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Barley powdery mildew populations on volunteers and changes in pathotype frequencies during summer on artificially inoculated field plots

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Abstract

Pathotype frequencies in barley powdery mildew populations were assessed in artificially inoculated barley plots. The field experiment was organised in eighteen 3 m × 3 m plots with different inoculum compositions, obtained by sequential inoculation with three isolates, gl-1, gl-2 and gl-3, shortly after seedling emergence. In the conidia populations before summer, pathotypes corresponding to the inoculated isolates were detected at frequencies in the range 11–42% for GL1, 0–14% for GL2 and 2–34% for GL3. On the volunteers appearing after harvest these three pathotypes were observed at lower frequencies: 0–37% for GL1, 0–12% for GL2 and 0–23% for GL3. The overall ranking of GL1, GL2 and GL3 frequencies was thus the same before and after summer. The populations on volunteers were influenced by both sexual and asexual populations present in the same field at the end of the previous growing season. However, at the small-scale level no simple correlation was found between the frequencies in the conidia populations on volunteers and those in the airborne population, or the conidia populations on the crop before summer, or the calculated expected frequencies in populations of ascospores. During the summer survival, chance events may also have a large influence on pathotype frequencies leading to a high variation between repeated events of transition from the crop to the volunteers.

Keywords: *Blumeria graminis* f. sp. *hordei*, summer survival, virulence

Introduction

During asexual epidemics (10–15 generations) on green leaves of the growing host, the composition of field populations of *Blumeria graminis* (Syn. *Erysiphe graminis*) f.sp. *hordei* is strongly influenced by the early inoculum and the importance of immigration is later reduced (O'Hara and Brown, 1996; Bousset et al., 2001). The selection exerted by resistance genes in the host on the avirulence genes in the pathogen is based on a gene-for-gene interaction (Jørgensen, 1988). On a regional scale, during a growing season the pathotype composition of the aerial powdery mildew population could be predicted from the composition of the initial inoculum and the selection pressures exerted by the host varieties grown (Hovmøller et al., 1993). For a given growing season, a global approximation of the composition of the initial inoculum at the beginning of the asexual epidemic could be obtained from the composition of the population at the end of the asexual epidemic in the previous growing season. This approximation is equivalent to assuming a global averaging of the inoculum composition over the whole region and it gives good predictions at the regional scale (Hovmøller et al., 1993).

However, this approximation may not apply to the predictions regarding the inoculum of a single field because it ignores both the influence of sexual reproduction during summer and the possible stronger influence of the inoculum present at the field scale (local) rather than at the regional scale (distant). Sexual reproduction starts on senescent plants by mating between mycelia of the two opposite mating types leading to the formation of cleistothecia which remain on dry leaf debris (Smedegård -Petersen, 1967). Rain induces maturation and discharge of the ascospores, which is maximal over several weeks in autumn (Smedegård -Petersen, 1967). Little information is available about the survival of the inoculum on volunteers appearing after harvest, either by sexual or asexual reproduction. The possibility of asexual summer survival has often been postulated (Brändle et al., 1997; Brown et al., 1997; Hovmøller et al., 1997). This possibility is supported by the observation that at a period when the pathotype Va22Vh (GL1) was dominant in regional aerial populations on winter barley in Northern France (Caffier et al., 1996a), molecular markers indicated that isolates sampled from powdery mildew populations in 1992 and 1995 belonged to the same clone (Caffier et al., 1999).

However, to date it is not clear whether conidia can survive the gap between senescence of the crop and the emergence of volunteers only through migration of spores between regions with a delayed barley growing season, or if the survival of mycelium or conidia is also possible at the single-field level, for example, through infection of side tillers.

The aim of our study was to examine the processes occurring during the summer period of the pathogen cycle in more detail. In a field experiment including 18 field plots inoculated with three *B. graminis* f. sp. *hordei* isolates we investigated the possibility for pathotypes to survive at a local scale from the crop to the volunteers, along with the sexual and asexual origin of the populations on volunteers.

Materials and methods

Field experiment

The study was performed at Versailles INRA Research Station, 20 km west of Paris, France. Eighteen 3m × 3 m plots of the barley variety Plaisant were sown in spring 1999 three to six metres apart in a winter wheat (cv. Isengrain) field. Shortly after the emergence of the barley plants, the plots were inoculated on two occasions by transplanting seedlings infected with three single-spore isolates gl-1, gl-2 and gl-3 (Table 1). On the 18 field plots (P1–P18), nine combinations of the three isolates and the two transplantation times (detailed in Bousset et al., 2001) were replicated twice (plots P1–P9 and plots P10–P18, respectively) to generate a range of frequencies of the corresponding pathotypes. The three isolates were chosen because before artificial inoculation the corresponding virulence associations were found to be rare in the local mildew population: 2.8% for GL1, and <1% for GL2 and GL3. Because a pathotype detected in the field could consist of spores issued both from the artificial inoculation and 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). A detailed description of the protocol and the analysis of pathotype frequencies during the asexual epidemics has been published previously (Bousset et al., 2001) and some of the results describing the asexual populations were included in both studies.

The winter barley variety Plaisant had a low vernalisation requirement, meaning that sowing in early spring did not affect its ability to grow, elongate and produce seeds. In contrast, plants of the winter wheat variety Isengrain did not elongate well and only few tillers bloomed, due to insufficient vernalisation. To favour the emergence of volunteers on each plot, the barley and wheat plants were shredded instead of being harvested normally. At the harvest date (July 8) the barley seeds were fully ripened, but no mature seeds were present on the wheat plants. Hence, only barley volunteer plants appeared on each of the field plots, and the ground was bare (no volunteers) where wheat was previously growing. This meant that for a given plot the volunteer population was always sampled at the same place as the previous crop. Volunteers emerged and powdery mildew populations developed on all the barley plots. A sufficient number of lesions occurred to enable sample collection from each plot in early October.

Table 1. Virulence pathotypes of the three barley powdery mildew isolates used in the experiment

Virulence pathotype*		Isolates		
		gl-1	gl-2	gl-3
P01	<i>Mla1</i>	0	1	0
P05B	<i>Mla7</i>	0	0	0
P10	<i>Mla12</i>	0	0	0
P12	<i>Mla22</i>	1	0	0
P16	<i>Mlk</i>	0	0	1
P21	<i>Mlg</i>	0	0	0
P23	<i>MlLa</i>	0	1	0
P24	<i>Mlh</i>	1	0	1

^a The virulence pathotypes of the isolates were assessed on a differential set of 8 Pallas near-isogenic lines (Kølster et al., 1986). ‘1’ or ‘0’ indicate virulence or avirulence of the isolate on the given line, respectively. The isolates gl-1, gl-2 and gl-3 are also avirulent on the three lines P03 (*Mla6*), P08B (*Mla9*) and P11(*Mla13*).

On each plot, conidia populations were sampled from the crop at the end of the asexual epidemics (June 14) and later (October 2–12) from the volunteers that appeared after harvest (Table 2). Leaf segments with single colonies were sampled from each plot along a 10×10 grid and placed on water agar (4g l⁻¹ agar, 0.3 g l⁻¹ benzimidazole). The isolates were transferred two days later onto leaf segments of cv. Igrî for multiplication, and tested for virulence after 10–12 days. Two samples from the aerial population (81 and 86 isolates, respectively) were obtained from 1–6 October to estimate the pathotype frequencies in the local aerial powdery mildew population outside the experimental field. Two batches of 10-day old seedlings of cv. Plaisant were exposed at two locations more than 300 m from the nearest barley field. Virulence tests were used to recognise the three inoculated pathotypes GL1, GL2 and GL3 (Table 1) from the local natural powdery mildew population. The numbers of isolates showing the same pathotype as one of the three inoculated isolates gl-1, gl-2 and gl-3 were counted.

Virulence tests

The pathotypes of the isolates gl-1, gl-2 and gl-3 (Table 1) were assessed on eight near-isogenic lines of cv. Pallas, each having one of the resistance alleles Mla1, Mla7, Mla12, Mla22, Mlk, Mlg, MILa and Mlh (Kølster et al., 1986). Three more lines Mla6, Mla9 and Mla13 were used only at the start of the experiment to assess the frequencies of the GL1, GL2 and GL3 pathotypes in the local inoculum. Each isolate was inoculated using a settling tower on segments, cut from first leaves of 10-day old plants of the Pallas near-isogenic lines, maintained on water agar. After incubation (7 days at 17°C, continuous light 14μEm⁻²s⁻¹), the infection types (IT) were scored according to a 0–4 scale (Moseman et al., 1965). The isolates were classified as avirulent (IT 0–3) or virulent (IT 4).

Expected pathotype frequencies after sexual recombination

The expected pathotype GL1, GL2, GL3 frequencies after sexual recombination were calculated according to Bousset and de Vallavieille-Pope (2003). Only the five virulences Va1, Va7, Va22, Vg and Vh were included in the analysis, since independence of the loci is required by the calculation method and genetic linkage groups have been reported including the avirulence genes Aa9-Aa22-Ak and Aa6-Aa12-Ala (Brown and Simpson, 1994; Brown et al., 1996; Caffier et al., 1996b).

We explicitly modelled the crosses for each of the 1024 pairwise combinations of the 2⁵ = 32 possible pathotypes. For each cross we calculated the expected proportion among the progenies of the pathotypes GL1, GL2 and GL3, respectively. For any given cross the proportion of progenies belonging to a specific 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 specific pathotype, respectively.

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 conidia population at the end of the asexual epidemic. For some of the crosses, additional constraints were included (see below). To obtain the proportions of the GL1, GL2 and GL3 pathotypes in the expected population of ascospores, the expected number of progenies in each cross were summed over all possible crosses and divided by the total number of progenies. Our calculation of the probability of occurrence of a given cross assumes that all crosses are possible if the two parental pathotypes are present. We made the hypothesis that both mating types were present in the airborne pathotypes, hence that all crosses were possible among the airborne pathotypes and between inoculated and airborne pathotypes. Because only one isolate and therefore one of the mating types was inoculated and the GL2 and GL3 pathotypes were not detected in the airborne population, sexual crosses were not possible within these two pathotypes – this was taken into account by setting the number of progenies from the crosses GL2×GL2 and GL3×GL3 to zero. In addition to the artificial inoculation of one mating type, isolates of pathotype GL1 were detected in the local inoculum. Therefore, a small proportion of the GL1 pathotype was assumed to be airborne and have the mating type opposite to that of the inoculated isolate gl-1. This was taken into account by considering only 2.8% or 6.6% of the crosses GL1×GL1 to be possible. The isolates gl-2 and gl-3 were of opposite mating type (Bousset and de Vallavieille-Pope, 2003), therefore all crosses were possible between GL2 and GL3. It was not known whether the inoculated isolate gl-1 had the same mating type as gl-2 or as gl-3, hence we tested both alternative hypotheses. When assuming that the isolate gl-1 had the same mating

type as gl-2, this was taken into account by setting the number of progenies from the cross GL1×GL2 to zero, and for the alternate hypothesis the number of progenies from the cross GL1×GL3 was set to zero.

To allow comparisons between the observed and the expected ascospore populations, the frequencies of pathotypes in the conidia population observed at the end of the asexual epidemics and in the populations established on volunteers were re-calculated using only the five relevant virulences to assess pathotypes.

Data analysis

Initially each of the 18 small plots was considered individually and the frequencies of each of the inoculated pathotypes GL1, GL2 and GL3 in different populations were compared by chi-square or Fisher exact tests. For the populations on the crop and on volunteers, the analyses were performed considering both the pathotypes defined by eight virulences or by five virulences. In a further analysis the 18 plots were considered as repetitions of a local summer survival and a correlation analysis (Spearman) was made for the three pathotypes GL1, GL2 and GL3 between the frequencies on volunteers and in (i) the airborne population, (ii) the conidia population present on the crop at the end of the asexual epidemics, and (iii) the expected populations of ascospores.

Table 2. Sample sizes of the barely powdery mildew conidia populations before (crop) and after (volunteers) harvest on 18 field plots. The frequencies of the pathotypes GL1, GL2, GL3 observed (crop, volunteers) and calculated after sexual recombination (expected 1×2 and expected 1×3) were compared by chi-square or Fisher exact tests and the probability associated with the hypothesis H0 of identical frequencies is indicated. The pathotypes were defined by five virulences

Comparisons ^a	Field plots																	
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
Sample size																		
Crop	46	51	57	45	52	64	65	56	65	57	65	56	63	64	59	65	67	75
Volunteers	41	43	37	41	40	44	43	41	43	40	38	34	42	10	43	43	85	133
GL1																		
Volunteers-crop	0.56	0.09	0.01	0.60	0.29	<0.01	0.02	0.02	<0.01	<0.01	<0.01	0.12	0.04	0.46	0.01	0.18	<0.01	0.43
Volunteers-airborne	0.02	0.37	0.25	<0.01	0.18	0.50	0.50	0.01	0.12	0.31	0.12	0.50	0.18	0.50	0.50	0.24	0.14	<0.01
Volunteers-expected 1 × 2	0.03	0.39	0.08	0.01	0.27	0.14	0.64	<0.01	0.04	0.20	0.04	0.54	0.28	0.93	0.47	0.86	0.04	0.01
Volunteers-expected 1 × 3	0.03	0.39	0.03	0.03	0.42	0.05	0.54	0.01	0.01	0.09	0.02	0.26	0.41	0.90	0.13	0.62	<0.01	0.08
GL2																		
Volunteers-crop	nc ^b	0.01	nc	0.04	0.57	0.59	0.48	0.01	0.07	0.59	0.01	0.62	0.07	0.87	0.62	nc	0.04	0.77
Volunteers-airborne	nc	nc	nc	nc	nc	nc	0.50	nc	nc	nc	nc	nc	nc	nc	0.50	nc	nc	<0.01
Volunteers-expected 1 × 2	nc	0.29	nc	0.52	nc	0.59	0.84	0.19	0.36	0.59	0.25	0.62	0.36	nc	0.83	nc	0.19	0.05
Volunteers-expected 1 × 3	nc	0.29	nc	0.52	nc	0.59	0.84	0.19	0.60	nc	0.27	nc	0.60	nc	0.82	nc	0.44	0.02
GL3																		
Volunteers-crop	0.53	0.29	<0.01	0.27	0.32	0.01	0.04	<0.01	0.01	0.39	0.49	<0.01	0.54	0.64	<0.01	0.08	<0.01	<0.01
Volunteers-airborne	nc	nc	0.50	nc	nc	0.50	nc	0.50	nc	0.03	0.25	nc	0.25	nc	nc	<0.01	0.01	0.03
Volunteers-expected 1 × 2	0.14	0.16	0.16	0.27	0.32	0.21	0.13	0.12	0.04	0.18	0.49	0.05	0.54	0.64	0.03	0.01	0.46	0.16
Volunteers-expected 1 × 3	0.14	0.16	0.07	0.14	0.18	0.04	0.07	0.05	0.01	0.82	0.38	0.02	0.42	0.55	<0.01	0.02	0.04	0.02

^aIsolates were sampled by collecting infected leaves on the crop (June 14) or volunteers (October 2) from the field plots, or by exposing two batches of seedlings to the local airborne population near the experimental field (October 16–23). From the airborne population, a total of 167 isolates were collected. Pathotype frequencies expected after sexual recombination were calculated (see text) under the hypothesis that the isolate gl-1 had the same mating type as either gl-2 (Expected 1 × 3) or gl-3 (Expected 1 × 2).

^bNot calculated.

^a Isolates were sampled by collecting infected leaves on the crop (June 14) or volunteers (October 2) from the field plots, or by exposing two batches of seedlings to the local airborne population near the experimental field (October 16–23). From the airborne population, a total of 167 isolates were collected. Pathotype frequencies expected after sexual recombination were calculated (see text) under the hypothesis that the isolate gl-1 had the same mating type as either gl-2 (Expected 1×3) or gl-3 (Expected 1×2).

^b Not calculated.

Results

Each of the 18 plots considered separately

For the populations on the crop and on volunteers, the values obtained in the analyses performed considering the pathotypes defined by either eight or five virulences were highly correlated ($p < 0.001$) for the three pathotypes (Table 3) and the conclusions of the comparisons were in most cases identical. We will present here only the values for the analysis with five virulences, which correspond to the illustration shown in Figure 1. In the populations of conidia on the crop at the end of the asexual epidemics, the inoculated pathotypes GL1 and GL3 were detected on all the plots, and GL2 on all but three plots. A broad range of frequencies was

observed for GL1 (11–42%) and GL3 (2–34%), whereas less variation was observed for GL2 (2–14% when detected) (Figure 1). In the populations established on volunteers, the inoculated pathotypes GL1, GL2 and GL3 were detected on 18, 3 and 9 plots, respectively, and on the small-plot scale a large amount of variation in their frequencies was observed among plots (Figure 1). In the populations on volunteers, the frequencies of the inoculated pathotypes GL1, GL2 and GL3 were in two cases higher than on the crop before harvest (1, 0 and 1 plot, respectively), and in 26 cases lower (10, 7 and 9 plots, respectively). In the remaining 26 cases no significant difference was found (Figure 1, Table 2).

In the airborne population sampled in October, the frequency of pathotype GL1 was 6.6% on average (6.2% and 7% in each of the two samples, respectively). The pathotypes GL2 and GL3 were not detected, indicating a frequency below 1%. In the populations on volunteers, the frequencies of the inoculated pathotypes GL1, GL2 and GL3 were in nine cases higher than in the airborne population in October (4, 1 and 4 plots, respectively), and not significantly different in the remaining 43 cases (Figure 1, Table 2).

The conclusions from the comparisons between pathotype frequencies of GL1, GL2 and GL3 observed on volunteers and those expected after sexual recombination were in most cases identical (Table 2) when calculated under the hypotheses that isolate *gl-1* had either the same mating type as *gl-2* or as *gl-3*. We present data for the latter assumption and mention those cases in which the conclusions differ depending on the hypothesis (for GL3). To set the constraints on the few GL1×GL1 crosses possible due to the isolates of the GL1 pathotype detected in the local inoculum (mixture of both mating types), we had only estimations in the airborne population before the inoculation (2.8%) and in October (6.6%). Because similar conclusions were obtained when using either 2.8% or 6.6% in the calculations (data not shown), only the latter are presented. In the populations on volunteers, the frequencies of the inoculated pathotypes GL1, GL2 and GL3 were in six cases higher than in the expected populations of ascospores (4, 1 and 1 plots, respectively), and in eight cases lower (5, 0 and 3 plots, respectively). In the remaining 40 cases the frequencies were not significantly different (Figure 1). For GL3, the frequency was lower in six additional plots only under the hypothesis that *gl-1* and *gl-3* were of the same mating type.

Table 3. Spearman correlation between the frequencies of three barley powdery mildew pathotypes measured on the crop at the end of the epidemics before summer and on the volunteers. The pathotypes were defined by either eight (crop-8; volunteers-8) or five (crop-5; volunteers-5; expected) virulences

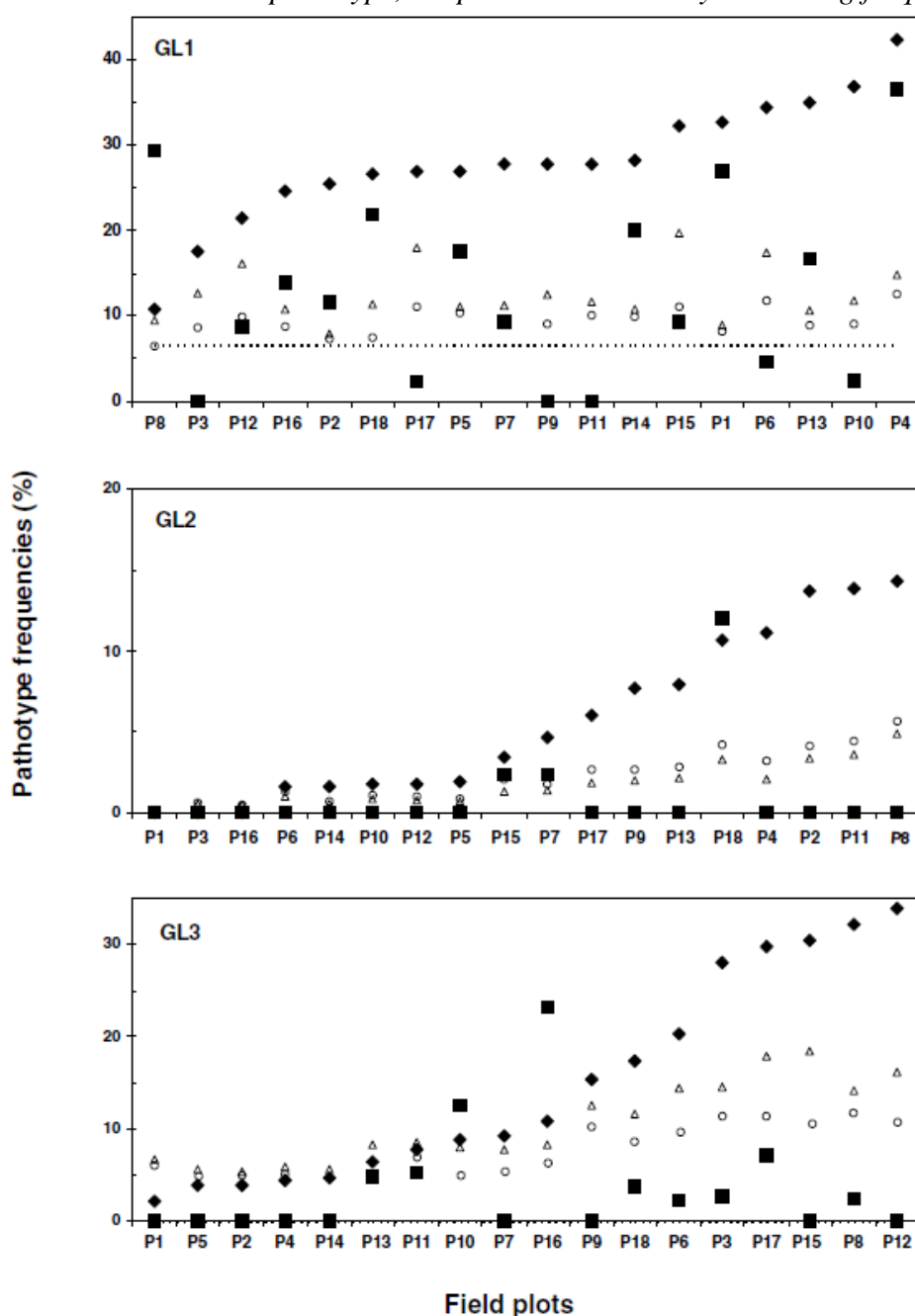
Pathotype	Spearman correlation	
	<i>r</i>	<i>p</i>
Crop-5/Crop-8		
GL1	0.89	<0.001
GL2	0.99	<0.001
GL3	0.99	<0.001
Volunteers-5/Volunteers-8		
GL1	0.70	0.001
GL2	0.81	<0.001
GL3	0.86	<0.001
Volunteers-5/Crop-5		
GL1	0.11	0.67
GL2	0.14	0.57
GL3	0.24	0.35
Volunteers-8/Crop-8		
GL1	0.32	0.20
GL2	0.20	0.44
GL3	0.16	0.54
Volunteers-5/Expected 1 × 3 ^a		
GL1	-0.52	0.03
GL2	0.18	0.49
GL3	0.29	0.24
Volunteers-5/Expected 1 × 2 ^a		
GL1	-0.23	0.37
GL2	0.21	0.41
GL3	0.29	0.25

^a The frequencies expected after sexual recombination were calculated (see text) for GL1, GL2, GL3 from the observed frequencies of all the pathotypes observed in the populations of conidia before summer. As the inoculated isolates consisted of only one of the mating types, we had to include additional constraints (see text) concerning the crosses between isolates of the pathotypes GL1, GL2 and GL3. We tested both alternative hypotheses that the isolate *gl-1* had the same mating type as either *gl-2* (Expected 1×3) or *gl-3* (Expected 1×2).

All the plots considered as 18 repetitions of a local summer survival

Spearman correlation analyses were performed with 167 isolates from the airborne population, 1072 from the populations on the crop and 841 from the populations on volunteers. For the three inoculated pathotypes, the number of isolates in the populations on volunteers was correlated neither to the airborne population nor to the conidia populations on the crop, nor to the expected populations of ascospores (Table 3). The only significant negative correlation was for the pathotype GL1 when the calculations of the expected frequencies were made under the hypothesis that isolate gl-1 was of the same mating type as gl-2

Figure 1. Frequencies of the three inoculated pathotypes GL1, GL2, GL3 observed in barley powdery mildew populations sampled on 18 small plots from the crop before harvest (diamonds), and from volunteers after the harvest (squares). The frequencies of these pathotypes expected after sexual recombination were calculated (see text) from the pathotype frequencies observed on the crop, considering that the isolate gl-1 has the same mating type as either gl-2 (triangles) or gl-3 (circles). The frequency of the GL1 pathotype in the local population is indicated (dashed line). The pathotype frequencies were determined on a differential set of five virulences. For each pathotype, the plots were sorted by increasing frequency on the crop.



Discussion

Summer survival and populations on volunteers

The three inoculated pathotypes were detected on volunteers on at least some plots, however only GL1 was detected in the airborne population, at a frequency of 6.6%. Because the isolates were not tested with molecular markers we cannot exclude the possibility that some isolates having the pathotypes GL1, GL2 and GL3 on volunteers were immigrants from distant locations and not progenies of the inoculated isolates. However, the frequency on volunteers was significantly higher than in the airborne population (6.6%) on four plots: up to 37% for GL1, and up to 23% for GL3, even though the frequency of GL3 was below 1% in the airborne population. We regard the distant airborne origin as much less likely than an origin from the experimental plots, hence we interpreted these results as an indication that the population on volunteers was influenced by the population present in the same field during the previous growing season.

On the other hand, the frequency on volunteers could not be directly predicted from the composition of the population present in the same field during the previous growing season. If the occurrence of sexual recombination could explain the absence of correlation between the frequencies on the crop and on the volunteers, sexual recombination was not sufficient to explain the observed frequencies. Since previous results (Bousset and de Vallavieille-Pope, 2003) indicated that biased mating type frequencies in some pathotypes may be taken into account in the calculations, we included additional constraints for the crosses within and between GL1, GL2 and GL3. In most cases (40 out of 54), the frequencies observed in the populations on volunteers were not different from the frequencies expected after sexual recombination, calculated taking into account that some crosses were not possible because only one mating type was inoculated for GL1, GL2 and GL3. However, these frequencies were not directly correlated and we interpreted these two results as an indication that both sexual recombination and some additional forces were acting on the populations during the over-summering of barley powdery mildew.

One of these forces is likely to be random drift, because during the summer the populations are reduced in size. Random drift could account for some of the variation, but on the volunteers of some plots the inoculated pathotypes were recovered at frequencies much higher than the frequencies expected in any of the 18 plots following sexual recombination (Figure 1). This might be an indication that asexual survival from the crop to the volunteers may occur at an experimental field level. The persistence of clones from one growing season to the next has been reported in pathogens of perennial crops. The occurrence of both sexual and asexual reproduction regimes in the same population, resulting in the presence of clonal lineages within a diverse population, was reported in a population of *Cryphonectria parasitica* on chestnut trees (Liu et al., 1996), however, this fungus also had a mixed reproduction regime (Taylor et al., 1999). In grapes, overwintering of *Uncinula necator* is possible either as mycelium in dormant buds or as ascospores on leaf debris, and differentiation between sexual and asexual populations was reported by Délye and Corio-Costet (1998). The possibility of clone persistence at the field level had not previously been tested for barley powdery mildew and our results provide a first indication that asexual survival might be possible at the field level. Additional studies should be designed to confirm these observations, in particular, a protocol should be developed to ascertain that the isolates observed on volunteers are clonal progenies of the inoculated isolates, and to exclude the hypotheses that they are immigrants or recombinant progenies of crosses between inoculated and immigrant isolates (Brown, 2000). Because the sequence of isolate arrival on the plot had a strong influence on the subsequent pathotype frequencies (Bousset et al., 2001), studies aimed at understanding the availability of the inoculum in the air depending on the spore source (sexual vs. asexual, local vs. distant) are desirable.

Benefits from experiments using artificial inoculations

Using artificial inoculation of field plots with three isolates belonging to pathotypes rare in the local airborne population allowed us to test hypotheses concerning the origin of spores that contributed to primary inoculum on volunteers. The study included 18 field plots, with a wide range in frequencies of the three pathotypes prior to summer. This allowed observation of the high variability in the resulting frequencies of the inoculated pathotypes on volunteers, among plots in similar environmental conditions. Previous field studies on natural populations allowed detection of changes in pathotype diversity (Welz and Kranz, 1987; Caffier et al., 1996a),

but did not permit recognition of differences in pathotype frequencies occurring over many repetitions of the transition from the crop to the volunteers. On a small scale, the variability in pathotype frequencies on volunteers was neither correlated to the frequencies in the populations of conidia on the crop before summer nor to the expected frequencies in ascospore populations. Although the dynamics of pathotypes in field populations during the growing season suggest that the population structure on field plots is mainly determined by the composition of the primary inoculum (Bousset et al., 2001), the results of this field experiment indicated that chance events such as bottlenecks might also have a large influence on the pathotype frequencies during the summer.

Artificial inoculations of field plots have been used to estimate the frequencies of recombination and migration in populations of ascomycete fungi (Zhan et al., 1998; 2000). However, in our experiment the overall ranking of GL1, GL2 and GL3 was the same before and after summer when the data from the 18 field plots were pooled but on the other hand we also clearly illustrated the high variability of pathotype frequencies observed on volunteers when the scale of the field considered is small. Even though it could be criticised that the sample sizes were limited and that virulence markers were not the most discriminating technique, the contribution of immigrants and recombinants to the population seems to be highly variable. Furthermore, this variation was observed among plots of the same barley variety, in the same year and in the same location. This underlines the concern about generalisations based on estimations issued from a single field. Taking into account sample sizes of several hundreds of isolates (Brown et al., 1997) it is surely not possible to repeat such experiment on many large fields.

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