VARIATION IN AGGRESSIVENESS AND GENETIC DIVERSITY OF GRAPE PHYLLOXERA IN SOUTHERN FRANCE

VARIATION DE L'AGRESSIVITÉ ET DIVERSITÉ GÉNÉTIQUE DU PHYLLOXÉRA DE LA VIGNE DANS LE SUD DE LA FRANCE

M. YVON[1] and J.-P. PÉROS[2]

1 : UMR BGPI, 2 : UMR DGPC Équipe Vigne, INRA, 2 place Viala,
34060 Montpellier cedex 1, France

Abstract: The aphid relative *Daktulosphaira vitifoliae* (Fitch) (*Homoptera : Phylloxeridae*) feeds on the leaves and roots of grapevine (*Vitis* spp.). The insect was introduced into France from North America around 140 years ago and caused severe damage until control of the root-infesting form was initiated by grafting European cultivars onto resistant rootstocks. The objective of our research was to analyze both the variation in pathogenicity and the genetic diversity of the insect in southern France. Twenty samples of phylloxera were collected from leaf galls throughout the region. Their aggressiveness was monitored by measuring the reduction in root dry mass of artificially inoculated potted plants. One trial was performed with three rootstocks (41B, 3309C and 140R) and another with the highly susceptible *Vitis vinifera* cv. Carignan. No evidence for preference was shown, but aggressiveness varied widely among phylloxera samples, especially in the Carignan trial. Genetic diversity was assessed using 30 Random Amplified Polymorphic DNA (RAPD) markers obtained with 15 primers. Wide diversity was found and all samples corresponded to distinct RAPD phenotypes. The genetic structure of phylloxera population in Southern France was not showed to be influenced either by the location or by the host.

Résumé: *Daktulosphaira vitifoliae* (Fitch) (*Homoptera: Phylloxeridae*) est un puceron inféodé aux feuilles et aux racines de vigne (*Vitis* sp). Cet insecte originaire d'Amérique du Nord, a été introduit en France il y a environ 140 ans et causa des dégâts considérables. Le greffage des vignes européennes sur des porte-greffe américains, résistants aux phylloxéras radicicoles, se révéla comme étant la méthode de lutte la plus efficace. L'objectif de notre recherche est d'étudier à la fois les différences d'agressivité et la diversité génétique de cet insecte dans le sud de la France où vingt échantillons de phylloxéra gallicoles sont récoltés. Leur agressivité est évaluée en mesurant la réduction du poids sec des racines de plants cultivés en pots et inoculés artificiellement. Un premier essai a été réalisé avec des plants de trois variétés de porte-greffes résistants (41B, 3309C, 140R) et un second avec des plants du cépage très sensible Carignan (*Vitis vinifera*). Aucune interaction statistique n'est mise en evidence entre les porte-greffe et les échantillons de phylloxera qui montrent d'importantes différences d'agressivité notamment dans l'essai réalisé avec le Carignan. Une diversité génétique importante est mise en évidence à l'aide de 30 marqueurs RAPD obtenus avec 15 amorces. Tous les échantillons présentent des phénotypes RAPD distincts. La structure génétique de la population de phylloxera dans le Sud de la France ne semble pas être influencée ni par la localité ni par l'hôte.

Key words: phylloxera, *Daktulosphaira vitifoliae*, grapevine, genetic diversity, preference, aggressiveness, RAPD, southern France

Mots clés: phylloxéra, *Daktulosphaira vitifoliae*, vigne, diversité génétique, préférence, agressivité, RAPD, sud de la France

INTRODUCTION

The grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (*Homoptera: Phylloxeridae*) is native to North and Central America. After introduction into Europe around 1860, it severely damaged grapevines by feeding on the roots (POUGET, 1990). It is also able to feed on leaves with differences that accord to the *Vitis* species (WAPSHERE and HELM, 1987; WILLIAMS and SHAMBAUGH, 1988). In Europe, cultivars of *Vitis vinifera* were grown on their own roots before the introduction of the insect. Subsequently the pest was controlled by grafting European cultivars onto phylloxera-resistant rootstocks derived from American *Vitis* species.

In France, the grape phylloxera has a breeding system that combines parthenogenesis and sexual reproduction (MAILLET, 1957). Briefly, the aerial portion of the cycle begins in the spring with the hatch of the

*Correspondance : yvon@ensam.inra.fr

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female fundatrice from an overwintering egg. The females then establish themselves on expanding leaves where several parthenogenetic generations occur in galls. From the leaf galls, crawlers wander to other leaves or into the ground to settle on roots. The root-feeding portion of the cycle also includes several parthenogenetic generations. In late summer, root-galling morphs produced alate sexuparae from which sexual morphs (females and males) originate. After mating the females each lay one overwintering egg on cracked bark on the trunk or on arms of the vines.

Variability in *D. vitifoliae* came to the attention of researchers when a failure in resistance of rootstock AXR 1 was observed in California (GRANETT *et al.*, 1985). Bioassays on excised roots, derived from the method developed by POUGET (1975), have been widely used to analyze the adaptive ability of the insect as well as the resistance of grapevine rootstocks. These studies have shown that feeding and reproductive performance varies among phylloxera collections worldwide (SONG and GRANETT, 1990; DE BENEDICTIS and GRANETT, 1993; MARTINEZ-PENICHE, 1993; HAWTHORNE and VIA, 1994; OMER et al., 1999a). In addition, host-adapted races or biotypes have been characterized indicating that particular phylloxera colonies were better able to utilize their hosts of origin than were populations from other hosts (SONG and GRANETT, 1990; HAWTHORNE and VIA, 1994; DE BENEDICTIS *et al.*, 1996).

Molecular markers have been developed to identify genetic differences between biotypes as well as to study the genetic diversity within phylloxera populations. In California, FONG *et al.* (1995) found as much polymorphism within biotypes as between biotypes in Random Amplified Polymorphic DNA (RAPD) patterns. In native North American populations of the aphid, more RAPD variation has been identified in populations from Missouri (DOWNIE, 2000) than in populations from New York and Arizona (LIN *et al.*, 1999). Molecular data are currently increasing and add considerable information about the population biology of the grapevine phylloxera. RAPD analysis and comparison of the sequence of the cytochrome oxydase I gene (CO1) from phylloxera samples taken throughout the native range have revealed a distribution of genetic variation that favors the hypothesis of geographic influence rather than the hypothesis of host effect (DOWNIE *et al.*, 2001). Based on the genealogy of the CO1 gene, DOWNIE (2002) further demonstrated that the population introduced into Europe represented a limited subsample of native American genetic diversity. In Europe, Amplified Fragment Length Polymorphism (AFLP) markers identified two distinct phylloxera groupings that roughly corresponded to northern and southern collection site locations (FORNECK *et al.*, 2000). In Australia, genotypes have been characterized with microsatellite markers and it has been suggested that sexual recombination and exchanges between leaf and root forms are limited (CORRIE *et al.*, 2002).

In this study, our first objective was to analyze the variation in aggressiveness in phylloxera samples taken from leaf galls in southern France. The second objective was to develop reproducible RAPD markers to assess the level of genetic diversity among samples from this region. The dominant inheritance of RAPD markers makes it difficult to draw final conclusions concerning population genetics but they could nevertheless provide a convenient tool for the initial appraisal of genetic diversity in the region we sampled.

MATERIALS AND METHODS

I - PHYLLOXERA SAMPLING

In August 1999, leaf galls were collected from different rootstocks or scion cultivars at twenty sites in southern France (table I). Galled shoots were taken from five adjacent vines, placed in hermetic plastic bags and maintained in cool conditions until reaching the laboratory. The number of eggs per gall was checked and found to be similar across the samples. For each sample, 240 galls from different leaves were immediately used for infection tests. The content (female and eggs) of five galls collected on the same leaf was checked for the absence of other insects and mites and then stored at –80°C until DNA extraction.

II - ASSESSMENT OF AGGRESSIVENESS

Canes of cv. Carignan (*Vitis vinifera* L.) and the rootstocks 41B (*Vitis vinifera* x *V. berlandieri*), 3309C (*V. riparia* x *V. rupestris*) and 140Ru (*V. rupestris* X V. berlandieri) were collected in November 1998. Single-bud cuttings were transferred in plastic pots (4x4 cm) containing a sterilized mix of peat moss, mould and volcanic scoria. The plants were grown in an insect-proof greenhouse at 25 ± 3°C under natural illumination.

Two experiments were set up in an insect-proof greenhouse in May 1999. In the first experiment, i.e. the rootstock trial, six plants, two from each rootstock (41B, 3309C and 140Ru) were transplanted in a 10-liter pot. In the second experiment, i.e. the Carignan trial, one plant from the cv. Carignan was transplanted in a three-liter pot. In both cases, the pots contained the soil mix described above. The phylloxera inoculation was performed in August at the date of sampling of each sample (table I) using fifty galls per pot for the

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Table I - Phylloxera samples from southern France compared for aggressiveness and genetic diversity in this study

Échantillons de Phylloxéra du Sud de la France comparés dans ce travail pour l'agressivité et la diversité génétique

Sample	Locality, district	Host	Habitat[a]	Date of sampling
AIG	Aigues-Vives, Aude	Alicante Bouschet	CV	09/08/99
ALB	Albaron, Bouches-du-Rhône	3309 C	N	23/08/99
ARN	Villeneuve les Mgl, Hérault	Fercal	GC	11/08/99
BOI	La Boissière, Hérault	110 R	N	24/08/99
CLE	Saint-Clément de Rivière, Hérault	3309 C	N	10/08/99
FLE	Villeneuve les Mgl, Hérault	140 Ru	GC	11/08/99
GAL	La Galère, Gard	140 Ru	N	19/08/99
GUI	La Guirlande, Hérault	Rupestris du Lot	AV	24/08/99
LAT	Lattes, Hérault	3309 C	N	17/08/99
LED	Lédignan, Gard	1103 P	N	19/08/99
MON	Montpellier/ENSA, Hérault	3309 C	GC	05/08/99
NAR	Narbonne, Aude	Carignan	CV	09/08/99
PAL	Palaja, Aude	3309 C	GC	16/08/99
PAR	Pargazan, Aveyron	3309 C	AV	15/08/99
PON	Le Ponan, Gard	44 53 M	AV	23/08/99
PUI	Puicheric, Aude	Alicante Bouschet	CV	09/08/99
QUI	Quissac, Gard	3309 C	N	12/08/99
RED	La Redorte, Aude	SO4	N	16/08/99
ROD	Rodilhan, Gard	1103 P	N	12/08/99
SAV	Savignargues, Gard	3309 C	N	19/08/99

a : Commercial vineyard (CV), nurseries (N), grapevine collections (GC), abandoned vineyards (AV)

rootstock trial and ten for the Carignan trial. The method of root inoculation was described in detail by YVON and LECLANT (2000). Briefly, galls were introduced in a plastic tube (150 mm in length, 30 mm in diameter) placed in the soil mix. The tube was perforated with holes (2 mm in diameter) to allow colonization of the root system by the crawlers. Several precautions were taken to prevent escape of phylloxera crawlers: 1) the soil mix was placed between two layers of sand, the top of the tube being sealed with a plastic cover before placing the second layer 2) the base of each pot was placed in a plastic bag, which was stuck in place with adhesive tape 3) a ring of glue was deposited at the top of the pot. Each trial included a negative control, i.e. plants without inoculation. Both trials were conducted in complete randomized designs with four replicates, each replicate corresponding to one pot. Plants were watered as needed with an equal volume for each pot and were fertilized every 15 days with a complete mineral solution (Mairol, Agro Végétal, Gif-sur-Yvette, France). The plants were then propped and trimmed each month to avoid contact between them.

Five months after infestation, the pots were destructively harvested. The roots were washed carefully in tap water and dried at 65°C to measure their dry mass. Data were subjected to analysis of variance using the PROC GLM procedure in SAS (SAS Institute, Cary, USA). Means were separated using the Tukey multiple range test of Tukey (P=0.05). The percentage of root dry mass from the negative control was calculated as %R = 100 x [(mean root dry mass of control)–(mean root dry mass of sample)] / (mean root dry mass of control).

III- DNA EXTRACTION AND AMPLIFICATION

DNA was extracted using a CTAB method as described by PÉROS et al. (1996) except that the step using chloroform with 10% CTAB was omitted. DNA was quantified on 0.8% agarose gel stained with ethidium bromide by visual comparison with different quantities of λDNA (Life Technologies, Cergy Pontoise, France). The amplification reactions were performed according to WILLIAMS e*t al.* (1990) with some modifications. Each reaction volume of 25 µl included 1 unit of Taq polymerase (Life Technologies, Cergy Pontoise, France), the buffer purchased with the enzyme, 3 mM de MgCl2, 100 µM of each dNTP, 0.5 µM of primer (Life Technologies, Cergy Pontoise, France) and 30 ng of template DNA. Amplifications were carried out in a PT100 thermocycler (MJ Research, Watertown, USA) programmed for a denaturation step of 4 min at 94 °C, followed by 36 cycles of 1 min at 93 °C, 1 min at 38 °C, 1 min at 72 °C, and then a final step of 6 min at 72 °C. Amplification products were separated by electro-

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Table II - Random amplified polymorphic DNA primers used, size and frequency of amplified alleles in a phylloxera population

Amorces RAPD utilisées, taille et fréquence des allèles amplifiés dans une popuation de Phylloxéra

Primer	Sequence (5'to 3')	Putative loci[a]
A02	TGCCGAGCTG	2530 (0.10)
A10	GTGATCGCAG	690 (0.10)
A16	AGCCAGCGAA	620 (0.10); 750 (0.80); 1960 (0.10); 2270 (0.10)
B08	GTCCACACGG	690 (0.65); 830 (0.95); 1730 (0.90)
C15	GACGGATCAG	1210 (0.20)
C18	TGAGTGGGTG	400 (0.10); 560 (0.85); 1070 (0.50); 1290 (0.15); 1580 (0.50)
G02	GGCACTGAGG	1250 (0.95)
G05	GTGAGACGGA	380 (0.95); 1070 (0.15); 1090 (0.15)
G12	CAGCTCACGA	760 (0.20)
G13	CTCTCCGCCA	1450 (0.95)
G16	AGCGTCCTCC	800 (0.05); 1180 (0.50)
G18	GGCTCATGTG	620 (0.05); 1740 (0.05)
J16	CTGCTTAGGG	480 (0.05)
122	GTAGACGAGC	720 (0.10); 810 (0.10)
183	CGTGATTGCT	1190 (0.95); 1350 (0.05)

a : For each RAPD primer used, alleles at the putative loci are listed according to the size of the amplicon in base pairs. The frequency of the amplified allele for each putative locus is given in backets.

phoresis on 1.5 % agarose gels in a 0.5x Tris-borate-EDTA buffer and were visualized using UV light after ethidium bromide staining.

IV- RAPD DATA ANALYSIS

A total of 30 decamer primers of random sequence were selected from the results obtained on phylloxera (MARTINEZ-PENICHE, 1993; FONG *et al.*, 1995) and other arthropods (TIXIER-GARCIN, 2000). Fifteen primers generated few, intense and reproducible bands that showed polymorphism (table II) and these were used for the analysis. The presence or absence of the amplification products was scored and binary matrices were constructed with the markers in columns and the samples in rows. The Jaccard's distance (JD) between phylloxera samples was calculated as JD=1-SJ where SJ is the coefficient of similarity defined by JACCARD (1908). In our case, SJ=a/(a+b+c) where a is the number of bands present for both individuals, b the number of bands present for individual 1, but not for individual 2 and c the number of bands present for individual 2, but not for individual 1. DARWIN software (version 4, CIRAD-Flor, Montpellier) was used to calculate JD and to construct a phenogram from the distance matrix using the algorithm of the neighbor-joining method developed by SAITOU and NEI (1987). Reliability of the resulting tree was tested by bootstrap analysis (FELSENSTEIN 1985) of 1,000 replicates with DARWIN. Genetic distance data were examined for possible correlation with geographic distances by performing a Mantel test between the genetic and geographic distance matrices using a program provided by J. GOUDET (University of Lausanne, Switzerland).

RESULTS

I - VARIATION IN AGGRESSIVENESS

In both experiments, the roots of control plants did not show either swellings of small rootlets (nodosities) or galls on larger roots (tuberosities) indicating that the method of infestation prevented the escape of phylloxera crawlers from inoculated pots. For rootstock cultivars, the formation of nodosities at the tip of their main roots explained why growth of these roots. There was no significant interaction between the rootstock cultivar and the phylloxera sample with respect to root dry mass (F=1.31, df=40,195, P=0.12). Conversely, there was a highly significant effect of the phylloxera sample (F=3.22, df=20,195, P=0.0001). Most of the samples were distinguished from the control but the Tukey test showed no significant differences betweens samples ((table III). However, the percentage of decrease in the dry mass of the control (%R) varied from 22 % for the GUI sample to 50 % for the MON sample. In addition, the effect of the rootstock cultivar was highly significant (F=8.74, df=2,195, P=0.0002). On average, 3309C had a higher level of root dry mass than the two other rootstocks (table III).

The MON sample was not included in the Carignan trial due to the lack of plant material. The infected roots

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Table III - Effect of phylloxera sample on the root dry weight (g) of potted plants

Effet de l'échantillon de Phylloxéra sur le poids sec (g) des racines de plantes cultivées en pot

	Rootstock trial				Carignan trial	
	3309C	140Ru	41B	Mean		
Control	6.34	5.39	4.94	5.56a	Control	6.00a
GUI	5.73	3.62	3.92	4.42ab	ALB	5.08a
ALB	4.30	2.98	5.62	4.30ab	PAR	4.69ab
PAR	4.77	3.70	3.93	4.13ab	ROD	3.35bc
PON	4.00	2.99	5.13	4.04ab	PAL	3.27bcd
SAV	4.34	3.69	3.61	3.88ab	GUI	3.25bcde
BOI	4.40	3.84	2.91	3.71ab	SAV	3.21bcdef
LED	3.17	3.80	3.87	3.61b	PON	3.01cdefg
PAL	3.63	3.73	3.37	3.58b	BOI	2.51cdefgh
LAT	5.47	3.44	1.81	3.57b	NAR	2.43cdefghi
AIG	3.62	3.65	3.24	3.50b	LAT	2.36cdefghi
ROD	3.80	2.97	3.72	3.49b	ARN	2.34cdefghi
RED	3.99	2.75	3.63	3.46b	CLE	2.22cdefghi
QUI	3.92	3.92	2.39	3.41b	LED	2.06cdefghi
GAL	4.84	2.42	2.91	3.39b	GAL	1.92cdefghi
FLE	3.58	2.48	4.07	3.38b	RED	1.62defghi
ARN	3.71	2.90	3.48	3.36b	PUI	1.43ghi
NAR	4.46	2.91	2.47	3.28b	FLE	1.11ghi
CLE	3.01	3.23	2.95	3.06b	QUI	0.89hi
PUI	2.88	2.82	2.85	2.85b	AIG	0.85i
MON	2.58	3.04	2.86	2.83b		
Mean	4.17	3.39	3.54			

Means followed by different letters are significantly different according to the Tukey's test at P=0.05.

of cv. Carignan showed not only nodosities but also tuberosities that caused necrosis and root death. A highly significant effect of the phylloxera sample was observed (F=20.43, df=19,59, P=0.0001). The samples clearly differed in their ability to decrease root dry mass (table III). The percentage of decrease varied from 15 % for the ALB sample to 86 % for the AIG sample.

II - GENETIC DIVERSITY

Among the 133 intense bands generated by 15 primers, 30 (22.6 %) were polymorphic and used for analysis (table II). The value for Jaccard's distance varied from 0.071 between AIG and PAL samples to 0.792 between PUI and FLE samples (data not showed). All phylloxera samples were distinguished and the NJ tree did not reveal any particular genetic structure, the clustering of samples being associated with low bootstrap values (figure 1). This indicated that the RAPD analysis did not allow the grouping of samples either according to their host origin, or their habitat, or their location. In addition, comparison of the genetic and geographic distance matrices did not reveal any positive significant correlation between the two data sets (r=-0.172, Pr=0.09). The FLE sample differed from the other samples in that Jaccard's distance and the number of marker differences among all samples averaged 0.438 and 6.9, respectively, whereas the means for FLE and other samples were 0.738 and 17.0, respectively.

DISCUSSION

Phylloxera samples are shown to differ in aggressiveness when tested on the established root system of potted plants. The variation in damage induces by different samples is detected more easily by inoculating the very susceptible plant material (cultivar of *Vitis vinifera*) than the resistant rootstock cultivars. In contrast to other studies, we do not find any phylloxera isolate x rootstock interaction. For example, SONG and GRANETT (1990) found that samples taken from 3309C in southern France were more aggressive when inoculated to this rootstock.

What happens in the vineyard? Phylloxera mainly lives in the upper layers of the soil. The insect could

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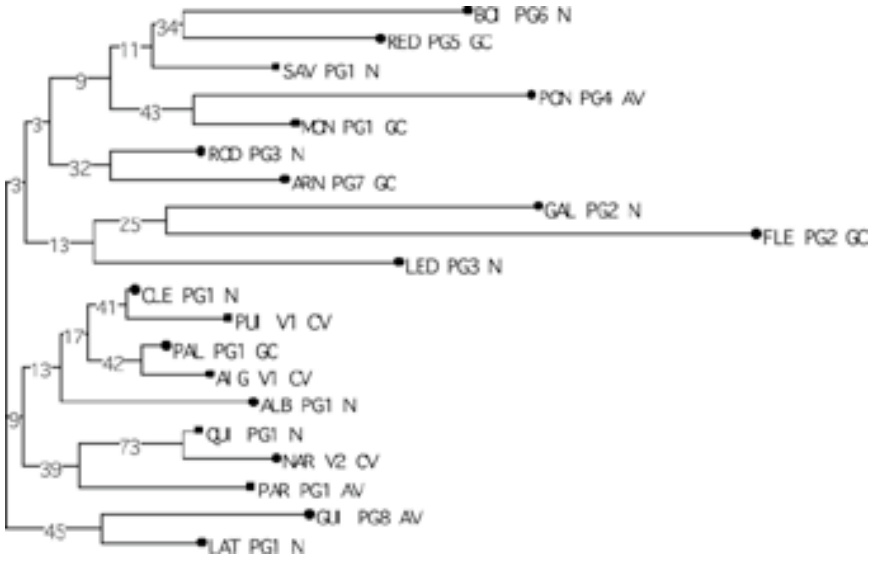


Fig. 1 - Relationships among phylloxera samples revealed by Neighbor-Joining analysis of the matrix of Jaccard's distance based on 30 RAPD markers (Boostrap values are given).

Name and habitat are indicated, the host of origin is coded from PG1 to PG7 for rootstocks and V1, V2 for *Vitis vinifera* cultivars, see table I.

Relations entre les échantillons de Phylloxéra révélées par l'analyse Neighbor-Joining de la matrice des distances de Jaccard obtenue avec 30 marqueurs RAPD.

Les valeurs de «bootstrap » sont données. Le nom et l'habitat des échantillons sont indiqués, la variété d'origine est codée de PG1 à PG7 pour les porte-greffe et V1, V2 pour *Vitis vinifera*, voir tableau I.

thus form destructive tuberosities in the upper part of the main roots of susceptible plants. However, only the extremity of roots and the rootlets of resistant rootstocks are damaged by infection. This effect is observed in the artificial conditions of our experiments performed with potted plants of resistant rootstocks. However, such damage is not observed in the field since the main roots develop rapidly and deeply in the soil. It is however possible that grafted grapevines may suffer from phylloxera in specific soil conditions (compact soil and shallow bed-rock) and climatic conditions (periods of drought). This phenomenon would explain the temporary weakening observed in Charente in grapevines grafted onto 41B by BRANAS (1963). Weakening of vines due to phylloxera has not been observed in southern France indicating that even the most aggressive samples are unable to induce damage on the rootstocks currently used. However, the variation in aggressiveness has to be taken into account when evaluating the resistance to *D. vitifoliae* of rootstocks, especially those with a *V. vinifera* parentage. Samples with the highest level of aggressiveness must be chosen for screening.

Analysis with RAPD markers demonstrates that diversity within the sample population is significant since all samples are found to be genetically distinct. In Europe, based on the use of AFLP markers, phylloxera samples have been grouped either in a northern cluster or in a southern cluster (FORNECK *et al.*, 2000). Northern haplotypes have also been recovered in southern Europe indicating that groups were not completely geographically isolated. It is therefore possible that the FLE sample, which is genetically different from the other samples, belongs to the northern cluster and all the others to the southern cluster. This hypothesis could be tested by further comparison between our samples and some representatives of southern and northern groups.

There is no correlation between genetic similarity and geographical location of the isolates. In addition, the host plant does not influence the genetic structure of the population. The latter result is consistent with data of FORNECK *et al.* (2000) who did not detect any significant effect of the host on the grouping of the phylloxera AFLP haplotypes. All these findings suggest that the population of grape phylloxera in southern France is well mixed and that new genotypes produced by sexual events have the ability to move between areas and between host genotypes. The alatae or the crawlers from leaf galls can ensure the gene flow at a regional scale.

Our results corroborate previous observations indicating wide diversity in European populations of phylloxera. For instance, DOWNIE (2002) found four mitochondrial haplotypes among six phylloxera samples from southern France and FORNECK *et al.* (2000) using AFLP markers distinguished all phylloxera samples from three areas in southern France.

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Based on the more limited variation they observed in phylloxera from the southern group compared to the northern group, FORNECK *et al.* (2000) suggested that more extreme winter temperatures might promote the sexual phase in the North. However, according to MAILLET (1957) the most severe winter conditions cannot kill the winter eggs. An alternative explanation could be a poor survival rate of alatae and their eggs during hot and dry summers. These unfavorable conditions would reduce the size of the population of sexual morphs and could explain the rarity of leaf galls in some regions like California (GALET, 1982) and South Africa (DE KLERK, 1974). In southern France we also observe that leaf galls are less frequent following a pronounced hot and dry summer (YVON, unpublished results). On the other hand, in southern France the leaf galling portion of the cycle is favored both by the abundance of susceptible plants in rootstock nurseries and by development of rootstocks after the dieback of the scions in abandoned vineyards.

In conclusion, the regular appearance of recombinant genotypes that may vary in aggressiveness in the population of *D. vitifoliae* from southern France does not seem to constitute an additional risk for current rootstock cultivars. Indeed, after more than one century of use, no failure of resistance has yet been observed. The RAPD markers develop for this study may be useful to study the persistence of genotypes in the same region as well as their movement among regions by performing more extensive sampling over successive years.

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REFERENCES

BRANAS J., 1963. Dépérissement du 41B en Charente, phylloxéra et court-noué. *Prog. Agric. Vitic.*, **161**, 178-186.

CORRIE A.M., CROZIER R.H., VAN HEESWIJCK R. and HOFFMANN A.A., 2002. Clonal reproduction and population genetic structure of grape phylloxera, *Daktulosphaira vitifoliae*, in Australia. *Heredity*, **88**, 203-211.

DE BENEDICTIS J. A. and GRANETT J., 1993. Laboratory evaluation of grape roots as hosts of California grape phylloxera biotypes. *Amer. J. Enol. Vitic.*, 44, 285-291.

DE BENEDICTIS J. A., GRANETT J. and TAORMINO S. P., 1993. Differences in host utilization by California strains of grape phylloxera. *Amer. J. Enol. Vitic.*, **47**, 373-379.

DE KLERK C.A., 1974. Biology of Phylloxera vitifoliae (Fitch) (*Homoptera Phylloxeridae*) in South Africa. *Phytophylactica*, **6**, 109-118.

DOWNIE D. A., 2000. Patterns of genetic variation in native grape phylloxera on two sympatric host species. *Mol. Ecol.*, **9**, 505-514.

DOWNIE D.A., 2002. Locating the sources of an invasive pest, grape phylloxera, using a mithochondrial DNA gene genealogy. *Mol. Ecol.*, **11**, 2013-2026.

DOWNIE D.A., FISHER J.R. and GRANETT J. (2001). Grapes, galls, and geography: the distribution of nuclear and mtDNA variation across host plant species and regions in a specialist herbivore. *Evolution*, **55**, 1345-1362.

FELSENSTEIN J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783-791.

FONG G., WALKER M. A. and GRANETT J., 1995. RAPD assessment of California phylloxera diversity. *Mol. Ecol.*, **4**, 459-464.

FORNECK A., WALKE M. A. and BLAICH R., 2000. Genetic structure of an introduced pest, grape phylloxera (*Daktulosphaira vitifoliae* Fitch), in Europe. *Genome*, **43**, 669-678.

GALET P., 1982. Phylloxera, 1059-1312. *In: Les maladies et les parasites de la vigne. Tome 2: Les parasites ani - maux.* ed. Galet P., Montpellier 1875 p.

GRANETT J., TIMPER P. and LIDER L. A., 1985. Grape phylloxera (*Daktulosphaira vitifoliae*) (*Homoptera: Phylloxeridae*) biotypes in California. *J. Econ. Entomol.*, **78**, 1463-1467.

HAWTHORNE D. J. and S. VIA, 1994. Variation in performance on two grape cultivars within and among populations of grape phylloxera from wild and cultivated habitats. *Entomol. Exp. Appl.*, **70**, 63-76.

JACCARD P., 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud Sci. Nat.*, **44**, 223-270.

KELLOW A.V., MCDONALD G., CORRIE A.M. and R. VAN HEESWIJCK, 2002. *In vitro* assessement of grapevine resistance to two populations of phylloxera from Australian vineyards. *Aust. J. Enol. Vitic.*, **8**, 109-116.

KOCSIS L., GRANETT J., WALKER M.A. and OMER A.D., 1999. Grape phylloxera populations adapted to *Vitis berlandieri* x *V. riparia rootstocks. Am. J. Enol. Vitic.*, 50, 101-106.

LIN H., DOWNIE D. A., WALKER A., GRANETT J., and ENGLISH-LOEB G., 1999. Genetic structure in native populations of grape Phylloxera (*Homoptera: Phylloxeridae*). *Ann. Entomol. Soc. Am.*, **92**, 376-381.

MAILLET P., 1957. Contribution à l'étude de la biologie du Phylloxéra de la vigne. *Ann. Sci. Nat. Zool. Biol. Anim.*, **19**, 283-410.

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MARTINEZ-PENICHE R., 1993. Influence de la variabilité du Phylloxéra (*Daktulosphaira vitifoliae* Fitch) et de la culture *in vitro* sur l'expression de la résistance génétique des racines de porte-greffes de vigne. *Thèse* de Doctorat, Ecole Supérieure Nationale Agronomique, Montpellier (1993).

PÉROS J.P., THIS P., CONFURON Y. and CHACON H., 1996. Comparaison by isosyme and RAPD analysis of some isolates of the grapevine dieback fungus *Eutypa lata*. *Am. J. Enol. Vitic.*, **47**, 49-56.

OMER D.A., GRANETT J., KOCSIS L. and DOWNIE D.A., 1999. Preference and performance responses of California grape phylloxera to different *Vitis* rootstocks. *J. Appl. Ent.*, **123**, 341-346.

OMER D.A., GRANETT J. and SHEBELUT C.W., 1999. Effect of attack intensity on host utilization in grape phylloxera. *Crop Prot.*, **18**, 341-347.

POUGET R., 1975. Méthode de contamination de racine de vigne *in vitro* par le Phylloxéra radicicole: Application à la recherche de porte-greffes résistants. *Connaissance Vigne Vin*, **3**, 165-176.

POUGET R., 1990. *Histoire de la lutte contre le Phylloxéra de la vigne en France*. ed. INRA, Paris.

SAITOU N. and NEI M., 1987. The Neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406-425.

SONG G.I. and J. GRANETT, 1990. Grape phylloxera (*Homoptera: Phylloxeridae*) biotypes in France. *J. Econ. Entomol.*, **83**, 489-493.

TIXIER-GARCIN M. S., 2000. La présence de Phytoséiides dans les abords non cultivés des parcelles a-t-elle un intérêt appliqué en viticulture ? *Thèse de doctorat*, Ecole Nationale Supérieure Agronomique de Montpellier.

WAPSHERE A.J. and HELMK.F., 1987. Phylloxera and *Vitis*: an experimentally testable coevolutionary hypothesis. *Am. J. Enol. Vitic.*, **38**, 216-222.

WILLIAMS J. G. K., KUBELIK A. R., LIVAK K. J., RAFALSKI J. A., and TINGEY S. V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, **18**, 6531-6535.

WILLIAMS R. N. and SHAMBAUGH G. F., 1988. Grape phylloxera (*Homoptera: Phylloxeridae*) biotypes confirmed by electrophoresis and host susceptibility. *Ann. Entomol. Soc. Am.*, **81**, 1-5.

YVON, M. and LECLANT F., 2000. Intraspecific variability in grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in France: development of rearing techniques. *Acta Hortic.*, **2**, 581-586.

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