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# Multiline varieties and disease control

## 6. Effects of selection at different stages of the pathogen life cycle on the evolution of virulence

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**Summary.** Existing theoretical models have led to conflicting predictions concerning the likely effect of the widespread use of dirty crop multilines on the evolution of virulence in pathogen populations. Here we attempt to clarify these problems by extending existing models to include selection against unnecessary genes for virulence at two different stages in the life cycle of the pathogen. The results of these studies indicate that the stage of the life cycle at which selection occurs can significantly influence the evolution of virulence in pathogen populations growing on multiline varieties.

**Key words:** Multiline varieties – Disease control – "Dirty crop" approach – Evolution – Pathogens

### Introduction

The effects of the widespread use of 'dirty crop' or 'partially resistant' multilines, where each of the components of the multiline carries one or more resistance genes but none of the resistances are effective against all known races of the pathogen (Marshall 1977) on the evolution of virulence in pathogen populations remains an open question. One viewpoint is that such multilines will stabilise the racial composition of pathogen populations with simple races, carrying one or few genes for virulence, predominant and as a result, will provide effective long-term disease control. The alternative viewpoint is that 'dirty crop' multilines will lead to the evolution of new and complex pathogen races, perhaps even a 'super-race' which can overcome all the resistance genes in the multiline, and will fail to control disease (Browning and Frey 1969; Marshall 1977).

This question cannot be answered empirically in small plot studies. Indeed, it can only be answered satisfactorily by cultivating multilines over large areas, so they are the major factor influencing the evolution of virulence in the local pathogen population, and for relatively long periods of time, so that the pathogen population approaches an evolutionary equilibrium. However, the dangers of large scale long-term field experiments, should the 'dirty crop' multiline concept prove invalid, are self-evident.

As an alternative, several authors have sought to answer this question theoretically. For example, Groth (1976) developed a simple discrete generation model which has been used to examine various aspects of the evolution of pathogen virulence on mixed host varieties (Groth and Person 1977; Marshall and Pryor 1978, 1979; Marshall and Burdon 1981a, b; Marshall and Weir 1985). However, Barrett and Wolfe (1978) criticised the model developed by Groth (1976) on a number of grounds. In particular, they suggested that the assumption of discrete pathogen generations was unrealistic since a pustule may produce spores over several pathogen generations. They therefore developed an alternative overlapping generations model which they have used in an extensive series of computer simulation studies of pathogen growth and evolution of multiline varieties (Barrett 1978, 1980).

On the bases of their analyses, Barrett and Wolfe (1978) concluded that their model led to "slightly different conclusions" to Groth's (1976) model. However, a closer comparison of these models indicates that they can differ substantially in their implications with respect to the evolution of virulence in pathogen populations. The reasons for these differences in the predicted outcomes of the models of Groth and Barrett/Wolfe remain obscure despite some discussion of their relative merits in the literature (Groth 1978; Leonard and Czochor 1980). While the models differ most obviously in their assumptions concerning the mode of pathogen reproduction (discrete versus overlapping generations) they also differ in their assumptions concerning the stage of the life of the pathogen at which selection against unnecessary genes for virulence acts to reduce pathogen fitness. It is unclear whether the differences in the outcomes of the two models are due to differences in one or both of these assumptions.

The aim of this paper is to clarify this issue. To do this we extend the overlapping generations model of

Barrett and Wolfe (1978) to include selection against unnecessary genes for virulence at different stages of the pathogen life cycle and compare the results obtained with those of the discrete generation model of Groth (1978). We also develop a more general model of the evolution of pathogen virulence on multiline varieties which simultaneously allows for selection against unnecessary genes for virulence at two different stages of the pathogen life cycle.

## Basic assumptions of the models

The model of Barrett and Wolfe (1978), as well as that of Groth (1976), are based on a large number of simplifying assumptions. Those shared in common between the two models are:

(i) The multiline is composed of equal proportions of n ($n \geqq 2$) host genotypes which are identical except that each carries a different homozygous gene conferring resistance to a specified pathogen;

(ii) A gene-for-gene relationship exists between resistance in the host and virulence in the pathogen (Flor 1956);

(iii) The multiline mixture is reconstituted annually so its composition is stable over time, and it is grown over a large area so that it is the major factor influencing the evolution of virulence in the local pathogen population;

(iv) Biotypes carrying unnecessary genes for virulence are less fit than biotypes carrying only effective genes for virulence and each unnecessary gene for virulence reduces their fitness by a constant amount, (s);

(v) Each generation, pathogen biotypes compete and reproduce at different rates (depending on the particular fitness model invoked) on susceptible hosts and the resultant spores are distributed at random over the host population and this procedure is repeated until the pathogen population reaches equilibrium;

(vi) Pathogen biotypes do not reproduce on non-corresponding (resistant) hosts;

(vii) Biotypes with all possible combinations of virulence genes exist initially, or can arise quickly, in the pathogen populations;

(viii) The pathogen population is large enough that only deterministic equilibria need to be considered.

Barrett and Wolfe (1978) and Groth (1976) differ in their assumptions concerning the fate of parental pathogen lesions. Groth assumed the parental lesions simultaneously produce spores and then die so that the pathogen generations are discrete. Barret and Wolfe assumed that all surviving pathogen lesions regardless of their age periodically and simultaneously produce spores and potentially can do so indefinitely. Under this model then, the pathogen generations overlap and there may be pathogen lesions of many different ages producing spores on the crop. Neither of these assumptions are particularly realistic – they represent contrasting extremes and the behaviour of most pathogens is intermediate between these extremes.

Barrett/Wolfe and Groth also differed in the assumptions they made concerning the way two or more unnecessary genes for virulence interact to reduce pathogen fitness. Groth considered both additive and multiplicative fitness effects models. Barrett and Wolfe limited their attention to the multiplicative model arguing that the additive model is not valid for large selection coefficients (s), presumably because of the problem of negative fitness values, while for small values of s the additive and multiplicative models are virtually identical. However, as shown by Groth (1976) and Marshall and Pryor (1979) the way in which unnecessary genes for virulence combine to reduce the relative fitness of complex pathogen races significantly influences the evolution of virulence in the pathogen population for moderate to large values of s ($\geqq 0.2$). Consequently, we consider both the additive and multiplicative models here. We overcome the problem of negative fitness values under the additive model by assuming that if $s > 1/(k-1)$ pathogen. For example, the greater relative fitness of simple have zero fitness on susceptible hosts.

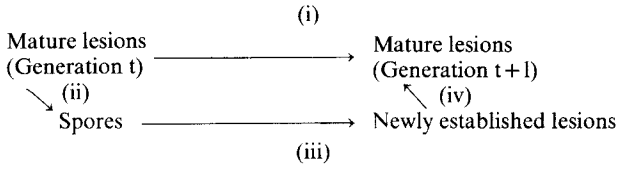
Finally, Barrett/Wolfe and Groth differed in their assumptions concerning the stage of the life cycle of the pathogen at which selection against unnecessary virulence genes acts. We consider this point in more detail below.

### Selection at different stages of the pathogen life cycle

Selection against unnecessary genes for virulence may operate at one or more of several different stages of the life cycle of the pathogen. For example, the greater relative fitness of simple pathogen biotypes compared to their more complex counterparts may be due to differences in:

(i) survival of mature, established, spore producing lesions;
(ii) fecundity (number of spores produced);
(iii) the ability of spores to disperse, germinate and establish new lesions;
(iv) survival of newly established lesions in competition with each other and mature lesions.

The schematic representation of the life cycle of the pathogen between generations t and t+1, below, illustrates these different stages at which selection may operate




Under Groth's discrete generation model all pathogen lesions are presumed to be the same age and to die simultaneously at the end of each generation. Consequently, his model does not allow the possibility of the differential survival of established lesions. Thus, under Groth's model, selection against unnecessary genes for virulence is assumed to be due solely to differential fecundity and/or differential establishment and survival of new lesions. However, under the Barrett/Wolfe overlapping generations model, lesions of different ages are presumed to coexist on the crop and their model allows selection to operate at all four stages of the pathogen life cycle above. In their 1978 paper, and all subsequent articles, Barrett and Wolfe chose to assume that selection against unnecessary genes for virulence was due solely to differential survival of mature spore producing lesions. To better understand the implications of this choice we now consider three variations of their model:

(a) Selection against unnecessary virulence genes is entirely due to differences in fecundity. This model is also valid if selective differences are due to differential establishment or differential survival of newly established lesions and is the overlapping generations equivalent of Groth's discrete generation model.

(b) Selection against unnecessary genes for virulence is entirely due to differences in the relative survival of mature spore producing lesions. This is the model of Barrett and Wolfe (1978) which we include for comparative purposes with (a) above.
(c) Selection against unnecessary genes for virulence involves differential fecundity and/or differential establishment and survival of new lesions as well as differential survival of established lesions. This model includes (a) and (b) as special cases.

*a) Differential fecundity and/or differential establishment and survival of new lesions*

Under the Barrett/Wolfe model the potential reproductive rate of all races is $(1+\alpha)$, where 1 represents the parental infection and $\alpha$ represents the number of daughter infections produced per parent per generation. If there are $N_1$ mature spore producing lesions of a simple race, carrying a single gene for virulence, on an n-line multiline at the end of the generation $t_0$, then at the end of the asexual generation $t_1$, the number of such lesions will be

$$N_1 + N_1\,\alpha/n = N_1(1+\alpha/n) \tag{1}$$

as the fitness of established infections is, by definition, 1, and the fitness of new infections is 1/n since all races are assumed to die on resistant hosts. The number of mature lesions at the end of generations $t_2, t_3, \ldots, t_g$ will be $N_1(1+\alpha/n)^2$, $N_1(1+\alpha/n)^3, \ldots, N_1(1+\alpha/n)^g$, respectively, so that the effective reproduction rate of simple races on the multiline is

$$R_1 = (n+\alpha)/n \tag{2}$$

and is constant each generation.

By analogy, the effective reproduction rate for races with two genes for virulence, assuming selection against unnecessary genes for virulence results in differential fecundity and/or differential establishment and survival of new lesions, is

$$R_2 = 1+2\,\alpha\,(1-s)/n = \{n+2\,\alpha\,(1-s)\}/n. \tag{3}$$

Again the fitness of established lesions is 1 and the fitness of new lesions is $2\,(1-s)\,\alpha/n$ since this class of pathogen biotype is virulent on two components of the multiline and their fitness is $(1-s)$ relative to biotypes with a single gene for virulence. Extending this argument, the effective reproduction rate of races with k genes for virulence is, under the assumption of additive fitness effects,

$$R_k = (n+k\,\alpha\,\{1-(k-1)\,s\})/n \tag{4}$$

and, assuming multiplicative fitness effects,

$$R_k = \{n+k\,\alpha\,(1-s)^{k-1}\}/n. \tag{5}$$

The fitness of complex races relative to simple races with a single virulence gene is,

$R'_k = R_k/R_1$, which for aditive fitness effects, is

$$R'_k = \{n+k\,\alpha\,[1-(k-1)\,s]\}/(n+\alpha) \tag{6}$$

and for multiplicative fitness effects,

$$R'_k = [n+k\,\alpha\,(1-s)^{k-1}]/(n+\alpha). \tag{7}$$

It should be emphasised at this point that an n-line multiline where each carries a different single gene for resistance, may be parasitised by many different pathogen races and in theory k may be very large. We may conveniently divide these many biotypes into 2n classes – those with 1, 2, . . . . , k, . . . . n effective genes for virulence (that is, virulence genes for which the corresponding resistance genes occur in the host population) with and without one or more redundant genes for virulence (that is, virulence genes for which the corresponding resistance genes are absent from the host population). For the types of models considered here, biotypes carrying redundant genes for virulence are less fit than those with only effective genes for virulence for all $s>0$ and will be eliminated from the pathogen population. Therefore, in defining the equilibrium racial composition of the pathogen population we can restrict our attention to the n classes of biotypes carrying only effective genes for resistance and hence, values of k in the range $1 \leqq k \leqq n$.

It is evident from (6) and (7) that $R'_k$ is a nonlinear function of k for both the additive and multiplicative fitness effects versions of this model. The number of virulence genes in the most fit class of pathogen biotypes can be determined as the value of k, symbolised $\hat{k}$, which maximises $R'_k$. For the additive fitness effects model

$$\hat{k} = (1+s)/2\,s \tag{8}$$

and for the multiplicative fitness effects model

$$\hat{k} = -1/\ln(1-s). \tag{9}$$

Because we have treated (6) and (7) as continuous functions, (8) and (9) usually yield non-integer solutions for k. Since $\hat{k}$ represents the number of virulence genes in the most fit class of biotypes, it must be an integer. Because (6) and (7) are monotonically increasing then decreasing, the integer solution to them is obtained by evaluating $R'_k$ at the integer values of k immediately greater and lesser than $\hat{k}$ and choosing the integer which yields the larger value of $R'_k$.

The above results given by (8) and (9) are identical to those obtained by Groth (1976) for his discrete generation model. Thus, the assumption of overlapping generations does not alter Groth's conclusions provided selection against unnecessary genes for virulence is due to differential fecundity of established lesions or differential establishment and survival of new lesions.

*b) Differential survival of established lesions*

Under this model, as in the previous case, the effective reproduction rate of simple races is

$$R_1 = (n+\alpha)/n. \tag{10}$$

The effective reproduction rate of races with two genes for virulence, assuming selection against unnecessary genes for virulence, is

$$R_2 = (1-s)\,[n+2\,\alpha]/n. \tag{11}$$

By analogy, the effective reproduction rate of races with k genes for virulence is, assuming additive fitness effects,

$$R_k = [1-(k-1)\,s]\,[n+\alpha\,k]/n \tag{12}$$

and, assuming multiplicative fitness effects,

$$R_k = (1-s)^{k-1}\,[n+\alpha\,k]/n. \tag{13}$$

The fitness of complex races relative to simple races with a single gene for virulence is, for additive fitness effects,

$$R'_k = [1-(k-1)\,s]\,[n+k\,\alpha]/(n+\alpha) \tag{14}$$

and for multiplicative fitness effects

$$R'_k = (1-s)^{k-1}\,[n+k\,\alpha]/(n+\alpha) \tag{15}$$

(15) is identical, except for minor differences in symbolism, to the expression first given by Barrett and Wolfe (1978).

In this case the value of k which maximises (14) is

$$\hat{k} = [\alpha\,(1+s) - n\,s]/2\,\alpha\,s = (1+s)/2\,s - n/2\,\alpha \tag{16}$$

while for the multiplicative fitness effects model

$$\hat{k} = -[\alpha + n \ln (1-s)]/\alpha \ln (1-s) \quad (17)$$

as shown by Barrett and Wolfe (1978).

Again it should be stressed that k is an integer and this fact must be taken into account when interpreting the results of (16) and (17).

Three points are worthy of further note with respect to this model. First it is clear, in sharp contrast to our findings with the previous model, that the question of whether the pathogen generations are discrete or overlapping is of fundamental importance if selection against unnecessary virulence genes acts via differential survival of established lesions. Indeed, this model is biologically meaningful only if the pathogen generations overlap – there can be no differential survival of established lesions if the generations are discrete because they all die at the end of each generation. Second, for the additive fitness effects model, as well as the multiplicative effects model as shown earlier by Barrett and Wolfe (1978), the equilibrium composition of the pathogen population is dependent upon the number of lines in the multiline (n), the growth rate of the pathogen population ($\alpha$) and the level of selection (s) against unnecessary virulence genes. Again, this result contrasts sharply with results for the previous model, where the outcome is dependent on only the level of selection (s) against unnecessary virulence genes and is independent of $\alpha$ and n. The dependence of the outcome of the present model on $\alpha$ and n is due to the fact that these parameters determine the effective level of selection against unnecessary genes for virulence, for a given value of s, through their effects on the proportions of new (unselected) versus established (selected) lesions each generation.

Finally, the comparison of (16) with (8) and (9) with (17), respectively, indicates that, for pathogens with low to moderate intrinsic rate of increase ($0 < \alpha < 10$), much lower levels of selection against unnecessary genes for virulence are required to prevent the development of complex pathogen biotypes on multiline varieties if selection is due to differential survival of established lesions rather than differential fecundity and/or differential establishment and survival of new lesions. To illustrate this point further we have calculated the levels of selection against unnecessary genes for virulence required to ensure any particular class of biotypes become fixed in the pathogen population (Marshall and Pryor 1978) for both the additive and multiplicative fitness effects versions of Groth's (1976) model (selection due to differential fecundity/establishment and survival of new lesions) as well as the Barrett and Wolfe (1978) model (selection due to differential survival of established lesions). These critical values of s are given in Table 1 for each model. We also give a number of numerical examples (Table 2) of the levels of selection required to stabilise the composition of pathogen populations with races carrying k ($= 1, 2, 3, 5, 10$) genes for virulence predominant assuming $n = 10$ and for a range of values of $\alpha$ (1, 10, 20 and $\infty$). These examples also illustrate the point that for large $\alpha$ the models of Groth and Barrett/Wolfe lead to similar results since, under this circumstance, the parent lesions make up a very small proportion of the total population size next generation.

### *c) Selection at two or more stages of the life cycle of the pathogen*

In the previous sections, we considered models where selection against unnecessary genes for virulence is assumed to occur at a single stage in the life cycle of the pathogen. There is, of course, no good reason why selection should be confined to any one particular stage of the pathogen life cycle. Such assumptions usually are made purely for mathematical convenience. Our aim here is to extend these models to consider selection at two or more different stages of the pathogen life cycle.

In developing such models, an important consideration is how the selective differences operating at the various life cycle stages combine to determine the net overall selective differentials for each genotype each generation. Unfortunately, there is little experimental data on life cycle components of selection to guide us in our choice of model. As a first step, therefore, we have

**Table 1.** Levels of stabilising selection(s)[a] required to ensure a particular class of pathogen biotype becomes fixed in the pathogen population under models of Groth (1976) and Barett and Wolfe (1978)

| No. of virulence genes in dominant pathogen biotype | Groth | Barrett and Wolfe |
|---|---|---|
| | Additive fitness effects | |
| 1 | $s > 0.5$ | $s > \alpha/(n+2\alpha)$ |
| k | $1/2\,(k-1) > s > 1/2\,k$ | $2/[n+2\alpha\,(k-1)] > s > \alpha\,(n+2\,k\,\alpha)$ |
| n | $s < 1/2\,(n-1)$ | $s < \alpha/[n+2\alpha\,(n-1)]$ |
| | Multiplicative fitness effects | |
| 1 | $s > 1/2$ | $s > \alpha/(n+2\alpha)$ |
| k | $1/k > s > 1/(k+1)$ | $\alpha/(n+k\alpha) > s > \alpha/[n+\alpha\,(k+1)]$ |
| n | $s < 1/n$ | $s < \alpha/n\,(1+\alpha)$ |

[a] If s equals any of the critical values in the body of the table the pathogen population will be polymorphic for two adjacent classes of pathogen biotypes (e.g. with k and k+1 genes for virulence) maintained in a neutral equilibrium

**Table 2.** Levels of stabilising selection(s) required to ensure the dominant pathogen biotype carries k virulence genes for the models of Groth and Barrett and Wolfe for a 10 line multiline

| No. of virulence genes (k) in predominant pathogen biotype | Groth | Barrett and Wolfe | | | |
|---|---|---|---|---|---|
| | | $\alpha=1$ | $\alpha=10$ | $\alpha=20$ | $\alpha=\infty$ |
| Additive fitness effects | | | | | |
| k= 1 | >0.50 | >0.08 | >0.33 | >0.40 | >0.50 |
| k≦ 2 | >0.25 | >0.07 | >0.20 | >0.22 | >0.25 |
| k≦ 3 | >0.17 | >0.06 | >0.14 | >0.15 | >0.17 |
| k≦ 5 | >0.10 | >0.05 | >0.09 | >0.10 | >0.10 |
| k=10 | <0.05 | <0.04 | <0.05 | <0.05 | <0.05 |
| Multiplicative fitness effects | | | | | |
| k= 1 | >0.50 | >0.08 | >0.33 | >0.40 | >0.50 |
| k≦ 2 | >0.33 | >0.08 | >0.25 | >0.29 | >0.33 |
| k≦ 3 | >0.25 | >0.07 | >0.20 | >0.22 | >0.25 |
| k≦ 5 | >0.17 | >0.06 | >0.14 | >0.15 | >0.17 |
| k=10 | <0.10 | <0.05 | <0.09 | <0.10 | <0.10 |

made the simplest possible assumption – that selection occurs independently at each stage of the pathogen life cycle and hence, fitness components can be combined multiplicatively.

*Multiplicative combination of fitness components*

Under this model, the fitness of a race with k genes for virulence relative to simple races with a single gene for virulence is

$$R'_k=[1-(k-1)s_1]\{n+k\,\alpha[1-(k-1)s_2]\}/(n+\alpha) \quad (18)$$

assuming genes of virulence combine additively to reduce pathogen fitness and

$$R'_k=(1-s_1)^{k-1}[n+k\,\alpha(1-s_2)^{k-1}]/(n+\alpha) \quad (19)$$

if genes for virulence combine multiplicatively. In (18) and (19), $s_1$ specifies the level of selection against unnecessary genes for virulence due to differential survival of established lesions and $s_2$ specifies the level of selection due to differential fecundity and/or differential establishment and survival of new lesions. As before, n is the number of lines in the multiline and $\alpha$ is a parameter describing the growth rate of the pathogen population.

Here, the value of k which maximises (18) is

$$\hat{k}=(a-b)/c \quad (20)$$

where $a=2\,\alpha[s_1(1+s_2)+s_2(1+s_1)]$

$$b=\{4\,\alpha^2[s_1(1+s_2)+s_2(1+s_1)]^2 - 12\,s_1\,s_2\,\alpha[\alpha(1+s_1)(1+s_2)-n\,s_1]\}^{1/2}$$

$$c=6\,\alpha\,s_1s_2$$

while for the multiplicative effects model $\hat{k}$ is given by

$$n\ln(1-s_1)+\alpha(1-s_2)^{k-1}[k\ln(1-s_1) + k\ln(1-s_2)+1]=0 \quad (21)$$

which must be solved numerically.

The numbers of virulence genes expected in the dominant pathogen biotype under this model for a range of values of $s_1$, $s_2$ and $\alpha$ are given in Table 3. These data reinforce the point that selection against unnecessary genes for virulence due to differential survival of established lesions is much more effective, particularly for low values of $\alpha$, in preventing the development of multivirulent pathogen biotypes on multiline varieties than selection due to differential fecundity and/or the differential establishment and survival of new lesions (cf. $s_1=0.10$ or 0.20 and $s_2=0$ with $s_2=0.10$ or 0.20 and $s_1=0$). These results also clearly demonstrate, as we might expect, that a given selection intensity operating at two different stages of the pathogen life cycle is more effective in preventing the build up of complex pathogen biotypes than the same selection pressure operating at a single life cycle stage (cf. $s_1=s_2=0.10$ with $s_1=0.10$, $s_2=0$ or $s_1=0$, $s_2=0.10$). Finally, the results in Table 3 indicate that for $\alpha\leqq 10$, a given selection intensity operating at two life cycle stages is more effective in preventing the development of multi-virulent pathogen biotypes than twice the selection intensity operating at a single stage if selection is due to differential fecundity, but it is equal to or less effective than twice selection intensity at a single life cycle stage if selection is due to differential survival of established lesions.

**Table 3.** Expected number of virulence genes in the dominant pathogen biotype growing on a 10 line multiline assuming selection against unnecessary genes for virulence may act at one or both of two different stages of the pathogen life cycle

| $\alpha$ | Additive fitness effects | | | | |
|---|---|---|---|---|---|
| | $s_1=0.10$[a] $s_2=0$ | $s_1=0$ $s_2=0.10$ | $s_1=0.10$ $s_2=0.10$ | $s_1=0.20$ $s_2=0$ | $s_1=0$ $s_2=0.20$ |
| 0.1 | 1 | 5, 6[b] | 1 | 1 | 3 |
| 1 | 1 | 5, 6 | 1 | 1 | 3 |
| 10 | 5 | 5, 6 | 3 | 2, 3 | 3 |
| 100 | 5 | 5, 6 | 4 | 3 | 3 |
| | Multiplicative fitness effects | | | | |
| 0.1 | 1 | 9, 10 | 1 | 1 | 4, 5 |
| 1 | 1 | 9, 10 | 1 | 1 | 4, 5 |
| 10 | 8, 9 | 9, 10 | 4 | 3, 4 | 4, 5 |
| 100 | 9 | 9, 10 | 5 | 4 | 4, 5 |

[a] $s_1$ specifies the level of selection due to differential survival of established lesions, $s_2$ specifies selection due to differential fecundity and/or differential establishment and survival of new lesions, and $\alpha$ specifies the rate of growth of the pathogen population

[b] Where two values are given it indicates that biotypes with these number of virulence genes are equally fit and will co-exist in the pathogen population in a neutral equilibrium

*General model*

The second approach we will adopt is to attempt to develop a more general model in which no explicit assumptions are made concerning the ways selective differences operating at two or more life cycle stages combine to determine net selection difference per generation. Under such a model the fitness of biotypes with k genes for virulence relative to simple races with one gene for virulence is, in the additive case,

$$R'_k = \{n[1-(k-1)\,s_1]+k\,\alpha[1-(k-1)\,s_2]\}/(n+\alpha) \quad (22)$$

and assuming virulence genes combine multiplicatively to reduce pathogen fitness,

$$R'_k = [n(1-s_1)^{k-1}+k\,\alpha(1-s_2)^{k-1}]/(n+\alpha). \quad (23)$$

Here, $s_1$ specifies the level of selection against unnecessary genes for virulence due to the differential survival of established lesions, as before. However, $s_2$ includes two components – one due to differential survival of established lesions and one due to differential fecundity and/or differential survival and establishment of new lesions. If $s_1=0$ the model is identical to the one considered in (a) above while if $s_1=s_2$ it becomes identical to that in (b) above. More importantly, if $s_1>s_2$ under this model, it signifies that selection against unnecessary virulence genes due to differential survival of established lesions is offset in part by selection in favour of unnecessary genes for virulence due to differential fecundity and/or differential survival and establishment of new lesions. That is, there is selection against unnecessary genes for virulence at one stage of the life cycle and selection in favour of such genes at another life cycle stage.

As before, we wish to establish the value or values of k which maximise $R'_k$. We consider the relationships between $R'_k$ and k for these models below.

*Additive fitness effects.* For this model it can be shown that the number of virulence genes, $\hat{k}$, which maximise pathogen fitness is

$$\hat{k}=[\alpha(1+s_2)-n\,s_1]/2\,\alpha s_2 \quad (24)$$

provided $1/(n+\alpha)\neq 0$.

As a check on these results note that if $s_1=s_2$ then (24) is identical to (16), while if $s_1=0$ it reduces to (8) as expected.

The expected numbers of virulence genes ($\hat{k}$) in the most fit pathogen biotype under this model are given in Table 4 for a range of values of $s_1$, $s_2$, and $\alpha$. We note that for low population growth rates ($\alpha \leqq 1$) the dominant pathogen biotype will carry only a single gene for virulence provided $s_1>0.1$ and $s_2>0.05$. However at higher pathogen growth rates much stronger selection against unnecessary genes for virulence ($s_1$, $s_2>0.4$) is required to ensure that simple races dominate the pathogen population. We also note that if $s_2>s_1$ then the most fit pathogen biotypes generally carry fewer genes for virulence than under the Barrett/Wolfe model ($s_1=s_2$). On the other hand if $s_1>s_2$ then the reverse is true. This latter result reflects the fact that if $s_1>s_2$ this implies that selection against unnecessary

**Table 4.** Number of virulence genes ($\hat{k}$) carried by the most fit pathogen biotype growing on a 10 line multiline assuming additive fitness effects under a general model of selection at two different stages in the pathogen life cycle

| | $s_2$ | | | | |
|---|---|---|---|---|---|
| $s_1$ | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
| | | | $\alpha=1$ | | |
| 0.1 | 1 | 1 | 1 | 1 | 1 |
| 0.2 | 1 | 1 | 1 | 1 | 1 |
| 0.3 | 1 | 1 | 1 | 1 | 1 |
| 0.4 | 1 | 1 | 1 | 1 | 1 |
| 0.5 | 1 | 1 | 1 | 1 | 1 |
| | | | $\alpha=10$ | | |
| 0.1 | 9 | 5, 6[a] | 3 | 2 | 2 |
| 0.2 | 4 | 4 | 2, 3 | 2 | 1, 2 |
| 0.3 | 3 | 3 | 2 | 2 | 1 |
| 0.4 | 2 | 2 | 2 | 1, 2 | 1 |
| 0.5 | 1 | 1 | 1 | 1 | 1 |
| | | | $\alpha=100$ | | |
| 0.1 | 9 | 5 | 3 | 2 | 2 |
| 0.2 | 4 | 4 | 3 | 2 | 2 |
| 0.3 | 3 | 3 | 3 | 2 | 2 |
| 0.4 | 2 | 2 | 2 | 2 | 2 |
| 0.5 | 1 | 1 | 1 | 1 | 1 |

$s_1$ and $s_2$ specify the levels of selection against unnecessary genes for virulence at different stages in the life cycle and $\alpha$ specifies the rate of growth of the pathogen population

[a] Where two values are given it indicates that biotypes with these numbers of virulence genes are equally fit and will co-exist in the pathogen population in a neutral equilibrium

genes for virulence at the first life cycle stage is partially offset by selection in their favour at the second life cycle stage.

*Multiplicative fitness effects.* As before, the relationship between $R'_k$ and k (23) is more complex for the multiplicative than the additive fitness effects model. Differentiating $R'_k$ with respect to k, and setting the derivative equal to zero, to find the optimum $\hat{k}$ which maximises $R'_k$, yields a complex equation

$$\{n(1-s_1)^{k-1}\ln(1-s_1)+\alpha(1-s_2)^{k-1}[1+k\ln(1-s_2)]\}/(n+\alpha)=0 \quad (25)$$

from which it is difficult to obtain an explicit expression for $\hat{k}$. However, it is relatively easy to show for this model that there is a simple upper bound, $k_\mu$, where

$$k_\mu = 1/\ln[1/(1-s_2)] \quad (26)$$

to the number of virulence genes carried by the most fit pathogen biotype.

Numerical values of this upper bound are given in Table 5 for a range of values of $s_2$. Two points should be noted here. First, it is clear that if $s_2 \geqq 0.5$ then simple races with only a single gene for virulence will be most fit and will dominate the pathogen population regardless of the value of $s_1$ ($0 \leqq s_1 \leqq 1$). Second, if $s_2 \geqq 0.2$ the most pragmatic means of finding $\hat{k}$ which maximises $R'_k$, seeing it will be an integer, is simply to evaluate $R'_k$ for k = 1, 2, 3, 4 and 5 and select k for highest $R'_k$.

It can be shown that the solution to (25) depends critically on three quantities

$$\Delta_1 = n(1-s_2)\ln[1/(1-s_1)]/\alpha(1-s_1) \quad (27)$$

$$\Delta_2 = \ln[1/(1-s_2)]/\ln[(1-s_2)/(1-s_1)] \quad (28)$$

$$\Delta_3 = \Delta_2[1+\ln(\Delta_1/\Delta_2)]. \quad (29)$$

We have not presented the details of the derivation of $\Delta_1$, $\Delta_2$ and $\Delta_3$ as quantities of interest, but in Table 6 we give the full solution to (25) in terms of $\Delta_1$, $\Delta_2$ and $\Delta_3$. We have given particular attention to establishing

**Table 5.** Upper bound of number of virulence genes ($\hat{k}$) carried by the most fit pathogen biotype under a general model of selection at two life cycle stages of the pathogen

| $s_2$[a] | Upper bound of $\hat{k}$ |
|---|---|
| 0.05 | 19.50 |
| 0.10 | 9.49 |
| 0.20 | 4.48 |
| 0.30 | 2.80 |
| 0.40 | 2.96 |
| 0.50 | 1.44 |
| 0.60 | 1.09 |
| 0.70 | 0.83 |
| 0.80 | 0.62 |
| 0.90 | 0.43 |

[a] $s_2$ specifies level of stabilising selection against unnecessary genes for virulence due to differential fecundity and/or establishment and survival of new lesions

**Table 6.** Number of virulence genes ($\hat{k}$) in most fit pathogen biotype under a general model of selection at two life cycle stages of the pathogen. $s_1$ and $s_2$ specify the level of selection against unnecessary genes for virulence at the two life cycle stages

| Condition | $s_2 \geqq s_1$ | | $s_2 < s_1$ |
|---|---|---|---|
| $\Delta_1 < 1$ | $\hat{k} \geqq 1$[a] | | $\hat{k} \geqq 1$[a] |
| $\Delta_1 = 1$ | $\hat{k} = 1$ | $\Delta_2 < 1$ | $\hat{k} \geqq 1$[a] |
| | | $\Delta_2 \geqq 1$ | $\hat{k} = 1$ |
| $\Delta_1 > 1$ | $\hat{k} = 1$ | $\Delta_3 < 1$ | $\hat{k} \geqq 1$[a] |
| | | $\Delta_3 \geqq 1$ | $\hat{k} = 1$ |

[a] $\hat{k}$ must be determined numerically in these cases from the equation (25), except when $s_1 = s_2$ when an explicit solution exists

$\Delta_1$, $\Delta_2$ and $\Delta_3$ are defined in the text

**Table 7.** Number of virulence genes carried by the most fit pathogen biotype growing on a 10 line mixture assuming multiplicative effects and selection (intensity specified by $s_1$ and $s_2$) at two different stages in the pathogen life cycle

| | $s_2$ | | | | |
|---|---|---|---|---|---|
| $s_1$ | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 |
| | | | $\alpha = 1$[a] | | |
| 0.1 | 1 | 1 | 1 | 1 | 1 |
| 0.2 | 1 | 1 | 1 | 1 | 1 |
| 0.3 | 1 | 1 | 1 | 1 | 1 |
| 0.4 | 1 | 1 | 1 | 1 | 1 |
| 0.5 | 1 | 1 | 1 | 1 | 1 |
| | | | $\alpha = 10$ | | |
| 0.1 | 8, 9[b] | 4 | 2 | 1 | 1 |
| 0.2 | 9 | 3, 4 | 2 | 1, 2 | 1 |
| 0.3 | 9 | 3 | 2 | 1 | 1 |
| 0.4 | 9 | 3 | 1, 2 | 1 | 1 |
| 0.5 | 9 | 3 | 1 | 1 | 1 |
| | | | $\alpha = 100$ | | |
| 0.1 | 9 | 4 | 3 | 2 | 1 |
| 0.2 | 9 | 4 | 3 | 2 | 1 |
| 0.3 | 9 | 4 | 3 | 2 | 1 |
| 0.4 | 9 | 4 | 3 | 2 | 1 |
| 0.5 | 9 | 4 | 3 | 2 | 1 |

[a] $\alpha$ specifies the rate of growth of the pathogen population
[b] Where two values are given it indicates that pathogen biotypes with these numbers of virulence genes are equally fit and will co-exist in the pathogen population in a neutral equilibrium

when $\hat{k} = 1$ or $\geqq 1$, because this is of prime interest to plant breeders who are considering using multiline varieties as a disease control strategy.

To further illustrate the properties of this model, the number of virulence genes carried by the most fit pathogen biotype are given in Table 7 for $n = 10$ and a range of values of $s_1$, $s_2$ and $\alpha$. As in the additive case it will be seen that for $\alpha \leqq 1$, simple races carrying a single gene for virulence will be most fit and dominate the pathogen population for all $s_1$, $s_2 > 0.1$. At higher population growth rates, much stronger levels of selection are required to ensure simple races dominate the pathogen population.

## Discussion

The results reported here clearly indicate that the stage of the life cycle of a pathogen at which 'stabilising selection' (Van der Plank 1963, 1968) against unnecessary genes for virulence operates can significantly influence the evolution of virulence in populations of that pathogen growing on multiline varieties. If selection against unnecessary genes for virulence is assumed to be due solely to differential fecundity and/or differential establishment and survival of new lesions, then pathogens growing on mixed host populations will evolve the same degree of racial complexity regardless of whether the pathogen generations are discrete or overlapping. This conclusion is a simple corollary of the fact that under this assumption there is no selection amongst established lesions.

On the other hand, if selection against unnecessary genes for virulence is assumed to be due solely to the differential survival of established lesions, then the mode of reproduction of the pathogen assumes critical importance. If the pathogen generations are discrete there can be no effective selection against unnecessary genes for virulence under this model because all pathogen lesions are assumed to simultaneously produce spores and then die. If there are no surviving lesions there can be no selection amongst them. If pathogen generations overlap and pathogen lesions can potentially survive more than one generation then this model is valid and is biologically meaningful. In this case, the effective level of selection against unnecessary genes for virulence, for a given value of s, will depend on both $\alpha$ and n since these parameters determine the reproduction rate of each race on the multiline and hence the proportions of new (unselected) versus established (selected) lesions each generation.

The present studies also provide further insight into the differences between the models of Groth (1976) and Barrett and Wolfe (1978). In particular, they suggest that the most important difference between the models proposed by these authors is the stage or stages in the life cycle of the pathogen at which selection against unnecessary genes for virulence is assumed to act. In Groth's (1976) model selection against unnecessary genes for virulence is assumed to be due to differential frecundity and/or establishment of new lesions while under the Barrett and Wolfe (1978) model selection is assumed to act via differential survival of established lesions. It is this latter assumption which leads to the dependence in the outcome of the Barrett/Wolfe model on $\alpha$ and n. It is also this assumption which is responsible for the fact that lower levels of selection are required under the Barrett/Wolfe model to prevent a development of complex pathogen biotypes on multiline varieties particularly at low population growth rates ($\alpha \leqq 1$).

The practical significance of the result reported here is difficult to ascertain because of the current lack of knowledge of the selective forces governing the evolution of virulence in pathogen populations. Although the concept of 'stabilising selection' against unnecessary virulence genes was introduced some 20 years ago by

Van der Plank (1963, 1968) its general validity is still a matter of some dispute (Parlevliet 1981). Further, there is still considerable doubt, even given that stabilising selection is a general phenomenon, that it will be strong enough to prevent the development of highly complex pathogen races on 'dirty crop' multilines. Recent studies have tended to cloud rather than clarify this issue. For example, Leonard (1977) using the laboratory data of Watson and Singh (1952) estimated the level of stabilising selection against the gene for virulence for the stem rust resistance gene *Sr6* in bread wheat to be $s=0.42$. On the other hand, Grant and Archer (1983), using field data, estimated unnecessary virulence for *Sr6* reduced fitness by only 4% (that is $s=0.04$). Which of these dramatically different estimates of the level of selection acting against unnecessary virulence at the *Sr6* locus is correct is a matter of conjecture which will only be resolved by further experimental studies. Indeed, until more information on the levels of 'stabilising' selection acting against unnecessary genes for virulence is available, and further, until we better understand the stage in the life cycle at which such selection operates and the ways unnecessary virulence genes interact to reduce the fitness of complex pathogen races, we cannot be sure that multilines offer a viable alternative to other forms of disease control.

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