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Erinea in the 'Ansonica' grapevine cultivar: trichome complement, histological effects and analysis of chlorophyll fluorescence in affected leaves

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Summary

Grapevine leaves are usually characterized by trichomes, specialized epidermal cells. They are interesting in ampelography and also important for the plant ecological responses in biotic and abiotic interactions. In nature, the trichome development is a genetic trait but it can be modified by pests as eriophyid mites. *Colomerus vitis* is quite common and its economic value is sometime substantial. Here, we studied the leaf erineum induced by *C. vitis* on 'Ansonica' ('Inzolia'), an important grapevine cultivar characterized by a low level of leaf trichome coating. To date, the interaction between *C. vitis* and grape has been investigated in few pedo-climatic conditions and no data are reported in 'Ansonica'. Therefore, our objectives were: (1) the analysis, in a Tuscan environment, of the morphology and histology of trichomes in 'Ansonica' leaves unaffected or affected by *C. vitis*; (2) evaluation, in mature leaves, of the effects of the mite both on pigment content and chlorophyll *a* fluorescence parameters. 'Ansonica' was devoid of glandular trichomes but it has been established the presence of few simple trichomes strictly associated with the veins. In the erineal sectors, a dense proliferation of simple trichomes in the abaxial epidermis and the development of hyperplasia in the adaxial surface were observed. Moreover, the leaf sections in the erineal regions were thicker due to an abnormal development of the lacunar parenchyma, and trichome proliferation was also extended to interveinal regions. Leaves with erinea showed a deficient content of carotenoids, in comparison to unaffected leaves. In 'Ansonica' leaves, *C. vitis* induced a decrease in the steady-state operational efficiency of photosystem II associated to a reduction in photochemical quenching and an increase in non-photochemical quenching values. In leaves with erinea, the reduction of photosystem II efficiency was extended to foliar areas not directly affected by galls. The collected results highlight that 'Ansonica' is susceptible to attacks by *C. vitis* and in the case of widespread leaf attacks the productive damage should not be underestimated.

K e y w o r d s : *Vitis vinifera*; *Colomerus vitis*; chloroplast pigments; leaf galls; photosystem II.

Introduction

The epidermis is the superficial layer of a plant in direct contact with the atmosphere. It includes epidermal cells and specialized cells such as stomata and trichomes or hairs (Fambrini and Pugliesi 2013 and 2019, Landi *et al.* 2017). The developmental control of the trichomatous complement in leaves highlights a regulatory network based on four fundamental elements: (i) genes that activate and/or modify the normal cell cycle of epidermal pavement cells; (ii) transcription factors that create an activator/repressor complex with a central role in determining cell fate, initiation and differentiation of an epidermal cell in a trichome; (iii) phytohormones that act at different levels; (iv) epigenetic mechanisms with a specific role in trichome development (reviewed in Fambrini and Pugliesi 2019). Trichomes play well-recognized roles in defence against insect herbivores forming a physical barrier that obstructs insect movement and mediating chemical defences (Riddick and Simmons 2014, Schmidt 2014, Hua *et al.* 2020). Trichomes act also as a mechanosensory switch transducing mechanical stimuli (e.g. insect movement) into physiological signals, thereby helping the plant to respond to insect attacks (Zhou *et al.* 2017). Hairs can also modulate plant responses to abiotic stresses as water loss, excess of temperature and light reflecting, for example, UV radiation (Karabourniotis *et al.* 1999).

The formation of trichomes begins at the base of the young leaf, a phase in which all the cells are potentially capable of developing trichomes, and the cells that generate the hair are arranged at regular intervals apart from each other (Pesch and Hülskamp 2009). Normally, no clusters of trichomes have ever been observed on the foliar epidermis: this implies the existence of a mechanism capable of regulating the spatial arrangement of the hairs. A similar phenomenon is observed in the development of stomata (Fambrini and Pugliesi 2013, Landi *et al.* 2017).

A recent study analysed the anatomical structure, ultrastructure and ontogeny of trichomes in the genus *Vitis* (Ma *et al.* 2016). The presence of glandular trichomes is not documented in grapevine, *Vitis vinifera* L., contrary to ribbon and simple trichomes (Ma *et al.* 2016). The ribbon type is prostrate and elongated, the simple type is erect and conically shaped (Ma *et al.* 2016, Gago *et al.* 2016). Their

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distribution regards predominantly petiole and leaf veins (Ma *et al.* 2016). The anatomical aspects and fluorescent properties of grape leaf trichomes have been investigated to improve ampelographic discrimination of some grapevine cultivars (Gago *et al.* 2016).

Experiments carried out on grapevine cultivars characterized by different amounts of leaf pubescence clarified that a dense covering of trichomes has a significant effect on the attenuation of the brightness of visible wavebands intercepted by the lamina. Furthermore, trichomes constitute an effective filter against UV-A radiations as well as against UV-B radiations. Indeed, leaf trichomes can modify the distribution of light radiation within the mesophyll parenchyma, a phenomenon linked to absorption, dispersion and reflection events (Karabourniotis *et al.* 1999, 2020).

Trichome types, distribution and density on *V. vinifera* leaves are a genotype-dependent trait (Boso *et al.* 2010; Gago *et al.* 2016) but abnormal trichome proliferations can be induced by the mite *Colomerus vitis* (Pagenstecher) (Eriophyoidea: Eriophyidae), which is very common in vineyards. In general, eriophyoid mites are tiny (on average 0.1-0.3 mm long), worm-like and with only four legs (Lindquist 1996). The mouthparts (chelicerae, pedipalps and labrum) are stylet-like (Alberti and Nuzzaci 1996), and their piercing and salivary injection cause suberification of epidermal cell walls and can stimulate plant deformations (Petanović and Kielkiewicz 2010). On the basis of the induced symptoms, three strains of *C. vitis* are distinguished: one attacks the buds, one causes leaf erinea (the most worldwide, called grape erineum mite, GEM) and another causes foliar rolling (Duso and de Lillo 1996). Usually, erinea consist of hypertrichosis on the abaxial surface of the leaves, which correspond to blisters on the adaxial surface delimited by ribs. Erinea can affect more or less extended portions of the leaf lamina. These hypertrophic hairs offer protection and food source for the mites. The grapevine cultivars with greater leaf tomentosity are the most subjected to the attack of GEM (Javadi Khederi *et al*. 2014). Meristematic tissues and immature leaves are usually preferential sites for *C. vitis* in grapevine. In particular, female overwinter under outer scales of buds and the colonization of new leaves occurs after the buds open. Here, mites induce the first erinea and soon reproduce (Duso and de Lillo 1996).

The damage caused by GEM is not always relevant (Duso and de Lillo 1996) but, in some instances, it is not negligible due to the negative consequences for plant development and productivity (Bernard *et al.* 2005, Javadi Khederi *et al*. 2014, 2018a, 2018b, de Lillo *et al.* 2018). Furthermore, this mite has been confirmed to be a virus vector (Malagnini *et al*. 2016, de Lillo *et al.* 2018, Morán *et al.* 2018). Recent investigations on GEM regarded the identification of specific host genes involved in the defence strategy in grapevine leaves (Javadi Khederi *et al.* 2018c), the analysis of parameters to characterize the biochemical base of grapevine susceptibility (Javadi Khederi *et al.* 2018d), the morphological, genetic and biological characterization of spring-summer and winter morphs of the mite (Valenzano *et al.* 2019, 2020).

Here, we studied the leaf erineum by *C. vitis* in 'Ansonica' ('Inzolia') for two main reasons: this cultivar is important for enology of Sicily and Tuscany islands (Giglio and Elba) and also, because this cultivar could be a representative model for plants genetically predisposed to develop a reduced density of trichomes on the leaves. In particular, our goals were: (1) to perform the morphological and histological analyses of trichomes in this grapevine unaffected or affected by *C. vitis*; (2) to evaluate the effect of the mite both on the content of pigments and chlorophyll fluorescence in mature leaves. The results obtained in a Tuscan vineyard, showed the alteration of trichome complement in mature leaves of 'Ansonica' affected by *C. vitis* attack and the negative effects on physiological parameters.

Material and Methods

Plant material: Leaf samples were collected from five randomly selected plants of 'Ansonica' in a vineyard at Campo Sperimentale in Colignola (Pisa; 43°43′32.02"N 10°27′37.66″E) of Department of Agriculture, Food & Environment (University of Pisa). The sampling was done directly in vineyards. Mature leaves (40 d old) affected by *C. vitis* or unaffected were isolated at the same time, from node 10-12 in plants with phenological state 73-75 based on the phenological card BBCH (http://cma.entecra.it/iphen/doc/vite/Scala_BBCH_vite_IPHEN.pdf). For this cultivar, general information, microsatellite profile, ampelography, ampelometry, trueness to type and accessions can be found at https://vitisdb.it/varieties/ampelography/8229 (D'Onofrio and Scalabrelli 2014, Scalabrelli *et al.* 2015).

Environmental Scanning Electron Microscope (ESEM) analysis: Samples of 'Ansonica' leaves at immature and mature stage of development were observed with an environmental scanning electron microscope (FEI QuantaTM 200, Hillsboro, OR, USA). The analysis was carried out in low-vacuum conditions by mounting small portions of freshly collected leaves on the support without the aid of any fixative, and a series of digital images through the control software of the instrument were imminently acquired.

Histological analysis and tissue clearing: Leaf samples collected from mature leaves in the central region of the lamina, were fixed for 24 h in formaldehyde/glacial acetic acid/ethanol/distilled water (10:5:50:35 v/v) at room temperature before being transferred into 70 % (v/v) ethanol (Fambrini *et al.* 2020). Water was removed by graded ethanol series while the dehydrated material was cleared in xylene with 5 steps according to Ruzin (1999). Paraffin-embedded tissues were sectioned using a manual rotary microtome (Reichert, Vienna, Austria). The serial transverse sections obtained (8 µm thick) were stained with a solution containing alcian blue 8GX, bismarck brown Y and safranin O according to Graham and Trentham (1998). Sections were observed with a light microscope (DMRB, Leica, Wetzlar, Germany) and a selection of digital images was recorded with a camera unit (PowerShot A590 IS, Canon, Tokyo, Japan). Photo editing of selected digital images was performed with respect to the brightness (Photoshop Elements, Adobe, San Jose, CA, USA). To further investigate the trichome morphology,

samples (1-2 cm of length) were detached in the central region of mature leaves affected or unaffected by *C. vitis* and fixed in FAA solution [5 % (v/v) acetic acid, 45 % (v/v) ethanol, 5 % (v/v) formaldehyde and 45 % distilled water] under a vacuum. After 24 h, the fixed samples were cleared by soaking in 5 % sodium hydroxide at 60 °C overnight, and placing in a saturated chloral hydrate solution until they became transparent. Hand-made sections of this clarified material were mount in glycerol and then analysed with binocular microscopy (Wild Makroskop M420, Heerbrugg, Switzerland). Finally, digital images were recorded with a camera unit (PowerShot A590 IS, Canon, Tokyo, Japan). Thickness analyses of leaf lamina and palisade parenchyma were made on cross sections using an eyepiece micrometer.

Pigment analysis: Pigments were extracted by tissue homogenization in 100 % acetone as previously described (Fambrini *et al.* 2004). In particular, mature leaves unaffected or affected by erineum were both used. Spectrophotometric analysis was performed using an UV-VIS Scanning Spectrophotometer (UV-2101PC, Shimadzu Italia, Milan, Italy). The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a* + Chl *b*) and total carotenoids (Car) were calculated according to the following equations (Lichtenthaler 1987):

$$\text{Chl } a = (11.24 \times A_{661.6}) - (2.04 \times A_{644.8}) \quad (1)$$

$$\text{Chl } b = (20.13 \times A_{644.8}) - (4.19 \times A_{661.6}) \quad (2)$$

$$\text{Chl } a + \text{Chl } b = (7.05 \times A_{661.6}) + (18.09 \times A_{644.8}) \quad (3)$$

$$\text{Car} = (1000\ A_{470} - 1.90\ \text{Chl } a - 63.14\ \text{Chl } b)/214 \quad (4)$$

where A is the absorbance measurement determined at 661.6, 644.8 and 470 nm. Five extracts were made for the grape cultivar from independent plants.

Chlorophyll *a* fluorescence parameters: 'Ansonica' leaves affected by the mite *C. vitis* (with erinea) and unaffected (control) were used for chlorophyll *a* fluorescence analysis. These two types of leaves were of comparable developmental stage. The imaging technique was performed by a chlorophyll fluorescence imaging system (IMAGING-PAM, Walz, Effeltrich, Germany). Details of the capture of chlorophyll fluorescence parameters are reported in Guidi *et al.* (2007). The current fluorescence yield (F_t) was continuously measured and the F_0 images were recorded in a quasi-dark state. The maximum fluorescence yield F_m was determined with a saturating pulse of 8,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (photosynthetic photon flux density, PPFD) for 1-2 s. The images of F_0 and F_m were subtracted and divided $[(F_m-F_0)/F_m]$ to generate the image of the maximum quantum efficiency of PSII photochemistry F_v/F_m. The F_t and the maximum light adapted fluorescence (F_m') were determined in the presence of an actinic illumination of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, then Φ_{PSII} was computed as the quotient $(F_m' - F_t)/F_m'$ (Genty *et al.* 1989). Non-photochemical quenching $[q_{NP} = (F_m-F_m')/(F_m-F_0')]$ was calculated according to Schreiber *et al.* (1995). Correct F_0' determinations require the application of a far red light, which would disturb the fluorescence imaging. Therefore, instead of measuring, F_0' was estimated using the approximation of Oxborough and Baker (1997) $[F_0' = F_0/(F_v/F_m + F_0/F_m)$. Images of the fluorescence parameters were displayed by means of a false colour code ranging from 0.00 (black) to 1.00 (purple). Single leaf in triplicate were used for the analyses. In every leaf analysed, an internal square portion (ISQ) of the lamina was chosen and, inside it, the value of each single parameter was measured in different points (five per leaves) and an average value was calculated. The area, the position, and the number of points were the same in all analysed leaves.

Statistical analysis: Thickness measurements were done in three slides with ten cross sections each, from five 'Ansonica' leaves affected or unaffected by erinea ($n = 5$). The means (± SD) were analysed by Student's *t*-test ($p \leq 0.05$). For leaf pigments, the values reported are the mean (± SD) of three internal round regions (12 mm diameter) of leaves picked randomly on each of the five biological replicas ($n = 5$). Differences between means were analysed by Student's *t*-test ($p \leq 0.05$). For chlorophyll fluorescence parameters, the values reported are the mean (± SD) of five internal spots randomly placed on each of the three biological replicates ($n = 3$). In affected plants, the parameters were calculated in both affected and not affected areas of the leaf lamina. Following evaluation of homogeneity of variance by Bartlett's test ($p \leq 0.05$), the mean values were compared with one-way ANOVA and the means separated by Tukey test as post-hoc for $p \leq 0.05$ comparing control leaves and both healthy and affected areas.

Results and Discussion

As recently pointed out, *C. vitis* is one of the less studied mites compared with few more economically impacting eriophyoids (de Lillo *et al.* 2018). Here we analysed some interactions between *C. vitis* and 'Ansonica' plants comparing the territory of the felty galls (erinea) on the lower leaf surface and blister-like swellings on the upper one, with ungalled territories of the leaves.

The ESEM analysis of abaxial epidermis of young (Fig. 1A) and mature (Fig. 1B-C) leaves of 'Ansonica' showed the absence of glandular trichomes and the presence of a reduced number of simple and erect trichomes. Length and size of simple trichomes were influenced by the leaf development stage, and their differentiation was rigorously associated with the veins (Fig. 1A-C). In addition to simple and erect trichomes, also ribbon and prostrate trichomes were identified (Fig. 1A). This type of trichomes was found to be less abundant than the erect trichomes and mostly localized on the main rib of the foliar lamina (data not shown). These results were in accordance with the 'Ansonica' ampelography card (D'Onofrio and Scalabrelli 2014, Scalabrelli *et al.* 2015). The histological analysis (Fig. 1D-F) confirmed that the trichome differentiation is associated with main and minor veins and, in addition, the cross sections demonstrated that erect trichomes were formed by a single column of cells. In general, the histological traits of trichomes observed in 'Ansonica' appeared similar to the results collected in a set of grape cultivars analysed by Gago *et al.* (2016).

As shown in Fig. 2A-D, 'Ansonica' leaves are susceptible to *C. vitis* attacks. In the erinea, the proliferation of masses of simple trichomes characterized by hyperplasia was observed (Fig. 2B, C and D). These erinea were similar to those documented by other authors following attacks of eriophyoid mites (Royalty and Perring 1996). When the

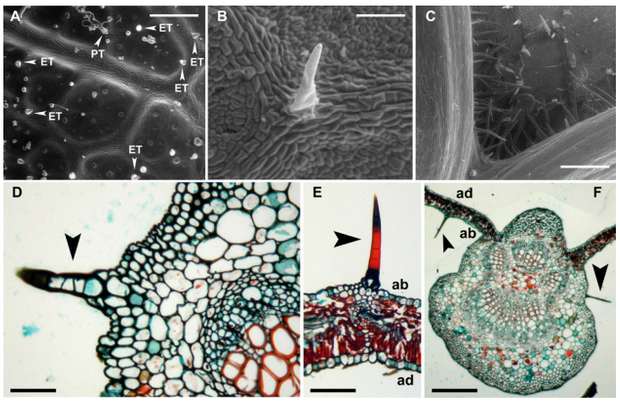

Fig. 1: Trichome analysis in 'Ansonica' leaves. (**A-C**) Environmental scanning electron microscope (ESEM) micrographs of leaves at different stages of development; (**A**) epidermis of young leaf at the abaxial side: differentiation of erect (ET) and prostrate (PT) trichomes in leaf veins; (**B**) simple trichome in a later stage of development on the abaxial side of a mature leaf; (**C**) intersection of primary vascular bundles with proliferation of simple and erect trichomes on the abaxial side, at the proximal end respect to petiole. (**D-F**) Histological analysis of 'Ansonica' leaves at mature stage, through cross sections. (**D**) Close up of a main leaf vein with a simple trichome showing a multicellular origin (black arrowhead); (**E**) a portion of a leaf blade with an analogous trichome, in correspondence to a minor vein; (**F**) cross section of leaf blade including the main midrib. Note the differentiation of two isolated simple trichomes (black arrowheads). ad = adaxial; ab = abaxial. Scale bars: A = 300 µm; B = 100 µm; C = 500 µm; D = 80 µm; E = 104 µm; F = 561 µm.

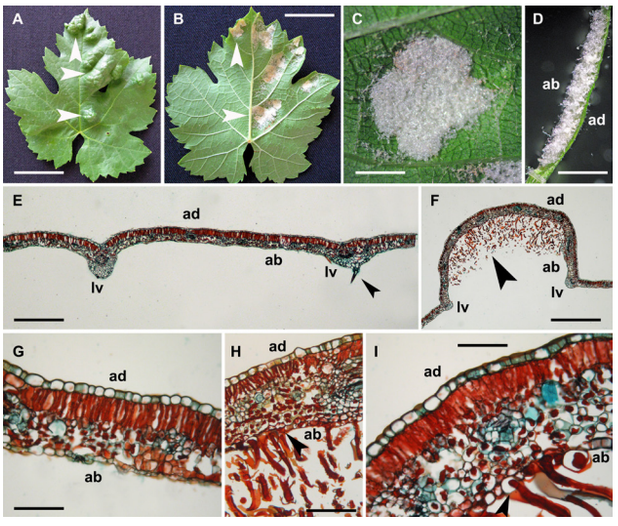

Fig. 2: Erinea in 'Ansonica' leaves attacked by *Colomerus vitis* (Pagenstecher). (**A**) Adaxial (ad) leaf surface with evident blister-like galls (white arrowheads). (**B**) Abaxial (ab) leaf surface with development of patchy erinea regions (white arrowheads) in perfect correspondence with the galls of the upper surface. (**C**) Close up of an abaxial erineum area. (**D**) Hand-made leaf section to show transversal view of an erineum. Note hypertrophic trichomes leaned to form a dense felt. (**E**) Cross section of a not affected leaf region. The black arrowhead indicates an isolated and erect trichome associated to the leaf vein (lv). (**F**) Cross section of a erineum region to show the abnormal proliferation of trichomes on the abaxial leaf surface (black arrowhead) and the greater and irregular thickness of leaf lamina in the erineum. (**G**) Cross section of unaffected leaf. (**H-I**) Close up of cross sections of erinea to show an ectopic initiation of hypertrophic trichomes in epidermal sectors (black arrowhead in Fig. 1**H**) and also the presence of some cells of the abaxial epidermis with increased volume (black arrowhead in Fig. 1**I**). Scale bars: A = 51.43 µm; B = 48 µm; C = 3.6 µm; D = 2.42 µm; E = 598 µm; F = 1496 µm; G = 111 µm; H = 166 µm; I = 83 µm.

leaf galls were studied at histological level, a change of the leaf structure in cross sections was evidenced (Fig. 2F), if compared to unaffected leaf areas (Fig. 2E). The leaf section of an erineum was thicker than the control and this trait was related to an abnormal development of the lacunar parenchyma because the palisade parenchyma remains monostratified as in the control (Fig. 2E, F; Table). At histological level, similar phenomena were observed by many authors after eriophyoid mites attacks, as reviewed by Petanović and Kielkiewicz (2010). Furthermore, the histological analysis showed an epidermal origin of the hypertrophic simple trichomes (Fig. 2H, I) suggesting an ectopic proliferation when compared to the control where the trichomes originated only from veins (Fig. 1E, F). We identified also some cells of the abaxial epidermis with increased volume (Fig. 2I). Trichome characteristics in 'Ansonica' leaves and in erinea regions were analysed also using hand-sections of cleared tissues (Fig. 3). The results confirmed the presence of isolated simple trichomes with multicellular nature in unaffected leaves (Fig. 3A). By contrast, we observed in erinea a dense proliferation of trichomes which were very elongated, variously folded, with a hypertrophic appearance and lacking a multicellular nature (Fig. 3B-D).

Several data demonstrated the alteration of chloroplast pigment content and photosynthetic parameters in leaves affected by galls development due to proliferation of insects (Jiang *et al.* 2018, Oliveira *et al.* 2016) or eriophyoid mites (Larson 1998, Gailite *et al.* 2005, Patankar *et al.* 2011, de Lillo *et al.* 2018, Jiang *et al.* 2018 and 2020).

Table

Histological analysis in mature leaves of 'Ansonica' affected by erinea due to *Colomerus vitis* (Pagenstecher). Cross sections (8 µm) of paraffin-embedded explants were used. Thickness measurements were done in three slides with 10 cross sections each, from five 'Ansonica' leaves affected or unaffected by erinea. Means (± SD) were analyzed by Student's *t*-test. ns = not significant; ** significant for $p \leq 0.01$

Character	Asymptomatic region	Erineum region
Thickness of the leaf lamina (mm)	0.1572 ± 0.0127	0.2364 ± 0.0114 **
Thickness of the palisade parenchyma (mm)	0.0452 ± 0.0025	0.0442 ± 0.0048 ns
Palisade parenchyma/leaf lamina ratio	0.2907 ± 0.0185	0.1907 ± 0.0223 **

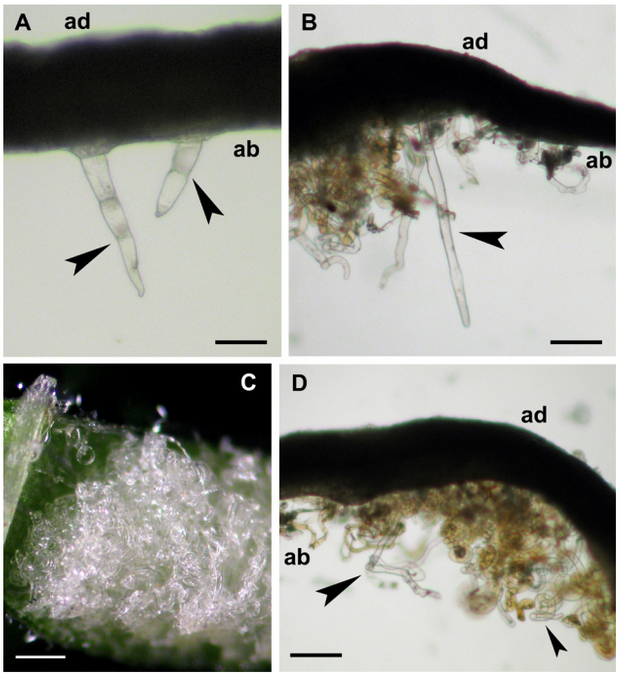

Fig. 3: Erinea in 'Ansonica' leaves after tissue clearing. (**A**) Hand-made section of a mature leaf with simple erect trichomes showing multicellular origin at abaxial side (black arrowheads). (**B**) Hand-made section of mature leaf with an erineum region. Note an elongated and hypertrophic trichome (black arrowhead). (**C**) Close up of an abaxial erineum area with a higher magnification than in Fig. 2C. Note the intricate skein of elongated and twisted trichomes that reflect light. This portion of lamina was not clarified (**D**) Hand-made leaf section of an erineum region with twisted and hypertrophic trichomes (black arrowheads). ad = adaxial; ab = abaxial. Scale bars: A = 0.072 mm; B = 0.144 mm; C = 2.5 mm; D = 0.164 mm.

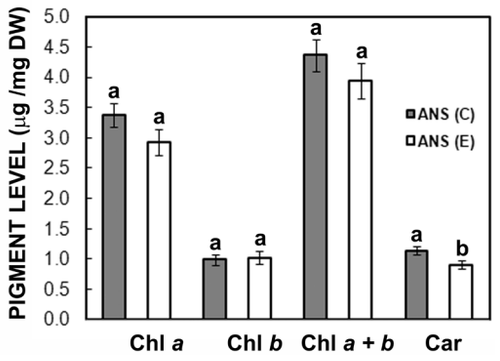

Fig. 4: Photosynthetic pigments content (µg/mg DW) in 'Ansonica' leaves unaffected (C) or affected (E) by erinea. Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl *a* + *b*, total chlorophyll; Car, total carotenoids. Statistical analysis was conducted separately for each pigment by Student's *t*-test ($p \leq 0.05$). Different letters indicate significant difference between mean (± SD) values ($n = 5$).

In particular, with specific regard to the physiological consequences of erinea in grapevine, previous data have been reported by some authors. In a study conducted in Iran on nineteen grapevine cultivars grown under greenhouse conditions, the induced infestation of *C. vitis* clarified that the depigmentation level was influenced by the intensity of the infestation (Javadi Khederi *et al.* 2018b). In fact, the most significant reduction in chlorophyll content was found in concomitance with the highest infestation rate in all the five cultivars tested (Javadi Khederi *et al.* 2018b). The interaction between the grape cultivar 'Muscat' and *C. vitis* in Swiss vineyards (*in vivo* conditions, during different years) was investigated by Linder *et al.* (2009) through the analysis of photosynthesis, stomatal conductance and chlorophyll content and these authors concluded that the negative effects for this grape cultivar were negligible. In this context, our investigations unveil that 'Ansonica' leaves with erinea showed a deficient level of carotenoids respect to unaffected leaves (Fig. 4). Modification of chloroplast pigment content in leaves of the plant host after mite attack were previously observed (e.g. Javadi Khederi *et al.* 2018b, Banerjee *et al.* 2019); however, it is not obvious how to explain the decrease of total content of carotenoids contrary to chlorophylls observed in the galled leaves. Further investigations will be necessary to describe the carotenoid profile in both types of leaves. The analysis of chlorophyll *a* fluorescence parameters demonstrated that *C. vitis* induced a reduction in the operational efficiency of photosystem II (PSII) at steady-state (ϕ_{PSII}) in 'Ansonica' galled leaves, accompanied by a reduction in photochemical quenching (qP) and an increase in non-photochemical quenching (NPQ) values (Fig. 5). By contrast, there were no differences in the potential photochemical efficiency of PSII (F_v/F_m; Fig. 5). Notably, in leaves affected by *C. vitis* the reduction of the aforementioned parameters was extended to foliar areas not directly affected by hyperplasia processes. In fact, healthy and affected areas in infected leaves had similar values of ϕ_{PSII}, qP and NPQ which were significantly different from leaves belonging to control plants (Fig. 5). These results showed similarities to what has been ascertained in other plant-eriophyoid interactions (Larson, 1998, Patankar *et al.* 2011) but to the best of our knowledge, these phenomena are yet to be demonstrated in grapevine plants affected by *C. vitis*. In conclusion, the pest caused a widespread damage to the photosynthetic apparatus by decreasing the amount of energy directly tunnelled in photochemistry (reduced values of qP) and thereby increasing the need to dissipate excess energy by non-photochemical quenching mechanisms (higher NPQ values).

In the adaxial side of lamina, the role that foliar trichomes may have on preserving the efficiency of PSII has been studied (e.g. Savé *et al.* 2000, Morales *et al.* 2002). However, the observed analysis of chlorophyll fluorescence in 'Ansonica' affected by erineum seems not to be specifically influenced by the proliferation of hypertrophic trichomes, given that they are located in the abaxial surface, thereby resulting ineffective in interacting to the light burden over the photosynthetic apparatus of mesophyll cells. Therefore, it is conceivable that eriophyids negatively affect the efficiency of PSII and the dissipation of absorbed light through alternative mechanisms, for example impairing other pivotal biochemical processes, e.g. the Calvin-Benson cycle. Indeed, similar levels of F_v/F_m in both infected and control leaves are supportive for the lack of structural damages to the PSII core, whereas lower values of ϕ_{PSII} may indicate an impairment of electron transport to photochemistry. In this context, the carboxylation phase might not be able to sustain

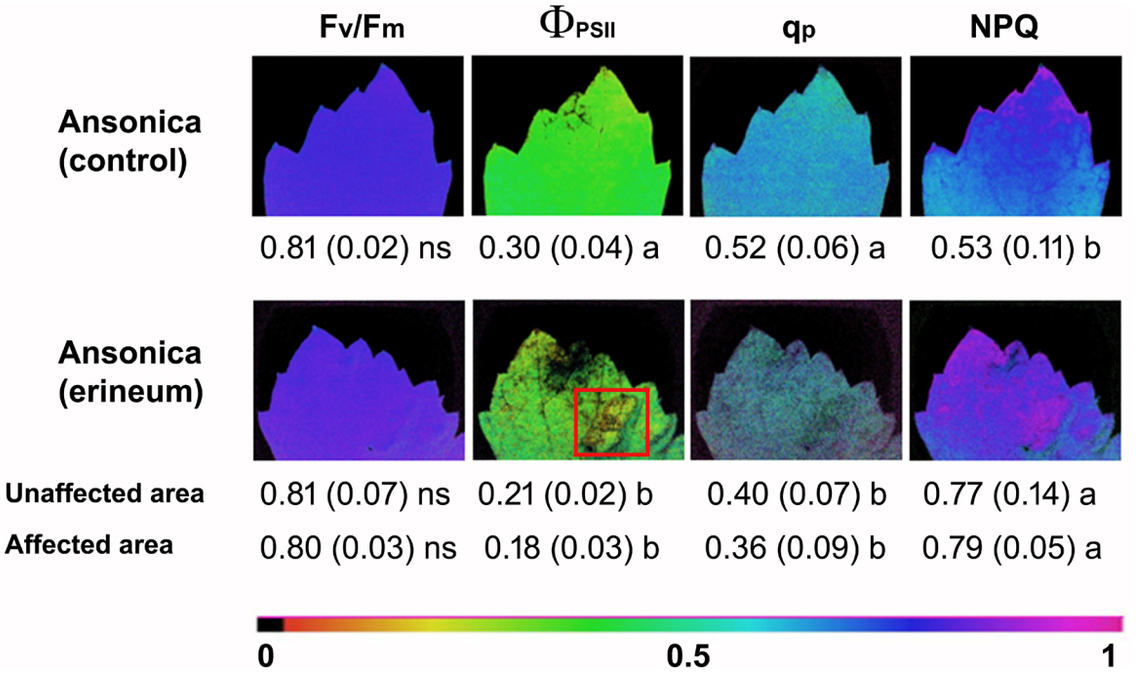

Fig. 5: Analysis of chlorophyll *a* fluorescence parameters: potential photosystem II (PSII) photochemical efficiency - F_v/F_m; quantum yield of PSII - Φ_{PSII}; photochemical quenching - qP; non-photochemical quenching - NPQ. These parameters were determined at saturating light (~ 500 µmol $m^{-2}s^{-1}$) in mature leaves of 'Ansonica' affected by *Colomerus vitis* (Pagenstecher) (erineum) or asymptomatic (control). The values are derived from the mean (± SD) of five areas within an internal square portion (ISP) randomly placed on each of the 3 biological replicas ($n = 3$). In affected plants, the parameters were calculated in both affected (square red) and not affected areas of the leaf lamina. Following evaluation of homogeneity of variance by Bartlett's test ($p \leq 0.05$) the mean values were compared with one-way ANOVA and the means separated by Tukey test as post-hoc for $p \leq 0.05$. Different letters indicate significant difference between mean values.

the electron flow promoted by PSII and PSI leading to the accumulation of energy (ATP) and reducing equivalents (NADPH) generated by the light phase of photosynthesis. In general, insects that alter host development are able to influence plant metabolism in very complex ways and relationships (Motta *et al.* 2005). In grapevine, the aggression of the phylloxera (*Daktulosphaira vitifoliae*, Fitch) increases the expression of genes involved in glycolysis, fermentation, and transport of nutrients, water and mineral salts while decreasing the activity of other genes that are important for the metabolic pathways of shikimate and phenylpropanoids (Nabity *et al*. 2013).

In conclusion, results of the present experiments offer for the first time the evidence that the effects of erineum attacks also occur in non-symptomatic areas contiguous to blister-like galls, as revealed by chlorophyll fluorescence analysis, in particular the decline of ϕ_{PSII} and qP, associated with the parallel increase in NPQ in both healthy and affected areas of infected leaves. However, we are aware that future experiments are necessary to understand the reason underlying the intimal biochemical and physiological changes induced by erineum attack in 'Ansonica' leaves.

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Conflict of interest

The authors declare that they have no competing interests.

References

Alberti, G.; Nuzzaci, G.; 1996: 1.6.5 SEM and TEM techniques, 399-410. In: E. E. Lindquist, M. W. Sabelis, J. Bruin (Eds): Eriophyoid mites - their biology, natural enemies and control, Vol. 6. Elsevier Science Publishers, Amsterdam, Netherlands. DOI: https://doi.org/10.1016/S1572-4379(96)80025-X

Banerjee, P.; Islam, M. M.; Laha, A.; Biswas, H.; Saha, N. C.; Saha, G. K.; Sarkar, D.; Bhattacharya, S.; Podder, S.; 2019: Phytochemical analysis of mite-infested tea leaves of Darjeeling Hills, India. Phytochem. Anal. **31**, 277-286. DOI: https://doi.org/10.1002/pca.2893

Bernard, M. B.; Horne, P. A.; Hoffmann, A. A.; 2005: Eriophyoid mite damage in *Vitis vinifera* (grapevine) in Australia: *Calepitrimerus vitis* and *Colomerus vitis* (Acari: Eriophyidae) as the common cause of the widespread 'Restricted Spring Growth' syndrome. Exp. Appl. Acarol. **35**, 83-109. DOI: https://doi.org/10.1007/s10493-004-1986-4

Boso, S.; Alonso-Villaverde, V.; Santiago, J. L.; Gago, P.; Dürrenberger, M.; Düggelin, M.; Kassermeyer, H. H.; Martinez, M. C.;

2010: Macro- and microscopic leaf characteristics of six grapevine genotypes (*Vitis* spp.) with different susceptibilities to grapevine downy mildew. Vitis **49**, 43-50. DOI: https://doi.org/10.5073/vitis.2010.49.43-50

D'Onofrio, C.; Scalabrelli, G.; 2014: The software for a 'Universal Grapevine Database'. Acta Hortic. **1046**, 665-672. DOI: https://doi.org/10.17660/ActaHortic.2014.1046.91

Duso, C.; de Lillo, E.; 1996: Damage and control of eriophyoid mites in crops: 3.2.5 Grape, 571-582. In: E. E. Lindquist, M. W. Sabelis, J. Bruin (Eds): Eriophyoid mites - their biology, natural enemies and control. Vol. 6. Elsevier Science Publishers, Amsterdam, Netherlands. DOI: https://doi.org/10.1016/S1572-4379(96)80036-4

de Lillo, E.; Pozzebon, A.; Valenzano, D.; Duso, C.; 2018: An intimate relationship between eriophyoid mites and their host plants - a review. Front. Plant Sci. **9**, 1786. DOI: https://doi.org/10.3389/fpls.2018.01786

Fambrini, M.; Bernardi, R.; Pugliesi, C.; 2020: Ray flower initiation in the *Helianthus radula* inflorescence is influenced by a functional allele of the *HrCYC2c* gene. genesis **58**, e23401. DOI: https://doi.org/10.1002/dvg.23401

Fambrini, M.; Pugliesi, C.; 2013: Usual and unusual development of the dicot leaf: involvement of transcription factors and hormones. Plant Cell Rep. **32**, 899-922. DOI: https://doi.org/10.1007/s00299-013-1426-1

Fambrini, M.; Pugliesi, C.; 2019: The dynamic genetic-hormonal regulatory network controlling the trichome development in leaves. Plants **8**, 253. DOI: https://doi.org/10.3390/plants8080253

Fambrini, M.; Castagna, A.; Dalla Vecchia, F.; Degl'Innocenti, E.; Ranieri, A.; Vernieri, P.; Pardossi, A.; Guidi, L.; Rascio, N.; Pugliesi, C.; 2004: Characterization of a pigment deficient mutant of sunflower (*Helianthus annuus* L.) with abnormal chloroplast biogenesis, reduced PS II activity and low endogenous level of abscisic acid. Plant Sci. **167**, 79-89. DOI: https://doi.org/10.1016/j.plantsci.2004.03.002

Gago, P.; Conéjéro, G.; Martínez, M. C.; Boso, S.; This, P.; Verdeil, J.-L.; 2016: Microanatomy of leaf trichomes: opportunities for improved ampelographic discrimination of grapevine (*Vitis vinifera* L.) cultivars. Aust. J. Grape Wine Res. **22**, 494-503. DOI: https://doi.org/10.1111/ajgw.12226

Gailite, A.; Andersone, U.; Ievinsh, G.; 2005: Arthropod-induced neoplastic formations on trees change photosynthetic pigment levels and oxidative enzyme activities. J. Plant Interact. **1**, 61-67. DOI: https://doi.org/10.1080/17429140500254728

Genty, B.; Briantais, J. M.; Baker, N. R.; 1989: The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87-92. DOI: https://doi.org/10.1016/S0304-4165(89)80016-9

Graham, E. T.; Trentham, W. R.; 1998: Staining paraffin extracted, alcohol rinsed and air dried plant tissue with an aqueous mixture of three dyes. Biotech. Histochem. **73**, 178-185. DOI: https://doi.org/10.3109/10520299809141108

Guidi, L.; Mori, S.; Degl'Innocenti, E.; Pecchia, S.; 2007: Effects of ozone exposure or fungal pathogen on white lupin leaves as determined by imaging of chlorophyll *a* fluorescence. Plant Physiol. Biochem. **45**, 851-857. DOI: https://doi.org/10.1016/j.plaphy.2007.07.001

Hua, B.; Chang, J.; Wu, M.; Xu, Z.; Zhang, F.; Yang, M.; Xu, H.; Wang, L. J.; Chen, X. Y.; Wu, S.; 2020: Mediation of JA signalling in glandular trichomes by the *woolly/SlMYC1* regulatory module improves pest resistance in tomato. Plant Biotechnol. J. **19**, 375-393. DOI: https://doi.org/10.1111/pbi.13473

Javadi Khederi, S.; de Lillo, E.; Khanjani, M.; Gholami, M.; 2014: Resistance of grapevine to the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) in western Iran and its correlation with plant features. Exp. Appl. Acarol. **63**, 15-35. DOI: https://doi.org/10.1007/s10493-014-9778-y

Javadi Khederi, S.; Khanjani, M.; Gholami, M.; de Lillo, E.; 2018a: Sources of resistance to the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) in grapevine cultivars. Syst. Appl. Acarol. **23**, 405-425. DOI: https://doi.org/10.11158/saa.23.3.1

Javadi Khederi, S.; Khanjani, M.; Gholami, M.; de Lillo, E.; 2018b: Impact of the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) on the development of plants of grapevine cultivars of Iran. Exp. Appl. Acarol. **74**, 347-363. DOI: https://doi.org/10.1007/s10493-018-0245-z

Javadi Khederi, S.; Khanjani, M.; Gholami, M.; Bruno, G. L.; 2018c: Study of defense-related gene expression in grapevine infested by *Colomerus vitis* (Acari: Eriophyidae). Exp. Appl. Acarol. **75**, 25-40. DOI: https://doi.org/10.1007/s10493-018-0255-x

Javadi Khederi, S.; Khanjani, M.; Gholami, M.; Panzarino, O.; de Lillo, E.; 2018d: Influence of the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) on grape (*Vitis vinifera*) defense mechanisms. Exp. Appl. Acarol. **75**, 1-24. DOI: https://doi.org/10.1007/s10493-018-0252-0

Jiang, Y.; Veromann-Jürgenson, L. L.; Ye, J.; Niinemets, Ü.; 2018: Oak gall wasp infections of *Quercus robur* leaves lead to profound modifications in foliage photosynthetic and volatile emission characteristics. Plant Cell Environ. **41**, 160-175. DOI: https://doi.org/10.1111/pce.13050

Jiang, Y.; Ye, J.; Veromann-Jürgenson, L. L.; Niinemets, Ü.; 2020: Gall- and erineum-forming *Eriophyes* mites alter photosynthesis and volatile emissions in infection severity-dependent manner in broad-leaved trees *Alnus glutinosa* and *Tilia cordata*. Tree Physiol. tpaa173. DOI: https://doi.org/10.1093/treephys/tpaa173

Karabourniotis, G.; Bornman, J. F.; Liakoura, V.; 1999: Different leaf surface characteristics of three grape cultivars affect leaf optical properties as measured with fibre optics: possible implication in stress tolerance. Aust. J. Plant Physiol. **26**, 47-53. DOI: https://doi.org/10.1071/PP98052

Karabourniotis, G.; Liakopoulos, G.; Nikolopoulos D.; Bresta, P.; 2020: Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. J. For. Res. **31**, 1-12. DOI: https://doi.org/10.1007/s11676-019-01034-4

Landi, M.; Basile, A.; Fambrini, M.; Pugliesi, C.; 2017: Transcription factors and hormone-mediated mechanisms regulate stomata development and responses under abiotic stresses: an overview, 239-283. In: G. Pandey (Ed.): Mechanism of plant hormone signaling under stress. Vol. 2, 1st ed. John Wiley & Sons, Inc., Haboken, NJ. DOI: https://doi.org/10.1002/9781118889022.ch27

Larson, K. C.; 1998: The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. Oecologia **115**, 161-166. DOI: https://doi.org/10.1007/s004420050503

Lichtenthaler, H. K.; 1987: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes, 350-382. In: S. P. Colowick, N. O. Kaplan (Eds): Methods in enzymology. Vol. 148. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto. DOI: https://doi.org/10.1016/0076-6879(87)48036-1

Linder, C.; Jermini, M.; Zufferey, V.; 2009: Nuisibilité de l'érinose sur le cépage Muscat. Rev. Suisse Vitic. Arboric. Hortic. **41**, 177-181.

Lindquist, E. E.; 1996: 1.1 External anatomy and systematics 1.1.1. External anatomy and notation of structures, 3-31. In: E. E. Lindquist, M. W. Sabelis, J. Bruin (Eds): World Crop Pests - Eriophyoid Mites Their Biology, Natural Enemies and Control Volume 6. Elsevier Science Publ., Amsterdam, The Netherlands. DOI: https://doi.org/10.1016/S1572-4379(96)80003-0

Ma, Z. Y.; Wen, J.; Ickert-Bond, S. M.; Chen, L. Q.; Liu, X. Q.; 2016: Morphology, structure, and ontogeny of trichomes of the grape genus (*Vitis*, Vitaceae). Front. Plant Sci. **7**, 704. DOI: https://doi.org/10.3389/fpls.2016.00704

Malagnini, V.; de Lillo, E; Saldarelli, P.; Beber, R; Duso, C; Raiola, A.; Zanotelli, L.; Valenzano, D.; Giampetruzzi, A.; Morelli, M.; Ratti, C.; Causin, R.; Gualandri, V.; 2016: Transmission of grapevine Pinot gris virus by *Colomerus vitis* (Acari: Eriophyidae) to grapevine. Arch. Virol. **161**, 2595-2599. DOI: https://doi.org/10.1007/s00705-016-2935-3

Morales, F.; Abadìa, A.; Montserrat, G.; Gil-Pelegrin, E.; 2002: Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions. Trees **16**, 504-510. DOI: https://doi.org/10.1007/s00468-002-0195-1

Morán, F.; Olmos, A.; Lotos, L.; Predajňa, L.; Katis, N.; Glasa, M.; Maliogka, V.; Ruiz-García, A. B.; 2018: A novel specific duplex real-time RT-PCR method for absolute quantitation of Grapevine Pinot gris virus in plant material and single mites. PLoS One **13**, e0197237. DOI: https://doi.org/10.1371/journal.pone.0197237

Motta, L. B.; Kraus, J. E.; Salatino, A.; Salatino, M. L. F.; 2005: Distribution of metabolites in galled and non-galled foliar tissues of

Tibouchina pulchra. Biochem. Syst. Ecol. **33**, 971-981. DOI: https://doi.org/10.1016/j.bse.2005.02.004

Nabity, P. D.; Miranda, M. J.; Berenbaum, M. R.; DeLucia, E. H.; 2013: Leaf-galling phylloxera on grapes reprograms host metabolism and morphology. Proc. Natl. Acad. Sci. USA **110**, 16663-16668. DOI: https://doi.org/10.1073/pnas.1220219110

Oxborough, K.; Baker, N. R.; 1997: Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of *qP* and *Fv'/Fm'* without measuring *Fo'*. Photosynth. Res. **54**, 135-142. DOI: https://doi.org/10.1023/A:1005936823310

Patankar, R.; Thomas, S. C.; Smith, S. M.; 2011: A gall-inducing arthropod drives declines in canopy tree photosynthesis. Oecologia **167**, 701-709. DOI: https://doi.org/10.1007/s00442-011-2019-8

Pesch, M.; Hülskamp, M.; 2009: One, two, three... models for trichome patterning in *Arabidopsis*? Curr. Opin. Plant Biol. **12**, 587-592. DOI: https://doi.org/10.1016/j.pbi.2009.07.015

Petanović, R.; Kielkiewicz, M.; 2010: Plant-eriophyoid mite interactions: specific and unspecific morphological alterations. Part II. Exp. Appl. Acarol. **51**, 81-91. DOI: https://doi.org/10.1007/s10493-009-9328-1

Riddick, E. W.; Simmons, A. M.; 2014: Do plant trichomes cause more harm than good to predatory insects? Pest Manag. Sci. **70**, 1655-1665. DOI: https://doi.org/10.1002/ps.3772

Royalty, R. N.; Perring, T. M.; 1996: 3.1 Nature of damage and its assessment, 493-512. In: E. E. Lindquist, M. W. Sabelis, J. Bruin (Eds): World crop pests - eriophyoid mites their biology, natural enemies and control, Vol. 6. Elsevier Science Publ., Amsterdam, The Netherlands. DOI: https://doi.org/10.1016/S1572-4379(96)80031-5

Ruzin, S. E.; 1999: Plant Microtechnique and Microscopy. Oxford University Press, New York, NY.

Savé, R.; Biel, C.; de Herralde, F.; 2000: Leaf pubescence, water relations and chlorophyll fluorescence in two subspecies of *Lotus creticus*. Biol. Plant. **43**, 239-244. DOI: https://doi.org/10.1023/A:1002704327076

Scalabrelli, G.; D'Onofrio, C.; Barbagallo, G.; Falco, V.; 2015: 'Ansonica'. In: Italian *Vitis* Database, www.vitisdb.it, ISSN 2282-006X.

Schmidt, R. A.; 2014: Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control: a review. Exp. Appl. Acarol. **62**, 1-17. DOI: https://doi.org/10.1007/s10493-013-9730-6

Schreiber, U.; Bilger, W.; Neubauer, C.; 1995: Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis, vol. **100**, 49-70. In: E. D. Schulze, M. M. Caldwell (Eds): Ecophysiology of photosynthesis. Springer, Berlin-Heidelberg. DOI: https://doi.org/10.1007/978-3-642-79354-7_3

Valenzano, D.; Bari, G.; Malagnini, V.; de Lillo, E.; 2019: Off-host survival of *Eriophyoidea* and remarks on their dispersal modes. Exp. Appl. Acarol. **78**, 21-33. DOI: https://doi.org/10.1007/s10493-019-00417-w

Valenzano, D.; Tumminello, M. T.; Gualandri, V.; de Lillo, E.; 2020: Morphological and molecular characterization of the *Colomerus vitis* erineum strain (Trombidiformes: Eriophyidae) from grapevine erinea and buds. Exp. Appl. Acarol. **80**, 183-201. DOI: https://doi.org/10.1007/s10493-020-00470-w

Zhou, L.; H, Liu, S. B.; Wang, P. F.; Lu, T. J.; Xu, F.; Genin, G. M.; Pickard, B. G.; 2017: The Arabidopsis trichome is an active mechanosensory switch. Plant Cell Environ. **40**, 611-621. DOI: https://doi.org/10.1111/pce.12728

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