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SEED PRODUCTION IN DIANTHUS
(Dianthus plumaris L.)

A thesis presented in partial fulfilment of the requirement for the
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ABSTRACT

Stigma receptivity, method of pollination and seed production under glass house and field conditions of ten Hammett Dianthus (*Dianthus plumaris* L.) cultivars were investigated in this study.

All of the cultivars had very low or nil receptivity to pollen at the time of flower opening. Stigma receptivity peaked on the third or fourth day after flower opening, and continued until the sixth day after flower opening before declining. However, the period of highest stigma receptivity was not affected by difference in pollen source (i.e. self or cross pollen).

All the cultivars produced highly viable pollen, suggesting that this was not a factor to causing low seed set. However, seed production potential was greatly affected by the pollen source; i.e. cultivars showed very high male selectivity. Cv. Crossover for example, produced 65 seeds per flower (57.8 % ovule fertility) in its best crossing combination (Crossover (♀) X Far North (♂), whereas the same cultivar produced only 16.6 seeds per flower (14.8 % ovule fertility) in its poorest crossing combination (Crossover (♀) X Spot On (♂) and 29 seeds per flower (26 % of ovule fertility) following self pollination.

Seed production of the ten cultivars under field conditions was very low, and no cultivar produced even a gram of seed per plant. However, hand pollination treatments (both cross and self) under glasshouse conditions produced significantly higher numbers of seeds than natural pollination. Although honey bees, bumble bees, and some flies were observed visiting the field trial, the accepted natural pollinators of *Dianthus* were not found. The implication of these variables in relation to the potential for commercial *Dianthus* seed production is discussed.

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CHAPTER ONE

1.1 INTRODUCTION

"The fairest flowers o' the season are the carnations" (Shakespeare 1601) .

The garden carnation (*Dianthus plumaris* L.) is variously known as dianthus, pink, garden pink, and grass pink (Allwood, 1954). Before being called carnations, they were known as carnarine, the cornontion, the clove gillyflower and by many other names. In England they became known as the divine flower because of their beauty and attractive fragrance (Kingman, 1983).

Popularity as a bedding or pot plant has largely driven the demand for commercial seed production. Unfortunately, erratic and often poor seed setting has been a problem for both floriculturilists and plant breeders. Under natural conditions the seed set of *Dianthus* species is very poor. On average more than 50% of the flowers (of *Dianthus caryophyllus* L.) are sterile (Shafi Bhat et al., 1991).

Dianthus was known to the world as far back as 300 B.C. as a five petaled single flower about one inch in diameter and pinkish in colour (Allwood, 1954). The plant was originally distributed in its wild state over the southern half of Europe, particularly in France and northern Italy, was introduced to England and from there to America by English immigrants (Kingman, 1983).

The development of American floriculture has contributed greatly to carnation production. For example the introduction of the world famous new red carnation "William Sam" during the second world war was a big step forward to the carnation industry (Kingman, 1983).

Carnation (*Dianthus plumaris* L.) belongs to the family Caryophyllaceae which is characterised by a delightful spicy clove-like fragrance, and conspicuous flowers of different colours. Because of the very elegant flowers, they are very impressive when planted in herbaceous borders, rock gardens or in pots.

Commercial carnations today are not nature's products, and many of them are totally different to the original plant (Allwood, 1954). Although they have been selected and cultivated by breeders for several hundred years, the plants are often propagated by layering and from cuttings to maintain the characteristics achieved by breeding and selection (Mansfield, 1951; Allwood, 1954). Selection criteria for *Dianthus* breeding include the flower colour, petal number and resistance to disease etc. (Hammett, 1996-pers.comm.). However, the literature does not suggest that seed production ability is included as a character in the selection process. This suggests that any commercial large scale seed production system should begin by testing the seed setting ability of existing clones. This is essential if there is to be any improvement in the commercial viability of the carnation industry.

Dianthus species are highly prone to virus infection. Since viruses are transmitted through cuttings and layering (Allwood, 1954; Holdings and Stone, 1972; Arthur, 1984) the maintenance of virus free stocks by vegetative means is practically difficult. Although techniques such as heat treatment and meristem-tip culture have been developed to produce virus free plants (Arthur, 1984) they need very sophisticated laboratory conditions to achieve good results. A further problem exists in that the clones derived from different meristem-tip cultures, even from adjacent buds on the same stem, can differ considerably in their horticultural characteristics due to mutation (Holdings and Stone, 1972). Even though it is possible to maintain virus-free stocks in this way, the maintenance of crop characteristics at the same time is not always reliable.

Although carnations are generally propagated by cuttings, the rooting ability of border carnations is very poor (Mansfield, 1951; Hobbs, 1997) making them less easy to propagate in this way. Also, the transport of cutting materials for propagation, and later the rooted cuttings is bulky (Arthur, 1984), generally resulting in plants propagated in this way being more expensive than seeds.

In *Dianthus*, the natural method of propagation from seeds does not transmit viruses (Holdings and Stone, 1963, 1965 and 1972; Hobbs, 1997) suggesting seed is a more reliable form of propagation despite the fact that many of the hybrid carnations show poor seed set under natural conditions (Mansfield, 1951; Shafi Bhat et al., 1991).

An essential feature for seed setting is successful pollination and fertilisation. Therefore, information on various aspects of morphology and floral biology such as time of anthesis and pollen fertility, stigma receptivity, etc. are essential prerequisites for any hybridisation and /or seed production programme. Studies on these aspects are likely to be helpful in explaining causes of low seed set and yield.

Hampton and Phetpradap (1992) have detailed information about the potential of flower seed production in New Zealand. According to them the New Zealand seed industry has a number of strengths including well educated and innovative growers, a highly developed infrastructure, and suitable agroclimatic conditions which provide vast scope for the production of a wide range of flower seeds of both tropical and temperate origin. Dr. K. R. W. Hammett (the pioneer private breeder of border carnation and Dahlia in New Zealand) and the interest of seed companies (eg: Excell Seeds Ltd) in producing flower seeds are also a good sign for future domestic and export markets. As flower seeds are in great demand in western countries, entering into an international flower seed market can be highly remunerative (Desai et al., 1997).

Very little published information can be found on pollination and seed setting in carnation (Shafi Bath, et al., 1991) especially *Dianthus plumaris*, so it is not possible to refer to previous publications. Though some seed companies produce seeds commercially, all their findings are kept "in house" due to the highly competitive nature of the flower seed production business (Hampton and Phetpradap, 1992). The present study was therefore planned to:

1. obtain information on the floral biology of *Dianthus*;
2. ascertain the effect of various pollination methods (selfing and crossing) on seed yield;

3. identify the likely mode and extent of pollination in the field;
and
4. determine the potential for commercial seed production under field conditions.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 HISTORY AND GENERAL BACKGROUND

Dianthus plants belong almost exclusively to the Old World. The genus consists of about 300 species (Perry and Greenwood, 1972; Selby, 1996), belongs to the family Caryophyllaceae (Greek, clove-leafed), and has been under cultivation in Europe for over two thousand years (Allwood, 1954). The generic name *Dianthus*, is derived from the Greek *Dios*; divine, and *anthos*; a flower (Allwood, 1954). The common name "Carnation" refers to any of the cultivated forms of *Dianthus* (Mansfield, 1951), although some authors today use the name to define the commercial forms of *Dianthus* species only.

Dianthus are valued as a floral or garden crop for its attractive flowers, with several varieties being grown under controlled conditions for cut flower production. The garden carnations (pinks) are used for flower beds, borders, and cut flowers as well. Most garden types are hardier than floral types (Desai et al., 1997) and are therefore easier to grow (Allwood, 1954).

The name "pink" causes endless confusion today, because it has nothing to do with the colour of the flower, as one would naturally suppose. It is a mediæval English word meaning an "eye or small eye" and was used this way by Shakespeare (Allwood, 1954).

2.2 GENERAL DESCRIPTION AND BOTANY

Dianthus plumarius (wild or garden pink), was first recorded in 1753. This was the origin of all the double-flowered pinks and the varieties so extensively cultivated in gardens. The plants are hardy and produce scented flowers. The Linnæan specific name

plumarius describes the plume-like segmentation of the petals (Allwood, 1954). Over time, due to abundant breeding and selection, the original plant has become hard to find in gardens and commercial nurseries. Considerable variation now exists in the size of the plant as well as flower colour etc.

Bailey (1968) reported that the carnation has been appreciated by many breeders and has been modified by breeding so that the flowers are of variable size, form and colour. It is a long way from the carnations of early days to the perfectly formed full blooms found today. The filling out of the blooms has evolved gradually and has been assisted by cross-pollination and selection by carnation breeders over many years from the native carnation of Mediterranean origin which flowers in early summer (Perry and Greenwood, 1972; Bunt et al., 1985).

The plant is a perennial with branching stems, with linear grass-like leaves in opposite and decussate pairs (Perry and Greenwood, 1972). This explains the use of the name "grass pinks" (Hawthorn and Pollard, 1954). Plant height varies from <5 cm. to 30-35cm. *Dianthus* produces perfect flowers with a superior ovary which is one-celled and has many ovules and two styles. The stamens are borne in two whorls of five, the outer whorl being superimposed on the sepals and the inner whorl on the petals (Thompson, 1942, Hawthorn and Pollard, 1954). *Dianthus* flowers are pedicellate, complete, hermaphrodite, actinomorphic, pentamerous, hypogynous and borne in terminal or solitary regions of the growing stems. Flower colour varies and includes red, pink, and white or combinations of these. Petal number also varies from 5 to 50 according to species or clone (Shafi Bhat et al., 1991). Petal margins may be either smooth or serrated (Perry and Greenwood, 1972).

2.3 BORDER CARNATIONS

Among the true *Dianthus* species, there are quite a reasonable proportion of interesting and pretty garden plants. However, there are also quite a large number which are intriguing only to the botanist (Mansfield, 1951). During the sixteenth century *Dianthus* formed one of the principal flowers of English gardens, and it is from this stock, with

occasional assistance of importation from the continent, (more especially in the case of yellow forms) that the British carnation section has sprung (Allwood, 1954).

Border carnations are front of the border plants, seldom much more than 30 cm tall when in bloom. The plant has a mat of narrow leaves, usually blue-green, which in milder regions are attractive all year round (Harper and McGourty, 1985). There was a strong interest in border carnations from World War II until the seventies (Hammet, 1997), but since then interest in them has slowly declined.

2.4 AGRONOMIC REQUIREMENTS

2.4.1 Soil

Dianthus prefer well-drained well-aerated fertile soils with plenty of sunshine (Perry and Greenwood, 1972). Sandy loams and alluvial soils with a pH of 6.3 - 6.8 are ideal (Desai et al., 1997). *Dianthus* does not grow well in saline soils, as increased salinity reduces flower numbers, weight and the size of the flower (Bass et al, 1995).

2.4.2 Nutrient requirements

With the recent development of commercial production of carnations, many studies have been done on the symptoms of mineral deficiencies in cultivated and green house crops. Under experimental conditions, clear visual deficiency symptoms of N and K have been observed, but not very clearly for P and Ca deficiencies (Medina, 1992). However, plants are very sensitive to deficiencies of boron and other minor elements (Desai et al., 1997). Holly and Baker (1963) reported that boron deficiency is common in carnation growing areas around the world, especially where the crop has been cultivated in the same soils for many years.

Experimental evidence has shown that different cultivars have different production abilities. They absorb different amounts of the same nutrients even when they are grown under the same environmental conditions. Among the two carnation cultivars Barbra and Starlight, the Barbra sap ionic balance was 1 compared with only 0.8 in Starlight (Zoronoza et al, 1989).

2.4.2.1 Nitrogen

As with many other plants, low nitrogen (N) supply hardens carnations (Holly and Baker, 1963) and causes low flower stem emission, followed by weak, brittle stems with shortened internodes. It also reduces the number of flowers, and flower size, and results in thin and narrow leaves (Medina, 1992). Conversely, the addition of exceptionally high amounts of nitrogen results in plants which become chlorotic, with burned spots on the foliage. Holly and Baker (1963) reported that green house grown carnations gave best yield and quality with two to three kilograms of actual N per 9 m² per year.

2.4.2.2 Potassium

Carnation plants use potassium (K) in large amounts, second only to nitrogen, Holly and Baker (1963) suggested that 1.25 to 1.5 kg of actual K per 9 m² per year is necessary for green house grown carnations. When potassium is lacking, plants become thin and weak. Lower foliage burns, seed ripens prematurely, and necrotic spots appear in the middle of aged leaves. The top leaves under the flower are often scorched or have dead spots on them (Holly and Baker, 1963; Medina, 1992).

2.4.2.3 Phosphorus

Phosphorus (P) is required in lesser quantities than either nitrogen or potassium. Holly and Baker (1963) have recommended 2.5 kg of superphosphate per 9 m² per year for green house grown carnations. Due to the nature of phosphorus, it is easily tied up or made unavailable to plants when the pH of the soil is highly alkaline.

Phosphorus hunger results in stunted or thin carnation plants. Foliage colour is not usually changed, but leaves become narrow, and the flowers small. Plant growth is greatly reduced. Phosphorus deficiency reduces flower production and tends to delay harvest (Medina, 1992). Despite this, no effect on flower yield has been observed from extremely high amounts of phosphorus (Holly and Baker, 1963).

2.5 EFFECT OF PHOTOPERIOD ON GROWTH AND FLOWERING

Flowering in many plants is not solely determined by the genetic composition of the plant. It is controlled by environmental factors which interact with genetic constitution in a specific manner (FAO, 1961; Zeevaart, 1976). For most crops, light and temperature are the primary factors controlling the change from vegetative to the reproductive stage. Short or long photoperiods, during the transition of the apex from the vegetative to the reproductive stage of development, maximise total flower production by increasing lateral shoot production on the primary shoot and by hastening floral initiation on lateral shoots (Healy and Wilkins, 1983)

Blake (1955) first showed that carnations were a long day plant, a character which has been exploited in glass house production. Long days detrimentally inhibit lateral branching and reduce flower production (Bunt et al, 1981; Healy, 1982). However, several workers have found that the use of a short photoperiod during the transition of the meristem from vegetative to reproductive development increases lateral branching even when a long photoperiod is subsequently applied. Healy and Wilkins (1983) found that there is only a narrow window for the short photoperiod essential for the promotion of lateral branching. If it is too long floral initiation may be inhibited.

2.5.1 Light intensity

Flower initiation is strongly affected by the daily intensity of incident radiant energy and delays in flower initiation caused by low intensity ($<3 \text{ mc day}^{-1}$) have been reported both under glasshouse (Harris and Harris, 1962; Bunt, 1973) and controlled

Environmental conditions (Abou Dahab, 1967). But under normal field conditions, flower initiation occurs earlier with the initiation of fewer leaf pairs before the flower is produced as the daily light increases (Abou Dahab, 1967; Bunt, 1973). Bunt (1973) concluded that the mean daily radiation flux density had more effect on flower initiation than daylength under natural conditions. At high daily radiation intensity, most if not all of the primary lateral shoots produced by young "stopped" or "pinched" carnation plants initiate flowers contemporaneously, resulting in a "flush" of flowers. Under low daily radiation intensity, flower initiation in the young shoots is spread over a longer period (Bunt, 1979).

2.5.2 Vernalization

Smith, (1989) reported that field planted hybrid pinks (crosses between *D. caryophyllus* x *D. plumaris*) in U.K. gave the highest flower yield and the highest vegetative growth from an August planting compared to a February or April planting. Hybrid pinks initiate flowering in long days and so remain vegetative in the autumn and winter. This indicates the importance of producing a well established plant in the autumn and growing it through winter and spring. Most carnation species require vernalization in order to flower (Cockshull, 1985).

2.6 PROPAGATION

The propagation of carnations can take place in three ways: from seeds; by layering or from cuttings. In the case of *Dianthus* species the most natural method of propagation is from seed, because *Dianthus* does not produce runners or any other vegetatively propagated organs (Mansfield, 1951). However though many plants reproduce from seed true to character, for hybrids which are clones, the seed cannot be relied upon to consistently produce plants resembling the seed parent. So hybrid carnations are only propagated by cuttings or layering (Mansfield, 1951).

2.6.1 Seeds

Seeds may be stored, usually in a cool dry place, through the winter months. But as a rule seedsmen advise to "sow as soon as ripe" suggesting this is by far the soundest and best method of ensuring reasonable success. Seeds can be sown thinly on a suitable soil medium. They should be lightly covered with the same soil by about three times the depth of the thickness of the seed (Mansfield, 1951).

2.6.2 Layering

As mentioned before, most *Dianthus* species today are breeder's selections. Hence the maintenance of the characters of seed bearing plants under open pollination is impracticable. Therefore, commercially they are cloned by layering or by cuttings (Mansfield, 1951; Allwood, 1954). The process of layering depends for its success upon the interruption of the flow of the sap of the plant, which encourages the quick formation of roots. The normal time taken for a good layer to root is from four to six weeks (Mansfield, 1951).

The ideal medium for rooting is a light, sandy soil mixed with compost. If the soil does not meet this requirement, it is advisable that five centimeters of the surface soil around the plant should be removed and compost placed around it. The layer should be bent down into the soil with an ordinary layering pin. Care should be taken to ensure that the tongue is not broken while it is being bent. As soon as the layer forms new roots, it is ready to be potted.

2.6.3 Cuttings

Cuttings can be taken from young growing shoots. They will be too small in mid winter in field grown carnations, but too large at some other seasons (Holly and Baker, 1963). This really does not apply to glasshouse grown plants.

Cuttings should contain at least six mature leaves: those with the 2-4 leaves at the bottom end removed are good for planting. As a rule cuttings will take from three to five weeks to root, and it is most important that during the early stages they should be adequately watered. Cuttings produced at low temperature are better than those produced at a higher temperature, because they root easily and have higher resistance to fungal (eg: fusarium wilt) diseases (Mansfield, 1951).

2.7 WEED CONTROL

Weeds compete with the crop for water, nutrients and space and ultimately reduce yield. It becomes an even a greater problem when the weed seeds are harvested along with the crop, becoming a part of the seed lot after threshing. In many cases these weed seeds cannot or can only be partially removed, most often resulting in the loss of good seed. The cleaning process entails much time and money so weed control in the field is of the utmost importance (Vis, 1980).

Weed control in field grown flowers is desirable for optimum flower yield and quality (Lamont and O'Connell, 1986). Most often this is accomplished by laborious and costly hand weeding. Some weeds such as bitter cress and willow herb have short life cycles and are difficult to control by hand weeding (Lamont, 1985). In Australia it has been estimated that the labour cost for manual weeding can exceed \$10,000 per hectare, depending on the severity of weed infestation (Lamont, 1985). Since many flower plants are small and slow growing, it is highly desirable to use land that is relatively free of weeds (Hawthorn and Pollard, 1954)

There are a number of options available for weed control in field grown flower crops today. Inter-row weed control can be achieved by cultivation, but with each cultivation, more weed seeds are exposed and this also make new seed beds for weed seeds to grow among the crop plants. Further there is an added risk of cultivation damage to the crop plants. Inter row weed control achieved by contact herbicides can prevent this problem (Vis, 1980).

Weed seed contamination through nursery soils is also a problem in field grown flowers. Thus, weed control is a necessary practice at the nursery stage too. Weeds present in container nursery crops can reduce plant density by 30 to 60% when compared to plants without weeds (Jacobson and Klett, 1988). So the use of herbicides to control weeds in nursery containers is equally important for field weed control in flower crops.

2.7.1 Herbicide formulation and phytotoxicity

The herbicides which are widely use to control weeds in all type of field crops are manufactured with different formulations, and for some crops, the chemical formulation of the herbicide can affect crop safety. Watkins and Heggens (1982) reported several ornamental plants were damaged when oxadiazon and oxyfluorfen were applied to ornamental plants as an emulsifiable concentrate formulation, but Creager (1982) observed little phytotoxicity when granular formulations of these same herbicides were applied to ornamental plants.

Several other workers have reported effective and safe weed control in ornamental plants using pre-emergent herbicides. Currey et al., (1977) found that granular forms of oryzalin, trifluralin, alachlor, napropamide and oxadiazone provided excellent weed control with no phytotoxicity. Herbicides applied as granules minimise contact of the active ingredient with the foliage, and crop damage can be expected to be less.

Herbicides often produce no significant reduction in shoot weight of transplanted species, but may inhibit either germination or early growth in the same species when direct seeded. For example chloroxaron, napropanide and oryzalin are not phytotoxic to transplants of Sweet William but severely retard the growth of direct seeded plants (Lamont, 1986).

2.7.2 Herbicide action on transplants

Application of pre-emergence herbicides such as simazine, oxydiazole, oxyfluorfen, and alachlor before transplanting carnations is advisable, and can give prolonged weed

control. These herbicides have low water solubilities and are considered to be safe to use on carnations (Lamont, 1986), although they can cause leaf burn if used at high rates. Application rates of oxydiazole 4.0 kg, oxyfluorfen 2.0 l, and alochlor 2.25 l per hectare are recommended for carnations.

2.8 HARVESTING AND POSTHARVEST OPERATIONS

This is the last stage of seed production and one in which many mistakes can be made, leading to spoiling of the seed quality and reduction or complete loss of yield, at a time when much money has already been spent (Vis, 1980). If crops are harvested too early, there may be a high percentage of immature seeds which will have to be removed later by seed cleaning machines. On the other hand if crops are harvested too late, some yield may be lost through shattering. Since flower crops are highly prone to shattering, correct time of harvest is vitally important (Salunkhe et al., 1987)

As with many other flower crops, carnation seeds are harvested before shattering begins. The pods are usually picked manually and dried in the shade. The seeds are extracted by hand or by threshing. After cleaning, seeds are stored in a dry, cool place in an air-tight container (Desai et al., 1997).

2.9 FLOWER SEED INDUSTRY

2.9.1 History

Even though flower gardening has a long history, the history of commercial flower seed production only goes back to the second half of last century. It was at that time that seed companies in Western Europe (France, Germany, UK, and Netherlands), started growing and marketing flower seeds species in addition to their production and commercialisation of vegetable seeds (Southward, 1997).

The production expansion occurred slowly between the wars until, following World War II, the production of flower seeds became world-wide, but was restricted locally to those regions with the most suitable climate and soil conditions for particular species (Vis, 1980). Today it has undergone unrestricted revolutionary growth in terms of both number of crops and varieties as well as production. This phenomenal development is the result of technological advancements in the production of flower seeds and developments in the areas of production, packaging, handling, transportation, and marketing of flower and ornamental plants all over the world (Desai et al., 1997).

2.9.2 Areas of production

Though flower seed production is carried out in many countries today, it is mainly confined to particular types of areas, where preferably as many species as possible can be produced and where climatic conditions give outstanding quality, particularly by constantly delivering a low rainfall at the time of flowering and maturing period. Also such areas have a rather mild climate without extremes of temperature, affording crops the correct environment for the best growing conditions. This is important since the length of the seed maturity period may be quite long, and harvest can be delayed to allow seeds to develop to full size without the risk of having to harvest too early (Vis, 1980).

2.9.3 Seed production

It is of paramount importance that the greatest care should be taken over production, as only seeds harvested under good conditions can be expected to have a high germination and to subsequently store well. In addition, the cost expended on an inferior seed lot is almost equal to that expended on a good lot. The area for commercial flower seed production typically comprises relatively small area plantings. The cost of production is also high, with 40% of the cost being for field labour, which must be of such manual skill that good work approaches craft status. Flower seed production is a more complicated process than crop seed production. Each species of flower crop grown for seed has its own specific planting time, cultural problems, pollination, harvesting, and storage requirements (Boder, 1961)

According to Desai et al., (1997) four types of flower seeds are produced commercially.

1. **Mixture of open pollinated seeds.**- This refers to a combination of more than one colour or plant type in each seed lot. Open pollination commonly produces natural mixtures in several flower crops. However it is not possible to obtain true-to-type progenies from open pollination.
2. **Pure inbred lines** - These are produced by repeated selfing to obtain uniform progenies. They are the basic genetic material for F₁ hybrid seed production
3. **F₁ hybrids** - Results from the crossing of two inbred lines.
4. **F₂ hybrids** - They are produced by selfing F₁ hybrids. They are less uniform than F₁ hybrids but they are cheaper to produce.

2.9.4 Climate

The climate is a major determining factor in the successful production of flower seeds. Most crops require a long growing season free from late spring and early autumn frosts, although flower crops vary in the extent to which they can withstand cool temperatures and frost. Mild climates with moderate rainfall favour the growth and development of most flowers and ornamental crops (Desai et al., 1997). According to Vis (1981) it is not the number of solar hours per annum which is of importance but their spread over the months. Similarly with rain, as although in some areas the total annual rainfall may be unacceptably high, a reliable presence of a rain free period during flowering and seed maturation still may be favourable for the crop. In areas with such climatic conditions it is possible to sow many crops directly in the field and to mature a satisfactory crop in the same season, thereby reducing the cost of production (Hawthorn and Pollard, 1954).

Seed recovery at harvest is a big problem in flower seed production. Although, dry weather conditions are a necessary prerequisite of producing high quality flower seeds, many crops tend to shatter seeds very easily. In the United States much of the flower seed area is located in California and often in areas adjacent to the ocean, or at least where the humidity tends to be higher at harvest time. These conditions are necessary to

prevent (or greatly reduce) shattering in a number of the flower seed crops (Hawthorn and Pollard, 1954).

2.9.5 Soil fertility and moisture

Soil fertility and texture have a great influence on the establishment of any crop. Most flower seeds prefer soils with high organic matter content, since it improves soil aeration and maintains light texture of the soil. Also it helps better plant rooting, which enables better absorption of nutrients from the soil. As a general rule seed crops are favourably influenced by: soil nutrient status, with no deficiency of either phosphorus or potassium, and suitable water conditions with either drainage or irrigation as required (Vis, 1980)

2.9.6 Sowing and planting

This is a point that may often be taken for granted but it is of the utmost importance to sow and plant at the optimum time. In the case of a late sowing, time can not be made up by forcing the crop and such plants become weak with a consequential influence on their seed setting (Vis, 1980). This is of a particular importance with biennials and perennials, because some of them need to undergo a minimum vegetative growing period before they start flowering. On the other hand, adjusting to the ideal time for sowing and transplanting avoids delays in the harvest and reduces the risk of less favourable late season weather conditions.

2.9.7 Weed control

Production of high quality seed requires thorough control of weeds in the plot. Many flower crops are very slow growing compared to other crops, so weeds can easily compete with them (Harrington, 1996). In addition to reducing seed yield, weeds are often a source of contamination by way of mixture at the time of harvest. Weeds in the seed plot or nearby areas may also harbour a number of pests and diseases. Effective control of weeds at all stages of crop growth is essential, to ensure they do not flower or

set seed. Planting seed crops into clean, fallow land or following a suitable crop rotation is generally recommended to keep weeds at a minimum. Hand weeding operations, or chemical weed control may be necessary (Desai et al, 1997).

2.9.8 Disease control

Just as in other types of seed crops, disease control is of paramount importance. In particular, fungal diseases and insect pests can have an extremely detrimental effect on seed yield and quality. The difficulty is that there are no specific chemicals registered as safe for use on flower seed crops, and consequently disease control must be carried out using products developed for use on other crops. Consultation with the local advisory services and trial authorities can be a great help (Vis, 1980).

2.10 SEED PRODUCTION METHODS

2.10.1 Seed to seed method

The seed to seed method involves sowing seeds directly in the field where the crop is to be produced. This eliminates the work and expense of raising of cuttings. Except for multiplication seeds, critical rouging is also not necessary in this method. As the production cost is low, most of the market seed is produced by the seed to seed method. Generally the seed is sown in the late spring or early summer depending upon the cultivar and locality. The planting date is adjusted so that the crop will attain sufficient growth, and seed can be harvested before the onset of winter (Hawthorn and Pollard, 1954). The only basic requirement in this method is to sow good quality seed that will give high field emergence.

2.10.2 Clone to seed method

This is slightly different from the seed to seed system because it involves the preparation of rooted cuttings in advance to plant in the field with the onset of suitable weather. Up to this stage the method of culture is much like that followed in the production of market plants (Hawthorn and Pollard, 1954). No rouging is necessary because the plants are very uniform due to their clonal source. But the genetic composition of the seeds produced may vary according to the method of pollination and the genetical stability of the clone (Desai et al., 1997).

The breeding of new *Dianthus* cultivars is not seed production oriented (Hammett pers com, 1996), so in order to get uniform progenies most of today's *Dianthus* have to have their homozygosity improved by inbreeding. In most vegetables and flowers, the inbreeding depression increases with each generation of an inbred line's maintenance, resulting in reduced vigour and seed yield per plant. Reduced vigour also leads to increased winter death in crops that have to be over-wintered in the field (Wills and North, 1978). This suggests that the clone to seed method will be more appropriate for multiplying *Dianthus*, because it avoids these problems.

2.11 SEX IN FLOWERS

A flower is defined as "a collection of essential organs, of stamens or pistils or both within a protective envelope". It is a specialised structure, in which the same tissue cannot produce both male and female organs and therefore a space barrier exists between them. It is for this reason that pollination is necessary (Percival, 1965). The gap may be greater or less depending upon the distribution of sex in the species. Thompson (1942) has clearly explained the formation of different floral parts of the Caryophyllaceae family and their possible role in pollination.

Flowers are among the most complex and diversified objects in the plant kingdom. Despite this there is much common ground for a large measure of unity and regularity in their structure (Proctor et al., 1996). So a study of floral structure helps to understand how flowers work and their requirements for pollination.

2.12 POLLINATION OF FLOWERING PLANTS

The transfer of pollen from the anther to the stigma is called pollination (Cornquest, 1968). Both angiosperm and gymnosperm plants need pollination for seed set. The plants of these species are either self-fertile, self-infertile or partially both. Self-fertile plants set fruit or seeds with their own pollen. Sometimes self-fertile species are automatically pollinated with the pollen produced by the same flower (auto-pollination). But self-infertile (cross pollinated) plants always need to receive pollen from other plants of the same species for seed set. Flowers of cross pollinated plants are so constructed that some external agent eg. wind, water or insects is needed to transfer pollen from anthers to stigmas (Free, 1993).

2.12.1 Self fertilization

Many plants are capable of self-fertilization, despite an enormous range of adaptations to attract pollinators and to disperse pollen onto the stigmas of other plants. Self fertilization is common in many plants, and for some it is the norm (Proctor et al., 1996). In such plants their own pollen can reach the ovules of the same individual. The ability of plants to produce seeds without the aid of any external pollinating agent is often an advantage; the production of any seed, even despite the possibility of producing disadvantageous mutations, is better than producing no seed at all. In the majority of British plants, perhaps two-thirds of the flowering species are capable of some or complete self-fertilisation, and this is probably true of most species in temperate regions (Proctor et al., 1996). For example, sweet pea is a completely self pollinated plant but it carries very scented and conspicuous petals.

Generally self-pollinating species have flowers which are smaller in all their parts than those requiring cross-pollination. The flowers are also usually fewer in number with less or no nectar, fewer pollen grains and ovules, and with less defined colour and guide marks (Proctor et al., 1996).

2.12.2 Partial self-fertilisation

Many species which do not automatically self-pollinate are, nevertheless, self-fertile. In such plants both selfed pollen and crossed pollen can land on the stigma at the same time. On such occasions the pollen tubes deriving from cross-pollination grow more quickly down the style, and outcompete the slower growing pollen tubes arising from self-pollination (Stephenson and Bertin, 1983). In some species, self-fertilisation serves as a 'back up' if cross-pollination fails (Proctor et al., 1996). If no crossed pollen reaches the stigma, it will then self-fertilise and produce seeds. One example of such a back-up system is shown in bellflowers (*Campanula* spp.) where the anthers mature first and the stigma pushes up through the anthers before opening. As they mature, the stigma lobes diverge, eventually totally reflecting so that they come into contact with any of the flower's own pollen remaining on the style. Thus, self-pollination can take place if cross-pollination fails (Faegri and van der Pijl, 1979). In some species, rain may also occasionally enhance self-pollination within a flower (Hagerup, 1950; Catling, 1980).

2.12.3 Cross pollination

Although in most flowering plants some selfing is possible, occasional or regular cross-fertilisation resulting from a transfer of pollen between two individuals is favoured. Cross-fertilisation provides at least two vital advantages. Firstly, the new plants bear a new combination of genes, so variation is reduced and the population can potentially adapt to a new changing environment. The second, and the more immediate advantage, is that each plant obtains a set of chromosomes from each parental plant. Having two different parents means that a mutation on one chromosome is unlikely to be present on the corresponding chromosome of the other plant. This combination of genes reduces the

risk of producing lethal mutants. Similarly this increases the chance of producing substances that are beneficial to the health of new plants.

So, flowering plants have developed various strategies to ensure cross pollination wherever possible. In hermaphroditic flowers the provision for cross-pollination may be made within the individual flower in one of two ways; either the stamens and stigmas are widely separated in space (style may be very long and the stamens very short or stamens may extend out of the corolla), or they ripen at different times (Cornquest, 1968). Both of these features may be present in one flower (Perry and Greenwood, 1972). *Dianthus* is generally a cross pollinated plant and it falls into the second group; ie cross pollination is favoured by the maturation of the anthers before the stigmatic surface become receptive (Buell, 1952).

2.13 ANEMOPHILOUS & ENTOMOPHILOUS PLANTS

Plant species can be categorised according to the method of pollen transportation for pollination. The pollen carrier of anemophilous plants is wind, compared with insects in entomophilous plants. Both types of plants have special modifications to increase the chances of receiving pollen for pollination (Percival, 1965).

Some plants are well adapted for pollination and they use both the selfing and crossing systems to reach their goal. Grasses are totally anemophilous, whereas orchids are almost totally entomophilous. Between these two extremes there are many species sharing both pollination types. *Erica orborea* is entomophilous at the onset of anthesis, with bees being attracted by nectar present in the disk at the base of the ovary. This phase lasts only two days, after which the anther filaments lengthen and any pollen grains still present are dispersed by wind (Pacini, 1992).

Pollination in flowering plants may be preferentially anemophilous or entomophilous, depending on where the plant originated, and the availability of natural pollinators. Generally anemophily decreases from the poles to the tropics, whereas entomophily decreases or is restricted to warm seasons from the tropics to the poles. Anemophilous species normally produce excessive amounts of pollen (Percival, 1965), while entomophilous species advertise many rewards (pollen, nectar, conspicuous flowers) to attract insects and other pollinating agents (Free, 1993).

2.14 FLORAL BIOLOGY AND POLLINATION ECOLOGY

2.14.1 Floral and apical morphology

In carnation, Cheng and Langhans (1971) defined four stages in the transformation of the shoot apex from the vegetative to the reproductive form. The first stage involves a rapid increase in the size of the apex, which becomes dome-shaped. This is followed by the initiation and growth of sepals - the apex now being double the size of a vegetative apex. In the third stage, the petals, stamen, and carpel primordia are initiated concurrently. In the final stage, the differentiation and development of the flower parts occurs. The apex is then at least four times the size of a vegetative apex. The development time from stage one to four being about 34 days (Bunt et al., 1985).

2.14.2 The calyx

The biology of the calyx depends on its role in the biology of the flower as a whole. Many botanists think the calyx protects the flower in the bud stage, but it may well also have a protective function at other stages of flowering. Its structure also differs depending on what use it is put to. In the Caryophyllaceae it is effective in preventing robbing of the nectar. However it also has another important function, that of preserving

the imbrication of the clawed petals and of holding them erect and close together, so that they form a 'tube'. The calyx is also retained in the fruiting stage (Percival, 1965).

2.14.3 The petals

Petals are the distinguishing mark of a flower to the layman, and yet they are not an essential part of it. They are inconspicuous or absent in many anemophilous flowers, and are the foremost feature of entomophilous and ornithophilous flowers. Despite this variable role, they have many attributes and may perform several functions. The chief role of the petals is the attraction of pollinators. Enhancing attributes of scent, colour and shape are all associated with this.

Petals and stamens have a somewhat similar nature and biology. They are both spherical organs, usually non-herbaceous, and generally more delicately textured than sepals or carpels. In many flowers, intermediate stages will be found between the two; petals bearing a pollen sac or petaloid stamens may be seen very commonly (Percival, 1965).

2.14.4 Structure of the pistils

The pistil is composed of two fused carpels with free central placentation and two separate styles. The placenta is a columnar structure extending about two third of the length of the maturing fruit. The ovules are arranged more or less regularly along its surface in four vertical rows. In the selected Hammett *Dianthus* the ovule number varies from 15-35 in a row. The microphytes of the ovules are directed towards the nearer of the two columns of transmission tissue located on the opposite side of the placenta (Buell, 1952)

2.14.5 Pollination ecology

The morphology of *Dianthus* strongly suggests it is insect pollinated, possibly by a Lepidoptera (Percival, 1965). However, very little information is reported in the literature about the pollinator /or pollinators and their activity. Müller (1981) stated that

Dianthus superbis (L.) is pollinated by day flying hawkmoths (*Maccroglossum atellatarum*). Later Oberdorfer (1983) stated it is also pollinated by butterflies. However more recent work by Erhardt (1991) has shown that even the butterfly with the longest proboscis (18 mm) in Europe is still incapable of reaching the hidden nectar of *Dianthus superbis*. He did find, however, that some other night flying hawkmoths pollinate the flower. Despite this, the natural pollinators of other *Dianthus* species are, as yet, unknown.

2.15 ANTHESIS

The opening of anthers to expose pollen is called anthesis, the duration of the process varying according to the pollination system (Pacini, 1992). It is very short in anemophilous species such as grasses (Dowding, 1987) whereas it is longer in ornamental plants. In orchids it can last for around 60 days (Clifford and Owens, 1988). In most cases pollen grains maintain their viability after anthesis, but in some species pollen viability decreases sharply during anthesis. Both pollen receptivity and anthesis pattern varies from one plant species to other. In species such as broad bean (*Vicia faba*), there are seven opening and closing rhythms of the flower (Perrymam and Marcellos, 1988) Bath et al. (1991) showed in *Dianthus caryophyllus* dehiscence started immediately after flower opening (8.00 a.m.); maximum dehiscence occurred between 10 a.m. and 11 a.m. and minimum anther dehiscence between 4 - 5 p.m.. The possible reason for the opening of maximum number of flowers during this interval (Fig. 2.1) may be attributed to the factors favouring phytochrome activity that results in anthesis, because in typical herbacious plants, the time of flower initiation and anthesis are controlled by phytochrome (Cathy, 1964).

Pollination occurs when the anthers open to expose the pollen, and the stigma is receptive. Anthers will dehisce over a considerable range of temperature and humidity, but both these factors may become limiting. In many plants a steady rise in the number of stamens ripening and presenting pollen occurs with increasing temperature (Percival 1965). Maturity of the anther is a necessary prerequisite for dehiscence. If an anther is fully "ripe" it will dehisce even if the surrounding air is virtually saturated with moisture. If it is not fully mature, hot sun, dry air or wind will not bring about dehiscence (Percival, 1965).

2.16. POLLEN

Pollen is a highly nutritious material. It contains 16- 30 % or more of protein, 1-7 % starch, 0-15 % free sugars and 3-10 % of fat by dry weight, as well as a significant amount of phosphate and other essential ingredients of living cells (Harbone, 1993). It is one of the staple foods of anthophilous insects and also their major source of protein for nectar (Percival, 1965).

2.16.1 Pollen formation

Pollen is the most important factor affecting the likely success of the process of pollination. Both gymnosperm and angiosperm plants produce pollen in stamens which germinate and develop to make contact with the egg cell, effecting pollination. Viable pollen is pollen which delivers two male gametes to the embryo sac. The viability of pollen grains varies greatly between different species (Percival, 1965). Pollen undergoes a sequence of events in flowering plants. This can be listed as follows.

A pollen mother cell must:

- go through meiosis,
- divide and
- differentiate into pollen grains

A pollen grain must:

- dehisce, and perhaps need a maturation period,
- attach to a stigma,

hydrate,
germinate and
produce a pollen-tube

A pollen tube must:

penetrate the stigma,
enter the pollen tube transmitting tract,

the generative nucleus must divide and the tube must grow through the style to the ovule and enter the embryo sac and release the gametes (Heslop-Harrison, 1992). Failure at any of these stages causes male-sterility, because then the pollen is unable to deliver the gametes to the embryo sac.

Anemophilous pollen is small and dry and is present as single separate units. Entomophilous pollen however, is bigger in size and is found in clusters. During the process of pollen synthesis, tryphine is deposited on the pollen grain before dehydration completes (Keijzer, 1987). This serves two purposes. One is to clump the pollen grains and to facilitate their adhesion to the pollinator's body. The other is to make pollen dormant for the time to take it to reach the stigma (Picini, 1992).

2.17 STIGMA

The stigma of flowering plants is an efficient structure with both morphological and physiological adaptations for the capture, hydration and germination of pollen. It is the receptive surface of the style, the tissue which connects the stigma to the ovule forming the pollen transmitting tract. The style is also involved in pollen tube guidance, nutrition and incompatibility responses (Heslop-Harrison, 1992).

Angiosperm stigmas are structurally very diverse and the surfaces adapted for pollen grain capture differ widely in the morphology of the receptive cells and in the amounts of surface secretion. According to Heslop-Harrison (1992) stigmas can be divided into two main groups depending on the presence or absence of fluid secretions. ie wet stigma-(bearing fluid secretions) and dry stigma -(no fluid secretions).

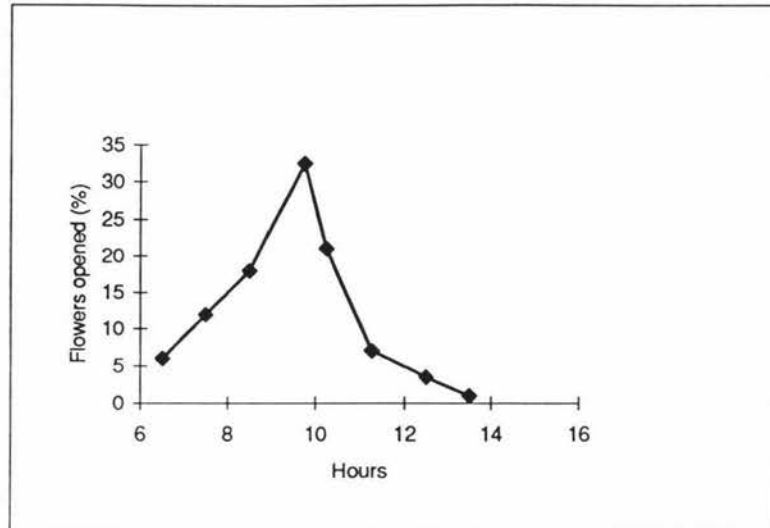


Fig. 2.1 Anthesis of *D. caryophyllus* var. Margueritae

Source: Shafi Bhat et al. 1991: Floral biology and seed setting studies in *Dianthus caryophyllus* (L.)

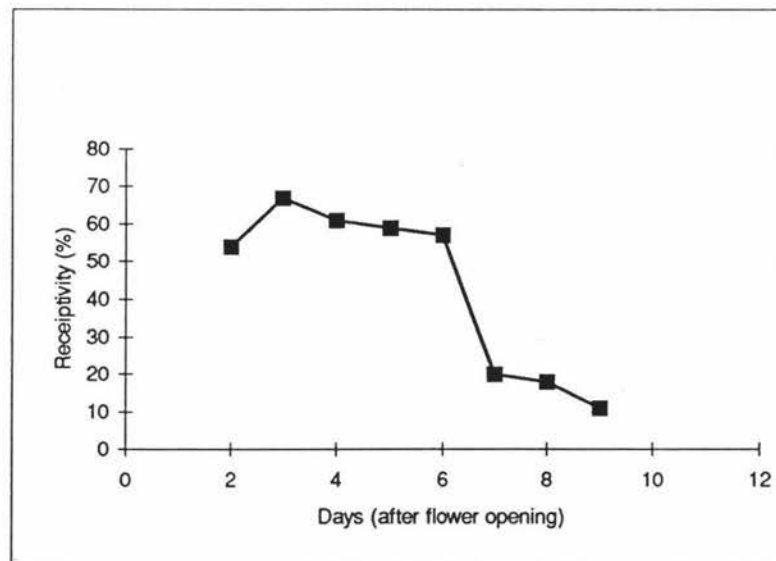


Fig. 2.2 Stigma receptivity in *D. caryophyllus* var. Margueritae

Source: Shafi Bhat et al. 1991: Floral biology and seed setting studies in *Dianthus caryophyllus* (L.)

Though this sexual selection is more prominent in the animal kingdom than in the plant kingdom, plants have also developed mechanisms to obtain this objective. The possibility of sexual selection in plants has been well documented during the last few decades. Haldane (1932) for example, noted that stigmas often contain more pollen than is needed to fertilise all ovules, leading to intraspecific competition of pollen to fertilize ovules and availability of pollen over an extended period. Huxley (1942) also mentioned that competition among pollen grains is likely, and suggested it may happen due to rapid growth of pollen tubes.

2.18.1 Male competition

Competition refers to the use of a resource by one individual that makes that resource less available to other individuals. Male competition in plants can be easily divided into that affecting pollen deposition and that affecting fertilization once pollination occurs. ie. the competition of pollen donors for access to a stigma, and competition of pollen grains on a stigma for access to egg cells. Charnov (1979) suggested that post-pollination competition may be more important than pre-pollination competition, because this increases the production of high vigour seeds (McKenna and Mulcahy, 1983).

Bateman (1948) seems to have been the first to suggest specially the existence of sexual selection in plants, as a result of the size difference in male and female gametes. He suggested that male competition should lead to increased production of pollen. The effect of such competition should be more apparent in monoecious or dioecious plants than in hermaphrodites with bisexual flowers.

Hill-Cottingham and Williams (1967) suggested that the low seed number of self-pollinated apples may result from cross-pollen reaching the ovules faster than self-pollen. But some plants selectively shed fruits from self-pollinated flowers. For example, *Macadamia ternifolia* matures fruits from self-pollinated flowers only when fruit set is low, while in some *Cucurbita* species, self-pollinated flowers produce mature fruits only when the fruits from cross-pollinated flowers are removed.

The plant then continues to supply reserves only to self pollinated fruits in the absence of cross-pollinated fruits (Stephenson and Bertin, 1981).

Gilbert (1975) suggested that sexual selection in dioecious plants lead to greater pollen and nectar production in male sporophytes, which compete for relatively rare female sporophytes. Jansen (1977) noted that selection pressure on male and female functions (pollen and fruit/seed production, respectively) differ greatly, that all pollen donors are not equally fit, and that female sporophytes should be selective in their production of offspring (seeds) with respect to pollen donors.

2.19 SELF INCOMPATIBILITY & POLLINATION

Plants have developed various mechanisms to encourage cross pollination and this phenomenon is referred as self-incompatibility. This is always found in hermaphrodite species, and a high percentage are pollinated by several species of insect and less by wind. In bisexual plants, when the anthers open stigmas are not receptive. Conversely, this does not occur when stigmas are receptive, thus reducing the chance of self pollination (Percival, 1965).

Different kinds of pollen can land on the stigma no matter whether it is self pollinated or cross pollinated. On such occasions some plants reduce the incidence of self pollination by controlling the speed of pollen tube growth along the style. In dianthus (*D. caryophyllus*) cross pollen tubes grow faster than self pollen tubes. This has been explained as being due to the stylet-resistance to self pollen (Shaf Bath et al., 1991). The ability of a pollen grain to fertilise depends either on its own genotype at the S-locus (gametophytic SI) or on the S-genotype of the pollen parent (sporophytic SI). Fertilization is possible when the S-alleles are expressed in the pistil. The S-gene products expressed in the pollen and the pistil are thought to interact and mediate this recognition process (Kaufmann et al, 1992)

2.19.1 Floral advertisement and rewards

Insects and animal pollinators obtain food from the flowers they visit, usually in the form of nectar or pollen. This is one side of a mutually beneficial relationship. The plant obtains in return the service of the pollinators in carrying pollen from one flower to another. Although food is generally the tangible benefit pollinators get from flowers, they are usually attracted to the flowers in the first place by the flowers' colour or scent. The interactions between the adaptations of insect pollinated flowers and those of the specialised flower- visiting insects are a classic instance of co-evolution (Proctor et al., 1996).

The production of pollen is a characteristic of all angiosperm plants and is an intergral part of the pollination process. Though plants produce pollen extensively, as a reward, pollen is very expensive to the plant, because it is rich in nitrogen and phosphorous- two elements often limiting growth by short supply in the natural habitat (Procter et al., 1996).

2.19.2 Nectar

Nectar is essentially an aqueous solution of sugars. It mainly contain sucrose, fructose and glucose in different proportions, varying from plant to plant.

2.20 TYPES OF POLLINATOR VECTORS AND THEIR EFFICIENCY

Many insects, birds and some other small mammals have been recorded as pollinators in flower crops (Free, 1993). According to foraging habits and morphology, insects are considered to be the most effective pollinators. To be an efficient pollinator an insect must; visit several flowers of the same species in succession; move frequently from one flower to the another; carry plenty of pollen on its body; and brush against stigmas of the flowers to effect pollen transfer.

Among the insect pollinators bumble bees and honey bees are by far the most important. Some Diptera species carry as much pollen on their bodies as bees, but they do not work flowers as consistently (Free, 1993). Other insects often recorded visiting flowers include, lepidoptera (butterflies), coleptera (beetles) and ants.

Under natural conditions, there is usually no very great concentration of one flower species in any one place, and thus the numbers in the native insect population that are visiting them are probably sufficient to pollinate them. However, many hectares may be occupied by a single flower crop, and where certain localities are favoured for growing particular plant species, there may be too few wild insect pollinators to effect adequate pollination. It is also possible that when a new crop is introduced into an area the natural pollinator may be absent. As a result, even though other factors involved in the production of a normal seed or fruit crop may be favourable, the yield may be limited by lack of pollination (Free, 1993).

The efficiency of insect pollinators varies according to many factors. Pollinator efficiency can vary with the distance from stamen to stigma (Percival, 1965). The greater the distance, the lesser the efficiency. Further, this may also vary with the type of insect. Bees are particularly efficient pollinators, because of their specificity and their socialness. Bees can carry viable pollen up to several thousand meters. Comparatively, wasps and flies are not as effective since they can only carry viable pollen shorter distances (several hundred meters). Generally most pollinating insects are most active in warm and sunny conditions. However, bumble bees retain their activeness in a wider range of climates and forage in misty, rainy and very cool weathers (even temperatures just above freezing point). This is because they can maintain the temperature of flight muscles well above the air temperature (Dijkgraaf, 1996).

2.21 INSECT POLLINATORS

Pollination is one of the key factors which determines the seed set of flowering plants. In commercial flower seed production, insect pollinators are usually employed to ensure a high percentage of pollination and high seed yields, because in most situations hand

pollination cannot be employed as it is time consuming and laborious. So bumble bees, honey bees, blowflies and more recently, solitary bees have all been used to pollinate flowers (Free, 1993) for commercial seed production.

The importance of insect pollinators varies and depends upon the degree of self-infertility. Some plants are completely self-infertile whereas some are partly self-fertile and partly self-infertile. So the efficiency of per insect visits may vary accordingly. Even the self pollinating flowers benefit from insect visits. Self-fertile plants may produce more fruits, or seeds of better quality when cross-pollinated than self pollinated, and various devices often favour cross pollination even when selfing can occur (Free, 1993). Besides increasing crop yield, an abundance of pollinators set a greater proportion of early flowers of some crops resulting in an earlier and more uniform seed crop (Free, 1993).

2.21.1 Pollen loading and unloading by insects

As already stated, most of the pollen of entomophilous species is sticky, so when pollinators visit and move around the flower, pollen grains adhere to the body parts and the hair of the insects and carry over to the styles. However, some insects can collect pollen without even touching the anthers (Corbert et al, 1982; Erickson and Buchman, 1983). During the flight their body become electrostatically charged, thus attracting pollen from the stamen.

2.21.2 Bees

Among hymenopterous pollinators, honey bees and bumble bees (*Apidae*) are undoubtedly the most skilled and capable visitors of the angiosperms (Varghese, 1977), because they visit a single species of plant as long as it blooms. Further they are social insects, and they collect more material than they need. This increases the number of visits to the crop, which ultimately increases the probability of pollination (Free, 1993).

The honey bee is of considerable economic importance, because it will pollinate a large variety of cultivated crops. The mean life of a honey bee is four to five weeks and it

starts to collect food at the age of three weeks. Honey bees will visit flowers up to 10 km from the hive, and they are sensitive to different colours. Bees collect twenty five times more nectar than they need and each one can visit several hundred flowers a day (Faegri and van der Pijl, 1979). According to the habit of food collection, bees can be divided into two kinds, polliniferous and nectariferous (Percival, 1965). The former actively collect pollen, some of which is dropped on the stigma in passing; the latter become dusted with pollen while collecting nectar and incidentally brush the stigma. Thus flowers may be visited by different kinds of insects, at different stages of receptivity, attracted by different rewards offered by the flower (Faegri and van der Pijl, 1979).

2.21.3 Bumble Bees

Bumble bees were introduced to New Zealand specially for pollination work. Some workers argue that bumble bees are superior pollinators to honey bees or other insects. They have larger bodies with more hairs which collect more pollen from the stamens and make better contact with pistils (Dijkgraaf, 1996)

Honey bees and other native bees do not work on some flowers (eg. tomatoes and kiwifruit) whereas bumble bees are very effective on these crops. Bumble bees have a longer tongue compared to honey bees so that they can reach the nectar at the bottom of tubular flowers. They also work in a wider range of environmental conditions than honey bees. They fly and forage in misty, rainy and temperatures just a few degrees above freezing point (Dijkgraaf, 1996)

There are four species of bumble bees found in New Zealand. Large earth bumble bee (*Bombus terrestris*), large garden bumble bee (*B. ruderatus*), small garden bumble bee (*B. hortorum*) and short haired bumble bee (*B. subterraneus*). Compared to *B. terrestris* all the other three species have longer tongues (Dijkgraaf, 1996).

2.21.4 Flies

Most flower visiting flies have sucking mouth parts for feeding on fluids, including nectar. Bombyliidae (bee-flies), Syrphidae (hover flies), and some other groups are highly specialised flower visitors. They have developed an elongated proboscis, which is adapted to feeding in long tubed flowers, and have the ability to hover when sucking nectar. According to their physical and behavioural capabilities, these flies can pollinate both primitive and advanced flowers (Varghese, 1977).

2.21.5 Butterflies and Moths

Survival of plants and insects is inter-related, and whereas flower scents are given by nature for the plant's fertilization, they are of equal importance to the survival of the pollinator. Flowers in which the nectar is deeply secreted in long tubes are usually heavily scented to attract the long-tongued Lepidoptera (Genders, 1977). Müller suggested that both butterflies and moths are attracted to flowers by scent rather than sight. The scents of flower adapted to pollination by Lepidoptera are usually sweet and some times heavy. *Dianthus* is one of the best known butterfly visited garden plants (Proctor, 1996), with its scented flowers, and nectar secreted in its tube like calyx.

(Crane, 1957) showed that colour also plays an important part in attracting butterflies. He noticed that nocturnal Lepidoptera prefer pale coloured flowers. The flowers mostly visited by butterflies (*eg. Dianthus*) bear flowers of pink colouring (Genders, 1977) who also noted that red-coloured dianthus were neglected by butterflies, but that they made use of them in the absence of pink blooms.

Both butterflies and moths prefer to visit flowers with long narrow tubes and flat-topped corollas. These two features are prominent in *Dianthus* (Percival, 1965). The tongue of the butterfly is dry, smooth and a very long tube. As a result it is unlikely to retain much pollen and transfer it to the stigma, especially where stigma and stamens are concealed in the corolla tube. But in flowers such as *Dianthus*, which have their anthers and stigmas

exposed above the corolla tube, the likelihood of pollination by other body parts is enhanced (Percival, 1965).

Butterflies and moths, in their perfect stage, restrict themselves almost entirely to nectar as their food (Percival, 1965). This adaptation has reduced their mouth parts to a thin, suctorial tube that can be inserted into deep nectaries. Their special flowers have long tubes or spurs and are generally, inaccessible to short-tongued insects. Lepidoptera, particularly the Sphingidae, can be observed sucking nectar from the long tube- yet, the evolutionary significance of Lepidoptera cannot be compared that of beetles, bumble bees or honey bees as pollinators (Varghese, 1977).

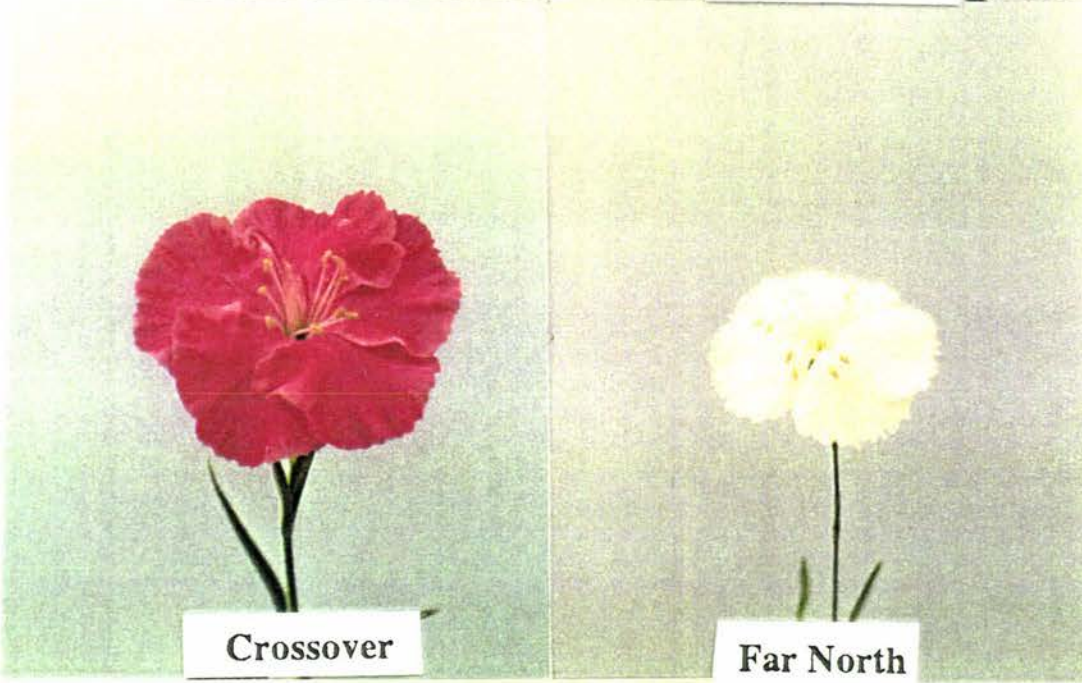
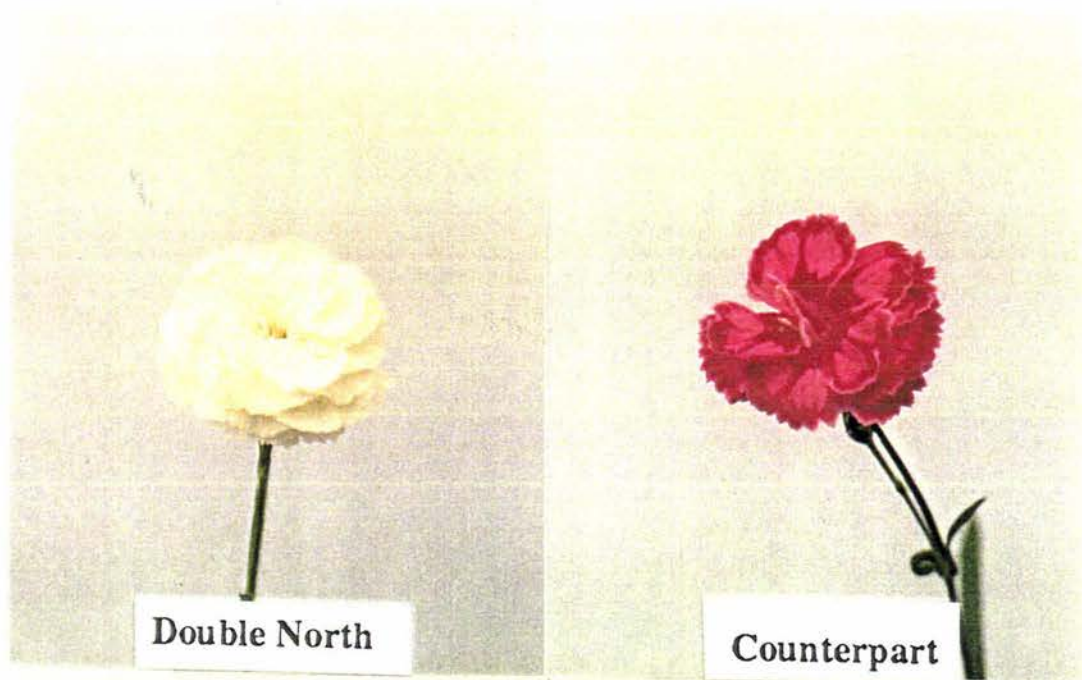


Plate 1. Flowers of ten cultivars used in this study



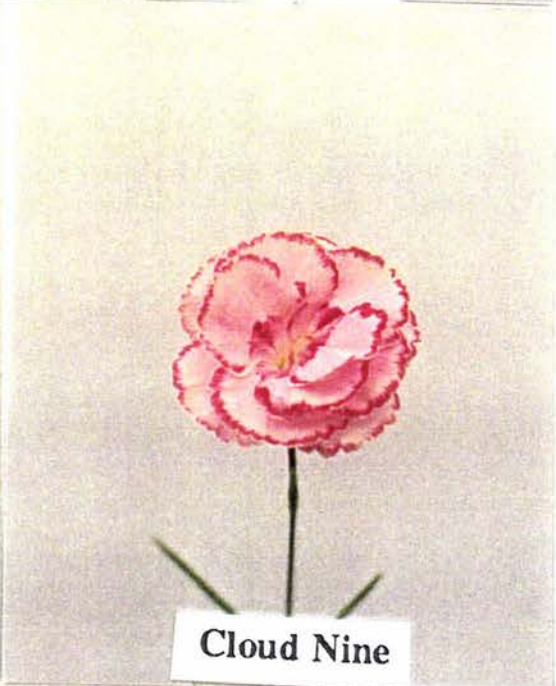
Neat 'N' Tidy



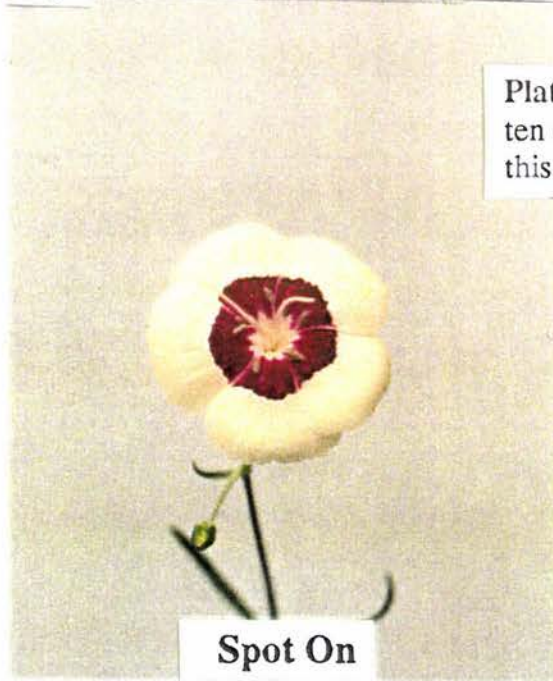
Royal Velvet



Mary



Cloud Nine



Spot On

Plate 1.1 Flowers of ten cultivars used in this study

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 Plant material

All the experiments were conducted using ten cultivars of Hammett Dianthus (*Dianthus plumaris* L.); ie Far North, Far Out, Double North, Counterpart, Cross Over, Spot On, Neat & Tidy, Mary, Royal Velvet and Cloud Nine (see plate 1 and 1.1). These cultivars were selected after a discussion with their breeder Dr. Keith Hammett. Experimental plants (clonal material) of each cultivar were purchased from Seaview Nurseries Ltd., Box 141, 139 Sykes Road, Manurewa, Auckland.

3.1.2 Grown of purchased plants

As plants were small at the time of purchase they were further grown on in a glasshouse at the Plant Growth Unit, Massey University until the shoots had up to eight to ten nodes. The glass house temperature was maintained at $19 \pm 3^{\circ}\text{C}$. Plants were watered manually (at two to three days intervals) with a hand held sprinkler. Pots were always kept wet to avoid moisture stress. The plants were liquid fertilised with Yates Liquid Lush (Appendix 4) (5ml product in two litres of water) at two-week intervals.

3.1.3 Taking of cuttings

As the original plant number was limited, further plants were propagated by cuttings. When plant shoots had reached 6-7cm (approximately 8-10 pairs of leaves) cuttings were obtained by cutting shoots with scissors. Three batches of cuttings were taken from the original plants, on 10th August, on 13th September and on 10th October 1996. Approximately 6-10 cuttings were taken from each plant at each time.

3.1.4 Rooting media

60% peat and 40% pumice potting mixture was used as the rooting medium with the addition of the following fertiliser mixture:

300g dolomite

100g Ag lime

100g pg mix 14:16:18 (N:P:K with the trace elements Boron, Copper, Molybdenum, Manganese, Zinc, and Iron)

The above quantities of nutrients were added to every 100-litres of mixture. The rooting medium was then placed in nursery trays (60 - plug trays) with 4.5x 5.0 cm plug holes.

3.1.5 Planting of cuttings and rooting

Immediately after taking cuttings, two to three pairs of leaves were removed from the basal end. This was done to provide wounding to encourage rooting. Cuttings were dipped in 8% Seradix 1 (root promotant from Rhone-Poulanic Limited) and planted immediately in the rooting media. The trays were transferred to a misting chamber (Langhans, 1954) with an additional capillary water supply from the bottom of the trays.

3.1.6 Hardening of plants

Cuttings had rooted after 2-3 weeks in the misting chamber. They were then transported on to an open bench in the same glasshouse for another two weeks. Then they were potted into 15 cm black polythene potting bags with the same potting mixture used for rooting cuttings.

3.1.7 Experimental sites

Three experiments were carried out in a glass house at the Seed Technology Centre, Massey University. The aim of the first experiment was to detect the time of stigma receptivity of each cultivar. The second experiment was a series of crossings between

each cultivar to determine potential seed production ability under glasshouse conditions. The third experiment was to reconfirm the results of the first two experiments. A fourth experiment was conducted in the field to assess the seed production ability of each cultivar under open pollination conditions. During this experiment insect visitors to the flowers were also observed.

3.2 GLASS HOUSE EXPERIMENT 1: Stigma receptivity to self pollen

3.2.1 Determination of stigma receptivity

As there was no published literature found regarding the stigma receptivity of *Dianthus plumaris* (L.), this preliminary experiment was conducted to determine the time when stigmas become most receptive to pollen. This was necessary, since one of the objectives of this study was to determine the seed production potential of the selected *Dianthus* cultivars. The experiment was set out as a completely randomised design (CRD), in the STC glasshouse of Massey University.

3.2.2 Plant management

Three similarly sized plants from each cultivar were randomly selected and then located randomly on the glasshouse bench. Each plant was treated as a replicate. Plants were watered manually every two to three days without wetting the foliage. Watering frequency varied depending upon the weather, and watering was always to runoff to ensure that pots did not dry out. Plants were given foliar fertiliser (Yates Liquid lush, 5 ml product in 2 litres of water) once a week throughout the experiment.

3.2.3 Emasculation of the flowers

At the time of flowering, plants were covered with iron framed cages covered with polyethylene mesh (9 holes/cm²). This was used to exclude pollinating insects. When the plants began to flower, they were emasculated carefully with a pair of forceps. Emasculation was done before anthesis (24 h prior to the flowers opening) to ensure no

pollen was released in the flower. Flowers were then tagged on the day they opened, and the date was recorded. As the flowers opened at different times of the day, emasculation was done 2-3 times a day, in the morning, at noon, and in the evening.

3.2.4 Pollination of the flowers

The flowers were hand pollinated with fresh pollen taken from another plant from the same cultivar (self pollen) at 1, 2, 3, 4, 5, 6, and 7 days after flower opening and allowed to set seeds. Five to six flowers were pollinated from each plant and each flower was taken as a sample. The covering cages were removed after sufficient number of flowers were pollinated.

3.2.5 Harvesting of seeds

Seed capsules were harvested by cutting them from the stem with a pair of scissors around 28-32 days after pollination. Generally by this time seed capsules had started to become brown (Plate 3.). The seeds were harvested by opening the capsules carefully and the number of good seeds (fully developed) (Plate 11.) were counted and recorded.

3.2.6 Ovule number

Six well-developed flowers from each cultivar were randomly selected and the ovaries were carefully dissected. This allowed the number of ovules per ovule to be counted under a binocular microscope and recorded.

3.3. GLASS HOUSE EXPERIMENT 2: Stigma receptivity to cross pollen

According to the results of experiment one, stigmas had their highest receptivity to self-pollen on the fourth day after the flower opened. Since only self pollen was used in this experiment a further study was done to check the stigma receptivity to cross pollen. The hypothesis was that stigma receptivity (maturation) might be vary with different pollen sources (self/cross pollen).

The cultivars Double North and Spot On were selected to test this hypothesis, because in experiment one they had shown a large variation in seed numbers produced when self pollinated or cross pollinated. This is illustrated by the fact that when Double North (♀) was crossed with Spot On (♂) on the fourth day after flower opening, 49 seeds were produced, whereas the same cultivars produced only 3 seeds when they were crossed in the opposite direction. Sixteen plants from each cultivar were randomly selected and arranged randomly on the bench in a glasshouse. The experiment was conducted using a completely randomised design and was carried out similarly to the first experiment.

3.3.1 Plant management

Plants were treated similarly (glass house condition, watering and fertilising etc.) as in the previous experiment.

3.3.2 Emasculation of the flowers

At the time of flowering, plants were covered with pollinator exclusion cages. Flowers were tagged on the date of opening and emasculated carefully with a pair of forceps, 24 h before anthesis. As the flowers opened at different times of the day, emasculation was done two to three times a day, in the morning, at noon and in the late afternoon.

3.3.3 Pollination of the flowers

Eight plants from each cultivar were self pollinated 1, 2, 3, 4, 5, 6, 7, and 8 days after flower opening, and the other eight plants were cross pollinated (pollen taken from the other cultivar) in the same way. Self pollinated plants were considered as the control, whereas cross pollination was the treatment.

The flowers were pollinated with fresh pollen taken from different plants of the cultivars (self pollen and cross pollen) and allowed to set seeds. Five to six flowers were pollinated from each plant for each treatment. The covering cages were removed after the required number of flowers were pollinated.



Plate 2. Field trial at the time of flowering



Plate 3. Maturing seed capsules (cv: Double North)

3.3.4. Harvesting

Four weeks after pollination seed capsules were separated by cutting the flower stalks. Then they were carefully opened to harvest seeds and the number of developed seeds were counted and recorded. Only fully developed black seeds were counted as in the former experiment.

3.4 GLASS HOUSE EXPERIMENT 3: Seed production potential of selected cultivars under glasshouse conditions

3.4.1 Experimental site and design

This experiment was conducted to assess the seed production potential of selected cultivars in the glass house during February - May 1997. The experiment was carried out using a completely randomised design.

3.4.2 Plant management

Ten plants from each of the ten cultivars were selected randomly, and arranged randomly on the glass house bench. The plants were treated equally throughout the experiment. They were watered manually every three to four days without wetting the foliage, and liquid fertilised (Yates Liquid lush 5 ml product in 2 litre of water) was applied at weekly intervals. The glass house temperature was adjusted to a minimum of 19⁰C and a maximum of 25⁰C.

3.4.3 Emasculation of flowers

Immediately before flowering all flower buds from each shoot were removed except the top one. This was selected because it was the first to appear on the shoot and matured first. Flowers were emasculated with a pair of forceps 24 h before anthesis (as explained in the first experiment) and tagged. Then the plants were covered with the same cages

used in the first experiment to prevent pollinating insects reaching the flowers.

3.4.4 Pollination of flowers

Flowers were pollinated on the fourth day after flower opening. Before actual crossing, stigmas were checked thoroughly with a magnifying glass to make sure that they were not contaminated with pollen at the time of pollination. Each cultivar was pollinated with pollen taken from every other cultivar, allowing 100 different combinations of crossings to be performed (Fig 3.1). Fresh pollen (just after anther opening) was used for all crossings, and pollen was obtained from extra plants grown in the glasshouse for the purpose.

One plant was allocated for each treatment and 5-6 flowers were pollinated on this plant. The remaining plants were left in the glass house but not hand pollinated, to see whether they would set seed under glasshouse conditions. Ten to fifteen growing flower buds from each cultivar were randomly selected and emasculated 24 h before flowering. These flowers were then covered with paper bags to prevent contamination (pollination) by foreign pollen, and left until seed maturity.

3.4.5 Harvesting of seeds

Seeds were harvested when seed capsules started to dry up on the plant (approximately 28-30 days after pollination). The number of good seeds (black and fully mature) was counted and recorded.

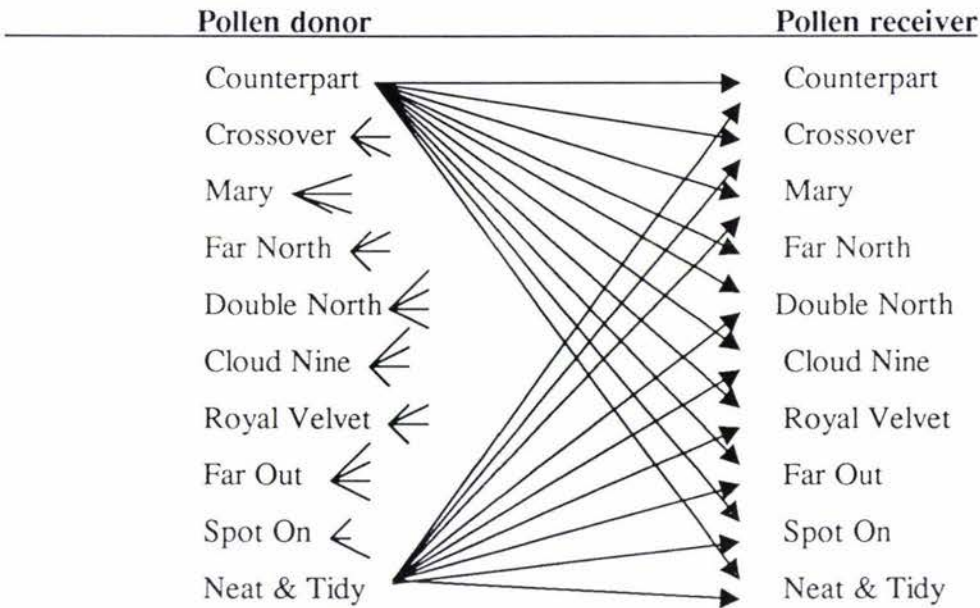
3.5 EXPERIMENT 4: Open pollination (field) trial

This experiment (November 1996-March 1997) was designed to test the seed production ability of all the cultivars under field conditions (Plate 2).

3.5.1 Plant material

Cuttings propagated at the Plant Growth Unit, Massey University were also used for this experiment. Thirty plants from each of the ten cultivars were used as field transplants.

Fig 4.1 Diagrammatic presentation of different crossing combinations



3.5.2 Experimental site

The trial was located about 200 m south of the Seed Technology Centre in the campus experimental plots of Massey University.

3.5.3 Soil type and land preparation

The soil type was an Ohakea silt loam, which is a flat soil which occurs in low terraces from old colluvium overlying stoney alluvium. It is considered to have slow internal drainage and is overall imperfectly to poorly drained. The natural nutrient status is low phosphorus, with medium calcium and potassium (Southward, 1997).

The land was ploughed in early November and power harrowed on 25 November 1996. No fertiliser or herbicide was applied to the soil before planting. A soil sample was taken

on 28th of November and send for analysis to the Department of Soil Science at Massey University. The result of the soil analysis is presented in Appendix 1.

3.5.4 Planting design

The experiment was laid out as a completely randomised block design. One hundred plants were planted in each block (rep), consisting of 10 plants from each cultivar. Plants were planted at 25 X 50 cm spacing. Each block was separated by a border row. The field lay out and the plant positions are given in Appendix 3.

3.5.5 Weed control

Since the soil was wet at the time of harrowing no herbicide was incorporated into the soil at that time. However 8 days after planting (6 December) Foresite[®] 380 was sprayed at the recommended rate of 65 ml in 10 litres of water per ha. Spraying was done along the rows and a mist-guard was used to avoid spray drift on to the plants. Further weed control was done with a push hand hoe on 15 January and this was continued at two to three week intervals as required until final harvest.

3.5.6 Irrigation

Plants were irrigated with an oscillating garden sprinkler as required throughout the experiment. This normally was done for 1-1 1/2 h every three to four days depending on weather conditions. When the seed capsules started to mature, the field was allowed to dry for a longer time by extending the watering interval to five to six days.

3.5.7 Harvesting

Seed capsules were harvested with a pair of scissors when they started to open naturally. The first harvest was on 8 February and it was followed by another two harvests at two week intervals. All the dried flowers were harvested in the third round. Each plant was harvested separately into a paper bag.

3.5.8 Seed drying

As the seed capsules were wet due to rainy weather during harvesting, bags were dried in an oven 30°C (± 1) for two weeks and then kept at room temperature until seed counting was completed.

3.5.9 Seed cleaning

Seed was hand removed from the capsule by rubbing. Seeds were then sieved with a 2.007 mm sieve and further cleaned with a micro blower (type 35) for 2 minutes at an air flow of 15 m s⁻¹.

3.5.10 Seed number and flower fertility

The number of fertilised flowers per plant was counted and, the percentage of fertilised flowers calculated and recorded. A capsule with at least one developed seed was counted as a fertilised flower. The number of seeds set per flower was also recorded.

3.6. OBSERVATION OF NATURAL INSECT VISITORS TO THE FIELD TRIAL

Insect visitations to the flowers in the field trial were observed during peak flowering. A preliminary observation was carried out over several mornings, afternoons, evenings and two nights. During the night, observations were done with a hand torch. On 14 February insect visitations to the trial plot were recorded from 6.00 am to 6.00 pm at two hour intervals.

3.6.1 Catching of insects

Suspected pollinators were caught with an insect catching net. They were collected in glass bottles and sent for identification to Dr. Wang Qiao (Entomologist, Massey University).



Plate 4 Plants growing in the glasshouse

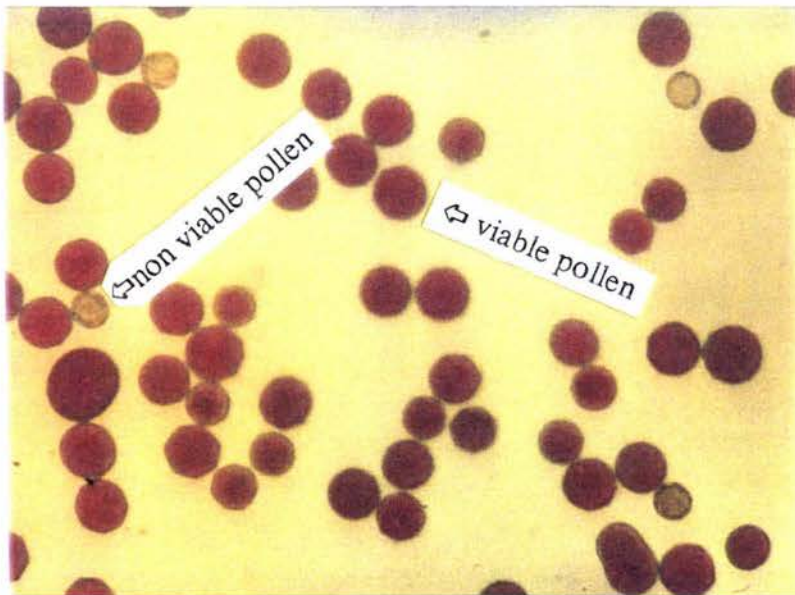


Plate 5. Viable and non-viable pollen grains

CHAPTER FOUR

4. RESULTS

4.1 STIGMA RECEPTIVITY

The stigmas of different Hammett *Dianthus* cultivars were receptive to pollen at different days after flower opening. The stigmas of cv. Mary, Far North, Double North, and Cloud Nine were receptive on day one (Table 1). They produced 7.0, 12.5, 9.0 and 12.2 seeds after being pollinated on the first day of flower opening. However, the rest of the cultivars were not receptive to pollen until the second day of flower opening (Table 1).

Stigma receptivity of all the cultivars increased with time, and was generally highest from the third to seventh day after flower opening. Cv. Counterpart produced its highest number of seeds per flower (37.8) on the fourth day but this was not significantly different from numbers on third and the sixth day after flower opening. Cv. Crossover produced 37.6 seeds on the fourth day and it was not significantly different from third to seventh day. Cv. Mary produced 37.6 seeds on the sixth day, and it was not significantly different from fourth to seventh day. Cv. Far North produced 36.8 seeds on the fifth day and it was not significantly different from third to seventh day. Cv. Double North produced 42.2 seeds on the fourth day and it differed significantly from any other treatments. Cv. Cloud Nine produced 45.0 seeds on the third day but it was not significantly different to the results of third to seventh day. Cv. Royal Velvet produced 29.2 seeds on the fourth day and it was not significantly different to third day. Cv. Far Out produced 34.0 seeds on the fourth day but it was not significantly different from third to seventh day. Cv. Spot on produced 8.4 seeds on the sixth day and it did not differ from four to seventh day. Cv. Neat & Tidy produced 41.3 seeds on the fifth day and it did not differ from fourth and seventh day of flower opening (Table 1 and Fig. 4.1).

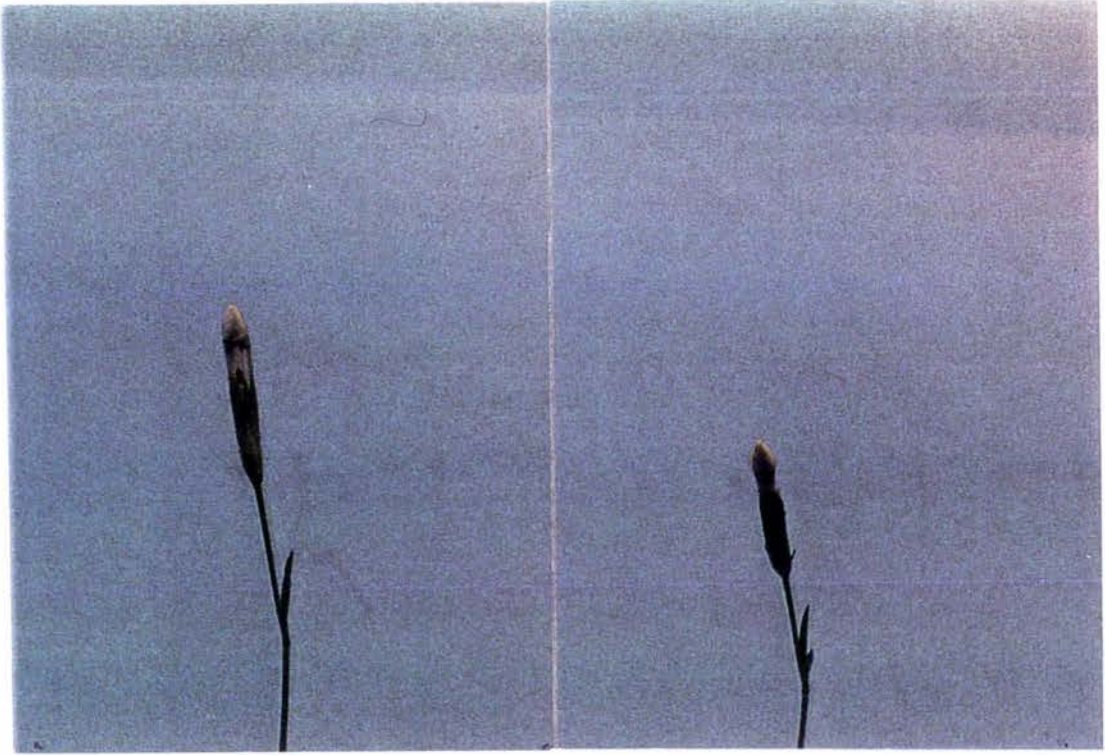


Plate 6a. Flower bud 24 hr before flowering
(cv. Spot on)

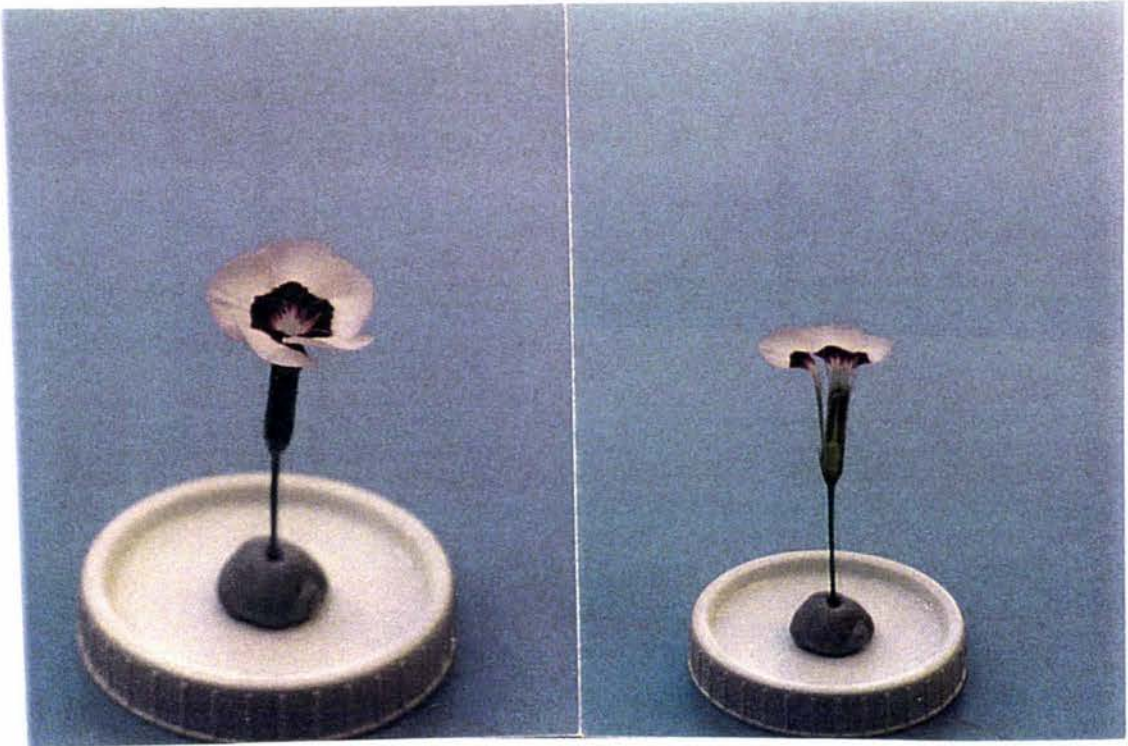


Plate 6b. Flower on the first day of flowering
(cv. Spot on)

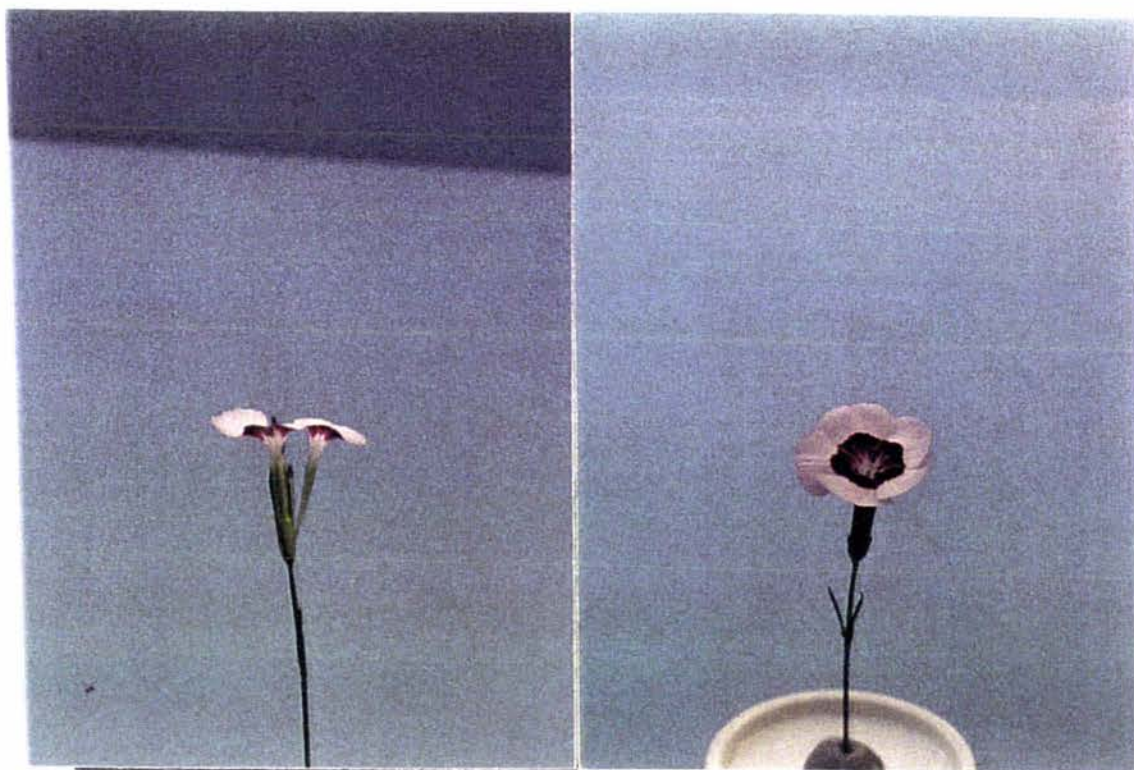


Plate 7a. Flower on the second day of flowering
(cv. Spot on)

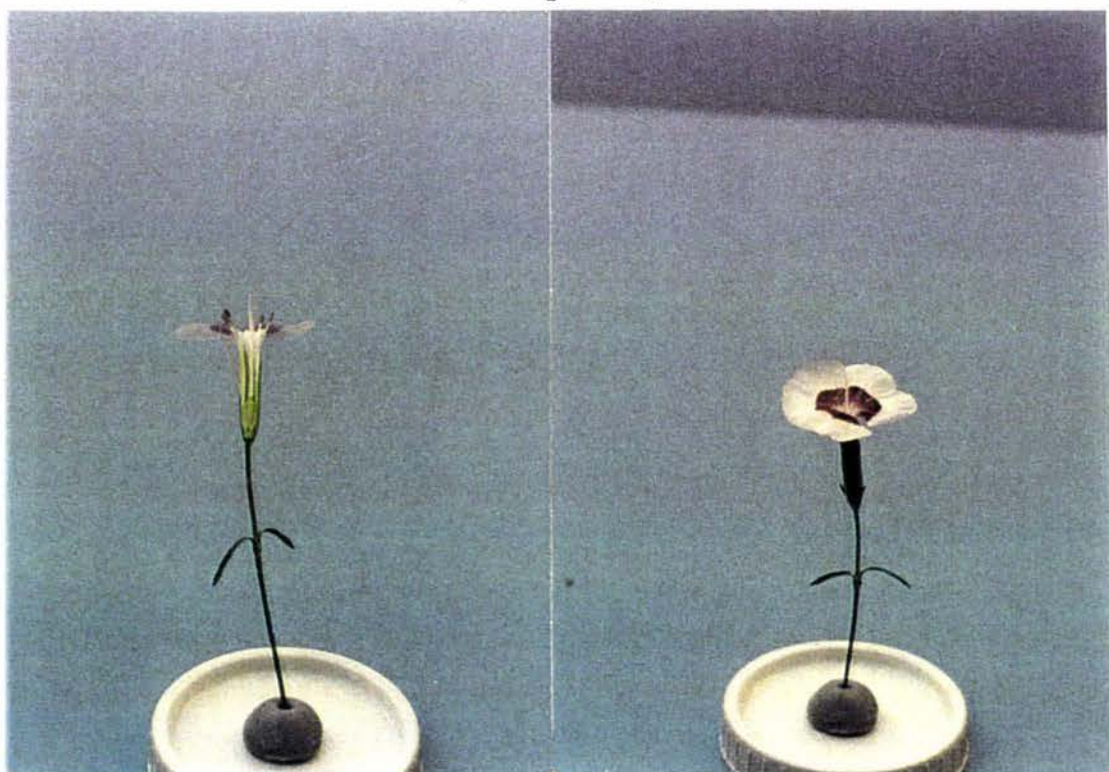


Plate 7b. Flower on the third day of flowering
(cv. Spot on)

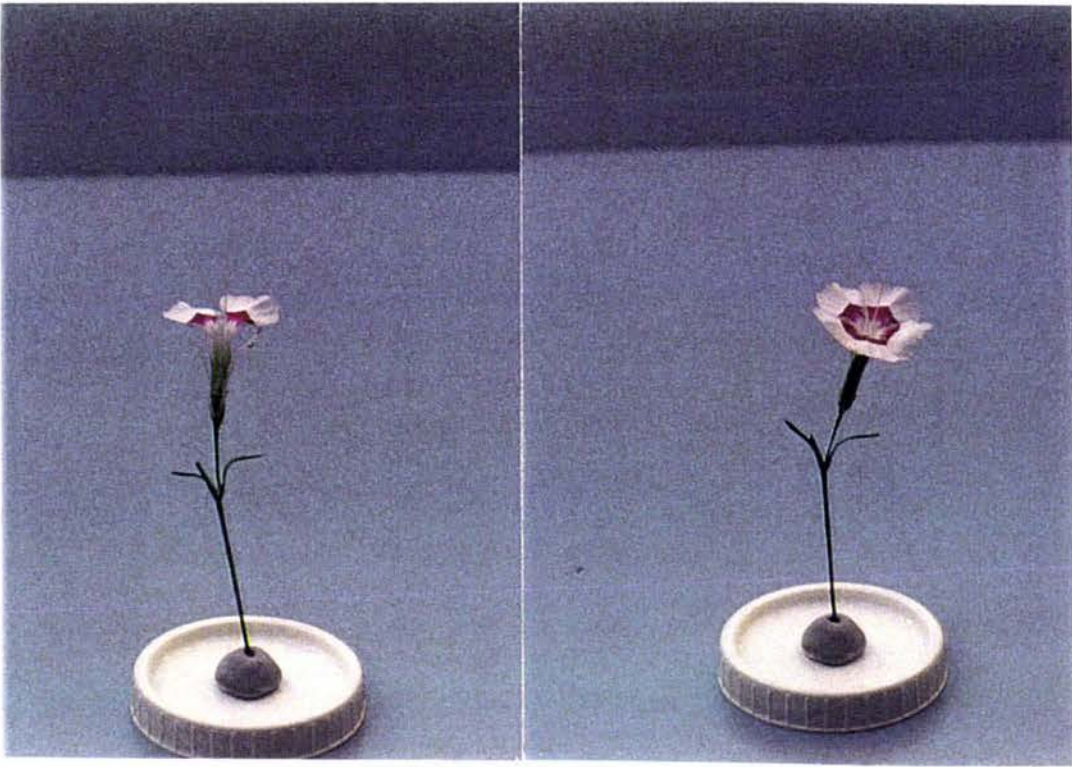


Plate 8a. Flower on the fourth day of flowering
(cv. Spot on)

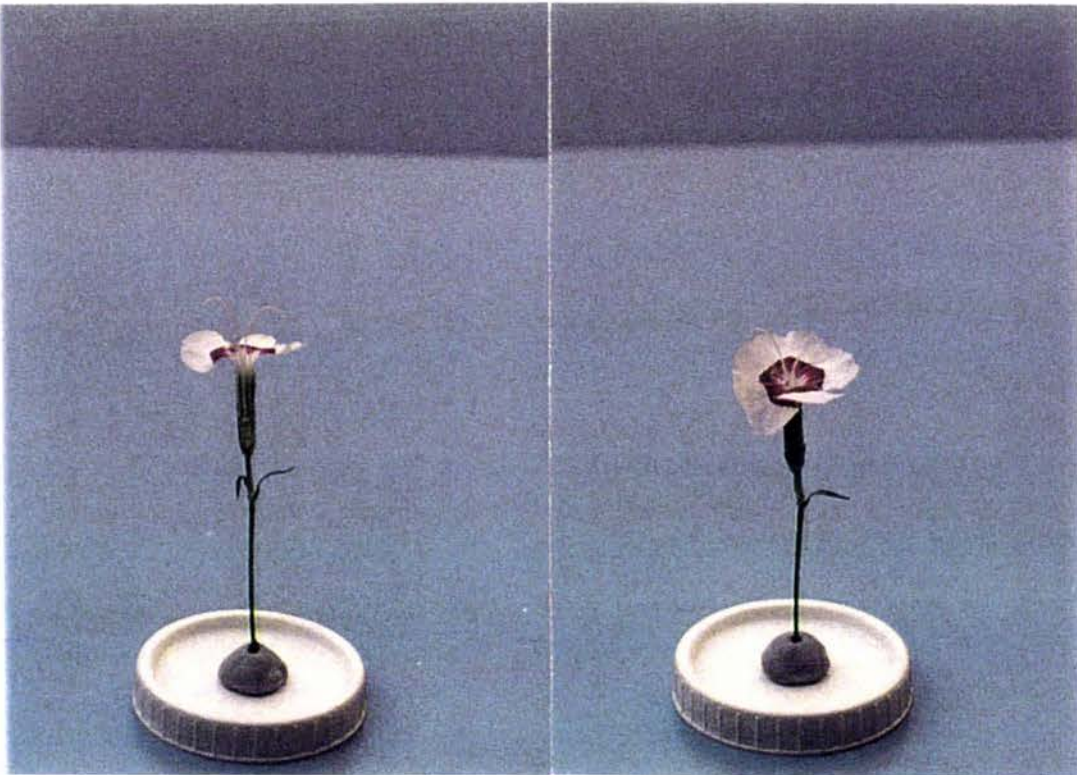


Plate 8b. Flower on the fifth day of flowering
(cv. Spot on)

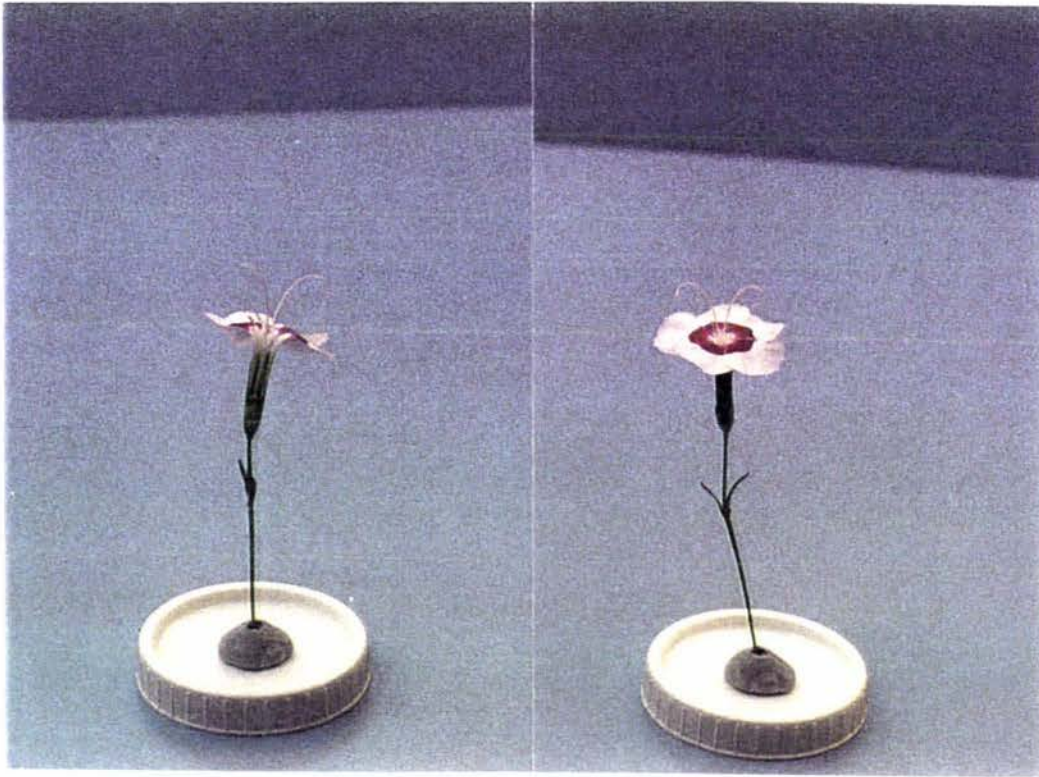


Plate 9a. Flower on the sixth day of flowering
(cv. Spot on)

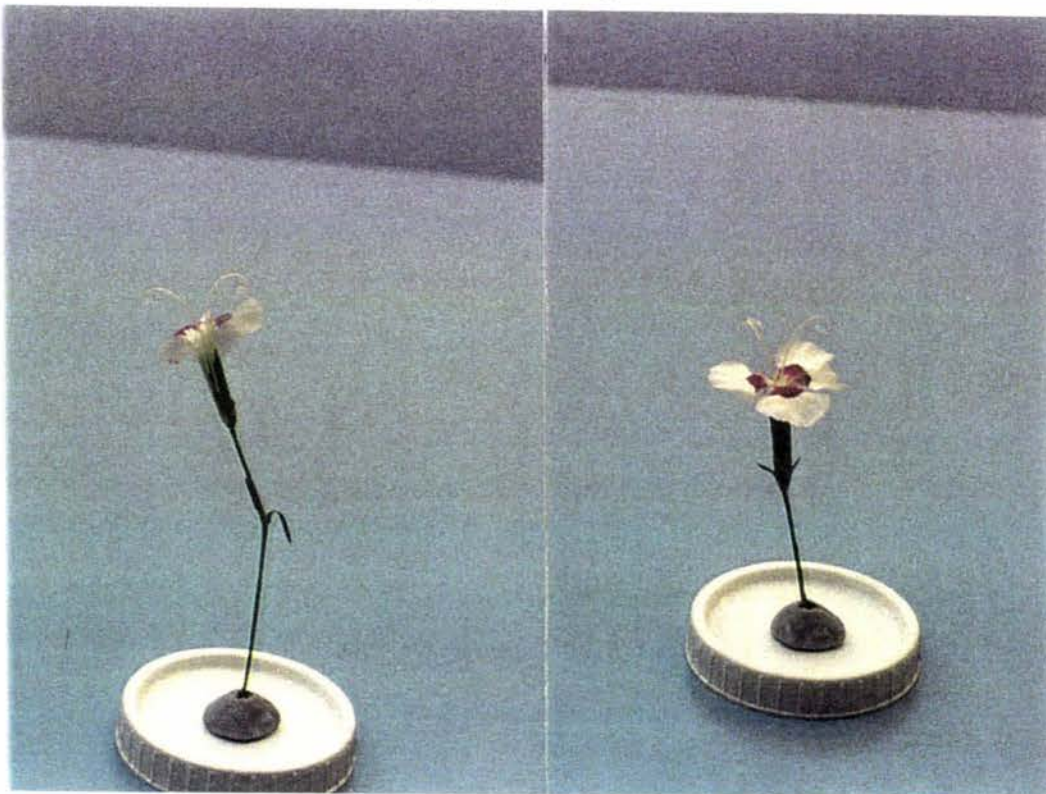


Plate 9b. Flower on the seventh day of flowering
(cv. Spot on)

Figure 4.1. Duration of peak receptivity of stigmas, and day after flower opening when the maximum number of seeds produced (▲).

Cultivar name	Days of peak receptivity after flower opening						
	1	2	3	4	5	6	7
Counterpart			████████████████				
				▲			
Crossover			████████████████				
				▲		▲	
Mary				████████████████			
						▲	
Far North			████████████████				
					▲		
Double North			████				
				▲			
Cloud Nine			████████████████				
			▲				
Royal Velvet			████				
				▲			
Far Out			████████████████				
				▲			
Spot On			████████████████				
						▲	
Neat & Tidy			████			████	
				▲			

The day on which the highest number of seeds was produced by different cultivars varied (Fig. 4.1). This suggests that stigma maturity varied from cultivar to cultivar. Generally, the stigmas showed their highest receptivity to pollen from the third to seventh day (Table 1, Fig. 4.1), whereas none of the cultivars produced their highest seed number after pollination on the first or second day after flower opening.

While the number of seeds set after pollination on the fourth day was not always the maximum for all cultivars, these values did not differ significantly from the maximum values (Table1).

4.2 POLLEN VIABILITY

All ten cultivars produced highly viable pollen (Table 2). Cv. Neat & Tidy had the poorest pollen viability (87.4%) whereas cv. Mary, Far North and Cloud Nine had 100% viable pollen (Table 2.). The rest of the cultivars had pollen viability within this range.

4.3 OVULE NUMBER

Ovule number per ovary in the cultivars was not constant, and varied widely among cultivars (Table 2). Cv. Mary had the lowest number of ovules per ovary (68.7) whereas cv. Far Out had the highest number of ovules per ovary (131.3). On the basis of ovule number per ovary the plants used in this study were categorised into three groups:

Cv. Crossover, Double North, Counterpart, and Far Out can be categorised as those that produced the highest number of ovules per ovary (>110 ovules/ovary). Cv. Far North, Royal Velvet and Cloud Nine had a medium number of ovules per ovary (80-110). The lowest ovule number per ovary (<80) was recorded in cv. Neat & Tidy, Spot On and Mary (Table 2).

4.4 1000 SEED WEIGHT

Seed weight also differed significantly among the cultivars (Table 2). Cv. Mary had the lowest 1000 seed weight (1.11g), while cv. Cross Over had the highest (2.55g). The seed weight difference between these two cultivars was more than 100 per cent. Cv. Counterpart, Royal Velvet and Far Out had 1000 seed weights of more than 2.26g, but they did not differ significantly. The 1000 seed weight of cv. Neat & Tidy, Spot On, Cloud Nine and Mary was less than 1.60g.



Plate 10. Non developed seeds and aborted ovules



Plate 11. Fully developed and viable seeds (scale 1cm)

Table 1. Effect of pollination time (days after flower opening) on seed set in *Dianthus*.

Days after flower opening	Mean number of seeds set for the different cultivars									
	Counterpart	Crossover	Mary	Far North	Double North	Cloud Nine	Royal Velvet	Far Out	Spot On	Neat & Tidy
1	0.0c	0.0b	7.0b	12.5b	9.0c	12.2c	0.0b	0.0b	0.0b	0.0c
2	5.4c	2.4b	15.2b	15.2b	25.8b	24.2bc	3.0b	8.0b	0.1b	5.4c
3	27.4ab	34.4a	16.2b	30.0a	30.6b	45.0a	20.2a	15.8ab	1.2b	21.6b
4	37.8a	37.6a	31.8a	35.2a	42.2a	40.8a	29.2a	34.0a	7.8a	35.4ab
5	18.2b	34.8a	34.4a	36.8a	29.8b	39.8ab	2.8b	21.4ab	7.4a	41.3a
6	30.3ab	37.6a	37.6a	23.4ab	29.0b	43.6a	4.0b	18.6ab	8.4a	20.4bc
7	15.2c	35.6a	33.2a	23.2ab	22.0b	44.8a	4.8b	14.6ab	8.2a	32.4ab

Means within columns with the same letter are not significantly different at $P < 0.05$.

Table 2. Pollen viability, ovule number and 1000 seed weight

Cultivar name	Pollen viability	No. of ovules per flower	1000 seed weight (g)
Counterpart	90.8e	125.8b	2.3b
Crossover	93.4d	112.5c	2.6a
Mary	100.0a	68.7g	1.1f
Far North	100.0a	83.5ef	1.4de
Double North	97.1c	115.2c	2.0c
Cloud Nine	100.0a	99.7d	1.3e
Royal Velvet	99.6ab	88.8e	2.3b
Far Out	97.6bc	131.3a	2.4b
Spot On	91.6de	78.3f	1.5d
Neat 'N' Tidy	87.4f	78.8f	1.5d

Means within columns with the same letter are not significantly different at $P < 0.05$.

4.5 EXPERIMENT 2

Different crossing combinations produced different numbers of seeds (Table 3) depending on the pollen source. Thus within the same mother plant the minimum and maximum seed number often varied 2-3 fold eg in cvs. Far North, Cloud Nine, Far Out and Neat & Tidy. It was 3 fold in cv. Counterpart, 4 fold in cv. Crossover and 10 fold in cv. Spot On.

Some cultivars preferred to be pollinated by self pollen rather than cross pollen (Tables 2, and 6.). Cv. Far North, Far Out and Neat & Tidy produced the highest number of seeds when they were self pollinated ie. 54.2 seeds for the Far North x Far North combination, 57.0 seeds for the Far Out x Far Out combination and 45.4 for the Neat & Tidy x Neat & Tidy combination. In the above crossings, these plants turned 64.9, 43.4 and 57.6 percent of their ovules into seeds.

Table 3. Seed production ability (seeds/flower) of cultivars with different pollen sources (glass house- hand pollination)

Pollen donor (σ)	Pollen receiver (ρ)									
	Counterpart	Crossover	Mary	Far North	Dble North	Cloud Nine	Royal Velvet	Far Out	Spot On	Neat & Tidy
Counterpart	33.8b★	24.6de	-	26.1ef	29.2e	49.2bcd	14.4d	31.2e	16.2cd	34.2def
Crossover	16.6e	29.4cd★	20.8d	32.6de	31.2de	31.8	31.2c	43.8cd	24.8ab	37.0cde
Mary	38.2b	57.6ab	-	54.6a	46.0a	56.2ab	50.4a	44.4cd	22.8b	38.6cd
Far North	29.4cd	65.0a	33.0ab	54.2a★	36.0cd	66.8a	43.8ab	55.0ab	18.6bc	44.2ab
Dbl. North	28.0cd	27.4d	28.2bc	21.6f	26.8e ★	37.6de	13.4d	34.0de	3.0e	20.2g
Cloud Nine	30.8c	58.4ab	37.4a	48.4a	44.8ab	52.6bc★	41.4b	60.6a	29.6a	36.0def
Royal Velvet	45.2a	54.2b	24.2cd	38.8cd	46.4a	49.6bcd	33.0c★	47.0bc	21.7bc	40.8bc
Far Out	31.4c	38.0c	-	47.6ab	39.4bc	50.6bc	30.4c	57.0ab★	16.4cd	31.8f
Spot On	24.0d	16.6e	22.4d	39.0cd	49.0a	42.0cde	27.2c	39.2cde	11.2d★	33.2ef
Neat & Tidy	31.6c	57.6ab	-	41.2bc	32.0de	47.4bcd	43.0ab	59.0a	29.6a	45.4a★

Means with the same letter within columns are not significantly different at $P < 0.05$

★ Combinations of self pollination. -Since cv. Mary did not produce adequate numbers of flowers complete data could not be obtained (applies also to Table 4 and 5).

Table 4. Comparison of seed setting of cultivars according to pollen donor and pollen receiving ability

Cultivar name	No. of seeds set as a mother plant (♀)	No. of seeds set as a pollen donor plant (♂)	No. of seeds set after cross pollination	No. of seeds set after self pollination
Counterpart	30.9 d	28.8 cd	30.6 c	38.1 cd
Crossover	42.9 abc	30.9 cd	44.4 ab	29.4 e
Mary	-	45.4 a	-	-
Far North	40.4 abcd	45.9 a	38.8 abc	54.2 a
Double North	38.1 bcd	23.6 d	39.3 abc	26.8 e
Cloud Nine	48.4 a	44.7 a	47.9 a	52.6 ab
Royal Velvet	32.8 d	41.9 ab	32.8c	33.0 de
Far Out	47.1 ab	38.0 abc	46.0 a	57.2 a
Spot On	19.4 e	31.3 bcd	20.3 d	11.2 f
Neat & Tidy	36.1 cd	43.0 a	35.1 bc	45.4 bc

Means with the same letter within a column are not significantly different at $P < 0.05$.

4.5.1 Self pollinating ability at pre flowering stage

None of the of the ten Hammett Dianthus cultivars produced seeds when the flowers were emasculated and bagged before flower opening.

4.5.2 Pollen donors

Though pollen viability was high in all cultivars, they did not all produce a high number of seeds per flower with every crossing (Table 3). Only a few cultivars produced pollen that was accepted by most of the other cultivars (Table 4). Good pollen donors among the cultivars were Mary, Far North, Cloud Nine, Neat & Tidy, Royal Velvet and Far Out

(Table 4). Cv. Double North was the poorest pollen donor in the series, followed closely by cv. Spot On, Counterpart, and Crossover.

4.5.3 Good mother (seed bearing) plants

As a seed bearing plant cv. Cloud Nine produced the highest number of seeds (48.4) when hand cross-pollinated in the glasshouse, and cv. Spot On was the poorest seed bearer even under the glasshouse conditions. It only produce 19.4 seeds per flower (Table 4). However, the number of seeds produced by cv. Crossover, Far North, and Far Out did not differ from that produced by cv. Cloud Nine.

4.5.4. Importance of crossing combinations

The crossing combination (♀ and ♂) was very important in influencing the number of seeds set (Table 3). The crossing combination of cv. Crossover (♀) x Far North (♂) produced 65 seeds whereas the same plants in the opposite combination (Far North (♀) x Cross Over (♂) produced only 33 seeds. This was true for many other combinations as well. eg. cv. Far Out (♀) x Neat & Tidy (♂) produced 59 seeds whereas cv. Neat & Tidy (♀) x Far Out (♂) produced only 32 seeds.

Cv. Spot On was the poorest seed bearing plant among all the cultivars (Table 4). Cv. Spot On (♀) X Double North (♂), the poorest combination, produced only 3 seeds per flower. The opposite combination, however produced 49 seeds per flower which is around 43 % ovule fertility (Table 3). Though cv. Spot On was not a good seed bearing plant its performance as a pollen donor was considerably better (Table 4).

Many of the cultivars were equally receptive to both self and cross pollen and on many occasions did not produce significantly different numbers of seeds either with self pollen or with cross pollen (Table 4). However, in cv. Spot On cross and self pollination (hand) and open pollination produced 20.3, 11.2 and 0.8 seeds per flower respectively. Though there was a significant difference between the hand cross pollination and open pollination treatments in the glass house, there was no significant difference either between hand

crossing and hand selfing or hand selfing and open pollination. Cv. Counterpart, Crossover, Cloud Nine, Royal Velvet, Far Out and Spot On did not differ significantly between hand selfing or hand crossing treatments. However, there was a significant difference between open pollination and the other two treatments (Table 5.).

Both cv. Far North and Neat & Tidy preferred to be self pollinated than cross pollinated (Table 5.). For both cultivars hand self pollination produced a significantly higher number of seeds than hand crossing or open pollination.

The only cultivar to prefer cross pollination was Double North. It produced 39.3 seeds (Table 5), in the hand crossing treatment and that was significantly higher than any of the other treatments. Open pollination (no hand crossing) always produced the lowest number of seeds per flower in the glasshouse.

The highest number of seeds was obtained from the following crossings.

Seed plant	x	Pollen donor	No.of seeds	% ovule fertility
Crossover	x	Far North	65.0	57.8
Cloud Nine	x	Far North	66.8	66.9
Far Out	x	Cloud Nine	60.6	46.2
Far Out	x	Neat & Tidy	59.0	45.0

Poorest yields were obtained from the following cross combinations

Seed plant	X	Pollen donor	No. of seeds	% ovule fertility
Cross Over	x	Spot On	16.6	14.8
Royal Velvet	x	Double North	13.4	15.1
Royal Velvet	x	Counterpart	14.4	16.2
Spot On	x	Double North	3.0	0.4
Spot On	x	Spot On	11.2	14.3
Spot On	x	Far Out	16.4	20.9
Spot On	x	Counterpart	16.2	20.7

Table 5. Comparison of seed set obtained using different pollination methods under glasshouse conditions

Method of pollination	Mean number of seeds set by the different cultivars									
	Counter part	Cross Over	Mary	Far North	Double North	Cloud Nine	Royal Velvet	Far Out	Spot On	Neat & Tidy
Hand Crossing	30.6 a	44.4 a	-	38.9 b	39.3 a	47.9 a	32.8 a	46.0 a	20.3 a	35.1 b
Hand Selfing	38.8 a	29.4 a	-	54.2 a	26.8 b	52.6 a	33.0 a	57.2 a	11.2 ab	45.4 a
No hand crossing	1.8 b	2.8 b	-	15.0 c	7.8 c	15.2 b	8.7 b	0.0 b	0.8 b	7.9 c

Means with the same letter in a column are not significantly different at $P < 0.05$.

Table 6. Comparison of seed set and yield under field and glasshouse conditions

Cultivar name	Field experiment (open pollination)				Glasshouse experiment (open pollination)	
	% of pollinated flowers per plant	Seed set (no.) per plant.	Seed yield per plant (mg)	Seed no. per flower	% of pollinated flowers per plant	Seed no. per flower
Counterpart	57.7 bc	111.7 e	22.1 e	8.3 c	42.8 bcde	1.8 cde
Crossover	57.2 bc	133.4 de	30.8 de	9.3 c	41.1 cdef	2.8 cde
Mary	42.0 d	236.6bc	33.4 cde	13.6 a	84.4 a	19.8 a
Far North	66.8 a	583.2 a	95.8 a	16.7 a	75.6 ab	14.1 ab
Double North	56.2 c	199.5 bcd	37.3 cd	12.8 ab	69.0 abcd	7.8 bcd
Cloud nine	58.7 abc	247.6 bc	41.4 cd	9.9 c	69.9 abc	15.2 ab
Royal Velvet	65. ab	221.3 bc	48.5 bc	14.3 a	30.1 efg	8.7 bc
Far Out	57.1 bc	269.9 b	63.7 b	14.4 a	0.0g	0.0 e
Spot on	28.7 e	38.4 f	07.1 f	4.5 d	7.7 fg	0.8 de
Neat & Tidy	59.2 abc	173.2 cd	29.6 de	9.7 bc	35.3def	7.9 bcd

Means with the same letter within the columns are not significantly different at $P < 0.05$.

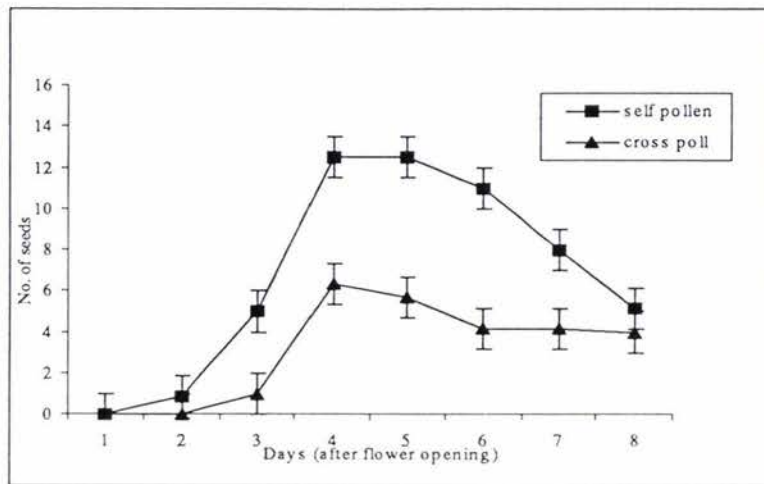


Fig. 4.2 Seed set in cv. Spot On with different pollen sources. Bars indicate standard errors of the mean.

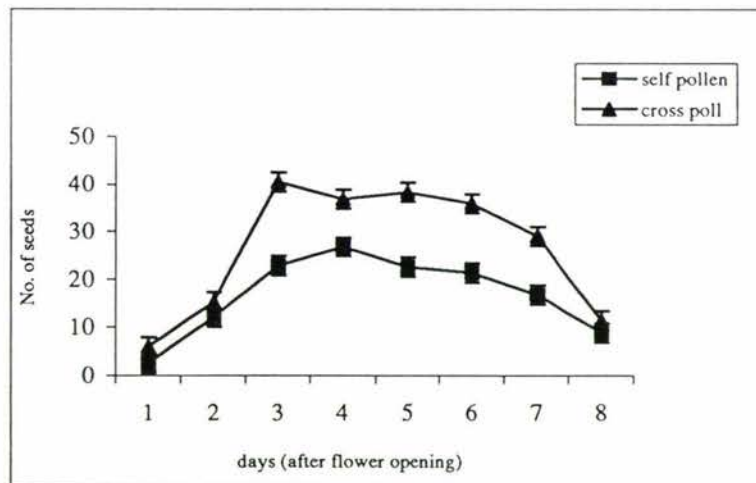


Fig. 4.3 Seed set in cv. Double North with different pollen sources. Bars indicate standard errors of the mean.

4.6. GLASSHOUSE EXPERIMENT 3

Stigma receptivity did not change due to the use of the different pollen sources for pollination. When cv. Spot On was pollinated with self and cross pollen the seed number produced varied greatly due to the pollen source, but in both treatments the highest number of seeds was produced on the fourth day of flower opening (Fig. 4.2). Cv. Double North also showed a similar trend. When it was cross pollinated, the highest number of seeds was produced on the third day of flower opening. In the same treatment, the numbers of seed produced on the fourth, fifth and sixth days of flower opening were less than that of third day, but they were not significantly different from each other. The same cultivar produced the highest number of seeds in the self pollinated flowers on the fourth day. However, on both occasions there was no significant difference between the seed numbers produced from the third to sixth day after flower opening (Fig. 4.3).

When approximately 15 flowers of each cultivar were emasculated before flowering and bagged, no seeds were produced (data not shown).

Table 7. Distribution of insect pollinator visits to the field trial site on 14/2/1997

Time	Number of visits /m ² /h				
	Bumble bees	Honey bees	Hover fly	Blow fly	House fly
6.00-7.00	8.0	4.0	-	-	1.3
8.00-9.00	2.7	1.3	1.3	4.0	8.0
10.00-11.00	4.0	1.3	-	4.0	8.0
12.00-13.00	-	2.7	-	4.0	4.0
14.00-15.00	-	-	1.3	2.7	2.0
16.00-17.00	-	-	-	-	2.7
18.00-19.00	-	-	-	-	2.7

4.7. FIELD EXPERIMENT

4.7.1 Climate

A summary of climate data is presented in Appendix 2. Palmeston North was not over wet over these five months. The highest rainfall (100.5 mm) was in November, and the lowest (58.0 mm) in February. The maximum / minimum temperature varied from 22.8 °C to 9.3 °C and it was windy during this time.

4.7.2. Floral fertility

Except for cv. Spot On all the other cultivars showed reasonable floral fertility under open pollination (field) conditions (Table 6). Cv. Far North had the highest floral fertility (66.8 %) compared with only 28.7 % in cv. Spot On. Both these cultivars were significantly different to the rest of the cultivars, Far North being at the top end and Spot On at the bottom end.

4.7.3 Seed set per plant

The seed number per plant was highest in cv. Far North (583.2) and lowest in cv. Spot On (38.4) (Table 6). Both the figures were significantly different to any of the other cultivars in the series. The seed number per plant of cv. Far North was twice as high as the next highest one, Far Out (269.9). Among the others the number of seeds per plant of cv. Mary, Double North, Cloud Nine, Royal Velvet, and Far Out (236.6, 199.5, 247.62, 221.3, 269.2) did not differ significantly.

4.7.4. Seed weight per plant

Cv. Far North produced the highest seed weight per plant (95.8 mg) while cv. Spot on produced the lowest (7.1 mg). None of the clones produced more than one gram of seeds per plant under field conditions (Table 6).

4.7.5. Seed number per flower

Cv. Far North produced the highest number of seeds per flower (16.7), but this was not significantly different to the seed numbers of cv. Mary (13.6), Double North (12.8),

Royal Velvet (14.3) and Far Out (14.4). Cv. Spot on only produced 4.5 seeds per flower and it was the lowest among the cultivars (Table 6.)

4.8. INSECT VISITATION TO THE FIELD TRIAL

Very few insects were observed visiting the trial field. However of those that did, more visited the flowers in the morning than in the afternoon (Table 7). Both bumble bees and honey bees visited the flowers in the morning but not after 1 pm. House flies were present all day.

Though *Dianthus* is supposed to be pollinated by butterflies none were seen in this study. The following insects were caught from the field and identified: honey bee (*Apis mellinifera* L.) bumble bee (*Bombus ruderatus* L.) hover fly (*Melangyna novae-zealandiae* L.) European blow fly (*Calliphora vicina* L.) and common house fly (*Musca domestica* L.).

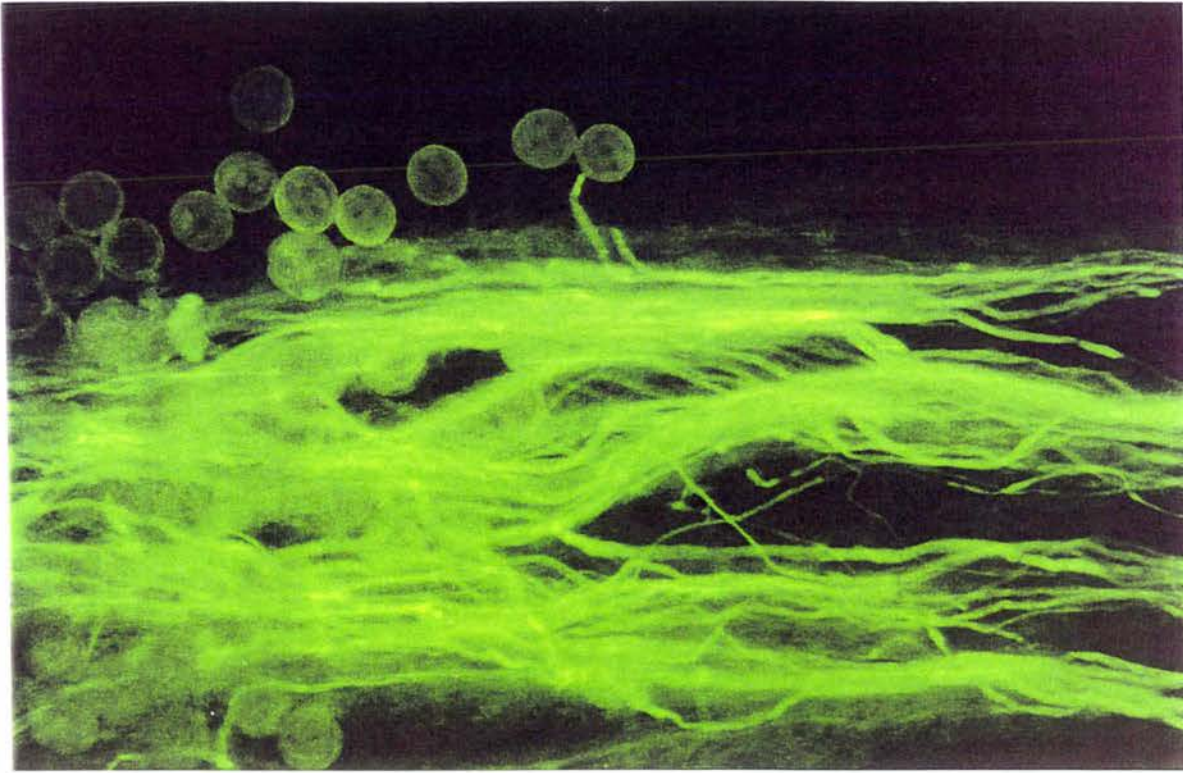


Plate 12. Pollen tube growth at the tip of stigma (self pollination cv. Spot on)



Plate 13. Growing Pollen tubes close to the top end of stigma
(self pollination cv. Spot on)

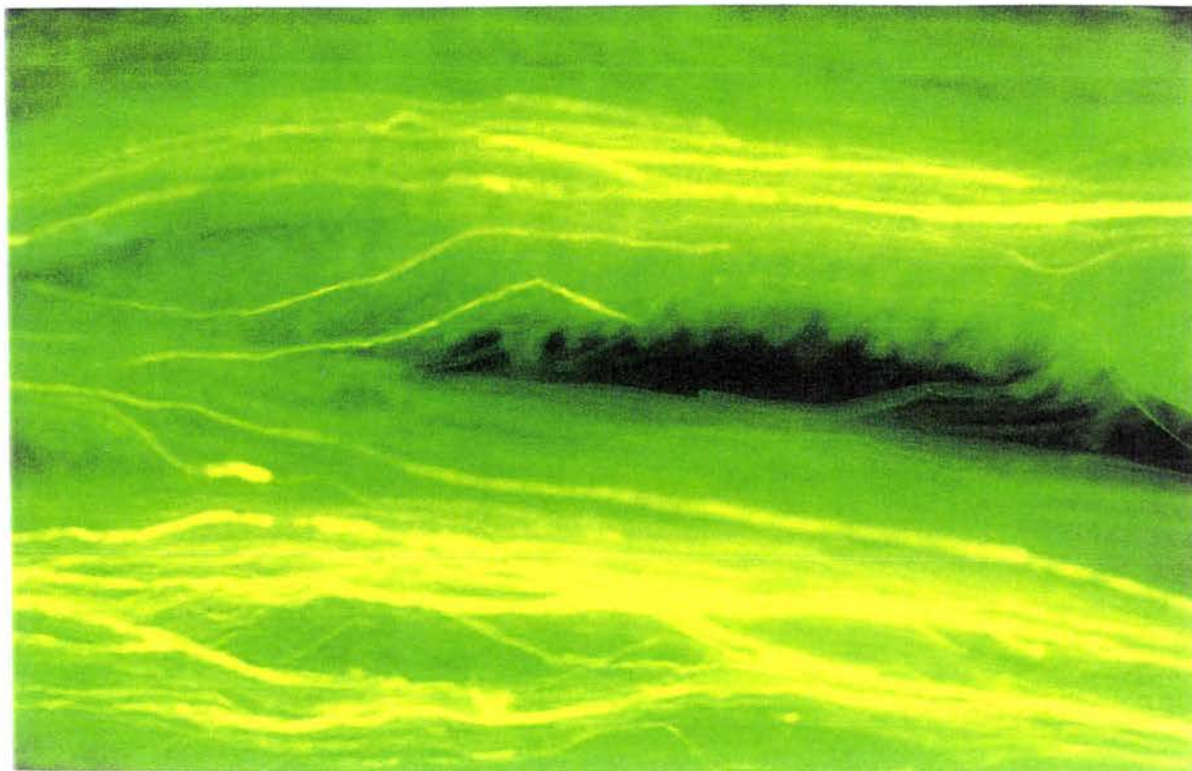


Plate 14. Growing pollen tubes close to the bottom end of stigma
(self pollination cv. Spot on)

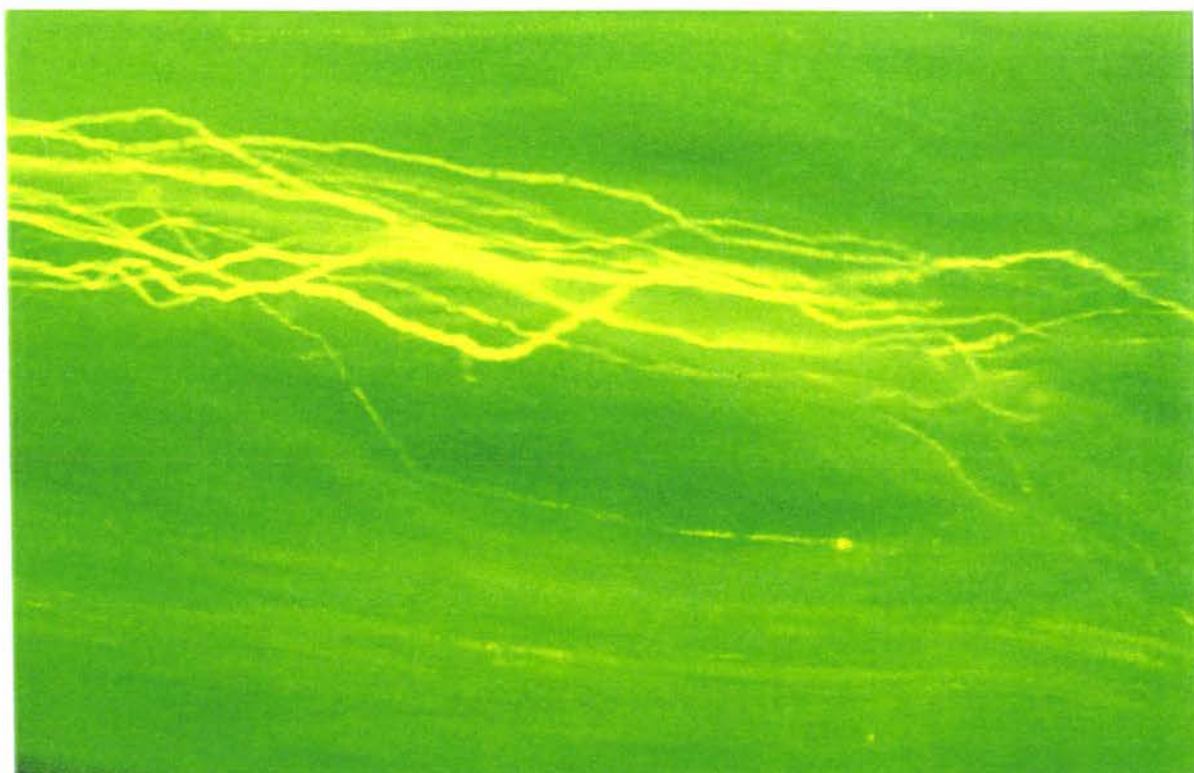


Plate 15. Growing pollen tubes at the bottom end of stigma
(self pollination cv. Spot on)



Plate 16. Insect pollinators from the field trial (1, 4 =bumble bee; 2,5 =honey bee; 3 =hover fly)

CHAPTER FIVE

5.1 GENERAL DISCUSSION AND CONCLUSION

Most of the Hammett *Dianthus* stigmas were not receptive to pollen at the onset of flower opening. They gradually increased their receptivity with maturation and were most receptive at and after the fourth day after flower opening. *Dianthus* are normally cross pollinated flowers, and the plant achieves this by maturing the anthers before the stigmas become receptive (Buell 1952; Shafi Bhat et al., 1991).

Shafi Bhat et al., (1991) further mentioned that the initial stage of stigma receptivity of *D. caryophyllus* is marked with the beginning of curling of the top of the stigma lobes. Though it was not one of the objectives in this study to observe the changes in the stigma, Plates 7b, 8a, and 8b show a quite similar stigma behaviour pattern. This suggests that these cultivars of Hammett *Dianthus* may also have the same behaviour as *D. caryophyllus*.

Stigma receptivity to pollen increased from the first day to the third or fourth day of flower opening. Stigmas remained highly receptive until the sixth day and then started to decline in receptivity under glasshouse conditions. This finding totally agrees with the findings of Shafi Bhat et al. (1991).

The stigma receptivity of cultivars Spot On and Double North did not change with pollen source (cross or self), and only changed with time after flower opening. This means the receptivity of the stigma is achieved with maturation of the stigma, which conditions the stigma to assist the capture, hydration, and germination of pollen (Heslop-Harrison, 1992). However the number of seeds produced per flower varied from day one to day seven, depending upon the pollen source. In this study, when cv. Spot On was crossed with self pollen and cross (Double North) pollen, self pollen always produced a higher number of seeds, and both treatments produced the highest number of seeds on the fourth to sixth day of flower opening. Similarly when cv. Double North was crossed with self pollen and cross (Spot On) pollen, the seed number

per flower was always higher in the cross pollinated treatments, but both treatments produced the highest number of seeds from the third to sixth day after flower opening. This result suggests that the receptivity of the stigma does not change due to different pollen sources. However, a reasonable number of seeds may be produced even when stigmas are at a low receptive stage, if the pollen is highly compatible with the stigma.

All the selected *Dianthus* cultivars produced highly viable pollen and therefore this does not seem to be a limiting factor if all other conditions are at an optimum for seed set. Further, the growth of pollen on the stigma also seems to be very active, but the self hand pollen of cv. Spot On only produced 11 seeds out of its 78 ovules per flower. Plate 12 and 13 clearly show that the pollen tubes which had started to grow on the tip of stigma was more than sufficient to produce this a low number of seeds provided they reached the ovary for fertilization, or if fertilized ovules were not aborted. Since these objectives were not included in this study, further investigation is necessary to confirm this is correct.

In the selected Hammett cultivars the number of seeds set per flower depended mainly on the compatibility of the pollen received by the stigma. In other words, these plants showed very high male selectiveness (Huxley, 1942) in the process of seed setting. Each cultivar had a big variation in the number of seeds produced per flower, and that depended upon the pollen source. Within a cultivar the maximum and minimum seed number per flower varied at least 2 fold, but in cultivar Spot On the figure was as high as ten fold. This strongly suggests that, although the plants have a higher seed setting potential, the pollen source contributed to a certain extent to the low seed production in some cultivars (Beatman, 1948; Trivers 1972). The rate of pollen tube growth often depends on the particular combination of pollen donor and pollen recipient (Pfahler, 1967). Since this biological phenomenon has been well understood, the identification of suitable pollinizer cultivars is a common practice in horticultural crops (Austin, 1995).

None of the cultivars produced seeds when their flowers were emasculated before flower opening. This suggests that there is no mechanism of self pollination before flowers open. Plants may achieve this either by not releasing pollen before the flowers open (Conquest, 1971) or the stigmas may not be receptive to pollen (Buell, 1952, Proctor, 1996) by that time. However some different *Dianthus* species (eg. *D. silvester* Wulf) produce cleistogamous flowers that do not bloom but produce seeds (Erhadot, 1988). It may be that different species of *Dianthus* have developed different pollination mechanisms due to geographical adaptation.

Aizen et al. (1990) found that *Dianthus chinensis* produced more seeds when cross pollinated than self pollinated. They suggested this was because of the inter-stylet resistance which makes self pollen grow more slowly through the style, causing low seed set in self pollination. Plates 12-15 also show a grate reduction of pollen tube growth from top to bottom along the styles of cv. Spot On when self pollination was done, but as there is no evidence to compare it with the cross pollen, a rational conclusion can not be made. However, in this study, the only cultivar which produced a significantly higher number of seeds after cross pollination was cv. Double North (Table 5). The seed numbers produced by the other cultivars by hand selfing or hand crossing did not differ significantly. Cv. Neat & Tidy even produced a significantly higher number of seeds in the selfing treatment. These findings make it difficult to totally agree that a self-incompatible mechanism exists in all Hammett *Dianthus* cultivars. It is probably safer to say that most of the cultivars of Hammett *Dianthus* are self compatible, but some still prefer cross pollination. Erhadot (1988) reported that self compatibility is a general trait in the Caryophyllaceae family.

Mating systems determine the pattern of genetic transmission and affect the organisation of genetic variation in a population. Inbreeding restricts heterozygosity and gene migration through pollen flow, reducing the variation within a population. In contrast, out crossing, which promotes gene flow, increases the likelihood of variation of plant populations by substructuring them. Self fertilisation has evolved in the plant kingdom. About 20% of higher plants are predominantly selfing (Brown, 1990) and in these species occasional out crossing has important consequences (Kearns and Inouye, 1993).

There was no real relationship between pollen viability (all high) and seed set. This suggests a failure in the female system is more likely than in the male system for controlling the seed set. However, successful pollination is essential for high seed production in *D. plumaris*. Both hand crossing and hand selfing always produced a significantly higher number of seeds than the non hand pollinated treatment in the glasshouse. However, to get the maximum number of seeds, the flowers should be pollinated when the stigmas are at their highest receptive stage to pollen.

Except for cv. Far Out all the other cultivars produced a small number of seeds even with out crossing the flowers. Since the flowers are not self pollinated before flowering (as explained above) and no insect pollinators were seen in the glasshouse, this suggests that the flowers may have had a self pollination mechanism at one stage of flowering to produce seeds. Most probably this may happen in the later stage of the flower's life span.

Flowers encourage cross pollination by maturing the stigmas after anthesis (Buell 1952; Shafi Bhat et al., 1991). If cross pollination fails, self pollination may serve as a backup system (Proctor et al., 1996). *Dianthus* may achieve this by curling the stigma lobes towards the stamens (Shafi Bhat et al., 1991) if they were not cross pollinated in the early stage of stigma receptivity. This may be an advantage to the plant to carry on its generations. Cross pollination may produce new offspring which are more adapted to the environment. But if it fails, it is still advantageous to the plant to produce some seeds, even with self pollen, rather than no seed. However, this should be further investigated.

Because today *Dianthus* are not natural selections, and are introduced plants in many parts of the world (Mansfield, 1951; Allwood, 1954), an understanding of the method of pollination has great importance to the commercial production of seeds. Since it is an introduced plant, distribution of its natural pollinator can not be expected in all the new *Dianthus* growing sites. So if the plant has developed a self pollinating mechanism in any stage of its life, it has great importance.

Different cultivars had different seed production potential. This varied from 78.3 seeds per flower in Spot On to 131.3 seeds per flower in Far Out, but no cultivar turned all its ovules into seeds even when hand pollinated under glasshouse conditions. Cv Counterpart converted 36%, Crossover 57.8%, Mary 54.4%, Far North 65.4% Double North 42.5%, Cloud Nine 67%, Royal velvet 58%, Far Out 46.2%, Spot On 39%, and Neat & Tidy 57.6% of their ovules into seeds in their best crossing combinations. Generally the upper limit to the seed set per flower is the number of ovules per ovary. However, the actual number of seeds set is determined by the number of fertilized ovules, weather condition, and the ability of maternal parents to provide the necessary resources for development (Stephenson, 1981).

Field seed production was much less than that in the glasshouse. This is quite a general phenomenon in seed plants. They produce more ovules than the number of seeds they can produce (Stephenson, 1981). The low seed set of field grown plants may be due to poor pollination, fertilization, or problems of assimilate partitioning following unsuitable environmental conditions. Since the effect of hand pollination was not tested in the field and the temperature variation in the field was not controlled their effects on seed set can not be assessed. However, as seed production varied widely among cultivars, an investigation should be done for individual cultivars to find out the real cause.

Except for cv. Spot On the other cultivars had a higher percentage of pollinated flowers under field conditions, but the seed number produced per flower was very low. Hand pollination in the glasshouse produced a comparatively higher number of seeds than the field trial. These data strongly suggest a problem of pollination in the field, and the following evidence supports the argument further.

Very few insects were observed visiting the flowers in the field trial. According to the literature, non of the observed insects are considered to be effective pollinators of *Dianthus*. Lepidopteras (butterflies and moths) are reported to be the most effective pollinators of *Dianthus* (Müller, 1881; Percival, 1965; Erhardt, 1988; Proctor et al., 1996). Though a few were seen in the nearby fields none of them were observed visiting the trial site.

Müller (1881) stated that *Dianthus* is pollinated by day flying hawkmoths (*M. stellatarum*), and Erhardt (1988) found large migratory hawkmoths (*Herse convolui*) and noctuid moths (*Autographa bractea*) also working in naturally grown dianthus in Europe. Since *Dianthus* are introduced plants to New Zealand, natural pollinators may be not available here. Even if present, maybe they were not active in this area or during the experiment. Further, the number, and the frequency of visits of the identified insects were low in the *Dianthus* trial, even though many of the same insects (bumble bees and honey bees) were seen visiting an adjoining clover trial. It may be possible that the observed insects were only casual visitors to the *Dianthus*, because of more attractive adjacent pollen sources.

Since *Dianthus* stigmas became receptive to pollen after anthesis (Buell 1952; Shafi Bhat, 1991), there is a possibility that pollen was washed off by rain before stigmas became receptive. Even though self pollen seems to be compatible in *Dianthus*, and also according to the structure of the flower, it is possible to transfer pollen to the stigma by rain splash, but there may be not enough pollen available under field condition when the stigma becomes receptive. Pollen load on the stigma has been identified as one of the critical factors of seed set. For most systems, more than one pollen grain per ovule is required to initiate maximum seed production (Kearns and Inouye, 1993). Sprat et al. (1992) found that 2.6 pollen grains per ovule were needed in *Hibiscus* for 100% seed set. Since the occasional insect visitors and the other possible agents may carry little pollen to the stigma, these may not sufficient for full seed set.

Also, the air temperature can affect pollination through its effect on both plants and animals. Flower development and opening, nectar secretion, anther dehiscence, and seed development are likely to be dependent on ambient temperature (Kearns and Inouye, 1993). Similarly, air temperature affects the activity of flower visiting insects (Free, 1993). Since there was a wide fluctuation of temperature in the field during the experiment, the temperature effect on low seed production cannot be estimated. Perhaps, the almost constant glasshouse temperature, which did not fall down below 16°C, could also be one reason for higher seed yield in the glasshouse pollinations.

5.2 Conclusion

1. Stigmas of Hammett dianthus are most receptive after the fourth day of flower opening.
2. Stigma receptivity does not change due to the source of pollen, and is determined by the stage of maturity of the stigma.
3. Pollen source is one of the factors that determines the number of seeds set in Hammett Dianthus
4. Cv. Double North prefers cross pollen whereas Neat and tidy prefers self pollen. The rest of the cultivars equally response to both self and cross pollen.
5. Pollination and pollinator effectiveness seem to be two of the factors causing low seed set in field grown Dianthus

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Appendix 1 Soil test result of the field experiment

Soil Sample	pH	Olsen P	SO ₄	Exch. K	Exch. Ca	Exch. Mg	Exch. Na	CEC	Soil volume correction factor
Campus Plot 9	6.1	16.7	14.0	0.33	7.6	1.15	0.15	13	1.15

Comment: Phosphate and sulphate values are expressed as $\mu\text{g/g}$ (air-dry). Exchangeable cation and CEC values are expressed as $\text{meq}/100\text{g}$ (air-dry). The soil volume correction factor is a measure of the weight of air-dry soil (g) per volume (ml) and can be used to convert results to a volume basis (e.g. $\mu\text{g/g} \times \text{soil volume correction factor} = \mu\text{g/ml}$).

Soil test completed on the 21/02/97 from sample taken two weeks earlier in the month.

Analysis performed by the :

Fertilizer and Lime Research Centre
 Massey University
 Palmerston North

Appendix 2 Table .2. The weather conditions in Palmston North during the field experiment.

Month	Tot. rainfall (mm)	Temperature		RH	Sun hr.	Wind (km.)
		Max.	Min.			
September	102.8	16.8	8.0	82	139.8	16.4
October	95.6	17.2	8.9	80	170.1	20.7
November	100.5	17.1	9.3	76	166.6	28.6
December	91.1	20.2	11.5	77	198.7	22
January	68	21.0	11.8	75	242.2	21.7
February	58	22.8	13.6	82	165.3	19
March	68.1	19.9	12.4	81	136.9	18.3

Appendix 3 Layout of the field experiment

	Rep 1										Rep 2										Rep 3												
A	I	C	G	D	E	J	A	B	H	F	A	G	E	B	D	A	H	C	J	I	F	A	A	H	G	I	E	D	F	D	D	F	H
C	I	B	J	A	F	D	G	H	E	C	H	F	B	G	D	C	H	J	A	E	I	B	D	A	C	B	H	F	J	J	G	I	E
A	H	D	F	B	J	E	I	G	C	A	G	C	G	F	I	H	D	B	J	A	E	D	J	B	F	I	E	A	H	C	D	G	F
F	D	E	A	J	B	H	G	C	I	F	I	H	J	A	C	D	F	G	E	B	I	H	F	I	G	D	B	I	C	H	J	A	J
E	F	A	B	J	H	E	C	I	D	G	E	J	D	I	A	B	C	H	G	F	E	I	G	F	B	I	J	C	D	A	E	H	I
D	C	F	A	D	H	I	B	E	G	J	A	I	G	F	E	H	J	B	D	A	C	F	I	J	H	F	B	G	E	D	C	A	H
G	B	J	J	A	G	E	D	H	C	F	B	E	H	D	C	I	G	F	A	B	J	E	A	D	G	E	H	I	C	J	F	B	C
I	H	G	C	I	E	B	J	F	D	A	F	D	F	J	H	B	I	G	C	E	A	J	H	B	D	G	F	I	A	C	E	J	G
B	H	A	J	B	C	D	I	E	F	G	C	I	E	G	H	D	B	J	F	A	C	G	C	A	B	I	H	E	F	J	D	G	B
J	B	F	E	D	G	C	H	J	A	I	D	F	B	D	E	I	H	A	C	J	G	A	F	D	H	G	A	C	J	E	I	B	A
H	F	G	H	C	A	J	D	I	B	E	J	G	A	J	C	E	D	H	B	I	F	C	E	I	J	F	D	H	B	G	A	C	D
A	D	C	G	J	H	E	A	F	I	B	C	A	B	C	D	E	F	G	H	J	I	G	A	H	B	C	G	F	J	I	D	E	C

Clone (plant) positions: A-Counertpart, B-Crossovet, C-Mary, D-Far Noth, E-Double North, F-Cloud nine, G-Royal Velvet, H-Far Out, I-Spot on, J-Neat & Tidy.