Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

80_15154

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:

- (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
ABST	TRACT		ii-iii
ACKN	NOWLEDO	GEMENTS	iv
TABI	LE OF (CONTENTS	v-x
LIST	r of ta	ABLES	xi-xii:
TABI	LE OF I	FIGURES	xiv-xv
LIST	r of Pi	LATES	xvi
SEC	rion or	NE : LITERATURE REVIEW .	1-48
CHAI	PTER O	NE : POLLINATION:	1-4
CHAI	PTER T	NO : FACTORS AFFECTING POLLINATION OF FRUIT CROPS:	4-34
Α.	Temper	rature:	4-8
	A.1.	Effects on anther dehiscence, pollen germination, pollen tube growth and ovule senescence:	4
	A.2.	Effects on pollen viability:	7 .
	A.3.	Effects on pollinating insects:	8
В.	Humid	ity, rain and wind:	8-11
C.	Orchai	rd pesticides:	11-20
*	C.1.	Importance of orchard pesticides:	11
	C.2.	Effects on pollen germination and pollen tube growth:	14
	C.3.	Effects on pollinating insects:	18
	C.4.	Factors affecting poisoning:	20
D.	Mechan	nisms of pollen transfer:	22-27
	D.1.	Wind pollination:	22
	D.2.	Insect pollination:	25
E.	Method	ds of promoting cross pollination:	27-34
	E.1.	Indirect methods:	28-32
	(a)	Planting pollinizers at effective distances:	28
	(b)	Amanaina nollinizens in the anahand:	29

		Page
	(c) Top working of the trees:	31
	(d) Placing bouquets in the trees:	31
	(e) Using hive inserts:	32
	E.2. Direct methods:	32-34
	(a) Hand method (pollen application by brush):	32
	(b) Spraying and dusting:	33
CHAP	TER THREE : FERTILIZATION AND FRUIT SET:	34-37
Α.	Fertilization:	34-35
В.	Fruit set:	35-36
C.	Seed number and fruit development:	36-37
СНАР	TTER FOUR : FACTORS AFFECTING FERTILIZATION AND FRUIT SET:	37-43
Α.	Variety:	38-41
В.	Weather condition:	41
С.	Nutrition:	41-43
D.	Other cultural factors:	43
CHAP	TER FIVE : TECHNIQUES IN POLLINATION EXPERIMENTS:	44-48
Α.	Pollen viability tests:	44-48
	A.1. By staining pollen:	44
	A.2. By growing pollen:	45-48
	(a) The growing media:	45
В.	Observation of pollen tubes in the styles:	48
SECT	TION TWO : EXPERIMENTAL	49-124
CHAP	PTER SIX : MATERIALS AND METHOD:	49-67
Α.	In Vitro Studies:	49-56
	A.1. Preparation of agar petridishes:	49
	A.2. Pollen collection and storage:	49
	A.3. Growing pollen:	50
	A.4. Counting of germinating pollen:	50
	A.5. Experiments:	50-56

			Page
Experiment	No.1.	A study of the effects of triforine (Saprol R) on apple pollen germination in vitro.	50
Experiment	No.2.	A study of the effects of mancozeb (Dithane $M45^R$) on apple pollen germination in vitro.	51
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.	51
Experiment	No.4.	A study of the effects of mancozeb 7% plus dinocap (Dikar ^R) on apple pollen germination in vitro.	51
Experiment	No.5.	A study of the effects of bayleton (BayletonR) on apple pollen germination in vitro.	52
Experiment	No.6.	A study of the effects of dichlofluanid (Euparen R) on apple pollen germination in vitro.	52
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.	52
Experiment	No.8.	A study of the effects of vinclosoline (Ronilan ^R) on peach pollen germination in vitro.	53
Experiment	No.9.	A study of the effects of Ethephon (Ethrel R) on peach pollen germination in vitro.	53
Experiment	No.10.	A study of the effects of dichlofluanid (Euparen R) on peach pollen germination in vitro.	53
Experiment	No.11.	A study of the effects of streptomycin on peach pollen germination in vitro.	54
Experiment	No.12.	A study of the effects of streptomycin plus triforine $(Saprol^R)$ on peach pollen germination in vitro.	54
		Jarmondon no norto.	77

				Page
		Experiment No.13.	A study of the effects of triforine (Saprol R) and triforine plus streptomycin sprays on the germinability of peach pollen.	54
		Experiment No.14.	A study of the effects of dichlofluanid (Euparen R) on kiwifruit pollen germination in vitro.	55
		Experiment No.15,	A study of the effects of captan on kiwifruit pollen germination in vitro.	55
		Experiment No.16.	A study of the effects of vinclozoline (Ronilan ^R) on kiwifruit pollen germination in vitro.	55
	*	Experiment No.17.	A study of the effects of dichlofluanid (Euparen ^R) sprays on the germinability of kiwifruit pollen.	55
		Experiment No.18.	A study of the effects of temperature on peach pollen germination in vitro.	56
		Experiment No.19.	A study of the effects of temperature on apple pollen germination in vitro.	56
В.	In Vi	vo Studies:		57-62
	В.1.	Collection and pr treatment:	eparation of flowers for	57
	(a)	Apples and peache	s:	57
	(b)	Kiwifruits:		57
	B.2.	Spraying and poll	ination of flowers:	57
	B.3.	Collection and pr	eservation of treated flowers:	58
	B.4.	Observation of po	llen tubes in the styles:	58
	B.5.	Experiments:		59-62
		Experiment No.20.	A study of the effects of bupirimate (Nimrod ^R) sprays on pollen germination and pollen tube growth in apple (Splendour) styles.	59
		Experiment No.21.	A study of the effects of triforine (Saprol R) on peach pollen germination and pollen tube growth in peach styles.	59
		Experiment No.21.	(Splendour) styles. A study of the effects of triforine (Saprol ^R) on peach pollen germination and pollen tube growth in peach	

				Page
	Experiment No.2	la.	A study of the pollen germination and the pollen tube growth in kiwifruit styles hand pollinated at different hours after dichlofluanid sprays.	60
	Experiment No.2	22.	A study of the effects of temperature and fungicides on kiwifruit pollen germination and pollen tube growth in vivo.	60
	Experiment No.2	23.	A study of the effects of temperature on pollen germination and pollen tube growth in kiwifruit styles.	61
*	Experiment No.2	24.	A study of the effects of flower age and temperature on pollen germination and pollen tube growth in kiwifruit styles.	61
C. Field	studies:			62-67
C.1.	Experiments:			62-67
	Experiment No.2	25.	A study of the effects of different fungicides on the fruit set of Golden Delicious.	62
	Experiment No.2	26.	A study of the effects of bupirimate (Nimrod R) on the fruit set of Splendour trees.	63
	Experiment No.2	27.	A study of the effects of different fungicides on the fruit set of Golden Queen peach trees.	63
	Experiment No. 2	28.	A study of the effects of different fungicides on the fruit set of Red Haven peach trees.	64
	Experiment No.2	29.	A study of the effects of different fungicides on the fruit set of kiwifruits.	65
	Experiment No.3	30.	A study of the effects of pollination method and fungicides on the fruit set, fruit weight and seed numbers of kiwifruits.	66

		Page
D.	Statistical Analysis:	67
СНА	APTER SEVEN : RESULTS AND DISCUSSION:	67-111
Α.	In Vitro Studies:	67-83
	A.1. Apple:	67
	A.2. Peach:	71
	A.3. Kiwifruits:	75
	A.4. Discussion:	77
В.	In Vivo Studies:	84-97
	B.1. Apple:	84
	B.2. Peach:	85
	B.3. Kiwifruits:	87
	B.4. Discussion:	95
С.	Field Studies:	97-111
	C.1. Apple:	97
	C.2. Peach:	100
	C.3. Kiwifruits:	103
	C.4. Discussion:	106
CHA	APTER EIGHT : FINAL DISCUSSION:	112-121
Α.	Therapeutant Effects:	112-119
В.	Temperature Effects:	119-120
С.	Hand Pollination Effects:	120-121
CHA	APTER NINE : CONCLUSION:	121-124
RTI	RITOGRAPHY	125-130

LIST OF TABLES

<u> Fable</u>		Page
1.	Optimum temperature range for in vivo pollen germination and pollen tube growth in selected fruits.	6
2.	Orchard pesticides for bloom season application in New Zealand.	13
3.	Effects of fungicides on in vitro pollen germination.	16
4.	Toxicity of some selected pesticides to honey bees.	24
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.	67
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°C on different dates in October 1978 and in November 1979.	68
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.	69
8.	Effect of temperature on the bursting of the cell walls of pollen grains grown in 1.5% agar and 10% sucrose media for 18 hours.	71
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.	71
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	Se2
	September 1979.	72

<u>Table</u>		Page
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.	74
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.	75
13.	In vitro germination of Matua (Kiwifruit) pollen collected from anthers sprayed with 100 ppm dichlofluanid compared with Matua pollen collected from unsprayed flowers.	77
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.	. 83
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.	84
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.	85
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.	87
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).	88
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	90

Table	· ·	Page
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).	91
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.	93
22.	Pollen germination and pollen tube growth 24 hours after pollination in Hayward kiwifruit styles from flowers of different ages held at different temperatures.	94
23.	Mean percentage fruit set in Golden Delicious and Splendour trees sprayed with different fungicides during the bloom season of 1978 and 1979.	98
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.	101
25.	Mean percentage fruit set in Hayward vines sprayed twice with different fungicides during the bloom season in 1979.	103
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.	105
27.	Comparisons of fruit set at 5 weeks 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.	106

TABLE OF FIGURES

rigure		Page
1.	Diagram showing the effective pollination period for normal and strong flowers.	3
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.	70
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.	73
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.	76
5.	Pollen germination and pollen tube growth 24 hours after pollination with fresh Matua pollen in Hayward kiwifruit styles from flowers of different ages.	86
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.	86
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.	92
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.	92
9.	Percentages of initial and final fruit set in Golden Delicious and Splendour trees sprayed with different fungicides during bloom season.	99
10.	Percentages of initial and final set in Golden Queen and Red Haven trees sprayed with different fungicides during bloom season.	102
11.	Percentages of fruit set at 5 weeks after full bloom and at harvest in Hayward vines sprayed with different fungicides during bloom season.	104

Figure		Page
12.	Mean fruit weights and mean seed numbers per fruit at harvest in fruits from bee pollinated and bee plus hand pollinated flower clusters from Hayward vines.	109
13.	Relationship between fruit weight and seed number per fruit in fruits bee pollinated and bee plus hand pollinated flower clusters in fungicide sprayed and unsprayed vines.	110

LIST OF PLATES

Plate No.		Page
1.	Kiwifruit (Matua) pollen grains. X5500.	78
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.	78
3.	Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose. X440.	81
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.	81
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.	82
6.	Kiwifruit (Matua) pollen germination after 20 hours incubation at 25°C in 1.5% agar media containing 10% sucrose and 10 ppm dichlofluanid. X440.	82
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.	89
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	89
	with 1000 ppm dichlofluanid, X77.	69