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Effect of sexual recombination on pathotype frequencies in barley powdery mildew populations of artificially inoculated field plots

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Abstract

Changes in pathotype frequencies due to sexual reproduction during summer were assessed in barley plots inoculated at the start of the growing season with powdery mildew isolates of the pathotype GL1 (Va22Vh) which belonged to a clonal lineage frequent in Northern France in 1992–1996 and was absent or rare in the local population at the time of the study. The capacity for recombination among isolates belonging to the pathotype GL1 was confirmed by performing crosses in the greenhouse. The field experiment was repeated over two years, with two field plots inoculated with either a single isolate (one mating type) or four isolates (two mating types). Following artificial inoculation, the frequency of GL1 increased to between 40% and 80% of the total conidia population at the end of the asexual epidemics before summer. When only one isolate (one mating type) of the pathotype GL1 was present, the frequency of the GL1 pathotype decreased to 4–5% of the ascospore population, following sexual recombination between the inoculated isolate and the local natural population. When isolates of both mating types of GL1 were inoculated, however, the GL1 pathotype remained dominant (28%) in the ascospore population. A pathotype dominant at the end of the summer might possibly have over-summered through asexual reproduction, or alternatively it may originate from sexual reproduction. The observed GL1 frequencies and numbers of virulences per isolate (complexity) observed on the field plots were compared to those calculated with a model assuming random or non-random mating

Keywords: ascospore, *Blumeria graminis* f. sp. *hordei*, over-summering, virulence

Introduction

The causal agent of powdery mildew on barley, *Blumeria graminis* (Syn. *Erysiphe graminis*) f. sp. *hordei*, is an haploid fungus capable of both asexual and sexual reproduction. Asexual reproduction (10–15 generations) takes place on green leaves of the growing host (Wolfe and McDermott, 1994). At the end of the asexual epidemic the fungus may go through one annual cycle of sexual reproduction (Wolfe and McDermott, 1994). The fungus is heterothallic and sexual recombination between mycelia of opposite mating types occurs on senescent plants in early summer (Götz et al., 1996). Cleistothecia are produced and spend the summer on the dry leaf debris. Ascospore discharge provoked by rain-induced humectation sometimes starts in summer but reaches a maximum over several weeks in autumn (Smedeg°ard-Petersen, 1967; Götz et al., 1996).

Avirulence genes in the pathogen are matched by corresponding resistance genes in the host according to a gene-for-gene relationship (Jørgensen, 1988). Aerial and field populations of *B. graminis* f. sp. *hordei* in Europe usually have a high pathotype diversity (Wolfe et al., 1992; Andrivon and de Vallavieille-Pope, 1993; Brändle, 1994;Müller et al., 1996). However, the presence of a dominant pathotype Va22Vh (GL1) was reported in regional aerial powdery mildew populations on winter barley in Northern France (Caffier et al., 1996a) and molecular markers indicated the existence of a clonal lineage within the dominant pathotype (Caffier et al., 1999). Even though not in equal frequencies, isolates of both mating types were detected within the clonal lineage. It was not known whether isolates belonging to the clonal lineage (Caffier et al., 1999) were able to undergo sexual crosses or not. In this paper, we use the designation ‘clonal lineage’ as defined by Caffier et al. (1999), even though the term ‘clonal’ might be contested if sexual crosses within this lineage appear possible.

This study was intended to investigate (i) the capacity of the clonal lineage to accomplish sexual reproduction, tested by greenhouse crosses; (ii) the influence of sexual recombination on pathotype frequencies in a population including dominant clones, studied in a field experiment including two plots artificially inoculated with either one isolate (no sexual recombination between isolates within the inoculated

pathotype) or four isolates of the same pathotype (presence of two mating types and potential sexual recombination between isolates of the dominant inoculated pathotype); (iii) the possibility to calculate the frequencies expected after sexual recombination in a case when mating types were not in equal frequencies.

Materials and methods

Greenhouse crosses

Single conidial isolates were used as parents in the crosses. Isolate CC52 came from the United Kingdom (Brown and Simpson, 1994; Caffier et al., 1996b) and the other isolates were collected in France in 1992 (gl-7), 1995 (gl-1, gl-4, gl-5, gl-6) and 1998 (gl-2, gl-3). Pathotypes of the isolates (Table 1) were assessed on 11 near isogenic lines (NILs) of cv. Pallas, each possessing one of the resistance alleles *Mla1*, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mla22*, *Mlk*, *Mlg*, *MlLa* and *Mlh* (Kølster et al., 1986). The isolates gl-1, gl-4, gl-5 and gl-6 belonged to the clonal pathotype GL1 (Va22Vh) that has been described in barley powdery mildew populations from Northern France (Caffier et al., 1996a; 1999). These isolates were chosen according to their haplotypes with five RAPD and three SCAR molecular markers (Caffier et al., 1999). With the SCAR marker SEGH V2, linked to the mating type locus (Brändle et al., 1997), the isolates gl-1 and gl-5 had the same allele whereas gl-4 and gl-6 had the other allele. To avoid possible confusion caused by several isolates belonging to the same pathotype or the fact that a pathotype detected in the field could consist of spores issued both from the artificial inoculation and from the local airborne inoculum, small type is used when referring to isolates inoculated (e.g. gl-1) and capitals when referring to the corresponding pathotypes (e.g. GL1). To confirm the mating types, crosses were made between CC52, used as a tester parent, and the four isolates gl-1, gl-4, gl-5 and gl-6 (Table 2). The isolate gl-7 was crossed with gl-1 and gl-6, whereas gl-2 was crossed with isolate gl-3. All possible crosses were attempted between the four isolates belonging to the clonal lineage. Crosses were carried out in the greenhouse (15–25 ◦C) in autumn 1998 on the spring cv. Proctor using the method of Brown et al. (1992).

Table 1. *Virulence pathotypes and mating type alleles of the eight isolates used in the experiment*

Isolate	*Blumeria graminis* f. sp. *hordei* isolates							
	Clonal lineage				Others			
	gl-1	gl-4	gl-5	gl-6	CC52	gl-2	gl-3	gl-7
Virulence pathotype[a]								
P01 *Mla1*	0	0	0	0	0	1	0	0
P03 *Mla6*	0	0	0	0	0	0	0	0
P05B *Mla7*	0	0	0	0	1	0	0	0
P08B *Mla9*	0	0	0	0	1	0	0	1
P10 *Mla12*	0	0	0	0	1	0	0	0
P11 *Mla13*	0	0	0	0	1	0	0	0
P12 *Mla22*	1	1	1	1	0	0	0	1
P16 *Mlk*	0	0	0	0	1	0	1	0
P21 *Mlg*	0	0	0	0	1	0	0	1
P23 *MlLa*	0	0	0	0	0	1	0	0
P24 *Mlh*	1	1	1	1	1	0	1	0
Mating type[b]	1	0	1	0	1	md[c]	md[c]	1

[a]The virulence pathotypes of the isolates were assessed on a differential set comprising 11 Pallas isolines (Kølster et al., 1986). '1' or '0' indicate virulence or avirulence of the isolate on the given line, respectively.

[b]The isolates were tested with the SCAR marker SEGH V2 linked to the mating type locus (Br¨andle et al., 1997) in a previous study (Caffier et al., 1999).

[c]Missing data. The isolates gl-2 and gl-3 were of opposite mating types, but the mating type of each isolate was not identified.

Recovery of ascospores and virulence tests

To ascertain the fertility of the cleistothecia obtained in the crosses, some progeny isolates were recovered on cv. Igri (*Mlra*) leaf segments maintained on water agar (4 g l−1 agar, 0.3 g l−1 benzimidazole) (Brown et al., 1992). After 10 days of incubation, the resulting colonies were transferred onto new leaf segments for multiplication, and tested for virulence after 10–12 days. To check that the cleistothecia resulted from the crosses made and not from contamination, five to ten colonies from each cross were tested for virulence on 11 Pallas NILs (Table 2). Each isolate was inoculated, using a settling tower, onto segments cut from first leaves of 10-day old plants of the Pallas NILs and maintained on water agar. After incubation (7 days at 17°C, continuous light 14µEm^{-2} s^{-1}), infection types (IT) were scored according to a 0–4 scale (Moseman et al., 1965). Isolates were classified as avirulent (IT 0–3) or virulent (IT 4). When both parents had the same virulence or avirulence allele for one of the tested virulences, it was checked that all the progenies also had this allele. In the crosses between isolates belonging to the clonal lineage, it was checked that all the progenies had the parental pathotype.

Table 2. *Crosses performed, occurrence of cleistothecia and virulence pathotype of the progenies*

Crosses	Cleistothecia[a]	Progenies GL1[b]
With other isolates		
CC52 × gl-1	No	—
CC52 × gl-4	Yes	0/8
CC52 × gl-5	No	—
CC52 × gl-6	Yes	0/7
gl-7 × gl-1	No	—
gl-7 × gl-6	Yes	0/6
gl-2 × gl-3	Yes	md[c]
Between isolates belonging to the clonal lineage		
gl-1 × gl-4	Yes	5/5
gl-1 × gl-5	No	—
gl-1 × gl-6	Yes	10/10
gl-4 × gl-5	Yes	7/7
gl-4 × gl-6	No	—
gl-5 × gl-6	Yes	9/9

[a]Absence (no) or presence (yes) of cleistothecia on the leaves inoculated with both isolates, 2–3 weeks after the inoculation.

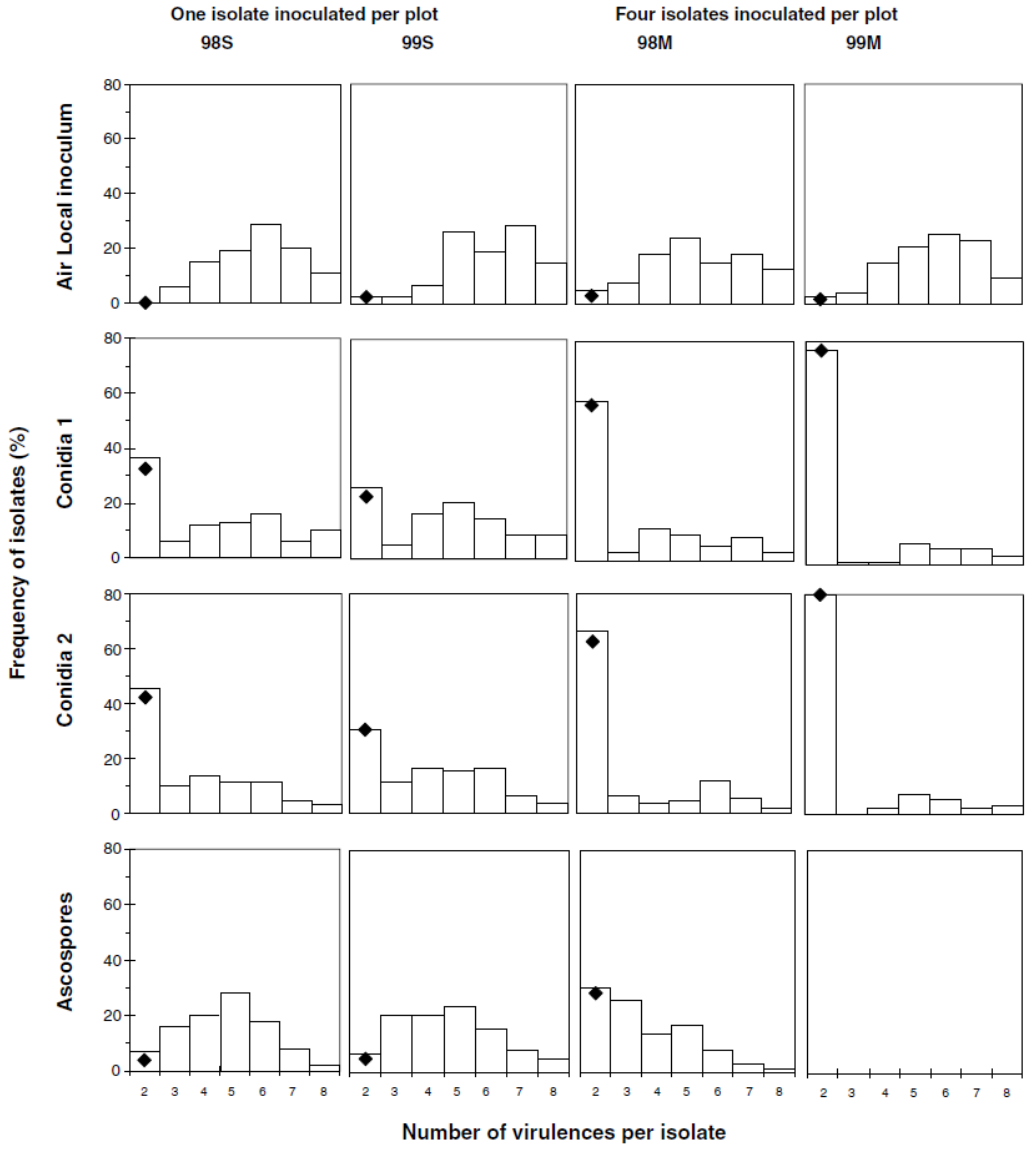
[b]The upper part of the ratio indicate the number of isolates having the same pathotype GL1 (Va22Vh) as the clonal lineage, and the lower part of the ratio indicate the number of isolates tested for virulence. (—) indicates that no progenies were obtained from the cross.

[c]Missing data. Cleistothecia were obtained from this cross, but the testing of progenies for virulence was not performed.

Field experiment

This field experiment was designed to compare ascospore (sexual progenies) populations coming from crosses between the natural population and a single inoculated isolate (gl-1) on plots S with those from crosses between the natural population and four isolates (gl-1, gl-4, gl-5 and gl-6, two of each mating type) inoculated in equal proportions on plots M. All the inoculated isolates, belonging to the clonal lineage previously identified by Caffier et al. (1999) had the same pathotype GL1 (Va22Vh), which was present at a very low frequency in the local airborne inoculum (Figure 1). The experiment was replicated in summer 1998 (plots 98M, 98S sown in February 1998) and summer 1999 (plots 99M, 99S sown in October 1998). 12×12mplots were sown at the Grignon INRA Research Station, 40 km west of Paris, with the winter barley cv. Plaisant (*Mlra*), at least 500m from the nearest barley field. Shortly after the emergence of the barley plants, infected seedlings prepared by blowing spores at low density onto 10-day old seedlings of cv. Plaisant were transplanted in 25 inoculation points and allowed to sporulate for two to three weeks before being removed from the plots.

Figure 1. *Distribution of* Blumeria graminis *f. sp.* hordei *isolates into complexity classes (histogram) and frequencies of the GL1 pathotype (diamonds). Note that most of the isolates having two virulences belong the GL1 pathotype. At the beginning of the asexual epidemics, populations were sampled from the four field plots (98S, 99S, 98M and 99M) before (Air local) and after (Conidia 1) artificial inoculation. At the end of the asexual epidemics, populations were sampled before (Conidia 2) and after (Ascospores) sexual reproduction. Pathotypes were defined on a differential set comprising 11 resistance genes.*



Four samples were collected from each field plot. A sample of the local airborne population at the beginning of the epidemics was obtained by exposing two batches per plot of seedlings (cv. Plaisant) for 7–12 days before the emergence of the barley plants (Table 3). The second and third samples of the conidial population were obtained after inoculation of the plots and just before summer, respectively, by collecting 110 leaves, each bearing a single colony, along a regular grid of 10 × 11 sampling points (Table 3). The fourth ascospore population sample was obtained by collecting cleistothecia, following the same protocol as for samples 2 and 3. After storage for four to six weeks at room temperature ascospores were hatched as described above and one colony per sampling point was retained. From each collected leaf or hatched progeny, conidia were transferred onto new cv. Igri leaf segments and tested for virulence 10–12 days later.

Table 3. *Sampling of powdery mildew populations from the air and the crop on barley field plots (cv. Plaisant) inoculated with the isolate gl-1 (plots 98S and 99S) or with the isolates gl-1, gl-4, gl-5 and gl-6 (plots 98M and 99M). The pathotypes were determined on a differential set comprising 11 resistance genes and pathotype diversity was measured using the modified Shannon index Hw' (Goodwin et al., 1992)*

Sample	1998			1999		
	Date	Sample size		Date	Sample size	
		98S	98M		99S	99M
Field plots						
Sowing	2/2			13/10		
Inoculation[a]	18/3–13/4			2–18/11		
Samplings[b]						
Air	7–19/3	119	141	16–23/10	120	120
Conidia 1	21/4	99	93	12/1	96	107
Conidia 2	2/6	88	110	22/4	105	95
Ascospores	1/7	99	88	9/6	63	md[c]
Pathotype diversity[b]						
Air		0.91	0.90		0.80	0.80
Conidia 1		0.73	0.48		0.71	0.26
Conidia 2		0.63	0.42		0.69	0.24
Ascospores		0.93	0.75		0.92	md[c]

[a]Plots were inoculated by transplanting sporulating seedlings, which were removed after 2–3 weeks.
[b]Samples from the local airborne population (air) were obtained by exposing batches of seedlings before artificial inoculation, whereas samples from the field plots were collected as leaf fragments bearing a single colony (conidia 1, conidia 2) during the asexual epidemics or bearing cleistothecia (ascospores) following sexual reproduction at the end of the asexual epidemics.
[c]Missing data. Despite intensive inspection, no cleistothecia could be found on this plot.

Data analysis

Initial analysis was performed on the pathotypes defined by 11 virulences. The frequencies of the GL1 pathotype and the distributions of isolates according to their number of virulences (complexity) were compared between the samples by chi-square tests of homogeneity. To avoid categories with null or very low sample size in the lowest and the highest complexity classes, those isolates with two or less virulences or with eight or more virulences, respectively, were pooled. The modified Shannon index of diversity Hw' (Goodwin et al., 1992), i.e. the normalized version of Shannon's H obtained by division through ln*(N)*, was calculated using the observed pathotype frequencies as follows:

$$\mathrm{Hw}' = -\frac{1}{\ln(N)} \times \sum_{i=1}^{m} p_i \ln(p_i)$$

where N = the sample size, pi = the frequency of the ith pathotype and m = the number of pathotypes.

Modelling the sexual recombination

The expected frequencies of (i) the complexity classes and (ii) the GL1 pathotype following sexual recombination were calculated as follows: of the 11 virulences assessed five were selected (see below) and the crosses for each of the 1024 pairwise combinations of the $2^5 = 32$ possible pathotypes were explicitly modelled.

The *expected distribution of complexity* among progenies was calculated for each of the crosses. For a given cross, the number of virulences segregating among the parents was used to calculate the number of categories in the progenies and their proportions. If no virulences were segregating among the parents, there was no segregation among the progenies. If one virulence was segregating among the parents, the progenies with and without this virulence segregated in a 1 : 1 ratio. Under the hypothesis of independent segregation of the five virulences, this calculation can be extended to more than one virulence segregating among the parents with a binomial segregation ratio, i.e. 1 : 2 : 1 for two virulences segregating among the parents, 1 : 3 : 3 : 1 for three, 1 : 4 : 6 : 4 : 1 for four and 1 : 5 : 10 : 10 : 5 : 1 for five virulences, respectively. The number of

virulences common to both parentswas then added to convert these segregations into the proportions of the complexity classes in which the sexual progenies would fall.

The *expected proportion of the GL1 pathotype* among the progenies was calculated for each of the crosses. For any given cross the proportion of progenies belonging to the GL1 pathotype is equal to the product over the five loci of a frequency *x*, with $x = 1$, 0.5 or 0 if for this locus both parents, one parent or none of the parents had the same allele as the GL1 pathotype, respectively.

This method assumes (i) the absence of genetic linkage between the loci, and (ii) the absence of differential selection among the gametes, because in either case the progenies would not segregate according to Mendelian expectations. Among the 11 resistance genes that we tested, genetic linkage groups including avirulence genes *Aa9-Aa22-Ak* and *Aa6-Aa12-ALa* were reported (Brown and Simpson, 1994; Brown et al., 1996; Caffier et al., 1996b). Therefore, only the five virulences *Va1, Va12, Va22, Vg* and *Vh* were included in the analysis. Because we aimed at modelling the result of sexual reproduction as a population of ascospores taken directly from the cleistothecia, this removed the problem of differential selection on the pathotypes during cycles of asexual reproduction. Differential selection among the ascospores, based for example on a differential germination depending on their pathotype, cannot be excluded, but such a phenomenon has not been documented for powdery mildew as far as we are aware.

The expected number of progenies was calculated for each cross as the product of the numbers of isolates of the two parental pathotypes observed in the population of conidia at the end of the asexual epidemics. To obtain the distribution of complexity in the expected population of ascospores, expected numbers of progenies in each cross were summed over all possible crosses and divided by the total number of progenies. Since each cross was individually modelled and its outcome weighted by its probability of occurrence, the gametic disequilibrium in the population before sexual recombination, i.e. non-random statistical association of the virulences due for example to selection on the pathotypes during the asexual epidemics, was taken into account in the frequencies of the parental pathotypes; hence the hypothesis of populations being in equilibrium is not required.

Additional constraints on *mating types frequencies* could be included as follows. Our calculation of the probability of occurrence of a given cross assumes that all the crosses are possible if the two parental pathotypes are present. We have to consider this hypothesis for the sexual crosses (i) among non-GL1 pathotypes, (ii) between GL1 and non-GL1 pathotypes, and (iii) within the GL1 pathotype. In samples of natural populations, it was reported that the mating type alleles were in 1 : 1 equilibrium when all pathotypes were considered (Br¨andle et al., 1997), and also within one pathotype when this pathotype was not a clonal lineage (Caffier et al., 1999). It is therefore reasonable to assume that the mating types in our field plots were in equilibrium for the non-GL1 pathotypes. This makes the hypothesis acceptable that all crosses are possible both among non-GL1 pathotypes and betweenGL1and non-GL1 pathotypes. Within the GL1 pathotype, two cases have to be studied, depending on the number of mating types inoculated.

When both mating types were inoculated (plots 98M and 99M), the protocol used ensured that the four isolates used for the inoculation were present in equal frequencies in the population in each of the 25 inoculation points at the start of the asexual epidemics. The hypothesis of all crosses being possible at the end of the asexual epidemics (random mating) is equivalent in this case to the assumption that there was no differential selection for one of the mating types within the GL1 pathotype during the epidemics.

When only one of the mating types was inoculated (plots 98S and 99S) and if the presence of GL1 isolates at the end of the asexual epidemics was due only to the artificial inoculation, only one mating type was present and no crosses within the GL1 pathotype were possible. To model this situation, we set the number of progenies of the cross GL1 × GL1 to zero. However, isolates of the GL1 pathotype were present at a low frequency in the local natural population, which also contributed to the field population (Figure 1). Assuming that mating types were in equal frequencies among these isolates, this situation was modelled by allowing a low proportion (5%, 10% or 20%) of the crosses GL1 × GL1 to be possible.

To allow comparisons between the observed and the expected populations, the frequencies of pathotypes and the distributions of complexity in the population of conidia at the end of the asexual epidemics were re-calculated when only the five virulences were considered in order to assess pathotypes. We calculated chi-squares to test (i) homogeneity between the observed proportions of GL1 when the pathotypes were

defined by 11 or 5 virulences; (ii) homogeneity between observed and expected proportions of the GL1 pathotype and (iii) homogeneity between observed and expected distributions of complexity.

Results

Greenhouse crosses

When cleistothecia were observed in the crosses attempted between two isolates, it was concluded that these two isolates had opposite mating type alleles (Table 2). The isolates gl-1, gl-5, gl-7 and CC52 had the same allele and gl-4 and gl-6 had the other allele. The isolates gl-2 and gl-3 had opposite mating types, but the alleles were not identified for each isolate. No cleistothecia were observed in any of the attempted crosses between isolates of the same mating type. Cleistothecia were observed in all crosses between isolates of opposite mating types. The cleistothecia were fertile and no discrepancies were observed between the pathotypes of the parents and those of the recovered progenies (ascospores). In the crosses between isolates of the clonal lineage, all the progenies had the parental pathotype Va22Vh. In the crosses CC52 × gl-4 and CC52 × gl-6, all the progenies were virulent on *Mlh* and avirulent on *Mla1, Mla6* and *MlLa*, as were both parents. Segregation was observed for the other loci. In the cross gl-7×gl-6, all the progenies were virulent on *Mla22* and avirulent on *Mla1, Mla6, Mla7, Mla12, Mla13, Mlk* and *MlLa*, as were both parents.

Field experiment

We first analysed the data with pathotypes defined by 11 virulences. In 1998 and 1999, before the inoculation of the field plots, the frequency of GL1 pathotype in aerial local populations was low (0–2.8%) and not significantly different between years (on S: $p = 0.08$; on M: $p = 0.53$) or plots (in 1998: $p = 0.13$; in 1999: $p = 0.69$). The distributions of the complexity of isolates in the local inoculum (airborne population at the start of the asexual epidemics) were homogeneous between years on the M plot ($p = 0.23$) but slightly different on the S plot ($p = 0.03$) and homogeneous between plots in 1999 ($p = 0.23$) but slightly different in 1998 ($p = 0.04$) (Figure 1). The pathotype diversity measured by the modified Shannon index Hw' was high, from 0.80 to 0.91 (Table 3).

The inoculations of the field plots were successful in all the plots and resulted in a frequency of the GL1 pathotype (23–77%) after inoculation (conidia 1) significantly higher ($p < 0.001$) than in the airborne inoculum (Figure 1). The GL1 frequencies in the M plots were higher than in the S plots in both years ($p = 0.001$) and higher in 1999 than in 1998 for the M plots ($p = 0.003$). The pathotype diversity was lower in the field plots than in the airborne population and higher on the S plots (Hw' = 0.71–0.73) than on the M plots (Hw' = 0.26–0.48) (Table 3). Between the first (conidia 1) and the second (conidia 2) samplings of conidial populations during the asexual epidemics, the GL1 frequency remained high and constant (on 98S: $p = 0.17$; on 99S: $p = 0.23$; on 98M: $p = 0.40$; on 99M: $p = 0.56$) (Figure 1), and the diversity decreased slightly in 1998 and remained constant in 1999 (Table 3). At the end of the asexual epidemics, the distributions of complexity were different in both years (in 1998: $p = 0.03$; in 1999:$p < 0.001$) between theMand S plots (Figure 1) and the pathotype diversity was higher on the S plots (Hw' = 0.63–0.69) than on the M plots (Hw' = 0.24–0.42).

In summer 1998, cleistothecia were abundant on both 98M and 98S plots. In summer 1999, cleistothecia were abundant on the 99S plot but none was found on the 99M plot, despite intensive (several hours) inspection of the plot. The frequency of the GL1 pathotype was lower ($p < 0.001$) in the populations of ascospores (4–28%) than in the populations of conidia at the end of the asexual epidemics (40–80%), and lower in the S plots (4–5%) than in theMplot (28%) (Figure 1). The distributions of the complexity of isolates were significantly different between the populations of conidia and the populations of ascospores in all the plots (on 98S and 98M: $p < 0.001$; on 99S: $p = 0.02$). Pathotype diversity was higher in the populations of ascospores (Hw' = 0.75–0.95) than in the populations of conidia (Hw' = 0.24–0.60) (Table 3).

Observed and expected populations following sexual recombination

For the comparison between the observed and the expected populations following sexual recombination, pathotypes were defined by five virulences and chi-square tests indicated that the proportions of GL1 defined by either 5 or 11 virulences were not significantly different (data not shown). In 1998, when both the frequency of the GL1 pathotype and the distributions of complexity were taken into account, the observed data showed a better fit to the hypothesis corresponding to the biological protocol (number of isolates inoculated) than to

the alternative hypothesis (Figure 2, Table 4). In the 98S plot, the observed frequency of GL1 was similar to the frequency expected under the hypothesis of one mating type within GL1, and different from the frequency expected assuming random mating of all pathotypes (Table 4). The distribution of isolate complexity was also in agreement with the hypothesis of a single mating type, and much less with the hypothesis of random mating (Figure 2, Table 4). In the 98M plot, the observed frequency of GL1 was different from the frequency expected under the hypothesis of one mating type within GL1, and in better agreement with the frequency expected assuming random mating (Table 4). The distribution of isolate complexity was also different from the hypothesis of one mating type and in agreement with the hypothesis of random mating (Figure 2, Table 4). On the other hand, the results obtained in 1999 were not in agreement with the biological expectations. In the 99S plot, even though only one isolate was inoculated, the observed data best fit the hypothesis corresponding to random mating (Figure 2, Table 4). The observed frequency of GL1 was different from the frequency expected under the hypothesis of one mating type within GL1, and similar to the frequency expected assuming random mating (Table 4). The distribution of isolate complexity was also different from the hypothesis of one mating type and in agreement with the hypothesis of random mating (Figure 2, Table 4).

Figure 2. *Distribution of Blumeria graminis f. sp. hordei isolates into complexity classes in ascospore samples from the four field plots (98S, 98M, 99S and 99M). 98S and 99S were inoculated with a single isolate, whereas 98M and 99M were inoculated with a mixture of four isolates (two mating types); all inoculated isolates had the same pathotype GL1 (Va22Vh). Pathotypes were defined on a differential set comprising five resistance genes. Curves correspond to the distributions expected (see text) assuming random mating in the presence of the two mating types (triangles) or only one mating type (circles) within the GL1 pathotype.*

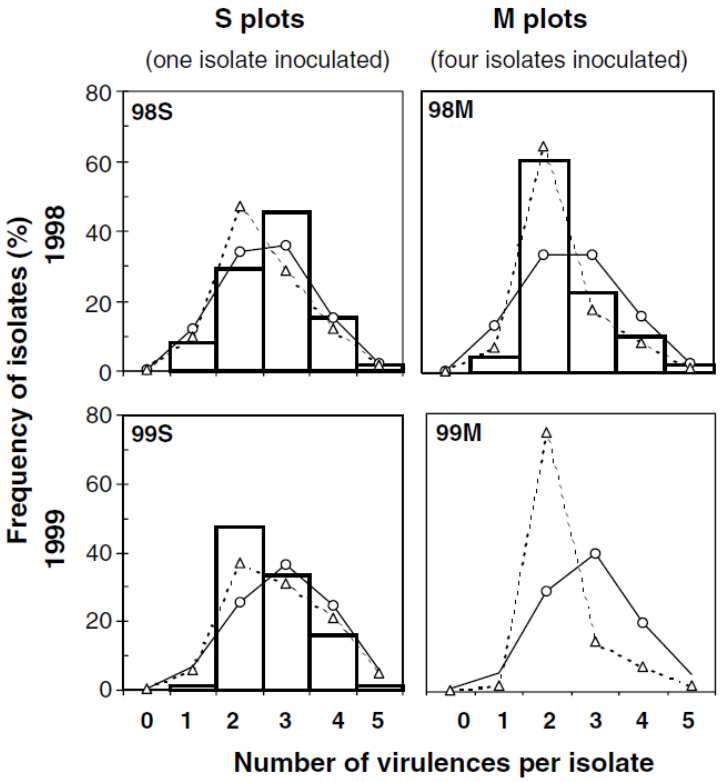


Table 4. *Observed and expected frequencies of the GL1 pathotype on barley field plots (cv. Plaisant) inoculated with the isolate gl-1 (plots 98S and 99S) or with the isolates gl-1, gl-4, gl-5 and gl-6 (plots 98M and 99M). The expected frequencies were calculated assuming either impossibility of mating within the GL1 pathotype due to the presence of only one of the mating types (1 mating type) or random mating among all the isolates (2 mating types). The probabilities associated with the chi-square tests of homogeneity between the observed and the expected frequencies of the pathotype GL1 and between the observed and the expected distributions of complexity (see Figure 2) are indicated. The pathotypes were determined on a differential set comprising 5 resistance genes.*

Plot		Observed in ascospores %	Expected			
			1 mating type		2 mating types	
			%	*p*	%	*p*
98S	GL1	13.1	14.3	0.75	31.2	0.003
	Complexity[a]			0.65		0.09
98M	GL1	42.1	18.8	0.001	56.5	0.05
	Complexity[a]			0.007		0.85
99S	GL1	30.2	9.93	0.001	23.7	0.36
	Complexity[a]			0.04		0.07
99M	GL1	md[b]	18.36	md[b]	70.6	md[b]
	Complexity[a]			md[b]		md[b]

[a]The observed and the expected distributions of isolates into complexity classes are given in Figure 2.
[b]Missing data. Despite intensive inspection, no cleistothecia could be found on this plot.

Discussion

Capacity of isolates belonging to the clonal lineage to cross sexually

In the laboratory crosses between CC52 and the four isolates belonging to the clonal lineage, mating types postulated with the SCAR marker SEGH V2 were confirmed. Both the crosses between isolates of the clonal lineage and those with isolates from other pathotypes were fertile. It was concluded that the *B. graminis* f. sp. *hordei* clonal lineage was not in reproductive isolation from the rest of the population. Large scale clonality in pathogen populations has been described when multiplication is strictly asexual due to the lack of sexual stage, as for example, in *Fusarium oxysporum* f. sp. *cubense* (Koenig et al., 1997). In these cases, the populations consisted of a few well-defined clones or clonal lineages. In fungi capable of sexual crosses, clonality was reported in the absence of opportunities for sexual crosses, for example, in *Magnaporthe grisea* (Zeigler, 1998), or *Phytophthora infestans* when only one of the two mating types was represented in populations outsideMexico (Goodwin et al., 1998), and in *Puccinia graminis* f. sp. *tritici* when the secondary host was not present (Groth and Roelfs, 1982; Wellings and McIntosh, 1990). Our results do not support the hypothesis that the clonal lineage observed in the *B. graminis* f. sp. *hordei* populations (Caffier et al., 1996a; 1999) was due to a lack of ability of the sexual lineage to undergo sexual crosses. The results were obtained on a limited number of isolates and under greenhouse conditions, so alone cannot provide information on whether or not the clonal lineage was able to take part in sexual crosses under the field conditions from 1992 to 1996. It was not possible to test this hypothesis retrospectively because the natural populations that occurred in the same field during this period were no longer available. However, further support for these laboratory results comes from the agreement with observations in the field that the isolates of the clonal lineage GL1 were able to mate with the current natural population.

Sexual recombination, mating types and persistence of a dominant pathotype

Contrasting results were obtained on the 98M and 98S plots following sexual recombination, i.e. on the 98M plot the frequency of GL1 decreased (from 63% to 28%) but GL1 remained the most frequent pathotype in the populations of ascospores, whereas on the 98S plot the frequency of GL1 decreased (from 42% down to 4%) and GL1 was no longer a frequent pathotype. When the frequencies were recalculated taking into account only five virulences, the frequencies were all slightly higher but the magnitude of the decrease were similar to the results with the 11 virulences pathotypes, for both the 98M (from 68% to 42%) and 98S (from 44% to

13%) plots. The observed frequencies of GL1 and distributions of complexity on these two plots fit the hypotheses of random mating on 98M and no mating between GL1 isolates on 98S better than the alternate hypothesis. These results were in agreement with the experimental protocol where 98M was inoculated with the two mating types in equal proportions, whereas 98S was inoculated with only one isolate and therefore only one mating type. We interpret these results as an indication that it is possible to predict the pathotype frequencies following sexual recombination from the frequencies in the population at the end of the asexual epidemics and the mating types present.

On the 99S plot, a decrease in frequency of GL1 was observed (from 31% to 5%), similar to the 98S plot results. However, when the pathotype frequencies were re-calculated with five virulences, the observed frequency of GL1 and the distribution of complexity were in agreement with the frequencies expected assuming random mating, and different from those expected assuming a barrier to crosses between GL1 isolates. This result was not in agreement with the biological expectations (only one mating type inoculated), hence we examined the following hypotheses: (i) The two mating types were present on the plot, (ii) the frequency of GL1 had been underestimated in populations of conidia at the end of the asexual epidemics, and (iii) the calculations of the frequencies were not appropriate.

The presence of the second mating type at high frequency on the plot could be due to a contamination either during the inoculation or during the asexual epidemics. The possibility that the infected plants used to inoculate the plots were contaminated is unlikely in the laboratory because the inoculated plants were prepared separately for the different isolates and maintained under plastic hoods until plantation in the field. Once the field plots were inoculated, some isolates of the second mating type may have immigrated with the natural inoculum, but high frequencies were unlikely because the proportion of the GL1 pathotype was low in the local airborne inoculum (Figure 1) and the two experimental plots 99S and 99M were more than 500m apart. Furthermore, simulations with a part of the population (5%, 10% or 20%) having both mating types did not alter the conclusions (data not shown). Finally, in the distributions of complexity for the pathotypes defined by 11 virulences, the shift towards lower complexity observed on the 98M plot (random mating) was not observed on the 99S plot (Figure 1).

Assuming that only one of the mating types was present, but the frequency of GL1 in the population of conidia before summer was underestimated, we made simulations using the same composition of the population at the end of the asexual epidemics and increasing the frequency of the GL1 pathotype. Even with a frequency up to 80%, only minor changes in the distribution of complexity were obtained (data not shown) which do not support the hypothesis of underestimation.

The expected pathotype GL1 frequencies after sexual recombination calculated using only the five independent virulence loci were for the 99S plot in the populations of conidia at the end of the asexual epidemics 40% versus 31% with 11 virulences, and in the observed populations of ascospores 30% versus 5% with 11 virulences. In the observed ascospore population, the reduction of the differential set also introduced a shift in the distribution of complexity from a bellshaped pattern with 11 virulences (Figure 1) to a distribution strongly biased towards lower complexity classes with 5 virulences (Figure 2). Even though we have no biological explanation for this bias, we cannot exclude the hypothesis that the sample of ascospores when only 5 virulences were used was not representative of the whole ascospore population in the field, therefore not fitting the expected frequencies.

Sexual recombination resulted in a decrease in frequency of the dominant pathotype GL1. Therefore, the pathotype diversity was higher in ascospore populations than in conidia populations. The increase in pathotype diversity following sexual recombination was in agreement with previously reported results (Welz and Kranz, 1987). In addition, our field experiment, provides experimental evidence that a pathotype could remain dominant in populations of ascospores. However, this occurred only when the frequency of this pathotype was high and both mating types were represented in the populations of conidia before summer. In all other cases the frequency of the dominant pathotype decreased following recombination with the rest of the population.

Consequences for the estimation of the proportions of recombination and immigration

Estimating recombination and migration frequencies in populations of ascomycete fungi has been attempted previously using changes in mating type frequency (Brändle et al., 1997; Brown et al., 1997) or gametic

disequilibria (Brown and Wolfe, 1990). This subject received increased attention recently when a new technique based on field inoculations was presented (Zhan et al., 1998; 2000; Brown, 2000). Our results reflect the concern mentioned by Brown (2000) regarding the consideration of (i) the occurrence of sexual progenies with parental pathotypes and (ii) non-random mating. On the plots 98S and 99S, isolates showing pathotypes similar to that of the inoculated clone were observed in the ascospore population, resulting from crosses between the inoculated isolates and the rest of the population. The frequencies were not excessive (4–5%) when 11 virulence were used to discriminate the pathotypes, but much higher (13–30%) when using only 5 virulences. This illustrates the confusion caused when the markers used do not provide adequate discrimination between inoculants, recombinants and immigrant (local population) categories. In our experiment, mating was not random in the 98S plot, and this generated discrepancies between the observed frequency of the GL1 pathotype and that expected under an assumption of random mating. Our results show on a simple case (only one of the pathotypes had biased mating type) that accounting for the unequal frequencies of mating types improves the calculation of the expected frequencies, and we propose a method to achieve this. This may not be realistic in a natural population, but it should at least be considered when designing an experiment including artificial inoculation.

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