# CHAPTER 6

# EFFECTS OF MIXTURES OF SUNFLOWER HYBRIDS CONTAINING DIFFERENT SOURCES OF RESISTANCE TO *PUCCINIA HELIANTHI* ON YIELD AND RUST DEVELOPMENT

## 6.1 INTRODUCTION

Modern agricultural practices are designed to minimize inputs to provide maximum monetary returns to the producer. These practices can involve intense cultivation, heavy fertilizer applications and the use of herbicides and irrigation to reduce the effects of enviromental constraints on crop production. Monotypic monocultures allow greater synchronization and specialization of these farming practices (Marshall,1977).

There are hazards associated with genetic homogeneity in cropping systems (Marshall,1977; Barrett,1981; Adams, Ellingboe and Rossman,1971). The reduction of interspecific and intraspecific diversity through the widespread culture of similar pure line cultivars exposes these lines to an increased risk of epidemics of plant diseases. The widespread use of genes conditioning high levels of resistance to diseases applies selection pressures on the pathogen populations and gives any virulent pathotypes which might evolve a competitive advantage over avirulent pathotypes (Day,1978).

Plant breeders have responded to the evolution of new pathotypes by introducing new genes for resistance which protect the new or established cultivars. If new cultivars are widely adapted and as a consequence are widely grown they may be exposed to a diversity of pathogen populations which again applies selection pressure for virulent pathotypes (Leonard and Czochor,1980). For example, Stevens and Scott(1950) estimated that the average life of rust resistant oat cultivars in the United States was only 4-5 years. The effect of the recurrent introduction of new sources of resistence on the appearance of new virulent pathotypes of plant rusts has been described by Johnson(1961) as "man-guided evolution".

Despite the often ephemeral nature of the pathotype specific types of resistance they have been widely used as a means of controlling plant rusts because they are usually oligogenic and inherited as a dominant character. They are therefore easy to manipulate in breeding programmes. Under conditions of disease pressure plants possessing this type of resistance produce superior yields over susceptible lines with similar yield potentials under disease-free conditions. Pathotype specific resistance conferred by a new resistance source cannot be considered to be ephemeral until a virulent pathotype appears.For example, the interspecific coffee hybrid "Hibrido de Timor", which was first discovered in 1927 in East Timor, was resistant to all the 30 known pathotypes of *Hemileia vastatrix* Berk.Br. recognized in 1984 (Rodrigues,1984).

On-farm profitability must be considered when deciding on possible control measures for rust diseases. Littlefield(1981) reviewed the success and limitations of quarantine and eradication, cultural practices, microbiologic control agents and chemical treatments. Fungicides can provide control when genetic mechanisms are overcome but their use imposes another economic cost as well as possible enviromental hazards. The other techniques are not of use or have not been shown to be effective against many rusts. Manipulation of the host through plant breeding currently offers the best approach of controlling rust and thereby obtaining reliable yields.

Oligogenic resistance to rust fungi in pure host lines has generally been ephemeral. It has however provided an insight into how the frequency of genes for virulence in populations of obligate pathogens respond to changes in the resistance genes in the host population (Person, Groth and Mylyk,1976; Kiyosawa,1980; Wolfe, Barrett, Shattock, Shaw and Whitbread,1976). This knowledge has raised the possibility that by controlling the genetic composition of the host population that the composition of the pathogen can be manipulated, even managed (Person *et al*,1976; Wolfe and Barret,1980). The general aim of managing genetic composition of host lines is to ensure that epidemics do not develop to levels that affect the economy of the crop. Approaches that can be used for disease management include i) cycling of resistance genes, ii) inter and intra-regional deployment of resistance genes, iii) the use of effective pathotype non-specific resistance, iv) tolerance, v) multigenic cultivars, vi) multiline cultivars and/or vii) combinations of any of these.

Cycling of resistance genes in time in the host population is based on the premise that once a gene has been overcome it should be withdrawn until the frequency of the corresponding virulence gene in the pathogen population diminishes to a level where the resistance gene can be reintroduced to provide effective disease control (Stevens,1949). For this strategy to work it is essential that the virulence gene does not become fixed in the pathogen population. Wolfe(1984) plotted the frequency of the gene Mla 12 for resistance in barley to *Erysiphe graminis* DC f.sp. *hordei* Marchal against the frequency of the matching pathogenicity gene Va 12 in the years 1967-1983. There were two cycles during that time where the increasing area grown to cultivars possessing the Mla 12 gene resulted in the rapid increase in frequency of the Va 12 virulence gene. The subsequent restriction in use of Mla 12 decreased the frequency of Va 12. This approach to disease management requires intensive analysis of the frequency of virulence genes. Virulence genes should not become fixed in a population in the absence of the corresponding specific resistance genes.

Regional deployment of resistance genes can be on an inter-regional or intra-regional basis. Many of the plant rusts have the capacity for long range aerial dispersal and can move from region to region during a season. If different resistance genes are used in cultivars of a crop in each region

then the moving inoculum can be exposed to disruptive selection if the virulence genes from one region are at a selective disadvantage in another. Inter-regional deployment of different resistance genes has been suggested as a means of reducing the danger of epidemics of cereal rusts along the "Puccinia path" of North America (Knott,1972; Littlefield,1981).

Intra-regional deployment of resistance genes may be useful where inoculum does not have the capacity for wide dissemination or serial plantings of crops within the region are common. The later crops should have different resistance genes to that used in the earlier crops. Problems with intra-regional gene deployment include the regulatory and economic impositions that may be neccessary to achieve co-operation.

Pathotype-nonspecific resistance is what van der Plank(1963) referred to as "horizontal resistance". Ideally this type of resistance should be effective against all pathotypes and act to reduce or delay the rate of epidemic development. In pathosystems involving rust fungi it is often referred to as "slow rusting" (Wilcoxson,1981). There is a basic resistance/susceptibility interaction in all hosts once pathotype specific resistance has been overcome which will condition damage to the host and the rate and extent of pathogen development (Browning,1981). The components of this interaction that influence the development of epidemics include, i) the latent period between infection and production of secondary inoculum from that infection ii) the infection efficiency iii) the sporulation rate and capacity and iv) the infectious period.

Pathotype-nonspecific resistance is generally considered to be polygenically inherited since so many different mechanisms are involved (Parlevliet,1978; Johnson and Wilcoxson,1979). It is also considered to be durable (Johnson,1978) since it is assumed that by not applying strong selection pressures the probability that a new more aggressive pathotype will predominate is reduced. Person *et al*(1976) and Nelson(1979) point out that populations of organisms will contain individuals with differing fitnesses to survive and among these may be some with a competitive advantage on hosts with pathotype-nonspecific resistance. Many workers have shown that some pathotypes or strains within pathotypes quickly predominate when a mixture of pathotypes is used to inoculate a susceptible host (Brown and Sharp,1970; Irish,1950; Browder,1965). Other workers have shown that some pathotype-specificity may be expressed on host lines thought to possess pathotype-nonspecific resistance (Clifford and Clothier,1974; Parlevliet,1977; Rouse, Nelson, MacKenzie and Armitage,1980). Mussell(1980) warned that fields that support some epidemic development may act as inoculum sources for nearby fields. Care must be used before deciding that breeding for pathotype nonspecific resistance is the panacea for all diseases.

Tolerance is implied when a cultivar supports compatible-type infections but suffers smaller yield reductions than another cultivar with a similar severity of disease (Schafer,1971). Simons(1969,1972) identified

tolerance to *Puccinia coronata* Cda. var. *avenae* Fraser and Led. among oat species. The utilization of tolerance in managing the losses caused by rust diseases would require education of growers to accept that the presence of disease in the tolerant crops would not be reducing yield significantly. As yet tolerance is poorly understood and difficult to conclusively identify and manipulate.

Multigenic resistance requires that several genes each conditioning pathotype-specific resistance are combined into one cultivar (Watson and Singh,1952). It is also referred to as "pyramiding" resistance genes (Green,1975). More genetic changes for acquisition of virulence genes are required in the pathogen before a virulent pathotype forms therefore the rate and probability that this happens should be less (Day,1974; van der Plank,1968). The multigenic resistant cultivar should remain resistant longer. Person, Groth and Mylyk(1976) argued that although a multigene cultivar should be initially effective it was also applying selection for the extremely rare pathotype with matching virulence. They believed that there was a danger of underestimating the variability within pathogen populations as many genotypes may be maintained at very low frequencies. The durability of any multigenic cultivar would be dependent on the capacity of the pathogen to recombine virulence genes and the ability of these recombinants to survive in the population until selection pressure is in their favour.

The multiline approach to managing plant diseases has its basis in avoiding intra-crop genetic homogeneity with respect to genes for resistance. This can be achieved by creating mechanical mixtures of near-isogenic lines each possessing different genes for rust resistance. Jensen(1965) defined multilines as " re-constitutable composites of phenotypically similar, genetically dissimilar lines". Multilines are created by back-crossing desirable genes into superior recurrent parents (Borlaug and Gibler,1953). The terms line mixtures, variety mixture or cultivar mixtures are used for mixtures of non-isogenic pure-lines, varieties or cultivars (Wolfe,1985).

Mixtures have been proposed as a means of attaining predictable and consistant yields by Simmonds(1962) and Jensen(1965). Allard and Bradshaw (1964) divided the mechanisms by which mixtures produce stability of performance into i) individual buffering which is the product of the developmental and physiological flexibility of individuals in a population and ii) population buffering which is that buffering resulting from interactions between different genotypes. Heterogenous populations are expected to use both forms and be more stable. Marshall and Brown(1973) concluded that when the yield of a mixtures surpassed the mean yield of the components then a nett positive interaction had occurred. To outperform the highest yielding component the interaction effects must be sufficient to compensate for the lower yielding components. Since the relative dominance of components within a mixture can vary in different enviroments (Harlan and Martin,1938) it is possible that the performance stability of a mixture of

lines with such dissimilar yield responses could surpass that of the most stable pure-line (Marshall and Brown,1973). In some situations better-adapted lower yielding components may "out-compete" higher yielding components to the detriment of the mixture. Wolfe(1985) described this as the "Montogomery effect" after Montogomery(1912) had recorded the phenomenon. Suneson(1949) also demonstrated this phenomenon when after repeated sowings of a mixture a line with better yield and lower leaf disease levels in pure stands became practically extinct.

Tozzetti(1767) made the earliest recorded observation of the efficacy of interspecific mixtures in reducing rust infection when he questioned " *why wheat growing seeded with rye, or with vetch, was not damaged by the rust, while a field of wheat alone, standing between one of rye and one of vetch yielded scarsely any seed*". Jensen(1952) proposed that multilines or mixtures may have a role in stabilizing the performance of cultivars in disease situations. Suneson(1960) and Browning *et al*(1964) demonstrated the yield superiority of mixtures of cereals over the expected yields when exposed to rust epidemics. Wolfe(1978) found that over a number of trials the mean yield advantage of barley mixtures over the component means increased from 1-2% to 3-4% when trials were infected with powdery mildew (*Erysiphe graminis* DC f.sp.*hordei* Marchal).

Multilines and mixtures have been divided into either "clean crop" or "dirty crop" depending on the susceptibilities of the components to the local pathogen populations. In the "clean crop" approach as advocated by Borlaug(1953) all components are resistant to all prevalent pathotypes of the pathogen to be controlled. If a new pathotype occurs to which one component is susceptible then only a fraction of the mixture is affected (Marshall,1977). Susceptible plants whose growth is retarded may also be at a competitive disadvantage against disease-free neighbours for exploitation of available resources. The disease-free plants, in an example of individual buffering (Allard and Bradshaw,1964), may then compensate for the diseased plants. At the earliest opportunity breeders could replace the susceptible component with a resistant one or remove it totally from the mixture. The "clean crop" approach therefore reduces the deleterious effect that the occurrence of a new virulent pathotype will have on yield but it also requires that breeders have access to a range of effective resistance genes. The effective resistance genes are also simultaneously exposed to the pathogen population. This may or may not favour selection for new pathotypes.

In the "dirty crop" approach proposed by Browning and Frey(1969) each component line possesses at least one gene conferring pathotype specific resistance but none of the lines are completely resistant to all known pathotypes of the pathogen. Theoretical simulation models have been used to predict the potential usefulness of the "dirty crop" approach for disease control (Marshall and Pryor,1978; Barrett,1981).

Success of the "dirty crop" approach depends on stabilizing

selection (*sensu* van der Plank,1963) operating to allow "simple" pathotypes with the least number of unnecessary virulence genes to predominate. Since each component would be infected by the pathotype with virulence to it the remaining components would act as spore traps to reduce inoculum spread. The advantages of the "dirty crop" approach include the extension of the usefulness of genes for resistance and because components need not be isolines then mixtures can be constituted of lines with varying levels of resistance to several diseases (eg. Aufhammer *et al*,1984). The disadvantages are that stabilizing selection may not operate and more complex pathotypes or "super races" (Groth,1976) virulent on more components will predominate and reduce the effectiveness of the mixture.

There are no published data on the efficacy of cultivar mixtures of sunflower hybrids to control sunflower rust and provide reliable yields. The studies reported in this chapter investigated: i) the yields of cultivar mixtures of sunflower hybrids compared to those of the component hybrids and ii) the efficacy of these cultivar mixtures in reducing the rate of epidemic development of *Puccinia helianthi*.

## 6.2 General Materials and Methods

Mixtures of different hybrid sunflower cultivars were used since isolines were not available for this study. The sunflower hybrids chosen all shared a common maternal parent and therefore had at least half their genomes in common. All cultivar mixtures were constituted to provide an equal field establishment of each component i.e. equiproportional. Seedling establishment tests were first conducted to achieve equal establishment of each component. Two hundred seeds of each component were divided into four replicates of fifty seeds. These were planted by hand at a depth of 5cm into field plots at Pacific Seeds, Toowoomba. The percentage of normal seedlings i.e. with a healthy growing tip was determined 14d after sowing. Establishment varied from around 60% to 85% between seed lots of each hybrid with low readings invariably coming from seed stocks held longest in storage. The establishment percentages were then used to determine the seed numbers necessary to give each component an equal opportunity of establishing in the experimental plots. Seeds of component lines were mixed mechanically before sub-packeting for sowing.

All trials were sown using a two-row cone seeder. Excess seed was used and the trials later thinned to the plant populations recommended for each trial area. Plant size was not considered during the thinning process in order to avoid biasing the composition of the plot in favour of components with greater or lesser seedling vigour. Each component should have then been present in the plot at an equal frequency and with a random distribution.

The datum rows were harvested using a small self-cleaning self-propelled header when all plants in the experimental plots had matured

and dried. The weight of grain from the datum rows of each plot was recorded in the field. Sub-samples of grain were taken back to the laboratory where they were cleaned of debris and dried for 3h at 130°C. The percentage oil content at dry weight was then determined on cooled samples using a Newport MKIV Nuclear Magnetic Resonance (NMR) Quantity Analyser.

Observed yields of the cultivar mixtures were also expressed as percentages of the yields predicted as calculated from the yields of the constituent components. The formula for estimating grain yield (Ey) was:

$$Ey = \frac{\sum Oi}{n}$$

where Oi = observed yield of the ith component and n = number of constituent components. Similarly the formula for estimated oil yield (Eo) was:

$$Eo = \frac{\sum Ooi}{n}$$

where Ooi = observed oil yield of the ith constituent component. Estimating oil content of the cultivar mixtures was complicated by the necessity to account for the grain contribution of each component and the percentage oil content of that grain. The formula for calculating estimated oil content (Ec) was:

$$Ec = \sum_{i=1}^{n} (Oi/T).Oo$$

where T = combined grain yield of n constituent components, Oo = observed oil content of the ith component and Oi = observed grain yield of the ith component.

Direct assessments of severity of rust infections were made during growth by using the pictorial field assessment keys developed by Siddiqui, Brown and Allen(1975). Twenty plants were randomly selected from the datum rows of each plot and the proportion of the abaxial surface of two leaves on each plant occupied by sori of *Puccinia helianthi* was determined. The severity of disease for each plot was obtained by summing all individual assessments and dividing by the total number of leaves assessed. The pictorial keys developed by Schneiter and Miller(1981) were used when determining the growth stages of plants.

Direct assessment of severity of disease in multilines and cultivar mixtures can be complicated by the wide variation in severity that may be present between different individuals in the mixture. Spore trapping was used in some experiments reported in this chapter as an indirect method of comparing the amount of disease in each plot.

The spore traps used were simple rods that trapped spores through wind impaction. The plastic rods were 6cm long and 4mm in diameter and a circumference of 12.566mm which were mounted in 16mm rubber stoppers (Figure 6.1). Gregory(1951) recommended rods of around 5mm for ease of handling.

Smaller diameter rods were used since it was also shown that the efficiency of rods as spore traps increased with decreasing diameter. The trapping surfaces used were clear cellophane rectangles 15 x 20mm which were mounted 5mm below the tip of the rods, the long axis of the rectangle parallel to the length of the rod. Adherence was obtained by first exposing a cellophane rectangle to high humidity and then wrapping it around the rod using thumb and forefinger. The 2mm overlap of cellophane adhered to itself. The humidity was provided by either a brief exposure to steam or longer exposure in the bottom of a Petri dish with a moist filter paper disc in the lid. Once mounted the trapping surface was coated with silicone grease (General Electric G-697) by rotating the rod between thumb and forefinger which were smeared with the lubricant. Excess grease was removed by repeating the procedure between thumb and middle finger to which no grease had been applied. Preparation of spore traps was conducted in a Laminer Flow Hood. Each prepared rod was inverted and sealed in a test tube using the rubber stopper.

In the field each spore trap was mounted in the end of a 75cm length of rigid 15mm PVC pipe (actual dimensions, 18mm internal and 22mm external diameters). The internal circumference of the end of the pipe was bevelled to aid insertion of the rubber stopper. To allow for height adjustment of the spore trap the mounting pipe was fitted into a 1m length of 20mm PVC pipe (actual dimensions, 23mm internal diameter). Rubber o-rings (15mm diameter) were stretched and fitted into grooves cut into the exterior of the inner pipe. The rubber rings provided sufficient friction to hold the inner mounting pipe in position while allowing freedom of movement for telescopic extension (Figures 6.1 and 6.2). The spore traps were positioned at the average height of the base of the capitula of neighbouring plants. This height varied between cultivars.

Reports in the literature described urediniospores of various rust fungi reaching peak concentration of the air spora during the morning (Kramer and Pady,1966) or around noon (Hirst,1953; Sreeramulu,1959; Savary,1986). Preliminary results using a Rotorod spore sampler (Edmonds,1972) showed that on five dates between December 1986 and March 1987 the concentration of urediniospores of *Puccinia helianthi* in field plots peaked between 0930 and 1130h (author, *unpublished data*). Spore traps were therefore exposed in field plots in a set sequence in the morning and collected in the same sequence later in the day. The time that the first trap was placed in its holder and later returned to its test tube were recorded.

On return to the laboratory the trapping surface was peeled from the rod and mounted sporiferous surface upwards on a clean microscope slide smeared with petroleum jelly. A clean 2cm square coverslip was then applied and gently pressed down to flatten the trap. The number of uredinospores was counted in five 2mm wide transects perpendicular to the long axis of the rectangle by using the field of view at 100x magnification of a compound

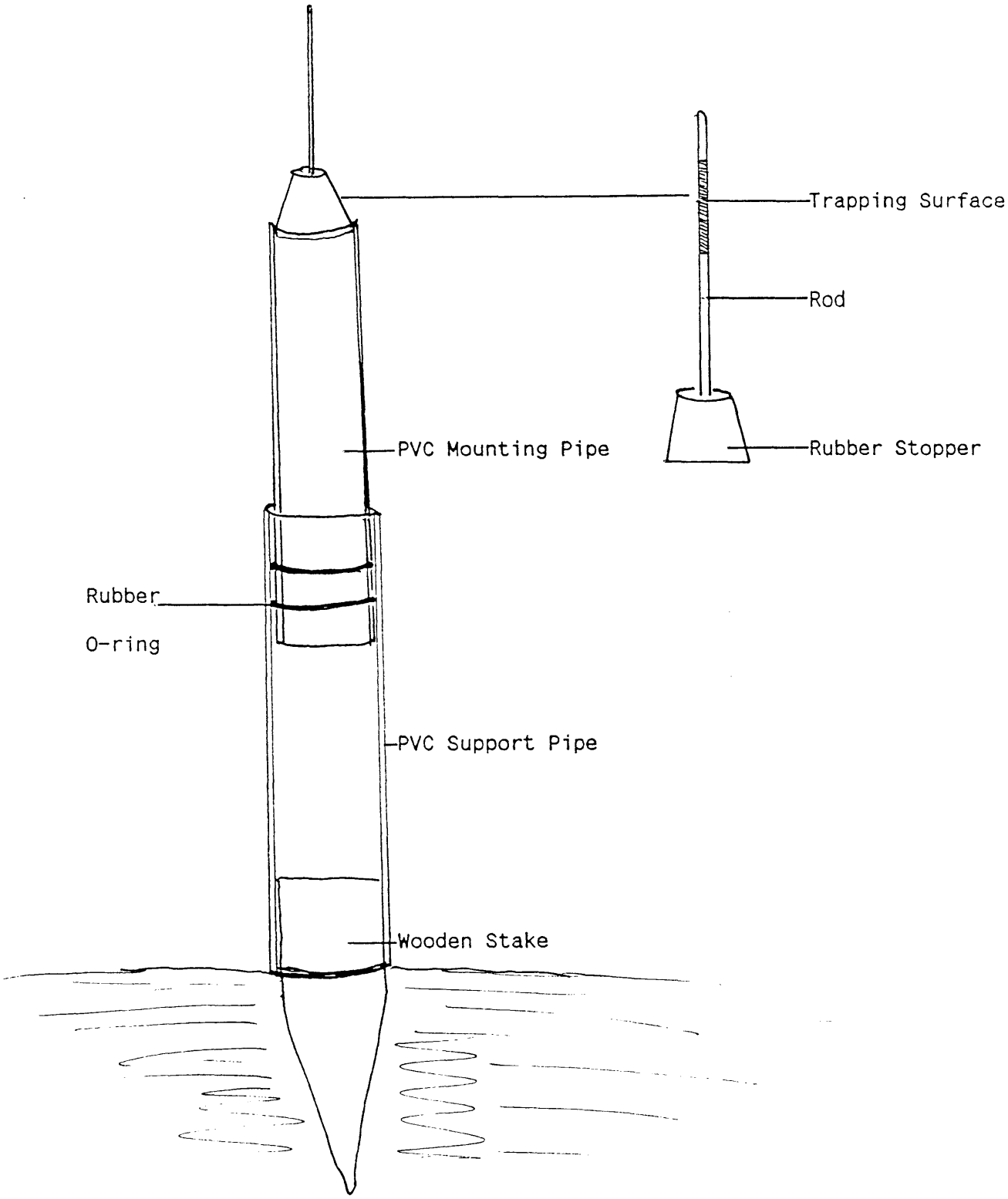



Figure 6.1 Design of the simple wind-impaction spore traps used.

(not to scale)

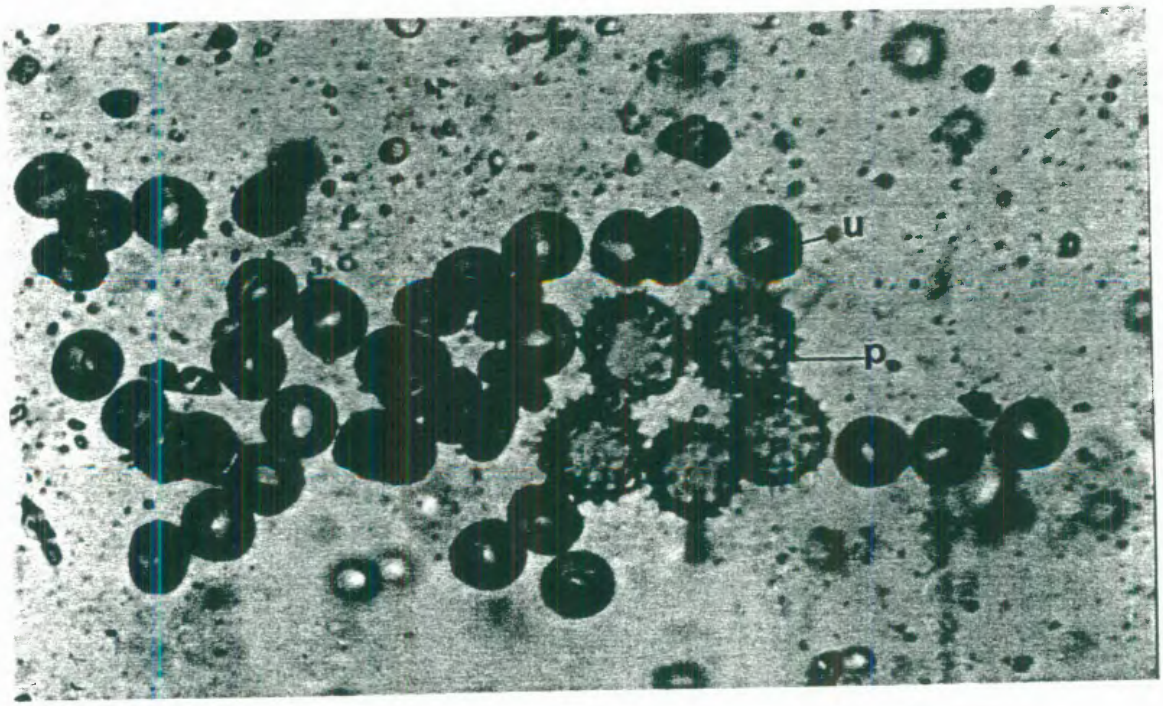



Figure 6.2. a). A simple spore trap in field plot b). A cluster of urediniospores trapped (u) + five sunflower pollen grains (p)

microscope. The first transect was positioned 1mm from the edge of the rectangle and a 2mm gap was left between each transect. This left the final transect 1mm from the other end of the rectangle. This resulted in the number of urediniospores in 125.66mm$^2$ being assessed (i.e. 5 transects by 2mm per transect by circumference of trapping surface around the rod - 12.566mm). The number of urediniospores counted on each occassion for each cultivar was converted to the average number per mm$^2$ per hour of exposure (Xm) using the formula:

$$Xm = \frac{Xi}{125.66 \times t \times n}$$

where Xi - total number of spores counted for n replicates of cultivar i

t - duration (h) of trapping

Data from repetitive trapping at intervals gave data for plotting curves of spores trapped against time. Comparison of the total spore trapping for each cultivar was made by determining an Area Under the Curve (AUC) calculated by adopting the Area Under the Disease Progress Curve formula of Shaner and Finney(1977). The formula derived by trapezoidal integration was:

$$AUC = \sum ( Y_i + Y_{i+1}) / 2 \times dt_i$$

where $dt_i$ = time interval between assessments $Y_i$ and $Y_{i+1}$

$Y_i$ = disease assessment; here spores trapped.

In the trials where epidemic development in the plots was to be monitored using spore trapping techniques it was desirable that interplot movement of urediniospores be minimal. Two rows of hybrid sunflower were planted between plots in the same block and each block was separated by a buffer of the same hybrid. Buffers of a similar length were placed on the front and back of the trials and six rows were planted on the outside of the trials. The hybrids were chosen as buffers because they had a large canopy that would help intercept urediniospore movement between plots. The hybrids chosen as buffers were also considered to be resistant to the prevalent pathotypes of sunflower rust. The buffer would not therefore contribute to the epidemic development in the plots. The rust-free buffer also allowed a means of moving between plots without disturbing the plants in the plots. Minimal interference with inoculum dispersal was therefore achieved.

Direct visual assessments of rust severity were made in conjunction with spore trapping in the Felton and Gowrie Junction trials. Visual assessments were made on the day following a spore trapping. The plots were not entered at any other time. This was done to avoid excessive movement in the plots which could affect inoculum spread and subsequent epidemic development.

In the analyses, the "Expected" or predicted value for the cultivar mixture was calculated from the mean of the results for the component cultivar in each replicate and was included as a treatment. Analyses were performed using the MSTAT Computer package (Michigan State University). Comparison and separation of means were obtained with an Analysis of Variance

and Range Test, respectively. Plots and regressional analyses were obtained with the Plot function.

## 6.3 EXPERIMENTAL

### 6.3.1. A comparison of the yielding abilities of mixtures of sunflower hybrid cultivars and of the component pure lines in the absence of disease.

Two field trials were conducted to obtain a comparison of the yielding ability of mixtures of sunflower hybrid cultivars and of the component pure-lines. It was hoped that an epidemic of sunflower rust would develop at one of the sites and preferably at only one site. This would allow comparison of the responses of the cultivar mixtures under diseased and non-diseased situations.

Materials and Methods.

The trials were conducted on the Central Queensland Highlands during Autumn 1986. One trial was conducted on the property "Homlea Downs" belonging to Mr. R. Otto at Clermont, 106km north of Emerald. The second trial was conducted on the property "Enderly" belonging to Mr. W. Gardiner at Gindie, 23km south of Emerald. Both trials were situated in commercial sunflower fields. The surrounding crops were Pacific Seeds Hysun 22 at Clermont and Pacific Seeds Hysun 33 at Gindie.

The four hybrids chosen to be components were all single-cross hybrids and had different phenotypes for reaction to sunflower rust (author, *unpublished data*). They were:

I Hysun 31 - susceptible to all pathotypes known at the time
II Hysun 32 - resistance to Races 0 and 3, susceptible to Race 1
III Pac 378 - resistance to Races 0,1 and 3
IV Pac 380 - resistance to Races 0 and 1, susceptible to Race 3

Eleven equiproportional mixtures of the four cultivars were prepared based on estimated seedling establishment (Table 6.1).

The trial at Clermont was planted on the 3rd March 1986 and at Gindie on 4th March 1986. The experimental design at each was identical and consisted of four replicates arranged according to a randomized complete block design. Each block consisted of fifteen treatments; the four component hybrids and the eleven mixtures. Individual treatment plots were of four rows with an inter-row spacing of 1m. Plot length was 9m at Clermont but only 6m at Gindie where plot length was reduced to allow the trial to fit between contour banks. On 24th March 1986 plots at both sites were thinned manually to one plant every 25cm to give a plant density of 40000/ha. Irrigation was not available at either site and rust epidemics were allowed to develop naturally.

Table 6.1 The composition of the eleven equiproportional cultivar mixtures used in this experiment.

| Treatment | % Component in Mixture | | | |
|---|---|---|---|---|
| | I(Hysun 31) | II(Hysun 32) | III(Pac 378) | IV(PAC 380) |
| I | 100 | — | — | — |
| II | — | 100 | — | — |
| III | — | — | 100 | — |
| IV | — | — | — | 100 |
| I-II | 50 | 50 | — | — |
| I-II-III | 33 | 33 | 33 | — |
| I-II-III-IV | 25 | 25 | 25 | 25 |
| I-II-IV | 33 | 33 | — | 33 |
| I-III | 50 | — | 50 | — |
| I-III-IV | 33 | — | 33 | 33 |
| I-IV | 50 | — | — | 50 |
| II-III | — | 50 | 50 | — |
| II-III-IV | — | 33 | 33 | 33 |
| II-IV | — | 50 | — | 50 |
| III-IV | — | — | 50 | 50 |

The plots were inspected at mid-bud (G.S.R3), flowering (G.S.R5.1 - 5.8) and physiological maturity (G.S.R9) stages of plant growth so that disease assessments could be made.

Results.

Rust epidemics had not developed in any of the treatments at either site by the time the plants reached physiological maturity. Trace levels (<0.5% leaf surface) were present but these levels were too low to permit accurate assessments. No other diseases were observed to be present. The results represent therefore the yield responses of the cultivar mixtures to two enviroments in the presence of minimal disease.

The yield data for the two sites are presented in Tables 6.2 and 6.3 together with the relative performances (expressed as a percentage) of the cultivar mixtures compared to that predicted from the observed results of the components. The results showed that, in the absence of disease, the yields of the cultivar mixtures of sunflower hybrids were competitive with pure line hybrids. Mixtures that contained Component III (Pac 378) in general yielded at or above the expected level (99-110% expected oil yield). Exceptions occurred when this component was in combination with Component II (Hysun 32) which performed poorly.

The data from the trial at Gindie supported the contention that the yield from cultivar mixtures can be competitive with the yield of pure lines. The lower equivalent yields obtained at Gindie were due to it being more

Table 6.2 Performance of eleven equiproportional cultivar mixtures and the four component sunflower hybrids grown at Clermont[1].

| Treatment | Yield (kg/ha) | | Percentage of Predicted | | Oil Content Observed (%) | Oil Content Predicted (%) | Oil Yield (kg/ha) | | Percentage of Predicted | Ranking |
|---|---|---|---|---|---|---|---|---|---|---|
| I Hysun 31 | 857.8 | *cde* | ___ | *a* | 47.4 | ___ | 406.6 | *bcdef* | ___ | |
| II Hysun 32 | 673.3 | *e* | ___ | *bcde* | 45.3 | ___ | 305.0 | *f* | ___ | |
| III Pac 378 | 1055.6 | *ab* | ___ | *e* | 44.0 | ___ | 464.4 | *ab* | ___ | |
| IV Pac 380 | 916.7 | *abcd* | ___ | *bc* | 45.6 | ___ | 418.0 | *abcde* | ___ | |
| I-II | 722.2 | *de* | 94.3 | *bcd* | 45.5 | 46.5 | 328.6 | *ef* | 92.4 | 10 |
| I-II-III | 913.3 | *abcd* | 106.0 | *bcd* | 45.5 | 45.5 | 415.6 | *abcde* | 106.0 | 4 |
| I-II-III-IV | 885.5 | *bcde* | 101.0 | *cde* | 44.6 | 45.5 | 394.9 | *bcdef* | 99.1 | 5 |
| I-II-IV | 725.6 | *de* | 88.9 | *bc* | 45.6 | 46.1 | 330.9 | *def* | 87.9 | 9 |
| I-III | 979.4 | *abc* | 102.3 | *bcd* | 45.5 | 45.5 | 445.6 | *abc* | 102.3 | 2 |
| I-III-IV | 937.8 | *abc* | 99.4 | *bcd* | 45.5 | 45.5 | 426.7 | *abcd* | 99.3 | 3 |
| I-IV | 843.3 | *cde* | 95.1 | *b* | 46.2 | 46.5 | 389.6 | *bcdef* | 94.5 | 6 |
| II-III | 830.0 | *cde* | 95.9 | *cde* | 44.9 | 44.5 | 372.7 | *bcdef* | 96.9 | 7 |
| II-III-IV | 833.3 | *cde* | 94.5 | *cde* | 44.6 | 44.9 | 371.7 | *cdef* | 93.9 | 8 |
| II-IV | 694.4 | *e* | 87.3 | *cde* | 45.0 | 45.5 | 312.5 | *f* | 86.4 | 11 |
| III-IV | 1100.6 | *a* | 111.6 | *de* | 44.3 | 44.7 | 487.6 | *a* | 110.5 | 1 |
| | | | | | | | | Mean | 97.4 | |

1. Means in each column followed by at least one lower case letter in common do not differ significantly ($P>0.05$).
2. Coefficients of Variation. cv. for equivalent grain yield = 15.4%
cv. for percentage oil content = 1.7%

Table 6.3. Performance of eleven equiproportional cultivar mixtures and the four component sunflower hybrids grown at Gindie[1].

| Treatment | Yield[2,3] (kg/ha) | Percentage of Predicted | Oil Content Observed (%) | Oil Content Predicted (%) | Oil Yield[3] (kg/ha) | Percentage of Predicted | Ranking |
|---|---|---|---|---|---|---|---|
| I Hysun 31 | 395.8 | ___ | 50.0 *ef* | ___ | 198.0 | ___ | |
| II Hysun 32 | 395.8 | ___ | 53.9 *a* | ___ | 213.1 | ___ | |
| III Pac 378 | 489.6 | ___ | 47.6 *h* | ___ | 233.3 | ___ | |
| IV Pac 380 | 510.4 | ___ | 51.5 *de* | ___ | 262.1 | ___ | |
| I-II | 520.8 | 131.6 | 53.1 *ab* | 52.0 | 278.9 | 135.7 | 2 |
| I-II-III | 479.2 | 112.2 | 51.5 *cde* | 50.3 | 246.7 | 114.9 | 7 |
| I-II-III-IV | 432.3 | 96.5 | 51.6 *cde* | 50.6 | 223.8 | 98.8 | 9 |
| I-II-IV | 427.1 | 98.4 | 52.9 *abc* | 51.8 | 225.7 | 100.6 | 8 |
| I-III | 380.2 | 85.9 | 49.1 *fgh* | 48.7 | 186.7 | 86.6 | 11 |
| I-III-IV | 432.3 | 92.9 | 49.5 *fg* | 49.7 | 214.9 | 92.9 | 10 |
| I-IV | 489.6 | 108.1 | 50.5 *def* | 50.8 | 246.2 | 107.0 | 5 |
| II-III | 546.9 | 123.5 | 51.2 *de* | 50.4 | 280.7 | 125.8 | 1 |
| II-III-IV | 489.6 | 105.2 | 51.3 *de* | 50.8 | 251.4 | 106.5 | 6 |
| II-IV | 520.8 | 114.9 | 51.9 *bcd* | 52.5 | 270.6 | 113.9 | 3 |
| III-IV | 520.8 | 104.2 | 48.3 *gh* | 49.6 | 251.5 | 101.5 | 4 |
| | | | | | Mean | 107.7 | |

1. Means in each column followed by at least one lower case letter in common do not differ significantly (P = 0.05)›
2. Coefficients of Variation cv. for equivalent grain yield = 19.96%
   cv. for percentage oil content = 1.96%
3. There were no statistically significant differences in these yield parameters at the 5% level of significance.

drought stressed than the trial at Clermont. The lower yields and other field variability resulted in the trial having a higher coefficient of variation for grain yield and subsequently no statistically significant differences ($P>0.05$) were found between mean yields. However it can be seen from Table 6.3 that only one mixture performed worse than the worst pure-line in regard to oil yield while three were better than the best pure-line. Eight of the cultivar mixtures produced a higher oil yield than predicted from the yields of the components. In the other three mixtures (I-III, I-III-IV, I-II-III-IV) the reduced oil yield was due to lower than expected grain yields.

Table 6.2 and Table 6.3 also give a ranking of the observed oil yields as a percentage of expected oil yield at the two sites. The rankings show that there was almost a complete reversal in order with some mixtures yielding poorly at Clermont but yielding well at Gindie and *vice versa*. Usually the mixtures that yielded best also yielded better than predicted. The mixtures with the greatest increase over the predicted oil yield at Clermont included hybrids III (Pac 378) or IV (Pac 380) or both as components. At Gindie hybrids I (Hysun 31) or II (Hysun 32) or both were components of the top three yielding mixtures.

### 6.3.2 The effect of cultivar mixtures of sunflower hybrids on the rate of disease development of *Puccinia helianthi* and on yield.

The use of multiline cultivars or cultivar mixtures comprised of pure-lines possessing different genes for specific resistance to rust fungi has been proposed as a means of producing "synthetic horizontal resistance" by Browning and Frey(1969). They suggested that the diversity of resistance genes in the host composite would reduce the rate of epidemic development in the composite as a whole and therefore be comparable to the effect of "slow-rusting" pure lines on epidemic development. Three field trials were conducted therefore to test whether a 'dirty crop' cultivar mixture of sunflower hybrids resistant and susceptible to sunflower rust reduced epidemic development of sunflower rust. A comparison was also made to examine the ability of the mixture to yield relative to the pure line components.

A single equiproportional cultivar mixture (here-in named Pacmix XV) was prepared from four component single-cross sunflower hybrids chosen because each had different phenotypes for rust reaction. The hybrids chosen also had greater reliability of yield than those used in the Central Queensland trials (Pacific Seeds, *unpublished data*). The components used were:

I Hysun 31 - susceptible to all pathotypes known at the time
II Pac 354 - resistance to Races 0 and 3, susceptible to Races 1 and 1,3.
III Pac 388 - resistance to Races 0,1 and 3, susceptible to Race 1,3
IV Pac 392 - resistance to all Races then identified

Also included in some of the trials were the commercial hybrids

Pacific Seeds Hysun 33, Cargill Dynamite and Cargill Thunder. Dynamite has been described as possessing some "slow-rusting" characteristics (J.K. Kochman, *pers.comm.*).

### 6.3.2.1 Clifton Trial

Materials and Methods.

One field trial was conducted on the eastern Darling Downs during Summer and Autumn 1987. Three potential sites were chosen in the Wyreema, Cambooya and Clifton areas south of Toowoomba. The trial was conducted on the property "Kia-Ora" belonging to Mr. K. Bange, 4km west of Clifton following sufficient planting rain. It was planted on 16 January 1987 on land that had been cropped to wheat the previous winter (i.e. 'double-cropped') so Di Ammonium Phosphate fertilizer (DAP) at the rate of 60kg/ha was incorporated as a side-band near the seed.

The seven treatments in the trial were the cultivar mixture, the four constituent components and two commercial hybrids Pacific Seeds Hysun 33 and Cargill Dynamite. The experimental design consisted of three replicates arranged according to a randomized complete block design. Individual plots within each block were of eight rows each 7m long with an inter-row spacing of 76cm. The sunflower hybrid used as buffer was Dekalb 610.

The plots were thinned on 5 February 1987 to give one plant every 25cm to give a plant density of approximately 50000/ha. Plants were also removed from the central 75cm section of the middle two rows of each plot to give a 2.3m x 0.75m clearing where the rod spore traps were to be positioned in each plot. Plants were also removed from a 60cm wide walkway orientated perpendicular to the rows. This walkway connected the central clearing to the buffer rows between plots.

Irrigation was not available and the trial was planted on negligible sub-soil moisture. The first rainfall after planting fell on 2 March 1987 and was recorded as 30mm at Clifton township. By that time (44 days after sowing) the plants were highly drought stressed and had differentiated buds. The rain allowed the plants to survive to maturity but was too late to allow significant increase in the size of the plants. Average plant height was only 60cm at anthesis. Spore trapping was not used because the small stature and reduced canopy of the plants was considered to be insufficient to reduce interference from external inoculum influxes. Visual assessments of the severity of leaf infections were therefore performed.

Disease severity assessments were made on 4 March 1987 when plants were at mid-bud (G.S.R3), 19 March 1987 when varieties varied from commencing anthesis (G.S.R5.0) to having completed 50% anthesis (G.S.R5.5) and 10 April 1987 as the plants approached physiological maturity (G.S.R8-R9).

Results.

The results are presented in Table 6.4. The severity of rust infection increased rapidly from budding to anthesis especially in the two hybrids Hysun 33 and Dynamite.

Table 6.4 Severity of rust caused by *P.helianthi* on leaves of six pure-line sunflower hybrids and a cultivar mixture at three growth stages [1]

| | Days After Sowing | | | | | |
|---|---|---|---|---|---|---|
| | 47 | | 62 | | 84 | |
| Cultivar | Growth Stage | Severity (%) | Growth Stage | Severity (%) | Growth Stage | Severity (%) |
| Hysun 31 | R3 | 3.03 *b* | R5.0 | 17.0 *b* | R8 | 8.13 *bc* |
| Pac 354 | R3 | 1.43 *b* | R5.5 | 3.10 *c* | R9 | 5.17 *bc* |
| Pac 388 | R3 | 1.13 *c* | R5.0 | 3.33 *c* | R8 | 5.0 *bc* |
| Pac 392 | R3 | 0.07 *c* | R5.4 | 1.57 *c* | R8 | 2.67 *c* |
| Pacmix XV | R3 | 0.53 *c* | R5.3 | 4.60 *c* | R8 | 3.0 *c* |
| Expected | — | 1.41 *b* | — | 6.25 *c* | — | 5.24 *bc* |
| Hysun 33 | R3 | 4.93 *a* | R5.0 | 24.0 *ab* | R9 | 11.0 *b* |
| Dynamite | R3 | 5.67 *a* | R5.5 | 22.33 *a* | R9 | 20.33 *a* |

1. Means in each column followed by at least one lower case letter in common do not differ significantly (P>0.05).

At each assessment the percentage severity for Pacmix XV was less than the mean of the four constituent components (0.53 vs 1.42% at day 47, 4.6 vs 6.25% at day 62 and 3 vs 5.24% at day 84). The decline in rust severity in Hysun 31, Hysun 33 and Dynamite at final assessment coincided with the senescence of the lower more heavily infected leaves.

The yield data taken from the four central rows revealed that the two most heavily infected hybrids Hysun 33 and Dynamite produced the lowest yields. Each yield parameter for Dynamite was significantly less (P<0.05) than the cultivar mixture Pacmix XV which showed the highest mean yield (Table 6.5). Variability in the data (coefficient of variation for equivalent yield = 22.5%) resulted in some large differences in mean yields for each cultivar not being statistically significantly different (P>0.05). The cultivar mixture Pacmix XV returned the highest equivalent grain yield and equivalent oil yield of all entries. Grain yield of Pacmix XV was 113.5% of

Table 6.5 Yield performance of six sunflower hybrids and a cultivar mixture grown at Clifton (Autumn 1987)[1]

| Cultivar | Equivalent Yield (kg/ha) | Oil Content (%) | Equivalent Oil Yield (kg/ha) |
|---|---|---|---|
| Hysun 31 | 296.0 *a* | 47.6 *b* | 140.8*ab* |
| Pac 354 | 263.0 *ab* | 48.5 *ab* | 127.6*abc* |
| Pac 388 | 285.9 *a* | 49.0 *a* | 140.1*ab* |
| Pac 395 | 306.8 *a* | 48.3 *ab* | 148.3*ab* |
| Pacmix XV | 326.7 *a*(113.5%)[3] | 47.8 *ab* | 156.4*a*(112.4%)[3] |
| Expected[2] | 287.9 *a* | 48.3 *ab* | 139.2*ab* |
| Hysun 33 | 225.2 *ab* | 44.9 *c* | 101.1 *bc* |
| Dynamite | 157.6 *b* | 45.9 *c* | 72.5 *c* |
| c.v.(%) | 22.5 | 1.69 | |

1. Means in each column followed by at least one lower case letter in common do not differ significantly ($P > 0.05$).
2. Expected results based on yields of the constituent components.
3. Difference in yield of Pacmix relative to that of the predicted yield.

that predicted from the mean of the grain yields of the four constituent components. Oil yield was 112.4% of that predicted. The slight depression in percentage improvement in equivalent oil yield as compared to that recorded for grain yield is the result of a lower than expected oil content of the grain.

6.3.2.2 Felton Trial

Materials and Methods.

This trial was planted on 28 October, 1987 on the property "Mayfield" belonging to Mr. R. Free at East Felton, South-East Queensland. The experimental design was alterred from that described for the previous trial by i) including four replicates,ii) increasing inter-row spacing to 90cm and row length to 8m and iii) using the sunflower hybrid Pacific Seeds Hysun 44 as buffer.

Plots were thinned 22d after sowing to give a plant density equivalent to 50000/ha. Since a wider row spacing was used plants were not removed from the central area of each test plot. Instead, leaves within 40cm of the spore trap were removed at a later date. Plants and leaves were removed from within the plot to provide a walkway from the buffer rows beside the plot to the site of the spore trap.

Spore traps were first exposed in the test plots on 4 January, 1988 when the first hybrid to flower, Cargill Dynamite, had reached 50% anthesis. Trapping continued at 6-7 day intervals for a total of 6 occasions (Table 6.6) and concluded when all treatments were senescing (G.S. R.9)

Table 6.6. Spore trapping dates and durations for Felton Cultivar Mixture Trial.

| Trap Date | Days after Sowing | 1st trap Exposed | 1st trap Collected | Exposure (h) |
|---|---|---|---|---|
| 4 Jan,88 | 68 | 0930 | 1630 | 7 |
| 11 Jan,88 | 75 | 0930 | 1630 | 7 |
| 18 Jan,88 | 82 | 0930 | 1630 | 7 |
| 26 Jan,88 | 89 | 0930 | 1600 | 6.5 |
| 2 Feb,88 | 96 | 0900 | 1630 | 7.5 |
| 8 Feb,88 | 103 | 0930 | 1630 | 7 |

Results.

A sunflower rust epidemic failed to develop. Since disease severity did not excede 1% leaf coverage in any of the plots direct assessments were not applied. The results from spore trapping are presented in Table 6.7.

Significantly more urediniospores ($P<0.05$) were trapped in plots of Dynamite than in any other treatment as illustrated by the calculation of the Area under the Curve (AUC) (Table 6.7). More spores were trapped in plots of Hysun 31 and Hysun 33 than in any of the remaining pure-lines. The AUC for spore production in Pacmix XV was 78.9% of that predicted but was not significantly different ($P>0.05$) from the AUC for the predicted estimate. Cumulative data of spore counts provided a better indication of differences in urediniospore production over time than individual assessments (Figure 6.3). The data was transformed by setting the greatest total spore count for all the cultivars at 100%. In this example the line was Dynamite with a grand total of 1.7123 urediniospores trapped per $mm^2$ per hr. The accumulated counts for each cultivar at each assessment were then calculated as a percentage of that count. At the last assessment therefore the percentage for each cultivar indicated the reduction in total urediniospores trapped as compared to Dynamite. It can be seen from Figure 6.3 that rust development proceeded most rapidly in Dynamite. The curves for Dynamite and Hysun 31 were similar up to 82 days post-sowing. The curves then become divergent with the curve for Dynamite increasing at a greater rate. The curve for the accumulation of urediniospores in plots of Hysun 33 progressed parallel to that of Hysun 31. It commenced at a lower level and consequently concluded at a lower level.

Table 6.7 Number of urediniospore trapped in six sunflower hybrids and a sunflower hybrid mixture grown at Felton, January-February, 1988.

| Cultivar | Flowering Time[1] | Days after Sowing 68 | 75 | 82 | 89 | 96 | 103 | AUC[4] |
|---|---|---|---|---|---|---|---|---|
| Hysun 31 | 73 | 13.4[2,3] | 116.4 | 113.4 *a* | 352.6 *b* | 486.2 *b* | 212.3 *ab* | 1103.3 *b* |
| Pac 354 | 73 | 3.1 | 73.6 | 50.3 *bc* | 168.3 *d* | 266.3 *c* | 125.0 *c* | 544.6 *d* |
| Pac 388 | 75 | 3.1 | 43.1 | 31.5 *c* | 162.5 *d* | 254.9 *c* | 102.0 *c* | 479.5 *d* |
| Pac 392 | 74 | 6.8 | 29.8 | 17.9 *c* | 124.6 *d* | 259.9 *c* | 116.8 *c* | 439.4 *d* |
| Pacmix XV | 74 | 3.4 | 30.5 | 32.7 *c* | 185.5 *d* | 237.7 *d* | 144.9 *abc* | 492.5 *d* |
| Expected | — | 6.6 | 65.7 | 53.3 *bc* | 202.0 *cd* | 316.8 *c* | 139.0 *bc* | 624.1 *cd* |
| Hysun 33 | 75 | 3.7 | 51.4 | 67.6 *abc* | 307.3 *bc* | 400.8 *bc* | 223.4 *a* | 829.9 *bc* |
| Dynamite | 69 | 18.2 | 118.4 | 88.4 *ab* | 543.9 *a* | 718.6 *a* | 224.8 *a* | 1395.4 *a* |
| c.v.% | — | 120.3 | 70.5 | 55.2 | 28.3 | 24.2 | 30.9 | 20.2 |

1. Flowering Time. Days after sowing to reach 50% anthesis.
2. Data $10^{-3}$ . $h^{-1}$ urediniospores
3. Means in each column followed by at least one lower case letter in common do not differ significantly ($P>0.05$)
4. AUC - Area under curve, spores trapped vs. time.

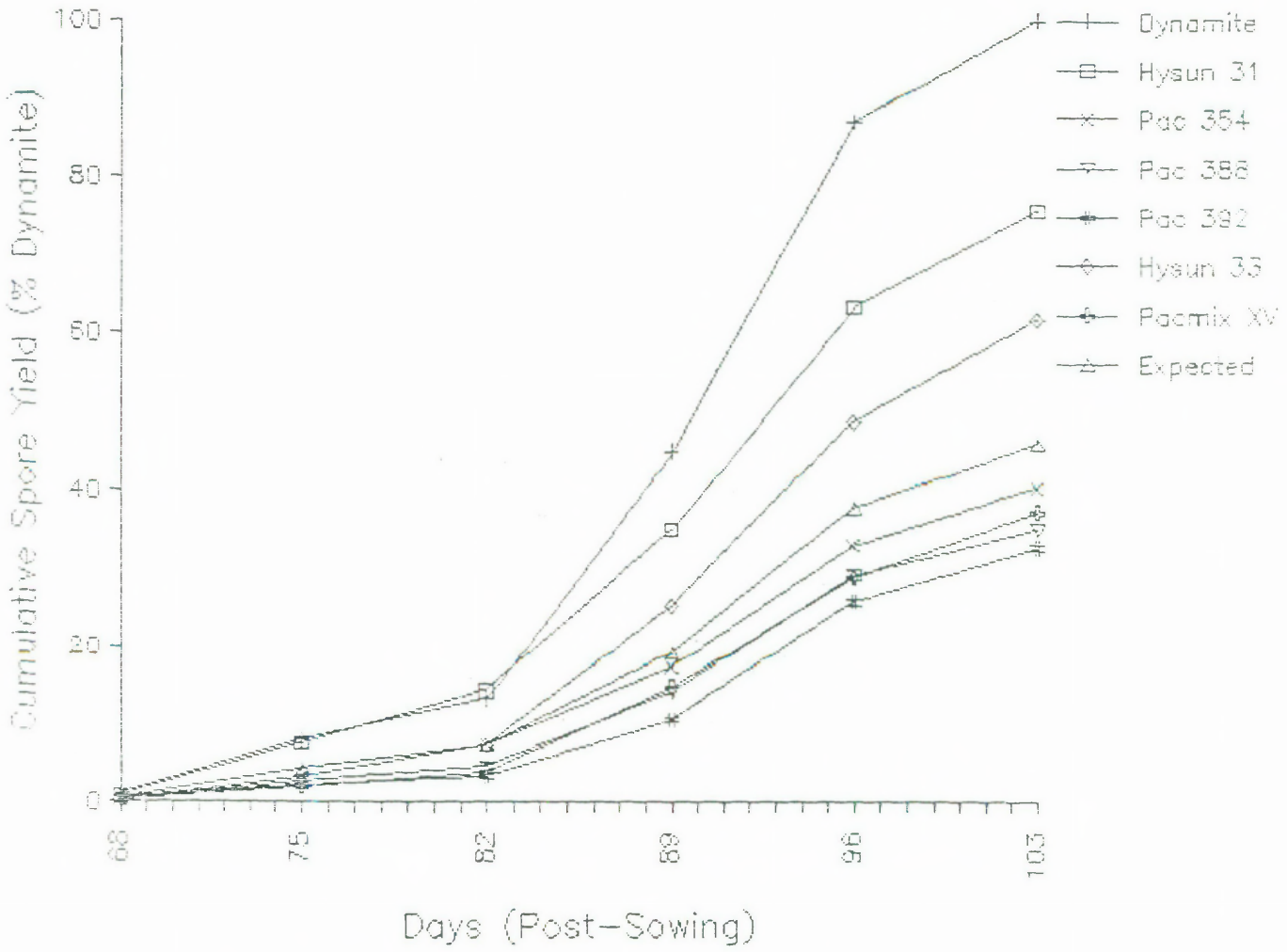



Figure 6.3. Graph of accumulated spore counts against time
Total spore count for Dynamite has been arbitarilly set at 100%

The level of disease development as assessed by spore trapping was lowest in the group of hybrids Pac 354, Pac 388 and Pac 392 and the cultivar mixture Pacmix XV. In each of these cultivars the cumulative spore count was between 20-30% that of Dynamite.

It can be seen by comparing Table 6.5 and Table 6.8 that the Felton site was a higher grain yielding site than the Clifton site. All treatments yielded well at the Felton site. Grain yield of the cultivar mixture was 97.6% of that predicted and was only greater than one (Hysun 31) of the pure-line components. The percentage oil content was also lower than expected and consequently oil yield was lower. The contribution of Pac 388 to oil content of grain samples of Pacmix XV may not have been as great as expected. The oil content of grain of at least one other component in the mixture may have been greater than expected. These hypotheses could not be tested since it was impossible to differentiate and separate the grain of each component from the sample of Pacmix XV.

Table 6.8 Yield performance of six sunflower and a cultivar mixture grown at Felton (Summer,1988).[1]

| Cultivar | Equivalent Yield (kg/ha) | Oil Content (%) | Equivalent Oil Yield (kg/ha) |
|---|---|---|---|
| Hysun 31 | 2204 ns | 50.4 *b* | 1113 ns |
| Pac 354 | 2362 | 50.7 *b* | 1197 |
| Pac 388 | 2344 | 52.2 *a* | 1222 |
| Pac 392 | 2385 | 50.8 *b* | 1216 |
| Pacmix XV | 2268 (97.6%) | 50.7 *b* | 1150 (96.9%)[3] |
| Expected[2] | 2324 | 51.0 *b* | 1187 |
| Hysun 33 | 2313 | 50.9 *b* | 1177 |
| Dynamite | 2191 | 51.2 b | 1150 |
| c.v.(%) | 8.4 | 1.29 | |

1. Means in each column where a significant difference existed followed by at least one lower case letter in common do not differ significantly (P>0.05)
2. Expected results based on yields of the constituent components.
3. Difference in yield of Pacmix XV and the predicted yield.

6.3.2.3 Gowrie Junction Trial

Materials and Methods.

This trial was planted on 2 February 1988 on a block of land share-farmed by Mr. G. Ingleton at Gowrie Junction, 10km from Toowoomba. The experimental design was the same as the previous trial except that interrow spacing was reduced to 76cm and an extra treatment, the sunflower hybrid Cargill Thunder, was included. This hybrid was considered to be highly susceptible to sunflower rust. The trial was surrounded by a crop of Pacific Seeds Hysun 24. Plots were thinned to give a plant density equivalent to 50000/ha.

More extensive data was collected at the Gowrie Junction trial site so that a better understanding of disease development and spore trapping could be obtained. Plant height, leaf number and leaf area assessments were made for each pure-line cultivar at anthesis. Plant height was measured by taking the mean distance from soil surface to the point of connection of peduncle to capitulum for ten plants. Leaf number and area was determined by harvesting four plants from each plot. A 1.2m stick was laid from each end of the rows second from the outside of each plot. The plant closest to the end of the stick in the plot was cut at ground level. In the laboratory the

number of pre-senescent leaves was counted. The position of the leaves were determined by counting leaf scars on the stem. Leaf area was estimated from the product of length by width by the correction factor of 0.7 (English,1976).

The leaf area occupied by sori and total leaf area for each cultivar was used to estimate the total area of sori per plant. Visual assessments of rust severity were made on six occasions between budding and completion of anthesis in each cultivar.

Spore traps were first exposed in the test plots on 8 April 1988 (67 days post sowing) and continued at 3 day intervals for a total of 16 occasions (Table 6.9). At the time of completion all cultivars were past the stage of physiologic maturity (G.S.R.9).

Table 6.9 Spore trapping schedule for Gowrie Junction cultivar mixture trial.

| Trap Date | Days after Sowing | 1st Trap Exposed | 1st Trap Collected | Exposure Time (h) |
|---|---|---|---|---|
| 8 Apr,88 | 67 | 0845 | 1600 | 7.25 |
| 14 Apr,88 | 73 | 0845 | 1630 | 7.75 |
| 17 Apr,88 | 76 | 0830 | 1645 | 8.25 |
| 21 Apr,88 | 80 | 0900 | 1545 | 6.75 |
| 24 Apr,88 | 83 | 0830 | 1630 | 8.0 |
| 27 Apr,88 | 86 | 0845 | 1615 | 7.5 |
| 30 Apr,88 | 89 | 0845 | 1645 | 8.0 |
| 3 May,88 | 92 | 0900 | 1615 | 7.25 |
| 6 May,88 | 95 | 0845 | 1645 | 8.0 |
| 9 May,88 | 98 | 0845 | 1630 | 7.25 |
| 12 May,88 | 101 | 0845 | 1645 | 8.0 |
| 15 May,88 | 104 | 0830 | 1630 | 8.0 |
| 20 May,88 | 109 | 0845 | 1645 | 8.0 |
| 23 May,88 | 112 | 0845 | 1645 | 8.0 |
| 26 May,88 | 115 | 0900 | 1600 | 7.0 |
| 29 May,88 | 118 | 0830 | 1645 | 8.25 |

Results.

The data in Table 6.10 shows that more lower leaves had senesced and died in some hybrids than others. This occurred in cultivars with the greatest number of leaves. Consequently at anthesis all cultivars had similar numbers of leaves. Total leaf area per plant differed between the cultivars. The greatest was 5680 $cm^2$ for Hysun 33 and the least 3386 $cm^2$ for Thunder.

Sunflower rust was most severe in Thunder at all assessments from budding onwards (Table 6.11). The average leaf area coverage in this

hybrid was 0.52% at budding (64 days after sowing). A similar disease severity was not recorded until flowering (77-84 days) in Hysun 31, Hysun 33

Table 6.10 Leaf area assessments at anthesis for cultivars in Gowrie Junction sunflower cultivar mixture trial

| Cultivar | Plant Height (cm) | Lowest Remaining Leaf | Highest Leaf | Number of Leaves | Total Leaf Area ($cm^2$/plant) |
|---|---|---|---|---|---|
| Hysun 31 | 115 | 12 | 31 | 19 | 4964.96 |
| Pac 354 | 104 | 6 | 26 | 20 | 4334.41 |
| Pac 388 | 130 | 11 | 30 | 19 | 4646.27 |
| Pac 392 | 120 | 8 | 28 | 20 | 4628.81 |
| Hysun 33 | 140 | 15 | 37 | 22 | 5682.99 |
| Dynamite | 104 | 6 | 24 | 18 | 3622.99 |
| Thunder | 105 | 13 | 30 | 17 | 3386.14 |

and Dynamite or until post-anthesis (90days) in Pac 354 and Pacmix XV. This level of disease was not obtained with Pac 388 and Pac 392. Disease severity in the cultivar mixture was identical to that predicted at 3 assessments (70, 77, 84 days) and was lower at 90 and 99 days.

The two most heavily rusted lines, Thunder and Dynamite, also had the smallest total leaf area per plant (Table 6.10). Thus the actual area occupied by sori per unit area of plot would have been reduced in these cultivars. The leaf area occupied by sori at anthesis gave the following results: Thunder 163$cm^2$, Dynamite 49.3$cm^2$, Hysun 33 31.3$cm^2$,Hysun 31 30.8$cm^2$, Pac 388 11.2$cm^2$, Pac 354 8.7$cm^2$ and Pac 392 8.3$cm^2$. The different areas of sporulative tissue were expected to be reflected in numbers of urediniospores released and trapped.

The results from the spore trappings at Gowrie Junction are presented in Table 6.12. Similar trends in Area under the Curve (AUC) were found at Gowrie Junction and Felton. The AUC's for Dynamite and Hysun 31 were significantly greater ($P<0.05$) than for the other cultivars except Thunder. The AUC for Pacmix XV was 98.4% of that predicted. Spore yields fluctuated between trapping dates. For example, spore yields at 95 days were lower than those at 92 days. Day 95 was a very calm day with persistent fogs whereas day 92 was a fine day with light breezes. These factors would account for the reduced liberation and dispersal of urediniospores.

The cumulative spore yields expressed as a percentage of the total for Dynamite are given in Figures 6.4 and 6.5. Figure 6.4 shows the high degree of susceptibility of Cargill Thunder relative to the other cultivars. Figure 6.5 shows the rapid accumulation of uredinic spores that occurred in Dynamite and Hysun 31. The curves for Hysun 33, Pac 354, Pacmix XV and the

Table 6.11 Severity of sunflower rust on leaves of seven pure-line sunflower hybrids and a cultivar mixture.

| | Days After Sowing | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 64 | | 70 | | 77 | | 84 | | 90 | | 99 | |
| | G.S. | Severity (%) | G.S. | Severity (%) | G.S. | Severity (%) | G.S. | Severity (%) | G.S. | Severity (%) | G.S. | Severity (%) |
| Hysun 31 | 3 | 0.11 | 4 | 0.2 | 4 | 0.31 | 5.5 | 0.62 | 6 | 1.52 | 6 | 2.68 |
| Pac 354 | 4 | 0.07 | 5.2 | 0.15 | 5.4 | 0.16 | 5.9 | 0.2 | 6 | 0.61 | 6/7 | 1.1 |
| Pac 388 | 2 | 0.01 | 3 | 0.05 | 4 | 0.11 | 5.1 | 0.24 | 5.8 | 0.31 | 6 | 0.39 |
| Pac 392 | 3 | 0.03 | 4 | 0.03 | 4 | 0.12 | 5.6 | 0.18 | 5.8 | 0.22 | 6 | 0.29 |
| Pacmix XV | 2/3 | 0.09 | 3/4 | 0.11 | 4/5.1 | 0.18 | 5.5/6 | 0.31 | 6 | 0.59 | 6 | 0.77 |
| Expected | _ | 0.06 | _ | 0.11 | _ | 0.18 | ___ | 0.31 | _ | 0.67 | _ | 0.87 |
| Hysun 33 | 2 | 0.07 | 3 | 0.1 | 4 | 0.25 | 5.2 | 0.55 | 5.9 | 1.0 | 6 | 1.22 |
| Dynamite | 4 | 0.14 | 5.2 | 0.29 | 5.5 | 0.89 | 5.9 | 1.36 | 6 | 3.85 | 6/7 | 7.88 |
| Thunder | 3 | 0.52 | 4 | 1.29 | 5.1 | 2.45 | 5.5 | 4.82 | 6 | 5.9 | 6 | 10.91 |

Table 6.12 Numbers of urediniospore trapped in seven sunflower hybrids and a sunflower hybrid mixture grown at Gowrie Junction, April-May 1988.

Days After Sowing

| Cultivar | Ft[1] | 67 | 73 | 76 | 80 | 83 | 86 | 89 | 92 | 95 |
|---|---|---|---|---|---|---|---|---|---|---|
| Hysun 31 | 84 | 106.5[2] *abc* | 203.8 *bc* | 472.2 *b* | 163.6 *bc* | 274.3 *bc* | 338.5 *c* | 523.0 *bc* | 648.2 *bc* | 211.4 bc |
| Pac 354 | 79 | 84.3[3] *ab* | 79.3 *bcd* | 262.2 *c* | 247.8 *b* | 235.0 *bc* | 236.4 *cd* | 218.2 *cd* | 401.5 *cd* | 59.4 d |
| Pac 388 | 86 | 47.2 *c* | 26.4 *d* | 158.2 *c* | 131.8 *c* | 106.9 *c* | 273.3 *cd* | 61.2 *d* | 272.5 *d* | 91.8 d |
| Pac 392 | 84 | 64.8 *bc* | 66.2 *cd* | 106.6 *c* | 86.3 *c* | 64.9 *c* | 84.1 *d* | 145.0 *cd* | 171.2 *d* | 40.3 d |
| PacmixXV | 84 | 71.9 *bc* | 67.8 *cd* | 234.2 *c* | 233.5 *b* | 237.7 *bc* | 245.9 *cd* | 139.0 *cd* | 403.1 *cd* | 75.8 d |
| Expected | __ | 73.8 *bc* | 66.7 *cd* | 236.6 *c* | 157.3 *bc* | 176.1 *bc* | 249.1 *cd* | 244.0 *cd* | 357.5 *d* | 101.2 cd |
| Hysun 33 | 86 | 73.3 *bc* | 81.1 *bcd* | 226.0 *c* | 110.5 *c* | 136.0 *c* | 285.7 *cd* | 201.0 *cd* | 407.2 *cd* | 84.1 d |
| Dynamite | 77 | 63.4 *bc* | 217.2 *ab* | 639.0 *b* | 241.1 *b* | 430.5 *b* | 697.4 *b* | 654.3 *b* | 896.5 *b* | 283.0 b |
| Thunder | 84 | 133.9 *a* | 359.2 *a* | 951.9 *a* | 367.0 *a* | 923.9 *a* | 1277.2 *a* | 1168.6 *a* | 1556.2 *a* | 540.9 a |
| c.v.% | | 37.8 | 66.0 | 34.8 | 29.4 | 55.8 | 37.1 | 65.4 | 28.1 | 44.1 |

Table 6.12 cont.

Days After Sowing

| Cultivar | 98 | 101 | 104 | 109 | 112 | 115 | 118 | AUC[4] |
|---|---|---|---|---|---|---|---|---|
| Hysun 31 | 574.9 *b* | 922.6 *bc* | 90.3 *bc* | 17.2 *b* | 67.4 *b* | 77.9 *a* | 45.7 *a* | 14874.2 *b* |
| Pac 354 | 258.8 *c* | 528.5 *cd* | 57.5 *c* | 23.1 *b* | 25.6 *b* | 17.9 *de* | 14.5 *b* | 8602.7 *c* |
| Pac 388 | 200.9 *c* | 493.4 *cd* | 33.6 *c* | 13.2 *b* | 41.0 *b* | 27.3 *cde* | 7.0 *b* | 6669.5 *c* |
| Pac 392 | 92.2 *c* | 192.5 *d* | 50.0 *c* | 22.6 *b* | 22.4 *b* | 7.7 *e* | 16.7 *b* | 4361.4 *c* |
| PacmixXV | 254.4 *c* | 523.0 *cd* | 82.3 *bc* | 18.9 *b* | 53.7 *b* | 31.8 *cd* | 32.9 *ab* | 8364.2 *c* |
| Expected | 295.3 *b* | 534.4 *cd* | 57.7 *c* | 20.4 *b* | 41.1 *b* | 29.9 *cd* | 19.1 *b* | 8497.7 *c* |
| Hysun 33 | 231.6 *c* | 721.5 *bc* | 58.7 *c* | 10.2 *b* | 68.4 *b* | 47.5 *bc* | 28.0 *ab* | 8626.9 *c* |
| Dynamite | 640.7 *b* | 1125.1 *b* | 128.6 *ab* | 14.4 *b* | 49.8 *b* | 68.5 *ab* | 13.3 *b* | 20283.3 *b* |
| Thunder | 1256.3 *a* | 2003.8 *a* | 181.1 *a* | 57.5 *a* | 136.3 *a* | 64.5 *ab* | 46.5 *a* | 34444.4 *a* |
| c.v.% | 33.0 | 42.9 | 44.6 | 74.4 | 53.3 | 32.8 | 63.4 | 26.5 |

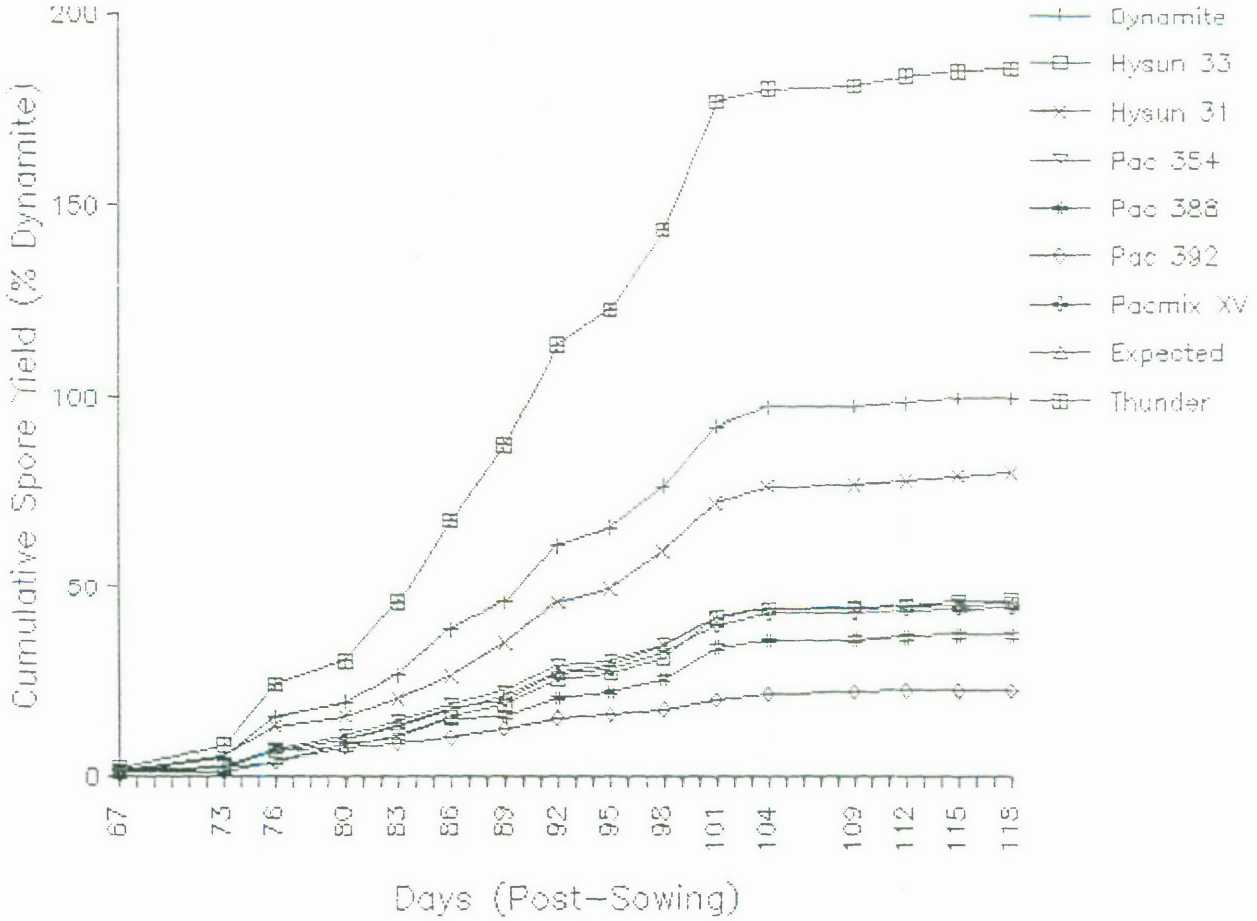



Figure 6.4 Graph of accumulated spore counts for all hybrids against time. Total spore count for Dynamite has been arbitarilly set at 100%.

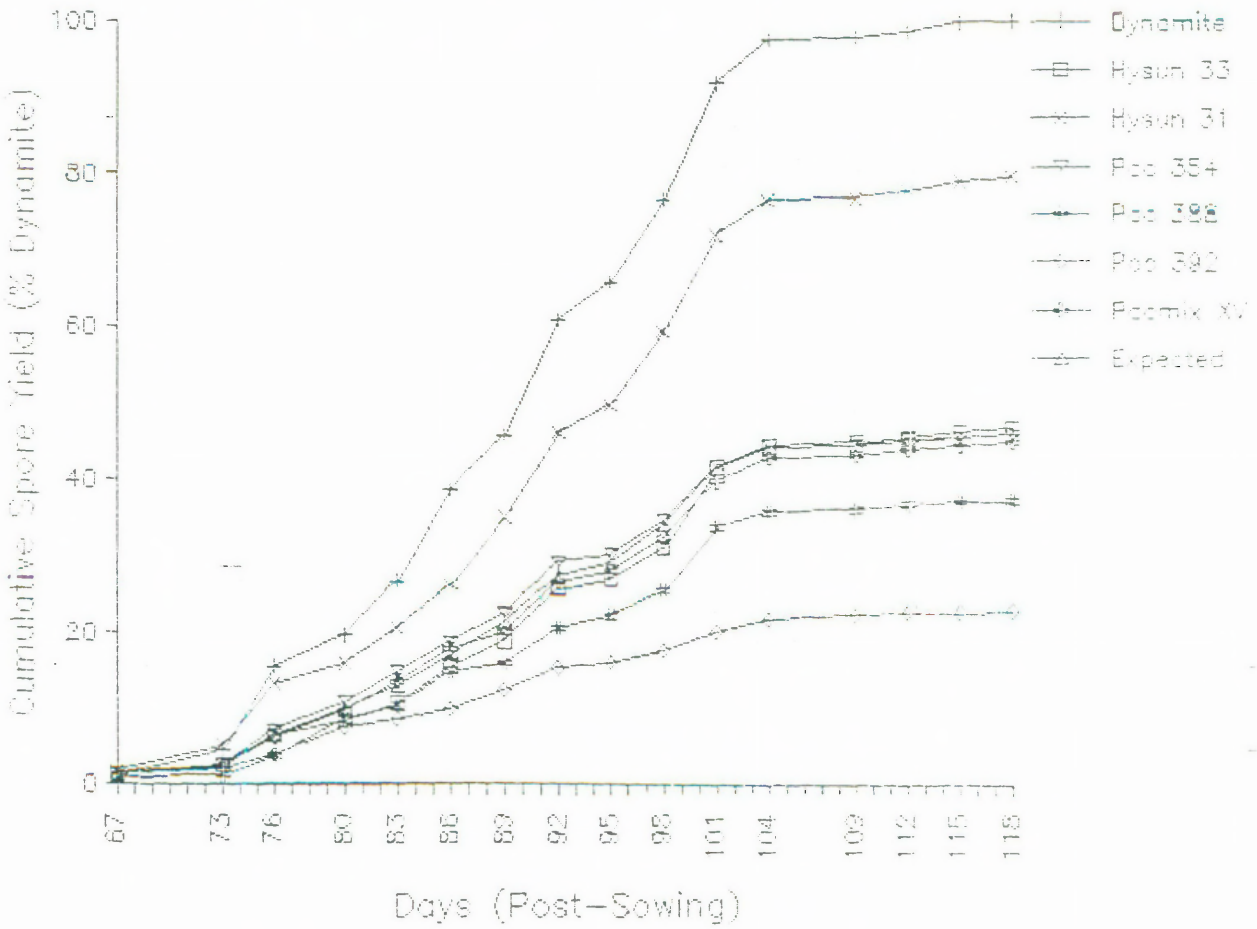



Figure 6.5 Graph of accumulated spore counts against time. Total spore count for Dynamite has been arbitarilly set at 100%.

"Expected" were all similar. Urediniospore accumulation were lowest in Pac 388 and Pac 392. The spore trapping data showed similar trends to the visual assessments of disease severity (Table 6.11). Calculations based on comparing estimated areas of sori with the mean number of urediniospores trapped on days 83 and 86 revealed a wide variation. For example, it was estimated that Dynamite had 30% of the area of sporulative tissue of Thunder but 51.3% of the spore count of Thunder.

The results for grain and oil yields (Table 6.13) revealed that Pacmix XV yielded almost exactly as predicted. In this trial the varieties with high levels of rust infection (Thunder and Dynamite) had significantly lower grain yields ($P<0.05$) than the resistant lines Pac 388 and Pac 392. The grain yield of Hysun 31 was also significantly lower ($P<0.05$) than that of Pac 392. This is in contrast to the grain yields at Felton (Table 6.8) where in the presence of low levels of sunflower rust all cultivars yielded comparably.

Table 6.13 Yield performance of seven sunflower hybrids and a cultivar mixture grown at Gowrie Junction (Autumn,1988)

| Cultivar | Eqivalent Yield (kg/ha) | Oil Content (%) | Equivalent Oil Yield (kg/ha) |
|---|---|---|---|
| Hysun 31 | 1158 *bc* | 53.3 *a* | 617.2 |
| Pac 354 | 1211 *abc* | 51.6 *b* | 624.9 |
| Pac 388 | 1322 *ab* | 50.8 *bc* | 671.6 |
| Pac 392 | 1453 *a* | 50.2 *bcd* | 729.4 |
| Pacmix XV | 1286 *ab* | 51.8 *ab* | 666.1(100.9%)[3] |
| Expected[2] | 1286 *ab* | 51.4 *b* | 660.0 |
| Hysun 33 | 1068 *bc* | 47.6 *f* | 508.4 |
| Dynamite | 980 *c* | 49.4 *cde* | 484.1 |
| Thunder | 594 *d* | 48.8 *def* | 289.9 |
| CV% | 11.05 | 1.49 | |

1. Means in each column followed by at least one lower case letter in common do not differ significantly ($P>0.05$).
2. Expected results based on mean of yields of constituent components.
3. Difference in yield of Pacmix XV and the predicted yield.

### 6.3.3 Comparison of cumulative yield of urediniospores and disease severity by using three mathematical models.

It was necessary to determine whether the cumulative spore yield curves presented in Figure 6.5 were related to the progress of sunflower rust

epidemics occurring in each plot before those curves could be used to provide an objective comparison of the rate of epidemic progress. The curves were similar to the sigmoidal curves representative of 'compound interest diseases'. Such curves were transformed by van der Plank(1963) using logarithmic equations to obtain a set of data points to which linear regression could be applied. Other transformations exist that might be more appropiate for certain pathosystems (Berger,1987).

Materials and Methods.

The direct visual assessments of the severity of sunflower rust in the plots were assessed at 64,70,77,84,90 and 99 days post-sowing (Table 6.11). The proportional severity data (0-1) as calculated from the percentage leaf severity was transformed by the Logistic, Gompertz and Weibull equations and plotted as the abscissa against the comparable transformations applied to the cumulative spore yields from days 67 to 98 as the ordinate.The formulae used for the transformations were:

i) Logistic $Y = \ln(y/(1-y))$ where y = proportion of disease severity or proportion of cumulative spore yield

ii) Gompertz $Y = -\ln(-\ln(y))$ where y = proportion of disease severity or proportion of cumulative spore yield

iii) Weibull Cumulative Distribution Function (WCDF)

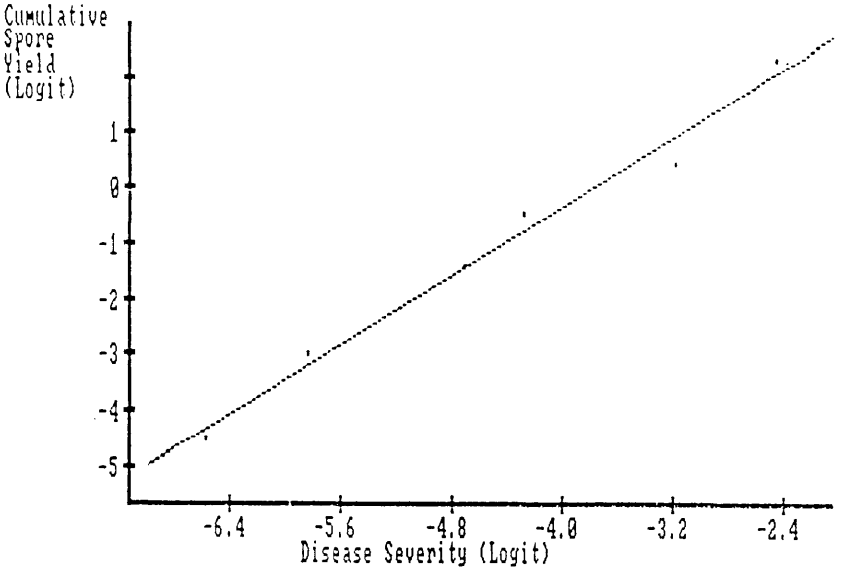
$$Y = 1 - \exp(-((t-a)/b)^c)$$ where a - Weibull location parameter
b - Weibull scale parameter
c - Weibull shape parameter
t - time

Estimation of the b and c parameters for the Weibull cumulative distribution function were obtained by using the computer program "NLIN" (Berger,1987). "NLIN" was iterative and required an initial estimate of each parameter to be entered before supplying the calculated estimates. To assess the range of estimates calculated each data set was therefore entered a number of times with different 'seed' parameters. The a or location parameter was set at day 1. Regression and correlation analyses were then performed by using the Plot function of MSTAT.

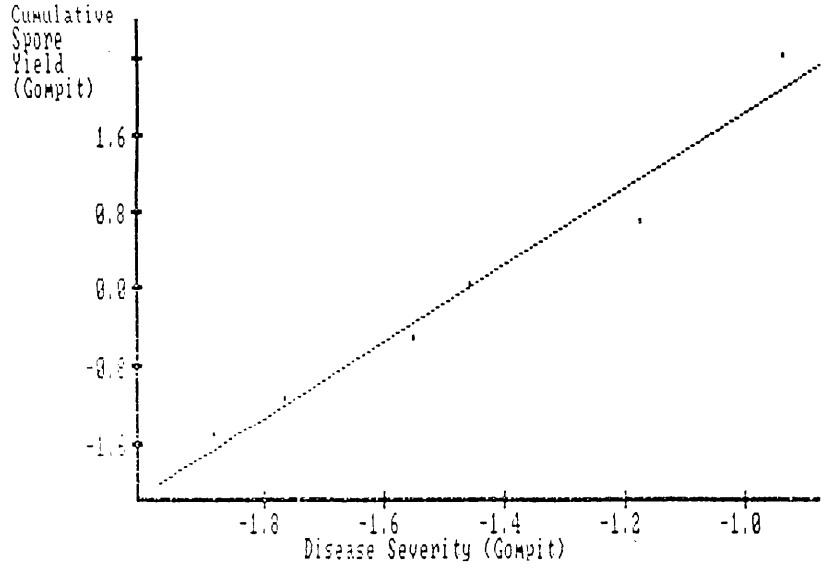
Results.

An example of the plots for the different transformations of the data for Dynamite is shown in Figure 6.6. The results for the regression analyses are in Table 6.14. In every combination of cultivar and

a)




b)




c)

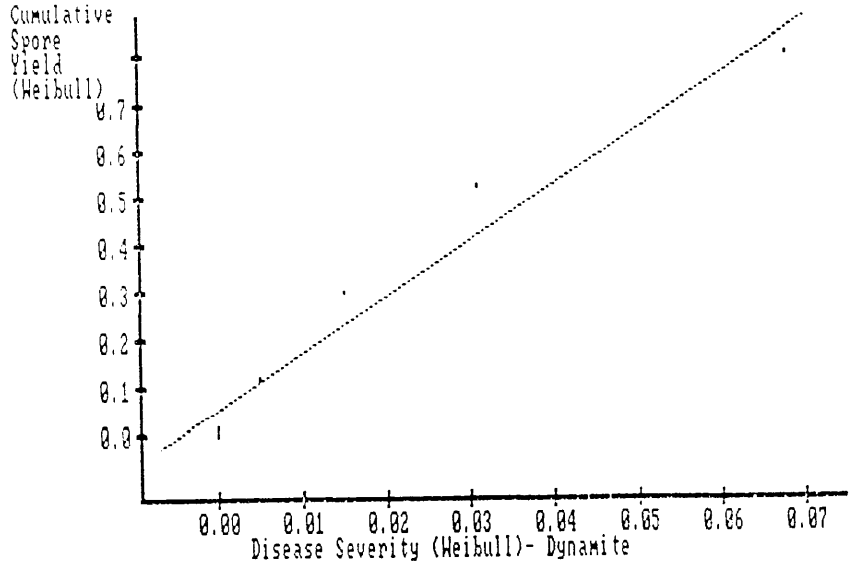



Figure 6.6 Plots of cumulative spore yield data against disease severity in Dynamite over a 35 day period transformed by a) Logistic b) Gompertz c) Weibull cumulative distribution function equations

Table 6.14 Regression analyses from three transformations applied to the comparison of cumulative spore yield against disease severity

| | Logistic | | Gompertz | | Weibull | |
|---|---|---|---|---|---|---|
| | r[1] | P[2] | r | P | r | P |
| Dynamite | 0.992 | <0.001 | 0.982 | <0.001 | 0.979 | <0.001 |
| Hysun 33 | 0.991 | <0.001 | 0.983 | <0.001 | 0.964 | 0.002 |
| Hysun 31 | 0.985 | <0.001 | 0.991 | <0.001 | 0.948 | 0.004 |
| Pac 354 | 0.908 | 0.012 | 0.936 | 0.006 | 0.925 | 0.008 |
| Pac 388 | 0.953 | 0.003 | 0.950 | 0.004 | 0.908 | 0.012 |
| Pac 392 | 0.980 | <0.001 | 0.983 | <0.001 | 0.901 | 0.014 |
| Pacmix | 0.972 | 0.001 | 0.987 | <0.001 | 0.974 | 0.001 |
| Expected | 0.981 | <0.001 | 0.987 | <0.001 | 0.980 | <0.001 |

1. r - coefficient of correlation
2. P - probability of correlation ie. goodness of fit based on Students t-test. All are highly significant

transformation there was a highly significant correlation between increasing disease severity and increasing accumulation of urediniospores of *Puccinia helianthi*. This supported the premise that the results of spore trapping reflected the amount of disease in the experimental plots.

### 6.3.4 Use of mathematical models to utilize cumulative spore yield curves to assess the rate of disease development.

The results of Section 6.3.3 indicated that the cumulative spore yield curves presented in Figure 6.5 were directly related to the progress of the sunflower rust epidemics occurring in each experimental plot. Analysis of the curves could be used to provide a quantitative comparison of the rate of epidemic progress.

Materials and Methods.

The three transformations (Logistic, Gompetrz and Weibull) were applied to the total curves of the cumulative proportion of urediniospores trapped (as a proportion of the total for Dynamite) against time. The slopes of the transformed lines were used to represent the rate of disease progress. The transformed data were subjected to linear and correlation analyses with the Plot function of MSTAT.

Results.

The Weibull Cumulative Distribution Function provided the best

model as determined by the correlation co-efficient (range 0.98-1.00) (Table 6.15). The Gompertz transformation provided better fits (range 0.925-0.984) than the Logistic equation (range 0.906-0.967) except for the curve for Dynamite. Each model revealed a highly significant relationship ($P<0.001$) between cumulative spore yield and time. An example of the plots of the three transformations for one cultivar is shown in Figure 6.7. In most cases the Logistic and Gompertz equation did not transform the sigmoid curves as effectively as the Weibull transformation.

The rate of progress of sunflower rust as estimated from the slope of the regression line (b) was greatest in Dynamite and least in Pac 392. The rankings of the other treatments varied slightly between transformations. The slopes of the regression lines for Pacmix XV did not differ from the "Expected".

## 6.4 DISCUSSION

The investigations reported in this chapter are the first comparisons known to the author of the yield responses of sunflower hybrid mixtures with that of their pure line components in the presence and absence of sunflower rust. Yield responses of mixtures compared to the mean yield of the constituent components have been classified by Fehr and Rodriguez(1980) as being either neutral, complementary, overcompensatory or undercompensatory. Ideally, overcompensation will operate in agricultural situations to produce yields greater than that predicted.

Eleven sunflower hybrid mixtures grown at two locations in Central Queensland showed that the yield of some mixtures surpassed those of the best pure-line components. Generally the yield performance of the mixtures was equal to or better than the yields predicted from the mean yields of the components. The mean oil yield in twenty two mixtures was 102.4% of that predicted. It would appear therefore that overcompensation operated in the mixtures. Only on one occasion did a mixture (Hysun 31-Pac 378, Gindie) yield less than the lowest yielding pure-line component. This may have been an example of undercompensation. These results are in agreement with the generalisations made by Wolfe(1985) who considered that the yields of mixtures could be competitive with pure lines. Other crops where the yields of mixtures have been found to equal or exceed the yields predicted from the pure-line components include oats (Shorter and Frey,1979), soybean (Fehr and Rodriguez,1980), rapeseed (Leon and Diepenbrock,1987), flax (Gubbels and Kanaschuk,1987), wheat (Stuke and Fehrmann,1987), barley (Parlevliet and van Ommeren,1988). Others have been reviewed by Marshall(1977).

The results obtained in the Central Queensland trials showed that components that were well adapted to the enviroment in which the mixture was grown strongly influenced the performance of that mixture. Similarly, components that were poorly adapted to the environment had a detrimental

Table 6.15 Comparison of transformations applied to cumulative spore yields obtained at the Gowrie Junction Trial.

| Cultivar | Logistic b[1] | Logistic $r^2$ | Logistic P[3] | Gompertz b | Gompertz r | Gompertz P | Weibull b | Weibull r | Weibull P |
|---|---|---|---|---|---|---|---|---|---|
| Hysun 31 | 0.102 | 0.965 | <0.001 | 0.062 | 0.984 | <0.001 | 0.019 | 0.996 | <0.001 |
| Pac 354 | 0.075 | 0.925 | <0.001 | 0.035 | 0.964 | <0.001 | 0.010 | 0.999 | <0.001 |
| Pac 388 | 0.081 | 0.916 | <0.001 | 0.033 | 0.960 | <0.001 | 0.013 | 0.999 | <0.001 |
| Pac 392 | 0.058 | 0.906 | <0.001 | 0.022 | 0.946 | <0.001 | 0.005 | 1.000 | <0.001 |
| Pacmix XV | 0.078 | 0.929 | <0.001 | 0.035 | 0.968 | <0.001 | 0.010 | 0.999 | <0.001 |
| Expected | 0.078 | 0.924 | <0.001 | 0.036 | 0.967 | <0.001 | 0.010 | 0.999 | <0.001 |
| Dynamite | 0.220 | 0.967 | <0.001 | 0.175 | 0.925 | <0.001 | 0.024 | 0.980 | <0.001 |
| Hysun 33 | 0.080 | 0.947 | <0.001 | 0.037 | 0.976 | <0.001 | 0.010 | 0.998 | <0.001 |

1. b - slope of regression line
2. r - coefficient of correlation
3. P - probability of correlation ie. goodness of fit based on Students t-test . All are highly significant.

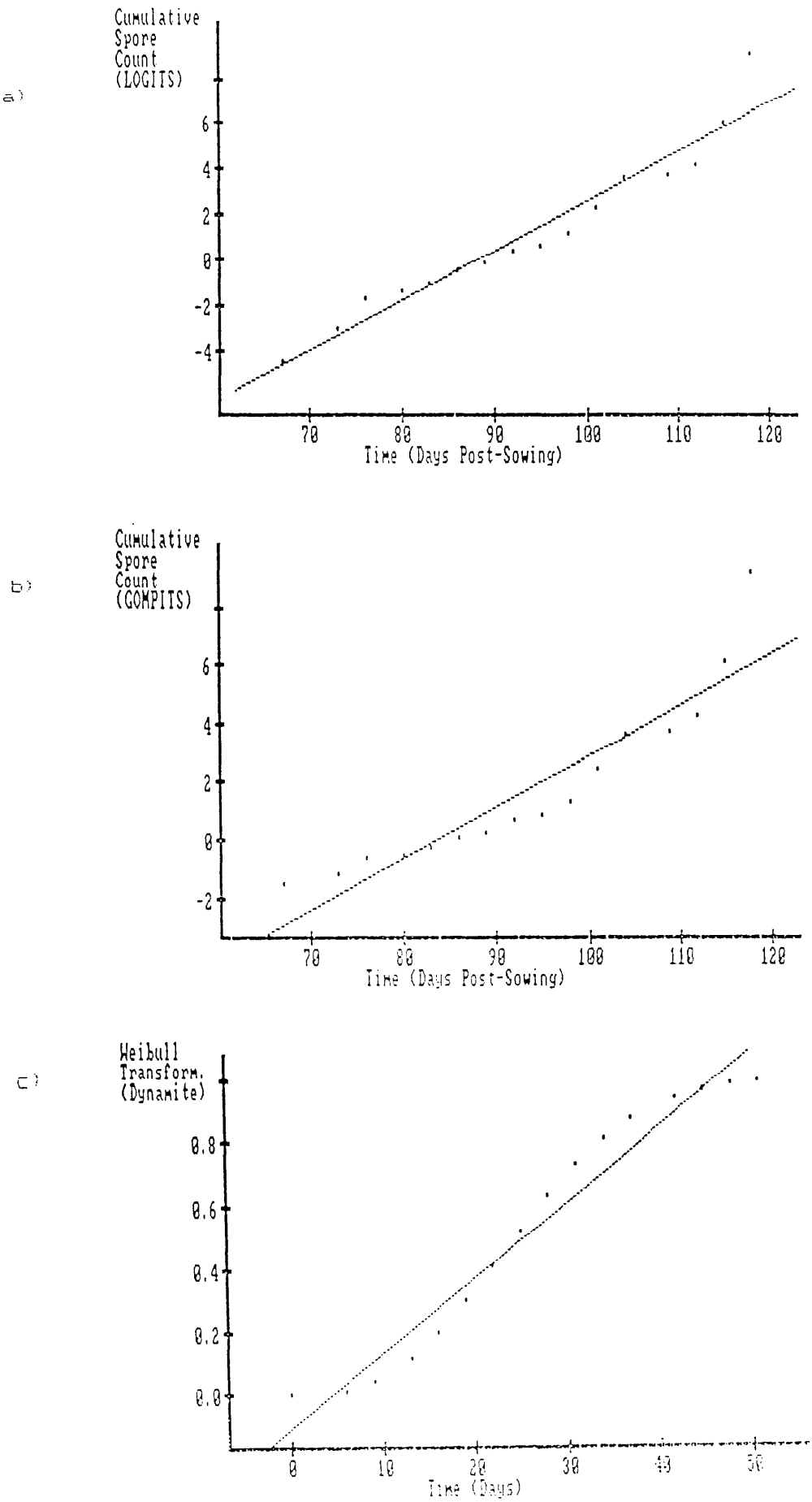



Figure 6.7 Epidemic progress of sunflower rust in Dynamite as assessed by cumulative spore counts transformed by a) Logistic b) Gompertz and c) Weibull cumulative distribution equations

effect on the performance of the mixture. All of the sunflower hybrid mixture combinations which contained Hysun 32 yielded below the predicted level (86.4-99% predicted oil yield) at Clermont. The only exception to this was mixture Hysun 31-Hysun 32-Pac 378 with 106% of predicted oil yield. This reflected the greater than predicted grain yield which may have been a consequence of Hysun 31 and Pac 378 compensating for the low contribution of Hysun 32. The mixtures that performed best relative to the predicted yields at Gindie contained Hysun 31 or Hysun 32 or both. This suggests that Hysun 31 and Hysun 32 are better adapted for the Gindie area than the Clermont area. Recent observations (Pacific Seeds, *unpublished data*) showed that in the Clermont area some sunflower hybrids showed poor seed set. Hysun 32 is one such hybrid. This may explain the poorer performance of mixtures containing Hysun 32 at Clermont.

The overall stability of yield of sunflower hybrid mixtures can only be assessed by more extensive trialling over a greater range of environments than was possible in this study. The limited results available make it difficult to differentiate between random error and true compensation effects (Fehr and Rodriguez,1980). The set of experiments using Pacmix XV may give some indication of how a sunflower hybrid mixture might perform under a range of circumstances. Little disease developed in the trial that was undertaken at Felton and no major environmental stresses were apparent. There were also no significant differences ($P>0.05$) between the yields of Pacmix XV and the pure line cultivars. This result contrasted with that obtained at the Clifton trial which was subjected to disease and drought stress. At Clifton the severity of rust on Pacmix XV was lower than predicted from the mean disease severities of the individual components. Pacmix XV out-yielded all of the pure-lines. It would appear that this was an example of overcompensation. The more heavily rusted pure-line hybrids Hysun 33 and Dynamite yielded poorly. At the third site (Gowrie Junction) the severity of disease in Pacmix XV was the same as predicted from the mean of the components and so was the yield. This suggests there had been a neutral response in the mixture at that site. The more heavily infected hybrids yielded poorly with the equivalent oil yield of the commercial hybrid Cargill Dynamite being 72.7% that of Pacmix XV.

Disease control through the use of multiline cultivars and cultivar mixtures can occur provided that the components differ in their susceptibility to disease (Wolfe,1985). Mixtures of cultivars possessing pathotype specific resistance reduce the level of disease through a reduction of initial effective inoculum which delays the onset of the epidemic. In addition the apparent infection rates would be reduced (Parlevliet,1979). Influxes of spores at the start of the season may not encounter a susceptible host and are subsequently wasted. The secondary spores produced by successful infections may not be deposited on a susceptible component of the mixture. Reduction of the initial effective inoculum will not occur if all components

are susceptible to the influx. The rate of epidemic development slows as the reproductive efficiency of the pathogen is reduced (Zadoks and Schein,1979).

The curve for the cumulative spore counts for Hysun 31 was higher but parallel to the curve for Hysun 33 at Felton. Both cultivars may have rusted at a similar rate but the quantity of initial inoculum that caused infections in plots of Hysun 33 may have been lower than in plots of Hysun 31. This may have been the case if the plots were exposed to equal loads of initial inoculum which consisted of a mixture of pathotypes, more of which were virulent to Hysun 31 than Hysun 33.

Assessment of disease levels in a heterogenous population of plants is difficult since it is difficult to obtain a truly random representation of all components. The indirect but objective assessment of spore output from the diseased plot has sometimes been used to assess disease levels within a crop. The results of Cournoyer(1970) are often cited (Frey, Browning and Simons,1979; Jowett, Browning and Cournoyer Haning,1974). She made relative cumulative counts of urediniospores of *Puccinia coronata* trapped outside plots of oat cultivars. Many more spores were trapped outside plots of susceptible lines than mixtures or resistent lines. Mundt and Leonard(1985) used urediniospore trapping as a means of comparing disease progress in mixtures of different crops. Jeger(1984) found a correlation between accumulation of spores trapped and progress of disease.

The spore trapping technique employed in the work reported in this chapter consisted of simple rods which depended on wind impaction for collection of spores. The traps were not volumetric and gave no indication of spore concentration in the air. Rods do have the advantages of being simple, cheap and easy to use. The Gowrie Junction trial required 33 traps. It would have been impossible to obtain or afford that number of more sophisticated traps. There were strong correlations between the severity of *Puccinia helianthi* in the sunflower plots and the accumulation of urediniospores trapped in those plots. The efficacy of spore trapping using rods is dependant on having large buffered plots to eliminate the effect of any interplot interference caused by inoculum movement between plots. It is also dependant on suitable weather conditions ~~being present~~. Measurement of disease levels should commence as early as practicable in the growing season so that the progress of disease may be better assessed.

Based on the assumption that the accumulation of urediniospores trapped was directly correlated to disease progress the cumulative spore yield curves were analysed to provide relative rates of r, the apparent infection rate as defined by van der Plank(1963). Jowett *et al*(1974) subjected the cumulative curves of Cournoyer(1970) to logistic transformation but did not obtain the expected straight line but rather inverted S-shaped curves. Roelfs, Dirks and Romig(1968) applied the logistic transformation to the cumulative spore counts obtained after rods had been used to trap urediniospores of rusts of wheat. Other models such as the Gompertz equation

(Berger,1981) and Weibull function (Campbell, Pennypacker and Madden,1980) have also been used to linearize the curves.

The outcome of application of the various models can be assessed subjectively from the plots or through analysis of the data. The coefficient of correlation (r) or the coefficient of determination ($R^2$) are most often used to assess the goodness of fit of empirical models (Cornell and Berger,1987). The Weibull function consistently gave better fit to the cummulative spore yield curves than the other two models tested. This was obvious from the plots of transformed data against time and the coefficients of correlation. Regression analyses of the data transformed by each model provided estimates of the slopes of the lines representative of the rate of disease progress.

Browning and Frey(1969) proposed that mixtures may be a means of producing 'synthetic horizontal resistance' or 'slow rusting'through their effects on the rate of disease development. In these trials the mixture Pacmix XV rusted more slowly than the commercial cultivars. This result was not suprising since three of the component cultivars in the mixture exhibited low rates of disease development. These low rates had not been anticipated since high infection type reactions to the predominant pathotype, Race 1,3, were produced on Pac 354 and Pac 388 in glasshouse tests.

At the Gowrie Junction site the rate of development of sunflower rust in the sunflower hybrid mixture did not differ from that predicted from the rates of disease development in the individual components. This may have been due to the presence of pathotypes of complex virulence which reduced the buffering effect of the mixture. Inoculum taken from the trial at anthesis showed that qualitatively the pathotype structure was similar in all ent ries. A quantitative assessment was not attempted. The exchange of inoculum of a single complex pathotype could have occurred between plants in the mixture. The dynamics of the pathotype structure in relation to sunflower hybrid mixtures needs to be examined further.

# CHAPTER 7

## INVESTIGATIONS INTO ASPECTS OF THE *PUCCINIA HELIANTHI : HELIANTHUS ANNUUS* PATHOSYSTEM RELEVANT TO THE EFFICACY OF SUNFLOWER HYBRID MIXTURES.

### 7.1 INTRODUCTION

Multiline or cultivar mixtures composed of components possessing a diversity of genes for disease resistance have been found to reduce the rate of epidemic development relative to the means of pure-line plots of the individual components (Jeger, Jones and Griffiths,1981; Mundt and Browning,1985; Priestley, Bayles and Parry,1988). It appears that four mechanisms may interact to cause this decrease in epidemic development. Burdon and Shattock(1980) suggested that in a given area, a mixture of resistant plants among susceptible plants reduced the amount of susceptible (target) tissue available for infection. This resulted in a reduction in the amount of inoculum produced for subsequent dispersal. Burdon and Shattock (1980) also suggested that the increased distance between susceptible plants decreased the concentration of inoculum that was deposited on other susceptible plants. A third mechanism is the barrier effect that resistant plants in the mixture have when intercepting the movement of propagules. Johnson and Allen(1975) suggested that cross-protection that resulted from the induction of a resistant reaction to infection by a virulent pathotype by the prior or simultaneous infection by an avirulent pathotype would act in multilines.

Few studies have been made to determine the relative importance of each of these different mechanisms that might operate in cultivar mixtures. The barrier effect of resistant plants was considered to be the most important by Browning and Frey(1969) and Trenbath(1977). However, Burdon and Chilvers(1977) attributed much of the decrease in the rate of disease development of *Erysiphe graminis* on barley in a mixture of wheat and barley ~~to be due~~ to a decline in the density of susceptible plants. They concluded that interception was negligble when the proportion of susceptible plants increased.

The density of the individual components interacts with the nature of the initial exogeneous inoculum to influence the effectiveness of cultivar mixtures to retard disease development. Mundt and Browning(1985) found that in the *Puccinia coronata* : *Avena sativa* pathosystem an increase in the susceptible genotype unit area ("clumping") from 0.003 to 0.84$m^2$ did not affect the efficacy of the mixture if the initial inoculum had a focal distribution. However, an increase in the genotype unit area did reduce the effectiveness of an oat mixture to reduce crown rust development when initial inoculum was uniformly distributed (Mundt and Leonard,1985b).

It is the destiny of the propagules that will influence the role that each mechanism has on reducing disease development. In the Uredinales, spores may be lost to the pathosystem by dispersal out of the plot or by deposition onto resistant hosts or by falling to the ground (Aylor and Ferrandino,1985). Spores may also be deposited on and infect the host of origin or be deposited on and infect other susceptible plants. Robinson(1976) used the term autoinfection to describe the reinfection of the host of inoculum origin. He used the term alloinfection to describe the infection of other susceptible hosts.

The rate of epidemic development in a mixture of resistant and susceptible plants relative to that in susceptible pure-lines is thought to decrease as the rate of spore liberation and movement between hosts in the mixture increases (Barrett,1978; Zadoks,1978). Autoinfection fails to expose spores to loss from the system and its importance relative to alloinfection changes with dispersal gradient of the propagule (Mundt and Leonard,1986), dispersal distance between susceptible plants (Leonard,1969; Burdon and Chilvers,1976) and host leaf area (Burdon and Chilvers,1982). It was theorized that the level of autoinfection would increase with plant size. If this is so mixtures may be less effective in crops with large plants than with small plants (Mundt and Leonard,1986). McCartney and Bainbridge(1984) estimated that 1-2% of particles the size of rust urediniospores would be deposited on the cereal plant on which they were produced.

Cross-protection through induced resistance has been shown to occur experimentally (Yarwood,1956; Littlefield,1969; Cheung and Barber,1972; Ouchi, Oku, Hibino and Akiyama,1974; Bahamish and Wood,1985). It may be a mechanism by which the rate of epidemic progress is slowed in cultivar mixtures (Kochman and Brown,1975; Johnson and Allen,1975; Chin and Wolfe,1984). Johnson and Taylor(1976) considered that to effectively suppress successful infections at least one component of the mixture must be heavily infected to provide sufficient propagules of the avirulent pathotype so that there is a likely chance of avirulent and virulent infections occurring in close proximity. The importance of cross-protection in mixtures is uncertain since no component in an effective mixture should be severely infected.

The diversity of resistance genes possessed by different components of a mixture influences the virulence structure of the pathogen population. The resistance genes used in pure-line crops impose directional selection for pathotypes with the corresponding genes for virulence (Leonard and Czocher,1980). In a heterogeneous mixture several independant and interdependant selection forces can act to affect the efficacy of the mixture for disease control. Two conflicting outcomes are possible. Stablizing selection (*sensu* van der Plank,1968) could lead to pathotypes with few virulence genes predominating in the pathogen population. Alternatively, a 'super race' with a complex virulence genotype could be selected which would reduce the effectiveness of the mixture.

In population genetics stabilizing selection is considered to result from a combination of opposing selection forces. This results in a stable equilibrium of allele frequency that provides optimum fitness for the population in a particular enviroment (Crill,1977). Van der Plank(1968) used the term stabilizing selection to describe how different pathotypes could become dominate in a population based on their virulence genotypes. He considered that genotypes possessing unnecessary genes for virulence would suffer a competitive disadvantage against simpler genotypes when directional selection was not applied. The action of stablizing selection in favour of less virulent pathotypes rather than the development of 'super-races' has been seen as critical to the ability of 'dirty crop' mixtures to reduce disease levels (Browning and Frey,1969; Leonard,1977; Barrett,1978; Wolfe and Barrett, 1980; Marshall and Weir,1985). The inclusion in an oat mixture of a completely susceptible component was theoretically necessary to assist maintenance of simple pathotypes in the equilibrium pathogen population (Leonard,1969; Leonard,1977).

For stablizing selection to function the simple pathotypes must be more parasitically fit than complex pathotypes. Fitness was defined by Browning(1981) as the probability that a population would leave descendents over a number of generations. Competition studies have been used under laboratory conditions to study the relative fitness of different pathotypes to reproduce in mixtures (Brown and Sharp,1970; Ogle and Brown,1970). Stahle(1986) assessed the reproductive capacity of pathotypes by measuring the latent period, infection efficiency and spore production in a single generation. Parlevliet(1981) measured stabilizing selection in the field by monitoring the virulence genes in the pathogen population and relating these to changes in the composition of resistance genes in the host cultivars grown. The general conclusion made by Ogle and Brown(1970), Nelson(1972), Crill(1977), Parlevliet(1981) and Stahle(1986) was that an inverse relationship between an increase in virulence genes and reproductive fitness was not a general phenomonen but may be present in specific examples.

Groth(1976) and Person *et al*(1976) predicted that cultivar mixtures would favour selection of pathotypes with complex virulence genotypes or 'super-races'. However, field data has not been available to test this prediction (Wolfe,1985). Chin and Wolfe(1984) studied the *Erysiphe graminis* f.sp *hordei*: barley mixture pathosystem and found that complex pathotypes capable of infecting many components in the mixture predominated early in the season whereas simple pathotypes became dominant as the season progressed. Chin and Wolfe(1984) also showed that pathotypes were not equally aggressive on different susceptible components in the mixture. Complex pathotypes were not as adapted to specific host lines as other more specialized pathotypes.

Once a gene conferring pathotype specific resistance has been overcome it may still have a residual effect on the reproductive development of virulent pathotypes (Nass, Pederson, MacKenzie and Nelson,1981; Brodny,

Nelson and Gregory,1986). Other factors in the genetic background may not favour the development of certain pathotypes (Andres, Wilcoxson and Roelfs,1986). Environmental conditions may favour the development of some pathotypes (Kiyosawa,1980).

Several theoretical models have been produced to examine the development of epidemics in cultivar mixtures (Ostergaard,1983; Gumpert, Geiger and Stahle,1987). Some of these have assumed that once an epidemic commences mutations towards greater virulence or recombinations of virulence genes do not occur in the pathogenic population. Recombination was attributed to the sexual process only (Gumpert *et al*,1987). New pathotypes have been shown to originate through somatic hybridization between existing pathotypes. Examples include *Puccinia recondita* Rob. ex Desm (Vakili and Caldwell,1957), *P.graminis* Pers.f.sp.*tritici* (Watson and Luig,1962), *Melampsora lini* (Ehrenb.) Lev. (Flor,1964), *Puccinia striiformis* Westend (Little and Manners,1969) and *Erysiphe graminis* DC f.sp.*tritici* Em. Marchal (Menzies and MacNeill,1986). It is possible that new pathotypes may arise from somatic recombinations between pathotypes present in 'dirty-crop' cultivar mixtures.

There are many factors that might affect the potential of using cultivar mixtures to control rusts. However, these have not been studied fully in established cultivar mixtures nor have they been examined at all with sunflowers. The objectives of the experiments reported in this chapter were to study aspects of (i) induced resistance, (ii) autoinfection, (iii) pathotype competition, (iv) somatic hybridization and (v) defeated gene and background effects in the *Puccinia helianthi* : sunflower pathosystem.

## 7.2 EXPERIMENTAL

### 7.2.1 Resistance induced by inoculating sunflower with an avirulent pathotype of *Puccinia helianthi*.

Two experiments were performed on two separate occasions to examine the effect of simultaneous and prior inoculation of sunflower with an avirulent pathotype of *P.helianthi* on subsequent infection by a virulent pathotype of the same pathogen.

Materials and Methods.

The sunflower hybrid Cargill Sunking and the rust differential sunflower line 69-17-8-1-1 were used in this study. The two pathotypes used were Race 0 which gave a resistant infection type (Reaction type $0_1$) on both sunflower lines and Race 1 to which both lines were susceptible (Reaction type 3-4).

Single sunflower seedlings which were grown in potting mix contained in 5cm square plastic pots, were kept in a controlled environment cabinet at the Queensland Department of Primary Industries Complex,

Toowoomba. Conditions in the growth cabinet were 18/22 ± 1°C night/day, 55/65% relative humidity night/day and light at the plant platform at 400uE.$m^{-2}$.$sec^{-1}$ supplied in 12h photoperiods. Seedlings were inoculated when the first true leaf had fully expanded. The orientation of these leaves was opposite and perpendicular to the axis of the stem.

Race 0 was increased on the 'universal suspect' S37-388 and Race 1 on the differential line 69-17-8-1-1 in separate growth cabinets. Prior to the first inoculation one of the first leaves of each seedling was marked with indelible ink and then covered with a piece of aluminium foil to prevent deposition of urediniospores. This allowed the first pair of true leaves of individual seedlings to be used for comparison. Plants were inoculated at 1600h in a spore settling tower designed by Brown and Kochman(1973).

The exposed primary leaf of all seedlings was inoculated with the avirulent pathotype Race 0. Twenty milligrams of dry urediniospores of Race 0 were discharged into the spore settling tower to uniformly inoculate all seedlings. The urediniospore density on filter paper discs that were placed in the settling tower was approximately 600 spores/$mm^2$.

The seedlings were then removed from the settling tower and the aluminium foil removed. Five seedlings were returned to the settling tower and exposed to a 20mg discharge of dry urediniospores of the virulent pathotype Race 1 to give an inoculum density of approximately 600 spores/$cm^2$ on the freshly exposed leaves. These plants provided the initial or simultaneous inoculation.

All seedlings were placed in a high humidity incubator in darkness at 20°±2°C for 16h before being returned to the growth cabinet. Inoculations using Race 1 were repeated using five different seedlings at 1600h on seven successive days. These were incubated overnight and returned to the growth cabinet.

The leaf dimensions were measured and the number of erumpent sori per leaf were counted at twelve days after the second inoculation. Leaf area was estimated by the formula; Leaf Area = length x breadth x 0.7 (English,1976), and density of sori was expressed as number/$cm^2$.

Results.

The density of sori produced in the respective inoculations are shown in Table 7.1. Simultaneous and prior inoculations with an avirulent pathotype, Race 0, on seedlings of two sunflower lines reduced the infection efficiency of the virulent pathotype, Race 1. Fewer sori (<100%) formed on leaves previously inoculated with Race 0. The suppression in number of sori increased with the interval between inoculations. At five to six days after inoculation with the avirulent pathotype, Race 0, hypersensitive flecking had commenced on leaves of both lines. Sori that subsequently developed on these leaves were of infection reaction type 2-3. The sori produced by Race 1 on the opposite leaves were of reaction type 4 (Figure 7.1).

Table 7.1 Effect of the interval between successive inoculations with two pathotypes of *Puccinia helianthi* on the density of uredinia on leaves of the sunflower line 69-17-8-1-1 and the hybrid Cargill SunKing.

| Interval Between Successive Inoculations (days) | Number of sori/cm² of leaf surface | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 69-17-8-1-1 | | | | SunKing | | | |
| | R0 | R1 | R0 + R1[1] | (%)[2] | R0 | R1 | R0 + R1 | (%) |
| 0 | 0 | 28.9 | 27.8 | 96 | 0 | 45.6 | 39.2 | 86 |
| 1 | | 35.6 | 32.1 | 90 | | 25.1 | 29.9 | 119 |
| 2 | | 15.3 | 12.7 | 83 | | 63.1 | 34.7 | 55 |
| 3 | | 40.9 | 15.8 | 39 | | 64.9 | 21.2 | 33 |
| 4 | | 19.5 | 10.8 | 55 | | 32.9 | 12.8 | 39 |
| 5 | | 19.4 | 6.5 | 34 | | 37.4 | 30.3 | 81 |
| 6 | | 26.5 | 7.8 | 29 | | 65.1 | 19.1 | 29 |
| 7 | | 20.6 | 5.9 | 29 | | 58.2 | 12.8 | 22 |

1. R1 - Race 1 only, R0 + R1 - Race 0 and Race 1, R0 - Race 0 only
2. % sori of control, Race 1.

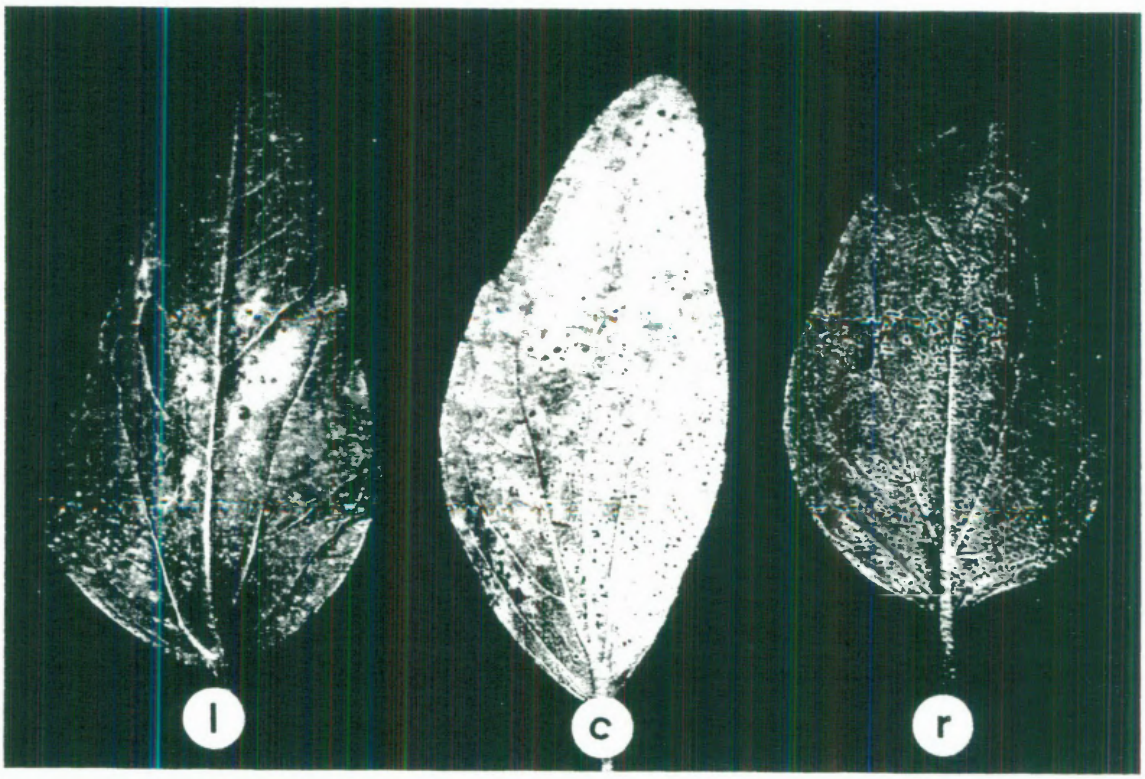



Figure 7.1. Effect of a 6 day interval between successive inoculations with two pathotypes of *P.helianthi* on the density of uredinia formed on leaves of the sunflower line 69-17-8-1-1. L-Race 0 C-Races 0+1 R-Race 1

The exception was SunKing at day 1 where an 19% increase in sori number was detected. Examination of the raw data suggested that one replicate plant may not have possessed the resistance gene/s and was susceptible to both pathotypes. The mean density of sori for SunKing at day 1 was reduced to 24.8, a 98.8% reduction compared to the control, if the highly susceptible plant was excluded from the calculation.

### 7.2.2 Rust development on rust susceptible sunflower plants surrounded by resistant or susceptible plants.

The objective of this experiment was to examine the effect of auto-infection on the development of rust in a sunflower hybrid mixture.

Materials and Methods.

The sunflower hybrids chosen were Cargill Thunder and Pacific Seeds Pac 5787. Thunder was chosen because of its high degree of susceptibility to sunflower rust. Pac 5787 was used as a barrier because it was resistant to the prevalent pathotypes of sunflower rust in the field in 1987. It was also known to be of an equivalent stature to Thunder. A third hybrid, Cargill Advance, was chosen to separate plots because it was resistant to the prevalent pathotypes of sunflower rust and was significantly taller than the other hybrids and would therefore retard urediniospore movement between plots.

This field experiment was conducted on the Pacific Seeds facility at Toowoomba during the Spring/Summer of 1987. Six experimental plots were planted manually on the 27 August. Three seeds of the respective hybrids were sown at each intersect of a 50cm grid. These were later thinned to leave a single plant at each point. Three plots were sown with the sunflower hybrid Cargill Thunder only. Three plots were sown with six plants of Thunder separated by two plants of the hybrid Pac 5787 (Figure 7.2). Each plot was surrounded by three rows of the hybrid Cargill Advance sown at 50cm interrow spacing and thinned to 25cm between plants.

On 27 October when the Thunder plants to be inoculated had reached growth stage R1 (Schneiter and Miller,1981) they were inoculated with urediniospores of Race 1,3. This pathotype was chosen because it was virulent on Thunder and avirulent on Pac 5787. A 1.5cm diameter disc of moist filter paper was dusted with urediniospores and affixed to the adaxial surface of the seventh true leaf with a paper clip. The leaf was then covered with a plastic bag to maintain a high relative humidity. Inoculations were made in the evening and the plastic bags and inoculation discs were removed the following morning. No rust sori were observed in the plots prior to inoculation. The inoculations provided six foci in each of the six plots. Observations made at 10 days after inoculation showed that each inoculation

had been successful. The epidemics were then allowed to develop undisturbed.

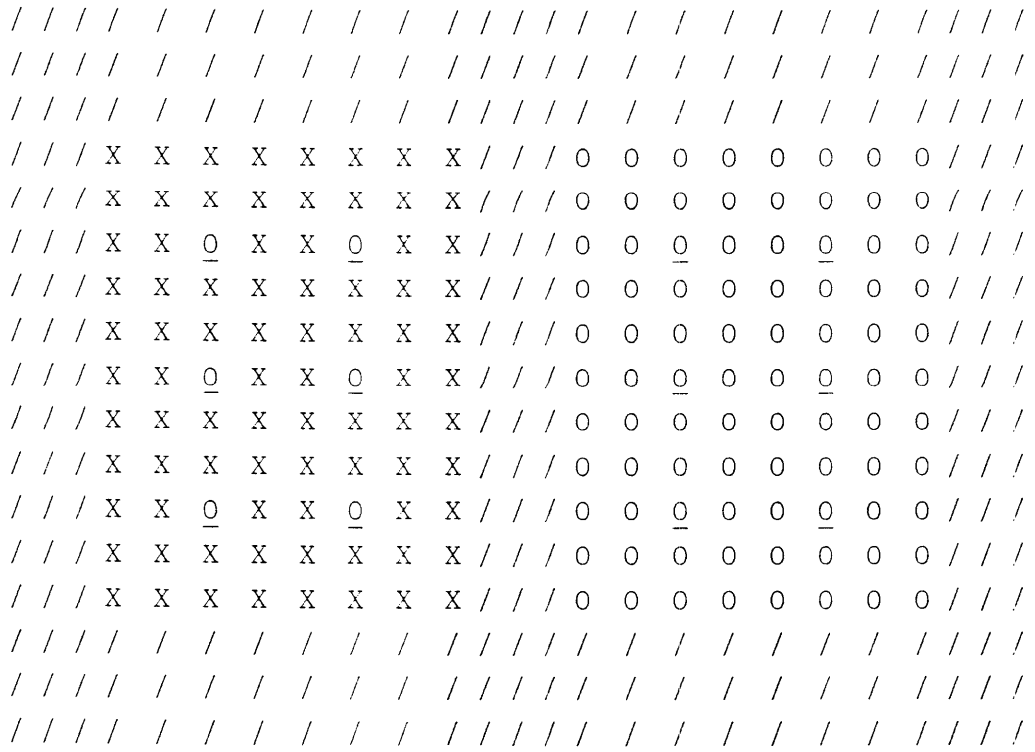


Figure 7.2 Design of experimental plots. O - Cargill Thunder, O - inoculated plants, X - Pac 5787, / - Cargill Advance

On 14 December 1987 when Thunder had attained physiological maturity (G.S. R9) the plants originally inoculated were tagged and removed. The 24th leaf was arbitarily chosen for assessment of rust severity. Counting leaves and leaf scars revealed that the average leaf number per plant was 32.4.

A cardboard template in the shape of a sunflower leaf was prepared. In it were cut 25 randomly positioned 1cm$^2$ holes. The template was laid over the abaxial surface of a leaf and the number of sori exposed in each of the holes completely over the leaf was counted.

Results.

The results were expressed as the mean number of sori per cm$^2$ (Table 7.2). It was assumed that no exogeneous inoculum entered the plots and that inoculum movement between susceptible check plants in the plots was minimal. The number of sori on the Thunder plants surrounded by Pac 5787 was 28% that of the Thunder plants surrounded by Thunder. This data suggests that autoinfection in sunflower may contribute to a disease severity of at least 28% of the combined auto- and alloinfections.

Table 7.2 Effect on rust severity on a susceptible sunflower hybrid surrounded with either resistant or susceptible plants.[1]

| | Surround | | | |
|---|---|---|---|---|
| | Susceptible | | Resistant | |
| Replicate | Number of Uredinia/cm$^2$ | Range | Number of Uredinia/cm$^2$ | Range |
| 1 | 8.9 | (8.0-10.6) | 1.6 | (1.0-2.8) |
| 2 | 7.6 | (6.0-12.9) | 3.5 | (0.5-7.0) |
| 3 | 12.7 | (9.0-19.7) | 3.0 | (0.7-5.7) |
| Mean | 9.7 | | 2.7 | |

### 7.2.3. Relative ability of the two pathotypes of *Puccinia helianthi* to survive in a mixture.

On a host line with no operative pathotype-specific resistance genes, the concept of stabilizing selection (*sensu* van der Plank,1968) predicts that pathotypes with few unnecessary genes for virulence will, over several generations, become predominate in a mixture with pathotypes whose reproductive fitness has been depleted by the presence of unnecessary virulence genes. The experiment reported here was designed to investigate the validity of the concept of stabilizing selection by comparing the ability of two pathotypes of *Puccinia helianthi* to survive in a mixture over several generations on a sunflower hybrid with no known genes for resistance.

Materials and Methods.

Race 0 was used in this study because it had no known genes for virulence. The second pathotype used was an isolate of Race 1 which was representative of the first major shift in the virulence structure of the *P. helianthi* population detected in Australia (Kochman and Goulter,1984). Race 1 was virulent on sunflower lines containing the $R_1$ gene for resistance. Inoculum of each pathotype was increased on the 'universal suscept' S37-388 in separate controlled environment cabinets at the Queensland Department of Primary Industries Complex, Toowoomba. The sunflower hybrid used to study the competitive ability of each strain in a mixture was Pacific Seeds Hysun 31. This hybrid was chosen because it was widely grown during the period that Race 1 was first detected and was susceptible to both Race 0 and Race 1.

Five seedlings at growth stage V4 (Schneiter and Miller,1981) were used for each serial inoculation. A 1:1 (w/w) mixture of Races 0 and 1 was

prepared from urediniospores collected the preceding day and tested overnight on 0.5% water agar to ensure germination exceeded 90%. A spore settling tower was used to inoculate plants at 1600h. After 16h incubation in darkness in a humidity chamber kept at 20°C the seedlings were returned to the growth cabinet. Subsequent serial inoculations were made using urediniospores collected from the five inoculated seedlings by tapping them into a Petri dish.

The proportion of each pathotype sporulating on Hysun 31 was determined by using a scalpel to transfer urediniospores from ten individual sori on each seedling to individual 1 cm diameter filter paper discs. The scalpel blade was rinsed in alcohol, flamed and cooled between each transfer. The inoculation discs were moistened and then placed (sporiferous surface in contact) onto the adaxial surface of the first or second true leaves of seedlings of the sunflower rust differential line 69-17-8-1-1. Six inoculation discs were placed on each leaf. The differential line was susceptible to Race 1 (Reaction type 3-4) and resistant to Race 0 (Reaction type $0_;$). The plants had been raised in a separate growth cabinet under the environmental conditions specified in section 7.2.1. Following incubation the inoculated seedlings were returned to the growth cabinet. At twelve to fourteen days after inoculation the number of sites with uredinia (Reaction type 3-4) or hypersensitive flecks (Reaction type $0_;$) were counted (Figure 7.3). The inoculation was deemed to have failed at a site if there was no indication of infection. Such sites were omitted to avoid biasing the results in favour of resistant reactions. This procedure was carried out for five generations.

Results.

From Table 7.3 it can be seen that in the first generation following the initial inoculation with a mixture of urediniospores, Race 1 dominated and remained predominant at a stable proportion in the four succeeding generations.

Table 7.3 The percentage of uredinia of two pathotypes in a mixture of *Puccinia helianthi* after five successive generations on the sunflower hybrid Hysun 31.

| | Generation | | | | |
|---|---|---|---|---|---|
| Pathotype | 1 | 2 | 3 | 4 | 5 |
| Race 0 | 30 | 32.6 | 34 | 36 | 31.8 |
| Race 1 | 70 | 67.4 | 66 | 64 | 68.2 |
| Inoculation Sites | 50 | 46 | 50 | 45 | 44 |

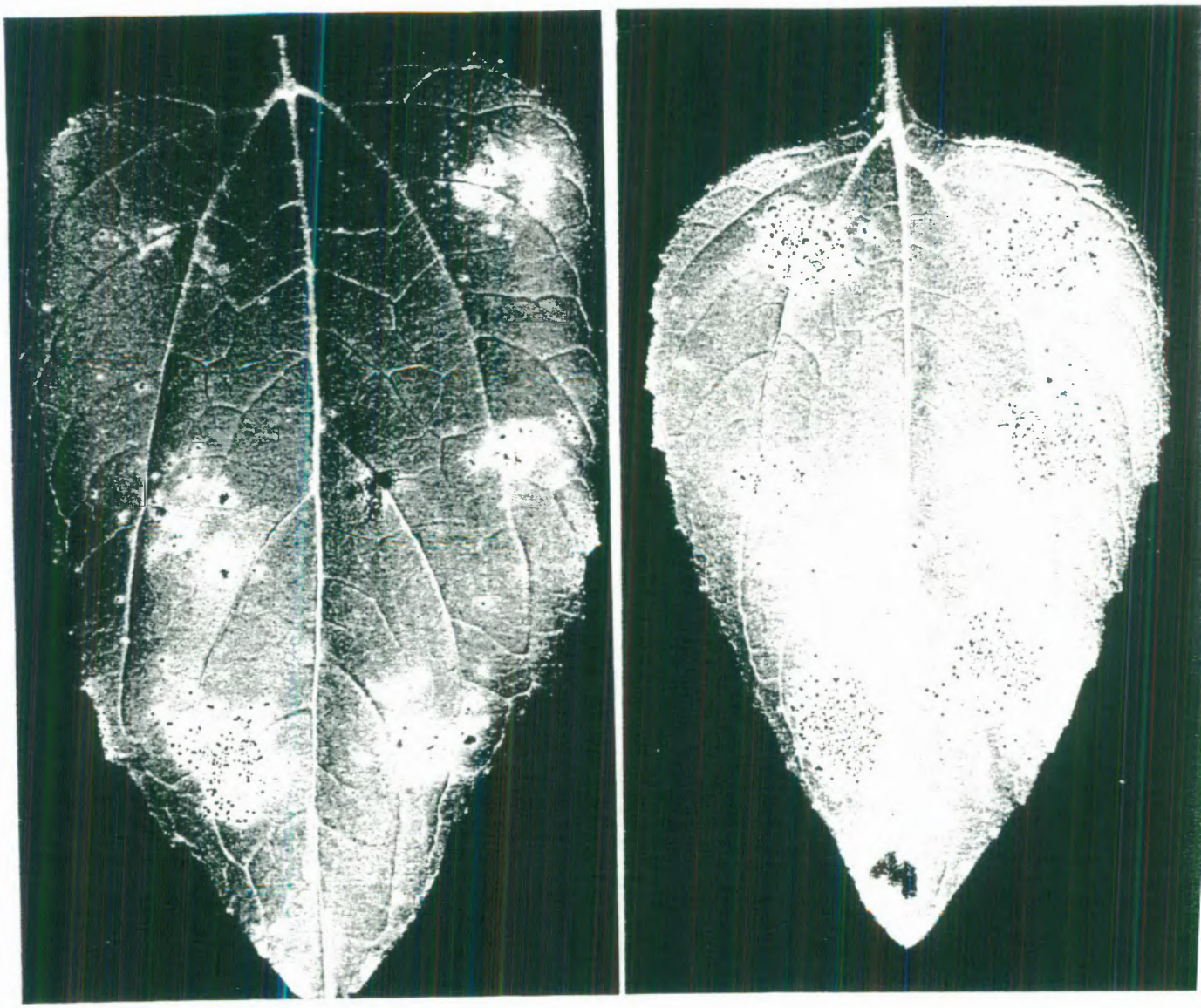


Figure 7.3. Reaction of the sunflower line 69-17-8-1-1 to mono-uredinial isolates taken from a mixture of two pathotypes showing the reaction to Race 0 (flecking) and Race 1 (sori).

### 7.2.4 An attempt to obtain asexual recombination of virulence genes through somatic hybridization of pathotypes of *Puccinia helianthi*.

The role of asexual recombination in the genesis of new pathotypes of *Puccinia helianthi* has not been reported although it has been reported for other rust fungi. The purpose of this study was to determine whether fusion of infection structures derived from urediniospores could be observed when mixtures of urediniospores of different pathotypes were used to inoculate water agar and host tissue. Subsequent generations of urediniospores produced on host tissue were examined for the presence of novel recombinations of virulence phenotypes.

Materials and Methods.

The pathotypes of *P.helianthi* used were Race 1, Race 1,3, Isolate 029 and Isolate 040. These pathotypes could be easily differentiated on the sunflower rust differential lines 69-17-8-1-1, 70019, 70096 and HA-R1 (Table 7.4). Each pathotype was increased in a glasshouse from single uredinia on the respective differential line ie. Race 1 on 69-17-8-1-1,

Table 7.4 Reaction of four sunflower rust pathotypes on a set of sunflower rust differential lines.

| Differential | Resistance Gene | Rust Pathotype | | | |
|---|---|---|---|---|---|
| | | Race 1 | Race 1,3 | Isol.029 | Isol.040 |
| S37-388 | -- | S[1] | S | S | S |
| 69-17-8-1-1 | R1 | S | S | R | S |
| 70019 | R1+R3 | R | S | R | R |
| 70096 | ? | R | R | S | R |
| HA-R1 | R4 | R | R | R | S |

1. Reaction types. Susceptible - 3,4 Resistant - 0,0;,1

Race 1,3 on 70019, Isolate 029 on 70096 and Isolate 040 on HA-R1. Increase of Race 1 was conducted in a separate glasshouse since 69-17-8-1-1 was susceptible to three of the pathotypes.

The following four potential somatic hybrid cross (SHX) mixtures were prepared with equal weights of each component; SHX-1 Race 1,3 and Isol.029, SHX-2 Race 1 and Isol.029, SHX-3 Isol.029 and Isol.040 and SHX-4 Race 1,3 and Isol.040. For the first part of the study, suspensions of each mixture in the light mineral oil Pegasol 3440 (Mobil Oil Ltd.) were prepared. Each suspension was sprayed using an airbrush onto five Petri dishes, four containing 0.5% water agar and one containing twelve 1cm leaf discs of S37-388 on moist filter paper. The open dishes were exposed to the air-flow of a Laminar flow hood until the mineral oil had evaporated and were then sprayed with atomized distilled water. The dishes were closed and placed in an darkened incubator kept at 20°C.

A Petri dish and three leaf discs inoculated with each mixture were removed after 4, 8, 12 and 24h incubation. The leaf discs were floated in small wide-mouth bottles on the whole leaf clearing and staining solution of Bruzzese and Hasan(1983) to avoid the loss of spores. The Petri dishes were sprayed with 0.1% lactophenol trypan blue to stain the germ-tubes. The leaf

discs were transferred after 24h to saturated chloral hydrate solutions for destaining. The material was then examined microscopically for evidence of fusion of infection structures.

The second part of the study consisted of a series of five co-infections of S37-388 seedlings with the four urediniospores mixtures and testing the subsequent urediniospore produced for the presence of new pathotypes. The new pathotypes tested for were specifically those combining the virulences of the two component pathotypes of the mixture. The procedure is shown schematically in Figure 7.4.

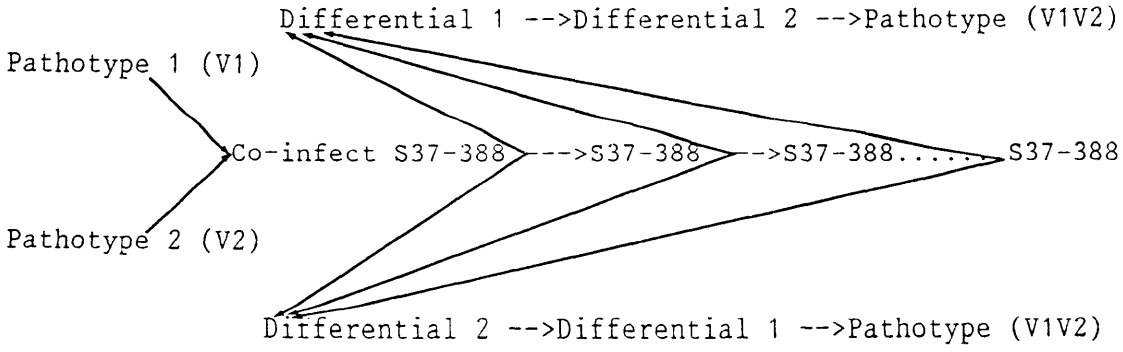



Figure 7.4. Schematic Representation of procedure for selecting recombinants derived from infection with a mixture of pathotypes.

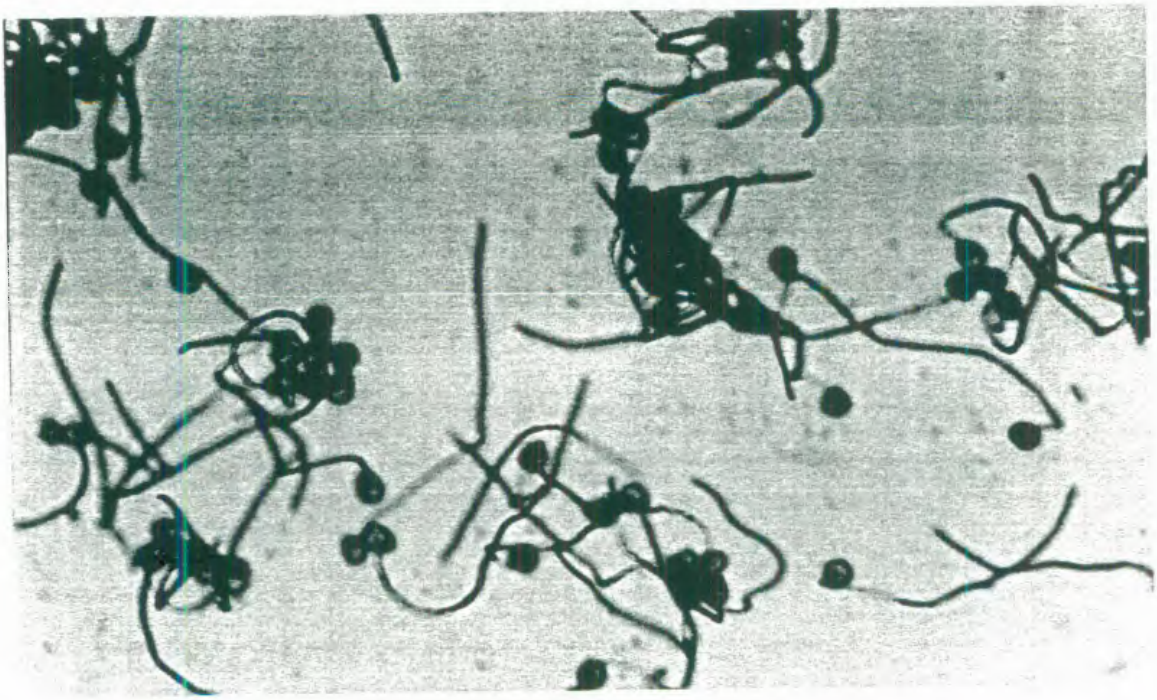
Seedlings of S37-388 were sprayed with suspensions of the urediniospore mixtures in Pegasol 3440 light mineral oil (Mobil Pty Ltd). The seedlings were then incubated in a humidity chamber overnight and then kept on a glasshouse bench. Once sporulation had commenced inoculation discs were prepared by pressing seed germination paper discs against the uredinia. These discs were then used to inoculate the differentials specific for the components of the mixture being used i.e. inoculum from S37-388 inoculated with SHX-1 was used to inoculate seedlings of 70019 and 70096, SHX-2 onto 69-17-8-1-1 and 70096, SHX-3 onto HA-R1 and 70096 and SHX-4 onto HA-R1 and 70019. Once those inoculations commenced sporulation urediniospores were taken and cross-inoculated onto seedlings of the other differential for each mixture. These were incubated for 14d and then examined for the occurrence of uredinia.

Results.

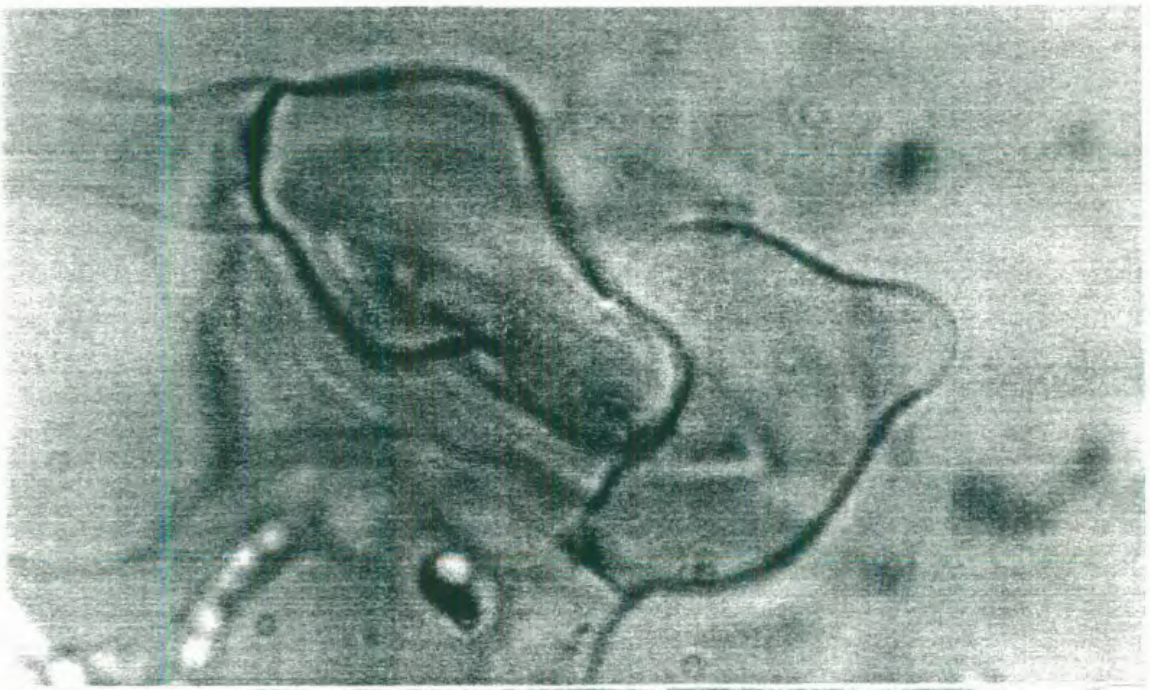
After 8h incubation there was a network of germ-tubes on both the agar and leaf discs. On the leaf discs appressoria were observed over stomata. Many of the germ-tubes had become intertwined but evidence that fusions had taken place was not seen (Figure 7.5). On one occasion two appressoria were observed to have formed over a single stoma (Figure 7.6a)

Figure 7.5 Germinated spores of SHX-2 mixture on water agar after 8h incubation (X400).

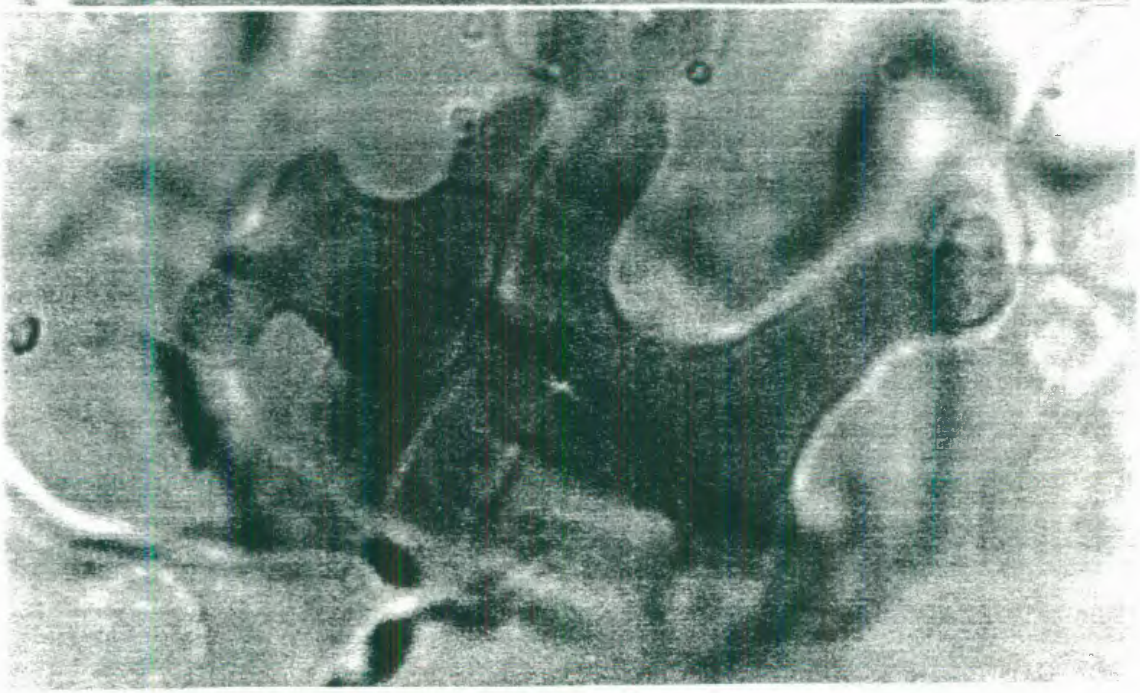
Figure 7.6 a). Two appressoria of SHX-1 mixture over a single stomate
b). Two sub-stomatal vesicles below the apressoria (X1000).



a)



b)



and focussing into the leaf revealed two sub-stomatal vesicles (Figure 7.6b). No fusion was observed.

It was anticipated that any sori formed from the cross-inoculations would represent colonies virulent on both differentials. That hypothesis would have been confirmed by further tests. For all four mixtures no recombinants were detected over five generations on S37-388.

### 7.2.5. Effect of a 'defeated gene' and genetic background on the resistance to *Puccinia helianthi* of six sunflower lines.

Once a gene conferring pathotype specific resistance has been overcome it may still have a residual effect on the reproductive development of virulent pathotypes. The purpose of this study was to examine the effect of the R1 gene for resistance to *Puccinia helianthi* against two pathotypes for which it did not confer immunity. To test the effect of a 'defeated gene' the reactions of S37-388 and its near-isogenic line possessing the R1 gene, S37-388RR were compared. The effect that the genetic background had on the development of virulent pathotypes was obtained by using the sunflower lines CM90RR, F164A, 69-17-8-1-1, S37-388RR and USDA RHA279. These were thought to possess only the R1 gene for resistance to rust.

Materials and Methods.

The experiment was conducted during April 1987. Seeds of each sunflower line were planted in five 12.5mm plastic pots in the glasshouse. The seedlings were thinned to two per pot when the cotyledons had extended fully. The plants were inoculated when the first true leaves were fully expanded. One of the pair of first leaves was marked with indelible ink to indicate the pathotype to be used to inoculate the leaf.

Inoculation consisted of collecting urediniospores of Race 1 and Race 1,3 and using each to uniformly dust 2cm discs of seed germination paper. This was done by discharging 20mg of urerdiniospores in a spore settling tower (Brown and Kochman, 1973). Race 1 was discharged first followed by Race 1,3 after a delay of 30 min to allow any spores in the tower to settle. Densities of urediniospores on the discs were 770/cm$^2$ and 740/cm$^2$ for Race 1 and Race 1,3 respectively. Two discs, one of each pathotype, were applied to each seedling. One disc was placed on the adaxial surface of one of the first leaves of each seedling and the second pathotype was placed on the other first leaf. The inoculation discs were then carefully moistened and gently pressed against the leaf to ensure contact. The seedlings were placed in a mist chamber overnight. The discs were then removed and the seedlings returned to a glasshouse bench. Post-inoculation temperatures in the glasshouse were maintained in the range 15-28°C night/day.

Assessment of quantitative resistance in monocyclic experiments can be made by assessing latent period, disease efficiency, colony growth,

sporulation rate and capacity and infectious period (Welz,1987). In this study the first three criteria were used. Erumpent uredinia at each inoculation site were counted daily for 8-11 days post-inoculation. Latent period was calculated by using regression analysis of probit percent of uredinia erupted on days post-inoculation to estimate the time that 50% of uredinia had erupted (Shaner,1980). Probit transformations were applied by using statistical tables (Fischer and Yates,1957) and regression analysis was performed using the PLOT function of MSTAT (Michigan State University). Probit transformation changed the sigmoidal percentage curve to a straight line.

Disease or infection efficiency was assessed as the number of colonies formed per inoculation site. The final counts of sori taken for the assessment of latent period were used to represent disease efficiency. The diameter of the erumpent uredinia was assumed to directly reflect the size of the colony within the leaf. At 14d post-inoculation the inoculated leaves were destructively sampled, taken to the laboratory and the average diameter of uredinia assessed by using an ocular micrometer in a stereomicroscope. The results for disease efficiency and size of uredinia were subjected to an Analysis of Variance and the means separated by Duncan's Multiple Range Test using MSTAT.

Results.

The results obtained are presented in Table 7.5. Comparison between

Table 7.5 A comparison of latent period, disease efficiency (number of sori) and colony growth (diameter of sori) produced by pathotypes of *P. helianthi* on six sunflower lines.

| | Pathotype | | | | | |
|---|---|---|---|---|---|---|
| | Race 1 | | | Race 1,3 | | |
| Line | Latent Period (days) | Uredinial Number | Uredinial Diameter (mm) | Latent Period (days) | Uredinial Number | Uredinial Diameter (mm) |
| S37-388 | 8.61 | 131.0*ab*[1] | 0.39*ab* | 8.64 | 125.6*ab* | 0.36*ab* |
| S37-388RR | 9.49 | 118.8*ab* | 0.37*ab* | 9.28 | 105.6*ab* | 0.37*ab* |
| 69-17-8-1-1 | 9.02 | 116.9 *b* | 0.36 *b* | 9.18 | 73.3 *b* | 0.31 *b* |
| CM90RR | 8.57 | 155.5*a* | 0.41*a* | 9.16 | 126.1*a* | 0.40*a* |
| F164A | 8.31 | 121.3*ab* | 0.38*ab* | 8.36 | 120.1*ab* | 0.37*ab* |
| RHA279 | 8.72 | 133.5*ab* | 0.37*ab* | 8.90 | 118.5*ab* | 0.36*ab* |

1. Means in each column followed by at least one letter in common do not differ significantly (P>0.05).

S37-388 and its near-isogenic line possessing the R1 gene S37-388RR showed that against two pathotypes of *P.helianthi* the presence of the gene resulted in delayed sporulation and did reduce, although not significantly, infection efficiency (Table 7.5). Among the lines possessing the R1 gene there were significant differences ($P<0.05$) in infection efficiency and size of uredinia. Significantly more and larger uredinia formed on CM90RR than on 69-17-8-1-1 for both pathotypes. Latent period was shortest on F164A and longest on S37-388RR for both pathotypes.

## 7.3 DISCUSSION

The studies reported in this chapter were designed to be preliminary examinations of several aspects of the *P.helianthi* : sunflower pathosystem that might influence the long term efficiency of cultivar mixtures. The phenomenon of induced resistance can lead to a reduction in the disease efficiency of virulent pathotypes. The reproductive fitness of different pathotypes and the selection pressure applied by host resistance will influence virulence structures of the pathogen population. The presence of several pathotypes in close proximity on susceptible hosts might promote the probability of asexual recombination of virulence genes through somatic hybridization and the development of a 'super-race'. Once resistance genes are overcome they may not necessarily be obsolete and may have a role in reducing disease. The ultimate susceptibility is the product of the total genetic make-up of the host. Host morphology can influence the ability of pathogen propagules to be disseminated and the number retained to infect the host of origin.

Prior infection with the avirulent pathotype Race 0 reduced subsequent infection by Race 1 in two sunflower lines and demonstrated that resistance can be induced in sunflower. Resistance of sunflower leaves to establishment of colonies of *P.helianthi* was shown to be an active defense that involved hypersensitive cell death following formation of fungal haustoria in pallisade mesophyll cells (Sood and Sackston,1970; Goulter,1983). The specific mechanism that elicits the response in the *P.helianthi* : sunflower interaction is not known.

It has been postulated that the outcome of many pathogen : host interactions involved molecular recognition factors (Keen,1985). Recognition may involve intimate contact of fungal structures and host cell plasma membranes. In the Uredinales specific molecular binding sites have been found in the walls of intercellular hyphae of soybean rust *Phakopsora pachryrhizi* Syd. (Ebrahim-Nesbat, Hoppe and Rohringrer, 1985) and polypeptides from the walls of the germ-tubes of *Puccinia graminis* f.sp. *tritici* bound selectively to wheat cell proteins (Kim and Reisener,1988). Macromolecules of fungal origin present in the intercellular fluids of cereal plants infected with rust fungi can elicit a hypersensitive response when infiltrated into

uninfected resistant cultivars (Deverall and Deakin,1975; Reiss,1986). The presence of such resistance-eliciting molecules moving in the intercellular fluids could explain the induced resistance. The virulent isolate fails to form a compatible relationship with host cells because the presence of an avirulent isolate has been recognized and defense mechanisms have been activated. Induced resistance may therefore involve an inducible active process and is not only due to blocking of stomata (Cheung and Barber,1972; Johnson and Allen,1975; Bahamish and Wood,1985).

Prior inoculation with a virulent pathotype followed by an avirulent isolate may induce susceptibility to the latter (Bahamish and Wood,1985). An inducible active factor was suspected to be involved since susceptibility of wheat leaves to a normally avirulent pathotype of *P.recondita* f.sp. *tritici* occurred up to 2mm from a virulent colony. Kogel *et al*(1985) were able to suppress the hypersensitive response of a resistant wheat line to an avirulent isolate of *P.graminis* f.sp. *tritici* by first applying a number of lectins which bound to the host cells. The response was attributed to the failure of the host cells to recognize an avirulent invader. The role of induced resistance in cultivar mixtures may be lessened by induced susceptibility (Chin *et al*,1984). The relative importance of induced resistance and induced susceptibility should be examined more fully for *P.helianthi* :sunflower pathosystems.

In the complexity of disease epidemiology within mixtures many factors interact. The degree of autoinfection with a virulent pathotype may influence subsequent induction of susceptibility. Autoinfection is detrimental to the efficacy of cultivar mixtures since there is a reduced likelihood that propagules will be lost from the system. The proportion of autoinfections to alloinfections is dependant on dispersal gradient of the propagule (Mundt and Leonard,1986), dispersal distance (Burdon and Chilvers,1976) and host leaf area (Burdon and Chilvers,1982). Sunflower plants have a large surface area compared to cereals and liberated urediniospores of sunflower rust could be expected to have a greater chance of being deposited on the plant of origin than similarly size propagules in cereal crops. Large plants may favour autoinfection but they also act as barriers to inoculum movement. Irrespective of the influence of autoinfection, it was obvious that a mixture of resistant and susceptible sunflower plants reduced development of sunflower rust on the susceptible plants.

Autoinfection increases as dispersal gradient increases (Mundt and Leonard,1985a; Mundt and Leonard,1986). Urediniospores are passively liberated (Ingold,1971) and are well adapted to air-borne dispersal. The shallow dispersal gradients of rust fungi were ~~attributed with~~ contributed to reducing rust diseases in cultivar mixtures with large plants (eg. *Zea mays* L.) and different canopy structure (eg.*Phaseolus vulgaris* L.)(Mundt and Leonard, 1985a). Liberation of clusters of propagules will alter the deposition

gradient (Ferrandino and Aylor,1987) with clusters being deposited sooner. In the spore trapping experiments used in Chapter 6 the author regularly trapped large clusters of urediniospores of *Puccinia helianthi*. One assessment (unpublished data) for the four replicates of Cargill Dynamite showed that the total number of spores trapped was comprised of 15.1, 22.5, 20.4, 27.4 and 14.6% of urediniospores in the five classes, single, 2-5 spores, 6-10 spores, 11-25 spores and more than 26 spores, respectively. More extensive study of the epidemiology of *Puccinia helianthi* is required before accurate predictions of disease increase in sunflower hybrid mixtures can be made.

Other predictions based on generalizations on the functioning of mixtures may only be valid for specific host pathogen combinations. Many studies on the factors affecting disease increase in 'dirty crop' mixtures have concentrated on the relative ability of pathotypes to compete on susceptible hosts (Browning and Frey,1969; Wolfe and Barrett,1980). The concept of stabilizing selection (*sensu* van der Plank,1968) predicts that in the absense of directional selection pathotypes with unnecessary genes for virulence will be at a selective disadvantage relative to pathotypes with simpler virulence genotypes. Competitive disadvantage of complex virulence genotypes cannot be applied generally but may be present in specific cases (Parleviet,1981). Information neccessary before predictions on the behaviour of sunflower hybrid mixtures can be made include assessment of the frequency of pathotypes in the sunflower rust population and the competitive abilities of each.

'Defeated' genes for resistance may express residual effects and reduce infection by virulent pathotypes. Brodny *et al*(1986) found that defeated genes significantly reduced pustule size and sporulation of *P.graminis* f.sp. *tritici* and the effect was increased when two or three genes were combined. The R1 gene for resistance to sunflower rust was found to extend the latent period and reduce the infection efficiency of two pathotypes infecting a sunflower line compared to its near-isogenic control line. Quantitative or background resistance will also influence the success of pathotypes to reproduce. Latent period, infection efficiency and colony growth for two pathotypes differed between five sunflower lines. Any resistance mechanism that reduces the rate of disease increase will be useful in both cultivar mixtures and pure-lines.

Cultivar mixtures may favour the evolution of pathosystems with complex virulence genotypes, the so-called 'super-races' (Groth,1979). Pathotypes virulent to a particular host line arise through mutation to virulence, sexual recombination of virulence genes and/or asexual recombination of virulence genes (Kiyosawa,1982). Mutation rates of $1x10^{-5}$ and $6x10^{-5}$ were obtained for virulence genes in *Melampsora lini* corresponding to resistance genes $L^6$ amd $M^3$ in flax (Flor,1958). The mutation rate in *Puccinia helianthi* is not known nor are the extents of sexual or assexual recombinations. Attempts to develop new combinations of virulence

through somatic hybridization of pathotypes of *P.helianthi* failed. Fusion of germ-tubes or infection structures was not observed. Brown(1935) found that haploid pycnial colonies could coalesce and hyphal fusions occur. The failure to obtain new virulence combinations was disappointing especially since the combinations sought have since occurred in the pathogen population in Australia (Chapter 5). Those results indicated that the respective virulences could be combined.

Failure to obtain evidence of the development of new pathotypes through somatic hybridization were recorded with *P.recondita* (Barr *et al*,1964) and formae specialis of *P.striiformis* (Newton *et al*,1986). It may be possible that new pathotypes of *P.helianthi* have been the product of mutation or sexual recombination. Mutation for virulence genes only without sexual recombination should result in pathotypes with similar genomes except for the virulence genes. Dry(1985;1988) reported great similarity in the isozyme and nucleic acid composition of isolates of *P.helianthi* collected in Australia. Sexually reproducing populations of *P.graminis* f.sp.*tritici* maintained diversity within enzyme systems. Similarity was typical of asexual reproduction (Burdon and Roelfs,1985). Sunflower rust can survive year round as uredinial infections in some parts of Australia and therefore the extent and role of sexual reproduction is not clear.

The virulence structure remained stable with regard to the R1 gene for many years (Kochman and Goulter,1984) although the pathogen had been recorded in Australia for a century and virulent mutants could have been expected to be present in the population. In the experiments reported in this chapter, Race 1 was found to survive at a stable equilibrium in a mixture with the prevalent pathotype Race 0. This could be explained if the infection efficiency (number of uredinia produced from application of a number of urediniospores) of Race 1 was greater than Race 0 and this was offset by uredinia of Race 0 having a greater sporulative capacity. These characteristics were not examined.

It would be desirable when making future examinations for somatic hybridization in *P.helianthi* to use other genetic markers beside virulence. Ellingboe(1961) used urediniospore wall colour and Newton *et al*(1986) used electrophoretic phenotypes to check that apparent recombinants were not mutants or recombinants. Colour mutants do occur in *Puccinia helianthi* (Brown,1940; Hennessy and Sackston,1972) but have not been recorded in Australia.

# CHAPTER 8

## GENERAL DISCUSSION

Diseases caused by rust fungi are among the most economically destructive of world crops (Littlefield,1981; Cummins and Hiratsuka,1983). Sunflower rust, *Puccinia helianthi*, can significantly reduce the yield of susceptible cultivars of sunflower. Many years of research has been devoted to developing sunflower lines with resistance to rust. Many of the cultivars released have possessed high levels of resistance. Plants of these cultivars exhibit low infection type reactions to infection by avirulent pathotypes. The resistance is often conferred by the action of simply inherited genes which are used because they are easily manipulated in breeding programs. The development or appearance in the rust population of new virulent strains can render the resistance ineffective.

It is necessary when developing a strategy for best utilizing host genes for resistance to understand the capacity of the pathogen population to undergo changes in the frequency of pathotypes. Frequency changes become apparent when a change in the host cultivars grown provides a certain extant pathotype with a selective advantage and it rapidly increases. Frequency changes also occur when new pathotypes develop that render previously resistant cultivars susceptible. New pathotypes of rust fungi develop by either sexual or asexual means. Studies of the sexual origin of new pathotypes of rust fungi are often hindered by difficulty in completing the sexual cycle especially obtaining the germination of teliospores.

Teliospores of *P.helianthi* collected from the field in Australia could be readily activated to germinate by using a technique described as cold-soak preconditioning. Freshly collected or stored leaf tissue bearing telia was kept moist in darkness in a refrigerator at 3-5°C for 3-6 weeks. When removed from this preconditioning environment and incubated at suitable temperatures sufficient germination of teliospores was obtained to allow studies to be made of teliospore germination and formation of metabasidia.

The optimum temperature for germination of teliospores and differentiation of metabasidia was at 17-18°C. Basidiospores were rarely formed at temperatures above 23°C . Abnormal metabasidia were formed instead. Incubation in a regime of exposure to light and then darkness was most favourable to germination. Exposure to continuous light did not prevent germination. Germination tended to peak at about 8h after the switch from light to dark. In nature this would result in most basidiospores being released at night.

Much of our knowledge of the sexual cycle in sunflower rust comes from the work of J.H. Craigie. Craigie(1927a) showed that *P. helianthi* is heterothallic. Crossing pycnia of different mating strains was necessary to

initiate the production of aecia. He later showed that fertilization of pycnia occurred following fusion of transferred pycniospores with the flexuous hyphae of the recipient pycnium (Craigie, 1933). Craigie (1959) then described the migration of nuclei from fertilizing pycniospores from the flexuous hyphae to the protoaecia and the creation of the dikaryotic state. Desriptions of karyogamy and the de-diploidization process in sunflower rust do not appear to exist in the literature and so were examined during the course of the studies reported in this thesis.

Teliospores were formed from the dikaryotic mycelia of the uredinia. The developing teliospores were initially dikaryotic but as the spores matured karyogamy occurred to produce monokaryotic diploid teliospore cells. Since it is the site of karyogamy the teliospore is then the probasidium in the terminology of Talbot(1973). Each cell of the two-celled teliospore can germinate to produce the site of meiosis, the metabasidium (Talbot,1973).

The cytoplasmic contents of the teliospore cell including the nucleus move into the elongating metabasidial germ-tube. The nucleus then divides to produce two daughter nuclei. Since individual chromosomes were not distinct it was not possible to observe whether the nuclear division was actually the first or reduction division of meiosis. The reduced size of the daughter nuclei suggested that the reduction division had taken place. The daughter nuclei were then separated by a septum which divided the cytoplasmic contents of the metabasidium into roughly equal volumes. The two nuclei then divided mitoticly to produce four haploid nuclei which were separated by the formation of two more septa. The metabasidium then contained four monokaryotic haploid cells. From each cell a peg-like sterigma emerged. Swellings at the tips of the four sterigmata expanded to become the basidiospores. The nucleus from each cell of the metabasidium then migrated into its respective basidiospore. Once within the basidiospore the nuclei were often seen to again divide to render the basidiospore binucleate.

The texts by Alexopoulus(1962), Talbot(1971) and Burnett(1975) which used the nuclear cycle of *Puccinia graminis* to typify the macrocyclic Uredinales described or illustrated the basidiospores as being uninucleate. Binucleate and even quadrinucleate basidiospores have been recorded for many species of rust fungi (Maire,1900; Blackman,1904; Allen,1933; Savile,1939; Kulkarni, 1958). Anikster(1983) considered that binucleate basidiospores were normal rather than exceptions among many rust fungi including *P.graminis* and *P.helianthi*. Nuclear staining showed that the binucleate state in the basidiospores of *P.helianthi* occurs after the metabasidial nuclei migrate into the basidiospores. The nuclei are assumed to be produced by a mitotic division.

The basidiospores infect a host by producing a short germ-tube from which a penetration peg directly penetrates the host epidermis. An intra-epidermal vesicle is then formed in the epidermal cell. The two nuclei

from the basidiospore migrate into the vesicle and quickly become separated by a septum. The monokaryotic state is then maintained until fertilization. Maires(1900) described a similar sequence of events in *Endophyllum sempervii*.

In a susceptible host the primary infection hyphae grow from the intra-epidermal vesicle and spread intracellularly. Rapid intercellular spread of the hyphae through the host tissue then occurs. A number of pycnia form in each colony. A pycnial cluster may therefore be derived from a single basidiospore and share the haploid genotype of that basidiospore. This shared genotype within a pycnial cluster allows the cluster to be used as a single haploid entity in genetic studies.

Resistance in sunflower to infections derived from basidiospores of sunflower rust is expressed in a number of ways. Epidermal cells containing intra-epidermal vesicles were seen to become necrotic before infection hyphae developed. Further growth of the fungus ceased. This rapid host reaction cannot be observed macroscopically because the death of solitary epidermal cells cannot be observed with the naked eye. The hypersensitive reaction of epidermal cells to penetration by rust fungi may not be a common phenomenon. Rohringer and Heitefuss(1984) reported that the Sr5 gene was the only gene known to condition a hypersensitive response to uredinial infection by stem rust in epidermal cells of wheat. The reaction of the leaf epidermal cells of the sunflower line HA-R3 to infections from basidiopores is another example of the expression of the hypersensitive re action of epidermal cells. Further studies may reveal whether the hypersensitive response of invaded epidermal cells is usual among direct basidiospore-derived infections.

Necrotic flecks were observed macroscopically when fungal colonies formed infection hyphae that spread through the leaf and elicited a hypersensitive response in numerous palisade mesophyll cells. Fungal colonies were also seen that became necrotic without any associated death of the invaded host cell. Some colonies also seemed to loose cell wall integrity. Recent research in other fungal:host interactions has shown that some plants are capable of producing enzymes that hydrolyse the cell walls of invading fungi (Netzer,Kritzman and Chet,1979; Boller and Metraux, 1988; Jondle, Coors and Duke, 1989). It is possible that these hydrolytic chitinases and glucanases are active in invaded sunflower cells. Growth of pycnial colonies of sunflower rust without necrosis of fungal or host cells was more rapid and extensive in some host lines than others. This was taken to indicate differences in host susceptibility.

These studies also provided information that contributes to our understanding of the role of the sexual cycle in the epidermiology of sunflower rust in Australia. Telia of sunflower rust are most common in the field in Australia during autumn but may be found at other times on mature crops. Formation of telia may be influenced by host cultivar or pathotype. Telia were not found on hybrid cultivars thought to possess the R$_1$ gene for resistance to rust despite the fact that these cultivars were

susceptible to the common field pathotype, Race 1. Mature teliospores of *P.helianthi* exhibit a germination dormancy which may be broken by field weathering or manipulation in the laboratory. Some teliospores collected in Southern Queensland from the field during autumn and early winter were found to germinate.

Teliospores capable of germinating may do so if temperature and moisture conditions are adequate. The basidiospores produced from the teliospores may germinate and infect the host under suitable environmental conditions. Teliospore germination and production of basidiospores occurs at temperatures below 22°C with an optimum around 17-18°C. Basidiospore germ-tube elongation was greatest between 14 and 16°C. Subsequent growth of pycnial colonies was greatest at 20 and 24°C and was completely inhibited at temperatures greater than 29°C. Lambert(1929) in a study of *P.graminis* found that the combined processes of teliospore germination, basidiospore formation, infection of the alternate host and development of aecia was retarded or prevented by temperatures greater than 22°C. Similarly, development of the sexual cycle of *P.helianthi* was prevented at temperatures greater than 22°C because of the failure of basidiospores to form. Direct infection by the abnormal sterigmata produced when teliospores of *P.helianthi* were germinated at temperatures greater than 22°C was not observed although Bailey(1923) had suggested that direct infection was possible.

In the field teliospores must survive periods when the host is not available for infection. Teliospores of *P.helianthi* do not require the presence of the host to stimulate germination. As the teliospores become activated to germinate they will do so when environmental conditions are suitable. Teliospores exposed on the soil surface at Toowoomba during August continued to germinate and by October very few viable teliospores remained. Planting seed into inoculated soil during September resulted in pycnia on the seedlings that grew but by October infections had ceased.

Sunflower seedlings available for infection in the field during spring might include new season crop plantings and volunteer seedlings from the previous crop. Under Australian agricultural practices it would be unusual for spring sunflower to be sown on land cropped to sunflower the previous summer. Volunteer seedlings would be cultivated during normal farm practices. The germination of teliospores in the absence of susceptible host tissue is probably wasted. Repetitive germination of basidiospores of *P. helianthi* may occur and allow wider dissemination of basidiospores. Further research is required to establish the ability of basidiospores to survive in the field.

Pycnia and aecia which have been seen to develop during autumn must establish effective uredinial infections for the progeny of the sexual cycle to have a chance of surviving the winter inter-crop period. Populations of wild sunflower in northern New South Wales and the Central Highlands of Queensland might provide the presence of a continuum of host tissue for

infection. The role that these roadside sunflower populations have on perpetuating sunflower rust during inter-crop periods and providing tissue for the completion of the sexual cycle needs to be examined further.

Wild sunflower populations may also act as reservoirs of genotype diversity of sunflower rust. Pathotypes of sunflower rust which are at a selective disadvantage on crop cultivars may be favoured on the wild populations. These pathotypes might be present at low frequency until a change occurs in crop cultivars grown.

New pathotypes might develop through sexual recombination of virulence genes, asexual recombination of virulence genes and mutations to virulence. Diversity of virulence phenotypes was found among basidiospores derived from a number of teliospore collections. Recombinations of the corresponding genotypes might be a means by which new pathotypes have developed. Asexual recombination through somatic hybridization was not detected in the studies reported in this thesis but the recombinations for virulence subsequently appeared in the rust population in the field. Further work is required to test whether somatic hybridization does occur in *P.helianthi* and whether the new pathotypes detected were the product of mutational changes to existing pathotypes or sexual recombinations.

The rapid~~ly changing~~ appearance of new pathotypes of sunflower rust has resulted in many well-adapted sunflower hybrids becoming susceptible to the fungus and losing favour with farmers. Adapted cultivars possessing genes conferring pathotype-specific resistance which is effective against some but not all pathotypes in a population may still have a use in 'dirty-crop' cultivar mixtures. Such a mixture will not be completely free of disease but the presence of a diversity of different genes for resistance may delay disease increase (Browning and Frey,1969). The 'dirty crop' approach was found to be an alternative means of using sunflower hybrid cultivars other than as pure-lines.

The use of mixtures of sunflower hybrids as a means of minimizing yield losses caused by sunflower rust can only be recommended if agronomic performance in the absence of disease is competitive with that of pure-line cultivars. Sunflower hybrids are usually compared by their stature, maturity (time from sowing to anthesis), grain yield and oil content of grain. The unifomity in flowering time and plant height of commercial pure-line sunflower hybrids has come to be expected by growers. Mixtures of sunflower hybrids may not display the same degree of uniformity. Sunflower plants which are of a certain height in pure stands may compensate in a mixture by elongating if growing next to a taller plant. This can result in a mixture of nearly-uniform height at anthesis. However, the flowering time of individual lines is not altered in a mixture with other lines and plant height does not increase after anthesis. If one component line therefore is later maturing and usually taller than the other components as a pure-line then that line may continue to grow above the other lines as they flower and cause

non-uniformity of height. Differences in post-anthesis inclination of the capitula among lines will also contribute to a mixture having a non-uniform appearance.

Sunflower hybrid mixtures may be composed of lines which will complement each other. Hybrids which are commercially unacceptible because of low oil content may have high grain yields. Conversely, some hybrids produce low yields of grain with a very high oil content. Mixtures of these lines may produce a sunflower hybrid cultivar mixture with acceptible yield characteristics.

Assesssment of disease levels in a heterogeneous population of plants is difficult since it is difficult to obtain a truly random representation of all components. The indirect but objective assessment of spore output from diseased plots by spore trapping has sometimes been used to compare disease progress (Jeger, 1984; Mundt and Leonard, 1985). The spore trapping technique employed in the experiments reported in this thesis consisted of simple rods which depended on wind impaction for collection of urediniospores. The traps were not volumetric. Rods do have the advantages of being simple, cheap and easy to use. Thirty-three traps were used in the trial at Gowrie Junction. It would have been difficult to obtain or afford that number of more sophisticated traps. Bromfield, Underwood, Peet, Grissinger and Kingslover(1959) and Burleigh, Romig and Roelfs(1969) also used simple rods. They found that the number of urediniospores impacted was correlated to the number of uredinia in plots of wheat. Similarly, there were strong correlations between the severity of *P. helianthi* in the sunflower plots and the accumulation of urediniospores trapped in those plots in the trials reported in this thesis.

Cumulative spore yield curves may be transformed and analysed to provide estimates of the rates of disease increase. The logistic and gompertz equations describe sigmoidal curves with inflection points, 0.5 and 0.37, respectively (Thal, Campbell and Madden, 1984). The Weibull function has an extra parameter which allows flexibility in the position of the inflection point (Pennypacker, Knoble, Antle and Madden, 1980) and can be used to describe other models. Berger(1982) warned that under certain circumstances the Weibull function could be inaccurate. The outcome of the application of the various models can be assessed subjectively from the plots or through regression analysis of the data. The coefficient of correlation (r) or the coefficient of determination ($R^2$) are most often used to assess the goodness-of-fit of empirical models (Cornell and Berger, 1987). The Weibull function consistently gave better fit to the cumulative spore yield curves for sunflower rust urediniospores than the other two models tested.

More extensive research on means of predicting changes in virulence of *P. helianthi* in Australia and formulating means of reducing the damage caused by sunflower rust are warranted.