

DEDICATION

This thesis is dedicated to the Lord who "has made everything beautiful in its time" and to my parents.

SELECTION AGAINST VIRULENCE GENES IN
POPULATIONS OF PLANT PATHOGENS

by

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ABSTRACT

The concept of selection against ^{unnecessary} virulence genes (sensu Van der Plank) is discussed and fresh evidence is presented from theoretical studies of pre-existing virulence gene frequencies and field data of declining virulence gene frequencies to show that this selective force does operate in pathogen populations.

Population genetics models of declining virulence gene frequencies are presented and these are used to estimate the selective coefficients, s for certain unnecessary virulence genes from field data. These s values are frequently lower than 0.1 and significantly less than values of s calculated from other methods and used in predictions of pathogen evolution in multilines and cultivar mixtures.

The significance of these lower, more reliable s values is discussed in relation to current models of pathogen evolution in heterogeneous host populations. In most circumstances complex races are predicted to predominate. However the general view that the success of these disease control strategies depends on the predominance of simple races is challenged.

Direct experimental evidence was sought for stabilizing selection against virulence to the Cf₄ gene in Fulvia fulva using sporulation counts and against virulence to the Mlg gene in Erysiphe graminis hordei using race competition experiments. Some evidence of stabilizing selection was discovered in the latter.

The concept of selection against virulence gene combinations and its detection is discussed. Theoretical and practical evidence is presented

that selection against such virulence pairs does occur. Special reference is made to the Puccinia graminis tritici populations in North America.

It is shown that the commonly used test for linkage disequilibrium is a valid means of detecting selection in large asexual populations in certain circumstances.

The restriction of variation and its causes in large asexual populations is discussed and the role of selection and the lack of recombination in contributing to linkage disequilibria is analyzed.

Finally a new two host component mathematical model of pathogen evolution in multilines and cultivar mixtures is presented, incorporating more selective forces than previous models. Computer simulations with the model have been conducted and the model is shown to be more realistic and hence useful than earlier models, in relating the theory to the field data.

Abbreviations and symbols used in the text

a	1. selective advantage of a race of a pathogen carrying a virulence gene, $\underline{V}_1$ when growing on a host with the corresponding resistance gene, $\underline{R}_1$
	2. a virulence gene carried by race $\underline{a}$, to a resistance gene, $\underline{R}_A$
α	realized reproductive rate
A	1. avirulence gene/allele
	2. cultivar carrying a resistance gene, $\underline{R}_A$
Aa ₆	avirulence in <u>Erysiphe graminis hordei</u> the cause of barley mildew to the <u>Mla₆</u> resistance gene in barley
A.D.A.S	Agricultural Development and Advisory Service
Ai-i	avirulence in <u>Pyricularia oryzae</u> the cause of rice blast to the <u>Pi-i</u> resistance gene in rice
A ₁	avirulence gene/allele to a resistance gene, $\underline{R}_1$
B	cultivar carrying a resistance gene, $\underline{R}_B$
b	a virulence gene carried by race $\underline{b}$, to a resistance gene, $\underline{R}_B$
BMV	Barley mildew virulence factor (gene)
c	1. selective disadvantage of a race carrying a virulence gene pair relative to a combination of their avirulence alleles or to a single virulence genotype
	2. a virulence gene carried by race $\underline{c}$, to a resistance gene, $\underline{R}_c$
Cf	resistance gene in tomato to <u>Fulvia fulva</u> , the cause of tomato leaf mould
Cf ₀	tomato genotype lacking known ^{major} /resistance genes
χ^2	chi square
cm	centimetre(s)

C.M.I.	Commonwealth Mycological Institute, Kew
cms-T	texas male sterile cytoplasm corn
D	Simpson's measure of diversity
d	a coefficient of selective advantage associated with a genotype
°C	degrees centigrade
°F	degree fahrenheit
d.f	degrees of freedom
D _t	delay time in appearance of a virulent race after first disease appearance
ϵ_{q_t}	the expected value of q after t generations
E.S.H.	elongating secondary hyphae of <u>Erysiphe graminis hordei</u>
F	F statistic or variance ratio
FAA, FAV, FVA, FVV	equal to PAA, PAV, PVA and PVV respectively
ft	foot/feet
g	1. selective disadvantage of a complex race when infecting any cultivar in a cultivar mixture compared with the appropriate simple race, due to a less fit background genotype 2. gram(s)
G.C.R.I.	Glasshouse Crops Research Institute, Littlehampton
>	greater than
≥	greater than or equal to
hr	hour(s)
"	inch(es)
I.P.O.	Instituut voor Plantenziektenkundig Onderzoek, Wageningen
ir	induced resistance factor

n	1. the proportion of hosts in a locality possessing a specific resistance gene
	2. the number of isolines or cultivars in a multiline or mixture respectively
N	pathogen population size : lesion number of a population or the number of spores produced by a population in a season
NaA	number of lesions of race <u>a</u> on host <u>A</u> carrying the <u>R_A</u> resistance gene in a multiline or cultivar mixture. <u>NaB</u> , <u>NabA</u> , <u>NabB</u> , <u>NbA</u> and <u>NbB</u> follow the same construction
9a,9b,9d,9e	virulence genes in <u>Puccinia graminis tritici</u> the cause of stem rust to corresponding <u>Sr</u> genes in wheat
N.A.C.A.M.	National Association of Corn and Agricultural Merchants
N.G.H.	neutral gene hypothesis
N.S.W.	New South Wales
N _t	number of lesions of a pathogen population at generation <u>t</u>
OMV	oat mildew virulence factor
P	probability
Pa	frequency of race <u>a</u> in a cultivar mixture or multiline. Race <u>a</u> carries virulence to the <u>R_A</u> resistance gene
Pab	frequency of race <u>ab</u> in a cultivar mixture or multiline. Race <u>ab</u> carries virulence to the <u>R_A</u> and <u>R_B</u> resistance genes
PAA	frequency of the double virulence genotype to the <u>R_A</u> and <u>R_B</u> or <u>R₁</u> and <u>R₂</u> resistance genes
PAV	frequency of a single virulence genotype to the <u>R_B</u> or <u>R₂</u> resistance gene
PVA	frequency of single virulence genotype to the <u>R_A</u> or <u>R₁</u> resistance gene
PVV	frequency of double virulence genotype to the <u>R_A</u> and <u>R_B</u> or <u>R₁</u> and <u>R₂</u> resistance genes

%	percentage
$\emptyset$	alloinfection frequency
$\emptyset_{\max}$	maximum value of $\emptyset$
Pi	resistance gene in rice to <u>Pyricularia oryzae</u>
Pi-a, Pi-i, Pi-k	specific <u>Pi</u> genes
Pi-o	avirulence allele to <u>Pi-i</u>
p	avirulence gene frequency
P_1, P_2, P_3	frequencies of races with 1, 2 and 3 virulence genes respectively in a cultivar mixture or multiline
$\underline{p}_t$	frequency of avirulence at generation <u>t</u>
q	virulence gene frequency
q_b	virulence gene frequency at balance
$\hat{q}_b$	estimated virulence gene frequency at balance
$\bar{q}$	$(\underline{s} + \underline{\mu a} + \underline{\mu v}) / 2\underline{s}$
$\hat{q}$	$\underline{\mu a} / (\underline{\mu a} + \underline{\mu v})$
q_t	frequency of virulence at generation <u>t</u>
r	apparent infection rate
R	1. resistance gene 2. ratio of the relative fitness of or reproductive potential of a complex race compared with that of a simple race (carrying one virulence gene)
R_A, R_B, R_C	resistance genes <u>A</u> , <u>B</u> , and <u>C</u> to a pathogen, respectively
R.H.	relative humidity
r_l	logarithmic infection rate
R_m	value of <u>R</u> (definition 2) for a complex race with <u>m</u> virulence genes
$\underline{R}_1, \underline{R}_2, \underline{R}_3$	1. resistance genes 1, 2 and 3, to a pathogen 2. specific resistance genes in potato to <u>Phytophthora infestans</u> , the cause of blight or in lettuce to <u>Bremia lactucae</u> the cause of downy mildew

s	selection coefficient against unnecessary virulence
s_a, s_b, s_1, s_2	selection coefficients against unnecessary virulence to resistance genes $\underline{R_A}, \underline{R_B}, \underline{R_1}$ and $\underline{R_2}$ respectively
7b	virulence to $\underline{Sr_{7b}}$ in <u>Puccinia graminis tritici</u>
sq	square
Sr	resistance gene in wheat to <u>Puccinia graminis tritici</u>
S.S	sums of squares
S.S.H	stabilizing selection hypothesis
t	generation number
Tmp	virulence to $\underline{Sr\ Temp}$ in <u>Puccinia graminis tritici</u>
Tt-1	virulence to $\underline{Sr\ Tt-1}$ in <u>Puccinia graminis tritici</u>
U.S.D.A.	United States Department of Agriculture
V	virulence gene/allele
Va ₆	virulence to the $\underline{Mla_6}$ resistance gene in <u>Erysiphe graminis hordei</u>
Vi-i	virulence to the $\underline{Pi-i}$ resistance gene in <u>Pyricularia oryzae</u>
Vg	virulence to the $\underline{Mlg}$ resistance gene in <u>Erysiphe graminis hordei</u>
V ₁	virulence to $\underline{R_1}$
x	1. proportion of disease 2. frequency of a virulence gene 3. number of samples in a virulence survey
Xi	the <u>i</u> th generation
X ₁	number of lesions in generation 1 in a pathogen population on a multiline or a cultivar mixture

x_{ov}	proportion of initial inoculum carrying virulence to a resistance gene
W	1. relative fitness of a pathogen 2. watts
W _a	relative fitness of races carrying an avirulence allele, <u>a</u>
W _{aA}	relative fitness of race <u>a</u> on host <u>A</u> carrying the <u>R_A</u> resistance gene in a multiline or cultivar mixture <u>W_{aB}</u> , <u>W_{aB}A</u> , <u>W_{aB}B</u> , <u>W_{bA}</u> and <u>W_{bB}</u> follow the same construction
W/m ²	watts per square metre
W _v	relative fitness of races carrying a virulence allele, <u>v</u>
y	frequency of a virulence gene
Y _i	mean percentage mutant at <u>i</u> th generation
z	selection coefficient against races with an avirulence allele on the hosts with corresponding resistance

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CHAPTER 1

SELECTION AGAINST UNNECESSARY VIRULENCE (STABILIZING SELECTION)

1.1. Literature Review

1.1.1 Introduction

The hypothesis of selection against unnecessary virulence genes within populations of plant pathogens was developed by Van der Plank (1963, 1968, 1975) for host pathogen interactions, where gene for gene relationships were established (Flor, 1956; Person 1959, 1966). Van der Plank proposed that unnecessary virulence genes (the term virulence is used to define a specific pathogen gene enabling its possessor to grow on a host with a corresponding resistance gene) which are not required for successful infection of a host may be subject to a selective force which he called 'stabilizing selection'. This term was originally applied to the genetics of quantitative characters, that is those whose expression is determined by many genes each with a small contributory effect to the phenotype (Grant, 1963). Stabilizing selection in the original sense of the term operates to select against the extremes of the character in populations, opposing directional selection. It therefore stabilizes shifts away from the mean. Because of the potential confusion with this term, stabilizing selection (sensu Van der Plank) is often referred to as 'selection against unnecessary virulence' or 'directional selection for avirulence' (Leonard and Czocher, 1980). Stabilizing selection (sensu Van der Plank) is very clearly a form of directional selection, for avirulence. The stabilization however occurs in that the original population frequencies of the avirulence and virulence alleles at any one locus are restored by stabilizing selection following the removal of the selective force for virulence, which causes the original departure from the initial balance.

The force of stabilizing selection (used hereafter sensu Van

der Plank) was considered to act in three ways : firstly to reduce the frequency of a virulence gene in a pathogen population following the mass removal from agricultural use of a host cultivar with the corresponding resistance gene. Secondly it may oppose the increase in frequency of pre-existing virulence genes by mutation, in pathogen populations growing on host populations which lack the corresponding resistance gene. Finally, in a mixed host population with the components carrying different resistance genes, stabilizing selection could select against races (a race is defined as a sub-population of the pathogen characterized by a particular virulence phenotype) with unnecessary virulence, so that those predominantly isolated from any one host would usually carry only necessary virulence genes required to infect that particular host. A variant of this would be the selection of an isolate without unnecessary virulence genes on a host in a race competition experiment with other isolates carrying unnecessary virulence genes.

Selection against unnecessary virulence was also considered to occur not only on hosts lacking specific resistance genes but also during the saprophytic phases of non-obligate pathogens (Van der Plank, 1968, Chapter 5).

The existence of stabilizing selection was thought to result from a lower fitness of races carrying unnecessary virulence genes, where fitness would be ultimately expressed (and defined) in terms of reproductive success.

Van der Plank (1968) postulated that biochemically a consequence of mutation from avirulence to virulence enabling the pathogen to overcome a particular resistance gene, was that some normal primary pathogen function conditioned by the avirulence gene was dismantled or achieved by another substitute biochemical pathway. As a consequence mutant pathogen races were able to avoid the effects of host resistance and therefore

confer on these virulent races a great advantage compared with avirulent races lacking necessary virulence. However when virulence genes are unnecessary this advantage is no longer present; instead the loss of normal primary pathogen function of the virulent races reduces fitness compared with avirulent races with an intact and normal function.

Van der Plank (1968, 1978) argued that the avirulence allele is the wild type allele with a normal primary function whilst the virulence allele is a mutant with an altered primary function. He noted that in pathogens of agricultural crops the pre-existing frequencies of most virulence genes effective against commercially used resistance genes are very low prior to the extensive use of the corresponding resistant hosts. This means that in natural, unselected pathogen populations avirulence is the normal wild type gene, with a normal primary function. This also suggests that where virulence is unnecessary, the avirulence gene is relatively fitter, implying the possession of a more efficient primary function. If the virulence allele is less common, because it is less fit, it must be associated with a less efficient primary function and ^{is} most likely to be a mutant.

Van der Plank (1978) has argued that the prime function of the avirulence gene is not avirulence; this is a secondary function or character. The gene has an unidentified primary function. It is the loss of this function, or adoption of a less efficient substitute pathway, that makes carriers of unnecessary virulence less fit.

The concept of stabilizing selection is controversial and has stimulated much debate amongst plant pathologists. The literature on the subject will now be described and the concept reviewed.

1.1.2 Origins of the concept of stabilizing selection

The roots of the concept of stabilizing selection pre-date Van der Plank (1963, 1968, 1975). Holton (1947) established that host selectivity of wheat was a major factor in establishing new races of smut fungi Tilletia caries and T. foetida produced by hybridization, especially the degree of resistance of the cultivars. A highly susceptible wheat cultivar such as Hybrid 128 promoted the increase and establishment of segregates with low numbers of virulence genes. Holton (1947) was uncertain why the most widely virulent races were less favoured on these hosts. The results were supported by observations that highly virulent races occurred mostly in regions in which smut resistant cultivars were grown.

Stevens (1949) stated that even though there is a rapid increase in frequency in new races of pathogens in response to the cultivation of new resistant hosts, old established races decline in frequency. Therefore old host cultivars may well be resistant to the new pathogens. If this were not so Stevens (1949) argued these new races would have appeared and dominated earlier. He concluded that the replanting of discarded cultivars should be contemplated as a means of disease control. Implicit in his argument is the assumption as races disappear following the removal of the selective host so will the frequency of virulence genes they carry.

Further experimental evidence for selection against unnecessary virulence was provided by the studies of Black (1952). He determined the relative survival values of isolates of six strains of Phytophthora infestans on a susceptible potato cultivar Craigs Royal (which lacks R (resistance) genes against blight) for ten pathogen generations. Certain genotypes of the pathogen had very low survival potentials. The race 1, 3, 4 isolate was absent by generation four, the race 1, 3 isolate

disappeared by generation six. Another race, race 1, 4 exhibited reduced sporulation by generation five. On the other hand Black's (1952) races 0; 4 and 2, 4 did not exhibit reduced sporulation. These results can be explained on the basis of selection against unnecessary virulence to the R_1 and R_3 genes. Black (1952) concluded "that the wider the host range of a strain the less prolific it is on recessive (lacking known R genes) varieties, and the lower its survival value in competition with less specialized forms".

A fourth author reporting probable stabilizing selection prior to Van der Plank's contributions (1963, 1968, 1975) was Flor (1953) who studied the population genetics of Melampsora lini the causal agent of flax rust. Flor (1953) found that in the north central states of the U.S.A. the principal races of the pathogen during each of the three periods 1931 - 1940, 1942 - 1947, 1948 - 1951 in which the predominant cultivars were those with increased numbers of resistance genes, were those carrying the least number of virulence genes compatible with survival. Further he noted that the proportion of isolates carrying genes for virulence having no apparent survival value (unnecessary, therefore) decreased in each successive period, and he concluded that the accumulation of the maximum number of avirulence genes had positive survival value.

Watson(1958)reported that races of Puccinia graminis tritici (causing stem rust of wheat) with wide host ranges could not maintain themselves in mixtures with races of narrow host range in glasshouse competition experiments. These observations are all consistent with the concept of selection against unnecessary virulence.

1.1.3 Development of the concept of stabilizing selection by Van der Plank

Van der Plank (1963, 1968, 1975) formally defined the concept of

stabilizing selection and presented much evidence to support it. In his 1968 book Van der Plank stated that "when the gene (for resistance) is absent, virulence to match it is unnecessary and stabilizing selection operates in favour of races of the pathogens without unnecessary virulence". The force was described as that causing the return of the pathogen population towards its unselected state once directional selection for virulence was stopped. Van der Plank (1968) then distinguished between resistance genes in the host with respect to stabilizing selection. A 'strong' resistance gene was one whose corresponding unnecessary virulence gene was subject to strong selection. On the other hand a 'weak' resistance gene was one where corresponding unnecessary virulence was subject to weak selection or no detectable selection. Van der Plank (1968) quoted the R_1 gene in potatoes as an example of a strong resistance gene because pre-existing virulence in the pathogen population occurred at very low frequencies, implying that selection against it was of sufficient magnitude to reverse the effect of continuous mutation to virulence.

In his 1975 book Van der Plank remarked upon the common occurrence of weak resistance genes. Cereals and potatoes in particular contain many resistance genes virtually useless commercially, due to the speed with which virulence against them appears and remains established in populations of rusts and Phytophthora infestans respectively. A possible explanation for the general absence of strong selection against unnecessary virulence was proposed by Van der Plank (1968). He thought most mutations from avirulence to virulence are likely to have little effect on the biochemistry and hence the fitness of the pathogen to survive in the absence of host resistance.

The inclusion in his hypothesis of weak and strong resistance genes in relation to selection against unnecessary virulence (Van der Plank 1968, 1975) represents a shift in thinking since the original, but brief

and undeveloped hypothesis was first proposed (Van der Plank, 1963). Initially he had stated "we take it as axiomatic that simple races are the fittest to survive on simple varieties", but his subsequent writings accommodated also, examples where selection against unnecessary virulence is either absent or weak. Presently therefore Van der Plank does not believe that selection will operate against every case of unnecessary virulence. Stabilizing selection may be detectable with some examples of virulence genes but not with others. Hence examples of the latter do not invalidate the concept because its validity is not dependent on its universal occurrence (Leonard and Czoher, 1980).

1.1.4 Evidence for the existence of selection against unnecessary virulence as presented by Van der Plank

The evidence can be summarized into several broad categories.

1.1.4.a Low frequencies of pre-existing virulence

Van der Plank (1968, 1975) demonstrated that populations of P. infestans initially had very low frequencies of pre-existing virulence to the $\underline{R}_1$, $\underline{R}_2$ and $\underline{R}_3$ resistance genes in cultivars of potato, when these newly bred cultivars were exposed to the natural populations of blight, in the late 1920s, 30s and 40s in Europe and the 1950s in North America. For several years virulence towards these resistant hosts was not found despite extensive searching. Eventually races virulent against cultivars with $\underline{R}_1$ or $\underline{R}_2$ or $\underline{R}_3$ were isolated and these rapidly spread as a result of the large acreages sown to the resistant cultivars. However the initial rarity of virulent races for several years in several countries indicated very low values of pre-existing virulence. This accounts for the 'boom' in the classical 'boom and bust cycle' of monogenic resistance. The low frequencies of pre-existing virulence accord-

ing to Van der Plank (1968) was due to selection against unnecessary virulence prior to the use of the resistant cultivars. This selection opposed the increase in the frequency of virulence in the population due to mutation pressure. Mutation to virulence in P. infestans is common (Van der Plank, 1968). The low frequency of pre-existing virulence especially to the R₁ gene has made this gene one of the most effective for resistance to blight and the most commonly used R gene for the breeding of resistant cultivars. Thus even today in regions where the R₁ gene has not been used, virulence towards it is rare, even though in nearby areas the virulence frequency may be much higher. For example in the early 70s in Holland R₁ virulence was common in the north but rare in the south (Van der Plank, 1975 p. 151).

Some virulence genes have been found at high pre-existing frequencies indicating the absence of any or strong stabilizing selection. The corresponding resistance genes for example Sr₈ and Sr₁₅ in wheat (against stem rust) in Australia and R₄ in potatoes are examples of weak resistance genes (Van der Plank, 1968).

1.1.4.b Race surveys and unnecessary virulence

Much of the evidence presented by Van der Plank (1963, 1968) for the hypothesis of stabilizing selection came from race surveys, from data similar to that of Holton (1947) and Flor (1953). Van der Plank (1963, 1968) indicated that simple races of P. infestans tend to be selected on hosts with no R genes, in Mexico and Germany. The frequency of races sampled with unnecessary virulence genes (apart from virulence to R₄ which is a very weak gene) declined progressively with each additional unnecessary virulence gene carried by the race. Thus for example Schick, Schick and Hausdörfer (1958) in Germany examined 209 isolates of P. infestans

samples in 1956 from cultivars lacking R genes. At that time R₁ cultivars were becoming popular, occupying about one half of the German potato acreage. Races with no unnecessary virulence genes (excluding virulence to R₄) constituted 72% of the isolates sampled, those with one, two and three unnecessary virulence genes accounted for 27%, 0.8% and 0.0% respectively of the isolates. This suggested that selection against virulence to the R₁, R₂ and R₃ genes was operating. Similar results from race surveys in Mexico were considered particularly significant by Van der Plank (1968) especially as an indigenous wild host Solanum demissum (a relative of Solanum tuberosum, the cultivated potato, which was brought in centuries ago (Van der Plank, 1968)), is very common at the high altitudes that potatoes are grown. This wild potato species was the source plant of R genes used for breeding monogenic resistance into S. tuberosum. Further, Mexico is thought to be the ancestral home of P. infestans (Bourke, 1964). The pathogen is present as both mating types in approximately a 1:1 ratio and sexual reproduction is common. To date throughout the rest of the world one mating type is very common whereas the other is rare or absent. Consequently, oospore production and sexual recombination are extremely limited (Alexopoulos, 1962).

Graham, Niederhauser and Romero (1959) sampled blight from S. tuberosum and S. tuberosum x S. demissum hybrids. From the hybrids, races of the pathogen possessing three or four virulence genes to the R genes carried by S. demissum were the most frequently isolated. Obviously, the commonest races have the necessary virulence genes to colonize clones of the hybrids possessing R genes. On the cultivated potato varieties, lacking known R genes the most commonly isolated race was race 0, lacking virulence to R₁, R₂, R₃ and R₄. The special significance of the Mexican data is that blight has been evolving on S. demissum for centuries and

yet the pathogen has been unable to overcome the effects of selection against unnecessary virulence. If it could then the complex races should predominate on the cultivars lacking R genes as well as on the hybrids which do. Van der Plank (1968) presented this evidence to counter the claim that the effects of selection against unnecessary virulence might be temporary, until a modification of the background genotype occurred in the pathogen circumventing the fitness loss caused by unnecessary virulence. Clearly this has not occurred in Mexico despite the many thousands of generations available for it to do so.

Van der Plank (1963, 1968) presented similar data and evidence of selection against unnecessary virulence in populations of P. graminis tritici in Australia. Watson (1958) found that the commonest races of P. graminis tritici were those with no unnecessary factors for virulence. Similar results were found during race surveys conducted in New Zealand, which is part of the same stem rust geographical area.

1.1.4.c Declining virulence gene frequencies

This evidence presented by Van der Plank (1968) is particularly significant since it shows stabilizing selection in action, unlike the above evidence which strongly implies it but does not demonstrate it directly. The data came from studies of P. graminis tritici populations in eastern Australia, between 1939 and 1962.

The initially resistant cultivar Eureka possessing the Sr₆ resistance gene was removed from cultivation due to the appearance and multiplication of rust races able to overcome it. Shortly afterwards races virulent on Eureka declined sharply. The decline in the acreage of wheat sown to Eureka occurred during the years 1945 - 1948 from about

19% of the acreage in northern New South Wales to about 1.5% in 1948 and for the next ten years or so. From 1945 to about 1956 the frequency of virulence to Sr₆ declined progressively from over 0.7 to 0.0. Virulence declined because on hosts lacking the Sr₆ gene, races carrying virulence were on average less fit relative to races avirulent on Sr₆.

This example of selection against unnecessary virulence in a population is very striking but this was the only one Van der Plank (1968) was able to cite. A number of other examples are found in later literature and these will be discussed below (section 1.3.6).

1.1.4.d Race competition experiments in glasshouses or small plots

The fourth line of evidence that Van der Plank (1963, 1968) presented to support the concept of stabilizing selection was from data of competition experiments between isolates of different races. Experiments differed in their numbers of unnecessary virulence genes. Mixtures of pathogen isolates were cultured on a susceptible host for a number of generations and the percentage of each race was determined at each generation. One of the best examples of approach was the study of Watson and Singh (1952) using Australian isolates of the related race groups 126 and 222 of P. graminis tritici in glasshouse experiments. Their data showed that isolates without unnecessary virulence to the Sr₆ resistance gene increased in the mixtures when in competition with isolates with unnecessary virulence on the susceptible wheat cultivar Federation. Isolates with unnecessary virulence to the Sr₁₁ resistance gene were also selected against.

Van der Plank (1968) recognized, however, the limitations of this kind of experiment. Firstly competition or interaction of races is likely to be much less intense in natural populations than in these artificial

experiments in which two or more isolates of different races are forced to grow at high densities on the same plants. Secondly it is vital to compare isolates of races which do not differ in their background genotype since the effects on fitness of unnecessary virulence might be confounded by the effects on fitness of the background genotype. Thirdly the sampling of the mixtures after discrete generations may result in incomplete measurements of fitness since some factors contributing to fitness might be expressed after the mixture is sampled each generation to estimate the percentage of the races in the mixture. Consequently natural populations in field conditions where there is a continuous epidemic are more valid experimental materials for testing for the existence of stabilizing selection.

1.1.5 Arguments against the concept of stabilizing selection and their refutation

Van der Plank's hypothesis of stabilizing selection has provoked one of the greatest debates in plant pathology, not least because of the significance of the concept to many current strategies of disease control. Much of the literature has been devoted to opposing the concept. Experimental evidence and arguments that apparently refute the concept have been reviewed, summarized and evaluated by Watson (1970), Nelson (1972, 1973) and Crill (1977) whilst many others have presented evidence opposing the hypothesis, for instance Martens, McKenzie and Green (1970), Ogle and Brown (1970), Green (1971) and Martens (1973). The arguments opposing the concept of stabilizing selection will now be discussed.

There are many cases of high pre-existing virulence gene frequencies cited in the literature. Further, examples where unnecessary virulence frequencies do not decline following the removal of the selective host

exist. These examples strongly imply that selection against unnecessary virulence genes is absent or weak.

Fleischmann (1963) reported the collection of a new race, race 332 of Puccinia coronata avenae causing crown rust of oats) from four Canadian provinces. This race which probably arose by mutation from race 216 was virulent on the oat cultivar Saia despite the absence of any known Saia type resistance in commercial cultivars at that time. Unnecessary virulence to this resistance therefore did not prejudice survival. Fleischmann (1963) concluded that other factors in addition to host resistance might confer selective advantages on certain biotypes within the P. coronata avenae population, and that within the range of races virulent on the prevailing commercial hosts there operates a secondary selection pressure screening for more aggressive biotypes.

Martens et al. (1970) showed the most successful races of Puccinia graminis avenae (causing oat stem rust) in Canada have for decades carried genes for virulence in excess of those required for successful parasitism.

Watson (1970) showed that one of the most prevalent strains of Puccinia recondita tritici (causing brown rust of wheat) 68-1,2,3,4 in Australia carried two unnecessary virulence genes and suggested that in the course of natural selection these genes have become associated with genes controlling aggressiveness and survival ability. Further Watson (1970) stated that the most prevalent races, races 21 and 34 of P. graminis tritici in Australia carry unnecessary virulence genes. However in some situations Watson (1970) recognised that stabilizing selection may result in the replacement of the gene for virulence by that for avirulence. He cited P. coronata avenae in Iowa as reported by Browning and Frey (1969) and some Australian wheat rust fungi as reported by Watson and Luig (1968)

as examples where clear stabilizing selection had occurred.

Green (1971) evaluated data on the Canadian races of P. graminis tritici collected from 1919 - 1969, and questioned Van der Plank's assertion that unnecessary virulence to the Sr₁₁ resistance gene reduces the fitness of races possessing it to survive (Van der Plank, 1969). In Canada from 1950 - 1969, various biotypes of race 15B all virulent on Sr₁₁ predominated despite a very minimal selection for virulence, caused by low acreages of Sr₁₁ cultivars in western Canada. Green (1971) also noted that the succession of main races in Canada in the 1960s except race 56 showed an increased range of virulence mostly unnecessary.

Some of the conclusions of Mackey (1973) support the work of Martens et al. (1970). He reported that in Sweden, race surveys had been conducted for cereal rusts and powdery mildews prior to the introduction of any race specific genes for resistance being introduced. Consequently there had been a total absence of race specific host selection pressure. At that time unnecessary virulence genes in the pathogens, P. graminis avenae and P. recondita tritici were common, suggesting that there was no obvious selection against these genes. However unnecessary virulence genes in Erysiphe graminis tritici which causes powdery mildew of wheat were much less common and Mackey (1973) suggested that these data supported the idea that unnecessary genes are somehow disadvantageous.

For Erysiphe graminis hordei (the cause of powdery mildew of barley) Jørgensen (1978) reported that in Denmark the frequency of unnecessary virulence to some resistance genes in barley e.g. Mla₁₀ was high, but in other cases, e.g. Mla₃, low.

Pre-existing virulence in Fulvia fulva (the cause of tomato leaf mould) to the Cf_{1,2,3,4,5} resistance genes in tomatoes has been found in varying amounts in France by Laterrot and Clerjeau (1979). They

suggested these Cf genes are not 'strong' (sensu Van der Plank 1968). Thus forces of selection against unnecessary virulence are weak or absent. However these authors could not reject the possibility that the existence of these virulent races was due to importation from other countries possibly Holland.

Evidence for stabilizing selection in Bremia lactucae (the cause of downy mildew of lettuce) is also lacking. Crute (1981) stated that "there is little evidence for selection against superfluous virulence against the genes R₁₋₁₁".

Leonard (1977a), Leonard and Czocher (1980) and Roelfs and Groth (1980) have challenged the conclusions that the existence or retention of unnecessary virulence genes in populations of pathogens automatically means that stabilizing selection cannot be operating. Leonard (1977a) showed using his model for selection in host populations that a small proportion of resistant plants may cause sufficient selection resulting in a high frequency of apparently unnecessary virulence. Further, Leonard (1977a) argued that the cereal rust fungi (where many examples of unnecessary genes at high frequency occur, (Browning and Frey, 1969; Watson, 1970)) have very large populations covering very large areas. Consequently unnecessary virulence genes in one part of the pathogen's geographical range may not be unnecessary in another area. Virulence genes may be necessary too for overwintering or oversummering on wild hosts between seasons. Further, new and superior genotypes of rust fungi may arise in one environment or subpopulation with necessary genes for virulence and these may spread into new areas, where the virulence genes are unnecessary. Because of the high fitness of these genotypes and the lack of sexual recombination to reshuffle these unnecessary virulence genes with a range of background genotypes, these unnecessary virulence may accumulate in the population.

In addition to the above examples where apparently unnecessary virulence genes are common in populations of plant pathogens either prior or subsequent to the use of the corresponding host resistance genes, many examples exist where in competition experiments isolates of races with unnecessary virulence gene predominated over isolates of races with none or fewer unnecessary virulence genes. Many of these experiments have been described by Watson (1970) and Nelson (1972, 1973) and examples include P. graminis tritici (Watson and Luig, 1968; Ogle and Brown, 1970; Osoro and Green, 1976), P. infestans (Thurston, 1961), Puccinia striiformis, causing yellow rust of wheat (Brown and Sharp, 1970) and P. graminis avenae (Martens, 1973), and E. graminis tritici (Mortensen, 1974). These experiments clearly show that complex races with unnecessary virulence genes may be good competitors when mixed with simple races. In other experiments conflicting results were obtained, either simple or complex races of the same pathogen predominating, even in trials involving the same virulence genes. For instance Thurston (1961) found that his race 0 of P. infestans predominated over various complex races in glasshouse mixtures. These complex races carried unnecessary virulence to the $\underline{R}_1$, $\underline{R}_2$ and $\underline{R}_4$ resistance genes. In some field experiments the same races multiplied more rapidly than some race 0 isolates indicating a superior aggressiveness of these former races.

Hence apparently contradictory results exist in the literature from race mixture experiments. Attempts to resolve these contradictions have been made by consideration of the distinction between the genes for virulence and the other genes which also influence fitness. It is evident that the fitness of an isolate or a race is determined both by the virulence and avirulence genes possessed, and the background genotype. In race competition experiments isolates with unnecessary virulence may occur in

a highly fit background genotype giving a greater competitiveness over an isolate of a race with no unnecessary virulence which happens to be in a less fit background genotype. Hence Thurston (1961) suggested that different isolates of a race may vary in aggressiveness. Watson (1970) showed that when spores of P. graminis tritici are treated with the mutagen Ethylmethanesulphanate cultures can be selected having the specific virulence gene required. However within these there was a great diversity in growth characters. Consequently selection could be made for cultures having the virulence gene plus a combination of characters which contribute to improved growth and survival in the laboratory. Watson (1970) asserted that in the field too, mutants can improve their competitive ability under natural selection. Watson (1970) concluded that "at present it seems that if the genes for virulence are independent of those for survival ability and aggressiveness, then in selection experiments it should be possible to improve the competitive ability once the requisite genes for virulence have been obtained". The genetic independence of virulence and aggressiveness has also been discussed by Van der Plank (1968).

An implication of these ideas is that selection against unnecessary virulence may be a temporary phenomenon until that virulence is combined with a highly fit background genotype (Watson, 1970; Nelson, 1972). This could occur either by a mutation to virulence in a fit genotype or an evolution of the background genotype to increased fitness which would nullify the effects of stabilizing selection. In other words stabilizing selection could be a temporary artifact.

However this argument does not refute the concept of stabilizing selection since an increase in fitness of a race with unnecessary virulence does not automatically mean that it will be as fit as a race with no unnecessary virulence genes, but possessing the same background genotype.

What the argument does show is the need to nullify the effects of the background genes of the isolates used in race competition experiments.

Van der Plank (1975) and Leonard (1977a) have pointed out that many of the race competition studies are of no value in evaluating the concept of stabilizing selection because the effects of the genes for virulence are masked by the effects of other genetic differences among the isolates. Leonard and Czocher (1980) stated that "the relative disadvantage associated with an unnecessary gene for virulence may not be apparent unless special efforts are made to isolate the effect of that gene or to nullify the effect of the background genetic variation". This argument applies also to experiments which support the concept of stabilizing selection. In only a few race competition experiments have the effects of the background genotype been nullified. The best examples are those of Leonard using P. graminis avenae (Leonard, 1969) and Bipolaris maydis (Leonard, 1977a,b). In the former he observed changes in the gene frequencies in a genetically diverse population derived from the sexual stage of the pathogen in which the background genes were randomly distributed with particular genes for virulence and avirulence. The population was cultured for eight generations on susceptible oats and sampled at each generation for avirulence and virulence on differential oat cultivars. Races virulent on seedlings of particular differential cultivars had survival values 14 - 46% lower than their avirulent counterparts on the same cultivars. Stabilizing selection was clearly operating. With B. maydis Leonard (1977a) combined 50 race 0 isolates with 50 race T isolates to provide sufficient genetic diversity to cancel or nullify the effects on fitness of the background genotype. Leonard (1977a) was interested to determine the effect on fitness of unnecessary virulence in race T to maize with texas male sterile cytoplasm, (cms-T cytoplasm) when both races were growing on

maize with normal cytoplasm. Race T was found to be 30% less fit than race 0. In another experiment Leonard (1977b) compared specially bred near isogenic lines of race 0 and race T in a race competition experiment. On maize lacking cms-T, race T was 12% less fit than race 0. Both of these results suggest the operation of stabilizing selection against race T in the absence of cms-T maize.

In most other race competition experiments no attempts were made to negate the effects of the different background genotype possessed by the competing isolates. Therefore it is likely that no valid statement as to the concept of stabilizing selection can be made from these studies. Some workers used isolates from the same race group of closely related race groups, for instance Watson and Singh (1952) and Ogle and Brown (1970). Whilst these are an improvement in reducing the effects of the background genes they do not nullify the contribution of these genes to the relative fitness of the competing races, since variants within each race group occur. Green (1975) for instance noted that single step changes within race 15B of P. graminis tritici in Canada could result in fairly large changes in aggressiveness.

There are other serious problems with race competition experiments compared with population studies, especially when each race is represented by a single isolate. Some of these problems have been discussed above. Nelson (1972) questioned the validity of glasshouse studies to evaluate fitness following his discussion of Thurston's (1961) contradictory results in the field and glasshouse. He suggested (Nelson, 1972) that concentrations of inoculum used in the glasshouse may be considerably higher than in natural conditions.

Further problems with race competition experiments concern environmental and ecological determinants of fitness which may override any

genetic causes of race predominance. Katsuya and Green (1967) found that temperature regime and inoculum density markedly affected the relative competitive abilities of races 15B and 56 of P. graminis tritici.

Falahati-Rastegar, Manners and Smartt (1981) similarly found that a mutant of Puccinia hordei (causing brown rust of barley) predominated over other races within 10 generations on each of five susceptible cultivars in a controlled environment at 14°C. However at 19°C each of four other races predominated. Inoculum density also influenced race predominance.

Nelson (1972) citing the work of Ogle and Brown (1970) stated that fewer transfer generations were required for the complex race of P. graminis tritici race 21-2,3,7 to predominate in their mixtures when the generation period was increased from 10 to 18 days. Consequently different sampling techniques and generation times influence the survival pattern. Hence survived trends observed by discrete generation glasshouse experiments may be artifacts of the techniques used.

The problem with these race competition experiments mean that mostly they cannot be used to evaluate the concept of stabilizing selection, except where suitable attempts have been made to eliminate the effects or fitness of the background genotypes of the competing races. More experiments of the kinds done by Leonard (1969, 1977a,b) are required.

In conclusion the evidence presented by opponents of the concept of stabilizing selection does not refute the concept. Leonard and Czoher (1980) concluded that the arguments propounded by Nelson (1972, 1973) and Crill (1977) do not directly dispute the concept of stabilizing selection but are based on examples and observations that seem not to conform to the concept. As discussed above, many of these examples and observations themselves may be invalid. However genuine examples and observations that do not show stabilizing selection, still do not disprove the concept, since its validity is not as some authors think conditional on its universal occur-

ence with every unnecessary virulence gene. Nelson (1972) expressed the sentiments of many opponents of stabilizing selection in the statement "Those who question it are reluctant to accept the irrevocable universal axiom that fitness equals simplicity" (few or no virulence genes). Van der Plank suggested this axiom in 1963, but thereafter he (1968, 1975) did not consider stabilizing selection to be universal. Nelson (1973) considered the 'weak-gene' philosophy was a buffer introduced by Van der Plank (1968) to cope with the examples where stabilizing selection did not seem to occur. Further, Nelson (1972, 1973) considered that this buffer was a built in escape making the hypothesis of stabilizing selection untestable. Any examples not supporting stabilizing selection were explained by the strong-weak gene rationale.

It is evident that there are two issues emerging from the literature. The first concerns the occurrence of stabilizing selection, the second concerns its extent. Whilst stabilizing selection is not universal its occurrence in some situations is less disputable.

1.2 Studies in pre-existing virulence gene frequencies

1.2.1 Introduction

In the development of the hypothesis of stabilizing selection, Van der Plank (1968, 1975) considered low frequencies of pre-existing virulence to be important evidence in favour of the concept (see also section 1.1.4.a). It is the purpose of this section to consolidate more formally this evidence by the application of some population genetics tools to Van der Plank's (1968, 1975) data and conclusions. Special reference will be made to P. infestans and E. graminis hordei populations in Europe, as examples of pathogens which have been evolving on their respective hosts for comparatively short and long periods, respectively.

1.2.2 Pre-existing virulence in *P. infestans* to the R_4 resistance gene

1.2.2.a High frequencies of pre-existing virulence to R_4

Van der Plank (1975) has commented upon the high incidence of virulence to R_4 in *P. infestans* populations in north west Europe, even though the R_4 gene has rarely been exploited commercially (Black, 1957; Van der Plank, 1968, 1975). The frequency of virulence (q) to R_4 in various countries in north west Europe in the 1950s is given in Table 1.

Table 1 Frequency of virulence (q) to the R_4 resistance gene in *Phytophthora infestans* populations in north west Europe¹

Country	q
Northern Ireland	0.96
England	0.86
Germany	0.95
Netherlands	0.72
Scotland	0.90
Sweden	large

¹From Van der Plank (1975)

The data originate from several workers at different times in the 1950s and from different countries. These values indicate high pre-existing virulence frequencies in *P. infestans* populations, and are not the effects of selection for virulence due to host selection pressure.

These high frequencies of pre-existing virulence to $\underline{R}_4$ restricted the use of $\underline{R}_4$ -containing cultivars and this gene has never assumed any great importance for blight control in Europe.

In Africa and North America virulence to $\underline{R}_4$ also occurs at high pre-existing frequencies (Black, 1957; Van der Plank, 1975). Also, virulence to certain resistance genes in other crops is similarly very high even though the genes concerned have never entered general commercial use, e.g. the $\underline{Sr}_8$ gene in wheat to P. graminis tritici (Day, 1974) in Australia and the $\underline{Pg}_3$ gene in oats to P. graminis avenae in Canada (Martens et al., 1970).

1.2.2.b Discussion of the reasons for high frequencies of pre-existing virulence to $\underline{R}_4$

In north west Europe P. infestans has been present since about 1845, (Reddick, 1928, 1939; Bourke, 1964). Thus P. infestans has been evolving on potatoes in Europe for about 137 years. The possible explanations for such high values of pre-existing virulence relate to :-

1. The relative fitness of the unnecessary virulence gene compared to avirulence in the period 1845 - 1982 in north west European conditions.
2. The nature of the inoculum of P. infestans that initiated the first disease in Europe in 1845.

Regarding the first point either :-

- a. the virulence gene $\underline{V}_4$ is selectively advantageous;
- b. or it is selectively disadvantageous;
- c. or it is selectively neutral, in comparison with the avirulence allele, $\underline{A}_4$, in the absence of the host gene, $\underline{R}_4$.

Regarding the second point either :-

- A. the frequency of $\underline{V}_4$ ($\underline{q}$) was zero, that is all the initial inoculum carried the avirulence allele, $\underline{A}_4$;
- B. or there was present in the initial inoculum a certain unknown frequency of $\underline{V}_4$.

The gene frequencies today depend on a number of factors such as the initial gene frequencies, the forces of selection acting on the two alleles $\underline{A}_4$ and $\underline{V}_4$, random genetic drift, migration effects and the mutation rates affecting $\underline{V}_4$ and $\underline{A}_4$. Sampling error further will distort a true measure of the gene frequency. For instance the number of samples used in these race surveys may have been quite small, e.g. the value of $\underline{q}$ for Scotland was based on just 20 samples of blight (Black, 1957). However, in all the north west European countries cited by Van der Plank (1975) the values of $\underline{q}$ are very high suggesting strongly that sampling errors only result in imprecision in the estimation of $\underline{q}$ but not its general size.

If $\underline{V}_4$ is assumed to be neutral (1c) then assumption 2B concerning the presence of $\underline{V}_4$ in the initial inoculum must hold; assumptions 1c and 2A cannot stand together. The reason concerns the forces that change the gene frequency, and the speed of these changes. Let the frequency of virulence to $\underline{V}_4$, $\underline{q}$ in 1845 be zero (assumption 2A) and suppose $\underline{A}_4$ and $\underline{V}_4$ are selectively neutral on hosts without the $\underline{R}_4$ resistance (assumption 1c). Then considering the Northern Ireland data, then in 1954 when Doling (1957) sampled the population for virulence to $\underline{R}_4$ (this was pre-existing virulence as Van der Plank (1975) reported that prior to 1954 in Northern Ireland the $\underline{R}_4$ gene had not been used in any commercial potato cultivar), $\underline{q}$ was 0.96. The change in $\underline{q}$ from 0.00 to 0.96 in 109 years (1845 - 1954) could only have occurred by differential rates of forward and backward

mutation, if $\underline{A}_4$ and $\underline{V}_4$ are neutral genes. Drift effects are surely negligible because the P. infestans populations are very large in Ireland.

We can predict the expected frequency of $\underline{q}$ in 1957 on these assumptions using Li's (1955) formula, which calculates the number of generations, $\underline{t}$ required to change a neutral gene from an initial frequency $\underline{q}_0$ to $\underline{q}_{\underline{t}}$ by mutation alone.

$$\underline{t} = \frac{1}{\underline{\mu}_a + \underline{\mu}_v} \cdot \log_e \left(\frac{\underline{q}_0 - \hat{\underline{q}}}{\underline{q}_{\underline{t}} - \hat{\underline{q}}} \right) \quad \ddagger \quad 1.2.2.1$$

where

$\underline{\mu}_a$ = forward mutation rate, $\underline{A}_4$ to $\underline{V}_4$

$\underline{\mu}_v$ = backward mutation rate, $\underline{V}_4$ to $\underline{A}_4$

$$\hat{\underline{q}} = \underline{\mu}_a / (\underline{\mu}_a + \underline{\mu}_v)$$

Using the Northern Ireland data, assume :

$\underline{t} = 13$ generations per year. Van der Plank (1968) assumed 1000 generations in 80 years; this is approximately 13 generations per year. In 109 years, $\underline{t} = 1417$ generations.

$$\underline{\mu}_a = 1 \times 10^{-6}$$

$$\underline{\mu}_v = 1 \times 10^{-8}$$

These rates are estimates based on Esser and Kuenen (1967) who cited forward mutation rates in fungi at $10^{-7} - 10^{-6}$ and backward mutation rates of $10^{-9} - 10^{-7}$.

$\ddagger$ Handwritten equations lack symbol underlining

$$\hat{q} = 0.990099$$

$$q_0 = 0.00$$

$$q_t = 0.96$$

Using equation 1.2.2.1 we can estimate an expected value of q_t , ξq_t after 1417 generations assuming the changes in q resulted from mutation alone.

$$1417 = \frac{1}{1.01 \times 10^{-6}} \cdot \log_e \left(\frac{0.990099}{\xi q_t - 0.990099} \right)$$

$$\xi q_t = 1.416 \times 10^{-3}$$

The real value of q_t was 0.96. Consequently the change in q from 0.00 to 0.96 is too great to be accounted for by mutation rates alone. Even if μ_a is increased to 1×10^{-5} , the expected value of q in 1954 would still only be 0.014. It can be concluded that either :

- 1) the gene V_4 is neutral and some V_4 was present in the introduced inoculum in 1845 or
- 2) there was no V_4 in the initial inoculum and the gene is not neutral in the absence of R_4 .

It can be shown using equation 1.2.2.1 that the number of generations, t , required to change the frequency of a neutral gene from $q_0 = 0.00$ to $q_t = 0.96$ is 3,458,725 assuming the same values of μ_a and μ_v . At 13 generations per year this change would take over 266,000 years. If μ_a is 1×10^{-5} , t is reduced to 323,992 generations taking a mere 24,900 years. Clearly these time periods are far in excess of the 109 years available.

If V_4 is neutral and its frequency increased to 0.96 from a q_0 value in 1845, in 109 years then q_0 can be estimated using equation

1.2.2.1. If $\mu_a = 1 \times 10^{-6}$, $q_0 = 0.95999$, if $\mu_a = 1 \times 10^{-5}$, $q_0 = 0.95954$. These values are only marginally smaller than q_t . These calculations strongly suggest that if V_4 is neutral in the absence of R_4 then the introduced frequency of V_4 must have been very high. Mutation pressure is simply too slow a force to produce large changes in gene frequencies in short time periods.

An alternative explanation for the observed high pre-existing virulence frequency to R_4 is that the unnecessary virulence gene, V_4 is selectively advantageous over the A_4 allele. Thus in 1845, q_0 could have been zero and it increased to its 1954 value by selection primarily. Alternatively, the value of q_0 could have been greater than zero and still be selectively advantageous, although the greater the value of q_0 assumed the lower the selective coefficient (s) required to raise q_0 to its 1954 value of 0.96. A mathematical model (UNA) was written to describe the changes in an advantageous virulence gene under mutation and selection.

The model UNA

Allele	:	A_4	V_4
Frequency	:	p_0	q_0
Average fitness	:	1	$1 + s$

$p_0 + q_0$, and generally $p_t + q_t$ sum to one, and it is assumed that the alleles A_4 and V_4 in the population occur randomly with the background genes making no differential contribution to fitness. This point is discussed in more detail in sections 1.3.6.a, 1.3.6.bi and 3.4.1.a.

In the next generation :

$$q_1 = \frac{q_0 \cdot (1+s) - q_0 \cdot \mu_v + p_0 \cdot \mu_a}{1 + s \cdot q_0} \quad 1.2.2.2$$

$$p_1 = 1 - q_1 = \frac{p_0 - p_0 \cdot \mu_a + q_0 \cdot \mu_v}{1 + s \cdot q_0} \quad 1.2.2.3$$

The denominator term is the sum of the two numerator terms and is a necessary correction factor to ensure that p_1 and q_1 sum to one. The formulae 1.2.2.2 and 1.2.2.3 can be used to calculate $q_2, q_3 \dots q_t$ given suitable values for the parameters.

The value of s required to change $q_0 = 0.00$ to $q_t = 0.96$ can be estimated using the model by empirical re-iteration of the above formulae, 1.2.2.2 and 1.2.2.3, 1417 times, and finding the value of s which simulates exactly the increase in q from q_0 to q_t . A computer programme of the model UNA was written to carry out the simulations and to estimate s . This programme is listed in Appendix 1. The maximum possible value of s was determined by setting q_0 to 0.00, μ_a to 1×10^{-6} and μ_v to 1×10^{-8} as above. In a second simulation, μ_a was set to 1×10^{-5} with all other parameter values unchanged.

When μ_a was set at 1×10^{-6} , s was 0.0087 and when μ_a was 1×10^{-5} , s was 0.0069. These are the approximate maximum selective advantages that the V_4 allele could have over the A_4 allele in the absence of R_4 hosts.

The possibility that the V_4 allele was selectively advantageous is interesting, since unnecessary virulence is normally considered to be neutral or disadvantageous. Black (1957) has summarized some experimental evidence that in certain countries such as Scotland, Holland and Canada in the 1950s a decline in the frequency of race 0 of *P. infestans* occurred, whilst race 4 (containing V_4) increased in prevalence. These observations could be due to the selective advantage of the V_4 allele. Black (1957) noted the fairly rapid replacement of race 0 by race 4 as the common biotype in commercial crops, almost simultaneously in several

countries but he was uncertain why the selection for race 4 occurred. However it was clear that the changes were not due to the selective influence of the R_4 resistance gene.

A third possible explanation for high values of pre-existing virulence frequencies to the R_4 gene in north west European populations of *P. infestans* is that the V_4 allele was selectively disadvantageous in the absence of R_4 and that from 1845 - 1957 its frequency declined from an even higher value at introduction.

In order to identify the most likely selective state of the V_4 allele in north west Europe in the absence of R_4 , it is necessary to consider the likely nature of the initial inoculum of *P. infestans* introduced into this area. Bourke (1964) has summarized the evidence as to the possible countries of origin of the pathogen. There are three possible locations : 1) North America - especially the east coast of the U.S.A. 2) Mexico and 3) South America. Bourke (1964) favoured the latter due to the trading links with north west Europe, which may have brought diseased tubers. However the evidence was incomplete and hence unconvincing especially as there is no reliable record of blight in South America in the 1840s or earlier (Bourke, 1964).

Van der Plank (1975) cites the frequencies of virulence to R_4 (q) in the New World countries as $q = 0.63$ in the U.S.A., $q = 0.57$ in Canada, $q = 0.38$ in Mexico and q as small in Columbia. All these values are typically lower than those for north west Europe, and if the frequency of q_0 in this area in the 1840s was similar to the moderately low values of q for Central and South America more recently than it is likely that unnecessary virulence to R_4 may have been selectively advantageous in Europe. However one must assume that the value of q in Central and South

America was moderately low in the 1840s, and that the frequency of V_4 in the inoculum entering north west Europe was similar. This latter assumption is only likely if a random sample of the population constituted the initial European inoculum.

Bourke (1964) described the spread of blight in north west Europe in 1845. The blight epidemic began in the Courtrai area of Belgium and spread in all directions from this area. The spread of blight in 1845 was completely atypical of the annual course of blight in Europe, where normally blight appears in the west, striking Ireland two months earlier than it did in 1845, and moving in an east or north easterly direction. This typical cycle became established in 1846. The evidence therefore points strongly to the arrival of blight shortly before the epidemic in 1845. Further the number of blight introductions must have been small and restricted to the region around the Courtrai district of Belgium, if all the blight in 1845 originated from this area.

However there is some evidence that blight was already present in parts of Europe in 1844 when the summer was mainly dry. The areas for which there are plausible reports of blight are Belgium, the Lille district of France, Kent and Sussex in England and Dumfriesshire in Scotland.

In addition claims from South Africa, Poland and Portugal stated that the only crops in which blight appeared in 1845 were grown from British seed potatoes of the 1844 crop implying blight was present in Britain prior to 1845.

The orderly progression of blight in 1845 had a few "hiccups" in its distribution. For instance in Great Britain blight first appeared in the Isle of Wight, off the south coast and spread north into England and Scotland. However some reports in 1845 indicated that blight appeared in Cheshire and Dumfriesshire some weeks earlier than an orderly advance

would indicate. This suggests that blight could have already been present in these areas prior to 1845.

The bulk of the evidence therefore suggests multiple introductions of the pathogens to Europe were likely. Consequently a random sample of P. infestans may have been introduced, bringing a moderately low frequency of virulence to R₄. Thus positive selection for the V₄ allele would be necessary to explain the high frequencies of virulence in Europe. The conclusion therefore of this section is that the unnecessary virulence gene, V₄ has been selectively advantageous in populations of P. infestans in Europe.

1.2.3 Pre-existing virulence to the R₁ gene

1.2.3.a Proof of selection against virulence

Van der Plank (1968, 1975) has shown that in contrast to virulence to R₄, pre-existing virulence to R₁ was at a low level both in Europe and in North America. As a consequence, and also in contrast to R₄, the R₁ gene has had much commercial use. The evidence for low frequencies^{of} pre-existing virulence to R₁ stems from the absence or rarity of blight on R₁ hosts during the years taken to develop the cultivars and the following 2 - 3 years of successful commercial cultivation (Thurston, 1979). This pattern is typical of the history of the use of single gene resistance in many other crops. Van der Plank has argued that such delays in the appearance of virulent races indicate their initial scarcity. The duration of these in the so called 'boom and bust cycle' (see Robinson, 1976 and Priestley, 1979 for a description) has varied from crop to crop and from gene to gene, and ^{is} determined in part by the popularity of cultivars with the resistance genes (Van der Plank, 1968). The majority of commercial cultivars with major resistance genes had some period of

'boom' before virulent races appeared and dominated with a consequent 'breakdown in resistance'. The occurrence of a 'boom' period is reasonable circumstantial evidence for a low frequency of pre-existing virulence.

Van der Plank (1968) concluded that the reason for ^{the} low pre-existing virulence frequency to $\underline{R}_1$ was that unnecessary virulence ($\underline{V}_1$) was selectively disadvantageous compared with the avirulence allele ($\underline{A}_1$). Hence despite the generation of $\underline{V}_1$ genes from $\underline{A}_1$ genes by forward mutation, stabilizing selection is of sufficient magnitude to oppose an increase in its frequency and keep it rare. The validity of this conclusion will now be discussed in detail.

Those who oppose the concept of stabilizing selection must believe that unnecessary virulence is as fit on average as avirulence. This means that $\underline{A}_1$ and $\underline{V}_1$ are neutral genes in the absence of the selective host genotype. Assuming the neutral gene hypothesis (NGH) the forces available to change gene frequencies in pathogen populations are random genetic drift, forward and backward mutation and migration. For most large isolated pathogen populations mutation is probably the most important. On this basis we can predict the gene frequencies of the $\underline{A}_1$ and $\underline{V}_1$ alleles at balance using a simple model :

Allele	:	$\underline{A}_1$	$\underline{V}_1$
Frequency	:	$\underline{p}_0$	$\underline{q}_0$
Average fitness	:	1	1

As above $\underline{p}_0 + \underline{q}_0$ and generally $\underline{p}_t + \underline{q}_t$ sum to one.

In the next generation,

$$\underline{p}_1 = \underline{p}_0 - \underline{p}_0 \cdot \mu_a + \underline{q}_0 \cdot \mu_v \quad 1.2.3.1$$

$$\underline{q}_1 = \underline{q}_0 + \underline{p}_0 \cdot \mu_a - \underline{q}_0 \cdot \mu_v \quad 1.2.3.2$$

where $\underline{p}_0, \underline{p}_1, \underline{q}_0, \underline{q}_1, \underline{\mu}_a, \underline{\mu}_v$ are defined as above (1.2.2).

At balance, on the NGH $\underline{p}_1 = \underline{p}_0$. Call this balance value $\underline{p}$. Therefore,

$$\underline{p} = \underline{p} - \underline{p} \cdot \underline{\mu}_a + \underline{q} \cdot \underline{\mu}_v$$

it follows,

$$\underline{p} \cdot \underline{\mu}_a = \underline{q} \cdot \underline{\mu}_v$$

$$\underline{q} = \frac{\underline{p} \cdot \underline{\mu}_a}{\underline{\mu}_v}$$

If $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$ then

$$\underline{q} = 100 \underline{p}$$

Since $\underline{p} + \underline{q} = 1$, it follows

$$\underline{p} = \frac{1}{101}, \quad \underline{q} = \frac{100}{101} = 0.990099 \text{ at balance.}$$

The time to reach balance depends on the initial gene frequencies, but it will be long since changes in frequency due to mutation occur slowly.

The time to balance can be estimated using equation 1.2.2.1 :

$$t = \frac{1}{\underline{\mu}_a + \underline{\mu}_v} \cdot \log_e \left(\frac{\underline{q}_0 - \hat{\underline{q}}}{\underline{q}_t - \hat{\underline{q}}} \right) \quad 1.2.2.1$$

However, since $\underline{q}_t = \hat{\underline{q}}$ the formula cannot be used exactly. Instead an estimate of $\underline{q}_t$ must be used, $\hat{\underline{q}}_t$. Using equation 1.2.2.1 and $\hat{\underline{q}}_t = 0.9$ the time ($\underline{t}$) required to change $\underline{q}_0 = 0.0$ to $\hat{\underline{q}}_t = 0.9$ by mutation alone is approximately 2,373,000 generations if $\underline{\mu}_a$ is 1×10^{-6} and $\underline{\mu}_v$ is 1×10^{-8} . This simulation can be used to support the NGH for the unnecessary virulence gene, $\underline{V}_1$ in the following way. It takes a very long time for values of $\underline{q}$ to change by mutation alone and to attain balance. Therefore if $\underline{V}_1$ was pre-existing at a very low frequency then it could be explained on the basis that the initial frequency of $\underline{V}_1$ in 1845 was low, and it has stayed low although increasing slightly due to mutation up to the mid 1920s (about 1925) when the $\underline{R}_1$ gene was first exploited by European potato breeders (Van der Plank, 1968). The first commercial cultivars

with $\underline{R}_1$ were probably produced in the late 1920s and early 1930s (Van der Plank, 1968). Assuming $\underline{q}_0$ was zero in 1845, then in 1925 after about 1000 generations (Van der Plank, 1968, p. 57) $\underline{q}_t$ would be 0.001, from equation 1.2.2.1, (Li, 1955), assuming $\underline{\mu}_a = 1 \times 10^{-6}$ and $\underline{\mu}_v = 1 \times 10^{-8}$, and an NGH. This value of $\underline{q}_t$ is very low, and Van der Plank (1968) evidently was unaware that low pre-existing virulences to the $\underline{R}_1$ gene in north west Europe (or North America) was explicable on a NGH, as opposed to a stabilizing selection hypothesis (SSH). Hence Van der Plank's (1968) evidence for selection against $\underline{V}_1$ in Mexico is sound (section 1.1.4.b) but not strictly so in north west Europe because of the much shorter time that P. infestans has been present there.

This alternative hypothesis about the selective state of the unnecessary virulence gene $\underline{V}_1$ from 1845 - 1925, assumes that the frequency of this virulence was very low in 1845 and hence also low in 1925. Van der Plank's (1968) stabilizing selection hypothesis also predicts the value of $\underline{q}$ to be low in 1925, its magnitude depending upon the initial value of $\underline{q}$ in 1845, the mutation rate to virulence and the size of the selective coefficient against unnecessary virulence, $\underline{s}$. Assume $\underline{q}_0$ in 1845 was zero, and $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{t} = 1000$ generations (as for the NGH calculations above) and $\underline{s} = 0.05$ (where the average fitness of the avirulence gene $\underline{A}_1$ is 1, the average fitness of $\underline{V}_1$ is $1 - \underline{s} = 0.95$). This value of $\underline{s}$ is similar to values calculated by Grant and Archer (1983), from examples of selection against unnecessary virulence genes in E. graminis hordei and P. graminis tritici, where declining virulence gene frequencies were observed from field data (see also section 1.3.6.h).

Using a mathematical model of declining virulence gene frequencies called DAVE 1 (see section 1.3.2), and the values given above for the parameters $\underline{q}_0$, $\underline{\mu}_v$, $\underline{\mu}_a$, $\underline{t}$ and $\underline{s}$ it can be shown that after 1000

generations q rises from zero (in 1845) to 2×10^{-5} (in 1925) under mutation and selection.

This means that if stabilizing selection was operating against V_1 in P. infestans populations prior to the use of R_1 then the value of q in 1925 would have been lower than predicted by the NGH, i.e. 0.00002 compared with 0.001 respectively.

In order to distinguish the NGH from the SSH we must examine the facts concerning the initial delays in the appearance and build up of races virulent on R_1 hosts when they were first introduced. Van der Plank (1968) argued that since it took so long for the virulent races to appear, i.e. seven years from 1925 - 1932, in Germany before Schick (1932) first isolated a new race of P. infestans from an R_1 potato cultivar, stabilizing selection must have been operating against unnecessary virulence. The delay was considerable especially considering that there were a series of blight years between 1927 - 1932 and that careful searching for blight on the resistant cultivars took place (Van der Plank, 1968). The delay is even more remarkable when it is considered that hybrids of S. tuberosum and S. demissum had been bred prior to 1925 when field trials in Germany with Müller's W varieties (probably hybrids between S. tuberosum and S. demissum possessing a single dominant gene for resistance, now called R_1 (Stevenson, Schultz, Akeley and Cash, 1952; Van der Plank, 1968)) began, following the initiative of British workers. Thus Salaman (1970) reported that in 1910 he had a family of seedlings of S. demissum (the source of R_1) in his trial grounds which were unaffected by blight. In 1911 he began to hybridize this species with S. tuberosum, and by 1914 he had a series of such families. By 1926 he possessed over a score of seedling varieties endowed with reasonably good economic characters which at all states of maturity

appeared to be immune. Some of these seedlings were sent to Ireland where they grew for over 30 years in the open, retaining a high degree of resistance to attack.

As in Germany, however, a new race virulent towards R_1 was discovered in England in 1932 (O'Connor, 1933). In 1936 the attack was more serious although it occurred a month later than that affecting neighbouring field crops at Cambridge.

In Germany breeding experiments along similar lines began in 1914 at Dahlem, and were greatly intensified in 1920 by Müller. It is remarkable that it took so long before a race virulent on R_1 was discovered despite the intensive searching.

The failure is even more remarkable when it is considered that extensive tests on R_1 hosts were done in research stations and breeder's nurseries with artificial as well as natural inoculation. Thus Katterman and Wenk (1933) described the testing done at the Bavarian Seed Breeding Institute at Weihestephan near Munich. The work began in 1927, and from 1930 - 1932 large scale artificial inoculations were conducted involving 5000 seedlings in 1931 and over 20,000 in 1932. Even under the exacting conditions of these tests, the resistant hybrid strains (which had been received from Berlin) were giving a large percentage of highly resistant seedlings in which even though infection spots developed in the autumn, there was no spread of the fungus in the tissues and no sporangia were produced. Since there was no sporulation, these infections were probably the infections of the A biotype of *P. infestans* described by Müller (1933a), the old race attacking the standard but not the W (R_1) strains. Müller (1929, 1931) had earlier mentioned that even the most resistant individuals of his W strains were attacked later in the growing season (presumably by the A biotype).

Katterman and Wenk (1933) described the appearance of a new race called the S race (Müller, 1933b) at the end of August 1932 which was highly virulent on all resistant ($\underline{R}_1$) strains and their progeny. This destructive form of blight probably came from some neighbouring susceptible (non $\underline{R}_1$) cultivars which had earlier been attacked by blight.

These studies indicate the frequency of virulence to $\underline{V}_1$ in $\underline{P}$. infestans to have been very low, in the artificial inoculum used by Katterman and Wenk (1933).

Müller's technique to test for blight resistance (Salaman, 1970) was to expose individually potted potato seedlings to artificial infection in a glass chamber. Artificial inoculation would then presumably have been to spray a suspension of sporangia or zoospores over the plants (inferred from the work of Salaman (1970) whose own testing involved the use of Müller's method; he sprayed a spore suspension onto the plants). Assuming in Katterman and Wenk's (1933) experiments that each plant was sprayed with 5 ml of a spore suspension at a concentration of 50,000 propagules per ml, then a total of 5×10^9 spores would have been sprayed onto the 20,000 seedlings. On the NGH the number of spores expected to carry virulence to $\underline{R}_1$ is $0.001 \times 5 \times 10^9 = 5,000,000$. Assuming only 1% of the zoospores successfully penetrate and produce a sporulating lesion then 50,000 lesions are expected on these 20,000 seedlings. At this frequency, blight would have been observed on the $\underline{R}_1$ plants prior to the end of August 1932. However on the SSH, where $\underline{q} = 2 \times 10^{-5}$ then only $2 \times 10^{-5} \times 5 \times 10^9 \times 0.01 = 1000$ lesions are expected. Such a number is more consistent with Katterman and Wenk's (1933) observations, concerning the absence of the virulent race prior to the end of August 1932.

The numerical details are disputable, but the scarcity of the virulent races until late August 1932 is much more easily explained by

the SSH rather than the NGH. Furthermore, under the SSH the value of q could potentially have been lower than 2×10^{-5} if s was greater than 0.05.

An additional numerical clue is the observations of Salaman noted above that in 1936 blight appeared on the seedlings growing in the open about one month later than on the neighbouring field crops which were susceptible to the old races to which the resistant seedlings were immune.

Van der Plank (1963) devised an equation relating the delay expected in the appearance of disease on resistant hosts in relation to the proportion of the incoming inoculum with virulence :

$$D_t = \frac{1}{r} \log_e \frac{x_o}{x_{ov}} \quad 1.2.3.3$$

where

D_t is the delay in appearance of disease on the resistant hosts,

r is the apparent infection rate,

x_o is the proportion of the inoculum which can infect the susceptible hosts (avirulent and virulent races),

x_{ov} is the proportion of the inoculum which can infect the resistant hosts.

D_t was a month, say 30 days. The value of r for blight in 1936 was assumed to be 0.4. This is high; however 1936 was a severe blight year (R.W. Polley, personal communication); in many regions being the worst since 1916. In Cambridge the tubers were infected as early as August 17th, indicating that r was very high. Van der Plank (1963) lists possible values of r for *P. infestans* determined by experiment ranging from 0.23 - 0.46. A value of 0.4 is therefore reasonable. The value of x_o is one, whilst x_{ov} is the unknown to be calculated. Therefore,

$$30 = 2.5 \log_e \frac{1}{x_{ov}}$$

$$= \frac{x_{oy}}{y} = 6.14 \times 10^{-6}.$$

This value is approximately the frequency of virulence to $\underline{R}_1$ at the beginning of the first spore showers on the immune seedlings in the Cambridge trials in 1936. This value is much lower than the minimum value calculated on the NGH of 0.001. Hence again the evidence strongly supports the SSH, as this is the only hypothesis which can give a realistically low value of $\underline{q}$ for 1932.

Thus it is concluded that even if it is assumed that the frequency of virulence to $\underline{R}_1$ was zero when blight was introduced into Europe in the 1840s (and there is no certainty of this) the gene frequency in the early 1930s expected on the NGH would not be low enough to account for the delays in the appearance of blight on the seedlings with the $\underline{R}_1$ gene. In order to account for the delays one must conclude that selection was operating against unnecessary virulence by reducing the frequency of $\underline{V}_1$ to very low levels.

1.2.3. b Calculation of the selective coefficient against $\underline{V}_1$

Accepting that unnecessary virulence to the $\underline{R}_1$ resistance gene was selectively disadvantageous it is possible to estimate the minimum value of $\underline{s}$ which would account for the low pre-existing virulence frequency. In 1936 in Cambridge the frequency of virulence to $\underline{R}_1$ at the start of the season was estimated as approximately 6.14×10^{-6} . Either the frequency of $\underline{V}_1$ was declining from its initial frequency in 1845 or alternatively its frequency could have been increasing by mutation from an initial value of zero or very close to zero. Whether decreasing or increasing its frequency would have continued to change in the absence of $\underline{R}_1$ hosts until the balance frequency was obtained. This balance frequency, $\underline{q}_b$ is $\mu a / s$ (section 1.3.2, equation 1.3.2.11), the classical

mutation, selection balance. If the introduced frequency of $\underline{v}_1$ ($\underline{q}_0$) in 1845 happened to be higher than $\underline{q}_b$, then $\underline{q}$ would have declined from 1845 - 1925. If however the value of $\underline{q}_0$ was less than $\underline{q}_b$ then $\underline{q}$ would have risen towards the $\underline{q}_b$ value. The former possibility is more likely, because the latter requires an extremely low value of $\underline{q}_0$. Assuming that $\underline{q}$ was declining therefore, then 6.14×10^{-6} was either the balance value or greater than the balance value, $\underline{q}_b$. If 6.14×10^{-6} is $\underline{q}_b$ then :

$$\underline{s} = \underline{\mu a} / 6.14 \times 10^{-6}$$

A range of $\underline{s}$ values is listed for a range of possible values of $\underline{\mu a}$, in Table 2.

Table 2 Values of the selection coefficients ($\underline{s}$) against unnecessary virulence given various mutation rates to virulence ($\underline{\mu a}$) and a constant balance frequency of $\underline{q}_b = 6.14 \times 10^{-6}$

$\underline{\mu a}$	$\underline{s}^1$
1×10^{-7}	0.016
3×10^{-7}	0.049
6×10^{-7}	0.098
9×10^{-7}	0.147
1×10^{-6}	0.163
5×10^{-6}	0.814

¹ Calculated from the equation $\underline{s} = \underline{\mu a} / \underline{q}_b$

The latter entry of $\underline{s} = 0.814$ is probably unrealistic (section 1.3.6.h). A measure of the mutation rate is not known but a range of

$1 \times 10^{-7} - 1 \times 10^{-6}$ is reasonable (Esser and Kuenen, 1967). The values of $\underline{s}$ in Table 2 are minima since $\underline{q}_b$ may be less than 6.14×10^{-6} . Since $\underline{s} = \underline{\mu}_a / \underline{q}_b$, the lower the value of $\underline{q}_b$, the greater the value of $\underline{s}$ for any given value of $\underline{\mu}_a$.

If on the other hand the value of $\underline{q}$ was increasing in 1936 from a very small value of $\underline{q}_0$ in 1845, then the maximum value of $\underline{s}$ would be that value that allows mutation to increase $\underline{q}$ from zero to 6.14×10^{-6} in 91 years (1845 - 1936) that is approximately 1183 generations (91×13). Using the model DAVE 1 (see section 1.3.2), and setting $\underline{\mu}_a$ to 1×10^{-6} , an almost identical value of $\underline{s}$ of 0.163 is obtained as in Table 2. The reason is that even for the smallest values of $\underline{s}$, $\underline{q}$ would have increased to a value greater than 6.14×10^{-6} by mutation pressure in much less than 1183 generations. For instance if $\underline{s} = 0.001$, $\underline{q}$ increases to a value greater than $\underline{q}_b = 6.14 \times 10^{-6}$ after 8 generations, if $\underline{q}_0$ is zero. Thus if $\underline{q}$ is as low as 6.14×10^{-6} after 1183 generations, it must be a balance value and hence $\underline{s}$ is the same as above, $\underline{\mu}_a / \underline{q}_b = 0.163$.

1.2.4 Pre-existing virulence in other pathogens with special reference to *Erysiphe graminis hordei* in Great Britain

In the studies on *P. infestans* in north west Europe there has been a limited period for pathogen evolution on its host. With other pathogens the time periods are larger. For example, barley has been grown widely in Great Britain for several thousand years since Neolithic times (Godwin, 1975). The mildew pathogen *E. graminis hordei* was either introduced at or soon after the time of initial cultivation or was present beforehand, growing on wild hosts. It is not unreasonable therefore to suppose the pathogen has been present for 2000 years in G.B., although there is little evidence to support or refute this hypothesis.

Prior to the intensive use this century of cultivars with specific single resistance genes to rusts and mildews, selection for virulence to these genes in the pathogen would be expected to be minimal or zero. The reason for this is that many of the resistance genes have been obtained from wild relatives from exotic sources, such as the Middle East, (the geographical centre of origin of wheat and barley). These wild barleys, e.g. Hordeum spontaneum, from which the Mla₆ gene for resistance to E. graminis hordei has been obtained (Wolfe and Schwarzbach, 1978a) are generally absent from Britain (Clapham, Tutin and Warburg, 1962). In addition the resistance genes Mlv, Mlas, Mla_{4/7}, Mla₉ and Mlar have been derived from exotic sources (Wolfe and Schwarzbach, 1978a). Some resistance genes however have been derived from European land cultivars. For instance Mlg was obtained from a German cultivar called Pflugs Intensiv and the Mlh gene from Ragusa B from Moravia (Wolfe and Schwarzbach, 1978a).

The cultivar Pflugs Intensiv was officially registered in Germany as an original cultivar in 1921 and although the percentage of the acreage on which it was grown was not known, it did not become widely used since it was only one of more than 50 which were officially recognized (Wolfe and Schwarzbach, 1978a). After the second world war, cultivars with Mlg derived from Pflugs Intensiv became widely used, reaching 10% of the acreage by 1948 and 70% by 1960. Although races with virulence to this gene were found as early as 1931, it was only in 1960 that Mlg cultivars were considered to be fully susceptible (Wolfe and Schwarzbach, 1978a). This means that virulence to Mlg must have been very rare at the time that Pflugs Intensiv was recognized as a potential source of mildew resistance, this probably being due to the rarity of the cultivar, preventing significant selection for virulence. For other Ml genes,

derived from exotic sources, selection (if any) for the corresponding V genes would have probably been even less, although it is not impossible that some of these resistance genes were present in wild hosts.

Effectively therefore virulence genes in E. graminis hordei to resistance genes in barley were unnecessary prior to the deliberate introduction of the latter into barley from their wild exotic relatives or indeed those from the European land cultivars. At this stage the frequency of these unnecessary virulences is likely to have been at or near their balance frequencies (q_b) assuming the operation of mutation and stabilizing selection. This means that q_b equalled or approximately equalled μa/s. This suggestion can be shown to be feasible if the approximate number of generations required to reach q_b is calculated. The time (t) in generations to change a virulence gene frequency from an initial value, q₀ to another value q by mutation and selection can be calculated from an equation :

$$t = 0.5 \log_e \left(\frac{B^2 - Q_0^2}{B^2 - Q^2} \right) + \frac{1 - s\bar{q}}{2sB} \log_e \left(\frac{B + Q_0}{B - Q_0} \cdot \frac{B - Q}{B + Q} \right) \quad 1.2.4.1$$

where

$$\bar{q} = \frac{s + \mu a + \mu v}{2s}$$

$$B^2 = \bar{q}^2 - (\mu a/s)$$

$$Q_0 = q_0 - \bar{q} \quad 1.2.4.2$$

$$Q = q - \bar{q} \quad 1.2.4.3$$

The equation is derived from the model DAVE 1 (section 1.3.2), and its derivation is shown in Appendix 2.

For E. graminis hordei, a forward mutation (A to V) rate, μ_a of 1×10^{-6} and a backward mutation (V to A) rate, μ_v of 1×10^{-8} are assumed. Consequently,

$$\underline{q_b} = \mu_a / \underline{s} = 1 \times 10^{-6} / \underline{s}$$

A range of $\underline{s}$ values from 0.1 - 0.0001 was chosen for the simulations in order to estimate the time taken to reach balance, $\underline{q_b}$ starting from a $\underline{q_o}$ value of either 0 or 1.

Calculations were performed using a computer programme called BALQ which calculates $\underline{t}$ from equation 1.2.4.1. The programme BALQ is listed in Appendix 3.

If $\underline{q_o}$ is set to 1 the time $\underline{t}$ to reach $\underline{q_b}$ exactly can be calculated (Table 3). However, when $\underline{q_o} = 0$, (equation 1.2.4.1) the time to reach $\underline{q_b}$ exactly, cannot be calculated, because if $\underline{q}$ is set as $\mu_a / \underline{s}$ ($\underline{q_b}$) in equation 1.2.4.3, both of the expressions in the brackets of equation 1.2.4.1 become negative, and the logarithm of a negative number is meaningless. However, various estimates of $\underline{q_b}$, $\hat{\underline{q_b}}$ could be used which give meaningful results. For instance when $\underline{s} = 0.1$, $\underline{q_b} = \mu_a / \underline{s} = 1 \times 10^{-6} / 0.1 = 1 \times 10^{-5}$; $\hat{\underline{q_b}}$ was taken as 0.999999×10^{-5} which is similar to $\underline{q_b}$. As smaller $\underline{s}$ values were assumed, less accurate values of $\hat{\underline{q_b}}$ had to be used, however none were smaller than $0.99 \underline{q_b}$. Estimates of $\underline{t}$ are given in Table 4.

Table 3 Number of generations, $\underline{t}$ for a virulence gene ($\underline{V}$) frequency ($\underline{q}$) to change from $\underline{q}_0 = 1$ to a balance frequency, $\underline{q}_b$ under mutation¹ and selection ($\underline{s}$)

$\underline{s}$	$\underline{q}_b$ ²	Number of generations, $\underline{t}$ ³
0.1	1×10^{-5}	421
0.01	1×10^{-4}	3,671
0.001	1×10^{-3}	29,948
0.0001	1×10^{-2}	232,147

¹ forward mutation ($\underline{A}$ to $\underline{V}$) rate = $\mu_a = 1 \times 10^{-6}$, backward mutation ($\underline{V}$ to $\underline{A}$) rate, $\mu_v = 1 \times 10^{-8}$,

² $\underline{q}_b = \mu_a / \underline{s}$,

³ calculated from equation 1.2.4.1 using the computer programme BALQ

Table 4 Number of generations, $\underline{t}$, for a virulence gene ($\underline{V}$) frequency ($\underline{q}$) to change from $\underline{q}_0 = 0$ to an approximate balance frequency, $\hat{\underline{q}}_b$ under mutation¹ and selection ($\underline{s}$)

$\underline{s}$	$\hat{\underline{q}}_b$ ²	Number of generations, $\underline{t}$ ³
0.1	0.999999×10^{-5}	139
0.01	0.9999×10^{-4}	922
0.001	0.99×10^{-3}	6924
0.0001	0.99×10^{-2}	46513

- 1 forward mutation (A to V) rate, $\mu_a = 1 \times 10^{-6}$, backward mutation (V to A) rate, $\mu_v = 1 \times 10^{-8}$
- 2 $\hat{q}_b \approx q_b = \mu_a/s$, where $\hat{q}_b = CF (\mu_a/s)$, where CF is a correction factor ≈ 1 .
- 3 calculated from equation 1.2.4.1 using the computer programme, BALQ

Clearly, the values of t are greater for any given value of s , when $q_0 = 1$ than when $q_0 = 0$, since q_b or $\hat{q}_b$ are always much closer to zero than to one. If the virulence allele is indeed the mutant form of the gene then, q_0 is more reasonably zero rather than one. However, in several natural host pathogen ecosystems in locations which are the likely centres of origin of the pathogen, resistance genes abound in wild hosts, and necessary virulence genes are common in many races of the pathogen. Examples are S. demissum and P. infestans in Mexico (Van der Plank, 1968), and Avena sterilis and P. coronata avenae in Israel (Browning, 1974). Consequently, if the European pathogen population originated from these pathogen centres of origin, initially in Europe q_0 would probably take some intermediate value between 0 and 1.

The values of t in Tables 3 and 4 are significant since many of these are within the likely number of generations that say E. graminis hordei has had available for reproduction in the U.K. Assuming 20 generations per year which may be an underestimate (see section 1.3.6.c) and Appendix 7), and that the pathogen has been present for 2000 years we can assume 40,000 generations to have elapsed. Consequently for selective coefficients against unnecessary virulence, greater than 0.001 there has been sufficient time to reach balance frequencies for these virulence genes. Furthermore a very small amount of selection against

unnecessary virulence, $\underline{s} = 0.001$ will result in the predominance of the avirulence gene and the scarcity of the virulence gene since an $\underline{s}$ value of 0.001 although small is large in comparison with $\underline{\mu_a} = 1 \times 10^{-6}$.

In the discussions above, stabilizing selection against unnecessary virulence has been assumed. However it is feasible that this kind of selection did not operate in pathogen populations prior to the use of host resistance genes, in which case an NGH would follow (provided of course the unnecessary virulence gene was not advantageous). If this was true then for E. graminis hordei using Li's(1955) equation (1.2.2.1), assuming $\underline{q}_0 = 0$, $\underline{\mu_a} = 1 \times 10^{-6}$, $\underline{\mu_v} = 1 \times 10^{-8}$ and $\underline{t} = 40,000$ generations it is possible to estimate an approximate minimal value of $\underline{q}_t$ just prior to the introduction of host resistance genes on a mass agricultural scale :

$$t = \frac{1}{\underline{\mu_a} + \underline{\mu_v}} \log_e \left(\frac{\underline{q}_0 - \hat{q}}{\underline{q}_t - \hat{q}} \right) \quad 1.2.2.1$$

$$40,000 = 990099.01 \cdot \log_e \left(\frac{-0.990099}{\underline{q}_t - 0.990099} \right)$$

$$\Rightarrow \underline{q}_t = \frac{-0.990099}{1.0412272} + 0.990099$$

$$= 0.0392$$

This value is the (approximate) minimum value of $\underline{q}_t$ assuming an NGH. Clearly, if $\underline{q}_0$ was greater than zero which is likely considering the pathogen's requirement for virulence at its centre of origin in the Middle East as discussed earlier, then $\underline{q}_t$ would have been much greater than approximately 0.04.

However, even a value of $\underline{q}_t = 0.04$ would be high enough to cause an earlier appearance of virulence in the breeder's trials prior to commercial introduction of corresponding resistance.

For example using equation 1.2.3.3 we can estimate the delay in appearance of virulence :

$$D_t = \frac{1}{r} \cdot \log_e \frac{x_o}{x_{ov}} \quad 1.2.3.3$$

Assuming $r = 0.21$ (Fried, Mackenzie and Nelson 1979, for E. graminis tritici), $x_o = 1$ and $x_{ov} = 0.04$ then

$$D_t = \frac{1}{0.21} \cdot \log_e 25 \approx 15 \text{ days}$$

This means that mildew would be expected to occur on resistant hosts just 15 days after the first appearance on susceptible hosts. This is too short to account for the delay in appearance in virulence on resistant cultivars throughout breeders trials and the first few years of commercial use. With a $\underline{D}_t$ value of 15 days and $\underline{q}_t = 0.04$ virulence would have increased rapidly within the first year of the breeder's trials, and an agricultural 'boom' period with the resistant cultivars would have been impossible. The resistance would not have survived the breeder's trials let alone commercial usage.

However experience has shown that the majority of resistance genes and in commercial crops undergo a 'boom and bust cycle'. In order for a 'boom' to occur there must be a low frequency of pre-existing virulence initially, certainly much lower than 0.04. The 'boom' period usually lasts only for a few years and within 3 - 5 years from the time of their widespread distribution single gene resistant cultivars must be replaced due to the rapid multiplication of virulent races (Agrios, 1978).

Thus for the barley powdery mildew pathosystem the successful use

of a number of M1 genes, each for several years is evidence of a low frequency of pre-existing virulence and the operation of stabilizing selection.

An NGH cannot account for the observed low frequency of pre-existing virulence to most commercially used resistance genes. This should be generally true for other host-parasite pathosystems where low pre-existing virulence frequencies have been found.

This conclusion indicates that stabilizing selection is probably more common than thought by many (Van der Plank, 1975; Crill, 1977), a point discussed later (section 1.3.4.ci).

1.2.5 Conclusions

The major conclusions are :

1. Unnecessary virulence to the R₄ gene of potatoes carried by European populations of P. infestans from 1845 - 1950s may have been selectively advantageous over avirulence.
2. Unnecessary virulence to the R₁ gene in potatoes in Europe from 1845 - 1925 was selectively disadvantageous.
3. In situations where it is unnecessary, virulence to commercially utilized resistance genes is likely to be selectively disadvantageous compared with avirulence.
4. Stabilizing selection is more common than previously thought.

1.3 Models of declining virulence gene frequencies

1.3.1 Introduction

In this section (1.3) models of declining virulence gene frequencies are described and discussed. Their relevance to an understanding of stabilizing selection in pathogen population genetics (especially the changes in virulence gene frequencies), and in their application to calculate the coefficient of stabilizing selection ($\underline{s}$) from field data are important.

1.3.2 DAVE 1

The model DAVE 1 is described also by Grant and Archer (1983), and in common with the others described in this section, includes modelling of selection and mutation, at one locus with two alleles, $\underline{A}$ and $\underline{V}$ defined as avirulence and virulence respectively toward a host resistance gene $\underline{R}$. The frequency of $\underline{A}$ and $\underline{V}$ in the pathogen population at generation $\underline{t}$ are $\underline{p}_{\underline{t}}$, $\underline{q}_{\underline{t}}$ respectively, where $\underline{p}_{\underline{t}} + \underline{q}_{\underline{t}} = 1$. The mutation rate from $\underline{A}$ to $\underline{V}$ is $\underline{\mu}_a$ and from $\underline{V}$ to $\underline{A}$, $\underline{\mu}_v$. $\underline{A}$ is considered the normal dominant allele, and $\underline{V}$ to be the recessive mutant gene. Consequently $\underline{\mu}_a > \underline{\mu}_v$. It is assumed that the alleles $\underline{A}$ and $\underline{V}$ are randomly distributed amongst the various pathogen genotypes. Hence on average, the relative fitnesses of the two alleles on hosts lacking the $\underline{R}$ gene but possessing the allele $\underline{R}_0$ conditioning susceptibility are, assuming the operation of stabilizing selection :

Allele	:	$\underline{A}$	$\underline{V}$
Average fitness	:	1	$1-\underline{s}$

However on hosts carrying the $\underline{R}$ gene the average relative fitnesses of the two alleles are :

Allele	:	$\underline{A}$	$\underline{V}$
Average fitness	:	$1-\underline{z}$	1

where $\underline{z}$ is a coefficient of selection against races lacking necessary virulence to grow on hosts with the $\underline{R}$ gene. Normally, the races with the $\underline{A}$ allele are unable to sporulate on hosts, with the $\underline{R}$ gene and therefore $\underline{z} = 1.0$. The average fitness of the $\underline{A}$ allele becomes zero.

If $\underline{m}$, $\underline{n}$ are the proportions of the two host genotypes, $\underline{R}_0$ and $\underline{R}$ respectively, and $\underline{m} + \underline{n} = 1$, then the overall average relative fitnesses ($\underline{W}$) are :

$$\begin{aligned} W_A &= m \times 1 + n \cdot (1 - z) \\ &= m + n - n \cdot z \\ &= 1 - n \cdot z \end{aligned} \quad 1.3.2.1$$

$$\begin{aligned} W_V &= m \cdot (1 - s) + n \times 1 \\ &= m - m \cdot s + n \\ &= 1 - m \cdot s \end{aligned} \quad 1.3.2.2$$

Consequently in generation 0 :

Allele	:	$\underline{A}$	$\underline{V}$
Average fitness ($\underline{W}$)	:	$(1 - \underline{n} \cdot \underline{z})$	$(1 - \underline{m} \cdot \underline{s})$
Frequency (generation 0)	:	$\underline{p}_0$	$\underline{q}_0$

and in the next generation

$$p_1 = \frac{p_0 \cdot (1 - n \cdot z) - p_0 \cdot \mu_a + q_0 \cdot \mu_v}{1 - p_0 \cdot n \cdot z - q_0 \cdot m \cdot s} \quad 1.3.2.3$$

and

$$\begin{aligned} q_1 &= 1 - p_1 \\ &= \frac{q_0 \cdot (1 - s \cdot m) + p_0 \cdot \mu_a - q_0 \cdot \mu_v}{1 - p_0 \cdot n \cdot z - q_0 \cdot m \cdot s} \end{aligned} \quad 1.3.2.4$$

The denominator term is a necessary division factor which ensures $\underline{p}_1 + \underline{q}_1 = 1$, and is obtained simply as the sum of the two numerator terms :

$$\begin{aligned}
 & p_0 \cdot (1 - n \cdot z) - p_0 \cdot \mu a + q_0 \cdot \mu v + q_0 \cdot (1 - m \cdot s) + p_0 \cdot \mu a - q_0 \cdot \mu v \\
 &= p_0 \cdot (1 - n \cdot z) + q_0 \cdot (1 - m \cdot s) \\
 &= p_0 - p_0 \cdot n \cdot z + q_0 - q_0 \cdot m \cdot s \\
 &= 1 - p_0 \cdot n \cdot z - q_0 \cdot m \cdot s
 \end{aligned}$$

The values of $\underline{p}_2$, $\underline{q}_2$ and $\underline{p}_t$, $\underline{q}_t$ are simply obtained from $\underline{p}_1$, $\underline{q}_1$ and $\underline{p}_{t-1}$, $\underline{q}_{t-1}$, respectively. Generally therefore,

$$\underline{p}_{t+1} = \frac{\underline{p}_t \cdot (1 - n \cdot z) - \underline{p}_t \cdot \mu a + \underline{q}_t \cdot \mu v}{1 - \underline{p}_t \cdot n \cdot z - \underline{q}_t \cdot m \cdot s} \quad 1.3.2.5$$

$$\underline{q}_{t+1} = \frac{\underline{q}_t \cdot (1 - m \cdot s) + \underline{p}_t \cdot \mu a - \underline{q}_t \cdot \mu v}{1 - \underline{p}_t \cdot n \cdot z - \underline{q}_t \cdot m \cdot s} \quad 1.3.2.6$$

If resistant hosts are absent, then $\underline{n} = 0$, $\underline{m} = 1$. This parallels the field situation prior to the commercial use of R gene cultivars when V genes are unnecessary. In these circumstances equations 1.3.2.5 and 1.3.2.6 reduce to

$$\underline{p}_{t+1} = \frac{\underline{p}_t - \underline{p}_t \cdot \mu a + \underline{q}_t \cdot \mu v}{1 - s \cdot \underline{q}_t} \quad 1.3.2.7$$

$$q_{t+1} = \frac{q_t \cdot (1-s) + p_t \cdot \mu_a - q_t \cdot \mu_v}{1 - s \cdot q_t} \quad 1.3.2.8$$

At balance, the gene frequencies are constant; therefore,

$$\begin{aligned} \underline{p}_t + 1 &= \underline{p}_t \\ \underline{q}_t + 1 &= \underline{q}_t \end{aligned}$$

Call these balance values, $\underline{p}$, $\underline{q}$ respectively.

It follows from equation 1.3.2.7, that at balance

$$p = \frac{p - p \cdot \mu_a + q \cdot \mu_v}{1 - s \cdot q}$$

$$p \cdot (1 - s \cdot q) = p - p \cdot \mu_a + q \cdot \mu_v$$

$$p - p \cdot q \cdot s = p - p \cdot \mu_a + q \cdot \mu_v$$

$$p \cdot q \cdot s = p \cdot \mu_a - q \cdot \mu_v$$

$$s = \frac{p \cdot \mu_a}{p \cdot q} - \frac{q \cdot \mu_v}{p \cdot q}$$

$$s = \frac{\mu_a}{q} - \frac{\mu_v}{p} \quad 1.3.2.9$$

At balance $p \approx 1$, $q \approx 0$ and $\underline{p} \gg \underline{q}$, because selective hosts ($\underline{R}$) are absent ($\underline{n} = 0$), and the pathogen population with the $\underline{V}$ allele is on average less fit than the population with the $\underline{A}$ allele on $\underline{R}_0$ hosts. Further $\mu_a > \mu_v$. Consequently, $\mu_a/\underline{q}$ is much larger than $\mu_v/\underline{p}$ and equation 1.3.2.9 reduces to :

$$\underline{s} \approx \mu_a/\underline{q} \quad 1.3.2.10$$

from which the classical mutation-selection balance equation

$$\underline{q} = \underline{\mu a} / \underline{s} \quad 1.3.2.11$$

is derived.

An equation derived from DAVE 1 which calculates the time (t generations) to reach balance is derived in Appendix 2.

1.3.3 DAVE 2

This is a variant of the model DAVE 1. DAVE 1 includes no selection against the mutants. DAVE 2 which is slightly more accurate biologically includes factors for selection against the mutants. Equations 1.3.2.5 and 1.3.2.6 for DAVE 1 become for DAVE 2 :

$$p_{t+1} = \frac{p_t \cdot (1 - n \cdot z) - p_t \cdot \mu a \cdot (1 - n \cdot z) + q_t \cdot \mu v \cdot (1 - m \cdot s)}{1 - p_t \cdot n \cdot z - q_t \cdot m \cdot s} \quad 1.3.2.12$$

$$q_{t+1} = \frac{q_t \cdot (1 - m \cdot s) + p_t \cdot \mu a \cdot (1 - n \cdot z) - q_t \cdot \mu v \cdot (1 - m \cdot s)}{1 - p_t \cdot n \cdot z - q_t \cdot m \cdot s} \quad 1.3.2.13$$

If n = 0, m = 1, i.e. in the absence of resistant hosts then equations 1.3.2.12 and 1.3.2.13 reduce to :

$$p_{t+1} = \frac{p_t - p_t \cdot \mu a + q_t \cdot \mu v \cdot (1 - s)}{1 - q_t \cdot s} \quad 1.3.2.14$$

$$q_{t+1} = \frac{q_t \cdot (1 - s) + p_t \cdot \mu a - q_t \cdot \mu v \cdot (1 - s)}{1 - q_t \cdot s} \quad 1.3.2.15$$

The difference between these latter two equations and the equivalent two for the model DAVE 1, 1.3.2.7 and 1.3.2.8, is that in the model DAVE 2 only there is a $(1-s)$ term reducing $q_{\mu v}$ in the numerator effectively reducing the number of back mutants from $\underline{V}$ to $\underline{A}$ in DAVE 2 compared with DAVE 1 provided that $\underline{s} > 0$.

1.3.4 Computer simulations on DAVE 1 and DAVE 2

Computer programmes of the two models have been written and utilized in simulation experiments. The programme of DAVE 2 is given in Appendix 4.

1.3.4.a DAVE 1 and DAVE 2 compared

The effects of the modification of DAVE 1 on the changes in frequency of the $\underline{A}$ and $\underline{V}$ alleles with time was studied using these programmes. In one simulation the parameter values were set at :

$$\begin{aligned} \underline{p}_0 &= 0.44, \quad \underline{q}_0 = 0.56, \quad \underline{\mu}_a = 1 \times 10^{-6}, \quad \underline{\mu}_v = 3.765 \times 10^{-9}, \\ \underline{m} &= 0.985, \quad \underline{n} = 0.015, \quad \underline{z} = 1.0, \quad \underline{s} = 0.04 \end{aligned}$$

and the frequency of the $\underline{V}$ allele after 105 generations was determined in each case :

Model	:	DAVE 1	DAVE 2
$\underline{q}_{105}$	:	0.085722615	0.085722617

These are very similar. Since DAVE 2 generates fewer back mutants, $\underline{q}$ declined very slightly slower in this model. Only a very slight difference in the $\underline{q}_{105}$ values resulted because $\underline{m} \approx 1$, $\underline{n} \approx 0$. This reduced in DAVE 2 equations 1.3.2.12 and 1.3.2.13, the selection factors against the mutants $(1 - \underline{n} \cdot \underline{z})$ and $(1 - \underline{m} \cdot \underline{s})$ to approximately 1 and $(1 - \underline{s})$ respectively. These are therefore very similar to the coefficients of $\underline{p}_t \cdot \underline{\mu}_a$ and $\underline{q}_t \cdot \underline{\mu}_v$ of DAVE 1 (equations 1.3.2.7 and 1.3.2.8) since $1 - \underline{s} \approx 1$. Further $\underline{t}$, the number of generations was small.

To test for differences resulting from larger values of $\underline{t}$ a second simulation was conducted with the following parameter values :

$\underline{p}_0 = 0.00$, $\underline{q}_0 = 1.00$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$. $\underline{m} = 1$,
 $\underline{n} = 0$, $\underline{z} = 1.0$, $\underline{s} = 0.05$. The programmes were run for 1000 generations. The results are shown in Table 5 :

Table 5 Decline in the frequency of virulence ($\underline{q}$) with time under selection¹ and mutation² using the models DAVE 1 and DAVE 2³

Time, $\underline{t}$ (generations)	Virulence gene frequency $\underline{q}$ Model	
	DAVE 1	DAVE 2
200	0.9946	0.9948
300	0.5221	0.5349
600	0.2022×10^{-4}	0.2023×10^{-4}
1000	0.1999×10^{-4}	0.1999×10^{-4}

¹ selective coefficient against unnecessary virulence, $\underline{s} = 0.05$

² Forward mutation rate, $\underline{\mu}_a = 1 \times 10^{-6}$
Backward mutation rate, $\underline{\mu}_v = 1 \times 10^{-8}$

³ $\underline{m} = 1.0$, $\underline{n} = 0$, $\underline{p}_0 = 0.0$, $\underline{q}_0 = 1.0$

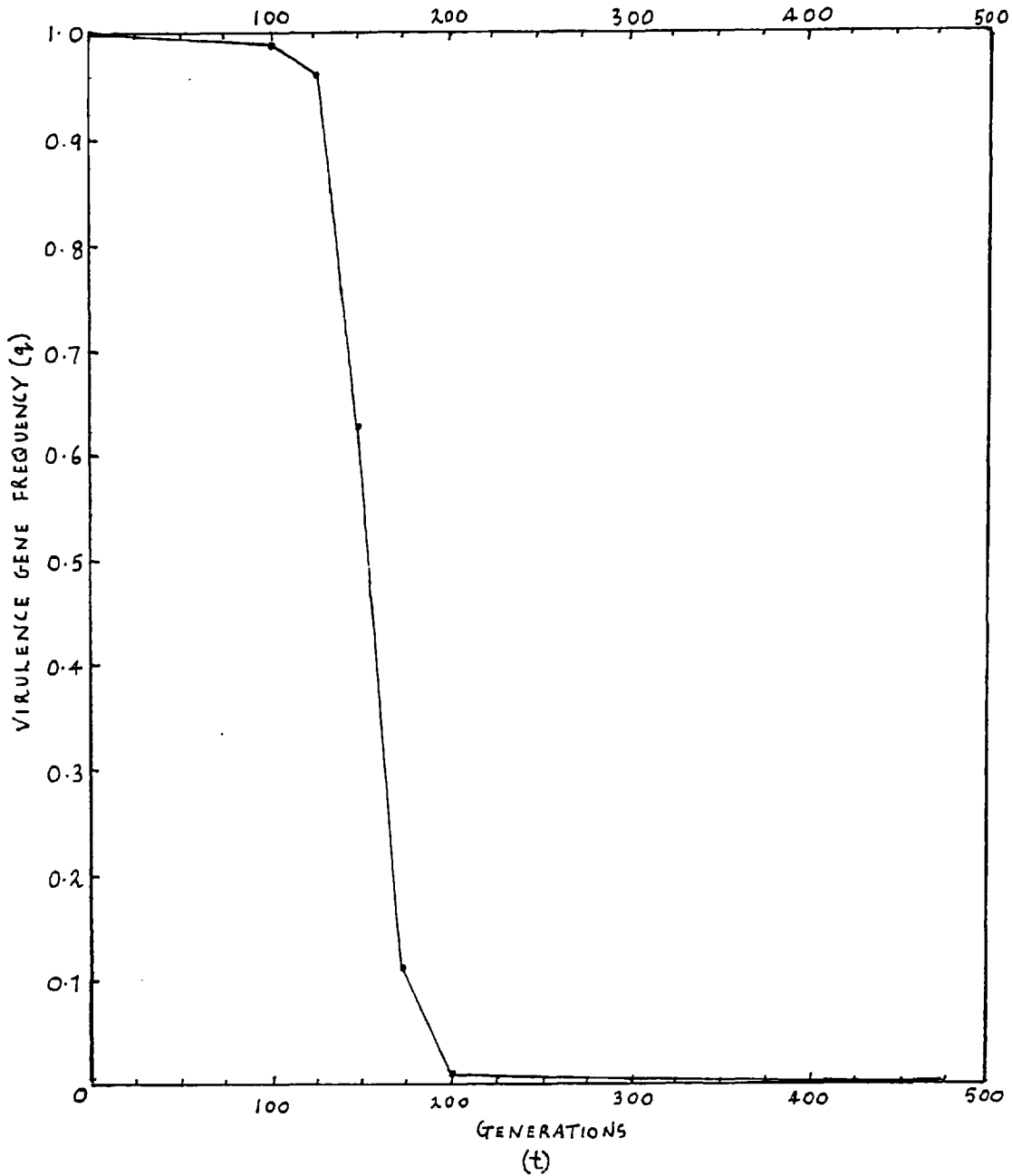
The differences in $\underline{q}_{\underline{t}}$ for DAVE 1 and DAVE 2 are still small even if $\underline{t} > 100$. The greatest difference of 0.01238, recorded in Table 5 occurred after 300 generations. Interestingly however, the values of $\underline{q}_{\underline{t}}$ converged again when $\underline{t}$ was increased to 600 or 100 generations. In conclusion

the extra accuracy gained by using DAVE 2 is negligible over the range of generations tested, and comparatively low $\underline{s}$ and $\underline{n}$ values. As will be seen $\underline{s}$ values are generally small, and $\underline{n}$ values small in cases of stabilising selection operating in the field.

1.3.4.b Asymptotic nature of the decline in the virulence gene frequency

Using the computer programmes of the models DAVE 1 and DAVE 2 the shape of the graph of the decline of a virulence gene frequency under stabilizing selection ($\underline{s} > 0$) can be determined. One such graph is shown in Figure 1. The parameter values chosen were $\underline{q}_0 = 1$, $\underline{p}_0 = 0$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{m} = 1$, $\underline{n} = 0$ and $\underline{s} = 0.1$. The model used was DAVE 2.

Figure 1 Asymptotic decline in a virulence gene frequency ($\underline{q}$)
under stabilizing selection using the model DAVE 2¹



¹ $\underline{p}_0 = 0.0$, $\underline{q}_0 = 1.0$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{m} = 1.0$, $\underline{n} = 0.0$,
 $\underline{s} = 0.1$

The graph of the decline in $\underline{q}$ with $\underline{t}$ is asymptotic, consisting typically of two tails where $\underline{q}$ declines very slowly and a short period where $\underline{q}$ declines very rapidly. The former occurs when $\underline{q} \approx 0$ or $\underline{q} \approx 1$. The fastest decline occurred between $\underline{q} = 0.96$ (at 125 generations) and $\underline{q} = 0.11$ (at 175 generations). The values of $\underline{p}$ and $\underline{q}$ at 25 generation intervals are shown in Table 6. The slowest decline in $\underline{q}$ occurs where $\underline{q} \approx 0$, rather than where $\underline{q} \approx 1$, because when $\underline{q} \approx 1$, there is considerable selection against the $\underline{V}$ gene, and little generation of $\underline{V}$ genes by mutation from the $\underline{A}$ allele, since $\underline{p} \approx 0$. However when $\underline{q} \approx 0$ the contribution of stabilizing selection to the decline is reduced considerably, whilst mutation to virulence is much more common than before, since $\underline{p} \approx 1$. Both of these factors combine to reduce the speed of the decline in $\underline{q}$, when $\underline{q} \approx 0$. The balance value of $\underline{q}$ is reached after 438 generations (between 425 and 450 generations from Table 6). An approximation of the balance frequency (1×10^{-5}) is predicted by the equation $\underline{q}_b = \mu \underline{a} / \underline{s}$ (1.3.2.11). The error between this estimate and the true value of $0.99999992 \times 10^{-5}$ is due to the approximation in equation 1.3.2.11. The precise value of $\underline{q}_b$ is derived from equation 1.3.2.9.

The very slow decline in $\underline{q}$ as it approaches zero means that it may take a very long time for stabilizing selection to return $\underline{q}$ to its balance value even if $\underline{s}$ is not small. This point is significant because it may not be possible to re-introduce a 'discarded' cultivar commercially (as Stevens, 1949 suggested) and not cause a very rapid re-selection for virulence, unless a very long period of time has elapsed.

An example of an unsuccessful attempt was the re-introduction of the $\underline{Sr}_6$ cultivar Eureka in Australia (see section 1.1.4.c). After the removal from general agricultural use of this cultivar, virulence to $\underline{Sr}_6$ declined rapidly in populations of *P. graminis tritici* in northern NSW and Queensland. In 1957 and 1958 the value of $\underline{q}$ determined by race

Table 6 Values of p and q at 25 generation intervals under stabilizing selection using the model DAVE2¹

Generation (<u>t</u>)	<u>p</u>	<u>q</u>
0	0.00000000	1.00000000
25	0.10382764 x 10 ⁻⁵	0.99999896
50	0.15625783 x 10 ⁻⁴	0.99998437
75	0.21877399 x 10 ⁻³	0.99978123
100	0.30399102 x 10 ⁻²	0.99696009
125	0.40743288 x 10 ⁻¹	0.95925671
150	0.37171114	0.62828886
175	0.89177764	0.10822236
200	0.99135380	0.86461954 x 10 ⁻²
225	0.99936498	0.63501637 x 10 ⁻³
250	0.99994510	0.54896956 x 10 ⁻⁴
275	0.99998678	0.13223357 x 10 ⁻⁴
300	0.99998977	0.10231410 x 10 ⁻⁴
325	0.99998998	0.10016612 x 10 ⁻⁴
350	0.99999000	0.10001192 x 10 ⁻⁴
375	0.99999000	0.10000085 x 10 ⁻⁴
400	0.99999000	0.10000005 x 10 ⁻⁴
425	0.99999000	0.99999996 x 10 ⁻⁵
450	0.99999000	0.99999992 x 10 ⁻⁵
475	0.99999000	0.99999992 x 10 ⁻⁵

¹ m = 1.0, n = 0.0, s = 0.1, μ_a = 1 x 10⁻⁶, μ_v = 1 x 10⁻⁸

surveys was zero (Watson and Luig, 1963). Eureka was considered once again to be effectively resistant and it was recommended that farmers reuse it. In 1960 - 61, 4.2% of the total acreage of wheat in northern NSW was Eureka. Although this was much less than the 19.2% maximum in earlier years it was sufficient to cause a very rapid selection again for virulence to Sr₆ in the pathogen population. The plan to re-introduce Eureka had to be abandoned, in its initial stages. These results are predictable from the models of declining virulence gene frequencies, DAVE 1 and DAVE 2. Watson and Luig (1963) stated that the national race surveys of P. graminis tritici comprised 400 - 500 samples annually. The minimum non zero virulence gene frequency, q that could be estimated from such surveys is $1/500 = 2 \times 10^{-3}$. Clearly, therefore, any estimate of q of zero may correspond to an actual population value of anything up to 2×10^{-3} at least. As shown above (Table 6) many generations of selection may be required to change q from 2×10^{-3} to its lowest (balance) value. Thus whilst race surveys may estimate q as zero for several years, the real value of q may still be declining. Consequently, if a host with a specific resistance gene is re-introduced, the longevity of successful commercial usage will vary depending on the real population value of q. In the case of Eureka evidently q had not reached q_b when this cultivar was re-introduced and thus a rapid rise in the virulence frequency was not surprising. Using DAVE 2, the number of generations required to change q from 2×10^{-3} to the balance frequency ($0.99999992 \times 10^{-5}$) for the set of parameter values given in Table 6 is about 224 (214 to 438) generations. If we assume 10 - 15 generations of the pathogen per year (see section 1.3.6.b) this decline may take 15 - 23 years to complete. Clearly then with s = 0.1, replanting of discarded cultivars on a large commercial scale is unlikely to be a successful venture unless

a very long term view is taken.

The asymptotic nature of the decline in virulence frequency also means that the time taken for $\underline{q}$ to change between the range 0.3 - 0.7 is very rapid and also rapid between 0.1 - 0.3 and 0.7 - 0.9. Thus in Table 6, $\underline{q}$ changed from about 0.96 - 0.11 in just 50 generations.

In another simulation with DAVE 2, $\underline{q}_0$ was set at 0.99, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{m} = 1.0$, $\underline{n} = 0.0$, and $\underline{s} = 0.1$. The generation times ($\underline{t}$) for $\underline{q}$ to decline from 0.99 - 0.90, 0.90 - 0.70, 0.70 - 0.30, 0.30 - 0.20, 0.10 - 0.01 are shown in Table 7. As an average, for the range 0.30 - 0.70 each change in $\underline{q}$ of 0.01 required only 0.4 generations. The rates of change in $\underline{q}$ were slower in the ranges of $\underline{q} = 0.90 - 0.70$ and 0.30 - 0.10.

Table 7 Numbers of generations for $\underline{q}$ to change from upper to lower limits under stabilizing selection using the model DAVE 2¹

Range in $\underline{q}$			Number of generations ($\underline{t}$)	
Upper		Lower	Actual	Number per 0.01 change in $\underline{q}$
0.99	-	0.90	23	2.55
0.90	-	0.70	13	0.65
0.70	-	0.30	16	0.40
0.30	-	0.10	13	0.65
0.10	-	0.01	23	2.55

¹ $\underline{q}_0 = 0.99$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{m} = 1.0$, $\underline{n} = 0.0$,
 $\underline{s} = 0.1$

Predictably therefore if selection is operating at a locus (not just stabilizing selection) gene frequencies will rarely be found in the range 0.30 - 0.70, but more frequently in the ranges 0.90 - 0.70, 0.30 - 0.10, 0.99 - 0.90 and 0.10 - 0.01. There have been few race surveys of sufficient size to test this prediction accurately but the hypothesis is supported by the data of Roelfs and Groth (1980) who cited the frequencies of 15 virulence genes in P. graminis tritici in each of two isolated U.S.A. populations. The data was from the 1975 race survey. The frequency of distribution of these 30 gene frequencies is shown below in Table 8. Only four gene frequencies were between 0.70 and 0.30. This low number is statistically lower than expected. All of these fell in the range 0.63 - 0.69. None occurred between 0.25 - 0.62. These data suggest selection of some kind on these virulence genes has been operating in the pathogen population.

Table 8 Frequency distribution of 30 virulence gene frequencies (q) in two isolated U.S.A. populations of Puccinia graminis tritici in 1975¹

Range (<u>q</u>)	0.99-0.90	0.90-0.70	0.70-0.30	0.30-0.10	0.10-0.01
Number of gene frequencies	5	8	4	8	5

¹ From Roelfs and Groth (1980)

1.3.4.c Variation in parameter values and changes in the rates of decline of the virulence gene frequency

The effect of $\underline{q}$ on the rate of change in the decline of $\underline{q}$ has already been discussed (1.3.4.b) and now the effect of other parameters is described.

1.3.4.ci The selective coefficient, $\underline{s}$

In these sets of computer simulations with DAVE 2 $\underline{q}_0$ was set at 0.9999, $\underline{p}_0$ was 0.0001. The mutation rates were $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-7}$; $\underline{m} = 1.0$, $\underline{n} = 0.0$ and $\underline{s}$ values were varied. In the first simulation, $\underline{s}$ was 0.1. A balance value of $\underline{q} = 0.99999911 \times 10^{-5}$ was reached between 350 and 375 generations.

When $\underline{s}$ was 0.01, after 1000 generations $\underline{q}$ had declined only to approximately 0.284. At 500 generations however $\underline{q}$ was still greater than 0.983. If $\underline{q}_0$ was set at 0.5, $\underline{q}$ declined to 0.270 after 100 generations.

Setting $\underline{s} = 0.001$ resulted in no experimentally detectable change in $\underline{q}$: after 1000 generations its value was 0.99955729. If however $\underline{q}_0$ was 0.5 then $\underline{q}$ declined to about 0.269 after 1000 generations.

These results show that selection against unnecessary virulence may be occurring slowly and significantly get undetectably using standard race survey techniques over short time periods, if $\underline{s}$ values are low. For instance in the last simulation with $\underline{s} = 0.001$ and $\underline{q}_0 = 0.5$, a decline in $\underline{q}$ from 0.5 - 0.4 took 400 generations, a period of many years for any known pathogen.

Further a $\underline{q}_0$ value of 0.5 occurs at the time of fastest decline in $\underline{q}$. If $\underline{q}_0$ was greater, say, in the range 0.7 to 0.9 then the equivalent decline of 0.1 would take even longer, than for a drop in $\underline{q}$ from 0.5 to 0.4.

This point is significant because opponents of the concept of stabilising selection may quote examples of non detection of a decline in a virulence gene frequency following the removal of the selective host as proof that selection is not operating. However unless the population is monitored over many years, weak selection may go completely undetected, and these conclusions may be erroneous. A decline in a virulence gene frequency (as occurred for virulence to Sr₆ (section 1.1.4.c)) may only be observable if s values are comparatively high (s $\geq$ 0.01), even if q₀ values are close to 0.5. It is also evident that low pre-existing virulence gene frequencies which have the benefit in some cases of thousands of years of selection (section 1.2.4) may be more sensitive indicators of selection against unnecessary virulence when s values are comparatively small (s $\leq$ 0.001).

Another consequence of the slow decline in q when s is small (s $\leq$ 0.001) is that selection against virulence may be much more common than was previously thought. Certainly detectable declines in q are not very common, but many undetectable declines in q may still be associated with weak forces of selection. Whilst an s value of 0.001 is small, it is large in comparison with mutation rates and is therefore significant at a population genetics level. However at a practical agricultural level, such low selective coefficients may be unimportant.

1.3.4.cii The mutation rates, μ_a , μ_v

In one set of simulations using the model DAVE 1, μ_a was set at 1×10^{-6} or 1×10^{-5} whilst keeping the other parameters constant at $\mu_v = 1 \times 10^{-7}$, s = 0.05, p₀ = 0.0 and q₀ = 1.0, m = 1.0 and n = 0.0. Both simulations were run for 500 generations and values of q were obtained every fifty generations. The results are shown in Table 9. Clearly,

the greater the value of μ_a , the slower the decline in q . This is expected since the greater the value of μ_a , the greater the generation of V alleles from A alleles by mutation. Hence the decline in the frequency of V is retarded. However the influence of μ_a on the rate of decline in q is very small up to about 300 generations when q has declined to about 0.098. From 300 - 500 generations the values of q diverge significantly.

Table 9 Effect of varying μ_a on the rate of decline in q using the model DAVE 1¹

Number of generations	q	
	$\mu_a = 1 \times 10^{-6}$	$\mu_a = 1 \times 10^{-5}$
50	0.99997731	0.99997752
100	0.99968121	0.99968144
150	0.99584905	0.99585385
200	0.94859253	0.94867151
250	0.58675211	0.58728242
300	0.9851×10^{-1}	0.9888×10^{-1}
350	0.8356×10^{-2}	0.8559×10^{-2}
400	0.6664×10^{-3}	0.8484×10^{-3}
450	0.6977×10^{-4}	0.2499×10^{-3}
500	0.2383×10^{-4}	0.2038×10^{-3}

¹ $\mu_v = 1 \times 10^{-7}$, $s = 0.05$, $p_o = 0.0$, $q_o = 1.0$, $m = 1.0$, $n = 0.0$

In a second set of simulations in which μ_v was set at three values 1×10^{-7} , 1×10^{-8} and 1×10^{-9} whilst all other parameter values were

kept constant at $\mu_a = 1 \times 10^{-6}$, $s = 0.05$, $m = 1.0$, $n = 0.0$, $p = 0.0$ and $q = 1.0$, the effect of varying the back mutation rate on the decline in q was studied. The results are shown in Table 10.

Table 10 Effect of varying μ_v on the rate of decline in q using the model DAVE 1

Number of generations	q		
	$\mu_v = 1 \times 10^{-7}$	$\mu_v = 1 \times 10^{-8}$	$\mu_v = 1 \times 10^{-9}$
50	0.99997731	0.99999773	0.99999977
100	0.99968121	0.99996811	0.99999681
150	0.99584905	0.99958335	0.99995832
200	0.94859253	0.99460996	0.99945837
250	0.58675211	0.93420481	0.99300635
300	0.985×10^{-1}	0.52212255	0.91614759
350	0.836×10^{-2}	0.776×10^{-1}	0.45674000
400	0.666×10^{-3}	0.645×10^{-2}	0.608×10^{-1}
450	0.698×10^{-4}	0.518×10^{-3}	0.497×10^{-2}
500	0.238×10^{-4}	0.583×10^{-4}	0.403×10^{-3}

As μ_v is increased so the speed of decline in q increases. This is expected, because the larger the back mutation rate, the lower the frequency of V alleles in the population.

For the first 150 generations the value of μ_v makes little difference to the rate of decline of q . However at and after 200 generations the values of q are significantly changed by varying μ_v . By 300 generations there is a very marked difference in the three values of q . This is in contrast

to the results in Table 9 when μ_a was varied. These results suggests μ_v has a greater effect on the decline in q than μ_a during the first 300 generations. This conclusion is somewhat surprising since forward mutation rates being 10-100 fold greater (Esser and Kuenen, 1967) are usually considered to be more significant than backward mutation in population genetics. In mathematical approximations of population genetics models, backward mutation may be ignored whilst factors for forward mutation are retained. An example is the approximation of the equation $q_b = \mu_a/s$ discussed above (section 1.3.2.a and equations 1.3.2.9 - 1.3.2.11. Strictly, q_b is derived from the equation $s = (\mu_a/q_b) - (\mu_v/p_b)$.

The results of the simulations varying the mutation rates are explicable if the equations utilized in calculating values of q in Tables 9 and 10 are examined. In both cases DAVE 1 was used and n was set at 0.0. Consequently the equation used to calculate successive values of q was :

$$q_{t+1} = \frac{q_t \cdot (1-s) + p_t \cdot \mu_a - q_t \cdot \mu_v}{1 - s \cdot q_t} \quad 1.3.2.7$$

Initially, and ^{approximately} for the first 150 generations (Table 10), $p_0 = 0.0, q_0 = 1.0$. Hence from equation 1.3.2.7 :

$$q_{t+1} \approx \frac{(1-s) - \mu_v}{1-s} \quad 1.3.4.1$$

Hence initially μ_v has an effect on q_{t+1} , whereas μ_a does not. However, since $1, s \gg \mu_v$, the rate of μ_v has only a small effect on the decline of q . Hence at 150 generations, the values of q are only slightly different for the three values of μ_v (Table 10). As the number

of generations increases these small differences in $\underline{q}$ are amplified.

This occurs when $\underline{p}$ and $\underline{q}$ are not approximately 0 or 1, and equation 1.3.2.7 approximates to :

$$q_{t+1} \approx \frac{q_t \cdot (1-s)}{1-s \cdot q_t} \quad 1.3.4.2$$

Small differences in $\underline{q}_t$ become greater due to the coefficient $(1-s)$ in equation 1.3.4.2, further reducing $\underline{q}_t$. At the same time the denominator becomes larger for a lower value of $\underline{q}_t$, further reducing $\underline{q}$.

After 500 generations the values of $\underline{q}$ have begun to converge (Table 10). This occurs when $\underline{q} \approx 0$. When $\underline{q}_t$ is small, equation 1.3.2.7 reduces to :

$$q_{t+1} \approx \frac{q_t \cdot (1-s) + p_t \cdot \mu_a}{1-s \cdot q_t} \quad 1.3.4.3$$

Since $s \cdot \underline{q}_t$ is very small this further reduces to :

$$q_{t+1} \approx q_t \cdot (1-s) + p_t \cdot \mu_a \quad 1.3.4.4$$

The smaller the value of $\underline{q}_t$, the larger the value of $\underline{p}_t$. Consequently, the greater the value of $\underline{p}_t \cdot \mu_a$. The extra number of mutants produced by forward mutation reduces the rate of decline of $\underline{q}$, in comparison with a higher value of $\underline{q}$ (associated with a smaller value of μ_v) where the $\underline{p}_t \cdot \mu_a$ term is smaller.

In the latter stages the decline in $\underline{q}$ is more affected by varying μ_a than μ_v . This is explicable from equations 1.3.4.1, 1.3.4.2 and 1.3.4.4 above. Initially, when $\underline{q} \approx 1$, and $\underline{p} \approx 0$ $\underline{q}$ is mostly influenced by the values of $\underline{s}$ and μ_v (equation 1.3.4.1), and barely by μ_a since the term $\underline{p} \cdot \mu_a$ (in equation 1.3.2.7) is virtually zero. When $\underline{p}$ and $\underline{q}$ are not

close to 0 or 1 equation 1.3.4.2 operates and changes in $\underline{q}$ are mostly influenced by $\underline{s}$ values. As $\underline{q}$ declines further and approaches a very small value, equation 1.3.4.4 best describes changes in $\underline{q}$. This equation carries a significant $\underline{p}.\underline{\mu}_a$ term which has a strong influence on $\underline{q}$, since $\underline{p} \approx 1$. On the other hand the $\underline{q}.\underline{\mu}_v$ of equation 1.3.2.7, significant when $\underline{q} \approx 1$, becomes virtually insignificant when $\underline{q} \approx 0$.

Consequently, varying $\underline{\mu}_v$ has more effect on $\underline{q}$, when $\underline{q}$ is not small, whilst varying $\underline{\mu}_a$ has much more influence on $\underline{q}$, when $\underline{q}$ is small, for example, $\underline{q} < 0.98 \times 10^{-1}$ (Table 9).

1.3.4.ciii The proportion of resistant hosts, $\underline{n}$

In the computer simulations described in sections 1.3.4.ci and 1.3.4.cii the proportion of resistant hosts $\underline{n}$ was assumed to be zero. Consequently, equations 1.3.2.7 and 1.3.2.8 for DAVE 1, and 1.3.2.14 and 1.3.2.15 for DAVE 2 were used to calculate successive values of $\underline{q}$. If $0 < \underline{n} < 1$ then equations 1.3.2.5 and 1.3.2.6 for DAVE 1 and 1.3.2.12 and 1.3.2.13 for DAVE 2 must be employed. These contain the parameters $\underline{m}$, $\underline{n}$ and $\underline{z}$ described in section 1.3.2.a, which are not found in the equations 1.3.2.7, 1.3.2.8, 1.3.2.14 or 1.3.2.15. In one simulation with DAVE 2, $\underline{n}$ was set at 0.05, $\underline{m}$ consequently was $1 - 0.05 = 0.95$. The value of $\underline{z}$ was taken as one, meaning that the avirulent races cannot sporulate on the resistant host. $\underline{\mu}_a$ was set at 1×10^{-6} , $\underline{\mu}_v$ at 1×10^{-8} , $\underline{p}_0$ at 0.0, $\underline{q}_0$ at 1.0, $\underline{s}$ at 0.1 and $\underline{t}$ was 475 generations. The results are shown in Table 11. They are directly comparable with those in Table 6 since all values except $\underline{m}$ and $\underline{n}$ are identical. Clearly as $\underline{n}$ increases so the rate of decline in $\underline{q}$ is reduced. This is expected since the virulent race reproduces fully on the resistant host whereas the avirulent race cannot sporulate on this host. This reduces the loss of $\underline{V}$ genes. Since $\underline{m} \gg \underline{n}$, $\underline{q}$ still declines, but even a small value of $\underline{n}$ may

Table 11 Values of p and q at 25 generation intervals under
stabilizing selection and mutation using the model DAVE 2¹
assuming a small proportion of resistant hosts²

Generation (<u>t</u>)	<u>p</u>	<u>q</u>
0	0.00000000	1.00000000
25	0.44340276 x 10 ⁻⁶	0.99999956
50	0.19670825 x 10 ⁻⁵	0.99999803
75	0.70928055 x 10 ⁻⁵	0.99999291
100	0.24335683 x 10 ⁻⁴	0.99997566
125	0.82337474 x 10 ⁻⁴	0.99991766
150	0.27740986 x 10 ⁻³	0.99972259
175	0.93308764 x 10 ⁻³	0.99906691
200	0.31325305 x 10 ⁻²	0.99686747
225	0.10461033 x 10 ⁻¹	0.98953897
250	0.34342713 x 10 ⁻¹	0.96565729
275	0.10685587	0.89314413
300	0.28697483	0.71302517
325	0.57517742	0.42482258
350	0.81996138	0.18003862
375	0.93871614	0.61283860 x 10 ⁻¹
400	0.98094790	0.19052097 x 10 ⁻¹
425	0.99424429	0.57557082 x 10 ⁻²
450	0.99826655	0.17334501 x 10 ⁻²
475	0.99946849	0.53151332 x 10 ⁻³

¹ $\underline{p}_0 = 0.0$, $\underline{q}_0 = 1.0$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{s} = 0.1$, $\underline{t} = 475$

² $\underline{n} = 0.05$, $\underline{m} = 0.95$, $\underline{z} = 1.0$

reduce significantly the rate of decline in $\underline{q}$. For example when $\underline{n} = 0.05$, $\underline{q}$ is 0.965 approximately, after 250 generations (Table 11), whereas if $\underline{n} = 0.00$, $\underline{q}$ is much lower at 0.549×10^{-5} after 250 generations (Table 6). The influence of $\underline{n}$ on the rate of decline of a virulence gene frequency has profound practical consequences and will be discussed in more detail later (section 1.3.6.biii).

1.3.4.civ The selective coefficient against avirulence, $\underline{z}$

In all the previous computer simulations with DAVE 1 and DAVE 2, $\underline{z}$ was taken as one. If $\underline{n} = 0$, then the $\underline{z}$ term disappears from the equation (1.3.2.7 and 1.3.2.8 for example). This is logical, since if hosts with the specific resistance gene are not grown then there can be no selection against avirulence.

In this simulation $\underline{n}$ was set at 0.05, and $\underline{z} = 0.98$, meaning that on the small percentage of hosts carrying the specific resistance gene genotypes with the avirulence gene have a mean relative fitness of $1 - \underline{z} = 0.02$ compared with the pathogen genotypes carrying the corresponding virulence gene, whose mean relative fitness is one. Sporulation on these hosts occurs in proportion to the mean relative fitnesses of the carriers of the avirulence and virulence alleles. The other parameter values were set at $\underline{m} = 0.95$, by difference, $\underline{p}_0 = 0.0$, $\underline{q}_0 = 1.0$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$ and $\underline{s} = 0.1$. The simulation was run for 475 generations. The results are shown in Table 12, and are directly comparable with the results in Table 11 of the simulation described in 1.3.4.ciii, where $\underline{z} = 1.0$. All other parameter values otherwise were identical in the two simulations. The results are not dissimilar, but clearly the small amount of sporulation of the avirulent races on the 5% of hosts with the specific resistance gene is significant. This can be seen if the values of $\underline{q}$

Table 12 Values of p and q at 25 generation intervals under stabilizing selection and mutation using the model DAVE 2¹ assuming a proportion of host resistance and some sporulation of the avirulent races on these hosts²

Generation (<u>t</u>)	<u>p</u>	<u>q</u>
0	0.00000000	1.00000000
25	0.44988629 x 10 ⁻⁶	0.99999955
50	0.20365310 x 10 ⁻⁵	0.99999796
75	0.75163190 x 10 ⁻⁵	0.99999248
100	0.26441511 x 10 ⁻⁴	0.99997356
125	0.91798317 x 10 ⁻⁴	0.99990820
150	0.31745674 x 10 ⁻³	0.99968254
175	0.10960350 x 10 ⁻²	0.99890396
200	0.37757240 x 10 ⁻¹	0.99622428
225	0.12921058 x 10 ⁻¹	0.98707894
250	0.43254765 x 10 ⁻¹	0.95674523
275	0.13505578	0.86494422
300	0.35034283	0.64965717
325	0.65064846	0.34935154
350	0.86544182	0.13455818
375	0.95690728	0.43092721 x 10 ⁻¹
400	0.98711370	0.12886300 x 10 ⁻¹
425	0.99621906	0.37809396 x 10 ⁻²
450	0.99888690	0.11131036 x 10 ⁻²
475	0.99966202	0.33798114 x 10 ⁻³

¹ $\underline{p}_0 = 0.0$, $\underline{q}_0 = 1.0$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 10^{-8}$, $\underline{s} = 0.1$, $\underline{t} = 475$

² $\underline{n} = 0.05$, $\underline{m} = 0.95$, $\underline{z} = 0.98$

are compared after 325 generations at which stage $\underline{q}$ is 0.425 when $\underline{z} = 1.0$ or 0.349 if $\underline{z}$ is 0.98. Hence the extra reproduction of the avirulent races here speeds up the decline in virulence, since more avirulence genes are generated. Leonard (1977a) has suggested that values of $\underline{z}$ (he calls $\underline{t}$) are likely to be between 0.98 - 1.00.

1.3.5 DAVE 4

The models DAVE 1 and DAVE 2 assume that the mean relative fitness of the avirulent races on hosts lacking the corresponding resistance gene is equal to the mean relative fitness of the virulent races on hosts with the corresponding resistance gene. The fitness matrix assumed in these models is :

	Pathogen allele	
	<u>A</u>	<u>V</u>
$\underline{R}_o(\underline{m})$	1	(1- <u>s</u>)
Host allele (proportions in brackets)		
$\underline{R}(\underline{n})$	(1- <u>z</u>)	1

In the model DAVE 4 this assumption is not made. Instead it is assumed the mean relative fitness of the races with a V gene on hosts with the R gene is greater than that of races with the A gene on hosts lacking the R gene. In some circumstances, there is good experimental evidence for this. In the history of the use of monogenic resistant cultivars, after the 'breakdown of resistance' had occurred in some cases there

appeared to be more disease on the formerly resistant cultivars compared with the original susceptible cultivars, grown in the same locality. This difference may have been due to differences in background genetic resistance possessed by the cultivars. An example of this is the potato cultivar *Vertifolia* described in detail by Van der Plank (1968) which lost background genetic resistance to late blight during breeding.

In the model DAVE 4 the fitness matrix becomes :

	Pathogen allele	
	<u>A</u>	<u>V</u>
$\underline{R}_0(\underline{m})$	1	(1- <u>s</u>)
Host allele (proportions in brackets)		
$\underline{R}(\underline{n})$	(1- <u>z</u>). (1+ <u>a</u>)	(1+ <u>a</u>)

a is a coefficient of selection that gives races with the V allele extra fitness on R hosts. The mean fitness of races with the A allele is (1 - z) multiplied by the fitness of races with the V allele on R hosts, i.e. (1-z). (1+a).

The overall mean relative fitness of avirulent races, W_a is given as :

$$\underline{W}_a = m \cdot x \cdot 1 + n \cdot (1 - z) \cdot (1 + a)$$

$$= m + n + n \cdot a - n \cdot z - n \cdot z \cdot a$$

$$= 1 + n \cdot (a - z - a \cdot z)$$

1.3.5.1

The overall mean relative fitness of virulent races, W_v is given as :

$$W_v = m.(1-s) + n.(1+a)$$

$$= m - m.s + n + n.a$$

$$= 1 - m.s + n.a \quad 1.3.5.2$$

If the gene frequencies of A and V alleles in generation zero are p_0 and q_0 respectively then in the next generation :

$$p_1 = \frac{p_0.(1+n.(a-z-az)) - p_0 \cdot \mu_a + q_0 \cdot \mu_v}{1 + p_0 \cdot n.(a-z-az) - q_0.(m.s+n.a)} \quad 1.3.5.3$$

and

$$q_1 = \frac{q_0.(1-m.s+n.a) + p_0 \cdot \mu_a - q_0 \cdot \mu_v}{1 + p_0 \cdot n.(a-z-az) - q_0.(m.s+n.a)} \quad 1.3.5.4$$

The denominator is a term that ensures $p_1 + q_1$ sum to one and is the sum of the two numerators :

$$p_0.(1+n.(a-z-az)) - p_0 \cdot \mu_a + q_0 \cdot \mu_v + q_0.(1-m.s+n.a) + p_0 \cdot \mu_a - q_0 \cdot \mu_v$$

$$= p_0 + p_0 \cdot n \cdot a - p_0 \cdot n \cdot z - p_0 \cdot n \cdot a \cdot z + q_0 - q_0 \cdot m \cdot s + q_0 \cdot n \cdot a$$

$$= 1 + p_0 \cdot n.(a-z-az) - q_0.(m.s+n.a)$$

If a is set at zero in equations 1.3.5.3 and 1.3.5.4 then DAVE 4 reduces to DAVE 1 and equations 1.3.5.3 and 1.3.5.4 reduce to equations 1.3.2.5 and 1.3.2.6 respectively.

1.3.6 Estimation of selective coefficients, $\underline{s}$, from field data
using the models DAVE 1, DAVE 2 and DAVE 4

1.3.6.a Introduction

Most calculations of the values of $\underline{s}$ or similar terms have come from mixed race experiments in the glasshouse (sections 1.1.4.d and 1.1.5) in which the proportion of two races of a pathogen, one possessing and one lacking the allele under study are monitored over successive generations (Leonard, 1969, 1977; Van der Plank, 1968). As discussed earlier (sections 1.1.4.d and 1.1.5) many studies of this type have limited value because the effects of the background genotype of the pathogen on fitness were disregarded. Methods discussed earlier (sections 1.1.4.d and 1.1.5) to eliminate such background genetic spuria are either to use isogenic or near isogenic pathogens differing only in the single virulence allele or by bulking large numbers of different isolates of the races. Leonard (1977a) used this later approach with 50 isolates for each of two races of B. maydis in order to provide sufficient background genetic diversity that differences other than for virulence would tend to be cancelled or nullified. The value of $\underline{s}$ Leonard (1977a) calculated was 0.30. However the highest $\underline{s}$ value he determined was 0.42 from data on races of P. graminis carrying unnecessary virulence to the Sr₆ gene in wheat (Leonard 1977a). I am unaware of any publication where $\underline{s}$ values have been determined from field data.

Using the models DAVE 1, DAVE 2 and DAVE 4 described above field data of several examples of declining virulence gene frequencies have been used to estimate values of $\underline{s}$ for certain unnecessary virulence genes. Field data are the most valid source of statistics for estimating values of $\underline{s}$ for several reasons. Firstly, large numbers of isolates are sampled

directly from natural populations rather than just a few isolates from the artificial population as in glasshouse experiments. Consequently a more random sample of the pathogen is studied, resulting in a more likely elimination of the effects of the background genotype as determinators of pathogen fitness. Secondly, field data are sampled from populations where natural epidemics have occurred and which have been exposed to natural levels of selection and competition. Further in the field there are continuous epidemics, whereas in glasshouse experiments unnatural discrete generations are created.

The basic technique for estimating values of $\underline{s}$ from field data is to estimate the number of generations ($\underline{t}$) required for the virulence gene frequency to decline from its starting value $\underline{q}_0$ to its final value $\underline{q}_t$. Since the number of generations may be estimated variously by different workers, and it clearly differs from year to year depending on the amount of initial inoculum at the start of the season, the length of the epidemic, the length of the host growing season, the availability of hosts for colonization between seasons and weather conditions, a minimum and maximum estimate of $\underline{t}$ is calculated. Consequently, two estimates of $\underline{s}$ are obtained representing the estimated upper and lower limits of a range in which the true value of $\underline{s}$ most probably lies. The upper limit is calculated by assuming the minimum generation number and the lower limit is calculated by assuming the maximum generation number, since a larger $\underline{s}$ value is required to change $\underline{q}_0$ to $\underline{q}_t$ if $\underline{t}$ is reduced. Once $\underline{q}_0$, $\underline{q}_t$ and $\underline{t}$ are known, then estimates of $\underline{z}$, $\underline{\mu_a}$, $\underline{\mu_v}$, $\underline{m}$ and $\underline{n}$ are required. The values of $\underline{z}$, $\underline{m}$ and $\underline{n}$ can be estimated accurately from experimental and agricultural data in the literature respectively whilst rough estimates of the mutation rates have to be made. However, the values of $\underline{\mu_a}$ and $\underline{\mu_v}$ are usually small in comparison with the values of $\underline{s}$, and further

variations in μ_a and μ_y do not usually influence significantly the rate of decline in q over the first few hundred generations (section 1.3.4.cii). Values of t in these calculations are usually much less than 200 generations.

The parameter values are entered into the computer programme of the appropriate model, and the value of s is determined by a trial and error method. Various values of s are used until one is found that causes q to decline from its q_0 value to its q_t value in exactly t generations. Two simulations are carried out one for each of the two estimates of t .

Since the field data may represent as many as 7 - 8 years of sampling of the pathogen population, the values of s determined are averages for this period. Virulence gene frequencies may not always decline steadily partly because their estimates are subject to sampling error, and consequently s values may vary from year to year within the sequence. The advantage of data from 7 - 8 seasons is that the variation in population size, weather and differences in selection between and within seasons for several years are taken into account. Further over several years consistent and hence genuine declines in q may be observed. If just two seasons' data are analysed the apparent decline from year one to year two may reflect sampling error rather than stabilizing selection. A trend of a decline in a virulence gene frequency over several years is strongly indicative of genuine selection as opposed to sampling error or indeed drift effects.

In another sense the values of s calculated using field data, the models and the technique described above are averages. They are average selective coefficients against the same unnecessary virulence gene in a whole range of background pathogen genotypes. Variation in the size of s occurs with differences in the other genes carried by a particular pathogen isolate. It is generally assumed that the range and frequency

of background genotypes with the V gene in a population is identical with the range and frequency of genotypes with the A gene in the same population. This assumption is valid in a large population because mutation to virulence will occur many times if N (the population size, lesions or spores produced) is much greater than μa . Since these mutants can and will occur in the full range of background genotypes which carry the A allele, so the range and frequency of genotypes with the A and V genes should be identical. This point was also made by Martens et al. (1970), and will be discussed in more detail later (sections 1.3.6.bi, 3.4.1.a and 3.4.2).

Several examples of the estimation of values of s from field data will now be described.

1.3.6.b Virulence to Sr_6 in *Puccinia graminis tritici* in Australia from 1948 - 1955

1.3.6.bi Calculation of s values

This example of stabilizing selection against unnecessary virulence was described in section 1.1.4.c.

In the mid-late 1940s the wheat cultivar Eureka was discarded by farmers in Australia following the failure of its former resistance to *P. graminis tritici* (Van der Plank, 1968; Watson and Luig, 1963). In 1945, the acreage of Eureka was about 18% of the total wheat acreage in northern New South Wales. About 70% of all collections of the fungus in northern N.S.W. and Queensland (which form a single ecological zone for stem rust of wheat, Luig and Watson, 1970), possessed virulence to the Sr_6 resistance gene carried by Eureka. By 1948 the acreage of Eureka had declined to about 1.5% of the total, but the percentage of isolates that could attack 'Eureka' had only dropped to about 56%. From 1948

there was a rapid decline in the frequency of Sr₆ virulence and by the 1956 rust race survey there were no isolates that had virulence to this gene in northern N.S.W. and Queensland. Thus Van der Plank (1968) postulated that stabilizing selection had caused the very dramatic change in frequency of the Eureka attacking races over a short period, i.e. from 56% to zero in just eight years in the near absence of selection for virulence. The percentage of Eureka remained roughly constant at about 1.5% of the total wheat acreage during this period. The decline in virulence occurred because the pathogen population with virulence against Sr₆ must have suffered a selective disadvantage compared with the population carrying the corresponding avirulence gene.

It could be argued that since the cultivar Eureka with Sr₆ was rapidly replaced by cultivars with the Sr₁₁ resistance gene (Watson and Luig, 1963), that the decline in virulence to Sr₆ was due to the increase in a pathogen genotype possessing virulence only to Sr₁₁ but not Sr₆. This would mean that the decline in virulence to the latter gene (and increase in frequency of the corresponding avirulence allele by a 'hitchhiking' effect (Wolfe and Knott, 1982)) would not be due to any inherent selective disadvantage of unnecessary virulence. This argument seems feasible since P. graminis tritici in Australia reproduces asexually, hence rapid sexual recombination of genes cannot occur. However the asexual populations of P. graminis tritici in Australia are very large and the number of uredospores produced annually is enormous. Many of these will carry combined virulence to two resistance genes, due to recurrent mutations. Day (1974) citing Sloodmaker in a personal communication stated that E. graminis hordei at the 10% level of infection may produce 10^{13} spores/hectare/day. Assuming a similar rate of production for P. graminis tritici and a mutation rate of 1×10^{-7} to virulence to

Sr₁₁ then daily 1×10^6 uredospores with this virulence gene are produced per hectare. The expected number carrying virulence to Sr₆ in addition, is $\underline{q} \times 10^6$ where $\underline{q}$ is the frequency of virulence to Sr₆ in the population. At the time of introduction of Sr₁₁, $\underline{q} > 0.5$ (Watson and Luig, 1963). Thus each hectare would have generated on average several hundred thousand uredospores carrying combined virulence daily and the likelihood of a pathogen genotype carrying only virulence to Sr₁₁ being selected is very small. Instead it is much more likely that pathogen genotypes were selected which carried virulence to Sr₁₁ combined with either virulence or avirulence to Sr₆ in proportion to the frequencies of the latter alleles. This indicates the decline in virulence to Sr₆ must have occurred by selection against the unnecessary virulence gene and not by a 'hitch hiking' effect during selection for virulence to Sr₁₁.

Values of $\underline{s}$ were calculated as the basis that the number of pathogen generations was 10 - 15 annually (K. Leonard, R. MacIntosh, personal communications). Therefore from 1948 to 1955 the virulence gene frequency ($\underline{q}$) fell from 0.560 to 0.085 (Van der Plank, 1968; Watson and Luig, 1963) in 70 to 105 generations. A seven year period was chosen rather than the eight period until 1956 when $\underline{q}$ was found to be zero, because of the asymptotic nature of the decline in $\underline{q}$ with time. As discussed above (section 1.3.4.b) when $\underline{q}$ is estimated as zero from a race survey, a small error in its estimation can cause a large error in the calculated number of generations of selection.

The model DAVE 1 was used to estimate $\underline{s}$. The simulations were run with the following numerical values for the parameters : $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$ (these were arbitrarily chosen from estimates cited by Esser and Kuenen (1967) and discussed in section 1.2.2.b since precise values are not known nor required. This is because the decline in $\underline{q}$ as given

by the model is very insensitive to changes in μ_a and μ_v when t values are relatively small (as discussed above in sections 1.3.4.cii and 1.3.6.a)), $\underline{m} = 0.985$, $\underline{n} = 0.015$, $\underline{p}_0 = 0.44$, $\underline{q}_0 = 0.56$. The common avirulent race 126 - 6 at the time of introduction of Eureka, in the early 1940s and the early 1950s when virulence was declining failed to sporulate on Eureka if it lacked the appropriate virulence gene (Watson and Luig, 1963), thus $\underline{z} = 1.0$. The value of $\underline{s}$ was varied until one chosen caused $\underline{q}$ to fall from 0.560 to 0.085 in exactly 70 or 105 generations. The required drop in $\underline{q}$ occurred in 70 generations when $\underline{s}$ was 0.0524 and in 105 generations when $\underline{s}$ was 0.0400. Thus the virulent races of P. graminis tritici were between 4.00 and 5.24% less fit on average than their avirulent counterparts in northern N.S.W. and Queensland between 1948 and 1955.

Leonard (1977) obtained a value of $\underline{s}$ of 0.42 for races virulent on Sr₆ cultivars, calculated using Australian data relating to mixtures of races grown in glasshouse competition experiments. The experiments were described by Watson and Singh (1952) and involved race groups 126 and 222, which were the predominant race groups in Australia up to 1956 (Watson and Luig, 1963). Thus Leonard's value of $\underline{s}$ and the ones calculated in this section are derived from data on the same races in the same country, and therefore the effect of the two techniques used to estimate $\underline{s}$, and the values of $\underline{s}$ obtained can be validly compared. K. Leonard (personal communication) has suggested that a possible reason for the large difference between the values obtained is that selection in the field may be less intense than it is in glasshouse experiments where pathogen population densities may be much greater. Another difference is that $\underline{s}$ values calculated from field data include the effects of selection during the survival phase between seasons unlike the glasshouse experiments.

1.3.6.bii Application of s values to the reintroduction of Eureka

In section 1.3.4.b the reintroduction of the wheat cultivar Eureka into commercial agricultural use in Australia after stabilizing selection had apparently reduced the frequency of virulence to Sr_6 to zero was described. The experiment failed because the incidence of virulence increased very rapidly again following the reintroduction. It was shown that although the frequency of virulence, q was estimated as zero, q in reality could have been at least as large as 2×10^{-3} . Further with $s = 0.1$ it was shown that many generations were required to change q from 2×10^{-3} to its balance value. Consequently the failure of stabilizing selection to protect sufficiently Eureka was not surprising, because insufficient time was allowed for selection to reduce q to a low enough level.

Using an s value of 0.0524 the highest calculated in section 1.3.6.bi the balance frequency and time to reach balance could be determined from the model DAVE 1. With the other parameter values set as above (section 1.3.6.bi), $\mu_a = 1 \times 10^{-6}$, $\mu_v = 1 \times 10^{-8}$, $m = 0.985$, $n = 0.015$, $z = 1.0$ and $q_0 = 0.085$ (the value of q in 1955 in northern N.S.W. and Queensland) the value of q_b was estimated as $0.27311950 \times 10^{-4}$ and the number of generations (t) required to reach this value is 676. With 10 - 15 generations per year balance would be achieved in 45 - 68 years assuming conditions remained constant. In Australia the frequency of virulence to Sr_6 was estimated as zero in 1957 and 1958 from sample sizes of 317 and 114 respectively (Watson and Luig, 1963). Eureka was then considered resistant and was reintroduced at 4.2% of the total wheat acreage in northern N.S.W. in 1960 - 1961 (Watson and Luig, 1963). The time period from estimating q as zero to the replanting of Eureka was clearly too

short to ensure that stabilizing selection reduced q to a low enough value to give Eureka another 'boom' period of agricultural use.

1.3.6.biii Application of s values to a challenge to the operation of stabilizing selection

Luig and Watson (1970) reported that in Western Australia (a rust zone geographically isolated from northern N.S.W. and Queensland) virulence to Sr_6 remained at frequencies greater than 0.9 despite the fact that less than 5% of the acreage was sown to plants with the Sr_6 resistance gene. They concluded the races of *P. graminis tritici* in Western Australia were different from those in northern N.S.W. and Queensland in the east of the country in that the former races combined the virulence gene with aggressiveness (a fit background genotype) unlike the races from the eastern states. If this hypothesis is true then it means that the selection against unnecessary virulence to Sr_6 in the east was due to a chance association of the virulence gene and a relatively unfit background genotype or range of background genotypes. Such an association might be envisaged to arise by mutation from avirulence to virulence in unfit background genotypes. In Western Australia however it must be assumed that mutation to virulence occurred in relatively fitter background genotypes.

This hypothesis is unreasonable for several reasons. Firstly, in a large population mutation to virulence will occur throughout a full range of background genotypes, as discussed in section 1.3.6.bi. If the daily rate of spore production is 10^{13} /hectare/day and this rate of production is maintained for just 30 days (the epidemic phase of stem rust in Australia last between six weeks and two months (R. MacIntosh, personal

communication)), in just 100,000 hectares of diseased wheat then during this period 3×10^{19} spores would be produced. If the mutation rate to virulence is 1×10^{-7} then 3×10^{12} of these spores would carry the virulence gene. Consequently, this large number of mutants will be expected by chance to carry the full range of background genotypes and not a limited number of very fit or unfit genotypes. In a small population of a pathogen Luig and Watson's (1970) suggestion is feasible. For instance if the annual spore production was 1×10^{10} then only 1×10^3 spores would be expected to carry the virulence gene. If the rate of survival and successful colonization is low say five in a 1000 then the surviving mutants might all share a limited range of background genotypes. By chance these could be very fit in which case no stabilizing selection would be expected, or very unfit in which case stabilizing selection would be expected.

A second point which makes Luig and Watson's (1970) hypothesis unreasonable concerns the frequency of background genotypes and their relative fitnesses in the pathogen population. The most prevalent races invariably will be the ones with the fittest background genotypes. Since mutation to virulence is random with respect to the background genotype, mutations should occur most frequently in the background genotype of the fittest and most common races. Consequently, virulence genes can be expected to be associated more frequently with advantageous genetic factors than with disadvantageous genetic factors. This point was also made strongly by Martens et al. (1970). The implications of this are that if a decline in the frequency of an unnecessary virulence gene occurs it is more likely to be due to a selective disadvantage associated with the virulence gene rather than a chance association of a neutral virulence gene (in the absence of the corresponding host resistance) with an unfit

background genotype. This is true even in a small population.

A third reason for discounting Luig and Watson's (1970) hypothesis is that a relatively small proportion of hosts with the resistance gene, $\underline{n}$ may cause sufficient selection for the virulence gene that observable stabilizing selection is prevented. This point was made earlier in section 1.3.4.ciii. The data on the frequencies of virulence to $\underline{Sr}_6$ in eastern and Western Australia can be used to illustrate this. The lack of stabilizing selection in the western state can be explained simply on the basis of a difference in $\underline{n}$, the proportion of Eureka grown in the two regions. In eastern Australia, $\underline{n}$ was on average about 0.015 whereas Luig and Watson (1970) estimated in Western Australia that $\underline{n}$ was less than 0.05. Using the model DAVE 1 and assuming a western value of $\underline{n} = 0.04$ it can be shown that if $\underline{q}_0 = 0.9$, $\underline{p}_0 = 0.1$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$ and $\underline{s}$ is set at its lowest value, $\underline{s} = 0.0400$ calculated from the eastern Australian data then the value of $\underline{q}$ (frequency of the virulence gene) rises very slightly to 0.914 in 100 generations, which represents 7 - 10 years. If however $\underline{s}$ is set at 0.0524 the maximum value of $\underline{s}$, then $\underline{q}$ would drop to 0.756 during the same period. In a third simulation $\underline{s}$ was kept at 0.0524 but $\underline{n}$ increased slightly to 0.045 ($\underline{n} < 0.05$); $\underline{q}$ declined in 100 generations to 0.842. Whilst in the latter two simulations with $\underline{s} = 0.0524$ a decline in $\underline{q}$ is predicted the largest decline 0.900 - 0.756 is still small in comparison with the decline in $\underline{q}$ in eastern Australia. Secondly the $\underline{s}$ value of 0.0524 may be too high; this is the maximum value in the range estimated from the eastern Australian data. In the first simulation with $\underline{s}$ set at 0.0400 $\underline{q}$ increased. In a fourth simulation with $\underline{n} = 0.04$ and $\underline{s} = 0.0462$ (the mean of 0.0524 and 0.0400) $\underline{q}$ declined slightly to 0.852 in 100 generations. This is a small decrease.

A third factor that must be borne in mind is the length of time that observations had been made on the pathogen population after virulence was no longer necessary. Luig and Watson (1970) reported in western Australia that there were major epidemics on Eureka in 1962 - 1964, principally caused by race 21 - 1,2. Between the 1963/1964 season to the time of writing (1969/1970) they reported that this race continued to comprise over 90% of the isolates examined. However it was not until the 1968/1969 season that wheats with Sr₆ occupied less than 5% of the total area (n < 0.05). Presumably, n declined progressively from about 1963 - 1968 indicating that prior to 1968, n may have been appreciably higher than 0.05. This would have caused selection for virulence (using DAVE 1), even if s was as high as 0.0524. It would seem the value of n only fell below 0.05 in 1968/1969. With s = 0.0524 and n = 0.04, in just one year, little decline in q would be expected (0.900 - 0.886 in 15 generations, from the second simulation). Therefore one can predict from these simulations using realistic parameter values of s and n, the continued maintenance of high values of q.

Thus Luig and Watson's (1970) observations on the prevalence of race 21 - 1,2 in Western Australia are explicable on the basis of selection for virulence on a small but significant proportion of Eureka and possibly other Sr₆ cultivars, rather than on the absence of stabilizing selection.

1.3.6.c Virulence to Mla₆ in Erysiphe graminis hordei in the United Kingdom from 1969 - 1975

In the early 1960s cultivars of barley carrying the Mla₆ gene for resistance to powdery mildew (caused by E. graminis hordei) became

popular in Europe. As a consequence Va₆ (virulence gene to Mla₆) genotypes of the pathogen increased in frequency. At this time barley cultivars with the Mlg resistance gene were also popular and consequently the corresponding virulence gene Vg was widespread within the pathogen population. Thus Wolfe and Schwarzbach (1978a) reported that in the U.K. physiologic race survey in 1968 all samples tested from Mlg and Mla₆ hosts possessed both virulence genes Vg and Va₆. The pathogen genotype with both these genes was genetically flexible in being able to infect cultivars with the genes Mlg and Mla₆ together or singly, conferring a large selective advantage over single virulence genotypes.

In the late 1960s, Mla₆ cultivars were rapidly withdrawn from the U.K. because they became unpopular due, amongst other reasons to the ineffectiveness of the resistance. This had the effect of reducing the selective advantage of the combined virulence and the frequency of Vg and Va₆ from Mlg hosts fell progressively and significantly from 100% in 1968 to 35% in 1975 (Wolfe and Schwarzbach, 1978a). Thus during this time the unnecessary virulence gene Va₆ was gradually lost from Mlg - attacking races. It was replaced by its avirulence allele which may be called, Aa₆. Such a decline in the virulence gene frequency is consistent with the causative force being stabilizing selection, Va₆ having an average relative fitness of $1 - \underline{s}$ compared with the allele Aa₆ with a mean relative fitness of 1.

The value of $\underline{s}$ can be calculated in a similar way to that described above (section 1.3.6.bi) for P. graminis tritici. The value of $\underline{q}_0$ was taken as 0.87 (the value of $\underline{q}$ for 1969 (Wolfe and Schwarzbach, 1978a)), $\underline{p}_0$ was thus 0.13, $\underline{m} = 0.957$, $\underline{n} = 0.043$ (an average for these years calculated from data from M.Wolfe personal communication and Cereal Seed Sales Surveys statistics - see Appendix 6), $\underline{z}$, $\underline{\mu_a}$ and $\underline{\mu_v}$ were as above for the P.

graminis tritici calculations (section 1.3.6.bi). The number of E. graminis hordei generations was calculated using meteorological data (Meteorological Office Bracknell, England) for the years 1969 - 1975 and data on the incubation period of barley mildew in the U.K. (Jenkyn, 1973). For the six years in question between 158 and 268 generations were estimated to have occurred (see Appendix 7). M. Wolfe (personal communication) stated that 28 - 43 asexual generations could be expected annually giving 168 - 258 in total. The two independent estimates are similar.

The simulation was run with various values of s until a drop in q from 0.87 to 0.35 was obtained in exactly 158 or 268 generations.

The required drop in q from 0.87 to 0.35 occurred in 158 generations when s was 0.0608 and in 268 generations when s was 0.0543. Thus the virulent races of E. graminis hordei were between 5.43 and 6.08% less fit than their avirulent counterparts.

One criticism of these calculations is a possible overestimation of the generation number, as calculated in Appendix 7. Whilst at 10°C, the incubation period for E. graminis hordei is 5 - 8 days (Jenkyn, 1973), colonies continue to sporulate after eight days and consequently generations will overlap. Prolonged sporulation after the eighth day will increase the mean generation time above 5 - 8 days, thus reducing the minimum number of generations per month.

The problem of overlapping generations is commonly applicable to studies of the population genetics of microorganisms. A widely used simplification is to assume discrete generations. The effect of overlapping generations on the estimates of t made in Appendix 7 cannot be determined. However the peak of spore production of a mildew colony may occur after eight days and these generation times increase the overall mean generation time. However many of the expected longer generations may not

occur because of inhibition of colony growth. This could happen by damage due to heavy rainfall or high temperatures (above 30°C, Jenkyn and Bainbridge, 1978), or due to leaf senescence. Further the early generations of spores may colonize fresh leaf material and sporulate prior to the later generations from the original parental colonies. These granddaughter spores reduce the overall mean generation time.

Values of $\underline{s}$ were recalculated using a range of 79 - 134 generations, half of the original figures, to give $\underline{s}$ values of 0.0674 - 0.0766. This represents only a small increase in $\underline{s}$ over the values determined with the initial estimates of $\underline{t}$, i.e. 0.0543 - 0.0608. These simulations indicate that error in the estimation of $\underline{t}$ may not necessarily have a very great influence on the final values of $\underline{s}$ determined. An approximate value of $\underline{s}$ may be sufficient for reasonable conclusions to be made about pathogen evolution in cultivar mixtures or multilines (see section 2.3). Variation in $\underline{s}$ of the order 0.01 - 0.02 will not influence these conclusions, but differences of greater magnitude (> 0.10) may do so markedly (section 2.3).

1.3.6.d Virulence to $\underline{Pg}_2$ in *Puccinia graminis avenae* in North America from 1951 - 1959

1.3.6.di Calculation of $\underline{s}$

The frequency of virulence in populations of *P. graminis avenae* to the $\underline{Pg}_2$ resistance gene in oats declined from 1951 to 1959 in Canada (Martens et al. (1970) Figure 1, p. 971). In 1951 the frequency of virulence to the $\underline{Pg}_2$ gene was about 0.70. By 1959 it had dropped to 0.08. This occurred in the ecological region of western Canada where barberry is rare and hence sexual reproduction is largely absent. In

eastern Canada, sexual reproduction is common.

Virulence to the Pg₂ resistance gene had risen dramatically in the early 1940s to the high value of 0.70 in 1951. The changes in virulence frequency both the increase and decrease were explained by changes in the host resistances used (Martens et al., 1970). According to these authors the widespread use of cultivars with the Pg₂ gene led to selection for virulence. In the late 1940s and early 1950s, hosts with the Pg₁ or Pg₄ resistance genes were introduced but they did not depress markedly the popularity of Pg₂ containing cultivars which constituted 85% or more of the acreage throughout the period 1951 to 1954.

By 1955 however the cultivar Rodney with the Pg₄ gene occupied 32% of the acreage in Manitoba (the most important part of the Canadian 'rust area' (J. Martens, personal communication) which meant that although the frequency of Pg₂ hosts declined to less than 70% of the acreage it was still sufficiently high to maintain a high frequency of virulence to Pg₂ in the pathogen population. This point will be discussed below (section 1.3.6.dii).

The decline in virulence to Pg₂ in Canada is more likely to have been due to a decline in virulence in the U.S.A. In the late 1940s there was a shift from Pg₂ to Pg₁ cultivars in the U.S.A. This resulted in a shift in race predominance from races 8, 10 and 11, virulent on Pg₂ cultivars, but avirulent on Pg₁ cultivars to races 3, 7 and 12 virulent on Pg₁ cultivars but avirulent on Pg₂ cultivars (J. Martens, personal communication and Johnson, 1949). The race structure of P. graminis avenae population of the U.S.A. has a profound influence on that of western Canada since the former supplies inoculum of the pathogen for the latter, from the overwintering area^{near} the Gulf of Mexico (Martens and McKenzie,

1979). The fungus does not overwinter in western Canada. Thus any changes in the U.S.A. population structure will be reflected in the frequency of virulence at the start of the oat stem rust season in western Canada. The Canadian host can only influence virulence frequencies within a rather short growing season. Only 4 - 5 generations of the pathogen occur in Canada (J. Martens, personal communication) and thus the amount of selection on virulence genes will be limited. At the start of the following season the initial frequency of virulence will be determined by the frequency of virulence in the incoming inoculum from the U.S.A., and not the frequency of virulence at the end of the previous season in Canada.

Thus the observed decline in frequency of Pg₂ virulence in Canada is probably more a reflection of the decline in the virulence frequency in the U.S.A. population of P. graminis avenae. It occurred because of the decline in Pg₂ cultivars in the U.S.A. rather than in Canada.

These observations are consistent with the operation of stabilizing selection; that as the Pg₂ cultivars were removed from mass cultivation in the U.S.A., races with virulence to the Pg₂ gene had to compete more with races with the avirulence allele on the non selective susceptible hosts lacking Pg₂. Evidently, the latter races were on average relatively fitter than their virulent counterparts and therefore increased in frequency at the expense of races with the unnecessary virulence gene.

An estimate of the average selective coefficient (s) against unnecessary virulence to the Pg₂ gene was calculated as before using the computer model DAVE 2. The parameter values were $\underline{q}_0 = 0.70$, $\underline{q}_t = 0.08$, $\underline{\mu}_a = 1 \times 10^{-6}$, $\underline{\mu}_v = 1 \times 10^{-8}$, $\underline{m} = 1.0$, $\underline{n} = 0.0$ (this assumes that there were no Pg₂ cultivars grown in the U.S.A. during 1951 - 1959. This is probably unrealistic but gives an underestimate of s rather than an

overestimate), $\underline{z} = 1.0$ and $\underline{t} = 108$ or 135 (nine years with 12 - 15 generations per year, J. Martens (personal communication)).

If 108 generations were assumed in the 9 years, $\underline{s}$ is 0.0302; if however 135 generations were taken $\underline{s}$ is 0.0242.

Thus between 1951 and 1959 in North America races of P. graminis avenae with virulence to Pg₂ were on average between 2.4 - 3.0% less fit than their avirulent counterparts on hosts lacking the Pg₂ gene.

1.3.6.dii The effect of selection for virulence to Pg₂ in Canada on the gene frequency

It was suggested above (section 1.3.6.di) that the changes in the frequency, of the Pg₂ virulence gene q were primarily due to the changes in the U.S.A. host populations; this explaining the decline in virulence to the Pg₂ gene in the Canadian population of P. graminis avenae from 1951 - 1959 even though the acreage of hosts with the Pg₂ gene remained very high in Canada. Using DAVE 2 and assuming the proportion of the acreage sown to Pg₂ cultivar, $\underline{n} = 0.5$ and the virulence gene frequency $\underline{q}_0 = 0.500$ (at the start of a Canadian oat stem rust season) with $\underline{s} = 0.0302$, and $\underline{\mu a}$, $\underline{\mu v}$ and $\underline{z}$ set as above, in five generations (maximum for Canada), q rises to 0.967 by the end of the season. Thus the effect of the high proportion of Canadian Pg₂ cultivars on the value of q would be to raise it through the season above its initial value determined by selection in the U.S.A. Virulence to Pg₂ declined in Canada from 1951 - 1959 presumably because $\underline{q}_0$ declined each year.

Another example of selection against a virulence gene (to Pg₉) in the Canadian population of P. graminis avenae occurred in eastern Canada from 1974 to 1977 and was reported by Martens and McKenzie (1979, Figure 3). Virulence to Pg₉ dropped dramatically from about 0.84 to 0.15

in just 3 years. Frequencies of hosts with Pg₉ in Canada and U.S.A. in these years were not reported so it is difficult to ascribe the reasons for the changes in the virulence frequency.

1.3.6.e Virulence to Pg₃ in Puccinia graminis avenae in the
United Kingdom from 1977 - 1979

Jones and Jones (1980) reported the decline in virulence to the Pg₃ resistance gene in oats in the U.K. from 1977 - 1979 following the decline in the popularity of the spring-sown cultivar Mostyn , which carries both Pg₁ and Pg₃. Virulence to Pg₁ is universally present in the pathogen population and hence the decline in Pg₃ virulence could not have been due to selection against Pg₁.

Virulence to Pg₃ between 1977 and 1979 occurred in two race types, one with the oat mildew virulence factors (OMV) 1 + 3 and one with the OMV 1 + 2 + 3 virulence formula. The frequencies of the two races in 1977 - 1979 is shown in Table 13.

Table 13 Percentages of OMV 1 + 3 and OMV 1 + 2 + 3 race types
of Puccinia graminis avenae in the U.K., 1977 - 1979

Virulence group	Percentage			
	Year	1977	1978	1979
<u>OMV</u> 1 + 3		35	3	0
<u>OMV</u> 1 + 2 + 3		35	52	38
Total		70	55	38

From Table 13 it can be seen that the decline in virulence to Pg₃ from 0.70 in 1977 to 0.38 in 1979 is consistent with the causal force being stabilizing selection.

The frequency of hosts with Pg₃ in the commercial oat acreage (n) was estimated as 0.050 in 1977, 0.025 in 1978 and 0.015 in 1979 from Silvey (1981). These values are the frequencies (as measured by the percentage of spring oat seed sales from N.A.C.A.M/M.A.F.F Seed Sales Surveys 1968 - 1980) of the cultivar Mostyn in those years. According to I. Jones (personal communication) Mostyn was the only cultivar during this period with the Pg₃ gene.

I. Jones (personal communication) indicated that races which lack specific virulence to Pg₃ are nevertheless capable of slight sporulation on Mostyn seedlings; but less on adult plants. Hence z was estimated as 0.99.

The mutation rates, μ_a and μ_v were taken as 1×10^{-6} and 1×10^{-8} respectively.

I. Jones (personal communication) estimated 20 - 35 generations of the pathogen per year in the U.K.

Using DAVE 2, values of s were estimated for the years 1977 - 1978 and 1978 - 1979, taking n = 0.025 and 0.015 respectively. For the former period, average maximum and minimum estimates of s were 0.059 if 20 generations were assumed, and 0.044 if 35 generations were assumed. The equivalent values for the years 1978 - 1979 were 0.051 and 0.035 respectively.

Thus on average races with unnecessary virulence to Pg₃ were about 3.5 - 5.9% less fit than their avirulent counterparts when growing on oats lacking this resistance gene in the U.K. from 1977 - 1979.

1.3.6.f Virulence to Pi-i in Pyricularia oryzae in Japan from
1959 - 1966

1.3.6.fi Introduction and race changes

The changes in the race structure of Pyricularia oryzae in Japan were recently summarized by Kozaka (1979). He concluded "the facts suggest the changes in race prevalence depends mainly on the genotypes of the cultivars grown, evolution taking place in steps from the basic races and that unnecessary genes (virulence) seem to have less fitness for survival". Kozaka (1979) thus advocated the operation of stabilizing selection against unnecessary virulence in P. oryzae, although he did not elaborate and identify which virulence genes were selected against in the absence of the corresponding resistance gene.

According to Kozaka (1979) up to 1960 the predominant races in Japan were N-2 with virulence to the Pi-a gene in southern Japan, and race N-1 with virulence to the Pi-a and Pi-i resistance genes and race C-1 with virulence to these two genes and the Pi-k gene in addition, in northern Japan. The prevalence of race N-1 in the north is attributed to the widespread cultivation of cool-temperate resistant cultivars with Pi-i. Therefore virulence was necessary and very common. In the south virulence to Pi-i was unnecessary and generally absent. These observations whilst not proving stabilizing selection are at least consistent with the hypothesis.

Up to 1960 no modern cultivar carrying the Pi-k was grown commercially and therefore Kozaka (1979) was uncertain why race C-1 co-predominated in the north. He noted however that foreign cultivars especially of Chinese origin did carry this gene and suggested that in ancient times when the cultivar Tobishi thought to be of Chinese origin was cultivated in Japan, races virulent to it may have become naturalized. Whatever the

origin of virulence, stabilizing selection against it evidently was weak, enabling the maintenance of a high frequency of pre-existing virulence. In contrast, the frequency of virulence to the Pi-k gene did decline significantly in various prefectures in Japan between 1959 and 1965 (Kiyosawa,1980), suggesting the possible operation of stabilizing selection.

Since 1960 several cultivars, newly developed from crosses with Chinese rice were released for blast control (Kozaka, 1979). As these new cultivars were planted in increasing acreages, minor races carrying virulence to the new cultivars increased rapidly, and after 2 - 4 years, a breakdown of resistance occurred. Since damage was more severe than the disease on the old cultivar (because they had less "field" resistance, Ezuka,1979), the new cultivars were replaced by the old. In addition, cultivars with the Pi-i gene also declined gradually in popularity because of poor eating quality. As a result races C-1, C-5, C-6, C-3 and N-1 all declined in frequency. Races C-1, C-6 and N-1 at least, carry virulence to the Pi-i gene. Thus unnecessary virulence to Pi-i declined once the selective hosts were removed, consistent with the operation of stabilizing selection.

1.3.6.fii Calculation of s

Kozaka (1979) presented graphically these race changes. From his graph the following data have been deduced. In 1959 races C-1 and N-1 both with virulence to the Pi-i gene occurred with a combined frequency of about 0.82. between 1959 - 1963 their frequency declined to about 0.22. Race C-1 then seemed to disappear whilst race N-1 further declined to about 0.04 by 1966. The frequencies of the main races in Japan from 1959 - 1966 is given in Table 14.

Table 14 Frequencies of main races of Pyricularia oryzae in Japan,
1959 - 1966¹

Year	Frequency				Total
	Race	<u>C</u> -1	<u>N</u> -1	<u>N</u> -2	
	Virulence to <u>Pi</u> -	<u>a,i,k</u>	<u>a,i</u>	<u>a</u>	
1959		0.48	0.32	0.18	0.98
1960		0.35	0.16	0.39	0.90
1961		0.00	0.44	0.50	0.94
1962		0.07	0.26	0.66	0.99
1963		0.00	0.21	0.75	0.96
1964		0.00	0.24	0.79	0.93
1965		0.00	0.05	0.78	0.83
1966		0.00	0.04	0.82	0.86

¹ from Kozaka (1979)

During the years 1959 - 1964 the races listed in Table 14, C-1, N-1 and N-2 accounted for 90% or more of the P. oryzae samples and in 1965 and 1966 at least 82%. Some of the differences in the races in 1965 and 1966 was accounted for by race C-8 which appeared in 1965 and by 1966 its frequency had risen to 0.10 (Kozaka,1979). This race lacks virulence to Pi-i. Because Table 14 is not complete we can only estimate the frequency of virulence to Pi-i in each year. However the estimates are reasonably accurate since a large percentage of the samples are accounted for each year except in 1965 (Table 15). The virulence gene frequency declined from $q_o = 0.82$ to $q_t = 0.04$ in seven years.

Table 15 Frequency of virulence to the Pi-i gene in Pyricularia oryzae
in Japan, 1959 - 1966

Year	Frequency (<u>q</u>) ¹
1959	0.82
1960	0.57
1961	0.47
1962	0.33
1963	0.22
1964	0.26
1965	0.06
1966 ²	0.04

¹ calculated by summing the frequency of C-1 and N-1 (from Table 14) and dividing by the total.

² The 1966 value was calculated from a total of 0.96 which includes the frequency of C-8 (not listed in Table 14).

The degree of selection for virulence to Pi-i during this period is difficult to judge, but it is probably small. Firstly the speed of the decline was very rapid. Secondly, although Kozaka (1979) stated that the Pi-i cultivars were withdrawn gradually, in a personal communication he indicated that the popularity of these cultivars declined rapidly in the early 1960s. This could mean that the decline was gradual up to about

1960 and thereafter more rapid. Thirdly Ezuka (1979) reported that the newly released cultivars after 1960 lacked the Pi-i gene.

To calculate the average selective coefficient, s of races with unnecessary virulence to the Pi-i gene, the model DAVE 4 (section 1.3.5) was utilized. This model was used instead of DAVE 1 and DAVE 2 because the relative fitness of races with virulence to Pi-i evidently was greater on average on rice cultivars with the Pi-i gene than the avirulent races when growing on cultivars lacking that gene (section 1.3.6.fi). The fitness matrix assumed in the model DAVE 4 is :

		Pathogen allele	
		<u>Ai-i</u>	<u>Vi-i</u>
Host Allele	<u>Pi-o</u>	1	(1- <u>s</u>)
	<u>Pi-i</u>	(1- <u>z</u>)(1+ <u>a</u>)	(1+ <u>a</u>)

where Ai-i and Vi-i are the avirulence and virulence allele to the Pi-i resistance gene respectively, and Pi-o (allelic to Pi-i) is the host gene conferring susceptibility to the Ai-i allele. The term a is as defined in section 1.3.5, a coefficient of selection for virulence on hosts carrying the corresponding resistance gene. In the models, DAVE 1 and DAVE 2, a is assumed to be zero. However, here, because there was more disease on the 'resistant' plants once resistance had been overcome compared with the earlier 'susceptible' cultivars, a is greater than zero. In three sets of simulations a was taken as 0.1, 0.3 and 0.5. These are estimated, in the absence of any experimentally determined values.

Since the avirulent races with the Ai-i gene can sporulate to a small degree on hosts with the Pi-i gene (Kiyosawa,1977), z was estimated

as 0.98. However since the value of $\underline{n}$ (the proportion of the resistant hosts carrying the $\underline{Pi-i}$ gene) is small, any error in the estimation of $\underline{z}$ will barely affect the calculation provided the generation number is relatively small (section 1.3.4.civ).

The mutation rates $\underline{\mu_a}$ and $\underline{\mu_v}$ were taken as 3.3×10^{-4} and 3.3×10^{-6} respectively. These are based on experimentally determined rates quoted by Kozaka (1979) for mutation to virulence to other resistance genes in rice. The value of $\underline{\mu_a} = 3.3 \times 10^{-4}$ is simply an average of those cited by Kozaka (1979). The backward mutation rate of $\underline{\mu_v} = 3.3 \times 10^{-6}$ was estimated as $0.01 \underline{\mu_a}$.

The number of generations of selection assumed in the 7 year period was 70 - 105. This range was calculated from T. Kozaka's (personal communication) estimate of about 10 - 15 generations per year.

Four sets of simulations were conducted with $\underline{n}$ (the proportion of $\underline{Pi-i}$ hosts) set at 0.00, 0.01, 0.03 or 0.05. A range of $\underline{n}$ values was chosen because no precise data were available.

Values of $\underline{s}$ were computed in the usual way, by finding those values which allow $\underline{q}$ to decline from 0.82 to 0.04 in exactly 70 or 105 generations. The results are shown in Table 16.

A range of $\underline{s}$ values emerges from Table 16 from $\underline{s} = 0.056$ as a minimum to $\underline{s} = 0.146$ as a maximum. The upper limit calculated from high values of $\underline{n}$ and $\underline{a}$ and a low generation number ($\underline{t}$) may be unrealistically high.

Table 16 Selection coefficients against unnecessary virulence to the
Pi-i gene, in Pyricularia oryzae in Japan, 1959 - 1966

Parameter	<u>n</u> ¹	<u>a</u> ²	<u>t</u> ³	<u>s</u> ⁴
	0.01	0	70	0.077
			105	0.056
		0.1	70	0.078
			105	0.057
		0.3	70	0.080
			105	0.059
		0.5	70	0.082
			105	0.061
	0.03	0	70	0.098
			105	0.077
		0.1	70	0.101
			105	0.080
		0.3	70	0.107
			105	0.086
		0.5	70	0.113
			105	0.092
	0.05	0	70	0.119
			105	0.098
		0.1	70	0.125
			105	0.103
		0.3	70	0.136
			105	0.114
		0.5	70	0.146
			105	0.125

- ¹ n = frequency of Pi-i hosts,
- ² a = selective coefficient of virulent races on Pi-i hosts,
- ³ t = generation number
- ⁴ s = selection coefficient against unnecessary virulence

It is important to emphasize again that the s values obtained are average relative fitness differences, between populations of avirulent and virulent genotypes with a variety of background genotypes in which the avirulence (Ai-i) and virulence (Vi-i) alleles are carried. Further they are averaged over a seven year period, during which the weather, predominant cultivars grown and crop husbandry techniques may have varied considerably. Consequently, the virulence gene frequency (q) did not decline smoothly from 1959 - 1966 (Table 15). If s values were calculated on an annual basis considerable variation in the s values would ensue. Calculations based on data collected over a longer period have the advantage that short-term variations will tend to cancel each other and an average s value will be obtained. Further, selection against unnecessary virulence between seasons may differ from selection within a season on the crop plant. Data collected over seven years will include the effects of selection during at least six intercrop periods, assuming a single crop per year.

1.3.6.g Virulence to cms-T corn in Bipolaris maydis in North
Carolina, U.S.A. from 1971 - 1972

1.3.6.gi Introduction

Bipolaris maydis (Helminthosporium maydis) was responsible for the southern corn leaf blight epidemic in the U.S.A. in 1970 causing an estimated 1 billion dollars of loss (Agrios,1978). The damage was caused by race T of the pathogen, which was highly virulent on corn with the Texas male sterile cytoplasm (cms-T) grown on about 85% of the acreage of corn that year (Leonard,1974). Race 0 up to the late 60s was the dominant race, and race T was thought to have arisen from race 0 by mutation (Yoder,1980). Race 0 was able to grow and sporulate on cms-T corn but its reproductive potential was less on this host compared with race T.

After the devastating 1970 epidemic, corn with cms-T cytoplasm was removed from agricultural use so that in North Carolina in 1971 50% of the corn acreage had normal cytoplasm and 50% consisted of blends of normal and cms-T corn. In 1972 nearly all dent corn in the south east U.S.A. including North Carolina had normal cytoplasm. By 1973 all the acreage in the corn belt had normal cytoplasm. As this replacement took place, there was a very rapid replacement in the pathogen population, of race T by race 0. Table 17 shows the relevant data for North Carolina.

Table 17 Frequencies of race 0 and race T of Bipolaris maydis in
North Carolina, 1970 - 1973¹

Year	No. of isolates	% race 0	% race <u>T</u>
1970	45	9	91
1971	250	8	92
1972	30	83 ²	17
1973	28	100 ²	0

¹ data from Leonard (1974)

² does not include isolates from corn known to have cms-T cytoplasm. None of the isolates of B. maydis obtained from corn with normal cytoplasm in 1973 was race T.

These results suggest that strong stabilizing selection was operating on race T when its virulence to cms-T corn was unnecessary. In its absence, race T was forced to grow on normal cytoplasm corn, and in competition with race 0, race T clearly had a selective disadvantage, of coefficient s and was rapidly replaced. The average relative fitness of race 0 and race T on normal cytoplasm corn was 1 and $1-s$ respectively.

1.3.6.gii Calculation of s

The value of s could be estimated for the period 1971 - 1972 when the frequency of race T fell from 0.92 to 0.17 in just one year.

The fitness matrix of the two races on the two host genotypes is similar to that for the rice blast system described in section 1.3.6.fii, and is shown below :

		Pathogen race	
		0	<u>T</u>
Host cytoplasm	Normal	1	$(1-s)$
	<u>cms-T</u>	$(1-z)(1+a)$	$(1+a)$

Race T on cms-T corn clearly has a greater fitness (reproductive potential) than race 0 on corn with normal cytoplasm as evidenced by the rapid replacement of race 0 by race T when cms-T corn was grown widely. Therefore $\underline{a} > 0$, and the model DAVE 4 (section 1.3.5) must be used.

A value of $\underline{z} = 0.43$ was obtained from Leonard (1977b), who calculated it from a race competition experiment.

In a personal communication, K. Leonard indicated that the average relative fitness of race 0 on normal cytoplasm and cms-T corn are approximately equal. Consequently, from the fitness matrix it follows :

$$(1-\underline{z}).(1+\underline{a}) \approx 1$$

$$\text{If } \underline{z} = 0.43$$

$$0.57 .(1+\underline{a}) \approx 1$$

$$\underline{a} \approx \frac{1}{0.57} - 1 \approx 0.75$$

The mutation rates μ_a and μ_v were estimated as above (sections 1.3.6.bi, 1.3.6.c, 1.3.6.di and 1.3.6.e) as 1×10^{-6} and 1×10^{-8} respectively.

The frequency ($\underline{n}$) of cms-T corn assumed to be present in North Carolina was taken as 0.000 and 0.005 in two separate simulations. These estimates are based on Leonard's (1974) claim that by 1972 nearly all dent corn in the S.E. U.S.A. had normal cytoplasm. In a personal communication, K. Leonard thought that for North Carolina in 1972, a maximum possible value of $\underline{n}$ would be 0.01 although he considered 0.005 to be more realistic. He also stated that in North Carolina there are on average about 6 - 10 generations of B. maydis each year. However in 1972 there were no records of blight in commercial fields before the end of July. If seed is planted in early April, blight can appear by the end

of the same month given suitable conditions. However the generation time is longer in the earlier part of the growing season than in the last 50 days or so, because sporulation is quicker on senescing tissue. Leonard however qualified the report of the absence of disease prior to the end of July, because the survey was not extensive and suggested that there had already been several cycles of pathogen reproduction before that time. A total of 5 - 9 generations for the whole season was estimated.

Using these data, s values were calculated from the model DAVE 4 in computer simulations of a decline in q from 0.92 to 0.17 in 5 or 9 generations. The results are shown in Table 18.

Table 18 Selection coefficients (s) against unnecessary virulence of race T of Bipolaris maydis to cms-T cytoplasm corn in North Carolina, 1971 - 1972

Frequency of <u>cms-T</u> corn (<u>n</u>)	Generation number	<u>s</u>
0.000	5	0.553
	9	0.361
0.005	5	0.559
	9	0.366

The values of s obtained 0.361 - 0.559 are exceptionally high but this is not surprising since the drop in q from 0.92 - 0.17 occurred very rapidly in just a single year. Race T evidently was very unfit on corn with normal cytoplasm in comparison with race 0.

The effect on the s values obtained of varying n from 0.000 to 0.005 is small (Table 18).

1.3.6.giii Implications of high s values

The minimum s value calculated for unnecessary virulence in race T of B. maydis to cms-T cytoplasm corn was 0.361 (Table 18).

Using equation 1.3.2.11

$$\underline{q_b} \approx \mu a / \underline{s}$$

the balance frequency of race T (q_b) is $1 \times 10^{-6} / 0.361 = 2.77 \times 10^{-6}$. Using the model DAVE 4 it can be shown that this frequency is reached starting from q₀ = 0.17 (the frequency of race T in North Carolina in 1972) in just 67 generations. With 6 - 10 generations per season this decline would take a maximum of 11 years. The actual time to reach balance in fact might be much less, since more than six generations might occur in a season, and the s value may be greater than 0.361 (Table 18).

The implications of the very short time necessary for stabilizing selection to cause the race T frequency to reach its low balance value, are that theoretically it should be possible to re-introduce cms-T cytoplasm corn in North Carolina after about 10 years of growing only normal cytoplasm corn. A number of years of use with these cms-T cultivars should be possible before race T is selected again in epidemic proportions. This suggestion is supported by the fact that cms-T corn was introduced commercially as early as the 1950s. There were at least 10 years of successful use of these cultivars without a race T epidemic, despite the claim of Nelson, Ayers, Cole and Peterson (1970) that race T was present widely long before the 1970 epidemic, even as early as 1955.

Nelson et al.'s (1970) claims about race T have been contended by Leonard (1972, 1973) who advocated an origin of race T just prior to the 1970 epidemic. However, even in the 1950s, race T must have been present at low levels (at least) simply due to mutation from race 0 to race T. Prior to the introduction of cms-T cytoplasm corn, race T probably occurred at a frequency equal to its balance value.

K. Leonard (personal communication) considered that μ_v might be greater than μ_a because race T virulence depends on the production of a host specific toxin (Yoder, 1980), therefore the gene for virulence might be dominant. If $\mu_a = 1 \times 10^{-8}$ and $\mu_v = 1 \times 10^{-6}$ the value of q_b becomes $1 \times 10^{-8} / 0.361 = 2.77 \times 10^{-8}$. The time to reach this balance value from $q_0 = 0.17$, is only 68 generations just one more than when μ_a was taken as 1×10^{-6} . Instinctively one might expect the time to reach the lower balance frequency to be much greater. However, since in the simulations with the lower balance frequency, μ_a is very small ($\mu_a = 1 \times 10^{-8}$) less race T is generated from race 0 by mutation, and more of race 0 is produced from race T by back mutation because μ_v is greater compared with the simulation where μ_a was greater and μ_v smaller. Consequently, the speed of the decline to the balance frequency of 2.77×10^{-8} (with $\mu_a = 1 \times 10^{-8}$, $\mu_v = 1 \times 10^{-6}$) is quicker than the speed of decline to a balance frequency of 2.77×10^{-6} (with $\mu_a = 1 \times 10^{-6}$, $\mu_v = 1 \times 10^{-8}$).

Hence altering the mutation rates in this hardly alters the time to reach the balance frequency.

If μ_a is set at 1×10^{-5} , whilst μ_v is kept at 1×10^{-8} the time to balance is reduced to 62 generations compared with the 67 generations when $\mu_a = 1 \times 10^{-6}$. Alternatively if μ_a is set at 1×10^{-7} the time to balance is increased to 71 generations. Consequently the time to reach balance increases as μ_a is reduced, provided μ_v and s are kept constant. Since there is only a nine generation difference

representing 1 to 2 years resulting from a 100 fold difference in μ_a , it is clear that error in the estimation of the mutation rates should not alter the basic conclusions reached in this section, that cms-T cytoplasm corn could be successfully replanted in North Carolina.

1.3.6.h Discussion

A summary of the results of section 1.3.6 are shown in Table 19.

Table 19 Summary of stabilizing selection coefficients (s) calculated in section 1.3.6

Pathogen	Unnecessary virulence gene to	Country	Year	Minimum <u>s</u>	Maximum <u>s</u>
<u>Puccinia graminis tritici</u>	<u>Sr</u> ₆	Australia	1948-1955	0.0400	0.0524
<u>Erysiphe graminis hordei</u>	<u>Mla</u> ₆	U.K.	1969-1975	0.0543	0.0608
<u>Puccinia graminis avenae</u>	<u>Pg</u> ₂	Canada	1951-1959	0.0242	0.0302
<u>Puccinia graminis avenae</u>	<u>Pg</u> ₃	U.K.	1977-1979	0.0350	0.0590
<u>Pyricularia oryzae</u>	<u>Pi-i</u>	Japan	1959-1966	0.0560	0.1460
<u>Bipolaris maydis</u>	<u>cms-T</u>	U.S.A.	1971-1972	0.3610	0.5530

Most of the selective coefficients lie between about 0.02 - 0.06. The coefficients of selection against race T of B. maydis are clearly exceptionally high. The implications of low s values to strategies for the use of resistance genes in agriculture will be discussed later (section 2.3).

All the examples in Table 19 and section 1.3.6 fit a classical stabilizing selection prediction that if an unnecessary virulence gene is relatively less fit on average compared with the avirulence gene when populations of pathogens carrying these alleles infect hosts lacking the corresponding resistance gene, then the removal of the selective host from mass agricultural use should be accompanied by a decline in the frequency of the virulence gene in the population. Further, all the pathogens have large population sizes and consequently the decline in the virulence gene frequency cannot be attributed to a 'hitch hiking' of a neutral gene because of linkage to another selectively disadvantageous gene (section 1.3.6.bi).

It could be argued that although the population sizes of these pathogens are large within a season, between seasons the overwintering or oversummering populations are much smaller; consequently the effects of genetic drift might be significant in changing virulence gene frequencies between seasons. Drift might then confound the effects of stabilizing selection and could provide an alternative explanation for declines in virulence. This possibility seems unlikely for several reasons. Firstly although the between-season population is much smaller than the within-season population, in absolute terms the former population size may still be too large for drift effects to be significant, especially if the area in which the overwintering or oversummering occurs is large. The second and most important reason is that if drift was significant in changing virulence gene frequencies between seasons, there should be marked and random fluctuations in these frequencies. This does not seem to occur. In many cases there are clear trends of progressive increases and decreases in the virulence gene frequencies over several years which can best be explained by directional selection. Each new season the

progressive increase or decline in virulence continues from the previous season; hence drift effects do not alter the basic trend. The decline in a virulence gene frequency may not always be totally smooth (see Watson and Luig, 1963; and Table 13, section 1.3.6.e, Table 15, section 1.3.6.fii), and it is possible that although drift may not alter or override the basic trend caused by selection it might affect virulence gene frequencies slightly in the short term. Although drift could be a source of variation, other factors could also be responsible for altering the smoothness of the trend, for example sampling variation, differences in selection within seasons and climatic factors.

This method of estimating values of the selective coefficient against unnecessary virulence is easily applicable to other pathogens, where suitable field data is available. Because of the increased use of race and virulence gene surveys by government agricultural establishments many data are available which show declining virulence gene frequencies. Some more likely examples of stabilizing selection include :

- 1) The decline in the frequency of virulence to the Pg₉ resistance gene of oats in P. graminis avenae in eastern Canada from 1974 - 1977 as reported by Martens and McKenzie (1979). The frequency of virulence declined from 0.84 to 0.15 in the three year period.
- 2) The decline in the frequency of virulence to the Sr₁₅ resistance gene of wheat in P. graminis tritici in Canada from 1964 - 1969 as reported by Mackenzie (1979). The frequency of virulence declined from about 0.39 to 0.11 in the five year period. This decline was significant statistically (Mackenzie, 1979), and Mackenzie although clearly not a supporter of the concept (of stabilizing selection)

was forced to conclude that the decline in virulence to the Sr₁₅ gene could well be an example of stabilizing selection. This is especially interesting since Van der Plank (1968) presented convincing evidence that this resistance gene was 'weak' in Australia. This means that s values for the same unnecessary virulence gene might vary considerably in different countries. Further s values theoretically might change with time within a country, although Van der Plank (1968) considered that pathogen evolution to reduce the effects of stabilizing selection was unlikely, based on P. infestans populations in Mexico (see also section 1.1.4.b). Further as stated earlier in section 1.3.6.biii virulence genes are more likely to occur in background genotypes that are relatively fit and common, and further evolution of the background genome is likely to be deleterious rather than beneficial.

The stability of s values is however a very important factor when considering strategies for the use of resistance genes, such as cultivar mixtures and multilines, and has not been investigated seriously. This is hardly surprising since the concept of selection against unnecessary virulence itself is still the subject of much controversy and is not accepted in many quarters.

- 3) The decline in the frequency of virulence to the Lr₁₆ resistance gene of wheat in P. recondita tritici in Canada from 1966 to 1974 as reported by Samborski (1975). This frequency declined from 0.56 to 0.06 from 1966 - 1974 following the removal of wheat cultivars, e.g. Selkirk with the Lr₁₆ resistance gene. By 1977 the frequency of virulence to Lr₁₆ had declined to zero (Samborski, 1978).

- 4) The decline in the frequency of virulence to the Sr_{9a} resistance gene of wheat in P. graminis tritici in the U.S.A. from 1972 to 1976. The data comes from Roelfs and McVey (1973) and Roelfs, Long, Casper and McVey (1977). The frequency of virulence declined from 0.45 to 0.10 in the four year period.

For various reasons it was not possible to estimate values of s from these examples, mainly due to lack of information on pathogen generation number (t) or the frequency of hosts with the resistance gene in question (n). However, the examples are further proof of the existence of stabilizing selection.

1.4 Experimental work to test for the existence of stabilizing selection in *Fulvia fulva* and *Erysiphe graminis hordei*

1.4.1 Introduction

As discussed earlier (section 1.1.4.d and 1.1.5) there are many problems and limitations associated with glasshouse experiments when used to determine the existence of stabilizing selection or to calculate values of $\underline{s}$. The two prime problems are that conditions (hence competition) in the field differ from those in the glasshouse and secondly in the latter experiments usually only a limited number of isolates of each virulence genotype are employed whereas in the field experiments involving natural inoculum, populations of many isolates of the pathogen are involved. Consequently, in glasshouse experiments the effects of unnecessary virulence on the relative fitness of pathogens may be masked or distorted by the effects of the differing background genotypes carried by the few isolates involved. However, as Leonard (1969, 1977a) has demonstrated the latter problem is not insurmountable if the effects of the background genotypes are nullified. Unfortunately but not surprisingly there is no solution to the first difficulty of the difference in environment between the glasshouse and the field. However, suitable glasshouse experiments may still be useful in determining the existence of stabilizing selection, if not the size of the selective coefficients involved. An example of this was discussed in section 1.3.6.bi with respect to unnecessary virulence to the $\underline{Sr}_6$ resistance gene of wheat. I estimated $\underline{s}$ from field data as between 0.0400-0.0524 whereas Lenoard (1977a) calculated $\underline{s}$ to be 0.42 from data from Watson and Singh (1952) which showed the decline in the frequency of the unnecessary virulence gene during several glasshouse race competition experiments. Whereas the glasshouse experiments and the field data both confirmed the existence of stabilizing selection,

the former yielded exaggerated values of $\underline{s}$, possibly due to the more intense selection in these experiments and the artificial creation of discrete generations (see section 1.1.5 for a discussion of this point). Thus these glasshouse experiments are useful in confirming the existence of selection against unnecessary virulence but not in calculating the magnitude of the selective force.

Two experimental techniques were employed to test for the existence of stabilizing selection. The first employs sporulation yields as an estimate of relative fitness of the pathogens; the second involved a traditional race competition experiment using a mixture of two isolates.

1.4.2 Experiments to determine the nature and magnitude of selection in respect of unnecessary virulence to the $\underline{Cf}_4$ resistance gene in the tomato leaf mould pathogen, Fulvia fulva

1.4.2.a Introduction to the disease and to the pathogen

Tomato leaf mould, caused by F. fulva (= Cladosporium fulvum) is the most severe foliar pathogen of glasshouse tomatoes (Dixon, 1981). Its distribution is world wide (Chapp and Sherf, 1960) and occasionally field crops in moist tropical or sub tropical areas have also suffered from destructive outbreaks of the disease (Dixon, 1981).

In temperate climates however it is only usually a serious disease in glasshouses where the humidity is very high through poor ventilation (Wheeler, 1969); it is rare in the open (Butler and Jones, 1955).

Small (1930) showed that the best development of the pathogen occurred at relative humidities exceeding 90% and at temperatures of about 22°C.

The biology of the pathogen and the disease have been summarized by Butler and Jones (1955). Sporulation occurs abundantly within a range of temperatures from 20° - 26°C. The maximum temperatures in which sporulation can occur is between 31° - 33°C, the minimum between 0° - 1°C.

Control of the pathogen is achieved by cultural methods especially cleaning out leaf debris and controlling the humidity in the glasshouse; by the use of specially bred resistant cultivars carrying one or more leaf mould resistance genes (Cf genes); or by the use of systemic and eradicant fungicides (Butler and Jones, 1955; Wheeler, 1969; Dixon, 1981). The pathogen typically has quickly overcome the resistant cultivars by the production and spread of new races which carry specific virulence genes to the corresponding resistance genes. Day (1956) demonstrated a gene for gene relationship between the factors for host resistance and pathogen virulence.

Because of the genetic adaptability of the pathogen the use of resistant cultivars has not been as successful as hoped for by breeders and pathologists. However some newer cultivars such as Sonatine and Nemato are resistant to all five race groups A, B, C, D and E of the fungus (ADAS Booklet 2243, 1980), and at present the pathogen is moderately well controlled in north west Europe.

1.4.2.b Introduction to the experiment

Johnson and Taylor (1976) argued that the use of spore yields for assessing the growth of pathogens and the susceptibility of hosts is extremely accurate and sensitive. Certainly, the data yielded is objectively numerical and hence easy to handle statistically. Further, sporulation differences between a pathogen isolate growing on two hosts result from a summation of the differences at various stages of pathogen development. Factors such as germination frequency, germ tube

development, internal tissue colonization, abundance of conidiophores, conidiation rate and latent period are all ultimately expressed each generation as spore output. The only factors for fitness escaping detection are likely to be spore longevity and the total period over which spores are being produced.

Consequently, it was considered that spore production could be used to detect the predicted loss in relative fitness conferred by an unnecessary virulence gene if stabilizing selection is operating. A race 4 isolate of the pathogen carrying virulence to the Cf₄ resistance gene (and possibly the Cf₁ and Cf₃ resistance genes, see section 1.4.2.c) was cultured on two near isogenic tomato lines one carrying the Cf₄ resistance gene, the other lacking any Cf genes. On the Cf₄ hosts the pathogen carried only necessary virulence to this resistance gene, whereas on the Cf₀ line, this virulence gene was unnecessary for successful infection. Therefore if unnecessary virulence to Cf₄ is selectively disadvantageous this should be reflected as a difference in sporulation, race 4 producing more spores on the Cf₄ plants.

There were several reasons for choosing the Cf₄, race 4 combination for this study. Firstly, the availability of suitable host and pathogen material namely seed of the two near isogenic host lines (described above and in section 1.4.2.c), and a healthy and aggressive isolate of the pathogen. Secondly, the Cf₄ resistance gene gives the carrier immunity to pathogens lacking corresponding virulence, unlike the Cf₁ or Cf₃ resistance genes (de Wit, 1977). This was important to help check the genotype of the Cf₄ plants. M. Gerlagh (personal communication) reported that at the I.P.O. in the Netherlands because of the difficulty in clearly identifying the virulence genes 1 and 3 they disregard them when naming races. Hence race 0 might be race 0 or race 1 or race 3 or race 1,3. Similarly the race 4 isolate supplied by Gerlagh

and used in these experiments might be race 4 or race 1,4 or race 3,4 or race 1,3,4. The problem arises because the genes Cf₁ and Cf₃ only encode partial resistance to the pathogen. The sporulating lesions are easily confused with those produced on fully susceptible genotypes (Laterrot and Clerjeau, 1979). For similar reasons the French workers too omit the Cf₁ and Cf₃ resistance genes from their range of differential hosts used to classify races of F. fulva (Laterrot and Clerjeau, 1979).

The possibility that the race 4 isolate might carry virulence to the Cf₁ and/or the Cf₃ resistance genes is not significant, since these resistance genes and their corresponding virulences are not considered important as differentials or in the classification of races, respectively. Further, since the race 4 isolate was used as the only pathogen isolate in these experiments, the effect of carrying virulence to Cf₁ and/or Cf₃ on relative fitness is cancelled. If instead of using two host genotypes (Cf₀ and Cf₄) and one pathogen genotype (race 4), the experimental design involved two pathogen genotypes (race 0 and race 4) and one host genotype (Cf₀) then the unknown virulences potentially carried by race 0 and race 4 might differ and the effects on pathogen fitness of unnecessary virulence to Cf₄ might be confused with the effects on the fitness of unnecessary virulence to Cf₁ and/or Cf₃. However in the experimental design used here, virulence to Cf₁ and/or Cf₃ cannot confound the effects on fitness of unnecessary virulence to Cf₄.

A third reason for using the Cf₄/race 4 combination was the suspicion that the Cf₄ resistance is a 'strong' resistance gene. This was deduced from the data of Kooistra (1964) on the longevity of resistance of certain cultivars carrying the Cf₄ gene. Thus in 1954 in Canada a cultivar called Vagabond was introduced, carrying the Cf₂ and Cf₄ resistance genes. The Cf₂ resistance gene had earlier proved to

be ineffective at conferring lasting resistance. In 1939 the cultivar Vetomold carrying the Cf₂ gene was introduced in Canada. Shortly afterwards virulent races appeared and the Cf₂ resistance gene became ineffective against these races. Consequently, at the time of the introduction (1954) of Vagabond (Cf₂ Cf₄) virulence to Cf₂ was probably common in Canadian populations of the pathogen and the longevity of resistance of Vagabond thus could not be attributed to a lack of virulence to the Cf₂ gene. Kooistra (1964) reported that up to the time of writing Vagabond had remained resistant not only in Canada but also elsewhere. However, Cf₄ resistance alone 'broke down' in Canada and Holland in the same year 1962 (M. Gerlagh, personal communication). Despite this the period of successful commercial use of the Cf₄ gene was comparatively long, especially when compared with the Cf₁, Cf₂ and Cf₃ genes where virulence to these resistances appeared very quickly after their respective introductions (M. Gerlagh, personal communication). The delay in appearance of races with virulence to Cf₄ may thus have been due to a rarity created by strong selection against unnecessary pre-existing virulence (section 1.2).

1.4.2.c Materials and methods

1.4.2.ci The pathogen

The isolate of race 4 of F. fulva used in the experiment was supplied by Gerlagh in April 1980. As stated above (section 1.4.2.b) this isolate could have been race 4; race 1,4; race 3,4 or race 1,3,4 but the unknown virulences were not significant. This strain R of the pathogen designated B10379 had been isolated from a Cf₄ tomato line called Purdue 135 . The culture B10379 was dated the 5th March and had been grown on an agar slope in a test tube. On arrival (25.4.80) it was

subcultured onto 0.25% malt agar (Bailey, 1950) on slopes in McCartney bottles and in petri dishes, and placed in a Gallenkamp illuminated incubator at a temperature of 22°C and a light intensity of 1.88 W/m². The pathogen was subcultured regularly, about every four weeks usually onto a medium of 0.25% malt agar. Sporulation on this medium was found to be moderate and acceptable for most purposes, such as inoculum preparation. Sporulation on plants is considerably more profuse and this proved to be the best way to maintain the pathogen. Culture maintenance was usually carried out on the tomato cultivar Moneymaker which lacks all known Cf genes.

Occasionally subcultures of the pathogen were prepared on other media such as 6% oat meal agar (Day, 1954), V8 juice agar and potato dextrose agar (P.D.A.). On the latter medium a race 0 isolate produced a high incidence of white cultural mutants, which were often sterile. Consequently, P.D.A. was rarely used. Sporulation on oat meal agar was quite abundant (as reported by Day, 1954), but not usually better than on the dilute malt agar. Sporulation on V8 agar as on potato dextrose agar was generally poor.

Subculturing was carried out using a sterile loop or needle in a lamina flow cabinet, whose surfaces had been cleaned with a 70% alcohol solution. Most cultures were kept on slopes in small McCartney bottles 7.6 x 2.5 cm (3" x 1"). Contamination (rare in McCartneys, occasional on petri dishes) was reduced by the use of chloramphenicol (100 mg/l) and by sealing petri dishes with "sellotape" .

1.4.2.cii Tomato plants

Seed of tomato lines T128, homozygous for the Cf₄ resistance gene, and lacking other known Cf genes, and seed of Craigella GCR 26 lacking all known Cf genes were supplied by Dr T. Hall from the G.C.R.I. Line T128 carries a background genome, 97% of which is based on the cultivar

Ailsa Craig (T. Hall, personal communication). Craigella is an Ailsa Craig type with a single recessive gene for uniform ripening. Consequently, Craigella and T128 are very similar genetically except for the differences at the Cf₄ locus.

Seed was stored in a refrigerator at 9°C. Plants were grown in Levington's potting compost in small trays 35.6 x 22.9 cm (14" x 9"), in five rows of ten seeds. The trays were kept in a greenhouse at South Kensington. The natural lighting was supplemented with eight Phillips mercury lamps (400 W) suspended over the benches. The artificial photoperiod was 16 hr and the approximate light intensity at bench level was 35.5 - 39.5 W/m². Despite thermostatic control plants were subjected to considerable diurnal and season temperature fluctuation, especially during periods of intense sunlight. As commercial crops would also be subject to similar influences no further attempts were made to reduce this variation.

After about two weeks when plants had reached about 5.1 cm (2") in height with the cotyledons fully expanded, individual seedlings were transplanted to a mixture of Levingtons compost, John Innes No. 2 and sand in 12.7 cm (5") square pots. After a few more weeks when the plant height was about one foot, and five or six leaves had expanded, they were supported by thin wooden stakes.

Occasionally the plants were watered with Long Ashton's nutrient medium.

1.4.3.ciii Inoculation and incubation of tomato plants
with F. fulva and subsequent development of
infection

Low humidity levels in many of the glasshouse compartments necessitated the use of techniques to increase and maintain high humidities around

the plants during incubation. Specially constructed chambers made from a wooden framework with dimensions of approximately 63.5 x 55.9 x 88.9 cm and covered with thick transparent polythene placed on the glasshouse bench top proved highly satisfactory. In some experiments the plants and chambers were placed in a glasshouse compartment similar to that described above (section 1.4.3.cii). Usually up to 16 individual plants in 12.7 cm (5") square pots fitted well into the chamber. Although the plants were closely packed it was sometimes necessary to water the chamber from the bottom, usually daily to help maintain a high relative humidity, necessary not only for initial infection but also for later development including sporulation (Small, 1930).

In other experiments automatic chamber irrigation was employed, using continuous bench top watering. This compartment was provided with both mercury lamps and fluorescent strip lights, providing a light intensity at bench level of approximately 19.8 W/m^2 .

The third location for incubating inoculated plants was in a glasshouse misting chamber. The periodic spraying from vertical water pipes above the bench top emitted a fine spray from a nozzle at the top. The polythene tent was placed over one of the pipes so that the plants inside were periodically sprayed. The advantage of the misting chamber was that relative humidities of 90 - 100% were easily maintained from inoculation to sporulation and harvesting of spores. This was not always the case in the plastic chambers in the other glasshouse compartments even with automatic bench top watering. At times the relative humidities dropped below 70%, a value at which infection is rarely observed (Small, 1930). Another advantage of the misting unit was that temperatures typically were close to 22°C (the optimum for pathogen development, Small, 1930). A disadvantage was the absence of supplementary illumination, resulting in

low growth rates especially in winter. Inoculation of the plants was achieved by spraying each plant with 5 - 10 ml of a spore suspension using a Shandon spray gun) onto the undersurfaces of individual leaves. The suspensions were prepared from either slope cultures in McCartney bottles or from dried infected leaves stored in plastic bags. From the former source spores were dislodged using distilled water poured into the bottles, the suspension was shaken and the surfaces gently scraped with a needle or loop. The suspensions were filtered through muslin (usually a double layer) and added to a bulk spore suspension. The concentration of the spore suspension was determined by haemocytometry, before loading onto the spray gun.

Infected leaves were used more commonly as an inoculum source due to the much higher spore yields obtained. Several dried leaves infected with the appropriate race were placed into a glass jar with 50 - 100 ml of distilled water, and shaken violently by hand and on a vortex mixer for two minutes. The resulting suspension was then filtered and the concentration of conidia determined as above.

After inoculation plants were immediately incubated at high humidities in the polythene chambers described above. Usually they were put into the chamber a few days prior to inoculation. The development of disease symptoms was typically similar to that reported by other authors (Butler and Jones, 1955; Wheeler, 1969 and Dixon, 1981), with yellow spots developing on the upper surface 7 - 10 days after inoculation. On the undersurface soon afterwards very light grey tufts of mould develop, which later turn pale buff and then brown as sporulation becomes abundant. Spores were usually harvested about three weeks after inoculation. Sometimes abscission of highly infected leaves from some plants had already occurred by this stage.

1.4.3.civ Testing the genotype of the race 4 isolate

Thirty ml of a spore suspension at a concentration of about 185,000 spores/ml were sprayed onto two seedlings possessing the Cf₄ gene. Fifteen days later both plants were well infected, several leaves had abscised and microscopically the presence of F. fulva was proved from conidium and conidiophore morphology, checked against the C.M.I. (1976) description of the pathogen. Plate 1 shows typical conidia of the pathogen. Virulence to Cf₄ was therefore present in the race 4 isolate.

In a second experiment the race 4 isolate was tested on a range of host genotypes namely plants carrying the Cf₁ or Cf₂ or Cf₅ or Cf₆ or Cf₁, Cf₃ or Cf₁, Cf₂ or Cf₂ Cf₃ or Cf₂ Cf₄ genes raised from seed provided by Dr T. Hall at the G.C.R.I. Plants carrying no Cf genes provided a control. Seedlings were transplanted two weeks after sowing into 12.7 cm (5") pots, two plants per genotype were used. After three days of growth under the polythene tent the plants were sprayed with 100 ml of a conidial suspension at 500,000/ml. The plants were then placed randomly in the polythene chamber. Fifteen days later the plants were examined and scored.

Results

Cf₀ plants

The control plants were very heavily infected with leaf mould. This was further verified by microscopic examination of spores washed from the leaf surface. The control confirmed that the conditions for infection were suitable and that the pathogen was viable.

Cf₁ plants

Some leaves were very chlorotic with a few light green spots

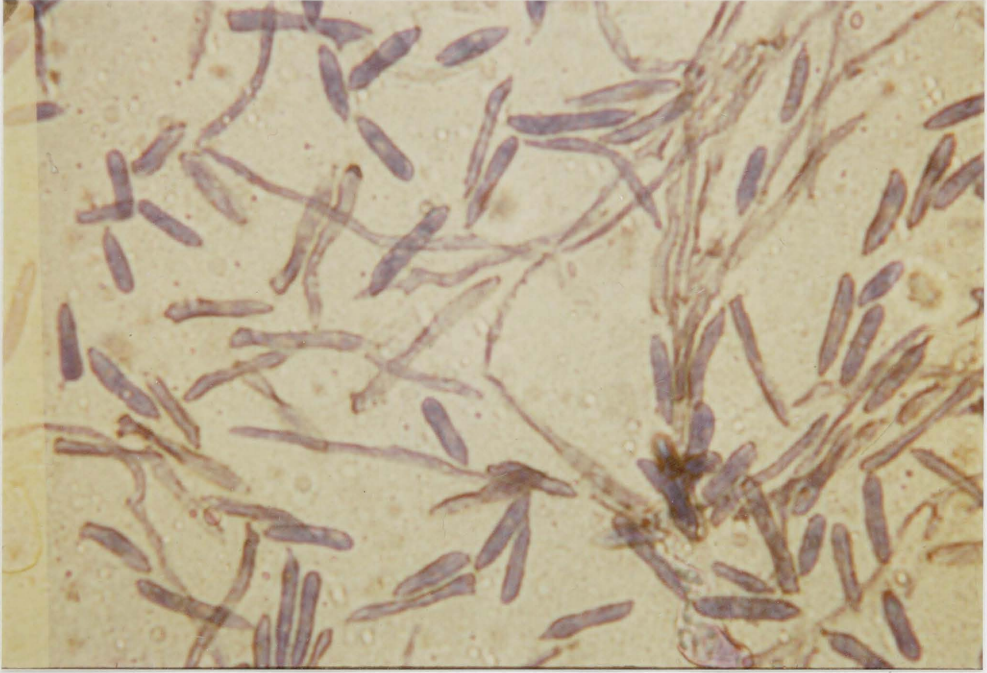


Plate 1. Conidia of Fulvia fulva.

400 x magnification.

remaining on most severely affected leaves. However typical leaf mould was not observed, even though some spores of the pathogen were found. These could have been left over from the initial inoculum. From these results, and the observations made above (section 1.4.2.b) concerning the reaction of a host with Cf₁ and an avirulent pathogen, it was concluded that the race 4 isolate lacks virulence to Cf₁.

Cf₂ plants

Leaves were yellowed and spotty but no infection was visible. Race 4 lacks virulence to Cf₂ therefore.

Cf₅ plants

Some leaves were yellowed at the edges on both the adaxial and abaxial surfaces. However there was no visible signs of infection, and no sporulation. The pathogen isolate appears to be avirulent on Cf₅ plants.

Cf₆ plants

There was some yellowing of the lower leaves, but again there were no signs of infection. The race 4 isolate carries avirulence to this resistance gene.

Cf₁, Cf₂ plants

Some leaves and leaflets were very yellow, and some leaflets were brownish with a dry black spotting. There was no visible leaf mould infection. A small amount of distilled water was washed over some leaflets and microscopically revealed some spores as from Cf₁ plants, though much less than from infected Cf₀ plants. The results confirm that the

race 4 isolate lacks virulence to Cf₁ or Cf₂ or both resistance genes.

Cf₁ Cf₃ plants

The results were similar to those for the Cf₆ plants. It was earlier concluded that the race 4 isolate lacks virulence to the Cf₁ gene. Because the reaction of race 4 on Cf₁ plants seemed more deleterious to the plants than on Cf₁ Cf₃ plants, it was concluded that the race 4 isolate, also lacked virulence to Cf₃. In support of this conclusion, both lines, T134 with Cf₁ and T131 with Cf₁ Cf₃ carry similar background genetic factors for resistance to leaf mould (T. Hall, personal communication). Unfortunately the seed of the Cf₃ line did not germinate and so a definitive test was not possible.

Cf₂ Cf₃ plants

Leaves were yellowed and spotted and possibly infected with another parasite. There was no evidence of leaf mould infection however. This test confirmed the earlier conclusion, that race 4 is avirulent on Cf₂ genotypes.

Cf₂ Cf₄ plants

All leaves were entirely green; an apparently immune response. This result confirms the lack of virulence to the Cf₂ resistance gene carried by the race 4 isolate. The background genotype of these Cf₂ Cf₄ plants differed from the Cf₂ plants (T. Hall, personal communication), and this could explain the very different symptoms on these two genotypes.

In conclusion, the race 4 isolate used in these experiments lacks virulence to the Cf₁, Cf₂, Cf₅, Cf₆ genes and possibly to the Cf₃ gene.

However, it possesses virulence to the Cf₄ resistance gene.

1.4.2.cv Experiments to compare sporulation of race 4
on Cf₀ and Cf₄ plants

Experiment 1

Four plants each of Craigella (Cf₀) and ^{T₁₂₈}/Cf₄ Cf₄) were grown in the usual way (section 1.4.2.cii) under identical conditions. At an age of about 3 - 4 weeks they were inoculated (section 1.4.2.ciii) with 10 ml of a spore suspension at a concentration of 155,000 conidia/ml. The inoculated plants were placed in a chamber in the misting unit of the glasshouse; each plant was assigned randomly a place in the chamber. After two weeks there was no sign of infection so the plants were re-inoculated using a much younger sample of race 4 spores than had been first used. The spore suspension had a concentration of 225,000 spores/ml and each plant was sprayed with 10 ml. The following day the chamber and the plants were moved to a illuminated chamber (section 1.4.3.ciii) to provide a slightly higher temperature than in the misting unit. Periodically, the plants were atomized with water in order to maintain a high relative humidity.

Five days later symptoms of leaf mould appeared which developed over the next eight days. On the eighth day the plants were atomized with water twice to help increase sporulation. The next day plants were removed from the glasshouse and sporulation on the leaves compared.

All infected leaflets of a plant showing visible symptoms were detached and placed in a small jar, 100 ml of distilled water was added and shaken by hand for exactly one minute. The mixture was filtered through a single layer of muslin and the spores were counted using a haemocytometer,

six counts per *suspension*. Before a drop was placed onto the slide, the filtered *suspension* was shaken to ensure even distribution of spores by preventing their settling to the bottom. The results are shown and discussed in section 1.4.2.cvi.

Experiment 2

Sixteen individually potted plants, eight of the cultivar Ailsa Craig (Cf₀) from Sutton's, Torquay and eight of line T128 (Cf₄) grown in similar conditions were inoculated 40 days after sowing with 10 ml of a race 4 suspension at a concentration of 150,000 spores/ml. The plants were incubated alternately in a 4 x 4 chess board pattern in a plastic chamber.

Nine days after inoculation early symptoms of leaf mould were noticed.

Twenty one days after inoculation the plants were removed from the glasshouse and scored. Plants were not heavily infected; leaves were silvery blue in colour. At the time of harvesting the typically velvety mould was absent, although present earlier. It is likely that lesions had dried somewhat.

The heights of the sixteen plants were measured, the number of expanded and fallen leaves were counted, the weights of the infected leaves taken, plus the number of leaves showing disease symptoms noted. The reasons for measuring these extra statistics were firstly to provide a means of comparing the growth of the two plant genotypes and secondly to estimate the amount of disease on them. The rationale for the first reason is that a difference in plant growth between the genotypes might alter the surface area of the leaves which in turn could influence the spore counts. One genotype could theoretically yield more spores not because of greater relative reproductive fitness but simply because the greater surface area would directly support greater sporulation. Plant

height and leaf weight were simple measures of plant growth. To have measured the leaf area would have been too laborious with healthy green leaves and impossible with brown senescent partly desiccated and highly convoluted infected leaves.

The rationale for the second reason is to provide by counting the number of infected leaves and dropped leaves a simple measure of the intensity of disease on a plant. This was considered to be useful in providing an alternative to sporulation as a basis for comparison of the two host genotypes.

Spore counts were also taken, from a suspension made by shaking each infected leaf with 40 ml of distilled water in a glass jar for 30 seconds on a vortex mixer. Unfortunately, the leaves yielded fewer spores than anticipated probably due to a failure to maintain high humidity and hence 40 ml of water was probably too large a volume for the suspensions. The results are given and discussed in section 1.4.2.cvi.

Experiment 3

This third experiment to repeat a comparison of sporulation of race 4 on Cf₀ and Cf₄ plants involved six Cf₀ plants and seven Cf₄ plants. Seedlings were transplanted into individual pots after 28 days and inoculated three days later with 10 ml/plant of a race 4 spore suspension at a concentration of 147,500 conidia/ml. Plants of the two genotypes were placed in a damp chamber alternately. Infection was visible 11 days later, and after a further 10 days when heavy and uniform infection was apparent, the plants were scored. All infected leaflets were removed from each plant in turn and weighed, including the abscised leaves. After weighing the leaves were placed in a jar with 80 ml of distilled water and shaken by hand for 30 seconds. Six haemocytometer counts were made from the resulting suspension. Because of the time required to score each plant

Cf₀ and Cf₄ plants were scored alternately.

1.4.2.d Results and discussion

Experiment 1

The results are shown in Table 20. The variation within each set of six haemocytometer counts for each plant is quite small, however between plants within and between different genotypes there is much more variation. The mean spore total for the Cf₀ plants is 4.00×10^6 , and for the Cf₄ plants, 7.58×10^6 . A summary of the analysis of variance or the spore data is shown in Table 21.

Table 20 Results of Experiment 1 (section 1.4.2.cv) to compare sporulation of a race 4 isolate of F. fulva on two near isogenic tomato lines, one carrying and one lacking the Cf₄ gene

Plant Number ¹	Gene	Haemocytometer counts ²	Spore total
2	<u>Cf₀</u>	2,3,4,3,9,4	4.167×10^6
3	<u>Cf₀</u>	6,3,6,3,6,2	4.333×10^6
8	<u>Cf₀</u>	5,5,5,12,6,8	6.833×10^6
7	<u>Cf₀</u>	0,1,0,1,1,1	0.666×10^6
4	<u>Cf₄</u>	6,10,11,8,10,27	12.000×10^6
1	<u>Cf₄</u>	11,12,10,11,9,9	10.333×10^6
5	<u>Cf₄</u>	0,0,2,2,4,0	1.333×10^6
6	<u>Cf₄</u>	12,6,5,5,6,6	6.666×10^6

¹ Randomly assigned for placement into damp chamber

² Counts were made from 100 ml spore suspensions from all infected leaves on the plant

Table 21 Analysis of variance of spore total data¹(Table 20) for Experiment 1 to compare sporulation of a race 4 isolate of F.fulva on two near isogenic lines of tomato one carrying and one lacking the Cf₄ gene

Item	d.f.	S.S.	M.S.	F	P
Between lines	1	25.56	25.56	1.85	> 5%
Within lines	6	82.82	13.80		
Total	7	108.38			

¹ 10⁶ powers omitted to simplify arithmetic

Clearly, there is no evidence of a significant difference in sporulation of the race 4 isolate on Cf₀ and Cf₄ plants. Even if the results for plant 5 (Table 20) are omitted from the analysis an F ratio of 4.87 results which is not significant at the 5% level. One obvious problem with this experiment was the lack of replicates and hence the within lines d.f. was small. In Experiments 2 and 3 larger numbers of plants were used.

Experiment 2

The mean height of the eight Cf₀ plants was 53.00 cm, for the eight Cf₄ plants it was 52.25 cm. The height data were analyzed by analysis of variance. An F ratio of 0.02469 resulted which corresponded to a P value much greater than 5%, with 14 d.f. within lines. Clearly, there was no difference in the height of the plants.

Similarly there was no significant difference in the leaf numbers between the two lines. On average the Cf₀ plants had 10.250 leaves/

plant, whereas the $\underline{Cf}_4$ plants had 10.125 leaves/plant. However, significantly, more leaves had dropped from the $\underline{Cf}_4$ plants than the $\underline{Cf}_0$ plants. With 14 d.f. within lines, an F ratio of 6.230 had a probability $0.02 < P < 0.05$. This could mean that race 4 caused more disease on the $\underline{Cf}_4$ plants than on the $\underline{Cf}_0$ plants, due to a greater relative fitness on the former.

There was no significant difference between the mean number of infected leaves for each line. The $\underline{Cf}_0$ plants had an average of 6.000 infected leaves per plant, whereas for $\underline{Cf}_4$ plants the equivalent figure was 5.375. The F ratio was 0.5485 which is very small, indicating that there was no difference in the mean number of infected leaves.

An analysis of the infected leaf weights again showed no significant differences between the two host lines.

The data and analysis of the plant heights leaf weights and leaf numbers all indicate that any differences between sporulation on the two lines is not due to differences in growth habits of the plants (section 1.4.2.cv Experiment 2).

A summary of the spore count data is shown in Table 22. Individual haemocytometer counts are not given, just the sum of the means for each of the infected leaves (column 4).

There is considerable within line variation of the total spore counts per plant (column 5). Infection generally was light, probably due to desiccation of lesions caused by periodic lowering of the relative humidity. A summary of the analysis of variance for the spore total data (column 5, Table 22) is shown in Table 23.

Table 22 Summary of spore count data for Experiment 2 to compare sporulation of a race 4 isolate of F. fulva on two near isogenic tomato lines, one carrying and one lacking the Cf₄ gene

Plant Number	Gene	No. of infected leaves	Sum of mean ¹ haemocytometer count for each leaf	Total spore count per plant
1	<u>Cf₀</u>	4	0.5	2.00 x 10 ⁵
3	<u>Cf₀</u>	4	0.0	0.00
5	<u>Cf₀</u>	7	25.5	1.02 x 10 ⁷
7	<u>Cf₀</u>	6	4.0	1.60 x 10 ⁶
9	<u>Cf₀</u>	11	31.0	1.24 x 10 ⁷
11	<u>Cf₀</u>	6	0.5	2.00 x 10 ⁵
13	<u>Cf₀</u>	5	9.0	3.60 x 10 ⁶
15	<u>Cf₀</u>	5	0.0	0.00
2	<u>Cf₄</u>	4	0.0	0.00
4	<u>Cf₄</u>	6	2.0	8.00 x 10 ⁵
6	<u>Cf₄</u>	6	1.0	4.00 x 10 ⁵
8	<u>Cf₄</u>	5	5.0	2.00 x 10 ⁶
10	<u>Cf₄</u>	5	0.5	2.00 x 10 ⁵
12	<u>Cf₄</u>	6	0.0	0.00
14	<u>Cf₄</u>	5	0.0	0.00
16	<u>Cf₄</u>	6	0.5	2.00 x 10 ⁵

¹ The suspension from each leaf was used to make two counts from which a mean was calculated. The sum in column four represents the sum of the mean of all the infected leaves.

Table 23 A summary table of the analysis of variance of the spore total data (Table 22) for Experiment 2 to compare sporulation of a race 4 isolate of F. fulva on two near isogenic tomato lines, one carrying and one lacking the Cf₄ gene

Item	d.f.	S.S.	M.S.	F	P
Between lines	1	3.78225×10^{13}	3.78225×10^{13}	3.2229	> 5%
Within lines	14	1.64294×10^{14}	1.17353×10^{13}		
Total	15	2.02116×10^{14}			

Table 23 shows that the difference in the mean spore counts on Cf₀ and Cf₄ plants was not significant. Although there was a larger within lines d.f. in Experiment 2 than in Experiment 1 (Table 21) the spore count variation within each host genotype resulted in a non significant mean spore count difference. Superficially, the difference in mean spore counts is large however. The mean spore count for the pathogen on each Cf₀ plant was 3,525,000, whereas on each Cf₄ plant the mean spore count was only 450,000.

Experiment 3

A summary of the results is shown in Table 24. Of the three experiments, plants in this one gave the greatest spore yields. The success of this experiment was due to the success in maintaining a high R.H. throughout the period of disease development.

Table 24 A summary of the results of Experiment 3 to compare sporulation of a race 4 isolate of F. fulva on two near isogenic tomato lines one carrying and one lacking the Cf₄ gene

Plant number	Gene	Leaf weight (g) ¹	Haemocytometer counts	Average ²
1	<u>Cf₄</u>	3.60	91,115,88,49,64,72	80
3	<u>Cf₄</u>	3.76	62,64,58,48,51,58	57
5	<u>Cf₄</u>	5.12	86,98,94,98 ³	94
7	<u>Cf₄</u>	5.45	89,136,100,104,106,135	112
9	<u>Cf₄</u>	2.40	64,60,84,59,74,58	67
11	<u>Cf₄</u>	2.93	130,100,65,59,34,53	74
13	<u>Cf₄</u>	6.24	28,24,21,21,11,14	20
2	<u>Cf₀</u>	3.93	60,85,65,81,75,72	75
4	<u>Cf₀</u>	3.81	122,118,94,108,125,120	115
6	<u>Cf₀</u>	4.81	214,116,116,118,157,163	147
8	<u>Cf₀</u>	4.12	235,167,255,215,198,211	214
10	<u>Cf₀</u>	4.93	199,197,175,170,240,185	194
12	<u>Cf₀</u>	3.42	62,70,47,40,58,59	56

¹ Total weight of all infected leaves on the plant

² Averages rounded up to the nearest integer

³ Only four counts made erroneously instead of six

The average haemocytometer scores (column 5, Table 24) were analyzed statistically. A summary of the analysis of variance is shown in Table 25. Again as in Experiments 1 and 2 there is no evidence of a significant difference between the mean spore counts of the Cf₀ and Cf₄ plants. The mean number of spores per plant was 53,400,000 for the Cf₀ line and 28,800,000 for the Cf₄ line. This difference is superficially quite substantial (as in Experiment 2), however the large variations in the haemocytometer counts 56 - 214 for the Cf₀ line and 20 - 112 for the Cf₄ line resulted in statistical insignificance. The mean leaf weights were very similar - 4.17g for the Cf₀ and 4.21g for the Cf₄ plants. This difference was not significant.

Table 25 A summary table of the analysis of variance of the mean haemocytometer counts (Table 24) for Experiment 3 to compare sporulation of a race 4 isolate of F. fulva on two near isogenic lines of tomato one carrying and one lacking the Cf₄ gene

Item	d.f.	S.S.	M.S.	F	P
Between lines	1	179.4	179.4	1.66	>5%
Within lines	11	1190.3	108.2		
Total	12	1369.7			

All three experiments although differing slightly in their experimental details, yielded the same basic conclusion; there is no evidence that F. fulva exhibits greater sporulation on Cf₄ plants compared with Cf₀ plants. This means there is no evidence here for the operation of stabilizing selection against unnecessary virulence to the Cf₄ gene in this pathogen. Indeed in two of the three experiments sporulation of race 4 was greater, although not significantly so, on Cf₀ plants than on the Cf₄ plants. The major difficulty with this approach was the very large variations in spore counts between plants within genotypes. One possible explanation for this lies in the impossibility of creating uniform conditions for infection and disease development. Some leaves are always drier than others due to their position on plants and environmental differences at the various blocks within the chambers.

As a method of assessing the existence of stabilizing selection it is simple technically, yields highly numerical results, but is rather laborious. With suitable host and pathogen material and adequate replication it could prove a useful tool for studying the effects on fitness of unnecessary virulence.

The results and conclusion of these experiments are at variance with the expectations as discussed in section 1.4.2.b. Russell (1978) reported that the cultivar Vagabond with the Cf₂ and Cf₄ genes, for 14 years remained resistant to leaf mould in Canada. However he suggested that the cultivar probably possessed other resistance genes which explains the unusual longevity of resistance of this cultivar. If this is true then it would mean that virulence to Cf₄, unnecessary prior to the first use of Vagabond in 1954 in Canada and indeed also unnecessary on other non Cf₄ cultivars grown after the introduction of Vagabond, was not necessarily at a selective disadvantage. This conclusion is also supported by the work reported by Laterrot and Clerjeau (1979), who concluded that Cf₄

is not a 'strong' gene in France. They sampled the F. fulva population in France from plants lacking known Cf genes. At that time resistant cultivars were rarely grown and hence there was no obvious selection for pathogen races carrying virulence genes to the Cf resistance genes. Virulence to the Cf₄ gene was found in four isolates out of a total of 65 sampled, two in Lorraine and two in Provence. Although the frequency of unnecessary virulence was not high ($4/65 = 0.0625$) it is not insignificant. If the frequency of virulence to Cf₄ in the pathogen population was at this level the introduction of Cf₄ cultivars on a large scale in France would result in rapid selection for virulence.

However it is possible that the comparatively high frequency of virulence to Cf₄ in France is due to migration of virulent races from nearby Holland rather than due to weak forces of selection against unnecessary virulence (Laterrot and Clerjeau, 1979).

On balance, however, we must conclude that the evidence to date suggests that selection against unnecessary virulence is absent, or weak and hence not detectable.

1.4.3 Experiments to determine the nature and magnitude of selection in respect of unnecessary virulence to the Mlg resistance gene in the barley powdery mildew pathogen Erysiphe graminis hordei

1.4.3.a Introduction to the disease and to the pathogen

Powdery mildews are probably the most common and widespread of plant diseases (Agrios, 1978). The volume of research on E. graminis hordei in consequence has been enormous. Russell (1978) stated that powdery mildew is probably the most important fungal disease of barley. It is

found wherever barley is grown, and it affects the crop at all stages of growth (Russell, 1978). Losses of up to 50% of normal grain yield have been reported in late sown crops of susceptible spring barley cultivars (Russell, 1978).

The biology of the pathogen and the disease has been extensively described (Butler and Jones, 1955; Jenkyn and Bainbridge, 1978), as have the genetics of resistance and breeding for resistance (Wolfe, 1972; McIntosh, 1978 and Russell, 1978). Much information has also accumulated on the pathogen population genetics (Wolfe and Barrett, 1977; Wolfe and Schwarzbach, 1978a,b).

1.4.3.b Introduction to the experiment

The prime motive for the experiment was the existence and availability of almost isogenic lines of barley differing genetically only in a single resistance gene, the Mlg factor (the differentials) and two almost isogenic isolates of E. graminis hordei differing by only a single virulence gene, the Vg factor (virulence to the Mlg resistance gene).

This plant and pathogen combination provides a means of determining the effect of unnecessary virulence (Vg) on relative pathogen fitness if the two pathogen isolates are grown in a mixture on plants lacking the Mlg resistance gene. Since the two isolates are thought only to differ by this one virulence gene, any significant difference in relative fitness between the isolates could be attributed to the possession or lack of the Vg allele. The near-isogenic lines of barley differing only in the Mlg locus were used to sample the mixture periodically, in order to estimate the frequency of the isolates in the mixture. This was done by shaking samples of the mixture over plants of the differential lines. The proportion of Vg in the mixture is simply the colony count on the Mlg

plants divided by a similar statistic for the mlg plants, since spores with the Vg allele can develop colonies only on the Mlg plants, whereas both pathogen isolates can develop on the fully susceptible mlg plants. Consequently, it was important for the Mlg/mlg tester lines to be otherwise isogenic since differences in their background resistance could result in erroneous estimates of the proportion of each isolate in the mixture.

This procedure is typical of race competition experiments (sections 1.1.4.d and 1.1.5), with the refinement that here the effects of background genes on pathogen fitness were nullified by the use of these near isogenic isolates (section 1.4.1).

1.4.3.c Materials and methods

1.4.3.ci The pathogen

The two near isogenic isolates of E. graminis hordei were supplied by Professor Hermansen from The Royal Veterinary and Agricultural University, Copenhagen, who described (Hermansen, 1980) the isolation of a spontaneous mutant to virulence to Mlg from an avirulent isolate. The two isolates mother and mutant were cultured on the cultivar Proctor which lacks any known major resistance genes to powdery mildew. The two isolates were maintained on potted seedlings in two separated growth cabinets under similar conditions. Temperatures ranged from 15 - 20°C; relative humidities were 70 - 75% and artificial illumination photoperiods were 15½ - 16 hours. The light intensities in the growth cabinets were about 7.6 - 8.3 W/m² at shelf level. The isolates were regularly transferred about every two weeks onto fresh plants usually about two weeks old by shaking infected plants over healthy plants in the growth cabinet.

Both isolates grew readily, visible colonies appearing after 5 - 6 days. Heavy sporulation was maintained for several weeks from primary and eventually secondary colonies. Care was taken to prevent cross-contamination and in occasional checks none was ever found.

1.4.3.cii Barley plants

Seed of the two near isogenic lines of barley differing only in the Mlg resistance gene was supplied by the U.S.D.A. and were described by Moseman (1972). One per cent of the background genotype of the two near isogenic lines was based on the cultivar Manchuria whilst the remainder (99%) and the resistance gene was provided from a donor cultivar Goldfoil.

The cultivar Proctor until recently commonly grown in the U.K. is probably not closely related genetically to the near isogenic lines, except that in common to one line it lacks the Mlg gene.

Plants were usually grown in 12.7 cm (5") round plastic pots in Levington's potting compost, sown with 5 - 10 seeds per pot. The plants were well watered and the pots were placed into a growth room well away from the two growth cabinets containing the stock cultures. This growth room was at a temperature of about 25°C, was illuminated for 16 hr/day, with a light intensity at bench level of 10.6 W/m². The R.H. was very low, about 25%. This very dry atmosphere prevented mildew developing on the plants through contamination, throughout the two year period that isolates of E. graminis hordei were maintained.

Plants were used for inoculation about 10 - 14 days after sowing. The plants grew rapidly.

Proctor leaves were narrow unlike those of the two near isogenic lines, which were considerably wider; This was beneficial for colony counting.

1.4.3.ciii Genotype testing

Seedlings of the two near isogenic lines were placed into each of the two growth cabinets carrying the mother and mutant isolate cultures, and spores shaken over these healthy tester plants. After about seven days these plants were scored for mildew. In the growth cabinet containing the mother isolate (supposedly lacking virulence to Mlg) only the barley line lacking the Mlg gene was infected with mildew. The line possessing Mlg remained symptomless to the naked eye. However all the plants in the other growth cabinet containing the mutant isolate were equally and heavily infected with mildew. This test confirmed that the mother isolate lacked virulence to the Mlg resistance gene, whereas the mutant culture possessed this virulence gene. Further no obvious contamination, at least from the mutant to the mother culture had taken place.

1.4.3.civ Experiment to compare the competitive ability of the Ag mother isolate and the Vg mutant isolate in a race mixture experiment

Experiment 1

A summary of the method is shown in Figure 2.

Seed of the cultivar Proctor was sown in 12.7 cm (5") square plastic pots in a 3 x 3 pattern giving nine seeds per pot. Six similar pots were prepared. After seven days three pots were placed into each of the growth cabinets containing a different isolate of the pathogen. Earlier on the same day Proctor seed was sown in a similar way in eight 12.7 (5") round pots. These were the first set of plants to be inoculated with both isolates. Two weeks later, four pots of Proctor seedlings infected with each race separately (two pots per isolate) were brought to the laboratory as were the second batch of two week old healthy

Figure 2 Outline of the method of Experiment 1 to compare the competitive ability of two isolates of Erysiphe graminis hordei, one with and one without virulence to the Mlg gene of barley, in a race competition experiment (section 1.4.3.civ)

TIME (days)	
0	Sow seed of Proctor barley (six pots).
7	a. Incubate these pots of plants in separate sets with the two isolates of the pathogen. b. Sow a further set of Proctor seed (eight pots).
21	a. Inoculate the two week old healthy plants with both isolates from the infections developed on the initial set of plants. This starts generation 1 of the mixture. b. Sow three sets of seed - one of Proctor and one for each of the tester lines of genotypes <u>Mlg</u> and <u>mlg</u> (eight pots per set).
22	Incubate the eight pots with the mixture.
34	Assemble pots of the mixture in the laboratory.
35	a. Assemble pots from day 21b in the laboratory. b. Inoculate trios of pots of plants, one of Proctor and one each of the tester lines with inoculum from the plants carrying the first generation of the mixture - one pot per trio. c. Repeat Day 21b.
36	Incubate plants from Day 35b.
42	Score the colonies on the tester lines. These results give the percentage of each isolate at the end of generation 1.
48/49	Repeat Days 34 and 35. Continue the cycle

Proctor seedlings. Four pots of these, labelled replicates 1a, 1b, 1c and 1d were placed at the bottom of a settling tower, 1.1m in height. One pot of infected Proctor seedlings carrying the mutant isolate and one pot with the mother isolate were shaken for a few seconds over the top of the settling tower. After a few minutes by when the spores had settled onto the healthy plants, the four pots were covered with close-fitting clear plastic, conical propagator tops. The process was repeated using the remaining four pots of two week old Proctor seedlings (labelled replicates 2a, 2b, 2c and 2d) and the remaining two pots of inoculum donors. Thus in total there were eight pots inoculated with both isolates.

Earlier in the same day another set of pots of Proctor seed for the next mixture generation was prepared as previously. At the same time eight pots with seed of the Mlg line (five seeds per pot) and eight pots with seed of the mlg isolate were also prepared; these to be used to assay the mildew mixture inoculated that day, two weeks later.

The following day the eight pots of the mixture were taken from the laboratory to another growth cabinet isolated from the two stock cultures and also from the growth room with healthy plants. The conditions in this growth cabinet were : a temperature of 15°C, a relative humidity of about 75% and a photoperiod of 14 hr. The approximate light intensity at shelf level was 9.1 W/m^2 . The propagator tops which were placed over the plants following inoculation, to maintain a high R.H. around the plants, to aid germination and penetration of the conidia, and to prevent any contamination in the laboratory, were removed.

Twelve days later the mixture plants were recovered with the tops and brought back to the laboratory. Old spores were shaken off in the process but under the high R.H. within the covers, abundant sporulation occurred overnight.

The following day, now two weeks after the Proctor seedlings were inoculated with both isolates, the Mlg/mlg tester lines and the Proctor plants for the next generation of the mixture epidemic (eight pots for each of the three genotypes) were assembled in the laboratory.

Three pots, one of each tester line and one of Proctor, labelled 1a, were placed at the bottom of the settling tower. The trio were inoculated by dusting with spores shaken for five seconds from one pot of plants of the previous generation of the mixed race artificial 'epidemic' over the top of the settling tower. The top of the tower was then covered and left for one minute. The three inoculated pots were then covered with propagator tops. Using the remaining seven pots of plants infected with the mixture of isolates, i.e. replicates 1b-2d, the whole process was repeated seven times. Thus the tester plants to estimate the percentage of Vg at the end of the first generation were inoculated at the same time as the plants for the next generation of the mixture. All 24 pots of plants were left overnight in the laboratory, in order to avoid shaking the plants and possibly detaching some of the inoculum.

Earlier in the same day as the inoculations, further batches of Proctor and the tester line were sown, to be ready for inoculation in a further 14 days.

The next day the inoculated sets of tester plants and Proctor plants were moved to separate growth cabinets, the latter set to the growth cabinet where the first generation of the mixture was incubated and the tester plants to a similar growth cabinet underneath the first. The propagator tops were then removed.

A week later the tester plants were brought to the laboratory and the percentage of the mutant isolate in the mixture at the end of the first generation determined. The first leaves of the tester plants

(3 - 5 leaves usually) for each replicate pair, for example Mlg 1a and mlg 1a were taken and a 10.2 cm (4") length was cut from the tip. The number of colonies visible with the naked eye on each leaf piece was counted on one side of the leaf, usually the adaxial surface. Sometimes the leaves were twisted so that colonies were counted on both sides of the 'twist' facing upwards, so that only a single 10.2 cm (4") span of leaf was assayed. If there were different numbers of Mlg and mlg plants assayed for mildew, the total counts on each genotype were adjusted accordingly. The process was repeated with the other seven pairs of pots of tester plants.

Generally, lesions were common on the uppermost facing leaf surface, and usually they were distinct. Occasionally infection was very heavy, with colonies coalescing. Counting precise numbers became difficult in this situation and rough estimates were taken.

Six days later the mixture plants inoculated at the same time as the tester plants were brought to the laboratory covered and a day later the new batch of two week old uninfected plants of the Mlg and mlg tester lines, and Proctor were inoculated in the same way as described above. Thus the third generation of the mixture was set up at the same time as the tester plants to assay the second generation were inoculated.

The whole process was repeated until five generations of culturing and assaying the mixture had been completed.

In the subsequent generations (2 - 5) the bottom 12.7 cm (4") of the leaves of the tester plants were used for counting the colonies because of considerable yellowing of the tips. Furthermore the proximal portion of the leaves was generally more heavily colonized, probably due to a more horizontal alignment at inoculation.

Experiment 2

Experiment 1 was carried out from late July 1981 to November 1981, largely during the autumn months. Experiment 2 was carried out in the following summer from late April to early September.

Essentially Experiment 2 was very similar to Experiment 1, but with the innovation that ^{the} initial set of Proctor plants inoculated with both isolates received a greater inoculum of the mutant so as to ensure a high initial proportion of this isolate in the mixture. This was to provide sufficient "headroom" to accommodate its expected reduction in frequency and was achieved by initially inoculating five pots of Proctor plants with the mutant isolate and only one with the mother isolate. The mildew which developed on these plants was used to inoculate seven pots of two week old Proctor with both isolates, and so set up the first generation of the mixture, with seven replicate pots of plants.

After two weeks the seven pots of plants carrying the mixture were sampled on Mlg and mlg tester plants as described in Experiment 1, at the same time as transferring the mixture onto a new batch of two week old

Proctor . The methods were as described above for Experiment 1 (see Figure 2).

Owing to absence generations 4 and 5 could not be sampled and hence results were obtained for generations 1, 2, 3 and 6.

1.4.3.d Results and discussion

Experiment 1

The results of Experiment 1 are shown in Appendix 8, and are further summarized in Table 26. A graph of the decline in the mean percentage of the mutant isolate over the five generations is shown in Figure 3. The data for the eight replicate estimates of the percentage of the mutant

at each generation were pooled to obtain Figure 3. This was justified statistically. The mean percentage of the mutant in the mixture for each group of replicates separately is shown below :

Generation :	1	2	3	4	5
% mutant - Rep. 1a - d :	28.00	21.75	9.00	5.00	5.00
% mutant - Rep. 2a - d :	27.50	23.75	7.25	10.00	1.67

The means are clearly similar at each generation except perhaps at generation 4. The significance of the difference between replicate means at generation 4 (data shown in Table 26) was examined using an analysis of variance after the eight percentages were first transformed using an arc sine transformation to normalize the data. An F value of 2.14 was obtained which is not significant at the 5% probability level with only 6 d.f. for the within replicates item.

Using a regression analysis a line of best fit through the data in Figure 3 was drawn. The regression equation obtained was :

$$\hat{\underline{Y}}_{\underline{i}} = 33.021 - 6.361 \underline{X}_{\underline{i}}$$

where $\underline{X}_{\underline{i}}$ represents the $\underline{i}$ th generation, and $\hat{\underline{Y}}_{\underline{i}}$ an estimate of the $\underline{i}$ th mean percentage of the mutant isolate. The significance of the regression coefficient of -6.361 was also tested statistically using an analysis of variance. An F value of 26.497 was obtained, significant at the $P=0.05$ level, with only 3 d.f. associated with the 'Remainder' item in the analysis. Thus the decline in the percentage of the mutant isolate was statistically significant.

The standard deviation of the regression coefficient was 1.236.

Table 26 Summary of results of Experiment 1 to determine the changes in percentage of a mutant¹ isolate of E. graminis hordei in a mixture with mother¹ isolate when grown on Proctor² barley for five two week generations

	Generation				
	1	2	3	4	5
	% mutant in mixture				
1a	37	12	5	4	1
1b	18	27	11	16	10
1c	20	33	12	0	0
1d	37	15	8	0	9
Replicate					
2a	28	18	6	3	1
2b	11	22	2	11	-
2c	51	26	13	14	3
2d	20	29	8	12	1
Average	27.75	22.75	8.12	7.50	3.57

¹ mutant isolate carries a single gene mutation to virulence to the Mlg resistant gene. Otherwise it is identical genetically with the mother isolate and was obtained by mutation from it.

² Proctor barley lacks the Mlg resistance gene.

Figure 3 Graphical representation of the results of Experiment 1 to determine changes in the mean percentage of a mutant isolate of E. graminis hordei in a mixture with its mother isolate when grown on Proctor barley for five two week generations

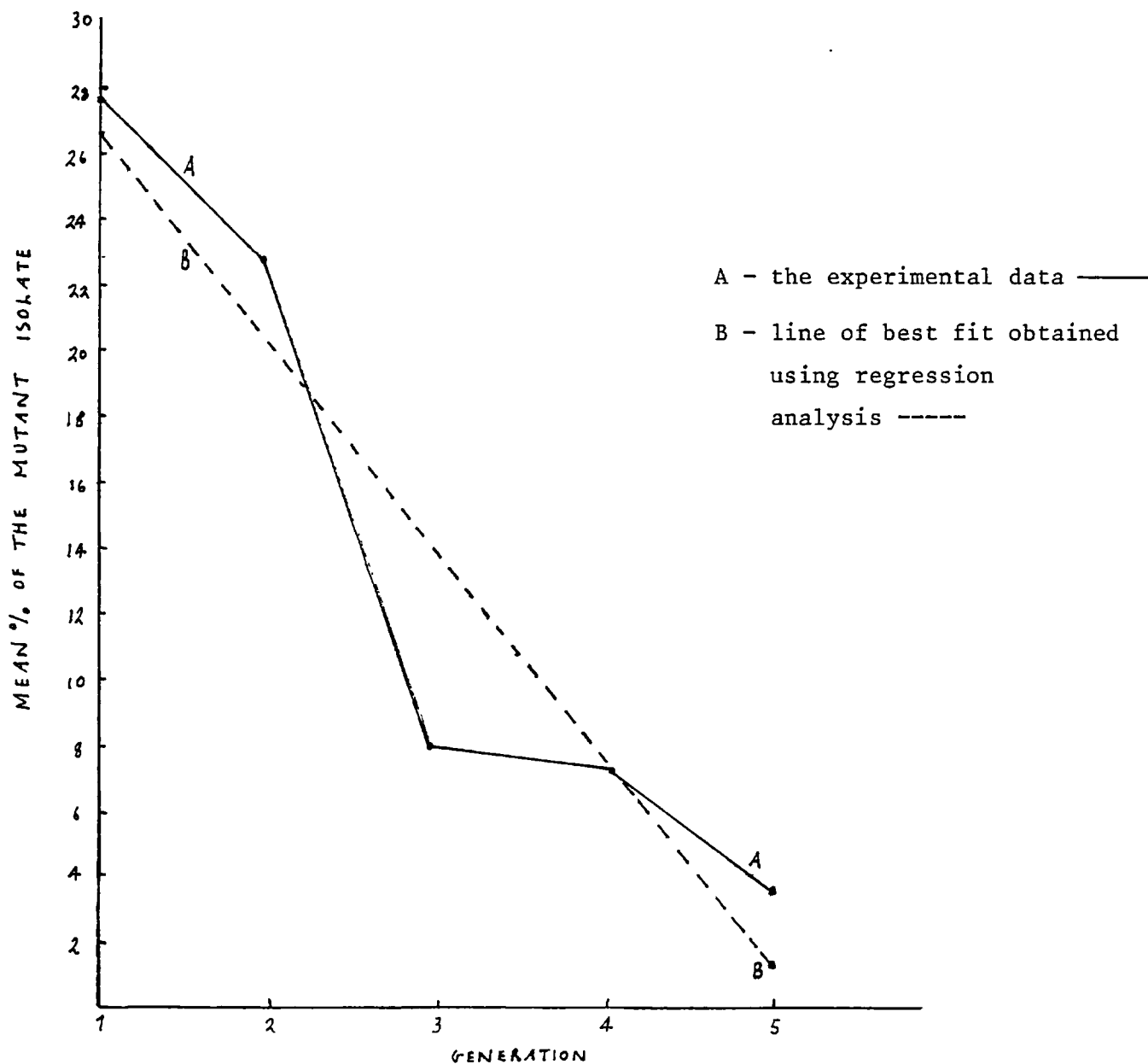


Table 26 shows for all seven (not 2b) replicates with five generations of results a decline in the percentage of the mutant with time. Only replicates 1a and 2a show a totally consistent decline with each generation. The other results show rather erratic declines with occasional increases from one generation to the next. The eight individual replicate scores are affected by considerable sampling error but when averaged for each generation (Table 26, Figure 3) the variation is significantly reduced. The overall result is a significant decline in the percentage of the mutant isolate in the mixture.

From these data it is concluded that the decline in the percentage of the mutant isolate is due to a reduced relative fitness compared with the mother isolate when both were grown in a mixture. Selection operated to reduce the frequency of the mutant isolate. Since genetically the isolates were assumed to be identical other than for virulence to Mlg it was concluded that virulence to this resistance gene when unnecessary is disadvantageous.

Calculation of the selective coefficient, $\underline{s}$

An estimate of an average selection coefficient against unnecessary virulence was calculated using the method of Leonard (1969). A graph of $\log_e (\underline{x}/1-\underline{x})$ is plotted against generation number where $\underline{x}$ is the proportion of the mother isolate in the mixture. The line of best fit is determined using regression analysis, and $\underline{s}$ is calculated from the slope $\underline{m}$ of this line where

$$\underline{m} = -\log_e (1 - \underline{s}) \qquad 1.4.3.1.$$

Appendix 9 shows the calculation of $\underline{s}$ from the data in Table 26 and Figure 3.

The $\underline{s}$ value obtained, 0.4495 is considerably greater than the typical values calculated from field data in section 1.3.6 and summarized in Table 19, and confirms the suspicion expressed in section 1.4.1 that whilst artificial race competition experiments in confined environments may be valid tests of the existence of stabilizing selection they are not suitable for accurate estimates of the size of $\underline{s}$. A value of $\underline{s} = 0.4495$ seems inflated, and must surely reflect greater competition between the isolates than under natural conditions (section 1.4.1).

Experiment 2

The results of Experiment 2 are shown in Appendix 10 and in summary form in Table 27. The average percentage of the mutant in the mixture remained high at about 89 - 92% and there was no consistent decline therefore of the mutant isolate in the mixture over six generations. The averages of generation 1 and 3, the lowest and highest percentages of the mutant respectively were compared using a one way analysis of variance on the data (Table 27) transformed using an arc sine transformation. An F ratio of 0.4 was non significant. Therefore statistically there is no evidence of changes in the percentage of the mutant in the mixture over six generations.

These results appear to contradict those of Experiment 1, where a clear decline in the frequency of the mutant isolate occurred. It is possible that since the mutant isolate was initially at a very high frequency and the mother isolate initially at a very low frequency, the lack of a decline is explicable on two accounts. Firstly, there would be a general lack of competition between the isolates since the mother

Table 27 Summary of results of Experiment 2 to determine the changes in percentage of a mutant¹ isolate of E. graminis hordei in a mixture with a mother¹ isolate when grown on Proctor² barley for six two week generations

		Generation			
		1	2	3	6
		% mutant in mixture			
Replicate	1	89	96	100	44
	2	68	100	100	100
	3	100	100	100	100
	4	89	87	100	100
	5	99	77	84	100
	6	100	85	91	100
	7	82	100	73	100
Average		89.57	92.14	92.57	92.00

¹ Mutant isolate carries a single gene mutation to virulence to the Mlg resistance gene. Otherwise it is identical genetically with the mother isolate and was obtained by mutation from it.

² Proctor barley lacks the Mlg resistance gene.

isolate was rare. Secondly the mother isolate could have been lost in some replicates by chance sampling effects in transfer of each generation. These possibilities cannot be ignored.

Independent evidence corroborates the suggestion that unnecessary virulence is disadvantageous, i.e. the mother isolate is fitter on susceptible hosts lacking Mlg, than the mutant isolate. Cheong (1982) compared microscopically the developmental stages of infection of the two isolates when infecting the mlg tester line. After 48 hr the mean percentage germination of the mother isolate (75.21%) was significantly greater ($P = 0.02$) than that for the mutant isolate (60.43%).

At 48 hours the formation of elongated secondary hyphae (E.S.H.) was significantly greater ($P = 0.05$) for the mother isolate but by 72 hours this had been negated. Conidiophore production was also examined on 20 colonies per replicate leaf examined. Four days after infection there were significant differences in conidiophore production for each isolate. The mother isolate had a greater mean number of young and old conidiophores, $P = 0.01$, 0.02 , respectively. Five days after infection the mean number of young conidiophores were similar for the two isolates, but the difference in the number of old ones had become highly significantly different ($P = 0.001$), the mean for the mother isolate was 32.26 per replicate leaf whereas for the mutant isolate the mean was only 13.74 per replicate.

Combining the individual means at each developmental stage in a components analysis led to the conclusion that the number of conidiospores produced by the mother isolate was 4.74 times that of the mutant isolate (Cheong, 1982, Table 4.32).

In a similar repeat of the experiment Cheong (1982) found the mother isolate had significantly higher rates of germination, appressoria formation, E.S.H. formation and conidiophore production than the mutant

isolate. Only the spore production per colony was the same for each isolate. Combining the component means resulted in a very large difference in spore production, the mother isolate being the fittest.

These results strongly support the hypothesis developed from Experiment 1, that unnecessary virulence to the Mlg is selectively disadvantageous compared with avirulence.

CHAPTER 2

SELECTION AGAINST UNNECESSARY VIRULENCE AND MODELS OF PATHOGEN EVOLUTION IN MULTILINES AND CULTIVAR MIXTURES

2.1 Introduction

In Chapter 1 stabilizing selection coefficients against unnecessary virulence (s) were calculated from field data.

In Chapter 2, values of s obtained from these studies are applied to published models of pathogen evolution in multilines and cultivar mixtures, and predictions of pathogen evolution are made from these simulations. The conclusions are relevant both to the validity and usefulness of the current models used by plant pathologists, and to the likely efficacy of multilines and cultivar mixtures in the control of plant pathogens.

For many years, the only strategy for the use of major resistance genes was to breed cultivars carrying them (usually singly) and grow these cultivars over large acreages until new virulent races appeared which rendered the previous resistant cultivars susceptible. These were then replaced with new cultivars into which a different resistance gene had been incorporated until the resistance was once again "broken" by a new race of the pathogen. This is the classical 'boom and bust' cycle (described by Robinson, 1976 and Priestley, 1978).

In the last 25 years or so, other strategies for the utilization of resistance genes have been described and in some cases employed (see section 2.2 for the relevant literature) largely due to the general failure of the previous simple strategy (Johnson, 1961; Van der Plank, 1963, chapter 15 and Marshall, 1977).

One of the most significant of the new strategies has been the use of mixtures of genetically different host lines marketed as a single cultivar.

One approach to this, multilines (proposed by Jensen, 1952) involves a series of almost isogenic host lines called isolines. Each isolate is genetically homogeneous save for the possession of a unique major resistance gene to the pathogen that the multiline is designed to control.

Another approach, cultivar mixtures (Wolfe and Barrett, 1976) involves the mixing of seed of different commercial cultivars, each of which is chosen to carry a different major resistance gene (as in the isolines of multilines) and a different (or heterogeneous) background genotype (unlike multilines).

Other strategies not discussed in this chapter include patterns of gene deployment in time and space, and superlines (see section 2.2 for the relevant literature).

The general failure of the strategy of the monoculture of single resistance gene cultivars in the 'boom and bust' cycle was largely predictable in retrospect from a knowledge of the selection of advantageous mutations in populations. Unfortunately, the theoretical understanding came too late to prevent further repetitions of the cycle.

In contrast the practical use of multiline cultivars and cultivar mixtures has been paralleled by theoretical studies designed to predict the long term performance of this approach based on modelling pathogen evolution on the plants. This theory is much more complex than the earlier theory of pathogen evolution in the 'boom and bust' cycle, due to (i) the large number of pathogen genotypes that can be supported in multilines and cultivar mixtures, and (ii) to the complex interplay of more selective forces operating on these genotypes.

Stabilizing selection is considered by pathogen population geneticists and modellers to be one of the most important forces acting on populations of pathogens in cultivar mixtures and multilines (section 2.2.2.a) since it was considered until recently to be the only force opposing the population expansion of super races (i.e. those races carrying virulence genes necessary to colonize all members of these host populations).

Stabilizing selection acts potentially against all races in multilines or cultivar mixtures which carry more than one virulence gene. In the infection of a single isoline or cultivar, a race with m virulence genes requires only one of these genes. The remaining $m-1$ genes are unnecessary for infection and may confer on their carriers an additive or multiplicative fitness loss.

Selection against unnecessary virulence genes helps to offset the large selective advantage that complex races (which carry many corresponding virulence genes to the host resistance genes) have over simple races (carrying one or a few virulence genes (Grant and Archer, 1983)).

The size of the stabilizing selection coefficients has a crucial role in determining pathogen evolution in mixed host populations, as predicted by the mathematical models (sections 2.2 and 2.3) and this is the principal topic of this chapter.

2.2 Literature review

The literature relevant to this chapter is extensive and sections of it have been reviewed in detail by Leonard and Czocher (1980) and therefore will not be discussed here.

2.2.1 Strategies for the use of resistance genes

The various strategies for the use of major resistance genes have been summarized and discussed by Frey, Browning and Simons (1977),

Marshall (1977) and Day (1978). The principal strategies together with the key literature are listed below :

1. Regional gene deployment (Van der Plank, 1968; Frey et al., 1977).
2. Cultivar diversification schemes (Priestley, 1979; Priestley and Bayles, 1980).
3. Multiple gene resistance cultivars or superlines (Watson and Singh, 1952; Nelson, 1973; Marshall, 1977).
4. Multilines (Browning and Frey, 1969, 1981).
5. Cultivar mixtures (Wolfe, 1978; Chin, 1979; Wolfe and Barrett, 1980 and Wolfe et al., 1981a).
6. Cultivar rotation (Person, 1966).

2.2.2 Models of pathogen evolution in multilines and cultivar mixtures

2.2.2.a Introduction

Many of the most important mathematical models of multiline cultivars have been reviewed by Leonard and Czocher (1980). Only a few relevant ones will be discussed here.

Browning and Frey (1969) and Frey, Browning and Simons (1973) discussed the mechanisms by which multilines could control plant pathogens, with special reference to Puccinia coronata avenae, the cause of oat crown rust. In their proposal, each isoline carried a unique single gene for resistance to rust, but none of the lines is completely resistant to all known races of the pathogen (known as the "dirty crop" approach as opposed to the "clean crop" approach, advocated by Borlaug (1959) in which all isolines of the multiline are resistant to all the prevalent races

of the diseases to be controlled). Multilines according to these authors should 'stabilize' the race structure of the pathogen populations, ultimately selecting for simple races carrying just one or a few corresponding virulence genes. Complex races would not be selected due to stabilizing selection operating against their unnecessary virulence genes. If simple races predominate in multilines each isoline acts as a spore trap to simple race with non corresponding virulence genes (known as the 'fly paper' effect). This should reduce the rate of disease increase. Consequently, multilines should have an effect similar to both vertical resistance and horizontal resistance (Van der Plank, 1963) by reducing the amount of inoculum available for infection, and reducing the apparent infection rate respectively. Marshall (1977) has stated that if these proposals are valid then the potential advantages of multilines are enormous especially in providing a permanent but flexible strategy for control, and in extending indefinitely the life of 'strong' (sensu Van der Plank) resistance genes.

Burdon (1978) and Chin (1979) discussed other ecological and genetic mechanisms operating in heterogeneous plant population which contribute to disease control, including the reduced density of susceptible plants and cross protection.

Marshall (1977) noted that the hypothesis of Frey et al. (1973) concerning the mode of disease control of multilines rests heavily on the assumption that sufficient stabilizing selection operates to select predominantly for simple races. Marshall (1977) considered that this assumption was questionable (citing Watson, 1970) and there was very little known about the magnitude of the selective forces against unnecessary virulence and for necessary virulence. He concluded "the question of the effectiveness of multilines in stabilizing and controlling pathogen populations remains shrouded in controversy".

Marshall (1977) believed that potentially either complex or simple races could be selected in multilines, but neither extreme outcome would be probable. Simple races would not predominate because of the substantial selection for complex races with the genetic capacity to attack many more of the component isolines. However, super races would be unlikely to arise and predominate since one cultivar would seldom be grown long enough for this evolution to occur. Instead, he envisaged the gradual evolution and predominance of complex races leading to a gradual erosion of the effectiveness of the disease control conferred by the multiline. The obvious question Marshall (1977) raised was how to protect the crops once a super race has arisen, and all the resistance genes have been overcome. This point will be discussed later in section 2.3.5.

2.2.2.b Groth's (1976) model

Groth (1976) proposed a simple model which described relative race fitness in multilines on the assumptions of (i) the gene for gene theory, between resistance genes in the host and matching virulence genes in the pathogen (ii) the multiline comprises n isolines each with a single unique major resistance gene. Each isoline occurs with a frequency of $1/n$, (iii) the existence of races with all possible combinations of the n corresponding virulence genes (iv) races which lack necessary virulence being incapable of sporulation on the resistant host (v) the occurrence of stabilizing selection against unnecessary virulence. Groth (1976) suggested two variants of the model. In the first, the additive model, the combined effects on the relative fitness of complex races of each unnecessary virulence gene are additive, in the second variant, the multiplicative model, they are multiplicative (see below).

Groth (1976) showed with the additive model that a complex race carrying $\underline{m}$ virulence genes (all corresponding to the resistance genes in the multiline unless otherwise stated) will be relatively fitter than a simple race with just one virulence gene, when $\underline{R} > 1$, where :

$$\underline{R} = \underline{m} \cdot [1 - (\underline{m} - 1) \cdot \underline{s}] \quad 2.2.2.1$$

and is defined as a ratio of the relative fitness of, or reproduction potential of, a complex race with $\underline{m}$ virulence genes compared with that of a simple race. The parameters $\underline{s}$ and $\underline{m}$ are defined as in section 1.3.2 as the stabilizing selection coefficient (assumed to be identical numerically for all unnecessary virulence genes in Groth's model) and the number of virulence genes carried by the complex race respectively.

Groth deduced that maximum fitness occurs in the race where :

$$\underline{m} = \frac{1 + \underline{s}}{2 \cdot \underline{s}} \quad 2.2.2.2$$

Using the multiplicative model which he considered to be more realistic Groth (1976) showed that :

$$\underline{R} = \underline{m} \cdot (1 - \underline{s})^{\underline{m} - 1} \quad 2.2.2.3$$

from which it could be shown that maximum pathogen fitness occurs when :

$$\left[\underline{m} \cdot \log_e (1 - \underline{s}) + 1 \right] \cdot (1 - \underline{s})^{\underline{m} - 1} = 0 \quad 2.2.2.4$$

This is true when

$$\underline{m} \cdot \log_e (1 - \underline{s}) + 1 = 0$$

Hence,

$$\underline{m} = -1 / \log_e (1 - \underline{s}) \quad 2.2.2.5$$

Alternatively equation 2.2.2.4 holds when,

$$(1 - \underline{s}) \underline{m}^{-1} = 0$$

This has no solution for $\underline{m}$, hence numerical values of this parameter can only be determined from equation 2.2.2.5.

Neither equation 2.2.2.2 and 2.2.2.5 contain the term $\underline{n}$, the number of isolines in the multiline. However in practice this is unrealistic. Consider the additive model where $\underline{n} = 3$, and $\underline{s} = 0.1$ for each unnecessary virulence gene. According to equation 2.2.2.2 the fittest race carries $\underline{m} = (1 + \underline{s})/2.\underline{s} = 1.1/0.2 = 5 - 6$ virulence genes. But since $\underline{m} > \underline{n}$ only three of these 5 - 6 virulence genes could possibly be needed. The remaining 2 - 3 virulence genes will always be unnecessary in this multiline and therefore a race with 5 - 6 virulence genes cannot possibly be fitter than a race with the three virulence genes corresponding to the three component multiline. Consequently, if the calculated value of $\underline{m}$ using equation 2.2.2.2 (or indeed equation 2.2.2.5) exceeds $\underline{n}$, the number of virulence genes carried by the fittest race is not independent of $\underline{n}$, but in fact must equal $\underline{n}$. In such cases the super race would be predicted to predominate the race structure of the multiline.

Groth (1976) recognized the need to be able to measure precisely pathogen fitness and lacking information on the size of $\underline{s}$ he did not attempt numerical calculations of $\underline{m}$. However he did conclude that if the levels of stabilizing selection were similar to those reported by Leonard (1969) or Van der Plank (1968) then there will be an upper limit to pathogen race complexity (see section 2.3).

Groth (1976) recognized the importance of understanding how fitnesses combine; hence his recognition of both multiplicative and additive models. Barrett and Wolfe (1978) however showed that if $\underline{s}$ is very small

(< 0.1), the differences between Groth's (1976) models are not significant, because equation 2.2.2.3

$$\underline{R} = \underline{m} \cdot (1 - \underline{s})^{\underline{m} - 1}$$

from the multiplicative model reduces to :

$$\underline{R} \approx \underline{m} \cdot [1 - (\underline{m} - 1)\underline{s}]$$

This is identical with equation 2.2.2.1 for the additive model. If $\underline{s} > 0.1$ however the two variations of Groth's (1976) model do differ significantly (Barrett and Wolfe, 1978).

Marshall and Prior (1978) deduced a similar model to that of Groth (1976). They concluded that unless selection against unnecessary virulence is high ($\underline{s} > 0.4$) a multiline will require more than ten isolines to provide a mixture with 80% of the plants resistant to the most fit races. For low selection coefficients ($\underline{s} = 0.1$) a successful multiline would require too many isolines (for breeding and management) if each carried a single unique resistance gene.

2.2.2.c Barrett and Wolfe's (1978) model

Barrett and Wolfe (1978) criticized Groth's (1976) model on the belief that even if $\underline{s}$ values are constant, the mean fitness of each pathogen genotype will vary during the season where mean fitness "is the fitness of each pathogen genotype averaged over all components and weighted by its frequency on each component at a given point in time." Components here refers to isolines within the multiline. Barrett and Wolfe (1978) believed that the lesions produced by the end of the first asexual generation in Groth's (1976) model would have fitnesses during the second generation of 1.0 and $1 - \underline{s}$ for the simple and complex races respectively. However the

spores released from these parental lesions reinfect the host at random and have relative fitnesses of $1/n$ and $\frac{m}{n} \cdot (1-s)$ for a two component model (Groth, 1976) which differ from those for the parental lesions. These differences in fitnesses in the second and subsequent generations arise because the mature lesions do not die immediately upon releasing spores, but continue to reproduce in subsequent generations, and continue to produce a proportion of inoculum which is not conditional on first landing on the 'correct' host line. This random element on dispersal, applicable only to the spores is absent for lesions since spores are dispersed, whereas lesions are not. Groth's (1976) model which assumes that the mean fitness of races is constant throughout the season only holds when complete redistribution of pathogen genotypes occurs over all hosts (i.e. 100% alloinfection) and hence the fitness of the simple and complex races are always $1/n$ and $\frac{m}{n} \cdot (1-s)$ respectively, for a two host component model. This is only true at the first generation or where the generations are discrete, that is lesions reproduce and then die.

To model the effects of changing mean fitnesses in plant pathogen populations Barrett and Wolfe (1978) introduced a constant, α , the realized growth rate where the population multiplies by a rate $(1 + \alpha)$ each generation. Thus if the population size after the first generation is $\underline{X}_1$, in the next generation, $\underline{X}_2 = \underline{X}_1 \cdot (1 + \alpha) = \underline{X}_1 + \underline{X}_1 \cdot \alpha$. $\underline{X}_2$ consists of the number of lesions in generation one ($\underline{X}_1$) plus those produced in the second generation ($\underline{X}_1 \cdot \alpha$). The use of the term α , the realized reproduction rate, takes into account the wastage of spores on dispersal.

Barrett and Wolfe (1978) then showed that the mean fitness of complex races ($\underline{R}$) relative to that of the simple races is given by :

$$\underline{R} = \frac{(1-s)^{m-1} \cdot (n + \alpha \cdot m)}{n \cdot (1 + \alpha)} \quad 2.2.2.6$$

where $\underline{R}$, $\underline{s}$, $\underline{m}$ and $\underline{n}$ are as defined above for Groth's (1976) model.

Further they deduced that $\underline{R}$ is maximized, assuming α and $\underline{n}$ positive, by a value of $\underline{m}$, $\hat{\underline{m}}$ where :

$$\hat{\underline{m}} = \frac{-\alpha - \underline{n} \cdot \log_e (1-s)}{\alpha \cdot \log_e (1-s)} \quad 2.2.2.7$$

Equation 2.2.2.7 becomes on division

$$\hat{\underline{m}} = \frac{-1}{\log_e (1-s)} - \frac{\underline{n}}{\alpha} \quad 2.2.2.8$$

If $\underline{n}$ is small and α large, $\underline{n}/\alpha$ becomes small, in comparison with the $-1/(\log_e (1-s))$ term. For instance if $\underline{n} = 2$, $\alpha = 10$, and $\underline{s} = 0.1$, $-1/\log_e (1-s) = 9.49$, whereas $-\underline{n}/\alpha = 0.2$. Thus $\hat{\underline{m}} = 9.49 - 0.2 = 9.29$.

Under these circumstances, the $\hat{\underline{m}}$ value is similar to the $\underline{m}$ value (9.49) calculated using Groth's (1976) multiplicative model (equation 2.2.2.5). Groth (1976), and Grant and Archer (1983) considered α values for rusts and mildews to be in the region of 5 - 10 or even greater than 10, by relating α to the more familiar terms, the apparent infection rate, $\underline{r}$ and the logarithmic infection rate, $\underline{r}_1$ (sensu Van der Plank). For example Groth (1978) equated an α value of 10, to an $\underline{r}_1$ value of 0.23 per day assuming a generation length of 10 days. Much higher rates of $\underline{r}_1$ may occur for the kinds of diseases multilines are designed to control (Van der Plank, 1963).

A higher value of $\underline{n}$, equal to 10 and a lower value of α equal to five may not yield an $\hat{\underline{m}}$ value as calculated from Barrett and Wolfe's (1978) model (equation 2.2.2.8) much smaller than the equivalent term calculated from Groth's (1976) model. Thus if $\underline{s} = 0.1$ the estimated number of virulence genes carried by the fittest race on the multiline are 7.49 and 9.49 respectively. However very low values of α , such as

0.1 and 0.5 as used by Barrett and Wolfe (1978) do alter significantly the estimation of $\hat{m}$ and yield large differences in $\hat{m}$ values between their model and that of Groth (1976).

Groth (1978) noted that values of α may be reduced by multilines which slow disease increase (Leonard, 1969a), and that the rates of disease increase may be very slow at the start and at the end of an epidemic (Van der Plank, 1963). In addition α values may be further reduced by fungicides or by the use of partial resistance genes and background 'horizontal' resistance in the isolines. However, Groth (1978) argued that values of α are still likely to be in the range 5 - 10 for rusts and mildew diseases.

Values of $\alpha = 0.1$ and 0.5 seem excessively and unrealistically small. Using an equation calculating $\underline{r}_1$ from α :

$$\underline{r}_1 = \frac{1}{t} \log_e (1 + \alpha) \quad 2.2.2.9$$

from Grant and Archer (1983) where $\underline{t}$ is the mean generation time, a value of 0.5 is equivalent to an $\underline{r}_1$ value of about 0.04 if $\underline{t} = 10$ days. Since $\underline{r}_1 > \underline{r}$ (Van der Plank, 1963; Grant and Archer, 1983), $\underline{r}$ will be even smaller. Values of $\underline{r} = 0.4$ have been reported for some rust diseases under favourable conditions (Van der Plank, 1963) whilst Fried et al. (1979) recorded values of $\underline{r}$ for powdery mildew of wheat in multilines to be about 0.20 to 0.23. Whilst more experimental data are required to estimate α values for rusts and mildews in multilines, the evidence to date is more consistent with α values of 5 - 10 rather than 0.1 - 0.5. If so, then the prediction of Groth's (1976) model will barely be altered by the extra complexity of Barrett and Wolfe's (1978) model in which the predicted number of virulence genes carried by the fittest race in a

multiline is determined not only by the value of $\underline{s}$ (as in Groth's (1976) model; equations 2.2.2.2 and 2.2.2.5) but also by the number of isolines, $\underline{n}$ and a measure of the realized reproductive rate, α .

The model of Barrett and Wolfe (1978) is more realistic than that of Groth (1976), and its predictions favour more the selection of simpler rather than complex races in multilines. Leonard and Czocher (1980) criticized the application of Barrett and Wolfe's (1978) model to the kind of pathogens and diseases that multilines have been designed to control, such as rice blast and cereal rusts, on the grounds that the causal pathogens reach an early peak of sporulation after which colonies gradually die, so concluding sporulation. In other words the assumption of Barrett and Wolfe (1978) that infections continue to sporulate at a constant rate is not correct.

Barrett and Wolfe (1978) calculated from equation 2.2.2.7 a range of values of $\hat{\underline{m}}$ using a range of estimates of α (0.1, 0.5, 1.0, 10) and a range of estimates of $\underline{s}$ (0.1, 0.25, 0.5 and 0.75). A two host component model ($\underline{n} = 2$) was assumed. The super race ($\underline{m} = 2$) was fittest when $s \leq 0.1$ and $\alpha \geq 0.5$ or when $s \leq 0.25$ and $\alpha \geq 10.0$; i.e. generally when α values are large and $\underline{s}$ values small. In all other cases except for $\underline{s} = 0.25$, $\alpha = 1.0$ where the fitnesses of the super race and the simple races ($\underline{m} = 1$) were equal, the simple races predominated. These results will be discussed in more detail later (section 2.3).

2.2.2.d Barrett's (1978, 1980) models

Barrett (1978, 1980) extended the model of Barrett and Wolfe (1978) to include a factor for the non random dissemination of spores. In all the previous models it was assumed that all spores entered an aerial pool from which they randomly infect all components of the multiline. This is

unrealistic, since some spores will be dispersed onto the same plant and hence infect the same host genotype as that supporting their parent lesions. This is referred to as autoinfection and is a non random component of spore dissemination. The random element is referred to as alloinfection. In Barrett's (1978, 1980) models the proportion of spores that result in alloinfection is denoted by $\emptyset$, whereas $1 - \emptyset$ is the proportion that lead to autoinfection. The value of $\emptyset$, will clearly vary with the crop density, degree of tillering, stage of growth of the plants, location of the infections on the plant and climatic conditions amongst other factors. The use of a constant value of $\emptyset$ is therefore simplistic but a necessary one until more accurate experimentally determined values are available.

The significance of the inclusion of autoinfection into the models of pathogen evolution in multilines is that it favours simpler races. The reason is the removal of the 'fly paper' effect that occurs under alloinfection when spores are deposited onto non matching host genotypes. Autoinfection thus ensures a matching host genotype is always available. In a pure stand, matching host genotypes are always available, hence all infections are effectively from autoinfection.

Barrett (1980) using his three host component model ($n = 3$) demonstrated that when $\emptyset = 0.1$ and $s = 0.1$ the simple races with one virulence gene dominated the pathogen race structure, becoming the most prevalent after 10 generations. Initially all eight races (one with no virulence genes, race 0; three with one virulence gene, race a, race b and race c (where a, b and c are virulence genes to the three host resistance genes, A, B and C respectively); three with two virulence genes, race ab, race ac and race bc; and the super race, race abc) were each set at a frequency of 0.125. If $\emptyset$ was increased to 0.5 when s was

0.1, the super race dominated very quickly. At an intermediate value of $\emptyset = 0.22$ Barrett (1980) found that simple races were ultimately favoured. In all these simulations Barrett (1980) assumed that $\alpha = 1.0$. Consequently as $\emptyset$ cannot exceed $\alpha/(1 + \alpha)$, a $\emptyset$ value of 0.5 was the maximum attainable. When α was 5 - 10 the complex races were favoured because the degree of alloinfection $\emptyset$ can exceed 0.5. Leonard and Czoher (1980) considered that proportions of autoinfection were likely to be negligible based on experimentally determined values of $\emptyset$ for oat stem rust and rice blast. For instance Leonard (1969a) found that for oat stem rust $\emptyset$ was about 0.90 - 0.95. Based on Barrett's (1980) model, with $\emptyset = 0.9$ and $\underline{s} = 0.1$, super races would rapidly predominate in multilines. Further if $\emptyset$ is 0.9, from the equation :

$$\emptyset_{\max} = \frac{\alpha}{1 + \alpha} \qquad 2.2.2.10$$

from Barrett (1978) it follows α may be as high as 9.0, confirming the earlier conclusion on the likely range of α values for rust fungi.

Barrett's (1980) model was also very useful in assessing the effect of multilines and cultivar mixtures on the population sizes of pathogens, as his equations enabled the calculation of the population size at each generation. It was found that the greater the alloinfection frequency, $\emptyset$ the smaller the pathogen population size, because as $\emptyset$ is increased so is the proportion and hence wastage of spores (especially of the simple races) landing on non corresponding host genotypes.

This finding is very significant because it means that the predominance of complex races in multilines that will occur if $\emptyset$ values are high, may not necessarily mean more disease in the multiline compared with the scenario when simple races predominate. Indeed the greatest population

sizes were predicted when $\emptyset = 0.0$ as in pure stands.

A commonly expressed or implied fear is that if super races predominate then the multiline will lose its ability to control disease. Clearly this may not be true. Consequently the assumption that the disease control superiority of multilines results only from a suppression of complex races as believed by Frey et al. (1977) is probably false.

2.2.2.e Trenbath's (1977, 1983) models

Trenbath (1977, 1983) examined theoretically the claim that multilines resist disease better than pure cultures. His models are more complex than the earlier ones discussed and include a greater ecological emphasis. In the first model, Trenbath (1977) used a selection coefficient of 0.415, based on what he considered to be consistent with experimental data. This value was deduced from race competition experiments of Watson and Singh (1952) discussed earlier (sections 1.1.4.d and 1.3.6.bi).

Trenbath (1977) concluded that "stabilizing selection can contribute significantly to the horizontal resistance of a multiline". This is hardly surprising when such large coefficients of selection are used.

Further, Trenbath (1977) compared simulated rates of breakdown of three strong resistance genes used in three different strategies - in multilines, all the three genes combined in a single cultivar (a superline) and using the genes singly in cultivars sequentially introduced. Interestingly the rate of breakdown of the resistance genes was approximately the same in all three cases.

In the second similar model (Trenbath, 1983), a selection coefficient of 0.34 was used, and a fourth strategy, that of rotation of cultivars with these separate resistances was considered. Again the predicted longevity of resistance was similar for all four strategies, but ludicrously

high at ∞ years. This absurdity led Trenbath (1983) to suggest that his selection coefficient was too high. These results will be discussed in the next section (2.3).

2.3 Multilines models and simulations using selection coefficients calculated from field data

2.3.1 Introduction

In the previous section (2.2) it was shown that the inclusion of stabilizing selection against unnecessary virulence in models of pathogen race dynamics in multilines, was essential to predict the suppression of complex race build up. The absence of stabilizing selection in multilines would otherwise result in rapid selection for virulence in the pathogen population, and ultimately in the predominance of super races.

Since the values of α and $\emptyset$ for pathogens that multilines are designed to control are of the order of magnitude that favour complex races (section 2.2), predictions of race dominance largely depend upon the values of $\underline{s}$ chosen. The values of $\underline{s}$ used in these models were typically higher than those calculated from field data and discussed in detail in section 1.3.6. Barrett and Wolfe (1978) utilized a range of $\underline{s}$ values of 0.10 - 0.75. Not surprisingly simple races were predicted to predominate in calculations using $\underline{s}$ values of 0.5 or 0.75. Trenbath (1977, 1983) similarly used very high selection coefficients of 0.415 and 0.340 respectively. Barrett (1978, 1980) assumed values of $\underline{s}$ of 0.1 or 0.5 in his simulations. In none of these simulations was $\underline{s} < 0.1$ in contrast to those calculated from field data, Table 19 (section 1.3.6.h). Further the data from which these $\underline{s}$ values were calculated involved observable declining virulence gene frequencies. Selection against these

unnecessary virulence genes (section 1.3.6.h) was therefore very strong and probably atypical of unnecessary virulence genes in general. Consequently, the values of s in Table 19 are in the upper range of likely s values. Typically s values are expected to be less than those listed in Table 19. Nevertheless it is instructive to use some of these s values in simulations with some of the models in order to predict more accurately the likely course of pathogen evolution in multilines.

For the pathogens causing rust and mildew diseases listed in Table 19, s values commonly were about 0.03 to 0.06. The range in s values for the P. oryzae data has a high upper limit of 0.146 (Table 16, section 1.3.6.fii) due to insufficient knowledge of the proportion of resistant hosts (n) with the Pi-i gene grown in Japan.

The very high s values calculated for the decline in race T of B. maydis are clearly atypical (Table 19). For various reasons this is likely to be a special case and unrepresentative of plant pathogens in general. For example the mode of pathogenesis depends upon a selective toxin, susceptibility to which is associated with a cytoplasmic determinant pleiotropic for male sterility. Ability to produce T-toxin in the pathogen is the unique attribute of race T, a feature rapidly lost (section 1.3.6.g) in the absence of cms-T hosts, implying a considerable metabolic penalty.

In the simulations described below, s values in the range of 0.05 - 0.06 have been used.

2.3.2 Groth's (1976) model

In section 2.2 for the multiplicative model it was shown that :

$$\underline{m} = -1/\log_e (1-\underline{s}) \qquad 2.2.2.5$$

If s values are typically in the range of 0.03 - 0.06 then m values

will vary from 16.16 - 32.83. Since the number of isolines in a multi-line and hence resistance genes would not normally exceed fifteen (Marshall, 1977), the fittest races in multilines would be predicted to carry all the necessary corresponding virulence genes confirming the genetic ability to infect every isolate. These are therefore super races.

The equivalent equation for the additive model was :

$$m = \frac{1 + s}{2 \cdot s} \quad 2.2.2.6$$

Thus if $s = 0.03$, $m = 17.17$ and if $s = 0.06$, $m = 8.83$. Whilst these m values are lower than those calculated for the multiplicative model, intermediate or complex races are predicted to be the fittest. The multiplicative model was considered to be the most realistic of the two (Groth, 1976; Barrett and Wolfe, 1978), so that high values of m are most likely.

2.3.3 Barrett and Wolfe's (1978) model

Barrett and Wolfe's (1978) equation (2.2.2.7) for estimating $\hat{m}$, the number of virulence genes possessed by the fittest race in a multi-line was :

$$\hat{m} = \frac{-\alpha - n \cdot \log_e(1-s)}{\alpha \cdot \log_e(1-s)} \quad 2.2.2.7$$

Grant and Archer (1983) calculated from this equation and using a value of $s = 0.061$, a series of estimates of $\hat{m}$ for a range of n and α values. The results are shown in Table 28.

Table 28 Values of $\hat{\underline{m}}$ required to maximize pathogen fitness
on a multiline cultivar for ranges of $\underline{n}$ and α with
 $\underline{s}$ set at 0.061¹

$\underline{n}$	$\hat{\underline{m}}^2$			
	$\alpha = 0.1$	$\alpha = 0.5$	$\alpha = 1.0$	$\alpha = 10.0$
2	- 4.102 ³	11.898	13.898	15.698
5	- 34.102 ³	5.898	10.898	15.398
10	- 84.102 ³	- 4.102 ³	5.898	14.898
15	-134.102 ³	- 14.102 ³	0.898 ³	14.398

1 where $\hat{\underline{m}}$ is the number of virulence genes which maximize the fitness of the pathogen, $\underline{n}$ is the number of equally represented isolines in the multiline, $\underline{s}$ is the selection coefficient acting against each virulence gene in the absence of a corresponding host resistance gene. α is the realized rate of reproduction (section 2.2.2).

2 where values of $\hat{\underline{m}}$ fall outside the range 1 to $\underline{n}$ take the true $\hat{\underline{m}}$ as 1 when the table value is negative, or less than one; and $\underline{n}$, when the table value exceeds $\underline{n}$.

3 simple races are fitter than complex races. In all other cases the most fit races are complex.

Before comment is made on the results of Table 28, clarification is first needed on the biologically absurd figures contained in it. In Table 28 $\hat{\underline{m}}$ may exceed $\underline{n}$ for example when $\underline{n} = 2$, $\alpha = 0.5$; $\hat{\underline{m}} = 11.898$.

Clearly $\hat{\underline{m}} = 11.898$ can only be a mathematical prediction, the fittest race biologically is the one with the two virulence genes corresponding to the two resistance genes in the multiline with two components ($\underline{n} = 2$). A race with $\underline{m} = 11.12$ virulence genes cannot possibly be fitter than the race with $\underline{m} = 2$, since 9 - 10 of these genes must always be unnecessary, and carry a selective handicap for the carrier. The opposing mathematical prediction that the fittest race carries 11 - 12 virulence genes arises from the equation from which equation 2.2.2.7 is derived (Barrett and Wolfe, 1978). This equation :

$$R_{\underline{m}} = \frac{(1-s)^{\underline{m}-1} \cdot (\underline{n} + \underline{m}\alpha)}{\underline{n}(1+\alpha)} \quad 2.2.2.6$$

calculates the relative fitness ($R_{\underline{m}}$) of a race with $\underline{m}$ virulence genes, given s , $\underline{n}$ and α .

The size of $R_{\underline{m}}$ is dependent only on the numerator terms $(1-s)^{\underline{m}-1}$ and $(\underline{n} + \underline{m}\alpha)$ for given values of $\underline{n}$ and α . In the former term which calculates the detriment to fitness of unnecessary virulence genes, $\underline{m}$ may exceed $\underline{n}$. However in the latter term, which calculates the benefit to fitness of necessary virulence, $\underline{m}$ cannot exceed $\underline{n}$ biologically since only $\underline{n}$ of these can be necessary. Mathematically, $\underline{m}$ may exceed $\underline{n}$ explaining why R_{11} calculated from equation 2.2.2.11 exceeds R_2 . Consequently if $\hat{\underline{m}}$ calculated from equation 2.2.2.7 exceeds $\underline{n}$, the fittest race is the super race with $\underline{m} = \underline{n}$ virulence genes. The same applies with Groth's (1976) model (section 2.2.2).

Similarly, a race with a negative number of virulence genes is meaningless biologically and arises from a similar mathematical quirk. If $\hat{\underline{m}}$ is calculated as negative or less than one, biologically the correct value is one.

From Table 28 it is evident that the super races are predicted to predominate the race structure of multilines when $\alpha = 10.0$ for all values of $\underline{n}$ and when $\alpha = 0.5$ and 1.0 for $\underline{n} = 2$ or 5 . For $\alpha = 1.0$ and $\underline{n} = 10$ a complex race is the fittest. For all other combinations of $\underline{n}$ and α , the simplest races where $\underline{m} = 1$ are the fittest.

Groth (1978) and Grant and Archer (1983) argued that values of α for rusts and mildews may equal or exceed $5 - 10$ (see also section 2.2.2). Further in commercial multilines $\underline{n}$ varies from 6 to 15 (Marshall, 1977). Because evidence of significant selection against most unnecessary virulence genes is lacking (Van der Plank, 1975) a value of $\underline{s} = 0.061$ is only likely to be realistic for a few of the $6 - 15$ corresponding unnecessary virulence genes, and for the rest $\underline{s}$ values may be much lower. Table 28 however was calculated on the basis that all $\underline{n}$ corresponding unnecessary virulence genes carry the same fitness loss, $\underline{s} = 0.061$. True values of $\underline{n}$ where $\underline{s} = 0.061$ (strong genes, sensu Van der Plank) are therefore lower than $6 - 15$.

Consequently, with higher values of α ($1 - 10$), lower values of $\underline{n}$ ($2 - 5$), and realistic estimates of $\underline{s}$ (< 0.061) complex races are predicted by Barrett and Wolfe's (1978) model to be selected in multilines. This is expected as it was shown in section 2.2.2 that when $\underline{n}$ is small and α is large, Barrett and Wolfe's (1978) model becomes similar to that of Groth (1976).

2.3.4 Barrett's (1980) model

Barrett's (1980) model differed from those of Groth (1976) and Barrett and Wolfe (1978) by the inclusion of a partition of infections into alloinfection (at a proportion $\emptyset$) and autoinfection (at a proportion $1 - \emptyset$). With low values of $\emptyset$, Barrett's (1980) model predicted the

selection of simple races in multilines even if $\underline{s}$ values are relatively small ($\underline{s} = 0.1$, Barrett, 1980). This result differed from simulations with earlier models which predicted the selection of complex races if $\underline{s}$ was small, unless α values were very low (Barrett and Wolfe, 1978).

Values of $\emptyset$ potentially may vary each generation within a single crop and within a season, and are difficult to measure. Leonard and Czocher (1980) thought values of $\emptyset$ would exceed $1 - \emptyset$ for the kinds of diseases multilines are designed to control (section 2.2.2), and thus the prediction of selection of complex races in multilines is again a reasonable expectation from Barrett's (1980) model, especially if smaller more realistic values of $\underline{s}$ than 0.1 are used in simulations. A computer programme of Barrett's (1980) model was written to perform such simulations. In this three host component and eight race model, values of $\emptyset$, $\underline{s}$, α , $\underline{z}$ and $\underline{t}$ (section 1.3.2.a) could all be varied. Initially, the frequency of each of the eight races (section 2.2.2.d) was set at 0.125, and the population total at 8.0.

In the first simulation, $\underline{s}$ was 0.05, and $\emptyset$ was 0.1. After one generation the frequency $\underline{p}_0$ of the race with only avirulence genes had declined from 0.125 (in generation zero) to 0.005 (in generation one). In the next and subsequent generation, $\underline{p}_0$ declined to and remained at zero respectively. The frequencies $\underline{p}_1$, $\underline{p}_2$, $\underline{p}_3$ of races with one, two and three genes respectively are shown below in Table 29.

Table 29 Frequencies p_1 , p_2 , p_3 of races with one, two and three virulence genes respectively in simulations with Barrett's (1980) model¹

Generation	p_1	p_2	p_3
0	0.375	0.375	0.125
1	0.268	0.495	0.233
2	0.268	0.499	0.233
6	0.285	0.498	0.217
20	0.348	0.486	0.166
280 ²	0.980	0.020	0.000

¹ $\underline{s} = 0.05$, $\emptyset = 0.1$, $\alpha = 1.0$, $\underline{z} = 0.98$

² At generation 280 the population size was approximately 8.805×10^{75}

Table 29 shows that from generations two to six, p_1 begins to rise steadily, whilst p_2 and p_3 gradually decline. This means that simple races are predicted to predominate when $\underline{s}$ and $\emptyset$ values are both small, but the time taken to reach $p_1 = 0.98$, 280 generations may be long. For example if there are only 10 generations/year, this change requires nearly 30 years.

The population sizes at generation 20 and 280 are 8.141×10^5 and 8.805×10^{75} respectively. The calculation of the latter population size relies on the unrealistic assumption that there are 280 successive generations in a season.

Changes in the value of α do not change the race frequencies

(providing other parameters are constant), only the population totals at each generation.

This simulation is unlikely to have much practical significance for cereal multilines as $\emptyset$ values are unlikely to be as small as 0.1 on average for rust and mildew pathogen. However multiline crops grown in protected environments under glass or plastic may theoretically select simple races, because the high wind velocity which favour allo-infection in cereal crops are eliminated, and hence $\emptyset$ values may be very small.

In a second simulation $\underline{s}$ was set at 0.01 whilst all other parameters were unchanged. At generation 20, $\underline{p}_3$ alone had increased to 0.372 whilst $\underline{p}_1$ and $\underline{p}_2$ were 0.150 and 0.478 respectively and declining. Thus a reduction of $\underline{s}$ to 0.01 from 0.05 reverses the selection for simple races. Ultimately, the super race with three virulence genes predominates. The population size at generation 20 was higher (1.888×10^6) in this second simulation because selection against unnecessary virulence genes was reduced, not because the selection of complex races automatically means more reproduction and hence disease.

In a third simulation $\underline{s}$ was set at 0.05 as in the first simulation whilst $\emptyset$ was increased to 0.2. As in the second simulation $\underline{p}_3$ was increasing steadily at generation 20 whilst $\underline{p}_1$ and $\underline{p}_2$ were steadily declining. The frequencies were also similar at 0.372, 0.140 and 0.489 respectively.

Thus for $\underline{s} = 0.05$ an increase from $\emptyset = 0.1$ to $\emptyset = 0.2$ changes the prediction of selection for simple races to one of selection for complex races. The population total at generation 20 was 4.086×10^5 the smallest of the totals for the three simulations. This observation is consistent with Barrett's (1980) observation that as propagule exchange ($\emptyset$) increases so does the degree of disease control achieved by the multiline.

In a final simulation, $\underline{s}$ was set at 0.05 and $\emptyset$ at 0.9. This $\emptyset$ value is a realistic estimate for cereal rusts and rice blast (section 2.2.2.d). The results of this simulation showed very rapid selection for the super race. At generation 20 its frequency was 0.993. Intermediate races with two virulence genes made up the difference. Further at generation 20 the population size was only 1.357×10^5 , the smallest for any of the four simulations.

2.3.5 Discussion and Conclusions

From these models, calculations and simulations it is evident using realistic estimates of $\underline{s}$, $\emptyset$ and α that super races or complex races are predicted to predominate the pathogen race structure of multilines. Simple races predominate with realistic $\underline{s}$ values only if α or $\emptyset$ values are very small.

The simulation with Barrett's (1980) model also confirms the suggestion (section 2.2.2.d) that the predominance of the super race may perhaps surprisingly be accompanied by smaller pathogen populations as can be seen by contrasting the results of the fourth and the first simulations (section 2.3.4), which differed only in the value of $\emptyset$ chosen. In the fourth simulation where $\emptyset$ was set at 0.9, the super race was rapidly selected in the multiline, whereas in the first simulation using a value of $\emptyset$ of 0.1 simple races predominated. However the population size was considerably reduced in the fourth simulation; after 20 generations the population total was nearly six times less than that where the simple races predominated.

In the second simulation with Barrett's (1980) model, the super race predominated eventually but at generation 20 the population size was 2 - 3 times that where the simple race predominated (simulation 1).

These results show that under different combinations of conditions super races can predominate with comparatively high or low population totals compared with simulation where simple races predominate. The key parameters determining the population totals of super races, are $\emptyset$ and $\underline{s}$. If $\underline{s}$ and $\emptyset$ values are low ($\underline{s} = 0.01$, $\emptyset = 0.1$) i.e. in the second simulation (section 2.3.4) the population size will be comparatively high (1.888×10^6) because there is little selection against simple races (as $\emptyset$ is low) and against complex races (as $\underline{s}$ is low). However if $\underline{s} = 0.05$ and $\emptyset = 0.9$ (simulation 4, section 2.3.4) complex races still predominate but the population size is much reduced (1.357×10^5) because there is considerable selection against both simple and complex races.

These findings are significant for the 'dirty crop' approach because they shift the emphasis in thinking from race predominance in multilines to an emphasis on disease levels. The domination of the super race in a multiline does not automatically lead to more disease. Evidently the assumption in multiline theory that the selection of simple races is the basis of disease control may be wrong. If some disease is tolerated in multilines, and simple races are acceptable then surely super races are acceptable, especially if they cause less disease. The philosophy that "simple races are O.K. but complex ones are not", is challenged. If the predominance of super races is acceptable in multiline strategy with a 'dirty crop' approach this raises the question - "what is the philosophy of the disease control of multilines?". Surely as a means of reducing disease levels by using major resistance genes so that selection against all races of the pathogen occurs, not just against complex races. In some senses it would not matter whether simple or complex races predominate, if disease is reduced. In other circumstances race predominance may be important. For instance if a superline strategy (involving the

same genes) replaced a multiline it would be advantageous if simple races predominated at the time of changeover.

The current philosophy of disease control using multilines that super races need to be suppressed at all costs largely stems from the experiences of the 'boom and bust' cycle where increased virulence led to a breakdown in resistance and the ultimate removal from cultivation of the formerly resistant cultivar. However in multilines, virulence gene accumulation may not mean more disease. Multilines theoretically should go through a 'boom, decay and stabilize' cycle where the initial boom in disease control is eroded somewhat (decay period) as races with corresponding virulence genes (whether carried singly as simple races or in combination as complex races) appear and increase on the multiline. Eventually, the pathogen should reach and maintain its maximum potential for reproduction and disease on the multiline as all isolines become infected at an intensity which should be considerably less than that in pure stands. A multiline's resistance will then act like horizontal resistance or partial resistance, in allowing disease, but at reduced amounts. A 'bust' should be avoided because the multiline's mechanisms of resistance both ecological (section 2.2.2.a) and genetic (stabilizing selection and fly paper effects) cannot be avoided by the pathogen. Other genetic mechanisms of resistance that operate in multilines are discussed in Chapter 4.

It is evident that many of the authors who have developed or commented on models of evolution of pathogens in multilines were influenced by earlier estimates of $\underline{s}$ values calculated by Leonard (1969b, 1977a) or from race competition data; for example the work of Watson and Singh (1952). Thus Trenbath (1977, 1983) and Barrett (1978) and Barrett and Wolfe (1978) all used high $\underline{s}$ values in their simulations,

ranging from 0.10 - 0.75. This was hardly surprising since, firstly very few experimental estimates of $\underline{s}$ were available, and secondly those available were high especially compared with the values listed in Table 19 (section 1.3.6.h). Thus Leonard (1969b) estimated selection coefficients ranging from 0.14 - 0.39 for four unnecessary virulence genes in *P. graminis avenae*, from race competition experiments. Similarly, Leonard (1977a) estimated $\underline{s}$ values of 0.12 or 0.30 for *B. maydis* again from race competition experiments. Finally, Leonard (1977a) calculated from Watson and Singh's (1952) race competition data an $\underline{s}$ value of 0.42 for unnecessary virulence to the $\underline{Sr}_6$ gene and 0.22 for unnecessary virulence to the $\underline{Sr}_{11}$ gene in *P. graminis tritici*.

As discussed earlier (sections 1.3.6.bi, 1.4.1 and 1.4.3.cv) race competition experiments even if correct controls are applied (sections 1.1.4.d, 1.1.5 and 1.4.1) may yield overestimates of $\underline{s}$. The application of $\underline{s}$ values calculated from field data (section 1.3.6) should prove more useful in predicting the effects of pathogen evolution on multilines from the models to date. The prediction from these models differ markedly depending upon the magnitude of $\underline{s}$ chosen. The consequences of low $\underline{s}$ values /^{on} simulation predictions have been fully discussed earlier (section 2.3). Complex races are predicted to predominate multilines.

For higher $\underline{s}$ values, simple races are predicted to be selected as by Barrett and Wolfe (1978, Table 1). They showed using equation 2.2.2.7

$$\hat{m} = \frac{-\alpha - n \cdot \log_e (1-s)}{\alpha \cdot \log_e (1-s)} \quad 2.2.2.7$$

that for $\underline{n} = 2$ the fittest races in the multiline were simple races, for all values of α (0.1, 0.5, 1.0 and 10.0) when $\underline{s}$ was 0.50 or 0.75.

Groth (1976) did not attempt estimates of $\underline{m}$ from his equation 2.2.2.2

and 2.2.2.5 (section 2.2.2.b). However using equation 2.2.2.5 for the multiplicative model :

$$\underline{m} = -1/\log_e (1 - \underline{s}) \quad 2.2.2.5$$

estimates of $\underline{m}$ can be calculated from a range of higher $\underline{s}$ values. They are shown in Table 30.

Table 30 Estimates of $\underline{m}$ ¹ from a range of $\underline{s}$ ² values from Groth's (1976) model³

$\underline{s}$	$\underline{m}$
0.2	4.48
0.3	2.80
0.4	1.95
0.5	1.44
0.6	1.09
0.7	0.83

¹ $\underline{m}$ = number of virulence genes possessed by the fittest race in the multiline

² $\underline{s}$ = stabilizing selection coefficient

³ model equation is $\underline{m} = -1/\log_e (1 - \underline{s})$

Table 30 again clearly shows that higher $\underline{s}$ values chosen in the estimation of the number of virulence genes carried by the fittest races in multilines result in the prediction that simple races are selected,

except perhaps if $\underline{s} = 0.2$. It must be remembered that the calculation of $\underline{m}$ is independent of $\underline{n}$ if $\underline{m} < \underline{n}$, but the effective degree of complexity of races with $\underline{m}$ virulence genes (as in Table 30) depends on the magnitude of $\underline{n}$. For example in a multiline with just two isolines ($\underline{n} = 2$), for $\underline{s} = 0.3$, $\underline{m} = 2.80$ (Table 30). In this case, the fittest race will be the super race with both corresponding virulence genes. However if $\underline{n} = 15$ for example, then a race with 2 - 3 virulence genes will be comparatively simple.

Thus the predictions of the likely evolution of races of pathogens in multilines from the models described varies enormously according to the $\underline{s}$ values chosen. It is therefore important that $\underline{s}$ values are accurately determined; this is best provided by estimates from field data.

Caution must be exercised in the usefulness of the predictions made from these models even with more realistic estimates of $\underline{s}$ for two reasons. Firstly, estimates of $\underline{s}$ calculated from field data may not be typical for virulence genes to many of the corresponding resistance genes in multilines. Further each unnecessary virulence has a different $\underline{s}$ value. Consequently, the models of evolution of pathogens in multilines are simplistic in assuming the same $\underline{s}$ value for every virulence gene. More realistic models must assign different $\underline{s}$ values to different virulence genes, but this makes the mathematics more complex. Secondly, the models are usually very simplistic in their exclusion of other selective forces that could operate in multilines against the pathogen population. This is the topic of later chapters and will not be discussed here. Suffice it to say that these other selective mechanisms include induced resistance and susceptibility, selection against virulence gene combinations (Chapter 3) and selection against the background genotype of complex races in cultivar mixtures.

Most of the models described here; those of Groth (1976), Barrett and Wolfe (1978) and Trenbath (1977, 1983) are models of pathogen evolution in multilines. Those of Barrett (1978, 1980) are of cultivar mixtures and multilines. In the above discussions (section 2.1, 2.2 and 2.3) I have refrained from applying conclusions on the evolution of pathogens in multilines to evolution in cultivar mixtures. Pathogen virulence dynamics are more complex in cultivar mixtures due to the heterogeneous nature of the components' background genotypes and as discussed in Chapter 4, the selection for complex races in cultivar mixtures should be less pronounced than in multilines.

CHAPTER 3

SELECTION AGAINST VIRULENCE GENE COMBINATIONS

3.1 Introduction

Selection may occur against particular pairs or combinations of virulence genes in pathogen populations as well as against individual unnecessary virulence genes. Such associated fitness losses may arise due to a biochemical interaction between the individual gene products, or between some subsequent products of the relevant metabolic chains. The fitness loss associated with a particular virulence gene combination may be independent of the host genotype. For example a virulence combination $\underline{V}_1\underline{V}_2$ may confer an equal selective disadvantage to its carrier on hosts of genotypes $\underline{R}_1\underline{R}_2$, $\underline{R}_1\underline{R}_2$ and $\underline{R}_0$ or on the genotype $\underline{R}_0$, compared with pathogen genotypes lacking the combination $\underline{V}_1\underline{V}_2$. This is in contrast to selection against unnecessary virulence where the virulence combination $\underline{V}_1\underline{V}_2$ is only selectively disadvantageous on $\underline{R}_0$, $\underline{R}_1$ and $\underline{R}_2$ host genotypes but not the $\underline{R}_1\underline{R}_2$ host genotype. The difference is that the biochemical interaction in stabilizing selection is between the host and pathogen genes (it is not an inherent property of the virulence gene) whereas in combination selection it is a biochemical interaction between pathogen genes (it is an inherent property of the virulence gene pair).

Naturally, on $\underline{R}_1\underline{R}_2$ genotypes, a race with the $\underline{V}_1\underline{V}_2$ combination is fitter on average than races with $\underline{V}_1$ or $\underline{V}_2$ singly, but may be less fit than $\underline{V}_1$ on $\underline{R}_1$ or $\underline{V}_2$ on $\underline{R}_2$ hosts if combination selection operates. Loss of fitness arises because of an interaction between the $\underline{V}_1$ and $\underline{V}_2$ genes. The epistasis is not evident however when $\underline{V}_1$ and $\underline{V}_2$ are present singly in separate genotypes.

Selection against a particular virulence gene combination may occur in addition to or independently of stabilizing selection, i.e. there may be selection against the V_1V_2 combination with or without selection against the genes V_1 and V_2 when unnecessary. Thus the fitness of V_1V_2 may be greater on R_1R_2 genotypes where only combination selection operates, than on R_0 or R_1 or R_2 genotypes where both combination and stabilizing selection may operate. Alternatively R_1 and R_2 may be weak genes in which case stabilizing selection would not operate and the average fitness of the combination V_1V_2 would be equal on R_0 , R_1 , R_2 and R_1R_2 hosts.

The concept of selection against virulence gene combinations is, like stabilizing selection, a controversial subject. Much of the controversy centres not so much on any inherent problems with the theoretical basis of the selective force, but more on the validity of methods to test for its existence in asexual pathogen populations.

In this chapter, evidence is presented to show firstly that this type of selection can operate in sexual and asexual populations, with particular emphasis on Puccinia graminis tritici populations in North America, and secondly that the commonly used test for linkage disequilibrium can be validly used in certain circumstances to test for combination selection in large asexual populations. The theoretical basis for the generation and selection of combinations of virulence genes is also discussed in detail.

3.2 Literature review

Wolfe and his colleagues (Wolfe and Wright, 1972; Wolfe and Schwarzbach, 1975; Wolfe, Barrett, Shattock, Shaw and Whitbread, 1976) developed a simple test for linkage disequilibrium (non random association) of virulence genes in pathogen populations. In the test the observed

frequency of combined virulence in a pathogen population is compared with the expected frequency, based on the product of the individual virulence gene frequencies. Thus if the frequency of the virulence gene $\underline{V}_1$ is $\underline{x}$ ($0 \leq \underline{x} \leq 1$) and the frequency of virulence gene $\underline{V}_2$ is $\underline{y}$ ($0 \leq \underline{y} \leq 1$) then the expected frequency of the pathogen genotype $\underline{V}_1\underline{V}_2$ is $\underline{x}.\underline{y}$ if the following assumptions are met :

- (i) the pathogen population sampled is large,
- (ii) reassortment of virulence genes is random and independent,
- (iii) there is no differential selection for the genes or gene combinations involved.

The significance of a deviation of observed frequency of combined virulence from the expected is assessed by a χ^2 test. Wolfe et al. (1976) reported that when the model was applied to data of Erysiphe graminis hordei numerous deviations from expectation were observed. Most were explicable because of differential selection, i.e. assumptions (i) and (ii) above held, but assumption (iii) did not (Wolfe et al., 1976). The selection could be explained in most cases from a knowledge of the hosts and the resistance genes they carried (Wolfe et al., 1976).

As an example Wolfe and Schwarzbach (1978a) stated that there are "consistently and unexpectedly low frequencies of virulence for the Mla_{4/7} resistance in pathogen populations on Mlas varieties. It is likely that the same virulence gene can simultaneously be essential for the pathogen to grow on one variety but reduce its ability to grow on the other". Wolfe and Schwarzbach (1978a) reported that about half the total spring barley area in the U.K. in recent years had been sown to Mlas and Mla_{4/7}

varieties ensuring a large selective advantage for combined virulence. The lack of this virulence combination was thus very surprising, and Wolfe and Schwarzbach (1978a) concluded that the virulence combination causes a severe limitation to the pathogen.

Lower than expected frequencies of combined virulence have been recognized in other cases (Wolfe and Barrett, 1977). Wolfe, Slater and Minchen (1981) for example reported twelve negative deviations involving barley mildew virulence genes 1 - 8 (BMV1-8).

MacIntosh (1978) commenting on a lack of combined virulence to Mlg and Mla₁₂ predicted from random recombination (reported by Wolfe and Schwarzbach, 1975), suggested that the reduced frequency of combined virulence may be temporary and that new variants with the expected combined virulence frequencies might occur in the future.

Wolfe and Wright (1978) provided some evidence of this for the virulence combination to the Mlas and Mla_{4/7} genes in populations of E. graminis hordei, where the U.K. cereal pathogen virulence survey of 1977 revealed some significant increases in the combined virulence frequency. However Wolfe et al., (1981b) noted that it is consistently observed that combined virulence to Mlas and Mla_{4/7} is selected against on hosts with either resistance gene; hence the disassociation has clearly not been broken down in recent years.

Wolfe et al. (1976) also noted that the observed frequency of some virulence combinations might exceed the expected frequency; an example being virulence to Mlg and Mla₆, and they suggested that this virulence combination may have increased its fitness with time. Hence selection in favour of virulence combinations is theoretically and practically as likely as selection against virulence gene pairs. According to Wolfe et al. (1981b) these positive deviations tend to occur among more recently selected virulence genes in populations on cultivars with the longest

exposed resistance genes (Mlh, Mlg and Mla₆). Negative deviations tend to occur among more recently selected virulence genes on cultivars with more recently introduced resistances, for example the Mla_{4/9} genes. However virulence to Mlv was disassociated from all other virulence genes i.e. Mlh, Mlg, Mla₆, Mlas, Mla_{4/7}, Mla and Mla_{4/9}, yet this resistance gene and corresponding virulence have been present in the U.K. since at least 1967 (Wolfe and Schwarzbach, 1978a, Figure 3). Wolfe et al. (1981b) suggested that for cultivar mixtures and diversification schemes it is best to concentrate on new cultivars with recently introduced resistances, especially those that show a reciprocal negative deviation. These, however, tend to be rare in E. graminis hordei populations in the U.K.

Van der Plank (1975) applied the test for linkage disequilibrium to data of P. graminis tritici populations in Canada during the years 1965 - 1969 and showed that combined virulence to Sr₆ and Sr_{9d} occurred at a frequency significantly lower than expected. He concluded that the pathogen had been unable to combine the two virulences in a phenotype adequately fit to survive. Further, Van der Plank (1975) asserted that the cultivar Selkirk for many years popular in Canada due to its durable disease resistance was resistant because it carried the corresponding resistance gene combination, Sr₆ and Sr_{9d}.

Later, Van der Plank (1978) analyzed similar data for the years 1970 - 1975 and again showed that in all these years the observed frequency of virulence to both genes was significantly less than expected. Other virulence gene pairs were found with similar deviations namely virulence to Sr_{9a} and Sr_{9d} (virulence genes called 9a and 9d respectively; hereafter with this notation), 9a and 9e, 9b and 9d and 9b and 9e. Van der Plank (1975, 1978) attributed these observations to selection against these virulence gene pairs.

Recently, Roelfs and Groth (1980) challenged the conclusions of Van der Plank (1975) about these selectively disadvantageous virulence gene combinations in P. graminis tritici, on the basis of their observations of the pathogen population in the United States. They noted that the populations of stem rust in central and eastern U.S.A. and Canada reproduce primarily asexually. Sexual reproduction is very rare since Barberry hosts on which the sexual phase occurs was eradicated many years ago. Due to the lack of sexual reproduction, they claimed there is effective 'linkage' of genes in these asexual populations. Some virulence genes might be linked in coupling, some in repulsion, resulting in pairs with higher and lower (respectively) observed frequencies than expected. Therefore they argued "for such populations, deviations of observed from expected combined frequencies are not a valid basis for conclusions about the fitness associated with virulence genes". They then stated that the assumption of independent gene distribution applies only to sexually reproducing populations as in the stem rust population west of the Rocky Mountains in the U.S.A. A more detailed description and evaluation of Roelfs and Groth's (1980) objection to Van der Plank's (1975, 1978) analyses and conclusions is given in section 3.3.2 and 3.3.4.

Recently, Wolfe and Knott (1982) discussed causes of linkage disequilibrium other than that due to selection. Disequilibrium could arise as a result of mixing populations that are genetically different. Thus if a virulence gene $\underline{V}_1$ is selected in one population and another virulence gene $\underline{V}_2$ is selected in another population, on mixing the two populations and testing for linkage equilibrium the observed frequency of combined virulence $\underline{V}_1\underline{V}_2$ will initially be much less than expected (see also section 3.4.3).

A second pair of factors causing linkage disequilibria was inbreeding

and drift. Inbreeding (in an asexual population) presumably could result in a restriction in the number of pathogen genotypes. In a small population where drift effects can be significant, drift could cause major genotype shifts resulting in a linkage disequilibrium. For example if there are three major genotypes in a small asexual population in linkage equilibrium, one carrying $\underline{V}_1$ another $\underline{V}_2$ and the third the $\underline{V}_1\underline{V}_2$ virulence genes and drift effects then result in a rapid increase in $\underline{V}_1$ and $\underline{V}_2$, and a decrease in $\underline{V}_1\underline{V}_2$, linkage disequilibrium will result. However drift effects are likely to be small in most pathogen populations which are very large.

Thirdly, selection for one locus may select for a linked gene which is selectively neutral. Pseudo selection for the latter gene ensues as a result and this can result in an abnormally large frequency of combined virulence. However this 'hitch hiking' effect is unlikely in a large population even if it is an asexually reproducing one because of the effect of recurrent mutation (sections 1.3.6.bi and 3.4.2).

Other factors causing linkage disequilibria include environmental factors and sampling methods.

Wolfe and Knott (1982) defined the conditions where the use of the test for linkage disequilibrium is a valid basis for detecting selection against a virulence gene combination. They concluded that if the isolates sampled were from a homogeneous sub population of a sexually reproducing pathogen (or an asexual population if there is random genetic exchange) collected from a limited area and only from susceptible hosts then the requirements for a valid analysis of selection are met.

These authors then concluded that a number of tests of linkage disequilibria have produced doubtful conclusions because of invalid use of the procedure. They considered the examples provided by Wolfe et al.

(1976) as being suspect since the data were collected from different hosts and regions, and were summed prior to analysis.

Van der Plank's (1975) analysis and conclusions also received criticism, since the data from which he had drawn his conclusions that virulence to Sr₆ and Sr_{9d} had a selective disadvantage came from collections from different hosts and different regions and were summed indiscriminately. Further Wolfe and Knott (1982) concluded that Van der Plank's (1975) interpretation of the analysis was invalid because he was not dealing with a randomly mating population.

However, on the other hand, Wolfe and Knott (1982) noted the commonness of combined virulence to Sr₆ and Sr_{9d} in P. graminis tritici populations in Texas, but not in northern U.S.A. in 1968. Van der Plank's (1975) analysis was of data from the years 1965, 1967, 1968 and 1969. By 1975 combined virulence was more common in the U.S.A. especially in the central states (Roelfs and McVey, 1976). Wolfe and Knott (1982) suggested that the wheat cultivar Selkirk remained resistant in Canada during the period when it was widely grown because the completely matching virulence combination was not selected in the south and never became established in the overwintering area.

3.3. Studies on the North American P. graminis tritici populations and evidence for selection against virulence combinations

3.3.1 Introduction

Considerable analysis of the virulence dynamics of P. graminis tritici has already been done (Green, 1971, 1975; Van der Plank, 1975, 1978, 1982; Roelfs and Groth, 1980 and Wolfe and Knott, 1982). However the conflicting assumptions and hence conclusions have resulted in confused evidence for the operation of selection against virulence combinations.

It is the intention in this section to abstract from the data, errors, misunderstandings and 'red herrings' that exist in the literature evidence that clearly indicates the operation of selection against some virulence gene pairs in these populations.

3.3.2 Evidence of Roelfs and Groth (1980) to support the contention that the test for linkage disequilibrium is not a valid basis for conclusions about the fitness associated with virulence genes in an asexual population

Roelfs and Groth (1980) analyzed data of the sexually reproducing population of P. graminis tritici in north west U.S.A. as well as data of the main isolated, asexual population in central and eastern U.S.A. This population causes stem rust in the wheat belt of the U.S.A., and is separated from the western population in which sexual reproduction is common by the Rocky Mountains. Little or no migration between the two populations occurs (J. Groth, personal communication), and stem rust is not a serious pathogen west of the Rocky Mountains unlike the situation in the main wheat belt.

In support of Roelfs and Groth's (1980) contention that the test for linkage disequilibrium is not a valid basis for conclusion about the fitness associated with virulence genes (see also section 3.2), they :

- (a) noted in the sexual population, 100 distinct phenotypes (with respect to 15 virulence genes studied) were identified in the 426 isolates sampled, giving a percentage of distinct phenotypes of 23.5%. Variation in the asexual population was much less with only 17 distinct phenotypes identified in 2377 isolates, giving 0.7% of distinct phenotypes.

(b) noted that the mean deviations of observed frequencies of virulence pairs from their expected frequencies for the asexual population were much larger than for the sexual population (Roelfs and Groth, 1980, Table 4). The mean deviations were calculated as follows. In their Table 2 and 3 respectively they presented data of the observed frequencies singly and in combination (pairs) of 15 virulence genes for the sexual and asexual populations separately. In addition they calculated the expected frequencies of all the combined virulences based on the individual observed virulence frequencies. These too were presented in Tables 2 and 3. In total there were 105 pair wise combinations of the 15 virulence genes for each population. The mean deviations for each virulence gene in each population in combination with the other 14 genes were determined with the standard deviation of the 14 deviations (Roelfs and Groth, 1980, Table 4). Use of t-tests showed that for the sexual population five of the virulence genes had significant mean deviations, four of these indicating selection against the gene, i.e. virulence genes 9a, 9b, 10 and 17. However for the asexual population only two of the mean deviations were significant for virulence genes 8 and 16. Both of these mean deviations were positive. The reason for fewer significant mean deviations in the asexual population data was the very large standard deviations associated with these mean deviations, unlike those for the sexual population. The main point, however, is that the size of the mean deviations were much larger for the asexual population, than for the sexual population. Roelfs and Groth (1980) did not report on the significance of the deviations of the individual gene pairs. I assessed the significance of all 105 combinations for each population using χ^2 tests. For

the sexual population, there were 33 significant deviations representing 31.4% of all the comparisons. Of these 16 were deviations where the observed frequency was significantly less than expected (disassociations). Individual deviations were small, even if significant; none being greater than 0.05. For the asexual population on the other hand 71 comparisons showed significant deviations and 23 of these were more than 0.10. Hence the asexual population's individual and mean deviations were greater than those in the sexual population.

- (c) noted that Simpson's measures of diversity, $\underline{D}$ was greater for the sexual population, 0.974, than for the asexual population, 0.501. Roelfs and Groth (1980) concluded the former population was the most diverse genetically, with respect to these 15 virulence genes.
- (d) examined the distribution of genes for virulence among the isolates. In their Figures 2 and 3 they plotted the number of isolates against the number of loci expressing virulence for each population. The data for the sexual population had a nearly symmetrical distribution, whereas the distribution of the data of the asexual population was assymetrical and bimodal. This study was extended to consider numbers of virulence loci pair differences in order to overcome a weakening of the comparison as a result of the hosts in the location of the two populations possessing different resistance genes. They plotted in Figures 4 and 5, a frequency distribution of number of gene differences for each population. They also plotted an expected distribution for each population based on random gene association. For the sexual population only was there a close fit of observed to

expected. The asexual population showed a clustering of isolates into groups. This however is not surprising since the asexual population was made up of just a few genotypes. This was noted by Wolfe and Knott (1982) in an analysis of the deviations in the asexual population. Further Wolfe and Knott (1982) pointed out that an unusual distribution of the deviations for each virulence gene in the asexual population occurred because one phenotype, race 15-TNM made up 69% of all the isolates, whilst four others made up another 24%. Thus if two virulence genes were either present or absent in race TNM a large positive deviation (association) would be likely.

- (e) plotted, for the two populations, a frequency distribution of the frequencies of all paired combinations of loci expressing virulence, i.e. a frequency distribution of the 105 virulence gene pair frequencies given in Tables 2 and 3. This is given in Roelfs and Groth (1980) Figure 7. For the asexual population the distribution was bimodal with locus pair frequencies either high (0.70 or greater) or low (0.25 or lower) with no intermediate values. On the other hand the sexual population's distribution was more spread over the range 0.00 - 0.99 but with most values occurring at the lower end of the scale.

Having presented these observations, Roelfs and Groth (1980) concluded "this difference in distribution is consistent with the principle that in the asexual population, the unit of selection is largely the genotype whereas in the sexual population it is largely the individual gene, since our earlier data indicate that genes are being nearly randomly

reshuffled in the sexual population". These conclusions then led Roelfs and Groth (1980) to state that "deviations of observed from expected combined frequencies (test for linkage disequilibrium) are not a valid basis for conclusions about the fitness associated with virulence genes".

3.3.3 Evidence for virulence gene pairs with reduced fitness in both the sexual and asexual populations

Wolfe and Knott (1982) argued that isolates drawn from a homogenous sub population of a sexually reproducing pathogen collected from a limited area and only from susceptible hosts meet the requirements for a valid analysis of selection of virulence gene combinations. The sexually reproducing population of P. graminis tritici described by Roelfs and Groth (1980) meets all these requirements. The data were collected from two neighbouring counties, one in Washington State and one in Idaho. Samples were taken from susceptible hosts and none of the Sr genes examined in the study were used in agriculture in the region (Roelfs and Groth, 1980; J. Groth, personal communication). Therefore the significant deviations described in section 3.3.2 themselves are evidence of selection against some virulence gene combinations. Roelfs and Groth (1980) noted that the mean deviations for the virulence genes 9a, 9b, 10 and 17 were all negative and significant. Further on analysis of the individual 105 pair-wise combination I noted 16 pair wise combinations which were disassociated. Clearly all the assumptions for a valid analysis of the data to detect combination selection have been met, and selection against these combinations is the most likely explanation for the observed deviations. The pairs of virulences which seem to be less fit are 9a and Tmp; 9a and 9e; 9a and 10; 9a and 11; 9a and 7b; 15 and 9e; 15 and 11; 15 and 7b; 17 and Tmp; 17 and 9e; 17 and 11.

Roelfs and Groth (1980) concluded that the test for linkage disequilibrium was not applicable to the asexual population (section 3.3.2) primarily because of the lack of random gene distribution. This assertion is not entirely true. It can be shown that in the asexual population too, selection against virulence combinations can be detected. The issue debated is not "does combination selection occur in the asexual population" but "can it be detected if it is there ?". According to Wolfe and Knott's (1982) specifications, a classical analysis is not valid because the pathogen population is not randomly mating and secondly because the data of Roelfs and Groth (1980) is pooled from eight different ecological zones. The effect of pooling from heterogeneous sub populations will be discussed later (section 3.4.3 and Appendix 11).

Detection of certain virulence pairs with reduced fitness in the asexual population is now described. As stated above in section 3.3.2 the significance of the deviations of all 105 individual pair wise combinations for each population was tested. The significant deviations are shown in Table 31. When this table was examined in detail it was noticed that nine of the 15 virulence genes fell into two association groups. One group consisted of genes 9a, 15 and 17. Every pair wise combinations of genes occurred at a frequency significantly greater than expected. The other group consisted of virulence genes 7b, 9e, 10, 11, Tt-1 and Tmp. Similarly every pair wise combination of these genes was an association. Further every pair wise combination between genes from different groups occurred at a frequency significantly lower than expected, i.e. they were disassociated. Other genes did not fall exactly into these groupings, but were more related to one group than another. For example virulences 6 and 9b tended to be associated with the first group and disassociated from the second group and vice versa for virulence gene 8. These groupings

Table 31 Significant disassociations (-) and associations (+)
between virulence genes from an asexual population of
P. graminis tritici in the U.S.A. in 1975¹

Genes	5	6	7 <u>b</u>	8	9 <u>a</u>	9 <u>b</u>	9 <u>d</u>	9 <u>e</u>	10	11	15	16	17	Tt-1	Tmp
5	-			+	+							+			
6		-		+		+	-	-			+		+	-	-
7 <u>b</u>				+	-	-		+	+	+	-		-	+	+
8					-	+	+	+	+	+	-	+	-	+	+
9 <u>a</u>						+		-	-	-	+		+	-	-
9 <u>b</u>								-			+		+	-	-
9 <u>d</u>											-	+		-	
9 <u>e</u>									+	+	-		-	+	+
10										+	-		-	+	+
11											-		-	+	+
15													+	-	-
16															
17														-	-
Tt-1															+
Tmp															

¹ The table was calculated from data from Roelfs and Groth (1980)

probably explain the bimodal distribution of the frequency of the number of virulence genes carried in the asexual population (Roelfs and Groth, 1980, Figure 3, and section 3.3.2). One mode was primarily due to race TNM which comprised 69% of all the asexual isolates (Roelfs and McVey, 1976, from which the original data has been abstracted). This race carries 10 of the virulence genes examined in the study (Roelfs and Groth, 1980 and Table 1 of Roelfs and McVey, 1976) which showed race TNM to carry virulence genes 5, 9d, 9e, 7b, 11, 8, Tt-1 and 10 but not 6, 9a or 9b. In addition race TNM carries virulences 16 and Tmp a total of 10 genes. Some isolates may also carry the virulence gene 17. All the group 2 genes noted above 7b, 9e, 10, 11, Tt-1 and Tmp are carried by race TNM. The other mode, for the asexual population in Roelfs and Groth's (1980) Figure 3, was much smaller consisting of about 200 isolates with six virulence genes. This mode probably consisted largely of race QCB carrying virulence genes 5, 9a, 9d, 15, 16, 17; which includes all the first group genes 9a, 15 and 17.

The association of genes 9a, 15 and 17 and their disassociation with genes from another association group of virulence genes 7b, 9e, 10, 11, Tt-1 and Tmp is not surprising therefore. However the relevant point here is that in the isolated sexual population, a similar disassociation pattern involving the same two groups of virulence was found. Table 32 shows the significant deviations of observed from expected frequencies for all pair wise combinations of genes from the two groups for the sexual population.

Whilst there are no associations (out of a possible three) within group 1 virulences and not every pair wise combination within group 2 is an association, there is remarkable similarity in associations and disassociations between the sexual population and the asexual population.

Table 32 Significant disassociations (-) and associations (+)
between virulence genes from the groups of virulences in a
sexual population of P. graminis tritici in the U.S.A.
in 1975²

	group 1			group 2					
	<u>9a</u>	15	17	<u>7b</u>	<u>9e</u>	10	11	<u>Tt-1</u>	<u>Tmp</u>
<u>9a</u>				-	-	-	-		-
15				-	-		-		
17					-		-		-
<u>7b</u>							+		
<u>9e</u>							+	+	
10									
11								+	+
<u>Tt-1</u>									+
<u>Tmp</u>									

¹ group 1 - 9a, 15, 17; group 2 - 7b, 9e, 10, 11, Tt-1, Tmp
Deviations for the other six virulences were not considered.

² The table was calculated from data from Roelfs and Groth (1980, Table 2).

Within group 2 there are 6+, 0- which is just a significant departure from a 1:1 ratio on a χ^2 test with 1 d.f. Between groups there were 11-, 0+ suggesting a very real basis for the argument that the genes of these virulence groups are disassociated in the sexual population, as in the

asexual population. Unless the similarities in both populations have arisen by chance the only alternative explanation is that the selection against the eleven virulence combinations noted above and in Table 31 in the sexual population also operates in the asexual population. The two populations are isolated and therefore selection in both is the most reasonable explanation. The probability of these patterns arising by chance is very small.

The size of the deviations in the asexual population were much greater (Roelfs and Groth, 1980, Tables 2 and 3) on average than those in the sexual population. Therefore it would seem that both selection against virulence gene combinations and other factors such as the lack of sexual recombination operates in the asexual population resulting in larger numbers of sizes of deviations.

The main conclusion of this section is that selection against virulence combinations in the asexual population is detectable. Roelfs and Groth's (1980) assertion that the test for linkage disequilibrium is not a valid basis for making conclusions about the fitness of virulence gene combinations is not true. However without comparison with the data for the sexual population these conclusions could not have been made about selection in the asexual population.

3.3.4 Variability in the sexual and asexual populations and causes of its restriction in the latter

In the previous section evidence of selection against some virulence gene combinations was presented for both the sexual and asexual population of P. graminis tritici in the U.S.A. (the Canadian population will be discussed later in sections 3.4.1.c, 3.4.4.b). However there were large differences between the two U.S.A. populations. Roelfs and Groth (1980) demonstrated that the populations differ greatly in their

genetic variability with respect to the 15 virulence genes studied; the asexual population was shown to be much less variable (section 3.3.2). These authors attributed the difference in variability to the lack of random genetic exchange through sexual reproduction in the asexual population. However it is evident the two populations differed in more respects than the presence or general absence of sexual recombination. These differences (outlined below) lead to the conclusion that the difference in variability in the two populations is not as great as Roelfs and Groth (1980) suggest, and secondly other factors such as selection may contribute to the restriction in the variation in the asexual population. The lack of sexual recombination may not be the only cause.

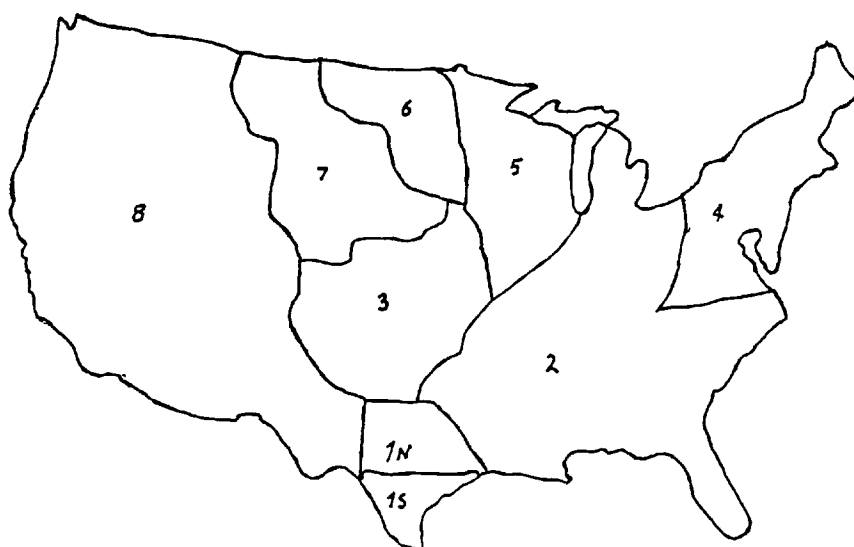
The asexual population was sampled from eight distinct ecological areas over two thirds of the United States. The sexual population on the other hand was sampled from just one ecological area, from two neighbouring counties in the states of Washington and Idaho. A second important difference was that many more samples were taken from the former population (2377) than the latter (426). In the sexual population 100 distinct phenotypes were noted amongst the 426 isolates sampled. However, if 2377 isolates were sampled instead, the percentage of distinct phenotypes of 23.5% ($100 \times 100/426$) most certainly would have been reduced. Thirdly, Sr resistance genes have been in common use in the wheat cultivars grown in the area east of the Rocky Mountains where the asexual population resides but not in the area where the sexual population is situated.

Thus in the sexual population there is no obvious selection for the virulence genes or combinations of them due to host pressure. Variation is not therefore restricted since all pathogen genotypes are genetically capable of survival. However in the area of the asexual population the

use of Sr genes for many years would have had a significant selective effect on the stem rust pathogen population reducing variation in the number of genotypes, a point also made by Green (1975) and Wolfe and Knott (1982). This is particularly true of the asexual population of P. graminis tritici in the U.S.A. where the annual movement of the pathogen over many of the eight ecological zones in the "Puccinia path" (Figure 4) would be expected to contribute to the selection of a few very fit genotypes over most of the area. The greater variation in the sexual population than the asexual population can therefore be explained in terms of selection rather than recombination alone. A number of facts from Roelfs and Groth (1980) support this hypothesis that selection has been greater in the asexual population :

- (a) the asexual population consisted of genotypes with more virulence genes than the genotypes of the sexual population. Race TNM in the asexual population carried 10 virulences (11 if virulence to Sr₁₇ is included). This was the predominant race. Other races carried principally 5 - 8 or 9 - 11 genes. Not a single race of the stem rust pathogen was found carrying less than five virulence genes. The modal number of virulence genes/genotypes in the asexual population was 10, however in the sexual population it was only six. This suggests that there has been more selection for the accumulation of virulence genes in the asexual population than in the sexual population.
- (b) The individual virulence gene frequencies in the asexual population are generally higher than in the sexual population. This can be seen if Tables 2 and 3 from Roelfs' and Groth (1980) are compared.

Figure 4 Ecological areas of Puccinia graminis tritici in the U.S.A.¹



¹ From Roelfs, Casper and Long (1981)

The relevant data is shown also in Table 33.

For all virulence genes except 9a, 10, 15 and 17 the frequency in the asexual population is greater than in the sexual population, however due to the wide variation in the individual frequencies for each population, the difference between the mean frequency for each population is not significant.

To counter the hypothesis that selection for virulence genes was greater in the asexual population than the sexual population, is the observation by Roelfs and Groth (1980) who noted the absence of a significant correlation between host resistance gene frequencies and pathogen virulence gene frequencies. They concluded that the former did not strongly influence the latter. If this is true, then there is little evidence that virulence genes are selected for in the asexual population due to host selection pressure. The lack of correlation noted by Roelfs and Groth (1980) can be explained on the grounds that most of the cultivars listed in their Table 1, which accounted for about 79% of the total wheat acreage in 1975, were considered resistant in the field. Therefore, host gene frequencies were high, whilst pathogen gene frequencies were low. A temporary lack of correlation is expected until resistance 'breaks down' and matching virulence gene frequencies correspondingly rise. This is a feature of the 'boom and bust' cycle; during the 'boom' a lack of correlation between host and pathogen gene frequencies is to be expected, but in the 'bust' phase virulence frequencies increase substantially leading to correlation. Furthermore, the lack of correlation noted by Roelfs and Groth (1980) does not mean host frequencies in the past have not influenced pathogen virulence gene frequencies today.

Table 33 Virulence gene frequencies in the asexual and sexual population of
P. graminis tritici in the U.S.A. in 1975¹

Population	Gene	Frequency														
		5	6	7 <u>b</u>	8	9 <u>a</u>	9 <u>b</u>	9 <u>d</u>	9 <u>e</u>	10	11	15	16	17	T <u>t</u> -1	T <u>mp</u>
Asexual		0.99	0.11	0.82	0.86	0.14	0.12	0.99	0.77	0.88	0.82	0.23	0.99	0.20	0.81	0.77
Sexual		0.68	0.07	0.18	0.15	0.63	0.01	0.69	0.04	0.96	0.04	0.93	0.64	0.75	0.19	0.03

¹ From Roelfs and Groth (1980)

Other factors may lead to a lack of correlation between host resistance and pathogen virulence genes. One is the absence of stabilizing selection. If the forces of selection against unnecessary virulence are very weak a pathogen virulence gene frequency may rise to a high level even if the frequency of the resistant host is comparatively small. An example is the V_4 virulence gene in P. infestans (section 1.2.2.a). In the P. graminis tritici populations of the U.S.A. some virulence gene frequencies are very high even though obvious host selection pressure has been very limited, for example virulence to Sr_{9d}.

A final argument that Roelfs and Groth (1980) used to support their claim for the greater variability of the sexual population, seems to be invalid. The analysis of the frequency distribution of all paired combinations of loci expressing virulence for the two populations was discussed in section 3.3.2 but the analysis is deceptive. It was noted that the distribution for the asexual population was bimodal with values either high (0.70 or greater) or low (0.25 or lower) with no intermediate values. On the other hand the distribution for the sexual population was more evenly spread over the range 0.00 - 0.99. From these distributions Roelfs and Groth (1980) concluded that "this difference in distribution is consistent with the principle that in the asexual population the unit of selection is largely the genotype whereas in the sexual population it is largely the individual gene". However the distributions noted were expected on the basis of the individual gene frequencies (Table 33). For the asexual population individual gene frequencies were either very high (0.77 or greater) or very low (0.23 or less). Consequently, expected frequencies for all pair wise combinations of virulence genes must also be very high or very low simply because of multiplication of two high frequencies gives a high expected frequency of the virulence combination,

whereas the multiplication of a high and a low or two low frequencies always gives a low frequency. Intermediate virulence gene frequencies are not expected. If Table 3 (Roelfs and Groth, 1980) is examined none of 105 pair wise combinations has an expected frequency between 0.23 and 0.59. Thus it is hardly surprising therefore that no intermediate frequencies were observed. For the sexual population (Roelfs and Groth, 1980, Table 2) intermediate as well as high and low frequencies for combined virulence are expected due to some individual gene frequencies being lower than 0.70 (Table 31). Hence the frequency distribution of the pair wise frequencies noted by Roelfs and Groth (1980) is an artefact of the individual virulence gene frequency data (Table 33).

The conclusion of this section (3.3.4) is that the extent of the variability difference is not as great as Roelfs and Groth (1980) suggest and that the effect of selection for restricted virulence gene groupings is a contributory factor at least to the indisputable restriction in variation in the asexual population.

It is important here to distinguish the operation of two kinds of selection. Firstly selection for virulence genes by the use of Sr resistance genes will restrict variation in any population, asexual or sexual, since some genotypes cannot survive. Secondly within the genotypes that can survive, selection against virulence combinations may prevent the existence of some combinations or reduce the expected frequencies of such combinations; this may result in the establishment of linkage disequilibria. The first kind of selection, for virulence genes may or may not result in linkage disequilibria depending on whether the population is sexual or asexual, homogeneous or heterogeneous. Selection for virulence genes in large homogeneous sexual populations should not lead to disequilibria (Wolfe and Knott, 1982) but may in heterogeneous

populations where selection for different genotypes, say $\underline{V}_1$ and $\underline{V}_2$ in relatively isolated sub-populations occurs. Isolation is a barrier preventing matings between the different genotypes and hence the generation of recombinants, $\underline{V}_1\underline{V}_2$ and the consequent linkage disequilibria cannot, therefore, be attributed to an unfit combination of the virulence genes $\underline{V}_1$ and $\underline{V}_2$, but due to selection for the genes separately.

In the absence of sexual reproduction linkage disequilibria may similarly arise in both homogeneous and heterogeneous populations through selection of different pathogen virulence genotypes. This is discussed in detail in sections 3.4.2 and 3.4.3.

Therefore in the asexual population of P. graminis tritici in the U.S.A. the large linkage disequilibria noted in section 3.3.3 are produced by a combination of both types of selection - selection for virulence genes due to host selection pressure and selection against particular virulence gene pairs, and are maintained in the absence of sexual recombination. Mutation in a large asexual population can produce new virulence combinations and thus oppose the restriction in variation and the establishment and maintenance of linkage disequilibria albeit minimally and slowly (section 3.4.1).

The differing effects of selection and lack of recombination are important factors to distinguish since the causes of linkage disequilibria depend upon them. If linkage disequilibria are caused by selection against virulence combinations but not selection for different virulence genes then the use of the corresponding resistance genes either in multi-lines and cultivar mixtures or in single cultivars might prove to be a satisfactory means of disease control. If however linkage disequilibria are due to selection for different virulence genes then the use of these strategies could prove unfruitful since the linkage disequilibria should

be temporary (even in an asexual population where mutation could combine any pair of virulence genes) and the observed frequency of combined virulence might rapidly increase under the influence of the host selection pressure. Even linkage disequilibria caused by selection against particular combinations of virulence genes may prove to be temporary especially if the pathogen can evolve a background genotype with a greater fitness, obviating the selective effects of the virulence combination. This was suggested in section 3.2, but the general lack of information on this prevents firmer predictions or conclusions.

It is evident that a more detailed understanding of the causes of linkage disequilibria, and the effects of selection, lack of sexual recombination and mutation are required for the asexual population and this is the topic of section 3.4.

3.4. Generation, recombination and selection of virulence genes in large asexual populations

3.4.1 Virulence gene accumulation and mutation

3.4.1.a Generation of mutants - a theoretical study

The potential to accumulate virulence genes in large asexual populations is great. This is because the population size N may greatly exceed the mutation rate to virulence μ .

Consider a virulence gene $\underline{V}$ at a frequency $\underline{q}$ ($0 \leq \underline{q} \leq 1$) in a large pathogen population which only reproduces asexually. Assuming there has been selection for $\underline{V}_1$, through host selection pressure so that $\underline{q} > 0.5$. Mutations to a virulence gene combination $\underline{V}_1\underline{V}_2$ from $\underline{V}_1$, where $\underline{V}_2$ is an unselected virulence gene to another resistance gene, $\underline{R}_2$, occur at a frequency of $\underline{q}.\mu_2$ where μ_2 is the mutation rate from avirulence to $\underline{R}_2$

($\underline{A}_2$) to virulence ($\underline{V}_2$). If $\underline{N}$ is the number of spores produced in a year then the number of $\underline{V}_1\underline{V}_2$ mutants generated from $\underline{V}_1$ genotypes is $\underline{N} \cdot \underline{q} \cdot \underline{\mu}_2$. If $\underline{N} \gg 1/\underline{q} \cdot \underline{\mu}_2$ then $\underline{N} \cdot \underline{q} \cdot \underline{\mu}_2 \gg 1$, hence the generation of the $\underline{V}_1\underline{V}_2$ combination will be frequent. A numerical example follows.

Consider the P. graminis tritici population in the U.S.A. From 1976-1981 there were approximately 68 million acres of wheat harvested each year in the U.S.A. (Mr F. Couledge, American Embassy in London, personal communication). Day (1974, p. 180) stated that barley mildew at a 10% level of infection (percentage leaf area diseased) produces about 10^{13} conidia per hectare per day. Assuming a similar level of disease for P. graminis tritici and the same rate of spore production then on 68 million acres of wheat (= 27.5 million hectares), approximately $27.5 \times 10^6 \times 10^{13} = 2.75 \times 10^{20}$ spores are produced daily in the U.S.A. Assuming this rate of production is maintained for 50 days then annually about 1.375×10^{22} spores are produced in the U.S.A. J. Groth (personal communication) independently estimated $10^{20} - 10^{25}$ uredospores per season in North America. Assuming mutation rates to virulence are approximately 1×10^{-6} , then mutants to virulence will be common. For example approximately $1 \times 10^{-12} \times 1.375 \times 10^{22} = 1.375 \times 10^{10}$ spores are expected to carry two mutations whereas $1 \times 10^{-18} \times 1.375 \times 10^{22} = 1.375 \times 10^4$ are expected to carry three mutations. These calculations provide ample evidence to support the idea that virulence genes can accumulate in single genotypes by mutation alone in large asexual populations.

Further, double and triple virulence genotypes can also be produced from single and double virulence genotypes respectively. As an example consider virulence to both Sr₆ and Sr_{9e}. This virulence combination had an expected frequency in 1975 of $0.11 \times 0.77 = 0.085$, where 0.11 was the frequency of virulence to Sr₆ and 0.77, the frequency of virulence to Sr_{9e}.

(Table 33). However the observed frequency for this combined virulence was zero in 1975 from 2377 samples. The frequency of spores with both virulence genes produced from either single virulence genotype, by single mutation was approximately $0.11 \times 10^{-6} + 0.77 \times 10^{-6} = 8.8 \times 10^{-7}$ per generation. If 1.375×10^{22} spores are produced annually then $8.8 \times 10^{-7} \times 1.375 \times 10^{22} = 1.21 \times 10^{15}$ will carry combined virulence. A further 1.375×10^{10} spores (calculated above) carry combined virulence produced by a double mutational event.

It is clear therefore that in large asexual populations, mutation may generate each season new combinations of virulence genes in pairs or triplets. Further step wise accumulation may also occur from single gene virulences. The future of these virulence combinations initially depends on drift effects if the combination is very rare. For instance the chances of survival of a triple mutant at a frequency of 1×10^{-18} in a population may be very low. M. Wolfe (personal communication) argued "the vast majority of uredospores are not dispersed more than a few centimetres from where they were formed so that the actual spore population subject to selection on different hosts is, relatively, very small. If a rare mutant does happen to land on a field of a matching resistant host, against huge odds, there is then only a very short period available for rapid increase of that mutant (I am assuming that its rate of increase in the place where it is formed, is, by definition, minimal or nil). Even if it does increase, the chances of it overwintering, by being blown to the south (overwintering area of Mexico and Texas and some other southern states) and then contributing significantly to the next season's population, are minimal. So in the following season, we return to the frequency provided by the mutation/selection equilibrium and the amount of field-to-field spore distribution". This argument may be applicable to extremely rare

mutants like triple mutants, but is unlikely to be relevant to double virulence genotypes generated by single mutation in a mono virulence genotype. The numbers of spores carrying two virulence genes generated each year in this way is enormous (see above) and the chances of survival are therefore large, even though migration to the overwintering area must take place first before any contribution can be made to the following year's epidemic. If this was untrue then there would be little basis for any matching virulence gene produced by mutation ever becoming established and selected for on hosts with matching resistance, or for virulence gene accumulation in asexual populations.

The future of mutants generated in large asexual populations once established will then depend upon selection; selection against unnecessary virulence, and selection for necessary virulence.

The operation of the parasexual cycle in asexual populations of pathogens is an alternative mechanism to mutation, for the generation of recombinants. However its demonstration in nature is difficult in the absence of methods for handling available genetic markers (Day, 1974).

Roelfs and Groth (1980) concluded from their observations on the asexual population of P. graminis tritici in the U.S.A. which was characterized by a restriction in variation in pathogen genotypes compared to a sexual population, that if parasexuality is operating in the population then the mechanism is providing only minimal genetic recombination.

3.4.1.b Generation of mutants - evolution within the
race 15 group of P. graminis tritici
in Canada

The previous section was a theoretical study of the U.S.A. stem rust population and the generation within it of mutants with accumulated

virulence gene combinations.

This second line of evidence shows that virulence gene accumulation has taken place in an asexual population of P. graminis tritici in Canada. The pathogen population in Canada is similar to that in the U.S.A. especially at the start of the short Canadian season because each year inoculum of stem rust fungus arrives from the central and northern U.S.A. wheat belt to start the Canadian epidemic. The pathogen does not overwinter in Canada, but arrives each year from the south (Van der Plank, 1968). Consequently, the races present and the frequencies of virulence genes in the Canadian population are initially determined by the nature of the population arriving from the U.S.A. Subsequent evolution will be determined by the cultivars of wheat grown in Canada, although the importance of this is reduced by the relatively few generations of the pathogen at high latitudes (Van der Plank, 1968).

Green (1975) specifically discussed evolution within the 15B race group of P. graminis tritici. Race C10 (15B-1) was first identified in Canada and suddenly became predominant in one year (1950, Green, 1975). This race carried virulence genes 5, 7b, 9a, 9b, 9d, 9e, 10, 11, 13, 14, 15, 17, Tt-1, Tt-2 (Green, 1981) and possibly others. It is interesting to speculate how this race evolved. Green (1975) noted that "it is not possible to develop lines of evolution for the 'standard' races in Canada". He also elaborated that whereas some of the standard races evolved by changing virulence on one differential or group of differentials at one time, other races including race 15 could not be accounted for in this way. Race 15 is rather dissimilar from many of the standard races and especially from the groups of races which have been detected almost every year in Canada since 1919 (Green, 1975).

Stakman and Harrar (1957) however stated that race 15 was present in the U.S.A. as long ago as 1918, and that race 15B was clearly

distinguished from it in 1939. Thereafter it rapidly replaced race 15 from which it is distinguished by virulence to Sr 11. In the U.S.A. (and possibly Canada) race 15B remained at very low levels until 1950. Thus race C10 (15B-1) may well have originated from race 15 after barberry eradication was complete by 1928 (Roelfs and Groth, 1980) and therefore by mutation in an asexual population, although the original race 15 evolved in a sexual population.

Green (1975) recorded the likely evolutionary pathway from race C10 after 1950 in his Figure 2. All of the changes recorded occurred in a largely asexual population. New strains of 15B appeared as early as 1952, but a major line of evolution was established in 1957 with the appearance of race C9 (15B-1L). This was initially considered to be like C10 except for accumulated virulence on the related durum wheats Yuma and Langdon. Other differences between 15B-1 type races (for example C10) and 15B-1L races (for example C9) have subsequently become evident. Race 15B-1 differed from race 56 in temperature adaptation (Katsuya and Green, 1967), and uredia of 15B-1 were darker and often did not sporulate as well as races 56 and 11. Race 15B-1L types however are more typical of the species (Green, 1975). In addition whereas C10 (15B-1) had at least 14 virulence genes, listed above, race C9 (15B-1L) lacked seven of these 9a, 9b, 10, 13, 15, 17 and Tt-2. Green (1975) was uncertain of the relationship between C9 and C10 but it would seem reasonable to assume that C9 evolved in an asexual population of P. graminis tritici.

Race C9 never became predominant and in 1962 race C18 also a 15B-1L type appeared with added virulence genes 7a and 10 (not carried by C9).

In 1969 a strain like C18 but virulent on cultivars with Sr₈ appeared. This race, C33 was also a 15B-1L type, and because of its aggressiveness

predominated over C18. Race C33 in Canada is probably identical with race TNM in the U.S.A.

Many other changes in the number of accumulated virulence genes in the C9 evolutionary line occurred during 1962 - 1973, and Green (1975) summarizing these stated there were 10 between these years in the race 15B-1L group, of which six were increases in virulence and four were decreases in virulence. Green (1975) thought mutation to be the most probable cause of these one step changes.

In this asexual population, therefore, the lack of sexual recombination has not hindered the pathogen in accumulating (or losing) new virulence genes, nor indeed prevented changes in race characteristics including aggressiveness. That the first named changes at least occurred by single step mutation indicates that recombination of virulence genes occurs in this way, without sexual recombination.

Considering the U.S.A. population of P. graminis tritici again Roelfs and Groth's (1980) Table 3 reveals that only four frequencies of the 105 pair wise combination of virulences were zero. Recombination mechanisms of which mutations must be very significant had evidently been successful in producing all 101 other virulence pairings. Further, as was discussed earlier (section 3.3.4) in the asexual population, complex races dominated the race structure, with a model number of 10 pathogen virulence genes. These observations support the conclusions made here that asexual populations are able to accumulate virulence genes by successive mutation.

3.4.1.c Resistance of multiresistant gene cultivars of
wheat in Canada

There has been considerable debate in the literature recently about the mechanisms by which the wheat cultivar Selkirk maintained durable resistance to P. graminis tritici in Canada. Van der Plank (1975, 1982) argued that Selkirk was largely resistant to stem rust for over 10 years (its withdrawal from Canadian wheat fields was for reasons other than loss of resistance to stem rust) because the pathogen has been unable to combine the virulence genes 6 and 9d into a fit genotype. Since Selkirk carries the Sr₆ and Sr_{9d} genes amongst others, the pathogen must combine the corresponding virulence genes to attack Selkirk. Van der Plank's (1975, 1982) conclusions stem from his analysis of observed and expected combined virulence gene frequencies. He showed that from 1972 - 1978 inclusive the expected frequency of combined virulence for these two genes ranged from 0.050 to 0.168, whereas in every year the observed frequency of combined virulence was zero except in 1975 when it was 0.009.

Wolfe and Knott (1982) criticized the validity of Van der Plank's (1975) analysis for the years 1965 - 1969, on the grounds that the data from which the virulence gene frequencies had been obtained had indiscriminately been summed (presumably over heterogeneous areas or from heterogeneous plants or sub populations of the pathogen), and because the population of the pathogen was not randomly mating (section 3.2). These criticisms will be discussed later (sections 3.4.2. and 3.4.3). However if they are valid and the linkage disequilibrium is not attributed to selection against this virulence gene combination but is due to sampling from heterogeneous sub populations or a lack of recombination then it is still difficult to explain the comparatively high frequency of combined virulence in the U.S.A. and Mexico from which Canada receives its inoculum

(Van der Plank, 1982; Wolfe and Knott, 1982).

Dr A. Roelfs (personal communication) stated that races QSH, RTQ and RHR carry combined virulence to Sr₆ and Sr_{9d} in the U.S.A. The most northern ecological zones of P. graminis tritici are zones 5, 6, 7 (Figure 4 and Roelfs, Casper and Long, 1981, Figure 1), which all border on Canada from the Great Lakes in the east to western Montana in the west. The frequency of races QSH, RTQ and RHR in these zones is shown for the years 1972 - 1978 inclusive, in Table 34. Only in ecological zone 7 for three of the years under study was the combined frequency of virulence to Sr₆ and Sr_{9d} zero and for two of those years a very small number of isolates were sampled. Whilst the distribution of sampling within these ecological zones is not given in the papers recording the data (see footnote of Table 34), and the three zones extend as far south as Colorado, Nebraska and Missouri, the inoculum entering Canada largely originates from these three zones. This means that races of P. graminis tritici with combined virulence genes 6 and 9d enter the wheat fields in Canada from the south each year. In 1977 and 1978 especially the frequency of this virulence combination was considerably higher than in previous years, except perhaps in 1975.

Van der Plank (1982, Table 2.2) stated that combined virulence was absent from Canada in every year except 1975, from 1972 - 1978 inclusive. For the seven years concerned, the number of isolates sampled ranged from 106 - 653 (Van der Plank, 1982, Table 2.1); but only one of these sample sizes was less than 282 isolates. In 1975, in Canada, the frequency of combined virulence was 0.009; and it is interesting that in this year in the U.S.A. the frequency of combined virulence to Sr₆ and Sr_{9d} was comparatively high.

Consequently, it is difficult to explain why combined virulence of

Table 34 Frequency of races QSH, RTQ and RHR of Puccinia graminis
tritici in ecological zones¹ 5, 6, 7 in the U.S.A. in
the years 1972 - 1978²

Frequency of races QSH, RTQ and RHR			
Zone	5	6	7
Year			
1972 ³	0.03(216) ⁴	0.02(431)	0.03(148)
1973	0.01(318)	0.03(198)	0.00(6)
1974	0.04(320)	0.03(682)	0.04(114)
1975	0.08(342)	0.12(914)	0.12(59)
1976	0.05(223)	0.02(597)	0.00(65)
1977	0.16(371)	0.27(238)	0.31(85)
1978	0.12(151)	0.17(391)	0.00(5)

¹ from Roelfs, Casper and Long (1981). See also Figure 4

² data from Roelfs and McVey (1973, 1974, 1975, 1976);
Roelfs, Long, Casper and McVey (1977); Roelfs, Casper and
Long (1978, 1979)

³ races QS, RT and RH summed in 1972

⁴ number of isolates on which the frequency was based

genes 6 and 9d is generally absent from Canadian populations of P. graminis tritici other than to assume selection against this combination is taking place as the pathogen moves northwards into Canada from the U.S.A. The detail of Van der Plank's (1975) analysis of the Canadian data may be invalid as Wolfe and Knott (1982) suggest (examined in sections 3.4.2 and 3.4.3) but there is no doubt that the 6, 9d virulence combination enters Canada from the U.S.A. and appears to be selected against during its northward migration onto Canadian wheats.

Even if the virulence combination 6 and 9d is selectively disadvantageous, some authors have argued that this does not mean that Selkirk's durable resistance can be totally attributed to this phenomenon. Green and Campbell (1979) stated that Selkirk carries three resistance genes other than Sr₆ and Sr_{9d} namely, Sr_{7b}, Sr₁₇ and Sr₂₃. Hare and McIntosh (1979) showed that Selkirk also carries the Sr₂ resistance gene. Thus Johnson (1981) argued that the durability of Selkirk's resistance could have been due to additional unidentified resistance genes carried by this cultivar, and that since it is difficult to be sure that the entire resistance genotype of a cultivar has been fully described, the underlying genetic basis of durability may not always be obvious. This argument is perfectly true but may not fully explain Selkirk's durable resistance. Race 15B-1 suddenly became predominant in a single season in Canada (Green and Campbell, 1979). It was able to attack the main commercial North American wheat cultivars, but disappeared soon after the release of Selkirk in 1954 (Green and Campbell, 1979). Race 15B-1 was designated race C10 (Green, 1975), and it carried virulence to the Sr genes 7b, 9d and 17 (amongst others) possessed by Selkirk (Green and Campbell, 1979). Further Van der Plank (1968, P.94) stated that in 1953 and 1954 race 15B-1 destroyed the batch of bread wheats which had derived

their resistance from the cultivar Hope and its relative 'H44'. Since the Sr₂ gene possessed by Selkirk is possessed also by and was probably derived from H44, which was an ancestor of Selkirk (Knott, 1968), this indicated that race 15B-1 carried virulence to Sr₂ as well as to Sr_{7b}, Sr_{9d} and Sr₁₇. This would also apply to the H gene possessed by H44 and inherited by Selkirk (Green and Dyck, 1979; Green, 1981). Green's (1981) Table 1 of virulence formulae of races of P. graminis tritici does not include details of virulence to Sr₂₃, but Roelfs and McVey (1979) stated that races 56 - MBC and 151 - QCB - QFB and QSH were avirulent to this resistance gene. By implication and in the context of the paper which lists the Sr genes and race reactions on cultivars possessing them race 15B must be virulent against the Sr₂₃ gene. Thus it appears that race 15B-1 carried virulence to Sr₂, Sr_{7b}, Sr_{9d}, Sr₂₃ and H. It lacked virulence to Sr₆ and it is tempting to conclude that the possession of this gene conferred Selkirk's resistance to race 15B-1. Virulence singly to this gene has been present at low but detectable frequencies; Van der Plank (1982) reported frequencies in most years, between 0.10 - 0.20. The resistance of Selkirk presumably could have been overcome, therefore by the accumulation of virulence genes in pathogen genotypes already carrying virulence to Sr₆. Alternatively and more simply from the evolution of a genotype able to attack Selkirk by the acquisition of virulence to Sr₆; say by race 15B-1. There is evidence that the latter change did occur after Selkirk was released (Green, 1975; Van der Plank, 1975). Races 15B-3 and 15B-5 appeared in the 1950s (and early 1960s) soon after Selkirk was released. Both races had the full genetic potential to attack Selkirk. Race 15B-3 appeared first and Green (1975) considered that it had evolved virulence to Sr₆ from race 15B-1 presumably by a single gene mutation. This would

confirm, if true, that race 15B-1 had all the genetic potential to attack Selkirk other than virulence to Sr₆. Race 15B-5 on the other hand according to Green (1975) acquired virulence to Sr₆ from race 15B-4 which was itself a mutant from race 15B-1 with additional virulence to the durum wheat cultivar Golden Ball .

However, the appearance in small proportions of races 15B-3 and 15B-5 did not result in destructive epidemics on Selkirk as these races disappeared soon after their appearance (or have only occurred rarely).

Katsuya and Green (1967) and Green (1971) thought that these races with virulence to Selkirk were unaggressive and could not maintain themselves in nature. This lack of fitness may have resulted from an unfit background genotype possessed by races 15B-3, 15B-4 and 15B-5 inherited from the 15B-1 parental strain. Evidence supporting this hypothesis was provided by Green (1975) who stated that all four of these races disappeared or appeared only rarely, soon after Selkirk was released, and a line of evolution from race 15B-1 was terminated. However, race 15B-1 itself did dominate the race structure of the Canadian pathogen population from 1950 to 1955 (Green, 1971) causing major epidemics in 1953 and 1954 and therefore it could not have been unfit, at that time. Races 15B-3 and 15B-5 which evolved from race 15B-1 by mutation should have had a similar background genotype and hence fitness to race 15B-1.

The overwhelming factor however which should have conferred on races 15B-3 and 15B-5 but not 15B-1 or 15B-4 greater fitness than any other race was the ability to attack Selkirk, which by 1956 covered 80%, and by 1961, 86% of the wheat area of Manitoba (Green and Campbell, 1979). A lack of background fitness compared with 15B-1L races which dominated the race structure of the pathogen after the decline of the

15B races, would have been adequately compensated for by the ability of races 15B-3 and 15B-5 to overcome Selkirk. The great unfitness of races 15B-3 and 15B-5 suggests a cause other than lack of background genetic fitness. These races could have evolved background genetic fitness in the same genotype as the extremely advantageous virulence combination of genes 2, 6, 7b, 9d, 17, 23, H which they possessed. It is not unlikely that a large, unavoidable and permanent fitness deficit is due to a harmful virulence combination of say 6 and 9d.

It seems likely that because the accumulation of virulence genes to attack Selkirk in populations of Puccinia graminis tritici

- (a) theoretically was not difficult
- (b) occurred in practice
- (c) conferred a large selective advantage, an epistatic fitness loss in the pathogen occurs with the necessary virulence combination.

Luig and Watson (1970) and MacIntosh (1977) reported the success in control of stem rust by the use of wheat cultivars in Australia possessing combinations of resistance genes. Green (1975) then noted "plant breeding in North America in the last 40 years has shown that combined resistance is an effective, although not perfect method for controlling stem rust of wheat and in the absence of the sexual stage of the rust can produce long lived resistance. Single step changes in the virulence of the rust readily overcome single gene resistance but apparently are not very effective in overcoming complex resistance". It is clear from the above discussions concerning races 15B-3 and 15B-5 that if the pathogen is limited in overcoming combined resistance it is not through the lack of a mechanism to produce the necessary recombinants. Thus the success of combined resistance may be due to a lack of combined virulence or more likely may stem from a lack of fitness of pathogen genotypes which carry the necessary combined virulence.

3.4.2 Linkage equilibria and selection in large homogeneous asexual populations

In section 3.4.1 it was argued that mutation is a powerful force in generating combinations of virulence genes in pathogen populations. The existence of a mechanism to generate virulence gene combinations however may not always ensure that linkage equilibria exist in large asexual populations. In this and the next section the existence of linkage equilibria in large asexual populations is discussed with relevance to the validity of the linkage disequilibrium test used to detect virulence gene combinations with reduced fitness.

Maynard-Smith (1968) challenged the generally accepted view that sexual reproduction always accelerates adaptation to a changing environment by combining in a single descendant, mutations originally occurring in distinct individuals. Maynard-Smith (1968) showed in a simple model with pairs of allelic genes that the rates of evolution in an asexual and a sexual haploid population are the same in a uniform environment. His model is directly applicable to populations of plant pathogens. Consider two virulence genes $\underline{V}_1$ and $\underline{V}_2$ with avirulence alleles $\underline{A}_1$ and $\underline{A}_2$ respectively. There are four genotypes with respect to these two gene pairs. $\underline{A}_1 \underline{A}_2$, $\underline{V}_1 \underline{A}_2$, $\underline{A}_1 \underline{V}_2$ and $\underline{V}_1 \underline{V}_2$. Assuming the operation of selection against unnecessary virulence, with coefficients of selection $\underline{s}_1$ and $\underline{s}_2$ respectively, then the relative fitness of the four pathogen genotypes in the absence of corresponding resistance genes $\underline{R}_1$ and $\underline{R}_2$ is :

Genotype :	$\underline{A}_1 \underline{A}_2$	$\underline{V}_1 \underline{A}_2$	$\underline{A}_1 \underline{V}_2$	$\underline{V}_1 \underline{V}_2$
Fitness :	1	$(1 - \underline{s}_1)$	$(1 - \underline{s}_2)$	$(1 - \underline{s}_1)(1 - \underline{s}_2)$
Equilibrium frequency :	$\underline{P}_{AA}$	$\underline{P}_{VA}$	$\underline{P}_{AV}$	$\underline{P}_{VV}$

Maynard-Smith showed that at equilibrium produced by a classical mutation/selection balance :

$$\frac{P_{AA} \cdot P_{VV}}{P_{VA} \cdot P_{AV}} = 3.4.2.1$$

This equilibrium equation can also be described in more simple mathematical terms. If back mutation is ignored (as in Maynard-Smith's, 1968, model (see also section 1.3.2)) at balance the gene frequencies are :

<u>Gene</u>	<u>Balance frequency</u>
$\underline{V}_1$	$\mu_1 / \underline{s}_1$
$\underline{A}_1$	$1 - (\mu_1 / \underline{s}_1)$
$\underline{V}_2$	$\mu_2 / \underline{s}_2$
$\underline{A}_2$	$1 - (\mu_2 / \underline{s}_2)$

where μ_1 and μ_2 are the forward mutation rates from $\underline{A}_1 \rightarrow \underline{V}_1$ and $\underline{A}_2 \rightarrow \underline{V}_2$ respectively, (avirulence is considered the normal allele and virulence the mutant allele (Day, 1974)). The genotype frequencies are expected to be :

<u>Genotype</u>	<u>Balance frequency</u>
$\underline{A}_1 \underline{A}_2$	$(1 - \mu_1 / \underline{s}_1) \cdot (1 - \mu_2 / \underline{s}_2) = P_{AA}$
$\underline{V}_1 \underline{A}_2$	$(\mu_1 / \underline{s}_1) \cdot (1 - \mu_2 / \underline{s}_2) = P_{VA}$
$\underline{A}_1 \underline{V}_2$	$(1 - \mu_1 / \underline{s}_1) \cdot (\mu_2 / \underline{s}_2) = P_{AV}$
$\underline{V}_1 \underline{V}_2$	$(\mu_1 / \underline{s}_1) \cdot (\mu_2 / \underline{s}_2) = P_{VV}$

The genotype frequencies at balance are simply the

products of the individual gene frequencies. It is reasonable to assume a random association of the alleles, at balance in the absence of selection for virulence for the following reason. In a large asexual population of size $\underline{N}$, $\underline{N}$ is much greater than $1/\mu$ (the reciprocal of the mutation rate to virulence). Consequently, each season single gene mutants at a locus are produced in large numbers ($\underline{N}\mu$). As $\underline{N} \gg 1/\mu$ so, $\underline{N}\mu \gg 1$. Consequently these mutants occur in a variety of genetic backgrounds. For instance consider mutants from $\underline{A}_2 \rightarrow \underline{V}_2$ occurring at a rate, μ_2 . These may occur in two genetic backgrounds with respect to the other gene pair $\underline{A}_1/\underline{V}_1$, in genotypes with $\underline{A}_1$ or genotypes with $\underline{V}_1$, to produce combined genotypes $\underline{A}_1 \underline{V}_2$ or $\underline{V}_1 \underline{V}_2$ respectively. As the number of mutants ($\underline{N}\mu$) with $\underline{V}_2$ is large, the proportion of $\underline{A}_1 \underline{V}_2 : \underline{V}_1 \underline{V}_2$ genotypes should by chance approximate to the same proportion as $\underline{A}_1 : \underline{V}_1$ in the population. The same is true of mutations from $\underline{A}_1$ to $\underline{V}_1$.

Consequently prior to the use of $\underline{R}_1$ and $\underline{R}_2$ in large asexual populations genotype frequencies are the products of the gene frequencies as the genes are randomly or independently distributed by recurrent mutation in the various genotypes, and the population is in linkage equilibrium.

Equation 3.4.2.1 derived by Maynard-Smith (1968) is now derived from the genotype frequencies above. Thus

$$\begin{array}{ccccccc} (1-\mu_1/\underline{s}_1) & \cdot & (1-\mu_2/\underline{s}_2) & \cdot & (\mu_1/\underline{s}_1) & \cdot & (\mu_2/\underline{s}_2) \\ \underline{P}_{AA} & \times & \underline{P}_{VV} & = & \underline{P}_{VA} & \times & \underline{P}_{AV} \end{array}$$

Maynard-Smith (1968) then showed if the environment changed such that the relative fitnesses of the four pathogen genotypes, assuming no epistatic fitness interactions (for example a fitness loss associated with a virulence combination) becomes

Genotype	:	$\underline{A}_1 \underline{A}_2$	$\underline{V}_1 \underline{A}_2$	$\underline{A}_1 \underline{V}_2$	$\underline{V}_1 \underline{V}_2$
Fitness	:	1	$(1+\underline{d}_1)$	$(1+\underline{d}_2)$	$(1+\underline{d}_1).(1+\underline{d}_2)$

where $\underline{d}_1$, $\underline{d}_2$ are selective advantages, then for any generation in the evolution of the population predominantly from $\underline{A}_1 \underline{A}_2$ to $\underline{V}_1 \underline{V}_2$ the following equation holds :

$$\underline{F}_{AA} \cdot \underline{F}_{VV} = \underline{F}_{VA} \cdot \underline{F}_{AV} \quad 3.4.2.2$$

where $\underline{F}_{AA}$, $\underline{F}_{VV}$, $\underline{F}_{VA}$ and $\underline{F}_{AV}$ are the genotype frequencies. Maynard-Smith (1968) proved this for both evolving asexual and sexual populations, and concluded that sexual reproduction would not alter this rate of evolution since all that sexual reproduction can do is to restore the independence relationship.

This means in a continuously homogeneous population selection for single gene virulence genotypes ($\underline{V}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$) and the double virulence gene genotype ($\underline{V}_1 \underline{V}_2$) by the use of corresponding $\underline{R}$ gene hosts will not create linkage disequilibria, provided the fitnesses of these three pathogen genotypes during selection are $(1+\underline{d}_1)$, $(1+\underline{d}_2)$ and $(1+\underline{d}_1).(1+\underline{d}_2)$ respectively. Under these conditions the fitness of the genotype $\underline{V}_1 \underline{V}_2$:

$$(1+\underline{d}_1).(1+\underline{d}_2) = 1+\underline{d}_1+\underline{d}_2+\underline{d}_1\underline{d}_2 \quad 3.4.2.3$$

exceeds $1+\underline{d}_1+\underline{d}_2$, the fitness of $\underline{V}_1 \underline{V}_2$ on a host population consisting only of $\underline{R}_0$, $\underline{R}_1$ and $\underline{R}_2$ but not $\underline{R}_1 \underline{R}_2$ genotypes. Consequently the conditions for linkage equilibrium in a large, homogeneous asexual population require that $\underline{V}_1 \underline{V}_2$ carries additional fitness lacking in the mono-virulence pathogen genotypes. This results from the cultivation

of $\underline{R}_1 \underline{R}_2$ genotypes as a proportion of the host population. When the host genotypes $\underline{R}_0$, $\underline{R}_1$, $\underline{R}_2$ and $\underline{R}_1 \underline{R}_2$ are grown in equal proportions, then the above fitness relationships ensue and $\underline{d}_1$, $\underline{d}_2$ and $\underline{d}_1 \cdot \underline{d}_2$ equal one.

Thus the use of Maynard-Smith (1968) analysis shows conclusively that in large asexual populations which are homogeneous linkage equilibria are expected prior to and during selection for virulence genes, provided there is sufficient selection for the $\underline{V}_1 \underline{V}_2$ genotype. In such populations a departure from linkage equilibrium is indeed good evidence for the operation of differential selection against the virulence gene combination, $\underline{V}_1 \underline{V}_2$.

In circumstances where there is insufficient selection for $\underline{V}_1 \underline{V}_2$ then linkage disequilibrium will result because of the strong selection for the single virulence genotypes $\underline{V}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$. However in a sexual population matings between these two genotypes generates the $\underline{V}_1 \underline{V}_2$ genotype rapidly opposing linkage disequilibrium (see also section 3.4.3).

3.4.3 Linkage disequilibria and selection in large heterogeneous asexual populations

Maynard-Smith (1968) discussed the advantages of sexual reproduction. He showed that if the environment was not uniform (heterogeneous) and initially there were two environments, in one the genotype $\underline{V}_1 \underline{A}_2$ being fittest and in the other the genotype $\underline{A}_1 \underline{V}_2$ being fittest (as might be caused by regional resistance gene deployment) then at balance

$$\underline{P}_{AA} \cdot \underline{P}_{VV} \ll \underline{P}_{VA} \cdot \underline{P}_{AV} \quad 3.4.3.1$$

Consequently, the frequencies of $\underline{V}_1$ and $\underline{V}_2$ singly are high ($\underline{P}_{VA}$ and $\underline{P}_{AV}$) whereas the frequency of $\underline{V}_1 \cdot \underline{V}_2$ ($\underline{P}_{VV}$) is much lower than expected on

an assumption of random gene distribution. This means an automatic linkage disequilibrium is created if the heterogeneous pathogen population is sampled and treated as a single homogeneous population even though in the individual regions linkage equilibria may exist. In such a case a proof of disequilibrium would not necessarily mean the particular virulence gene combination is selectively disadvantageous.

If the environment changes and becomes homogeneous so that $\underline{V}_1 \underline{V}_2$ now becomes the fittest pathogen genotype, sexual reproduction rapidly speeds up the evolution of $\underline{V}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$ to $\underline{V}_1 \underline{V}_2$ compared with asexual reproduction because of the recombination that occurs generating the $\underline{V}_1 \underline{V}_2$ genotype from $\underline{V}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$ matings. Sexual reproduction rapidly restores the relationship in equation 3.4.2.2, quickly establishing linkage equilibrium. The establishment of a linkage equilibrium requires much longer time periods in an asexual population.

This scenario of the re-establishment of linkage equilibrium by sexual reproduction in an initially heterogeneous environment is similar to that described in the last paragraph of section 3.4.2 for a homogeneous asexual population, where there is strong selection for the pathogen genotypes $\underline{V}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$ but not for $\underline{V}_1 \underline{V}_2$. Similarly, it can be shown (Appendix 11), that even in a heterogeneous asexual population, where there is equal selection for these three genotypes in isolated sub populations linkage disequilibrium does not result, if the whole population is sampled, provided equation 3.4.2.1 holds prior to selection (i.e. there is initial equilibrium). This is identical to the conditions for equilibrium in homogeneous populations described in section 3.4.2.

These considerations clearly show that it is the nature of selection for the pathogen genotypes that determines linkage equilibrium or disequilibrium and not whether the population is homogeneous or heterogeneous.

An intermediate situation, the effect on linkage equilibria of selection for only one of the mutant genes (virulence alleles) was not discussed by Maynard-Smith (1968). Consider a simple numerical example. Assume hosts $\underline{R}_1$ resistance gene are grown in one half of the acreage of an agroecosystem and $\underline{R}_0$ hosts are grown in the other half. Further, assuming linkage equilibrium prior to the introduction of $\underline{R}_1$ hosts, the pathogen population is in balance, with the genotype frequencies given by :

<u>Genotype</u>	<u>Frequency</u>
$\underline{A}_1 \underline{A}_2$	$P_{AA} = (1-\mu_1/\underline{s}_1)(1-\mu_2/\underline{s}_2) = 0.9998$
$\underline{V}_1 \underline{A}_2$	$P_{VA} = (\mu_1/\underline{s}_1) \cdot (1-\mu_2/\underline{s}_2) = 9.9999 \times 10^{-5}$
$\underline{A}_1 \underline{V}_2$	$P_{AV} = (1-\mu_1/\underline{s}_1) \cdot (\mu_2/\underline{s}_2) = 9.999 \times 10^{-5}$
$\underline{V}_1 \underline{V}_2$	$P_{VV} = (\mu_1/\underline{s}_1) \cdot (\mu_2/\underline{s}_2) = 1 \times 10^{-8}$

where $\mu_1 = \mu_2 = 1 \times 10^{-6}$ and $\underline{s}_1 = \underline{s}_2 = 1 \times 10^{-2}$

Assuming two subpopulations develop in the two halves of the agroecosystem due to selection in the two localities on $\underline{R}_0$ and $\underline{R}_1$ hosts, then in one subpopulation after selection on the former genotype pathogen frequencies are expected to be as above :

Genotype	$\underline{A}_1 \underline{A}_2$	$\underline{V}_1 \underline{A}_2$	$\underline{A}_1 \underline{V}_2$	$\underline{V}_1 \underline{V}_2$
Frequency	0.9998	9.999×10^{-5}	9.999×10^{-5}	1×10^{-8}

However on $\underline{R}_1$ plants, genotypes $\underline{A}_1 \underline{A}_2$ and $\underline{A}_1 \underline{V}_2$ lacking necessary virulence to $\underline{R}_1$ cannot survive. After selection the frequency of $\underline{V}_1 \underline{A}_2$ and $\underline{V}_1 \underline{V}_2$ are expected in the same proportion as prior to selection, i.e.

$9.999 \times 10^{-5} : 1 \times 10^{-8}$. Their final frequencies after selection become $(9.999 \times 10^{-5}) / (9.999 \times 10^{-5} + 1 \times 10^{-8}) = 0.999$, and 0.0001 respectively.

If the heterogeneous population is now sampled as a single population proportionally from each subpopulation then the overall genotype frequencies are :

<u>Genotype</u>	<u>Frequency</u>
$\underline{A}_1 \underline{A}_2$	$\frac{1}{2} \times 0.9998 = 0.4999$
$\underline{V}_1 \underline{A}_2$	$\frac{1}{2} \times 0.999 + \frac{1}{2} \times 9.999 \times 10^{-5} = 0.5$
$\underline{A}_1 \underline{V}_2$	$\frac{1}{2} \times 9.999 \times 10^{-5} = 4.9995 \times 10^{-5}$
$\underline{V}_1 \underline{V}_2$	$\frac{1}{2} \times 0.0001 + \frac{1}{2} \times 1 \times 10^{-8} = 5.0005 \times 10^{-5}$

Therefore the frequency of $\underline{V}_1 = 0.50005$ and the frequency of $\underline{V}_2 = 1 \times 10^{-4}$. If the population is in linkage equilibrium then the expected frequency of $\underline{V}_1 \underline{V}_2$ will be $0.50005 \times 1 \times 10^{-4} = 5.0005 \times 10^{-5}$ which is identical with the estimated observed frequency.

Consequently, selection for one virulence gene only in a large asexual population and the creation of heterogeneous pathogen subpopulations does not result in linkage disequilibrium. The conclusion therefore, is that the test for linkage disequilibrium is a valid method to detect selectively disadvantageous virulence combinations in large asexual populations in these circumstances. In practice the test would prove too difficult since the expected frequency of combined virulence is very small, and an unwieldy number of samples to detect it is required. Thus to ensure a 95% probability of detection in a virulence survey, the number of samples, $\underline{x}$ required would be :

$$1 - (1 - 0.00005)^{\underline{x}} = 0.95$$

$$(0.99995)^{\underline{x}} = 0.05$$

$$\underline{x} \log_e 0.99995 = \log_e 0.05$$

$$\underline{x} = \log_e 0.05 / \log_e 0.99995$$

$$= 59,914 \text{ samples}$$

To detect disequilibrium would require even more samples.

This calculation has wider implications as well to the earlier discussions on the variability of the asexual and sexual population of P. graminis tritici in the U.S.A. (section 3.3.4). It is evident that many rare mutants will be undetectable using conventional race sampling methods. Even the massive collections of stem rust isolates in the U.S.A. wheat fields (2000 or more samples annually) would not be sufficient to detect rare mutants at frequencies of about 0.00005. Consequently, the estimated variability of a pathogen population is only as good as the efficiency and size of the virulence survey. The asexual population was undoubtedly more variable than Roelfs and Groth's (1980) analysis suggested because rare genotypes remained undetected.

3.4.4 Puccinia graminis tritici population in North America - homogeneous or heterogeneous ?

3.4.4.a The U.S.A. population

Instinctively, one might suppose the U.S.A. asexual population of P. graminis tritici is heterogeneous, with distinct ecological zones and different cultivars grown within the individual zones. Roelfs and Groth (1980) and Wolfe and Knott (1982) for example considered the asexual

population to be genetically heterogeneous. Empirically, however, a demonstration of heterogeneity should be sought in a study of the population genetics of the pathogen rather than from a study of the potential selective forces such as regions, climatic and geographical factors and selective hosts. The salient question is, do clearly distinct pathogen subpopulations exist in different regions or zones ? The rationale for this question is : heterogeneity in the pathogen population, created by different selective forces in different zones results in linkage disequilibria, whereas heterogeneity of the selective forces per se in different zones may not necessarily lead to heterogeneity in the pathogen population. This was noted in section 3.3.4 where a few races only, dominated the race structure of the asexual population in the U.S.A., despite the fact that the selective forces in the different zones were very different (Roelfs and Groth, 1980). It was suggested that due to movement of inoculum between zones, a few fit races were selected over most of the pathogen's range, resulting in a more homogeneous population than would be predicted from a knowledge of the potential selective forces in the various ecological zones. This suggestion is further supported by the observation of Roelfs and Groth (1980) of a lack of correlation between host resistance and pathogen virulence gene frequencies and they suggested that the former do not influence the latter (see also section 3.3.4). Thus there was little evidence that the different host selective forces in different zones actually selected different pathogen subpopulations.

The data on the asexual population of P. graminis tritici in the U.S.A. in 1975 is now examined in more detail. It exists in eight ecological zones (Figure 4). Zones 1S and 1N are in south and central Texas respectively (Roelfs et al., 1981). Annual collections of stem

rust from these zones tend to be small, for example in 1975 only 43 isolates were sampled from 18 collections, whereas from zone 1N only nine isolates were sampled from five collections (Roelfs and McVey, 1976). Further only 1.5% of the total wheat acreage of the U.S.A. in 1975 was grown in these zones, most being in zone 1N (Roelfs and Groth, 1980). In contrast apart from zone 7, with 59 samples tested for virulence, the number of samples tested in other zones was much larger ranging from 146 to 914 (Roelfs and McVey, 1976). Due to the small number of isolates from zone 1N, this zone's data will be excluded from the discussions. Zone 2 is a large area of eastern U.S.A. and contained 16.1% of the total wheat acreage. Zones 3, 5, 6 and 7 cover a very large area in the central and central north of the U.S.A., and contained 80.5% of the total wheat acreage in 1975, mostly in zones 3 and 6. Zone 4 in the north east is isolated from the main wheat belt in the central and northern regions, and only 1.9% of the wheat acreage was grown there in 1975. Zones 3, 5, 6 and 7 covering most of the wheat growing area of the U.S.A. are in the 'Puccinia Path' - the annual movement of the pathogen northwards in spring and southwards in autumn (Van der Plank, 1968). The pathogen populations in these zones are therefore expected to be similar. Roelfs and McVey's (1976) summary of the pathogen races in these zones in 1975 confirms this expectation. Race TNM occurred at a frequency of 0.79, 0.77, 0.75 and 0.74 in zones 3, 5, 6 and 7 respectively. Related races TDM and TLM occurred at combined frequencies of 0.06, 0.06, 0.10 and 0.05 in the four zones. The final main race QSH occurred at frequencies of 0.08, 0.07, 0.11 and 0.12 respectively. The races structure in each zone was very similar, and therefore are homogeneous.

The population in zone 2 was rather different (Roelfs and McVey, 1976). Race TNM occurred at a lower frequency of 0.44, whilst races TDM and TLM had a combined frequency of 0.1 similar to that in zones

3, 5, 6 and 7. Race QCB which was never found at a frequency of more than 0.03 in zones 3, 5, 6 and 7, had a frequency of 0.20 whilst other minor races RCR and RPQ occurred at frequencies of 0.09 and 0.04 respectively in zone 2 (Roelfs and McVey, 1976).

In the more isolated zone 4, the population was even more distinct from those in the other zones. TNM did not dominate the race structure as in zones 2, 3, 5, 6 and 7, occurring at a frequency of just 0.09. The dominant race was QCB at a frequency of 0.63. A series of minor races made up the remainder of the population.

Thus in 1975, race TNM dominated the main central wheat belt and the eastern zone 2. However race QCB was predominant in the north east (zone 4) and in zone 2 in the eastern and south eastern areas it was common without predominating. It would appear that race QCB is more favoured to the east and south of the main wheat growing areas.

Linkage disequilibria result, therefore, irrespective of the selective state of virulence gene pairs simply because of selection for one race, TNM in one large area (zones 3, 5, 6, 7 and partly zone 2) and selection for another race, QCB in another large area (zones 1N, 4 and partly zone 2). It is interesting to note the virulence genes carried by these two main races TNM and QCB, Dr. A. Roelfs (personal communication) stated that race TNM carries amongst other virulences, genes 7b, 9e, 10, 11, Tt-1 and Tmp whilst race QCB carries genes 9a, 15 and 17 amongst others (Roelfs and McVey, 1976). These are the genes of the two association groups identified from an analysis of the data in Roelfs and Groth's (1980) Tables 2 and 3a (section 3.3.3). The groupings are therefore explicable partly on the basis of selection for races QCB and TNM in different regions. Large linkage disequilibria are therefore understandable. This further explains the difference in magnitude of

observed from expected differences between the asexual and sexual population. In the former the deviations were much larger. However selection against some virulence gene combinations was detected (section 3.3.3).

Whilst the asexual population as a whole in the U.S.A. is heterogeneous, it is homogeneous throughout large regions in zones 3, 5, 6 and 7. Thus a valid check for the existence of selection against virulence gene combinations by the use of linkage disequilibrium test is possible provided the fitness relations of the various pathogen isolates meets the conditions described in section 3.4.2. Alternatively, the data from a single zone could be analyzed separately. This has yet to be attempted. Linkage disequilibrium may then be confidently attributed to selection against virulence gene combinations.

3.4.4.b The Canadian population

As in the U.S.A. wheat is grown in a wide area of the country. Green (1971) reported frequencies of the "standard" races of P. graminis tritici in Canada from 1956 - 1969. Data were given for all provinces in four groups (i) the Maritime Provinces, (ii) Quebec and Ontario, (iii) The Prairie provinces (Manitoba, Saskatchewan and Alberta) and (iv) British Columbia. The pathogen population is several thousand miles wide during the rust season, even though the pathogen does not overwinter in Canada, and the infection season is short (Van der Plank, 1968). The Prairie Provinces of western Canada are part of the North American Great Plains, one of the world's greatest cereal growing areas (Green and Campbell, 1979) and an integral part of the North American epidemiological system for rust (Martens and McKenzie, 1979). Manitoba is the heart of the Canadian rust area, although rust is also severe in

south eastern Saskatchewan (Green and Campbell, 1979).

Green (1971) noted that even though main races of the pathogen were found throughout Canada, there were differences in the relative amounts of these races in eastern and western Canada. Races 17, 38 and 48A were more common in eastern Canada whilst race 15B-1LX (Can.) was more common in the west. Some rare races also were found only in the west or the east, notably races 15B-3 and 15B-5 in the west as described earlier (section 3.4.1.c), which could attack Selkirk. Rust in British Columbia differed from that in the other three areas.

Green (1971) considered that the similarities of the races found in eastern and western Canada indicate a common source of inoculum, in the United States. The differences were explained by differences in climatic conditions and the cultivars grown in the areas. However, the British Columbia population was so different, that a different source of inoculum was supposed.

Thus it would seem that although the population as a whole is heterogeneous as in the U.S.A. large sections are homogeneous. Tests for selection against virulence gene combinations using tests for linkage disequilibrium may therefore yield valid conclusions.

3.5 Summary of main conclusions of the chapter

1. Evidence exists in the sexual and asexual population of P. graminis tritici in North America of virulence gene combinations which are selectively disadvantageous. These are listed in Table 32.
Virulence to Sr₆ and Sr_{9d} also appears to be selectively disadvantageous in Canada.
2. Variability in the asexual population in the U.S.A. is restricted

by several selective forces - selection for virulence genes and selection against virulence gene combinations.

3. Virulence gene accumulation and recombination can freely occur by recurrent mutation in large asexual populations. The failure of the pathogen in Canada to overcome the resistance of the wheat cultivar Selkirk is probably due to epistatic fitness loss conferred by the virulence genes required to overcome Selkirk's resistance, rather than unidentified resistance which the pathogen has not been able to overcome.
4. Providing virulence genes are initially at equilibrium frequencies in large homogeneous asexual populations, linkage equilibria will exist and equal selection for virulence genes singly and in combination, or for only one virulence gene in such homogeneous populations will not alter these. Equivalent selection for the same genotypes under conditions where a heterogeneous population will arise, similarly will not lead to linkage disequilibria. In these situations the classical test for selectively disadvantageous virulence gene combinations by tests for linkage disequilibrium is valid.
5. The asexual populations of P. graminis tritici in the U.S.A. and Canada are heterogeneous. In the former at least large linkage disequilibria are caused by selection of different genotypes (carrying exclusive virulence gene combinations) in different zones, and maintained by the lack of sexual recombination. However, within large sub-sections of these populations the race structure is remarkably homogeneous, and it is possible that within these sub-populations, tests for linkage disequilibrium may be valid indicators of selection against virulence gene combinations.

3.6 Application

A major finding of the chapter was that linkage equilibria between two virulence genes in a large asexual population should exist in the absence of selection for both or either virulence gene, or if there is equal host selection for both virulence genes singly and in combination, assuming the combination is not differentially unfit or superior (sections 3.4.2 and 3.4.3). An identification of such virulence pairs in large asexual populations could provide evidence of the operation of combination selection.

The evidence suggesting the existence of some selectively disadvantageous virulence gene combinations in both the asexual and sexual populations of Puccinia graminis tritici in the U.S.A. (section 3.3.3) warrants further attention. In the asexual population it was suggested that for such genes the large linkage disequilibria noted by Roelfs and Groth (1980) (see also section 3.3.2) were attributable to a combination of selection against the virulence pair, reinforced by selection for the genotypes carrying these genes singly, and maintained in the absence of sexual recombination, although recurrent mutation opposes disequilibria to an extent by generating recombinants.

An application of this phenomenon would be to cultivate wheats of genotype carrying resistance genes whose corresponding virulence genes in the pathogen population are disassociated. Thus Roelfs and McVey (1976) noted that combinations of Sr₆ and 9e, 6 and Tmp, 9a and Tmp, and 9b and Tmp would be resistant to all cultures of the pathogen identified in 1975 in the U.S.A. Mass cultivation of such cultivars may select rapidly for the corresponding virulence genotypes that undoubtedly exist in the pathogen population at low frequencies (produced by mutation) but this may be offset by selection against the virulence pair (as described for virulence to Selkirk in Canada (section 3.4.1.c)).

CHAPTER 4

PATHOGEN EVOLUTION IN CULTIVAR MIXTURES AND MULTILINES : A NEW MODEL

4.1 Introduction

The mathematical models of pathogen evolution in cultivar mixtures and multilines were discussed in Chapter 2. Simulations with these models utilizing more realistic values of the parameters s , $\emptyset$ and α yielded the prediction that complex races are generally favoured in multilines and cultivar mixtures, even though there would be less disease when compared with pure stands, or even compared with when simple races predominate in heterogeneous host populations.

The conclusion of complex race predominance is not always consistent with the results from field experiments or even from commercial multilines or cultivar mixtures.

Chin (1979) monitored race changes in cultivar mixtures of barley in field trials. In one set of experiments using mixtures in varying proportions of the cultivars Hassan (H) with the Mlas resistance gene and Wing (W) with the Mla_{4/7} genes Chin (1979) found that although the relative frequency of races with both virulence genes hw almost doubled in a H : W mixture (1:1) compared with pure stands, the absolute frequency (colonies per tiller) did not increase to the same degree. The smaller increase in absolute frequency was largely attributed to the reduction in the amount of infection in mixtures compared with pure stands. Nonetheless the selection for hw races was significant. In pure stands the final relative frequency of hw virulence was 15.5 - 16.3%. The corresponding simple races with virulence genes h or w dominated the pure stands. However on a H : W mixture the frequency

of all three virulences h, w and hw was approximately equal, and hence there was positive selection for the hw virulence during the season. Chin (1979) stated that if the same cultivar mixture is sown repeatedly the pathogen population may become increasingly pre-adapted to the same range of host genotypes each season with the result that complex genotypes predominate. Chin (1979) thought that control would be lost in these circumstances and that different mixtures, or rotation of the resistance genes would result in better disease control. This is similar to the strategy for the use of single R gene cultivars (section 2.1), except that replacement with mixtures may be less frequent.

From the results on pure stands of both H and W where simple virulences predominated at the end of the experiments; Chin (1979) concluded the fitnesses of h on H and w on W were greater than that of hw on either cultivar, and that these results were consistent with previous laboratory and field studies which indicated that the virulence combination (hw) was disadvantageous in comparison with the single virulence genotypes on their corresponding hosts. Whilst the virulence combination may be unfit, a loss of fitness of hw on H or W compared with h and w races respectively could be partly or totally due to selection against unnecessary virulence genes w and h when hw races are growing on H and W cultivars respectively. Unfortunately, Chin (1979) did not estimate the frequency of the virulences at the start of the experiment and therefore the low frequencies of the hw combination on H and W pure stands may have been due to low initial frequencies rather than due to selection. However, M. Wolfe (personal communication) stated that for the next three years the hw virulence combination repeatedly proved to be 'awkward' to the pathogen.

In a second set of experiments Chin (1979) examined pathogen evolu-

tion in three component mixtures, of Hassan (H) and Wing (W) and an additional cultivar Midas (M) carrying the Mla₆ resistance gene. More data were collected for this second set of experiments in that the virulence gene frequencies were scored at different times (31 and 53 days after sowing) during disease development and thus trends in race and virulence gene frequencies could be identified. At a time of 53 days after sowing, on pure stands of H, W and M, Chin (1979) found that the simple virulence genotypes h and w dominated at high frequencies on H and W respectively whilst on M the double virulence genotype hm dominated, although m the single virulence genotype was increasing. Between 31 and 53 days on the pure line of M strong selection against the wm genotype (which dominated at 31 days) occurred.

On the mixture of H : W : M the frequency of races was sampled individually on each component. On H again the single virulence genotype, h predominated at and was increasing between 31 and 53 days. On W, w genotypes predominated after both 31 and 53 days but declined between the two sampling dates. The next most common virulence genotype at 53 days was the complex genotype hwm which had increased markedly between 31 and 53 days.

On M, the double virulence genotype wm predominated at 53 days but not at 31 days. The complex race with hwm was declining and was the third most common genotype at 53 days.

Thus Chin (1979) provided evidence that complex races or genotypes do not necessarily predominate or are not selected rapidly in cultivar mixtures. However there was clear evidence of greater selection on average for the complex genotypes hw, wm and hwm in the mixtures than in the pure stands. However, these increases in complex races were offset by the large reduction in population size (and hence disease

levels) in the mixture.

These results contradict the conclusion of Chapter 2 that complex races are normally selected rapidly. However the experimental data confirms the model prediction that there will be less disease in cultivar mixtures than in pure stands even if complex races are selected.

Wolfe et al. (1981a) indicated that growing mixtures can lead to an increase in the frequency of pathogen races of intermediate complexity, but also noted that complex races may not be highly pathogenic to all of the cultivars that they can attack. Instead mixtures may be composed of pathogen genotypes differentially adapted to each cultivar.

Wolfe et al. (1981a) however recognized that although cultivar mixtures have given consistently good disease control they have been used only over a small area for a short period of time so that predictions about the response of the pathogen population to a very widespread mixture are difficult.

Browning and Frey (1981) noted that in plots of their oat multilines in Texas the spore populations carried more virulence genes in more combinations than did those infecting pure lines. However the more diverse pathogen population had not led to more disease. There was no indication of rapid selection for complex or super races in their multilines.

Thomas and Clifford (1981) studied the disease intensities of barley powdery mildew and the virulence dynamics of E. graminis hordei in a number of barley multilines with up to four isogenic lines over two successive years, where the pathogen population was maintained between seasons on a winter barley bridge. In most cases the multilines reduced epidemic development and initial inoculum levels compared with pure stands but some increases in the rates of epidemic development were apparent.

The four near isogenic lines contained mildew resistance from the following cultivars :

- 1) CI 1243 (A)
- 2) Spiti (B)
- 3) Halle Hohenthurm (C)
- 4) Mianwali (D)

Eleven multilines were developed, AB, AC, AD, BC, BD, CD, ABC, ABD, ACD, BCD and ABCD.

Corresponding virulence genes are denoted a, b, c, d respectively.

In the 1980 trials a susceptible winter barley cultivar Hoppel was sown in a five acre field, and a large natural population of mildew allowed to develop on it. This served as a natural pool of inoculum for infecting the nearby plots of multilines and pure stands. The pathogen population on Hoppel was dominated by the virulence genotype ab, whilst c and d were rare, and always were found in the combinations abc and abd.

In the pathogen population on multilines and pure stand plots (cultivar Universe) genotypes c and d remained at low frequencies except on cultivars providing directional selection for these virulences. The overall pathogen populations however tended towards greater complexity. On the ABCD multiline, abcd races (super races) dominated the population at the end of the season. Unnecessary virulence dominated the Universe populations.

In the 1981 trials the trend to increased complexity continued even though some virulences were unnecessary on certain lines. Extensive testing confirmed complex races dominated most populations. Unnecessary virulence declined in certain cases especially the virulence d in populations of C.

Despite the observation of Thomas and Clifford (1981) that rapid increases in relevant virulences and race complexity occurred, some of the multilines retained most of their disease control and yield benefit.

In summary the experimental data on race evolution in cultivar mixture and multilines suggest that rapid selection of complex genotypes does not necessarily occur. Although many more experimental data are required over longer periods of observation, apparent discrepancies exists between some of these experimental data and the mathematical models which predict the likely evolution of progressively more complex races of pathogens when growing on multilines and cultivar mixtures. Chapter 4 resolves some of these discrepancies.

4.2 Selective forces against complex races in cultivar mixtures and multilines

It is evident that the old models of pathogen evolution in cultivar mixtures and multilines are simplistic in only assuming one kind of selective force against complex races viz stabilizing selection. It is equally evident that three other selective forces may operate against complex races but not simple races in cultivar mixture. Two of these forces may also operate against complex races in multilines. Whilst the existence of these forces and indeed their operation in heterogeneous host populations has been recognized they have hitherto not been incorporated into mathematical models. These forces are reviewed briefly and then a new model incorporating them described. The four forces that may operate in cultivar mixtures which specifically select against complex races are :

1. Stabilizing selection.
2. Selection against virulence gene combinations.
3. Selection against the background genotype of complex races.
4. Induced susceptibility.

The first two and the last force may operate in multilines but not selection against the background genotype of complex races for reasons discussed below (section 4.2.1).

Stabilizing selection has already been discussed in detail in Chapter 1, whilst selection against virulence gene combinations was the topic of Chapter 3. The last two forces will now be considered.

4.2.1 Selection against the background genotype of complex races

This selective force can only operate in cultivar mixtures and not in multilines. In a multiline a complex race can develop a fit background genotype adapted to the background genotype of every isoline in the multiline, giving the pathogen a high fitness. Similarly the simple races can also evolve a similar fit matching background genotype giving them high fitness on the one isoline they can infect.

However in cultivar mixtures, each cultivar possesses a different background genotype as well as possessing a different major resistance gene. Each simple race can develop a fit matching background genotype as in multilines. However a race with two or more virulence genes, although capable of infecting two or more cultivars in the mixture, cannot develop a fit matching background genotype to all of these simultaneously. Hence a complex race infecting a cultivar may be considerably less fit than the corresponding simple race not only because of stabilizing selection but also because of a less fit matching background genotype. For complex races in cultivar mixtures there is a

necessary sacrifice of fitness for flexibility. In order to infect several cultivars complex races need several virulence genes. They are genetically flexible, but the flexibility necessarily results in a sacrifice of fitness, i.e. the ability to maximize reproduction on any one cultivar is reduced compared with the highly adapted simple races.

In samples of E. graminis hordei with virulence to both Hassan, H (Mlas) and Wing W (Mla_{4/7}) Chin (1979) detected differences in aggressiveness between isolates taken from either H or W growing in pure stands and isolates recovered from mixtures. Isolates of hw races from pure stands were more aggressive than those taken from the mixtures indicating that adaptation to the host background genotype had occurred to a greater extent in the pathogen population growing on pure stands. In a pure stand the flexibility of complex races such as hw is unnecessary and therefore fitness is not sacrificed. Consequently, complex races with hw developed a fully fit matching background genotype like the simple races. Further in the pure stands hw isolates from H were more aggressive on H than W and vice versa, strongly indicative of adaptation to the host background genotype. Isolates of hw from H or W plants in the mixture did not show this adaptation.

Wolfe et al. (1981a) also reported that barley cultivar mixtures with three components each with the same identified resistance genes, but with different unidentified background genotypes gave significant disease control compared with pure stands. In one experiment the three barley cultivars used were Aramir, Athos and Porthos all carrying the identified resistance genes Mlg + Mlas. This was therefore an unusual cultivar mixture in that only the unidentified background genotype contributed to the mixture effects and not the major resistance genes. On June 4th, the powdery mildew score on the mixture relative to that of the

means of the components grown alone was 116%. By the 28th of the same month, this had been reduced to 34%. The disease control can only be explained by the lack of adaptation of the pathogen to the three different background genotypes in the mixture, a problem absent in pure stands.

These experiments provide support to the theoretical expectation of this selective force acting against complex races in cultivar mixtures. Modelling this force, if the average relative fitness of simple races is 1, then the average relative fitness of complex races is reduced by a factor $(1 - \underline{g})$ where $\underline{g}$ ($0 \leq \underline{g} \leq 1$) is a coefficient of selection against the less fit background genotype of the complex race.

4.2.2 Selection against the super race by induced susceptibility

Trenbath (1977, 1983) included factors for induced resistance or cross protection in his models of multilines. Chin (1979) demonstrated the importance of induced resistance in cultivar mixtures of barley against powdery mildew and demonstrated that induced susceptibility could also occur but was much less significant than induced resistance.

While induced resistance acts against all races of the pathogen and contributes to disease control, induced susceptibility selects differentially against the super races in multilines or cultivar mixtures in the following way. Every race in such heterogeneous host populations can induce normally resistant hosts to become susceptible to normally avirulent races. Further every race except the super race can benefit from induced susceptibility by becoming virulent on normally resistant plants. The super race cannot by definition benefit from induced susceptibility because it is already virulent on every host component in the multiline or cultivar mixture. Consequently, induced susceptibility selects differentially against the super race by default, as a

consequence of benefit being conferred on every other race.

4.3 Pathogen evolution in cultivar mixtures and multilines : the new model

A new model has been written incorporating all four selective forces against complex races discussed in section 4.2. All four operate in cultivar mixtures, but one, selection against the background genotype is absent from pathogen populations in multilines.

The model is described for cultivar mixtures but is applicable to multilines with some modification.

4.3.1 Model conditions and parameters

1. The model assumes just two cultivars A and B at proportions m and n respectively where $\underline{m} + \underline{n} = 1$.
2. There are three pathogen races in the cultivar mixture with respect to the two cultivars, races a, b and ab at initial proportions $\underline{Pa}_{(1)}$, $\underline{Pb}_{(1)}$ and $\underline{Pab}_{(1)}$ respectively. In any generation t
$$\underline{Pa}_{(t)} + \underline{Pb}_{(t)} + \underline{Pab}_{(t)} = 1 \quad 4.3.1.1$$
3. There is a gene for gene relationship between the resistance genes $\underline{R_A}$ and $\underline{R_B}$ carried by A and B respectively and the pathogen virulence genes a, b carried by races a and b respectively, and by race ab.
4. The relative fitnesses of the various combinations are given in Table 35.

Table 35 Relative fitnesses¹ of pathogen races in a two host
cultivar mixture model

Pathogen	Host	
	<u>A</u>	<u>B</u>
<u>a</u>	$\underline{W}aA^2 = 1$	$\underline{W}aB = 0^3$
<u>b</u>	$\underline{W}bA = 0$	$\underline{W}bB = 1$
<u>ab</u>	$\underline{W}abA = (1-\underline{c})^4 \cdot (1-\underline{g})^5 \cdot (1-\underline{s}_b)^6$	$\underline{W}abB = (1-\underline{c}) \cdot (1-\underline{g}) \cdot (1-\underline{s}_a)^6$

- ¹ Fitness is assumed to be a measure of relative viable spore production (where infection capacities are equal). For example on A for every 100 spores that race a produces, race ab produces $100 \cdot (1-\underline{c}) \cdot (1-\underline{g}) \cdot (1-\underline{s}_b)$ spores
- ² $\underline{W}aA$ means the relative fitness ($\underline{W}$) of race a on cultivar A etc.
- ³ If a spore of race a lands on cultivar B it is assumed not to infect or sporulate ($\underline{z} = 1$, where $\underline{z}$ is the coefficient of selection against avirulence). Thus the fitness of race a on B is zero. However induced susceptibility may cause normally avirulent spores to be infectious and induce the formation of sporulating colonies.
- ⁴ $\underline{c}$ = a coefficient of selection against the virulence combination ab. If the average relative fitness of simple races is 1, combination selection reduces the average relative fitness of race ab by $(1-\underline{c})$.
- ⁵ $\underline{g}$ = a coefficient of selection against the less adapted background genotype of race ab. If the average relative fitness of

simple races is 1, this selection reduces the average relative fitness of race ab by $(1-g)$.

- 6 $\underline{s}_a, \underline{s}_b$ = selective coefficients against unnecessary virulence carried by race ab on cultivars B and A respectively. See section 1.3.2.
-

5. Initially there are $\underline{N}_{(1)}$ pathogen individuals in the cultivar mixture where

$$\underline{N}_{(1)} = \underline{Na}_{(1)} + \underline{Nb}_{(1)} + \underline{Nab}_{(1)} \quad 4.3.1.2$$

the sum of the numbers of the races a, b and ab in generation 1 respectively. Generally in any generation (t)

$$\underline{N}_{(t)} = \underline{Na}_{(t)} + \underline{Nb}_{(t)} + \underline{Nab}_{(t)} \quad 4.3.1.3$$

and

$$\underline{Na}_{(t)} = \underline{NaA}_{(t)} + \underline{NaB}_{(t)} \quad 4.3.1.4$$

the numbers of race a on A and B respectively and

$$\underline{Nb}_{(t)} = \underline{NbA}_{(t)} + \underline{NbB}_{(t)} \quad 4.3.1.5$$

and

$$\underline{Nab}_{(t)} = \underline{NabA}_{(t)} + \underline{NabB}_{(t)} \quad 4.3.1.6$$

It follows that

$$\underline{Pa}_{(t)} = \underline{Na}_{(t)} / \underline{N}_{(t)} \quad 4.3.1.7$$

$$\underline{Pb}_{(t)} = \underline{Nb}_{(t)} / \underline{N}_{(t)} \quad 4.3.1.8$$

$$\underline{Pab}_{(t)} = \underline{Nab}_{(t)} / \underline{N}_{(t)} \quad 4.3.1.9$$

6. The alloinfection frequency = $\emptyset$, where $0 \leq \emptyset \leq (\alpha/1+\alpha)$ (see section 2.2.2.d and equation 2.2.2.10). The autoinfection frequency = $1 - \emptyset$.

7. The induced resistance factor ir and the induced susceptibility factor = is, and represent arbitrary numerical measures of induction . They are always greater or equal to zero and may exceed one.
8. The realized growth rate of the pathogen is $(1 + \alpha)$ where α is a reproductive rate (section 2.2.2.c).

4.3.2 Model equations

The equations calculating numbers of each race on each host genotype are :

For race a

$$N_{aA(t)} = (1 + \alpha) \cdot (W_{aA} \cdot (N_{aA(t)} \cdot (1 - \sigma) + N_{aA(t)} \cdot \sigma \cdot m + N_{aB(t)} \cdot \sigma \cdot m)) \cdot (1 - (\alpha \cdot ir \cdot W_{bB} \cdot (N_{bA(t)} \cdot (1 - \sigma) + N_{bA(t)} \cdot \sigma \cdot m + N_{bB(t)} \cdot \sigma \cdot m))) / N_{(t)} \quad 4.3.1.10$$

$$N_{aB(t)} = N_{aB(t)} + is \cdot \alpha \cdot (N_{aB(t)} \cdot (1 - \sigma) \cdot W_{aA} + N_{aB(t)} \cdot \sigma \cdot n \cdot W_{aA}$$

$$+ N_{aA(t)} \cdot \sigma \cdot n \cdot W_{aA}) \cdot (N_{bB(t)} \cdot (1 - \sigma) \cdot W_{bB} + N_{bB(t)} \cdot \sigma \cdot n \cdot$$

$$W_{bB} + N_{bA(t)} \cdot \sigma \cdot n \cdot W_{bB} + N_{aB(t)} \cdot (1 - \sigma) \cdot W_{aB} + N_{aB(t)} \cdot$$

$$\sigma \cdot n \cdot W_{aB} + N_{aB(t)} \cdot \sigma \cdot n \cdot W_{aB}) / N_{(t)} \quad 4.3.1.11$$

For race b

$$N_{bB(t)} = (1 + \alpha) \cdot (W_{bB} \cdot (N_{bB(t)} \cdot (1 - \sigma) + N_{bB(t)} \cdot \sigma \cdot n + N_{bA(t)} \cdot \sigma \cdot n)) \cdot (1 - (\alpha \cdot ir \cdot$$

$$W_{aA} \cdot (N_{aB(t)} \cdot (1 - \sigma) + N_{aB(t)} \cdot \sigma \cdot n + N_{aA(t)} \cdot \sigma \cdot n)) / N_{(t)} \quad 4.3.1.12$$

$$NbA_{(2)} = NbA_{(1)} + (1-\alpha) \cdot (NbA_{(1)} \cdot (1-\theta) \cdot W_b B + NbA_{(1)} \cdot \theta \cdot m \cdot W_b B$$

$$+ NbB_{(1)} \cdot \theta \cdot m \cdot W_b B) \cdot (NaA_{(1)} \cdot (1-\theta) \cdot W_a A + NaA_{(1)} \cdot$$

$$\theta \cdot m \cdot W_a A + NaB_{(1)} \cdot \theta \cdot m \cdot W_a A + NaB_{(1)} \cdot (1-\theta) \cdot W_a A$$

$$+ NaB_{(1)} \cdot \theta \cdot m \cdot W_a B + NaB_{(1)} \cdot \theta \cdot m \cdot W_a B) / N_{(1)} \quad 4.3.1.13$$

For race ab

$$NaB_{(2)} = (1+\alpha) \cdot (NaB_{(1)} \cdot (1-\theta) \cdot W_a B + NaB_{(1)} \cdot \theta \cdot m \cdot W_a B + NaB_{(1)} \cdot \theta \cdot m \cdot W_a B).$$

$$(1 - (\alpha \cdot ir \cdot W_b B \cdot (NbA_{(1)} \cdot (1-\theta) + NbA_{(1)} \cdot \theta \cdot m + NbB_{(1)} \cdot \theta \cdot m)) / N_{(1)}) \quad 4.3.1.14$$

$$NaB_{(2)} = (1+\alpha) \cdot (NaB_{(1)} \cdot (1-\theta) \cdot W_a B + NaB_{(1)} \cdot \theta \cdot n \cdot W_a B + NaB_{(1)} \cdot \theta \cdot n \cdot W_a B).$$

$$(1 - (\alpha \cdot ir \cdot W_a A \cdot (NaB_{(1)} \cdot (1-\theta) + NaB_{(1)} \cdot \theta \cdot n + NaA_{(1)} \cdot \theta \cdot n)) / N_{(1)}) \quad 4.3.1.15$$

The equations calculating $\underline{NaA}_{(2)}$ and $\underline{NbB}_{(2)}$ take into account infections both from cultivars normally susceptible, i.e. A and B respectively, as well as infections originating from the normally resistant cultivar which have become infected because of induced susceptibility. The induced resistance factor (ir) reduces $\underline{NaA}_{(2)}$ and $\underline{NbB}_{(2)}$,

by an amount, dependent not only on $\underline{ir}$, but also the number of spores of the avirulent race landing on the appropriate cultivar and inducing resistance, and the size of the population of the normally virulent race, conditioned to be avirulent.

The equations calculating $\underline{NaB}_{(2)}$ and $\underline{NbA}_{(2)}$ likewise take into account infections originating from both cultivars and induced susceptibility of amount $\underline{is}$. If $\underline{is} = 0$, and $\underline{NaB}_{(1)}$ and $\underline{NbA}_{(1)}$ are zero also, then there is never a population of non matching races on normally resistant cultivars.

The equations calculating $\underline{NabA}_{(2)}$ and $\underline{NabB}_{(2)}$ are constructed similarly to those of $\underline{NaA}_{(2)}$ and $\underline{NbB}_{(2)}$.

4.3.3 Computer simulations

A computer programme of the model, called SELIN 6 (see Appendix 12) was written and various simulations of pathogen evolution were carried out with it.

4.3.3.a The effect of varying the induced resistance factor $\underline{ir}$

Initially in all simulations $\underline{Pa}_{(1)} = \underline{Pb}_{(1)} = 0.25$, $\underline{Pab}_{(1)} = 0.50$ and $\underline{N}_{(1)} = 4$. In these simulations the following values for the parameters were used : $\underline{c} = 0.05$, $\underline{g} = 0.05$, $\underline{s}_a = 0.05$, $\underline{s}_b = 0.05$, $\underline{m} = 0.5$, $\underline{n} = 0.5$, $\alpha = 1.0$, $\emptyset = 0.5$ and $\underline{is} = 0.0001$. The estimates of $\underline{c}$ and $\underline{g}$ were based on known values of $\underline{s}$ (section 1.3.6.h) in the absence of data of direct measurements. The value of $\underline{is}$ was also an estimate. Values of $\underline{ir}$ were set at 0.000, 0.001, 0.100 and 0.900. After 50 generations the race frequencies and population sizes were obtained. Results are shown in Table 36.

Table 36 The effect of varying the induced resistance factor
 (ir) on race frequencies and population sizes after 50
 generations

<u>ir</u>	0.000	0.001	0.100	0.900
<u>Pa</u> (50)	0.00071	0.00071	0.00071	0.00071
<u>Pb</u> (50)	0.00071	0.00071	0.00071	0.00071
<u>Pab</u> (50)	0.99858	0.99858	0.99858	0.99858
<u>N</u> (50)	0.599083	0.598677	0.559754	0.322197
	x 10 ¹²	x 10 ¹²	x 10 ¹²	x 10 ¹²

Clearly in this model, varying ir does not alter the race frequencies, only the population sizes. The greater the value of ir, the smaller the value of N. These results are not surprising since induced resistance acts against all races in a cultivar mixture equally, it is not race specific.

4.3.3.b The effect of varying the induced susceptibility factor (is)

All parameter values were as set in the previous section (4.3.3.1) except ir was set at 0.0 and is was set at 0.001, 0.100 and 0.900 in three simulations. The results are shown in Table 37.

Table 37 The effect of varying the induced susceptibility factor (is) on race frequencies and population sizes after 50 generations

<u>is</u>	0.001	0.100	0.900
<u>Pa</u> (50)	0.000712	0.001014	0.034625
<u>Pb</u> (50)	0.000712	0.001014	0.034625
<u>Pab</u> (50)	0.998576	0.997973	0.930749
<u>N</u> (50)	0.599085 x 10 ¹²	0.599447 x 10 ¹²	0.641517 x 10 ¹²

Varying the value of is affects both race frequencies and population sizes. The higher the value of is the greater the frequency of simple races and the larger the final population total. These results are expected since induced susceptibility selects against the super race ab and increases the disease level.

However it is evident from these simulations that varying ir and is in the range 0.0 to 0.9 has little effect on the race frequencies and population sizes. Induced resistance and susceptibility are difficult to define or quantify.

4.3.3.c The effect of varying the alloinfection frequency ($\emptyset$)

Parameter values for c, g, s_a, s_b, m, n and α were as above

(sections 4.3.3.a and 4.3.3.b), ir was set at 0.001 and is at 0.0001. Two values of $\emptyset$ were chosen, 0.1 and 0.9 (strictly $\emptyset_{\max} = 0.5$, but the prime purpose is to test the model). The results are shown in Table 38.

Table 38 The effect of varying the alloinfection frequency ($\emptyset$) on race frequencies and population sizes after 50 generations

$\emptyset$	0.1	0.9
$\underline{Pa}_{(50)}$	0.496741	0.000000
$\underline{Pb}_{(50)}$	0.496741	0.000000
$\underline{Pab}_{(50)}$	0.006517	1.000000
$\underline{N}_{(50)}$	0.916945 x 10^{14}	0.597987 x 10^{12}

Varying the value of $\emptyset$ has a marked effect on race frequencies and population sizes. The lower the value of $\emptyset$ the greater the selection for the simple races. This is consistent with the results of Barrett's (1980) simulations. At $\emptyset = 0.1$, the super race is virtually absent; at $\emptyset = 0.9$, the reverse is true, the simple races are maintained only by back mutation.

The population size is considerably larger when $\emptyset = 0.1$ than when $\emptyset = 0.9$, again consistent with the simulations of Barrett (1980). This

predicts that the predominance of simple races is associated with higher disease levels.

4.3.3.d The effect of varying the selective coefficients

- (i) against the virulence gene combination, $\underline{c}$ and
- (ii) against the background genotype of the complex
race, $\underline{g}$

The parameters, $\underline{c}$ and $\underline{g}$ were set at :

- (i) $\underline{c} = \underline{g} = 0.0$
- (ii) $\underline{c} = \underline{g} = 0.2$
- (iii) $\underline{c} = 0.2, \underline{g} = 0.0$

in three simulations.

In the absence of experimentally determined data, these values were arbitrarily selected to test the effects of varying these parameters.

Values of $\underline{s}_a$, $\underline{s}_b$, $\underline{m}$, $\underline{n}$, α , $\underline{ir}$ and $\underline{is}$ were as in section 4.3.3.c, whilst $\emptyset = 0.5$.

The results are shown in Table 39.

Table 39 The effect of varying the selective coefficients (i) against the virulence gene combination, c and (ii) against the background genotype of the complex race, g, on race frequencies and population sizes after 50 generations

<u>c</u> , <u>g</u>	0.0, 0.0	0.2, 0.2	0.2, 0.0
<u>Pa</u> (50)	0.000005	0.499983	0.171640
<u>Pb</u> (50)	0.000005	0.499983	0.171640
<u>Pab</u> (50)	0.999991	0.000034	0.656719
<u>N</u> (50)	0.911565 x 10 ¹⁴	0.845630 x 10 ⁹	0.247095 x 10 ¹⁰

When c and g are both zero the complex race predominates and the population size is comparatively high. When c and g are both 0.2, simple races predominate and the population size is very low. In these two simulations complex race domination and high population size are positively associated unlike the results of section 4.3.3.c (Table 38). Clearly, combination selection and background genotype selection against the complex race, reduces N(50), but if c = g = 0.0 then selection against the complex race is reduced hence the population size is higher.

For comment on the results of the third column see section 4.3.4.

4.3.3.e The effect of varying host frequencies (m, n)

In all simulations to date, m and n were assumed to be equal. In this simulation m was set at 0.75 and n at 0.25. Values of c, g, s_a and

$\underline{s}_b$ were set at 0.05, $\alpha = 1.0$, $\emptyset = 0.5$, $\underline{i}_r = 0.001$ and $\underline{i}_s = 0.0001$.

The results are shown in Table 40.

Table 40 The effect of varying the host frequencies ($\underline{m}$, $\underline{n}$) on
race frequencies and population sizes after 50 generations

$\underline{m}$, $\underline{n}$	0.50, 0.50	0.75, 0.25
$\underline{Pa}_{(50)}$	0.000710	0.575553
$\underline{Pb}_{(50)}$	0.000710	0.000000
$\underline{Pab}_{(50)}$	0.998580	0.424446
$\underline{N}_{(50)}$	0.598677×10^{12}	0.140810×10^{13}

Changing $\underline{m}$ from 0.50 to 0.75 selects the corresponding simple race, and the final population total is increased. Therefore the disease control of a cultivar mixture is greatest when host frequencies are equal because the mixture effects are maximized.

4.3.4 Discussion and conclusions

From these simulations it is clear that the selective coefficients $\underline{c}$ and $\underline{g}$, and the value of $\emptyset$ have most influence on the race frequencies and pathogen population sizes in cultivar mixtures, over the range of values chosen. High values of $\underline{c}$ and $\underline{g}$ favour simple races and low disease levels (Table 39) whilst high $\emptyset$ values favour the complex races and low disease levels (Table 38). A final simulation was performed to see the

effects of combining high values of $\underline{c}$, $\underline{g}$ and $\emptyset$. The parameter values were set at :

$$\underline{c} = \underline{g} = 0.2$$

$$\emptyset = 0.9$$

$$\underline{s}_a = \underline{s}_b = 0.05$$

$$\underline{m} = \underline{n} = 0.5$$

$$\alpha = 1.0$$

$$\underline{ir} = 0.9$$

$$\underline{is} = 0.0001$$

These values maximize the selective effects against the pathogen population in the cultivar mixture, over the range of values chosen in the simulations. After 50 generations the following results were obtained :

$$\underline{Pa} = 0.003670$$

$$\underline{Pb} = 0.003670$$

$$\underline{Pab} = 0.992660$$

$$\underline{N} = 0.664957 \times 10^4$$

The complex race ultimately predominates. The predominance of simple races in the earlier simulations of section 4.3.3.d (Table 39) which occurred when $\underline{c} = \underline{g} = 0.2$ and $\emptyset = 0.5$ is reversed due to the higher alloinfection frequency of $\emptyset = 0.9$.

The most significant feature of this simulation is the massive reduction in population size, $\underline{N}_{(50)}$ compared with the results in other simulations (Tables 36 - 40 inclusive). The previous lowest population total was 0.845630×10^9 (Table 39); the reduction to $\underline{N}_{(50)} =$

0.664957×10^4 represents over a 100,000 fold decrease.

The reduction occurs, when the selective coefficients, $\underline{c}$ and $\underline{g}$ and the alloinfection frequency $\emptyset$ are high ($\underline{c} = \underline{g} = 0.2$; $\emptyset = 0.9$) because there is strong selection against all races in the cultivar mixture, complex and simple ones alike.

The point raised in Section 2.3.5 that complex race domination does not necessarily mean more disease is further supported by these simulations. In this final simulation massive disease reduction and complex race domination are associated.

Realistic predictive simulations are not feasible as accurate experimentally determined values of $\underline{c}$, $\underline{g}$, $\underline{ir}$ and $\underline{is}$ are unavailable at present. On the other hand, experimentally determined values of $\underline{s}$, α , $\emptyset$, $\underline{m}$ and $\underline{n}$ are available.

The chosen values of the parameters $\underline{s}$, $\underline{c}$ and $\underline{g}$ are not unrealistically high nor the values of $\emptyset$ unrealistically low (see Table 39), and yet simple races are selected. This is in contrast to the very high values of $\underline{s}$ and/or very low values of $\emptyset$ required by the older models (Barrett, 1978, 1980; Wolfe and Barrett, 1978) to ensure simple race predominance. This new model is therefore more realistic than the older models in that simulations with realistic parameter values lead to predictions similar to the field evidence (section 4.1) which shows that complex races are not necessarily selected in cultivar mixtures. The older models cannot accommodate these field observations unless excessively high values of $\underline{s}$ or excessively low values of $\emptyset$ are assumed. These models were too simplistic in assuming stabilizing selection as the only differential selective force acting against complex races. The new model represents a considerable improvement therefore.

The difference between the disease control potential of multilines and cultivar mixtures can be seen from Table 39. For a multiline, $\underline{g} = 0$ since the background genotype of all isolines is identical. This is the situation represented in column 3. The results are directly comparable with those in column 2 which is representative of cultivar mixtures. The inclusion in the simulation of selection against the background genotype of complex races at high enough levels ($\underline{g} = 0.2$) alters the race dominance. For multilines ($\underline{g} = 0.0$) the complex race dominated at generation 50, whereas in cultivar mixtures ($\underline{g} = 0.2$) the simple races dominated.

The main advantage of cultivar mixtures is their predicted superior disease control, as shown by a lower population total at generation 50. The population total in the cultivar mixture was 2.9 times smaller. This means that cultivar mixtures are superior strategies for (a) opposing complex races build up and (b) reducing disease. It is the opinion of the author that to date the use of cultivar mixtures is the best strategy for using major resistance genes.

Greater disease control theoretically should result from their use due to the great exploitation of the selective forces against all races of the pathogen.

Summary

The content and conclusion of chapter 4 is the culmination of this thesis. Chapter 4 incorporates the findings of chapters 1 and 3 which discuss selection against unnecessary virulence genes and selection against virulence gene combinations, into a new model of pathogen evolution in heterogeneous host populations and shows the older models described in chapter 2 to be less realistic. Further the model shows

cultivar mixtures to be a superior disease control strategy than multilines.

The potentially massive reduction in the pathogen population in heterogeneous host populations should be greatly encouraging to plant pathologists. Further, the disease control of multilines and cultivar mixtures was predicted in chapters 2 and 4 to be independent of race dominance, so that complex races might predominate the pathogen population but with less disease than when simple races predominate. This finding if substantiated in practice is an encouragingly devastating blow to the previous assumption that accumulation of virulence automatically results in more disease.

Appendix 1

Computer Programme UNA

PROGRAM UNA (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)

C

COMMENT ALL READ STATEMENTS USE A "FREE FORMAT" INPUT.

C

```
WRITE(6,10)
READ(5,*)P,Q,S,UA,UV
99 WRITE(6,11)
READ(5,*)NG
N=1
26 P=(P-P*UA+Q*UV)/(1.0+S*Q)
Q=1.0-P
N=N+1
IF (N.GT.NG) GO TO 25
GO TO 26
25 WRITE(6,27)P,Q
27 FORMAT(1X,3HP =,F8.6,5X,3HQ =,F8.6)
GO TO 20
20 WRITE(6,12)
10 FORMAT(1X,29H ENTER VALUES OF P,Q,S,UA,UV )
11 FORMAT(1X,30H ENTER THE NO. OF GENERATIONS )
12 FORMAT(1X,28H ENTER A REVISED VALUE OF S )
READ(5,*)S
P=0.9999999
Q=1E-6
GO TO 99
END
```

Appendix 2

Derivation of equation 1.2.4.1

$$t = 0.5 \log_e \left(\frac{B^2 - Q^2}{B^2 - Q^2} \right) + \left(\frac{1 - sq}{\lambda s B} \right) \cdot \log_e \left(\frac{(B + Q) \cdot (B - Q)}{(B - Q) \cdot (B + Q)} \right)$$

The equation is derived from the model DAVE 1 (section 1.3.2) which describes mathematically the changes in an avirulence (A) and virulence (V) allele pair under mutation and stabilizing selection. The model is described below

Allele	:	<u>A</u>	<u>V</u>
Average Relative fitness	:	1	1-s
Initial frequency	:	<u>p</u> _t	<u>q</u> _t
q_{t+1}	=	$\frac{(1-s) \cdot q_t + Ma \cdot p_t - Mv \cdot q_t}{1 - s \cdot q_t}$	

It follows that

$$q_{t+1} = \frac{(1-s - Ma - Mv) \cdot q_t + Ma}{1 - s \cdot q_t}$$

This is a difference equation for q_t which can be replaced by a differential equation by making the approximation :

$$\frac{dq}{dt} \approx \frac{q_{t+1} - q_t}{\Delta t} \quad \text{where } \Delta t = 1$$

$$\therefore \frac{dq}{dt} \approx q_{t+1} - q_t \approx \frac{s \cdot q_t^2 - (s + Ma + Mv) \cdot q_t + Ma}{1 - s q_t}$$

$$\frac{dt}{dq} = \frac{1 - s \cdot q_t}{s \cdot q_t^2 - (s + Ma + Mv) \cdot q_t + Ma}$$

where $\gamma = \mu_a + \mu_v$ and $\alpha = \mu_a$

$$t(q) = \int_{q_0}^{q_k} \frac{(1 - s \cdot q_k) \cdot dq}{s \cdot \left(q_k - \frac{s+\gamma}{2s} \right)^2 - \frac{(s+\gamma)^2}{4s} + \alpha}$$

Let $Q = q_k - \bar{q}$, where $\bar{q} = \frac{s+\gamma}{2s}$, and

$$Q_0 = q_0 - \bar{q}$$

$$\Rightarrow dQ = dq$$

$$\therefore t(q) = \int_{Q_0}^Q \frac{(1 - s \cdot Q - s \cdot \bar{q}) \cdot dQ}{s \cdot Q^2 - s \cdot B^2}$$

where

$$s B^2 = \frac{(s+\gamma)^2}{4s} - \alpha$$

$$B^2 = \left(\frac{s+\gamma}{2s} \right)^2 - \frac{\alpha}{s} = \bar{q}^2 - \frac{\alpha}{s}$$

$$t(q) = \int_{Q_0}^Q \frac{1 - s \cdot Q - s \cdot \bar{q}}{s(Q-B) \cdot (Q+B)} \cdot dQ$$

$$= \frac{1}{s} \int_{Q_0}^Q \left[\frac{1 - s \cdot B - s \cdot \bar{q}}{2 \cdot B \cdot (Q-B)} - \frac{1 + s \cdot B - s \cdot \bar{q}}{2 \cdot B \cdot (Q+B)} \right] \cdot dQ$$

$$= \frac{1}{s} \int_{Q_0}^{Q_0} \frac{1 - s \cdot B - s \cdot \bar{q}}{2 \cdot B \cdot (B-Q)} \cdot dQ + \frac{1}{s} \int_Q^{Q_0} \frac{1 + s \cdot B - s \cdot \bar{q}}{2 \cdot B \cdot (B+Q)} \cdot dQ$$

$$= \left[- \frac{1 - s \cdot B - s \cdot \bar{q}}{2s \cdot B} \cdot \log_e (B-Q) + \frac{1 + s \cdot B - s \cdot \bar{q}}{2s \cdot B} \cdot \log_e (B+Q) \right]_Q^{Q_0}$$

$$= \left[\frac{1}{2} \log_e (B-Q) + \frac{1}{2} \log_e (B+Q) + \frac{1 - s \cdot \bar{q}}{2s \cdot B} \cdot \log_e \left(\frac{B+Q}{B-Q} \right) \right]_Q^{Q_0}$$

$$\therefore t(q) = \frac{1}{2} \log_e \left(\frac{B^2 - Q_0^2}{B^2 - Q^2} \right) + \frac{1 - s \cdot \bar{q}}{2s \cdot B} \cdot \log_e \left(\frac{(B+Q_0) \cdot (B-Q)}{(B-Q_0) \cdot (B+Q)} \right)$$

where

$$\bar{q} = \frac{s + \sigma}{2s} = \frac{s + \mu a + \mu v}{2s}$$

$$\beta^2 = \bar{q}^2 - \frac{\alpha}{s} = \bar{q}^2 - \frac{\mu a}{s}$$

$$Q_0 = q_0 - \bar{q}$$

$$Q = q_t - \bar{q}$$

Appendix 3

Computer Programme BALQ

```
PROGRAM BALQ(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
WRITE(6,53)
```

```
C
COMMENT    THE NEXT READ STATEMENT USES "FREE FORMAT" INPUT
C
COMMENT    QS,QF ARE STARTING,FINISHING FREQUENCIES OF Q RESPECTIVELY
C
      READ(5,*)UA,UV,S,QS,QF
      QBAR=(S+UA+UV)/(2*S)
      A2=(QBAR*QBAR)-(UA/S)
      A=SQRT(A2)
      Q0=QS-QBAR
      Q=QF-QBAR
      Q02=Q0*Q0
      Q2=Q*Q
      VAR1=A2-Q02
      VAR2=A2-Q2
      VAR3=A+Q0
      VAR4=A-Q
      VAR5=A-Q0
      VAR6=A+Q
      VAR7=2*S*A
      VAR8=VAR1/VAR2
      VAR9=(VAR3*VAR4)/(VAR5*VAR6)
      IF(VAR1.EQ.0.0)WRITE(6,54)
      IF(VAR2.EQ.0.0)WRITE(6,55)
      IF(VAR3.EQ.0.0)WRITE(6,56)
      IF(VAR4.EQ.0.0)WRITE(6,57)
      IF(VAR5.EQ.0.0)WRITE(6,58)
      IF(VAR6.EQ.0.0)WRITE(6,59)
      IF(VAR7.EQ.0.0)WRITE(6,60)
      IF(VAR8.EQ.0.0)WRITE(6,61)
      IF(VAR8.LT.0.0)WRITE(6,62)
      IF(VAR9.EQ.0.0)WRITE(6,63)
      IF(VAR9.LT.0.0)WRITE(6,64)
      IF(VAR1.EQ.0.0.OR.VAR2.EQ.0.0.OR.VAR3.EQ.0.0.OR.VAR4.EQ.0.0.OR.
+ VAR5.EQ.0.0.OR.VAR6.EQ.0.0.OR.VAR7.EQ.0.0.OR.VAR8.LE.0.0.OR.
+ VAR9.LE.0.0)STOP
      X=((1.0-S*QBAR)/(2*S*A))*ALOG(((A+Q0)*(A-Q))/((A-Q0)*(A+Q)))
      Y=0.5*ALOG((A2-Q02)/(A2-Q2))
      T=Y+X
      WRITE(6,52)T
52 FORMAT(1X,3HT =,F10.1)
53 FORMAT(1X,23H TYPE IN UA,UV,S,QS,QF      )
54 FORMAT(1X,14H VAR1 IS ZERO      )
55 FORMAT(1X,14H VAR2 IS ZERO      )
56 FORMAT(1X,14H VAR3 IS ZERO      )
57 FORMAT(1X,14H VAR4 IS ZERO      )
58 FORMAT(1X,14H VAR5 IS ZERO      )
59 FORMAT(1X,14H VAR6 IS ZERO      )
60 FORMAT(1X,14H VAR7 IS ZERO      )
61 FORMAT(1X,14H VAR8 IS ZERO      )
62 FORMAT(1X,18H VAR8 IS NEGATIVE )
63 FORMAT(1X,14H VAR9 IS ZERO      )
64 FORMAT(1X,18H VAR9 IS NEGATIVE )
      STOP
      END
```


Appendix 4Computer Programme DAVE 2

```

PROGRAM DAVE2 (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
DIMENSION P(2000),Q(2000)
WRITE (6,200)

```

```

C
COMMENT  Y,Z ARE THE PROPORTIONS OF SUSCEPTIBLE AND RESISTANT HOSTS
C         RESPECTIVELY.T IS THE SELECTIVE COEFFICIENT AGAINST AVIRULENCE
C         ON THE RESISTANT HOSTS.N IS THE GENERATION NUMBER.
COMMENT  ALL READ STATEMENTS USE A "FREE FORMAT" INPUT.

```

```

C
200 FORMAT (/ ,4X, *ENTER VALUES OF P(1),UA,UV,Y,Z,T,S...*,/)
   READ (5,*)P(1),UA,UV,Y,Z,T,S
  15 WRITE (6,300)
300 FORMAT (/ ,4X, *ENTER NO. OF GENERATIONS AND PRINT INTERVAL...*,/)
   READ (5,*)NG,NP
   WRITE(6,400)
400 FORMAT (///,X, *GENERATION*,6X, *P*,20X, *Q*)
      N=1
      20 Q(N)=1.0-P(N) * (1.0-Z*T)
      P(N+1)=(P(N)*(1.0-Z*T)-P(N)*UA+Q(N)*UV*(1.0-Y*S))/(1.0-Q(N)*S*(1.0
+      -Z)-P(N)*T*Z)
      IF (MOD(N,NP).EQ.0)WRITE (6,100) N,P(N),Q(N)
      N=N+1
      IF (N.GT.NG) GO TO 10
      GO TO 20
  10 WRITE(6,150)
 150 FORMAT (/ ,4X, *REVISED VALUE OF S...*,/)
      READ (5,*) S
      GO TO 15
 100 FORMAT (I6,2G20.8)
      END

```

Appendix 5Computer Programme DAVE 4

```

PROGRAM DAVE4 (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
DIMENSION P(2000),Q(2000)
WRITE (6,200)

```

```

C
COMMENT      Y,Z ARE THE PROPORTIONS OF THE SUSCEPTIBLE AND
C            RESISTANT HOSTS RESPECTIVELY.T IS THE SELECTIVE
C            COEFFICIENT AGAINST AVIRULENCE ON THE RESISTANT
C            HOST.A IS THE SELECTIVE ADVANTAGE OF VIRULENCE
C            ON THE RESISTANT HOST.N IS THE GENERATION NO.
COMMENT      ALL READ STATEMENTS USE A "FREE FORMAT" INPUT.
200 FORMAT (/,4X,*ENTER VALUES OF P(1),UA,UV,Y,Z,T,S,A...*,/)
      READ (5,*)P(1),UA,UV,Y,Z,T,S,A
      15 WRITE (6,300)
300 FORMAT (/,4X,*ENTER NO. OF GENERATIONS AND PRINT INTERVAL...*,/)
      READ (5,*)NG,NP
      WRITE(6,400)
400 FORMAT (///,X,*GENERATION*,6X,*P*,20X,*Q*)
      N=1
      20 Q(N)=1.0-P(N)
      P(N+1) =(P(N)*(1.0+Z*(A-T-A*T))-P(N)*UA+Q(N)*UV)/(1.0+P(N)*Z*(A-T-
+      T*A)-Q(N)*S*Y+Q(N)*Z*A)
      IF (MOD(N,NP).EQ.0)WRITE (6,100) N,P(N),Q(N)
      N=N+1
      IF (N.GT.NG) GO TO 10
      GO TO 20
      10 WRITE(6,150)
150 FORMAT (/,4X,*REVISED VALUE OF A,S...*,/)
      READ (5,*) A,S
      GO TO 15
100 FORMAT (I6,2G20.8)
      END

```

Appendix 6

Estimation of an average proportion of the acreage of barley with the
Mla₆ resistance gene between 1969 and 1975 in the U.K.

M. Wolfe (personal communication) provided data on the approximate frequencies of Mla₆ cultivars (n) taken from the A.D.A.S. Plant Pathology Laboratory Surveys of foliar diseases, based on approximately 250 randomly selected fields in England and Wales. They were :

Year	Proportion of <u>Mla₆</u> (<u>n</u>)
1969	0.03
1970	0.03
1971	no survey
1972	0.00 (10% fields described as unknown)
1973	0.04
1974	0.05
1975	0.03

For the years 1969 - 1975 this represents an average of n = 0.03. However, M. Wolfe (personal communication) thought since 1970 the figures were probably underestimated, because from that time the cultivar, Midas (with Mla₆) was becoming established in Scotland and Northern Ireland which were not included in the above figures. M. Wolfe stated that the cultivar Midas occupied 12% of the Scottish acreage in 1972. However, the amount of barley grown in Scotland is very small in comparison with England and Wales. D. Yajnik (M.A.F.F. Librarian,

London, personal communication) supplied data for the amount of grain produced in England and Wales (spring and autumn sown barley) in 1980 as $10,320 \times 10^3$ tonnes. The equivalent figure for Scotland was 133×10^3 tonnes. Hence the increase in the use of Midas in Scotland in the early 1970s probably contributed very little to an increase in n for the whole of the U.K.

A second source of data used to estimate n is the M.A.F.F./A.D.A.S. Cereal Seed Sales Surveys 1970 - 1975, a voluntary survey amongst seed merchants. The amount of Midas sold in comparison with the total is shown below in Table 41.

Table 41 Sales of the barley cultivar Midas in England and Wales
1970 - 1975¹

Year ²	Tonnage Midas sold	Tonnage Total sold	% Midas
1970	846	267,650	0.3
1971	10,519	196,576	3.5
1972	8,374	237,293	3.5
1973	12,388	240,495	5.0
1974	15,012	219,423	6.8
1975	18,030	260,848	6.9

¹ from M.A.F.F./A.D.A.S. Cereal Seed Sales Surveys 1970 - 1975

² from 1st May - 30th April

The average percentage of Midas for these six years is 4.3%. Due to

the possible underestimation of $\underline{n}$ from the data from M. Wolfe (above)
 $\underline{n}$ was taken as 0.043 for the simulations described in section 1.3.6.c.

Appendix 7

Estimation of the number of generations of *Erysiphe graminis hordei* between 1969 and 1975 in the U.K.

As with the *P. graminis tritici* data (section 1.3.6.bi) a minimum and maximum estimate of the generation number was calculated.

Basic data

In England spring sown barley is planted in February or March and harvested in August. Autumn sown barley is sown in October and harvested in July. Thus apart from September mildew is present on barley all the year round, and is a persistantly endemic disease. In September the pathogen probably grows on unharvested barley or on wild barley relatives and perhaps other grasses but more likely on volunteer barley plants.

The incubation time or generation time (inoculation to the first appearance of symptoms, which marks also the start of sporulation (J. Jenkyn, personal communication)) is known to vary with environmental conditions. During hot weather where temperatures are 30°C or greater the incubation time is extended as mildew growth is reduced or stopped at such temperatures (Jenkyn and Bainbridge, 1978).

At mean air temperatures at or above 10°C it is found that the generation time is approximately 5 - 8 days (Jenkyn, 1973); however at lower temperatures it is variable, generally increasing with a fall in temperature (Jenkyn, 1973). This author recorded the experimentally determined incubation periods from spring 1970 to winter 1971 in the south of England. A summary of his results is shown below :

Month	J	F	M	A	M	J	J	A	S	O	N	D
Incubation period (days)	29-36	36+	17-20	9-16	5-8	5-8	5-8	5-8	5-8	7-12	16-36+	16-20

The early part of the winter of 1971 was mild, hence incubation periods were low for December. Several replicates were used for each month's data.

Weather in 1968 - 1975 Data of the average monthly temperatures for England and Wales for the period of 1968 - 1975 were obtained from the Meteorological Office. They are shown below :

Table 42 Mean monthly temperatures ($^{\circ}\text{F}^1$) for England and Wales
1968 - 1975

	Month												Year
	J	F	M	A	M	J	J	A	S	O	N	D	
<u>Year</u>													
1968	41.0	37.0	44.6	47.3	50.5	58.8	59.2	60.8	58.1	55.6	45.1	39.4	50.0
1969	43.2	35.1	39.4	46.2	52.9	57.7	62.6	61.9	58.3	55.9	43.2	39.6	49.8
1970	39.9	38.8	39.7	44.8	55.4	61.5	60.1	61.5	58.8	52.3	47.1	41.0	50.1
1971	41.4	41.7	42.3	46.6	53.6	55.2	62.6	61.2	58.6	53.8	44.2	45.1	50.7
1972	40.1	41.0	44.6	47.8	52.0	54.3	60.3	60.1	54.3	52.2	44.4	43.7	49.6
1973	41.4	41.0	44.4	45.7	53.2	59.5	60.6	62.4	58.6	49.6	44.1	42.3	50.2
1974	43.7	43.0	43.2	46.9	52.3	57.2	59.9	60.3	54.7	46.8	45.0	46.9	50.0
1975	45.0	41.4	41.5	47.5	50.2	58.6	63.5	65.7	57.0	50.9	44.1	42.1	50.7

¹ Nb 50°F = 10°C

Generally winters were mild especially toward the end of the period although there were some individually cold months for example February 1969. The summer of 1975 was warm; most summers however were generally not hot, apart from certain months for instance July 1969 or June 1970.

Estimation of generation number each month

Estimates for a minimum and maximum generation number for each month from July 1969 to July 1975 are shown below in Table 43. The estimates are reckoned on the basis of the incubation periods given above (from Jenkyn, 1973).

Where the mean temperature was 50°F or greater an incubation period of 5 - 8 days was assumed. This leads to an estimate of approximately 4 - 6 generations per month. For October 1973 and 1974 in which the mean temperatures were less than 50°F, reduced generation numbers of 3-5 and 2-4 respectively were estimated.

April temperatures were fairly constant and with incubation periods of 9 - 16 days, 2-3 generations are obvious estimates.

For March months, Jenkyn's (1973) estimates of 17-20 days for the incubation periods are probably underestimates since March 1970, 1971 were generally colder months than usual especially compared with March 1972 - 1974. Therefore 1-2 generations were estimated for these three months whilst 0-1 generations were assumed for the other March months.

February temperatures were cold and 0-1 generations were assumed except 1974 where the average temperature was similar to the March value, thus 1-2 generations were assumed.

For January, 0-1 generations were reckoned except 1974 and 1975 when 1-2 were assumed because of higher average temperatures.

Table 43 Estimates of minimum and maximum generation number of
E. graminis hordei per month from July 1969 - July 1975 in
England and Wales

Year	Month	Generations	
		Minimum	Maximum
1969	J	4	6
	A	4	6
	S	4	6
	O	4	6
	N	1	2
	D	0	1
1970	J	0	1
	F	0	1
	M	0	1
	A	2	3
	M	4	6
	J	4	6
	J	4	6
	A	4	6
	S	4	6
	O	4	6
	N	2	3
	D	0	1
1971	J	0	1
	F	0	1
	M	0	1
	A	2	3
	M	4	6
	J	4	6
	J	4	6
	A	4	6

Year	Month	Generations	
		Minimum	Maximum
1971	S	4	6
	O	4	6
	N	1	2
	D	1	2
1972	J	0	1
	F	0	1
	M	1	2
	A	2	3
	M	4	6
	J	4	6
	J	4	6
	A	4	6
	S	4	6
	O	4	6
	N	1	2
	D	0	1
1973	J	0	1
	F	0	1
	M	1	2
	A	2	3
	M	4	6
	J	4	6
	J	4	6
	A	4	6
	S	4	6
	O	3	5
	N	1	2
	D	0	1

Year	Month	Generations	
		Minimum	Maximum
1974	J	1	2
	F	1	2
	M	1	2
	A	2	3
	M	4	6
	J	4	6
	J	4	6
	A	4	6
	S	4	6
	O	2	4
	N	2	3
	D	2	3
1975	J	1	2
	F	0	1
	M	0	1
	A	2	3
	M	4	6
	J	4	6
	J	4	6
Totals		174	284

For November, 1-2 generations were assumed except 1970 and 1974 where the mean temperatures were very high, and 2-3 were taken.

Similar values for December 1974 to November 1970 and 1974 were taken, otherwise 0-1 generations were assumed, except 1971 when a 1-2 range was chosen.

Total generation number for the six year period

The totals in Table 43 of minimum and maximum generation numbers for the six year period July 1969 - July 1975 are 174 and 284 respectively. These values have to be corrected somewhat for days where the temperature exceeded 30°C or 86°F. Mildew growth is retarded or stopped at such high temperatures (Jenkyn and Bainbridge, 1978).

In May and June for this period the temperature did not exceed 86°F on any day (Meteorological Office data of maximum temperatures). In July on at least one day in five of the years; in August on at least one day in two of the years and once in a September month the temperature exceeded 86°F. Assume for each 'hot' day or period two generations are lost so with eight such days, 16 generations can be omitted giving a final estimate of 158 - 268 generations for the six year period. This represents 27 - 45 generations annually.

Independently, M. Wolfe (personal communication) estimated that in British conditions about 28 - 43 asexual generations of mildew occur annually. The two independent estimates are similar.

Appendix 8

Results of Experiment 1 to determine the changes in percentage of a mutant¹ isolate of *E. graminis hordei* in a mixture with a mother¹ isolate when grown on Proctor² barley for five two week generations

Generation 1

Replicate	Tester line	Colony counts	% mutant
1a	<u>Mlg</u>	23,19	37
	<u>mlg</u>	60,53	
1b	<u>Mlg</u>	14,5,14,11	18
	<u>mlg</u>	73,44,48,73	
1c	<u>Mlg</u>	12,16,14	20
	<u>mlg</u>	89,47,70	
1d	<u>Mlg</u>	25,33,11	37
	<u>mlg</u>	71,73,42	
2a	<u>Mlg</u>	26,15,12	28
	<u>mlg</u>	39,65,83	
2b	<u>Mlg</u>	9,6,(7½) ³	11
	<u>mlg</u>	97,66,36	
2c	<u>Mlg</u>	22,8,19,9,11	51
	<u>mlg</u>	26,28,27,27,27	
2d	<u>Mlg</u>	11,11,11	20
	<u>mlg</u>	104,25,40	

continued

Appendix 8(continued)

Generation 2

Replicate	Tester line	Colony counts	% mutant
1a	<u>Mlg</u>	12,3,0,18	12
	<u>mlg</u>	55,43,61,121	
1b	<u>Mlg</u>	0,6,10,4	27
	<u>mlg</u>	30,9,19,16	
1c	<u>Mlg</u>	15,17,6	33
	<u>mlg</u>	30,36,50	
1d	<u>Mlg</u>	16,10,3,34	15
	<u>mlg</u>	71,116,157,85	
2a	<u>Mlg</u>	8,16,8	18
	<u>mlg</u>	54,69,56	
2b	<u>Mlg</u>	7,22,19	22
	<u>mlg</u>	43,132,47	
2c	<u>Mlg</u>	23,18	26
	<u>mlg</u>	71,87	
2d	<u>Mlg</u>	23,13,17	29
	<u>mlg</u>	75,17,93	

continued

Appendix 8 (continued)

Generation 3

Replicate	Tester line	Colony counts	% mutant
1a	<u>Mlg</u>	2,4,3,5	5
	<u>mlg</u>	140,42,30,51	
1b	<u>Mlg</u>	3,7,10,4	11
	<u>mlg</u>	53,80,84,53	
1c	<u>Mlg</u>	2,3,2,5	12
	<u>mlg</u>	23,28,4,49	
1d	<u>Mlg</u>	10,3,11,1	8
	<u>mlg</u>	67,68,80,100	
2a	<u>Mlg</u>	6,3,2,4	6
	<u>mlg</u>	68,46,60,69	
2b	<u>Mlg</u>	0,3,3	2
	<u>mlg</u>	80,148,54	
2c	<u>Mlg</u>	18,13,21	13
	<u>mlg</u>	112,126,160	
2d	<u>Mlg</u>	9,13,10	8
	<u>mlg</u>	148,105,156	

continued

Appendix 8 (continued)

Generation 4

Replicate	Tester line	Colony count ⁴	% mutant
1a	<u>Mlg</u>	1	4
	<u>mlg</u>	27	
1b	<u>Mlg</u>	3	16
	<u>mlg</u>	19	
1c	<u>Mlg</u>	0	0
	<u>mlg</u>	33	
1d	<u>Mlg</u>	0	0
	<u>mlg</u>	41	
2a	<u>Mlg</u>	1.25 ⁵	3
	<u>mlg</u>	47	
2b	<u>Mlg</u>	31	11
	<u>mlg</u>	295	
2c	<u>Mlg</u>	6	14
	<u>mlg</u>	42	
2d	<u>Mlg</u>	2	12
	<u>mlg</u>	17	

continued

Appendix 8 (continued)

Generation 5

Replicate	Tester line	Colony count ⁶	% mutant
1a	<u>Mlg</u>	0,0,1,0	1
	<u>mlg</u>	15,21,15,20	
1b	<u>Mlg</u>	10,12,3,3,3	10
	<u>mlg</u>	72,59,76,31	
1c	<u>Mlg</u>	0,0,0,0	0
	<u>mlg</u>	Abundant	
1d	<u>Mlg</u>	3,2,1,4	9
	<u>mlg</u>	30,28,29,30	
2a	<u>Mlg</u>	0,0,2,0	1
	<u>mlg</u>	43,20,50,49	
2b	<u>Mlg</u>	9,12,76,19,9	-
	<u>mlg</u>	Too abundant to count colonies	
2c	<u>Mlg</u>	1,0,0,1	3
	<u>mlg</u>	10,23,13,15	
2d	<u>Mlg</u>	1,2,0,0	1
	<u>mlg</u>	50,94,60,92	

¹ mutant isolate carries a single gene mutation to virulence to the Mlg resistance gene. Otherwise it is identical genetically to the mother isolate and was obtained by mutation from it.

² Proctor barley lacks the Mlg resistance gene.

³ An average of counts 9 and 6. Only two leaves were scored.

⁴ All scores for 3-5 leaves per tester line were summed, because colony counts per individual leaf were so small due to incubation of the mixture in a low humidity (RH = 30-40%) growth room.

- 5 Four leaves were scored giving a sum of only one colony. Since the counts on the mlg line summing to 47 were based on five leaves, the total count on the Mlg leaves was adjusted accordingly.
- 6 Tester plants were scored after 10 days resulting in very heavy mildew on some leaves.

Appendix 9

Calculation of the selection coefficient, s against unnecessary virulence to the Mlg resistance gene of barley, in E. graminis hordei from the results of race mixture Experiment 1 (sections 1.4.3.civ, v)

A graph of $\log_e (\underline{x}/1-\underline{x})$ against generation number ($\underline{t}$) is plotted where $\underline{x}$ is the proportion of non mutants. The calculation of $\log (\underline{x}/1-\underline{x})$ is shown is shown in Table 44.

Table 44 Calculation of $\log_e (\underline{x}^1/1-\underline{x})$ from race mixture
Experiment 1²

Generation ($\underline{t}$)	% mutant	Proportion of non mutants ($\underline{x}$)	$\underline{x}/(1-\underline{x})$	$\log_e (\underline{x}/1-\underline{x})$
1	27.75	0.7225	2.604	0.96
2	22.75	0.7725	3.396	1.22
3	8.12	0.9188	11.315	2.43
4	7.50	0.9250	12.333	2.51
5	3.57	0.9643	27.011	3.30

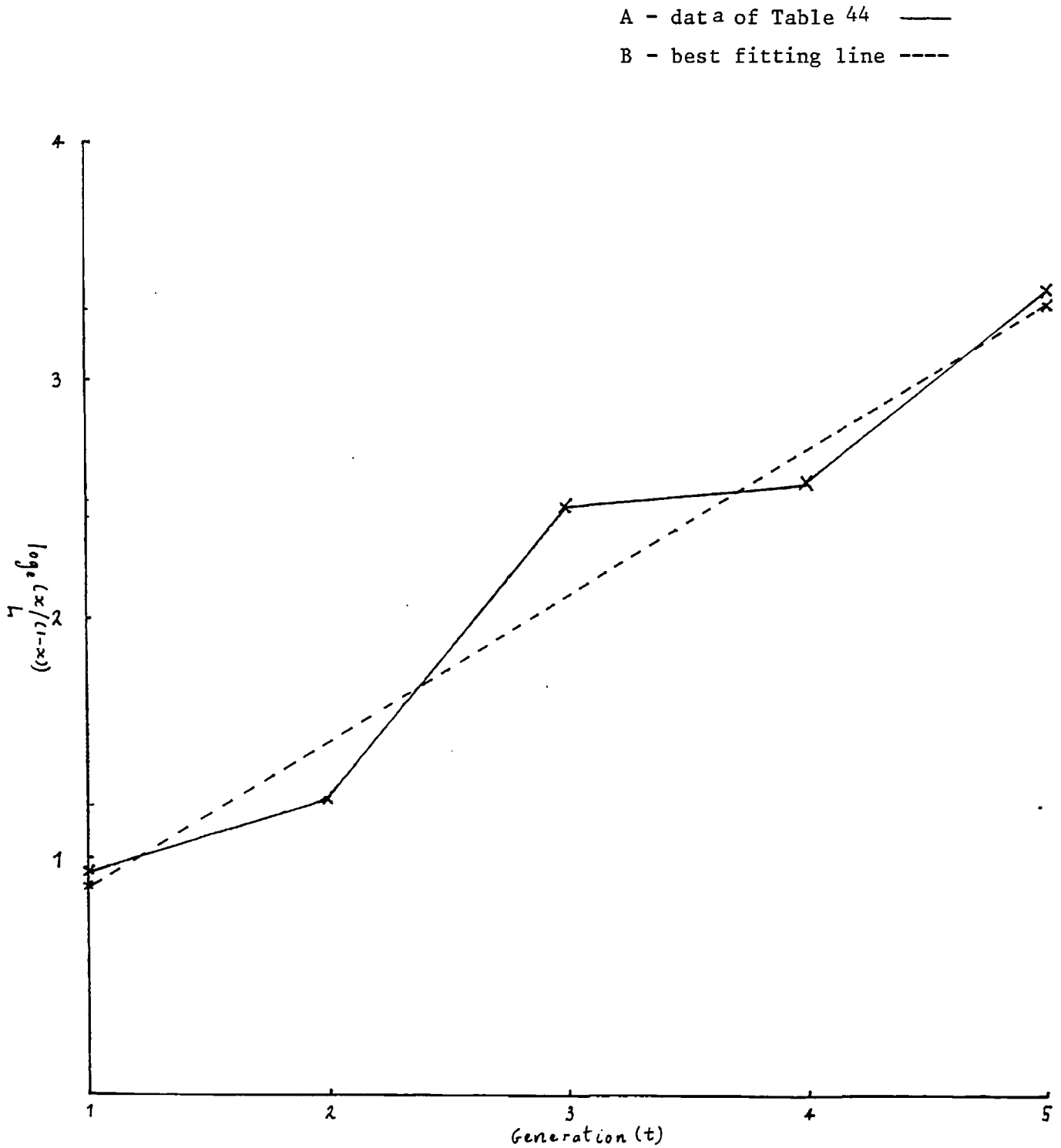
¹ $\underline{x}$ = proportion of the non mutant isolate (mother) in mixture at each generation ($\underline{t}$)

² sections 1.4.3.civ and 1.4.3.cv

The graph of $\log_e (\underline{x}/1-\underline{x})$ against $\underline{t}$ is shown in Figure 5.

A regression analysis is applied to the data as follows :

Figure 5 Graph of $\log_e(\underline{x}^1/1-\underline{x})$ against $\underline{t}^2$ for race mixture
Experiment 1³ and best line fit



- 1 $\underline{x}$ = proportion of non mutants in the mixture
- 2 $\underline{t}$ = generation
- 3 sections 1.4.3.civ,v

Generation	$\log_e (x/1-x)$	$\underline{t}'_{\underline{i}}$	$\underline{L}'_{\underline{i}}$	$\underline{t}'_{\underline{i}}{}^2$	$\underline{L}'_{\underline{i}}{}^2$	$\underline{t}'_{\underline{i}} \cdot \underline{L}'_{\underline{i}}$
$\underline{t}_{\underline{i}}$	$\underline{L}_{\underline{i}}$					
1	0.96	-2	0.96	4	0.9216	-1.92
2	1.22	-1	1.22	1	1.4884	-1.22
3	2.43	0	2.43	0	5.9049	0
4	2.51	1	2.51	1	6.3001	2.51
5	3.30	<u>2</u>	<u>3.30</u>	<u>4</u>	<u>10.8900</u>	<u>6.60</u>
		0	10.42	10	25.5050	5.97

where $\bar{\underline{t}}_w = 3$ and $\bar{\underline{L}}_w = 0$ are working means, $\underline{i}$ is the $\underline{i}$ th value, $\underline{t}'_{\underline{i}} =$

$$\underline{t}_{\underline{i}} - \bar{\underline{t}}_w, \text{ and } \underline{L}'_{\underline{i}} = \underline{L}_{\underline{i}} - \bar{\underline{L}}_w.$$

Consequently,

$$\text{Mean of } \underline{L}_{\underline{i}} = \bar{\underline{L}} = 2.084$$

$$SS(\underline{t}) = 10$$

$$SS(\underline{L}) = 25.505 - (10.42)^2/5 = 3.78972$$

$$SP(\underline{t}, \underline{L}) = 5.97$$

The regression equation is :

$$\hat{\underline{L}}_{\underline{i}} = \underline{a}' + \underline{b} (\underline{t}_{\underline{i}} - \bar{\underline{t}}) \quad \text{A.4.1.}$$

$$\text{where } \underline{a}' = \bar{\underline{L}}$$

$$\underline{b} = SP(\underline{t}, \underline{L})/SS(\underline{t}) = 5.97/10 = 0.597$$

$$\bar{\underline{t}} = \text{mean of } \underline{t}_{\underline{i}} = 3$$

Hence from equation A.4.1 it follows :

$$\hat{\underline{L}}_{\underline{i}} = \underline{a}' - \underline{b} \cdot \bar{\underline{t}} + \underline{b} \cdot \underline{t}_{\underline{i}}$$

$$= 2.084 - 0.597 \times 3 + 0.597 \cdot \underline{t}_1$$

$$= 0.293 + 0.597 \cdot \underline{t}_1$$

Therefore, the co-ordinates of the best fitting line are :

$\underline{t}_i$	$\hat{\underline{L}}_i$
1	0.890
2	1.487
3	2.084
4	2.681
5	3.278

These co-ordinates have been plotted in Figure 5.

From equation 1.4.3.1

$$\underline{m} = -\log_e (1-\underline{s})$$

$\underline{s}$ can be calculated from $\underline{m}$, the slope of the best fitting line.

$$\begin{aligned} \underline{m} &= \frac{\underline{L}_5 - \underline{L}_1}{\underline{t}_5 - \underline{t}_1} = \frac{3.278 - 0.890}{5 - 1} \\ &= \frac{2.388}{4} = 0.597 \end{aligned}$$

Therefore,

$$\begin{aligned} \log_e (1-\underline{s}) &= -0.597 \\ 1-\underline{s} &= 0.5505 \\ \underline{s} &= 0.4495 \end{aligned}$$

Appendix 10

Results of Experiment 2 to determine the changes in percentage of a mutant¹ isolate of *E. graminis hordei* in a mixture with a mother¹ isolate when grown on Proctor² barley for six two week generations

Generation 1³

Replicate	Tester line	Colony counts	% mutant
1	<u>Mlg</u>	93,119,158,50	89
	<u>mlg</u>	58,121,112,182	
2	<u>Mlg</u>	56,23,20,40	68
	<u>mlg</u>	69,55,38,43	
3	<u>Mlg</u>	75,55,108,23	100
	<u>mlg</u>	54,104,53,44	
4	<u>Mlg</u>	178,100,60,55	89
	<u>mlg</u>	76,136,116,111	
5	<u>Mlg</u>	108,77,59,31	99
	<u>mlg</u>	40,65,57,117	
6	<u>Mlg</u>	71,164,61,104	100
	<u>mlg</u>	58,79,105,95	
7	<u>Mlg</u>	86,40,47,29	82
	<u>mlg</u>	78,68,56,45	

continued

Appendix 10 (continued)

Generation 2⁴

Replicate	Tester line	Colony count	% mutant
1	<u>Mlg</u>	28,38,19,21	96
	<u>mlg</u>	24,27,24,35	
2	<u>Mlg</u>	32,33,33,28	100
	<u>mlg</u>	13,62,15,13	
3	<u>Mlg</u>	152,69,75	100
	<u>mlg</u>	109,46,107	
4	<u>Mlg</u>	14,5,43,0	87
	<u>mlg</u>	46,4,17,4	
5	<u>Mlg</u>	29,5,4,2	77
	<u>mlg</u>	22,8,14,8	
6	<u>Mlg</u>	2,59,16,20	85
	<u>mlg</u>	24,19,21,50	
7	<u>Mlg</u>	48,35,39,30	100
	<u>mlg</u>	7,17,30,17	

continued

Appendix 10 (continued)

Generation 3

Replicate	Tester line	Colony counts	% mutant
1	<u>Mlg</u>	37,27,19	100
	<u>mlg</u>	21,19,21	
2	<u>Mlg</u>	13,16,6,22	100
	<u>mlg</u>	17,16,4,18	
3	<u>Mlg</u>	8,40,35,30	100
	<u>mlg</u>	33,19,19,17	
4	<u>Mlg</u>	4,10,28,36	100
	<u>mlg</u>	14,10,26	
5	<u>Mlg</u>	13,15,31	84
	<u>mlg</u>	35,25,10	
6	<u>Mlg</u>	19,9,10,2	91
	<u>mlg</u>	8,9,10,17	
7	<u>Mlg</u>	10,11,4,2	73
	<u>mlg</u>	11,11,5,11,8	

continued

Appendix 10 (continued)

Generation 6

Replicate	Tester line	Colony counts	% mutant
1	<u>Mlg</u>	9,10,15	44
	<u>mlg</u>	41,31,7	
2	<u>Mlg</u>	25,22,25	100
	<u>mlg</u>	17,12,10	
3	<u>Mlg</u>	34,19,31,33	100
	<u>mlg</u>	36,25,17,9	
4	<u>Mlg</u>	83,26,49,43	100
	<u>mlg</u>	32,49,30,54	
5	<u>Mlg</u>	31,41,48,35	100
	<u>mlg</u>	11,19,32,28,16	
6	<u>Mlg</u>	13,23,33,24	100
	<u>mlg</u>	13,18,33	
7	<u>Mlg</u>	73,36,15	100
	<u>mlg</u>	26,18,10	

1 mutant isolate carries a single gene mutation to virulence to the Mlg resistance gene. Otherwise it is identical genetically to the mother isolate and was obtained by mutation from it.

2 Proctor barley lacks the Mlg resistance gene

continued

- 3 bottom 3" of first leaves scored because of very heavy infection
- 4 top 6" of second leaves scored because of very heavy infection on the first leaves except for replicates 3 and 7 where the bottom 3" of the first leaves were scored

Appendix 11

Selection for virulence genes in a large heterogeneous asexual population and linkage equilibria

Consider two virulence genes, $\underline{V}_1$ and $\underline{V}_2$ with alleles $\underline{A}_1$ and $\underline{A}_2$ respectively in a large, heterogeneous asexual population. Prior to selection for virulence the virulence genes are in a mutation/selection balance of frequency $\mu/\underline{s}$ where μ is the mutation rate from avirulence to virulence (at a rate of $\mu = 1 \times 10^{-6}$ per gene per generation) and $\underline{s}$ is the selective coefficient against unnecessary virulence ($\underline{s} = 0.01$). It can be shown see section (3.4.2) that at balance the genotype frequencies are :

<u>Genotype</u>	<u>Frequency</u>	
$\underline{A}_1 \underline{A}_2$	$(1-\mu/\underline{s})^2$	<u>PAA</u>
$\underline{V}_1 \underline{A}_2$	$(\mu/\underline{s}) \cdot (1-\mu/\underline{s})$	<u>PVA</u>
$\underline{A}_1 \underline{V}_2$	$(1-\mu/\underline{s}) \cdot (\mu/\underline{s})$	<u>PAV</u>
$\underline{V}_1 \underline{V}_2$	$(\mu/\underline{s})^2$	<u>PVV</u>

and that PAA.PVV = PAV.PVA

3.4.2.1

If $\mu = 1 \times 10^{-6}$, $\underline{s} = 0.01$ then

$$\underline{PAA} = 0.9998, \underline{PVA} = \underline{PAV} = 9.999 \times 10^{-5} \text{ and } \underline{PVV} = 1 \times 10^{-8}$$

Prior to selection the population is in linkage equilibrium, and it is homogeneous. Assume that now instead of the total acreage of the crop being sown to $\underline{R}_0$ hosts (lacking $\underline{R}_1$ and $\underline{R}_2$ genes the corresponding resistances), four host genotypes $\underline{R}_0$, $\underline{R}_1$, $\underline{R}_2$ and $\underline{R}_1\underline{R}_2$ are grown in equal proportions in four distinct and separated localities so that four heterogeneous (to each other) populations of the pathogen develop,

by selection.

- (a) On R_0 hosts, the genotype frequencies after selection are expected to be as above : $\underline{PAA} = 0.9998$, $\underline{PVA} = \underline{PAV} = 9.999 \times 10^{-5}$, $\underline{PVV} = 1 \times 10^{-8}$.
- (b) On R_1 hosts only $\underline{V_1A_2}$ and $\underline{V_1V_2}$ genotypes survive assuming races with non corresponding virulence genes do not sporulate on resistant hosts. Their final frequencies are expected to be $(9.999 \times 10^{-5}) / (9.999 \times 10^{-5} + 1 \times 10^{-8}) = 0.9999$ and 0.0001 respectively.
- (c) On R_2 hosts only $\underline{A_1V_2}$ and $\underline{V_1V_2}$ genotypes survive at frequencies of 0.9999 and 0.0001 respectively.
- (d) Finally on R_1R_2 hosts only $\underline{V_1V_2}$ survives at a frequency of 1.0 .

Since the frequency of each host genotype is 0.25 , proportional sampling from the four heterogeneous sub populations would give overall genotype frequencies as follows :

- (a) $\underline{A_1A_2}$

$$0.25 \times 0.9998 = 0.24995$$

- (b) $\underline{V_1A_2}$

$$0.25 \times 9.999 \times 10^{-5} + 0.25 \times 0.9999 = 0.25$$

- (c) $\underline{A_1V_2}$

$$0.25 \times 9.999 \times 10^{-5} + 0.25 \times 0.9999 = 0.25$$

- (d) $\underline{V_1V_2}$

$$0.25 \times 1 \times 10^{-8} + 0.25 \times 0.0001 + 0.25 \times 0.0001 + 0.25 \times 1.0 = 0.25005$$

The frequency of genes $\underline{V_1}$ and $\underline{V_2}$ are $0.25 + 0.25005 = 0.50005$. The expected frequency of $\underline{V_1V_2}$ assuming no differential selection is thus $(0.50005)^2 = 0.25005$, which is identical to the observed frequency above after selection. In this case selection for virulence genes creating

heterogeneous populations does not result in linkage disequilibrium.

If the frequency of $\underline{R}_1\underline{R}_2$ hosts is less than that of $\underline{R}_1$ and $\underline{R}_2$ hosts linkage disequilibrium results. The most extreme example is where the frequency of $\underline{R}_1\underline{R}_2$ is zero. This situation was discussed in section 3.4.3.

On the other hand if there is only selection for the $\underline{V}_1\underline{V}_2$ genotype, say by culture of $\underline{R}_1\underline{R}_2$ hosts in one half of the area whilst the other half is sown to $\underline{R}_0$ hosts then the frequency of the four pathogen genotypes after selection becomes :

(a) $\underline{A}_1\underline{A}_2$

$$0.5 \times 0.9998 = 0.4999$$

(b) $\underline{V}_1\underline{A}_2$

$$0.5 \times 9.999 \times 10^{-5} = 4.9995 \times 10^{-5}$$

(c) $\underline{A}_1\underline{V}_2$

$$0.5 \times 9.999 \times 10^{-5} = 4.9995 \times 10^{-5}$$

(d) $\underline{V}_1\underline{V}_2$

$$0.5 \times 1 \times 10^{-8} + 0.5 \times 1.0 = 0.500000005$$

The frequency of genes $\underline{V}_1$ and $\underline{V}_2$ are both approximately 0.5 in which case the expected frequency of $\underline{V}_1\underline{V}_2$ is about 0.25 which is much less than the observed frequency. Linkage disequilibrium results. However the virulence genes are apparently associated rather than disassociated. The size of the deviation will be reduced if separate $\underline{R}_1$ and $\underline{R}_2$ genotypes are grown.

Appendix 12

Computer Programme SELIN 6

```

PROGRAM SELIN6(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
C
COMMENT      N = NUMBER OF;A,B,AB = RACES A,B,AB RESPECTIVELY.
C            R1,R2 = HOST LINES WITH R1,R2 GENES RESPECTIVELY.
C            T = THE COEFFICIENT OF SELECTION AGAINST THE
C            BACKGROUND GENOTYPE OF THE COMPLEX RACE.
C            ZM,ZN = THE RESPECTIVE HOST LINE FREQUENCIES.
C            A = THE REALIZED REPRODUCTIVE RATE.
C            G = THE ALLOINFECTION FREQUENCY.
C            NOGMAX,NOGINT = THE GENERATION NUMBER AND INTERVAL
C            REQUIRED.
C            RIR,RIS = MEASURES OF THE AMOUNT OF INDUCED
C            RESISTANCE AND SUSCEPTIBILITY RESPECTIVELY.
COMMENT      ALL READ STATEMENTS USE A "FREE FORMAT" INPUT.
REAL N,NA,NB,NAB,NAR1,NAR2,NBR1,NBR2,NABR1,NABR2
SNAR1=1.0
SNAR2=0.0
SNBR1=0.0
SNBR2=1.0
SNABR1=1.0
SNABR2=1.0
SN=4.0
NAR1=0.0
NAR2=0.0
NBR1=0.0
NBR2=0.0
NABR1=0.0
NABR2=0.0
N=0.0
NA=0.0
NB=0.0
NAB=0.0
PA=0.25
PB=0.25
PAB=0.5
WRITE(6,90)
90 FORMAT(1X," TYPE IN C,T,SA,SB" )
READ(5,*)C,T,SA,SB
WAR1=1.0
WBR2=1.0
WABR1=((1.0-C)*(1.0-T)*(1.0-SB))
WABR2=((1.0-C)*(1.0-T)*(1.0-SA))
WRITE(6,91)
91 FORMAT(1X," TYPE IN ZM,ZN,A,G,NOGMAX,NOGINT,RIR,RIS" )
READ(5,*)ZM,ZN,A,G,NOGMAX,NOGINT,RIR,RIS
NP=1
NG=1
10 NAR1=(1.0+A)*(WAR1*(SNAR1*(1.0-G)+SNAR1*G*ZM+SNAR2*G*ZN))*((1.0-(A*
+ RIR*WBR2*(SNBR1*(1.0-G)+SNBR1*G*ZM+SNBR2*G*ZN))/SN)
NAR2=SNAR2+RIS*A*(SNAR2*(1.0-G)*WAR1+SNAR2*G*ZN*WAR1+SNAR1*G*ZN*
+ WAR1)*(SNBR2*(1.0-G)*WBR2+SNBR2*G*ZN*WBR2+SNBR1*G*ZN*WBR2+
+ SNABR2*(1.0-G)*WABR2+SNABR2*G*ZN*WABR2+SNABR1*G*ZN*WABR1)/SN
NBR2=(1.0+A)*(WBR2*(SNBR2*(1.0-G)+SNBR2*G*ZN+SNBR1*G*ZN))*((1.0-(A*
+ RIR*WAR1*(SNAR2*(1.0-G)+SNAR2*G*ZN+SNAR1*G*ZN))/SN)
NBR1=SNBR1+RIS*A*(SNBR1*(1.0-G)*WBR2+SNBR1*G*ZM*WBR2+SNBR2*G*ZM*WB

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+ R2)*(SNAR1*(1.0-G)*WAR1+SNAR1*G*ZM*WAR1+SNAR2*G*ZM*WAR1+SNABR1*
+ (1.0-G)*WABR1+SNABR1*G*ZM*WABR1+SNABR2*G*ZM*WABR2)/SN
NABR1=(1.0+A)*(SNABR1*(1.0-G)*WABR1+SNABR1*G*ZM*WABR1+SNABR2*G*ZM*
+ WABR2)*(1.0-(A*RIR*WBR2*(SNBR1*(1.0-G)+SNBR1*G*ZM+SNBR2*G*ZM))
+ /SN)
NABR2=(1.0+A)*(SNABR2*(1.0-G)*WABR2+SNABR2*G*ZM*WABR2+SNABR1*G*ZM*
+ WABR1)*(1.0-(A*RIR*WAR1*(SNAR2*(1.0-G)+SNAR2*G*ZM+SNAR1*G*ZM))
+ /SN)
NA=NAR1+NAR2
NB=NBRI+NBRI2
NAB=NABRI+NABRI2
N=NA+NAB+NB
PA=NA/N
PB=NB/N
PAB=NAB/N
NP=NP+1
NG=NG+1
IF(NP.GE.NOINT)GO TO 22
SNAR1=NAR1
SNAR2=NAR2
SNBR1=NBRI
SNBR2=NBRI2
SNABR1=NABRI
SNABR2=NABRI2
SN=N
GO TO 10
22 WRITE(6,53)NG,PA,PB,PAB,N
53 FORMAT(1X,5HGEN =,I4,2X,4HPA =,F8.6,2X,4HPB =,F8.6,2X,5HPAB =,F8.6
+ ,2X,3HN =,E12.6)
NP=0
IF(NG.GE.NOIMAX)GO TO 23
SNAR1=NAR1
SNAR2=NAR2
SNBR1=NBRI
SNBR2=NBRI2
SNABR1=NABRI
SNABR2=NABRI2
SN=N
GO TO 10
23 CONTINUE
END

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