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Abstract

A field experiment with barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) was designed in order to study how the time of arrival of inoculum in the field influenced pathotype frequencies in the resulting populations. Three isolates belonging to pathotypes that were absent or rare in the local aerial inoculum were used to inoculate field plots of winter barley cv. Plaisant. Two successive inoculations with different combinations of the three isolates were performed with an approximately two-generation delay, and frequencies of inoculated pathotypes were assessed four and nine generations after the first inoculation. Pathotypes of the first inoculated isolates generally persisted throughout the period of sampling; this is described as an 'early arrival' effect. During the epidemics the inoculated isolates were not replaced by isolates from the natural airborne inoculum. Pathotype frequencies depended mainly on the time of arrival of inoculum in the plot, but frequencies also depended on the isolate that had been inoculated. The most frequent isolate, GL1, belonged to the clonal lineage dominant in powdery mildew populations on winter barley in the north of France. These results confirmed that the composition of a powdery mildew population in a field is largely determined by the composition of the initial inoculum.

Keywords: *Blumeria graminis*, epidemiology, *Erysiphe graminis*, immigration, population genetics, primary inoculum

Introduction

Understanding pathogen population structure and evolution is necessary for the design of management practices aimed at limiting the appearance and spread of virulent and fungicide-insensitive genotypes of the pathogen. To date, populations of *Blumeria graminis* (syn. *Erysiphe graminis*) f.sp. *hordei*, have become rapidly adapted to all but the *mlo* (Schwarzbach, 1998) resistance alleles used in barley varieties, and to several groups of fungicides (Brown *et al*., 1997). Epidemiological characteristics, including recombination and efficient dispersal, contribute to this high potential for adaptation. The life cycle of the fungus allows 15–20 asexual generations over the course of a barley-growing season, while one nonobligatory sexual generation in summer could favour the production of new pathotypes (Wolfe & McDermott, 1994). Airborne migration of spores also favours the spread of new pathotypes, as they can travel over large distances (Hermansen *et al*., 1978; Limpert, 1987).

In Europe, surveys have shown a high level of pathotype diversity within powdery mildew populations (Wolfe *et al*., 1992; Andrivon & de Vallavieille-Pope, 1993; Müller *et al*., 1996). Between two successive growing seasons, large changes in the pathotype diversity can occur rapidly (Brown *et al*., 1993). During the barley-growing season, the selection of pathotypes by the resistance alleles used in barley varieties is strong (Hovmøller & Østergård, 1991; Hovmøller *et al*., 1993). Once the populations are established on a field, few changes in pathotype frequencies are observed over one growing season (Welz *et al*., 1990; Huang *et al*., 1995). There are contradictory opinions about the period of time during which the population structure in a field is influenced by the airborne spores. Based on the spore population in the air, it was postulated that most weeks of the season might be important for migration (Limpert *et al*., 1999). Based on the proportions of virulent and avirulent spores sampled above a field, the relative importance of immigration rapidly decreased when the epidemics started (O'Hara & Brown, 1996a). How strongly the population structure (i.e. pathotype frequencies) in one field could be determined by the composition of the primary inoculum was unclear.

To understand how a specific population structure, with a clone dominant in a diverse population, as in Northern France during the early 1990s (Caffier *et al*., 1996, Caffier *et al*., 1999), could be maintained over several years, the influence of the arrival of inoculum during the establishment of epidemics in field plots on the resulting population structure was studied. In a field experiment two successive artificial inoculations with three *B. graminis* f.sp. *hordei* isolates were used to assess the importance of time of arrival of inoculum in the plot. The advantage of the early isolates, and the influence of the isolate used on pathotype frequencies in resulting populations, were quantified at the start of the epidemics. A second sample was taken at the end of the epidemics to test for the persistence of the two effects during the whole barley-growing season.

Materials and methods

Field design and creation of powdery mildew populations

Four replicates with nine 3×3m plots each of the winter barley cv. Plaisant, with the resistance allele *Mlra*, were grown at the Versailles INRA Research Station, 20km west of Paris. Two replicates (locations A1, A2) were sown in autumn 1998 and the other two (locations S1, S2) in spring 1999. Plots were sown in a winter wheat (cv. Isengrain) field, at least 300m from the nearest barley field. They were arranged along three rows and three columns, 3–6m apart.

Two successive inoculations were performed with three single-spore isolates (GL1, GL2, GL3, described below), one shortly after the emergence of the barley plants, the other approximately two pathogen generations later. The central plot was left uninoculated as a control for infection by natural, airborne inoculum. The eight remaining plots received 30 sporulating powdery mildew colonies of each isolate by transplanting three pots with infected seedlings into the middle of each plot. There were eight inoculation sequences. The first inoculation was done with no, one, two or three isolates, and the second inoculation with the remaining isolates (Table1). The number of possible powdery mildew generations (Table2) which elapsed between the first and second inoculation, or between the two samplings, was estimated using the relationship between temperature and latent period (Friedrich, 1995). The temperature was recorded at 1m height, with a 3h step by the local weather station. Each 3h period contributed to a fraction of latent period according to its mean temperature. The sum of these fractions over time gave the number of generations completed.

Table 1. *Two successive inoculations performed with three Blumeria graminis f.sp. hordei single-spore isolates (GL1, GL2, GL3) to create various powdery mildew populations on nine plots of barley in each replicate*

	Inoculation sequence								
Inoculation	1	2	3	4	5	6	7	8	Control 9
1st	GL1[a]	GL2	GL3	GL1 + GL2	GL1 + GL3	GL2 + GL3	GL1 + GL2 + GL3	none[b]	none
2nd	GL2 + GL3	GL1 + GL3	GL1 + GL2	GL3	GL2	GL1	none	GL1 + GL2 + GL3	none

[a] GL1, GL2, GL3 corresponded to pathotypes Va22 Vh, Va1 VLa and Vk1 Vh, respectively.
b No infected seedlings were transplanted.

Table 2. *Growth stages, dates and estimated numbers of Blumeria graminis f.sp. hordei generations[a] elapsed between the first and second inoculations, or the two samplings, in the autumn- and spring-sown replicates*

		Autumn-sown (A1, A2)		Spring-sown (S1, S2)	
	Growth stages[b]	Date	Generations	Date	Generations
Sowing		1/10		16/3	
First inoculation	12–13 (2–3 unfolded leaves)	26/10	0	7/4	0
Second inoculation	13–14 (3–4 unfolded leaves)	16/11	1·7	26/4	1·6
First sampling	20–23 (early tillering)	6/1[c]–5/3[d]	4·2[c]/5·8[d]	10/5	3·9
Second sampling	33–37 (stem elongated)	24/3	8·7	14/6	9·3

[a] Number of generations estimated using a relationship between temperature and latent period (Friedrich, 1995).
[b] Growth stages according to Zadoks *et al*. (1974).
[c] For replicate A1.
[d] For replicate A2, sampled later than A1.

Build-up of artificial inoculum

The three isolates were multiplied from single conidia isolated from colonies collected in unsprayed fields of winter barley cv. Plaisant in 1995 (GL1) and 1998 (GL2 and GL3). Their pathotypes were assessed on 11 near-isogenic lines of cv. Pallas (Kølster *et al*., 1986), with the resistance alleles *Mla*1, *Mla*6, *Mla*7, *Mla*9, *Mla*12, *Mla*13, *Mla*22, *Mlk*1, *Mlg*, *MlLa* and *Mlh*. The isolate GL1 was virulent on *Mla*22 and *Mlh*, GL2 on *Mla*1 and *MlLa*, and GL3 on *Mlk*1 and *Mlh*. The three isolates were chosen because their pathotypes were rare in the local mildew population at the start of the experiment. In a sample of 107 single-colony isolates collected on seedlings of cv. Plaisant exposed at the trial site in October 1998, the frequency of the GL1 pathotype was 3%. The GL2 and GL3 pathotypes were not detected, indicating a frequency below 1%. The isolate GL1 belonged to the clonal lineage detected on winter barley in the north of France in 1992–95 (Caffier *et al*., 1996, Caffier *et al*., 1999).

GL1, GL2 and GL3 were multiplied on leaf segments of winter barley cv. Igri, with the resistance allele *Mlra*. Pots of 9-day-old cv. Plaisant seedlings were inoculated at low density, then incubated for 10days in a growth chamber (14h day, 17°C, 110μEm^{-2}s^{-1}/10h night, 14°C) followed by 3–6days at low temperature (8–10°C) to adapt the seedlings to field conditions. At 13–16days after inoculation, the infected seedlings were thinned to three to nine per pot, to standardize the inoculum load (30±3 sporulating colonies per pot) over the plots.

Assessment of powdery mildew populations in the plots and virulence tests

Frequencies of pathotypes in the powdery mildew populations were assessed approximately four and nine generations after the first inoculation (Table2). In each plot, leaf segments with a single colony were sampled along a regular grid of 10×10 sampling points, and placed on water agar. Conidia were transferred 2days later onto leaf segments of cv. Igri for multiplication, and tested for virulence 10–12 days later.

A differential set of eight Pallas isolines P01 (*Mla*1), P05 (*Mla*7), P10 (*Mla*12), P12 (*Mla*22), P16 (*Mlk*1), P21 (*Mlg*), P23 (*MlLa*), and P24 (*Mlh*) was used, with cv. Igri (*Mlra*) as a control for inoculation density. This subset allowed the three pathotypes GL1, GL2 and GL3 to be distinguished from the local population. The virulences matching *Mla*7, *Mla*12 and *Mlg* were absent in GL1, GL2 and GL3, and frequent in the natural population (49, 75 and 80%, respectively, in the sample from trap plants at the trial site in October 1998). Each isolate was inoculated using a settling tower on leaf segments of 10-day-old seedlings of the Pallas isolines, maintained on water agar. After incubation (7days at 17°C, continuous light 14μEm^{-2}s^{-1}), the infection types (IT) were scored on a 0–4 scale (Moseman *et al*., 1965) and classified as avirulent (IT 0–3) or virulent (IT 4).

Statistical analysis

Generalized linear models were used to analyse the pathotype frequencies. A multinomial analysis (McCullagh & Nelder, 1990) was done to compare the frequencies of four categories of pathotype (GL1, GL2, GL3 and other) using the genmod procedure of sas (SAS Institute Inc., 1997) with a log link. The differences between pathotype frequencies were regarded as significant if the P value for the χ-square test was <0·05. Analyses by pathotype were done to compare the frequencies of individual pathotypes in plots given different inoculations, at different sampling times and at different sowing times. Here, the probability distribution was binomial and the analyses were performed with the genmod procedure of sas using a logit link.

Results

Artificial field inoculations

In autumn 1998 and spring 1999, barley powdery mildew epidemics were observed in all 36 field plots. Although disease severity on the plots was not quantified, no obvious discrepancies in disease level were observed among the four replicates. At the first sampling time the disease severity was low, but colonies were present at each of the sampling points. At the second sampling time the disease severity was higher, some leaves were covered with mildew, but the disease level on the plots was far from saturation as mildew-free leaves were frequent.

During the whole experiment, 5737 isolates were tested for virulence, 3181 isolates (74–94 per plot) during the first sampling and 2556 isolates (38–96 per plot) during the second sampling. Isolates in the samples

exhibiting the same pathotype as GL1, GL2 or GL3 were assumed to result from artificial inoculation, and to be clonal progeny of the three inoculated isolates. Isolates with other pathotypes resulted from natural infection of the plots by the local airborne spore population. The GL1, GL2 and GL3 pathotypes were found in all samples from the 32 inoculated plots, indicating the success of the artificial inoculations. The mean frequencies of these three pathotypes on the artificially inoculated plots ranged from 1 to 28%.

Effect of pathotype

Although the choice of the three isolates used was based on their pathotype so that they could be recognized, rather than on specific competitive abilities, differences between their frequencies were detected using the multinomial analysis (Table3). The frequency of GL1 was the highest and that of GL2 the lowest (Fig.1, plots 7–9; Table4). Interactions between pathotype and inoculation sequence, sampling time and location were all significant. Pathotype effect was also significant for each of the eight individual inoculation sequences and for the control plot (data not shown).

Figure 1 *Effects of number of isolates inoculated at the same time and of pathotype (inoculated isolate) on pathotype frequencies on the barley plots. The three isolates GL1, GL2 and GL3 were inoculated in sequences described in Table1. The delay between the first and second inoculations was approximately two generations (Table2). The frequencies are means over the two sampling times at four locations, and the standard error of the mean is indicated. Different letters indicate a significant difference (P<0·05), tested using the contrast statement in the multinomial analysis for each of the nine plots (see text).*

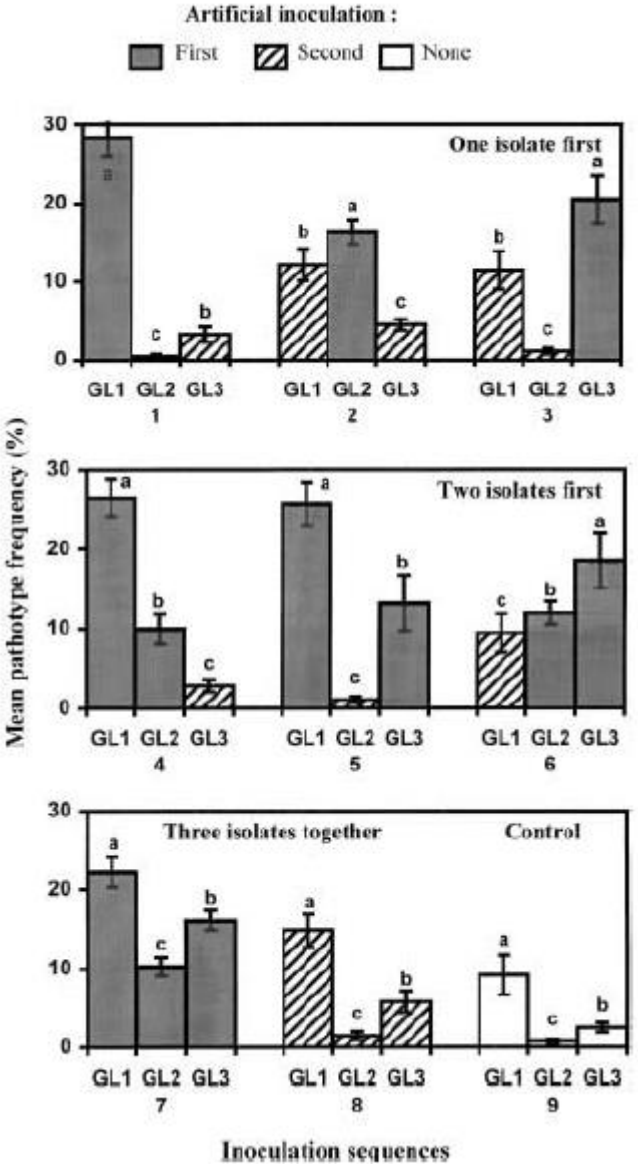

Table 3. *Effects of the pathotype (inoculated isolate) and interactions with the inoculation sequence, the sampling time and the location, assessed using a multinomial analysis performed on the observed numbers of each of the four categories (GL1, GL2, GL3 and others)*

Source	df	χ-square	*P* value
Pathotype	3	4968·0	0·0001
Pathotype × inoculation sequence	24	921·6	0·0001
Pathotype × sampling time	3	30·2	0·0001
Pathotype × location	9	165·4	0·0001

Table 4. *Significance of the differences between the pathotypes, assessed using contrast statements in the multinomial analysis*

Contrast	df	χ-square	*P* value
GL1–GL2	1	410·6	0·0001
GL2–GL3	1	59·0	0·0001
GL1–GL3	1	169·2	0·0001

Effect of time of inoculation

Time of inoculation, whether at the start of the experiment or approximately two generations later, had a substantial effect on the resulting pathotype frequencies. Inoculation sequence had a significant effect on the frequencies of the three genotypes (Table5). When an isolate had been transplanted at the first inoculation, its frequency in the sampled population was always significantly higher than when transplanted at the second inoculation (Fig.1; Table6). The pathotype frequencies resulting from the first inoculation were significantly different from those in the uninoculated control plot in each case, while pathotype frequencies resulting from the second inoculation were significantly higher than in the control only for GL1 (Table6).

Table 5. *Effects of inoculation sequence, sampling time and location in analyses of pathotype frequencies for each of the three pathotypes (inoculated isolates)*

Pathotype	Source	df	χ-square	*P* value
GL1	Inoculation sequence	8	230·8	0·0001
	Sampling time	1	11·4	0·0007
	Location	3	78·8	0·0001
GL2	Inoculation sequence	8	382·2	0·0001
	Sampling time	1	0·9	0·35
	Location	3	2·3	0·52
GL3	Inoculation sequence	8	307·0	0·0001
	Sampling time	1	14·1	0·0002
	Location	3	60·3	0·0001

For each inoculation sequence, the isolates that had been transplanted first were most frequent in each case (Fig.1). The magnitude of this early arrival effect was influenced by pathotype, but early arrival effect prevailed over pathotype effect in the six treatments in which the three isolates were not transplanted at the same time (Fig.1, plots 1–6). The environmental conditions might have influenced the success of the inoculations (see Discussion), but the early arrival effect was observed in both autumn and spring.

Table 6. *Effect of inoculation time, assessed using contrast statements in analyses of pathotype frequencies performed for each of the three pathotypes (inoculated isolates)*

Pathotype	Contrast[a]	df	χ-square	*P* value
GL1	Early–late	1	168·6	0·0001
	Early–none	1	106·2	0·0001
	Late–none	1	6·0	0·014
GL2	Early–late	1	299·0	0·0001
	Early–none	1	117·0	0·0001
	Late–none	1	0·6	0·44
GL3	Early–late	1	226·5	0·0001
	Early–none	1	115·7	0·0001
	Late–none	1	3·6	0·059

[a] Plots were grouped according to the time when they were inoculated: early, at the start of the experiment; late, approximately two generations later; none, uninoculated plot (control).

Effect of time of sampling

The persistence of the effects of inoculation time and genotype over the growing season was assessed by comparing pathotype frequencies four or nine generations after the first inoculation. The sampling time had a significant effect on pathotype frequencies for the two most frequent pathotypes, GL1 and GL3, but not for the least frequent pathotype, GL2 (Tables 3 and 5). The mean frequencies, averaged over all plots, increased between the two sampling times, from 16·2 to 19·3% for GL1, and from 8·2 to 11·1% for GL3. The frequency of GL2 remained stable, with mean frequencies of 6·2 and 5·7%. The increases in frequencies between the sampling times for GL1 and GL3 were small compared to the initial differences between the three pathotypes at the first sampling time.

For each inoculation sequence, the pathotype frequencies are presented as a mean over the two sampling times (Fig.1). The relative frequencies of the pathotypes, shown in Fig.1, also applied when the frequencies at the two sampling times are analysed separately (data not shown). The magnitude of the early arrival effect was not reduced after nine generations, when the first-arrived isolates were still more frequent than those transplanted two generations later. In six of the nine inoculation sequences, pairwise comparisons indicated no significant differences between the frequencies at first and second sampling times for all three pathotypes (data not shown). In plots given inoculation sequences 5 and 6, the frequencies of GL3 were significantly higher in the second than in the first sample. In the control plot, the frequency of GL1 was significantly higher in the second than in the first sample.

Effect of sowing time

The experiment included four locations, two of which were sown in autumn (A1, A2) and two in spring (S1, S2). The number of degrees of freedom was not sufficient to introduce both location and sowing time into the model. Only location was introduced as an effect, and the autumn-sown locations were compared to the spring-sown ones using contrast statements. The interaction between pathotype and location was significant (Table3). Location had a significant effect on the pathotype frequencies for GL1 and GL3, but not for GL2 (Table5). For GL2 and GL3, the two locations sown at the same time (in autumn and spring, respectively) did not differ significantly in the frequency of either pathotype (Table7). It was therefore possible to assess the effect of sowing time. For GL3, the frequencies were significantly higher in the spring-sown than in the autumn-sown locations, whereas for GL2 the frequencies were the same for both sowing times. For GL1, the frequencies were significantly different in the two autumn-sown locations (Table7). Therefore it was not possible to interpret the difference between the autumn- and spring-sown locations.

Table 7. *Effects of sowing time and location, assessed using contrast statements in analyses of pathotype frequencies performed for each of the three pathotypes (inoculated isolates)*

Pathotype	Contrast[a]	df	χ-square	*P* value
GL1	Autumn–spring	1	63·9	0·0001
	A1–A2	1	16·7	0·0001
	S1–S2	1	0·5	0·49
GL2	Autumn–spring	1	0·006	0·94
	A1–A2	1	2·25	0·13
	S1–S2	1	0·009	0·92
GL3	Autumn–spring	1	56·0	0·0001
	A1–A2	1	2·2	0·14
	S1–S2	1	1·7	0·19

[a] Location and sowing time of plots: autumn sowing included locations A1 and A2; spring sowing included locations S1 and S2.

The first sample from the two autumn-sown plots was taken 4·2 and 5·8 generations after the first inoculation in A1 and A2, respectively (Table2). When the results from the first and second sampling times were separated in the analysis, similar conclusions were obtained (data not shown). It was concluded that the significant difference between the frequencies of GL1 in the two autumn-sown locations was not due to the first sample being taken slightly later.

Discussion

Effect of time of inoculation

Most experiments on the population genetics of powdery mildew fungi have been performed using natural populations, and there was no way of knowing when each pathotype had arrived on the field. Here, the time of infection by each pathotype was controlled by using two successive inoculations. In each plot, the first-arrived clones were the most frequent in the resulting populations. There were differences between the three isolates used, but the early arrival effect always prevailed over pathotype effect when the population was established. This result was consistent in all plots and locations and at both sampling times.

With the protocol used, lower spore production by the infected seedlings transplanted at the second inoculation could have led to the same observation. The number of sporulating colonies transplanted was counted on each pot and it was identical for both inoculations. However, the climatic conditions encountered after the transplantation, such as temperature and the occurrence of rain, might have influenced the spore production of the 30 colonies. Mean temperature and cumulative rainfall were calculated during the estimated two generations after each transplantation. In the autumn experiment, mean temperatures after the first and the second inoculations were 5·6 and 3·8°C, and cumulative rainfall was 108 and 77mm, respectively. In the spring experiment, the mean temperatures were 9·2 and 15·1°C, and the cumulative rainfall was 74 and 2mm. Within this range, spore production increases with temperature. Therefore spore production by the seedlings transplanted at the second inoculation might have been reduced in the autumn experiment because of the weather conditions, but not in the spring experiment. It was concluded that the observed effect of the inoculation time on pathotype frequencies was not due to the climatic conditions.

Previous work on the early phases of barley and wheat powdery mildew epidemics showed that epidemics are usually initiated by a large number of infections (O'Hara & Brown, 1998). The resulting foci overlap and merge very quickly, so that the pattern of pathotypes observed in the field appears random very quickly (Rouse *et al*., 1981; O'Hara & Brown, 1997). O'Hara & Brown (1996a) observed a rapid decrease in the relative proportion of immigrant spores in the field and postulated that immigration of spores is most important early in the epidemics. The results obtained in this study were in agreement with this hypothesis. Here, the importance of the immigrant spores was not estimated as the number of spores, but as the contribution of those spores to pathotype frequencies in the field population. A delay of no more than two generations was sufficient to create a shift in frequencies in favour of the first-arrived pathotypes. The confirmation that immigration is most important early in the epidemics gives support to a model which aims

to predict population evolution by regarding the dispersal of the airborne spore population on the host varieties as a single event at the beginning of the growing season (Østergård & Hovmøller, 1991; Hovmøller *et al*., 1993). This model assumes that the *B. graminis* f.sp. *hordei* population in a field is essentially determined by the population arriving from the previous crop or volunteers, followed by selection. This hypothesis is supported by the data presented from this study.

Effect of pathotype

Although the epidemics were all started from the same number of lesions of the inoculated isolates, there were significant differences between the frequencies of GL1, GL2 and GL3 in all treatments and replicates, even at the first sampling time.

The establishment of a population on a susceptible host is likely to be sensitive to the short-term environmental conditions (O'Hara & Brown, 1996a), such as temperature (Eckhardt, 1987; Ohl & Kranz, 1989), disease density (O'Hara & Brown, 1996b) and the age of the plants. A significant interaction was observed between the pathotype and sowing time, but the ranking of pathotype frequencies was the same for the first and second inoculations in both autumn and spring experiments, despite differences in the mean temperatures during the first two generations. In wheat powdery mildew competition experiments, the relative fitness of isolates changed at different densities (O'Hara & Brown, 1996b). Additional experiments, including studies on life-cycle components such as latent period, infection efficiency and spore production, are necessary to understand the causal factors of the observed differences and the conditions under which they are expressed. In barley powdery mildew, a selective advantage has been observed between isolates due to different abilities to overcome new resistance alleles such as *Mla*13 (Brown, 1994; Brändle *et al*., 1997). On a field of any one variety, all isolates need the virulence alleles matching the resistance alleles present in the variety. This study, however, was concerned with competition in the absence of any such strong selective force. Dominant pathotypes were observed in diverse field populations on single varieties, with no indication of clonality within these pathotypes (Welz *et al*., 1990). The isolate GL1 belonged to a clonal lineage that was dominant on winter barley in the north of France for previously unknown reasons (Caffier *et al*., 1996, Caffier *et al*., 1999). This study showed that differences in the fitness of clones, as shown by the ranking of their frequencies, GL1>GL3>GL2 (Fig.1), might occur on the same variety under field conditions.

Two-generation delay and population structure

In studies of airborne inoculum, from March to October the spore contents of the air fluctuated over time, but viable spores were present in the air even after the harvest in summer (Limpert *et al*., 1999). Based on these observations, it was postulated that most weeks of the season might be important for migration (Limpert *et al*., 1999). By contrast, results of this study indicate that the contribution of the inoculum to a population, in terms of pathotype frequencies, depends strongly on the time of first arrival on the plot. Although natural infection was possible during the whole barley-growing season, in experimental plots the isolates GL1, GL2 and GL3 were not replaced by natural airborne pathotypes. This emphasizes the crucial role of the first-arrived inoculum in the epidemic. The fact that spores are present in the air does not imply that they have much influence in fields where powdery mildew populations are already established.

The early arrival effect may explain the persistence of the clonal lineage represented by GL1 on winter barley in the north of France (Caffier *et al*., 1996). On autumn-sown fields, mildew epidemics begin in autumn. On these fields the clonal lineage would therefore have been established a long time before the immigration in spring of isolates selected by spring barley varieties. The persistence of the early-arrival effect over the whole growing season might have prevented the replacement of the established clonal lineage in autumn-sown fields by isolates from spring-sown fields. This hypothesis could account for the maintenance of the clonal lineage on autumn-sown barley over several years, but it does not explain how it became established initially. The persistence of a clone on winter barley might not be possible in all situations, and the effects of oversummering in the sexual and asexual phases on a population structure in which a clonal lineage is dominant in an otherwise diverse population are being tested (Bousset, 2000). Much work has been done to understand the structure and evolution of powdery mildew populations during the epidemic phase. The results described here imply that studies of the summer survival phase and early phases of the establishment of

populations on the crop are necessary to complete our understanding of the evolution of powdery mildew populations over years.

Field studies of plant pathogen populations

In this study, artificial inoculations were successful on all 32 field plots and the influence of airborne inoculations was limited by the protocol used. Low doses of inoculum (30 colonies per isolate), and having all fields infected to start epidemics, helped to reduce interplot interference (O'Hara & Brown, 1998) sufficiently to measure the inoculation time effect, despite variation in climatic conditions in the two seasons. Inoculation of field plots by transplantation of infected seedlings was also used in studies of dispersal gradients (Welham *et al*., 1995). However, as far as the authors are aware, field inoculations have not previously been described for population genetic studies of barley powdery mildew, and airborne inoculum is regarded as a problem in field experiments. This study demonstrated that, even for a pathogen with frequent long-distance dispersal and a large amount of natural airborne inoculum, it was possible to set up a field experiment that combined artificial populations in which evolution was easier to follow than in diverse natural populations, with real environmental conditions. The use of this kind of field experiment is a matter of current debate, and further studies are needed to improve the methodology and analysis (Zhan *et al*., 1998, Zhan *et al*., 2000; Brown, 2000).

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