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Evolution of a pathogen population in host mixtures: rate of emergence of complex races

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Abstract The development of a pathogen population in a crop varietal mixture was studied with an epidemic simulator based on the model EPIMUL. The pathogen population was composed of simple races, able to develop on only one genetic component of the mixture, and a complex race, which developed on all mixture components. The complex race was modelled with a very low initial frequency compared to simple races, to simulate the emergence of a complex race in the field. Several successive epidemics were simulated as if the pathogen population reproduced on the same plot for several years. The development of the complex race on the simulated plot was either focal or uniform. The effects of the cost of virulence, of density dependence and of differential adaptation to host genetic background on the simple race-complex race competition were studied. Experimentally measured values of the cost of virulence and differential adaptation were incorporated into the model, and both factors were shown to greatly reduce the increase in frequency of the complex race over time. Density dependance also influenced race competition, but mainly for high values of the parameter. Our results suggest that the cost of virulence is probably not the only mechanism that may influence the simple race-complex race competition in host mixtures. In our simulations, differences in the spatial distribution of the initial inoculum between parasite races led to large differences in their final frequencies. Thus, more investigations, including randomized disease distributions, would be of interest to judge the potential importance of spatial effects in the field.

Key words Varietal mixture · Cost of virulence · Density dependance · Differential adaptation · Epidemic control

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Introduction

Host mixtures are considered to be an important disease-control method, particularly for air-borne pathogens of cereals (Browning and Frey 1969; Jeger et al. 1981; Wolfe 1985; Mundt 1989). In a mixture of cultivars having different resistance genes, pathogens are unable to multiply on part of the host population, and spore losses on resistant plants result in large reductions in disease severity. The question has been raised, however, of the consequences of their use on a large scale for the evolution of pathogen populations. Different models (reviewed by Marshall 1989) have been proposed to describe the conditions in which complex races, able to develop on several components of the mixture, are selected and erode the resistance of mixtures. Most of these models (Groth 1976; Barrett 1980; Østergård 1983) are based on the existence of a selective disadvantage, or cost, associated with virulences that are unnecessary in a host-pathogen interaction. These models describe competition between simple and complex races: simple races have a high specificity for a given host component but are unable to develop on other hosts; complex races develop on several mixture components but are affected by a fitness disadvantage proportional to the number of virulences they carry.

Other mechanisms, like density dependence or differential adaptation of the pathogen to host genetic background, could also be involved in simple race-complex race competition (Lannou and Mundt 1996). Density dependent fitness is a general phenomenon in

population biology (Silvertown 1987) and has been described for wheat stem rust by Katsuya and Green (1967) and for bean rust by Kardin and Groth (1989). These authors measured a decrease in the pathogen multiplication rate for increasing lesion density on host leaves and showed that the magnitude of this effect varied among isolates. Thus, infection density could affect simple and complex races differentially during an epidemic and reduce selection for complex races. Differential adaptation has been described by Chin and Wolfe (1984) for powdery mildew on barley. These authors observed that, at the end of an epidemic, isolates of a complex virulence genotype sampled from two different varieties grown in pure stands had a greater multiplication rate on the variety from which they were isolated than on the other one. This differential adaptation to host genotype did not occur when the two varieties were grown in a mixture. A possible explanation for this result is selection for increased reproductive ability on a given host genotype within pathogen populations of the same virulence type, but physiological aspects could also be involved. As simple races reproduce always on the same host genotype, whereas complex races can infect different host genotypes successively during an epidemic, differential adaptation could result in a higher increase of the reproduction rate for simple races than for complex races.

In a previous study (Lannou and Mundt 1996) we tested the effects of the cost of virulence, of density dependence and of differential adaptation in simulated epidemics where two simple races and a complex race were inoculated on a two-component mixture. The simple and complex races were inoculated at the same initial frequency and the evolution of the complex race was observed during the development of epidemics. The frequency of the complex race increased in almost every situation. Our objective in the present paper was to simulate the emergence of a complex race in the pathogen population, starting at a very low frequency, and to observe the consequences of the cost of virulence, density dependence and differential adaptation to host genotype on the dynamics of simple and complex races and on the severity of disease during a succession of epidemics. We used a model designed for the simulation of the spread of an air-borne parasite in a plot.

The model

The model employed is a modification of the epidemic simulator EPIMUL (Kampmeijer and Zadoks 1977), which was initially developed to study the progression of epidemics in time and space. In its initial structure, the simulator included a module that numerically computed disease progression on individual hosts and a function that accounted for pathogen dispersal between hosts. Mundt et al. (1986) modified the dispersal function and Lannou et al. (1995) altered the model to simulate epidemics caused by a population of different genotypes (races) of the same pathogen species. For the present study, we used further modifications of the model (Lannou and Mundt 1996) to describe interactions that can occur between different pathogen races during an epidemic.

Structure of the model

The epidemic develops on a simulated plot, divided into square compartments which represent individual plants. Different resistance levels can be assigned to the compartments, in order to simulate a heterogeneous host population such as a varietal mixture. There is no host growth function in the model. Rather, the leaf surface area of a plant is constant and divided into infection sites, which represent the host surface that can be occupied by a lesion. The course of the epidemic is described with a daily time step on each plant by computing the evolution of the sites from vacant to latent, latent to infectious, and infectious to removed.

Both the pathogen and the host populations can include multiple genotypes (Lannou et al. 1995). The interaction between a host genotype and a pathogen genotype is described by the spore efficacy (SE, or the probability for a spore to produce a lesion when deposited on the host), the daily spore production rate per infectious lesion (SP), the latent period, and the infectious period. The value of each of these parameters depends on both the host and the pathogen considered in the interaction. The multiplication rate of a pathogen on a given host is defined by the daily multiplication factor Dmfr:

$$\mathrm{Dmfr} = \mathrm{SE} \times \mathrm{SP}. \tag{1}$$

Spores are dispersed among plants according to a function proposed by Mundt and Leonard (1985, see also Mundt et al. 1986), which relates the spore density (y) to the distance (x) from the spore source by:

$$\mathrm{y} = (\mathrm{x} + c)^{-b}, \tag{2}$$

where c is a constant that is approximately equal to the radius of a plant in two-dimensional space and b represents the spore-dispersal gradient. A low value of b gives a shallow dispersal gradient; a high value of b gives a steep dispersal gradient.

Modelling of simple race-complex race interactions

Three mechanisms were investigated as having a possible effect on the competition between races in the pathogen population:

Cost of virulence

The cost of virulence represents a reduction in the pathogen multiplication rate Dmfr, due to the presence of unnecessary virulence genes. We defined a corrected multiplication rate (CDmfr) as:

$$\mathrm{CDmfr} = \mathrm{Dmfr}\,(1 - \mathrm{Cv}), \tag{3}$$

where Cv represents the total cost of virulence for a pathogen genotype.

Density dependence

A density dependent multiplication rate was computed as:

$$\mathrm{CDmfr} = \mathrm{Dmfr}\left(1 - \mathrm{Dd}.\frac{occ}{frsi}\right), \tag{4}$$

where Dd is the magnitude of the effect, *occ* is the number of occupied sites (i.e. latent, infectious or removed) on the plant for which CDmfr is calculated, and *frsi* is the total number of infection sites on a plant. For each day and for each plant, the CDmfr values of the races were re-computed according to equation (4). The lowest possible value for CDmfr was 0.

Differential adaptation

To account for Chin and Wolfe's (1984) data, we considered that pathogens were selected for increased multiplication rate when they reproduced on a given host. We assumed that the spores of a pathogen race produced on a host genotype had a mean multiplication rate greater than the previous spore generation that had been produced on the same host genotype, due to the selection within the lesion population for the individuals best adapted to the genetic background of a given host. Our model did not allow us to keep track of individuals, or even sub-populations, having different reproductive parameters within the same pathogen race. Therefore, we limited ourselves to the description of an increase in the mean reproductive rates of the races without trying to model these increases at the individual level. In the model, the lesions on a plant are distributed into different age classes, corresponding to 1 day of the latent or infectious periods, to allow their dynamic classification as latent, infectious or removed lesions. To each lesion age class, we associated a parameter (Ngh) representing the mean number of successive generations the pathogen has reproduced on the same host genotype. When spores were deposited on a plant of the same genotype as the plant from which they were produced, their Ngh was increased by 1. If the source and target plants were of different genotypes, the spore Ngh was re-set to 0. Spores released by a plant had the same Ngh as the sporulating lesions which produced them. Since the different lesions within the same age class could not be distinguished, the Ngh of all spores deposited on a plant on the same day was averaged, and the mean value was attributed to the new lesions. Lesions created the same day on different plants could still have a different Ngh, depending on the origin of the spores. With this simplification, the model did not really differentiate sub-populations within a pathogen race, but allowed different lesions of the same race to have different multiplication rates. The consequence was that, for a pathogen reproducing always on the same host genotype, recent lesions had on average a higher Ngh than older lesions. The effect of differential adaptation was computed as:

$$\mathrm{CDmfr} = \mathrm{Dmfr}\ (1 + \mathrm{Da.Ngh}), \qquad (5)$$

where Cdmfr is the daily multiplication factor corrected for the host adaptation effect, and Da is the magnitude of the host adaptation effect. For instance, Da = 0.01 means that the mean Dmfr of a spore population increased by 1% for each successive generation that a pathogen race was on the same mixture component. A maximal value for CDmfr was fixed for Ngh = 10 which, as described by Chin and Wolfe (1984), is a reasonable value for a powdery mildew epidemic.

Since the multiplication rate Dmfr depends on both SE and SP (Eq.1), Cv, Dd and Da could affect either SE or SP. We chose to modify the spore production SP and to use a constant spore efficacy SE = 1 for all host-pathogen interactions.

Outputs of the model

Disease severity

The disease severity on a plant was computed as the number of infectious and removed sites (visible disease) divided by the total number of sites per plant. This measure was averaged in order to give a mean disease severity for each host-pathogen combination.

Race frequencies

Race frequencies were computed as the frequencies of each pathogen genotype in the population of infectious lesions. For example, a frequency of 25% of a race at a given time meant that 25% of the lesions producing spores at this time were of that genotype.

Disease reduction in mixtures

The epidemic in each host mixture was compared to an epidemic caused by the same pathogen population on a pure susceptible stand. The disease severities in mixed and pure stands were compared to calculate a relative disease reduction, at the first date at which severity reached, or exceeded, 90% in the pure stand. For instance, if the disease severity was 50% in the mixture on the day that it was 91% in the pure stand, the relative disease reduction in the mixture was calculated as (91−50)/91 = 45.1%.

Simulations

The pathogen population included two simple races (SR1 and SR2), virulent on only one host genotype, and a complex race (CR), virulent on all of the mixture components. Simulations were performed on a 1:1 mixture of host h1 and h2 distributed in the plot according to a regular pattern:

h1 h2
h2 h1.

In all simulations, the simulated plot was of 3600 plants with 1875 infection sites on each of them (Mundt et al. 1986). The latent and infectious periods were of 5 days and 10 days, respectively, for all races on all susceptible hosts. The parameters of the dispersal function were also identical for all races ($c = 0.03$ and $b = 2.5$), which gave a dispersal gradient of medium steepness. The effect of changes in the dispersal gradient on the competition between races is described in Lannou and Mundt (1996).

Our objective in the present paper was to study the simple race-complex race competition in the long term. Therefore, we simulated a succession of epidemics on the same plot over several years. At the beginning of a new epidemic, the frequency of the different races in the initial inoculum was identical to the frequencies of the races at the end of the previous epidemic, but the total amount of inoculum was reset to 3600 lesions. For example, if an epidemic ended with SR1, SR2 and CR in frequencies of 0.4, 0.4 and 0.2 respectively, the next epidemic was initiated with 1440 lesions of each simple race and 720 lesions of the complex race. In addition, the host surface area available for infection was re-set to 100% at the beginning of each new epidemic. This simulated a situation where the same mixture is grown on the same plot over several years. However, in order to test the consequences of a change in the host population, additional simulations were performed. When the frequency of the complex race exceeded 10% in these simulations (i.e. after 5–6 epidemics), host h2 was replaced in the mixture by host h3, which was considered to be resistant to all races. Simulations with the h1-h3 mixture were repeated for 11 or 12 successive epidemics.

In order to simulate the emergence of the complex race in a population of simple races, the first epidemic was initiated by placing one spore of each simple race on each plant of the plot, and one spore of the complex race on one plant of each host genotype at the plot center. This first epidemic thus started with 1799 lesions of a simple race and one lesion of the complex race on each of the two host mixture components, which gave an initial frequency of the complex race equal to 5.56×10^{-4}. The first epidemic was then a combination of two general epidemics, caused by the two simple races, and a focal epidemic, caused by the complex race. Since the simple races did not reproduce on the same host, they did not interact together, but only

with the complex race. A major difficulty in our simulation design was to decide on the spore distribution of the complex race at the beginning of each epidemic. Two options were tested. In the first one, the complex race was inoculated in a focus at the plot center. Spores of the complex race were distributed on central plants so that each plant would receive between one and two spores. If the complex-race frequency increased during a season, the plot surface initially inoculated with the complex race in the next epidemic also increased from the plot center to its borders. In the second option, spores of the complex-race were distributed over the whole plot as soon as the complex race severity on the plot was greater than 1%, each plant receiving then a fraction of the complex-race inoculum. In these generalised epidemics, we sometimes used lesion numbers lower than 1, considering this as an approximation of a general distribution of discrete lesions.

The effects of the cost of virulence, density dependence and differential adaptation were simulated separately according to the following design. Values used for the cost of virulence were Cv = 0, 0.01, 0.05, 0.1, and 0.2. Density dependence did not affect the simple races, Dd(SR) = 0, but only the complex race with Dd(CR) = 0, 0.1, 0.2, 0.5, 1.0. Differential adaptation affected both simple and complex races with values of Da = 0, 0.01, 0.023 and 0.05. All these values were based on a previous study (Lannou and Mundt 1996).

In our simulations, differences in the spatial distribution of the initial inoculum had an effect on race competition. In order to distinguish this effect from others, an additional set of simulations was conducted by inoculating at the same time two races with identical multiplication rates on a pure susceptible stand. The first race initiated an epidemic with 0.25 lesions on each of the 3600 plants of the plot, whereas the second one started with 900 lesions distributed on 9, 100, 256, 400, 900, 1800, 2025, or 3600 plants. The inoculated plants were situated at the plot center. For example, in the case of nine plants inoculated, a central square of 3×3 plants was inoculated with 100 spores per plant. In three other simulations, the second race was also inoculated on 4, 9 or 25 isolated plants, distant from each other by 30, 20 or 10 plant radii, respectively, to create multifocal epidemics.

Results

The length of the simulated epidemics was between 32 days (when the proportion of complex race was high) and 36 days (when the proportion of complex race was low). Since the latency period lasted 5 days, this corresponded to a maximum number of pathogen cycles of six or seven. However, the mean number of pathogen cycles per epidemic estimated by the parameter Ngh (mean number of successive generations on the same host) for the simple races was between 3 and 3.2. In all epidemics, the progression of both simple races was identical.

For all values of the cost of virulence Cv, density dependence Dd and differential adaptation Da, the complex race increased in frequency, whether it was focally or generally inoculated (Fig. 1). When the complex race developed focal epidemics, however, its rate of progression was strongly reduced by the three selective effects. When the multiplication rate of all races was constant (Cv = 0, Dd = 0, Da = 0), the complex race frequency was 0.85 after 12 simulated focal epidemics. It was reduced to 0.52 with a cost of virulence Cv = 0.1, to 0.31 with a differential adaptation effect Da = 0.023, and to 0.34 with a density dependence effect Dd = 0.5. When the complex race inoculum was generally distributed, its frequency increased much faster. After 11 simulated epidemics, the complex race frequency was 0.99 in the absence of selective effects, 0.94 for Cv = 0.1, 0.82 for Da = 0.023, and 0.91 for Dd = 0.5. The distribution of the complex race inoculum on the plot had a major influence on the simple race-complex race competition.

When the mixture composition was changed, the complex race developed only on host h1, in competiton with simple race SR1. For a focal distribution of its inoculum, the complex race was represented at the beginning of the sixth epidemic by 10–161 spores distributed over 8–128 central plants of the plot, depending on the values of parameters Cv, Da and Dd. For these simulations, the complex-race frequency decreased asymptotically, but at a slower rate than it had previously increased. After six more epidemics, its frequency was reduced from 0.169 to 0.048 in absence of selective effects, from 0.063 to 0.006 for Cv = 0.1, from 0.053 to 0.015 for Da = 0.023, and from 0.054 to 0.006 for Dd = 0.5. When the complex race was generally distributed, its frequency was stable in the absence of selective effects; it decreased continuously, but slower than in the case of a focal distribution, when the cost of virulence or density dependence was simulated. Since the complex race reproduced always on the same host genotype in the new mixture, its multiplication rate was increased by differential adaptation until, after three to four successive epidemics, it equalled the simple-race's multiplication rate.

As the complex race increased in frequency, disease control due to mixing decreased (Fig. 2). Changes in mixture efficacy were attributable only to the changes in the complex-race frequency. They were calculated only for simulations in which Cv = 0, Da = 0, and Dd = 0. For the first epidemic, disease was reduced by 48% in the mixture, relative to a pure susceptible stand. The disease reduction dropped to 39% after six successive epidemics for a focal distribution of the complex race, and to 38% after five successive epidemics for a general distribution of the complex race. When the host mixture composition was changed, the disease reduction again reached its initial value of 48%.

The mean number of successive generations that races reproduced on the same host genotype (Ngh) differed for simple and complex races (Fig. 3). For simple races, Ngh increased regularly during the first four epidemics until it reached the maximal value (arbitrarily fixed to 10). According to Eq. 5, the simple-race's multiplication rate increased at the same pace. In the case of the complex race, the parameter Ngh increased much slower and stabilized around 1.65. In the beginning of the first epidemic, the multiplication rate of all races was Cdmfr = 5. During the fourth epidemic, it reached a maximal value of 6.15 for simple races and 5.19 for the complex race.

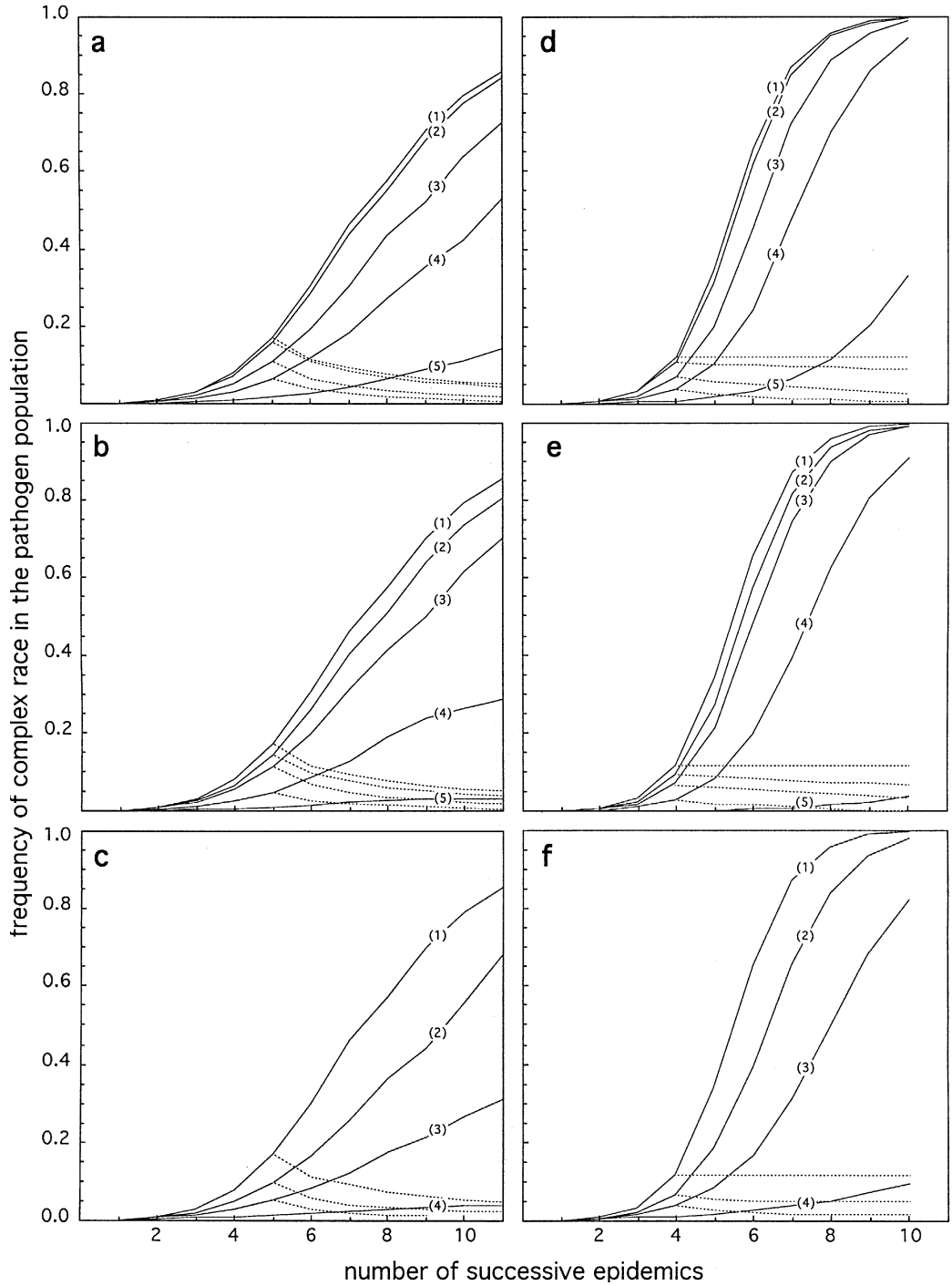

Fig. 1a–f Frequency of the complex race in the pathogen population for a succession of epidemics on the same host mixture (*continuous lines*) and when the mixture composition is changed (*dotted line*). In **a, b** and **c**, the complex race develops focally; in **d, e** and **f** it develops a general epidemic (see text). In **a** and **d**, the effect of the cost of virulence is simulated with Cv = 0 (*1*), 0.01 (*2*), 0.05 (*3*), 0.1 (*4*), 0.2 (*5*); in **1b** and **1e**, the effect of density dependence is simulated with Dd = 0 (*1*), 0.1 (*2*), 0.2 (*3*), 0.5 (*4*), 1.0 (*5*); in **1c** and **1f**, the effect of differential adaptation is simulated with Da = 0 (*1*), 0.01 (*2*), 0.023 (*3*), 0.05 (*4*)

Simulations of the competition between two races with identical multiplication rate, but different initial distributions on the plot, showed that initial inoculum distribution had a large effect on the final race frequencies (Fig. 4 a). This could be explained by considering that inoculum losses during epidemics were greater for focal epidemics (Fig. 4 c and d). Spore losses were of two different kinds: some were transported out of the plot; others were deposited on previously infected

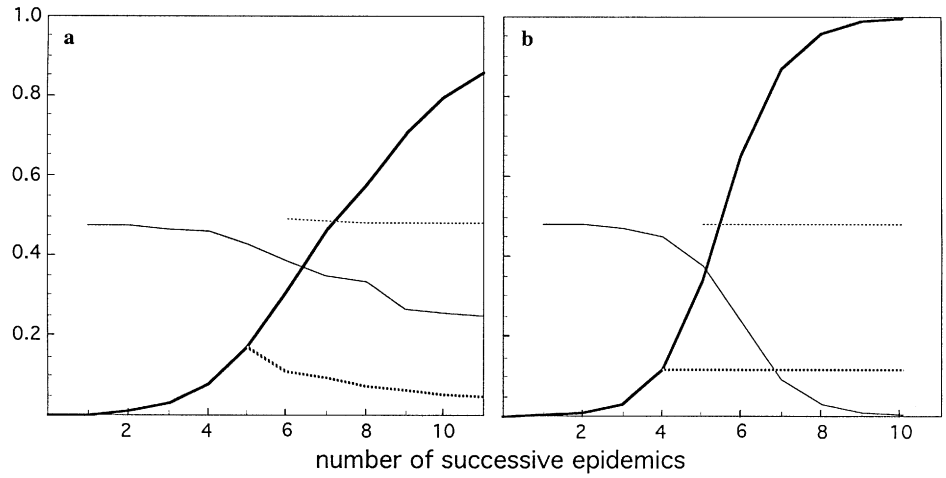

Fig. 2a, b Change of mixture efficacy (*thin solid lines*) in reducing disease severity relative to the mean of components in pure stands when the complex race increases in frequency over time (*thick solid lines*) for focal epidemics (**a**) and general epidemics (**b**). Data are from the same simulations as in Fig. 1, when Cv = 0, Dd = 0 and Da = 0. *Dotted lines* indicate the effect of a change in mixture composition on the frequency of the complex race (*thick dotted lines*) and mixture efficacy (*thin dotted lines*)

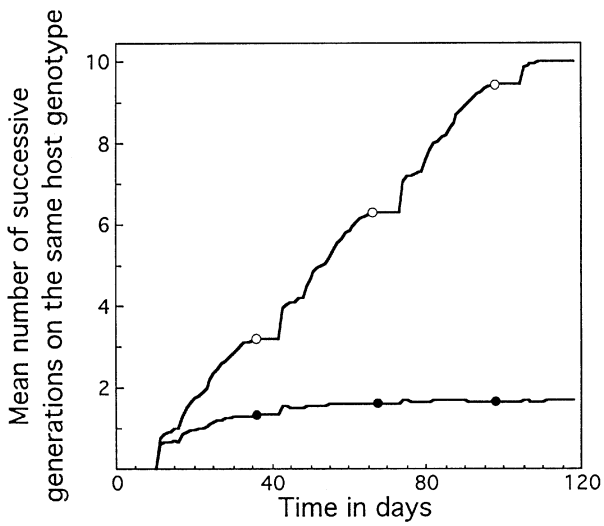

Fig. 3 Mean number of successive generations the pathogen reproduces on the same host genotype. Abscissa: time in days. *White circles*: simple race; *black circles*: complex race. *Circles* indicate the start of a new epidemic. Data are from the same simulations as in Fig. **1c** with Da = 0.01

tissue. The first mechanism predominated in general epidemics but the second one was of much greater importance in focal epidemics (Fig. 4 b).

Discussion

The model we used in this study has been shown to give qualitatively similar responses to epidemic development in a host mixture as has been found in the field. Particularly, the effects of inoculum distribution on simulated epidemics have been compared with experimental results (Mundt et al. 1986). The effects of weather variability and host growth are not accounted for by this simulator, which is considered to model an epidemic in a stable environment (Kampmeijer and Zadoks 1977). Its main advantage, compared to differential equations (Van der Plank 1963; Østergård 1983) describing disease progression in time, is that it allows simulation of an epidemic spread in space, particularly the effect of the spore-dispersal gradient around an infected plant and the distribution of the initial inoculum. These parameters are of importance for a correct description of epidemic progression in host mixtures (Mundt 1989) and also of the simple race-complex race competition for host tissue (Lannou and Mundt 1996). Other models describe with more realism the spread of the inoculum in space (Zavolek and Zadoks 1989; Ferrandino 1993), but they are probably too sophisticated to be used at the same time as the simulation of interactions within the pathogen population.

The choice of the three selective effects introduced in our model was based on a literature review. The cost of virulence is almost always used in models of complex races in host mixtures. It is often described as a cumulative (additive or multiplicative) effect of individual costs associated with each virulence gene in a gene-for-gene (Flor 1946) relationship. We chose here to use a global cost instead, because we did not want to speculate on the nature of the cost or its additivity. To be consistent with other studies (Marshall 1989), our parameter Cv can be decomposed into:

$$Cv = (k - 1)\, s \quad \text{(additive model)} \tag{6a}$$

or,

$$Cv = 1 - (1 - s)^{(k-1)} \quad \text{(multiplicative model)}, \tag{6b}$$

with k representing the number of virulences and s the cost per virulence gene.

Though density dependent fitness has been demonstrated for plant pathogens (Katsuya and Green 1967;

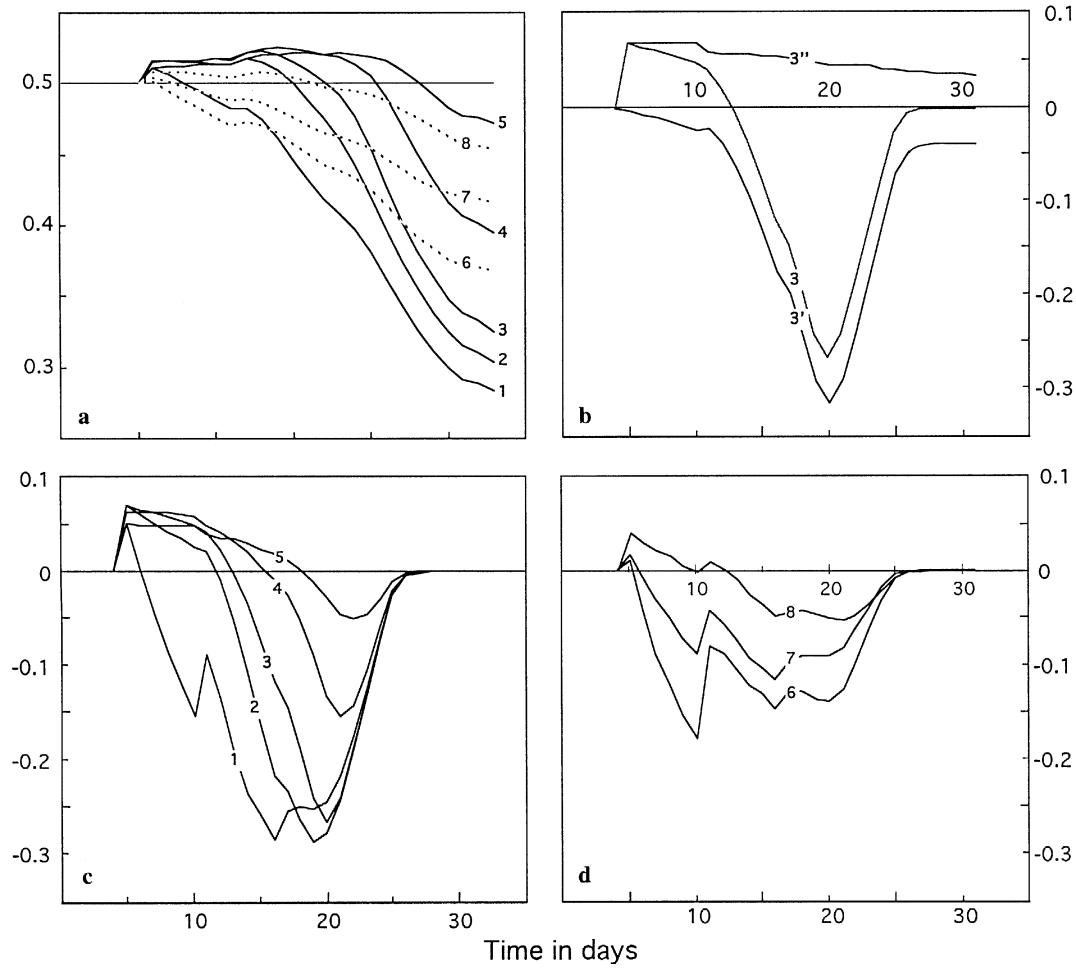

Fig. 4a–d Effect of the spatial distribution of the initial inoculum on the competition of two simple races inoculated on a pure susceptible stand. One race is generally inoculated on each plant and the other is focally inoculated with a single focus of 9 (*1*), 100 (*2*), 256 (*3*), 900 (*4*), 2025 (*5*) plants or with 4 (*6*), 9 (*7*) or 25 (*8*) multiple foci of one plant each, separated by 30, 20 or 10 plant radii respectively. **a** Frequency of the focally inoculated race; **b** differences in the proportion of spores lost during the epidemic (the generally inoculated race minus the focally inoculated race) when the size of the initial focus is 256 plants. *Curve* (*3″*) is for the spores lost out of the plot; *curve* (*3′*) is for the spores lost on previously infected tissue; **c** differences in the proportion of spores lost during the epidemic for single initial foci of various size; **d** differences in the proportion of spores lost during the epidemic for multi-focal inoculations

Kardin and Groth 1989), it is unclear how general such a phenomenon may be. Since our model computed the lesion density on individual plants, we can assume that our parameter Dd was accurate as long as our model's dispersal function is considered realistic.

In Chin and Wolfe's (1984) study, the differential adaptation effect was measured 54 days after sowing, which represents probably 5–10 pathogen generations. In our model we considered that differential adaptation no longer changed the mean reproductive rate of the pathogen after ten generations on the same host (after which the most fit individuals are assumed to have been largely selected within the races). In the field, selection for increased pathogenicity on a host genotype can be faster or slower, and this would influence the rate of increase of the complex race.

An artificiality in our simulations was the transition from year to year. Differences in the survival capacity between races can occur in the field. Also, sexual reproduction is likely to change genotype frequencies or to produce new genotypes for some pathogens. This was not taken into account in our simulations. There are no available data on the inoculum distribution of an airborne pathogen in a plot from one year to another. Since our model has been designed to describe airborne pathogens, and since we did not include immigration effects in our study, we could consider that the inoculum initiating a new epidemic survived on volunteer plants or on plant debris. Considering also that the complex-race epidemic started from two spores deposited at the plot center, it was logical to assume that it took time for the spores of the complex-race to be distributed over the whole plot. At the end of the first

epidemic, the density of complex race spores was higher at the plot center than near the borders. Since the survival rate during summer was supposed to be very small (the total inoculum was reduced to 3600 spores), we considered that the inoculum for the next epidemic should be placed on central plants, where the spore density was previously the highest. In the field, it is likely however that secondary foci of a complex race would occur randomly, and that the complex race would spread throughout the plot faster than in our simulations with a focal spore distribution. To model random spatial distributions of simple and complex races would have required that we replicate simulation runs, which would have been too costly. Data presented in Fig. 4 show that the effect of initial spore distribution on race competition decreased when the focus size increased and when the number of foci increased. However, even in the case of multi-focal inoculations, race frequencies were affected greatly by initial spore distribution. Therefore, we consider that our simulations with a focal and a general distribution of the complex race represented two extreme situations between which the spatial distribution of the pathogen population could be expected.

In a previous study (Lannou and Mundt 1996), we found that high values of the cost of virulence, density dependence or differential adaptation were necessary to obtain a decrease in the complex-race frequency in epidemics where spore distribution was identical for simple and complex races. In the present study, the complex race was disadvantaged by a focal inoculation, in comparison with the simple races. This can explain the very low rate of increase of the complex-race frequency with $Cv = 0.2$ or $Dd = 1$. This could also explain why differential adaptation had a large effect here whereas this parameter had little impact on the progression of the complex race in our previous study. We should also note that, in our earlier paper (Lannou and Mundt 1996), only one epidemic was simulated, during which the mean number of simple-race generations (Ngh) increased up to 3.28 (for the same spore dispersal gradient, $b = 2.5$, as in the present paper). This probably did not allow differential adaptation to express a significant effect. In the present study, Ngh reached its maximal value of 10 during the fourth epidemic.

Few experimental data are available to determine which values are realistic for density dependence, differential adaptation, or even the cost of virulence. Most of the measured values for the cost of virulence range between 0.05 and 0.1 (Leonard 1977; Grant and Archer 1983) but higher values have also been measured (0.30 to 0.39 in Leonard 1977). We found here that, even though a cost of virulence of 0.1 is unable to account for a simple race-complex race equilibrium (Lannou and Mundt 1996), it can reduce significantly the rate of increase of complex-race frequency over a long time. Density dependence had a noticable effect on the complex-race frequency in our simulations for Dd values higher than 0.2. From figure 2 in Kardin and Groth (1989), we calculated a differential density effect between two isolates of $Dd = 0.121$ (isolates U2-1 and S1-1 with a Dd magnitude of 0.998 and 0.877 respectively). This level of density dependence had little effect in our present study. The lack of other data makes it difficult to judge the importance of density dependence in the simple race-complex race competition. Moreover, we assumed here that density dependence affected simple races more than the complex race, although there are no experimental data to support this.

Differential adaptation showed a large effect for values greater than 0.01. From Chin and Wolfe's data (1984, table 6), a value of 0.023 can be calculated for Da by considering that the effect is maximum after ten generations ($Ngh = 10$). Simulations with $Da = 0.023$ resulted in a strong slowing of the complex race's increase in frequency. If more experimental data confirm Chin and Wolfe results, this could mean that differential adaptation is a mechanism of great importance for pathogen evolution in host mixtures.

The disease reduction in the host mixtures relative to pure stands declined with an increasing frequency of the complex race in the pathogen population. Disease reduction in host mixtures is mainly accounted for by inoculum loss on resistant plants (Wolfe 1985) and therefore did not affect the complex race. When the complex race was focally inoculated, a large part of its inoculum was lost on previously infected tissue during the epidemic (see Fig. 4 b) and did not contribute to new infections. This explains why mixture efficacy tended to be higher when the complex-race was focally inoculated in comparison with epidemics where it was generally distributed. Depending on the complex-race inoculum distribution in the host mixture, disease reduction due to mixing could be eroded more or less quickly. When the host composition was changed, the mixture recovered its initial efficacy since part of the complex-race inoculum was then lost on host h3, as for the remaining simple race. At the same time, the frequency of the complex race decreased slowly when a selective effect reduced its multiplication rate or when its inoculum distribution was not generalised. This led to the conclusion that regular changes in host-mixture composition can preserve the efficacy of this epidemic control method in the long term. We must note, however, that the complex race decreased asymptotically and did not disappear after six successive epidemics. Therefore, a complex race is likely to become more frequent if a regular rotation of the same host mixtures is used over a long period. It is difficult to discuss this point in more detail from our results, because it depends mainly on the complex-race's capacity to survive at low frequency during over-seasoning, which is not taken into account in our model. Our simulations were only for two-component mixtures. In three- or four-component mixtures, and in the absence of a "super-race" (Groth 1976) virulent on all the mixture

components, it is likely that the mixture efficacy in controlling epidemics would be less sensitive to an increase in the frequency of races of intermediate complexity.

Until now, most theoretical studies on the simple race-complex race equilibrium in host mixtures were based on the existence of a cost of virulence (Groth 1976; Barrett 1980; Østergård 1983; Marshall 1989). We have here investigated other mechanisms that could also be of importance, particularly differential adaptation, and have shown that differences in spatial distribution between simple and complex races could lead to a strong slowing of the complex-race increase. In a previous study (Lannou and Mundt 1996), we have shown how the complex-race frequency depends on disease dispersal parameters such as the spore dispersal gradient or the initial spore distribution. In the field, it is likely that multiple mechanisms combine to influence the complex-race progression. In addition to the effects tested here, environmental interactions (Welz et al. 1990) and induced resistance (Chin and Wolfe 1984; Lannou et al. 1995) might also limit the development of complex races, as has been suggested in experimental studies (Dileone and Mundt 1994). Further, the strong influence of the spatial pattern of the inoculum in our studies suggests a need for a stochastic treatment of the initial inoculum, and subsequent dispersal, in studying competition between simple and complex pathogen races.

SR	simple race
CR	complex race
b	spore-dispersal gradient [ ]
c	parameter of the spore-dispersal function [m]
SE	spore efficacy [lesions/spore]
SP	daily spore production rate [spores/day/lesion]
Dmfr	daily multiplication rate [lesion/lesion]
CDmfr	daily multiplication rate corrected for density dependance or host adaptation effects [lesion/lesion]
Ngh	number of successive generations on the same host genotype [ ]
Cv	magnitude of the cost of virulence [ ]
Dd	magnitude of the density dependence effect [ ]
Da	magnitude of the differential adaptation effect [ ]
s	cost of virulence in Marshall's (1989) models [ ]
k	number of virulences in a pathogen genotype in Marshall's models [ ]
occ	number of occupied sites in a compartment [ ]
frsi	total number of sites in a compartment [ ]

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