Oecologia (Berlin) (1987) 73:257–267

Oecologia

Phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* in populations of *Glycine canescens*

J.J. Burdon
Division of Plant Industry, CSIRO, G.P.O. Box 1600, Canberra City, A.C.T. 2601 Australia

Summary. Phenotypic patterns of resistance to nine races of the pathogen *Phakopsora pachyrhizi* (soybean rust) in two natural populations of *Glycine canescens* were determined. In both populations there was considerable variability both within and between different host lines in their resistance or susceptibility to the nine different pathogen races. The genetic basis of these patterns of resistance was analyzed through an extensive series of crosses. In both host populations resistance was conditioned by single dominant genes with major phenotypic effects. One, two or three such genes were present in each host line. Using the principles of the gene-for-gene hypothesis, knowledge about the number of resistance genes present in each host line and by cross comparison of the phenotypic patterns of disease resistance detected in each line, estimates were made of the number of resistance genes or alleles present in each population of *G. canescens*. The two populations contained a minimum of 10 and 12 resistance genes. The relevance of these results to agriculture is discussed briefly.

Key words: Glycine canescens – Phakopsora pachyrhizi – Plant pathology – Population genetics – Pathogen resistance

Pathogens are potentially major selective forces affecting the size and structure of plant populations. By reducing the fecundity of affected plants (Alexander and Burdon 1984; Parker 1986), weakening their ability to compete with neighbours (Burdon et al. 1984) or simply by killing them directly (Augspurger 1983; Augspurger and Kelly 1984), pathogens may affect the size of current and future plant populations. At the same time, if these effects are borne disproportionately by a subset of individuals in the population that are particularly susceptible to attack, pathogens may also affect the genetic structure of their host populations (Burdon 1985, 1987).

Various large scale geographic surveys of disease resistance in wild plants have shown very general associations between the frequency of occurrence of resistance and those physical environmental conditions that are necessary for the rapid growth, development and spread of the pathogen concerned (e.g. Dinoor 1970; Moseman et al. 1984; Jarosz 1984). Fewer studies have investigated the disease resistance structure of individual populations in detail (e.g. Kinloch and Stonecypher 1969; Dinoor 1977; Burdon et al. 1983). Moreover, while the latter studies have clearly shown the diversity in phenotypic disease resistance response that may be encountered in plant populations, none have tried to analyze the genetic basis of the observed patterns.

Certainly, a variety of studies aimed at uncovering resistance for use in agricultural plant improvement programs have detected individual plants carrying race-specific resistance that is apparently controlled by single genes. However, to date we have no direct evidence concerning the genetic basis of disease resistance in whole populations of wild plants.

The present paper reports on a study of phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* Syd. in populations of *Glycine canescens* F.J. Herm. *Glycine canescens* is one of a number of perennial species of the genus *Glycine* that are native to Australia. Earlier studies (Burdon and Marshall 1981a, b; Burdon and Speer 1984) detected the occurrence of both race specific and race non-specific resistance in these species to *P. pachyrhizi* (the causal agent of a rust disease of many legume species including soybean (*G. max*)). At a very early stage in the current study it became apparent that attempts to measure race non-specific resistance would be extremely difficult because of the relatively high frequency of race specific resistance in the populations studied. For this reason, this study concentrated on characterizing the distribution and genetic basis of the race specific resistance present in these populations.

Materials and methods

Phenotypic analysis of disease resistance

Collection and maintenance of plants. The populations of *Glycine canescens* investigated in this study were both found in the North-West Slopes Division of New South Wales, Australia. Population G1514[1] (C) was growing in open dry sclerophyll forest 52 km south of Coonabarabran (31°49′S, 149°09′E) while population G1500 (W) was growing in scattered eucalypt woodland on the outskirts of the township of Warialda (29°32′S, 150°35′E).

Plants were not seeding at either site when visited in April 1982 and, consequently, a random sample were carefully dug up (22 individuals at G1514; 14 at G1500), placed in individual bags and returned to the laboratory. The

[1] CSIRO Division of Plant Industry *Glycine* sp. germplasm accession number

plants were then placed in 15 cm pots filled with a 3:1 mixture of loam and river sand and kept in a naturally lit, heated (18°/24° C, night/day) glasshouse and watered as necessary. Within a few weeks all plants began to grow vigorously.

Selfed-seed for use in the subsequent analysis of disease resistance was individually collected from each of these plants. This was made particularly easy by the occurrence on *G. canescens* of cleistogamous and chasmogamous self-fertile flowers that were prevented from outcrossing through the exclusion of any insect pollen vectors.

Collection and maintenance of pathogen isolates. The nine isolates of *Phakopsora pachyrhizi* used were collected from various species of wild *Glycine* and *G. max* growing within the main distributional range of this pathogen along the eastern Australian coast from Walkamin, Queensland (17°08′S, 145°26′E) to Taree, New South Wales (31°54′S, 152°29′E). On receipt in the laboratory, each isolate was inoculated onto the susceptible soybean cultivars 'Clarke' or 'Dare' by dusting leaves with dry spores. Inoculated plants were then sprayed with a fine water mist and kept at 100% relative humidity overnight. Approximately 12 days after inoculation developing uredia erupted. The purity of each isolate was ensured at this stage by transferring spores from a single uredium to a new, previously uninfected, plant. Each purified isolate was then increased and maintained on either 'Clarke' or 'Dare'.

The racial identity and relative pathogenicity of the nine isolates of *Phakopsora pachyrhizi* was assessed on a standard set of wild *Glycine* hosts that carry different genes for resistance to this pathogen (Burdon and Speer 1984). The isolates/races encompassed a range of pathogenicity from individual races that were avirulent on most, or all, of the differential lines (for example, R1 and R2) to races that were able to overcome a wide range of resistance genes (for example, R5 and R6). Isolates of the pathogen were not collected from the site of the original host populations because of a lack of opportunity to repeatedly revisit the sites and the low probability of detecting the pathogen on any given occasion.

Screening for resistance to Phakopsora pachyrhizi. Seed of each plant collected from the field was chipped to promote germination, planted in 5 cm tubes filled with a sandy loam and placed in a naturally lit, heated (18°/24° C night/day) glasshouse. Approximately eight weeks after emergence all plants possessed three to four leaves and were ready for screening for resistance to *Phakopsora pachyrhizi.* Ten to twenty seedlings of each host plant line were inoculated with each race of the pathogen. Because of a general tendency in *G. canescens* for increasing resistance with increasing ontogenetic age, sequential inoculation of the same plants with the nine different pathogen races was not possible. Instead a fresh set of seedlings was used in the assessment of the infection type response to each race. Seedlings were inoculated as described earlier for the inoculation of soybean cultivars. After being held overnight at 100% relative humidity (to promote spore germination) seedlings were moved to a naturally lit, heated glasshouse (18°/24° C night/day). Twelve to fourteen days later, when disease symptoms had fully developed, the infection types on each seedling were recorded.

Infection types were graded according to a scale modified from those typically used in the investigation of resistance in cereals to rust fungi (e.g. Simons, 1970). The scale used in this study was as follows:

Infection type	Symptoms
;	Necrotic flecks or spots only; no sporulation whatsoever.
1	Minute uredia surrounded by relatively large regions of chlorotic or necrotic tissue.
2	Small uredia still associated with hypersensitive areas of chlorotic or necrotic tissue.
3	Large profusely sporulating uredia, with very little or no associated chlorosis or necrosis of tissue.

Where symptoms that overlapped two or more infection types occurred on the one plant, this was designated by the combination of both infection types. For example, ;1 was used to indicate a common infection type in which some infections gave rise to necrotic flecks while others were characterized by minute uredia. Similarly, intermediate infection types were designated as $1^{=}$, 1^{-}, 1^{+} and so forth. Finally, differences in the colour and appearance of hypersensitive necrotic flecks were also recorded. Phenotypically, these infection type responses can be broadly classified into highly resistant (;), resistant (1), moderately resistant (2) and fully susceptible (3) categories. Examples of some of these infection types are shown in Figure 1.

Genetic analysis of disease resistance

Following the phenotypic analysis of disease resistance in the two populations of *G. canescens* an investigation of the genetic basis of the observed resistant phenotypes was intiated.

Hybrid production. Plants for hybridization were grown and crossed in a naturally lit, heated (18°/24° C night/day) glasshouse. In all this work the original field-derived plants from the two *G. canescens* populations were crossed with a standard, 'universally susceptible' line (G1232.1) isolated in earlier work (Burdon and Speer 1984). In most of these crosses, line G1232.1 was used as the female parent. On the assumption that most resistant genes were likely to the dominant, this approach was likely to simplify the verification of the hybrid nature of putative crosses. The larger chasmogamous flowers borne in racemes were used in making crosses. Young chasmogamous flowers were emasculated prior to the dehiscence of anthers and pollination was immediately effected. The female plant was then enclosed in a plastic bag overnight to help prevent the drying of the exposed stigma and ovary.

Verification of hybrids. The actual parentage of putative hybrids was determined through comparisons of: (i) morphological characters (particularly the shape of the first leaf), (ii) phenotypic patterns of isozymes extracted from young leaves, and (iii) infection type responses educed by inoculation with avirulent races of *Phakopsora pachyrhizi.* Horizontal starch-gel electrophoresis of crude enzyme extracts was carried out using one of two buffer systems. (System A: electrode buffer – 0.4 M sodium citrate pH 8.0; gel buffer – 5.0 mM Histidine pH 8.0. System B: electrode

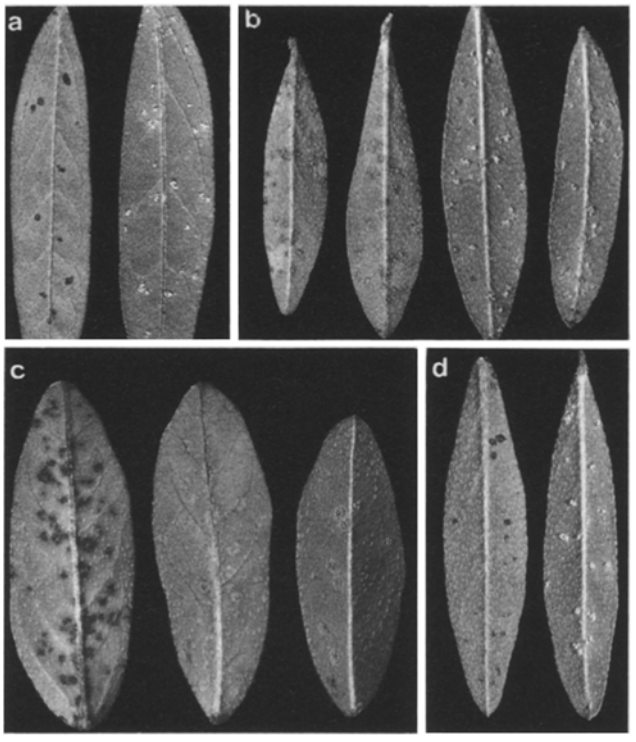


Fig. 1a–d. Representative examples of the visual appearance of some infection type responses observed in segregating F_2 families of seedlings derived from crosses between various lines of *Glycine canescens* populations G1514 & G1500 and the universal susceptible line G1232.1 when infected with the pathogen *Phakopsora pachyrhizi*. **a** G1232.1 × 1514(15) showing (left) a resistant (;) and (right) a susceptible (3) reaction; **b** G1232.1 × 1500(8) showing (left) a pair of resistant (;1⁻) and (right) a pair of susceptible (3) reactions; **c** G1232.1 × 1514(7) showing left to right, two resistant (;) & (;1⁻) reactions and one susceptible (2⁺) reaction. **d** 1232.1 × 1514(8) showing (left) a resistant (;) and (right) a susceptible (3) reaction

buffer – 0.3 M borate, 0.1 M sodium hydroxide; gel buffer – 3.0 mM citrate, 15.2 mM Tris). On completion of electrophoresis gel slices were stained for the following enzymes: aspartate aminotransferase (AAT, EC 2.6.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), menadione reductase (MDR, EC 1.6.99.2), glucosephosphate isomerase (GPI, EC 5.3.1.9) and phosphoglucomutase (PGM, EC 2.7.5.1). The full procedure for the preparation of material for electrophoresis and the subsequent staining of gels is given by Broué et al. 1977 and Brown et al. 1978.

The hybrid nature of many putative crosses was also confirmed by their infection type response to appropriate races of *P. pachyrhizi*. Where the susceptible line G1232.1 was used as the female parent, F_1 hybrids were generally recognized by a resistant infection type response. However, individuals that gave susceptible reactions were not discarded without electrophoretic testing as these could have been hybrids involving recessive resistance genes. When the line G1232.1 was used as the male parent, hybrids could not be verified by their infection type responses.

F_2 seedling analysis. Plants confirmed to be F_1 hybrids were planted in 20 cm pots filled with sandy loam, placed in an insect-free glasshouse and allowed to flower and set seed. Selfed F_2 seed was collected from both cleistogamous and chasmogamous flowers on these plants. When sufficient F_2 seed was accumulated this, together with representative samples of seed of the original parents, was germinated and later inoculated with an appropriate race of *P. pachyrhizi*. The actual identity of the race or races used in the analysis of any particular cross depended on the resistance phenotype of the original parent (only races producing a resistant phenotype were used) and on the immediate availability of suitable inoculum. The infection type responses of these seedlings were classified 12 to 14 days later when symptoms were fully expressed.

To confirm the genetic basis of the observed segregation patterns, representative examples of different infection type categories from some of these F_2 seedling populations were grown through to maturity. Seed collected from these plants was used to produce families of F_3 seedlings that were again tested with an appropriate race of *P. pachyrhizi*.

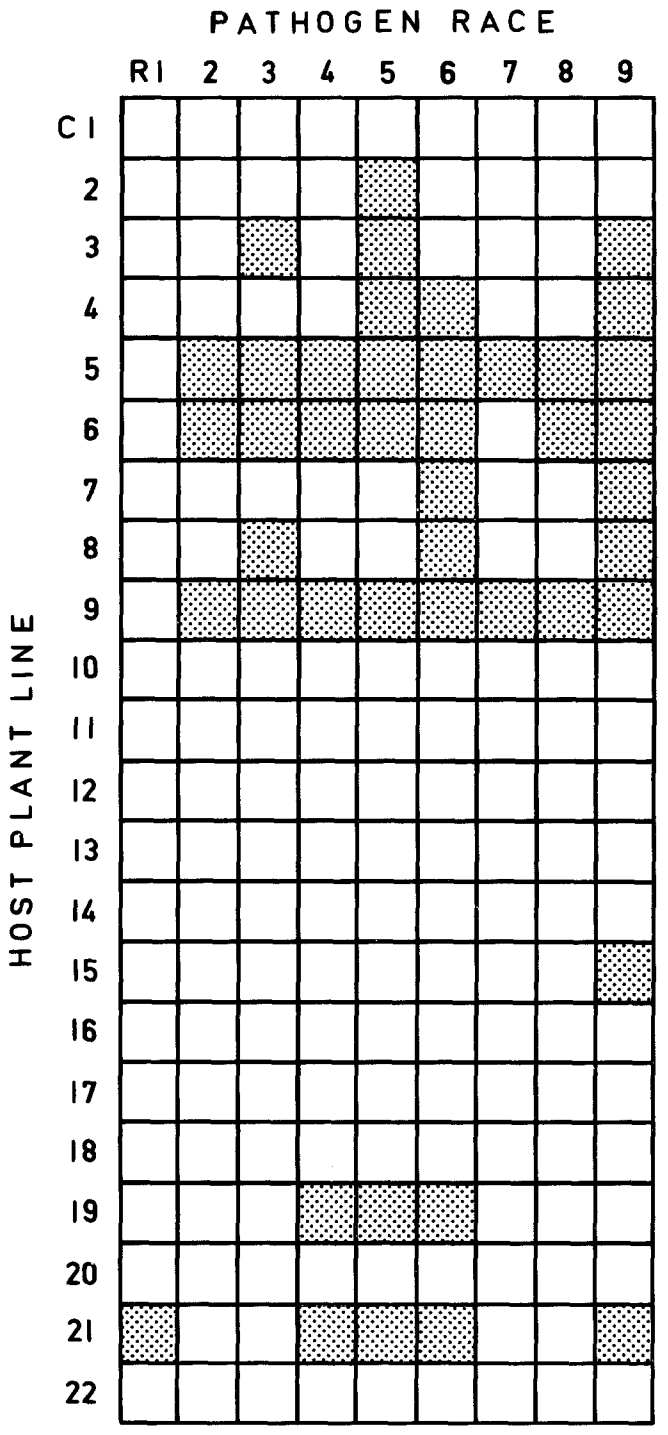
Results

The phenotypic patterns of resistance to *Phakopsora pachyrhizi* of the two populations of *Glycine canescens* are shown in Figs. 2 and 3. Population G1514 (Fig. 2) was characterized by a high frequency of resistance with 78% (154/198) of individual host line-pathogen race combinations giving a resistant reaction. Half of the host lines were resistant to all nine races of the pathogen, while others possessed resistance effective against from one to eight of the races used. No individual was susceptible to all races, nor was any pathogen race virulent on all host lines. Race 1 was the most avirulent and produced a susceptible reaction on only one host line. At the other extreme, races R5, R6 and R9 produced susceptible reactions on over 35% of the host lines. Indeed, no two pathogen races had the same reaction spectrum. The nine races defined 11 different host phenotypes in population G1514.

In addition there were noticeable differences in the visual appearance of the resistant infection type response produced by different lines of G1514. In some cases these were characterized by black (eg. C2, C7, C19 & C22) or brown (eg. C18 and C20) necrotic flecks or lesions. In these responses the pathogen failed to sporulate even if such host individuals were monitored for a further 10 days. In other cases, resistant reactions were characterized by a reduced level of pathogen reproduction (relative to that occurring in fully susceptible reactions).

Overall, the phenotypic pattern of disease resistance in population G1500 (Fig. 3) was one of greater susceptibility than that of population G1514. In contrast to G1514, only 47% (60/126) of all host line-pathogen race combinations in population G1500 gave a resistant reaction. Indeed, no individual host was resistant to all races (the least susceptible being W8 which was resistant to seven of the nine races). Conversely, two lines (W1 and W2) were susceptible to seven to the nine races. The other host lines showed a range of resistant and susceptible responses. The pathogen races also showed a similar diversity in their virulence. Race 1 was avirulent on all but one host line while R7 was virulent against all individuals.

In contrast to the resistant infection type responses shown by population G1514, those of population G1500 were typically associated with a light to moderate level of pathogen sporulation. Total suppression of pathogen sporulation was evident in only one host line (W1).



Range of resistant infection type responses

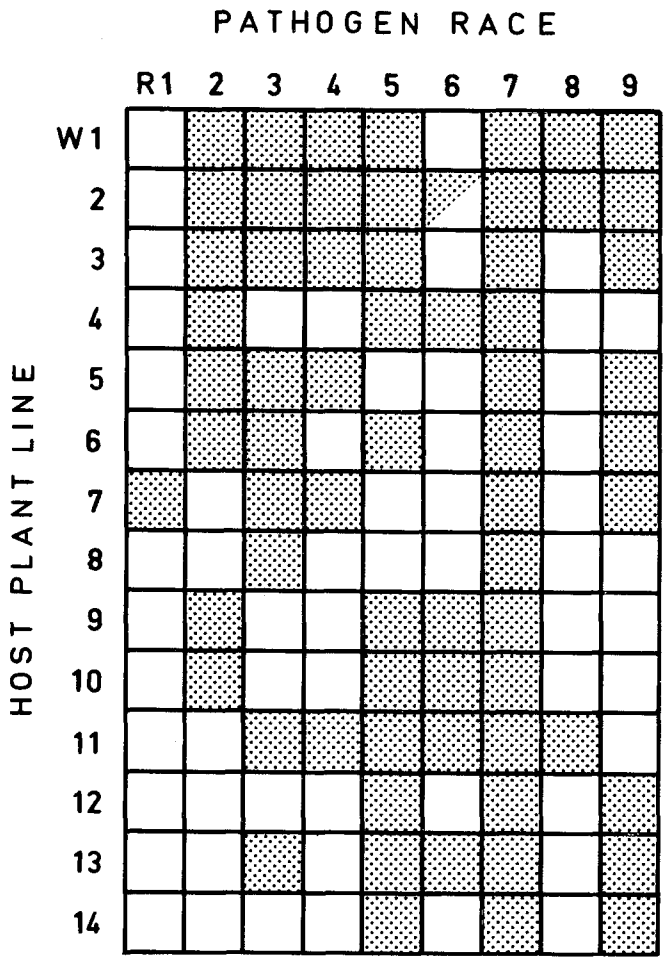
; $1^{=}$	mid to light tan fleck; very little sporulation; much chlorosis
;	black fleck, no sporulation
;	black fleck, no sporulation
;	black fleck; no sporulation
; 1	dark brown fleck; light sporulation; some chlorosis
; 2^{-}	light tan flecks; moderate sporulation; chlorosis
;	black fleck; no sporulation
;	black fleck; no sporulation
; 1	light tan flecks; light sporulation; some chlorosis
; $1^{=}$	mid brown fleck; very little sporulation
$1^{-}2^{=}$	light tan pustules; moderate sporulation; much chlorosis
; 1^{-}	light tan flecks; light sporulation; some necrosis
; $1^{=}$	dark brown flecks; very little sporulation
; $1^{=}$	mid brown fleck; very little sporulation; much chlorosis
;	black fleck; no sporulation
; $1^{=}$	mid brown fleck; very little sporulation
; $1^{=}$	dark brown fleck; very little sporulation
;	mid to dark tan fleck; no sporulation
;	black fleck; no sporulation
;	mid brown fleck; nor sporulation
; 1^{+}	light tan fleck; moderate sporulation chlorosis
;	black fleck; no sporulation

Fig. 2. Phenotypic patterns of resistance to nine different races of *Phakopsora pachyrhizi* shown by *Glycine canescens* population G1514. The infection type response to each line is also given. Open squares, resistant reaction; closed squares, susceptible reaction

The genetic basis of these observed patterns of disease resistance was examined by crossing resistant host lines with a common susceptible host and scoring F_2 segregation patterns (Tables 1 and 2). No data are available for host lines C6 and C19 in population G1514 as both of these died before crosses were made. In population G1500, data are available for only seven of the 14 lines. This was caused by the premature death of a few lines and the very low fertility of some F_1 hybrids.

In many cases more than one F_1 hybrid was made and kept for the production of F_2 seed. Equally, in many analyses the particular pathogen race used, varied between different batches of F_2 seedlings. Before such data were pooled, heterogeneity χ^2 values were calculated and data were combined only where these were non-significant. In three cases in the analysis of population G1514 (C15, C20 and C22), and two cases in population G1500 (W8 and W9), χ^2 values were significant and the different sets of data are presented separately (Tables 1 and 2).

Depending upon the particular cross involved, the infection type responses of the F_2 seedlings to particular races of *P. pachyrhizi* could be classified into two, three or even



Range of resistant infection type responses

;	mid tan fleck; no sporulation
; 1	mid tan fleck; light sporulation, some chlorosis
;1⁻1⁺	light-moderate sporulation, much chlorosis
; 1	light sporulation, chlorosis
; 1 1⁺	moderate sporulation, some chlorosis/necrosis
; 1 2⁻	moderate sporulation, little chlorosis
1	light sporulation, some chlorosis
;1⁻2⁻	mid tan fleck; light-moderate sporulation, some chlorosis
; 1	mid tan fleck; light sporulation, some necrosis
; 1 1⁺	mid tan fleck; light sporulation, some necrosis
; 1 2⁻	mid tan fleck; light-moderate sporulation, some chlorosis
;1⁻1⁺	mid tan fleck; light sporulation
; 1	mid tan fleck; light sporulation, chlorosis
; 1 1⁺	mid tan fleck; light-moderate sporulation; some chlorosis

Fig. 3. Phenotypic patterns of resistance to nine different races of *Phakopsora pachyrhizi* shown by *Glycine canescens* population 1500. The infection type response of each line is also given. Open squares, resistant reaction; closed squares, susceptible reaction; split square, segregating reaction

(in one case: C20(b)) four distinct phenotypes. The number of individuals in each of these different categories are given in Tables 1 and 2. In no case was a continuous distribution of infection types encountered.

In population G1514, with one exception (C22(b)), the distribution of F_2 seedlings between the resistant and susceptible infection type categories could be explained by the presence, in the original parental line, of one, two or three dominant genes for resistance each of which is inherited independently. In most cases where the presence of more than one resistance gene was implicated, these all had similar infection type responses resulting in the occurrence of 15:1 or 63:1 ratios of resistant to susceptible individuals among the F_2 seedlings. However, in eight cases more than one resistant infection type was observed. In crosses involving host lines C3, C7 and C8, two such responses were recorded and the distribution of F_2 seedlings among these categories best fitted a 12:3:1 ratio – indicative of the presence of two dominant resistance genes, one epistatic to the other. In all cases the epistatic resistance gene conferred a more pronounced resistance phenotype. In four other crosses (those involving C4, C14, C18 and C20(a)) although more than one resistant infection type response occurred, best fit to a recognized segregation ratio was obtained by pooling these categories. Finally, in one cross involving host line C22 (C22(b) in Table 1) the ratio of resistant to susceptible individuals among the F_2 seedlings was intermediate between a 3:1 and a 15:1 ratio. The reason for this is unknown.

During the initial analysis to determine whether data from different F_2 segregating families could be pooled, three cases involving population G1514 were uncovered where significant heterogeneity existed (C15, C20 and C22). In the case of crosses involving host C15, the two sets of F_2 segregation data fitted 15:1 and 3:1 ratios respectively. This suggests that one of the two resistance genes present in the original parental line was in a heterozygous state. In the case of C20, although both sets of data can be explained by a three-gene model, the occurrence of more than one resistant phenotype in cross C20(b) is associated with the use of the 'universal' susceptible G1232.1 as the female parent. In the two crosses pooled in C20(a), G1232.1 was the male parent. As noted above, the reasons for differences between crosses C22(a) and C22(b) are unknown.

The more restricted data available for the segregation patterns found among F_2 seedlings derived from crosses involving population G1500 (Table 2) are in general agreement with that for population G1514. In the original parental lines of population G1500, one, two or three resistance genes were present. These generally induced the same resistance phenotype but in the case of crosses involving W14, two distinct resistant phenotypes occurred and the data fit-

Table 1. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_2 seedling families derived from crosses between individual lines of *Glycine canescens* population G1514 and the 'universal' susceptible line G1232.1

Plant number	Number of crosses[a]	Testing races[b]	Hetero-geneity[c] χ^2	Total number of F_2 individuals	Observed number F_2 individuals				Ratio of best fit	χ^2	Appro-priate P
					R			S			
					;[d]	;1⁻	;2⁻	3			
C1	5(3)	R2,5	1.695[(4)]	565	–	533	–	32	15:1	0.331	0.75–0.50
C2	2(1)	R3	0.054[(1)]	189	147	–	–	42	3:1	0.778	0.50–0.25
C3	1(1)	R4	–	136	101	26	–	11	12:3:1	0.723	0.75–0.50
C4	4(3)	R3,4	4.303[(6)]	327	263	49	–	15	15:1	1.543	0.25–0.10
C5	2(2)	R1	0.125[(1)]	234	–	177	–	57	3:1	0.051	0.90–0.75
C7	2(2)	R1	0.180[(1)]	267	209	46	–	12	12:3:1	2.029	0.50–0.25
C8	4(4)	R3,4	1.807[(6)]	446	329	92	–	25	12:3:1	1.226	0.75–0.50
C9	3(3)	R1,2	4.028[(6)]	477	–	370	–	107	3:1	1.678	0.25–0.10
C10	3(3)	R5	0.657[(2)]	269	–	264	–	5	63:1	0.154	0.75–0.50
C12	3(2)	R1	1.718[(3)]	263	–	256	–	7	63:1	2.044	0.25–0.10
C13	5(3)	R3	1.881[(5)]	483	–	474	–	9	63:1	0.284	0.75–0.50
C14	1(1)	R3	–	90	65	24	–	1	63:1	0.119	0.75–0.50
C15 (a)	4(3)	R7,8	2.403[(3)]	300	–	280	–	20	15:1	0.089	0.90–0.75
(b)	1(1)	R7,8	1.623[(2)]	138	–	110	–	28	3:1	1.633	0.25–0.10
C16	3(1)	R3	0.446[(2)]	182	–	177	–	5	63:1	2.364	0.25–0.10
C17	3(3)	R3	2.111[(2)]	448	–	–	442	6	63:1	0.145	0.75–0.50
C18	2(2)	R3,8	1.621[(1)]	128	112	12	–	4	63:1	2.016[e]	0.25–0.10
C20 (a)	2(2)	R7	0.385[(1)]	238	232	–	–	6	63:1	1.422	0.25–0.10
(b)	1(1)	R7	–	87	49	28	8	2	63:1	0.307	0.75–0.50
C21	4(1)	R2,3	2.788[(3)]	378	–	283	–	95	3:1	0.004	>0.90
C22 (a)	2(2)	R5	1.023[(1)]	227	–	217	–	10	15:1	1.318	0.50–0.25
(b)	1(1)	R5	–	188	138	18	–	32	?	–	–

[a] Number of F_1 hybrid plants used to produce seed for F_2 testing. Values in parentheses are the number of different pollination events represented by these F_1 plants
[b] Designation of races follows Burdon and Speer (1984) and Burdon unpublished
[c] Superscript values in parentheses are the numbers of degrees of freedom
[d] Infection type responses, see text for details
[e] Observed values fit both a 63:1 and a 15:1 ratio. Here the ratio with the minimum χ^2 value is given

Table 2. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_2 seedling families derived from crosses between individual lines of *Glycine canescens* population G1500 and the 'universal' susceptible line G1232.1

Plant number	Number of crosses[a]	Testing races[b]	Hetero-geneity[c] χ^2	Total number of F_2 individuals	Observed number F_2 individuals				Ratio of best fit	χ^2	Appro-priate P
					R			S			
					;[d]	;1⁻	;2⁻	3			
W2	1(1)	R6	–	107	–	82	–	25	3:1	0.153	0.75–0.50
W3	1(1)	R6	–	65	–	63	–	2	63:1	0.969	0.50–0.25
W5	1(1)	R6	–	90	–	88	–	2	63:1	0.257	0.75–0.50
W8 (a)	3(2)	R1,6	0.536[(2)]	155	–	122	–	33	3:1	1.138	0.50–0.25
(b)	1(1)	R6	–	69	–	–	48	21	3:1	1.087	0.50–0.25
(c)	2(2)	R6	1.033[(1)]	124	–	–	120	4	63:1	1.821	0.25–0.10
W9 (a)	2(1)	R3,4	1.655[(1)]	87	–	75	6	6	15:1	0.185	0.75–0.50
(b)	2(1)	R4	0.753[(1)]	229	–	212	–	17	15:1	0.538	0.50–0.25
W10	3(2)	R3,4	1.123[(3)]	192	–	–	141	51	3:1	0.250	0.75–0.50
W14	2(2)	R1	0.101[(1)]	184	144	–	32	8	12:3:1	1.507	0.50–0.25

[a] Number of F_1 hybrid plants used to produce seed for F_2 testing. Values in parentheses are the number of different pollination events represented by these F_1 plants
[b] Designation of races follows Burdon and Speer (1984) and Burdon unpublished
[c] Superscript values of parentheses are the numbers of degrees of freedom
[d] Infection type responses, see text for details

ted a 12:3:1 ratio. Somewhat variable results were obtained in crosses involving host lines W8 and W9. In both cases, where the original host line from population G1500 was used as the male parent (Table 2: W8(b) and (c); W9(a)), the infection type response of the most resistant phenotype showed a greater degree of sporulation (;2⁻) than when the original G1500 host lines were used as the female parents (Table 2: W8(a); W9(b)). In the case of W8, however,

Table 3. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_3 seedling families derived from crosses between individual lines of *Glycine canescens* populations G1415 and G1500 and the 'universal' susceptible lines G1232.1

Cross	F_2 phenotype	Number of families	Heterogeneity χ^2	F_3 phenotype		F_3 ratio of best fit	χ^2	Appropriate P
				R	S			
G1232.1 × G1514/C9	R	3	–	62	0	–	–	–
		3	0.976	39	15	3:1	0.222	0.75–0.50
	S	2	–	0	35	–	–	–
G1232.1 × G1514/C21	R	4	–	78	0	–	–	–
		3	1.937	43	18	3:1	0.661	0.50–0.25
	S	2	–	0	38	–	–	–
G1232.1 × G1500/W2	R	8	–	229	0	–	–	–
		5	0.325	106	25	3:1	2.445	0.25–0.10
G1232.1 × G1500/W10	R	4	–	76	0	–	–	–
		8	6.178	97	30	3:1	0.129	0.75–0.50
	S	3	–	0	57	–	–	–
G1232.1 × G1514/C4	R	3	–	119	0	–	–	–
		6	4.180	213	20	15:1	2.166	0.25–0.10
		4	0.771	96	24	3:1	1.600	0.50–0.25

the data from one of the groups of crosses (W8(c)) best fitted a three dominant, resistance gene model, while for the other two groups a one-gene model was adequate. The simplest explanation for this result is that two of three resistance genes present in the original parental line W8 were in a heterozygous state.

The genetic basis of the observed phenotypic differences in resistance was confirmed by progeny testing selected F_2 seedlings that were grown through to maturity (Table 3). In all cases, families of F_3 seedlings, derived from resistant F_2 individuals, were either uniformly resistant or segregated for resistance and susceptibility at the F_3 stage. For each set of families derived from any particular cross, heterogeneity χ^2 values were calculated and found to be non-significant before data were pooled. In four crosses, those involving G1514 C9 and C21 and C1500 W2 and W10 with G1232.1, ratios of resistant to susceptible individuals within the F_3 segregating families all fitted a 3:1 ratio. This was indicative of the presence of a single dominant gene for resistance (Table 3). These results were in agreement with those from the F_2 seedling testing (Table 1 and 2). Finally, in the cross G1232.1 × C4 (Table 3) three groups of resistant phenotypes were detected among the F_3 seedling families. In one of these groups, all F_3 individuals were phenotypically resistant. The other two groups of families segregated for resistance and susceptibility. χ^2 heterogeneity testing confirmed the distinct nature of these two groupings, one in which the frequency of resistant to susceptible segregants fitted a 15:1 ratio and one which fitted a 3:1 ratio. Again, this result agreed with that previously obtained at the F_2 stage (Table 1).

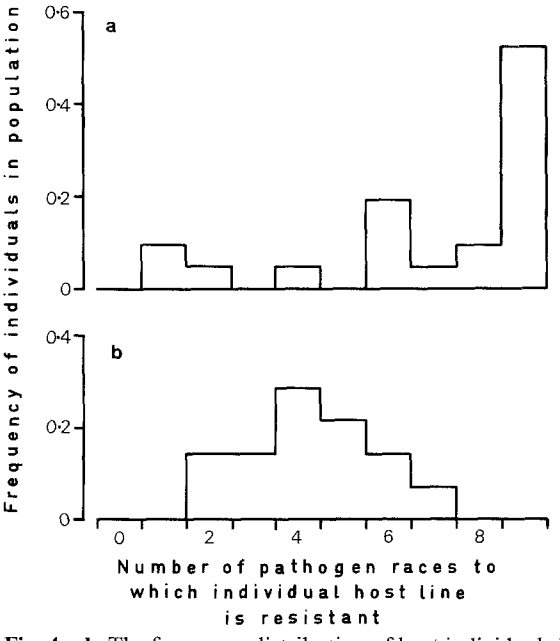


Fig. 4a, b. The frequency distribution of host individuals in *Glycine canescens* population (**a**) G1514 and (**b**) G1500 with respect to the number of pathogen races to which they are resistant

Discussion

Phenotypic pattern of disease resistance

In the broadest outline, the structure of resistance to the leaf rust pathogen *Phakopsora pachyrhizi* was similar in *Glycine canescens* populations G1514 and G1500. Both showed a two-dimensional mosaic of resistance and susceptibility. Not only did the response of hosts vary according to the pathogen race with which they were challenged, but individual hosts varied with respect to one another in these responses. As a result, the frequency of resistant or susceptible individuals in the population as a whole varied according to the pathogen race involved.

However, when examined in more detail, the phenotypic pattern of disease resistance differed markedly between the two populations. In population G1514 most host individuals were resistant to most of the pathogen races. Indeed, the average host line was resistant to seven races (Fig. 4a), with just over half being resistant to all nine races. By contrast, the average host line in population G1500 was resistant to only 4.3 races of the pathogen (Figure 4b). This difference between the two *G. canescens* populations appears to be due largely to the high frequency of a single "genotype" in population G1514 that had a fully resistant

phenotype based on three resistance genes. However, definitive proof that the same three genes are involved in all of these individuals has not been obtained. Furthermore, the resistance expressed by individuals of population G1514 was generally more complete than that occurring in population G1500 (Figs. 2 and 3). In the latter population, resistance was generally associated with light to moderate sporulation. In G1514, on the other hand, resistance was frequently characterized by the total suppression of sporulation. Where sporulation occurred, this was generally more limited than that occurring on host lines from population G1500.

The highly diverse nature of the phenotypic pattern of disease resistance and susceptibility in these two populations of *G. canescens* is in keeping with that observed in a number of other wild plant populations. A number of examples are available concerning phenotypic patterns of disease resistance in individual populations. Many of these provide only a partial picture of the interactions occurring between hosts and their pathogens as they report the response of particular populations to only one or two pathogen races (e.g. Dinoor 1970; Zimmer and Rehder 1976; Burdon 1980) or to mixed cultures (e.g. Fischbeck et al. 1976; Moseman et al. 1984). However, even these indicate that complex patterns of disease resistance might be expected to occur in many host populations. Only a few examples are available that document in any detail the response of individual plant populations to a range of pathogen races each with different pathogenicity characteristics and each applied separately (e.g. Dinoor 1977; Burdon et al. 1983). However, the use of many pathogen races is necessary to prevent an oversimplified view of the resistance structure of the host population (Burdon 1985, 1987).

Burdon, Oates and Marshall (1983) found that a series of *Avena fatua* populations showed a range of patterns of disease resistance ranging from the uniform to the highly diverse. In one population, all individuals were uniformly resistant to four different races of *Puccinia coronata*. In several other populations, however, the response of individual host lines differed markedly according to the pathogen race involved. Particularly extreme examples of such diversity were found by Dinoor (1977) in a study of a similar host-pathogen interaction in Israel. In one population of *Avena* sp. in which 109 were plants challenged with six different races of *P. coronata*, Dinoor detected 18 different distinct patterns of resistance and susceptibility. In two other populations of 13 or 14 individuals, eight to nine different resistance phenotypes were observed! Such extreme patterns of diversity are not restricted solely to interactions between graminaceous hosts and their pathogens. In a study of the resistance of a natural population of the perennial legume *Stylosanthes guianensis* to the pathogen *Colletotrichum gloeosporoides*, Miles and Lenné (1984) also found considerable variation in the response of individual hosts to different isolates of the pathogen. Several plants were uniformly susceptible to eight different isolates of the pathogen; one or two others were uniformly resistant or highly resistant while still others showed marked differential responses depending upon the pathogen isolate concerned.

Genetic basis of disease resistance

F_2 segregation data, reinforced with a limited amount of progeny testing of F_3 seedling families, indicates the presence of one, two or three resistance genes (3:1, 15:1, 63:1 ratios respectively) in lines of both of these *G. canescens* populations. In all cases the genes were dominant in effect and, with the exception of gene(s) in host lines C15 and W2 & W8, appear to be in a homozygous state.

Table 4. Hypothetical data showing the response of three host lines to three different races of a fungal pathogen (*R*, resistant reaction; *S*, susceptible reaction)

		Pathogen race		
		R1	R2	R3
Host genotype	H1	S	S	R
	H2	S	R	S
	H3	S	R	R

Currently no direct evidence is available concerning the relative identity of the different resistance genes in the different host lines. That is, whether they are the same allele, different alleles at the same locus, or whether they are entirely different genes. Such evidence would require an extensive diallel in which the expected frequency of susceptible individuals in some crosses (assuming independent genes) could be as low as 1 in 5096! However, by comparing phenotypic patterns of resistance and susceptibility for each host line in a population with all other lines, (and assuming, as has been found here that differences in resistance and susceptibility are controlled by single genes), we can apply the principles of the gene-for-gene theory (Flor 1955; Person 1959) to make an estimate of the minimum number of resistance genes or alleles that must be present.

This approach is perhaps best illustrated with a highly simplified set of hypothetical data that shows the response of three host lines to three races of a pathogen (Table 4). In this example, the simplest explanation for the pattern of resistance and susceptibility shown by host H1 is the presence of a single resistance gene effective against race R3 of the pathogen. Similarly, the pattern observed for H2 can be explained by postulating the occurrence of another resistant gene or allele that is effective against R2. Finally, two solutions can be proposed to explain the pattern of resistance and susceptibility observed for H3. Either a single additional resistance gene is present or the host line carries both the genes present in H1 and H2. The latter explanation is the most conservative and is hence the one to accept without evidence to the contrary. Using this approach it is necessary to postulate the presence of a minimum of 12 and 10 resistance genes in the *G. canescens* populations G1514 and G1500 respectively in order to explain the observed phenotypic resistance patterns. The identity and distribution of those genes postulated for population G1514 is shown in Table 5.

This high level of diversity for resistance is paralleled by a similar diversity in isozyme multilocus phenotypes. Analysis of both populations for seven different enzymes uncovered 15 distinct multilocus isozyme phenotypes among the 22 members of population G1514 and six in population G1500 (Burdon and Brown, unpublished work).

Data concerning the genetic basis of phenotypic resistance patterns in wild plant populations is very limited. Certainly, many resistance genes that have been used in agriculturally important crop varieties have been derived from wild relatives (eg. *Sr26* for wheat stem rust resistance

Table 5. The identity of the different genes or alleles for resistance to *Phakopsora pachyrhizi* that must be postulated as being present in *Glycine canescens* population G1514 in order to explain the phenotypic and genetic disease resistance data presented in Fig. 2 and Table 1

Host line	Postulated resistance genes
C1, 22	A, B
C2	C
C3	C, D
C4	C, E
C5, 9	F
C6	(G)[a]
C7	E, H
C8	E, D
C10, 12, 13, 14 16, 17, 18, 20	A, B, I
C11	–[b]
C15	J, K
C19	(L)[a]
C21	K

[a] Estimated on the basis of the phenotypic disease resistance pattern only

[b] Genotype could not be estimated as no crossing data was available to permit distinction between occurrence of genes A, B or I either singly or in combination

derived from *Agropyron elongatum*, Luig 1983). However, studies which report on the usage of resistance genes in this way provide little insight into the genetic structure and distribution of disease resistance in the individual wild populations from which they were derived. Indeed, the only comparable data available is that from a study of three populations of *Avena* sp. growing in Israel (Dinoor 1977). Challenging members of these populations with six different races of *Puccinia coronata* allowed Dinoor to use the approach described above to estimate the number of resistance genes in each population. In two of the populations three resistance genes were postulated. In the third, the presence of a minimum of six resistance genes had to be invoked to explain the phenotypic patterns of resistance and susceptibility obtained in response to the six races of *P. coronata*. Although Dinoor (1977) provided no evidence, he did state that some of the host individuals carried more than one resistance gene.

The development and maintenance of disease resistance in plant populations

Current theory views the resistance structure of plant populations as being determined by a process of reciprocal selection of host and pathogen individuals that favours novel resistance and virulence genotypes, balanced by 'fitness' costs associated with the possession of such resistance or virulence (Jayakar 1970; Leonard 1969, 1977, 1984; Leonard and Czochor 1980). In situations where the selective pressure exerted by the pathogen has been high in the relatively recent past, host populations would be expected to show a greater diversity and higher frequency of resistant individuals than would host populations growing in sites unfavourable for the pathogen. In this light, the simplest explanation for the considerable diversity in the disease resistance phenotypes encountered in the *Glycine canescens* populations G1514 and G1500 is that these have arisen as a result of selective pressure exerted by the pathogen population over a period of time. The difference between the resistance structure of the two populations may be accounted for by micro-environmental differences that affect the growth and development of the pathogen or by local differences in the genetic structure of the pathogen population.

Unfortunately, the time scale involved in interactions of this nature is measured in many host generations. As a consequence, our current knowledge of the resistance structure of host populations is built on static pictures that provide no indication of the dynamism of particular host-pathogen interactions. This problem is particularly acute in the present study where the host *G. canescens* is a long-lived perennial species capable of regenerating from a substantial tap-root after periods of adverse conditions. This together with the production of cleistogamous seed that will tend to preserve inbred lines, and seed that has prolonged mechanical dormancy and long-term viability, makes it very likely that the observed disease resistance structure of the two populations reflects selective pressures exerted some time ago.

In such circumstances a detailed knowledge of the current pathogen population would not necessarily be useful. More important, is the knowledge that both populations G1514 and G1500 fall within the potential distribution of the pathogen *Phakopsora pachyrhizi* in eastern Australia. Although the main current region of distribution of *P. pachyrhizi* is a zone extending from the Great Dividing Range to the coast, in wet years that are particularly favourable to the pathogen, it has been recorded as far west as St. George, southern Queensland (Kochman 1977) – considerably further west than the two *G. canescens* under consideration.

Relevance to agriculture

In recent years considerable controversy has developed over the use of multilines or varietal mixtures for disease control in agriculture (eg. Browning and Frey 1969; Marshall 1977). Originally such mixtures, were proposed as a means of mimicing the perceived diversity of wild host-pathogen interactions and, hence, of obtaining freedom from the devastating disease epidemics that periodically sweep the genetically uniform crops of modern agriculture (Jensen 1952; Browning and Frey 1969). It was believed this could be done through the construction of multi-genotype mixtures, each component of which carried a single, different resistance gene. The pathogen population when confronted with the range of conflicting selection pressures represented by such a mixture would remain pathogenically simple. This view has been contested by various workers (Groth 1976; Marshall 1977; Marshall and Pryor 1978) who have argued that unless a marked net fitness penalty is associated with the possession of inappropriate virulence, such mixtures may in fact favour the emergence of pathogen races carrying virulence to most if not all of the resistance genes present in the mixture. However, using a mathematical model Marshall and Pryor (1978, 1979) showed such a 'super-race' was less likely to arise if the individual lines of the host population were protected by several genes, many of which were different from those in other host lines, than if all the resistance genes were deployed singly.

To date no data have been available concerning the genetic basis of phenotypic differences in disease resistance

in wild plant populations. The data presented in this paper certainly suggest that the diversity and complexity of the resistance structure of such populations may far exceed that occurring in the relatively simple mixtures currently being used in agriculture. This is particularly emphasized by Table 5 which indicates that the resistance genes present in these wild *Glycine* populations occur in a complex mixture of overlapping and disjoint subsets.

The data presented here also suggest that race specific resistance characterized by simply inherited, dominant genes with major phenotypic effects is relatively common in non-agricultural plant-pathogen interactions. This contrasts strongly with suggestions that gene-for-gene interactions are artifacts of agricultural breeding practices (Day, Barrett and Wolfe 1983; Barrett 1985). The proponents of this view suggest that the predominant form of genetically based resistance in wild plant populations is race non-specific resistance that is characterized by many genes with small effects that are effective against all races of the pathogen.

Only the accumulation of further knowledge will show whether the results presented here are typical of wild plant populations that have been subjected to pathogen selection. If they are, agriculturally based mixtures may have a to be made much more complex before they can be expected to prevent the development of pathogen races with wide virulence.

Acknowledgements. The assistance of Mr. S.S. Speer during the course of this study is acknowledged with considerable gratitude. The research program of which this study is part, has variously been supported by the Oilseeds Research Committee, the Rural Credits Development Fund and the International Board of Plant Genetic Resources.

References

Alexander HM, Burdon JJ (1984) The effect of disease induced by *Albugo candida* (white rust) and *Peronospora parasitica* (downy mildew) on the survival and reproduction of *Capsella bursa-pastoris* (shepherd's purse). Oecologia (Berlin) 64:314–318

Augspurger CK (1983) Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. J Ecol 71:759–771

Augspurger CK, Kelly CK (1984) Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. Oecologia (Berlin) 61:211–217

Barrett JA (1985) The gene-for-gene hypothesis: parable or paradigm. In: Rollinson D, Anderson RM (eds) Ecology and genetics of host-parasite interactions. Academic Press, London, pp 215–225

Broué P, Marshall DR, Muller WJ (1977) Biosystematics of subgenus *Glycine* (Verdc): Isoenzymatic data. Aust J Bot 25:555–566

Brown AHD, Nevo E, Zohary D, Dagan O (1978) Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). Genetica 49:97–108

Browning JA, Frey KJ (1969) Multiline cultivars as a means of disease control. Ann Rev Phytopathology 7:355–382

Burdon JJ (1980) Variations in disease resistance within a population of *Trifolium repens*. J Ecol 68:737–744

Burdon JJ (1985) Pathogens and the genetic structure of plant populations. In: White J (ed) Studies on plant demography: A Festschrift for John L Harper. Academic Press, London, pp 315–325

Burdon JJ (1987) Diseases and plant population biology. Cambridge University Press, Cambridge

Burdon JJ, Marshall DR (1981a) Inter- and intra-specific diversity in the disease-response of *Glycine* species to the leaf-rust fungus *Phakopsora pachyrhizi*. J Ecol 69:381–390

Burdon JJ, Marshall DR (1981b) Evaluation of Australian native species of *Glycine* for resistance to soybean rust. Plant Disease 65:44–45

Burdon JJ, Speer SS (1984) A set of differential hosts for the identification of pathotypes of *Phakopsora pachyrhizi* Syd. Euphytica 33:891–896

Burdon JJ, Oates JD, Marshall DR (1983) Interactions between *Avena* and *Puccinia* species. I. The wild hosts: *Avena barbata* Pott ex Link, *A. fatua* L and A *ludoviciana* Durieu. J Appl Ecol 20:571–584

Burdon JJ, Groves RH, Kaye PE, Speer SS (1984) Competition in mixtures of susceptible and resistant genotypes of *Chondrilla juncea* differentially infected with rust. Oecologia (Berlin) 64:199–203

Day PR, Barrett JA, Wolfe MS (1983) The evolution of host-parasite interaction. In: Kosuge T, Meredith CP, Hollaender A (eds) Genetic engineering in Plants. Plenum Press, New York, pp 419–430

Dinoor A (1970) Sources of oat crown rust resistance in hexaploid and tetraploid wild oats in Israel. Can J Bot 48:153–161

Dinoor A (1977) Oat crown rust resistance in Israel. Ann NY Acad Sci 287:357–366

Flor HH (1955) Host-parasite interaction in flax rust – its genetics and other implications. Phytopathology 45:680–685

Fischbeck G, Schwarzbach E, Sobel S, Wahl I (1976) Types of protection against barley powdery mildew in Germany and Israel selected from *Hordeum spontaneum*. Barley Genetics, Proc 3rd Intern Barley Genetics Symp 1975, pp 412–417

Groth JV (1976) Multilines and "super races": a simple model. Phytopathology 66:937–939

Jarosz AM (1984) Ecological and evolutionary dynamics of *Phlox-Erysiphe cichoracearum* interactions. Ph D thesis, Purdue University

Jayakar SC (1970) A mathematical model for interaction of gene frequencies in a parasite and its host. Theor Pop Biol 1:140–164

Jensen NF (1952) Intra-varietal diversification in oat breeding. Agronomy J 44:30–34

Kinloch BB jr, Stonecypher RW (1969) Genetic variation in susceptibility to fusiform rust in seedlings from a wild population of loblolly pine. Phytopathology 59:1246–1255

Kochman JK (1977) Soybean rust in Australia. In: Ford RE, Sinclair JB (eds) Rust of soybean. INTSOY Series Number 12, University of Illinois, pp 44–48

Leonard KJ (1969) Selection in heterogeneous populations of *Puccinia graminis* f. *sp. avenae*. Phytopathology 59:1851–1857

Leonard KJ (1977) Selection pressures and plant pathogens. Ann NY Acad Sci 287:207–222

Leonard KJ (1984) Population genetics of gene-for-gene interactions between plant host resistance and pathogen virulence. In: Chopra VL, Joshi BC, Sharma RP, Bansal HC (eds) Genetics: New Frontiers, vol 4. Oxford & IBH Publishing Co, New Delhi, pp 131–148

Leonard KJ, Czochor RJ (1980) Theory of genetic interactions among populations of plants and their pathogens. Ann Rev Phytopathology 18:237–258

Luig NH (1983) A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Adv Plant Breeding 11:1–198

Marshall DR (1977) The advantages and hazards of genetic homogeneity. Ann NY Acad Sci 287:1–20

Marshall DR, Pryor AJ (1978) Multiline varieties and disease control. I. The "dirty crop" approach with each component carrying a unique single resistance gene. Theor Appl Genetics 51:177–184

Marshall DR, Pryor AJ (1979) Multiline varieties and disease control. II. The "dirty crop" approach with components carrying two or more genes for resistance. Euphytica 28:145–159

Miles JW, Lenné JM (1984) Genetic variation within a natural *Stylosanthes guianensis, Colletotrichum gloeosporioides* host-pathogen population. Aust J Agric Res 35:211–218

Moseman JG, Nevo E, El Morshidy MA, Zohary D (1984) Resistance of *Triticum dicoccoides* to infection with *Erysiphe graminis tritici*. Euphytica 33:31–47

Parker MA (1986) Individual variation in pathogen attack and differential reproductive success in the annual legume, *Amphicarpaea bracteata*. Oecologia (Berlin) 69:253–259

Person C (1959) Gene-for-gene relationships in host:parasite systems. Can J Bot 37:1101–1130

Simons MD (1970) Crown rust of oats and grasses. Monograph No 5. American Phytopathological Society, Mass

Zimmer DE, Rehder D (1976) Rust resistance of wild *Helianthus* species of the north central United States. Phytopathology 66:208–211

Received January 5, 1987