultural Science 67, 258-264.

Nogler, G. A. (1984). Gametophytic apomixis. In 'Embryology of Angiosperms', (B. M. Johri ed.), Springer-Verlag Berlin, Germany.

O'Brien, T. P. and M. F. McCully (1981). The study of plant structure, principles and selected methods. Termarcarphi Pty. Ltd., Melbourne.

Ohta, K., N. Ito, T. Hosoki and Y. Sugi (1991). Influence of relative humidity on fruit cracking of cherry tomato grown in hydroponics culture and its control. Journal of the American Society for Horticultural Science 60, 337-343.

O'Neill, P. (1986). Self-incompatibility in rape. In 'Pollination 86', Plant Cell Biology Research Centre, School of Botany, The University of Melbourne, Australia.

Okamoto, G. and S. Imai (1982). Horticultural study of berry setting in Muscat of Alexandria grapes. Journal of the Japanese Society for Horticultural Science 50, 436-444.

Okamoto, G., M. Chibuichi and K. Shimamura (1989). Inhibition of pollen tube growth by diffusate and extract of grape pistils. Journal of the Japanese Society for Horticultural Science 58, 515-521.

Olesen, J. M. (1979). Floral morphology and pollen flow in the heterostylous species *Pulmonaria obscura* Dumort. (Boraginaceae). New Phytologist 82, 757-767.

Olien, W. (1987). Apomictic crab apples and their potential for research and fruit production. HortScience 22, 541-546.

Ornduff, R. (1980). Heterostylous population composition, and pollen flow in *Hedyotis caerulea*. American Journal of Botany 67, 95-103.

Pandey, K. K. (1958). The time of S-allele action. Nature, 181, 1220-1221.

Pandey, K. K. (1962). Interspecific incompatibility in *Solanum* spp. American Journal of Botany 4, 874-882.

Pandey, K. K. (1979). Overcoming incompatibility and promoting genetic recombination in flowering plants. New Zealand Journal of Botany 17, 645-663.

Pandey, K. K. (1980). Evolution of incompatibility systems in plants: origin of 'independent' and 'complementary' control of incompatibility in angiosperms. New Phytologist 84, 381-400.

Pastogi, G. R. and L. Singh (1989). Effect of alar-58 and cycocel on pollen viability and germination in *Lycopersicum esculentum* Mill. cv. Pusa Ruby. Horticultural Abstracts 59, 9145.

Pearson, T. C., D. S. Slaughter and H. E. Studer (1994). Physical properties of pistachio nuts. Transactions of the American Society of Agricultural Engineering 37, 913-918.

Peebles, R. H. and C. Hope (1937). The influence of different pollens on the development of the pistachio nut. Proceedings of the American Society for Horticultural Science 34, 29-32.

Peterson, C. E. and R. E. Foskett (1953). Occurence of pollen sterility in seed fields of Scott County Globe onions. Proceedings of the American Society for Horticultural Science 62, 443-448.

Philipson, M. N. (1978). Apomixis in *Cartaderia jubata* (Gramineae). New Zealand Journal of Botany 16, 45-59.

Pimienta, E. and Polito, V. S. (1982). Ovule abortion in 'Nonpareil' almond (*Prunus dulcis* [Mill.] D. A. Webb). American Journal of Botany 69, 913-920.

Pimienta, E. and V. S. Polito (1983). Embryo sac development in almond [(*Prunus dulcis*, Mill. ) D. A. Webb] as affected by cross-, self- and non-pollination. Annals of Botany 51, 469-479.

Polito, V. S. and N. Y. Li (1985). Pistilate flower differentiation in walnut (*Juglans regia* L.): a developmental basis for heterodichogamy. Scientia Horticulturae 26, 333-336.

Polito, V. S. and J G. Luza (1988a). Longevity of pistachio pollen determined by *in vitro* germination. Journal of the American Society for Horticultural Science 113, 214-217.

Polito, V. S. and J. G. Luza (1988b). Low temperature storage of pistachio pollen. Euphytica 39, 265-269.

Pontikis , K. A. (1977). Contribution to study on the effect of pollen of different species and cultivars of the genus *Pistacia* on nut development and quality. Horticultural Abstracts 47, 10270.

Popov, M. A. (1984). Stimulating pollen germination in apple under self-incompatibility. Horticultural Abstracts 56, 8539.

Porlingis, I. C. (1974). Flower bud abscission in pistachio (*Pistacia vera* L.) as related to fruit development and other factors. Journal of the American Society for Horticultural Science 99, 121-125.

Porlingis, I. C. and D. G. Voyiatzis (1993). Delaying anthesis of staminate pistachio with paclobutrazol. HortScience 28, 814-816.

Procopiou, J (1973). The induction of early blooming in female pistachio trees by mineral oil-DNOC winter sprays. Journal of Horticultural Science 48, 393-395.

Quinlan, J. D. and A. P. Preston (1968). Effects of thinning blossoms and fruitlets on growth and cropping of Sunset apple. Journal of Horticultural Science 43, 373-381.

Rana, A., G. Bitaradwaj and S. V. S. Chauhan (1989). Effects of some growth regulators on *in vitro* pollen germination in *Impatiens balsamina* (balsam). Horticultural Abstracts 61, 2134.

Rhodes, M. C. C. (1980). The maturation and ripening of fruits. In, 'Senescence in Plants', (K. V. Thimann, ed.), CRC Press, Florida, USA.

Rick, C. M. (1978). The tomato. Scientific American 239, 77-87.

Roggen, H. P. J. R. and A. J. van-Dijk (1972). Breaking incompatibility of *Brassica oleracea* by steel-brush pollination. Euphytica 21, 424-425.

Roggen, H. P. J. R. and A. J. van-Dijk (1976). Thermally aided pollination: a new method of breaking self-incompatibility in *Brassica oleracea*. Euphytica 21, 181-184.

Roggen, H. P. J. R., A. J. van-Dijk and C. Dorsman (1972). Electric aided pollination: a new method of breaking incompatibility in *Brassica oleracea*. Euphytica 25, 643-646.

Rowley, J. R. and J. J. Skvarla (1975). The glycocalyx and initiation of exine spinules on microspores of *Canna*. American Journal of Botany 62, 479-485.

Russell, S. D. (1982). Fertilisation in *Plumbago zeylanica*: entry and discharge of the pollen tube in the embryo sac. Canadian Journal of Botany 60, 2219-2230.

Sage, G. C. M. (1972). The inheritance of fertility restoration in male sterile wheat carrying cytoplasm derived from *Triticum timopheevi*. Theoretical and Applied Genetics 42, 233-243.

Sarkar, K. R. and E. R. J. Coe (1966). A genetic analysis of the origin of maternal haploids in maize. Genetics 54, 453-464.

Schroeder, C. A. (1947). Rootstock influence on fruit-set in the *Hachiya persimmon*. Proceedings of the American Society for Horticultural Science 50, 149-160.

Schwabe, W. W. and J. J. Mills (1981). Hormones and parthenocarpic fruit set. Horticultural Abstracts 51, 661-698.

Seavey, S. R. and K. S. Bawa (1986). Late acting self-incompatibility in angiosperms. Botanical Review 52, 195-219.

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

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

Sedgley, M. (1979). Intervarietal pollen tube growth and ovule penetration in the avocado. Euphytica 28, 25-35.

Sedgley, M. (1980). Anatomical investigation of abscised avocado flowers and fruitlets. Annals of Botany 46, 771-777.

Sedgley, M. (1981a). Early development of the *Macadamia* ovary. Australian Journal of Botany 29, 185-193.

Sedgley, M. (1981b). Storage of avocado pollen. Euphytica 30, 595-599.

Sedgley, M. (1982). Floral anatomy and pollen tube growth in the quandong (*Santalum acuminatum* (R. Br.) A. DC.). Australian Journal of Botany 30, 601-609.

Sedgley, M. and D. P. S. T. G. Attanayake (1988). The breeding system of rubber (*Hevea brasiliensis)*: a complete evaluation of controlled hand pollination methods. Euphytica 39, 83-91.

Sedgley, M. and A. R. Griffin (1989). Sexual reproduction of tree crops. Academic Press, London.

Sedgley, M., M. A. B. Blesing and H. I. M. V. Vithanage (1985). A developmental study of the structure and pollen receptivity of the macadamia pistil in relation to protandry and self-incompatibility. Botanical Gazette 146, 6-14.

Sedgley, M. (1994). Self-incompatibility in woody horticultural species. In 'Genetic Control of Self-incompatibility and Reproductive Development in Flowering Plants', (E. G. Williams, A. C. Clarke and R. B. Knox eds.), Kluwer Academic Publishers Dordrcht, The Netherland, pp 141-163.

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

Shaulp, P. M. (1986). Handbook of fruit set and development, CRC press, Florida, USA.

Shear, C. B. (1975). Calcium-related disorders of fruits and vegetables. HortScience 10, 361-365.

Shoup, J. R., J. Overton and M. Ruddat (1980). Ultrastructure and development of the exine in the pollen wall of *Silene alba* (Caryophyllaceae). Botanical Gazette 141, 379-388.

Shuraki, Y. D. and M. Sedgley (1994). Effect of pistil age and pollen parent on pollen tube growth and fruit production of pistachio. Journal of Horticultural Science 69, 1019-1027

Singh, A., Y. Ai and T. H. Kao (1991). Characterization of ribonuclease activity of three S-alleles associated proteins of *Petunia inflata*. Plant Physiology 96, 61-69.

Sinha, M. M. (1972). Pollen viability and pollen storage studies in Jackfruit (*Artocarpus heterophyllus* Lam.). Horticultural Abstracts 44, 9128.

Skene, D. S. (1980). Growth stresses during fruit development in Cox's Orange Pippin apples. Journal of Horticultural Science 55, 27-32.

Skvarla, J. J. and D. D. Larson (1966). Fine structural studies of *Zea mays* pollen 1: cell membranes and exine ontogeny. American Journal of Botany 53, 1112-1125.

Sniezko, R. and T. Visser (1987). Embryo development and fruit set in pear induced by untreated and irradiated pollen. Euphytica 36, 287-294.

Sommer, N. F., J. R. Buchanan and R. J. Fortlage (1986). Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. Phytopathology 76, 692-694.

Soost, R. K. and J. W. Cameron (1975). Citrus. In 'Advances in Fruit Breeding', (J. Janick and J. N. Moore, eds.), Purdue University, West Lafayette, IN., USA.

Spiss, L. and D. J. Paolillo (1969). Semi-vitro methods in the study of compatibility in birdsfoot Trefoil (*Lotus corniculatus* L.). Crop Science 9, 173-176.

Stanley, R. G. and H. F. Linskens (1974). Pollen: Biology Biochemistry Management. Springer-Verlag, New York.

Stanton, M. L. (1994). Male-male competition during pollination in plant populations. The American Naturalist 144, S40-S67.

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 cause and ultimate function. Annual Review of Ecology and Systematics 12, 253-279.

Steyn, E. M. A., P. J. Robbertse and D. Smith (1993). An anatomical study of ovary-to-cuke development in consistently low-producing trees of the 'Fuerte' avocado (*Persea americana* Mill.) with special reference to seed abortion. Sexual Plant Reproduction 6, 87-97.

Stone, C. L., L. E. Jones and W. E. Whitehouse (1943). Longevity of pistachio pollen under various conditions of storage. Proceedings of the American Society for Horticultural Science 42, 305-313.

Storey, W. B. (1975). Figs. In 'Advances in Fruit Breeding', (J. Janick and J. N. Moore, eds.), Purdue University, West Lafayette, IN., USA.

Stösser, R., H. P. Rasmussen and M. J. Bukovac (1969a). Histochemical changes in the developing abscission layer in fruits of *Prunus cerasus* L. Planta 86, 151-164.

Stösser, R., H. P. Rasmussen, and M. J. Bukovac (1969b). A histological study of abscission layer formation in cherry fruits during maturation. Journal of the American Society for Horticultural Science 94, 239-243.

Stösser, R. and S. F. Anvari (1982). On the senescence of ovules in cherries. Scientia Horticulturae 16, 29-38.

Swamy, N. R. and B. Bahadur (1984). Pollen flow in dimorphic *Turnera subulata* (Turneraceae). New Phytologist 95, 205-209.

Swingle, W. T. (1928). Metaxenia in the date palm, possibly a hormone action by the embryo or endosperm. Journal of Heredity 19, 257-268.

Tadeo, F. R., M. Talon, E. Germain and F. Dosba (1994). Embryo sac development and gibberellins in pollinated and unpollinated ovaries of walnut (*Juglans regia*). Physiologia Plantarum 91, 37-44.

Takayama, S., A. Isagai, T. Chise, Y. Ueda, K. Hinata, K. Okazaki and A. Suzuki (1987). Sequence of S-glycoprotein products of the *Brassica campestris* self-incompatibility locus. Nature 326, 102-106.

Takeda, F., K. Ryugo and J. C. Crane (1980). Translocation and distribution of $^{14}C$-photosynthates in bearing and non-bearing pistachio branches. Journal of the American Society for Horticultural Science 105, 642-644.

Takeno, K. and H. Ise (1992). Parthenocarpic fruit set and endogeneous indol-3-acetic acid content in ovary of *Cucumis sativus* L. Journal of the American Society for Horticultural Science 60, 941-646.

Therios, I. N., V. M. Tsiracoglou and K. N. Dimassi-Theriou (1986). Physiological aspects of pistachio (*Pistacia vera* L. ) pollen germination. Horticultural Abstracts 1979, 861.

Thompson, M. M. (1967). Genetics of incompatibility in *Corylus avellana* L. Theoretical and Applied Genetics 54, 113-116.

Thompson, M. M. and L. J. Liu (1973). Temperature, fruit set and embryo sac development in 'Italian' prune. Journal of the American Society for Horticultural Science 98, 193-197.

Tilton, V. R. and H. T. Horner (1980). Stigma, style, and obturator of *Ornithogalum caudatum* (Liliaceae) and their function in the reproductive process. American Journal of Botany 67, 1113-1131.

Tisser, B. E., C. W. Green and S. E. Bergquist (1987). Apomixis and sexuality in three species of *Amelanchier* shade bush (Rosaceae: Maloideae). American Journal of Botany 24, 321-328.

Tomas, I. A., D. H. Wallace, P. M. Ludford and J. L. Ozbun (1979). Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. Plant Physiology 64, 620-622.

Tomer, E., S. Gazit and D. Eisenstein (1980). Seedless fruit in 'Fuerte' and 'Ettinger' avocado. Journal of the American Society for Horticultural Science 105, 341-346.

Torabi, M. (1980). The effect of ethephon on ripening and splitting of pistachio nuts. HortScience 15, 521.

Tucker, G. A. and D. Grierson (1982). Synthesis of polygalacturonase during tomato fruit ripening. Planta 155, 64-67.

Turcotte, E. L. and C. V. Feaster (1967). Semigamy in pima cotton. Journal of Heredity 58, 55-57.

Visser, T. and C. M. Marcucci (1986). The performance of double or mixed pollinations with compatible, self-incompatible and incongruous pollen of pear and apple. Euphytica 35, 1011-1015.

Visser, T. and M. C. Marcucci (1983). Pollen and pollination experiments. IX. The pioneer pollen effects in pear and apple related to the interval between pollination and temperature. Euphytica 32, 703-709.

Vitagliano, C. and R. Viti (1989). Effect of some growth substances on pollen germination and tube growth in different stone fruits. Acta Horticulturae 239, 379-382.

Vithanage, H. I. M. V. and McE. Alexander (1985). Synchronous flowering and pollen storage techniques as aids to artificial hybridization in pistachio (*Pistacia* spp). Journal of Horticultural Science 60, 107-113.

Warmke, H. E. and M. A. Overman (1972). Cytoplasmic male sterility in *Sorghum*. Journal of Heredity 36, 103-108.

Weis, K. and V. S. Polito (1990). Cytochemistry and ultrastructure of the dehiscence zone of almond (*Prunus dulcis*) fruits. Canadian Journal of Botany 68, 63-72.

Went, V. and M. T. M. Willemse (1984). Fertilization. In 'Embryology of Angiosperms' (B. M. Johri, ed.), Springer-Verlag, New York.

Wet, E. D, P. J. Robbertse and H. T. Groeneveld (1990). The influence of temperature and boron on pollen germination in *Mangifera indica* L. Horticultural Abstracts 60, 7769.

Wetzstein, H. Y. and D. Sparks (1986). Flowering in pecan. Horticultural Reviews 8, 216-251.

Whitehouse, H. L. K. (1950). Multiple-allelomorph incompatibility of pollen and style in the evolution of the angiosperms. Annals of Botany, 54, 201-215.

Whitehouse, W. E. and C. L. Stone (1941). Some aspects of dichogamy and pollination in pistachio. Proceedings of the American Society for Horticultural Science 39, 95-100.

Whitehouse, W. E., E. J. Koch, L. E. Jones and C. L. Stone (1964). Influence of pollens from diverse *Pistacia* species on development of pistachio nuts. Journal of the American Society for Horticultural Science 84, 224-229.

Williams, E. G., R. Anderson, S. L. Mau and A. E. Clarke (1982). The isolated components of the *Prunus avium* L. style on *in vitro* growth of pollen tubes. Planta 156, 517-519.

Williams, E. G., J. L. Rouse, V. Kaul and B. F. Palser (1986). Overgrowth of pollen tubes in the embryo sac of rhododendron following inter specific pollination. Australian Journal of Botany 34, 314-323.

Wolper, J. A. and L. Ferguson (1990). Inflorescence bud retention in 'Kerman' pistachio: effects of defruiting date and branch size. HortScience 25, 919-921.

Woodbridge, C. G. (1973). Effect of rootstocks and interstock on nutrient levels in "Bartlett" pear leaves, on tree growth and on fruit. Journal of the American Society for Horticultural Science 98, 200-202.

Xu, R. Y., R. M. Chen, J. L. Chen, K. G. Wang and Z. L. Kang (1984). A study on the effect of the microelement boron on litchi production. Horticultural Abstracts 83, 2064.

Yamashita, K. and S. Tanimoto (1985). Studies on self-incompatibility of Hassaku (*Citrus hassaku* Hort. ex Tanaka). II. Glycoprotein composition in pistil soon after self pollination, and several trials to overcome self-incompatibility. Journal of the Japanese Society for Horticultural Science 54, 178-183.

Yamashita, K., H. Saita and N. Hashimoto (1990). Pollen-stigma interaction which might be critical to the gametophytic incompatibility of Japanese pear. Journal of the Japanese Society for Horticultural Science 59, 83-89.

Yano, F., S. Tokumasu and M. Kato (1975). The behaviour of pollen tubes and the developmental process of ovules in *Pelargonium*. Euphytica 24, 251-259.

Yates, I. E. and D. Sparks (1990). Three-year-old pecan pollen retains fertility. Journal of the American Society for Horticultural Science 115, 359-363.

Yates, I. E. and D. Sparks (1994). Anatomy differs for aborting and nonaborting pistilate flowers in pecan. Journal of the American Society for Horticultural Science 119, 949-955.

Yueh-Jiang, H., H. Okubo and K. Fujieda (1992). Pollen tube growth, fertilization and embryo development of *Camellia japonica* and *Chrysantha tuyama*. Journal of the Japanese Society for Horticultural Science 60, 955-961.

Zavada, M. S. (1984). Pollen wall development of *Austrobaileya maculata*. Botanical Gazette 145, 11-21.

Zhang, Y. X. and Y. Lespinasse (1991). Pollination with gamma irradiated pollen and development of fruits, seeds and parthenocarpic plants in apple. Euphytica 59, 101-109.

Zohary, M. (1952). A monographical study of the genus *Pistacia*. Palestine Journal of Botany Ser. 5, 187-238.

# 9 Appendix

**Published paper:**

Shuraki, Y. D. and M. Sedgley (1994). Effect of pistil age and pollen parent on pollen tube growth and fruit production of pistachio. Journal of Horticultural Science 69, 1019-1027.

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