

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.

POLLINATION PATTERNS IN SAFFLOWER

(*Carthamus tinctorius* L.)

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

PETER WILLIAM WOODS

1981

885.41.58

ABSTRACT

The influence of environmental conditions on safflower (*Carthamus tinctorius* L.) floret characters and insects were studied in relation to pollination in this species.

Insect activity was studied in a field experiment using part of the world germplasm collection of safflower. Honey bees were the most likely cross-pollinators. Activity of honey bees did not vary between genotypes studied. Correlations between insect and weather data were mainly non-significant.

A sample of 12 genotypes from the world collection were intensively studied in controlled environment rooms. Single plants were used as plots in a randomised complete block design, in each of four environments (day/night temperature treatments of 28/22°C and 24/18°C in combination with vapour pressure deficit treatments of -1.0 and -0.4 kPa). Environments reflected New Zealand summer conditions.

Coefficients of variation were acceptable for most characters. Considerable genotypic, environmental and genotype-environment interaction variances were observed for most characters. Standardised partial regression coefficients (path coefficients) and principal factors were utilized to determine the characters most important in self-pollination of safflower. These characters were: the length of the style-stigma; the rate of style-stigma growth; the rate of corolla tube growth and amounts of viable

pollen present during floret expansion.

Pollen viabilities remained high for the longest time in higher humidity environments. Large amounts of pollen were produced at the lower humidity. Floral parts were largest in the cool dry environment, however rates of style-stigma and corolla expansion were greater at lower temperatures. It was concluded that synchronization of the rates of style-stigma and corolla tube growth were important in maintaining the stigma in close proximity to viable pollen, and thus promoting the possibility of self-pollination. Self-pollination was greatest at the lower temperature and lower humidity.

The basic self-pollination mechanism observed was in agreement with previous authors.

A number of improvements for future controlled environment experiments involving safflower were suggested.

The implications of pollination of safflower on germplasm collection and maintenance, artificial crossing and breeding plans were discussed.

ACKNOWLEDGEMENTS

I am indebted to my supervisor Dr I.L. Gordon, and co-supervisor Mr E. Roberts for their guidance, assistance, patience and encouragement during the course of this study, and preparation of the manuscript.

I wish to express my gratitude to the D.S.I.R., Plant Physiology Division, for the use of their controlled environment facilities, and for the co-operation and helpful discussions I received from many of the staff. In particular I would like to thank Mr I.J. Warrington and Ms H. Turnbull for their assistance.

Special thanks are also due to the many staff and students of the: Agronomy Department; Seed Technology Centre; Farm Machinery Hall; Computer Centre; Library; Photographic Unit and Horticulture Department, who assisted in one way or another at various stages of this study.

Finally I would like to thank Mr and Mrs R. Cave for their considerable time, effort and patience in typing the manuscript.

Financial assistance from the D.S.I.R. Genotype-environment Interaction grant was greatly appreciated.

TABLE OF CONTENTS

Title Page	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	xii
LIST OF FIGURES	xix
LIST OF PLATES	xxi
LIST OF APPENDICES	xxii
INTRODUCTION	1
1. REVIEW OF LITERATURE	3
1.1 Taxonomic background of safflower	3
1.2 Overview of pollination	5
1.2.1 Introduction	5
1.2.2 Plant factors to be considered in pollination studies	5
1.2.2.1 Pollen	7
1.2.2.1.2 Pollen viability assay	9
1.2.2.1.2.1 Germination assays	9
1.2.2.1.2.2 Non-germination assays	10
1.2.2.2 The stigmatic surface	12
1.2.2.2.1 Sporophytic incompatibility response mechanism	13
1.3 Factors influencing pollination in safflower	14
1.3.1 Morphology of the capitulum	14
1.3.2 Floral differentiation	14
1.3.3 Floral morphology	14
1.3.4 Flowering	16
1.3.5 Safflower microspore development	16
1.3.6 Pollination mechanism	17

1.3.7	Safflower pollen and pollen tube cytology and endosperm development	18
1.3.8	Pollination controls	19
1.3.8.1	Closed flower condition	19
1.3.8.2	Thin-hull gene	21
1.3.8.3	Male sterility	21
1.3.8.4	Complete sterility	22
1.3.8.5	Bagging treatments	23
1.3.8.6	Artificial crossing	24
1.3.8.7	Isolation of breeding fields	25
1.3.8.8	Pollination/Fertilization control: self-incompatibility	25
1.4	The influence of pollen vectors on safflower	27
1.4.1	Natural crossing in safflower	27
1.4.2	Pollen vectors of safflower	27
1.4.2.1	Abiotic vectors: wind	28
1.4.2.2	Biotic vectors: insects	29
1.4.3	Response to safflower yield to insects	29
1.4.4	Relative efficiency of insect pollinators	31
1.4.5	Insect activity patterns	33
1.4.5.1	Activity of the domestic honey bee <i>(Apis mellifera)</i>	33
1.4.5.2	Activity of other pollinators	37
1.5	Plant factors influencing pollinator activity	38
1.5.1	Visual cues	38
1.5.1.1	Visual cues to the crop from a distance	38
1.5.1.2	Visual cues on safflower petals	39
1.5.2	Nectar	40
1.5.3	Pollen	43
1.6	Scales of measurement	45
1.6.1	Nominal or classificatory scale	45

1.6.2	Ordinal or ranking scale	45
1.6.3	Interval scale	46
1.6.4	Ratio scale	46
1.7	Parametric and non-parametric statistics	47
1.7.1	Crosstabulations	47
1.7.1.1	Chi-square test (χ^2)	47
1.7.1.2	Cramer's V (V)	48
1.7.1.3	Contingency coefficient (C)	49
1.7.1.4	Lambda (λ)	49
1.7.1.5	Uncertainty coefficient (U)	51
1.7.2	Spearman and Kendall rank correlations	52
1.7.3	Techniques of missing data estimation	53
1.7.4	Multiple regression	54
1.7.4.1	Dummy variables in regression	55
1.7.4.2	R ² : coefficient of multiple determination	56
1.7.4.3	Standardised partial regression coefficients (β')	
1.7.5	Principal factor analysis	57
1.7.6	Analysis of variance procedure	59
1.7.7.1	Homogeneity of error variances	60
1.7.7	Adaptation analysis	61
2.	FIELD EXPERIMENTS	62
2.1	Introduction	62
2.2	Time of effective cross-pollination	62
2.2.1	Experimental design	62
2.2.1.1	Treatments	62
2.2.1.2	Measurements and analysis	63
2.2.2	Results and associated discussion	64

	viii.	
2.3	Timing of insect activity and influence of weather	67.
2.3.1	Introduction	67
2.3.2	Methods	67
2.3.2.1	Statistical analysis	68
2.3.3	Results and associated discussion	71
2.3.3.1	Weather correlations	90
2.4	Effects of safflower genotype on insect activity	93
2.4.1	Introduction	93
2.4.2	Methods	93
2.4.3	Results	95
2.5	General discussion of field experiments	97
2.5.1	Pollinators and their activity	97
2.5.2	Potential for commercial use of bees in safflower	98
2.5.3	Effects of plant genotype on pollinator activity	98
2.5.4	Implications	100
3.	MAIN STUDY MATERIALS AND METHODS	102
3.1	Plant materials	102
3.2	Trial design	104
3.3	Controlled environment room conditions	104
3.3.1	Environmental conditions	104
3.3.2	Cultural conditions	105
3.4	Plant characters examined	107
3.4.1	Amount and viability of pollen	107
3.4.2	Length of floret parts	107
3.4.3	Number of pollen tubes reaching the stigma base	109
3.4.4	Stigma receptivity	110
3.4.5	Timing of plant development	112
3.4.6	Flower and nectar guide colour	115
3.4.7	Dead flower colour	115
3.4.8	Width of open capitula	115

3.4.9	Amount of nectar in a single floret	115
3.4.10	Floret density	118
3.4.11	Uniformity of floret emergence	118
3.4.12	Amount of pollen extruded	120
3.4.13	Positioning of pollen adhesion	120
3.4.14	Number of stigmas with pollen present	120
3.4.15	Degree of stigmatic extrusion	120
3.4.16	Timing of stigma presence and full stigmatic extrusion	120
3.4.17	Number of capitula	123
3.4.18	Plant height	123
3.4.19	Seed set	123
3.4.20	Character abbreviations	125
3.5	Data handling	128
3.5.1	Missing data	128
3.5.2	Statistical analysis	129
3.5.2.1	Populations of inference	129
3.5.2.2	Multiple regression analysis	129
3.5.2.3	Principal factor analysis	132
3.5.2.4	Analysis of variance	132
3.5.2.4.1	Heritability estimates	138
3.5.2.5	Adaptation analysis	139
4.	MAIN STUDY RESULTS AND ASSOCIATED DISCUSSION	142
4.1	Multiple regression analysis	142
4.2	Principal factor analysis	148
4.3	Analysis of variance	154
4.3.1	Homogeneity of errors	154
4.3.2	Coefficients of variation	161
4.3.3	Components of variance	161

4.3.3.1	Introduction	161
4.3.3.2	Blocks (within environments) variance	168
4.3.3.3	Environmental variance	168
4.3.3.4	Genotypic variance	169
4.3.3.5	Genotype-environment interaction variance	169
4.3.4	Heritability estimates	170
4.3.5	Examination of means and adaptation statistics	173
4.3.5.1	Pollen characters	206
4.3.5.2	Corolla characters	209
4.3.5.2.1	Lengths	209
4.3.5.2.2	Rates of floral part expansion	212
4.3.5.3	Days to: flowering; flowering median. The length of flowering within heads and the spread of flowering. Total number and width of capitula	213
4.3.5.4	Flower colour, floret density, floret emergence	214
4.3.5.5	Self-pollination under bags and other seed set characters	214
4.3.5.6	Stigma turgidity and esterase activity	215
5.	GENERAL DISCUSSION	218
5.1	Experimental objectives	218
5.2	Experimental design	219
5.2.1	Field experiments	219
5.2.2	Controlled environment study	219
5.3	Experimental methods	223
5.4	Experimental results	225
5.4.1	Pollination mechanism	225
5.4.2	Dynamics of the pollination mechanism	226

5.5 Implications for plant breeding	229
5.5.1 Germplasm	229
5.5.1.1 Collection	229
5.5.1.2 Seed increase and maintenance	230
5.5.2 Recommendations for artificial crossing	232
5.5.3 Breeding plans for safflower	235
5.5.3.1 Breeding plans utilising additive genetic variance	236
5.5.3.2 Breeding plans utilising dominance and epistatic genetic variance	238
5.5.3.3 Induced polyploidy	239
CONCLUSIONS	241
BIBLIOGRAPHY	243
APPENDICES	265

LIST OF TABLES

Table 1.1	Relations of incompatibility type with pollen and pistil characteristics and sites of pollen inhibition.	8
Table 1.2	Pollination of caged safflower by confined insects.	32
Table 1.3	Times of peak honey bee activity on safflower, reported by various authors.	34
Table 1.4	Number of honey bee visitors observed on five 4-square-yard plots related to nectar sugar concentration, as measured in the stomach using a portable refractometer.	35
Table 1.5	Sugar concentration in the nectar of flowers pollinated by various agents.	41
Table 2.1	Analysis of variance for time of effective cross-pollination experiment, based on seed counts.	65
Table 2.2	Table of ranked means for time of effective cross-pollination experiment, based on seed counts.	65
Table 2.3	Timing of pollen presence in the field	72

Table 2.4	Summary of weather data for days (24 hour period) on which insect counts were made.	73
Table 2.5	Total numbers of insects visiting safflower over the course of the day for various insect families.	84
Table 2.6	Summary of insect foraging behaviour.	86
Table 2.7	Summary statistics for crosstabulations of day by time of day for each insect type.	87
Table 2.8	Summary statistics of crosstabulations for insect by time of day tables for each day.	89
Table 2.9	Kendall rank correlations of daily total insect counts with daily weather.	91
Table 2.10	Kendall rank correlations of daily percentage of insect type with daily weather.	92.
Table 2.11	Genotypes used in the study of safflower genotype influences on insect activity.	94
Table 2.12	Summary statistics of crosstabulations for genotype by insect tables for each day and time of day combination.	96

Table 3.1	Genotypes used in the main study.	103
Table 3.2	Main features of environments.	105
Table 3.3	Abbreviations used for character names.	126
Table 3.4	Expectations of mean squares for the single environment model.	135
Table 3.5	Expectations of mean squares for the pooled environments model.	136
Table 4.1	Total coefficients of determination (R^2) for regression equations with dummy variables present.	143
Table 4.2	Standardised partial regression coefficients (β_k^*) for the independent characters entered into the regression equation (with dummy variables present).	144
Table 4.3	Ratios of standardised partial regression coefficients for each variable over the value for LS2 i.e. importance relative to LS2.	146
Table 4.4	Percentages of variation taken into account by the successive factors.	149

Table 4.5	Factor structure matrix.	149
Table 4.6	Factor scores for each character.	150
Table 4.7	Chi-square values and probabilities of tests of homogeneity of error variance among environments.	155
Table 4.8	Analysis of variance tables for character AVP1 for standardised data and non-standardised data.	157
Table 4.9	Genotype means (pooled across environments) with ranks for non-standardised and standardised data for character AVP1.	159
Table 4.10	Environment means with ranks for non-standardised and standardised data for character AVP1.	160
Table 4.11	Coefficients of variation in each environment.	162
Table 4.12	Estimates of block, environment, genotype and genotype-environment interaction variance components together with their standard errors (in brackets) and significance in the F-test.	164
Table 4.13	Blocks, environments, genotypes and genotype-environment interaction variance components ratio to the error variance.	166
Table 4.14	Full and restricted broadsense heritability estimates with standard errors (in brackets) and coefficients of variation, from the pooled analysis	171

Table 4.15	Environment means with standard errors (in brackets) and significance of the environments F-test.	174.
Table 4.16	Significance groupings of single environment means with least significant differences given where appropriate.	176.
Table 4.17	Genotype means in the pooled environment with their standard error, and F-test significance for genotypes.	178
Table 4.18	Significance groupings of genotype means in the pool environment, based on least significant differences.	180
Tables 4.19.1 to 4.19.12	Regression coefficients, their standard errors, significance from 0 and 1, ratio to least ecovalence, and coefficients of determination for the various genotypes. Statistics were derived from adpatation analysis. F-test significance of genotype-environment interaction is also given.	182-205
Table 4.20	Significance groupings across times of pollen viability percentages within each environment (based on individual t-tests).	207

Table 4.21	Significance groups across times of amounts of viable pollen within each environment (based on individual t-tests).	207
Table 4.22	Significance groupings across times of amounts of pollen within each environment. (based on individual t-tests).	207
Table 4.23	Significance groupings across times for pollen viability percentages in each genotype mean in the pool environment (based on individual t-tests).	208
Table 4.24	Significance groupings across times for amounts of viable pollen in each genotype mean in the pooled environment (based on individual t-tests).	208
Table 4.25	Comparisons of time differences between floret length measurements (using individual t-tests) for each environment.	210
Table 4.26	Comparisons among genotype means for floral lengths at the two times in the pooled environment (based on individual t-tests) and the genotype means of pollen presence in the pooled environment.	211
Table 4.27	Percentage of total style-stigma length exposed above the anther column.	212

Table 4.28	Significance groupings of genotype means in the pooled environment across seed set treatments (based on individual t-tests).	216
Table 4.29	Environment means of duration of stigma turgidity for emasculated and non-emasculated florets.	217
Table 4.30	Genotype means of duration of stigma turgidity for emasculated and non-emasculated florets.	217

LIST OF FIGURES

Figure 1.1	Reaction of tetrazolium salt to form a formazan compound.	11
Figure 1.2	Types of closed flower in safflower.	20
Figures 2.1 to 2.9	Graphs of the numbers of insects visiting safflower capitula versus time of day, for days on which observations were made.	75-83
Figure 3.1	Absorbance of filters.	111
Figure 3.2	Longitudinal section of a safflower floret. The nectar droplet represents a score of 5.5.	117
Figure 3.3	Diagrammatic representation of positioning of pollen adhesion.	122
Figure 3.4	Diagrammatic representation of the branching pattern of safflower, and consequential categories of capitula.	124
Figure 3.5	The standardised regression model, and the related path coefficient diagram.	131
Figure 3.6	The relationship of genotype regression coefficient and genotype mean in terms of 'adaptability'.	141

Figure 4.1 Graph of factor scores for factors
1 and 2.

LIST OF PLATES

Plate 3.1	Florets at various stages of development up to full extrusion.	108
Plate 3.2	A freshly opened head showing variation in the size of ovaries in the outer ring of florets.	113
Plate 3.3	A head with developing seeds clearly showing the outer female sterile florets.	113
Plate 3.4	Safflower head at a suitable stage of growth for emasculation.	114
Plate 3.5	Emasculated safflower head prior to elongation of the stigmas.	114
Plate 3.6	Flower colour scores for safflower heads.	116
Plate 3.7	Scores of dead flower colour.	116
Plate 3.8	Scores for floret density.	119
Plate 3.9	Scores of uniformity of floret emergence.	119
Plate 3.10	Score of amount of pollen extruded.	121
Plate 3.11	The range of stigma extrusion represented by scores.	121

LIST OF APPENDICES

Appendix 1	Counts of insects visiting 135m of safflower commencing at 7.30 a.m. (standard time) for days on which observations were made	265-274
Appendix 2	Climate-lab-modified half-strength Hoagland's nutrient.	275
Appendix 3	Insect vision of safflower.	276
Appendix 4	Summary statistics for the regression of SELF1% with independent variables including dummy variables (G,E,GE) for the single and pooled environments.	280-291
Appendix 5	Factor score coefficients	292
Appendix 6	Coefficients of variation, estimates of variance components and their ratios to error variance, heritability estimates, environment means, genotype means in the pooled environment, and adaptation statistics for characters studied but not presented in the text.	293-313
Appendix 7	Estimates of block and genotypic variance components with their standard error, and significance in the F-test for each single environment.	314-318

Appendix 8	Estimates of error variance with standard errors (in brackets) for each single environment and the pool Chi-square values and probabilities for tests of homogeneity of error variances.	319-322
Appendix 9	Genotype means within single environments for all characters studied.	323-335
Appendix 10	Coefficients of variation, estimates of variance components with standard errors, heritability estimates and genotype means for standardised data in the pooled environment.	336-343
Appendix 11	Stigma receptivity in safflower.	344-346

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an erect annual thistle-like herb adapted to semi-arid areas. It has a deeply penetrating taproot (Henderson, 1962) and strong stem with many appressed or spreading branches (Leon and Knowles, 1964) each terminating in a capitulum. Lower leaves of most genotypes are entire and free of spines. Upper leaves vary from spineless to strongly spined (Rao, 1943; Claassen, 1952). A short rosette phase may exist (Zimmerman, 1973). Height at maturity ranges from 0.5m to 1.5m (Purseglove, 1968). Considerable genotypic variation in morphology exists (Ashri *et al.*, 1976). Environmental changes also have considerable impact on morphology (Beech and Norman, 1966).

Safflower is known only in the cultivated form (Wiess, 1971), and is thought to have evolved in the area bounded by the Eastern Mediterranean and the Persian Gulf (Ashri and Knowles, 1960; Hanelt, 1963). Widespread domestication and dispersion have resulted in the plant having had many varied uses, e.g. as a dye, vegetable, cosmetic and medicinal herb (Wiess, 1971; Knowles, 1960a).

Current interest centres on safflower's hydrophobic oil which is light coloured and easily clarified. Non-yellowing properties have led to its widespread use in paints and varnishes. Meal made from seed is also utilised as a protein supplement in animal diets (Knowles, 1958). Cultivars exist today with improved oil quality and quantity, disease and insect resistance (Wiess, 1971).

Interest in New Zealand centres on its use as a summer growing oilseed crop for North Island areas prone to drought. Such environments are also associated with high humidities, fogs and late summer rainfall, which lead to disease problems in the crop, and sprouting damage to the seed. Resistance of safflower to *Botrytis cinerea* (the most troublesome disease in N.Z.) and sprouting damage are thought to exist (Knowles, 1958; Kotecha and Zimmerman, 1978).

An effective breeding program is needed to produce cultivars suited to the New Zealand environment. A knowledge of population gene structure within the species is required if selection methods and breeding plans are to be utilized efficiently. To this end a study of pollination patterns in safflower was initiated as past and present pollination patterns influence population structure. The present study consisted of two parts:

A. A field study was conducted to observe insect activity on the crop. The objective was to acquire knowledge of the role played by insect pollinators. The opportunity was taken to study floral characters such as floret morphology, pollen presence and stigmatic extrusion which might be of interest in subsequent studies.

B. The second part consisted of a controlled environment study of plant and floral characters. The objectives were to observe genotypic and environmental differences in characters potentially related to self-pollination; and to determine characters most important in self-pollination. Examples of such characters include amounts and viabilities of pollen, stigma receptivity, corolla characteristics and lengths of flowering.