THE DEVELOPMENT OF THE SEXUAL STAGE, PATHOGENIC SPECIALIZATION AND A POTENTIAL MEANS OF CONTROLLING THE UREDINIOPHASE OF SUNFLOWER RUST, *PUCCINIA HELIANTHI* SCHW.

by

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CERTIFICATE

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 any help recieved in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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SUMMARY

Sunflower rust has been present in Australia for over a century. The uredinial stage is common and is the cause of serious yield losses in commercial sunflower crops. The complete life cycle of this macrocyclic autoecious rust has only rarely been seen in Australia and has not been studied in detail. The importance of the sexual cycle in the epidemiology of sunflower rust in Australia is therefore uncertain. The role of the sexual cycle in the origin of new pathotypes of sunflower rust in Australia is also not known. The purpose of the studies reported in this thesis were to i) investigate and describe aspects of the sexual cycle of *Puccinia helianthi* in Australia and ii) study a means of controlling the disease through the manipulation of host genes for resistance.

Teliospores of sunflower rust (*Puccinia helianthi*) were activated to germinate by a cold pre-soak conditioning treatment. This consisted of storing wet telial material at 3-5°C for at least 14 days. This conditioning was suitable for telia freshly collected during summer and for material that had been kept dry at between 3-5°C for up to 6 years. The morphology of metabasidial formation was consistant with 'classical' descriptions for the Puccineacae. Temperature optima for teliospore germination were in the range 17-20°C. The optimum temperature for basidiospore formation was around 17°C. Basidiospore formation at temperatures below this optimum was delayed, while at high temperatures, abnormalities in metabasidial formation and a reduction in production of basidiospores were observed. Germination of teliospores occurred in complete darkness, continuous light and combinations of both light and dark. Germination of teliospores and subsequent metabasidial formation occurred best when the spores were first exposed to 8 or 12h photoperiods before imposition of darkness. Teliospore germination was delayed and decreased when incubation was conducted under continuous darkness or short photoperiods. Exposure to longer photoperiods before imposition of darkness reduced the number of teliospores that produced aberrant metabasidia.

Basidiospores of *Puccinia helianthi* incubated at a range of temperatures on water agar germinated laterally (in relation to the apiculus) to form germ-tubes. Elongation of germ-tubes was greatest between 14-16°C. On sunflower leaves the tip of the germ-tubes appressed against the host cuticle and a narrow penetration peg directly enterred the epidermal cell. An intra-epidermal vesicle formed from which hyphae emerged. The primary hyphae continued to grow intracellularly or intercellularly. The colony derived from a single basidiospore produced a cluster of pycnia. The rate of development

of pycnial colonies was greatest at temperatures between 20-24°C with pycnia erupting after 7 days. Attempts to obtain direct infection of leaf tissue by teliospores incubated at temperatures which suppressed basidiospore formation were not successful.

Expressions of resistance of sunflower lines to infections from basidiospores varied. Colony development in the sunflower line HA-R3 was usually arrested at the stage of intra-epidermal vesicle formation or primary branching. Single infected epidermal cells became necrotic. In the sunflower line HA-R1 colonies were aborted at various stages of development.

Infection of sunflower seedlings was obtained by sowing seed into soil inoculated with teliospores. Teliospores exposed on leaf material on the soil surface during late winter - early spring germinated readily . By October over 90% of teliospores had germinated *in situ*.

Nuclear staining and light microscopy was used to study the cytology of teliospore formation, karyogamy, teliospore germination and infections from basidiospores. The karyotype of *Puccinia helianthi* was not observed and actual chromosomal behaviour could not be determined accurately. The sequence of events during karyogamy and meiosis followed the published reports for other Pucciniaceae. Basidiopsores were binucleate but the uninucleate condition was quickly attained in the intra-epidermal vesicles formed after infection.

Forty teliospore accessions collected from the field were used to produce basidiospores which were inoculated onto 14 sunflower lines. The collections produced a range of infection types on the sunflower lines. It was concluded that on the basis that virulence genes in basidiospores are the same as in urediniospores that it could be postulated that the imminent susceptibility of certain sunflower hybrids could have been predicted if they had been screened by infections from basidiospores.

Preliminary selfing studies within three collections of teliospores showed that the collections were homozygous for avirulence to S37-388RR and CM29 and homozygous virulent on a selection of Hysun 33. Aeciospores obtained by bulk crossing pycnia on 70096 segregated for virulence when re-inoculated on 70096.

The introduction in recent years of sunflower hybrids with new sources of rust resistance has been associated with the identification of new pathotypes of sunflower rust. One such hybrid was used in the breeding of a new rust differential sunflower inbred line in a backcrossing program. Eleven distinct pathotypes of sunflower rust were identified by using thirteen public and proprietry inbred sunflower lines.

Eleven sunflower hybrid cultivar mixtures were assessed in two field trials in Central Queensland for their ability to yield compared to the pure-line components in the absense of sunflower rust. At Clermont, equivalent oil yields for the mixtures ranged from 86.4 to 110.5% of that of

the means of the respective pure-line components. The combined mean yield advantage for all eleven mixtures was 97.4% of that of the mean of individual components. The highest yielding mixture out-yielded the best pure-line. None of the mixtures provided lower yields than the lowest yielding pure-line. At Gindie, the range in equivalent oil yield was 86.6 to 135.7% of that predicted with a combined mean advantage for the eleven mixtures of 107.7% of that predicted. The different rankings of the mixtures between sites suggested that some adaption to the environments at each site may have been involved.

The yield of one sunflower hybrid mixture, Pacmix XV, was compared to its pure-line components and a number of commercial sunflower hybrids in a further three field trials at Clifton, Felton and Gowrie Junction in Southern Queensland. At Clifton, where the trial was stressed by drought Pacmix XV outyielded each component and the two commercial checks. Equivalent oil yield was 112.4% of that predicted. In the absence of obvious environmental stresses at Felton there were no significant differences ($P>0.05$) in equivalent oil yield among the treatments. However, Pacmix XV yielded 96.9% of that predicted. At Gowrie Junction the yield of Pacmix XV was 100.9% of that predicted.

The severity of rust on plants was assessed in the trials at Clifton, Felton and Gowrie Junction either by direct estimation of the percentage of leaf area covered by uredinia and/or indirectly by using simple impaction rods for trapping urediniospores in the air surrounding the plants. Poor plant growth at Clifton precluded the use of spore trapping. Direct assessment of disease levels showed that percentage of leaf area affected by rust was lower on Pacmix XV than that predicted from the mean of the leaf severities for the four pure-line components. At Felton disease epidemics did not develop to levels that made differences in leaf severity of rust among lines easily distinguishable. Calculation of the 'Area under the Curve' (AUC) as estimated by the number of spores trapped on each occasion showed that significantly more urediniospores ($P<0.05$) were trapped in plots of the commercial hybrid Cargill Dynamite than in plots of any other variety. The AUC for Pacmix XV was not significantly different from that predicted ($P>0.05$) although it was actually 78.9% of the predicted AUC.

More extensive data was collected from the trial at Gowrie Junction. The total leaf area of the plants of each treatment was estimated. Direct assessments of the severity of rust on leaves were made at weekly intervals from budding to completion of anthesis. Spore trappings were conducted at 3 day intervals from budding to physiologic maturity. AUC's calculated from urediniospores trapped showed that the cumulative spore count for Pacmix XV was not significantly different ($P>0.05$) from that predicted. Cumulative counts of urediniospores trapped was strongly correlated ($r>0.9$) with severity of leaf symptoms.

The cumulative spore yield curves were transformed using three mathematical models and regression analyses were applied. The logistic equation, Gompertz equation and Weibull frequency function provided coefficients of correlation(r) >0.9. The slopes of the transformed lines (b) were used to represent the rate of disease increase. The rate was greatest for Dynamite and least for Pac 392 and that of Pacmix XV no different from the rate predicted.

In the studies reported in Chapter 7 it was shown that a simultaneous or prior inoculation with an avirulent pathotype of *P. helianthi* reduced the infection efficiency of a virulent pathotype. This reduction in infection efficiency increased as the interval between the two inoculations increased. The virulent pathotype produced less than 30% of the number of sori formed on the control when the interval between the two inoculations was six days. In a competition study on a cultivar to which both pathotypes were virulent it was found that the two pathotypes quickly reached a stable frequency equilibrium that was maintained over five successive generations.

The residual effects of a 'defeated' resistance gene were examined by comparing the reaction of two near-isogenic lines to two virulent pathotypes of *P. helianthi*. The latent period was longer for both pathotypes on the isoline that contained the defeated R1 gene for resistance, S37-388RR, than its isoline S37-388 which does not possess any known genes for resistance. A non-significant reduction ($P>0.05$) in infection efficiency was recorded in S37-388RR for both pathotypes. The effect of genetic background on infection efficiency of *P. helianthi* was examined by inoculating five different sunflower lines all of which were thought to possess the R1 gene for resistance. Significant differences ($P<0.05$) in infection efficiency and uredinial diameter occurred among the lines. Uredinia were larger and more numerous on CM90RR and smallest and fewest on the sunflower line 69-17-8-1-1.

An attempt was made to obtain asexual recombinations for virulence by inoculating susceptible sunflower lines with mixtures of urediniospores of different pathotypes. The progeny of all four mixtures that went through five generations of co-infections were screened for the presence of recombinants. None were detected.

A small field trial was conducted to obtain an indication of the importance of autoinfection by rust in sunflowers. Susceptible sunflower plants were grown either surrounded by immune plants or other susceptible plants. At physiological maturity the density of sori on the susceptible plants surrounded by immune plants was 28% that of those surrounded by susceptible plants.

PREFACE

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K.C. Goulter.

TABLE OF CONTENTS