Chapter 8

General Discussion

The overall aim of the work reported in this thesis, was to develop a reliable system for screening sunflower for resistance to *A. helianthi* . A substantial part of acheiving this involved the development of a standard screening procedure that could be used in the greenhouse. This greenhouse assay was intended to supplement field screening, by confirming resistance reactions, increasing the speed of selection and reducing the number of lines that had to be screened in the field. The greenhouse assay was then used to measure components of resistance in a number of lines that ranged in their susceptibility to *A. helianthi.* In conjunction with this, epidemic parameters for the same lines were examined in the field and analysed to determine their ability to differentiate levels of QR. Components of QR measured in the greenhouse were then correlated with parameters of resistance measured in the field, to determine which components and epidemic parameters could be used for selection purposes. Finally, inbred restorer lines that were selected using both field and greenhouse experiments, were evaluated in hybrid combinations using the greenhouse assay and field experiments.

In developing a system for selecting for resistance to *A. helianthi,* consideration was given to the basic concepts that characterise quantitative traits. In many host-pathogen systems, it is more difficult to screen and select for quantitative resistance (QR) than it is for qualitative resistance (Parlevliet, 1986). The main reason for this is that QR is generally controlled by many genes, whose small, similar and supplementary effects on the expression of the phenotype give rise to continuous variation (Mather and Jinks, 1977).

Breeding for QR requires that the many genes that contribute to resistance are accumulated in a single genotype. The segregation of a large number of genes whose individual effect on the phenotype may not be distinguishable, make this task extremely difficult. In addition, other factors such as environment, nutrition and age of the host, inoculum load and any factor that affects these, add to the amount of phenotypic variation expressed in a population of plants. The variation caused by these non- genetic factors is therefore, non-heritable. In a breeding program, these factors mask genetic effects and reduce the reliability of selection, such that each cycle of selection will have a component of 'genetic drag' due to the presence of undesirable genotypes (Falconer, 1989). Progress in this system will be proportional to the level of 'drag' and the heritability of the characters being selected. Since heritability is a genetic fixture, screening procedures must focus on reducing the level of 'drag' by improving the accuracy of selection. The extent to which this can be done depends largely on the host-pathogen system and the genetic basis of resistance.

In developing the greenhouse assay (Chapter 3), procedures and techniques were used that would help reduce and control non-genetic variation. It must be accepted, that variation arising from experimental procedures or environmental conditions cannot be eliminated and at best, may only be reduced and controlled to a relatively consistent level. Experiments were conducted to establish optimum environmental conditions, such as the temperature used for inoculation and incubation, the duration of the dew period used for inoculation and incubation and methods of maintaining leaf wetness during the post-inoculation dew period. Inoculation procedures were strictly controlled to ensure that inoculum quality was standardised and that plants were inoculated with a known spore concentration. Inoculum was directed at leaves of a specific age on host plants at a particular growth stage. Experimental designs aimed at minimising variation were recommended and methods for assessing resistance were established.

Among researchers who have screened sunflower for resistance to *A. helianthi* in the greenhouse (Morris and Yang, 1983; Lipps and Herr, 1986; Block, 1990), few gave any

consideration to minimising the non-heritable portion of variation. Since the expression of resistance to *A. helianthi* is quantitative, it must follow that it can only be measured by using quantitative procedures, yet few researchers, if any, have applied quantitatve methods when screening sunflower for resistance to *A. helianthi.* Such procedures require that the factors contributing to the disease phenotype are equal for all plants or lines being evaluated. Clearly, equal application of these factors cannot be attained, but steps can be taken to minimise deviations. The work described in Chapter 3 has gone some way towards meeting these requirements. A basic greenhouse assay was recommended (section 3.4) and used in further experiments to measure components of QR.

Selection for QR is usually conducted in the field. This is because the correlations between greenhouse and field reactions are often poor and inconsistent (Simmonds, 1991). The reason for this may be partly due to the complexity of some host-pathogen systems, but may also be due to the use of inadequate greenhouse screening procedures (Rotem, 1988a). Verification of the greenhouse assay was dependent on 'feedback' from field data. Therefore, field and greenhouse screening should ideally, have proceeded together, but seasonal restrictions and the failure of disease development hindered the rate at which field experiments could proceed. The severe five-year drought that was experienced throughout eastern Australia while this work was being conducted, constrained the development of field epidemics of *A. helianthi.* In some field experiments, it was only luck and perseverence that resulted in disease development at all (see Chapter 5). Hence, development of the greenhouse assay was forced to proceed somewhat independently of field screening at first, as germplasm had not been sufficiently characterised in the field.

Screening for QR in the field must contend with a number of problems that generate variation which is not easily controlled. Interplot interference, influences of plant habit on micro-environment, method and timing of disease assessment, differences in maturity between various lines, disease pressure, type of epidemic and orientation of plants or plant parts to prevailing winds are all factors that generate variation in experimental plots.

Experimental procedures can be adjusted to account for some of the effects of these factors and various analytical methods can be employed that minimise their impact on the selection process, but overall, control of this type of variation is limited.

In most field experiments, designs that specifically minimise the impact of interplot interference were not used. The reason for this is that the designs that lessen interference between plots require a great deal of space. This places logistical demands on management of the site and limits the number of lines that can be included in a single experiment. The use of flanking spreader rows of the susceptible line B89 (Chapters 4, 7) attempted to provide uniform disease levels and while this may have lessened interference, it might not have prevented it entirely.

The experimental design that was described in Chapter 5 did however, attempt to reduce the amount of interference between plots. Observations of the epidemics that developed in each plot suggested that the level of interference might not be large, because allo-infection of adjacent rows appeared to be quite low despite high levels of infection at the disease focus. Auto-infection appeared to contribute most to rapid epidemic development and infection patterns on leaves tended to support this contention. These patterns indicated that under optimal weather conditions, many new infections occur as local lesions, which develop when spores from a sporulating lesion are washed across the leaf surface during periods of high moisture. Lesions tend to concentrate around an existing 'parent' lesion and towards the tips of leaves where moisture levels are high due to leaf orientation (Figure 8.1). Under optimal weather conditions, lesions expand, coalesce and give rise to successive cycles of local infection. Blighting is probably caused by infections resulting from extremely high numbers of spores concentrated in a small area around lesions. It is therefore possible that only a small proportion of spores from any given lesion contribute to allo-infection, but a much larger proportion are involved in auto-infection.

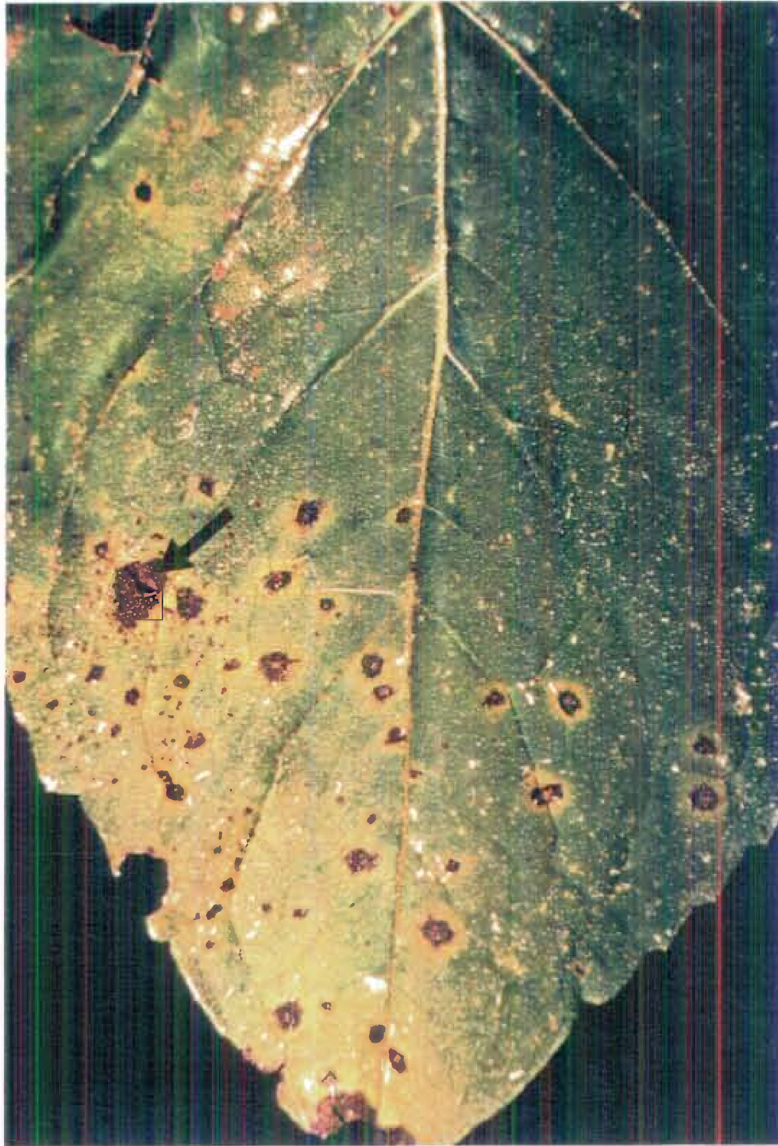


Figure 8.1. *Patterns of local infection appear as new infections surrounding a mature lesion of* A. helianthi (arrowed). *Lesions tend to concentrate towards the tip of the leaf where moisture levels are high.*

Some preliminary spore-trapping experiments (not reported in this study) indicated that spore dispersal during the early epidemic phase and during periods of wet weather were extremely low. Also, attempts to induce sporulation on attached leaves in the laboratory (Chapter 7) and in the field (not reported) indicated that sporulation is not simply a function of temperature and leaf wetness, but may also require other host or pathogen related signals. Sporulation occurs easily on detached tissue, such as sunflower residues (Jeffrey *et al.*, 1984) and senesced leaves, particularly those that have fallen from plants

and lie on moist soil. It may be that air-borne spore dispersal and allo-infection are greatest at the late stages of the epidemic when large amounts of dead tissue contribute to the available inoculum load. Interference would be greatest under these conditions but, epidemics of this magnitude are not always experienced in experimental field plots. In this study, only one epidemic (Site1, Chapter 5) approached these proportions.

The timing and method of disease assessment are two factors over which there can be some control. Since susceptability of sunflower to *A. helianthi* increases at anthesis, using this as the critical point for disease assessment (Allen, 1981) would seem to be logical. However, if plants are not assessed for resistance until flowering or post-flowering, then cross pollination would occur unless all plants were bagged prior to flowering. This imposes high demands on available resources, particularly where large plant populations are used. In studies conducted in Chapter 5, good correlations were found between disease severity ratings (DSRs) taken prior to flowering and those taken at and after flowering. If this were a general condition which applied also to other sources of germplasm and other epidemic patterns, then selection for resistance to *A. helianthi* could be carried out before flowering and only selected plants would have to be bagged for controlled pollination.

Estimation of disease severity using pictorial keys that contain a limited set of infection types is a common method of disease assessment The pictorial key devised by Allen (1983c) contained few classes between 5% and 25 % leaf area infected. The key was modified so that more classes were represented in this range (Appendix III), however, it must be acknowledged that the key presented a poor guide for matching infection patterns. This is understandable when one considers the infinite number of patterns that lesions can form following successive cycles of infection. No key could realistically account for even a small percentage of these patterns. There is therefore considerable opportunity for inaccurate assessment of disease severity, particularly at very low levels of disease where the relative bias and imprecision is highest (Parker *et al.*, 1995). Disease in this range (< 25%) tends to be overestimated (Forbes and Jerger,1987).

Providing that any bias is consistent, this factor would not pose a problem, but differences are likely to occur between researchers and even between years for the same researcher. The relatively high correlation obtained for estimates of DSR between years and sites in the experiments described in Chapters 5, 6 and 7 indicate that at least, disease estimations were consistent. However in Chapter 5, the flat disease gradients that were observed at very low levels of disease may have been due to the failure to accurately estimate very low levels of infection. Similarly, flat disease gradients were observed at low levels of disease caused by *Peronospora manshicura* (Naum.) Syd.ex Gaum.(Lim 1978) in soybean and *S. nodorum* in wheat (Jerger *et al.*, 1983), but no explanations were given for these observations. It is possible that they were due to inaccurate disease estimates. The use of relative rather than actual disease severity ratings may help overcome the problem of variation in disease assessment. Preliminary work using digital imaging to assess disease due to *A. helianthi* (Tucker, *et al.*, 1994) shows promise, particularly for greenhouse assessments. This system has the potential to measure disease quickly, accurately and consistently. Automation and portability of the imaging system will be of great benefit for field assessments.

A further problem encountered in disease assessment is that of distinguishing between necrosis due to disease and necrosis due to nutritional/physiological disorders and senescence. Certain genotypes are predisposed to physiological disorders resulting in tissue necrosis and necrotic lesions can occur on plant tissue as a result of nutritient deficiencies or natural senescence. The line ARpop// Rx677 (P_{17}) developed necrotic lesions on leaves, as a result of a physiological disorder (Figure 2.1). Necrotic lesions surrounded by chlorotic halos can occur on leaves due to phosphorous deficiency (Figure 2.2).

Disease assessment can also be biased if differences in susceptibility among sunflower lines is caused by differences in maturity. Cultivars that exhibit early maturity relative to other cultivars are often highly susceptible (Wolfe and Gessler, 1992). For example, no 'early' cultivars of potato with QR to late blight have been observed (Parlevliet, 1992). It

is likely that the rapid increase in disease severity for the line Rpop (P_{18}) late in the epidemic (Tables 5.2 and 5.7), was due to early maturity. Similarly, the line 10008-3-3-5 (P_9) was late to mature, which may account for its high level of resistance. The problem of different maturities is difficult to resolve in field experiments. One solution might be to only test lines of similar maturity together, but this may create some logistical problems. Moreover, differences in maturity between lines may not be sufficiently known during the early generations of a breeding program.

The epidemics examined in this study (Chapter 5) were probably typical of *A. helianthi* epidemics experienced in Australia. They were characterised by a long lag phase followed by a rapid increase in disease. This pattern may reflect the relationship between age-induced susceptability and weather patterns in Australia. The increase in disease during the early growth stages of the crop is dependent on successive periods of rainfall. The intensity of an epidemic resulting from an extended rainfall event at anthesis depends on the length of the wet period and the level of disease that developed prior to anthesis. In Australia, severe epidemics of *A. helianthi* occur infrequently, perhaps once in every five years. In India however, *A. helianthi* epidemics occur more frequently due to the regular periods of rainfall experienced during the monsoon season (Borkar and Patil, 1992). Epidemics can develop early in the life of a crop, causing seedling death and/or severe defoliation throughout the life of the crop, particularly at anthesis. Whether the lines selected under Australian conditions would maintain their resistance when subjected to the kinds of conditions experienced in India is unknown. Experimental evidence suggests that there is an infection threshold above which resistance fails to be effective (Chapter 3). It may be that the levels of resistance required to withstand the kinds of environmental conditions experienced in India are unattainable by conventional breeding methods. Application of the methods described in this thesis, to conditions experienced in India, might enable more efficient selection of resistant germplasm.

Other factors that can bias field assessments include plant density or plant architecture which can modify the micro-climate close to the infection court (Burdon, 1982; Carson,

1986). Plant densites higher than those normally used for the commercial production of sunflower in Australia were used in all field experiments. It was hoped that this would increase the chances of epidemic development by creating a highly favourable micro-environment for disease development. However, equal plant densities does not mean equal micro-environmental effects, because of differences in plant architecture (leaf number, size and orientation; plant stature) between lines. For example, lines 10020.11.1.18 (P_1) and Rx 677 (P_{17}) exhibited 'open' plant habits and had good levels of resistance, but it is difficult to separate resistance due to physical attributes that diminish micro-environmental effects and resistance due to post-infection defence mechanisms. Greenhouse screening using seedling plants fails to differentiate resistance due to these factors. For example, based on mean lesion size, the line Rx677 was rated as susceptible to *A. helianthi* in greenhouse screenings (Tables 7.3 and 7.4, Chapter 7) but was resistant in the field.

One of the major difficulties in evaluating QR is that the phenotypic expression of resistance is often affected by interactions between genotype and the environment. In order to take account of the average effect of this interaction, lines must be evaluated in different environments (locations) over time. In this study, it was expected that the impact of environment on the expression of QR could be determined from the degree of correlation for disease reaction, between lines tested at different locations and /or times. This was based on the assumption that environment and growing conditions at the two sites were inherently different. Using DSRs and/or audpcs, Spearmans' rank correlations gave generally good coefficients of correlation for lines tested at different sites in the same year (Chapters 5 and 6) and lines tested at the same site in different years (Chapter 7). This suggests that the evaluation of resistance was consistent and reliable under the epidemic conditions experienced at the respective locations and in different years.

Epidemic parameters that were well correlated with disease severity ratings (DSRs) were considered to be the best indicators of resistance in the field (Chapter 5). The area under the disease progress curve (audpc) and the volume (GV) under the three-dimensional surface

plot of disease progress in space and time, were well correlated with DSRs. The lack of correlation between DSR and apparent infection rates (*r*), disease gradients (*b*) and velocities of spread (*v*) may indicate that these parameters are inadequate for differentiating QR among sunflower lines, but are useful for studying epidemic patterns in individual lines. Three-dimensional surface plots of disease progress in space and time provided a rapid way of visualising epidemic parameters, DSR, *r*, and *b* together. Sunflower lines could be easily compared using the surface plots because the relationships between epidemic parameters were easily evaluated.

There was good correlation between mean lesion size (MLS) measured in the greenhouse and DSRs, audpcs and GVs measured in the field. MLS is a direct measure of the rate at which *A. helianthi* colonises tissue. It correlates well with measures of resistance in the field because disease severity ratings (DSRs) are a function of infection frequency and lesion size. Infection frequency was not as well correlated with DSR and therefore is not a reliable indicator of resistance. Nonetheless, it could be used in conjunction with MLS in a two-stage screening procedure where seedlings are initially selected according to their infection frequencies, then examined more closely using MLS. Other components of QR, incubation period and spore production were poorly correlated with DSRs. These components are more sensitive to environmental conditions and experimental techniques and consequently have high coefficients of variation (Rotem, 1992).

The cytoplasmic male sterile (*cms*) system used to produce sunflower hybrids further complicates the process of breeding for QR, because commercial hybrids are produced by crossing two or more inbred parents. The resistance phenotype of the resulting F_1 hybrid can be predicted, more or less, from the midparental pattern of inheritance. This pattern suggests that the F_1 will have a phenotypic value equal to the mean of the parents. However, deviations from thre midparent value, (towards greater resistance or susceptibility) can occur, because we do not know how the genes affecting the character combine to produce their effects. Studies could be conducted to determine heritabilities and combining abilities, but often, these merely confirm the adage, 'breed from the best'.

Furthermore, 'It does not pay a breeder to examine a genetical situation in detail, if he has to wait a long time for the answers' (Gilbert, 1989). The testing of F_1 hybrids is therefore, the most important phase of a breeding program for commercial sunflower breeders in Australia and is the final test of the value of inbred lines. F_1 phenotypic values that are less than the midparent value indicate a level of dominace. The F_1 hybrids made from resistant restorer lines and susceptible female lines (Chapter 6) were more resistant than suggested by their midparent values, indicating a high level of dominance among the gene(s) controlling resistance. More of the lines selected and developed in this study need to be tested in this way.

In this study, a system for identifying sunflower for resistance to *A. helianthi* was established. This system could potentially improve the accuracy and speed of selection of resistant germplasm. However, it remains constrained in some ways by the many variables that affect QR and which, when acting collectively and at different intensities, force selection errors. It was hoped that the procedures developed in this study would help reduce these errors, but it is acknowledged that they will not be eliminated. Nevertheless, the procedures developed here form the basic framework from which further research can proceed. For example, resistant germplasm identified using this system can be used to improve existing techniques or help to develop new techniques, such as those described below.

Future Research

(i) Further refine the greenhouse screening procedure by developing techniques for mass innoculation using large numbers of plants at a younger growth stage.

(ii) Investigate alternative methods for identifying resistant germplasm. For example, a study of host defence and the mechanisms of resistance could be used to develop assays using defence-related compounds as markers for selection. Similarly, assays could be developed pending an investigaton of the relationship between sensitivity to phytotoxins

produced by *A. helianthi* and resistance to infection. Likewise, sensitivity to ethylene could be investigated as a method of identifying resistant germplasm

(iii) Resistance to *A. helianthi* is known to occur among various species of *Helianthus* as well as related genera, *Tithonia* and *Viguiera*. Interspecific and intergeneric hybridisation provides a difficult, but feasible breeding option to improve levels of resistance in *H. annuus*.

(iv) It is likely that the levels of resistance attained through conventional breeding methods will be inadequate under some epidemic conditions. It may be possible to increase resistance by transforming resistant plants with genes that encode for specific antifungal proteins (AFPs). Novel AFPs have been identified and shown to have bioactivity against *A. helianthi* (Kong *et al*., 1996).

(v) The genetics of resistance could be studied using germplasm identified with this screening technique. Heritability, combining ability estimates and the nature of gene action may be useful for deciding further breeding strategies.

(vi) Digital image analysis could be used to provide faster, more accurate estimates of disease in both the greenhouse and the field.

References

Abbas H K, Egley G H, Paul R N. 1995. Effect of conidia production temperature on germination and infectivity of Alternaria helianthi. Phytopathology 85:677-682.

Abraham K J, Menon M R, Nair M C. 1976. Comparative efficacy of fungicides in the control of Alternaria blight of sunflower. *Madras Agricultural Journal* **63**: 421-423.

Acimovic M. 1969. *Alternaria* sp. - a new parasite of sunflower in Yugoslavia (Preliminary comment). *Zastita Bilja* **19**: 305-309.

Acimovic M. 1976. Sunflower trials for susceptibility and intensity of disease infection. *Proceedings VII International Sunflower Conference* II: 244-247.

Acimovic M. 1979a. Pathogenicity of *Alternaria helianthi* (Hansf.) Tub. and Nish. in sunflowers and other plant species. *International Sunflower Association Newsletter* **3**: 9-12.

Acimovic M. 1979b. Uticaj nekih ekoloskih faktora na sporulaciju *Alternaria helianthi* (Hansf.) Tub. and Nish. I na infekciju suncokneta.(English summary) *Zastita Bilja* **30**(1): 59-63.

Agrawat J M, Chippa, H P and Mathur, S J. 1980. Screening of sunflower germplasm against *Alternaria helianthi. Indian Journal of Mycology and Plant Pathology* **9**: 85-86.

Alcorn J L, Pont W. 1972. *Alternaria helianthi* on sunflower. *Australian Plant Pathology Society Newsletter* **1**: 30.

Allen S J. 1981. Alternaria blight of sunflowers in Australia. Ph.D. Thesis. Department of Botany, University of New England, NSW, Australia.

Allen S J, Brown J F, Kochman J K. 1982. The control of *Alternaria* blight of sunflowers in eastern Australia. In *Proceedings of the Tenth International Sunflower Conference,* Surfers Paradise, Queensland, Australia, pp. 149-151.

Allen, S.J., Brown, J.F. and Kochman, J.K. 1983a. The infection process, sporulation and survival of *Alternaria helianthi* on sunflowers. *Annals of Applied Biology* **102**: 413-419.

Allen S J, Brown J F, Kochman J K. 1983b Effects of leaf age, host growth stage, leaf injury and pollen on the infection of sunflowers by *Alternaria helianthi. Phytopathology* **73**: 896-898.

Allen S J, Brown, J F and Kochman J K. 1983c. Production of inoculum and field assessment of *Alternaria helianthi* on sunflower. *Plant Disease* **67**: 665-68.

Allen S J, Brown J F and Kochman J K. 1983d. Effects of temperature, dew period and light on the growth and development of *Alternaria helianthi. Phytopathology* **73**(6): 893-95.

Allen S J, Kochman J K, Brown J F. 1981. Losses in sunflower caused by *Alternaria helianthi* in southern Queensland. *Australian Journal of Agriculture and Animal Husbandry* **21**: 98-100.

Anahosur H J 1978. *Alternaria helianthi.* CMI descriptions of pathogenic fungi and bacteria, No. 582., Kew, Commonwealth Mycological Institute.

Anonymous 1981. Introduction. **In:** *Sunflowers in Australia.* (Ed. Warmington, C.R.), pp. 7-11. Pacific Seeds, Toowoomba, Qld, 4350.

Aylor D E and Ferrandino F J. 1989. Temporal and spatial development of bean rust epidemics initiated from an inoculated line source. *Phytopathology* **79:** 146 -151.

Balasubrahmanyam N, Kolte S J. 1980a. Effect of different intensities of Alternaria blight on yield and oil content of sunflower. *Journal of Agricultural Science, Cambridge* **94**: 749-751.

Balasubrahmanyam N, Kolte S J. 1980b. Effect of Alternaria blight on yield components, oil content and seed quality of sunflower. *Indian Journal of Agricultural Science* **50**: 701-706.

Bashi E, Rotem J, Pinnschmidt, H and Kranz J. 1983. Influences of controlled environment and age on development of *Alternaria macrospora* and on shedding of leaves in cotton. *Phytopathology* **73**: 1145-1147.

Berger R D and Luke H H. 1979. Spatial and temporal spread of oat crown rust. *Phytopathology* **69**: 1199 -1201.

Bhaskaran R and Kandaswamy TK 1977. Epidemiology of the leaf spot disease of sunflower. *East Africa Agriculture and Forestry Journal* **43**(1): 5-8.

Bhaskaran R and Kandaswamy T K. 1979. Evaluation of fungicides for the control of Alternaria leaf spot on sunflower. *Indian Journal of Agricultural Science* **49**(6): 48-82.

Birman R K, Singh B P 1995. Path-coefficients and genetic parameters of the components of field resistance of potatoes to late blight. *Annals of Applied Biology* **127**:353-362.

Block C C. 1990. Screening of sunflower germplasm for *Alternaria helianthi* resistance. In *Proceedings of Sunflower Research Workshop*, Bismark, ND: National Sunflower Association, pp. 22-24

Borkar S G and Patil, B S. 1992. Epidemiology of Alternaria leaf spot disease of sunflower. *Indian Phytopathology* 84-85

Box G E P. 1954. Some theorms on quadratic forms applied in the study of analysis of variance problems. I Effect of inequality of variance in the one-way classification. *Annals of Mathematical Statistics* **25**: 290-302.

Brown J F. 1974. A simple laboratory technique for screening `slow rusting' cultivars of cereals. *Australian Plant Pathology Society Newsletter* **3**(1): 25.

Burdon J J. 1982. Host density as a factor in plant disease ecology. *Annual Review of Plant Pathology* **20**: 143-166.

Byther R S, Steiner G W. 1972. Use of helminthosporoside to select sugarcane seedlings resistant to eyespot disease. *Phytopathology* **62**: 466-470.

Carson M L. 1981. Reactions of *Helianthus* to seedling blight caused by *Alternaria helianthi. Phytopathology* **71**: 1115 (Abstr.)

Carson M L, Mehdi S S. 1983. Infectivity of *Alternaria helianthi* on sunflower as influenced by host genotype, pathogen isolate and conidial concentration. In

Carson M.L. 1985a. Epidemiology and yield loss associated with Alternaria blight of sunflower. *Phytopathology* **75**: 1151-1155.

Carson M L. 1985b. Reaction of sunflower inbred lines to two foliar diseases. *Plant Disease* **69**: 986-988.

Carson M L. 1986. Effects of row spacing and plant stature on the development of alternaria blight of sunflower. In *Proceedings of Sunflower Research Workshop,* Bismark, ND: National Sunflower Association, p. 7

Carver R P. 1978. The case against statistical significance testing. *Harvard Educational Review* **48**: 378-398.

Chakraborty S, Cameron D F, Irwin J A G, Edye L A. 1988. Quantitatively expressed resistance to anthracnose (*Colletotrichum gloeosporiodes*) in *Stylosanthes scabra. Plant Pathology* **37**: 529-537

Clark C J, Tucker A M, Turton J A. 1971. Sampling technique for estimating roundworm burdens of sheep and cattle. *Experimental Parisitology* **30**: 181-186.

Cliff A D, and Ord J K. 1981. Spatial processes: Models and application. Pion Ltd, London. pp266.

Day R W, Quinn G P. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**: 433-463

De Aquino M L N, Bezerra J L, De Andrade Lira M. 1971. Sunflower blight in Perumbuco. *Revista de Agricultura* **46**: 151-156

Dhingra O D, and Sinclair J B. 1985. Culture of Pathogens. **In:** *Basic Plant Pathology Methods* Chapter II, pp. 11-48.

Ellis M B. 1968. *Alternaria chrysanthemi*, Simmons and Crosier, CMI Description No. 164.

Falconer D S. 1989. Introduction to Quantitative Genetics. Third Edition. John Wiley and Sons Inc., New York

Ferradino F J. 1993. Dispersive epidemic waves:I. Focus expansion within a linear planting. *Phytopathology* **83:** 795-802.

Fick G N. 1978. *Breeding and Genetics*. In: Sunflower Science and Technology. Ed J F Carter. Agronomy 19. American Society of Agronomy.

Fick G N, Zimmer D E. 1974. Monogenic resistance to Verticillium wilt in sunflower. *Crop Science* **14**: 895-896.

Forbes G A, Jerger M J. 1987. Factors affecting estimation of disease intensity in simulated plant structures. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **94**: 113-120.

Fry W E. 1978. Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato blight. *Phytopathology* **68:** 1650-1655.

Gardner,C O. 1963. Estimates of genetic parameters in cross-fertilizing plants and their implications in plant breeding. In *Statistical Genetics and Plant Breeding*. (Ed W D Hanson and H F Robinson). National Academy of Sciences-National Research Council, Washington D.C.

Gilbert N 1989. *Biometrical Interpretation: Making Sense of Statistics in Biology* Oxford University Press, New York.

Goosen P G, and Sackston W E 1968. Transmission and biology of sunflower downy mildew. *Canadian Journal of Botany* **46**(5): 5-10.

Gregory P H. 1968. Interpreting plant disease dispersal gradients. *Annual Review of Phytopathology* **6**: 189-212.

Griffiths H M, Jones D G 1987. Components of partial resistance as criteria for identifying resistance. *Annals of Applied Biology* **110**: 603-610.

Habgood R M. 1977. Resistance of barley cultivars to *Rhynchosporium secalis*. *Transactions of the British Mycological Society* **69**: 281-286.

Hansford C G. 1943. Contributions towards the fungal flora of Uganda. V. Fungi Imperfecti. *Proceedings of the Linnaean Society* **1**: 34-67.

Headrick J M, Pataky J K. 1988. Spatial and temporal development of common rust in susceptible and partially resistant sweet corn hybrids. *Phytopathology* **78**: 227 - 233.

Heiser, C B. 1957. A revision of the South American species of *Helianthus*. *Brittonia* **8**: 283-295.

Heiser, C B Smith, DM Clevenger, S Martin, W C. 1969. The North American Sunflowers (*Helianthus*). (Ed. Delevoryas, T.) *Memoir* **22**(2): 1-218.

Heiser, C B. 1978. Taxonomy and origin. **In:** *Sunflower Science and Technology*. (Ed. Carter, J.F.), pp. 31-53, American Society of Agronomy, Inc., Wisconsin, USA.

Herr, L J, Lipps, PE. 1981. Occurrence of *Alternaria helianthi* on sunflower in Ohio. (Abstr.) *Phytopathology* **71**: 880.

Hirano, S S Upper, C D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. *Annual Review of Phytopathology* **21**: 243-269.

Hunter M N, Kochman J K. 1985. Severe phosphorousdeficiency in sunflower - the cause of foliar symptoms similar to those produced by some fungal pathogens. *Helia* **8**: 57-62.

Ingold, C T. 1965. Rhythms of spore liberation in fungi. **In:** *Spore Liberation.* Chapter V: 96-116, Oxford University Press, London.

Islam, U Maric, A Cuk, L Skoric, D. 1976. Studies of Alternaria black spot (*Alternaria helianthi* Hansf.) of sunflowers in Yugoslavia. **In:** *Proceedings of the 7th International Sunflower Conference,* **1**: 230-237.

Islam U, Maric A. 1978. *Alternaria helianthi (*Hansf.) Tub. and Nish. on sunflower in Yugoslavia and some cultural characteristics of the fungus. *Zastita Bilja* **24**: 219-236.

Islam U, Maric A. 1980. Contribution to the studies on biology, epidemiology and resistance of sunflower to *A. helianthi* (Hansf.) Tub. and Nish. *Zastita Bilja* **31**: 35-39.

Jeffrey K K, Lipps P E, Herr L J. 1984. Effect of isolate virulence, plant age and crop residues on seedling blight of sunflower caused by *Alternaria helianthi. Phytopathology*, **74**: 1107-1110

Jeffrey, K K Lipps, P E Herr, L J. 1985. Seed-Treatment Fungicides for Control of Seedborne *Alternaria helianthi* on sunflower. *Plant Disease* **69**(2): 124-126.

Jerger, M J, Jones, D and Griffiths, E. 1983. Disease spread of non-specialised fungal pathogens from inoculated point sources in intraspecific mixed stands of cereal cultivars. *Annals of Applied Biology* **102**: 237 -244.

Jerger, M J. 1983. Analysing epidemics in time and space. *Plant Pathology* **32**: 5 -11.

Jovicevic B. 1976. Contribution to the research on fungus flora in sunflower seed. In *Proceedings of the Seventh International Sunflower Conference*, II: 200-204.

Kinman M L. 1970. New developments in the USDA and state experiment station sunflower breeding programmes. In *Proceedings of the 4th International Sunflower Conference*, Memphis, TN USA, pp. 181-183.

Kochman J K, Goulter K C. 1983. Wild sunflower *Helianthus argophyllus* - a source of resistance to rust (*Puccinia helianthi*) and Alternaria blight (*Alternaria helianthi*). In *Proceedings of Fourth International Congress of Plant Pathology*. Abstr., pp. 203. Rowprint Services Pty Ltd, Vic.

Kong G A. 1994. A technique for estimating the number of spores in a sample. In Proceedings of the Fifth Graduate Seminar Symposia. University of New England, 24 September, 1994. Botany Department, UNE.

Kong G A, Kochman J K, Brown J F. 1995. A greenhouse assay for screening sunflower for resistance to *Alternaria helianthi*. *Annals of Applied Biology* **127**: 463-478.

Kong G A, Kochman J K, Lawson W , Goulter K C, Engle, B E. 1996. An overview of sunflower disease research in Australia. In Proceedings of the XIV International Sunflower Association Conference, Beijing, China June 14-22, 1996.

Kong, G A, Kochman, J K and Brown, J F. 1997. Phylloplane bacteria antagonistic to the sunflower pathogen, *Alternaria helianthi. Australasian Plant Pathology* **26**: 85-87.

Kong, G A, Kochman, J K and Brown, J F. 1997. Components of quantitative resistance to *A. helianthi in sunflower. Annals of Applied Biology* **128**: In Press

Kolte, S J, Balasubrahmanyam N, Tewari A N, Awasthi R P. 1979. Field performance of fungicides in the control of alternaria blight of sunflower. *Indian Journal of Agricultural Sciences* **49**: 555-559.

Kranz, J. 1974. The role and scope of mathematical analysis and modelling in epidemiology. In *Epidemics of plant diseases: Mathematical analysis and modelling* Springer, NY.

Kumar L S, Prakash H S, Shetty H S. 1991. Effect of various factors on the phytotoxicity of culture filtrate of *Alternaria. helianthi. Plant Disease Research* **6**: 74-76.

Lamarque C, Kochman J K. 1988. Essai de standardisation des techniques d' inoculation du torunesol avec L' *Alternaria helianthi.* In *Proceedings of the 12th International Sunflower Conference.* Novi Sad, Yugoslavia, **1**: 566-570.

Last F T. 1960. Longevity of conidia of *Botrytis fabae* Sardina. *Transactions of the British Mycological Society* **43**(4): 673-80.

Last F T, Deighton FC. 1965. The non-parasitic microflora on the surfaces of living leaves. *Transactions of the British Mycological Society* **48**(1): 83-99.

Leclercq P. 1969. Une sterilite male cytoplasmique chez le tournesol. *Annals of Amelior Plant* **19**: 99-106.

Levine M N, Bramberg R H, Atkinson R E. 1936. Microorganisms antibiotic or pathogenic to cereal rusts. *Phytopathology* **26**: 99-100, Abstract.

Lim S M. 1978. Disease severity gradient of soybean downy mildew from a small focus of infection. *Phytopathology* 68: 1774-1778.

Lipps P E, Herr L J. 1986. Reactions of *Helianthus annuus* and *H. tuberosus* plant introductions to *Alternaria helianthi. Plant Disease* **70**: 831-35.

Luke H H, Berger R D 1982. Slow rusting in oats compared with the logistic and gompertz models. *Phytopathology* **72:** 400 -402.

Mackenzie D R. 1976. Application of two epidemiological models for the identification of slow stem rusting in wheat. *Phytopathology* **66:** 55 -59.

Madden L V, Knoke J K, Louie, R. 1982. Considerations for the use of multiple comparison procedures in phytopathological investigations. *Phytopathology* **72**: 1015-1019.

Mather, K, Jinks, J L 1977. Introduction to Biometrical Genetics Cornell University Press, New York.

Mehdi S S, Carson M L, Lay C L. 1984. Genetic and phenotypic associations of resistance to four diseases in two sunflower populations. In *Proceedings of 6th Sunflower Research Workshop* Bismark, ND: National Sunflower Association, pp. 13

Mehdi S S, Carson M L, Lay C L. 1984. Genetic and phenotypic associations of resistance to four diseases in two sunflower populations. In *Proceedings of 6th Sunflower Research Workshop* Bismark, ND: National Sunflower Association, pp. 13

Minogue K P, Fry W E. 1983. Models of spread of disease: Model description. *Phytopathology* **73:** 1168 -1173.

Minogue K P. 1986. Disease gradients and spread of disease. In *Plant disease epidemiology: Population dynamics and management*, Vol. 1. Ed. K J Leonard and W E Fry. MacMillan, NY.

Mirza M S, Ahmed Y, Beg A. 1984. First report of *Alternaria helianthi* on sunflower from Pakistan. *Pakistan Journal of Agricultural Research* **5**: 157-59

Morris J B, Yang S M, Wilson L. 1983. Reaction of *Helianthus* species to *Alternaria helianthi. Plant Disease* **67**: 539-540.

Morgan F L. 1963. Infection inhibition and germ tube lysis of three cereal rusts by *Bacillus pumilus. Phytopathology* **53**: 1346-1348.

Muirhead I F. 1990. Plant protection in sunflowers : Quarantine. In *Proceedings of the 8th Australian Sunflower Association Workshop*, Kooralbyn, Qld, 1990, pp. 159-161, Australian Sunflower Association, Qld.

Mukadam, D S. 1982. Studies on self inhibition of *Alternaria brassicicola* (Schw.) Wiltshire. *Indian Botanical Reporter* **1**: 37 -39.

Narain U, Saksena H K. 1973. Occurrence of Alternaria leaf spot of sunflower in India. *Indian Journal of Mycology and Plant Pathology* **3**: 115-116.

Neher, D A and Campbell, C L. 1992. Underestimation of disease progress rates with the logistic, monomolecular and gompertz models when maximum disease intensity is less than 100 percent. *Phytopathology* **82:** 811 -814.

Nishimura S, Kohmoto K. 1983a. Host-specific toxins and chemical structures from *Alternaria* species. *Annual Review of Phytopathology* **21**: 87-116.

Nishimura S, Kohmoto K. 1983b. Roles of toxins in pathogenesis. In *Toxins and Plant Pathogenesis*. (Ed. Daly, J.M. and Deverall, B.J.), pp. 137-57, Academic Press Aust.

Orellana R G. 1970a. Resistance and susceptibility of sunflowers to downy mildew and variability in *Plasmopara halstedii*. *Bulletin Torrey Botanical Club* **97**: 91-97.

Orellana R G. 1970b. The response of sunflower genotypes to natural infection by *Macrophomina phaseolina*. *Plant Disease Reporter* **54**: 891-893.

Parker S R, Shaw M W, Royle D J (1995). The reliability of visual estimates of disease severity on cereal leaves. *Plant Pathology* 44: 856-864.

Parlevliet J E. 1976. Evaluation of the concept of horizontal resistance in the barley/*Puccinia hordei* host-pathogen relationship. *Phytopathology* **66**: 494-497.

Parlevliet J E. 1979. Components of resistance that reduce the rate of epidemic development. *Annual Review of Phytopathology* **69**:281-286

Parlevliet J E. 1986. Identification and evaluation of quantitative resistance. *In: Plant Disease Epidemiology: genetics resistance and management* Vol II. Ed. K J Leonard and W E Fry. Mcgraw-Hill.

Parlevliet J E. 1992. Selecting components of partial resistance. In *Plant Breeding in the 1990s.* Eds H.T. Stalker, and J.P Murphey. Wallingford, UK: CAB International.

Payne R W, Lane P W, Ainsley A E, Bicknell K E, Digby P G,N, Harding S A, Leech P K, Simpson H R, Todd A D, Verrier P J, White R R. 1987. Genstat 5 (Computer Program).

Pelletier J R, Fry W E. 1989. Characterisation of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate and spore production. *Phytopathology* **79**: 511-517.

Pennypacker, S P, Knoble, H D, Antle, C E and Madden, L V. 1980. A flexible model for studying plant disease progression. *Phytopathology* **70:** 232 -235.

Phelps K 1991. Some problems in the statistical analysis of trials for resistance screening. *Plant Pathology* **40**:340-341

Phelps K, Cole R A, Entwistle A R, Mead A ,Pink D A C. 1991. Design of trials for resistance screening in semi-controlled environments. *Plant Pathology* **40**:161-165

Pon D S, Townsend C E, Weesman GE , Schmitt C G. Kingsolver 1954. A *Xanthomonas* parasitic on Uredia of cereal rusts. *Phytopathology* **44**: 707-710.

Putt E D. 1964. Breeding behaviour of resistance to leaf mottle or Verticillium in sunflowers. *Crop Science* **4**: 177-179.

Putt E D. 1978. History and present world status. **In:** *Sunflower Science and Technology.* (Ed. Carter, J.F.), pp. 1-25. American Society of Agronomy Inc, Wisconsin, USA.

Putt E D, Sackston W E. 1963. Studies on sunflower rust. IV. Two genes, R_1 and R_2 for resistance in the host. *Canadian Journal of Plant Science* **43**: 490-496.

Reddy P C, Gupta B M. 1977. Disease loss appraisal due to leaf blight of sunflower indicated by *Alternaria helianthi. Indian Phytopathology* **30**: 569-570.

Reynolds, K M and Madden, L V. 1988. Analysis of epidemics using spatiotemporal autocorrelation. *Phytopathology* **78:** 240 -256.

Robeson D J, Strobel G A. 1982. Deoxyradicinin, a novel phytotoxin from *Alternaria helianthi. Phytochemistry* **21**: 1821-23.

Robeson D J, Strobel G A. 1985. Deoxyradicinin and 3-epideoxyradicinol production by the sunflower pathogen *Alternaria helianthi. Annals of Applied Biology* **107**: 409-415.

Robeson D J, Gray G R, Strobel G A. 1982. Production of the phytotoxins radicinin and radicinol by *Alternaria chrysanthemi. Phytochemistry* **21**(9): 2359-2362.

Rogers, C E, Thompson, T E and Seiler, G J. 1982. Sunflower species of the United States. National Sunflower Association, Bismark, ND.

Rotem J. 1988a. Techniques of controlled condition experiments *In: Experimental Techniques in Plant Disease Epidemiology*. Ed. Kranz J and Rotem J. Springer Verlag

Rotem J. 1988b. Quantitative assessment of inoculum production, dispersal, deposition, survival and infectiousness in airborne diseases. *In: Experimental Techniques in Plant Disease Epidemiology*. Ed. Kranz J and Rotem J. Springer Verlag

Rotem, J. 1978. Climatic and weather influences on epidemics. *In: Plant Disease: An Advanced Treatise, Vol. 2* Ed. J G Horsfall and E B Cowling. pp 317-337 Academic Press NY.

Rudolf K. 1976. Non-specific toxins. **In:** *Physiological Plant Pathology*. (Ed. Heitefuss, R. and Williams, P.H.), pp. 270-311, Springer-Verlag, Berlin, Heidelberg, New York.

Russell G E. 1978. General principles and methods of breeding for resistance. In *Plant Breeding for Pest and Disease Resistance*, pp. 15-41. Butterworths & Co, London-Boston.

Sackston W E. 1981. The sunflower crop and disease : Progress, problems and prospects. *Plant Disease* **65**: 643-648.

Sackston W E. 1988. First report of *Alternaria helianthi* on sunflower in Canada. *Plant Disease* **72**: 801

Schneiter A A, Miller J F. 1981. Description of sunflower growth stages. *Crop Science* **21**: 901-3.

Scheffer R P. 1976. Host-specific toxins in relation to pathogenesis and disease resistance. **In:** *Physiological Plant Pathology*. (Ed. Heitefuss, R. and Williams, P.H.), pp. 247-265, Springer-Verlag, Berlin, Heidelberg, New York.

Scheffer R P. 1983. Chemical determinants of disease. In *Toxins and Plant Pathogenesis*. (Ed. Daly, J.M. and Deverall, B.J.), pp. 11-25, Academic Press Inc., Australia.

Scott G E, Futrell M C. 1970. Response of maize seedlings to *Fusarium moniliforme* and a toxic material extracted from this fungus. *Plant Disease Reporter* **54**: 483-486.

Shane W W, Baumer J S, Sederstrom S G. 1981. *Alternaria helianthi* : A pathogen of sunflower New York to Minnesota. *Plant Disease* **65**: 269-271.

Shaner G, Finney R E. 1977. The effect of nitrogen fertilization of the expression of slow-rusting resistance in Knox wheat. *Phytopathology* **67:** 1051-1056.

Simons M D. 1972. Polygenic resistance to plant disease and its use in breeding resistant cultivars. *Journal of Environmental Quality* **1**(3): 232-239.

Simmons E G. 1986. Alternaria themes and variations (17-21), *Mycotaxon* **XXV**(1): 203-216.

Simmons E G. 1965. *Alternaria chrysanthemi. Mycologica* **57**: 140-143.

Simmonds N W. 1991. Genetics of horizcntal resistance to diseases of crops. *Biological Reviews* **66**:189-241

Sleesman J P, Leben C. 1976. Microbial antagonists of *Bipolaris maydis. Phytopathology* **66**: 1214-1218.

Strandberg, J O. 1987. Isolation, storage and inoculum production methods for *Alternaria dauci. Phytopathology* **77**: 1008-1012.

Somasundara B, Anilkumar T B. 1987. Factors affecting spore germination of *Alternaria helianthi. Zentralblatt Mikrobiologie* **142**: 619-20.

Straley C S, Straley ML, Strobel G A. 1974. Rapid screening for bacterial wilt resistance in alfalfa with a phytotoxic glycopeptide from *Corynebacterium insidiosum. Phytopathology* **64**: 194-196.

Swinburne, T R Barr, J and Brown, A E 1975. Production of antibiotics by *Bacillus subtilis* and their effect on fungal colonists of apple leaf scars. *Transactions of the British Mycological Society* **65**: 211-217.

Takano K. 1963. A disease of sunflower caused by a species of *Alternaria. Annals of the Phytopathological Society of Japan* **28**: 300.

Tal B, Robeson D J, Burke BA, Aasen, A J. 1985. Phytotoxins from *Alternaria helianthi* : Radicinin and the structures of deoxyradicinol and radianthin. *Phytochemistry* **24**: 729-731.

Tosi, L and Zazzerini, A. 1991. *Alternaria helianthi* - A new sunflower parasite in Italy. *Informatore Fitopatologico* **41**: 54 -58.

Toxopeus H.J, 1959. Notes on the inheritance of field resistance of the foliage of *Solanum tuberosum* to *Phytophthora infestans*. *Euphytica* **8**: 117-124.

Toxopeus H,J. 1961. On the inheritance of tuber resistance of *Solanum tuberosum* to *Phytophthora infestans* in the field. *Euphytica* **10**: 307-314.

Tubaki K, Nishihara N. 1969. *Alternaria helianthi* (Hansf.) comb. nov. *Transactions of the British Mycological Society* **53**: 147-149.

Tucker, C.C., Chakroborty, S., Kong, G.A. and Kochman, J.K. (1995). Qualitative assessment of disease severity using digital image processing. Abstract 254 p109, Proceedings of the 10th Biennial Australasian Plant Pathology Conference, Lincoln NZ, August, 1995.

van der Bosch, F, Zadoks, J C and Metz, J A J. 1988a. Focus expansion in plant disease I : The constant rate of focus expansion. *Phytopathology* **78:** 54 -58.

van der Bosch, F. Frinking, H D, Metz, J A J and Zadoks, J C. 1988b. Focus expansion in plant disease III: Two experimental examples. *Phytopathology* **78:** 919 -925.

van der Plank, J F. 1963. Plant Diseases: Epidemics and Control. Academic Press, NY

van der Westhuizen G C A, Holtzhausen M A. 1980. *Alternaria helianthi* on sunflower in South Africa. *Phytophylactica* **12**: 49-52.

Waggoner, P E, 1986. Progress curves of foliar diseases: their interpretation and use. *In: Plant Disease epidemiology: Population dynamics and Management, Vol 1.* (Ed. K J Leonard and W E Fry). Macmillan, NY.

Wallace G B, Wallace M M. 1950. *Tanganyika Fungus List : Recent Records* No. 14, Mycological Circular, No. 19, Tanganyika Department of Agriculture.

Warner J N. 1952. A method of estimating heritability. *Agronomy Journal* **44**: 427-430.

Wilcoxson, R D, Skovmand, B and Atif, A H, 1975. Evaluation of wheat cultivars for ability to retard development of stem rust. *Annals of Applied Biology* **80:** 275-281.

Wilkinson L, Hill M, Vang E. 1992. *Systat: Statistics, Version 5.2 Edition*. Evanston, IL: Systat, Inc., 742pp.

Wolfe, S M and Gessler, C, 1992. The use of resistance genes in breeding: epidemiological considerations. *In: Genes involved in plant defence* Ed. T Boller and F Meins. Springer-Verlag Wien, NY.

Yarwood C E. 1976. Modification of host response : Predisposition. **In:** *Physiological Plant Pathology.* (Ed. Heitefuss, R. and Williams, P.H.), pp. 703-714, Springer-Verlag, Berlin, Heidelberg, New York.

Zad J. 1978. A note on the mycoflora of sunflower seeds in Iran. *Seed Science and Technology* **6**: 953-956.

Zadoks, J C and Shine, R D. 1979. Epidemiology and plant disease management. Oxford University Press.

Appendix I

Recipes of media used in this study

1. Potato-Dextrose Agar (PDA)

Potato	200 g
Dextrose	20 g
Agar	17 g
Distilled Water	1000 mL

Add peeled and diced potato to 500 mL of distilled water; add agar to 500 mL of distilled water. Place both in a steamer for 1 hour. After steaming, strain potatoes with cheesecloth and add the liquid obtained to the agar. Add dextrose and bring volume to 1 litre. Autoclave at 120°C/138KPa for 20 minutes.

2. Potato-Carrot Agar (PCA)

Potato	20 g
Carrot	20 g
Dextrose	17 g
Tap Water	1000 mL

Procedure is the same as that used to make PDA.

3. Sunflower Leaf Extract Agar (SLEA)

Fresh Sunflower Leaves	100 g
Distilled Water	1000 mL
Agar	17 g

Place leaves in water and steam for 1 hour in a steamer. Pour off the liquid and add agar. Return to steamer until agar has melted. Autoclave as prescribed for PDA.

4. Malt-Extract Agar (MEA)

Malt Extract	20 g
Agar	17 g
Distilled Water	1000 mL

Place malt extract in water and steam until dissolved. Add agar and steam until melted. Autoclave as prescribed for PDA.

5. Oatmeal Agar (OMA)

Oatmeal	50 g
Agar	17 g
Distilled Water	1000 mL

Add oatmeal to water and steam for 1 hour. Strain through cheesecloth. Add agar and steam until dissolved. Autoclave as prescribed for PDA.

6. Cornmeal Agar (CMA)

Cornmeal Agar (Difco)	17 g
$CaCO_3$	4 g
Distilled Water	1000 mL

Add cornmeal agar to water and steam until dissolved. Add $CaCO_3$ and stir until dissolved. Autoclave as prescribed for PDA.

7. V-8 Juice Agar (V-8)

Campbell's V-8 Juice	200 mL
Agar	17 g
Distilled Water	800 mL
$CaCO_3$	

Add agar to water. Steam both V-8 juice and water/agar for 20 minutes or until the agar is dissolved. Add V-8 juice to water/agar and adjust the pH 6.0 with $CaCO_3$. Autoclave as prescribed for PDA.

8. **Synthetic Media (SM)** (after Islam and Maric, 1978)

$Mg\,SO_4 \cdot 7\,H_2O$	3 g
$NH_4\,Cl$	3 g
$KH_2\,PO_4$	3 g
$Fe\,Cl_3 \cdot 6\,H_2O$	0.24 mg
$Zn\,Cl_2$	0.15 mg
$H_3\,BO_3$	0.06 mg
$Cu\,Cl_2 \cdot H_2O$	0.05 mg
$Mn\,Cl_2 \cdot 2\,H_2O$	0.04 mg
$Na\,Mo\,O_4 \cdot 2\,H_2O$	0.03 mg
Thiamine	0.1 g
Dextrose	20.0 g
Agar	17 g
Distilled Water	1000 mL

Add all ingredients to 1000 mL of water and place in a steamer until dissolved. Autoclave as prescribed for PDA.

Appendix II

A technique for estimating the number of microorganisms in a sample

Most of us have at some time, been faced with the problem of estimating the the total population of some microorgasnism from a representative sample of the population. Such samples might contain bacteria, spores or sclerotia per unit volume of soil, or the number of spores produced per unit area of diseased tissue etc. Whatever they might be, population samples are often made into a suspension and counts of the organism are performed on subsamples or aliquots drawn from the main sample. An estimate of the total sample is thus derived. Obviously, as the number of microorganisms per sample increases, and as the size of the subsamples increase as a proportion of the total sample volume, the accuracy of the estimate also increases.

Sampling in this way is usually very time consuming and is often undertaken without knowing either the level of accuracy that is required or even expected of the technique that is employed. The following is a simple method which allows the operator to set the sampling requirements needed to attain a certain level of accuracy, thereby saving time and effort.

Accuracy of an estimated total

The following assumes that the organism is uniformly distributed throughout the sample. This assumption can be checked after the aliquots have been counted, and is referred to later.

Example: An aliquot representing $p\%$ of the total sample contains r spores. Then an estimate of the total number N is:

$$N = 100 \times (r/p)$$

Then the standard deviation (SD) is:

$$SD(N) = 10/p \sqrt{r\,(100-p)}$$

and the percent SD is:

$$\% \text{ SD (N)} = 10 \sqrt{(100-p)/r}$$

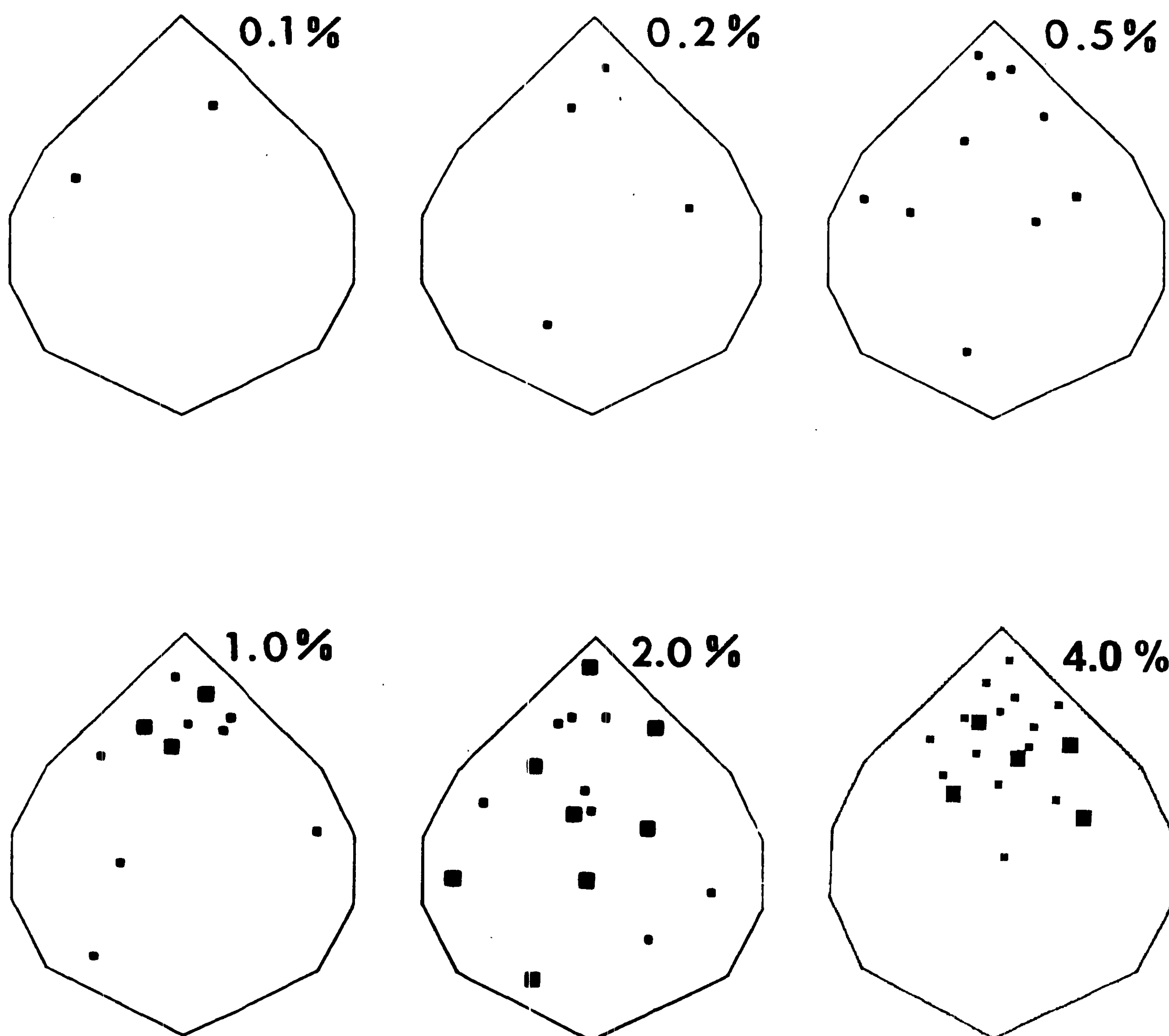
The criterion for accuracy is the % SD.

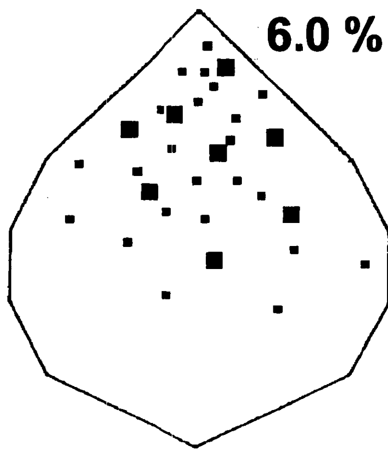
As an example, if 10 spores were found in a 1% sample, N = 1000 and the SD is 320 or 32%. The true number will, 95 % of the time, lie within 2 SD of the estimate. ie. 1000 ± 640.

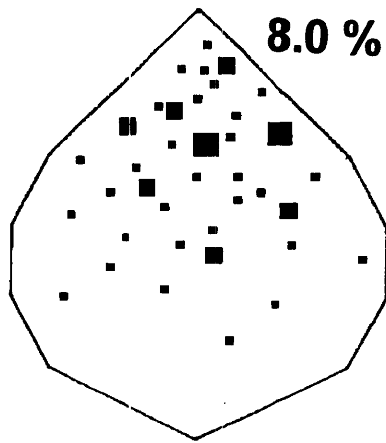
Thus, counting of aliquots should continue until a certain specified % SD is reached.

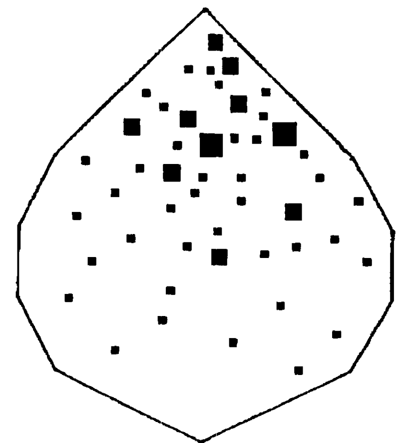
Appendix III

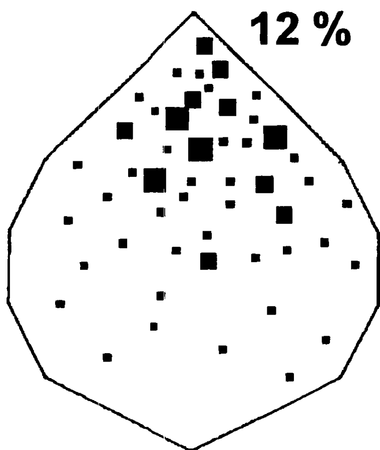
A pictorial key for assessing the proportion of leaf area infected with A. helianthi *.*

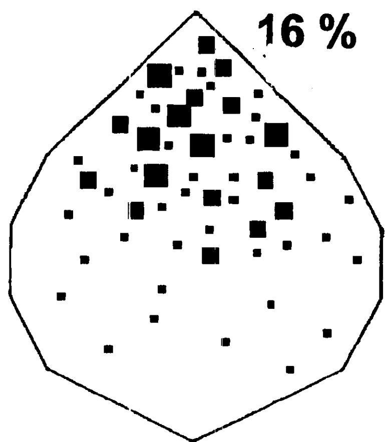


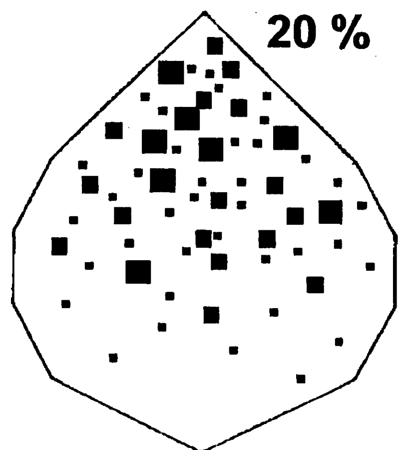

6.0 %

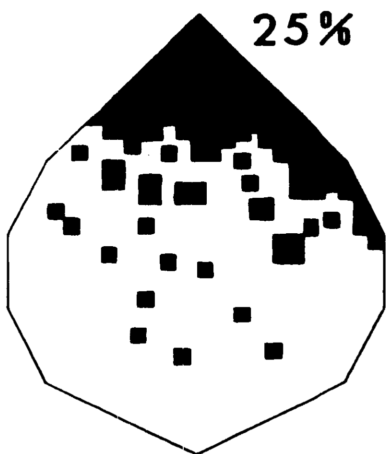

8.0 %

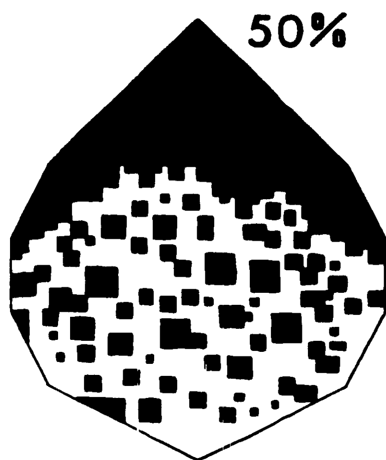


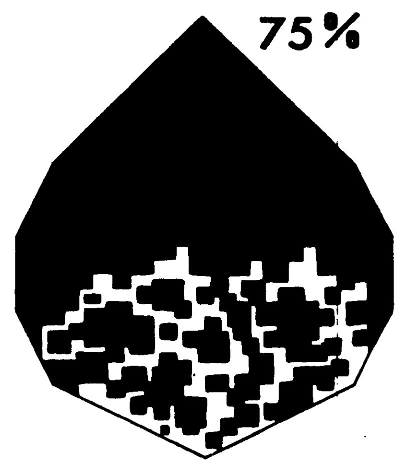

12 %


16 %


20 %


25%


50%


75%

Appendix IV

Publications arising from the research reported in this thesis.

Kong G A, Kochman J K, Brown J F. 1995. A greenhouse assay for screening sunflower for resistance to *Alternaria helianthi. Annals of Applied Biology* **127**: 463-478.

Kong, G A, Kochman, J K and Brown, J F. 1997. Phylloplane bacteria antagonistic to the sunflower pathogen, *Alternaria helianthi. Australasian Plant Pathology* **26**: 85-87.

Kong, G A, Kochman, J K and Brown, J F. 1997. Components of quantitative resistance to *A. helianthi in sunflower. Annals of Applied Biology* **128**: In Press