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Presence of symptoms caused by parasitic species described in grapevine herbaria from Georgia, Spain and the United Kingdom.

Presencia de síntomas causados por especies parásitas en herbarios de vid de Georgia, España y Reino Unido.

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Abstract

The present paper focuses on the presence of symptoms caused by parasitic species in samples of Eurasian grapevine (*Vitis vinifera* L.) belonging to different herbaria from Spain, Georgia and the United Kingdom. In the Spanish case-herbaria were collected by Clemente at the beginning of the 19th century and preserved at the Royal Botanical Garden of Madrid. This material was used as the basis for the publication in 1807 of the famous book "*Ensayo sobre las variedades de la vid común que vegetan en Andalucía*" (*Essay on the varieties of common grapevine that vegetate in Andalusia*). The Georgian herbaria originated from three institutions: National Museum of Georgia, National Herbarium of Georgia and Batumi Botanical Garden. The time period of the Georgian herbaria dates from mid-19th century. The UK herbaria came from Kew Royal Botanical Gardens and contain samples dated as of 1843. According to observations, in the Spanish herbarium, 98 samples out of

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185 show erinea, symptoms caused by *Colomerus vitis* (Pagenstecher) (Acari, Eryophiidae), from a total of 185 samples there. Meanwhile in other herbaria its presence was detected in 3 samples, which also showed spots caused by powdery mildew, *Erysiphe necator* (Schweinitz). Symptoms caused by downy mildew, *Plasmopara viticola* Berl. & De Toni, were only detected in one sample collected in Iran. Results attest the frequent presence of this monophagous mite pest on Southern Spanish cultivars and wild exemplars for more than two centuries.

Palabras clave: Andalusian cultivars, *Colomerus vitis, Erysiphe necator, Plasmopara viticola, Vitis vinifera*.

Resumen

El presente trabajo se centra en la presencia de síntomas causados por especies parásitas en muestras de vid euroasiática (*Vitis vinifera* L.) pertenecientes a diferentes herbarios de España, Georgia y Reino Unido. En el caso del español fue recogido por Clemente a principios del siglo XIX y conservado en el Real Jardín Botánico de Madrid. Este material sirvió de base para publicar en 1807 el famoso libro "*Ensayo sobre las variedades de la vid común que vegetan en Andalucía*". Los herbarios georgianos pertenecen a tres instituciones: Museo Nacional de Georgia, Herbario Nacional de Georgia y Batumi Botanical Garden. Los herbarios georgianos comienzan a recogerse a mediados del siglo XIX. Los del Reino Unido provienen de Kew Royal Botanical Gardens y contienen las muestras desde 1843. Según las observaciones, en ese herbario español, 98 muestras de un total de 185 mostraron erineos, causados por *Colomerus vitis* (Pagenstecher) (Acari, Eryophiidae). En los otros herbarios se observó la presencia de únicamente 3 casos de infestación por el ácaro y oidio, *Erysiphe necator* (Schweinitz). La presencia de síntomas causados por mildiu, *Plasmopara viticola* Berl. & De Toni, únicamente se detectó en una muestra recolectada en Irán. Los resultados atestiguan la frecuente presencia de esta plaga de ácaros monófagos en cultivares del sur de España y ejemplares silvestres a lo largo de más de dos siglos.

Key words: *Colomerus vitis*, *Erysiphe necator*, *Plasmopara viticola*, variedades andaluzas, *Vitis vinifera*.

Laburpena

Lan honek Espainiako, Georgiako eta Erresuma Batuko hainbat herbariotako mahatsondo-lagin eurasiarretan (*Vitis vinifera* L.) espezie bizkarroiek eragindako sintomak biltzen ditu. Espainiakoari dagokionez, Clementek bildu zuen XIX. mendearen hasieran, eta Madrilgo Errege Lorategi Botanikoan izan zen gordeta. Material hori oinarritzat hartu zen 1807an "*Ensayo sobre las variedades de la vid común que vegetan en Andalucía*" liburu ospetsua argitaratzeko. Georgiako herbarioak hiru erakunderen esku daude: Georgiako Museo Nazionala, Georgiako Herbarium Nazionala eta *Batumi Botanical Garden*. Georgiako herbarioak XIX. mendearen erdialdean hasi ziren biltzen. Erresuma Batukoak Kew Royal Botanical Gardensetik datoz eta 1843tik aurrerako laginak dituzte. Behaketen arabera,

Espainiako herbario horretan, 185 laginetatik 98k erineoak erakutsi dituzte, *Colomerus vitis*-ek (Pagenstecher) (Acari, Eryophiidae) eragindakoak. Beste herbarioetan, akaroen eta oidioaren hiru kasu baino ez dira ikusi, *Eryisiphe necator* (Schweinitz) hain zuzen. Mildiuk eragindako sintomak, *Plasmopara viticola* Berl. & De Toni, Iranen bildutako lagin batean baino ez dira detektatu. Emaitzek erakusten dute akaro monofagoen izurrite hori maiz agertu izan dela Espainiako hegoaldeko labore lurretan eta mahatsondoetan bi mende baino gehiagoko epean.

Gako hitzak: *Colomerus vitis*, *Erysiphe necator*, *Plasmopara viticola*, barietate andaluziarrak, *Vitis vinifera*.

Introduction

Nowadays the grapevine constitutes the most important fruit species from an economical point of view (Vivier and Pretorius, 2002). Around the world, especially in the Mediterranean climatic areas (South Africa, South America in Chile and Argentina, California and Australia), vineyard are present and a lot of varieties are cultivated. In the area of origin, southwest Asia and the European Mediterranean countries, the grapevine is one of the most important farming.

The autochthonous Eurasian wild grapevine (*Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi) is dioecious and constitutes the parental of the present cultivars belonging to the taxon *Vitis vinifera* L. subsp. *vinifera*. These are usually hermaphrodite and have their origin in the scarce hermaphrodite wild vines which appeared from mutation in male exemplars, which were selected by man due to their high production of bunches, as a consequence of their self-pollination (Forni, 2006).

There are morphological differences between wild and cultivated seeds, so they can be distinguishable by proportions and bigger size of the beck (Terral *et al.*, 2010). Archaeological investigations carried out in Georgia (Southern Caucasus Region) indicate that this geographical area constitutes, according to present data, the first domestication focus and, in consequence, the cradle of winemaking and the initial point for ancestral viticulture. It is located in the hills of Shulaveri Gora and Gadachrili Gora, dated back around 8,000 BP. Also, ceramic receptacles from that period, decorated by grape bunches (Chilashvili, 2004) and containing traces of wine, have been discovered in this region (McGovern, 2003; McGovern *et al*., 2017).

On the other hand, the great genetic affinity between wild and cultivated grapevines in that country would confirm it as the origin of domestication (Riaz *et al.*, 2018), a fact reinforced by the high biodiversity of cultivars present in the Georgian gene pool, which accounts for between 525 to 607 original names (Ketskhoveli *et al.*, 1960; Ujmajuridze *et al*., 2018).

Multidisciplinary works endorse the idea that grape cultivation radiated from the Triangle of the Fertile Grapevine. It has a vertex in the Zagros mountains (Iran), another in the Taurus mountains (Turkey) and one on the coast of the Caspian Sea, very close to the Caucasus mountain range, and covering the territory of Transcaucasia located between the Black and Caspian seas (Ocete *et al*., 2021). This triangle contains the Ararat mountain situated in its central area, mentioned in the biblical Genesis, that narrates the Noah's epic with his consumption of wine included, after stranding the ark in that place.

In Georgian language the word ღვინო (/ǵi:nƏu/) means wine. It is the original 'term' for the fermented grape must and it is the origin of the word because its similar pronunciation for several languages derived from Latin and also in English and German ones, following the journey of grapes throughout the old World (Tsereteli, 1947). Spain constitutes at present the biggest vineyard in the world (OIV, 2019). On the other hand, there are some possible links between both territories: Spain was known as 'Iberia' by Greeks and also a kingdom of Eastern Georgia had the same name 'Iberia' in the period of c. 302 BC – 580 AD (Toumanoff, 1969). Both geographical areas were the limits of the territory known as Oikumene in the classic Greek period. The Basque country (Euskal Herria), divided between Spain (Hegoalde) and France (Iparralde), has its own language with some words which share similar roots with the Georgian one (Personal communication of Xabier Kintana, Euskaltzaindia).

Viticulture expanded towards nearby regions, like Mesopotamia and the Middle East (Vavilov, 1926). Later, it moved towards Western Mediterranean areas (Negrul, 1938; Forni, 2012), where Phoenicians constituted its main vector of propagation. Due to this fact, in Andalusia (Southern Spain), concretely in Huelva town, seeds of cultivated grapevine have been found, with an antiquity situated around 800 years BC (González de Canales *et al*., 2020). According to the list of Clemente y Rubio (1807a; 1807b), this region probably contained the highest diversity of cultivars in Spain before *Phylloxera* infestation, discovered in this southern Spanish region in 1878.

The author cited in the previous paragraph, Simón de Roxas Clemente y Rubio (1777-1827), constitutes the prototype of the Spanish man of the Enlightenment. He was a librarian and director of the Royal Botanical Garden in Madrid (Spain), where he installed the pergolas for the maintenance of all the cultivars collected by him. He was an Honorable Member of the Royal Economic Societies of Granada and Sanlúcar de Barrameda.

Clemente was entrusted with the study of grapevine varieties from vineyards and natural habitats situated in Andalusia (Southern Spain). Gradually, he published his ampelographic descriptions in the *Semanario de Agricultura y Artes dirigido á los Párrocos* (Weekly of Agriculture and Arts for Parish Priests). In 1807 he finished his work, entitled *Ensayo sobre las variedades de la vid común que vegetan en Andalucía* (*Essay on*

the varieties of common grapevine that vegetate in Andalusia), where he described 117 cultivars and 2 wild grapevines found in sandy soils close to the mouth of the Guadalquivir river. This book was translated later into the French and German languages.

The history of Georgian herbaria starts in the first part of the 19th century with the establishment of Tbilisi National Botanical Garden in 1845 (Khutsishvili, 2011) founded on the basis of the previous Royal Garden of the 17th century. This botanical garden contains unique collections of up to one million herbarium sheets. Among others there are 90 herbarium sheets of grapevine from Georgia, Azerbaijan and Russia (North Caucasus and Black Sea Regions) from 1890 and containing mainly accessions of Eurasian wild grapevine *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

The second herbarium from Georgia takes its history from the 'Caucasian Natural Museum' (now Georgian National Museum) from 1852 and became active under the leadership of Gustave Radde (1831-1903) in Tbilisi from 1867. The herbaria are the result of the expeditions in the Caucasus, organized by G. Radde (Zhordania, 1984) and other researchers after him. This museum holds 43 herbarium sheets of grapevine from Georgia, Armenia, Azerbaijan, Turkey and Russia (North Caucasus and Black Sea Regions) dated from 1867.

The third herbarium studied is available in Batumi Botanical Garden, established in 1881 close to the Black Sea region (BBG, 2021). The herbarium collection contains 46.051 sheets mostly from West Georgia. Among those 3 samples of regional wild grapevine dated from 1962 can be found.

The herbarium of the Royal Botanical Gardens, Kew was founded in 1853. There are currently over 7,000,000 specimens in the Herbarium, representing nearly 98% of all of the genera in the world. It has the largest collection of historical plant specimens (including types), and plant specimens found in all regions of the world (Kew, 2021). This herbarium holds 49 sheets of *V. vinifera* subsp. *sylvestris* (Gmelin) Hegi from Italy, Austria, Hungary, Balkans, Transylvania, Moldova, Ukraine, Russia, Armenia, Azerbaijan, Turkey, Turkmenistan, Iran, Iraq and Afghanistan.

Four new grapevine pathogens from America spread in Europe in the second part of the 19th century: powdery mildew *Erysiphe necator* (Schweinitz, 1834) in 1845; phylloxera [*Dactylosphaera vitifoliae* (Fitch, 1855)] in 1863; downy mildew [*Plasmopara viticola* (Berk & Curtis, 1888) Berl. & De Toni)] between 1878-80 and black rot [*Guignardia bidwellii*) (Ellis, 1861)] in 1885. As a result of this invasion, the vineyard area and wine production dramatically decreased in Europe in the 19th century. Soon afterwards, all these parasitic species were discovered in Georgia: powdery mildew in 1857, phylloxera in 1881 and downy mildew in 1880 (Ramishvili, 2001), but not black rot.

Recent discovery of resistance to powdery mildew in some Caucasian grapevine varieties like 'Shavtsitska' and 'Tskhevdianis Tetra' from Georgia and 'Kishmish Vatkana'

and 'Dznandzhal Kara' from Central Asia allow researchers to provide some hypotheses such as: "the resistance trait might have been inherited from a *V. vinifera* progenitor thousands of years ago and conserved in Caucasian cultivars until today" (Possamai *et al*., 2021) or downy and powdery mildew "were indeed present in Asia for longer than thought, possibly since the continental split separated it from America" (Vezzulli *et al*., 2022). That is why to check the presence of some symptoms of downy mildew in the herbaria collected in the Caucasus and Central Asia regions became intriguing. On the other hand, resistance to downy mildew has been recently discovered in the Georgian cultivar Mgaloblishvili (Sargolzaei *et al.*, 2021).

The presence of the phytophagous mite *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae), a monophagous species, has been detected on the vineyards of both hemispheres (Keifer *et al*., 1982). In spite of direct damage to the plants modern study has recognized this mite as a vector of Grapevine pinot gris virus (GPGV) and Grapevine inner necrosis virus (GINV) (Valenzano *et al.*, 2020). It also colonizes all the wild grapevine populations studied by Ocete *et al.* (2020) in Europe (from Portugal to Romania), Africa (Morocco) and Asia Minor (Southern Caucasus).

The aim of the present paper is the identification of the parasitic species of Eurasian grapevine cultivars and wild grapevines as well, and their distribution observed by their symptoms on samples belonging to grapevine herbaria from Georgia, Spain and UK. They were dated before and after the arrival of North American fungal diseases (powdery and downy mildews) in Eurasia in order to follow the historical distribution of these parasites in viticultural areas.

Materials and Methods

To study the historical presence of distribution of pathogens hosted by European grapevines, the data was gathered from 5 herbaria: I) collected by Clemente y Rubio (1807a, 1807b) in the case of Spain, it is conserved in the Royal Botanical Garden in Madrid; II) National Museum of Georgia, Tbilisi Botanical Garden and Batumi Botanical Garden of Georgia; III) Royal Botanical Gardens, Kew from UK.

Different numbers of herbarium sheets were studied. In all the cases, each sample (185 from Spain, 136 from Georgia and 49 from UK) of grapevine from the different herbaria was examined meticulously in order to detect possible symptoms caused by parasitic arthropods and aerial fungal species from North American origin: powdery and downy mildews.

The presence of the phytophagous mite *Colomerus vitis* (Pagenstecher, 1857) (Acari, Eryophiidae) and powdery mildew was evaluated based on the macroscopic observation of 1,222 wild grapevine samples from different geographical regions of Europe, Southern Caucasus and Northern Africa by Ocete *et al*. (2020) (Table 1, Fig. 1).

Continent	Country	Number of vines sampled
Europe	Portugal	131
	Spain	367
	France	142
	Italy	104
	Germany	79
	Switzerland	23
	Hungary	84
	Romania	39
	Greece	27
Southern Caucasus	Georgia	92
	Azerbaijan	67
	Armenia	21
Northern Africa	Morocco	46
	TOTAL	1,222

Table 1.- Number of actual Eurasian and African wild grapevines sampled in different countries.

Tabla 1.- Número de vides silvestres actuales muestreadas en diferentes países de Eurasia y África.

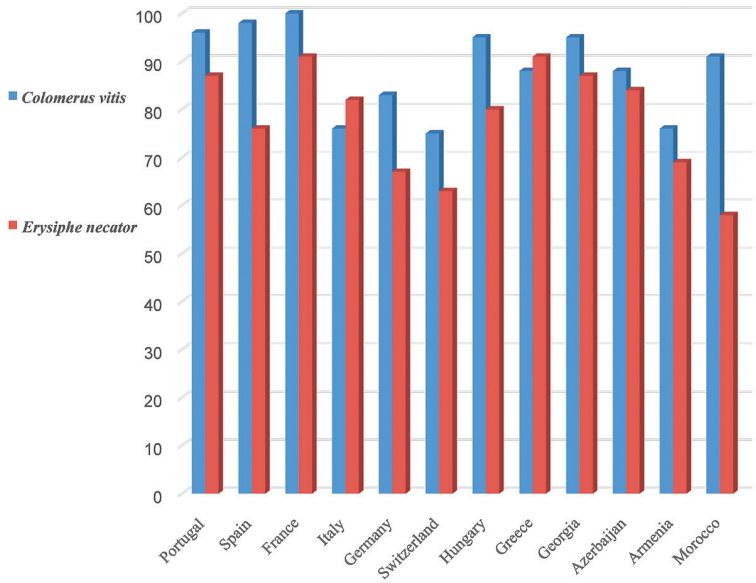


Fig 1.- Percentages of infestation / infection of erineum strain (*Colomerus vitis*) / powdery mildew (*Erysiphe necator*).

Fig 1.- Porcentajes de infestación / infección de erinosis (*Colomerus vitis*) / oidio (*Erysiphe necator*).

Type of materials No of samples	Names of cultivars and grape samples
Cultivars 86	Agracera (2 samples), Albillo castellano (nº 23), Albillo de Huelva, Albillo loco Veldiva, Albillo negro, Albillo pardo, Albillo pardo (nº 26), Albillos Castellano, Almuñecar. Beba, Beba Bequillete, Botón de gallo negro. Cabriel, Cepa comenta, Cordovi, Corovera. De Beguillet (nº 25), De Boutelou, De Columela (ferax). Fercoma. Galana (2 samples), Gallega (2 samples), Garabatona (2 samples). Heben. Jaén negro, Jaén negro de Sevilla, Jetubí loco. Lagrimosa, Langleya, Listán común, Listán ladrenado, Listán morado, Listan de paupérrima. Malvasía, Mantuo bravio (2 samples), Mantuo bravio (silvatica), Mantuo laeren (2 samples), Mantuo morado, Mantuo de Pilas (2 samples), Martinecia (2 samples), Melonera, Moscatel gordo blanco, Moscatel gordo morado, Moscatel menudo, Moscatel menudo morado (2 samples), Moscatelon, Mollar de Cádiz, Mollar negro (2 samples), Mollar negro (nº 20). Palomino bravío (2 samples), Palomino común, Pedro Ximénez, Pellucida (Cordovi), Pensiles fallax, Perrunos. Quintiniea. Rabo de Baca. Santa Paula, Santa Paula de Xerez. Terana, Torrontes (2 samples), Tempranillo (2 samples), Teta de vaca blanca. Valida, Verdaguilla, Virgiliana. Ximenez, Ximenez loco. Zedoti
Variations of *Vitis vinifera* 12	fragilis, ignobilis, mollis 2ª, pauperrima, prolifera, rotundifolia, rubella, subacida, subcompressa, sumosa, sulcata, var. (*no named*)

Table 2.- List of cultivars showing symptoms caused by the erineum strain (*Colomerus vitis*) in Clemente's herbarium (1807)

Tabla 2.- Lista de cultivares que muestran síntomas de erinosis (*Colomerus vitis*) en el herbario de Clemente (1807)

Results

Two main parasitic species were discovered during the observation of herbarium sheets of grapevine kept in Spain, Georgia and UK herbaria: the erineum strain of *Colomerus vitis* and powdery mildew, *Erysiphe necator*. Only one sample showed symptoms caused by downy midew, *Plasmopara viticola*. No symptoms caused by *Guignardia bidwellii* were found. They are very frequent on samples of different grapevine species of North America kept in the New York Botanical Garden. Data on the pest and both fungal diseases observed are indicated below.

Colomerus vitis (Pagenstecher, 1857) (Acari, Eryophiidae)

Table 2 shows those cultivars available with symptoms of the erineum strain identified on Clemente's Andalusian herbarium, mostly collected from vineyards belonging to Arcos de la Frontera, Bornos, Pago de Pajarete (Villamartín-Prado del Rey), Jerez de la Frontera, Sanlúcar de Barrameda, Trebujena and Lubrín (Fig. 2-3A).

Of the 185 sheets of *Vitis vinifera* cultivars and Eurasian wild grapevines, *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi observed from the herbarium of Clemente, 98 show symptoms of erineum strain in different degrees, which represents 53% of specimens infested by this pest. Consequently, at that time it must have been a very widespread

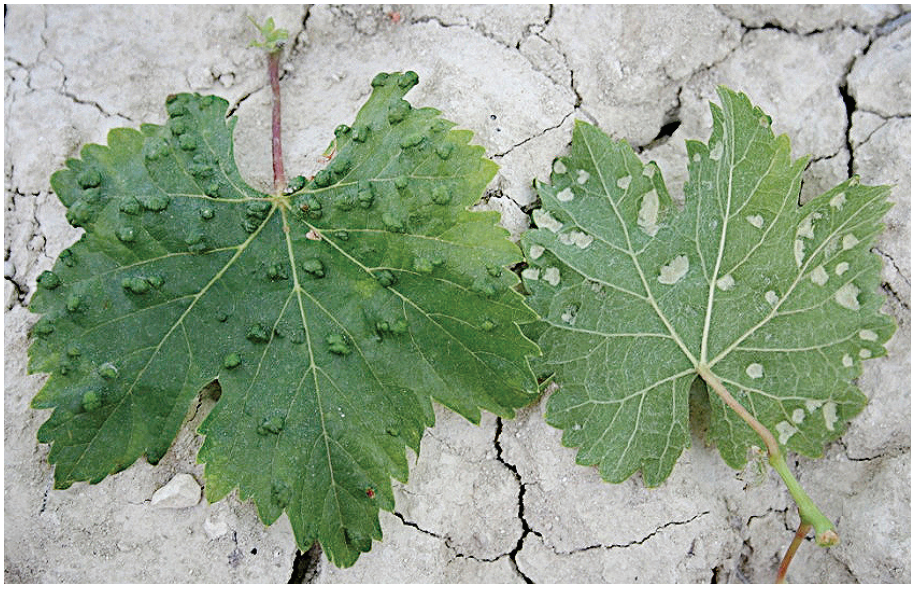
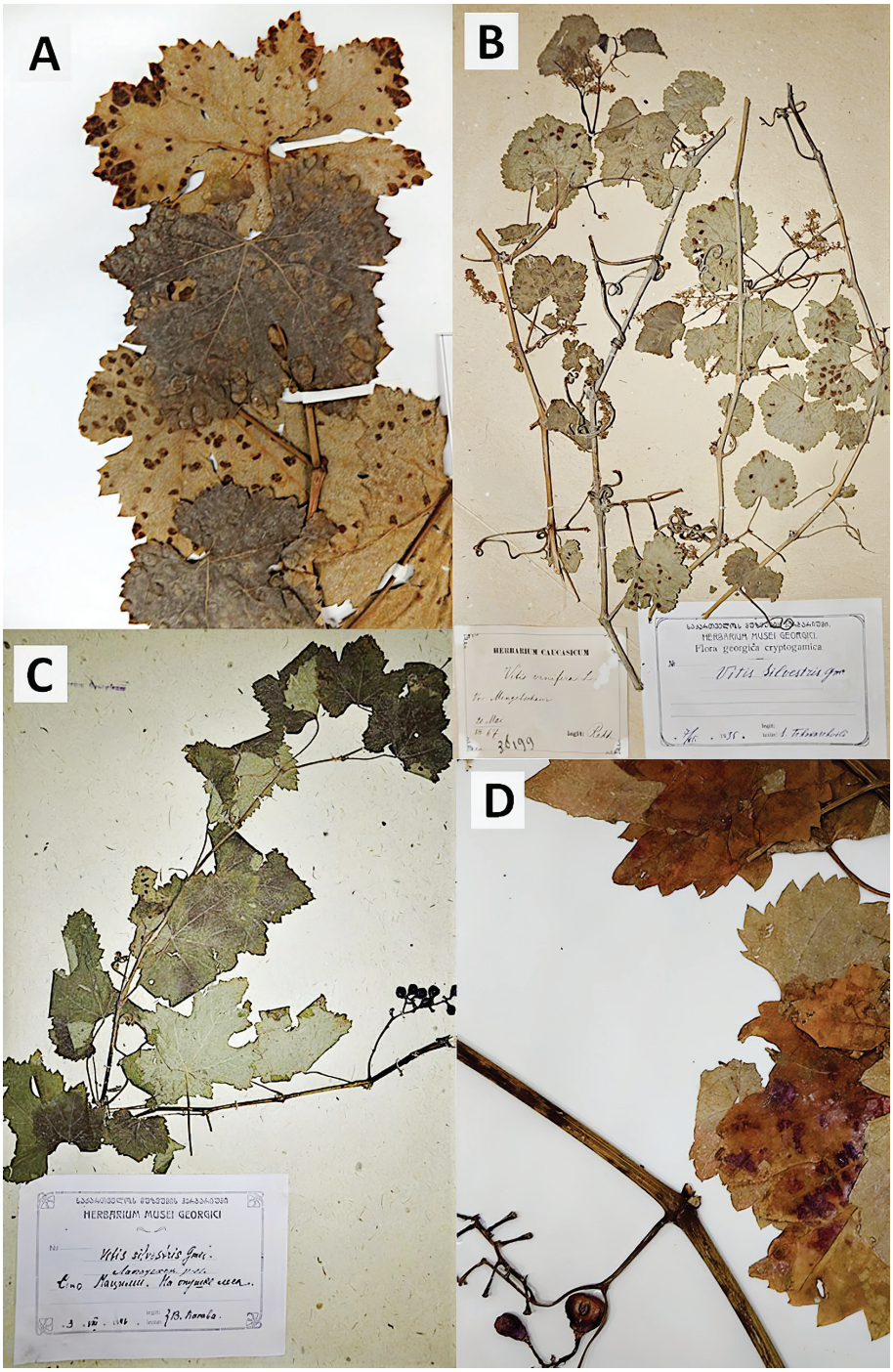


Fig. 2.- Actual symptoms of the erineum strain (*Colomerus vitis*) on fresh leaves from a cultivar (Jerez de la Frontera, Spain).

Fig 2.- Síntomas actuales de erinosis (*Colomerus vitis*) en hojas verdes de un cultivar (Jerez de la Frontera, España).


A
B
HERBARIUM CAUCASICUM
HERBARIUM MUSEI GEORGICI
Flora georgica cryptogamica
Vitis Silvestris Gmel
C
HERBARIUM MUSEI GEORGICI
D

◄ Fig. 3.- A) Symptoms of the erineum strain (*Colomerus vitis*) on Mantuo de Pilas cultivar (Clemente s herbarium); B) Symptoms caused by the erineum strain (*Colomerus vitis*) on an Azerbaijan wild grapevine, collected in the village of Mingechaur by Gustave Radde in 1867, from the herbarium belonging to Museum of Georgia; C) Presence of powdery mildew (*Erysiphe necator*) on a wild grapevine from a forest close to the village of Matsmi, Lagodekhi district of Georgia in 1946 (Museum of Georgia); D) Spots of downy mildew (*Plasmopara viticola*) on the top of the stems of an Iranian wild grapevine collected in 1965 (Kew herbarium).
Fig. 3.- A) Síntomas de erinosis (*Colomerus vitis*) en el cultivar Mantuo de Pilas (herbario de Clemente); B) Síntomas de erinosis (*Colomerus vitis*) en una vid silvestre de Azerbayan, recolectada en el pueblo de Mingechaur por Gustave Radde en 1867, del herbario del Museo de Georgia; C) Presencia de oidio (*Erysiphe necator*) en una vid silvestre de un bosque próximo al pueblo de Matsmi, Lagodekhi, distrito de Georgia en 1946 (Museo de Georgia); D) Manchas de mildiu (*Plasmopara viticola*) en el extremo del tallo de una vid silvestre de Irán recolectada en 1965 (herbario de Kew).

pest. In the Georgian herbaria only two accessions showed the presence of *Colomerus vitis* (a sample from. Mingechavir, Azerbaijan, collected in 1867 (Fig. 3B), and another one from Mukhrani, Eastern Georgia, collected in 1937). In the Kew herbarium only one accession collected in 1968 from Kopet-Dag, Turkmenistan, showed the presence of symptoms caused by this mite.

However, it became clear that the mite *Columerus vitis* habits in all vineyards from Portugal and Morocco to Azerbaijan (Southern Caucasus region) and to Turkmenistan (Central Asia).

Erysiphe necator (Schweinitz, 1834).

Symptoms caused by powdery mildew are very frequent in all non-treated cultivars and vineyards. From them, the spores have come to cause also infection on wild grapevine populations (Ocete *et al.*, 2007; 2012) (Table 3, Fig. 3C).

#	Herbarium name	Holding city & country	No of sheets	Years of range	Sheets with *Colomerus vitis*	Sheets with *Erysiphe necator*
1	Royal Botanical Garden	Madrid. Spain	185	1807	98	0
2	National Museum of Georgia	Tbilisi. Georgia	43	1867-1957	1	1
3	National Herbarium of Georgia	Tbilisi. Georgia	90	1890-2006	1	0
4	Batumi Botanical Garden	Batumi. Georgia	3	1962-2017	0	0
5	Royal Botanical Gardens Kew	UK	49	1843-2003	1	2
	Total		**370**	**1807-2017**	**101**	**3**

Table 3.- Number of herbarium sheets of *Vitis vinifera* L. with symptoms caused by erineum strain (*Colomerus vitis*) and powdery mildew (*Erysiphe necator*) from Georgian and British collections.

Tabla 3.- Número de muestras de herbario de *Vitis vinifera* L. con síntomas de erinosis (*Colomerus vitis*) y oidio (*Erysiphe necator*) de colecciones de Georgia y Gran Bretaña.

Plasmopara viticola (Berk & Curtis, 1888) Berl. & De Toni.

The first presence of this fungal disease, downy mildew, on wild grapevine populations was detected in Andalusia (Spain) (Ocete *et al.*, 2007). The presence of spots caused by this pathogen is detected mainly at the top of the stems. Some infection coming from nearby vineyards can also affect wild grapevines (Fig. 3D).

Discussion

The mite has three physiological races: race of the false galls (erineum strain); leaf curl strain and bud strain (Fig. 2, 3A, 3B). The latter causes the greatest economic damage, by preventing the development of buds and, consequently resulting in a lower production of grape bunches (Cordero *et al.*, 1991; Duso *et al.*, 2010).

This mite hibernates as imago state, under the previous year's wood bark and under the outer bracts of the buds. In spring, during budding, the adults begin to peck the new leaves on the underside causing the formation of false galls (erinea), due to the development of trichomes in the cells of the foliar epidermis, giving it a felt appearance and the subsequent deformation of the parenchyma (Ravaz, 1988; Ribéreau-Gayon and Peynaud, 1982). In these nests, females carry out the laying, developing up to seven generations (Arnaud and Arnaud, 1931). Generally, the damage caused by this strain is not usually significant, since it only causes a slight decrease in the photosynthetic capacity of the plant, even in areas with very humid weather (James *et al.*, 1995).

Parallel to the insignificant damage on grapevine caused by *Colomerus vitis* it should be accounted that grape growers take care against this pest by chemical treatment in vineyards. Contrarily, the wild grape populations are not so fortunate to have human protection. Due to this reason the populations of *Vitis vinifera* subsp. *sylvestris* living in natural areas can be an indicator for natural geographical distribution of this mite. On the other hand, data obtained in the present study demonstrated its presence from the beginning of the 19th century to the present day.

Modern survey of symptoms of powdery mildew on wild grapevine demonstrated that it is spread in the same geographical area like *Colomerus vitis*, from Portugal and Morocco to the South Caucasus region (Fig. 1), but the rate of distribution is lower in comparison to the mite s one: only 62% in Switzerland and a top of infection of 90% in France and Greece.

It is almost impossible to make comparisons of the infection situation between wild and cultivated grapevine, since grape growers are doing very intensive chemical treatments against powdery mildew using pesticides based mainly on sulfur. But it is well established that environmental climatic conditions play an important role in geographic and annual distribution of this pathology on vineyards (Maghradze *et al.,* 2015).

In reference to downy mildew, the first news of this fungal disease on wild grapevine populations appeared in Andalusia (Spain) (Ocete *et al.*, 2007). In vineyards, this pathogen is controlled by chemical treatments based on copper sulphate. According to the results of the present paper, its presence is lower than that of powdery mildew. In fact, only some symptoms have been found on a unique exemplar from Iran (Kew herbarium).

Conclusions

Observation of grapevine parasites like the mite *Colomerus vitis* and powdery mildew *Erysiphe necator* on the different sheet samples can be done successfully in spite of the fact that these herbariums were not focused on these organisms, but were collected for general botanical purposes. This method of observation can also be useful for the study of other grapevine diseases and pests. The limitation of this study is clear, but its contributions are precise and important to understand the fact that North American mildews colonized wild grapevine from affected cultivars in nearby vineyards. Both pathogens probably had reduced the size of the wild populations, eliminating the most sensitive specimens. These sanitary problems constitute another channel of human impact, together with the planting of orchards, groves, the cleaning of riparian vegetation and public works. So, it could be necessary to take protective measures in order to conserve this threatened phytogenetic resource, like in France, Germany, Austria, Hungary and the autonomous community of La Rioja (Spain).

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