The Identification and Development of Sunflower Germplasm with Resistance to Alternaria Blight

Gary Allen Kong B.App.Sc. (University of Queensland); Hons. (University of New England)

A thesis submitted for the degree of Doctor of Philosophy of the University of New England, Armidale, New South Wales.

Department of Botany
August, 1997

Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that the assistance received in preparing this thesis and all other sources used, have been acknowledged in this thesis.

Abstract

The purpose of this study was to develop a system for identifying sunflower germplasm with resistance to Alternaria blight, which is caused by the necrotrophic fungus, *Alternaria helianthi*. Field and greenhouse screening procedures were studied, so that a complementary system could be developed. Components of resistance measured in the greenhouse were tested for their correlation with epidemic parameters obtained from field experiments.

The following procedures are recommended to screen sunflower lines for their resistance to alternaria blight under greenhouse conditions. The first or second pair of leaves of seedling plants at the V8 growth stage were inoculated using inoculum grown on sunflower leaf extract agar for 5-10 days. Approximately 30 spores were applied to each leaf. Inoculated plants were misted and subjected to a dew period of 48h inside a sealed plastic tent. A dew period temperature of 26/26°C night/day and a post-dew period temperature similar to the average temperature experienced under local growing conditions should be applied. All lesions were measured 7 days after inoculation and lesion size (area mm^2) was calculated. The lesion size of lines being tested was expressed as a proportion of the mean lesion size of a susceptible standard included in each screening experiment. The susceptible line B89 was used as a standard line in all the screening experiments described in this study. Infection frequency of each line was expressed as a proportion of the infection frequency of the susceptible standard and was used in conjunction with mean lesion size to assess lines for resistance.

Bacteria found contaminating cultures of *A. helianthi* inhibited the germination of conidia, sporulation of cultures and the growth of germ-tubes and mycelium. Germ-tube swelling causing vesicle formation, excessive germ-tube branching and lysis of germ-tubes, were observed in biosaays using bacterial isolates obtained from healthy and

diseased sunflower leaves. Some bacteria appeared to be endoparasitic, persisting inside the lumina of conidia and causing erosion of the conidium wall which resulted in the destruction of conidial cells. The decrease in germination and infectivity of ageing conidia and the attenuation of ageing cultures of *A. helianthi* was attributed to the presence of antagonistic bacteria.

Sources of resistance to *A. helianthi* were not available at the time this research began. Therefore, locally developed inbred restorer lines (F_4) were accessed from a public breeding program and screened for resistance to *A. helianthi* in the field using a generated epidemic. Of the 37 lines evaluated, 32 had less disease than the susceptible line B89. Twelve lines had approximately half the amount of disease of B89 and 7 lines had less than one third of the disease found on B89. Variation for disease reaction within lines was high. Although no lines were immune, plants with very low levels of disease were observed in many of the lines. Single plant selections were selfed. Selections from eighteen lines (P_1 –P_{18}) were used in field and greenhouse experiments to evaluate methods of differentiating resistance. These lines were selected because they expressed disease reactions that ranged from highly resistant to susceptible.

Epidemic development was examined in a subset of ten lines (P_1 –P_{10}) using an artificially generated epidemic of *A. helianthi* that was replicated at two field sites. Experiments were designed to study epidemic development from a line source in plots surrounded by a buffer of forage sorghum that was included to reduce interplot interference. The epidemics that developed in each line were characterised by calculating parameters that described disease spread in either space or time or both space and time. Parameters were evaluated primarily for their usefulness in differentiating quantitative resistance (QR), however they were also examined to detect aspects of epidemic development which may help further in the characterisation of QR or be useful for future decisions of experimental design and disease assessment methods. Disease severity ratings (DSRs), area under the disease progress curves (audpcs) and volumes beneath the plot of disease progress in space and time (GVs) were good indicators of QR, while apparent infection rates (r),

disease gradients (*b*) and velocities of spread (*v*) were limited in their ability to differentiate QR. Three- dimensional surface plots of disease progress in both space and time allowed epidemic parameters to be viewed simultaneously. Inferences about the interrelationships between epidemic parameters could be drawn from these surface plots.

Allen (1981) proposed that anthesis was the critical point for disease assessment. In a breeding program, this would mean that large numbers of plants would need to have their flowers covered to prevent outcrossing prior to selection. This imposes constraints on time and labour. In this study, the DSRs taken prior to flowering were found to be well correlated with DSRs taken at flowering and post-flowering. Therefore, selection could be carried out prior to flowering and only resistant plants covered to allow controlled pollination. Thus, much time and effort was saved.

Disease assessment in a mature sunflower crop is an arduous task, particularly if the lowest leaves are used for assessment. If disease levels in the upper parts of plants could be used for disease assessment, then disease assessment could be made easier. However, the DSRs obtained for leaves in the middle and upper plant positions were poorly correlated with the DSRs of lower leaves and therefore could not be used for evaluating resistance.

Ten selected restorer lines (R-lines) were test-crossed with A89 (A-line) to produce F_1 hybrids that were evaluated in the field for resistance to *A. helianthi*. Two of the ten R-lines were selected for further evaluation based on the performance of these hybrids. Five commercial A-lines and the public line A89 were crossed with two restorer lines (R_1 and R_2), to produce twelve F_1 hybrids. These hybrids, their parental lines and three commercial hybrids were exposed to generated epidemics of *A. helianthi* at two field sites. Area under the disease progress curves (audpcs) were calculated for each line, from disease severity ratings (DSRs) taken from budding to late flowering. The commercial A-lines were as susceptible to *A. helianthi* as the susceptible line A89. R_1 and R_2 had high levels of resistance. Generally, the F_1 hybrids had much smaller audpcs than their

midparent estimates, indicating a high degree of dominance for genes controlling resistance in the resistant parents R_1 and R_2. Overall, the F_1 hybrids had the same resistance to *A. helianthi* as the commercial hybrids.

Eighteen sunflower lines (P_1–P_{18}) were inoculated with conidia of *Alternaria helianthi* under controlled conditions and components of QR, (spore production, incubation period, infection frequency and mean lesion size) were measured. The disease severity ratings (DSRs) of lines P_1 –P_{14} were measured in the field in 1994 and 1995, using a generated epidemic. The DSRs of the lines varied from highly susceptible to highly resistant. Spearman's ranking of the DSRs was highly correlated (R = 0.9) for both years. The rankings of lines by components of QR measured under controlled conditions were poorly correlated with the rankings of lines by DSRs. However, the linear regression of components with DRSs showed that mean lesion size was highly correlated (R = 0.74) and infection frequency was moderately correlated (R = 0.58) with the DSRs observed over the two years. Infection frequency was also well correlated (R = 0.75) with mean lesion size. Spore production and incubation period were poorly correlated with the DSRs for both years. An index based on infection frequency and mean lesion size gave a better correlation with the DSRs determined in 1995 than either component alone. However, in 1994 the index was not as well correlated with the DSRs as mean lesion size alone.

Mean lesion size and infection frequency were determined under controlled conditions, for twenty-three sunflower lines, comprising six parental lines and their F_1 hybrids and three commercial cultivars. The area under the disease progress curves (audpcs) of the same lines were previously determined at two field sites (Chapter 6). Both Spearman's ranking and linear regression analysis showed that on average, mean lesion size was highly correlated and infection frequency was moderately correlated with the audpc. There was also a high correlation for the audpcs between field sites.

It is recommended that mean lesion size, determined from seedling plants 7-9 days after inoculation could be used to select for resistance to *A.helianthi* in the greenhouse. Infection frequency could also be used as a predictor of resistance, but only in conjunction with mean lesion size. In the field, disease severity ratings (DSRs), area under the disease progress curves (audpcs) or volumes (GVs) below the surface plot of disease progress in space and time, can be used to evaluate resistance to *A. helianthi* .

Acknowledgements

I wish to extend my gratitude to Dr Joe Kochman for his advice, support and encouragement throughout the course of this research. I wish also to thank Associate Professor John Brown for the suggestions and guidance he has given me throughout the course of this study and during the preparation of this thesis. There was a number of people from whom I also received assistance and I gratefully acknowledge them. They included Dr Malcolm Ryley (Senior Plant Pathologist, DPIQ), Mr Don Gowanloch (Electron Microscopist, UQ), Mr Lex Diatloff and Mrs Barbara Stubbings (Bacteriologists, DPIQ), Dr John.Alcorn (Mycologist, DPIQ), Dr Bob Bray (Plant Breeder, formerly of CSIRO), Dr Doug George (Plant Breeder, UQ), Mr Gordon Simpson (Experimentalist, DPIQ).

This study would not have been possible without the financial support of the Grains Research and Development Corporation (GRDC). Their support is greatly appreciated.

Finally, the support and understanding given by my wife and family has been invaluable and I thank them wholeheartedly.

Table of Contents

THE IDENTIFICATION AND DEVELOPMENT OF SUNFLOWER GERMPLASM WITH RESISTANCE TO ALTERNARIA BLIGHT ..... **I**

CERTIFICATION ..... **II**

ABSTRACT ..... **III**

ACKNOWLEDGEMENTS ..... **VIII**

TABLE OF CONTENTS ..... **IX**

CHAPTER 1 ..... **1**

GENERAL INTRODUCTION 1
1.1 Sunflower Production in Australia 1
1.2 The Host : Helianthus 4
1.2.1 Origins and Taxonomy 4
1.2.2 Disease and Disease Resistance 5
1.3 The Pathogen 7
1.3.1 Origins and Distribution 7
1.3.2 Taxonomy and Morphology 8
1.3.3 Biology 9
(i) Disease symptoms 9
(ii) The Infection Process 11
(iii) Toxin Production 12
(iv) Dispersal of *A. helianthi* 14
(v) Survival of *A. helianthi* 15
1.4 Resistance to A. helianthi *in* Helianthus 16
1.5 Economic Importance and Control 18
1.6 Objectives of this Study 19

CHAPTER 2 ..... **22**

GENERAL MATERIALS AND METHODS 22
2.1 Collection of Isolates of A. helianthi 22
2.2 Culture and Storage of Isolates of A. helianthi 22
2.3 Growth of Sunflower Plants 23
2.4 Production and Preparation of Inoculum 23
2.4.1 Conditions for Culturing *A. helianthi* 23
2.4.2 Preparation of Inoculum 24
(i) Single spore inoculum 24
(ii) Spore suspension 24
2.5 Inoculation Procedures 25
2.5.1 Single spore inoculum 25
2.5.2 Spray inoculation 25
2.5.3 Incubation of Inoculated Plants 25
2.6 Disease Assessment Methods 26
2.7 Statistical Analyses 28

CHAPTER 3 ..... **29**

DEVELOPMENT OF A GREENHOUSE ASSAY TO SCREEN SUNFLOWER FOR RESISTANCE TO *ALTERNARIA HELIANTHI* 29
Summary 29
3.1 Introduction 29
3.2 Materials and Methods 34
3.2.1 Statistical Analyses of Data 34
3.2.2 Experiments to Standardise the Production of Inoculum 35
(i) Determining the effect of culture media on mycelial growth, sporulation and conidial infectivity 35
(ii) Determining the germination and infectivity of conidia obtained from sporulation bands of different age 36
(iii) Determining the effect of spore morphology and germination characteristics on infectivity of conidia 37
(iv) Determining the effect of successive subculturing on the infectivity of conidia 38
(v) Examination of bacteria contaminating cultures of *A. helianthi* 39
(vi) Determining the variation in pathogenicity among isolates 40
3.2.3 Experiments to Establish Optimal Host and Environmental Conditions for Infection 40
(i) Determining the effect of leaf position on lesion size 40
(ii) Determining the effect of the method used to maintain leaf wetness on lesion size 41
(iii) Determining the effect of dew period temperature regimes and duration of dew period on infection 41
(iv) Determining the effect of post-dew period temperature regimes on lesion size 42
(v) Determining the effect of plant density on infection 42
(vi) Determining the effect of inoculum dose on infection of susceptible and resistant lines. 42
3.3 Results 42
3.3.1 Experiments to Standardise the Production of Inoculum 43
(i) Effect of culture media on mycelial growth, sporulation and conidial infectivity 43
(ii) Germination and infectivity of conidia obtained from sporulation bands of different age 44
(iii) Effect of spore morphology and germination characteristics on infectivity of conidia 45
(iv) Effect of successive subculturing on infectivity of conidia 46
(v) Inhibition of *A. helianthi* caused by bacteria isolated from sunflower leaves and *A. helianthi* cultures. 46
(vi) Variation in pathogenicity among isolates 50
3.3.2 Experiments to Establish Host and Environmental Conditions 50
(i) Effect of leaf position on lesion size 50
(ii) Effect of the method used to maintain leaf wetness on lesion size 51
(iii) Effect of dew period temperature regimes and duration of dew period on infection 51
(iv) Effect of post dew period temperature on lesion size 54
(v) Effect of plant density on infection 54
(vi) Effect of inoculum dose on infection of susceptible and resistant lines 54
3.4 Discussion 55

CHAPTER 4 ..... **64**

INITIAL SELECTION OF RESISTANT GERMPLASM 64
Summary 64
4.1 Introduction 64
4.2 Materials and Methods 65
4.3 Results 67
4.4 Discussion 69

CHAPTER 5 ..... **72**

SPATIAL AND TEMPORAL DEVELOPMENT OF ALTERNARIA BLIGHT EPIDEMICS IN SELECTED SUNFLOWER LINES 72
Summary 72
5.1 Introduction 72
5.3 Materials and Methods 78
5.3.1 Field trial sites and trial design 78
5.3.2 Inoculation of plants and assessment of disease 81
5.3.3 Data Analysis 83

5.4 Results 83
5.4.1 Rainfall and temperature data 83
5.4.2 Plant phenologies throughout the rating period 85
5.4.3 Disease progress in time 85
(i) Disease Severity 85
(ii) Disease progress curves 87
(iii) Apparent infection rate 90
(iv) Area under the disease progress curve (audpc) 92
5.4.4 Disease progress in space 93
(i) Disease gradients 93
5.4.5 Disease progress in space and time 94
(i) Surface response maps 94
(ii) Velocity of disease spread and grid volumes 98
5.4.6 Correlations between epidemic parameters 99
5.4.7 Determining the relationship between DSRs for different leaf positions 102
5.5 Discussion 103

CHAPTER 6 ..... **112**

EVALUATION OF RESISTANT RESTORER LINES IN HYBRID COMBINATIONS 112
Summary *112*
6.1 Introduction 112
6.2 Materials and Methods 116
6.2.1 Preliminary selection of R-lines 116
6.2.2. Hybrid production and field trial design 117
6.2.3 Analysis of data 119
6.3 Results *119*
6.3.1 Audpcs of parental lines and F_1 hybrids 119
6.3.2. Differences between F_1 hybrids and midparent audpcs 121
6.3.3. Combined analysis of Variance for Sites 1 and 2. 122
6.3.4. Thousand grain weights and oil contents of hybrids 122
6.4 Discussion 123

CHAPTER 7 ..... **126**

CORRELATION BETWEEN GREENHOUSE AND FIELD RATINGS OF RESISTANCE TO A. HELIANTHI 126
Summary 126
7.1 Introduction 126
7.2 Materials and Methods 128
7.2.1 Growth, inoculation and incubation of plants 128
7.2.2 Genotypes 128
7.2.3 Design of Greenhouse Experiments 130
(i) Experiments involving lines P_1–P_{18} 130
(ii) Experiments involving R-lines, A-lines and their F_1 hybrids. 130
7.2.4 Measuring Components of Quantitative Resistance 131
7.2.5 Field Screening of Sunflower Lines 133
(i) Experiments involving lines P_1–P_{18} 133
(ii) Experiments involving R-lines, A-lines and their F_1 hybrids. 133
7.2.6 Statistical Analysis 133
(i) Experiments involving lines P_1–P_{18} 133
(ii) Experiments involving R-lines, A-lines and their F_1 hybrids. 134
7.3 Results 134
7.3.1 Uniformity of experimental conditions 134
7.3.2 Components of resistance and field severity of alternaria blight 135
(i) Lines P_1–P_{18} 135
(ii) R-lines, A-lines and their F_1 hybrids. 137
7.3.3 Correlations between components of resistance measured in the greenhouse and resistance measured in the field 139
(i) Lines P_1–P_{18} 139

(ii) R-lines, A-lines and their F_1 hybrids 140
6.4 Discussion 141

CHAPTER 8 ..... **148**

GENERAL DISCUSSION 148
Future Research 158

REFERENCES **160**

APPENDIX I **180**

RECIPES OF MEDIA USED IN THIS STUDY 180

APPENDIX II **183**

A TECHNIQUE FOR ESTIMATING THE NUMBER OF MICROORGANISMS IN A SAMPLE 183

APPENDIX III **185**

A PICTORIAL KEY FOR ASSESSING THE PROPORTION OF LEAF AREA INFECTED WITH *A. HELIANTHI* 185

APPENDIX IV **187**

PUBLICATIONS ARISING FROM THE RESEARCH REPORTED IN THIS THESIS. 187