Considerations on the European wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) and Phylloxera infestation

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K e y   w o r d s : artificial infestation, edaphic conditions, phylloxera, roots, symptoms.

Figure: Location of the sites were the survey was conducted.

Introduction: The Eurasian wild grapevine, *Vitis vinifera* subspecies *sylvestris* (Gmelin) Hegi, has been a common dioecious plant in different ecosystems from Central and Southern Europe. It grows thus on fluvisol regularly flooded and is also currently found on colluvisols. Both soils are regularly renewed with fresh rough material either brought by flooding or by gravity (Arnold 2002).

The human impact on wild grapevine habitats had direct and undirect implications. The progressive canalization of rivers, dam and levee constructions, forestry and horticulture exploitations of floodplain forests, cleaning of riverbanks, and the continuous extension of the road network led to direct eradications of wild grapevine populations throughout Europe (Arnold *et al.* 1998). Other undirect repercussions derived from the importation of North American diseases and pests such as powdery and downy mildews and respectively phylloxera led to the current status of the wild grapevine as threatened taxon in the European territory (Thorsell and Sigaty 1997).

The aim of the current paper was to compare symptoms caused by phylloxera on roots in several wild areas and in a experience carried out under artificial infestation.

Material and Methods: The first step of this work was to confirm the dioecious character of the wild grapevines during the flowering time, usually spread from May to July, depending on the geographical position of each site.

The prospecting of leaves and roots to find possible symptoms caused by phylloxera was carried out in 55 sites: 25 in Spain, 5 in Portugal, 4 in France, 1 in Switzerland, 2 in Germany, 5 in Hungary and 13 in Italy (Figure). To detect possible subterranean symptoms, roots were unearthed up
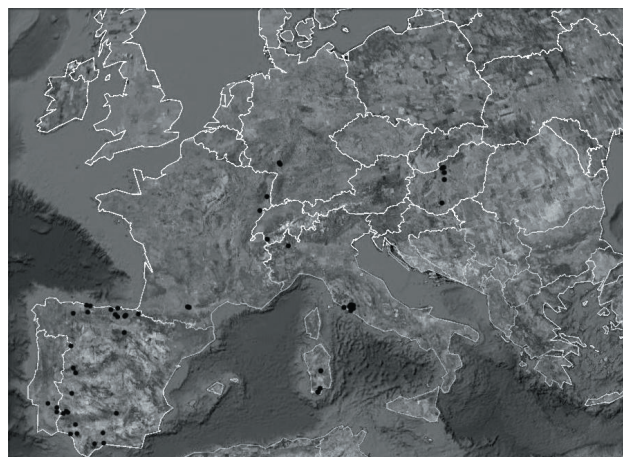
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to 40-50 cm of depth. Samples of capillary roots were observed under binocular. To verify if wild grapevines did not have an intrinsic resistance to phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera, Phylloxeridae), a test using 9 months old vines obtained from shoots placed in 2 litre pots was carried out. The substrate was composed by one part of perlite, two of sand and three of peat. The wild materials used for investigation were shoots of 3 vines per population, collected in Cádiz (Spain), La Rioja (Spain), Basque country (France), Germany (Baden-Wütenberg) and Hungary (Visegrad). The experiment was carried out with 5 repetitions. On the other hand, 15 plants of 'Chasselas' and 'Cabernet Sauvignon', the 333-EM (*V. vinifera* 'Cabernet Sauvignon' x *V. berlandieri*) and the 41B rootstock ('Chasselas' x *V. berlandieri*) were also tested. The artificial infestation on each pot was made following the procedure of Boubals (1966), using 10 leaves of *Vitis rupestris* which contained about 60 galls per leaf. The evaluation of damages caused by the root form of phylloxera was observed after 15 months of the infestation, according to the criteria given by IPGRI, UPOV and OIV (1997).

The dying cuts of the cited different roots, following the methodology of Curtis Patiño (1986), were observed under microscope to evaluate the depth of the damages caused by phylloxera referring to the cortex thickness.

The degree of infestation was evaluated in terms of number of tuberosities and nodosities per vine. Data were statistically processed via one-way analysis of variance (anova) with genotype as the main factor. Means were separated with Tukey's studentised range test ($P < 0.05$) using SPSS statistical software (version 16.0; SPSS Inc. Chicago, Illinois).

Results: The 55 populations of *V. vinifera sylvestris* with a total of 980 vines were studied (Figure). In its natural environment, no damages caused by phylloxera were found, even though some populations where close and even bordering to vineyards. As it is well known, this pest is largely spread to all European grape producing areas. Tuberosities and nodosities are the main entrance for soil fungi and bacteria leading to vine death (Granett *et al.* 2001). The damages caused in pots during artificial infestation are summarised in the Table. The two Euro-ameri-

Average number of tuberosities and nodosities caused by phylloxera in six samples of wild grapevine populations compared to two cultivars

Sample	Nodosities		Tuberosities	
	Means[x]	St. Dev.	Means[x]	St. Dev.
Chasselas	32.87 a	13.85	9.53 a	4.29
Cabernet Sauvignon	41.13 a	14.90	9.27 a	3.67
France 1	43.13 a	14.92	24.27 bc	7.35
France 3	36.67 a	11.29	23.20 bc	7.44
Germany 1	40.67 a	13.00	22.20 bc	10.25
Hungary 1	41.53 a	9.13	26.00 c	7.23
Spain 1	36.87 a	11.69	20.07 bc	5.55
Spain 2	61.47 b	18.17	18.13 b	5.62

[x] Means followed by the same letter are not statistically different according to Tukey's test.

can rootstock hybrids did not show any symptoms, so they were excluded by the data processing. According to the application of the Tukey's test, there is no significative difference in the case of nodosities. On the other hand, some significant differences were found in tuberosities data. As shown there are no differences in the infestation degree of the two cultivars, however wild grapevines were affected by a higher number of tuberosities. These data proved to be statistically significant.

The insect induced a higher number of tuberosities and nodosities on roots of wild vines than on those belonging to the 2 tested cultivars: 'Chasselas' and 'Cabernet Sauvignon', while no damages were scored on 41B and 333-EM rootstocks. This result was expected as these rootstock varieties are selected hybrids issued from crossing between *Vitis vinifera* and *Vitis berlandieri*.

It is interesting to remark that the intensity of the injuries among wild roots was in the range from 1 to 3. These values are very low (localized necrosis, punctures) or low (necrosis isolated within bark). In the case of the two tested cultivars damages were much more serious: from 7 (medium = necrosis penetrates central cylinder, isolated) to 9 (high = necrosis penetrates cylinder, isolation complete), as verified under microscope inspection.

Discussion: The results of the experiment based on artificial infestation by phylloxera agree with previous observations reported by Ocete *et al* (2007). The absence of damages in rootstock hybrids during the same experiments have reference to well establishment fact that the majority of North American native *Vitis* species have co-evolved with the insect and thus show various levels of resistance. The intensity of injuries in tested cultivars roots higher than that in wild roots is probably due to a thinner cover of cork in cultivar than wild plants.

The absence of injuries on wild roots in natural habitats may be linked to edaphic conditions. Indeed, this aphid like insect cannot live in permanent or temporary anoxic conditions or in soils with rough material such as gravel or sand.

Conclusion: This phytosanitary survey of European wild grapevines demonstrates that phylloxera is not a problem for the survival of the relictic populations. Wild grapevine populations only remain in sites where the edaphic conditions are currently unsuitable for the development of phylloxera such as permanent or temporary anoxic conditions and on gravel or sandy soils. Basing on these considerations we can suppose that the major trouble for wild grapevine survival could derive from the several grapevine hybrid species, used as rootstocks, which share a similar ecology with the European wild grapevines, growing in floodplain forests.

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