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Abstract

Aerial populations of *Blumeria graminis* f.sp. *hordei* were studied in two French and two Danish regions from 1991 to 1999, at a time of year when only winter barley was present. A high frequency of genotypes not able to grow on the spring-sown crop of the previous growing season (denoted 'spring-avirulent') was observed in most years and regions. This frequency increased with increasing proportion of winter barley; it was highest in France and decreased in general over the 8-year period. Most of the spring-avirulent genotypes possessed the V_{a22} virulence gene, matching a resistance that has never been present in barley cultivars grown in Europe. A hypothetical cropping system, including winter- and spring-sown crops with three resistance genes altogether, was constructed to mimic the utilization of host cultivars in the four regions. Results from a mathematical model simulating changes in the composition of the pathogen population in this system, demonstrated that selection solely due to host resistance genes, i.e. without assuming any cost of virulence, might lead to such results as those observed. The changes in frequency of spring-avirulent genotypes and the frequency of unnecessary virulence genes may be predicted from the proportion of the barley area sown with winter barley, the use of resistance genes in the cultivars, the initial composition of the pathogen population, and hitch-hiking due to gametic disequilibria.

Keywords: avirulence, *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *Hordei* , gametic disequilibrium, hitch-hiking, mathematical model, population genetics, unnecessary virulence genes

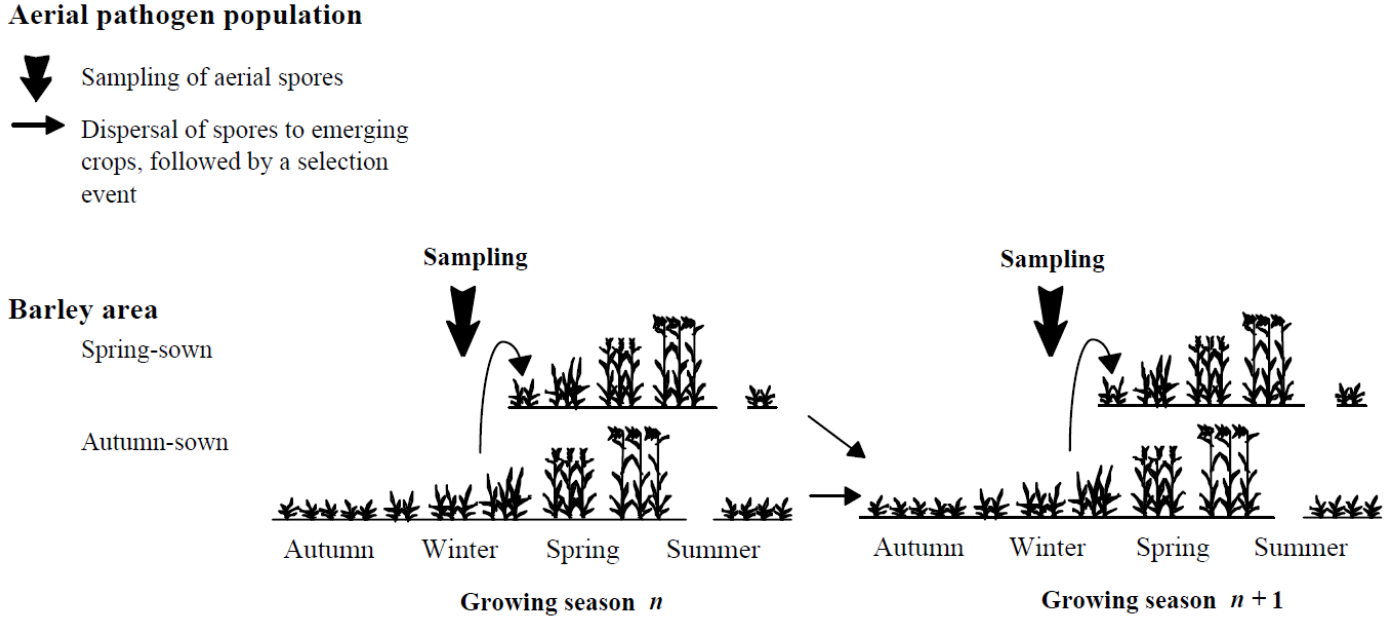
Introduction

The biotrophic fungus *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *hordei*, causing powdery mildew on barley, is common all year round in many areas of Europe, where barley is grown both as an autumn-sown crop (winter barley) and as a spring-sown crop (spring barley). The fungus reproduces asexually on green leaves of the growing host; but may also go through one annual cycle of sexual reproduction, where cleistothecia are produced on senescent host tissue of both spring and winter barley and ascospores are released in autumn (Smedegård-Petersen, 1967; Wolfe & McDermott, 1994). The aerial population at any time of the year consists of a mixture of spores from subpopulations on different host cultivars (Fig. 1). In autumn, spores from both winter and spring cultivars grown in the previous season may infect the newly sown crop.

The composition of the aerial powdery mildew population is strongly influenced by selection caused by host cultivars – a process that is fairly well understood. Virulence genes in the pathogen that are matched by resistance genes in the host according to a gene-for-gene relationship (Jørgensen, 1988) are subject to direct selection (Wolfe, 1984; Hovmøller *et al.*, 1993; Brown, 1994). Large-scale use of a specific resistance gene in an area leads to an increase in frequency of the corresponding 'necessary' virulence gene in the pathogen population, which results in the resistance gene becoming largely ineffective for powdery mildew control in that area. If all cultivars with this resistance gene are then withdrawn from the area, the frequency of the corresponding virulence gene, which is now 'unnecessary', may decrease. This decrease may to some extent be explained by negative two-locus gametic disequilibria and subsequent hitch-hiking due to direct selection for virulence gene(s) matching resistance gene(s) in the new introduced cultivars (Hovmøller & Østergård, 1991; Østergård & Hovmøller, 1991; Hovmøller *et al.*, 1993; Brown, 1994; Hovmøller *et al.*, 1997). Also, a fitness cost of unnecessary virulence genes has been suggested for explaining decreasing gene frequencies in populations of fungal plant pathogens (Vanderplank, 1968; Leonard, 1969; Grant & Archer, 1983; Bronson & Ellingboe, 1986). Unnecessary virulence genes may evolve differently, depending on whether the gene

became unnecessary in the area because cultivars with the corresponding resistance gene were withdrawn (as described above), or whether the corresponding resistance gene has never been utilized in cultivated barley (Munk *et al.*, 1991). In the latter case, the gene is called 'universally' unnecessary. Finally, a specific virulence gene may be alternatively unnecessary, e.g. if it corresponds to a resistance gene that is present only in spring barley cultivars and is unnecessary during winter.

Figure 1. *Schematic description of the population dynamics of host crops and aerial pathogen populations in the barley/powdery mildew pathosystem.*



Observed frequencies of unnecessary virulence genes vary considerably across geographical region and time (Munk *et al.*, 1991; Caffier *et al.*, 1996; Hovmøller *et al.*, 2000). In a study of the aerial population in northern part of France in 1991–93, high frequencies of an unnecessary virulence gene and high frequencies of 'simple genotypes' were found (Caffier *et al.*, 1996). A simple genotype was defined as one possessing few virulence genes and many avirulence genes when assayed on a core set of 10–12 differential cultivars, which have been used in many European virulence survey programmes (reviewed by Hovmøller *et al.*, 2000). Caffier *et al.* (1996) suggested two hypotheses to explain the changes in frequencies of simple genotypes: seasonal changes in aerial spore composition with different proportions of spores originating from winter and spring cultivars where different resistance genes introduce different frequencies among the isolates growing there; or different fitness (differential adaptation) of simple and complex genotypes when growing on any susceptible host cultivar (cost of virulence or genetic background). These hypotheses were based on the observation that winter barley generally possessed no or few (known) resistance genes (e.g. Jensen & Jørgensen, 1981; Caffier & de Vallavieille-Pope, 1996) as compared to spring barley with more complex combinations of resistance genes (e.g. Jensen *et al.*, 1992).

The objectives of the present study were to investigate and expand the hypotheses formulated by Caffier *et al.* (1996) about local evolution of virulence gene combinations in barley powdery mildew populations growing in a cropping system with autumn-sown and spring-sown barley. For this purpose, the expected effects of the cropping system were analysed, based on the theoretical model developed by Østergård & Hovmøller (1991) and modified by Hovmøller *et al.* (1993), and an extended data set was analysed including: (i) powdery mildew data from Denmark, where the distribution of autumn-sown and spring-sown barley is very different from that in France and the use of resistance genes in spring barley is more diverse; and (ii) additional French data from 1994 to 1998. Instead of focusing on the dynamics of simple genotypes, isolates were studied that would not have been able to grow on spring barley in each region in the previous growing season, designated 'spring-avirulent'. The reason for this choice is that spring-avirulent and not

simple genotypes relate directly to the selection regime and the relative fitness of the genotypes when infecting winter and spring crops, respectively. Changes in frequency of spring-avirulent genotypes and unnecessary virulence genes were simulated in a hypothetical cropping system including winter and spring sown barley with three resistance genes altogether. They were analysed to determine whether they could occur without selection forces other than those induced by host-resistance genes.

Materials and methods

Powdery mildew sampling

Samples of aerial powdery mildew populations in France and Denmark were collected as single-colony isolates from autumn to early spring at a time when no spring barley was present (Fig. 1). A barley-growing season (the time from emergence of the winter crop until harvest of the spring crop) comprises two calendar years, and the samples were designated accordingly. A total of 3235 single-colony isolates were analysed during the study (Table 1). In France, the isolates were collected on detached leaves of cv. Igri (possessing resistance gene *Mlra*) using a car-mounted spore trap moving along highways in two regions, north of Paris (Paris–St Omer) designated 'F-N', and east of Paris (Paris–Metz or Verdun) designated 'F-E'. Samples were collected in the seasons 1991/92 to 1994/95, and 1996/97 to 1998/99. Samples from 1991/92 and 1992/93 have been analysed in part elsewhere (Caffier *et al.*, 1996). In Denmark, samples were collected on trap plants of the same cultivar (Igri), which were exposed in fixed trays at two locations, Foulum in the west of Denmark (Jutland), designated 'DK-W', and Risø in east Denmark (Danish islands), designated 'DK-E'. The trap plants were exposed at a distance of at least 500 m from the nearest barley field.

Table 1. *Basic data on pathogen populations and crop. Sample sizes and percentage of spring-avirulent isolates (sp-avr) in populations of Blumeria graminis f.sp. hordei sampled from autumn to early spring in different growing seasons and regions, and proportion of winter barley in spring and of spring barley with unknown resistance genes (spring-unknown)*

		Pathogen population		Proportion of crop[b] (%)	
Region[a]	Years	Sample size	sp-avr (%)	Winter	Spring-unknown
France					
F-N	1990/91	–	–	91·6	0·7
	1991/92	196	69·4	84·3	0·8
	1992/93	106	67·0	72·6	0·0
	1993/94	67	23·9	69·7	0·1
	1994/95	21	23·8	71·3	0·0
	1996/97	39	7·7	md[c]	md
	1997/98	108	6·5	73·7	0·1
	1998/99	46	23·9	–	–
F-E	1990/91	–	–	69·0	1·3
	1991/92	260	28·8	66·8	0·6
	1992/93	83	34·9	58·5	0·1
	1993/94	35	5·7	58·8	0·1
	1994/95	64	15·6	61·6	1·5
	1996/97	91	0·0	md[c]	md
	1997/98	135	5·9	61·0	0·3
	1998/99	87	4·6	–	–
Denmark					
DK-E	1990/91	–	–	20·7	0·0
	1991/92	46	2·2	25·9	0·1
	1992/93	259	4·2	36·7	0·1
	1993/94	160	5·6	33·8	0·5
	1994/95	405	2·0	39·2	0·4
	1995/96	96	12·5	41·7	0·0
	1996/97	168	0·0	33·6	0·5
	1997/98	41	4·9	–	–
DK-W	1990/91	–	–	11·2	0·0
	1991/92	21	0·0	12·7	0·2
	1992/93	183	1·6	18·8	0·2
	1993/94	253	0·0	26·2	0·5
	1994/95	215	0·5	23·9	0·6
	1997/98	50	2·0	–	–

[a] The French samples are north of Paris (F-N) and east of Paris (F-E), respectively, and the Danish samples are Danish islands (DK-E) and Jutland (DK-W), respectively.
[b] From the proportion of winter crop (Winter) the proportion of spring crop can be calculated as 1-Winter
[c] Missing data for the crop 1996/97 in F-N and F-E. For the determination of spring-avirulent pathotypes in 1996/97, data from 1995/96 and 1997/98 were used to estimate the gene combinations present in the 1996/97 spring-sown crop.

Deployment of barley cultivars and distribution of resistance genes

Four regions were studied: in France, north of Paris designated 'F-N' and east of Paris designated 'F-E'; and in Denmark, west Denmark (or Danish islands) designated 'DK-W' and east Denmark (or Jutland) designated 'DK-E'. Information about areas of barley cultivars possessing different resistance gene combinations has been collected for winter and spring barley crops, respectively, in the regions where barley powdery mildew was sampled (Torp *et al.*, 1978; Jensen & Jørgensen, 1981; Anon., 1983–84; Rasmussen, 1985–87; Rasmussen, 1988; Jensen & Boesen, 1989–90; Anon., 1990–97; Anon., 1991–99; Deneken & Boesen, 1991–96; Brown & Jørgensen, 1991; Jensen *et al.*, 1992; Caffier & de Vallavieille-Pope, 1996; Deneken, 1997). Bousset *et al*. (2000) compiled this information, and the proportion of winter barley crops was calculated for each season. The resistance gene combinations included the resistance genes *Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mla22*, *Mlk*, *Mlg*, *MlLa* and *Mlh*. The four regions were different with respect to proportions of winter barley: 10–25% (DK-W), 20–40% (DK-E), 55–70% (F-E) and 70–90% (F-N) (Table 1). Most winter cultivars had no resistance genes, or had genes that were ineffective for powdery mildew control due to corresponding virulence frequencies close to 100% in the pathogen population (Bousset *et al.*, 2000). In France, resistance combinations in winter barley cultivars often included *Mlh* (up to 47% of the winter barley area); in Denmark, combinations often included *Mla7* (up to 26%), *Mlg* (up to 24%) and *Mla6* (up to 9%). In the spring crops, many different combinations were present at the same time, often including at least one allele from the *Mla* locus and one or more of the genes *Mlg*, *Mlk*, *MlLa* and *mlo*. Cultivars with specific combinations appeared and disappeared from the regions during the course of the study. No cultivar possessed the resistance gene *Mla22*. For a few cultivars grown in a limited area, the resistance genes were not identified (Table 1).

Virulence assays

Sampled isolates were assayed for virulence on a differential set of 12 near-isogenic Pallas lines reflecting most of the known barley powdery mildew resistance genes utilized in either France or Denmark in the period considered: P01 (*Mla1*), P02 (*Mla3*), P03 (*Mla6*), P04B (*Mla7*), P08B (*Mla9*), P10 (*Mla12*), P11 (*Mla13*), P12 (*Mla22*), P16 (*Mlk*), P21 (*Mlg*), P23 (*MlLa*) and P24 (*Mlh*) (Kølster *et al.*, 1986). The line P12 (*Mla22*) was not used in Denmark in 1991/92 and 1992/93. The line P02 (*Mla3*) was not used in France in 1997/98, and P24 (*Mlh*) was not used in France in 1991–94 and 1996/97.

The virulence tests were performed on detached leaf segments of the Pallas lines maintained on water agar 4–5 g L^{-1} and 0·3–0·35 g L^{-1} benzimidazole. Inoculated leaves were incubated for 8 days (16°C, continuous light 10 μE m^{-2} s^{-1}) in France, and 10–12 days (16–18°C, 18 h light 20 μE m^{-2} s^{-1}) in Denmark. Infection types were assessed using a 0–4 scale (Moseman *et al.*, 1965), and genetic interpretation in terms of virulence and avirulence genes corresponding to the considered resistance genes was carried out according to Hovmøller *et al*. (1995). For simplicity, observed genotypes (also designated pathotypes) were named according to the presence of virulence genes only – an isolate of genotype $V_{a6}V_{a22}$ was able to grow on P03 and P12 and on no other line in the differential set. As a substantial number of isolates were not tested on P24, the corresponding virulence gene was not included in the genotype, i.e. only 11-locus genotypes are considered in the following. However, 60 French isolates of specific 11-locus genotypes (V_{a22} or $V_{a6}V_{a22}$) from these years have subsequently been assayed on P24.

Spring-avirulent isolates

For each sample of airborne spores, a specific class of isolates (denoted spring-avirulent isolates) was considered. Based on their virulence and avirulence patterns, could not have originated from spring barley in the region studied of the previous growing season. Consequently, these isolates would have been produced on the previous winter crop or would have been immigrants. For each sample, spring-avirulent genotypes were defined using information about resistance genes in the grown cultivars in the four regions (Bousset *et al.*,

2000). The actual observed number of spring-avirulent isolates was then calculated for each sample. Note that a spring-avirulent genotype in a certain region and year may not be spring-avirulent in another region or year.

The model

The model describes evolution in a pathogen population in an area where both winter and spring crops are grown. A complete description of equations and basic features of the general model has been given by Østergård & Hovmøller (1991); Hovmøller *et al*. (1993); Hovmøller *et al*. (1997). Briefly, the features of the model are (i) pathogen reproduction is asexual only; (ii) mutation and migration of spores to and from the considered barley area are ignored; (iii) selection for virulence takes place mainly twice a year when aerial spores are dispersed on the emerging host crop, that is, in autumn on winter barley and in spring on spring barley (Fig. 1), and selection coefficients are given by the relative area of each cultivar at the time of selection; (iv) spores of different genotypes able to infect the same cultivar are assumed to produce the same number of offspring on that cultivar; (v) avirulent spores are not able to reproduce; (vi) the relative proportion of green-leaf area of different cultivars is constant from season to season; (vii) the number of spores per unit area infecting the emerging spring barley crop is of the same order of magnitude as the number infecting the emerging winter crop; and (viii) the total number of colonies produced by the pathogen during the lifetime of a susceptible host crop is of the same order of magnitude for winter and spring crops, respectively.

Let m cultivars be grown, and the relative area of a cultivar j in spring time in season n designated $s_{j(n)}$ Then $S_{(n)} = \Sigma_{j=\text{winter}}\, s_{j(n)}$ is the relative area of the winter crop in that spring, and $s_{j(n)}/S_{(n)}$ is the relative area in winter of season n of winter cultivar j. Let $h_{i(n)}$ denote the frequency of spores of genotype i in the aerial population being released from the winter barley crops during winter time in season n ($i = 1, \ldots, L$), where L equals the number of possible virulence gene combinations corresponding to the resistance genes in the cultivars grown ($\Sigma_i h_{i(n)} = 1$).

The genotype frequencies in the aerial population *after selection* by the winter crop of season $n + 1$, $h_{i(n+1)}$, can be expressed as follows (elaborated from Hovmøller *et al*. (1993), where frequency of spores of genotype i in the aerial population *infecting* the winter crop was considered):

$$h_{i\,(n+1)} = h_{i(n)} \times (S_{(n)} + \Sigma_{j=\text{spring}}\, u_{ij} \times s_{j(n)}) \times (\Sigma_{j=\text{winter}}\, u_{ij} \times s_{j(n+1)}/S_{(n+1)})/w_{(n)} \qquad (1)$$

where $u_{ij} = 1$ if genotype i is virulent on cultivar j and $u_{ij} = 0$ if genotype i is avirulent. The normalizing factor $w_{(n)}$ is defined such that $\Sigma_i h_{i(n+1)} = 1$.

Simulations in a hypothetical cropping system

A simplified cropping system including two winter cultivars and one spring cultivar was developed, being the most simple system in which the evolution of spring-avirulent genotypes and genotypes with an unnecessary virulence gene could be studied. Three resistance genes, R_x, R_y and R_z, were considered (Table 2). The winter crop consisted of cultivar A with resistance gene R_y and cultivar B with no resistance gene. The spring crop consisted of cultivar C with resistance gene R_z. Cultivars with resistance gene R_x were not grown. In the pathogen population, the three corresponding virulence genes, V_x, V_y and V_z, were initially found in frequencies p_x, p_y and p_z, respectively. Eight virulence genotypes were present (for convenience these are designated by the full combination of alleles) and the compatibility matrix (u_{ij}) between the three cultivars and the virulence genotypes is shown in Table 2. The virulence gene V_x was unnecessary and genotypes without V_z were spring-avirulent. Gametic disequilibria values that would give the largest changes in genotype frequencies were calculated using formulae in Robinson *et al*. (1991). Initial genotype frequencies were calculated from virulence gene frequencies and gametic disequilibria (D_{ab} = gametic disequilibrium between virulence genes V_a and V_b), as in Østergård & Hovmøller (1991). Virulence genotype frequencies in the following season were calculated according to Eqn 1. From these, the new frequencies of virulence genes, frequencies of certain virulence combinations, and gametic disequilibria were calculated. The long-term composition of the pathogen population will consist of the genotypes $V_xV_yV_z$ and $A_xV_yV_z$ only, but until this occurs the frequencies of the eight different genotypes, and also those of the spring-avirulent genotypes and genotypes with

unnecessary virulence genes, might increase or decrease depending on the initial composition of the population. The local evolution of the pathogen population was considered for 10 growing seasons.

Table 2. *Compatibility matrix between three hypothetical cultivars and eight pathogen genotypes used in the simulations. Virulence gene V_x corresponding to resistance gene R_x was universally unnecessary*

		Pathogen genotype and frequency							
Cultivars[a]	Area in spring	$V_xV_yV_z$ $h_{1(n)}$	$V_xV_yA_z$[c] $h_{2(n)}$	$V_xA_yV_z$ $h_{3(n)}$	$A_xV_yV_z$ $h_{4(n)}$	$V_xA_yA_z$[c] $h_{5(n)}$	$A_xV_yA_z$[c] $h_{6(n)}$	$A_xA_yV_z$ $h_{7(n)}$	$A_xA_yA_z$[c] $h_{8(n)}$
Winter	$S_{(n)}$								
A (R_y)	$s_{A(n)}$	1[b]	1	0	1	0	1	0	0
B (no)	$s_{B(n)}$	1	1	1	1	1	1	1	1
Spring	$1–S_{(n)}$								
C (R_z)	$s_{C(n)}$	1	0	1	1	0	0	1	0

[a] Resistance alleles are indicated in brackets.
[b] Value indicates virulence (1) or avirulence (0) of the genotype on the cultivar and corresponds to the compatibility u_{ij} of isolate i on cultivar j.
[c] Spring-avirulent genotypes.

The frequency of spring-avirulent genotypes $h_{sp\text{-}avr(n)}$ in season n is (see Table 2):

$$h_{sp\text{-}avr\ (n)} = h_{2(n)} + h_{5(n)} + h_{6(n)} + h_{8(n)} \quad (2)$$

In the following season $n + 1$, the frequency can be calculated from Eqns 1 and 2 and Table 2:

$$h_{sp\text{-}avr\ (n+1)} = [S_{(n)}h_{2(n)} + S_{(n)}(s_{B(n+1)}/S_{(n+1)})h_{5(n)} + S_{(n)}h_{6(n)} + S_{(n)}(s_{B(n+1)}/S_{(n+1)})h_{8(n)}]/w_{(n)} \quad (3)$$

with

$$w_{(n)} = h_{1(n)} + h_{4(n)} + S_{(n)}(h_{2(n)} + h_{6(n)}) + (s_{B(n+1)}/S_{(n+1)})(h_{3(n)} + h_{7(n)}) + S_{(n)}(s_{B(n+1)}/S_{(n+1)})(h_{5(n)} + h_{8(n)}) \quad (4)$$

From Eqns 3 and 4 it can be concluded that the changes in total frequency of the spring-avirulent genotypes do not depend on the frequencies of genotypes with the unnecessary virulence gene V_x, as such genotypes cancel out when $h_{sp\text{-}avr(n+1)}$ is calculated.

In the case when the areas of all cultivars are kept constant in successive generations:

$$S = S_{(n+1)} = S_{(n)}$$
$$s_A = s_{A(n+1)} = s_{A(n)}$$
$$s_B = s_{B(n+1)} = s_{B(n)}$$
$$s_C = s_{C(n+1)} = s_{C(n)}$$

the proportion of spring-avirulent genotypes on the winter crop in the following season is (see Eqns 3 and 4):

$$h_{sp\text{-}avr\ (n+1)} = [S(h_{2(n)} + h_{6(n)}) + s_B(h_{5(n)} + h_{8(n)})]/w_{(n)} \quad (5)$$

with

$$w_{(n)} = h_{1(n)} + h_{4(n)} + S(h_{2(n)} + h_{6(n)}) + s_B(h_{5(n)} + h_{8(n)}) + (s_B/S)(h_{3(n)} + h_{7(n)}) \quad (6)$$

Equations 5 and 6 show that, even in this simple case with three resistance genes, the frequency of spring-avirulent genotypes changes with the proportion of the winter crop, S, in a complex way. However, when further s_B/S is kept constant, $h_{sp\text{-}avr(n+1)}$ is an increasing function of S.

Changes in $h_{sp\text{-}avr}$ from one season to the next can be expressed as:

$$h_{sp\text{-}avr\ (n+1)} - h_{sp\text{-}avr\ (n)} = K_{(n)}/w_{(n)} \quad (7)$$

where

$$K_{(n)} = (1 - h_{sp\text{-}avr(n)})\,[S(h_{2(n)} + h_{6(n)}) + s_B(h_{5(n)} + h_{8(n)})] - h_{sp\text{-}avr(n)}\,[(h_{1(n)} + h_{4(n)}) + (s_B/S)\,(h_{3(n)} + h_{7(n)})] \quad (8)$$

The frequency of spring-avirulent genotypes decreases between seasons if $K_{(n)}$ is negative and increases if $K_{(n)}$ is positive. The conditions that determine what happens in general are complex. For a special case, when there is no resistance gene in the winter crop ($s_A = 0$ and thus $S = s_B$), Eqn 8 may be simplified to:

$$K_{(n)} = s_B(1 - h_{sp\text{-}avr(n)})h_{sp\text{-}avr(n)} - h_{sp\text{-}avr(n)}(1 - h_{sp\text{-}avr(n)}) \quad (9)$$

which is negative ($s_B < 1$), or null if $s_B = 1$, that is, if no spring cultivars are grown. Therefore, in a cropping system with both winter and spring cultivars and with no resistance gene in winter cultivars, the frequency of spring-avirulent genotypes will always decrease. This also holds true if the area with the resistant winter cultivar is small. In conclusion, $s_A > 0$ is a necessary condition for the total frequency of spring-avirulent genotypes to increase.

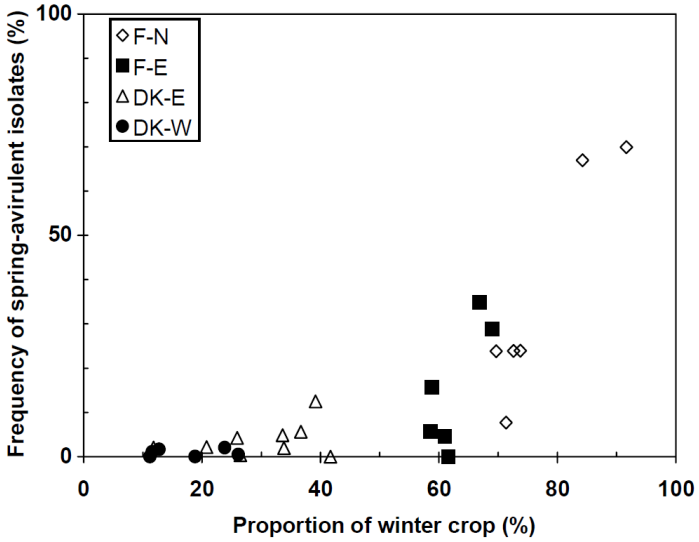
With calculations similar to Eqns 5–8 for $h_{5(n)}$ and $h_{8(n)}$, respectively, it can be shown that, independently of the proportion of winter cultivars, the two spring-avirulent genotypes avirulent on *Ry* will always decrease in frequency.

Results

Observations of spring-avirulent genotypes in France and Denmark

At the time of the year when only winter barley crops were present in each of the four regions, isolates avirulent on all spring barley cultivars of the previous growing season (spring-avirulent) were observed in 22 out of 26 samples constituting all together 13% (425 isolates) of the 3235 isolates sampled (Table 1). The frequencies of these isolates varied with location and year of sampling and were generally higher in France than in Denmark, higher in F-N (up to 70%) than in F-E (up to 35%), and higher in DK-E (up to 13%) than in DK-W (up to 2%). No frequencies as high as those observed in France in 1991/92 and 1992/93 were observed later in France, or at any time in Denmark. The highest proportion of winter barley was observed in the northern part of France in 1990/91 (92%) and 1991/92 (84%). In general, the frequency of spring-avirulent isolates increased with increasing proportion of winter barley in the previous season. This trend was most pronounced within regions (Fig. 2).

Figure 2 *Relationship between the frequencies of spring-avirulent isolates and the proportion of winter barley in the previous growing season in powdery mildew populations sampled in the two French and two Danish regions.*



Fifty-five per cent of spring-avirulent isolates possessed the universally unnecessary virulence gene corresponding to *Mla22* and had avirulence genes corresponding to 10 (or maybe nine for the few French isolates from 1997/98) other resistance genes that were used for assaying the isolates (first genotype given in Table 3). This overall most frequent spring avirulent genotype was found only in France. Twenty-four isolates of this genotype sampled from 1994/95 to 1998/99 were, in addition, tested on P24(*Mlh*), being present in many winter barley cultivars grown in France prior to 1994 (Bousset *et al.*, 2000). All isolates but one (96%) were virulent on P24, indicating that most of these spring-avirulent isolates had the genotype $V_{a22}V_h$ (data not shown).

Table 3. *Number and 11-locus virulence genotype of spring-avirulent isolates over years in powdery mildew aerial populations sampled from the four regions in autumn to early spring*

Genotype[a]													Season[c]							
V_{a1}	V_{a3}	V_{a6}	V_{a7}	V_{a9}	V_{a12}	V_{a13}	V_{a22}	V_k	V_g	V_{La}	Total	Region	91/92	92/93	93/94	94/95	95/96	96/97	97/98	98/99
–	–	–	–	–	–	–	V_{a22}	–	–	–	232	F-N	89	45	12	5	m	1	4[d]	6
												F-E	42	18	0	7	m	0	1[d]	2
												DK-E	0	0	0	0	0	0	0	m
												DK-W	0	0	0	0	m	m	0	m
–	–	V_{a6}	–	–	–	–	V_{a22}	–	–	–	52	F-N	10	3	3	0	m	1	2[d]	3
												F-E	7	0	0	1	m	0	5[d]	1
												DK-E	0	0	5	0	8	0	2	m
												DK-W	0	0	0	0	m	m	1	m
–	–	–	–	–	–	–	V_{a22}	–	–	V_{La}	25	F-N	12	11	0	0	m	0	0	0
												F-E	2	0	0	0	m	0	0	0
												DK-E	0	0	0	0	0	0	0	m
												DK-W	0	0	0	0	m	m	0	m
–	–	V_{a6}	–	–	–	–	V_{a22}	–	V_g	V_{La}	20	F-N	6	1	0	0	m	v	v	v
												F-E	7	1	0	0	m	v	v	v
												DK-E	v	2[d]	2	1	0	v	v	m
												DK-W	v	0	0	0	m	m	v	m
–	–	V_{a6}	–	–	–	–	V_{a22}	–	V_g	–	9	F-N	1	1	0	0	m	v	v	v
												F-E	3	4	0	0	m	v	v	v
												DK-E	0	0	0	0	0	v	v	m
												DK-W	0	0	0	0	m	m	v	m
–	–	V_{a6}	–	–	–	–	–	–	–	–	8	F-N	0	0	0	0	m	0	0	1
												F-E	0	0	0	2	m	0	1[d]	0
												DK-E	0	0	1	0	3	0	0	m
												DK-W	0	0	0	0	m	m	0	m
–	–	–	–	–	–	–	V_{a22}	V_k	–	–	8	F-N	4	3	0	0	m	0	0	0
												F-E	1	0	0	0	m	0	0	0
												DK-E	0	0	0	0	0	0	0	m
												DK-W	0	0	0	0	m	m	0	m
–	–	–	–	–	–	–	–	–	V_g	V_{La}	7	F-N	0	0	0	0	m	v	v	v
												F-E	0	0	0	0	m	v	v	v
												DK-E	v	0	0	7	0	v	v	m
												DK-W	v	0	0	0	m	m	v	m
–	–	–	–	–	–	–	V_{a22}	–	V_g	–	5	F-N	0	1	0	0	m	v	v	v
												F-E	2	1	1	0	m	v	v	v
												DK-E	0	0	0	0	0	v	v	m
												DK-W	0	0	0	0	m	m	v	m
Other genotypes[b]											59	F-N	14	6	1	0	m	1	1	1
												F-E	11	5	1	0	m	0	1	1
												DK-E	1	9	1	0	1	0	0	m
												DK-W	0	3	0	1	m	m	0	m

[a] –, Avirulence gene; V_{a6}, virulence gene corresponding to *Mla6* etc. The virulence gene V_h is not indicated in this table, but as the resistance gene *Mlh* was not present in any spring barley cultivars in both countries, this gene does not contribute to the definition of spring-avirulent isolates.

[b] Sum of the isolates belonging to genotypes (defined on the 11 loci) that occurred less than five times when all samples were considered.

[c] m, Missing data; v, genotype not spring-avirulent in this season.

[d] For the season 97/98, the isolates were not assessed for V_{a3} in France. For the seasons 91/92 and 92/93 the isolates were not assessed for V_{a22} in Denmark. As the resistance gene *Mla3* was not present in any spring barley cultivars in France, and the resistance gene *Mla22* was not present in any spring barley cultivars in Denmark, this has no consequences for the definition of spring-avirulent isolates.

The overall second most frequent genotype was $V_{a6}V_{a22}$, which was also the most frequent spring-avirulent genotype in Denmark. All Danish isolates of this genotype were virulent on *Mlh*, and all French isolates that were retested on P24(*Mlh*) (28 isolates) were also virulent (data not shown). Again, a high frequency of the combination $V_{a22}V_h$ was found. The remaining seven most frequent genotypes possessed from one to four virulence genes. Some genotypes were detected in both countries, e.g. $V_{a6}V_{a22}$, and some in only one country (e.g. $V_{a22}V_{La}$ in France and V_gV_{La} in Denmark). The less frequent spring-avirulent genotypes constituted 14% of the spring-avirulent isolates and possessed from zero to six virulence genes among the 11 listed (data not shown).

Predicted local evolution of spring-avirulent genotypes in northern France

The most frequent spring-avirulent 11-locus genotype in F-N possessed the universally unnecessary virulence gene V_{a22} (Table 3), and in most cases, isolates of this genotype also possessed the virulence gene V_h (see above). The evolution of genotypes with this characteristic – spring-avirulent and possessing an unnecessary virulence gene – were analysed by simulations of the changes in composition of a pathogen population in the hypothetical cropping system (Table 2). The three resistance genes considered in the hypothetical cropping system corresponded to *Mla22*, *Mlh* and *Mla12*, respectively; *Mla22*(*Rx*) has never been used, *Mlh* (*Ry*) has been widely used in winter barley, and *Mla12*(*Rz*) has been much used in spring barley. The initial virulence gene frequencies for $V_x = V_{a22}$, $V_y = V_h$ and $V_z = V_{a12}$ were taken from the F-N sample 1991/92 (Caffier *et al.*, 1996), and gametic disequilibria values that would give the largest changes in genotype frequencies were calculated using formulae given by Robinson *et al*. (1991) (Table 4, population 1). The initial frequencies of isolates belonging to genotypes defined as spring-avirulent were $h_{2(0)} = 0{\cdot}43$; $h_{5(0)} = 0{\cdot}06$; $h_{6(0)} = 0{\cdot}19$ and $h_{8(0)} = 0{\cdot}01$ for $V_xV_yA_z$, $V_xA_yA_z$, $A_xV_yA_z$ and $A_xA_yA_z$, respectively, representing a total frequency of $h_{sp\text{-}vr(0)} = 0{\cdot}69$. Of these, $V_xV_yA_z$ mimicking $V_{a22}V_hA_{a12}$ (or $V_{a22}V_h$ using the simple notation) was interesting according to the observations. Initially it had the highest frequency of the four spring-avirulent genotypes.

Table 4. *Values of virulence gene frequencies and gametic disequilibria in initial pathogen populations used in Figs 3–5*

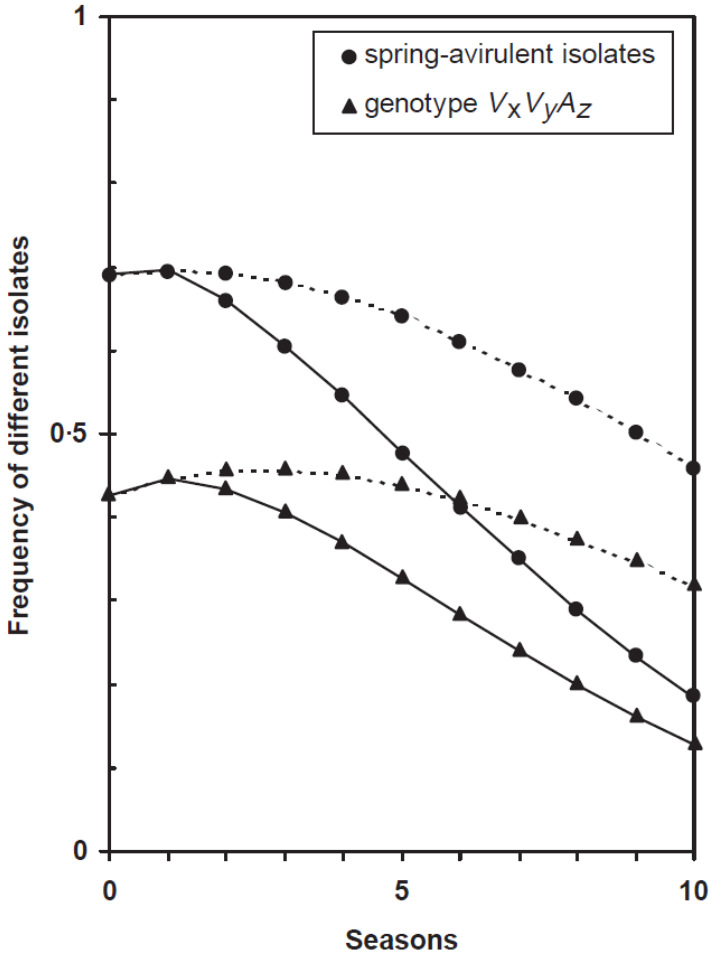
	Virulence gene frequencies			Gametic disequilibria[a]			
Population	$p(V_x)$	$p(V_y)$	$p(V_z)$	D_{xy}	D_{xz}	D_{yz}	D_{xyz}
1	0·62	0·75	0·31	0·030	−0·064	−0·103	0·023
2	0·48	0·49	0·51	0·235	−0·235	−0·240	−0·004
3	0·17	0·17	0·20	0·112	−0·005	−0·005	−0·012

[a] Gametic disequilibria were chosen to give large changes in genotype frequencies: D_{xy}, large positive; D_{yz} and D_{xz}, large negative values using formulae given by Robinson *et al*. (1991).

Data for cultivar use in this region were found in Bousset *et al*. (2000). The winter crop constituted 84% of the barley area in F-N in 1991/92 ($S = 0{\cdot}84$). The resistance gene *Mlh* was present at 35% of the winter barley area (here, cultivar A with the area s_A in the simulation, $s_A/S = 0{\cdot}35$). In addition to cultivar A, the winter barley crop consisted of a susceptible cultivar B (area s_B) referring to cultivars with *Mlra*, for which the virulence frequency was close to 100% in the aerial population, or to cultivars with no identified resistance genes. The resistance gene in the spring crop, *Mla12*, was used in only 50% of the actual spring barley area, the remaining area grown with cultivars having other resistance genes. Simulations were made with cultivar C (area s_C with *Rz*) equal to 50% of the spring barley and the remaining 50% with no resistance genes, as well as with cultivar C grown all over the spring barley area ($s_C/1 - S = 1{\cdot}0$); in both cases a similar pattern of pathogen evolution was obtained (data not shown) so it was decided to show only the latter results subsequently (cf. Table 2). The simulations over 10 growing seasons, with the winter crop proportion fixed as in the first season, demonstrated a slight increase followed by a decrease in frequency of all spring-avirulent isolates and of genotype $V_xV_yA_z$ with the unnecessary virulence gene (Fig. 3). Similar patterns were observed in simulations with proportions of winter barley decreasing over seasons according to actual proportions observed in F-N (Fig. 3). However, the decrease in frequencies occurred earlier when the proportion of winter crop was decreasing over years. This result can be predicted from the mathematical analysis (Eqns 5 and 6). These predictions, were to a large extent, in accordance with the expected dynamics of genotype $V_{a22}V_h$ based on available incomplete data (above and unpublished; Andrivon, 1991).

Figure 3 *Simulated evolution over 10 seasons showing frequency of spring-avirulent isolates and frequency of the genotype $V_xV_yA_z$ with an unnecessary virulence gene, with initial values corresponding to data in the north of France (F-N) in 1991/92. Proportion of winter crop was kept constant at its initial value (0·84, broken lines), or evolved according to the observed data (solid lines). The winter crop consisted of cultivars with the resistance gene Ry mimicking Mlh (35% of the winter barley area) or without resistance genes. The spring*

crop consisted of cultivars with the resistance gene Rz mimicking Mla12. Resistance gene Rx mimicking Mla22 was not present in the crops. See Table 4 for values of virulence frequencies and gametic disequilibria in the initial pathogen population (population 1).

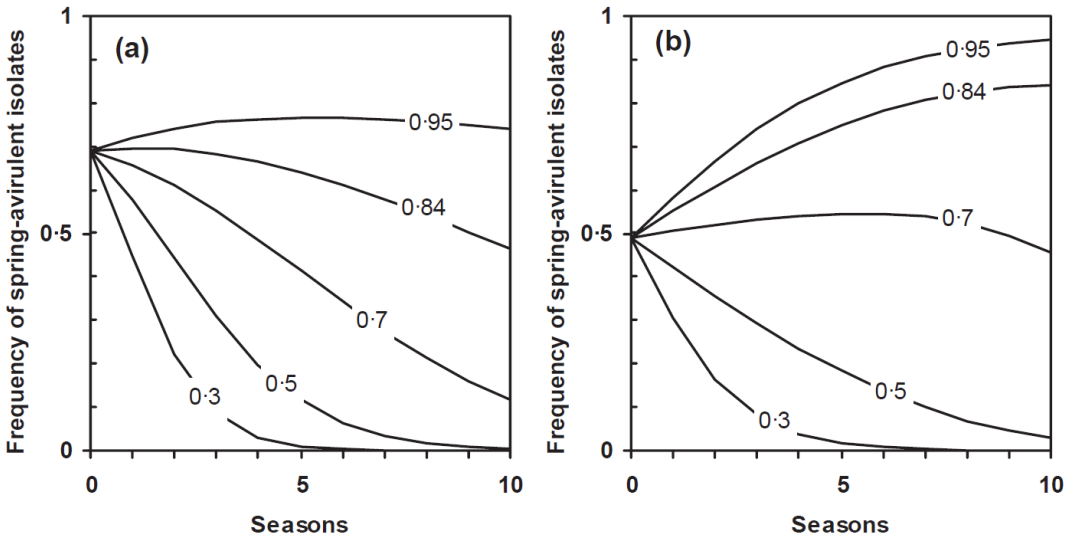


Simulations were also made with decreasing proportions of *Ry* according to the observed data for *Mlh*. The frequency of winter barley cultivars possessing *Mlh* constituted in 1991/92 35% of the winter area and decreased to 19% in 1993/94 and 4% in 1997/98 (Bousset *et al.*, 2000). The resulting changes in genotype frequencies of the pathogen population were similar to those with *Ry* constant (data not shown).

Predictions for other compositions of host crops and pathogen populations

Further analysis was done to demonstrate how the evolution would have occurred with different proportions of the winter crop. Different simulations were made for 10 seasons with the same initial pathogen population as described previously (population 1), the same constant use of resistance genes in the host cultivars (s_A/S = 0·35), and proportions of the winter crops (S) with fixed values of 0·95, 0·84, 0·7, 0·5 and 0·3, respectively. For decreasing values of S, the decrease in frequency of spring-avirulent isolates occurred earlier and the slope of the curve was steeper (Fig. 4a).

Figure 4 *Simulated evolution over 10 seasons showing frequency of spring-avirulent isolates for proportion of winter crops of 0·95, 0·84, 0·7, 0·5 and 0·3 respectively. The use of cultivars was as described in Fig. 3. Initial pathogen population was as population 1 (Table 4) in (a) and as population 2 (Table 4) in (b).*

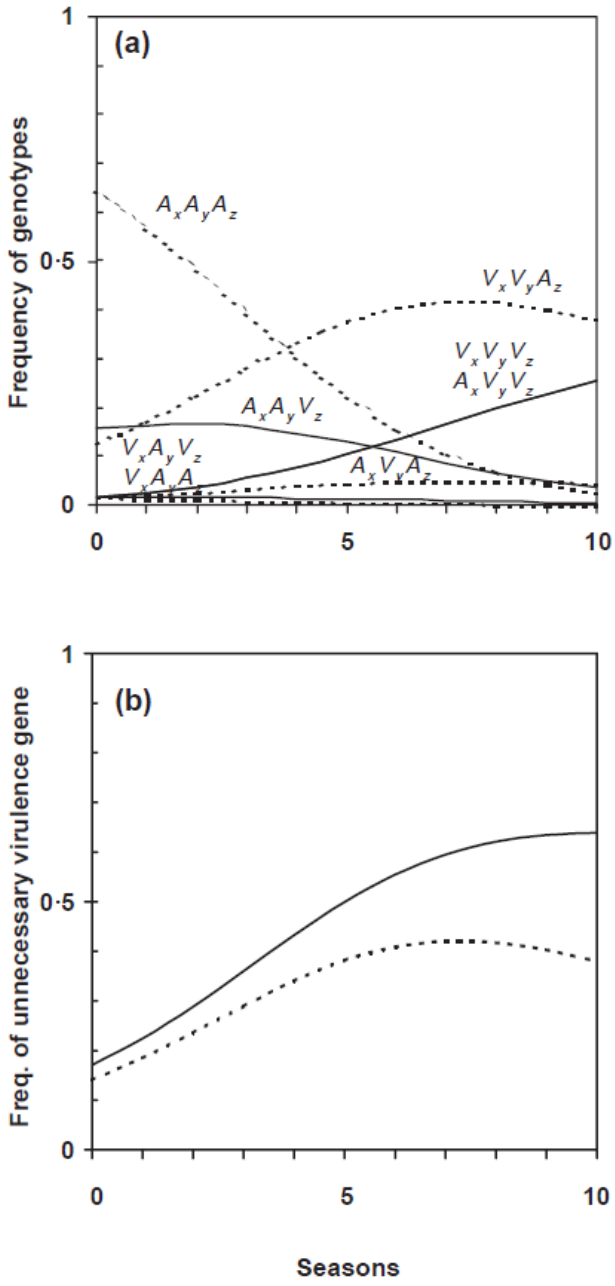


The evolution of several pathogen populations with different initial composition was also simulated, keeping the proportion of resistant cultivars in winter the same. In all cases, decreasing the proportion of winter crops resulted in a quicker decrease in frequency of spring-avirulent genotypes. But, depending on the initial values of virulence frequencies and gametic disequilibria in the pathogen population, the frequency of spring-avirulent genotypes might either decrease from the first season, or increase for some seasons before decreasing. In some cases, a large increase in frequencies of spring-avirulent isolates was observed, at least for large proportions of winter crops. To illustrate this, an example was selected with another pathogen population (population 2, Table 4). In this example, the proportion of spring-avirulent genotypes, initially 0·49, showed a large increase over the 10 seasons when large proportions of winter crops were present, e.g. for S = 0·84 or 0·95 (Fig. 4b).

Predictions for frequencies of unnecessary virulence genes

According to the presence of resistance genes, the virulence genes V_{a1}, V_{a3}, V_{a6}, V_{a7}, V_{a9}, V_{a12}, V_{a13}, V_k, V_g, V_{La} and V_h were often necessary in at least one region, whereas V_{a22} was universally unnecessary. The observed data at the beginning of the sampling period in F-N were characterized by high frequencies of genotypes with V_{a22} (up to 82%; Caffier *et al.*, 1996). High frequencies of genotypes with unnecessary virulence genes could also be obtained in the hypothetical cropping system, depending on the initial composition of the pathogen population. To illustrate this, a case was defined with a use of resistance genes in host cultivars as before (s_A/S = 0·35 and S varying), and large negative gametic disequilibrium between the unnecessary gene, V_x, and the virulence gene, V_y, being subject to direct selection by winter barley cultivars (population 3, Table 4). In this example, the total frequency of spring-avirulent genotypes decreased from the first generation. The frequency of the spring-avirulent genotype with the unnecessary virulence gene $V_xV_yA_z$ decreased from the first generation for low proportions of winter crops (e.g. S = 0·3, data not shown), but for high values of S the frequency increased in the first five to six seasons until it finally decreased. This was due to a large increase in frequency of the two genotypes $V_xV_yA_z$ and $V_xV_yA_z$, which possess virulence for the resistance gene *Ry* being used in the winter crop (Fig. 5a). Correspondingly, the frequency among spring-avirulent isolates of the unnecessary virulence gene V_x increased for several seasons, despite no direct selection for this gene (Fig. 5b). In addition, the frequency of the unnecessary virulence in the total population increased in frequency over the first 10 seasons, again despite no direct selection for this gene (Fig. 5b). In conclusion, strong gametic disequilibria and selection (hitch-hiking) may lead to high frequencies of genotypes carrying unnecessary virulence genes, even in a case when these genotypes generally are unable to infect spring barley (spring-avirulent). This may explain the observations in the first samples from F-N where the frequency was very high for genotypes combining the universally unnecessary virulence gene V_{a22} with the necessary virulence gene on the winter crop V_h.

Figure 5 *Simulated evolution over 10 seasons showing frequencies of the eight possible genotypes; spring-avirulent genotypes (dotted lines) and other genotypes (solid lines) (a), and frequencies of the unnecessary virulence gene V_x among spring-avirulent isolates (dotted line) and among all isolates (solid line) (b). Values for the crops were as in Fig. 4, with 84% of the crop being autumn-sown. Values for the initial pathogen population were as population 3 in Table 4.*



Discussion

In the present study, we have investigated the hypotheses of Caffier *et al*. (1996) about local evolution of virulence gene combinations in barley powdery mildew populations growing in a cropping system with autumn-sown and spring-sown crops. Frequency dynamics of multilocus virulence genotypes growing only on winter crops (spring-avirulent) have been the focus of the work. Very detailed data on the distribution of cultivars and on virulence gene combinations are required for analysing the dynamics of spring-avirulent genotypes.

In the present study, the deployment of resistance genes in host crops was known in detail, for all the barley cultivars grown in Denmark, whereas for the French spring barley cultivars information was missing

on the resistance genes for eight French spring barley cultivars, covering a maximum of 1·5% of the barley area in any region and year (Bousset *et al.*, 2000). As there was no reason to believe that resistance genes in these cultivars were different from those in the spring barley cultivars tested, and taking into account the small area of cultivars with unknown resistances genes, the spring-avirulent isolates could be classified. Isolates were characterized on a differential set representing most known barley powdery mildew resistance genes present in the four regions, except *Mlra*, *MlAb* and *Mla8*. The latter did not influence the interpretation of results because the corresponding virulence gene frequencies were very close to 100% in the aerial population in north-west Europe in that period (Limpert *et al.*, 1990; Hovmøller *et al.*, 1995; Hovmøller *et al.*, 2000). The results reported here show that spring-avirulent isolates could be found not only when the proportion of winter barley was high as in France, but also when the proportion of winter barley was much lower, as in Denmark. This suggests that a part of the pathogen population in all regions remains on winter barley throughout the growing season. This conclusion holds true even if some isolates characterized as spring-avirulent in our analysis may be a product of sexual reproduction (recombination) or may have migrated from an area where spring barley cultivars possessed either no resistance genes, or resistance genes identical to those in winter barley. The prevalent spring-avirulent isolates often had a low number of virulence genes, from zero to four virulence genes out of the 11 genes listed. This number reflects the fact that only a few resistance genes were used in winter cultivars. The dynamics of spring-avirulent genotypes relate directly to the selection regime and the relative fitness of the genotypes, whereas the dynamics of simple genotypes (genotypes possessing one virulence gene according to the differential set used) reflect the choice of differential set. In the previous analysis (Caffier *et al.*, 1996), genotypes were classified as simple or complex. All genotypes described as simple were also spring-avirulent according to the present analysis, but the opposite was not the case.

Observations of changes in virulence gene and genotype frequencies in aerial pathogen populations are difficult to interpret, particularly in complex cropping systems involving both autumn-sown and spring-sown crops. To help understand the dynamics in such systems, mathematical models have been developed (for review see Hovmøller *et al.*, 1997). Simulation models based on multilocus virulence genotypes were used to predict virulence dynamics of barley powdery mildew, depending on the distribution of host cultivars and various strategies for the deployment of resistance genes (Hovmøller *et al.*, 1993, 1997). In this study, the expected dynamics of spring-avirulent genotypes were simulated, as well as of genotypes with a 'universally' unnecessary virulence gene (mimicking V_{a22}), in a hypothetical cropping system with autumn-sown and spring-sown crops with one resistance gene present in winter barley and one present in spring barley, and an additional resistance gene not used. The mathematical model was based on the model system developed by Østergård & Hovmøller (1991), where host-induced selection was the only evolutionary force, and mutation, migration and genetic drift were not considered. Simulations based on this simple cropping system, and with the pathogen reproducing only asexually, were sufficient to explain most of the changes observed in the pathogen populations: the different frequencies of spring-avirulent isolates in the French and Danish samples; the potential increase in frequency of such isolates; their decrease in frequency from 1992 to 1998 in French samples; and the frequency dynamics of the unnecessary V_{a22} gene. Taking into account the actual complex selection regime including more than 14 resistance genes, and 51 combinations of them, when considering all seasons and regions (Bousset *et al.*, 2000), the accuracy of the predictions was striking. This indicates that the most important characteristics of the biological system were included in the model, and that other evolutionary forces were of only minor importance over the time and area considered. As selection forces in the model were determined only by host-resistance genes, there was no support for the hypothesis of a strong differential adaptation of simple and complex genotypes (cost of unnecessary virulence genes) when growing on a susceptible host cultivar, as discussed by Caffier *et al*. (1996). In conclusion, frequencies of genotypes in the pathogen populations evolved in relation to the proportions of winter and spring barley grown, and in relation to the resistance genes present in winter or spring cultivars under the influence of the gametic associations induced in the previous history of the pathogen populations. According to the model, when assuming no change in the use of resistance genes, spring-avirulent isolates will survive in the pathogen population for a limited period, depending on the proportion of winter barley. However, during this period spring-avirulent isolates may increase in frequency in specific cases. Such an increase was predicted only for genotypes which possessed a virulence gene subject to selection by a resistance gene in winter barley cultivars. The case of F-

N fitted these predictions. The very frequent 'spring-avirulent' genotype in France in the beginning of the sampling period often possessed the V_h virulence gene (matching *Mlh* resistance present in winter barley only), and *Mlh* was found over a large area in the beginning of the sample period. The use of *Mlh* increased from about 1% of the winter barley area in France in 1987/88 to about 47% in 1990/91 (Caffier & Vallavieille-Pope, 1996; Bousset *et al.*, 2000). The most frequent spring-avirulent genotype in Denmark possessed V_h and V_{a6}. The corresponding resistance genes *Mla6* and *Mlh* were present only in winter barley. However, in Denmark there was only weak direct selection for V_h since *Mlh* was present in only a small proportion of the winter barley area.

A common feature of many simple genotypes in the present study, and in the previous French study in particular (Caffier *et al.*, 1996), was the presence of the universally unnecessary V_{a22} gene in relatively high frequencies. However, the corresponding resistance gene, *Mla22*, has never been used in any listed barley cultivar in Europe or elsewhere (Torp *et al.*, 1978; Jensen & Jørgensen, 1981; Anon., 1983–84, Rasmussen, 1985–87; Rasmussen, 1988; Jensen & Boesen, 1989–90; Anon., 1990–97, Brown & Jørgensen, 1991; Deneken & Boesen, 1991–96; Anon., 1991–99; Jensen *et al.*, 1992; Caffier & de Vallavieille-Pope, 1996; Deneken, 1997). This could be taken as evidence for selection of an unnecessary virulence gene, in contrast to the 'fitness cost' of unnecessary virulence genes as suggested elsewhere (Vanderplank, 1968; Leonard, 1969; Grant & Archer, 1983; Bronson & Ellingboe, 1986). However, the simulations in the present study clearly demonstrate that genotypes possessing universally unnecessary virulence genes may increase or decrease in frequency due to hitch-hiking facilitated by gametic disequilibria. Strong gametic disequilibria between virulence genes have been shown to exist in many other barley powdery mildew populations (e.g. Østergård, 1982; Wolfe & Knott, 1982; Grant & Archer, 1983). Gametic disequilibria may arise and evolve for several reasons, e.g. by selection induced by host-resistance genes, either in past generations or in the growing season in which the samples were collected, and they are expected to remain in the barley powdery mildew population despite an annual cycle of sexual reproduction (Østergård & Hovmøller, 1991; Brown, 1995). Hitch-hiking may also take place between a virulence gene and genes other than virulence genes, e.g. genes influencing the general fitness of the pathogen. Therefore, it may be difficult to distinguish the effect of a single virulence gene from the effect of the gene background of the isolates in the pathogen population (Østergård, 1987). However, the fact that V_{a22} has been present in the aerial powdery mildew population in Europe in relatively high frequencies, and under very different selection regimes, for many years may be most consistent with the hypothesis that there is no fitness cost associated with this universally unnecessary gene. This does not exclude the possibility that other unnecessary virulence genes may be associated with cost of virulence.

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