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Greek epidemics of grapevine downy mildew are driven by local oosporic inoculum: a population biology approach

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Epidemics of grapevine downy mildew are usually assumed to be initiated by oospores, but the spread of the disease is commonly attributed to the secondary, asexual inoculum. Evidence from the investigation of population structures observed in 15 natural downy mildew epidemics which occurred in seven Greek vineyards and for three consecutive years, however, showed that primary infections from oospores play a major role in both the initiation and subsequent development of the epidemic. Furthermore, oosporic infections were found responsible also for the disease recrudescence in September. The role of secondary infections in the epidemic, although it differs depending on the region and on the sampling date, is mostly not important for the epidemic growth and pathogen dispersal. However, the clonal multiplication of some genotypes can, in rare instances, be of significance for the spread of the epidemic. The epidemic patterns that appeared can be grouped into a) the "continuous" epidemic pattern in humid areas, b) the "two-peak" epidemic in areas with dry summer, where bottleneck events during summer halt the disease and c) the "predominance-of-one-clone" pattern in islands/coasts, where the secondary infections have greater impact on disease growth than the oosporic ones. The present results, using both epidemiological and population genetics data, in combination with similar research accomplished under Central European conditions, constitute a first approach to *Plasmopara viticola* population biology.

Key words: microsatellite, genotypic diversity, genetic divergence, bottleneck, parentage analysis.

INTRODUCTION

The oomycete *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni (Order: Peronosporales, Family: Peronosporaceae), the causal agent of grapevine downy mildew disease, constitutes the most destructive pathogen in viticulture for continental climates with spring or autumn rains. The pathogen infects young inflorescences and berries as well as leaves, producing yellowish lesions (oilspots). Losses are caused through fruit destruction, killing of leaf tissue and weakening of shoots (Agrios, 2004). Earlier concepts regarding the pathogen's epidemiology have postulated that the disease starts from a few germinating sexual spores (oospores) that cause primary/oosporic infections early in the grapevine vegetative season. Oospores are considered to play a role only in the initiation of the disease. The first infections are followed by successive asexual cycles and the secondary sporangia produced cause secondary/clonal infections. The explosive disease progress and dispersal are attributed to the asexual spores (secondary sporangia), which are assumed to migrate long distances within a short time (Zahos, 1959; Lafon & Clerjeau, 1988; Blaise *et al*., 1999).

Two conditions affect Greek vineyards and give particular traits to *P. viticola* populations and epidemic growth compared with those in central Europe. The first exclusive condition is the Mediterranean climate, where, due to absence of prolonged rains

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early in spring, limited primary infections are expected (Zahos, 1959). Later in the season, during summer, long periods of heat and drought often restrict or even interrupt the disease (bottleneck event). Regularly, the epidemic revives in autumn, after the first September rainfalls. The disease revival is considered as the result of the re-sporulation of the spring/summer oilspots, of the germination of oospores that did not infect in spring or, less probably, of the migration of sporangia from northern/western regions of the country where the epidemic may last all summer (Zahos, 1959). The second feature of the Greek environment is the topography, characterized by numerous islands and mountainous mainland that isolate the populations and may drive them to diverge genetically.

The possibility to investigate *P. viticola* populations emerged with the development of species-specific, co-dominant, highly variable microsatellite markers that allowed the genotyping of the pathogen (Gobbin *et al*., 2003a). Preliminary research greatly challenged the importance of the secondary inoculum for the epidemic growth (Gessler *et al*., 2003). Occasional clonal multiplication was observed in four central European countries (Gobbin *et al*., 2002, 2003b, 2005), in contrast to an epidemic driven by one predominant clone in a Greek coastal vineyard (Rumbou & Gessler, 2004). Clonal but genetically rich populations were found in isolated vineyards in three Greek islands (Rumbou *et al*., 2002; Rumbou & Gessler, 2006). Those novel results necessitated an investigation for the role of the local oosporic inoculum in the *P. viticola* epidemic development. For the particular study, both epidemiological traits (source and types of inoculum, dispersal, disease progress) and population genetics data (population structure, migration, recombination, genetic drift) were required. Integration of epidemiological and population genetic analysis from 16 Greek *P. viticola* populations in total represents an analysis of the pathogen's population biology, which allows a broader and more encompassing perspective of the disease dynamics (Milgroom & Peever, 2003).

MATERIALS AND METHODS

Samplings were carried out during 2000-2002 in seven vineyards (Table 1). Six vineyards were not treated against downy mildew to follow uninterrupted natural epidemics of *P. viticola*, while one vineyard was treated (Lefkada) (Fig. 1). Four vineyards were located in central Greece (N. Aghialos, Damassi, Messenikolas and Preveza), while the three remaining plots were on islands (Kephalonia, Zakynthos and Lefkada). Applying a uniform sampling strategy, part of every oilspot detected on the leaves was collected as soon as it was visible and the rest of the lesion was left on the vine. Collected lesions were marked so as to avoid recollecting them in a subsequent sampling. Total number of lesions was collected in the early stages of the epidemics; when disease severity increased to more than five lesions per vine, the samplings were partial (five lesions/vine randomly selected). A total of 4872 oilspots were collected. For genotyping of all the collected lesions, an automated high-throughput DNA extraction method was used followed by PCR amplification of four *P. viticola*-specific microsatellite loci, according to Gobbin *et al*. (2003a). Selected loci were characterized by high polymorphism: GOB, 86 alleles; ISA, 5 alleles; CES, 22 alleles; and BER, 4 alleles. Isolates presenting the same allele pattern were considered as clones (derived from the same oospore), whereas those presenting a different allele pattern were assumed to have derived from different oospores. Using this procedure, the primary infections could be differentiated from the secondary infections.

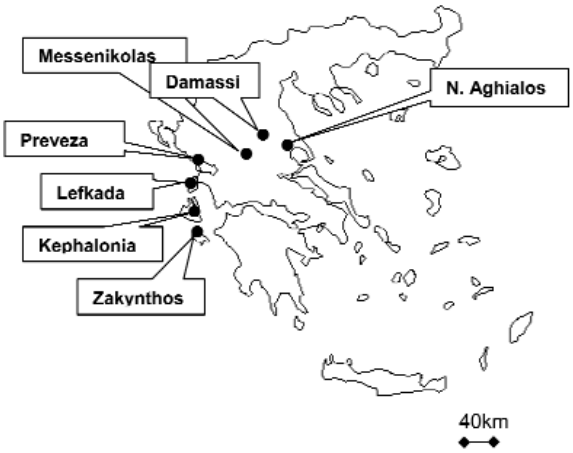


FIG. 1. Map of Greece where the sites of the seven selected plots are marked.

For population genetic analysis, the 52 collections of lesions (hereafter a collection of lesions will be called "sample") were used without having been corrected for clones (for estimates of genotypic diversity and spatial distribution) and also after clone correction (for estimates of gene diversity and Hardy-Weinberg equilibrium tests). After each of the samples was clone corrected, the samples collected from the same plot during one season were subjected to a pairwise test of differentiation by applying the log-likelihood statistic G for

TABLE 1. Description of the 52 *P. viticola* samplings and of the seven collection plots

Plot	Cultivar	No. of vines	Sampling period	No. of samplings/No. of epidemics	No. of lesions	No. of genotypes
N. Aghialos (Magnisia)	white Roditis	100	28/05/01-02/07/02	9/2	632	77
Damassi (Larissa)	″	60	29/05/01-15/11/02	10/2	1017	214
Messenikolas (Karditsa)	″	30	23/05/00-20/11/02	12/3	1117	403
Preveza	Managatiko & Korinthi	~250	07/06/00-13/11/01	7/2	689	230
Kephalonia	Moscatella & Vostylidi	170	13/06/01-16/07/02	5/2	199	32
Zakynthos	Skylopniktis	45	14/06/01-03/06/02	3/2	366	137
Lefkada	Agiorgitiko, Muscat, Robola etc.	320	31/05/01-03/10/02	6/2	852	204
Total		~975	2000-2002	52/15	4872	1297

TABLE 2. The 16 subpopulations of *P. viticola* collected from seven plots in Greece during the 2000, 2001 and 2002 grape-growing seasons (E_H: normalized Shannon's index, for details see text)

Subpopulation	Plot		Sampling period	No. of samplings	No. of lesions	No. of genotypes	E_H
agh	N. Aghialos	2001-2002	28/05/01-02/07/02	9	632	77	0.26
dam 01	Damassi	2001	29/05-15/10/01	6	656	130	0.70
dam 02a-c	Damassi	spring/summer 2002	16/05-19/06/02	3	280	50	0.61
dam 02d	Damassi	autumn 2002	15/11/02	1	81	37	0.69
mes 00	Messenikolas	spring/summer 2000	23/05-27/06/00	4	278	84	0.60
mes 01a-d	Messenikolas	spring/summer 2001	8/06-11/07/01	4	507	195	0.80
mes 01e	Messenikolas	autumn 2001	19/10/02	1	167	34	0.59
mes 02	Messenikolas	2002	7/06-20/11/02	3	165	92	0.80
pre 00	Preveza	2000	7/06-17/09/02	3	139	80	0.82
pre 01	Preveza	2001	31/05-13/11/01	4	550	150	0.65
kef	Kephalonia	2001-2002	13/6/01-16/07/02	5	199	33	0.50
zak 01	Zakynthos	2001	14/06/01 & 18/07/01	2	152	23	0.52
zak 02	Zakynthos	2002	03/06/02	1	214	114	0.83
lef 01a-c	Lefkada	spring/summer 2001	31/05-05/07/01	3	359	79	0.63
lef 01d	Lefkada	autumn 2001	22/11/01	1	166	53	0.59
lef 02	Lefkada	2002	29/05/02 & 05/10/02	2	327	75	0.59

diploid populations (Goudet *et al*., 1996) with the use of FSTAT software (ver. 2.9.3.1; Goudet, 1995). Samples with no significant difference were pooled together, as they all were partitions of the same population. Again, they were subjected to clone correction. The samples showing significant differences were treated separately. Sixteen subpopulations were finally obtained and used for the genetic analysis (Table 2).

Each of the 16 populations was studied separately for genetic diversity. The methods for genetic analysis (estimation of genotypic diversity, number of alleles, allele frequencies, expected and observed heterozygocity, Hardy-Weinberg tests) are described by Rumbou & Gessler (2004). The parentage analysis was performed with the use of IDENTITY (ver. 1.0; Wagner & Sefc, 1999); the software gives all possible parent-offspring combinations, assuming co-dominant Mendelian inheritance of alleles. The genotypes of 2000 were tested for being the potential parents of the genotypes of 2001 and the same was done for the genotypes of 2001 in relation to the genotypes of 2002.

The population genetic structure was first examined by testing the null hypothesis that the distribution of alleles is not significantly different across all 16 populations. Pairwise tests for allelic differentiation were performed by using the overall loci G-statistic, and their significance was evaluated after applying the sequential Bonferroni correction for multiple tests. The degree of differentiation among the plots was quantified using Weir & Cockerham's (1984) estimator (θ) of Wright's F_{ST}, as calculated by FSTAT.

The spatial distribution of the disease was represented with the use of SYSTAT software (ver. 10, SPSS Inc., 2000). Gaussian bivariate confidence ellipses for probability $p = 0.05$, based on the unbiased sample standard deviations of x (number of row) and y (number of vine in the row) (where x and y represent the coordinates of the vine where a particular lesion belonging to a particular genotype was found) were drawn. They described the dispersal of the total genotypes, of the most frequent genotypes (the ones that showed genotype frequency $p > 0.075$ in all samples) and of the remaining genotypes (the lesions remaining after subtracting from the total lesions the ones belonging to the most frequent genotypes). Clonal spread of the remaining genotypes is not presented because they produced very small numbers of clones and they were firmly focused on one/two vines. Only the spatial distribution of the epidemics in the mainland plots of Damassi, Messenikolas and Preveza are presented here (for N. Aghialos, see Rumbou & Gessler, 2004; for Kephalonia, Zakynthos and Lefkada, see Rumbou & Gessler, 2006).

RESULTS

Contribution of primary infections to epidemics

A large number of different genotypes were identified during the disease growth period in *P. viticola* populations in most regions. In all mainland epidemics the disease started with a high number of primary infections in the end of May/beginning of June (Damassi: dam 29/05/01: $N_{gen} = 44$, $E_H = 0.74$; dam 27/05/02: $N_{gen} = 31$, $E_H = 0.61$; Messenikolas: mes 23/05/00: $N_{gen} = 47$, $E_H = 0.70$; mes 08/06/01: $N_{gen} = 79$, $E_H = 0.86$; mes 07/06/02: $N_{gen} = 65$, $E_H = 0.82$; Preveza: pre 07/06/00: $N_{gen} = 21$, $E_H = 0.86$; pre 31/05/01: $N_{gen} = 44$, $E_H = 0.71$). The only exception among mainland epidemics was in the N. Aghialos plot, where the epidemic started with very few primary infections (agh 28/05/01: $N_{gen} = 5$, $E_H = 0.11$; agh 23/05/02: $N_{gen} = 5$, $E_H = 0.12$) (Rumbou & Gessler, 2004). In the islands, abundant primary infections were found in the epidemics in Lefkada in both years and in Zakynthos only in 2002 (Lefkada: lef 31/05/01: $N_{gen} = 27$, $E_H = 0.67$; lef 29/05/02: $N_{gen} = 53$, $E_H = 0.62$; Zakynthos: zak 03/06/02: $N_{gen} = 114$, $E_H = 0.83$) (Rumbou & Gessler, 2006). However, the populations from the Kephalonia and Zakynthos plots in 2001 showed a limited number of primary infections at the onset of the epidemics (Kephalonia: kef 13/06/01: $N_{gen} = 13$, $E_H = 0.59$; kef 01/06/02: $N_{gen} = 17$, $E_H = 0.52$; Zakynthos: zak 14/06/01: $N_{gen} = 16$, $E_H = 0.63$). The values of genotypic diversity, which account for both richness and evenness of the genotype distribution, were generally raised in epidemics with numerous primary infections ($E_H max = 0.86$). E_H represents the Shannon's index H' normalized for variable sample size by scaling the index by the value of H'_{max}. When E_H values are close to 1.00, this reveals high genotypic diversity and, thus, high significance of primary infections compared with the clonal infections.

At the same time, a continuous introduction of new individuals to the population, in terms of new genotypes, was observed (Fig. 2). In 32 of the samples collected, more than 40% of the genotypes in one sample were not present in the previous samples. Despite the high presence of new genotypes, the proportion of their lesions in the samples was not high: in 23 samples they covered less than 40% of the total lesions, while only in 14 samples they occupied more

than 40% of the sample. The "new" genotypes in each sample could constitute either new primary infections from oospores that overwintered in the particular plot (formed during the last five years of the epidemic or even earlier) or secondary infections migrating from neighbouring plots. The hypothesis that the "new" genotypes would be migrants was considered unlikely for two reasons. First, recent results on *P. viticola* epidemics (Rumbou & Gessler, 2004; Gobbin *et al*., 2005, 2006; Rumbou & Gessler, 2006) demonstrated that the great majority of secondary infections remain localized on a few vines during the whole epidemic. Second, in the cases of Preveza and the island plots, the probability of sporangia migrat-

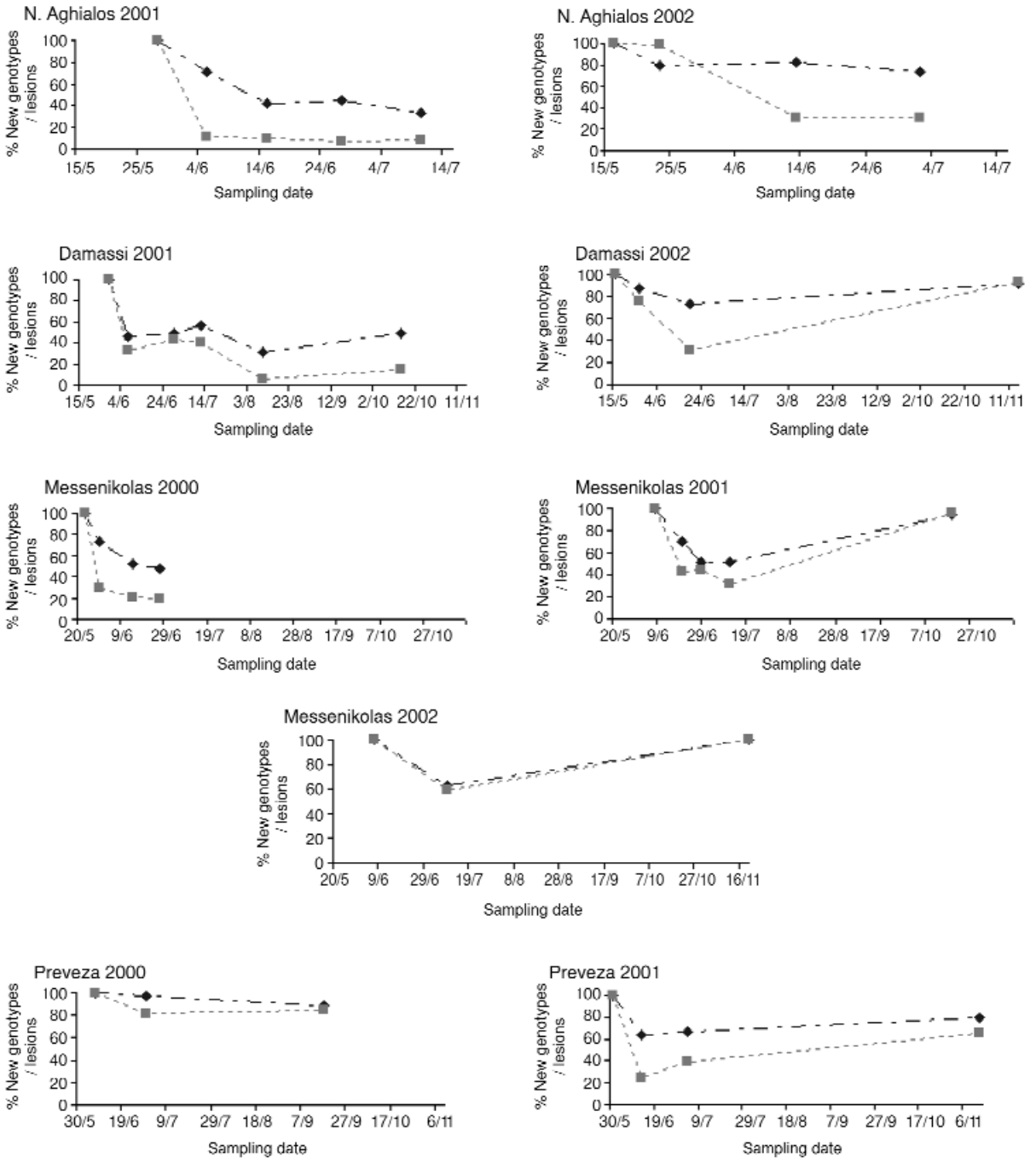


FIG. 2. Qualitative and quantitative incidence of new genotypes/primary infections in each sample collected from four Greek plots in one-three consecutive years (black rhombus: percentage of new genotypes, grey squares: percentage of lesions belonging to new genotypes).

ing to the plot is considered unfeasible as the plots were isolated from other vineyards. We consider, therefore, the "new" genotypes in one sample as new oosporic infections.

The contribution of oosporic infections to the epidemic was not equal over time. In the early samplings in all epidemics primary lesions were profuse. However, both qualitative and quantitative importance of primary infections clearly reduced with time, especially after the middle of June; this was mostly obvious in the epidemics of Damassi and Messenikolas (Fig. 2) as well as in Lefkada (Rumbou & Gessler, 2006). The percentage of oosporic infections in the population remarkably increased in the autumn samples in all plots where autumn samplings occurred (Damassi, Messenikolas, Preveza and Lefkada).

Considering the sexual events that gave birth to the oospores that caused the epidemics studied, it was found that, in considerable percentage, they occurred in the same plot. The highest percentage was found in Lefkada, where 45 out of 65 genotypes in 2002 (60%) could be recombinants derived from the 129 genotypes collected in 2001. In Messenikolas, the contribution of offspring from the 2001 to the 2002 epidemic was 51 genotypes (55.5%), while in Preveza, 61 (41%) of the total genotypes in the 2001 subpopulation could have been recombinants of the genotypes identified in 2000. The percentage was lower in Damassi (22 of 84 genotypes in 2002 could be recombinants of the 130 genotypes collected in 2001, 26%) and in Zakynthos (20 of 114 genotypes found in 2002 could be the progeny of the 23 genotypes found in 2001, 17.5%), while in N. Aghialos only four out of 54 genotypes (7.4%) of 2002 could have been recombinants of the 2001 population (one of them however was the predominant-2002 genotype). This fact allows us to assume that in the particular plot, the genotypes of autumn 2001 –which give birth to the oospores that produced the 2002 epidemic– were different than the summer genotypes.

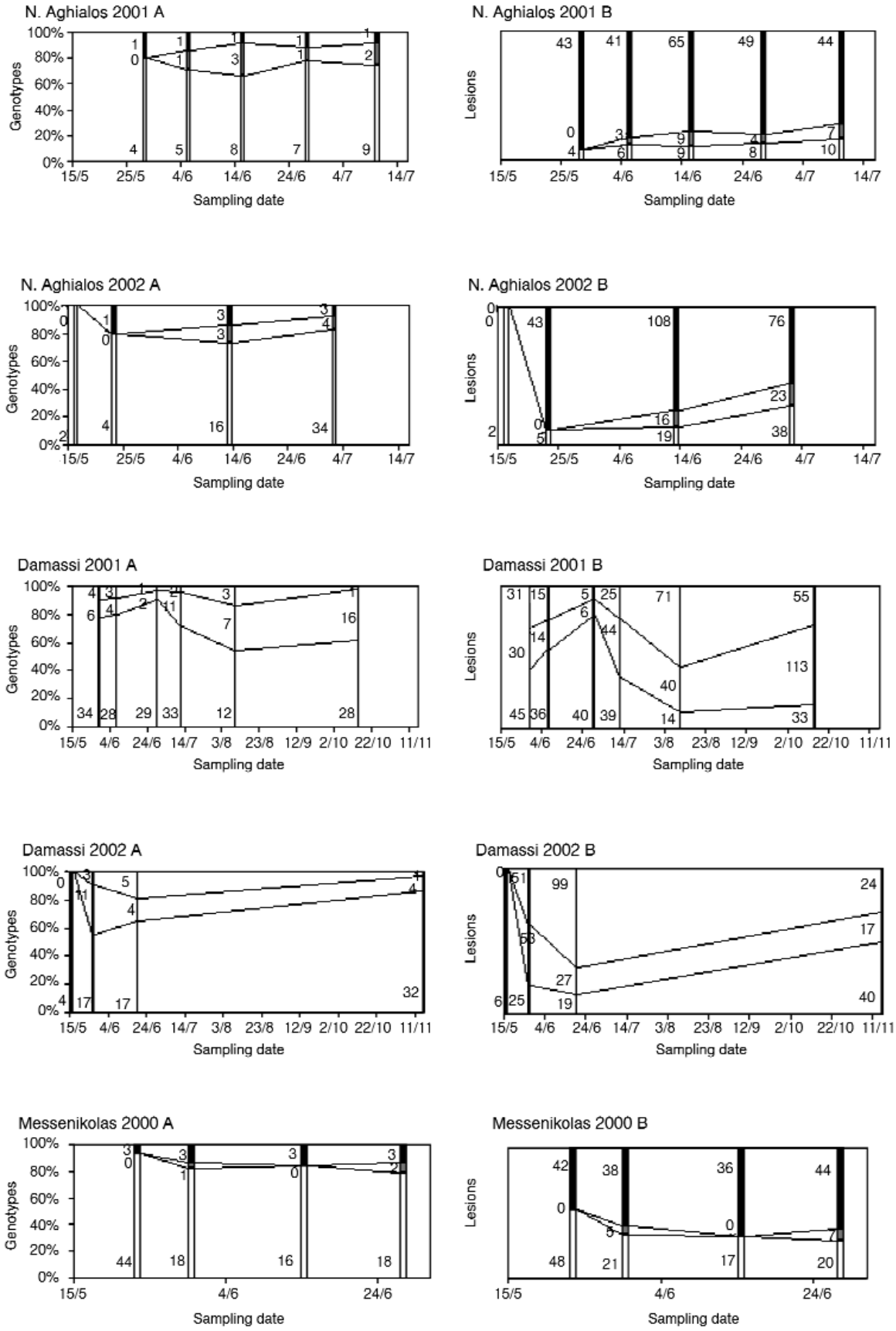
Regarding the contribution of different samples to the genetic material of the offspring, in Damassi 38% of the genotypes in 2001 that were likely to have been the parents of the recombinants in 2002 belonged to the autumn sample (2001f). Similar observations were made in the remaining populations that included autumn collections. We perceive therefore, that one year's primary inoculum is basically produced by the previous years' epidemic and in high proportion by the ones in autumn.

Finally, random recombination was shown to occur within most of the 16 subpopulations. This result comes from the Hardy-Weinberg (HW) tests which were satisfied for all loci in N. Aghialos, Messenikolas and in the island populations (data not shown). However, the test was not satisfied for one or more loci in the cases of Damassi 2001, in Preveza 2001 (the equilibrium was only accomplished when the sample 2001d was excluded from the population) and in Lefkada 2001a-c subpopulations. The HW equilibrium achieved for most of the 16 subpopulations suggests that each epidemic, either summer or autumn, derives from the local oosporic pool and probable migration events are not relevant.

Contribution of secondary infections to epidemics

Considering the asexual component of reproduction, the great majority of genotypes in each population was identified only once or twice throughout the survey period (occurring with genotype frequency $< 1\%$ in one sample). The genotypes that reached frequency $> 7.5\%$ in one sample represented 4-8% of the total oosporic population in one plot (one out of 18 oospores on average) in the cases of the N. Aghialos, Kephalonia and Zakynthos 2001 plots, where the oosporic pool was poor (class c in Fig. 3A). However, those genotypes represented an even lower part of the oosporic population (1-2% or one out of 75 oospores) in mainland plots like Messenikolas and Preveza, despite the high amount of oosporic infections that were found there. Therefore, consistently only a small fraction of the primary infections gave rise to successful secondary clonal infections.

Despite the low qualitative impact of the genotypes that contributed numerous clones to the population, their significance for the epidemic was not low. At the epidemics of N. Aghialos, Kephalonia and Zakynthos 2001, where a low number of oosporic infections occurred (class c in Fig. 3B), they mostly occupied more than half of each sample. In the rest of the plots, the amount of clonal infections was limited until the middle of July and increased to a significant level only late in summer, while in the autumn samples of Messenikolas 2001, Preveza 2001, and Lefkada 2001 and 2002 they played a very important role. In Zakynthos, the 2001 epidemic (zak 01) was driven by clonal infections, while in the consecutive year (zak 02) the primary infections were remarkably greater.


N. Aghialos 2001 A
N. Aghialos 2001 B
N. Aghialos 2002 A
N. Aghialos 2002 B
Damassi 2001 A
Damassi 2001 B
Damassi 2002 A
Damassi 2002 B
Messenikolas 2000 A
Messenikolas 2000 B
Genotypes
Lesions
Sampling date
100%
80%
60%
40%
20%
0%
15/5
25/5
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12/9
2/10
22/10
11/11

FIG. 3.

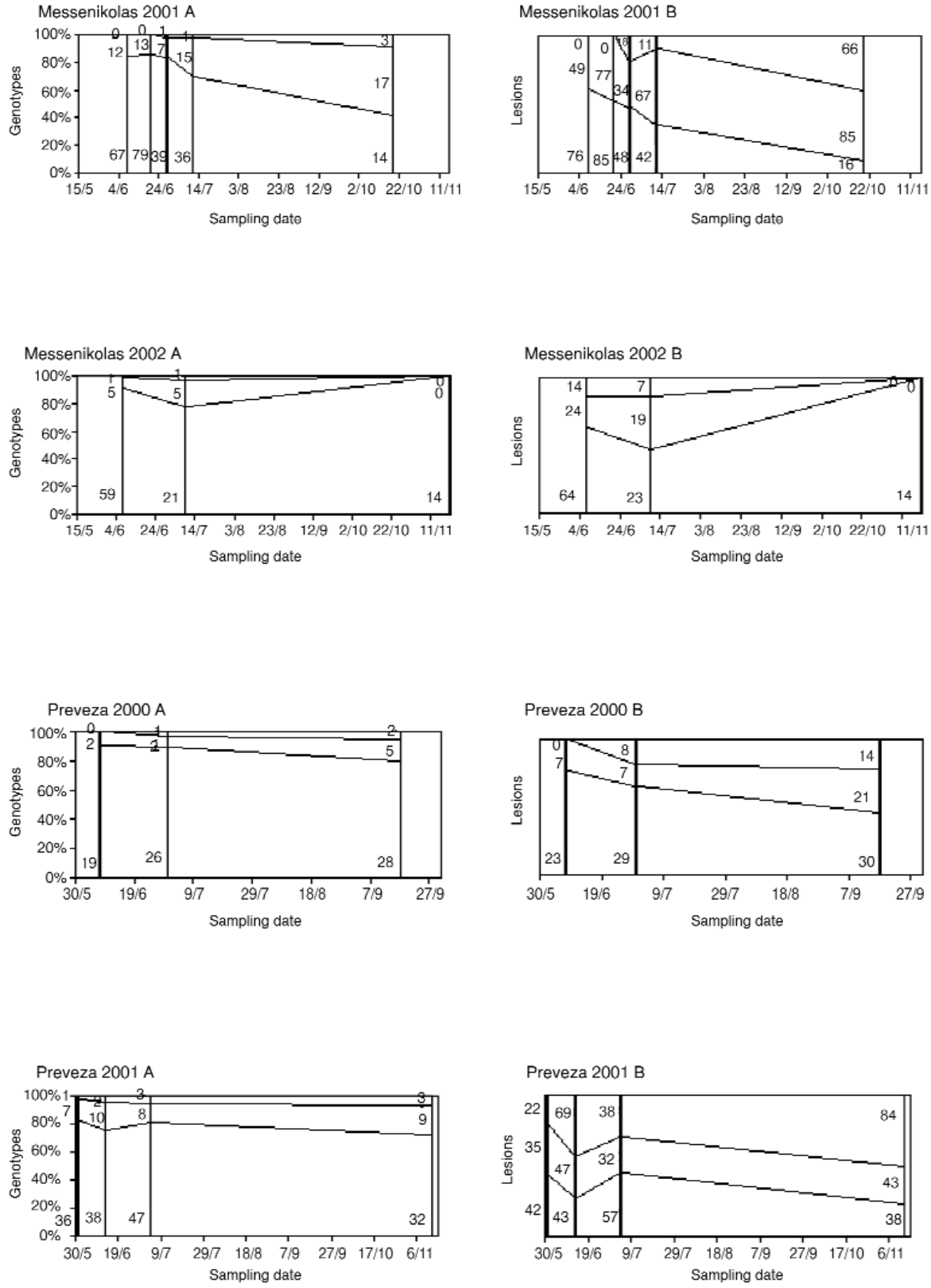

FIG. 3. Qualitative (A) and quantitative (B) incidence of genotypes with frequency < 1% (class a, empty bars), with frequency 1-7.5% (class b, grey bars) and with frequency > 7.5% (class c, black bars) in one sample. A: Percentage of genotypes in each frequency class; B: Percentage of lesions belonging to genotypes in each frequency class (numbers next to the bars correspond to numbers of genotypes –in A– and numbers of lesions –in B– of classes a and c).

Spatial distribution of clonal infections

The spread of the few genotypes in each epidemic that caused multiple clonal infections was shown to be restricted and followed in most cases a stepwise mode. In the Damassi plot in 2001, the clones of the most frequent genotypes were few in the first three samples, but increased and spread remarkably from the fourth sample on (g1, g2 and g3 in Fig. 4A). In 2002, the three most abundant genotypes (g4, g5 and g6 in Fig. 4B) spread in a strictly stepwise pattern, from vine to vine, and were much localized, especially in the early samples, around the vine where the first oosporic infection occurred. However, even at the later stages of the epidemic, despite the increased number of clones, they did not infect more than half of the vines in the 60-vine plot. In Messenikolas, a similar mode of limited and localized dispersal was shown by the frequent genotypes in 2001 (g7, g8 and g9 in Fig. 5A) and in 2002 (g12, g13 and g14 in Fig. 5C), although the first samples were collected relatively late (in June) and represented later stages of the disease. The difference with the Damassi epidemic was that the number of clones totally produced by each genotype did not increase much with time. This is also shown by the low percentage of class c (genotypes with frequency > 7.5% in one sample) in Fig. 3B, graphs of Messenikolas 2001 and 2002. However, the Messenikolas autumn sample in 2001, which shared only two genotypes with the previous samples (Fig. 2, Messenikolas 2002), was dominated by only two genotypes (g10 and g11 in Fig. 5B), which were widely spread. Finally, the highly clonal genotypes in the Preveza epidemic (g15, g16 and g17 in Fig. 6) followed a stepwise dispersal and ended up in having infected a large part of the vines. The autumn sample, which shared only 20% of its genotypes with the summer samples (Fig. 2, Preveza 2001), was dominated by one genotype. This genotype originated from an autumn oosporic infection and showed a rapid clonal increase and, consequently, a fast dispersal in the plot.

Genetic subdivision among populations

A significant genetic differentiation among the 16 Greek *P. viticola* subpopulations was revealed. After 120 pairwise tests of population differentiation with F_{ST} estimates, 111 (92.5%) pairs of populations showed a significant differentiation, while only nine population pairs were not significantly different among the 16 subpopulations (Table 3). The subpopulations agh, dam 02a-c, mes 01a-d and pre 00, showed significant differences from all other populations either from the same or from other plots. The non-significant differences obtained came basically either from populations of the same plot, e.g., zak 01 with zak 02, lef 01a-c with lef 02 or neighbouring plots, e.g. Damassi-Messenikolas, Preveza-Lefkada and Kephalonia-Zakynthos. The only population that showed similarities to the other ones (three) was the zak 01, apparently due to its small sample size. Fixation index (F_{ST}) estimates ranged from a minimum of 0.003 (comparison: pre 01/lef 01d) to a maximum of 0.0843 (comparison: pre 00/lef 02), with an average of 0.0364. Therefore, despite the clear genetic subdivision among the 16 subpopulations, genetic distances were small or moderate.

Regarding the genetic substructure within the plots, either one or two subpopulations were found during one growing season. The first case was only observed in Preveza where both epidemics were uninterrupted by bottleneck events. In most of the epidemics that lasted until autumn, two subpopulations existed in the vineyards in one year. This situation was found in Damassi in 2002, in Messenikolas in 2001 and in Lefkada both in 2001 and 2002. The only exception was the epidemic in Damassi in 2001, where the same population that caused the summer epidemic caused also the autumn epidemic. In the later case, the proportion of the disease attributed to new primary infections was only 15%. Concerning the N. Aghialos, Kephalonia and Zakynthos plots, due to lack of autumn samples, we cannot clearly determine the presence of an autumn population; however, the absence of genetic substructure among the different years suggests the presence of a single population during one growing season. In the rest of the plots (Damassi, Messenikolas, Preveza and Lefkada), among the samples of two consecutive grape-growing seasons, a clear genetic differentiation was revealed. In the case of the Zakynthos plot, particularly, the test for differentiation showed a significant difference between the zak 01 and the zak 02 samples, when all the Zakynthos samples were compared to each other, while the difference was estimated as non-significant for the comparison among the 16 subpopulations (Table 3).

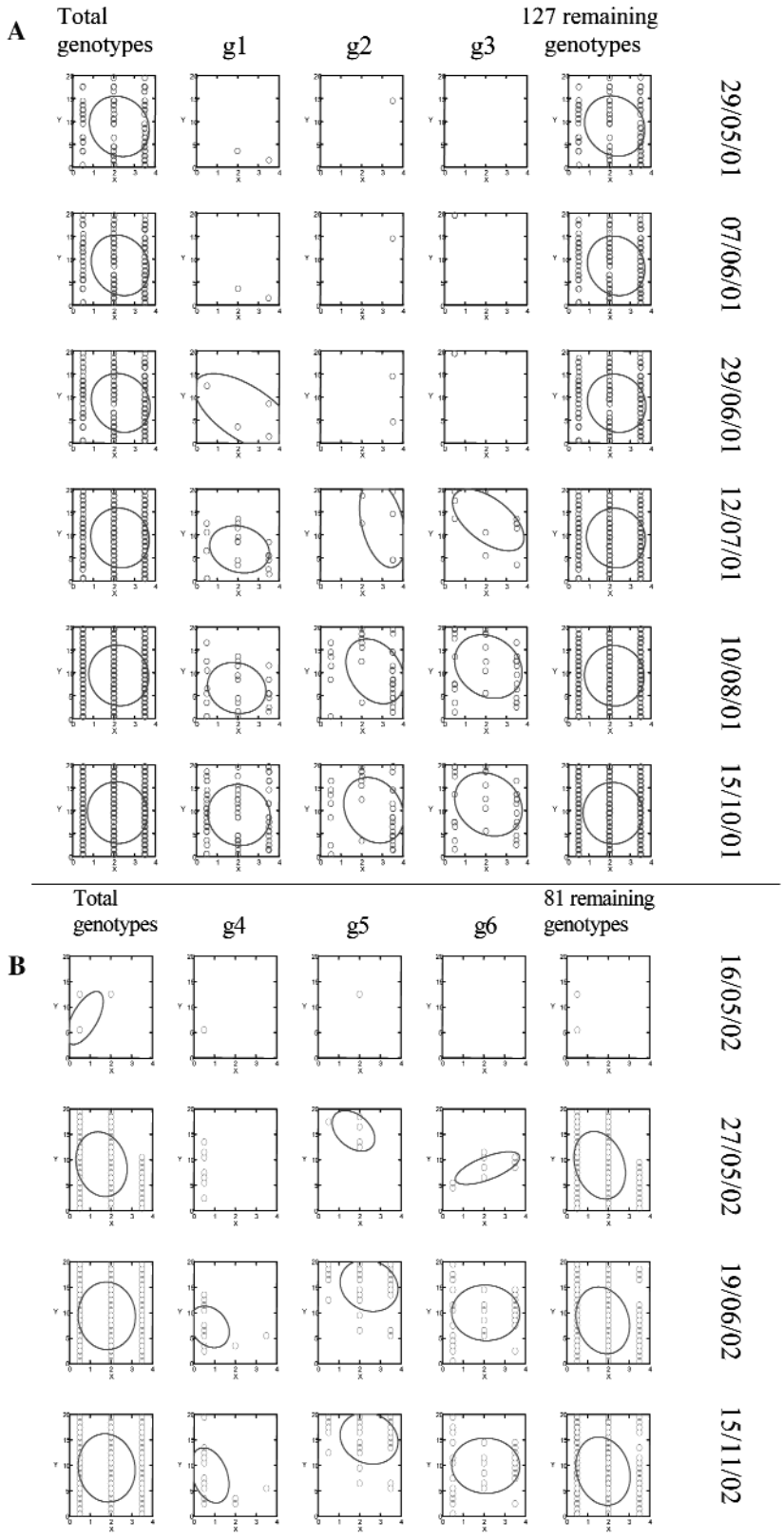

FIG. 4. Genetic and spatial distributions of the *P. viticola* epidemic in a 60-vine plot in the Damassi region in 2001 (A) and 2002 (B); total genotypes, three most frequent genotypes in each epidemic (g1/g2/g3 and g4/g5/g6, respectively) and remaining genotypes are shown in two-dimensional representation. The confidence ellipses are drawn for $p = 0.05$.

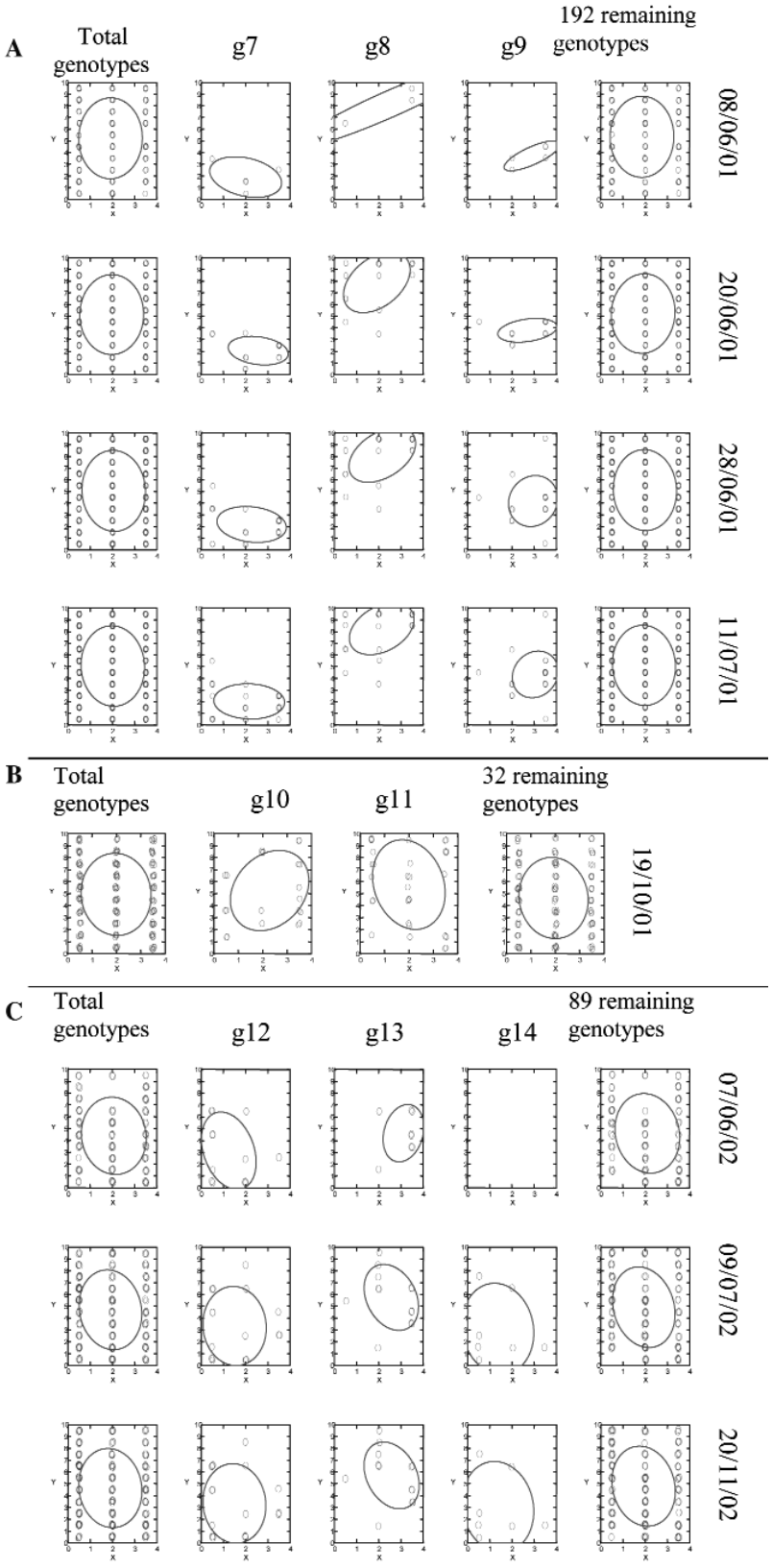

FIG. 5. Genetic and spatial distributions of the *P. viticola* epidemic in a 30-vine plot in the Messenikolas region in 2001 spring/summer (A), in 2001 autumn (B) and in 2002 (C); total genotypes, most frequent genotypes in each epidemic (g7/g8/g9, g10/g11 and g12/g13/g14, respectively) and remaining genotypes are shown in two-dimensional representation. The confidence ellipses are drawn for $p = 0.05$.

TABLE 3. Pairwise test of population differentiation among the 16 subpopulations collected from seven Greek vineyards in 2000, 2001 and 2002. Non-adjusted *p*-values for each pair of samples obtained after 12,000 permutations are shown. *: significance at the 5% nominal level; **: significance at the 1% nominal level (indicative adjusted nominal level for multiple comparisons = 0.000417); NS: not significant

	dam01	dam02 a-c	dam02 d	mes 00	mes 01 a-d	mes 01 e	mes 02	pre 00	pre 01	kef	zak 01	zak 02	lef 01 a-c	lef 01 d	lef 02
agh	8E-05**	8E-05**	0.00042*	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
dam01		8E-05**	8E-05**	8E-05**	8E-05**	0.52208NS	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
dam02 a-c			8E-05**	8E-05**	8E-05**	0.00017*	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
dam02 d				0.0006NS	0.00017*	8E-05**	0.0609NS	0.0002*	8E-05**	8E-05**	0.0016NS	8E-05**	8E-05**	8E-05**	8E-05**
mes 00					8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
mes 01 a-d						8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
mes 01 e							8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
mes 02								8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
pre 00									8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**	8E-05**
pre 01										8E-05**	8E-05**	8E-05**	8E-05**	0.1665NS	8E-05**
kef											0.0223NS	0.027NS	8E-05**	8E-05**	8E-05**
zak 01												0.07042NS	8E-05**	8E-05**	8E-05**
zak 02													8E-05**	8E-05**	8E-05**
lef 01 a-c														8E-05**	0.00275NS
lef 01 d															8E-05**

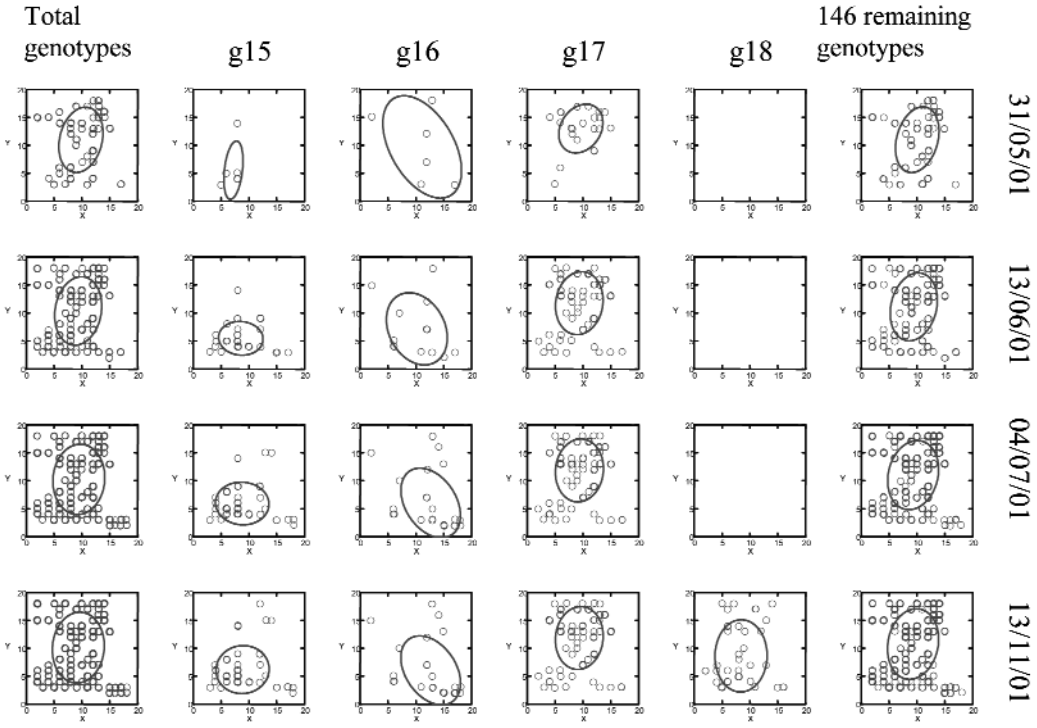

FIG. 6. Genetic and spatial distributions of the *P. viticola* epidemic in a 250-vine plot in the Preveza region in 2001; total genotypes, most frequent genotypes in each epidemic (g15/g16/g17/g18, respectively) and remaining genotypes are shown in two-dimensional representation. The confidence ellipses are drawn for $p = 0.05$.

DISCUSSION

Plasmopara viticola sexual spores were shown to probably constitute a major source of inoculum for downy mildew epidemics in Greek vineyards. The abundant genotypes identified in most of the populations studied most likely reflect the massive occurrence of oosporic infections and, consequently, a large pool of oospores in the soil. In contrast to the current belief that primary infections occur only at early disease stages and in limited scale, still they play a main role in the initiation of the disease in May, continue to occur throughout the epidemiological season and are responsible for the disease regeneration in September. Furthermore, they hold the genetic variability, which is critical for the population's survival. The local oosporic pool was genetically rich even in plots regularly and heavily treated against downy mildew during the years before the samplings (Damassi, Messenikolas, Lefkada). Limited oosporic infections were observed in the island plots of Kephalonia and Zakynthos (only in 2001) and in the N. Aghialos plot. These regions are very dry and the disease usually appears in low severity. Principally, oosporic inoculum and infections are of high threat for the vines and of high value for the pathogen.

Considering the asexual spores, their role in the epidemic had been overestimated until now. The great majority of the genotypes in each population (85%, on average) did not cause detectable secondary infections. Only a few genotypes per epidemic underwent relevant asexual reproduction, with their contribution to the disease severity depending on the epidemic type and stage. In general, their contribution increased only in late summer and in the autumn samples. Disease growth, therefore, was not triggered by the few clonal genotypes, but by the remaining genotypes, which were not clonal but abundant (Figs 4, 5 and 6). However, secondary infections played a leading role in epidemics where a small number of oosporic infections occurred (N. Aghialos, Kephalonia and Zakynthos). Clone dispersal followed a similar trend with clonal multiplication; it was limited for the majority of the genotypes. In one asexual cycle, the dispersal of the clones usually covered an area within a few vines around the site where the oosporic infection was first identified, spreading mostly along the row. The only case of widespread dispersal was

observed in the rare dominant genotypes. These findings are in agreement with the situation in central Europe, where 99% of the genotypes studied followed minor clonal multiplication and dispersal (Gobbin *et al*., 2005; Rumbou & Gobbin, 2005). Therefore, long-distance sporangia dispersal was shown not to be massive and the stepwise spatial pattern of spread is more likely than the long-distance dispersal.

The contribution of primary versus secondary infections to epidemic development showed two general patterns. The case where the role of primary infections was major while the role of secondary infections was minor throughout the growing season was met in the mainland populations of Damassi, Messenikolas, Preveza and in the island population of Lefkada. In these epidemics, the amount of primary infections was large throughout the growing season, while their quantitative role was high at the beginning of the epidemic, reduced with time and increased again in autumn. The role of the secondary infections followed an anti-parallel pattern; low contribution at the beginning, higher as the clonal cycles progressed and high again in the autumn. The other epidemic pattern was characterised by an increased clonality of one/few genotypes and a low qualitative role of the primary infections; it was found in low-severity epidemics in the islands of Kephalonia and Zakynthos (only in 2001) and in the coastal plot of N. Aghialos (called "predominance-of-one/few-clones" epidemic). In central European epidemics, a prevalence of the primary infections was most commonly observed, while the "predominance-of-one-clone" pattern of epidemic was only met in the isolated plots, and was connected to the absence of a primary inoculum in the soil or exceptionally arid conditions during particular years (Gobbin *et al*., 2003b, 2005).

A phenomenon that does not occur in central Europe is the bottleneck events, which highly influence the epidemics. In most of the Greek island and mainland plots, the disease grew until mid-summer, and then ceased because both primary and secondary infections were not possible due to unfavorable climatic conditions. In the regeneration of the disease in autumn, the percentage of new genotypes in the autumn samples compared with the summer ones was over 80% (except for the Damassi 2001 with 50% new genotypes), meaning that the majority of the spring/summer lesions died. The disease disruption due to the bottleneck was so severe that the autumn populations diverged genetically from the spring/summer ones in the same plot. These epidemics were called "two-peak" epidemics, as the bottlenecks during the summer prevented a continuous epidemic growth. The only case where a bottleneck did not occur was in the Preveza plot (called "continuous" epidemic).

The different contributions of the primary versus secondary infections in combination with the presence or absence of bottlenecks during an epidemic led to different genetic substructures among samples within the same plot. During a single grape-growing season, either one (in "continuous" epidemics) or two (in "two-peak" epidemics) *P. viticola* populations were responsible for the epidemic. Among samples of two or more consecutive grape-growing seasons either one or more populations were responsible for the epidemics. The first case occurred only in the Kephalonia and N. Aghialos plots and was characterized by the "predominance-of-one-clone" epidemic pattern and low disease severity, while the second case was more common and was found in all plots where the epidemic pattern was the "two-peak" type (Damassi, Messenikolas, Lefkada).

The new concepts obtained imply a site-related population structure and, consequently, pattern of epidemic. The local, indigenous oosporic pool in the soil most likely generates the epidemics. Even in the autumn, the disease restarts due to the local inoculum and not to the migration of sporangia from regions where the epidemic lasted all summer. The high genetic variability of the oosporic pool indicates high levels of sexual reproduction. Applying these outcomes, the disease control measures should not target just the asexual propagules attacking the growing vegetation. The importance of the oosporic inoculum demands that oogamy, which is especially abundant in the autumn, should be inhibited by effective control of the autumn epidemic. A practice like this would, at the same time, profit from the destruction of the secondary inoculum due to summer disease disruption, which occurs in any case under the Greek conditions. Simultaneously, new questions arise; the exact conditions under which oogamy can occur in nature, as well as the conducive conditions for oosporic and clonal infections, are some of the aspects that require further investigation. Current knowledge on those subjects is based on classical epidemiology or biology experiments. Considering the results obtained from the present study, we propose that a population biology view, which merges epidemiology and population genetics, is at the moment essential for evaluating existing beliefs.

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