EFFECTS OF THERAPEUTANTS AND TEMPERATURE
ON
POLLEN GERMINATION, POLLEN TUBE GROWTH AND FRUIT SET
IN
FRUIT CROPS

A thesis presented in partial
fulfilment of the requirements for the degree
of Master of Horticultural Science at
Massey University

DWARIKA NARAYAN MANANDHAR
1980

ABSTRACT

This study involved assessments of therapeutant effects on pollination and fruit set; of temperature effects on pollen germination and pollen tube growth in apples, peaches and kiwifruits; of flowerage effects on pollen germination and pollen tube growth in kiwifruit styles; and of hand pollination effects on fruit set, fruit weight and seed numbers per fruit in kiwifruit vines.

From the point of view of their *in vitro* effects on pollen germination therapeutants can be listed in order of decreasing inhibitory effect as follows:

(a) for apples - mancozeb plus dinocap, triforine, dichlofluanid, bupirimate, bayleton, citowett^R, captan and mancozeb;

(b) for peaches - triforine, vinclozoline, streptomycin plus triforine, mancozeb, captafol, iprodione, dichlofluanid, streptomycin and benomyl; and

(c) for kiwifruits - dichlofluanid, captan and vinclozoline.

However, it appeared that while *in vitro* a therapeutant was very inhibitory, the application of a spray to anthers in an intact flower did not affect the germination of pollen subsequently released by those anthers.

Fungicides proven to be toxic to pollen *in vitro* did not necessarily produce similarly toxic effects *in vivo*. Reasons for such varying effects of fungicides on pollen germination and pollen tube growth *in vitro* and *in vivo* are suggested.

At 24 hours after pollination the number of pollen tubes growing more than 1/2 the style length of kiwifruit (Hayward) flowers one to two days old were 38% and 27% higher than in flowers less than one day old and more than three days old respectively.

In apples 5 sprays of either bayleton or captan, 3 sprays of either of the therapeutants mancozeb, mancozeb plus dinocap, and triforine and a wetting agent citowett^R applied during bloom period caused no effect in fruit set in Golden Delicious trees; and 3 bupirimate sprays during bloom period had no effect on the fruit set in Splendour trees.

In peaches 6 sprays of either captafol, or benomyl or iprodione, 5 sprays of either mancozeb or vinclozoline or streptomycin or triforine during bloom period caused no effect on fruit set in Golden Queen trees. Three sprays of streptomycin or dichlofluanid caused no effect but 3 sprays of triforine, triforine plus streptomycin or of ethephon caused 50%, 70% and 90% reduction in fruit set in Red Haven peach trees.

In kiwifruits 3 sprays of either dichlofluanid, captan or vinclozoline reduced seed numbers per fruit by 37% but did not affect fruit set or fruit weight.

Thus *in vitro* and *in vivo* studies were useful in determining therapeutant effects on pollen but did not necessarily provide information on their effects on fruit set in apple, peach and kiwifruit and on fruit weight and seed numbers per fruit in kiwifruit.

The germination of both apple and peach pollen was higher at 24°C than at 16°C, 20°C, 28°C and at 32°C. After 18 hours incubation pollen tubes were longer at 28°C and at 32°C than at 16°C, 20°C and 24°C. *In vivo* studies with kiwifruit (Matua) pollen showed that pollen germination at 14°C, 18°C, 22°C and 26°C did not differ significantly. Temperatures both higher and lower than the temperature range 18 to 22°C were found to be inhibitory to pollen tube growth in kiwifruit (Hayward) styles.

Hand pollination of flowers did not influence percentage fruit set but increased fruit weight and seed numbers per fruit in kiwifruit (Hayward) vines. Because fruit weight was positively correlated with seed number per fruit in fruits from both bee pollinated and bee plus hand pollinated flower clusters, supplementary pollination may play an important role in the production of fruit in kiwifruit orchards.

ACKNOWLEDGEMENTS

Grateful thanks are given to my supervisor Dr G.S. Lawes for his guidance and counsel for this thesis.

Thanks are acknowledged to:

Professor J.A. Veale for teaching me the techniques of stylar squash preparations.

Messrs H. Neilson, D. Anderson and A. Watson for technical assistance.

Mrs E.V. Oram for the great care she took in typing this thesis.

Special thanks are extended to my own family for their encouragement, support and tolerance.

Gratitude expressed to His Majesty's Government of Nepal and New Zealand Government for providing the support necessary to undertake this study.

TABLE OF CONTENTS

Page

LIST OF TABLES

TABLE OF FIGURES

Figure Page

LIST OF PLATES

SECTION ONE : LITERATURE REVIEW

CHAPTER ONE : *POLLINATION:*

The term "pollination" is defined as the transfer of pollen from an anther into a stigma (Robbins *et al* (1962), Childers (1976), Brown (1975) and Janick (1963)) which is ready to receive it (Anonymous (1972)). Because in most fruits, pollination is a pre-requisite to fruit set (Westwood (1978)) the knowledge of the factors influencing orchard pollination becomes important.

In practice it is difficult to separate pollination from the events which immediately follow it such as - pollen germination, tube growth, fertilization, seed development and fruit set. Faegri and van der Pijl (1966) went on so far to state - "pollination includes the process of: (i) release of pollen from the male part of a flower; (ii) transfer from the paternal to the maternal part; (iii) successful placing of pollen on the recipient surface of the latter followed by germination of the pollen grain. This introduces the next phase which is fertilization". All these aspects have been included in this review because they are important to the question of fruit set.

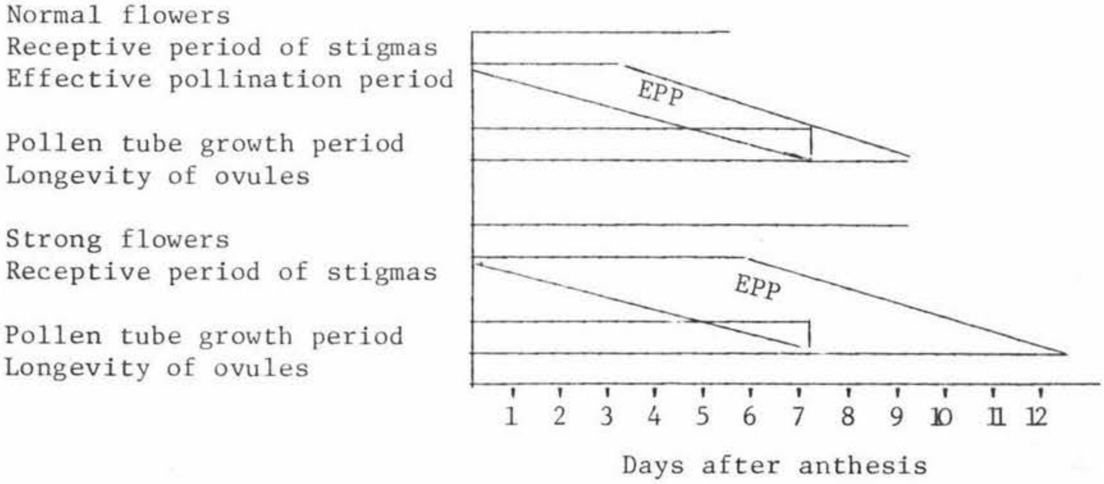
Self and cross pollinations are the two kinds of pollination determined by the genetic similarity of the plants involved. The pollination between genetically identical trees/plants is called self pollination and the pollination between genetically different trees is called cross pollination (Reuther *et al* (1968), Robbins *et al* (1962), Childers (1976)). In simple terms self pollination may be defined as the pollination of a pistil/pistils of a flower of one variety by pollen from a flower of the same variety and cross pollination as the pollination of a pistil or pistils of a flower of one variety by pollen from a flower of another variety. Self pollination is usually accomplished by gravity or by the actual contact of the shading anther with the sticky stigmatic surface. In cross pollination wind and insects are the agents of pollen transfer (Janick (1963)).

Most varieties of apricot, peach and Morello cherry (Teskey and Shoemaker (1978), Free (1964) and citrus (Chandler (1958)) set fruit when self pollinated and most varieties of apple (Childers (1978), Anonymous (1972), Free (1964)) pear, plum and sweet cherry need cross pollination (Free (1964), Childers (1976)). To obtain a maximum crop in almond 100% of the flower should be cross pollinated (Kester and Grigg (1959)). Walnut, pecan and filbert trees need cross pollination because male and female flowers in a tree fail to mature at the same time (Griggs (1953), Childers (1976)). Griggs (1953) showed that pecan nuts resulting from cross pollination filled better, were larger and were less likely to drop before they matured compared to those from self pollination. In avocado, male and female flowers of a variety do not open during the same part of the day (Papademetriou (1976)) and thus cross pollination becomes important. According to Winkler (1974), self pollination is the rule of *Vinifera* grapes; however, cross pollination is not only possible but under certain conditions desirable and that still under other conditions it is necessary. In kiwifruits male and female flowers are produced on different plants (Anonymous (1979); Schroeder and Fletcher (1967)). Both staminate and pistillate flowers produce pollen but the pollen from pistillate flowers is produced in little amount (Lawes (1980)) and are non viable (Schroeder and Fletcher (1967)). For these reasons, kiwifruit varieties are cross pollinated.

Cooper (1938) observed that after the first 24 hours the pollen tubes of cross pollinated flowers travelled down the styles faster than did those of self pollinated flowers. In citrus Lange (1973) found that with cross pollinated Minneola trees pollen tube growing down the style reached the ovules in 6 days whereas with self pollination only 40% of the total stylar length had been penetrated after 9 days. These results may justify the observations of Griggs (1953), Kester and Griggs (1959) (see above), Anonymous (1967) and of Dayton (1974), who found that cross pollination produced better results than with self pollination. According to Anonymous (1967), even normally self compatible top fruit varieties may show an increased set of fruit when cross pollinated and there are indications that the same may be true of soft fruits. Dayton (1974) found that while self pollination of emasculated Golden Delicious flowers produced a small set Delicious pollen set nearly 4 times as many fruits. Thus adequate provisions for cross pollination may be considered as an effective means of improving fruit setting in an orchard.

It is sometimes assumed that flowers are fully receptive and capable of setting until there are signs of senescence of the stigmas or petal abscission (Williams (1966)). But Dorsay (1930) recognized that these features were less important than the longevity of ovules. According to Williams (1970) effective pollination is restricted to a period immediately following the opening of the flower and in practice the duration of this period (Effective pollination period) equals the longevity of egg apparatus minus the time required for the pollen tube to reach the egg sacs. Strong apple flowers whose ovules remain viable for more than 12 days have longer effective pollination periods (EPP) than those of normal flowers whose ovules remain viable for only 9 days (see Fig 1; Williams (1965)). Williams (1965) found that varieties of apples and pears may vary considerably in their EPP and there is evidence that there may be large variations from year to year in many apple varieties. Williams also noted that if EPP is comparatively long then rapid pollen transfer is not important but if the period is short then the crop might be limited, if pollen transfer does not take place soon after anthesis. Therefore, it can be said that knowledge of the EPP may be of great value when assessing the value of a pollinating agent.

Fig. 1 : Diagram showing the effective pollination period for normal and strong flowers (After Williams (1965)).



According to Williams (1966), the duration of EPP can be assessed in two ways: (i) by hand pollinating a limited number of flowers at varying intervals after flower opening and recording the subsequence presence or absence of fruitlets (see also expt No.24) or (ii) by a morphological examination of the egg apparatus in flowers preserved in sequence of opening.

From the definition of the EPP as mentioned above it is evident that acceleration of pollen tube growth and/or prolongation of ovule longevity would cause an increase in the EPP in a flower. The factors that influence the pollen tube growth and ovule longevity in a flower are discussed under the Chapter Two. Increasing EPP can be considered as a worthwhile goal to increase chances of fruit set in an orchard.

CHAPTER TWO : *FACTORS AFFECTING POLLINATION OF FRUIT CROPS:*

A. *Temperature:*

A.1. *Effects on anther dehiscence, pollen germination, pollen tube growth and ovule senescence:*

Working with apples Snyder (1942) found that when temperatures were high (20°C) the anthers began to dehisce before the flowers opened but in cool conditions (20°C) the flowers were sometimes open before dehiscence occurred. Seaton and Kramer (1939) determined that in fairly still air condition the air temperature increased approximately 0.6°C (1F) with every 3 m above ground level and the flowers at the top of a tree presented pollen and were visited by insects before those on the lower branches. Percival (1955) noted that the temperature range for free anthesis for cherry and pear was about 5-14°C and for apple 10-19°C. Percival also observed that low temperatures seldom limit anther dehiscence, and in apples it occurred even at 5°C.

However, according to Winkler (1974), in grapes when temperature is below 15.5°C few if any of the anthers open. A rise above 15.5°C is accompanied by an acceleration in the opening of the anthers especially when the air is dry.

According to Williams (1970), within the range of mean temperature to which flowers are exposed in an apple orchard (7-15°C) compatible pollen tube growth was correlated with temperature and gave a linear relationship. Fertilization took place by the tenth day after pollination at 7°C and for each rise of 1°C in one day less was required to effect fertilization, leading to a requirement of only two days at 15°C. But at high temperatures (15-25°C) compatible, partially compatible and incompatible pollen tubes no longer followed the simple temperature/growth relationship demonstrated at lower temperatures. Growth inhibition or retardation of incompatible and partially compatible pollen tubes was increased and some cultivars fully self compatible in the 7-15°C range exhibited some self incompatibility at the higher temperatures. In avocado also, the rate of pollen tube growth increased with a rise in temperature but abnormal growth was frequently observed at 33/28°C (day/night temperature) and tubes failed to reach the ovary at 17/12°C (Sedgley (1977)). The optimum temperature range for pollen germination and pollen tube growth of some fruits are presented in Table 1 below.

Apple pollen fails to germinate below 4.5°C and pollen tubes make slow growth in the styles unless the temperature exceeds 5.4°C (Percival (1955)). Mature pollen from frost damaged flowers takes longer to germinate than those from undamaged flowers (Field (1942)). In cider apples the rate of growth of yellow styre pollen tubes in Michalin flowers at 5°C was 1 mm/day. At 14°C it was 1.33 times as fast and at 24°C it was eight times as fast as at 5°C. The rate of pollen tube growth in the joint style and ovary was faster than in the free style. This ratio decreased with increase in temperature for example it was 3 at 10°C but less than 2 at 20°C (Child (1967)). Temperatures either above 35°C or below 20°C seemed to retard growth somewhat (Cooper (1938)).

Mellentheim (1972) observed that in d'Anjou pears at 21°C pollen tube growth was completed within 24 hours while at 15.5 and 10°C growth was completed only by 72 and 102 hours respectively. Lombard *et.al* (1972) found that at 5°C tube growth required 12 days while at 15^{o}C required only 2 days. Thus, because pear ovule was viable for 11 days, the EPP at 5^{o}C was zero, while at 15^{o}C it was 9 days.

The slow growth of tubes at low temperatures is thought to be the principal reason for low set of fruits and nuts during cool spring season, even though no freezing of ovules occur (Westwood (1978)). Thompson and Liu (1973) concluded that the cause of erratic fruit setting in 'Italian prune' is attributed to its genetically determined sensitivity to cool weather in the post blossom period. They found that cool temperature (10^{o}C) delayed pollen tube growth, and early embryo and endosperm development so long that the ovule began to degenerate.

Table No.1. *Optimum temperature range for in vivo pollen germination and pollen tube growth in selected fruits.*

Kinds of fruits	Optimum temperature (^{o}C) range for in vivo Pollen germination	Pollen tube growth	References
Apple	21.1-26.6^{+}	22.2-26.6^{x}	$^{+}$Childer (1976) ^{x}Anonymous (1967)
Pear	23-25^{c}		Nyeki (1973)
Plum		22^{c}	Stott *et al* (1974)
Almond		15^{c} a 25^{c} b	Kester and Bradley (1976)
Avocado		25/20^{c}	Sedgley (1977)
Grape	26.6-32.2^{d}	26.6-32.2	Winkler (1974)

Note:
^{a} = when selfed
^{b} = when crossed
^{c} = day/night temperature
^{d} = highest germination

According to Williams (1970) ovule senescence in detached apple flowers was accelerated by high temperatures (15-25^{o}C) and this is probably a feature of orchard flowers also, though obviously to a lesser degree. He found that detached flowers kept in the 7-15°C range exhibited a lower sensitivity of the maternal parts to temperature. Thus it can be assumed that EPP of a flower can be modified through high sensitivity of pollen tube growth to temperature. In other words, pollen tube growth rate can be considered as the critical factor in determining the EPP of a flower at lower temperatures (7-15°C).

A.2. *Effects on pollen viability:*

According to Westwood (1978), under field conditions at moderate temperatures and high light intensity pollen has a short life and normally is viable for only a few hours. However, at low humidity, low light intensity and sub freezing temperatures pollen may remain viable for several years. Frankel and Galun (1977) have noted that temperatures above the optimum for the growth of the species decreases pollen longevity. Viability is extended progressively with a decline in temperature.

The results of the temperature experiments have indicated that pollen is not damaged by low temperatures (Sachan and Patro (1970) citrus - up to -20°C; Griggs (1953) apple, peach, plum, sour cherry - about 2-8°C; Olmo (1942) grape - up to -12°C). Nebel (1939) found that pollen viability can be substantially extended at a temperature range of 2-8 C under the condition of 50% relative humidity and observed that apple, pear, plum, peach, apricot and sour cherry pollen remained viable for 4, 3, 4½, 2 and 5½ years respectively under the storage conditions as mentioned above. However water content also plays an important role in the viability of pollen. Olmo (1942) observed that germination capacity of grape pollen preserved at -12°C was higher at 28% relative humidity (R.H.) than at 56% R.H. Stanley and Linskens (1974) have cited Aldrich and Crawford (1941) as having stated that for many pollen predrying is essential before storage at temperature below 0°C (see also Chapter Two B).

A.3. *Effects on pollinating insects:*

Low temperatures reduce the number and kinds of insects visiting the blossom of fruit plants (Anonymous (1967)). There is little insect activity below 10°C but above this temperature activity progressively increases (Williams and Wilson (1970); Westwood (1978)). Fox Wilson (1933) found that most bees, especially hive bees, are affected by low temperatures even when there is bright sunshine, whereas some wild bees (*Andrena sps*) are active when the temperature is low provided that the sun is bright. He also found that bumble bees are not unduly deterred by inclement weather.

It is reported that in spring hive bees collected pollen at temperatures as low as 7.7-11.1°C but foraging was confined to only those bees whose foraging areas were close to the hives (Rashad (1957) as cited by Anonymous (1967)). Free (1970) has cited Sax (1922) as having observed no bees in cool windy weather and Nevkryta (1957) as having observed no bees at 12 to 15°C in *Prunus avium* trees (cherry trees) more than 125 miles away from the hives. Hootman and Cale (1930) also observed only few bees further than 60 metres from their hives in fruit orchard during cool windy weather. Thus bee activity probably remains restricted (Anonymous (1967)) or is not at its greatest until temperatures are well above 15°C (Jackson (1974)). Brittain *et al* (1933) are cited by Anonymous (1967) as having stated that in Nova Scotia the optimum temperature for hive bees and the species of *Halictus* and *Andrena* is considered to be approximately 20°C.

Traynor (1966) has suggested that clearing an area around the hives or placing hives on black tar paper extending a foot or two in front will enable the hives to warm up faster and give the bees a bit more working time during the day.

B. *Humidity, rain and wind:*

High humidity or rain tends to delay the opening of anthers (Wauchop (1968); Teskey and Shoemaker (1978); Winkler (1974); Jackson (1974); Childer(1976) and Williams (1970)) because opening depends on stress from drying of the outer surface of the anther. Wauchop (1968) has noted that pear anthers will dehisce normally up to 80% R.H. Between 80% and 90% R.H. the anthers split but the walls do not recurl to expose the pollen load. Above 90% R.H. the anthers remain intact. At any effective humidity temperature combinations the outer whorl of stamens dehisce more rapidly than the middle whorl which in turn dehisce more rapidly than the inner whorl. The rate of dehiscence increases with temperature (see also Chapter Two A). Therefore it can be said that cloudy days and fog may delay anther dehiscence because of their tendency to increase air humidity and lower the temperature.

Rain may have deleterious effect on pollination. Not only may pollen be washed off stigmas and out of pollen sacs, but ripe pollen grains, because of high osmotic concentration of sugar within them, will readily burst in contact with water (Anonymous (1967; Winkler (1974)). Winkler (1977) has noted that only one third as many grape pollen grains germinate in a 5% sugar solution as in 15% solution. Thus it seems probable that when abundant rain falls while the flowers are opening the stigmatic fluid may become so diluted that the number of pollen grains that germinate will greatly be reduced. Burchill (1963) showed that rain removed most of pollen from the atmosphere but within a few hours of its cessation pollen was again detected. However, low humidity conditions are also not advantageous because such conditions may cause drying of the stigmas and reduction in pollen germination (Jackson (1974); Childer (1976); Teskey and Shoemaker (1978).

According to Frankel and Galun (1977) most pollen retain viability longer at low relative humidity (0-40%) than at higher ones but usually not below a distinct critical level which is species specific. For example, for apple and pear pollen a relative humidity of 10% is optimal; both higher and lower humidities severely shortening storage life. Stanley and Linskey (1974) have cited the results of many investigators (Kesseler (1930); Keinburg (1950); Visser (1955) and Lanner (1962)) as having indicated that pollen longevity, in general, is negatively correlated with the relative humidity during storage. Longevity usually increases with the reduction of relative humidity to about 6% during storage. Many pollen lose viability at either very high or very low humidities. Gollmick (1942) is cited by Linskens (1964) and Stanley and Linskens (1974) as having stated that longevity is best at a relative humidity between 6% and 60%; and for most species the optimum condition is below 60%. According to King *et al* (1930) relative humidity consistently enhanced the storage life of apple, quince, pear, peach, apricot and cherry pollens even at limiting temperatures such as room temperature.

After predrying, pollen longevity was generally increased at low humidity and temperature below $0^{\circ}C$ (Layne and Qua (1975)). Linskens (1964) and Stanley and Linskens (1974) have cited O'Konuki (1930) and Bunning and Hardtle (1946) as having given the following reasons for the resistance or tolerance of pollen to damage by desiccation:- pollen has only a small vacuole which makes mechanical damage difficult. As a consequence of low water content vital processes are active and respiration is reduced. For the same reason, diffusion is reduced, the chemical reactions involved in respiration are inhibited and the activity of enzymes is reduced.

Both rain and lack of sunshine reduce the number and kinds of insects visiting blossom (Anonymous (1967); Williams and Wilson (1970)). Flower visiting by insects usually ceases during heavy rain or showers; bumble bees are the exception but it is doubtful if they are then effective pollinators because anthers dehiscence is prevented by water and pollen can be washed off anthers (Williams and Wilson (1970), see also page 9 paragraph 2). Excessively wet weather may also cause flooding of the over wintering quarters of those wild bees which hibernate underground (Anonymous (1967).

Although wind acts as a pollinating agent (see Chapter Two D) wind may also cause an adverse effect on pollination by desiccating the stigmas, by hastening style death and petal fall and by limiting bee activity (Anonymous (1967);
Wind speeds above 11 miles per hour reduce the activity of pollen gatherers, while at 21 miles per hour bees remain in the hive

(Rashad (1957) as cited by Anonymous (1967)). If sufficiently strong, wind also influences the direction in which bees forage (Anonymous (1967)). Thus it is probable that windbreaks to a certain extent may reduce the adverse effects of wind on pollination.

C. *Orchard pesticides:*

C.1. *Importance of orchard pesticides:*

Crop protection programmes in the orchards are of special importance because all kinds of pome and stone fruits and most small fruits are long lived crops which need constant and intense care if they are to maintain their yield capacity over many years. According to Cramer (1967), the annual world loss of fruits (excluding citrus) from insect pests, diseases and weed infestation amount to $1,900 million. This is equivalent to a 23.4% loss of returns with insects accounting for 7.8%, diseases accounting for 12.6% and weed infestation accounting for 3%. Green (1976) has cited the Food and Agriculture Organisation of the United Nations as having estimated that 30% of the total potential apple production is lost to pests and diseases.

According to Kolbe and Kramer (1969), commercial fruit growing in its present day form would not be possible without the use of chemical pesticides for the protection of crops. In a long term (nine years, 1967 to 1975) trial laid at the Laacherhof Experiment Station to determine the losses of yield of some apple varieties from diseases and pests Kolbe (1975) obtained the following results:-

(i) In the complete absence of crop protection measures, yield was reduced by 40% and the proportion of market grade apples decreased from 80-30% as compared with the figures for a normal crop protection programme.

(ii) In the absence of fungicide and acaricide applications, yield was reduced by 40% and the proportion of market grade apples decreased by 35%.

(iii) In the absence of insecticide applications yield was reduced by 20% and the proportion of market grade fruits by 40%.

(iv) When measures were applied for the control of mildew and no scale fungicides, acaricides or pesticides were applied, yield was reduced by 20% and the proportion of market grade fruits reduced by 40%.

According to Green (1976), from grower's point of view crop protection chemicals when properly used have proved to be a very rewarding investment. Good appearance and substantial freedom from blemish or damage are a prerequisite now-a-days for a grower to obtain a reasonable price for his crops, and they can be assured economically only by intensive use of crop protection chemicals. In a series of experiments with apples over a period of ten years Green (1976) observed $5.17 average return for each $1 expenditure on pesticides.

The first use of chemical substances in the fight against pest and diseases occured during the middle of nineteenth century (Brown (1978), Green (1976)). Since that time a whole range of synthetic products have been discovered and developed and about 6000 novel compounds have been patented of which about 600 have found some commercial use. Some 80 different herbicides and fungicides and 40 insecticides are in common use in New Zealand (Anonymous (1979)). Out of these 120 pesticides only a small number are important from the point of view of the control of major pests and diseases during bloom period in New Zealand orchards. A list of such pesticides accompanied by the major pests and diseases controlled during bloom period in different kinds of fruit trees is presented in Table No.2.

However, it is evident that for fruit production, use of pesticides has proved very rewarding financially, it is true that the returns obtained from use of pesticides will vary from crop to crop and from locality to locality (see Chapter Two C.2.) and are greatly influenced by the skill and efficiency with which the farmer uses crop protection methods. Because pesticides have been deliberately developed to be toxic to some living organisms there is a possibility that some

Table No.2. *Orchard pesticides for blossom season application in New Zealand.* (a)

Name of the pesticides	Major pests and diseases	Crop
Bayleton (Bayleton^{R})	Powdery mildew	Apple
Benomyl (Benlate^{R})	Black spot, Powdery mildew	Apple
	Brown rot	Stone fruits
Binapacryl (Morocide^{R})	Powdery mildew, Two spotted mite	Apples
Bupirimate (Nimrod^{R})	Powdery mildew	Apples
Captan	Black spot, Glomerella ripe spot	Apples and pears
	Brown rot	Stone fruits
	Botrytis	Kiwifruits
Captafol (Difolatan^{R})	Black spot	Pears
	Brown rot	Stone fruits
	Alternaria	Tangelo
Chinmethionate (Morestan^{R})	Powdery mildew, European red mite	Apples
Dichlofluanid (Euparen^{R}) (b)	Botrytis, Downy mildew	Grapes
	Botrytis	Berry fruits, Kiwifruits (b)
Dinocap (Karathane^{R})	Powdery mildew	Apples and stone fruits
	European red mite	Apples
Iprodione (Rovral^{R}) (b)	Brown rot	Stone fruits
	Botrytis	Berry fruits, Kiwifruits (b)
Mancozeb (Dithane M45^{R})	Black spot	Apples
	Downy mildew	Grapes
Mancozeb 7% plus Dinocap (Dikar^{R})	Black spot, powdery mildew, E. red mite	Apples
Polyram	Black spot	Apples
	Downy mildew	Grape
Streptomycin (Agrimycin ^{R})	Fire blight	Apples and pears
	Bacterial spot, blast	Stone fruits
Triforine (Saprol^{R}) (c)	Brown rot, powdery mildew, rust	Stone fruits
Vinclozoline (Ronilan^{R}) (b)	Brown rot	Stone fruits
	Botrytis	Berry fruits, Kiwitfruits (b)

Note: (a) After McKenzie (1979)
(b) Not yet registered for use in Kiwifruits
(c) Fully registered in 1977 for brown rot control in stone fruits

pesticides may attack non target as well as target species. They are hazardous to bees (see Chapter Two C.3.). Some pesticides may be toxic to pollen (see Chapter Two C.2.). Thus it becomes necessary to confirm the safety with which pesticides may be applied.

C.2. *Effects on pollen germination and pollen tube growth:*

Very little information is available on the effects of different pesticides on pollen germination and on the rate of pollen tube growth. The available information shows that pollen from different varieties may differ in their reaction to a fungicide. For example, in invitro tests sulphur reduced the germination of apple pollen by 40% (Ries (1975)) but did not reduce the germination of cherry pollen (Eaton (1961)). Church and Williams (1978) observed that in apples, pollen germination of Grenadier variety was affected by fungicides such as triforine, sulphur, dithianon and dinocap liquid, and those of varieties Golden Delicious, Lord Lambourne and Worcester were affected by only one or two of those fungicides, perhaps because the anthers were more matured than those of *Malus purpurea* cv Aldenhamensis and *Malus sps* cv Winter Gold whose pollen germination were not affected by all those fungicides.

The results of invitro studies show that some pesticides such as dinocap, thiophanate methyl (Church and Williams (1977)), sulphur, zineb, 77% mancozeb + 7% dinocap (Dikar^{R}), polyram (Ries (1975)), captan and benomyl (Church and Williams (1977) and Ries (1975)) reduced the germination of apple pollen and triforine reduced the germination of apple (Church and Williams (1978)) and blueberry pollen (Bristo (1978) as cited in Ispray Ltd Report New Zealand (1979)). However a large reduction in the pollen germination under laboratory conditions may not indicate a similar level of effect in the field. For example, Bristo (1978) (as cited in Ispray Ltd Report New Zealand (1979)) found that blueberry pollen germination was completely arrested when pollen was placed on agar medium containing a concentration of 50 ppm Funginex^{R} (Triforine). But if flowering blueberry plants were sprayed with Funginex^R (Triforine) and the pollen collected from treated blossom placed on a PDA medium Funginex^R (Triforine) did not exhibit an inhibiting effect until the dosage was increased up to 2000 ppm.

According to Eaton and Chen (1969) spraying anthers before dehiscence causes less damage to pollen than spraying dehisced anthers. Church *et al* (1978) observed that captan and dinocap sprays on undehisced anthers did not affect the weight of pollen releasėd but average percent pollen germination and pollen tube growth in vitro were significantly lower than the unsprayed controls. When dehisced anthers were sprayed germination and pollen tube growth were reduced. More recently, in a trial to study the effects of commercial fungicides containing captan, dinocap, sulphur and triforine on apple pollen in the anthers Church and Williams (1978) observed that captan, dinocap and triforine reduced the amount of pollen released. All fungicides reduced the viability of pollen when sprayed on undehisced anthers and killed pollen when sprayed onto dehisced anthers of Cultivar Crawley Beauty.

In invivo studies with apples MacDaniels and Hildebrand (1939) found that Bordeaux mixture (2-6-100), 20-80 copper lime dust and sulphur reduced the pollen germination but did not inhibit the growth of pollen tubes upon the stigma. Church and Williams (1976) found that commercial fungicides containing dinocap, captan, binapacryl and diathianon reduced pollen germination on stigmas sprayed two hours after pollination but did not prevent fruit set. Spraying with thio-phenate methyl or benomyl formulations, or commercial fungicides containing dinocap, captan, binapacryl and diathianon one day before or after pollination, had no effect on pollen germination. Lovelidge (1976) has noted that wetting agents have an adverse effect on possibly through osmotic effects on pollen grains/ tubes.

The evidence on the effects of Agrimycin 17W and triforine on stone fruits pollen are conflicting. In U.S.A., Ries (1975) noted that Agristep 17W had no adverse effect on apple pollen in vivo. In New Zealand, until 1976, triforine was not noted to cause poor fruit set in peaches and apricots. Since 1977,

Table No.3. *Effects of fungicides on vitro pollen germination.*

Fungicides	Name of the crop	Rate	% pollen germination	Reference
Benomyl	Apple	Control 250 ppm	100) 59)	Ries (1975)
Benomyl	Apple	Control 250 ppm	79) 72)	Church and Williams (1978)
Benomyl	Apple	Control 250 ppm	100) 48)	Church and Williams (1977)
Thiophanate methyl	Apple	Control 500 ppm	79) 72)	Church and Williams (1978)
Thiophanate methyl	Apple	Control 370 ppm	100) 78)	Church and Williams (1977)
Bupirimate	Apple	Control 600 ppm	79) 63)	Church and Williams (1978)
Captan	Apple	Control 940 ppm	79) 2)	do
Captan	Apple	Control 1000 ppm	100) 13)	Ries (1975)
Captan	Apple	Control 1250 ppm	100) 0)	Church and Williams (1977)
Dikar^{R} + Trition^{R}	Apple	Control 2000 ppm a.i + 187 ppm a.i	100) 1)	Ries (1975)
Polyram	Apple	Control 2000 ppm	100) -)	do
Triforine	Apple	Control 240 ppm	79) 8)	Church and Williams (1977)
Triforine	Blueberry	Control 50 ppm	100) 0)	Bristo (1978) as cited by Ispray Ltd (1979)
Sulphur	Apple	Control 3800 ppm	79) 0)	Church and Williams (1978)

three reports have been received where poor fruit set in cherries and peaches occurred following the application of triforine over the blossom period (Anonymous (1978)). The report from Motueka (New Zealand) shows no clear evidence of damage by triforine to cause loss of crop when used on its own. Some damage was suspected when triforine (Saprol^R) was applied with triforine/agrimycin. Celamerck GmbH & Co's report indicates that triforine was very well tolerated using three applications at the rate of 0.1% to 0.2% but agrimycin and the combination of agrimycin and triforine (Saprol^R) were not tolerated by sweet cherry flowers (Agricultural Chemicals Board, New Zealand unpublished report (1979)). However, the effects of both triforine and agrimycin on cherry pollen were not studied, there are possibilities that agrimycin or triforine alone or the combination of agrimycin and triforine may be harmful to stone fruits pollen under New Zealand conditions.

From the review presented above some of the conclusions that can be drawn may be as follows:

(a) None of the pesticides examined so far are harmless to pollen. Benomyl is least toxic and sulphur and captan are the most toxic of those tested (see Table No.3.). But unfortunately it is reported that benomyl is not effective against black spot in some apple orchards where resistance has been developed (MacKenzie (1979)). Therefore, a study of the effects of many other fungicides particularly wide spectrum fungicides such as mancozeb 77% plus dinocap 7% (Dikar^R), captafol, on apple pollen in New Zealand orchards becomes important.

(b) A study on the effects of triforine and agrimycin 17 alone and the combination of triforine and agrimycin 17 on the pollen germination and pollen tube growth of stone fruits is important because of the following reasons:

   (i) triforine in combination with agrimycin 17 was suspected to be involved in cherry fruits thinning in New Zealand.

(ii) both triforine and agrimycin 17 are important fungicides for stone fruits. According to Ispray Ltd, triforine is probably the most effective and cheap fungicide of those currently available for the control of brown rot in stone fruits

(iii) the effects of triforine and triforine plus agrimycin on stone fruits pollen under New Zealand conditions have not been studied so far.

(c) The effects of captan on pome, stone and berry fruits pollen under New Zealand conditions have not yet been studied. Because captan is a wide spectrum fungicide (MacKenzie (1979)) and overseas studies have shown it to be inhibitory to apple pollen germination and pollen tube growth, the study of the possibilities of its use as a fruit thinner in heavy bearing apples and peaches may be important.

(d) The study of the effects of fungicides which have been reported to have affected fruit set such as dichlofluanid (Euparen^R) which reduced fruit set in stone fruit (Rough (1977)), bayleton (Bayleton^R) which increased fruit set in apple (Lawes personal communication) on the pollen of different fruit crops may be important. It may also be beneficial to compare the thinning effects of dichlofluanid (Euparen^R) with that of other/established thinning agents in stone fruits.

(e) The studies of the effect of pesticides such as bupirimate (Nimrod^R) on apples and vinclozoline (Ronilan^R) and Iprodione (Rovral^R) on stone fruits, kiwifruits and berry fruits pollen may be beneficial because these pesticides are important for the crops mentioned.

C.3. *Effects on pollinating insects:*

Many orchard pesticides have been found to either reduce the activity of or destroy bees. Wetting of flowers by spraying can reduce bee activity (Traynor (1966)). Fruit blossoms can be made unwittingly less attractive to bees by spraying during the bloom period with certain materials, e.g. lime sulphur (Anonymous (1967).

Many orchard pesticides are poisonous to bees and to other pollinating insects (Johansen (1969), Johansen (1977); Palmer-Jones (1967); Palmer-Jones (1965)). The most common symptom of bee poisoning is the appearance of excessive numbers of dead bees in front of hives. When the poisoning is severe thousands of bees will accumulate in front of a hive each day. Such a reduction in bees number will reduce pollination in the orchard. The toxicity of some selected pesticides to honey bees is presented in Table 4.

Any type of poisoning is likely to cause the bees to become agitated and agressive. When the hive cover is removed they fly off the top bar, sometimes straight at the bee keeper's head. Other signs include stupefaction, paralysis and abnormal, jerky or spinning movements. However, bees behaving as if they are chilled, crawling around in front of the hive is an almost sure sign of carbaryl poisoning. Bees exposed to this chemical quickly lose the ability to fly; they slow down and may take as long as 2-3 days to die (Johansen (1977); Johansen (1979)). Such disturbances of the foraging behaviour of bees reduces their effectiveness as pollinating agents.

According to Johansen (1979), if dead and dying light-coloured, newly emerged workers are seen it is a sure sign of pollen contamination because newly emerged bees feed actively on pollen and begin to produce royal jelly a few days later. However, the queen may remain alive and apparently healthy for up to two weeks before she is superseded. Lack of brood is not related to supersedure, but the supply of queen phermone is (Chaudhary and Johansen (1971)), and when it falls below a critical amount (which takes 10-14 days) the workers will get rid of the queen.

Queens may also be affected by contaminated pollen and behave abnormally. She may begin to lay erratically or she may simply die (Johansen (1977); Johansen (1979)). Johansen (1977) has noted that queenlessness has been associated with the use of a wide variety of insecticides, including arsenicals, dieldrin, carbaryl, malathion and parathion. Severely weakened and queenless colonies do not survive the following winter.

Thus, as a result of the death of bees from the use of insecticides on crops in flower, under the Apiaries Act 1927 the Apiaries Protection Regulations 1957 were introduced in New Zealand. The Act states that preparations injurious to bees shall not be applied in fruit trees including strawberry, raspberry, boysenberry and loganberry plants when the trees/plants are in bloom, unless most of the blossom has fallen (Palmer-Jones (1965)).

C.4. *Factors affecting poisoning:*

Dust formulations of pesticides are usually more hazardous to bees than sprays because powder residues remaining on a treated plant readily adhere to the body of a foraging bee and when she combs her hairs and packs the pollen on to her corbiculae, the insecticide becomes a contaminant in the pollen loads. (Johansen (1977); Johansen (1979)). Johansen (1979) has shown that powder formulation killed six times more bees than did the liquid formulations, even when treated foliage contained more of the active ingredient following liquid formulation applications. Free *et al* (1967) have noted that granular formulations tend to be quite low in hazard to bees, as might be expected.

Persistent fungicides are more dangerous, contaminating both nectar and pollen in the hive and so destroying developing bees and queen (Palmer-Jones (1965).
Johansen (1977) and (1979) have noted that a material such as naled (emulsifiable concentrate) can be applied with relative safety in late evening because it has a short residual toxic effect on honey bees, even though the initial hazard at application time is high. Johansen (1979) has found that wettable powders have a longer residual effect than emulsifiable concentrates.

Unusually cold nights following hot summer days cause condensation of copious dew on foliage, and the residual action of insecticides is then increased and many more bees may be killed the following day. According to Johansen (1977), an average $-12^{o}C$ lower mean temperature resulted in a four to five day increase (approximately two times) in residual toxicity to bees from short-residual chemicals. Johansen (1979) has cited several investigators as having shown that both DDT and carbaryl (Sevin^{R}) are considerably more toxic to honey bees at low than at high temperature.

Timing of application is obviously related to factors discussed above. Johansen (1979) has noted that application during late evening, night or early morning provides relative safety to bees from short-residual chemicals. However, the results may vary from one geographical region to another.

The strength of honey bee colony and the age of bees also affect the toxicity. Populous colonies always suffer greater losses than small colonies because more foragers are exported to the pesticidal residues. Often the kill is four times as great with the same applications. Newly emerged bees are much more susceptible to poisoning than the older bees that have been active in the field for three or four weeks (Johansen (1979)). It is true that reduced bee numbers and weaker colonies means low bee density in a period of perhaps few crop flowering days and thus leads to low bee activity and lower fruit set.

Flower structure may have some effect on the toxicity of pesticides to bees. Sprays would enter more readily in open flowers than in closed or less open ones (Palmer-Jones (1965)).

It is reported that addition of solvents and oily substances to spray materials tend to make them safer to bees. When Johansen (1972) increased the amount of the formulating solvent, xylene, demeton emulsifiable concentrate became less toxic to the alfalfa leaf cutting bee (*Megachile rotundata*). Johansen also tested the addition of powdered plastics, resins and spray oils to insecticides known to be hazardous to bees.

Many of these sprays became less toxic to bees. Johansen *et al* (1974) have cited Connola *et al* (1966) as having found that stickers such as Pinolene 1882 used with carbaryl allowed reduced rates and decreased hazard to bees. Johansen (1977) has cited Standifer (1972) and Moffett and Morton (1973) as having found that low dosages of surfactants were not toxic to bees. Foam additives that have been used to reduce the problem of spray drift do not increase the residual hazard to bees.

According to Johansen (1972) there are two possible explanations for observed safening effects; oils, stickers, emulsifiers and solvents may bind the residue more tightly to the plant. The result is less run off (and subsequently less environmental contamination) and more contact or ingestion by the pest, but decreased pick-up by pollinators or entomophagous insects. Resins, plastics and encapsulating materials coat insecticide particles making them less available to insects by reducing contact. These same materials may also increase absorption by plant and may increase toxicity to piercing and sucking insects by increasing systemic action.

Use of repellents to reduce the hazard of insecticidal sprays has been investigated since the early 1900s. Laboratory screening tests of 143 chemicals showed compounds containing nitrogen, short side chain substituted phenyl acetates and tolyl derivatives had greatest promise (Atkins *et al* (1975)). Aromatic five-, six- and seven membered ring structures containing nitrogen in the ring, straight chain amides, and phenyl ring structures with short-chain length amide substitution were among the most repellent compounds tested.

D. *Mechanisms of pollen transfer:*

D.1. *Wind pollination:*

It is reported that wind disseminates the pollen of apple (Burchill (1963); Smith and Williams (1967)), pear (Stephen (1958)), grape (Gladwin (1937) reported by Free (1970)) and nuts (Childers (1976) and Westwood (1978)).

According to Westwood (1978) wind borne pollen is only three hundredth of a millimeter in diameter and even in a mild breeze such pollen can travel hundreds of yards. Childers (1978) has noted that pecan pollen may be carried 909 m by the wind. Free (1970) has cited Hockey and Harrison (1930) and Brittain (1933) as having found that apple pollen was deposited on slides placed up to 61 m from apple trees; Stephen (1958) collected pear pollen on slides up to 1.2 km away from pear trees; Free (1970) has reported Gladwin (1937) as having observed that grape pollen can be carried up to 4.9 m by gentle fresh air currents but none was carried 7.3 m. In all observations, the amount of pollen disseminated decreased with the increasing distance from the pollen source trees. According to Smith and Williams (1967) dissemination of pollen is also affected by the turbulance of the air. They have noted more turbulance would increase the chances of deposition of pollen on stigma. Thus Springer (1916) and Auchter and Knapp (1937) (as reported by Free (1970)) supposed that wind sometimes affects self pollination of *Prunus* and *Pyrus*, or even cross pollination when the branches of adjacent trees are interwoven. However, all the workers mentioned above have concluded that wind pollination of fruits (except nuts and grapes) is not important. Stephen (1958) supposed that pollen must be forcibly brushed into a stigma before it adheres significantly to germinate and that wind borne pollen is therefore ineffective.

Olmo (1943) caged half of each of five 'Almeria' grape vines to exclude large insects such as honey bees and syrphids and found that 8.5% of the caged flowers set fruit compared to 10.5% of those in the uncaged halves and Olmo concluded that wind was the most important pollinating agent. Westwood (1978) noted that mulberry, chestnuts, pecans, filberts and walnuts have wind pollinating systems.

According to Robbins *et al* (1962), wind pollination is common in plants with inconspicuous flowers producing enormous quantities of pollen. Proctor and Yeo (1970) noted that wind pollinated plants usually have pollen grains with a smooth,

Table No.4. *Toxicity of some selected pesticides to honey bees.*[*]

Not harmful.	Should not be applied directly on bees visiting flowers but in early morning or evening when they are not flying.	Highly toxic : Must not be applied to crops in flower at any time.
Binapacryl	Chlorpyrifos	Aldrin
Bordeaux mixture	DDT[+]	Aminocarb
Chinmethionate	Demeton	Azinphosmethyl
Cyhexatin	Endosulfan[+]	Dichlorvos
Dicofol	Malathion	Lindane
Mancozeb plus dinocap	Methyldemeton[+]	Parathion
Dinoseb plus dinosam[+]	Oil sprays (superior type)	Phosdrin[+]
Sulphur	Carbaryl	Phosmet
Thiram		Carbaryl
Zineb		

[*] After Palmer-Jones (1965) and Johansen (1979).

[+] Pesticides field tested in New Zealand.

dry, surface in contrast with the sticky and often highly ornamented grains that are common in entomophilus species. Consequently their pollen grains are dispersed singly or in twos and threes, rather than sticking together in large groups.

Thus it can be said that although wind disseminates pollen of most fruit plants, wind pollination is controlled by factors such as - adoptability of flowers to wind borne pollen, turbulance of the air created by the wind and the distance from where the pollen was carried by the wind. This shows an understanding of the effect of pesticides on bees is important as this other vectar (the wind) plays little or no part in set.

D.2. *Insect pollination:*

Insects pollinate flowers of cherry, plum, prunes, peaches (Westwood (1978), Free (1970)), apples (Free (1966), Free (1970); Bulatovic and Konstantinovic (1960), MacDaniels and Heinicke (1929)) grape (Olmo (1943)), avocado (Papademetrious (1976)), citrus (Free (1970)) and pear (Bulatovic and Konstantinovic (1960); Stephen (1958)) trees. According to Westwood (1978) all plants with showy flowers are insect pollinated. Some apple (Langridge (1975); Palmer and Clinch (1968)), pear, peach, cherry (Langridge (1975)) and avocado (Papademetrious (1976)) varieties need insects for cross pollination to occur in them. Therefore, it can be said that the knowledge of the factors influencing the population and the pollinating efficiencies of insects in the orchard becomes important.

It is reported that honey bees and other races of hive bees depend for their existence on the pollen and nectar they collect from flowers (Anonymous (1967)). Pollen becomes trapped in the branched hairs which cover their bodies and although the bees clean their bodies of pollen which they place in pollen baskets on their rear legs, many scattered pollen grains are left. It is this remaining pollen that effects pollination (MacDaniels and Heinicke (1929), Anonymous (1967), Anonymous (1972)).

Free (1970) has cited many workers (Menke (1952), 75%; Smith (1962), 50%; Tsygankov (1953), 85%; Roberts (1956), 99%; Free (1966), 87%) as having found that honey bees form a high percentage of insects visiting *Prunus* and *Pyrus* flowers. In Egypt more than 45% of the insect visitors were bees. In Trinidad, however, avocado flowers were rarely visited by honey bees (Papademetrious (1976)).

Bees visiting flowers collect either pollen or nectar or both. Pollen collecting bees are more efficient than nectar collectors since they contact the stigma more frequently (Free (1966)). Free discovered that James Grieve and Cox's Orange Pippin (apple) flowers that received single visits from bees that scrabbled for pollen set averages

17.3% and 13.5% more flowers respectively than those visited by bees that did not scrabble. In citrus bees that foraged for both pollen and nectar collected, on average, less of each commodity than bees that collected one or other only. However, because the relationship between the length of pistils and stamens is constant within a variety (Forshey (1953)) even pollen gatherers may not pollinate certain varieties whose stigmas are too far above or below the stamens (Roberts (1945)).

Although honey bees may travel 3 km or more to visit flowers (Wilson (1929) as reported by Free (1970) pollen collection is normally limited to only 0.4 km (Percival (1947)). According to Free (1966) the pollinating efficiency of honey bees decreases as the distance from the source of compatible pollen increases.

Individual honey bees do not forage over the whole area within flight range but restrict their activities to a small portion of it to which they repeatedly return (Anonymous (1967)). Particularly during unfavourable weather, bees tend to forage near their hives. This has been frequently noticed in orchards where weather during flowering is likely to be changeable (Free (1970)). Free (1970) has cited Nevkryta 1957) as having found that at 12 to 15°C bees did not visit *Prunus avium* trees more than 125 m from their hives. Butler (1943) observed that bees visiting distant dishes of sugar syrup were more easily deterred by unfavourable weather than bees working to their hives; and that such bees did not work nearer home in these circumstances.

The rate at which bees visit fruit flowers depends on the amount of nectar (Free (1970); William and Wilson (1970)) and pollen present and these vary with the type of flower and stage of its development with climatic conditions and with the number of foraging insects present (Free (1970)).

The attractiveness of fruit flowers to bees increases with the increase in the concentration of sugar in nectar (Anonymous (1967); Butler (1945) as reported by Williams and Wilson (1970)) which varies with the species (Free (1970), Vansell (1934), with the stage of development of flowers (Ewert (1940) as reported by Free (1970)) with environment particularly humidity (Anonymous (1967), Vansell (1934), Vansell (1942)) and with soil moisture conditions (Traynor (1966)).

Nectar secretion occurs only above a threshold temperature which varies with the species concerned. Wild cherries secrete nectar at temperatures of 8°C or above only (Free (1970)). Vansell (1934) observed that plum nectar concentration varied from 62% early in the morning (100% R.H.) to 25.8% at 2 p.m. in the afternoon (55% relative humidity). Vansell also found that in some flowers with an open structure notably apricot, nectar was diluted by dew or rain at night. According to Free (1970), Vansell and Grigg (1952) reported that a dry wind produced a marked increase in apricot nectar concentration and bee visitation.

Thus, it can be said that the beneficial effects of honey bees in pollinating fruit flowers depends on factors such as foraging behaviour, flight range, and population of honey bees, the amount of pollen and nectar produced and the concentration of sugar in nectar produced by flowers. These factors may directly or indirectly be influenced by weather conditions and pesticides sprayed in the orchard (see also Chapter Two, A, B and C).

E. *Methods of promoting cross pollination:*

The discussion on the different aspects of pollination as presented above in the preceding sections shows that successful transfer of pollen in fruit flowers primarily depends on the availability of compatible pollen, activity of the pollinating agents (except for the autopollinated flowers e.g. citrus) and synchronization of the bloom period and the pollinating agent activity period (particularly with those flowers whose pollens and/or ovules remain viable for a relatively shorter period of time) in the orchard. Therefore the methods which are or can be used to increase the supply of pollen at proper time, and to improve the pollinating efficiency of the pollinating agents in the orchard will be dealt in this section as follows:

E.1. *Indirect methods:*

(a) *Planting pollinizers at effective distances:*

That the distance from pollinizers influences both the set and yield of main varieties has long been demonstrated (Tukey (1925), cherry; Free (1962), plum and apple; Westwood and Grim (1962), pear; Free and Spencer Booth (1964), dwarf apple; Williams (1959), cider apple; Anonymous (1967), apple; see also Chapter Two D.2.). Tukey (1925) showed a sharp reduction in both set and yield where trees were more than one row from a source of foreign pollen, with yield further reduced to 50% at 60 ft. Williams (1959) showed in an orchard of cider apple variety, Michalin, a self incompatible diploid, that the useful effect of the pollinating source can extend to approximately 90 ft. Free (1962) demonstrated that, in the plum orchard (variety 'Wydale') which had two rows of a pollinizer variety (Victoria) bisecting it, about 7% of the flowers on trees adjacent to the pollinizer set fruit, but only 1% or less did so on trees on four rows or more away. Free and Spencer Booth (1964) suggested that to ensure maximum pollination pollinator varieties should be at intervals of not more than 10 ft in the same rows. According to Anonymous (1967) any estimate of effective distance of pollinators is of limited practical value because the effective distance which is dependent on the pollinating insects will vary with local conditions and climate (see also Chapter Two A, B and C).

Thus however none of the workers (mentioned above) have provided precise information on the effective distances for cross pollination, the results of their work show that when planting trees in the orchard, the importance of distance from pollinizer variety should not be ignored.

(b) *Arranging pollinizers in the orchard:*

It is reported that following inclement blooming season, old orchards containing many varieties mixed together yielded heavily compared to more modern orchards containing fewer varieties (Anonymous (1967)). Stephen (1958) also found that cross pollination was greater, the greater there was intermixture of varieties throughout an orchard. However, while there is no proof that mixed pollen is preferable for cross pollination purposes, the inclusion of more than one pollinator variety reduces the risk of occasional insufficient cross pollination due to a small amount of blossom on the pollinator, or to a seasonal variation in overlap of flowering periods (Anonymous (1967)) or to the self/cross incompatibility of the pollinizer variety. For example, in an apple orchard of a triploid variety it may be necessary to include two diploid varieties as pollinators because most diploid varieties of apples are partly or wholly self incompatible (Anonymous (1972)). Thus it can be said that not only a high pollinator ratio but also the provision of more than one pollinator variety may be advantageous. But it is true that intermixing of many different varieties in the same orchard may not be usually commercially acceptable because : firstly all the varieties may or may not have equal commercial importance, secondly orchard operations such as pruning, spraying and harvesting etc. in such orchards become more costly in time and labour compared to those with minimum number of varieties.

In general practice, the pollinator variety can be introduced as single isolated trees at regular intervals throughout the main variety or it may form whole rows at intervals across the plantation (Anonymous (1972), Anonymous (1967)). In single tree arrangement systems, the pollinators can be spaced evenly throughout the orchard, thus aiding a uniform distribution of pollinator trees. In addition, although single tree arrangements may differ widely in pollinator ratios, they provide each of the main trees with a pollinator immediately adjacent on at least one side either directly or diagonally. Row system generally necessitates a fairly high pollinator ratio, for with bush trees at the common planting distance of 24 feet square low ratios lead to distances between pollinator and main variety that are too wide for efficient cross pollination. To provide every tree of the main variety with a pollinator on at least one side as in single tree systems every third row must be planted entirely with pollinators (Anonymous (1967)). Though the single tree arrangement system may result in more satisfactory pollination, work such as pruning spraying, particularly picking the fruits from those isolated trees is more costly in time and labour than when pollinator trees are grown in row system (Anonymous (1972)). Row pollinator arrangement system is often accepted because this arrangement gives increased ease of management in orchards.

In row pollinator arrangement, recommendations on the number of rows of one self unfruitful variety which can be planted together without preventing cross pollination vary from two to six (Free (1970)). In alternative row arrangement, the distance between pollinators and main variety should not be greater than twice the planting distance. Four rows of one variety may alternate well up to four of another variety (Anonymous (1967)). However, it is obvious that the number of rows of one self unfruitful variety to be planted together should be decided on the basis of the favourable conditions of pollination.

Another system of row arrangement is continuous row arrangement in which pollinating varieties are also placed in continuous rows. This system is usually convenient for those modern plantings where the trees are generally planted in continuous rows on a rectangular system so that the space between the trees is less than between the rows. This arrangement may not give the best pollinating conditions as the influence of the pollinator is seen on the rows adjacent to the main variety, but such systems have usually been successful in commercial practice (Anonymous (1972)).

Thus it can be said that to ensure adequate pollination, pollinator varieties (pollinizers) should be planted at suitable intervals in the orchard. The arrangement of pollinizers should be made in such a way that, if there is any thinning out of the orchard, there is still provision for adequate pollination.

(c) *Top working of the trees:*

Many workers (Anonymous (1972); Free (1970); Overley and Bullock (1947)) have noted that the grafting of the scions of the pollinating variety onto branches of the trees of main variety (top working) aids pollination in an orchard. According to Griggs (1953), top working of the branches led to more cross pollination than grafting of the whole tree. But the effect of top working is localized (Free and Spencer Booth (1964)). There is less success and more difficulty in harvesting the crop when one or two branches per tree are grafted (top worked) rather than the whole tree (Free (1970)). Free has noted that if grafts are used their fruits should be distinguishable from the main variety fruit so that no confusion arises during picking. Overley and Bullock (1947) have suggested that care must be taken while pruning, training and thinning the top worked trees.

(d) *Placing bouquets in the trees:*

This method has proved satisfactory when the bouquets of known satisfactory pollinizing varieties are properly handled and insects are present (Overley and Bullock (1947); Free (1962)). By placing bouquets of compatible pollen at intervals along rows of dwarf pyramid pears (variety Williams Bon Chretien) planted at 1.8 m x 3.6 m and which touched in rows, Williams and Smith (1967) found a gradient of decreasing fruit set and seed content over a distance of 24 trees (4 trees). However, records taken in adjacent row of trees gave no indication that the bouquets had affected set or seed content.

(e) *Using hive inserts:*

In this method previously collected pollen is placed in a device attached to the hive in such a manner that outward going bees become dusted with the pollen, thus carrying it to the trees they visit. While testing a modified hive insert in a pear orchard inadequately furnished with pollinators Townsend *et al* (1958) obtained, over two seasons, large increases in the quantity and uniformity of fruit set.

Hive inserts show obvious promise, particularly in orchards where cross pollination is normally inadequate. However, this method demands not only a readily available source of pollen, but frequent attention to the hives; it is necessary to replenish the pollen hourly to avoid loss of viability.

E.2. *Direct methods:*

(a) *Hand method (pollen application by brush):*

This method gives the most favourable results (Overley and Bullock (1947) but is time consuming and costly (Anonymous (1967); Teskey and Shoemaker (1978)). The operator applies the pollen to individual flower with a small stiff pig hair brush or with rubber end of a lead pencil. Care must be taken that contact is made with gentle pressure of the brush or pencil containing pollen to all stigmas of the pistil. Flowers opened only a few hours previous to operation are most receptive. Attention must be given to shady sides and tops of the trees, since in most orchards native insects or bees work best in the warmest and protected portion of the trees.

(b) *Spraying and dusting:*

Many labour saving methods for applying pollen have been developed and tested. Overley and Bullock (1947) tried a method of applying pollen water but were not too successful. They noted that the pollen in water solution does not adhere to the stigmatic surface of the pistil and pollen grains are apparently injured after 30 minutes. Teskey and Shoemaker (1978) have observed that apple pollen is seriously macerated when subjected to the pumps and fan of modern sprayers. In New Zealand, when kiwifruit pollen was mixed in a liquid medium containing algin (Sodium algenate) 0.005%, carboxymethyl cellulose 0.1%, boron (H_3Bo_4) 0.01%, and calcium nitrate ($Ca(No_3)_2$) 0.01% and applied as a spray in kiwifruit flowers Hopping (1978) observed that the number of pollen tubes in 22.5 mg/13 ml medium treatment was about half that found in hand pollinated styles and suggested that an improved method of application must be found. However, he noted that if each of the pollen grains observed with 22.5 mg/13 ml medium treatment reached ovules with the ovary there would have been sufficient number of pollen tubes/ovules (3 tubes/ovules) to ensure pollination and maximum seed development. More recently Hopping (1979) found that in the Black Doris plum the results of pollen application as a spray at the rate of 2.5 mg/ml of CBC medium (calcium nitrate 0.1%: boric acid 0.01%; calcium chloride 0.13%) was equivalent to that of hand pollination. CBC and CMC (calcium nitrate, mercuric chloride, calcium chloride) media maintained pollen viability in the liquid and also after pollen being sprayed. Hopping noted that the media may replace bees.

Bellows type small hand dusters (Overley and Bullock (1974)), pole dusters or puff gun (Williams and Wilson (1970)), bombs (Overley and Bullock (1947), Teskey and Shoemaker (1978) shotgun shells (Teskey and Shoemaker (1978), and aeroplanes (Overley and Bullock (1947) and Teskey and Shoemaker (1978) have been employed to dust pollen in fruit blossoms. The use of bellows type hand dusters resulted in heavy set requiring thinning but aeroplane method increased the set of fruit by only 1 percent (Overley and Bullock (1947)).

For application of pollen by dusting, pollen can be mixed with diluents such as powdered milk, egg albumen, and lycopodium powder etc. However, according to Overley and Bullock (1974), lycopodium, the best of pollen carriers studied, being an explosive organic material, does not produce good results when bomb is employed to dust pollen. Overley and Bullock have also noted that talc when used as a pollen carrier has a tendency to cover the stigmatic surface of the pistil the same as dust and apparently interferes with pollen germination.

CHAPTER THREE : *FERTILIZATION AND FRUIT SET:*

A. *Fertilization:*

With the completion of processes following pollination, two male nuclei are liberated from the rupturing pollen tube into the ovule. One of these nuclei fuses with the egg nucleus to form a zygote whilst the other fuses with the large central nucleus to form a primary endosperm cell of the embryo sac. (Anonymous (1967), Robbins *et al* (1962), Gardener *et al* (1952), Janick *et al* (1974), Chandler (1965)). These fusions that occur in the embryo sac are described or defined as *fertilization*. Following fertilization a number of changes are initiated in the parts of a flower, particularly in the ovary, as follows:

1. The stigma and style become withered and in many flowers the petals drop (Robbins *et al* (1962)).
2. The zygote develops to form plant embryo (Anonymous (1967), Robbins *et al* (1962), Gardener *et al* (1952); Chandler (1965)).
3. The primary endosperm cell develops to form the endosperm - the reserve food supply of the seed (Robbins *et al* (1962); Anonymous (1967); Chandler (1965)).
4. The embryo sac begins to enlarge rapidly, crowding and destroying many cells of the nucellus (Gardener *et al*

(1952)). In some plants, however, a portion of the nucellus may become the storage tissue of the seed rather than the endosperm. Such tissue of the nucellar origin is called perisperm (Robbins *et al* (1962), Gardener *et al* (1952)).

5. The integuments of the ovule develop to form the seed coat (Robbins *et al* (1962)).
6. Fertilization provides a stimulus which sets in motion the mechanism for the enlargement of the ovary (Gustafson (1961)). The ovary tissue to which ovules are attached develops/develops to form the *fruit*. (Gardener (1952); Robbins *et al* (1962); Chandler (1965)). According to Weaver (1972), the ovary fails to enlarge following anthesis unless fertilization occurs and unless parthenocarpy is initiated. Winkler (1974) noted that typical fruit of Thompson seedlers (grape) does not set without fertilization.
7. Accessory flower parts such as the receptacle or sepals or petals may also be stimulated to increased growth and become incorporated in the fruit (Robbins *et al* (1962).

Thus fertilization stimulates the growth of certain floral parts and brings about a withering of others. The net result is usually the development of the ovary and/or other flower parts into the fruit, and the ovules into the seed.

B. *Fruit set:*

Fruit set has been described or defined as the change over from the static condition of the flower/flower ovary to the rapidly growing condition of the fruit (Leopold and Scott (1952); Leopold and Kriedemann (1975)). The term is also used often in a somewhat narrower sense to indicate whether or not the fruit remains attached to the plant for any considerable time after flowering. Williams and Wilson (1970) have coined the terms 'Initial fruit set' and 'Final fruit set' to describe fruit set. According to these authors initial fruit set is assessed as the number or proportion of flowers that set fruit at 21 days after 80% petal fall and final set is assessed as the number or proportion of flowers that set fruit after June (December) drop.

However, it may be correct to note that a count of mature fruit is a less accurate indication of fruit set because factors other than pollination and fertilization in the post flowering period may influence the retention of fruitlets during or after June drop. Therefore, the term fruit setting or fruit retention may be defined as those changes which mark the transition of the flower into a young fruit and which involves the inhibition of abscission in a special zone at the base of pedicel of the young fruit that has survived the second drop period.

C. *Seed number and fruit development:*

It is generally considered that the developing seeds have an influence on fruit set and on fruit growth. That seeds are important in the development of sour cherry, early cherries and peaches was demonstrated by Tukey (1936b). Tukey found that when the embryo was destroyed at an early stage the fruit dropped, but if it was destroyed at a later date the fruit remained on the trees and ripened. According to Tukey (1936a), the influence of the seed on the growth of fruit can be seen quite distinctly in peaches, which like all stone fruits have an ovary containing two ovules. Following fertilization it is normal for one ovule to develop at the expense of the other, leading to a one seeded stone. As a result the fruit develops asymetrically, the side on which the growing seed is situated being slightly larger than the other. Gustafson (1961) has cited Heinickle (1917) as having stated that in general the weight of the fruit is proportional to the number of seeds in the fruits. Working with kiwifruits Hopping (1976) established a correlation between seed number and fruit weight and between seed distribution and fruit shape. But in apples, Brettain and Eidt (1933) found no correlation between the number of seeds and the weight of fruits. However, if in varieties having normally high seed content there was a lower number of seeds on one side this made less growth and the fruit was deformed. Schander (1955) (as cited by Anonymous (1967)) has noted that although fertilization is essential for fruit development, it is not necessary for all ten ovules to develop and fruits (apples and pears) may be produced with only a few seeds. In some varieties of apples such as Early Victoria and Grenadier, and in pears, fruits with low seed contents are more elongated in shape than those having the full complement of seeds.

It is reported that the development of the ovary and surrounding tissue into a fruit is dependent on hormonal stimuli chiefly from developing seeds (Luckwill (1949) as reported in Anonymous (1967)). However the mechanism of fruit set is unknown, gibberellins (GA), auxins, cytokinins, ethylene and abscisic acid (ABA) seem to have a role in the process (Dennis (1973)). Bukovac (1963) found that fruit set and growth of unpollinated ovules treated with gibberellins (GA) were similar to fruit set and growth of seeded ovules. Bukovac noted that as seeds develop following fertilization they produce GA which in turn triggers the production of IAA (a xin) which is related to fruit set. Bukovac's findings seem to be supported by the work of Sastri and Muir (1963) and Dennis (1967). According to Sastri and Muir, Studies with the tomato showed that diffusible auxin was not present in the flowers at open bloom but a significant amount could be obtained after the plants are treated with GA. Similar amounts of diffusible a xin were present in both GA treated and pollinated plants after 22 days. Dennis found that extracts from immature apple seeds contained substances with GA activity. When such extracts were applied to unpollinated blossoms of the same variety seedless fruits set and developed to maturity.

Thus it can be said that developing seeds play an important role in fruit setting by producing stimulus essential for the development of an ovary and its surrounding tissues into a fruit.

CHAPTER FOUR : *FACTORS AFFECTING FERTILIZATION AND FRUIT SET:*

Most apples (Teskey and Shoemaker (1978), Anonymous (1967), Westwood (1978)), peaches, cherries (Teskey and Shoemaker (1978), Westwood (1978)), prune, plum, apricot, almonds, berry fruits (Westwood (1978)) fruits develop as a result of pollination and fertilization. Some triploid varieties (Leopold and Kriedemann

(1975)) and Black Corianth (grape) (Weaver (1972)) fruits develop as a result of stimulative parthenocarpy i.e. pollination alone. Some varieties of persimmon, fig and pear (Weaver (1972) and Washington Navel orange (Leopold and Kriedemann (1975), Weaver (1972)) fruits develop as a result of vegetative parthenocarpy i.e. without both pollination and fertilization. Thus it is obvious that any factor that affects pollination, fertilization and parthenocarpy may influence fruit set in an orchard. The factors affecting pollination have already been presented in the preceding Chapter (Chapter Two). Some of the factors affecting fertilization and fruit set will be discussed below.

A. *Variety:*

Fruit species/varieties differ in their blossom morphology, in their pollen viability and ovule longevity periods; in their pollen and ovule fertility, and in their rate of pollen tube growth in the styles.

In most varieties of apple and pear the stigmas are held slightly above the level of the dehiscing stamens, an arrangement which favours cross pollination by insects. Varieties such as Cox's Orange Pippin, where the stigmas are well above the level of the anthers, are particularly dependent on insects for pollination and are therefore likely to set poor crops in seasons or situations unfavourable for insect activity. In those varieties where the styles are shorter than the stamens (e.g. Early Worcester, Merton Worcester) self pollination even in the absence of insects is facilitated and chances of cross pollination are reduced. Whilst this may have advantages in poor pollination years when insects are scarce, it is not generally beneficial in fruits such as apple and pear where cross pollination is usually necessary for maximum cropping where the variety is highly self incompatible (see below) it may even lead to crop failure, the stigmas becoming so thickly coated with self pollen that foreign compatible pollen is likely to have a reduced chance of germination (Anonymous (1967)).

The relative attractiveness of different flowers to insects is to some extent determined by flower structure, and may vary even between varieties. For example, it has been observed that hive bees are more attracted to the flowers of Worcester Pearmin which has spreading stamens than to those of Bramley's Seedling in which the close, stout filaments of the upright stamens make nectaries more difficult of access (Preston (1949)). The flower structure of "Delicious" apple (*M. domestica*) differs from that of most apple cultivars by having gaps at the base of stamens. Honey bees learn to collect nectar through these caps and avoid the flowers sexual parts. Consequently visits to Delicious flowers are less than half as effective in pollination as are visits to other cultivars (Robinson (1979)).

Pollen longevity i.e. the time during which the pollen retains its viability under natural conditions may be a matter of interest particularly in crops like avocado and nuts, because of the occurrence of dichogamy in them. It is reported that under natural conditions, avocado pollen remained viable for at least 6 days and 7 hours after dehiscence of anthers (Papademetriou (1975)) whereas walnut pollen when stored under natural condition lost viability 2-3 days after collection. In some cases, freshly collected walnut pollen lost its viability 1-3 hours after exposure to sunlight at 25-30^{o}C (Rud and Zhadan (1973)).

Williams (1970) has noted that because the differentiation of the egg apparatus in triploid cultivars (apples and pears) takes place 2 or 3 days after the flowers open the triploid cultivars have extended ovule longevity as compared to diploid cultivars in which the differentiation of the egg apparatus coincides with the opening of the flower.

Some varieties of fruit produce infertile pollen, which germinates poorly if at all (Westwood (1978)). According to Chandler (1965) triploid apple varieties such as Stayman, Winesap, Baldwin, Rhode Island Greening, Fall Pippin and Gravenstein with 51 chromosomes in the somatic cells usually produce a pollen having rather low viability and triploids of some other kinds of plants tend toward sterility. Gigilov (1976) studied the viability of 14 almond cultivars and found that cultivars such as Drake, Ne plus ultra, Nikitskii-6Z and Nikitskii Pozdonotsvetuschchi had the highest pollen viability (70.5-73.7% germination). Slade

(1977) noted that all sweet cherry varieties are self sterile and one peach variety, J.H. Hale, is male sterile. Krezdorn (1973) has cited Osawa (1912) as having reported that Navel sweet orange set fruit poorly because they are both pollen and ovule sterile and only weekly parthenocarpic.

The pollen tubes produced by certain varieties are either - incapable of growing down (Anonymous (1967)) or are unable to develop in their own styles (self incompatible varieties) or in the styles of other varieties (cross incompatible varieties) rapidly enough to effect fertilization (Griggs (1953), Gardener *et al* (1952), Anonymous (1967)). For examples, most apple and pear varieties are either self incompatible or only partially self incomptible (Anonymous (1967)). Pumelos (*C. grandis*), palestine sweet lime (*C. limettroids)* (Soost (1964)) and certain interspecific hybrids of grape fruit (*C. paradisi*) and mandarin (*C. reticulata*) and clementine mandarin are self incompatible (Reece and Register (1961)). In sweet cherry the variety Early River is probably incompatible with Bedford prolific, Roundel and at least ten other varieties (Anonymous (1967)).

However, the pollen tubes produced by certain self incompatible varieties may be capable of growing down the styles and effecting fertilization in some other groups of varieties (cross incompatible varieties). For example, if pollen of sweet cherry variety Early River is placed on the style of the variety Waterloo, it grows normally and brings about fertilization, and in the same way the pollen of Waterloo can fertilize Early Rivers (Anonymous (1967)).

The rate of pollen tube growth may vary from one variety to another. In citrus Lange (1973) observed that it took one more day for the pollen tube to reach the ovules of the Minneola flowers (6 days) than for those of Ortanique flowers (5 days). In avocado Papademetriou (1975) found that pollen of Nabal variety took 66 hours whereas Fuerte took only 44 hours to grow from the stigma to the egg cell. Child (1967) has noted that the rate of pollen tube growth in the joint style and ovary is faster than in the free style.

Thus the seriousness of the fruit set problem may differ from one variety/species to another. The problem may be acute with those varieties/species whose blossoms are unattractive to pollinating insects, and have stigmas well above the level of anthers; which are self sterile, self incompatible and non parthenocarpic in nature; and which have shorter pollen viability and/or ovule longevity periods.

B. *Weather condition:*

The effect of temperature, rainfall, humidity and wind on pollen viability, pollen germination and pollen tube growth rate, ovule longevity and bee activity has been discussed (see Chapter Two A and B). Working with Italian prune Thompson and Liu (1973) have found that cool temperatures ($10^{\circ}C$) delayed the fertilization process. In the grape vine, Dragnov and Dragnov (1975) observed that the minimum temperature for fertilization ranged from 14.6 to $16.5^{\circ}C$ and it was depressed at over 20 and $25.8^{\circ}C$ depending on the cultivar. Thus it can be said that weather conditions may affect fruit set by influencing pollination and/or fertilization of blossom in an orchard.

C. *Nutrition:*

Studies by different workers show that nitrogen (Williams and Reninson (1963), Williams (1963), Jackson (1974), Childer (1976), Howlett (1931), Hill Cottingham and Williams (1967), McDaniels and Heinicke (1929), Lenz (1966)), phosphorous (Taylor and Goubran (1975), Taylor (1975)), potassium (Ludders and Bunnemann (1975)), boron (Chanturiya (1974), Batjer and Thompson (1949)), iron, zinc (Austin (1974)) and magnesium (Greenham and White (1959)) influence fruit set in an orchard.

The relationships between nitrogen and fruit set has been summarized in Table No.5 below. The effect of nitrogen on fruit set is probably an indirect one - main effects of the nitrogen being (i) to stimulate leaf growth, thereby leading to increased production of carbohydrates which in turn assists in the retention of the developing fruitlets through the period of June drop (Anonymous (1967)) and (ii) to increase the rate of pollen tube growth (Williams (1963)) and ovule longevity (Williams (1963) and Williams (1965)) leading to fertilization and fruit set.

Table No.5. *Relationship between nitrogen and fruit set**

		Female Parent	
		High Nitrogen	Low Nitrogen
Male	High Nitrogen	High initial set Low first drop Moderate June drop	Low initial set Low frist drop Heavy June drop
Parent	Low Nitrogen	Low initial set Heavy first drop Low June drop	Low initial set Heavy first drop Moderate June drop

*After Anonymous (1967)

Dorsay (1930) has stated that "with greater tree or spur vigour the organization of the embryo sac is carried further and persists longer". Howlett (1936) reported that low nitrogen increased degeneration of embryo sacs, and hastened the time of such degeneration. Williams (1963) observed that in apple flowers pollen tube growth rate was increased by the summer application of nitrogen. Williams also noted that apple flowers from trees in low nitrogen the short lived embryo sacs were usually disorganized before pollen tubes resulting from self pollination at anthesis reached the nucellus. More recently Williams (1965) found that soil application of nitrogen in late summer resulted in strong embryo sacs the following spring which continued cell division and remained viable twice as long as those of controls. Therefore it can be said that cultural practices may be adjusted to compensate for low temperature and slow pollen tube growth, permitting longer effective pollination period.

According to Taylor and Goubran (1975) phosphate deficient trees showed delayed bud burst and retarded development of a reduced number of vegetative and floral meristems. They have observed that increasing the rate phosphate application to 9.5 kg/ 100 trees stimulated apple fruit set. Taylor (1975) also found that application of superphosphate resulted in a positive fruit set on peach trees. Taylor and Goubran (1975) have concluded that the pronounced effect of phosphorous on fruit set was at least partially attributable to its effect on the rate of flower cluster emergence since delayed emergence reduced the opportunity for cross pollination and fruit set.

Batjer and Thompson (1949) observed that boric acid sprays during the bloom period resulted in a 9-22% increase in the fruit set of Anjou pear. Such an effect of boric acid sprays on fruit set may be due to the stimulatory effect of boron on pollen germination (see Chapter Five A.2.).

D. *Other cultural factors:*

Cultural factors such as pruning (Cooper (1938), Batjer (1963)), pinching (Coombe (1962)), defoliation (Mullins (1967)), ringing (MacDaniels and Heinicke (1929)), girdling (Krezdorn (1960), Jackson (1974)), scoring (Batjer (1963), root stocks and fruit stripping (El-Zeftawi and Thornton (1975), Jackson (1974)), irrigation (Cooper (1938), Powell (1974), MacDaniels and Heinicke (1929)), thinning (Jones *et al* (1974), Cooper (1938), Coombe (1973)) and harvesting (Hilgeman *et al* (1967)) also influence fruit set in an orchard. Among these cultural practices, only thinning is important from the point of view of directly effecting pollen germination, pollen tube growth and fertilization of ovules. Some thinning materials such as sodium 4,6-dinitro-orthocresylate (DNOC) and Dinitro-6-S-Butyl phenol (DNBP) thin blossom. DNOC kills petals, pollen, and pistils (MacDaniels and Hildebrand (1940) as cited by Edgerton (1973), Teskey and Shoemaker (1978) and Westwood (1978)) and reduces the pollen tube growth in the styles of flowers (McKenzie (1979)).

CHAPTER FIVE : *TECHNIQUES IN POLLINATION EXPERIMENTS:*

A. *Pollen viability tests:*

A.1 *By staining pollen:*

A survey of literature on staining of pollen pertaining to its viability shows that nuclear dyes and vital stains are commonly used. Acetocarmine in glycerine jelly (Alexander (1969); Cockerham and Galleta (1976)) or cotton blue in lactophenol (Alexander (1969)) are the dyes commonly used for staining the non aborted pollen. In an experiment to examine the pollen characteristics in certain *Vaccinium sps* Cockerham and Galleta (1976) used the nuclear dyes for staining blueberry pollen as follows: Anthers were macerated in a drop of Galleta's acetocarmine consisting of 4 parts iron acetocarmine to 3 parts melted glycerine jelly. After sealing the preparations were stored at $4^{o}C$ for 48 to 72 hours to intensify the stain. Pollen was considered viable and potentially fertile if it appeared normal and stained strongly. However, Cockerham and Galleta have cited Rovsi (1966) as having found that *Vaccinium* pollen fertility estimates were higher when judged by stainability than by pollen germination. Alexander (1969) has noted that nuclear dyes do not stain the aborted pollen at all and they do not produce satisfactory staining of thick walled pollen. For this reason Alexander prepared stain for differential staining of aborted and non aborted pollen. The stain was prepared by adding the various constituents in the order given below, shaking after addition and storing in a coloured bottle:- 95% alcohol (ethanol) 10 ml; malachite green (10 mg) (1 ml of 1% solution in 95% alcohol); distilled water 50 ml; glycerol 25 ml; phenol 5 gm; chloral hydrate 5 gm; acid fuchsin 50 mgm (0.5 ml of 1% solution in water); glacial acetic acid (1-4 ml depending upon the thickness of the pollen wall. For very thin walled as in Balsam add 1 ml of acid; for thick walled and spiny pollen add 3 ml to produce good differentiation; for pollen inside non dehiscent anthers add 4 ml of acid). Alexander has noted that staining is hastened by flaming the slide (for loose thin walled pollen) or by immersing thick walled pollen or anthers for 24-48 hours at 50°C. In the typical stain aborted pollen grains are green non aborted red. The stain is very durable especially if the coverslips are covered with sealed paraffin wax.

Commonly used vital stains are Tetrazolium chloride (TTC) and Tetrazolium red (Alexander (1969)). According to Sarvello (1964) both Tetrazolium red and 2,3,5 - Triphenyl tetrazolium chloride were effective with the former reaching more quickly. They obtained differential staining of both viable and non viable pollen. The stain was kept in a 10% stock solution and diluted one part to five parts of a 60% sucrose solution. Suitable staining was obtained after a minimum period of one hour. A 0.01 percent stock solution of crystal violet also found effective but required a longer period to stain than did the Tetrazolium chloride (TTC). However, Overley and Watson (1953) found that TTC stained to a varying degree certain fruit pollen (apple, peach, pear, and grape) known to be non viable. I also tried staining peach and apple pollen with TTC but could not find reliable compared to the growing pollen in the medium. Most of the time even the viable pollen grains were not found to be stained.

A.2. *By growing pollen:*

(a) *The growing media:*

The results of many workers show that pollen grains can be germinated in vitro in both liquid (Hopping (1979), Krinko (1970), Kwack (1964), Thompson and Batjer (1950), Ries (1975)) and solid (Griggs (1953), Church and Williams (1978), Lawes (1978) growing media. While growing pollen on liquid medium Thompson and Batjer (1950) used double depression microslides; and Ries (1975) used depressions on porcelain plates for culturing pollen. Two drops of the medium were placed on each depression. After adding a small amount of pollen to each depression the slides were placed in covered petridishes or plastic boxes containing moist filter paper or moistened kein pack cellulose paper in the bottom. The petridishes or plastic boxes were then incubated at 20-25°C for 24 to 48 hours depending upon the species. Hopping (1979) cultured pollen in Erlenmyer flasks which during incubation were placed over a shaking water bath. Hopping has noted that the higher pollen germination percentage achieved by growing pollen in shake culture may be due to the better aeration in the medium than in the non shake culture.

A solid medium for growing pollen is prepared by adding one (Church and Williams (1978)) to two percent (Griggs (1953)) agar solution to the liquid medium (see below). I tried growing pollen on both the liquid and the solid medium and found that growing pollen in solid medium (i.e. medium containing agar) gave better results (produced higher germination). It was easier to handle and allowed more time period to observe the pollen under the microscope. Growing pollen in agar media may also require less pollen than in shake culture. In shake culture, while shaking there are possibilities of some pollen grains being wasted by being stuck to the wall of the flask. For these reasons, for all the experiments presented in this paper (see Chapter Six) solid growing media containing agar were used.

Many different kinds of substances have been reported to be used as the medium constituents for growing pollen in vitro. Thompson and Batjer (1950) noted that the addition of 10 ppm boron to the medium increased germination of several deciduous fruits pollen by 2 to 10 fold. Working with onion pollen Kwan *et al* (1969) found that boric acid stimulated pollen germination and tube growth at 100 mg/litre. According to Kirko (1970) the addition of fresh malt from germinating oats, wheat, barley or maize to a sugar solution stimulated the germination of fresh and particularly of stored apple, pear and apricot pollen. Potapov *et al* (1975) observed that the best medium for pear pollen germination was the stigma when compared with 15% sucrose solution or similar solution plus 1% agar.

According to Brewbaker and Kwack (1963) the presence of calcium ion in the media completely overcomes the pollen population effect - a phenomenon in which a small pollen population germinates and grows poorly, if at all, compared to a large pollen population. Kwack (1964) noted that the effect of calcium ion was much improved by the addition of some other cations such as magnesium and potasium. Thus Kwack (1965) recommended the following medium for general use in pollen culture of horticultural species.

Kwack's Medium:

Sucrose	10%	
Boric acid	100 ppm (100 mg/l)	
Calcium nitrate	$(Ca(No_3)_2)\ 4H_2O$	300 ppm
Magnesium sulphate	$MgSo_4\ 7H_2O$	200 ppm
Potassium nitrate	KNo_3	100 ppm

This medium is adjusted to a pH of 7.3 and may be used with or without 1% agar.

However, working with kiwifruit pollen Hopping (1978) found satisfactory results by using 10% sucrose plus 0.01% boric acid as a growing medium. Lawes (1978) found that Kwack's medium gave results similar to the medium containing 1.5% agar and 10% sucrose (cane sugar). Lawes observed that with both media the pollen germination was 90% where grains were fairly thickly spread and was less than 10% where grains were sparsely spread. I have also observed similar results with apple and peach pollen. Other workers (Church and Williams (1978) apple; up to 86% germination; Griggs (1953) apple; up to 93% germination; almond 87% germination) also have observed satisfactory results with the medium containing agar and 10 to 20% sucrose solution. Therefore, the medium containing 10% sucrose and 1.5% agar was used for the experiments presented in this paper (see Section Two Chapter Six).

Pollen collected both from fresh anthers (Lawes (1978); Church and Williams (1978) and from dried anthers (Lawes (1978); Ries (1975); Hopping and Jerram (1979)) have been reported to be used for in vitro studies. Hopping and Jerram (1979) found that kiwifruit pollen grains collected from anthers dried overnight at 25°C showed 87% germination. However, Lawes (1978) noted that oven dried kiwifruit pollen was unsatisfactory. With a view to comparing their utilities both fresh and oven dried pollen were used in the experiments presented in this paper (see Chapter Six).

For testing their viability in vitro Hopping (1979) hydrated apple, peach, plum and kiwifruit pollen grains until their moisture content reached 14%. Hopping noted that the hydration of pollen grains prior to being dusted on to the growing medium may reduce the possibility of bursting of their cell walls due to osmosis. For this reason, hydrated pollen grains were used for some of the peach and kiwifruit experiments presented in this paper (see Chapter Six).

B. *Observation of pollen tubes in the styles:*

Since the demonstration by Currier (1957) that callose in either living or dead tissue can be stained selectively with water soluble aniline blue and similar dyes which fluoresce in ultraviolet light, many workers (Martin (1959), tomato; Kho and Baer (1968), onion, brussels sprout, tomato, freesia; Tomer and Gotterich (1975), avocado; Sedgley (1976), avocado; Hopping and Jerram (1979), kiwifruit; Young and Sherman (1978), blueberry;) have used fluorescence microscopy to observe pollen tubes in the styles. Because the techniques described by Kho and Baer (1968) were found to be reliable, rapid, and flexible with respect to time scheduling, his techniques were applied in the experiments presented in this paper (see Chapter Six B).

SECTION TWO : EXPERIMENTAL

CHAPTER SIX : *MATERIALS AND METHOD:*

A. *In Vitro Studies:*

A.1. *Preparation of agar petridishes:*

Calculated amounts of agar (Davis Bacteriological Agar) and sugar were weighed and added to the measured volume of distilled water to prepare a growing medium containing 1.5% agar and 10% sucrose in a flask. The flask was then auto claved at 15 pounds pressure for 15 minutes during which period the agar and the sugar dissolved in the water to form a solution which when cooled was used as the growing media for pollen. Immediately after being taken from the autoclave, the solution in the flask was topped up with the required volume of fungicides formulation in sterile water. After the addition of fungicide formulation the resultant solution in the flask was well shaken and poured to form a thin layer into the sterilized petridishes. Hence varying concentrations of fungicides formulation were included in a 1.5% agar media containing 10% sucrose.

Immediately after being poured with growing media, the petridishes were transferred to a cool store at 5°C. The growing media in the petridishes were considered suitable/ ready enough for growing pollen when they were solidified and had been cooled to room temperature.

A.2. *Pollen collection and storage:*

For some experiments (see Chapter Six A.5.) fresh pollen grains were used. Fresh pollen grains were collected by shaking newly opened (1 to 3 days old) flowers into vials or into petridishes. For others (see Chapter Six A.5.) oven dried pollen was used. The anthers from one day old flowers were removed and dried at 25°C for 20 hours. Then the pollen released from the dried anthers was collected in petridishes or vials.

Apple pollen was collected from Golden Delicious and Splendour flowers (see also Chapter Six A.5.), peach pollen was collected from Golden Queen and Red Haven flowers (see also Chapter Six A.5.) and kiwifruit pollen was collected from Matua flowers in Massey University orchards. Immediately after their collection, the pollen grains were stored at 1.5°C by keeping them in vials enclosed in sealed plastic bags containing silica gel.

A.3. *Growing pollen:*

The pollen grains collected as mentioned above in A.2. (Chapter Six) were evenly dusted on agar by means of a brush over the surface of the media in the petridishes prepared as mentioned above in A.1. (Chapter Six). The petridishes were then incubated at 24 or 25°C (24°C for peach pollen; 25°C for apple and kiwifruit pollen). After 20 hours of incubation the germinating pollen grains and pollen tubes were observed through an olympus light microscope.

In some experiments (see Chapter Six A.5.), before their dusting on to the growing media, the pollen grains were hydrated at 25°C. The hydration was achieved by keeping open vials of pollen in a water saturated environment for 2 hours.

The pollen grains were grown in two petridishes per treatment.

A.4. *Counting of germinating pollen:*

The percentages of pollen germination were calculated on the basis of the counts taken at 4 or 5 (4 in experiment Nos.6, 9, 10 and 12; and 5 in other experiments - see Chapter Six A.5.) different representative microscopic field/areas in each of two petridishes. A grain was considered to have germinated if it had produced a tube longer than the grain's diameter. Forty or more pollen grains were examined per replicate.

A.5. *Experiments:*

Experiment No.1. *A study of the effects of triforine (Saprol^R) on apple pollen germination in vitro.*

On 30/10/78 petridishes containing 0 ppm, 10 ppm, 50 ppm, 100 ppm and 500 ppm triforine (Saprol^R) were prepared. On 31/10/78 within one hour of their collection oven dried Golden Delicious pollen was dusted on to the surface of the media on the petridishes. On 1/11/78 the germinating pollen grains in the petridishes were assessed.

Experiment No.2. *A study of the effects of mancozeb (Dithane M45^R) on apple pollen germination in vitro.*

On 30/10/78 petridishes containing 0 ppm, 10 ppm, 50 ppm, 100 ppm and 500 ppm mancozeb were prepared. On 1/11/78 one day old Golden Delicious pollen collected from fresh anthers was dusted on to the surface of the growing media on the petridishes. On 2/11/78 the germinating pollen grains in the petridishes were assessed.

Experiment No.3. *A study of the effects of captan, bupirimate (Nimrod^R), and a wetting agent (Citowett^R) on apple pollen germination in vitro.*

On 28/10/79 petridishes containing 0 ppm, 10 ppm, 50 ppm, 100 ppm and 500 ppm of any one of the fungicides namely captan, bupirimate (Nimrod^R) and Citowett^R were prepared. On 30/10/79 one day old pollen collected from fresh anthers of Golden Delicious flowers was dusted on to the surface of the growing media on the petridishes. On 31/10/79, the germinating pollen grains in the petridishes were assessed.

Experiment No.4. *A study of the effects of mancozeb 7% plus dinocap (Dikar^R) on apple pollen germination in vitro.*

On 28/10/79 petridishes containing 0 ppm, 10 ppm, 50 ppm, 100 ppm and 500 ppm mancozeb 7% plus dinocap (Dikar^R) were prepared. On 29/10/79 one day old oven dried Golden Delicious pollen was dusted on to the surface of the growing media on the petridishes. On 30/10/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.5. *A study of the effects of bayleton (Bayleton^{R}) on apple pollen germination in vitro.*

On 23/10/79 petridishes containing 0 ppm, 10 ppm, 50 ppm, 100 ppm and 500 ppm bayleton were prepared. On 31/10/79, within one hour of their collection oven dried Golden Delicious pollen was dusted on to the surface of the growing media on the petridishes. On 1/11/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.6. *A study of the effects of dichlofluanid (Euparen^{R}) on apple pollen germination in vitro.*

On 5/11/79 petridishes containing 0 ppm, 10 ppm, and 100 ppm dichlofluanid (Euparen^{R}) were prepared. On 6/11/79, within one hour of their collection from fresh anthers of Splendour flowers pollen grains were dusted on to the surface of the growing media on the petridishes. On 7/11/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.7. *A study of the effects of triforine (Saprol^{R}), benomyl (Benlate^{R}), iprodione (Rovral^{R}) and mancozeb (Dithane M45^{R}) on peach pollen germination in vitro.*

On 30/8/78 petridishes containing 0 ppm, 1 ppm, 10 ppm, 50 ppm and 100 ppm of any one of the fungicides treatment, namely triforine (Saprol^{R}), benomyl (Benlate^{R}), iprodione (Rovral^{R}) and mancozeb (Dithane M45^{R}) were prepared. On 31/8/78, within one hour of their collection from fresh anthers of Golden Queen flowers pollen grains were dusted on to the surface of the growing media on the petridishes. On 1/9/78, the germinating pollen on the petridishes was assessed.

Experiment No.8. *A study of the effects of vinclozoline (Ronilan^R) on peach pollen germination in vitro.*

On 30/8/78 petridishes containing 0 ppm, 1 ppm, 10 ppm, 50 ppm and 100 ppm vinclozoline (Ronilan^R) were prepared. On 1/9/78, within one hour of their collection from fresh anthers of Golden Queen the pollen grains were dusted on to the surface of the growing media on the petridishes. On 2/9/78, the germinating pollen on the petridishes was assessed.

Experiment No.9. *A study of the effects of Ethephon (Ethrel^R) on peach pollen germination in vitro.*

On 11/9/79 petridishes containing 0 ppm, 10 ppm, 100 ppm and 500 ppm ethephon (Ethrel^R) were prepared. On 13/9/79, within one hour of their collection from oven dried anthers of Red Haven flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. On 14/9/79, the germinating pollen on the petridishes was assessed.

Experiment No.10. *A study of the effects of dichlofluanid (Euparen^R) on peach pollen germination in vitro.*

On 11/9/79 petridishes containing 0 ppm, 10 ppm, 100 ppm and 500 ppm dichlofluanid (Euparen^R) were prepared. At 11 a.m. on 12/9/79 pollen collected from fresh anthers of Red Haven flowers was hydrated at 25°C. After 2 hours of hydration, the pollen grains were dusted on to the surface of the growing media. On 13/9/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.11. *A study of the effects of streptomycin on peach pollen germination in vitro.*

On 16/9/79 petridishes containing 0 ppm, 1 ppm, 10 ppm, 50 ppm and 100 ppm streptomycin were prepared. On 17/9/79 one day old pollen collected from fresh anthers of Red Haven flowers was dusted on to the surface of the growing media on the petridishes. On 18/9/79, the germinating pollen on the petridishes was assessed.

Experiment No.12. *A study of the effects of streptomycin plus triforine (Saprol^R) on peach pollen germination in vitro.*

On 16/9/79 petridishes containing 0 ppm, 10 ppm, 100 ppm and 500 ppm triforine (Saprol^R) plus 0 ppm, 10 ppm, 100 ppm and 500 ppm streptomycin were prepared. On 17/9/79, within one hour of their collection from fresh anthers of Red Haven flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. On 18/9/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.13. *A study of the effects of triforine (Saprol^R) and triforine plus streptomycin sprays on the germinability of peach pollen.*

On 16/9/79 six petridishes containing 1.5% agar media and 10% sucrose were prepared. At 9 a.m. on 19/9/79 flowers on three Red Haven trees were applied with any one of the treatments, namely 200 ppm triforine, 200 ppm triforine plus 100 ppm streptomycin, and no sprays. At 11 a.m. pollen grains from flowers on each tree were collected on to three different vials. Within one hour of their collection, pollen grains from each vial were dusted on to two petridishes. At 9.30 a.m. on 20/9/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.14. *A study of the effects of dichlofluanid (Euparen^R) on kiwifruit pollen germination in vitro.*

On 25/11/79 petridishes containing 0 ppm, 1 ppm, 10 ppm, 100 ppm and 250 ppm dichlofluanid (Euparen^R) were prepared. On 27/11/79, within one hour of their collection from fresh anthers of Matua flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. On 28/11/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.15. *A study of the effects of captan on kiwifruit pollen germination in vitro.*

On 25/11/79 petridishes containing 0 ppm, 1 ppm, 10 ppm, 100 ppm and 250 ppm captan were prepared. On 26/11/79, within one hour of their collection from fresh anthers of Matua flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. On 27/11/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.16. *A study of the effects of vinclozoline (Ronilan^R) on kiwifruit pollen germination in vitro.*

On 25/11/79 petridishes containing 0 ppm, 1 ppm, 10 ppm, 100 ppm and 250 ppm vinclozoline (Ronilan^R) were prepared. On 28/11/79, within one hour of their collection from fresh anthers of Matua flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. On 29/11/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.17. *A study of the effects of dichlofluanid (Euparen^R) sprays on the germinability of kiwifruit pollen.*

On 3/12/79 four petridishes containing 1.5% agar and 10% sucrose were prepared. At 9 a.m. on 4/12/79 two Hayward vines were applied with any one of the two treatments, namely 1000 ppm dichlofluanid sprays, and no sprays. At 11 a.m. pollen grains were collected from flowers on each vine on to two different vials and hydrated for two hours. At 1 p.m. the pollen grains from each vine were dusted on to two petridishes. At 9 a.m. on 5/12/79, the germinating pollen grains on the petridishes were assessed.

Experiment No.18. *A study of the effects of temperature on peach pollen germination in vitro.*

On 30/8/78 six petridishes containing 1.5% agar and 10% sucrose were prepared. On 2/9/78, within one hour of their collection from the fresh anthers of Golden Queen flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. Then the petridishes were randomly divided into three groups of two. Petridishes belonging to one group were incubated at any one of the temperature treatments, namely 24°C, 28°C and 32°C. After 18 hours of incubation, the germinating pollen grains were assessed.

Experiment No.19. *A study of the effects of temperature on apple pollen germination in vitro.*

On 30/10/79 ten petridishes containing 1.5% agar and 10% sucrose were prepared. On 31/10/79, within one hour of their collection from fresh anthers of Crab apple flowers the pollen grains were dusted on to the surface of the growing media on the petridishes. Then the petridishes were randomly divided into five groups of two. Petridishes belonging to one group were incubated at any one of the temperatures, namely 16°C, 20°C, 24°C, 28°C and 32°C. On 1/11/79, the germinating pollen grains on the petridishes were assessed.

B. *In Vivo Studies:*

B.1. *Collection and preparation of flowers for treatment:*

(a) *Apples and peaches:*

On one morning (see Chapter Six B.5.) few (more than twenty) nearly ready to open flowers on two shoots of a tree at Massey University orchards were covered with fine mesh bags in order to prevent bees from pollinating them. Next morning, the unopened flowers on each shoot were removed. Ten fully opened flowers on each shoot were selected for the application of spray treatments.

(b) *Kiwifruits:*

30 to 40 cm long shoot cuttings with nearly ready to open flowers were prepared from Hayward vines at Massey University orchards. Immediately after their removal from mother vines the shoots were planted in water filled 20 to 30 cm long glass jars kept inside a controlled environment cabinet calibrated to maintain a temperature of $22^{\circ}C$, 16 hours daylight of intensity 6000 flux, and 75% relative humidity. The shoots were prevented from being dessicated by regularly topping up with water in the jar.

B.2. *Spraying and pollination of flowers:*

The flowers that had opened in the last 24 hours (i.e. one day old flowers) were used (except in Experiment No.21.) for the application of spray and/or pollination treatments. Just prior to spraying, the mesh bag coverings on apple and peach flowers were removed; and the styles in each kiwifruit flowers were reduced to five or six. Apple and peach flowers were sprayed with the help of a 4.5 litre capacity hand compression sprayer and kiwifruit flowers were sprayed with the help of a spray gun atomizer attached to a pump.

Apple and kiwifruit flowers were hand pollinated but peach flowers were left to be pollinated by bees or by gravity. The flowers (except those in Experiment No.21.) were not hand pollinated until the spray droplets had dried (until about 2 hours after spraying). Hand pollination was accomplished by gently rubbing a pollen laden brush over the surface of the stigmas.

B.3. *Collection and preservation of treated flowers:*

Twenty four hours after their pollination (except those in Experiment No.21.) 10 apple and peach flowers per treatment were plucked from the shoots. Flowers belonging to one treatment group were preserved in one jar containing (FAA) 90 parts by volume 70% ethyl alcohol, 5 parts by volume 40% formaldehyde, and 5 parts by volume glacial acetic acid.

B.4. *Observation of pollen tubes in the styles:*

Kho and Baer's (1968) technique was used to observe pollen tube growth in the styles. The style samples detached from flowers and fixed in FAA (see Chapter Six B.3.) were washed in running water for 10 to 15 minutes and macerated overnight (18 hours for peach and kiwifruit styles; 20 hours for apple styles) in 8 N (NaOH) sodium hydroxide solution. Macerated styles were washed in distilled water for about one hour, stained with 0.2% aniline blue in 0.1 N potassium orthophosphate for about 30 minutes mounted in a drop of glycerine in a slide and squashed under a cover slip. A mercury illuminating apparatus fitted with ultra violet and blue violet excited filters, a high pressure mercury lamp (AHL-250-UV) and with a basin for observing fluorescing pollen tubes in the styles. While using the apparatus, DV-1 and BG12 filters were used for the excitation of ultra violet and blue violet lights; and FY4 eyepiece filters were used for protecting eyes from being damaged by ultra violet light.

While observing through ultra violet light, the number of germinating and non germinated pollen grains were recorded. Each style was divided into six different imaginary segments from top portion (stigmatic surface) to the bottom portion (style base) and the approximate number of pollen tubes in each segment were counted. Thus, percentages of pollen germination and percentages of pollen tubes reaching to less than 1/10th of style length, at 1/10 to 1/4 of style length, at 1/4 to 1/2 of style length, at 1/2 to 3/4 of style length, at 3/4 to less than full style length and at full style length (i.e. at the base of the style) were calculated.

Altogether, 10 styles were examined for each treatment. Thus, in kiwifruits, 2 styles were randomly selected from each flower sample.

B.5. *Experiments:*

Experiment No.20. *A study of the effects of bupirimate (Nimrod^R) sprays on pollen germination and pollen tube growth in apple (Splendour) styles.*

At 9 a.m. on 27/10/79 one day old Splendour flowers were sprayed with 100 ppm bupirimate (Nimrod^R). At 11.a.m. both sprayed and unsprayed flowers were pollinated with one hour old pollen (i.e. pollen collected at 10 a.m.) collected from fresh anthers of Golden Delicious flowers. At 11.a.m. on 28/10/79, the flowers were picked from the shoots and preserved in FAA. On 1/11/79, the styles from flower samples were prepared for fluorescence microscopy. On 2/11/79, pollen germination and pollen tube growth on the styles were assessed.

Experiment No.21. *A study of the effects of triforine (Saprol^R) on peach pollen germination and pollen tube growth in peach styles.*

On 19/9/1979 one day old Red Haven flowers were sprayed with 200 ppm triforine (Saprol^R). On 20/9/79, both sprayed and unsprayed flowers (i.e. 24 hours after their pollination) were preserved in FAA. On 10/11/79 the styles from flower samples were prepared for fluorescence microscopy. On 11/11/79, the pollen germination and the pollen tube growth in the styles were assessed.

Experiment No.21a. *A study of the pollen germination and the pollen tube growth in kiwifruit styles hand pollinated at different hours after dichlofluanid sprays.*

At 9 a.m. on 21/11/79 one day old Hayward flowers were sprayed with 1000 ppm dichlofluanid (Euparen^R). At 9.30 a.m. pollen grains were collected from fresh anthers of Matua flowers.

At 10.30 a.m., 12.30 p.m., and 4.30 p.m. on 21/11/79 and at 9.30 a.m. on 22/11/79, i.e. 1, 3, 7 and 24 hours after being sprayed with Euparen^R the flowers were hand pollinated.

At 5.30 p.m., 7.30 p.m., and 11.30 p.m. on 21/11/79 and at 4.30 p.m. on 22/11/79, i.e. 7 hours after their pollination, the flowers were plucked from the shoots and preserved in FAA.

On 7/1/80 the styles from flower samples were prepared for fluorescence microscopy. On 8/1/80 and 9/1/80 the assessments of pollen germination and pollen tube growth in the styles were made.

Experiment No.22. *A study of the effects of temperature and fungicides on kiwifruit pollen germination and pollen tube growth in vivo.*

At 8.a.m. on 22/11/79 sixty one-day old Hayward flowers were selected and randomly divided into four groups of fifteen. The flowers belonging to one group were applied with any one of the main treatments, namely 1000 ppm dichlofluanid (Euparen^R) sprays, 250 ppm vinclozoline (Ronilan^R) sprays, 1000 ppm captan sprays and no sprays. At 9.30 a.m. pollen grains were collected from fresh anthers of Matua flowers. At 10.30 a.m. all the flowers were hand pollinated. Immediately after being pollinated, five flowers from each of spray treatment groups were transferred into any one of the controlled environment cabinets callibrated to maintain temperatures of 22°C, 18°C and 14°C.

At 10.30 a.m. on 23/11/79, i.e. 24 hours after their hand pollination, the flowers were plucked from the shoots and preserved in FAA.

On 9/1/80 the styles from flower samples were prepared for fluorescence microscopy. From 10/1/80 onward, the assessments of pollen germination and pollen tube growth on the styles were made.

Experiment No.23. *A study of the effects of temperature on pollen germination and pollen tube growth in kiwifruit styles.*

At 9.a.m. on 23/11/79 pollen grains were collected from fresh anthers of Matua flowers. At 9.30 a.m., twenty one day old Hayward flowers were hand pollinated. Immediately after their pollination, the flowers were divided into four groups of five. The different groups of flowers were then transferred to different controlled environment cabinets calibrated to maintain temperatures of 26°C, 22°C, 18°C and 14°C. At 9.30 a.m. on 24/11/79 the flowers were plucked from the shoots and preserved in FAA.

On 19/1/80 the styles from flower samples were prepared from fluorescence microscopy. On 20/1/80 and on 21/1/80 the assessments of pollen germination and pollen tube growth in the styles were made.

Experiment No.24. *A study of the effects of flower age and temperature on pollen germination and pollen tube growth in kiwifruit styles.*

At 9.a.m. on 24/11/79 pollen grains were collected from fresh anthers of Matua flowers. At 10 a.m., fifteen fully opened nil day old flowers (i.e. flowers opened in the last few (2 to 3 hours), fifteen one day old flowers, fifteen two days old flowers and fifteen three days old flowers were hand pollinated. Immediately after their pollination, the flowers from each age group were randomly divided into three sub groups of five. The flowers belonging to one sub group of each age group were transferred to any one of the controlled environment cabinets calibrated to maintain temperatures, namely 22°C, 18°C and 14°C.

At 10 a.m. on 25/11/79, i.e. 24 hours after their pollination, the flowers in each cabinet were plucked from the shoots and preserved in FAA.

On 8/2/80 the styles from flower samples were prepared from fluorescence microscopy. On 9/2/80 and on 10/2/80 the assessments on pollen germination and pollen tube growth in the styles were made.

C. *Field studies:*

C.1. *Experiments:*

Experiment No.25. *A study of the effects of different fungicides on the fruit set of Golden Delicious.*

Seven Golden Delicious trees on the same row of an orchard block at Massey University were selected. Each of the seven trees were applied with any one of the treatments, namely five sprays of 1000 ppm captan, four sprays of 150 ppm mancozeb plus dinocap (Dikar^R), three sprays of 1200 ppm mancozeb, three sprays of 200 ppm citowett, three sprays of 200 ppm triforine (Saprol^R), five sprays of 200 ppm bayleton and no sprays. The first sprays were applied when about 10% of the flowers were opened and the last sprays were applied during full bloom period in each tree. The other sprays were applied at intervals of 3 days after the first sprays.

Spray operations for this experiment were carried out within the period of 14/10/78 and 27/10/78.

Percentages of fruit set were determined on the basis of the flowers/fruits counts taken from twelve selected branches on each tree. The selected branches contained fifteen or more flower clusters during full bloom and were from all sides of the trees.

The initial fruit set was assessed by comparing the flowers count at full bloom (25/10/78 to 27/10/78) and the fruits count at five weeks after full bloom (7/12/78 to 12/12/78) in the trees.

The final fruit set was assessed by comparing the flowers count at full bloom (25/10/78 to 27/10/78) and the fruits count at harvest (23/3/79 to 31/3/79).

Experiment No.26. *A study of the effects of bupirimate (Nimrod^R) on the fruit set of Splendour trees.*

Two Splendour trees on the same row of an orchard block at Massey University were selected. One of them was applied with two sprays of 125 ppm bupirimate (Nimrod^R) and the other left unsprayed. The first spray was applied when about 10% of the flowers were opened (21/10/79) and the second spray was applied during full bloom (27/10/79).

Percentages of fruit set were determined on the basis of the flowers/fruits count taken from selected six branches on each tree. The selected branches contained thirty or more flower clusters during full bloom and were from all the sides of the trees.

The initial fruit set was assessed by comparing the flowers count at full bloom on 27/12/79 and the fruits count on 10/12/79, i.e. 6 weeks after full bloom.

Experiment No.27. *A study of the effects of different fungicides on the fruit set of Golden Queen peach trees.*

Twenty four Golden Queen peach trees growing on two rows of an orchard block at Massey University were selected and randomly divided into three groups of eight. One tree in each group was applied with any one of the treatments, namely five sprays of 200 ppm triforine (Saprol^R), six sprays of 800 ppm captafol (Difolatan^R), five sprays of 1200 ppm mancozeb (Dithane M45^R), five sprays of 250 ppm iprodione (Rovral^R), five sprays of

100 ppm streptomycin, six sprays of 160 ppm benomyl (Benlate^R), five sprays of 250 ppm vinclozoline (Ronilan^R) and no sprays. Thus each treatment was applied to 3 of the 24 trees.

The first sprays were applied when less than 5% of the flowers were open and the last sprays were applied during full bloom in each tree. The sprays within the period of 5% bloom and full bloom were applied at intervals of 2 days.

Spray operations were carried out within the period of 1/9/78 and 18/9/78.

Percentages of fruit set were determined on the basis of the flowers/fruits count taken from five selected branches on each tree. The selected branches contained eighty or more flowers during full bloom and were from different sides of the trees.

The initial fruit set in all the trees was assessed by comparing flowers count at full bloom (12/9/78 to 18/9/78) and fruits count at 5½ weeks after full bloom (20/10/78 to 27/10/78).

The final fruit set in all the trees was assessed by comparing flowers count at full bloom (12/9/78 to 18/9/78) and fruits count at harvest (15/3/79 to 22/3/79).

Experiment No.28. *A study of the effects of different fungicides on the fruit set of Red Haven peach trees.*

Six Red Haven trees on the same row of an orchard block at Massey University were selected. Each tree was applied with any one of the treatments, namely three sprays of 1000 ppm dichlofluanid (Euparen^R), three sprays of 100 ppm ethephon (Ethrel^R), three sprays of 200 ppm triforine (Saprol^R), three sprays of 100 ppm streptomycin, three sprays of 200 ppm triforine plus 100 ppm streptomycin and no sprays.

The first sprays were applied when about 10% of the flowers were open, second sprays applied when 50% of the flowers were open and the last sprays applied during full bloom on the trees. The sprays were applied within the period of 12/9/79 and 19/9/79.

The percentages of fruit set were determined on the basis of the flowers/fruits count taken from four selected branches on each tree. The selected branches contained sixty or more flowers and, were from different sides of a tree.

The initial fruit set in all the trees was assessed by comparing the flowers count at full bloom (19/9/79) and fruits count at 4 weeks after full bloom (16/10/79).

The final fruit set in all the trees was assessed by comparing the flowers count at full bloom (19/9/79) and fruits count at harvest (20/12/79).

Experiment No.29. *A study of the effects of different fungicides on the fruit set of kiwifruits.*

Four Hayward vines from two rows of the same orchard block at Massey University were selected. One vine from each row was applied with any one of the treatments, namely two sprays of 1000 ppm dichlofluanid (Euparen^R), two sprays of 1000 ppm captan, two sprays of 250 ppm vinclozoline (Ronilan^R), and no sprays. Sprays were applied during 25% bloom (i.e. when 25% of the flowers were open) on 26/11/79 and during full bloom on 1/12/79.

The percentages of fruit set were determined on the basis of flowers/fruits count taken from four selected vines on each tree. The vines selected for fruit set count contained thirty or more flowers during full bloom and were from both sides of trees canopy.

The initial fruit set was assessed by comparing the flowers count at full bloom (29/11/79 to 1/12/79) and fruits count 5 weeks after full bloom (5/1/80 to 10/1/80) in the trees.

The final set in all the trees was assessed by comparing the flowers count at full bloom (29/11/79 to 1/12/79) and fruits count at harvest (5/5/80).

Experiment No.30. *A study of the effects of pollination method and fungicides on the fruit set, fruit weight and seed numbers of kiwifruits.*

On 28/11/79 fifty nearly ready to open flowers on four Hayward vines at Massey University orchards were tagged and covered with fine mesh bags. At 9 a.m. on 29/11/79 the fine mesh bag coverings were removed and the newly opened flowers on each tree were applied with any one of the treatments, namely 1000 ppm dichlofluanid sprays, 1000 ppm captan sprays, 250 ppm vinclozoline sprays and no sprays. At 11 a.m. about 50% of the tagged flowers on each tree were hand pollinated by gently rubbing a pollen laden brush over the surface of the stigmas. The pollen used for this experiment was collected at 9 a.m. the same morning (i.e. on 29/11/79) from fresh anthers of Matua flowers as in Experiment No.20.

After being sprayed, the flowers were left uncovered to allow bee visits in them. Thus, each vine consisted of both bee pollinated and bee plus hand pollinated flowers.

The percentages of initial fruit set in both hand plus bee pollinated and bee pollinated flower clusters was determined by comparing flowers count on 29/11/79 (full bloom) and fruits count on 13/1/80 (5½ weeks after full bloom).

On 8/4/80 the fruits on both bee pollinated and hand plus bee pollinated clusters from each of the vines were picked, weighed and bagged separately. Prior to bagging the fruits were dipped in 0.1% Ethrel^{R} in order to hasten their ripening. On 12/4/80 the ripened fruits were fed into a Braun juice extractor which separated their juice from pulp and seeds. After the juice extraction was over the pulp and seeds were found to be collected in the form of a thin coating over a plastic sheet inside the juice extractor. The coating of pulp and seed was thin and sparsely spread enough to allow counting of dark seeds on the plastic sheet. Thus seed numbers in each fruit were recorded

D. *Statistical Analysis:*

Results were statistically analysed on a Burroughs B6700 computer using the programme "Teddy Bear" by J.B. Wilson 1978. Where data was in percentage, an arcsin transformation was used, otherwise raw data was used without transformation. Analysis was by analysis of variance, with tests of significance being made using Duncan's Multiple Range test at both 1% and 5% levels.

CHAPTER SEVEN : *RESULTS AND DISCUSSION:*

A. *In Vitro Studies:*

A.1. *Apple:*

Golden Delicious pollen collected from fresh anthers showed higher germination (see Table No.5) than those collected from oven dried anthers. Fresh pollen (pollen dusted on to the growing media within one hour of collection) showed more germination (see Table No.5) than one day old pollen (pollen grains stored at 1.5°C for 24 hours). Pollen grains showed higher germination (germination percentage 90% or more) where thickly spread than (germination percentage about 10%) where sparsely spread in the growing media containing 1.5% agar and 10% sucrose.

Table No.5. *In vitro germination of apple (Golden Delicious) and peach (Red Haven) pollen grown for 20 hours in 1.5% agar and 10% sucrose media on different dates in August and October 1978 and in September and November 1979.**

Incubation temperature for apple pollen:- 25°C
Incubation temperature for peach pollen:- 24°C

Kind of pollen	Mean percent germination of pollen collected from			
	Fresh anthers		Oven dried anthers	
	Fresh	One day old	Fresh	One day old
Apple	95.5	84.4	71.4	58.8
Peach (Red Haven)	86.0	63.4	70.0	-

*The data was not analysed statistically.

Golden Delicious pollen germination was inhibited by all the fungicide treatments, namely triforine, mancozeb, mancozeb plus dinocap, captan, bayleton, bupirimate, dichlofluanid and a wetting agent citowett^{R}. Mancozeb plus dinocap was the most effective inhibitor of germination but triforine and citowett^{R} gave over 95% reduction at 50 ppm. Captan, mancozeb, bupirimate and dichlofluanid caused 95% or more reduction in germination at 500 ppm, 500 ppm, 100 ppm and 100 ppm respectively (see Table No.6). Pollen tube growth at higher concentrations of fungicides was less than at lower concentrations. With mancozeb plus dinocap treatment the pollen tube growth at 0 ppm was 2.5 mm whereas at 10 ppm it was only 0.05 mm after 18 hours of incubation.

Table No.6. *Effects of different fungicides on in vitro germination of apple (Golden Delicious) pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 25^{o}C on different dates in October 1978 and in November 1979.*

Treatment	Mean percent pollen germination at various concentrations of fungicides				
	0 ppm	10 ppm	50 ppm	100 ppm	500 ppm
Bayleton[1]	71.4a	32.3b	18.7b	9.5c	2.6c
Bupirimate[2]	84.4a	20.8b	9.9c	4.5c	0.0c
Captan[2]	84.4a	57.1b	41.5b	15.5c	3.1c
Citowett^{R}[2]	84.4A	5.6B	1.8C	1.0C	4.0C
Dichlofluanid[3]	95.5A	62.5B	-	0.0C	-
Mancozeb[4]	81.9a	30.3b	32.1b	38.3b	3.9c
Mancozeb plus dinocap[5]	58.8A	1.2B	0.9B	0.0B	0.0B
Triforine[6]	70.7a	16.8b	3.3c	0.0c	0.0c

Note: 1 : 1/11/79 : Oven dried pollen : (Experiment No.5)
2 : 31/10/79 : One day old fresh pollen : (Experiment No.3)
3 : 7/11/79 : Fresh pollen : (Experiment No.6)
4 : 2/11/78 : One day old fresh pollen : (Experiment No.2)
5 : 30/10/79 : One day old oven dried pollen : (Experiment No.4)
6 : 1/11/78 : Oven dried pollen : (Experiment No.1)

Table No.6 (Contd)

Treatment means within each row denoted by the same small letter are not significantly different at 5% level and treatment means within each row denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.

The germination percentages of crab apple pollen at 20°C, 24°C, 28°C and 32°C were not significantly different from each other. The germination at 16°C was 58% less than at 24°C but was not significantly different from that at 20°C, at 28°C and at 32°C ($P = 0.01$; see Table No.7). However, the speed of germination was faster and the lengths of pollen tubes were higher at higher temperatures (28°C and 32°C) than at lower temperatures (16°C and 20°C). The lengths of pollen tubes after 18 hours incubation at 32°C, 24°C and 16°C were recorded to reach up to 3.0 mm, 2.5 mm and 2.0 mm respectively. At higher temperatures, (28°C and 32°C), prior to their germination, more pollen grains showed bursting of their cell walls than at lower temperatures (see Table No.8).

Table No.7. *In vitro germination of crab apple and Golden Queen peach pollen grown for 18 hours in 1.5% agar and 10% sucrose media on different dates in August 1978 and October 1979.*

Pollen	Mean percent pollen germination at different temperatures				
	16°C	20°C	24°C	28°C	32°C
Apple[1]	42.3a	57.0ac	72.6c	60.7ac	48.1ac
Peach[2]	-	-	76.7a	21.7b	7.3c

Note: *Treatment means within each row denoted by the same letter are not significantly different at 5% level when analysed by Duncan's Multiple Range test.*

1 : Fresh pollen : (Experiment No.19)
2 : Fresh pollen : (Experiment No.18)

Fig. 2 : Effects of fungicides on in vitro germination of apple (Golden Delicious) pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 25°C.

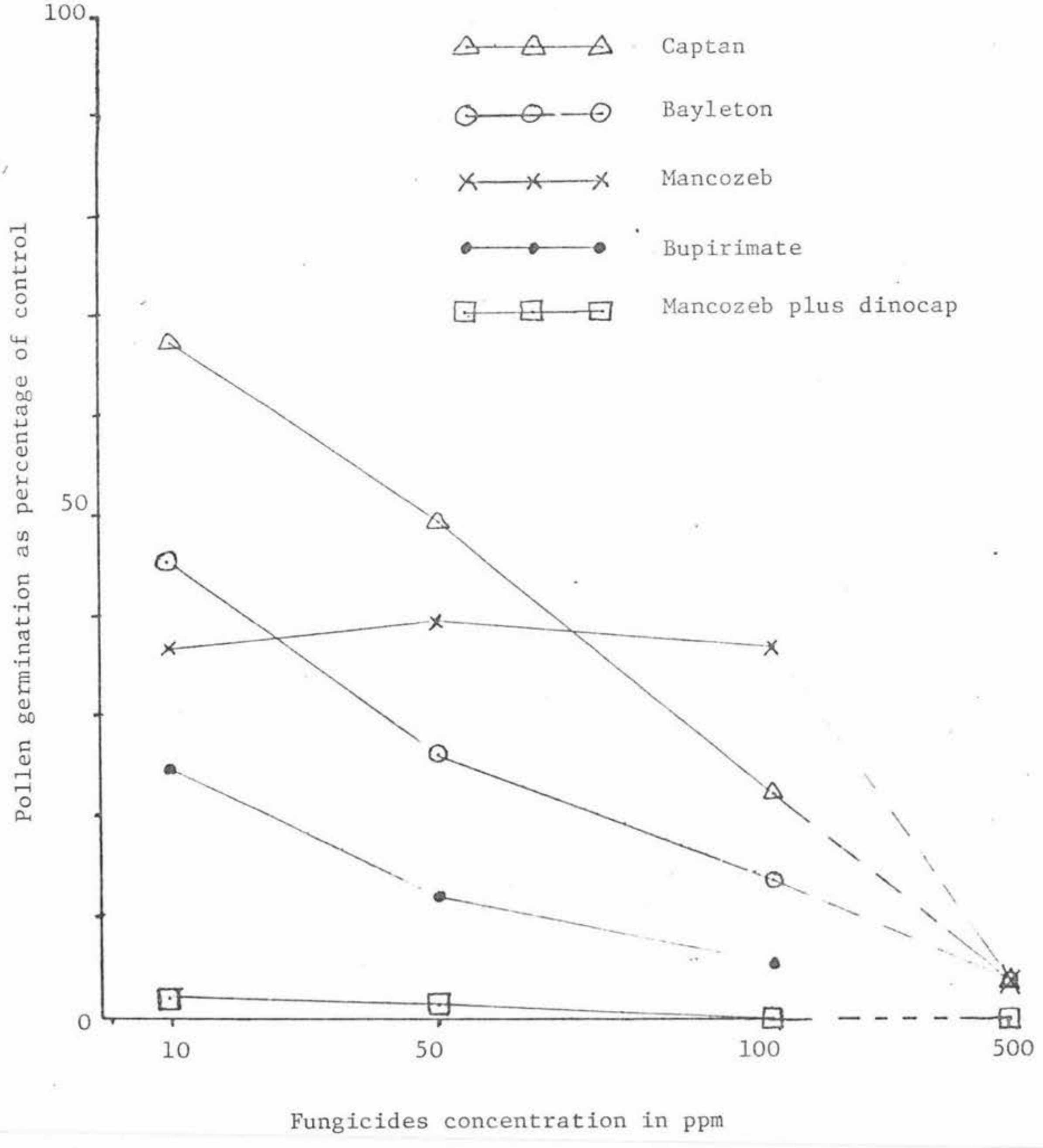


Table No.8. *Effects of temperature on the bursting of the cell walls of pollen grains grown in 1.5% agar and 10% sucrose media for 18 hours.*

Temperatures (°C)	Percentages of pollen grains with bursting cell walls	
	Apple	Peach
16	5.7	-
20	7.9	-
24	8.0	11.4
28	27.2	28.5
32	31.7	18.0

A.2. *Peach:*

The germination of Red Haven pollen collected from fresh anthers was higher than that collected from oven dried anthers. Fresh pollen showed higher germination than the stored pollen (see Table No.5). The germination of both Golden Queen and Red Haven pollen was higher where pollen grains were thickly spread (90% germination) than where pollen grains were sparsely spread (10% germination) in the growing media. The hydrated pollen grains tended to have higher germination than the non hydrated ones (see Table No.9).

Table No.9. *In vitro germination of hydrated and non hydrated Red Haven (peach) and Matua (kiwifruit) pollen grown for 18 hours in 1.5% agar and 10% sucrose media on different dates in August 1978 and November 1979.**

Incubation temperature for peach pollen:- 24°C
Incubation temperature for kiwifruit pollen:- 25°C

Pollen	Mean percent germination of	
	pollen hydrated for 2 hrs at 25°C	non hydrated pollen
Peach	97.5[1]	86.0[2]
Kiwifruit	89.5[3]	86.1[4]

*The data was not analysed statistically.

Table 9 (Contd)

1 : Fresh pollen : (Experiment No.10)
2 : Fresh pollen : (Experiment No.12)
3 : Fresh pollen : (Experiment No.17)
4 : Fresh pollen : (Experiment No.16)

Among the different fungicide treatments, namely triforine, streptomycin, streptomycin plus triforine, vinclozoline, iprodione, mancozeb, benomyl, captafol and dichlofluanid, triforine was the most effective and benomyl was the least effective inhibitor of peach pollen germination. At 10 ppm treatment triforine reduced pollen germination by more than 90% but benomyl reduced germination by only 68%. At 100 ppm triforine, triforine plus streptomycin, vinclozoline and ethephon completely arrested germination but mancozeb, captafol, iprodione, dichlofluanid, streptomycin and benomyl caused 94%, 94%, 93%, 90%, 88% and 76% respectively over a period of 20 hours incubation at 24°C (see Table No.10). The lengths of pollen tubes were shorter at higher concentrations than at lower concentrations. For example, after 18 hours incubation pollen tubes at 0 ppm, and 50 ppm triforine treatments reached lengths up to 2.5 mm and 0.05 mm respectively.

Table No.10. *Effects of different fungicides and a thinning agent (Ethephon) on in vitro germination of peach pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 24°C on different dates in August and September 1978 and August and September 1979.*

Treatment	Mean percent pollen germination at various concentrations					
	0 ppm	1 ppm	10 ppm	50 ppm	100 ppm	500 ppm
Benomyl[1]	77.2A	52.0B	24.6C	14.9C	20.3C	-
Captafol[1]	77.2A	48.1B	17.1C	22.3C	4.3D	-
Dichlofluanid[2]	97.5A	-	54.7B	-	10.3C	1.1C
Ethephon[3]	70.0A	-	53.2B	-	0.0C	0.0C
Iprodione[1]	77.2A	56.8B	32.2C	16.8D	5.1E	-
Mancozeb[1]	77.2a	45.8b	17.0c	4.1d	3.5d	-
Streptomycin[4]	63.4A	26.0B	24.9B	6.5C	7.1C	-
Streptomycin plus triforine[5]	86.0A	-	30.1B	-	0.0C	0.0C
Triforine[1]	77.2A	14.1B	7.1B	1.7C	0.0C	-
Vinclozoline[6]	74.8A	34.9B	8.8C	1.0C	0.0C	-

Fig. 3 : Effects of fungicides on in vitro germination of peach (Golden Queen) pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 24°C.

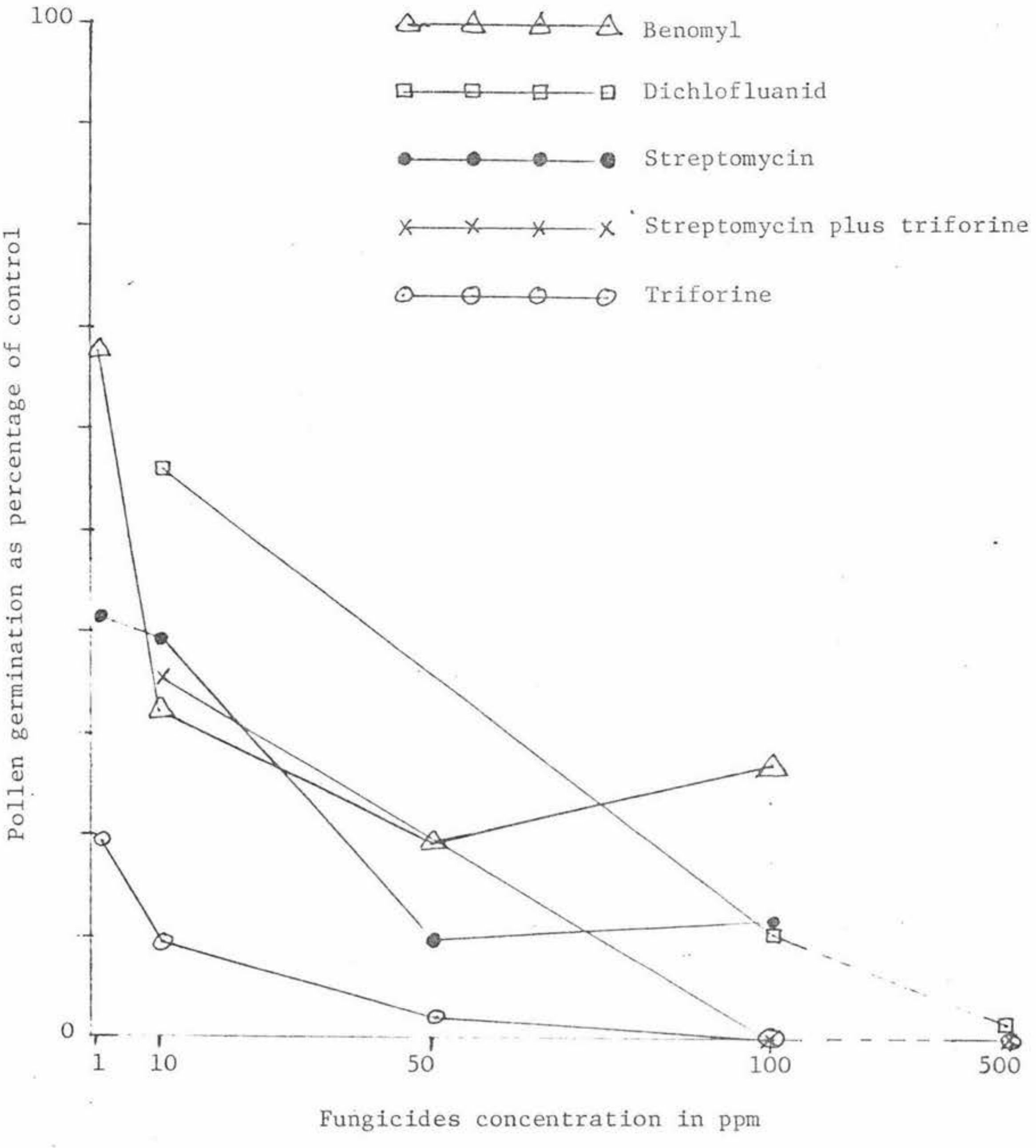


Table No.10 (Contd)

Note: *Treatment means within each row denoted by the same small letter are not significantly different at 5% level and treatment means within each row denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

1 : 1/9/78 : Fresh Golden Queen pollen : (Experiment No.7)
2 : 13/9/79 : Fresh hydrated Red Haven pollen : (Experiment No.10)
3 : 14/9/79 : Oven dried Red Haven pollen : (Experiment No.9)
4 : 18/9/79 : One day old fresh Red Haven pollen : (Experiment No.11)
5 : 18/9/79 : Fresh Red Haven pollen : (Experiment No.12)
6 : 2/9/78 : Fresh Golden Queen pollen : (Experiment No.8)

However, the germination of Red Haven pollen collected from anthers sprayed with 200 ppm triforine or 200 ppm triforine plus 100 ppm streptomycin was not significantly different from that from unsprayed anthers. ($P = 0.05$; see Table No.11).

Table No.11. *In vitro germination of Red Haven (peach) pollen collected from flowers sprayed with 200 ppm triforine or 200 ppm triforine plus 100 ppm streptomycin compared with Red Haven pollen collected from unsprayed flowers.*

Germinating media used:- 1.5% agar media containing 10% sucrose
Incubation temperature:- 24°C
Incubation period:- 20 hours
Date:- 20/9/1979

Pollen collected from	Mean percent pollen germination
200 ppm triforine sprayed anthers	68.06 NS^{o}
200 ppm triforine plus streptomycin sprayed anthers	70.10 NS^{o}
Unsprayed anthers	81.10 NS^{o}

NS : *Treatment means within column are not significantly different at 5% level when analysed by Duncan's Multiple Range test.*

^{o} : *See Experiment No.13 for other details.*

The germination percentages of Golden Queen pollen at 24°C, 28°C and 32°C were significantly different from each other (P = 0.05; see Table No.7). At 24°C the germinate was recorded to be 3.5 and 10.5 times higher than at 28°C and at 32°C respectively. As with apple pollen the number of pollen grains with bursting cell walls were higher at higher temperatures (at 28°C and at 32°C).

A.3. *Kiwifruits:*

Dichlofluanid, vinclozoline and captan had no significant effects on Matua pollen germination at concentrations of 1 ppm, 10 ppm, and 1 ppm respectively, but these materials completely inhibited germination at 100 ppm, 250 ppm and 100 ppm (see Table No.12 Fig.10).

Table No.12. *Effects of fungicides on in vitro germination of kiwifruit (Matua) pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 25°C on different dates in November and December 1979.*

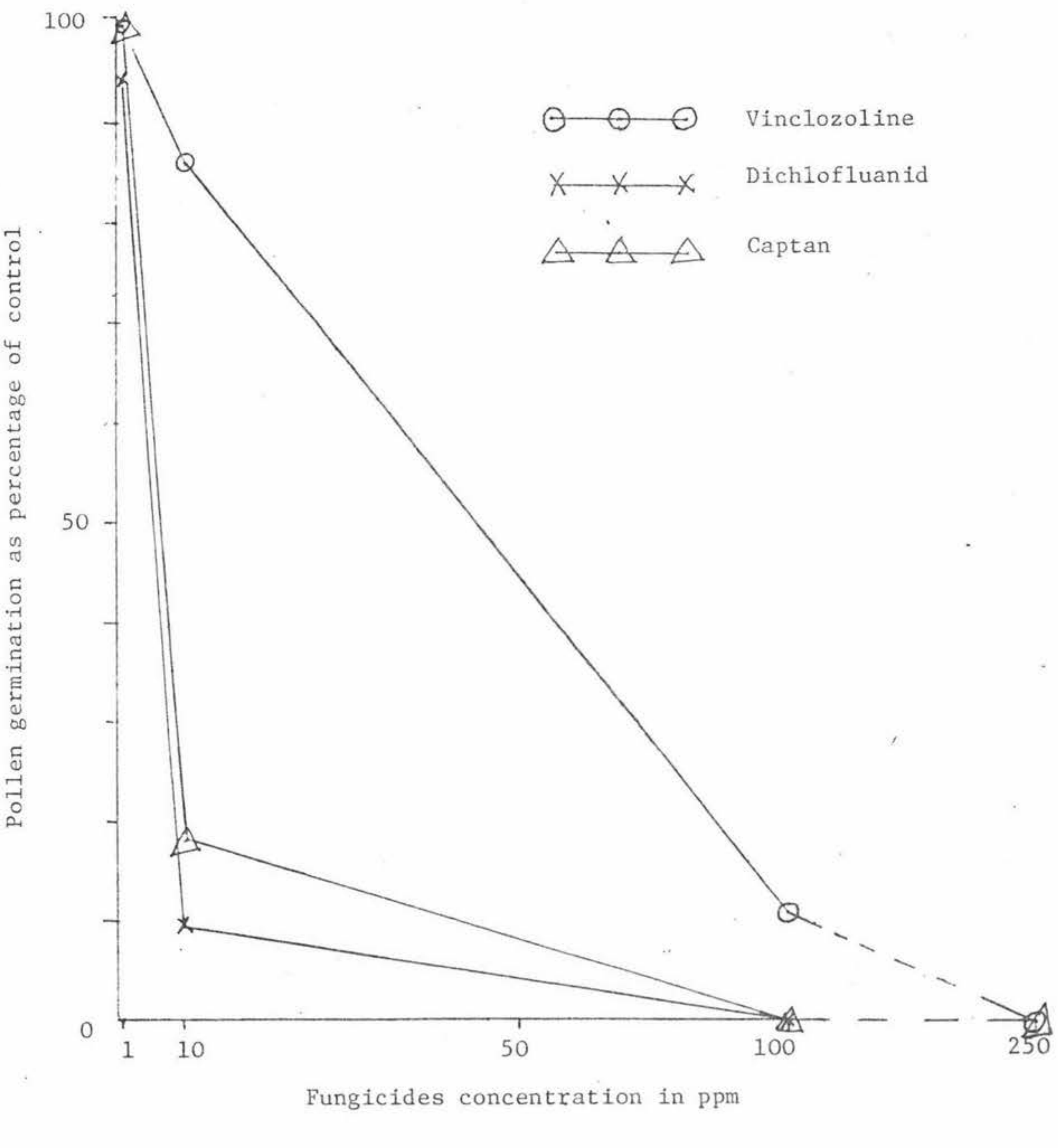
Treatment	Mean percent pollen germination at various concentration of fungicides				
	0 ppm	1 ppm	10 ppm	100 ppm	250 ppm
Dichlofluanid[1]	83.6A	78.3A	7.6B	0.0B	0.0B
Vinclozoline[2]	86.1a	88.2a	73.9a	10.0b	0.0b
Captan[3]	83.9a	84.0a	14.6b	0.0c	0.0c

Note: *Treatment means within each row denoted by the same small letter are not significantly different at 5% level and treatment means within each row denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

1 : 28/11/79 : Fresh Matua pollen : (Experiment No.14)
2 : 27/11/79 : Fresh Matua pollen : (Experiment No.15)
3 : 29/11/79 : Fresh Matua pollen : (Experiment No.16)

Dichlofluanid at 10 ppm caused over 90% reduction in the germination of Matua pollen (significant at 1% level;

Fig. 4 : Effects of fungicides on in vitro germination of kiwifruit (Matua) pollen grown for 20 hours in 1.5% agar and 10% sucrose media at 25°C.



see Table No.12). But one field spray of 1000 ppm dichlofluanid had no effect on the germinability of Matua pollen. The germination of Matua pollen collected from 1000 ppm dichlofluanid sprayed anthers was not significantly different from unsprayed anthers (P = 0.01, see Table No.13).

Table No.13. *In vitro germination of Matua (kiwifruit) pollen collected from anthers sprayed with 1000 ppm dichlofluanid compared with Matua pollen collected from unsprayed flowers.*

Experiment No.17.

Germinating media used:- 1.5% agar media containing 10% sucrose
Incubation temperature:- 25°C
Incubation period:- 20 hours
Date:- 5/12/1979

Pollen collected from	Mean percent pollen germination*
1000 ppm dichlofluanid sprayed anthers	59.0 NS
Unsprayed anthers	89.0 NS

NS : *Treatment means within column are not significantly different at 5% level when analysed by Duncan's Multiple Range test.*

* : *The difference in treatment means within column appears big (30%). The reason for such a difference to become statistically insignificant may be due to few (only 8) number of replications.*

The lengths of pollen tubes also were less at higher concentrations than at lower concentrations of fungicide. After 18 hours of incubation at 25°C the pollen tube growth under 10 ppm dichlofluanid treatment was 0.5 mm whereas under 0 ppm dichlofluanid treatment it was 2.5 mm.

A.4. *Discussion:*

The results presented above show that fungicides vary in their inhibitory effects on apple, peach and kiwifruit pollen. In apples, 10 ppm mancozeb plus dinocap (Dikar^R)

Plate No.1 : Kiwifruit (Matua) pollen grains. X5500.

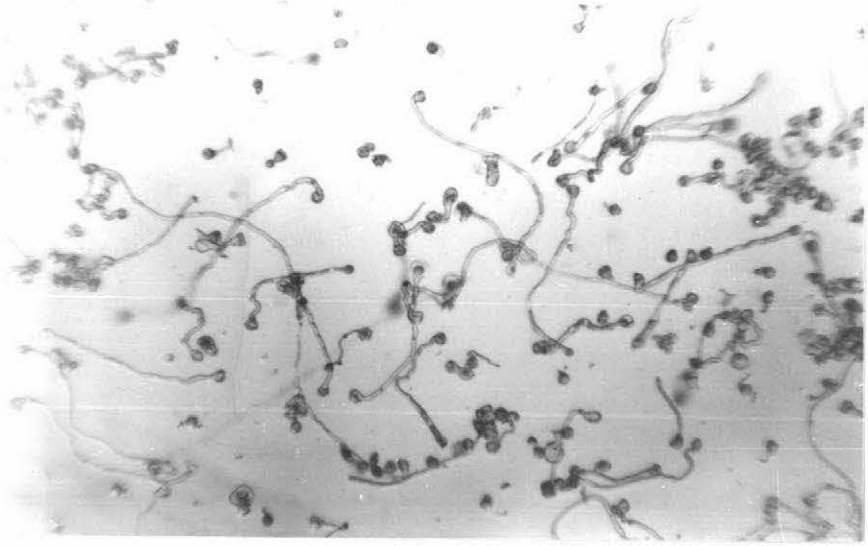


Plate No.2 : Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C when collected after spraying flower with 1000 ppm dichlofluanid. (Grown in 1.5% agar media containing 10% sucrose). X440.

caused 98% reduction but 10 ppm citowett, triforine, bupirimate, mancozeb, bayleton, dichlofluanid and captan caused only 93%, 76%, 75%, 63%, 55%, 35% and 32% reductions in pollen germination respectively. From the point of view of their concentrations required to completely arrest the apple pollen germination in vitro the fungicides can be listed in descending order of their importance as follows: mancozeb plus dinocap (Dikar^{R}), triforine, dichlofluanid, bupirimate, bayleton, citowett, captan and mancozeb. Mancozeb plus dinocap was the most effective and mancozeb was the least effective inhibitor of apple pollen germination in vitro (see Fig. 2). Hence dinocap seems to be toxic material for apple pollen. Church and Williams (1978) also found that dinocap killed pollen when sprayed on to dehisced anthers of Crawley Beauty apples.

In vitro studies showed that 10 ppm triforine caused over 90% reduction in peach pollen germination but 10 ppm vinclozoline, 10 ppm mancozeb, 10 ppm captafol, 10 ppm streptomycin plus 10 ppm triforine, 10 ppm streptomycin, 10 ppm iprodione and 10 ppm dichlofluanid caused only 88%, 78%, 76%, 65%, 61%, 58%, 44%, and 24% reductions in pollen germination respectively (see Table No.10). From the point of view of their concentrations required to completely arrest peach pollen germination in vitro the fungicides can be listed in descending order of their importance as follows: triforine, vinclozoline, streptomycin plus triforine, mancozeb, captafol, iprodione, dichlofluanid, streptomycin and benomyl (see Fig. 3). So far the effects of these fungicides on peach pollen germination in vitro have not been studied by any other workers.

Table No.9 shows that triforine, triforine plus streptomycin, vinclozoline and ethephon at 100 ppm completely inhibited peach pollen germination in vitro. Thus, it appears that if the in vitro conditions were to resemble the field condition triforine, triforine plus streptomycin and vinclozoline could be used as blossom thinners in peaches.

However, a large reduction in pollen germination under laboratory condition may not indicate a similar level of effect in the field (see also Chapter Two C.2). Table No.10 shows that at 100 ppm triforine and triforine plus streptomycin completely inhibited peach pollen germination in vitro but Table No.11 shows that 200 ppm triforine and 200 ppm triforine plus 100 ppm streptomycin did not affect peach pollen when sprayed on to anthers from Red Haven flowers. A 1000 ppm dichlofluanid spray on Matua (kiwifruit) anthers did not affect the germinability of pollen released but 100 ppm dichlofluanid caused a complete inhibition of pollen germination in vitro (see Table No.13 and Table No.14).

For kiwifruit (Matua) pollen dichlofluanid was the most effective and vinclozoline was the least effective inhibitor of germination. The reduction in Matua pollen germination by 10 ppm dichlofluanid was 5 and 6 times more than of those caused by 10 ppm captan and 10 ppm vinclozoline respectively (see Table No.12). However, while such trends were observed, the experimental design does not allow direct comparisons, and further comparisons would seem desirable.

Some fungicides namely dichlofluanid, triforine, mancozeb, captan and vinclozoline were tested with more than one kind of pollen. 100 ppm dichlofluanid caused over 90% reduction in germination of apple, peach and kiwifruit pollen, 100 ppm triforine completely arrested the germination of apple and peach pollen. 100 ppm mancozeb caused 97% reduction in the germination of peach pollen but caused only 62% reduction in the germination of apple pollen.

Captan at 100 ppm completely arrested the germination of kiwifruit pollen but caused only 85% reduction in the germination of apple pollen. 100 ppm vinclozoline caused over 90% reduction in germination of both peach and kiwifruit pollen. Thus, however the experimental design does not allow the direct comparison, it appears that dichlofluanid is equally toxic to apple, peach and kiwifruit pollen; triforine is equally toxic to apple and peach pollen; mancozeb is 1.5 times more toxic to peach than to apple pollen, captan is 1.2 times more toxic to kiwifruit pollen than to apple pollen and vinclozoline is equally as toxic to peach pollen as to kiwifruit pollen.

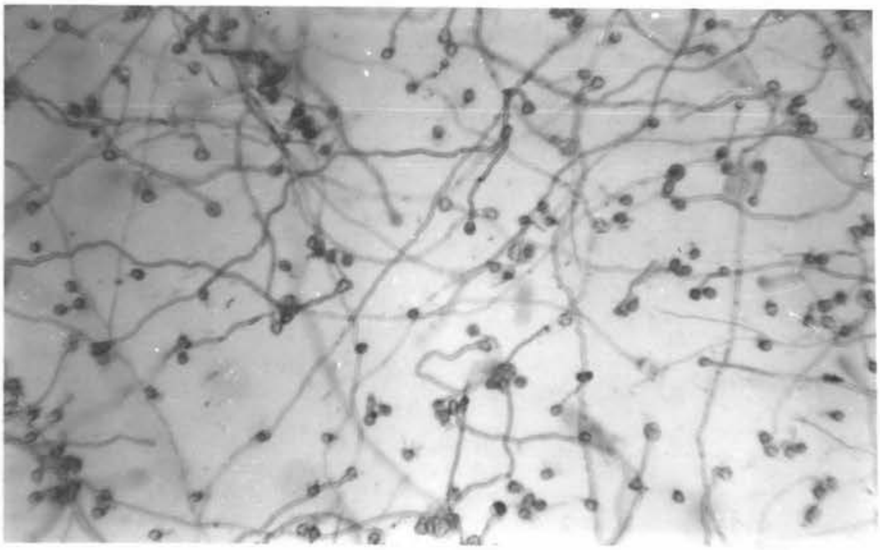


Plate No.3 : Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose. X440.

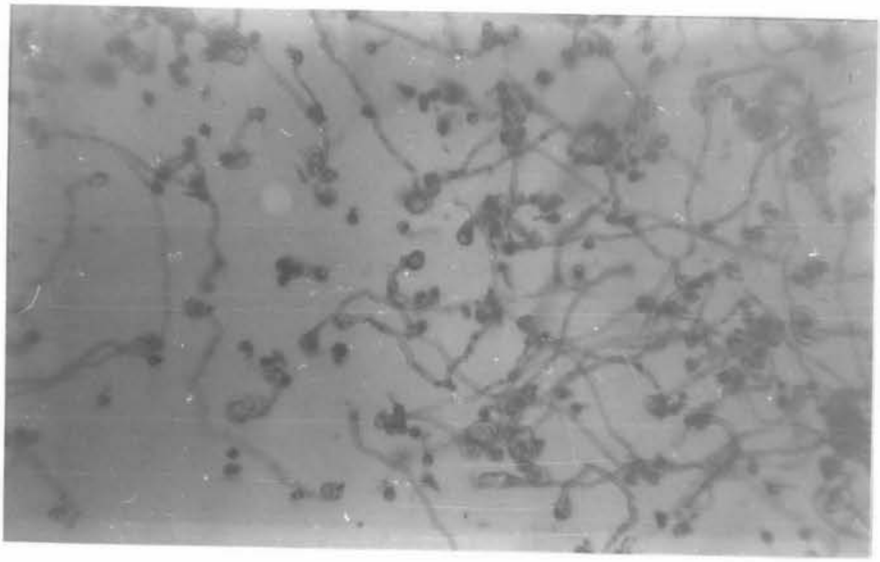


Plate No.4 : Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose and 1 ppm dichlofluanid. X440.

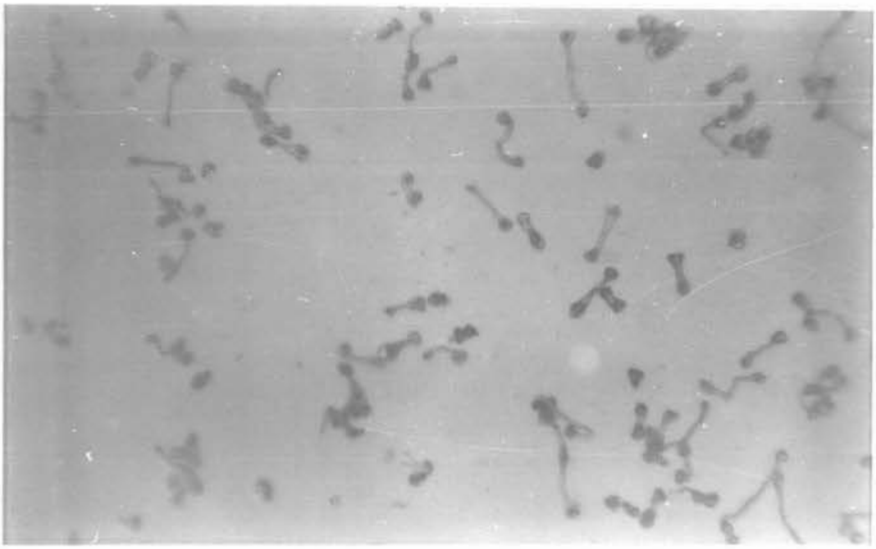


Plate No.5 : Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose and 10 ppm dichlofluanid. X440.

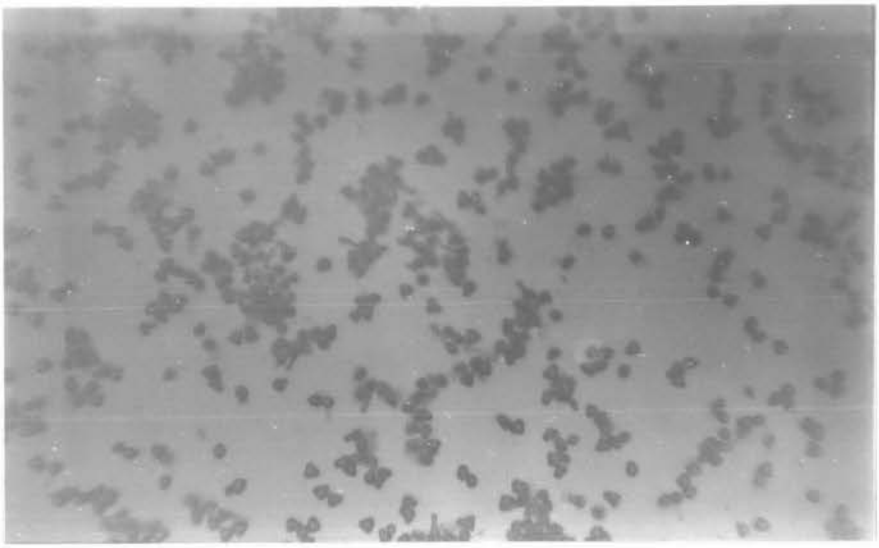


Plate No.6 : Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose and 100ppm dichlofluanid. X440.

Table No.14. *Inhibition of in vitro germination of apple, peach and kiwifruit pollen at 100 ppm dichlofluanid, 100 ppm triforine, 100 ppm mancozeb, 100 ppm captan and 100 ppm vinclozoline.**

Treatment	Mean percent reduction in germination of		
	Apple pollen	Peach pollen	Kiwifruit pollen
100 ppm dichlofluanid	100.0	90.0	100.0
100 ppm triforine	100.0	100.0	-
100 ppm mancozeb	62.0	96.5	-
100 ppm captan	85.0	-	100.0
100 ppm vinclozoline	-	100.0	90.0

*From Tables No.6, No.10 and No.12.

The results of temperature experiments showed that the germination of both apple and peach pollen was highest at 24°C; and pollen tubes were longer after 18 hours incubation at 28°C and at 32°C than at 16°C, 20°C and 24°C. At higher temperatures more pollen grains showed bursting of their cell walls than at lower temperatures. While the mechanism of the bursting of the pollen grains was not examined it appeared that the walls of the pollen grains burst at higher temperatures and thus affect pollen germination (see Tables No.7 and No.8). The reason for the lower germination percentages at lower temperatures may be that at lower temperatures metabolic activities of the pollen grains may be either reduced or stopped.

It was observed that pollen germination percentages decreased with an increase in the concentration of the fungicides. The bursting of the wall of the pollen grains also happened more at higher concentrations than at lower concentrations of fungicides treatment. Though the modes of action of fungicides were not examined it can be said that the substances in the fungicides may have adverse effect on the metabolic activity or on the physical condition, such as elasticity, membrane permeability or both of the pollen grains.

B. *In Vivo Studies:*

B.1. *Apple:*

At 24 hours after pollination, the percentages of pollen germination in the stigmatic surface of 1000 ppm bupirimate sprayed styles were half of that in those of unsprayed ones ($P = 0.01$; see Table No.15). But the pollen tube growth in the styles of sprayed flowers was not significantly different from that in unsprayed ones. Callose was distributed evenly along the walls of each pollen tube rather than in discrete plugs as in petunia (Cresti and van Went (1976)). The pollen tubes had a tendency to grow towards the central region to form a pollen tubes canal along the length of the styles. At 24 hours after pollination, up to 3.5% of the pollen tubes had grown to reach 1/4 to 1/2 way down the style (Table No.15).

Table No.15. *Pollen germination and pollen tube growth 24 hours after pollination, in styles from flowers sprayed with 1000 ppm bupirimate and in styles from unsprayed Splendour (apple) flowers.*

Experiment No.20. Date:- October 1979
Time of pollination:- Within 2 hours of spraying

Treatment	Mean percentage pollen germination	Mean percentages of pollen tubes reaching		
		< 1/10 of style length	1/10 to 1/4 of style length	1/4 to 1/2 of style length
Bupirimate sprayed	95.6A	87.0a	9.5a	3.5a
unsprayed	46.0B	85.0a	14.0a	1.0a

Note: *Within each column treatment means denoted by the same small letter are not significantly different at 5% level and treatment means denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

B.2. *Peach:*

The percentages of pollen germination in the stigmatic surfaces of the styles of Red Haven flowers sprayed with 200 ppm triforine were not significantly different from those in the stigmas of styles of unsprayed Red Haven flowers ($P = 0.01$; see Table No.16). But the pollen tube growth in the styles from triforine sprayed flowers was significantly less than in those from unsprayed flowers. At 24 hours after pollination in triforine sprayed flowers 4% of their pollen tubes were 1/2 to 3/4 down the style whereas in unsprayed flowers only 23% of their pollen tubes had grown that distance (Table No.16).

Callose was distributed evenly along the walls of each pollen tube rather than in discrete plugs as in petunia (Cresti and van Went (1976)). The pollen tubes had tendencies to grow towards the central region to form a pollen tubes canal along the length of the styles.

Table No.16. *Pollen germination and pollen tube growth at 24 hours after pollination in styles from flowers sprayed with 200 ppm triforine, and in styles from unsprayed Red Haven (peach) flowers.*

Experiment No.21. Date:- September 1979

Treatment	Mean percentage pollen germination	Mean percentages of pollen tubes reaching	
		< 1/2 of style length	1/2 to 3/4 of style length
Triforine sprayed	86.1A	96.2A	3.8A
unsprayed	93.8A	75.0B	22.7B

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

Fig. 5 : Pollen germination and pollen tube growth 24 hours after pollination with fresh Matua pollen in Hayward kiwifruit styles from flowers of different ages.

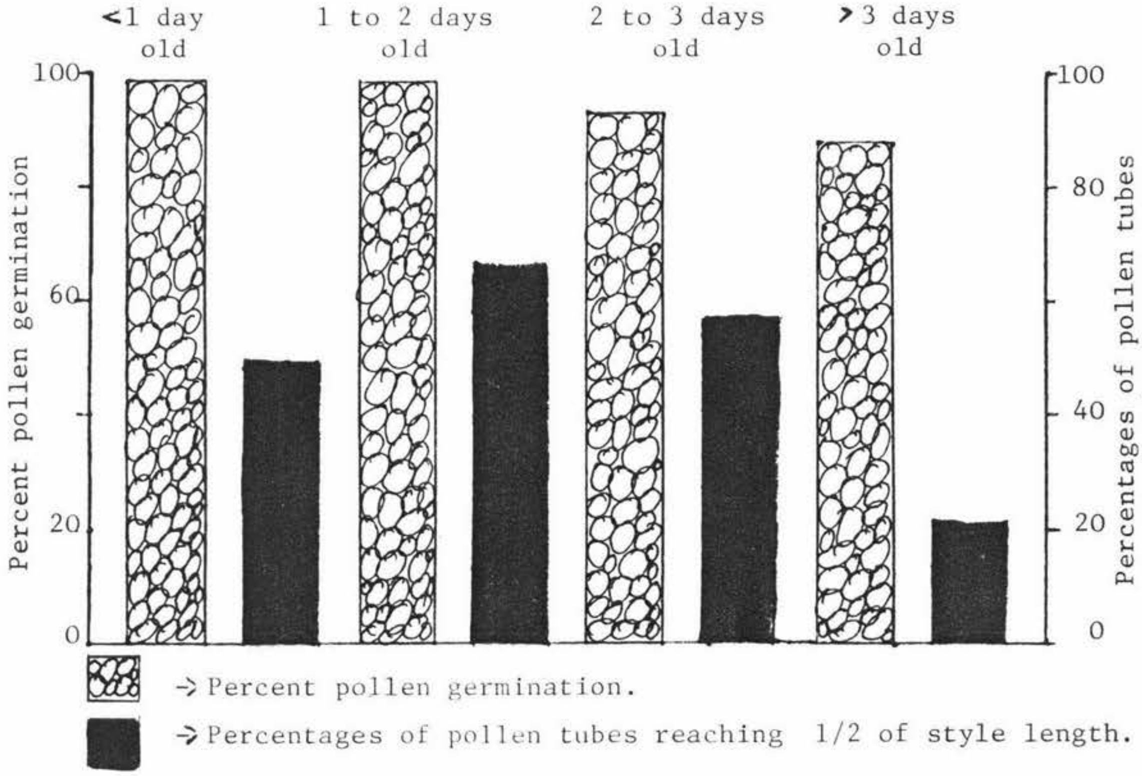
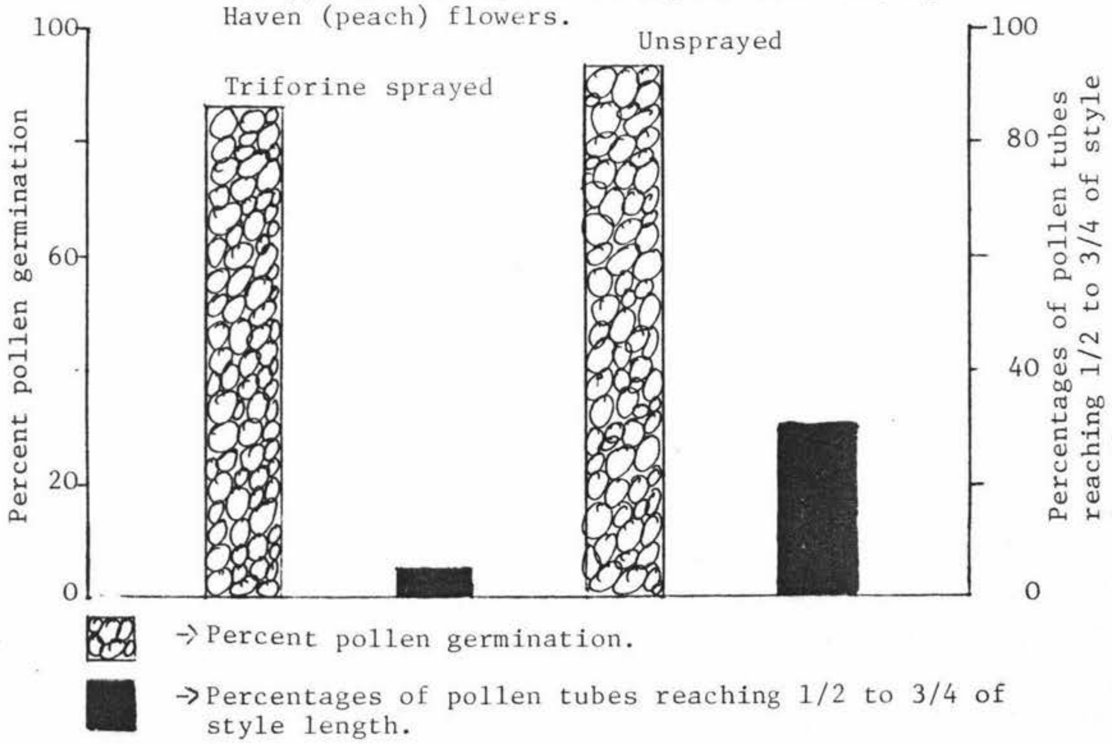


Fig. 6 : Pollen germination and pollen tube growth 24 hours after pollination in styles from flowers sprayed with 200 ppm triforine, and in styles from unsprayed Red Haven (peach) flowers.



B.3. *Kiwifruits:*

The percentages of pollen germination in the stigmatic surfaces of Hayward flowers sprayed with 1000 ppm dichlofluanid, 250 ppm vinclozoline, or 1000 ppm captan were not significantly different from those in the stigmatic surfaces of unsprayed styles (P = 0.01; see Table No.17). The pollen tube growth on dichlofluanid sprayed styles was not significantly different from those on vinclozoline sprayed and captan sprayed styles but was significantly different from those on unsprayed ones. The pollen tube growth on vinclozoline sprayed and captan sprayed styles was not significantly different from that on unsprayed ones (P = 0.01; see Tables No.17 and No.19). At 24 hours after pollination at 22°C, the mean percentages of pollen tubes reaching 3/4 to full style length of dichlofluanid sprayed, vinclozoline sprayed, captan sprayed and unsprayed styles were 26, 29, 44 and 69 respectively. At 24 hours after hand pollination 19 to 36% (see Table No.17) of the pollen tubes had grown less

Table No.17. *Effects of one spray application of fungicides on pollen germination and pollen tube growth in the styles of Hayward flowers at 22°C, 24 hours after hand pollination.*

Experiment No.22.	Date:- November 1979
Time of pollination:-	2 hours after spraying
Pollen used:-	Fresh Matua pollen

Treatment	Mean percentage pollen germination	Mean percentages of pollen tubes reaching	
		1/4 of style length	3/4 to full style length
1000 ppm dichlofluanid spray	94.0A	36.5A	26.0a
250 ppm vinclozoline spray	95.8A	34.4A	29.0ac
1000 ppm captan spray	82.0A	21.0A	44.0ac
Control (no spray)	96.1A	19.3A	68.5c

Table No.17 (Contd)

Note: *Within each column treatment means denoted by the same small letter are not significantly different at 5% level and treatment means denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

than 1/4 down the length of the styles. When observed 7 hours after pollination pollen germination and pollen tube growth in the styles hand pollinated after 1, 3, 7 and 24 hours of spraying with 1000 ppm dichlofluanid at 22°C were not significantly different from each other (P = 0.01; see Table No.18). However, the trend for one hour treatment shows that it may possibly be worth investigating the effects of dichlofluanid spraying with higher concentrations or closer to pollination.

Table No.18. *Pollen germination and pollen tube growth at 22°C in Hayward kiwifruit styles 7 hours after hand pollination where the flowers were sprayed with 1000 ppm dichlofluanid at different periods before pollination (pollinated with fresh Matua pollen).*

Experiment No.21a. Date:- November 1979

Period (Hours) between spray and pollination	Mean percentage pollen germination	Mean percentages of pollen tubes reaching		
		$<$1/10 of style length	1/10 to 1/4 of style length	1/4 to 1/2 of style length
One	82.0	96.5 NS	3.5 NS	0.0 NS
Three	83.0	87.5 NS	12.5 NS	0.0 NS
Seven	93.5	83.0 NS	14.0 NS	3.0 NS
Twenty four	91.5	83.5 NS	13.0 NS	3.5 NS

NS : *Within each column treatment means are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

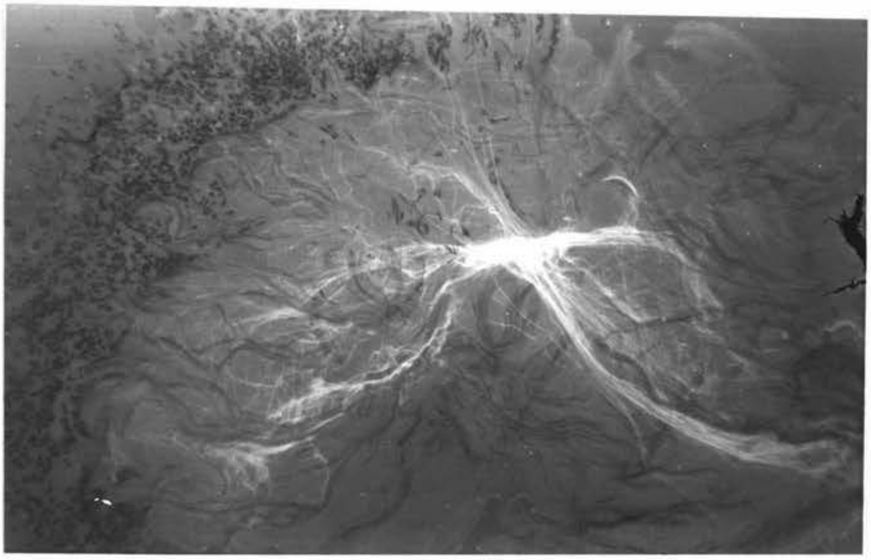


Plate No.7 : Pollen germination and pollen tube growth 24 hours after hand pollination at 22°C in a style from one day old kiwifruit (Hayward) flower. X77.

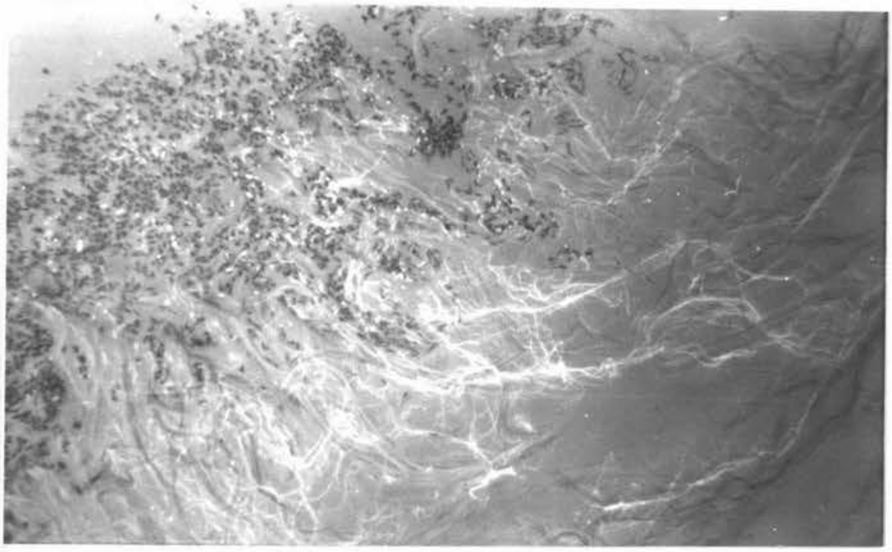


Plate No.8 : Pollen germination and pollen tube growth 24 hours after hand pollination at 22°C in a style from one day old kiwifruit (Hayward) flower sprayed with 1000 ppm dichlofluanid. X77.

The effects of fungicides on pollen germination and pollen tube growth were not influenced by temperature. The effects of 1000 ppm dichlofluanid spray and 250 ppm vinclozoline spray on the pollen germination and on the pollen tubes reaching 3/4 to full length of the styles at 22°C were not significantly different from those effects on the styles at 18°C ($P = 0.01$; see Table No.19).

The percentages of pollen germination on the styles at 26°C, 22°C, 18°C and 14°C were not significantly different from each other ($P = 0.01$, Table No.20). When the percentages of the pollen tubes growing less than 1/10 down the length of the styles were compared, 26°C and 14°C

Table No.19. *Effects of fungicides and temperature on pollen germination and pollen tube growth in Hayward styles 24 hours after hand pollination with fresh Matua pollen.*

Experiment No.22. Date:- November 1979

Temperature (°C)	Fungicides	Mean percent pollen germination	Mean percentages of pollen tubes reaching 3/4 to full length of the styles
22	1000 ppm dichlofluanid	94.0A	29.0A
	250 ppm vinclozoline	100.0A	28.8A
	Control	96.1A	76.1B
18	1000 ppm dichlofluanid	89.0A	21.0A
	250 ppm vinclozoline	100.0A	10.0A
	Control	95.6A	62.0B

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

temperature treatments were not significantly different from each other, and 22°C and 18°C temperature treatments were not significantly different from each other ($P = 0.01$; see Table No.20). From the point of view of their effects on pollen tube growth in the styles, temperature treatments in the descending order of their importance may be listed as follows: 22°C, 18°C, 26°C and 14°C (see Table No.20).

Table No.20. *Pollen germination and pollen tube growth 24 hours after pollination in the styles of one day old Hayward kiwifruit flowers grown at four different temperatures (pollinated with fresh Matua pollen).*

Experiment No.23. Date:- November 1979

Temperature (°C)	Mean percentage pollen germination	Mean percentages of pollen tubes reaching	
		< 1/10 of style length	1/2 to full style length
26	88.0A	38.5A	58.5A
22	100.0A	5.0B	87.5B
18	95.6A	10.0B	81.0AB
14	98.4A	40.5A	31.0C

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

Twenty four hours after pollination the percentages of pollen germination in the stigmatic surfaces of the styles from less than one day old, one to two days old, two to three days old and more than three days old Hayward flowers were not different from each other (Table No.21). When percentages of pollen tubes at different distances along the styles were compared pollen tube growth in the styles of one to two days old flowers was found to be significantly higher than the pollen tube growth in the styles of less than one day old and more than three days old flowers but was not found to be significantly different from that in the styles of two to three days old flowers.

Fig. 7 : Effects of one spray application of fungicides on pollen germination and pollen tube growth at 24 hours of hand pollination in the styles from Hayward flowers at 22°C.

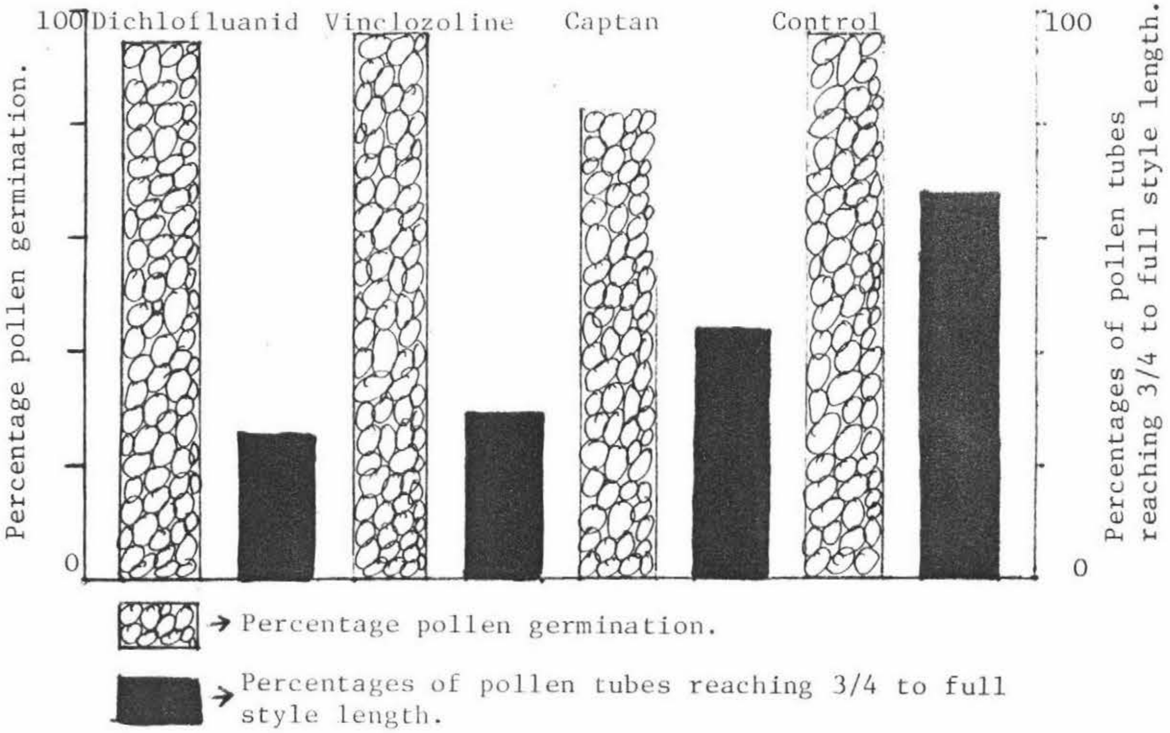


Fig. 8 : Pollen germination and pollen tube growth at 24 hours after pollination in Hayward (kiwifruit) styles from one day old flowers grown at 26°C, 22°C, 18°C and 14°C temperatures.

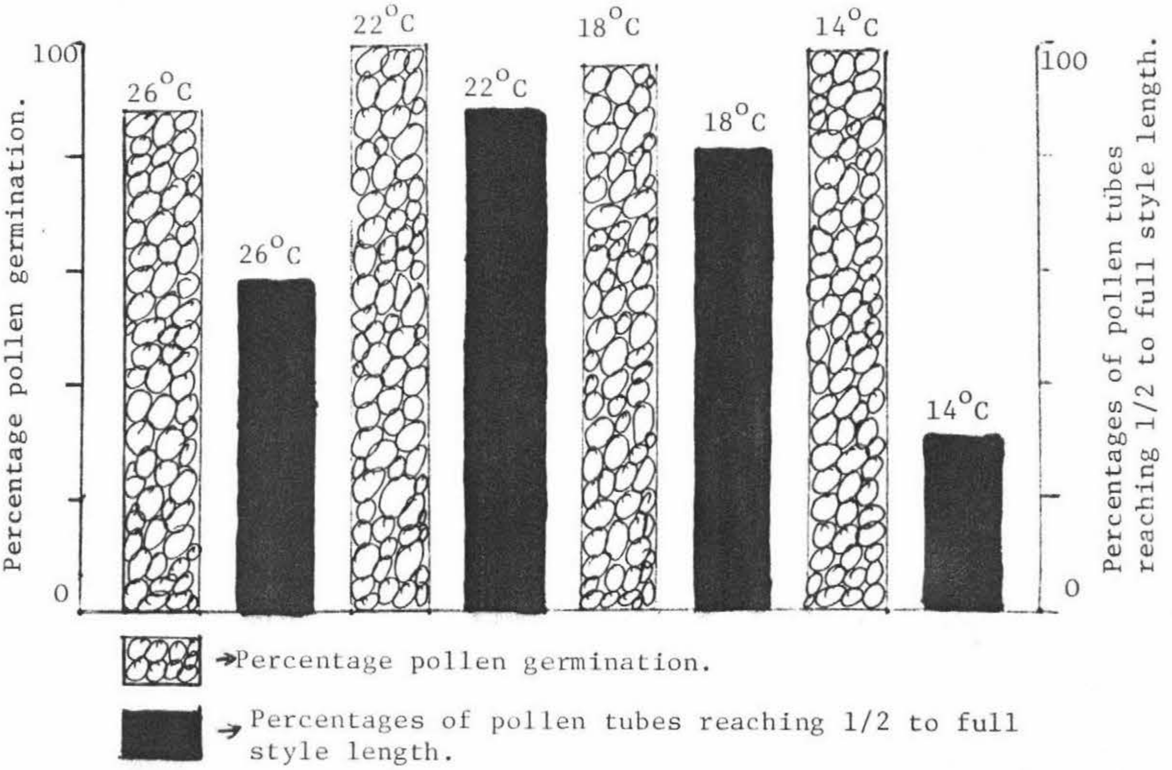


Table No.21. *Mean pollen germination and pollen tube growth 24 hours after pollination with fresh Matua pollen in Hayward kiwifruit styles from flowers of different ages.*

Experiment No.24. Date:- November 1979

Flowerage	Mean percentage pollen germination	Mean percentages of pollen tubes reaching	
		<1/2 of style length	> 1/2 of style length
1 day old	98.6	51.0A	49.0A
1 to 2 days old	98.0	33.5B	66.5B
2 to 3 days old	93.7	43.1AB	56.9AB
3 days old	88.1	76.9C	21.1C

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

Temperature did not influence the effects of flower age on pollen germination but influenced the effects of flower age on pollen tube growth in Hayward styles. When the percentages of the pollen tubes reaching 1/2 to full style length were compared, at 22°C styles from one day old and two days old flowers were not significantly different from each other and had more pollen tubes than the styles from less than one day old and more than three days old flowers; at 18°C styles from less than one day old, one day old and three days old flowers were not significantly different from each other and had more pollen tubes than the styles from more than three days old flowers; at 14°C styles from less than one day old and one day old flowers were not significantly different from each other and had more pollen tubes than the styles from two days old and more than three days old flowers (P = 0.01; see Table No.22).

Table No.22. *Pollen germination and pollen tube growth 24 hours after pollination in Hayward kiwifruit styles from flowers of different ages held at different temperatures.*

Experiment No.24. Date:- November 1979

Temperature (^{o}C)	Flowerage	Mean percentage pollen germination	Mean percentages of pollen tubes reaching	
			1/2 of style length	1/2 of style length
22	1 day old	100.0	36.5AB	63.5CD
	1-2 days old	100.0	12.5A	87.5D
	2-3 days old	100.0	12.5A	87.5D
	3 days old	90.0	55.5BC	44.5BC
18	1 day old	98.0	40.0BC	60.0CD
	1-2 days old	95.6	19.0A	81.0D
	2-3 days old	95.0	22.0A	78.0D
	3 days old	83.5	76.8C	23.2B
14	1 day old	97.8	76.5C	23.5B
	1-2 days old	98.4	69.0C	31.0B
	2-3 days old	86.1	95.0D	5.0A
	3 days old	90.8	98.5D	1.5A

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

As in apples and in peaches, the pollen tubes had tendencies of growing towards the central region to form pollen tube canal in Hayward styles. Callose was distributed evenly along the walls of each pollen tube rather than in discrete plugs as in petunia (Cresti and van Went (1976)). Callose like patches were observed in 1000 ppm dichlofluanid sprayed styles (30% of the styles) in 250 ppm vinclozoline sprayed styles (10% of the styles) and in styles at 14^{o}C.

B.4. *Discussion:*

In vivo studies with apples showed that 1000 ppm bupirimate sprays caused 50% reduction in the germination of the pollen grains on the stigmatic surface but did not influence the growth of pollen tubes in Splendour styles. Thus it appears that where pollen supply is abundant, bloom application of 1000 ppm bupirimate sprays may not be hazardous to the fertilization of Spendour flowers in the orchard.

Sprays of triforine at 200 ppm did not inhibit the germination of pollen grains on stigmatic surfaces but inhibited the growth of pollen tubes in peach styles. When the percentage of the pollen tubes reaching 1/2 to full length of styles at 24 hours of pollination was compared, triforine sprayed Red Haven styles had 20% less pollen tubes than the unsprayed ones. However, because only one pollen tube grows through the micropyle and effects fertilization (Stosser and Anwari (1979)) it appears that bloom application of triforine cannot be considered a hazard except under adverse weather conditions in peach orchards.

Sprays of 250 ppm vinclozoline and 1000 ppm captan had no effect both on pollen germination and on pollen tube growth in Hayward styles. 1000 ppm dichlofluanid had no effect on pollen germination but inhibited pollen tube growth in the styles. At 24 hours after pollination the numbers of pollen tubes at 3/4 to full length dichlofluanid sprayed styles were 40% less than in the unsprayed styles (see Table No.17). Thus it appears that dichlofluanid sprays during bloom can cause adverse effect on the fertilization of ovules in Hayward flowers in the orchard. Dichlofluanid sprays should be considered cautiously as compared with vinclozoline and captan sprays during bloom in a kiwifruit orchard.

Sedgley (1976) has suggested two possible mechanisms for control of pollen tubes in the styles as follows: (a) the availability of nutrients for tube growth and (b) the production of inhibitor by the successful tubes. Thus, although their modes of action were not examined, it is likely that triforine sprays are either inhibitory to or influence the availability of nutrients for tube growth in peach styles; and dichlofluanid sprays are either inhibitory to or influence the availability of nutrients for tube growth in kiwifruit styles.

The effects of temperature on pollen germination and on pollen tube growth in kiwifruit styles have not been studied so far by any other workers. The results presented in Chapter Seven B.3. show that temperature did not influence pollen germination but influenced the number of pollen tubes growing down to the base of Hayward styles. At 22°C the number of pollen tubes reaching 3/4 to full length of the styles was 57% and 29% more than at 14°C and at 26°C respectively. Thus temperatures both higher and lower than the temperature range 18 to 22°C can be considered inhibitory to pollen tube growth in Hayward styles. It appears that provisions for orchard heating may be important in the orchards where spring temperatures fall below 18°C.

Callose was observed in 1000 ppm dichlofluanid sprayed Hayward styles, in 250 ppm vinclozoline sprayed Hayward styles and in Hayward styles at 14°C temperature treatment. Working with avocado, Sedgley (1977b) suggested that the presence of callose in the pistil may reduce the availability to the pollen of substances necessary for germination and tube growth and contributes to the female sterility of male floral stage. Thus, it is likely that the presence of callose substances may be one of the reasons for less number of pollen tubes growing down the base of the styles of flowers sprayed with dichlofluanid and of the styles of flowers at 14°C. Currier (1957) has noted that callose appears as a result of the wounding in parenchymatous cells and is considered to function by gross plugging of tissue.

At 24 hours after pollination, the number of pollen tubes growing more than 1/2 the length of styles from one to two days old flowers was 38% and 27% higher than those from less than one day old and more than three days old flowers respectively. However, the substances present in the styles from different age groups were not analysed; it may be possible that styles from one day old and two days old flowers may contain more nutrients necessary for pollen tube growth than the styles from less than one day old and more than three days old flowers (see also page 95 paragraph 4). Hayward flowers pollinated within one to two days of their opening may set better than those pollinated less than one day and more than three days of their opening.

C. *Field Studies:*

C.1. *Apple:*

The percentage initial fruit set (fruit set at 5 weeks after full bloom) in Golden Delicious trees sprayed with captan, mancozeb plus dinocap (Dikar^R), mancozeb, citowett, or triforine was not significantly different from that in unsprayed trees but was significantly less than that in bayleton sprayed trees ($P = 0.01$; Table No.23). Hence whereas bayleton sprayed trees had 56% set, all other treatments including the unsprayed trees had 25 to 43% set.

The percentage final fruit set (fruit set at harvest) of bayleton sprayed Golden Delicious tree was 11%, 23%, 31%, 42%, 44% and 62% more than in citowett sprayed, unsprayed, mancozeb sprayed, captan sprayed, mancozeb plus dinocap sprayed and triforine sprayed Golden Delicious trees (Table No.23). Thus it appears that effects of fungicides on final set were same as on initial set.

The percentages of initial fruit set in a Splendour tree sprayed with bupirimate was not significantly different from that in an unsprayed tree ($P = 0.01$; see Table No.23). Also the percentage of final set in bupirimate sprayed tree did not appear different from that in unsprayed tree.

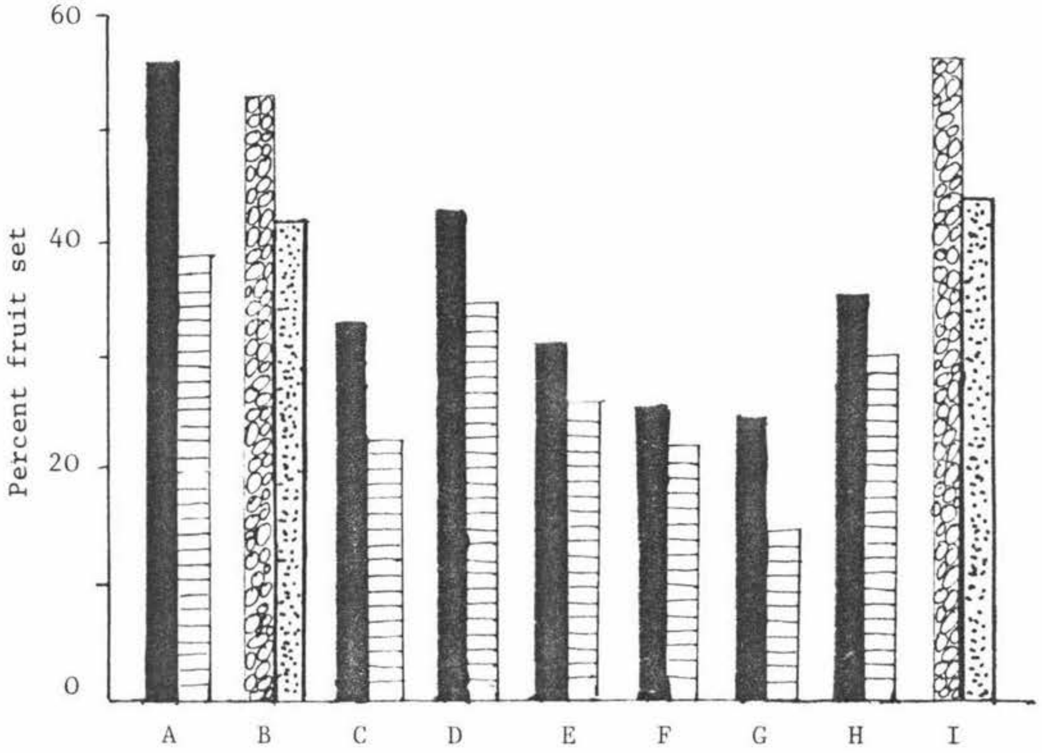
Table No.23. *Mean percentage fruit set in Golden Delicious and Splendour trees sprayed with different fungicides during the bloom seasons of 1978 and 1979.*

Experiments No.25 and No.26.

Treatment	Mean percentages of fruit set at			
	5 weeks after full bloom		harvest	
	Golden Delicious	Splendour	Golden Delicious	Splendour
200 ppm bayleton (5 sprays)	56.3A	-	38.8	-
100 ppm bupirimate (2 sprays)	-	52.7A	-	42.3
1000 ppm captan (5 sprays)	33.0B	-	22.6	-
200 ppm citowett (3 sprays)	42.8AB	-	34.5	-
1200 ppm mancozeb (3 sprays)	31.6B	-	26.9	-
1200 ppm mancozeb plus dinocap (4 sprays)	25.8B	-	21.8	-
200 ppm triforine (3 sprays)	24.9B	-	14.7	-
Unsprayed	35.1AB	55.8A	30.0	40.8

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test. Fruit set at harvest data was not statistically analysed.*

Fig. 9 : Percentages of initial and final fruit set in Golden Delicious and Splendour trees sprayed with different fungicides during bloom season.



A - Bayleton; B - Bupirimate; C - Captan; D - Citowett;
E - Mancozeb; F - Mancozeb plus Dinocap; G - Triforine;
H and I - Control.

■ → Initial set (Golden Delicious)

▤ → Final set (Golden Delicious)

▩ → Initial set (Splendour trees)

░ → Final set (Splendour trees)

C.2. *Peach:*

With Golden Queen peaches, the percentage fruit set 5 weeks after full bloom in benomyl sprayed, captafol sprayed, mancozeb sprayed, streptomycin sprayed, triforine sprayed, and vinclozoline sprayed trees were not significantly different from that in unsprayed trees ($P = 0.01$; see Table No.24). The percentage of fruit set at harvest in fungicide sprayed trees did not appear different from that of unsprayed trees. Hence fruit set at 5 weeks after full bloom and at harvest in different trees was 55-68% and 2-10% respectively (Table No.24).

With Red Haven peaches, the percentage of fruit set 4 weeks after full bloom in dichlofluanid sprayed trees and 100 ppm streptomycin sprayed trees was not significantly different from that in unsprayed trees, whereas the percentage fruit set 4 weeks after full bloom in triforine sprayed trees, triforine plus streptomycin sprayed trees and in ethephon sprayed trees was significantly lower than that in unsprayed trees ($P = 0.05$; Table No.24). Hence whereas unsprayed trees, dichlofluanid sprayed trees and streptomycin sprayed trees had 63-79%, triforine sprayed and triforine plus streptomycin sprayed trees had 31-33%, and ethephon sprayed trees had only 7% set. However, the final set (fruit set at harvest) in fungicide sprayed trees and ethephon sprayed trees did not appear different from that in unsprayed (Table No.24).

Table No.24. *Mean percentage fruit set in Golden Queen and Red Haven peach trees sprayed with different fungicides and ethephon during the bloom seasons of 1978 and 1979.*

Experiments No.27 and No.28.

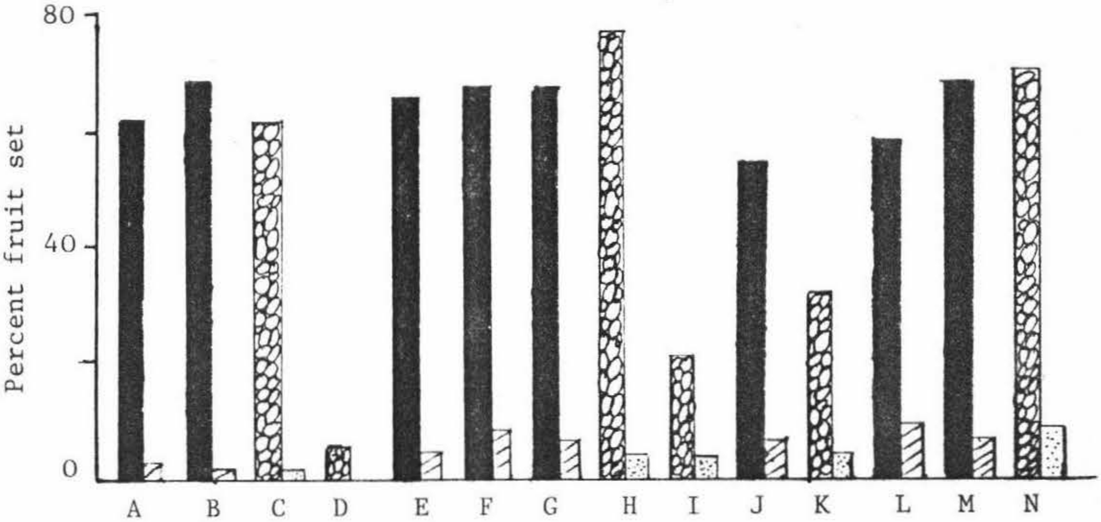
Treatment	Mean percentage fruit set			
	5 weeks after	4 weeks after	at harvest	
	full bloom in Golden Queen trees (1978 Season)	full bloom in Red Haven trees (1979 Season)	in Golden Queen trees (1978)	in Red Haven trees (1979)
160 ppm benomyl (6 sprays)	63.0AB	-	2.2	-
800 ppm captafol (6 sprays)	69.1A	-	2.0	-
1000 ppm dichlofluanid (3 sprays)	-	63.3a	-	1.0
100 ppm ethephon (3 sprays)	-	7.3c	-	0.0
250 ppm iprodione (5 sprays)	66.4AB	-	4.3	-
1200 ppm mancozeb (5 sprays)	67.8AB	-	8.6	-
100 ppm streptomycin*	67.7AB	76.9a	6.0	3.7
100 ppm streptomycin plus 200 ppm triforine (3 sprays)	-	21.2b	-	3.3
200 ppm triforine+	55.1B	32.5b	6.9	2.2
250 ppm vinclozoline (5 sprays)	58.2AB	-	8.9	-
Unsprayed	68.1AB	71.3a	10.0	8.6

Note: *Within each column treatment means denoted by the same small letter are not significantly different from each other at 5% level and treatment means denoted by the same capital letter are not significant at 1% level when analysed by Duncan's Multiple Range test. Mean percentage fruit set at harvest data was not analysed statistically.*

* *Three sprays in Red Haven and 5 sprays in Golden Queen.*

\+ *Three sprays in Red Haven and 5 sprays in Golden Queen.*

Fig.10 : Percentages of initial and final fruit set in Golden Queen and Red Haven trees sprayed with different fungicides during bloom season.



A - Benomyl; B - Captafol; C - Dichlofluanid;
D - Ethephon; E - Iprodione; F - Mancozeb;
G and H - Streptomycin; I - Streptomycin plus triforine;
J and K - Triforine; L - Vinclozoline;
M and N - Control.

■ → Initial set (Golden Queen)

▨ → Final set (Golden Queen)

▩ → Initial set (Red Haven)

⬚ → Final set (Red Haven)

C.3. *Kiwifruits:*

Two blossom sprays of captan, dichlofluanid or vinclozoline to Hayward vines did not affect the percent initial set (fruit set at 5 weeks after bloom) or final set (fruit set at harvest) or harvest fruit weight ($P = 0.05$; Tables No.25 and No.26).

The number of seeds per fruit in fruits from captan sprayed, dichlofluanid sprayed and vinclozoline sprayed vines was significantly less than that in fruits from unsprayed vines ($P = 0.01$; Table No.26). Hence whereas fruits from unsprayed vines had on average 716 seeds per fruit, fruits from all other treatments had 440-461 seeds per fruit (Table No.26). The seed numbers in fruits sample from unsprayed trees ranged from 121 to 1344 whereas those in fruits sample from all other fungicide sprayed trees ranged from 37 to 965.

Table No.25. *Mean percentage fruit set in Hayward vines sprayed twice with different fungicides during the bloom season in 1979.*

Experiment No.29.

Treatment	Mean percentage fruit set	
	5 weeks after bloom	at harvest
1000 ppm captan	80.4 NS	53.8 NS
1000 ppm dichlofluanid	73.3 NS	69.1 NS
250 ppm vinclozoline	78.1 NS	71.9 NS
Unsprayed	85.4 NS	79.1 NS

NS : *Within each column treatment means are not significantly different at 5% level when analysed by Duncan's Multiple Range test.*

Fig. 11 : Percentages of fruit set at 5 weeks after full bloom and at harvest in Hayward vines sprayed with different fungicides during bloom season.

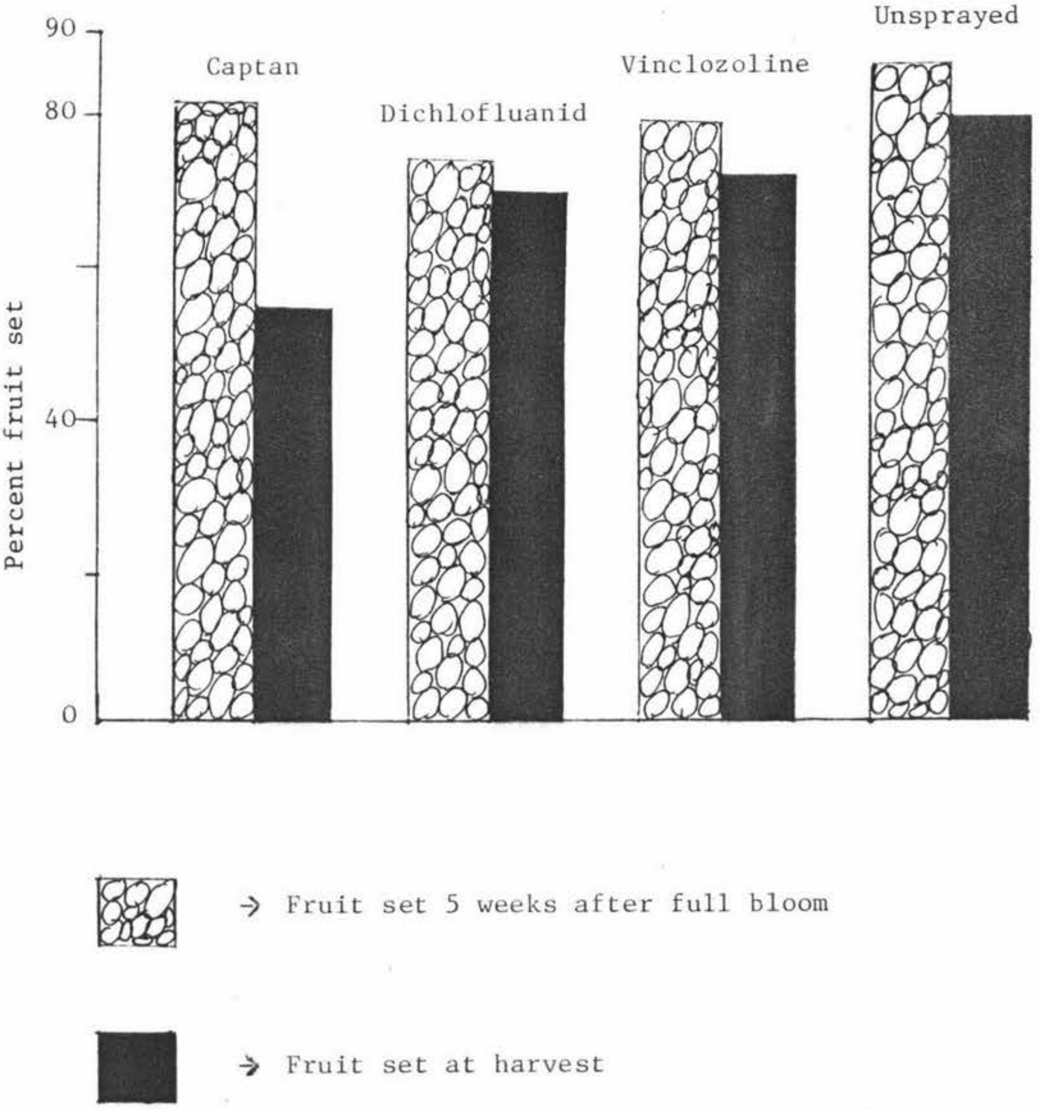


Table No.26. *Mean fruit weights and seed numbers per fruit at harvest in fruits from Hayward vines sprayed twice with different fungicides during the bloom season in 1979.*

Experiment No.29.

Treatment	Mean fruit weight at harvest (gm)	Mean Seed numbers per fruit at harvest
2 @ 1000 ppm captan	75.30a	461.31A
2 @ 1000 ppm dichlofluanid	70.47a	452.83A
2 @ 250 ppm vinclozoline	68.58a	440.17A
Unsprayed	78.00a	716.17B

Note: *Within each column treatment means denoted by the same small letter are not significantly different at 5% level; treatment means denoted by the same capital letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

The percentage initial fruit set (fruit set at 5 weeks after full bloom) and final fruit set (fruit set at harvest) in bee pollinated flower clusters was not significantly different from that in bee plus hand pollinated flower clusters from Hayward vines. But the mean fruit weight and the seed number per fruits were significantly less in fruits from bee pollinated flower clusters than in those from bee plus hand pollinated flower clusters (P = 0.01 Table No.27). The mean weights of fruits from bee pollinated and hand pollinated flower clusters were 61 gm and 82 gm respectively and the mean seed numbers per fruit in fruits from bee pollinated and bee plus hand pollinated flower clusters were 412 and 623 respectively (Table No.27).

Table No.27. *Comparisons of fruit set at 5 weeks after full bloom, fruit set at harvest, fruit weight at harvest and seed numbers per fruit at harvest in bee pollinated and bee plus hand pollinated flower clusters from Hayward vines at Massey University, New Zealand.*

Experiment No.30.

Treatment	Mean percentage fruit set at		Mean fruit weight (gm)	Mean seed numbers per fruit
	5 weeks after full bloom %	harvest %		
Bee pollination	84.6A	78.1A	61.05A	412.18A
Bee plus hand pollination	88.6A	83.7A	82.62B	623.06B

Note: *Within each column treatment means denoted by the same letter are not significantly different at 1% level when analysed by Duncan's Multiple Range test.*

When the fruits from sheltered trees (Experiment No.30) were examined fruit weights and seed numbers per fruit were found to be positively correlated with each other (P = 0.0001 Fig.13). The coefficients of correlation for bee pollinated and bee plus hand pollinated fruits were +0.84 and +0.78 respectively.

C.4. *Discussion:*

So far the effects of bayleton, bupirimate, captan, citowett^{R}, mancozeb, mancozeb plus dinocap and triforine on the fruit set of apples have not been reported by any other workers. The results presented in Table No.23 show that spray application during the bloom period with bayleton, captan, citowett^{R}, mancozeb, mancozeb plus dinocap or triforine did not affect the fruit set at 5 weeks after full bloom and at harvest in Golden Delicious trees. Bupirimate sprays during bloom did not affect fruit set 5 weeks after full bloom and at harvest in Splendour trees. Because such results were obtained with more bloom sprays than a grower would usually use it can be said that spray applications of bayleton, captan, citowett^R, mancozeb, mancozeb plus dinocap, and triforine during the bloom period should not be considered to pose any problem for fruit set in Golden Delicious trees. However, these results show only the direct effect of fungicides on fruit set. These materials (fungicides) could cause an effect on fruit set if they repel or kill bees. Palmer-Jones (1965) and Johansen (1979) have found that captan and dinocap are not harmful to hive bees.

Benomyl sprays, captafol sprays, iprodione sprays, mancozeb sprays, streptomycin sprays, triforine sprays and vinclozoline sprays during bloom period caused no effect on the fruit set 5 weeks after full bloom and at harvest in Golden Delicious peaches. Dichlofluanid sprays and streptomycin sprays, during bloom period caused no effect but triforine sprays, triforine plus streptomycin sprays during bloom period 50% and 70% reductions respectively in fruit set at 5 weeks after full bloom in Red Haven peach trees. Thus although the experimental designs do not allow the direct comparison of the observations made in Red Haven trees with that in Golden Queen trees it appears that Golden Queen and Red Haven trees differ in their response to spray application of triforine during their bloom periods (see Fig.10). Triforine plus streptomycin and triforine sprays during bloom period should be used cautiously in Red Haven trees but not in Golden Queen trees.

So far the evidence on the effects of triforine and triforine plus streptomycin on the fruit set in stone fruits are conflicting. Since 1977, three reports have been received where poor fruit set in cherries and peaches occurred following the application of triforine over the blossom period in New Zealand (Anonymous (1978)). New Zealand Ivon Watkin Dow Ltd's report indicates that triforine was very well tolerated but agrimycin and the combination of agrimycin and triforine were not tolerated by sweet cherry flowers (Agriculture Chemicals

Board, New Zealand 1979). Unfortunately all but one report show the list/name of peach varieties on which the effect of triforine and/or triforine plus streptomycin was studied. New Zealand Ispray Ltd's Report (1979) shows that triforine and triforine plus streptomycin sprays during bloom period did not cause reduction in fruit set in Golden Queen peach trees.

Ethephon sprays during the post blossom period cause the release of ethylene which stimulates the abscission of fruits and thus affects fruit set (Westwood (1979)). But the effects of ethephon sprays during bloom period on fruit set in peaches has not been reported by any other workers. Table No.24 shows that ethephon sprays during bloom period caused 90% reduction in fruit set at 5 weeks after full bloom. Thus it appears that ethephon sprays during bloom should be used cautiously in peaches.

Field studies with kiwifruits show that although mean seed number per fruit was reduced by 37%, mean fruit weight, mean fruit set at 5 weeks after full bloom and mean fruit set at harvest were not affected by captan sprays, dichlofluanid sprays and vinclozoline sprays during bloom period in Hayward vines. Because such results were obtained with more bloom sprays than a grower would usually use it appears that captan sprays, dichlofluanid sprays and vinclozoline sprays may safely be applied during bloom period in Hayward vines. However, these results show only the direct effect of fungicides on fruit set. These materials (fungicides) could cause an effect on fruit set if they kill or repel bees. Palmer-Jones (1965) has found that captan is not harmful to hive bees.

Hand pollination of flowers did not influence fruit set but increased both fruit weight and seed numbers per fruit in Hayward vines. The mean fruit weight was 1.35 times higher and the mean seed numbers per fruit was 1.5 times higher in fruits from bee plus hand pollinated flowers than in those from bee pollinated flowers. Hopping (1979) observed that hand pollination increased both fruit weight and seed numbers per fruit in fruits from kiwifruit vines. Hopping has also noted that

Fig. 12 : Mean fruit weight at harvest and mean seed numbers per fruit at harvest in bee pollinated and bee plus hand pollinated flower clusters from Hayward vines.

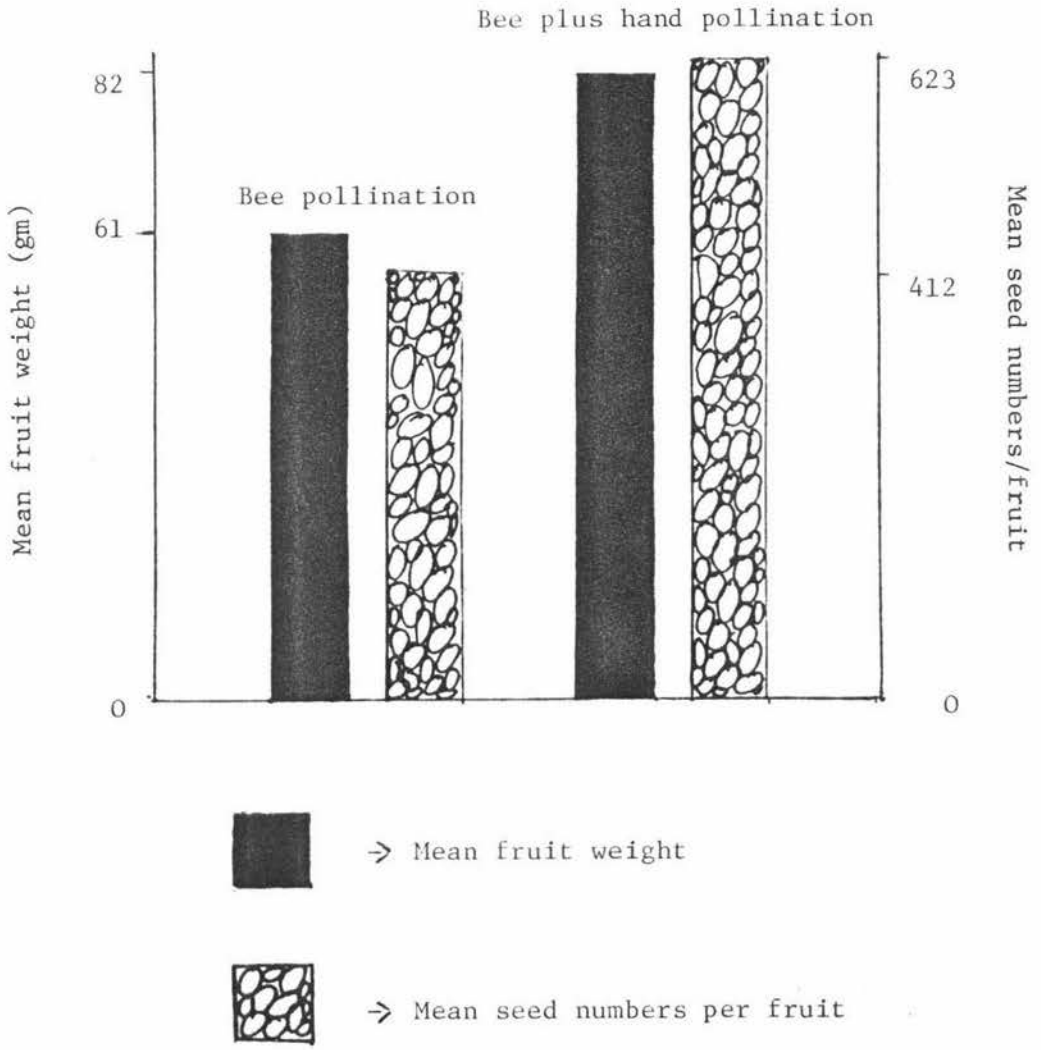
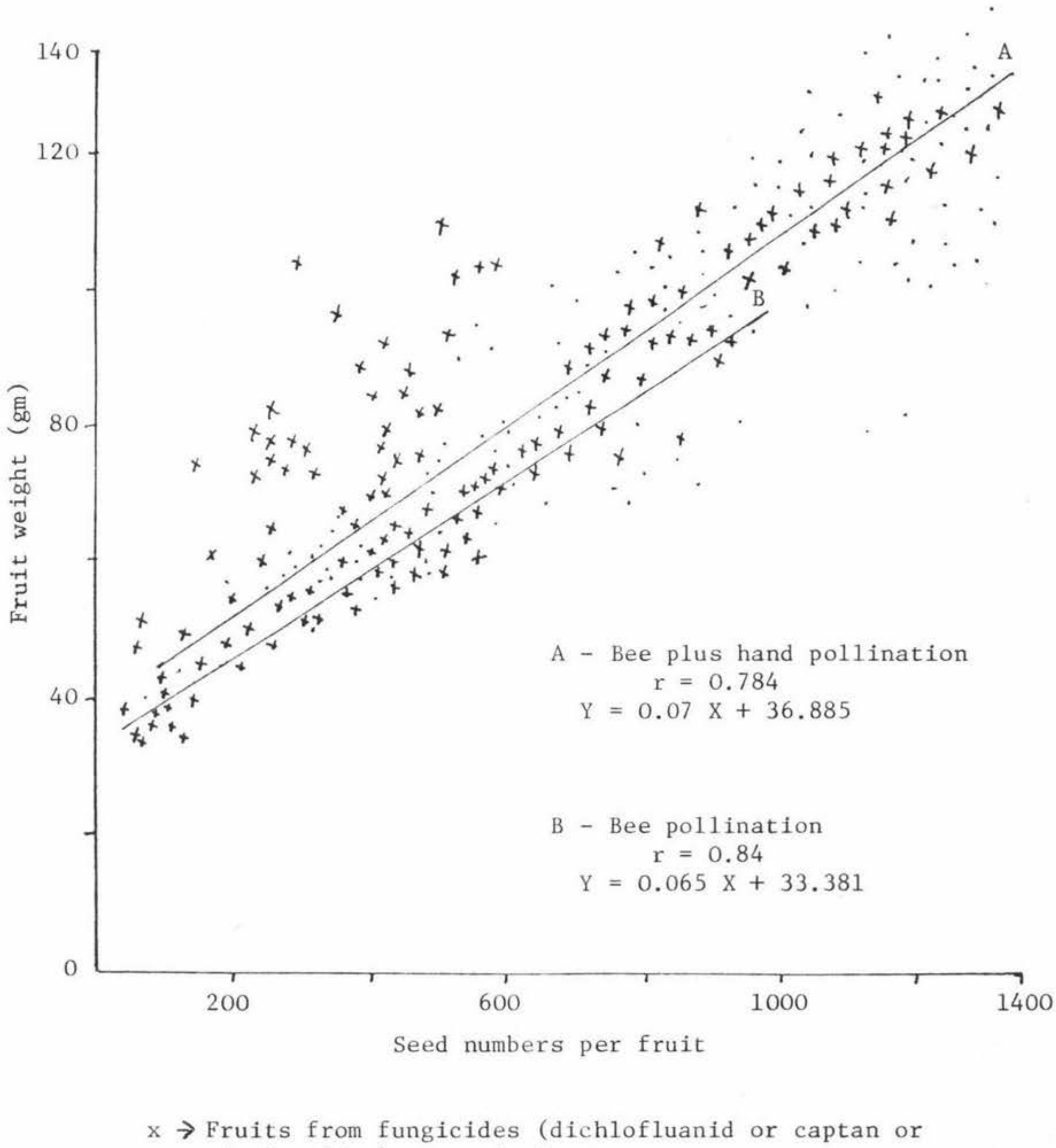


Fig. 13 : Relationship between fruit weight of and seed number in fruits from bee pollinated and bee plus hand pollinated flower clusters in fungicide sprayed and unsprayed vines.



x → Fruits from fungicides (dichlofluanid or captan or vinclozoline) sprayed vines.

· → Fruits from unsprayed vines.

supplemental pollination had detrimental effects on the fruit set of kiwifruits. He found that kiwifruit pollen can be suspended in aqueous media without loss of viability and then sprayed on to open flowers to increase seed number and hence fruit weight and number of fruits per vine. He suggested that for efficient use of valuable pollen 1.0 gm of pollen/litre of calcium nitrate, boric acid and calcium chloride (CBC) suspension medium (see page 33) should be applied twice to vines (at 40-50% and at 85-100% flowering) at rates of about 500 ml medium per vine per application time.

Hopping (1976) has shown that seed number is proportional to fruit size in kiwifruits. He found that kiwifruits containing 1000 seeds weighed about 90 gm while those containing 1300 seeds weighed up to 150 gm. The results from Experiment No.30 show that the fruit weight was positively correlated with seed numbers per fruit in fruits from both bee pollinated and bee plus hand pollinated flowers in Hayward vines. But the results from Experiment No.29 show that increased seed number was not reflected in an increase in fruit weight. The reason for such a response from the vines may be:

(a) vines under Experiment No.29 were not as sheltered and as vigorous as those under Experiment No.30;

(b) vines under Experiment No.29 were young with first crop.

Hopping (1980) also found that in some treatments where supplemental pollination increased seed number per fruit it did not increase fruit weight as much as would have been expected from such an increase. Thus it appears that the number of seeds is not the only factor that influences weight of the fruits.

CHAPTER EIGHT : *FINAL DISCUSSION:*

In most fruits, pollination is a prerequisite to fertilization and fruit set. This is also true with apples, peaches and kiwifruits. A survey of literature indicated that therapeutants and temperature may influence pollination and fruit set in fruit trees. Knowledge of the effects of therapeutant sprays and of temperature during bloom period would, therefore, be valuable to orchardists.

A. *Therapeutant Effects:*

So far the effects that many therapeutants used during the bloom period in New Zealand orchards have on the pollination of fruit trees have not been examined. The therapeutants that may currently be applied during bloom in New Zealand orchards are

(a)	in apple	- bayleton, bupirimate, dichlofluanid, mancozeb, mancozeb plus dinocap, triforine and a wetting agent citowett^R;
(b)	in peaches	- benomyl, captafol, dichlofluanid, iprodione, mancozeb, streptomycin, triforine and vinclozoline; and
(c)	in kiwifruits	- dichlofluanid, captan and vinclozoline.

This study involved the assessment of

(i) the effect of these therapeutants on pollen germination in vitro

(ii) the effect of some of these therapeutants (bupirimate in apples; triforine in peaches; and dichlofluanid, vinclozoline and captan in kiwifruits) on pollen germination and pollen tube growth in vivo; and

(iii) the effect of these therapeutants on initial and final fruit set on the trees.

The result of *in vitro* studies showed that therapeutants varied in their inhibitory effect on pollen germination. In apples mancozeb plus dinocap was the most effective inhibitor of pollen germination in vitro whereas mancozeb was the least effective inhibitor. While dinocap alone was not studied for its effects on pollen germination, it appears it may be a particularly effective inhibitor. Triforine and dichlofluanid were the most effective inhibitors and benomyl and vinclozoline were the least effective inhibitors of peach and kiwifruit pollen germination *in vitro* respectively. From the point of view of their effects on pollen germination *in vitro*, therapeutants can be listed in the order of decreasing inhibitory effect as follows:

(a) for apples - mancozeb plus dinocap, triforine, dichlofluanid, bupirimate, bayleton, citowett^{R}, captan and mancozeb;

(b) for peaches - triforine, vinclozoline, streptomycin plus triforine, mancozeb, captafol, iprodione, dichlofluanid, streptomycin and benomyl; and

(c) for kiwifruits - dichlofluanid, captan and vinclozoline.

It appeared that dichlofluanid was equally toxic to apple, peach and kiwifruits pollen; triforine was equally toxic to apple and peach pollen, vinclozoline was equally toxic to peach and to kiwifruit pollen and captan was slightly more toxic (1.2 times) to kiwifruit pollen than to apple pollen. However, while such trends were observed the experimental design does not allow direct comparisons and further comparisons would seem desirable. Direct comparison would be more helpful in selecting therapeutants least toxic to pollen.

More pollen grains burst their walls at high therapeutant concentrations than at low concentrations. Though the mode of action of the therapeutants was not examined it appears that substances in the therapeutants may have adverse effects on the pollen's metabolic activity or on the pollen's physical characteristics such as its wall elasticity and membrane permeability.

While *in vitro* therapeutants are very inhibitory the application of a spray to anthers does not affect the germination/viability of pollen subsequently released (2 hours after spraying) by those anthers. Triforine and triforine plus streptomycin at 100 ppm completely inhibited peach pollen germination *in vitro* but 200 ppm triforine and 200 ppm triforine plus 100 ppm streptomycin did not affect peach pollen germination *in vitro* when sprayed on to anthers from Red Haven (peach) flowers. A 1000 ppm dichlofluanid spray on Matua (kiwifruit) anthers did not affect the germinability of pollen released 2 hours after spraying, but 100 ppm dichlofluanid caused a complete inhibition of pollen germination *in vitro*. Bristo (1978) (as cited in New Zealand Ispray Ltd Report (1979)) found that blueberry pollen germination was completely arrested when placed on agar medium containing a concentration of 50 ppm triforine but if flowering blueberry plants were sprayed with triforine and the pollen collected from treated blossom placed on a PDA medium triforine did not exhibit an inhibitory effect until the dosage was increased up to 2000 ppm. It is obvious that growing pollen collected from fungicide sprayed anthers resembles the actual field situation. Therefore such tests with other fungicides, namely mancozeb plus dinocap, triforine, dichlofluanid, and bupirimate effects on apple pollen, vinclozoline, captafol, dichlofluanid and ethephon effects on peach pollen and vinclozoline and captan effects on kiwifruit pollen would be desirable.

In *in vitro* studies triforine at 100 ppm completely inhibited peach pollen germination but *in vivo* (spraying on the styles) triforine at 200 ppm caused no effect. In *in vitro* dichlofluanid and captan at 100 ppm completely inhibited kiwifruit pollen germination whereas *in vivo* (spraying on the styles) at 1000 ppm they had no effect.

In *in vitro* at 100 ppm bupirimate in apples, triforine in peaches, and dichlofluanid, vinclozoline and captan in kiwifruits caused more than 80% reduction in pollen tube length in 20 hours incubation, whereas *in vivo* at concentrations usually applied in the field bupirimate (100 ppm), vinclozoline (250 ppm) and captan (1000 ppm) caused no reduction; triforine (200 ppm) caused 19% reduction and dichlofluanid (1000 ppm) caused 40% reduction in the length of pollen tubes. Thus it appears that fungicides proven to be toxic *in vitro* may not necessarily produce similarly toxic effects *in vivo*.

The reason for such varying effects of fungicides on pollen germination and pollen tube growth *in vitro* and *in vivo* may be that

(a) in *in vitro* tests the therapeutant is spread uniformly throughout the growing medium and thus is in total contact with pollen tube grains whereas in *in vivo* tests therapuetants form only a thin layer on the stigma and are in limited contact with the pollen grains and tubes;

(b) stigmatic fluid may be a better medium producing very rapid germination and therefore provides less time for pollen to be affected by fungicides;

(c) the stigmatic surfaces may contain substances that nullify the toxic effect of the fungicides;

(d) pouring the fungicide solution into the hot (autoclaved) agar plus sucrose media may have resulted in the production of substances toxic to pollen; and

(e) under *in vivo* condition the substances in the therapeutants may be affected by the air and light and so their toxic effects may not persist as long as under *in vitro* conditions.

However, the *in vitro* germination of pollen collected 2 hours after spraying with triforine and dichlofluanid was not different from the germination *in vivo* of pollen in triforine or dichlofluanid sprayed styles.

Callose was observed in Hayward styles under 100 ppm dichlofluanid spray treatment, and in Hayward styles under 14°C treatment, whereas it was absent in styles under other fungicide and temperature treatments. Working with avocado, Sedgley (1977b) suggested that the presence of callose in the pistil of flowers may reduce the availability to the pollen of substances necessary for germination and tube growth and contribute to the female sterility of the male floral stage. Therefore, it is possible that the presence of callose substances may be one of the reasons for less pollen tubes growing down the base of the styles sprayed with dichlofluanid and of the styles at 14°C.

Sedgley (1976) has suggested two possible mechanisms for control of pollen tubes in the styles as follows:

(a) the availability of nutrients for tube growth; and

(b) the production of an inhibitor by the successful tubes.

Thus although their modes of action were not examined it is likely that triforine sprays in peaches, and dichlofluanid sprays in kiwifruits are either directly inhibitory, or influence the availability of nutrients for pollen tube growth in the styles.

At 24 hours after pollination, the number of pollen tubes growing more than 1/2 the length of styles of Hayward (kiwifruit) flowers one to two days old was 38% and 27% higher than in flowers less than one day old and more than three days old respectively. While the substances present in the styles from different age groups were not analysed it is possible that styles from flowers one day to two days old may contain more nutrients necessary for pollen tube growth than the styles of older or younger flowers.

In apples 5 sprays of either bayleton or captan, 3 sprays of either of the therapeutants mancozeb, mancozeb plus dinocap and triforine and a wetting agent citowett^R applied during the bloom period had no effect on fruit set 5 weeks after full bloom in Golden Delicious trees. Similarly 3 bupirimate sprays during the bloom period had no effect on fruit set 5 weeks after full bloom in Splendour trees. But Lawes (1980) has noted that bupirimate sprays are claimed to have reduced fruit set in Red Dougherty trees in New Zealand. However, while the number of bupirimate sprays applied to the Red Dougherty trees was not known it appears possible that Splendour and Red Dougherty trees differ in their response to bupirimate sprays during the bloom period. The effects of other therapeutants on the fruit set of apple trees have not been reported by any other workers. Because results of the present study were obtained with more sprays than growers would normally use it appears that mancozeb- mancozeb plus dinocap, triforine, bayleton, captan and citowett^R applied individually do not influence fruit set in apple trees.

In peaches 6 sprays of either captafol or benomyl or iprodione, 5 sprays of either mancozeb or vinclozoline or streptomycin or triforine during bloom period caused no effect on fruit set 5 weeks after full bloom and at harvest in Golden Queen trees. Three sprays of streptomycin or dichlofluanid caused no effect, but 3 sprays of triforine, 3 sprays of triforine plus streptomycin, caused 50% and 70% reduction in the fruit set 4 weeks after full bloom and at harvest in Red Haven trees. Thus although the experimental designs do not allow the direct comparison of the observations made in Red Haven trees with that in Golden Queen trees it appears that Golden Queen and Red Haven trees differ in their response to spray application of triforine during the bloom period. While these results were obtained with more sprays than growers would normally use it would seem wise to use triforine and triforine plus streptomycin sprays cautiously during the bloom period in Red Haven trees.

So far the evidence on the effects of triforine and triforine plus streptomycin on fruit set in peaches is conflicting. Since 1977, three reports have been received where poor fruit set in cherries and peaches occurred following the application of triforine over the blossom period in New Zealand orchards (Anonymous (1978)). Ivon Watkin Dow Ltd's report indicates that in cherries, triforine did not reduce fruit set but the combination of triforine and agrimycin did (New Zealand Agriculture Chemicals Board (1979)). Unfortunately, all but one report show the name of peach varieties on which effect of triforine and/or triforine plus streptomycin was studied. Ispray Ltd's Report (1979) shows that triforine and triforine plus streptomycin spray during bloom period did not cause reduction in fruit set in Golden Queen peach trees.

Ethephon sprays during the post blossom period cause the release of ethylene which stimulates the abscission of fruits and thus affects fruit set (Westwood (1979)). So far the effects of ethephon sprays during bloom period on fruit set in peaches have not been reported by any workers. The results of field studies show that 3 ethephon sprays during bloom period caused 90% reduction in fruit set 5 weeks after full bloom and at harvest. Thus it appears that ethephon sprays during bloom should be used cautiously in peach trees. It is also possible that ethephon sprays when used at less than 100 ppm concentration or when used less than 3 times during bloom period may become a means of chemical thinning in peaches.

In kiwifruits dichlofluanid sprays, captan sprays and vinclozoline sprays reduced seed numbers per fruit by 37% but did not affect fruit weight at harvest and fruit set 5 weeks after full bloom in Hayward vines. Thus it appears that these fungicides may safely be applied during bloom period in kiwifruit vines. However, it would be prudent to go carefully on such spraying until further experience shows the reduced seed number is not detrimental to fruit size and fruit set.

Many therapeutants that inhibited pollen germination *in vitro* and/or inhibited pollen germination and/or pollen tube growth *in vivo* did not reduce fruit set at 5 weeks after full bloom in apple and peach trees and in kiwifruit vines. The list of such fungicides for:

(a) apple, includes - bayleton, bupirimate, captan, citowett^{R} dichlofluanid, mancozeb, mancozeb plus dinocap and triforine;

(b) peach, includes - benomyl, captafol, iprodione, mancozeb, streptomycin, triforine, and vinclozoline in Golden Queen and streptomycin and dichlofluanid in Red Haven; and

(c) kiwifruit, includes - captan, dichlofluanid and vinclozoline.

Although the number of pollen tubes reaching the ovary were not examined it is possible that these fungicides either:

(a) did not influence fertilization or

(b) influenced fertilization

in so few ovules that it did not affect fruit set. In apples although fertilization is essential for fruit development it is not necessary for all ten ovules to develop, and fruits may be produced with only a few seeds (Anonymous (1969)). In peaches only one pollen tube grows through the micropyle and effects fertilization. In kiwifruits three pollen tubes/ovule is sufficient to ensure full pollination and maximum seed development (Hopping (1978)). Thus it appears that the inhibition of pollen germination and/or pollen tube growth caused by bupirimate sprays in apple (Splendour) styles, triforine in peach (Golden Queen) styles and dichlofluanid in kiwifruit (Hayward) styles was not strong enough to affect the percentage fruit set. However, it is possible that these therapeutants may cause adverse effect on fruit set in orchards with poor pollen supply and under adverse weather conditions.

Triforine sprays caused a 50% reduction and triforine plus streptomycin sprays caused a 70% reduction in fruit set 4 weeks after full bloom in Red Haven peach trees. Because

(a) triforine sprays reduced the number of pollen tubes reaching 1/2 to 3/4 style length by only 19%; and

(b) only one pollen tube is required to grow through the micropyle and effect fertilization and fruit set

it appears that the reduction in fruit set may be not only due to inhibitory effect of triforine on pollen tube growth, but also may be due to an adverse effect of triforine on ovary or on fertilization.

Thus it can be concluded that *in vitro* and *in vivo* studies were useful in determining effects of therapeutants on pollen germination and pollen tube growth but did not necessarily provide information on fruit set in apple and peach trees and on fruit set, fruit weight and seed numbers per fruit in kiwifruit vines.

B. *Temperature Effects:*

It appears that the effects of temperature on apple and peach pollen germination and pollen tube growth *in vitro* and *in vivo* have not been reported by any other workers. The results showed that the level of germination of both apple and peach pollen was higher at 24°C than at 16°C, 20°C, 28°C and 32°C. The reason for the lower germination percentage at lower temperatures may be that at lower temperatures metabolic activities of the pollen grains may either be reduced or be stopped. While the mechanism of the bursting of the pollen grains was not examined it was observed that the walls of the pollen grains burst at higher temperatures

(28°C and 32°C) and thus affect pollen germination. However after 18 hours incubation pollen tubes were longer at 28°C and at 32°C than at 16°C, 20°C and 24°C.

In vivo studies showed that the levels of apple and peach pollen germination at 14°C, 18°C, 22°C and 26°C were not significantly different from each other. But temperatures both higher and lower than the temperature range 18°C to 22°C were found to be inhibitory to pollen tube growth in Hayward (kiwifruit) styles. Thus a temperature range of 18 to 22°C appears to be optimum for kiwifruit pollen germination and pollen tube growth. So far the effects of temperature on pollen germination and pollen tube growth in kiwifruit styles have not been studied by any other workers.

It is reported that slow pollen tube growth at low temperatures is an important factor in limiting fruit set in almond trees (Lawes (1980)). Westwood (1978) has noted that the slow growth of tubes at low temperatures is thought to be the principal reason for low set of fruits in nut trees. Thompson and Liu (1973) concluded that in prunes cool temperatures (10°C) delayed pollen tube growth and early embryo and endosperm development so long that the ovule began to degenerate and reduced fruit set. However, while the effect of temperature on fruit set was not examined in this present study it appears that provisions for orchard heating may be important in kiwifruit orchards where the spring temperature falls as low as 14°C. In cool areas within gullies and by tall shelter trees the temperature effect may account for effects on fruit size in kiwifruit vines.

C. *Hand Pollination Effects:*

Hand pollination of flowers did not influence fruit set but increased fruit weight and seed numbers per fruit in Hayward kiwifruit vines. Hopping (1979) also observed that hand pollination increased both fruit weight and seed numbers per fruit in fruits from Hayward vines.

It was observed that fruit weight was positively correlated with seed number per fruit in fruits from both bee pollinated ($r = + 0.84$) and bee plus hand pollinated ($r = + 0.78$) vines.

Hopping (1976) also showed that Hayward fruits containing 1000 seeds weighed about 90 gm while those containing 1300 seeds weighed up to 150 gm. Thus it appears that supplementary pollination plays an important role in the production of kiwifruits in the orchards.

CHAPTER NINE : *CONCLUSION:*

Therapeutants varied in their inhibitory effects on apple, peach and kiwifruit pollen germination *in vitro*. Mancozeb plus dinocap, triforine and dichlofluanid were the most effective inhibitors and mancozeb, benomyl and vinclozoline were the least effective inhibitors of apple, peach and kiwifruit pollen germination *in vitro* respectively. From the point of view of their effects on pollen germination *in vitro* therapeutants can be listed in the order of decreasing inhibitory effect as follows:

(a) for apples - mancozeb plus dinocap, triforine, dichlofluanid, bupirimate, bayleton, citowett^{R}, captan and mancozeb;

(b) for peaches - triforine, vinclozoline, streptomycin plus triforine, mancozeb, captafol, iprodione, dichlofluanid, streptomycin and benomyl; and

(c) for kiwifruits - dichlofluanid, captan and vinclozoline.

It appeared that dichlofluanid was equally toxic to apple, peach and kiwifruit pollen; triforine was equally toxic to apple and peach pollen, vinclozoline was equally toxic to peach and to kiwifruit pollen and captan was slightly more toxic (1.2 times) to kiwifruit pollen than to apple pollen.

More pollen grains burst their walls at high therapeutant concentrations than at low concentrations. Though the mode of action of the fungicides was not examined it appears that the substances in the therapeutants may have adverse effects on the pollen's physical condition such as its wall elasticity, and/or on the membrane permeability of the pollen grains.

Triforine and triforine plus streptomycin at 100 ppm completely inhibited peach pollen germination *in vitro* but 200 ppm triforine and 200 ppm triforine plus 100 ppm streptomycin did not affect peach pollen germination *in vitro* when sprayed on to anthers from Red Haven (peach) flowers. A 1000 ppm dichlofluanid spray on Matua (kiwifruit) anthers did not affect the germinability of pollen released 2 hours after spraying but 100 ppm dichlofluanid caused complete inhibition of pollen germination *in vitro*. Thus it appears that while *in vitro* the fungicide is very inhibitory, the application of a spray to anthers does not affect the germination/viability of pollen subsequently released by those anthers.

In *in vitro* studies triforine at 100 ppm completely inhibited germination but at 200 ppm triforine had no effect on peach pollen germination *in vivo*. In kiwifruit dichlofluanid and captan at 100 ppm completely inhibited pollen germination *in vitro*, whereas *in vivo* they caused no effect. In *in vitro* bupirimate in apples, triforine in peaches, and dichlofluanid, vinclozoline and captan in kiwifruits at 100 ppm caused more than 80% reduction in pollen tube length whereas *in vivo* bupirimate, vinclozoline, and captan caused no reduction, triforine caused 19% reduction and dichlofluanid caused 40% reduction in the lengths of pollen tubes. Thus it appears that fungicides proven to be toxic to pollen *in vitro* may not necessarily produce toxic effects *in vivo*.

The germination of both apple and peach pollen was higher at 24°C than at 16°C, 20°C, 28°C and at 32°C. After 18 hours incubation pollen tubes were longer at 28°C and at 32°C than at 16°C, 20°C and 24°C. While the mechanism of the bursting of pollen grains was not examined it appeared that walls of pollen grains burst at higher temperatures (28°C and 32°C) and thus affect pollen germination. *In vivo* studies with Matua (kiwifruit) pollen showed that pollen germination at 14°C, 18°C, 22°C and 26°C were not significantly different from each other. But the temperatures both higher and lower than the temperature range 18 to 22°C were found to be inhibitory to pollen tube growth in Hayward styles. It appeared that provisions for orchard heating may be important in kiwifruit orchards where spring temperatures fall as low as 14°C.

At 24 hours after pollination, the number of pollen tubes growing more than 1/2 the length of styles of flowers one to two days old was 38% and 27% higher than in flowers less than one day old and more than three days old respectively. However, while the substances present in the styles from different age groups were not analysed it is possible that styles from flowers one day to two days old may contain more nutrients necessary for pollen tube growth than the styles of older or younger flowers.

In apples 5 sprays of either bayleton or captan, 3 sprays of either of the therapeutants mancozeb, mancozeb plus dinocap, and triforine, and a wetting agent citowett^R applied during bloom period caused no effect on the fruit set at 5 weeks after full bloom and at harvest in Golden Delicious trees; and 3 bupirimate sprays during bloom period had no effect on the fruit set at 5 weeks after full bloom and at harvest in Splendour trees.

In peaches 6 sprays of either captafol, or benomyl or iprodione, 5 sprays of either mancozeb, or vinclozoline or streptomycin or triforine during bloom period caused no effect on fruit set at 5 weeks after full bloom and at harvest in Golden Queen trees. Three sprays of streptomycin or dichlofluanid caused no effect, but 3 sprays of triforine and 3 sprays of triforine plus streptomycin caused 50% and 70% reduction in the fruit set at 4 weeks after full bloom and at harvest in Red Haven trees. Thus although the experimental design does not allow the direct comparison of the observations made in Red Haven trees with that in Golden Queen trees it appeared that Golden Queen and Red Haven trees differed in their response to spring application of triforine during bloom period. Triforine, triforine plus streptomycin and ethephon sprays during bloom period should be used cautiously in Red Haven trees.

In kiwifruits dichlofluanid sprays, captan sprays and vinclozoline sprays reduced seed numbers per fruit by 37% but did not affect fruit weight at harvest and fruit set 5 weeks after full bloom and at harvest in Hayward vines. Thus it appears that these fungicides may safely be applied during bloom period in kiwifruit vines. However, it would be prudent to go carefully on such spraying until further experience shows the reduced seed numbers is not detrimental.

Many therapeutants that inhibited pollen germination *in vitro* and/or inhibited pollen germination pollen germination and/or pollen tube growth *in vivo* did not reduce fruit set at 5 weeks after full bloom and at harvest in apple and peach trees and in kiwifruit vines. Although the number of pollen tubes reaching the ovary were not examined it is possible these therapeutants either

(a) did not influence fertilization or
(b) influenced fertilization

in so few ovules that it did not affect fruit set.

Thus it can be concluded that *in vitro* and *in vivo* studies were useful in determining effects of therapeutants on pollen germination and pollen tube growth but did not necessarily provide information on fruit set in apple and peach trees and on fruit set, fruit weight and seed numbers per fruit in kiwifruit vines.

Hand pollination of flowers did not influence fruit set but increased fruit weight and seed numbers per fruit in Hayward vines. Because fruit weight was positively correlated with seed number per fruit in fruits from both bee pollinated and bee plus hand pollinated flower clusters, supplementary pollination may play an important role in the production of kiwifruits in the orchards.

BIBLIOGRAPHY

Alexander, M.P. (1969) Differential staining of aborted and non aborted pollen. *Stain Technology., 44* : 117-122.

Anonymous (1967) "Pollination of fruit crops" The Horticultural Education Association. Gibbs and Sons, Orange Street, Canterbury, England.

Anonymous (1972) "Apples" Ministry of Agriculture, Fisheries and Food. Bulletin 207. Her Majesty's Stationery, London : 34-39.

Anonymous (1978) Saprol and fruit thinning. *Orchardist of New Zealand.,* June 1978 : 165.

Anonymous (1979) "Kiwifruit - A strange phenomenon" Martin Hill Design Institute Press. Auckland Printing. New Zealand.

Atkins, E.L.; R.L. MacDonald; T.P. MacGovern; M. Beroza; and E.A. Greywood Hale (1975) Repellent additives to reduce pesticide hazards to honey bees. Laboratory testing. *Journal of Apiculture Research., 14* : 85-97.

Atwood, C.E. (1933) Studies on the *Apoidea* of Western Nova Scotia with special reference to visitors to apple bloom. *Canadian Journal Research., 9* : 443-457.

Austin, M. (1974) Routine foliar feeding has a part to play in plum nutrition. *Commercial Grower No.4089* : 858-860. (Abstract in Horticultural Abstract *45* : 2191).

Batjer, L.P. (1963) Effects of pruning, nitrogen and scoring on growth and bearing characteristics of Valencia orange trees. *Journal of Horticultural Science., 50* : 219-226.

Batjer, L.P. and A. Thompson (1949) Effect of boric acid sprays applied during bloom upon the set of pear fruits. *Proceedings of American Society for Horticultural Science., 53* : 141-142.

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

Brown, A.G. and J.N. Moore (1975) "Advances in fruit breeding" Purdue University Press. West Lafayatte. Indiana., : 5-6.

Brown, A.W.A. (1978) "Ecology of pesticides" A Wiley-Interscience Publication. John Wiley and Sons. New York. Chichester. Brisbane. Toronto.

Bukovac, M.J. (1963) Induction of parthenocarpic growth of apple fruits with gibberellins A_3 and A_4. *Botanical Gazette., 124* : 191-195.

Bulatovic, S. and B. Konstantinovic (1960) The role of bees in the pollination of the more important kinds of fruits in Siberia. *Proceedings of the first International Symposium on pollination held in Copenhagen. Swedish Seed Grower's Association., Communication No.7* : 167-172.

Burchill, R.T. (1963) Airborne pollen in apple orchards. *Annual Report East Malling Research Station for 1962* : 109-111.

Butler, C.G. (1943) Work on bee repellents. Management of colonies for pollination. *Annals of Applied Biology., 30* : 195-196.

Chandler, W.H. (1958) "Evergreen Orchards" 2nd Edition. Henry Kimpton, London : 533.

Chandler, W.H. (1965) "Deciduous Orchards" Third Edition. Lea and Feligar, Philadelphia.

Chanturiya, I.A. (1974) The effect of minor nutrients on the percentage fruit set in citrus crops. *Subtropicheskie Kul'try No.2* : 48-50. (Abstract in Horticultural Abstract *45* : 7080).

Chaudhary, M. and C.A. Johansen (1971) Management practices affecting efficiency of the honey bee *Apis mellifera. Melanderia., 6* : 1-31.

Child, R.V. (1967) Pollination studies in fruit trees. V Pollen tube growth in relation to temperature and ovule longevity in the cider apple, Michalin. *Annual Report Long Ashton Research Station 1966* : 115-120.

Childers, N.F. (1976) "Modern Fruit Science" Seventh Edition. Horticultural Publications. Rutgers University, New Jersey.

Church, R. and R.R. Williams (1977a) Pollination of pome fruits : the toxicity of fungicides to apple pollen. *Annual Report Long Ashton Research Station 1976* : 23.

Church, R.M. and R.R. Williams (1977b) The toxicity to apple pollen of several fungicides as demonstrated by in vivo and in vitro techniques. *Journal of Horticultural Science., 52* : 429-436.

Church, R.M. and R.R. Williams (1978) Fungicide toxicity to apple pollen in the anther. *Journal of Horticultural Science., 53* : 91-94.

Church, R.: R.R. Williams, N.G. Morgan, and B.K. Cooke (1978) Toxicity of fungicides to apple pollen in the orchard. *Annual Report Long Ashton Research Station 1977* : 22-23.

Cockerham, L.E. and G.J. Galletta (1976) A survey of pollen characteristics in certain *Vaccinium* species. *Journal of American Society for Horticultural Science., 101* : 671-676.

Coombe, B.G. (1962) The effect of removing leaves, flowers and shoot tips on fruit set of *Vitis venifera.* L. *Journal of Horticultural Science., 37* : 1-15.

Coombe, B.G. (1973) The regulation of set and development of the grape berry. *Acta Horticulturae. No.34* : 261-274.

Cooper, J.R. (1938) Factors influencing fertilization of apple blossoms and setting of fruit. *Proceedings of American Society for Horticultural Science., 35* : 27-35.

Cramer, H.H. (1967) Plant protection and world crop production. *Pflanzenschutz Nachrichten., Bayer., 20* : 3-524.

Cresti, M. and J.L. van Went (1976) Callose deposition and plug formation in petunia pollen tubes in situ. *Planta., 133* : 35-40.

Currier, H.B. (1957) Callose substance in plant cells. *American Journal of Botany., 44* : 477-487.

Dayton, D.F. (1974) Overcoming self incompatibility in apple with killed compatible pollen. *Proceedings of American Society for Horticultural Science., 99* : 190-192.

Dennis, F.G. Jr (1967) The role of seeds in apple fruit development. *Farm Research., 33* : 12-13.

Dennis, F.G. Jr (1973) Physiological control of fruit set and development with growth regulators. *Acta Horticulturae No.34* : 251-257.

Dorsay, N.J. (1930) The relation between embryo sac development and the set of fruits in the apple. *Proceedings of American Society for Horticultural Science., 26* : 56-61.

Dragnov, D. and G. Dragnov (1975) The effect of materiological conditions on grapevine in flowering and fertilization. *Grandinauska i Lozarska Nanka., 12* (3) : 74-83. (Abstract in Horticultural Abstracts *46* : 3159).

Eaton, G.W. (1961) Germination of Sweet Cherry (*Prunus avium.* L.) pollen in vitro as influenced by fungicides. *Canadian Journal of Plant Science., 41* : 740-743.

Eaton, G.W. (1963) Germination of apple pollen as influenced by captan sprays. *Proceedings of American Society for Horticultural Science., 83* : 101-106.

Eaton, G.W. and L.I. Chen (1969) The effect of captan on strawberry pollen germination. *Journal of American Society for Horticultural Science., 94* : 558-560.

Edgerton, L.J. (1973) Chemical thinning of apples. In T.T. Kozlowski (Ed) shedding of plant parts. Academic Press, New York : 435-474.

EL-Zeftawi, B.M. and I.R. Thornton (1975) Effects of rootstocks and fruit stripping on alternate bearing of Valencia orange trees. *Journal of Horticultural Science., 50* : 219-226.

Faegri, K. and L. van der Pijl (1966) "The principles of pollination ecology" 1st Edition. Pergamon Press Ltd : pp13 and 25.

Field, C.P. (1942) Low temperature injury to fruit blossom. II A comparison of the relative susceptibility and effect of environmental factors on three commercial apple varieties. *Annual Report East Malling Research Station 1941* : 29-35.

Forshey, C.G. (1953) Blossom structure and its relation to fruit set in Delicious and several other commercially important varieties. *Proceedings of American Society for Horticultural Science., 62* : 154-158.

Fox-Wilson, G. (1933) Contributions from the Wisley laboratory. LXV Pollination in orchards. VIII Insect visitors to fruit blossom. *Journal Royal Horticultural Society., 58* : 125-138.

Frankel, R. and E. Galun (1977) "Pollination mechanisms, reproduction and plant breeding" Monographs on theoretical and applied genetics 2. Springer - Verlag Berlin. Heidelberg. New York.

Free, J.B. (1962) Effect of distance from pollinizer on trees in plum and apple orchards. *Journal of Horticultural Science., 37* : 262-271.

Free, J.B. (1964) Studies on the honey bee pollination of fruit flowers. *Proceedings of the Second International Symposium on pollination held in London 1964.* London Bee Research Association : 95-96.

Free, J.B. (1966) The pollinating efficiency of honey bee visits to apple flowers. *Journal of Horticultural Science., 41* : 91-94.

Free, J.B. (1970) "Insect pollination of Crops" Academic Press. London and New York.

Free, J.B. and Y. Spencer Booth (1964) The foraging behaviour of honey bees in an orchard of dwarf apple trees. *Journal of Horticultural Science., 39* : 78-83.

Free, J.B.; P.H. Needham; P.A. Racey; and J.H. Stevenson (1967) The effect on honey bee mortality of applying insecticides as sprays or granules to flowering field beans. *Journal of Science Food and Agriculture., 18* : 133-138.

Gardener, V.R.; F.C. Bradford; and H.D. Hooker (1952) "The fundamentals of fruit production" McGraw Hill Book Company Inc.

Gigilov, B.K.H. (1976) The viability of almond pollen. *Sub Tropicheskie Kul'tury No.3/4* : 125-128. (Abstract in Horticultural Abstracts *45* : 9067).

Green, M.B. (1976) "Pesticides boon or bain" Westview Press, Colorado, U.S.A.

Greenham, D.W.P. and G.C. White (1959) The control of magnesium defficiency in dwarf pyramid apples. *Journal of Horticultural Science., 34* : 238-247.

Griggs, W.H. (1953) Pollination requirements of fruits and nuts. *California Agriculture Experiment Station Extension Service Circular 424.*

Griggs, W.H. (1953) The storage of hand collected and bee collected pollen in a home freezer. *Proceedings of American Society for Horticultural Science., 62* : 304-305.

Gustafson, F.G. (1961) Development of fruits. *Encyclopaedia Plant Physiology., 14* : 951-956.

Hilgeman, R.H.; J.A. Dunlap and G.C. Sharples (1967) Effect of time of harvest of Valencia oranges on leaf carbohydrate content and subsequent set of fruit. *Proceedings of American Society for Horticultural Science., 90* : 110-116.

Hill-Cottingham, D.G. and R.R. Williams (1967) Effect of time of application of fertilizer nitrogen on the growth, flower development and fruit set of maiden apple trees Var Lord Lambourne and on the distribution of total nitrogen within the trees. *Journal of Horticultural Science., 42* : 319-338.

Hirano, R.T. and H.Y. Nakasone (1969) Pollen germination and compatibility studies of some *Psidium species*. *Journal of American Society for Horticultural Science., 94* : 287-289.

Hootman, H.D. and G.H. Cale (1930) A practical consideration of fruit pollination. *American Bee Journal Bulletin 579.*

Hopping, M.E. (1976) Effect of exogenous auxins, gibberellins and cytokinins on fruit development in Chinese gooseberry (*Actinidia chinensis* planch). *New Zealand Journal of Botany., 14* : 69-75.

Hopping, M.E. (1978) Supplemental pollination of kiwifruit. Personal communication.

Hopping, M.E. (1979) "Supplemental pollination of fruit crops" Department of Horticulture and Plant Health Seminar. Massey University, New Zealand.

Hopping, M.E. (1980) Personal communication.

Howlett, F.S. (1936) Factors affecting fruit set. I Winesap. *Ohio Agriculture Experiment Station Bulletin 483.*

Jackson, D.I. (1974) "Temperate and sub tropical fruit production" Bulletin 15. Department of Horticulture, Lincoln College, New Zealand.

Janick, J. (1963) "Horticultural Science" W.H. Freeman and Company, San Francisco : 463.

Janick, J.; R.W. Schery and V.W. Rutton (1974) "Plant Science. An introduction to world crops" W.H. Freeman and Company, San Francisco.

Johansen, C.A. (1972) Toxicity of field weathered insecticide residues to four kinds of bees. *Environmental Entomology., 1* : 393-394.

Johansen, C.A. ( ) Spray additives for insecticidal selectivity to j v beneficial insects. *Environmental Er* : 51-54.

Johanser esticides and pollinators. *Annual Review* ., *22* : 177-192.

J) Honey bee poisoning by chemicals; signs, ting factors, current problems and prevention. *rld., 60* : 109-125.

.A.; M.G. Kleinschmidt; L.I. Butler; L.M. McDonough and D.S. Jackson (1974) "Adjuvants decrease insecticide hazard to honey bees" Bulletin 501. College of Agriculture Research Centre, Washington State University, U.S.A.

Jones, W.W.; T.W. Embleton; E.L. Barnhart and C.B. Cree (1974) Effect of time and amount of fruit thinning on leaf carbohydrates and fruit set in Valencia oranges *Hilgardia 42* : 441-449. California Agriculture Experiment Station, Riverside, California, U.S.A. (Abstract in Horticultural Abstract *45* : 7830).

Kendall, D.A. and M.E. Soloman (1970) Report Zoology Section. *Annual Report Long Ashton Research Station for 1969.*

Kester, D.E. and W.H. Griggs (1959) Fruit setting in the almond. The effect of cross pollinating various percentages of flowers. *Proceedings of American Society for Horticultural Science., 74* : 206.

Kester, D.E. and M.V. Bradley (1976) Effects of temperature and genotype on pollen tube growth in some self incompatible and self compatible almond cultivars. *Journal of American Society for Horticultural Science., 101* : 490-493.

Kho, Y.O. and J. Baer (1968) Observing pollen tubes by means of fluorescence. *Euphytica., 17* : 298-302.

King, J.R. and C.O. Hesse (1938) Pollen longevity studies with deciduous fruits. *Proceedings of American Society for Horticultural Science., 36* : 310-319.

Kolbe, W. (1975) Effect of different crop protection programmes on yield and quality of apple varieties. *Pflanzenschutz Nachrichten., Bayer, 28* : 232-241.

Kolbe, W. and F.W. Cramer (1969) Development of chemical crop protection in fruit farming. *Pflanzenschutz Nachrichten., Bayer., 22* : 104-156.

Krezdorn, A.H. (1960) The influencing of girdling on the fruiting of Orlando tangelos and Navel oranges. *Proceedings of Florida State Horticultural Society., 73* : 49-52.

Krezdorn, A.H. (1973) Effect of growth regulators on set and development of citrus fruits. *Acta Horticulturae No.34* : 283-285.

Krinko, N.P. (1970) Malt as a constituent of artificial media for germinating pollen. *Sel.hoz.Biol. 5* : 781-782. (Abstract in Horticultural Abstract *41* : 5533).

Kwack, B.H. (1964) On the role of calcium and other cations in pollen germination and growth. *Botanical Magazine. Tokyo; 77* : 327-332.

Kwack, B.H. (1965) The effect of calcium on pollen germination. *Proceedings of American Society for Horticultural Science., 86* : 818-823.

Kwan, S.C.; A.R. Hamson and W.F. Campbell (1969) The effects of different chemicals on pollen germination and tube growth in *Allium cepa* L. *Journal of American Society for Horticultural Science., 94* : 561-562.

Langdridge, D.F. (1975) Pollination of fruit crops. *Victorian Horticulture Digest No.66* : 14-16.

Lange DE, J.H. (1973) Pollen tube growth in citrus. *Agroplantae 5* (3) : 737. (Abstract in Horticultural Abstract *44* : 10043).

Lawes, G.S. (1978) Kiwifruit pollen germination tests 1977/78. Massey University, New Zealand.

Lawes, G.S. (1980) Personal communication.

Layne, R.E.C. and H.A. Quamme (1975) "Apples" Advances in fruit breeding. Purdue University Press, West Lafayette, Indiana : 44.

Lenz, F. (1966) Flower and fruit development in Valencia late orange as affected by type of influorescence and nutritional status. *Horticulture Research., 6* : 65-78.

Leopold, A.C. and F.I. Scott (1952) Physiological factors in tomato fruit set. *American Journal of Botany., 39* : 310-317.

Leopold, A.C. and P.E. Kriedemann (1975) "Plant growth and development" Second Edition. McGraw Hill Book Company.

Linskens, H.F. (1964) Pollen physiology. *Annual Review of Plant Physiology., 15* : 255-270.

Lovelidge, B. (1976) Spraying's effect on pollination. *New Phytologist., 77* : 149-152.

Luckwill, L.C. (1976) Growth regulator in top fruit. *Outlook on Agriculture., 9* : 46-68.

Ludders, P. and G. Bunnemann (1975) The influence of seasonal differences in potassium availability on the vegetative and reproductive growth of apple trees. *Erwerbso 17* : 83-87. (Abstract in Horticultural Abstract *46* : 1976).

MacDaniels, L.H. and A.J. Heinicke (1929) "Pollination and other factors affecting the set of fruit with special reference to apple" Bulletin 497. Cornell University Agriculture Experiment Station, U.S.A.

MacKenzie, D.J. (1979) "Growers Guide" Bulletin. New Zealand Fruit Growers Federation Ltd, New Zealand.

Marks, G.E. (1954) An acetocarmine glycerol jelly for use in pollen fertility counts. *Stain Technology., 29* : 277.

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

Massee, A.M. (1937) Notes on some interesting mites observed on fruit trees in 1936. *Annual Report East Malling Research Station for 1936* : 222-228.

Medlar, J.T. (1966) Factor limiting the increase of bumble bees. *Proceedings of the Second International Symposium on pollination held in London 1964.* London Bee Research Association : 141-143.

Mellenthein, W.M.; C.Y. Wang and S.Y. Wang (1972) Influence of temperature on pollen tube growth and initial fruit development in d'Anjou pears. *Horticulture Science., 7* : 557.

Mittler, T.E. (1960) Preliminary studies on apple pollination. *Proceedings of the First International Symposium on pollination held in Copenhagen. Communication No.7; Swedish Grower's Association.*

Mommers, J. (1966) The concentration and composition of nectar in relation to honey bee visits to fruit trees. *Proceedings of the Second International Symposium on pollination held in London 1964.* London Bee Research Association : 141-143.

Mullins, M.G. (1967) Regulation of fruit set in grape vines. *Australian Journal of Biological Sciences., 20* : 1141-1147.

Nebel, B.R. (1939) Longevity of pollen in apple, pear, plum, peach, apricot and sour cherry. *Proceedings of American Society for Horticultural Science., 37* : 130-132.

New Zealand Agriculture Chemicals Board (1979) Unpublished Reports.

New Zealand Ispray Limited (1978) Saprol on stone fruit.

New Zealand Ispray Limited (1979) Unpublished Reports.

New Zealand Statistics Department (1979) New Zealand Official Year Book. 84th Annual Edition. Department of Statistics, Wellington.

Nyeki, J. (1973) The relationship between pollen tube development in the pear cultivars Clapp's Favourite and Williams and the sugar concentration, time of tube development and temperature. *Kiserlatugyi Kozlenmenyek. 64* : 23-31. (Abstract in Horticultural Abstract *44* : 167).

Olmo, H.P. (1942) Storage of grape pollen. *Proceedings of American Society for Horticultural Science., 41* : 219-224.

Olmo, H.P. (1943) Pollination of the Almeria grape. *Proceedings of American Society for Horticultural Science., 42* : 401-406.

Overley, F.L. and R.M. Bullock (1947) Pollen diluents and application of pollen to fruit trees. *Proceedings of American Society for Horticultural Science., 49* : 163-169.

Overley, G.D. and R. Watson (1953) The use of 2,3,5 - triphenyl tetrazolium chloride in viability tests of fruits pollen. *Proceedings of American Society for Horticultural Science., 61* : 299-303.

Palmer, J. and P.G. Clinch (1968) Observations on the pollination of apple trees. III Varieties Granny Smith, Kidd's Orange Red and Golden Delicious. *New Zealand Journal of Agriculture Research*

Palmer-Jones, T. (1965) Toxicity of pesticides to honey bees. *Wallaceville Animal Research Centre Publication No.111*, Department of Agriculture, New Zealand.

Palmer-Jones, T. (1967) Pesticides and New Zealand bee keeping. *New Zealand Science Review. 25* : 23-26.

Papademetriou, H.K. (1976) Some aspects of the flower behaviour pollination and fruit set of avocado in Trinidad. *California Avocado Society Year Book 1976* : 106-152.

Papademetrious, M.K. (1975) A study of the viability of avocado pollen under natural conditions. *California Avocado Society Year Book 1974-75., 58* 74-76.

Percival, M.S. (1947) Pollen collection by *Apis mellifera*. *New Phytologist., 46* : 142-173.

Percival, M.S. (1955) The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. *New Phytologist., 54* : 353-368.

Potapov, S.P.; L.I. Dufora and N.G. Stepchenko (1975) Determination of pear pollen quality. *Izvestiya Timiryazevskoi Selskokhozyaistvennoi Akademii No.3* : 217-221. (Abstract in Horticultural Abstract *46* : 1976).

Powell, D.B.B. (1974) Some effects of water stress in late spring on apple trees. *Journal of Horticultural Science., 49* : 257-272.

Preston, A.P. (1949) An observation on apple blossom morphology in relation to visits from honey bees (*Apis mellifera*). *Annual Report East Malling Research Station for 1968* : 64-67.

Proctor, M. and P. Yeo (1970) "The pollination of flowers" Collins, St James Place, London.

Reece, P.C. and K.O. Register (1961) Influence of pollination on fruit set of Robinson and Osceola tangerines. *Proceedings Florida State Horticultural Society., 74* : 104-106.

Reuther, W.; L.D. Batchelor and H.J. Webber (1968) "The Citrus Industry Vol. II" Publication. University of California, U.S.A.

Ries, S.M. (1975) Studies of apple pollen grain germination as influenced by different fungicides in vitro. *Transaction of the Illinois State Horticultural Society., 108* : 95-100.

Robbins, W.W.; E.T. Weier and R.L. Stocking (1962) "Botany. An introduction to plant science" John Wiley and Sons Inc. New York. London. Second Edition : 34-39.

Roberts, D. (1956) Sugar sprays encourage fertilization by honey bees. *New Zealand Journal of Agriculture., 93* : 206-211.

Roberts, R.H. (1945) Blossom structure and setting of Delicious and other apple varieties. *Proceedings of American Society for Horticultural Science., 146* : 87-90.

Robinson, S.W. (1979) Effect of apple cultivar on foraging behaviour and pollen transfer by honey bees. *Journal of American Society for Horticultural Science., 104* : 596-598.

Rough, B.F.A. (1977) "Thinning of stone fruit" Henry H. York and Co., Ltd., Wellington, New Zealand

Rud, G.Y.A. and V.M. Zhadan (1973) Studies on some problems of the flowering and fruiting biology of walnuts. *Rhferativnyal Zhurnal* 1.55.686. (Abstract in Horticultural Abstract

Sachan, B.P. and K.D. Patro (1970) Studies on pollen storage of some citrus species. *Progressive Horticulture 1* (4) : 13-16. K.A. College of Agriculture, India. (Abstract in Horticultural Abstract *42* : 230).

Sarvello, P. (1964) Vital stain testing of pollen viability in cotton. *Journal of Heredity., 55* : 154-157.

Sastri, K.K.S. and R.M. Muir (1963) Gibberellins : effects on diffusible auxin in fruit development. *Science., 140* : 494-495.

Schroeder, C.A. and W.A. Fletcher (1967) The Chinese gooseberry in New Zealand. *Economic Botany., 21* : 81-92.

Seaton, H.L. and J.C. Kramer (1939) The influence of climatological factors on anthesis and anther dehiscence in the cultivated cucurbits - A preliminary report. *Proceedings of American Society for Horticultural Science., 36* : 627-631.

Sedgley, M. (1976) Control by the embryo sac over pollen tube growth in the style of the avocado (*Persea americana* Mill). *New Phytologist., 77* : 149-152.

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

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

Sharples, G.C.; F.E. Todd; S.E. McGregor and R.L. Milne (1965) The importance of insects in the pollination and fertilization of the cardinal grapes. *Proceedings of American Society for Horticultural Science., 46* : 87-90.

Slade, D.A. (1977) "Pollination of fruit crops" Department of Horticulture, Massey University, New Zealand.

Smith, B.D. (1966) Pollination studies in fruit trees. (I) Some preliminary observations on pollen transfer. *Annual Report Long Ashton Research Station for 1965* : 123-128.

Smith, B.D. and R.R. Williams (1967) Pollination studies in fruit trees. VI Methods of pollen transfer at Long Ashton. *Annual Report Long Ashton Research Station for 1966* : 120-125.

Snyder, J.C. (1942) Commercial hand pollination methods for apples in the north-west. *Proceedings of American Society for Horticultural Science., 41* : 183-186.

Soost, R.K. (1964) Unfruitfulness in *C. grandis. Proceedings of American Society for Horticultural Science., 77* : 194-201.

Stanley, R.G. and H.F. Linskens (1974) "Pollen biology, biochemistry and management" Springer-Verlag. Berlin. Heidelberg. New York.

Stephen, W.P. (1958) Pear pollination studies in Oregon. *Agriculture Experiment Station. Oregon State College Bulletin No.43.*

Stosser, R. and S.F. Anvari (1978) Pollen tube growth and ovule longevity in relation to fruit set. *Abstract 20th International Horticultural Congress. Abstract No.1489.*

Stott, K.G.; C.J. Jefferies and O.C. Jag (1974) Pollination and fruit set in plums. *Annual Report Long Ashton Research Station for 1973* : 21-22.

Taylor, B.K. (1975) Response of newly planted peach and apple trees to superphosphate. *Australian Journal of Agriculture Research., 26* : 521-528.

Taylor, B.K. and F.H. Goubran (1975) The phosphorous nutrition of the apple trees. I Influence of rate of application of superphosphate on the performance of young trees. *Australian Journal of Agriculture Research., 26* : 843-853.

Teskey, B.J.E. and J.S. Shoemaker (1978) "Tree fruit Production" 3rd edition. The A.V.I. Publishing Company, INC. U.S.A.

Thompson, A.H. and L.P. Batjer (1950) The effect of boron in the germinating medium on pollen germination and pollen tube growth for several deciduous tree fruits. *Proceedings of American Society for Horticultural Science., 56* : 227-230.

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

Tomer, E. and M. Gottreich (1975) Observations on the fertilization process in avocado with fluorescent light. *Enphytica., 17* : 298-302.

Townsend, G.F.; R.T. Riddell and M.V. Smith (1958) The use of pollen inserts for tree fruit pollination. *Canadian Journal of Plant Science., 38* : 39-44.

Traynor, J. (1966) Increasing the pollinating efficiency of honey bees. *Bee World., 47* : 101-109.

Tukey, H.B. (1925) An experience with pollinizers for cherries. *Proceedings of American Society for Horticultural Science., 21* : 69-73.

Tukey, H.B. (1936a) A relation between seed attachment and carpel symmetry and development in prunus. Science., *84* : 513-515.

Tukey, H.B. (1936b) Development of cherry and peach fruits as affected by destruction of the embryo. *Botanical Gazette., 98* : 1-24.

Vansell, G.H. (1934) Relations between the nectar concentrations in fruit blossoms and the visit of honey bees. *Journal of Economic Entomology., 27* : 943-945.

Vansell, G.H. (1942) Factors affecting the usefulness of honey bees in pollination. *Circular 650 United States Department of Agriculture.*

Wauchope, D.G. (1968) Dehiscence of anthers in pear flowers. *Australian Fruit Research Conference. Mildura. Victoria. June 1968* : 171-172.

Weaver, R.J. (1972) Growth substances in agriculture. W.H. Freeman and Company, San Francisco, U.S.A.

Wellington, R.; R.G. Hatton and J. Amos (1921) The running off of black currants. *Journal of Pomology., 2* : 160-198.

Westwood, M.N. (1978) "Temperate zone pomology" W.H. Freeman and Company, San Francisco, U.S.A.

Westwood, M.N. and J. Grim (1962) Pollinizer placement and pear yields. *Proceedings of American Society for Horticultural Science., 81* : 103-107.

Williams, R.R. (1959) The effective distance of a pollen source in a cider apple orchard. *Annual Report Long Ashton Research Station for 1958* : 61-63.

Williams, R.R. (1963) The effect of nitrogen on the self fruitfulness of certain varieties of cider apples. *Journal of Horticultural Science., 38* : 52-60.

Williams, R.R. (1965) The effect of summer nitrogen applications on the quality of apple blossom. *Journal of Horticultural Science., 40* : 31-41.

Williams, R.R. (1966) Pollination studies in fruit trees. III The effective pollination period for some apple and pear varieties. *Annual Report Long Ashton Research Station for 1965* : 136-138.

Williams, R.R. (1970) "Factors affecting pollination in fruit trees" In physiology of tree crops. Proceedings of Symposium held at Long Ashton Research Station. Academic Press. London. New York.

Williams, R.R. (1979) "Chemical thinning of apples" In Horticultural Review Vol. I. The A.V.I. Publishing Company, INC. Westport. Connecticut.

Williams, R.R. and R.W. Reninson (1963) Summer nitrogen. A new method of bringing apple trees into bearing. *Experimental Horticulture., 9* : 34-38.

Williams, R.R. and B.D. Smith (1967) Observations on factors influencing the effective distance of pollinator trees in 1966. *Annual Report Long Ashton Research Station for 1966* : 126-133.

Williams, R.R. and D. Wilson (1970) "Towards regulated Cropping. A report of recent fruit set experiments in British experiments" Grower Books, 49 Dougherty Street, London W.C.1.

Wilson, G.F. (1926) Insect visitors to fruit blossoms. *Journal of Royal Horticultural Society., 51* : 225-251.

Winkler, A.J. (1974) "General Viticulture" University of California Press. Berkley. Los Angeles. London.

Young, M.J. and W.B. Sherman (1978) Duration of pistil receptivity, fruit set and seed production in rabbiteye and tetraploid blueberries. *Horticultural Science., 13* : 278-279.