EVOLUTION OF WILD AND FERAL VINES FROM THE EGA RIVER GALLERY FOREST (BASQUE COUNTRY AND NAVARRA, SPAIN) FROM 1995 TO 2015

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Abstract

Aims: The present paper is focused on wild and feral grapevines from the Ega River gallery forest (Alder grove), one of the most important tributaries of the Ebro River. Since this area was preliminary prospected in 1995, it was our intention to assess the evolution of the ecosystem during this time.

Methods and results: Ampelographic description of wild grapevines shows that all male plants had flowers with fully developed stamens and no gynoecium. No hermaphrodite individuals were detected. No virus infection and symptoms caused by Phylloxera on roots were detected. The Erineum strain of *Colomerus vitis* constitutes the main phytophagous arthropod. Damages caused by powdery and downy mildew were not lethal for the vines. Around 73 % of the wild grapevine individuals found in 1995 had disappeared due to human impacts. The number of rootstocks and producer hybrids (French hybrids) had increased in this period by about 30 %. Microvinification of wild berries yielded a wine with very high color intensity and total polyphenol index with a low pH.

Conclusion: The wild grapevine population described has suffered a dramatic regression in the 20-year period between prospections, together with a significant increase of feral accessions thus endangering the endurance of the ecosystem and remaining wild grapevine.

Significance and impact of the study: Wild grapevines are a valuable genetic resource for the genetic improvement of cultivated vines. However, our study shows that human impacts and increasing pressure of feral accessions are threatening wild grapevine populations.

Key words: Alder grove, endangered taxon, loss of biodiversity, sanitary status, *Vitis vinifera* subsp. *sylvestris*

Résumé

Objectifs : Le présent article est centré sur les vignes sauvages qui se trouvent sur la forêt-galerie de la rivière Ega (Aulnaie), un des plus importants affluents de l'Èbre. Puisque la première étude sur cette région a été réalisée en 1995, notre intention était d'étudier l'évolution de l'écosystème pendant cette période.

Méthodes et résultats: Une description ampélographique des vignes sauvages a montré que toutes les plantes mâles ont des fleurs avec des étamines bien développées et pas de pistil. Aucune plante hermaphrodite n'a été détectée. Aucune infection virale ou symptômes causés par le phylloxéra ont été trouvés. La variété Erineum de *Colomerus vitis* est la plus importante des arthropodes phytophages. Les dommages causés par l'oïdium n'ont pas été mortels pour les vignes. Près de 73 % des spécimens de vigne sauvage trouvés en 1995 ont disparu à cause de l'action de l'homme. Le nombre de rhizomes et d'hybrides productifs (hybrides français) a augmenté d'environ 30 % pendant cette période. La microvinification des baies sauvages a produit un vin d'une couleur très intense, un taux élevé de polyphénol et un pH bas.

Conclusion: La population de vigne sauvage décrite ici a subi une grave régression dans cette période de 20 ans entre les deux prospections, en même temps qu'une intensification des variétés férales mettant ainsi en danger l'équilibre de l'écosystème.

Signification et impact de l'étude: Les vignes sauvages représentent une ressource génétique utile à l'amélioration des vignes cultivées. Cependant, notre étude montre que l'action de l'homme et la pression croissante des variétés férales sont une menace pour les populations de vignes sauvages.

Mots clés: Aulnaie, taxon en danger, perte de biodiversité, statut sanitaire, *Vitis vinifera* subsp. *sylvestris*

manuscript received 14th January 2015 - revised manuscript received 19th April 2016

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J. Int. Sci. Vigne Vin, 2016, **50**, 2, 65-75

INTRODUCTION

The only Eurasian wild grapevine, *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, is considered an endangered subspecies in Europe (Di Vecchi-Staraz *et al.*, 2009 ; Bodor *et al.*, 2010). This is due to diverse anthropic impacts on natural habitats, like the exploitation of river-banks, public works, the infestation of downy and powdery mildew as well as the introduction of invasive vines, such as American rootstocks and producer hybrids used as a cultural method to prevent phylloxera infestation from the 19th century (Issler, 1938 ; Arnold, 2002 ; Ocete *et al.*, 2007 ; Iriarte-Chiapusso *et al.*, 2013).

During the COST Action FA-1003 East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding, developed between 2011 and 2014 (http://www.cost.eu/COST_ Actions/fa/FA1003), the objectives were to increase the knowledge on wild grapevine populations by studying its ampelography, genetic pool, sanitary status, and enological characteristics. One of the main targets was to find some vines with potential resistance to pest and diseases, mainly to downy and powdery mildew.

However, current viticulture is affected by an alarming genetic erosion process (This *et al.*, 2001 ; González-Moreno *et al.*, 2004 ; Esquinas-Alcázar, 2005 ; Gago *et al.*, 2009) in the framework of climatic warming and markets globalization (Mira de Orduña, 2010 ; Mozell and Thach, 2014). In modern vineyards there are only a small number of clones with sanitary passport of the cultivars allowed within each Guarantee of Origin, so the genetic pool is declining alarmingly. Global warming will result in loss of acidity and color and together with globalization and open borders will increase the risk of introducing new pests and diseases, which might spread easily in vineyards with low genetic diversity. Due to those facts, Eurasian wild grapevine constitutes a useful phytogenetic resource to be used in breeding of cultivars and rootstocks for those new requirements, previously mentioned (Jones *et al.*, 2005 ; Ocete *et al.*, 2007 ; Popescu *et al.*, 2013). On the other hand, this liana had important uses in the past (Ocete *et al.*, 2011c) and nowadays it constitutes a relic element of river-bank forests, colluvial positions and coastal cliffs (Arnold, 2002).

According to that idea, our attention was focused on Ega River, a tributary of Ebro River, situated in Northern Spain, because it conserves an extensive riparian vegetation with some disseminated population sites of wild grapevine, as it was shown in a preliminary prospection carried out by Ocete and Pérez (1995). The source of this river is located in Álava province (Basque Country) south of the Cantabrian Mountain-Range, its length is 113 km, the

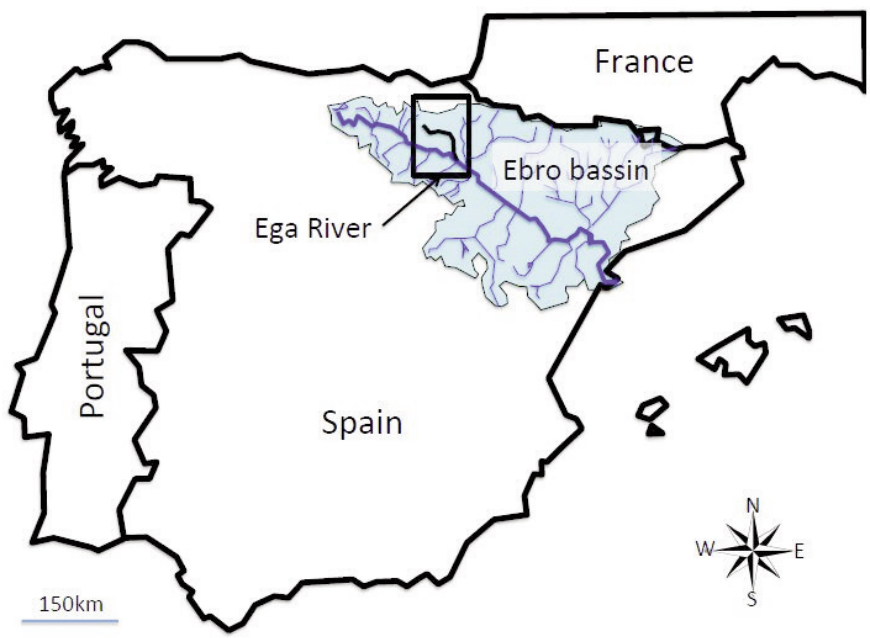

Figure 1. Localization of Ega River.

average flow is 13.76 m^3/s, draining an area of 1,497 km^2 (fig. 1). Other plant populations within the Ebro River basin have been described previously (Martínez De Toda and Sancha, 1999 ; Ocete *et al.*, 2003 ; Ocete *et al.*, 2011b)

The aim of the present paper was to compare the number of wild vines registered in 1995 with those sampled in 2014 and to denounce the lack of a specific legislation for the conservation of this endangered taxon in the Iberian Peninsula. Also, to complete the description of the characteristics of their habitats according to associated plant communities, their sanitary status and their oenological profile. Some information on feral vinifera is also included.

MATERIALS AND METHODS

1. Study area

The coordinates of the relic sites with wild vines were marked by GPS in a new prospection on the gallery forests, from the source to its flows into the Ebro River. Those wild grapevines and feral plants, such as rootstocks, producer hybrids (French hybrids) and cultivars were also counted.

2. Ampelographic study

The ampelographic study on wild grapevine was based on the list of descriptors published by O.I.V. (2009).

Male and female pollen samples were obtained by brushing mature anthers from both kinds of flowers. Pollen grains were introduced in DPX (Fluka) and observed under optical microscope Olympus BX 61 to study the morphological structure of the grains, according to Gallardo *et al.* (2009).

Main botanical supporting trees and bushes of the vines as well as the associated vegetation were identified using various botanical keys (Rameau *et al.*, 2008) and the study carried out on several botanical communities from Navarra (Peralta *et al.*, 2013). An evaluation of the active limestone was conducted by a Bernard calcimeter following the procedure of Álvarez-Iglesias *et al.* (2003).

The observation of symptoms caused by pests and diseases was carried out every two weeks from spring to autumn 2014 on the aerial parts of the plants (shoots, leaves and bunches), from the floor up to 4 m of height. On the other hand, roots of each wild vine were unearthed up to 40-50 cm of depth. Samples of rhizosphere of each vine were assessed under a binocular loupe to detect possible damages caused by phylloxera and root rot fungi. The procedures of Fenwick (1943) and Flegg (1967) were followed in the case of nematodes.

Leaves from two vines from each population were used to test the presence of Grapevine Fan Leaf, Grapevine Leafroll and Grapevine Fleck viruses using the Bioreba ELISA test kit (Martelli, 2000).

3. Microvinification process

Harvest took place on the 29th of October 2014. On the 31st, we began vinification with a volume of 6 kg of grape. Following hand destemming, 1000 berries (for a total weight of 314.6 g) were mashed and color parameters were identified on the juice.

The remaining berries (about 3 kg) were crushed using a pestle, inside a plastic bucket. Then microvinification was conducted at a temperature of 18ºC. As cap management 2 to 3 pumping over were applied daily. The fermentation activity started on the 3rd day. On day 6, a density of 1038 mg/l was measured. That day volatile acidity recorded was 0.87 g/l. For that reason, 0.8 g of metabisulphite were added to the ferment to prevent a further increase of the volatile acidity.

On day 7, the fermentation stopped at a density of 1032 mg/l and a volatile acidity of 0.50 g/l. It was necessary to add commercial yeast (F33 Laffort, 0.3 g/l) and an activator (Nutristar, 0.2 g/l) to resume fermentation.

On day 8, the bucket was warmed. The density dropped to 1030 mg/l.

On day 9, biggest fermentation activity was noticed and density decreased down to 1015 mg/l. The process stopped on day 10, at a final density of 1011 mg/l, volatile acidity of 0.67 g/l and 36 g/l of reducing sugars.

Different analytical parameters were obtained following the procedures described by O.I.V. (2015): near infrared for the determination of ethanol concentration; automatic potentiometry in the cases of pH and total acidity ; FCSA autoanalyser for volatile acidity ; and the OIV method for the determination of the color intensity.

RESULTS AND DISCUSSION

The ampelographic characters of the wild grapevine are shown in Table 1. These descriptors are similar to those described in other populations from the Ebro basin (Ocete *et al.*, 2011b). The coordinates and number of vines per location, observed in 1995 and 2014 are shown in Table 2.

Table 1. Ampelographical characters of the Rio Ega wild grapevine population.

Descriptors	female	male
Young Shoot: aperture of tip (OIV 001)	3 (half open)	5 (fully open)
Young Shoot: distribution of anthocyanin coloration on prostrate hairs of tip (OIV 002)	1 (absent) - 2 (piping)	2 (piping)
Young Shoot: intensity of anthocyanin coloration on prostrate hairs of tip (OIV 003)	3 (low)	3 (low)
Young Shoot: density of prostrate hairs on tip (OIV 004)	3 (low)	3 (low)
Young Shoot: density of erect hairs on tip (OIV 005)	1 (none or very low)	2 (none or very low)
Shoot: attitude (before tying) (OIV 006)	3 (semi-erect)	3 (semi-erect)
Shoot: color of dorsal side of internodes (OIV 007)	2 (green and red)	1 (green)
Shoot: color of ventral side of internodes (OIV 008)	2 (green and red)	1 (green)
Shoot: color of dorsal side of nodes (OIV 009)	2 (green and red)	1 (green)
Shoot: color of ventral side of nodes(OIV 010)	2(green and red)	1(green)
Shoot: density of erect hairs on nodes (OIV 011)	1 (none or very low)	1 (none or very low)
Shoot: density of erect hairs on internodes (OIV 012)	1 (none or very low)	1 (none or very low)
Shoot: density of prostrate hairs on nodes (OIV 013)	1 (none or very low)	1 (none or very low)
Shoot: density of prostrate hairs on internodes (OIV 014)	1 (none or very low)	1 (none or very low)
Shoot: intensity of anthocyanin coloration on bud scales (OIV 015-2)	1 (none or very weak)-3 (weak)	2 (none or very weak)-3 (weak)
Shoot: number of consecutive tendrils (OIV 016)	1 (2 or less)	2 (2 or less)
Shoot: length of tendrils (OIV 017)	3 (short)	3 (short)
Young leaf: color of the upper side of blade (4th leaf) (OIV 051)	4 (copper-reddish)	1 (green) - 3 (bronze)
Young leaf: density of prostrate hairs between main veins on lower side of blade (OIV 053)	3 (low)	4 (low)
Young leaf: density of erect hairs between main veins on lower side of blade (OIV 054)	1 (none or very low)	2 (none or very low)
Young leaf: density of prostrate hairs on main veins on lower side of blade (OIV 055)	1 (none or very low) - 3 (low)	3 (low)
Young leaf: density of erect hairs on main veins on lower side of blade (4 th leaf) (OIV 056)	1 (none or very low)	1 (none or very low)
Mature leaf: size of blade (OIV 065)	5 (medium)	1 (very small) - 3 (small)
Mature leaf: shape of blade (OIV 067)	2 (wedge-shaped) - 3 (pentagonal)	3 (pentagonal)
Mature leaf: number of lobes (OIV 068)	1 (entire leaf) - 2 (three)	2 (three) -3 (five)
Mature leaf: color of the upper side of blade (OIV 069)	5 (medium green)	6 (medium green)
Mature leaf: area of anthocyanin coloration of main veins on upper side of blade (OIV 070)	1 (absent)	1 (absent)
Mature leaf: area of anthocyanin coloration of main veins on lower side of blade (OIV 071)	1 (absent)	1 (absent)
Mature leaf: goffering of blade (OIV 072)	1 (absent or very weak)	2 (absent or very weak)
Mature leaf: undulation of blade between main and lateral veins (OIV 073)	1 (absent)	1 (flat)
Mature leaf: profile of blade in cross section (OIV 074)	1 (flat) - 3 (involute)	1 (flat) - 3 (involute)
Mature leaf: blistering of upper side of blade (OIV 075)	3 (weak)	4 (weak)
Mature leaf: shape of teeth (OIV 076)	1 (both side concave)- 2 (both side straight)	2 (both side concave)- 2 (both side straight)
Mature leaf: size of teeth in relation to blade size (OIV 077)	3 (short)	3 (short)
Mature leaf: length of teeth compared with their width (OIV 078)	4 (short)	4 (short)
Mature leaf: degree of opening / overlapping of petiole sinus (OIV 079)	3 (open)	1 (very wide open)
Mature leaf: shape of base of petiole sinus (OIV 080)	1(U-shaped)	1(U-shaped)
Mature leaf: teeth in the petiole sinus (OIV081-1)	1 (none)	2 (none)
Mature leaf: petiole sinus base limited by veins (OIV 081-2)	1 (not limited)	2 (not limited)
Mature leaf: degree of opening / overlapping of upper lateral sinus (OIV 082)	1 (open)	2 (open)
Mature leaf: teeth in the upper lateral sinuses (OIV 083-2)	1 (none)	2 (none)
Mature leaf: density of prostrate hairs between the main veins on lower side of blade (OIV 084)	1 (none or very low)	1 (none or very low)
Mature leaf: density of erect hairs between the main veins on lower side of blade (OIV 085)	1 (none or very low)	1 (none or very low)
Mature leaf: density of prostrate hairs on main veins on lower side of blade (OIV 086)	1 (none or very low) - 3 (low)	4 (low)
Mature leaf: density of erect hairs on main veins on lower side of blade (OIV 087)	1 (none or very low)	2 (none or very low)
Mature leaf: prostrate hairs on main veins on upper side of blade (OIV 088)	1 (absent)	1 (absent)
Mature leaf: erect hairs on main veins on upper side of blade (OIV 089)	1 (absent)	1 (absent)

Descriptors	female	male
Mature leaf: density of prostrate hairs on petiole (OIV 090)	1 (none or very low)	1 (none or very low)
Mature leaf: density of erect hairs on petiole (OIV 091)	1 (none or very low)	1 (none or very low)
Mature leaf: length of petiole compared to length of middle vein (OIV 093)	3 (slightly shorter)-5 (equal)	4 (slightly shorter)-5 (equal)
Mature leaf: depth of upper lateral sinuses (OIV 094)	1 (absent or very shallow	5 (medium)
Woody shoot: cross section (OIV 101)	2 (elliptic)	3 (elliptic)
Woody shoot: structure of surface (OIV 102)	2 (ribbed)	3 (ribbed)
Woody shoot: main color (OIV 103)	2 (brownish)	3 (brownish)
Woody shoot: lenticels (OIV 104)	1 (absent)	1 (absent)
Woody shoot: erect hairs on nodes (OIV 105)	1 (absent)	1 (absent)
Woody shoot: erect hairs on internodes (OIV 106)	1 (absent)	1 (absent)
Flower: sexual organs (OIV 151)	4 (reflexed estamens and fully developed gynoecium)	1 (fully developed stamens and no gynoecium)
Inflorescence: number of inflorescences per shoot (OIV 153)	2 (1,1 to 2 inflorescences)	3 (2,1 to 3 inflorescences)
Bunch: length (peduncle excluded) (OIV 202)	3 (short)	4 (short)
Bunch: width (OIV 203)	3 (narrow)	
Bunch: density (OIV 204)	1 (very loose)-3 (loose)	
Bunch: length of peduncle of primary bunch (OIV 206)	3 (short)	
Bunch: lignification of peduncle (OIV 207)	1 (at the base only)	
Bunch: shape (OIV 208)	2 (conical)	
Bunch: number of wings of the primary bunch (OIV 209)	2 (1-2 wings)	
Berry: length (OIV 220)	1 (very short)-3 (short)	
Berry: width (OIV 221)	1 (very narrow)-3 (narrow)	
Berry: uniformity of size (OIV 222)	1 (not uniform)	
Berry: shape (OIV 223)	2 (globose)	
Berry: color of skin (OIV 225)	6 (blue black)	
Berry: uniformity of color of skin (OIV 226)	1 (not uniform)	
Berry: thickness of skin (OIV 228)	7 (thick)	
Berry: hilum (OIV 229)	2 (visible)	
Berry: intensity of the anthocyanin coloration of flesh (OIV 231)	1 (none or very weak)	
Berry: juiciness of flesh (OIV 232)	2 (slightly juicy)	
Berry: must yield (OIV 233)	3 (little)	
Berry: firmness of flesh (OIV 235)	2 (slightly firm)	
Berry: particularity of flavor (OIV 236)	1 (none)	
Berry: length of pedicel (OIV 238)	1 (very short)	
Berry: ease of detachment from pedicel (OIV 240)	2 (easy)	
Berry: formation of seeds (OIV 241)	3 (completed)	
Berry: length of seeds (OIV 242)	1 (very short)	
Berry: weight of seeds (OIV 243)	1 (very low)	
Berry: transversal ridges on dorsal side of seeds (OIV 244)	1 (absent)	
Time of bud burst (OIV 301)	5 (medium)	
Time of full bloom (OIV 302)	5 (medium)	
Time of beginning of berry ripening (veraison) (OIV 303)	5 (medium)	
Time of physiological stage of full maturity of the berry (OIV 304)	7 (late)-9 (very late)	
Time of beginning of wood maturity (OIV 305)	7 (late)	7 (late)
Time of autumn coloring of leaves (OIV306)	8 (late)	8 (late)
Vigor of shoot growth (OIV 351)	5 (medium)	7 (strong)
Growth of axillary shoots (OIV 352)	10 (late)	10 (late)
Length of internodes (OIV 353)	11 (late)	11 (late)
Diameter of internodes (OIV 354)	12 (late)	12 (late)

Table 2. Comparison of the number of wild grapevines observed in 1995 and 2014 in each location.

Region	Location		Coordinates(*)	Nº of vines		Reduction (%)
				1995	2014	
Basque country	Santa Cruz de Campezo	B	2°23'12.2"W 42°39'25.5"N	31	12	61,3
		E	2°20'13.8"W 42°40'26.3"N			
Navarra	Marañón	B	2°27'48.9"W 42°37'37.6"N	7	3	57,2
		E	2°27'5.6"W 42°37'39.9"N			
	Arquijas	B	2°16'51.7"W 42°40'56.1"N	20	2	90
		E	2°16'6.7"W 42°40'57.0"N			
	Zubielqui	B	2° 4'24.4"W 42°40'18.6"N	27	10	63
		E	2° 3'34.8"W 42°40'51.8"N			
	Estella	B	2° 1'30.0"W 42°40'12.4"N	16	0	100
		E	2° 1'12.4"W 42°39'49.2"N			

(*) "B" and "E" stand for the two extreme points along the course of the river: Beginning and End.

1. Pollen

Pollen grains were tricolporated in male and acolporated in female flowers.

2. Evolution of the number of wild grapevines

As shown in Table 2, over the last twenty years wild plants were reduced between 57 % and 100 % depending on the site. The main causes were deliberate removal of vines, which was reported earlier in other geographical areas (Martínez de Toda, 1991). Natural lianas climb up bushes and trees seeking maximum sunlight exposure (Carter and Teramura, 1988) and are therefore often considered as weeds by forest managers (Lutz, 1943) mainly due to sunlight competition.

Site by site: in Santa Cruz de Campezo, Marañon and Zubielqui the losses were mainly due to cleaning works at the river-bank forest, whereas in Arquijas, the road enlargement and the subsidiary cleaning of the ditches resulted in the almost complete removal of the vines. The situation was even worse in Estella, where the whole population was destroyed by the construction of a new walkway following Saint James' Way between the church of the Holy Sepulcher and the camping site.

3. Accompanying vegetation

A phytosociological study performed in the area (Arnold, 2002) revealed that wild grapevines are also present at hillsides in a vegetation type called maquis. This type of vegetation is dominated by *Quercus ilex* L. and *Arbutus unedo* L. The understory vegetation is often composed of a dense coverage of *Ruscus aculeatus* L. Along the Ega River, wild grapevines are present in remnants of gallery forests, at their external edges or in the direct vicinity of water. These forests are woods or groves of alders, poplars and willows (Arozena Concepción and Ferreras Chasco, 2007). Rivas Goday (1964) described this vegetation as belonging to alliances of Alno-Ulmion and *Populion albae*. In some cases the presence of wild grapevines is reported in upper alluvial terraces dominated by *Fraxinus excelsior* L. This kind of habitat is named "*Alder grove from the Ebro river Basin*". It covers two kinds of subtypes: Subcantabric, in the northern riverbed, with *Fraxinus excelsior* L., and Submediterranean, along the southern one, with *Fraxinus angustifolia* L. It includes hybrids and introgressed individuals between both cited species, according to the terminology of Peralta *et al.* (2013).

The main frequent species of trees and bushes are: *Acer campestre*, *Acer monspessulanum*, *Acer pseudoplatanus*, *Alnus glutinosa*, *Arbutus unedo*, *Buxus sempervirens*, *Cornus sanguínea*, *Corylus avellana*, *Crataegus monogyna*, *Euonymus*

Table 3. Incidence of symptoms caused by parasitic species on all grapevines.

Parasitic species	% of infestation/infection	
	1995	2014
Colomerus vitis	100	100
Calepitrimerus vitis	59,4	40,7
Empoasca vitis	20,8	22,2
Thrips angusticeps	0	48,2
Erysiphe necator	93,1	92,6
Plasmopara viticola	78,2	77,8

europaeus, *Ficus carica*, *Fraxinus angustifolia*, *Fraxinus excelsior*, *Ligustrum vulgare*, *Populus nigra*, *Populus tremula*, *Pteridium aquilinum*, *Quercus ilex*, *Rosa sempervirens*, *Rubus ulmifolius*, *Ruscus aculeatus*, *Salix alba*, *Salix triandra*, *Sambucus nigra*, *Viburnum lantana*, and *Ulmus minor*.

In these gallery forests, the most frequent lianae are*:* *Bryonia cretica*, *Clematis vitalba*, *Hedera helix* and *Humulus lupulus*.

Having a closer look at the different strata of woods and groves of willows and alders, it becomes obvious that the tree strata is formed by pioneer species (Rameau *et al.*, 2008) such as *Salix alba* L., *Salix triandra* L., and *Populus tremula* L. These species are able to colonize bare soils and constitute the first step of colonization by vegetation after flooding. The bush and herbaceous strata are reflecting what Roulier (1998) called a floristic shift of the vegetation. The lack of flooding allows post-pioneer (Rameau *et al.*, 2008) species such as *Fraxinus angustifolia* Vahl. or *F. excelsior* L. *Ulmus minor* Mill. to establish and replace the *Salix* forests. These species are fast growing and light demanding species.

We also observed the presence of xerophile or mesoxerophile trees in the bush and herbaceous strata such as *A. monspessulanum*, *A. unedo*, *Q. ilex*, *B. sempervirens* and *F. carica*.

The soil is a typical Fluvisol (Arnold, 2002) with a total lime content of 22.9 % due to its development from calcareous rocks from the Mesozoic Era.

Soil environments and accompanying vegetation provided valuable information about the natural habitats of *V. vinifera* (Morano and Walker, 1995).

4. Pests

The incidence of symptoms caused by parasitic species on vines is shown in Table 3.

The main symptoms of infestation by arthropods are felty galls caused on the leaves by the erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae). Vines with a higher sensitivity/ susceptibility to this mite suffer a premature defoliation. The presence of this mite was observed in all locations, affecting 100 % of the vines in both sampling years. No symptoms caused by bud and curl leave strains were detected. The presence of another mite, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae), is lower, affecting 20 % and 22 % of

Table 4. Characteristics of the wine obtained after microvinification. Analytical data and procedure are compiled.

Parameter	Value		Method
Color intensity	26,57	Q-12	UV-VIS Spectrometry
Absorbance 420 nm	6,829	Q-12	UV-VIS Spectrometry
Absorbance 520 nm	18,401	Q-12	UV-VIS Spectrometry
Absorbance 620 nm	1,34	Q-12	UV-VIS Spectrometry
Total polyphenol index	141	Q-22/E-012	UV Spectrometry
Anthocyanins (mg/l)	516		UV-VIS Spectrometry
Total acidity (tartaric acid g)	19,3		Valoration
pH	2,96	Q-27	OIV compendium
Potassium (mg/l)	1410		Atomic Absorption
Ethanol (% v/v)	6,75	Q-38	MA-F-AS2-02
Tartaric acid (g/l)	4,2	Q-45	Color
L-Malic acid (g/l)	3	Q-45	Enzymatic

Table 5. Comparison of the number of escaped rootstocks and cultivars observed in 1995 and 2014.

	1995	2014
Rootstocks	35	43
Direct producer hybrids	23	31
Cultivars	13	10

grapevines in both years. The presence of both monophagous mites was detected in every wine grapevine population from the Iberian Peninsula to the South Caucasian region (Ocete *et al.*, 2011a; Ocete *et al.*, 2012).

Occasionally, the presence of the leafhopper *Empoasca vitis* Goethe (Homoptera, Cicadellidae) was observed. *Thrips angusticeps* Uzel (Thripidae, Thripinae) and their bite marks were found at shoot tips and on very young leaves. It is remarkable that this Thrips was not present during the first sampling. Its presence was detected in nearly 50 % of the vines in 2014. This species was also discovered only in a few wild grapevine populations from the nearby Basque Country (Ocete *et al.*, 2008).

5. Diseases

Symptoms of powdery mildew, *Erysiphe necator* (Schwein.) Burriel, were observed on leaves, branches, bunches of flowers of both sexes and also on bunches of berries in female individuals. On leaves "oil spots", together with other damages on shoots and bunches were caused by downy mildew, *Plasmopara viticola* (Berlese and de Toni). There was a minimal difference of infection in both sampling years. Powdery mildew affected nearly 93 % of the exemplars in both 1995 and 2014. At the same time, downy mildew affected about 78 % of wild grapevine lianas.

No virus infection was detected by ELISA tests.

In all cases it is necessary to emphasize that the level of infestation or infection of the different parasitic species varied from one wild grapevine to another within the same population, probably due to the genetic differences caused by the sexual reproduction by seeds.

No symptoms of infestation or infection attributable to Phylloxera (*Daktulosphaira vitifoliae* Fitch) (Homoptera, Phylloxeridae), root-knot nematodes or mycelium of root rot fungi were detected on roots, as it was reported previously in wild European grapevine populations from Portugal to Hungary (Ocete *et al.*, 2011a) and also in Transcaucasia (Ocete *et al.*, 2012). The absence of both pests might be prevented by edaphic conditions specific of fluvisols, in spite of some *Vitis* and *Muscadinia* species showing resistance to nematodes (Walker *et al.*, 1994). In spite of this, some nematode species, which were reported as possible virus vectors (longidorids), were detected in soil of some Austrian populations of wild grapevines along the Danube River. The most common species strictly linked to grapevines was *Longidorus elongatus* (Regner *et al.*, 2004).

It is necessary to mention that several roots belonging to *Populus nigra* and *Populus tremula*, which sometimes constitute the supporter for wild grapevine, often show clear symptoms of *Armillaria mellea* (Vahl.). Its mycelium can be observed on poplar roots and occasionally mushrooms of this species can be also found in autumn. Wild grapevines do not seem to be affected by this fungal disease. Thus, it would be necessary to carry out additional tests on the possible resistance or tolerance of wild grapevine roots against this important fungal disease.

6. Microvinification

Analytical data and procedures are compiled in Table 4.

The process did not develop any sanitary problem. No strange aromas were detected, and the wine obtained was strongly colored and had a nice fruity flavor. It had low concentration of ethanol, about 7 % (v/v) in spite of a potential alcohol level of 12.4 %. The taste of the wine was unbalanced, due to its low pH (values below the normal range between 3.2-3.8), high concentration of tartaric and malic acids and high index of polyphenols in the taste limit. Due to this, yeasts could not completely metabolize all the sugars in the must, resulting in a lower alcoholic wine than could be expected. Both previous characters had a synergistic effect worsening the wine taste. Additionally, an oxidation of the polyphenols led to a final production of acetaldehyde. Data on anthocyanin and absorbance, at the three wavelengths, and the intensity of color and concentration of polyphenols do not keep an adequate equilibrium. It is due to the fact that wild grapevine bears fruit to reproduce itself not to make good wine. Thus, the story of wine is the story of the taming of vine. Man intervened and diverts the vine from its original purpose, he had a fair bit of work on his hands (Clarke, 2002).

Both parameters, intensity of color and acidity, are the two most important characteristics of this microvinification, because they highlight the crescent importance of wild grapevine for breeding of red cultivars in areas under a temperate climatology. In these, the majority of the varieties show low values for both parameters. In consequence, the Syrah cultivar, for example, has been widely introduced to produce red wines in vineyards of Andalusia (Spain). According to the current process of global warming, wild grapevines constitute a remarkable phytogenetic resource in red cultivar breeding, which could increase the concentration of polyphenols and antioxidant compounds in a new generation of future wines.

The number and kind of the different feral grapevines found is shown in Table 5.

These vitaceae appeared from Santa Cruz de Campezo to San Adrián towns. Their presence is higher in the last stretch of the Ega River, between Zubielqui and the Ebro River.

In 2014, the percentage of rootstocks increased up to 34.8 % compared to 1995. Their ampelographic identification indicated that 17 individuals were phenotypically close to Rupestris of Lot (*Vitis rupestris*) and 9 were close to *Vitis riparia*. The percentage of producer hybrids (French Hybrids) grew up to 23.9 % in the same period. Cultivars diminished by 23.1 %, probably due to the presence of both kinds of mildews as well as the cleaning of river-bank forest. Among these cultivars, 6 were Garnacha tinta, 1 Garnacha blanca and 3 Calagraño (Jaén blanco).

The presence of feral vines from American origin is common in river-bank forests of other European rivers (Laguna, 2003 ; Arrigo and Arnold, 2007 ; Zecca *et al.*, 2010). Terpó (1962, 1988) gave the alarm in the case of the Danube. Within Spain, in the Valencia region, rootstocks are practically the only current vitaceae existing along the majority of gallery forests of rivers and creeks. A similar situation was found in several Spanish reserves of the Biosphera and Natural parks, such as Montseny (Catalonia), Urdaibai (Basque country) and Cazorla, Segura y Las Villas (Andalusia) and natural ecosystems from Portugal (Lara *et al.*, 2012).

Producer hybrids and rootstocks are particularly competitive and present a high risk of invasion. They are issued from interspecific breeding processes including several different North American *Vitis* species. They are selected to perfectly fit local soil and climatic conditions. They show high tolerances to downy and powdery mildew, and phylloxera (Laguna, 2003).

The presence of Garnacha, as the most frequent escaped *Vitis vinifera* cultivar found, is probably due to its relatively high level of tolerance to downy mildew. It was thus imported/introduced from the Aragon region to Navarra, La Rioja and Castilian and Leon vineyards in the end of the 19th century (Ocete *et al.*, 2006).

Due to pollen exchanges and seed dispersal by birds the cultivars and rootstocks were able to invade large territories and often hybridize and introgress with native wild grapevines. In the eighties, rootstocks were already suspected to hybridize with wild grapevines (Terpó, 1988). In addition, rootstocks are able to rapidly establish and spread. They are displacing autochthonous wild grapevine specimens from their natural habitats in Europe (Bodor *et al.*, 2010) including in different sites from the Iberian Peninsula (Lara *et al.*, 2013). Due to these facts and to the lack of suitable sexual reproduction conditions linked to floodings, wild populations decreased in size, leading to severe erosion of the gene-stock (Csepregi, 1992).

CONCLUSION

Our results highlight the importance of conserving, both *in situ* and *ex situ*, the few remaining exemplars of these relic populations, in particular as these Iberian wild parents have provided the A chlorotype to autochthonous grapevine cultivars (Arroyo-Garcia *et al.*, 2006).

An essential tool for improving the situation would be to develop and enforce a legal protection and its communication to all organizations involved in the conservation of natural ecosystems, as it is already the case in France, Austria, Germany and Hungary.

Acknowledgements : The authors would like to thank Mr. Ramón Vaca for his proofreading and enological evaluation and Mrs. Nicole Ortega for her help in the French version of the abstract.

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