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Impact of the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) on the development of plants of grapevine cultivars of Iran

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Abstract The present experiment was aimed at determining the influence of the grape erineum strain of *Colomerus vitis* (GEM) (Acari: Eriophyidae) on responses of local grapevine cultivars. GEM was applied at five density levels to each of five cultivars, i.e. Shahani, Sahebi Uroomie, Khalili Bovanat, Rishbaba and Sezdang Ghalat (listed from early to late grape ripening). The experiment was performed in a full factorial design (12 replicates each) and effects of the mite on the relative content of leaf chlorophyll, internode and cane length, leaf area and weight, number and size of the erinea, and percentage of leaves with erinea were investigated. Also mite density on leaves and in buds was assessed. Data were analyzed with a two-way ANOVA followed by Tukey's test to separate means among treatment levels and cultivars. The relative content of chlorophyll (expressed in Spad units) in infested leaves was reduced along with an increase in mite density and it was shown to be highly significant at the two higher mite density levels for Khalili Bovanat, Rishbaba and Sezdang Ghalat; Shahani and Sahebi Uroomie leaves appeared to be less affected by mite infestation. The highest mite density treatment displayed a strong correlation with weight (positive correlation) and size (negative correlation) of the leaves of four cultivars; leaves of Sahebi Uroomie appeared to be less affected. The reduced internode length was weak in infested plants. Most infested plants produced

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shorter canes and their lengths appeared to have a strong negative correlation with the highest mite density in four cultivars; canes of Sahebi Uroomie did not appear affected. At the highest mite density, canes of Khalili Bovanat and Sahebi Uroomie displayed the most and the least shortening effects, respectively. The percentage of leaves with erinea, as well as the number of erinea per leaves and the diameter of erinea increased along with the mite population density. The mite densities in buds (April 2014) and on leaves with erinea (in November 2013) were higher at the highest treatment level in the medium-late (Rishbaba) and late ripening (Sezdang Ghalat) cultivars, than in the early and early-medium ripening ones. Almost all data collected in the current experiment allowed the conclusion that Sahebi Uroomie and Shahani were less affected than the other cultivars (Khalili Bovanat, Rishbaba and Sezdang Ghalat).

Keywords Grape · Mite density · Plant responses · Plant morphology · Correlation

Introduction

Grapevine (*Vitis vinifera* L., Vitaceae) is cultivated in nearly all regions of Iran, but vineyards are more common in West and South Iran (Anonymous 2011). The intensive cultivation of grapevine often leads to injuries by pathogens or noxious animals and may require the implementation of pest control measures (Khanjani and Hadad Irani-Nejad 2009).

Among the mites, eriophyids (Acari, Eriophyoidea, Eriophyidae) are fairly common and significant pests (Khanjani and Hadad Irani-Nejad 2009). *Calepitrimerus vitis* (Nalepa) (*Ca. vitis*) and *Colomerus vitis* (Pagenstecher) (*Co. vitis*) are cosmopolitan, obligate plant feeders and can cause severe risks for grapevine (Duso and de Lillo 1996). Three strains of *Co. vitis* can be identified based on the feeding injuries they induce: a bud strain, an erineum strain (grapevine erineum mite = GEM) and a leaf-curling strain (Smith and Stafford 1948; Duso and de Lillo 1996). Generally, GEM is the most common and gives rise usually to patchy infestations on a few vines or on a few rows of plants. It causes obvious erinea mainly on the lower leaf surface which appears to become blister-like on the upper leaf side. They are whitish at first, later turn yellow and, finally, reddish brown (Duso and de Lillo 1996). Another species, *Colomerus oculivitis* (Attiah), has been reported in some areas and differs from *Co. vitis* by only a few morphological traits; it infests mainly buds and causes leaf reddening and desiccation, and yield reduction (Craemer and Saccaggi 2013). The taxonomic status of *Co. vitis* strains and *C. oculivitis* has not been well clarified until now (Craemer and Saccaggi 2013). A study to separate *Co. vitis* strains by bio-molecular means was carried out to gain further insight into the breeding biology and population structure of this species (Carew et al. 2004).

Little information is available on the direct and indirect impact of GEM on growth and yield parameters of grapevine (Whitehead et al. 1978; Králíková 1990; Dellei and Szendrey 1991; Avgin and Bahadiroğlu 2004; Walton et al. 2007; Malagnini et al. 2016). Their tiny size and cryptic behavior allow deep intimate interactions between eriophyids and the related host plants. Recently, Javadi Khederi et al. (2014a, b, 2018) pointed out that the grape quality and quantity were affected by high densities of GEM populations, that the injuries varied among cultivars (Shahani was the least affected, Gazne the most), and they demonstrated that leaf area, leaf weight, and the length of canes and internodes were influenced by high mite infestations.

In most of West-Iranian vineyards GEM was recognized as an actual economic threat affecting grape and leaf production (e.g., Khanjani and Hadad Irani-Nejad 2009; Javadi

Khederi et al. 2014a, 2018). Injuries caused by GEM have not been sufficiently studied on cultivars commonly cultivated in Iran, and their susceptibility to mite infestation as well as the crop cultivation system and the pesticide control methods require further investigations.

This study aims at the impact that GEM can have on a selected group of grapevine cultivars commonly cultivated in West and South Iran. The effects of various GEM densities on several vegetative growth features and leaf alterations were investigated.

Materials and methods

The experiment was conducted in a greenhouse located at the experimental site of the Bu-Ali Sina University, Hamedan, Iran, from January to November, 2013, and in the field from November 2013 to April 2014. During the experiment, the mean temperature in the greenhouse ranged from 16.6 ± 3.5 to 32.4 ± 4.1 °C, and the mean relative air humidity was $70.6 \pm 15.5\%$.

Preparation of plant material

The experiment was carried out on the grape cultivars Khalili Bovanat (cultivated for both fresh and raisin production), Sahebi Uroomie, Sezdang Ghalat, Rishbaba (all cultivated for fresh market) and Shahani (used for juice production). Sahebi Uroomie, Khalili Bovanat and Rishbaba are also cultivated for leaf production. With regard to the grape maturation and harvesting time, Shahani is an early cultivar, Sahebi Uroomie and Khalili Bovanat are early-medium cultivars, Rishbaba is a medium-late cultivar and Sezdang Ghalat is late (Karami 2012). The cultivars were also selected on the basis of recorded data on the impact of GEM infestation on the plants (Javadi Khederi et al. 2014a, 2018). Cuttings of the selected cultivars came from the Vine Research Institute of Shiraz (Iran) and were kept in the greenhouse since early January 2013 in order to stimulate rooting. In mid-July 2013, they were planted singly in 2.5-kg pots containing two parts of sand and one part of sheep manure. These potted plants were arranged in stands allowing them a uniform sunlight exposure. They were drip-irrigated 3 × per week (at 8:30 pm) for 30 min.

Before beginning the experiment, the potted plants were sprayed with sulphur (Sulfore 750 g/L, 100 mL per plant) at a concentration of 13 mL per 5 L of water to control a few thrips, and with tebuconazole (Folicur, Bayer Crop Science; 1:1000 vol/vol) to control powdery mildew. Five days later, sulphur and tebuconazole residues were removed by washing the leaves with water and each potted plant was fertilized with 100 mL of Hoagland and Arnon (1950) solution (0.2 g/L ammonium phosphate, 0.6 g/L potassium nitrate, 0.8 g/L calcium nitrate, 0.04 g/L Fetrilon Combi, 0.05 g/L iron chelated water).

In late August 2013, after chemical control and before beginning the experiment, the potted plants were severely pruned in order to induce the production of new leaves because young and tender leaves are much more sensitive to GEM. Soon after bud breaking, two potted plants per cultivar were randomly selected. All their green organs were washed with a water solution containing bleach (2% vol/vol) and detergent (0.5% vol/vol), sieved and the sediments remaining on the 500 mesh sieve were examined under a stereomicroscope (de Lillo 2001; Monfreda et al. 2007) in order to verify the lack of GEM and other organisms, including predatory mites and insects.

Induced infestation of plants

Leaves with erinea were collected from 20-year-old grapevines of the cultivar Fakhri, cultivated in the area of Siagoonag village (Hamadan, Iran; 34°48′N, 48°28′E, 1830 m above sea level), 1 day before starting the experiment. The leaf sample was packed in plastic bags, stored in a portable cooler and immediately transported to the laboratory (about 2 km from the collection site). The leaves were checked under a stereomicroscope for mite vitality. All organisms were removed with the exception of those apparently belonging to *Co. vitis*. A few erinea were selected haphazardly within the leaf sample and these were used to estimate mite density following the above-mentioned washing and sieving method.

The plants of each cultivar (60 potted plants per cultivar) were divided in five groups: four were infested with a selected dose (g) of erinea and one group was left uninfested (Table 1). Consequently, a full factorial experimental design was arranged in order to assess the main effects of GEM density and cultivar on various plant response parameters. Twelve replicates were used for each treatment. Specifically, five groups of 12 plants (one for each cultivar) were treated with one of five mite density levels (i.e., 0 mites per plant [uninfested control], 1500 [treatment I], 4800 [II], 7500 [III], or 13,000 [IV] mites per plant; Table 1).

The infestation was induced by tying eriocards (Javadi Khederi et al. 2014a) at the tip of the canes. Eriocards consisted of GEM-infested erinea glued on carton cards of about 4 cm^2. They were removed 2 weeks later, when most eriophyids should have moved to the youngest leaves of plants at 5–6 leaf stage.

Plant response

Three months after the infestation of potted plants with GEM, in late November 2013, the complete set of leaves per plant was examined in order to calculate the percentage of leaves per plant on which erinea were developed. At the same time, a subsample of leaves was collected from all assayed plants and the area, fresh weight, and relative content of chlorophyll of leaves were assessed as response variables, as well as number and diameter of the erinea. The subsamples of leaves were obtained from nodes 8–13 along the cane (starting from the basal one). Each fresh leaf blade (clipped from the stalk) was weighed with a precision balance (readability scale of 0.001 g) soon after collection. The relative content of chlorophyll was evaluated with SPAD-502 Plus (Konica-Minolta, Osaka, Japan) and was expressed in Spad meter unit which is known to be proportional to the amount of chlorophyll (Uddling et al. 2007). The leaf area was measured using a planimeter (AJP Model; Koizumi, Nagaoka, Japan) on leaves stored at 4 °C, 2 days after their collection. The erinea were counted on each leaf, and their diameter was measured with a caliper.

Table 1 Levels of *Colomerus vitis* mite infestation in the experiment: erinea were collected, weighed and administered to the plants, corresponding to various mite density levels (mean ± SE number of individuals applied per plant)

Density treatments	Erineum weight (g)	Mite density (number of eggs, juveniles and adults/plant)
Uninfested	0	0
I	1	1469 ± 10.0
II	3	4803 ± 7.3
III	5	7475 ± 15.9
IV	9	12,995 ± 80.4

Soon after collecting leaves (late November 2013), all assayed plants were transferred to outdoor natural conditions in order to allow mites to move into overwintering sites. Plants were not pruned and after bud break (April 2014), cane and internode lengths (from nodes 1–2 up to 9–10; hence, nine internodes per cane) of the previous season's growth were measured.

Mite density

Mite density was evaluated twice during the experiment by extraction of all stages (eggs included) following the above-mentioned washing and sieving procedure. Mites from leaves provided with erinea were counted in November 2013 on the leaf subsamples. Mites from buds were counted in April 2014 on the basal 12 buds of the three largest canes of each potted plant. This selection was based on preliminary observations of intra-plant mite distribution that indicated more mites in the buds of the more basal nodes than in those more distal on the canes (Javadi Khederi et al. 2018).

Statistical analysis

Plant responses and mite density for each cultivar and applied density level (established as experimental factors), as well their interaction, were analyzed by two-way 5×5 ANOVA using Proc GLM (SAS 2003), after verifying normal distribution and equal variance of the data. Percentages of leaves with erinea were $\sqrt{(x+1)}$ transformed (Mohiseni et al. 2011) before analysis. Means were separated using Tukey's multiple range test for post hoc comparison ($\alpha = 0.05$). Pearson's correlation coefficient was calculated to assess the interaction of mite infestation densities and grapevine features (leaf area and weight, cane and internode length, relative chlorophyll content, erineum number, diameter and percentage of leaves with erinea) using SPSS 13.0 (2004) (SPSS, Chicago, IL, USA).

In order to summarize the main effect of the experimental factors on the plant responses and mite density, corrected indexes (%) were calculated based on Abbott's (1925) formula. A density index for each applied density level was calculated as follows:

$$\text{Density index} = \left(1 - \frac{\text{Sum of means for each cultivar}}{\text{Sum of means for unifested level for each cultivar}}\right) \times 100.$$

This index describes the main effect induced by a GEM density.

A cultivar index was calculated as follows:

$$\text{Cultivar index} = \left(1 - \frac{\text{Sum of means per density level of a cultivar}}{\text{Means at unifested level for the same cultivar}}\right) \times 100.$$

This index describes the main effect induced by a cultivar.

Results

Plant responses

Overall, leaves of plants treated with the highest mite density (treatment IV) showed the lowest relative content of chlorophyll for all assayed cultivars (Table 2, Fig. 1a). The leaves of untreated plants revealed the highest relative content of chlorophyll, but the difference

Table 2 Mean (±SE) relative contents of chlorophyll (expressed as Spad units) in leaves of five grapevine cultivars treated with five *Colomerus vitis* mite densities (12 replicates per cv. and mite density), and cultivar and density indexes per factor level (see Materials and methods section Statistical analysis for explanation)

Density treatments	Cultivars					Density index
	Sahebi Uroomie	Khalili Bovanat	Sezdang Ghalat	Rishbaba	Shahani	
Uninfested	31.02 ± 0.63 g	38.61 ± 1.00a	37.20 ± 0.87bc	35.92 ± 0.59 cd	29.61 ± 0.56hi	–
I	29.42 ± 0.38hij	37.54 ± 0.30ab	32.93 ± 0.29f	35.30 ± 0.59de	29.69 ± 0.59hi	3.67γ
II	29.47 ± 0.29hij	34.54 ± 0.64e	33.05 ± 0.44f	34.68 ± 0.69de	28.91 ± 0.59ijk	5.39γ
III	29.51 ± 0.23hij	28.29 ± 0.24jkl	30.24 ± 0.69gh	27.34 ± 0.45 lm	28.64 ± 0.53ijkl	15.02β
IV	27.98 ± 0.27klm	23.97 ± 0.30n	26.71 ± 0.68n	27.82 ± 0.97klm	27.41 ± 0.37 lm	20.85α
Cultivar index	5.81γ	18.44α	16.65α	12.58β	2.70δ	

Means followed by the same Latin letters, and indexes with the same Greek letters within a row or within a column, do not differ significantly (factorial ANOVA followed by Tukey's test: $P < 0.05$)

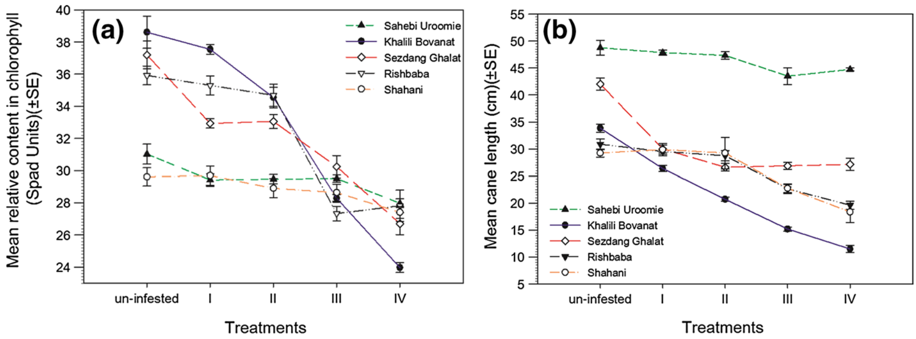

Fig. 1 Mean (± SE) **a** relative contents of chlorophyll (expressed as Spad units) in leaves and **b** cane length (cm) of five grapevine cultivars

with the lowest level of infestation (I) was significant only for Sahebi Uroomie and Sezdang Ghalat—the difference with the two highest levels of infestation (III and IV) was significant for all cultivars (Table 2).

The density index was significantly higher in treatment IV followed by treatment III, whereas no significant difference was found between treatments I and II (Table 2). The cultivar index was highest for Khalili Bovanat and Sezdang Ghalat, and lowest for Shahani (Table 2). Finally, interaction effects were detected between the factors cultivar and mite density level ($F_{24,275}=96.43$, $P<0.0001$) (Fig. 1a).

Although overall the internodes became shorter going from uninfested to the highest level of infestation, differences among treatment levels were not significant for any of the cultivars ($F_{24,275}=0.49$, $P=0.95$) (Table 3). Cultivar index was found to be significantly different among cultivars ($F_{4,4}=143.90$, $P<0.0001$)—post hoc comparison among means indicated that it was highest for Sahebi Uroomie, lowest for Shahani, Rishbaba and Khalili Bovanat, and intermediate for Sezdang Ghalat (Table 3). Density index was clearly lowest for treatment I and highest for treatment IV (Table 3; $F_{24,275}=8.63$, $P<0.0001$). No interaction effect was detected for internode length between cultivar and density levels.

The shortest canes within each cultivar were measured at the two highest levels of infestation (Table 3). Canes were longest in the untreated plants for four of the five cultivars. Cane length in Sahebi Uroomie appeared to be least affected by mite infestation levels. Density index separated the four infestation treatments, emphasizing that cane length depended on the applied mite density. Similarly, cultivar index separated the cultivars; Khalili Bovanat and Sezdang Ghalat exhibited the highest index value, Sahebi Uroomie the lowest (Table 3). Interaction effects between cultivar and mite density were detected ($F_{24,275}=10.39$, $P<0.0001$) (Fig. 1b).

Overall, leaf area gradually reduced going from uninfested to the highest level of mite infestation; plants of all cultivars exposed to the highest mite density (treatment IV) had significantly smaller leaves than in all other treatments (Table 4). Density index separated the treatments, index values growing proportionally with the applied mite density level. Similarly, cultivar index separated the cultivars—it was highest for Sezdang Ghalat and lowest for Sahebi Uroomie. Interaction effects among the cultivars and mite densities were detected ($F_{24,275}=24.70$, $P<0.0001$) (Table 4).

Leaf weight gradually increased with increasing level of mite infestation; plants assayed with the two highest mite density levels produced the heaviest leaves in all cultivars

Table 3 Mean (±SE) internode and cane length (cm) of five grapevine cultivars treated with five *Colomerus vitis* mite densities (12 replicates per cv. and mite density), and cultivar and density indexes per each factor level (see "Materials and methods" section Statistical analysis for explanation)

Variable	Density treatments	Cultivars					Density index
		Sahebi Uroomie	Khalili Bovanat	Sezdang Ghalat	Rishbaba	Shahani	
Internode length	Uninfested	5.58 ± 0.15a	3.42 ± 0.10bc	3.54 ± 0.17bc	3.71 ± 0.14bc	3.96 ± 0.14b	–
	I	5.50 ± 0.14a	3.37 ± 0.11bc	3.42 ± 0.13bc	3.71 ± 0.13bc	3.96 ± 0.14b	0.40γ
	II	5.04 ± 0.11a	3.37 ± 0.15bc	3.33 ± 0.11bc	3.54 ± 0.17bc	3.92 ± 0.15b	2.91β
	III	5.04 ± 0.11a	3.37 ± 0.12bc	3.21 ± 0.22bc	3.54 ± 0.19bc	3.87 ± 0.12b	3.49β
	IV	4.83 ± 0.14a	3.04 ± 0.19c	3.04 ± 0.21c	3.33 ± 0.14bc	3.50 ± 0.12bc	9.97α
	Cultivar index	7.77α	2.85β	6.10αβ	1.92β	1.32β	
Cane length	Uninfested	48.75 ± 1.36a	33.85 ± 0.75c	42.02 ± 1.14b	30.87 ± 1.00 cd	29.25 ± 0.75 cd	–
	I	47.87 ± 0.51a	26.37 ± 0.55def	30.00 ± 0.73 cd	29.50 ± 0.50 cd	29.87 ± 1.14 cd	10.73δ
	II	47.35 ± 0.72ab	20.72 ± 0.39fgh	26.60 ± 0.71de	28.75 ± 0.95 cd	29.25 ± 2.93 cd	17.05γ
	III	43.50 ± 1.55ab	15.20 ± 0.38hi	26.87 ± 0.63de	22.65 ± 0.83efg	22.75 ± 0.78efg	29.92β
	IV	44.75 ± 0.32ab	11.50 ± 0.67i	27.12 ± 1.14de	19.62 ± 0.72gh	18.37 ± 1.95gh	36.46α
	Cultivar index	5.65ε	45.42α	34.12β	18.33γ	14.17δ	

Within a variable, means followed by the same Latin letters, and indexes with the same Greek letters within a row or within a column, do not differ significantly (factorial ANOVA followed by Tukey's test: $P < 0.05$)

Table 4 Mean (±SE) area (cm^2) and weight (g) of leaves of five grapevine cultivars treated with five *Colomerus vitis* mite densities (12 replicates per cv and mite density), and cultivar and density indexes per each factor level (see Materials and methods section Statistical analysis for explanation)

Variable	Density treatments	Cultivars					Density index
		Sahebi Uroomie	Khalili Bovanat	Sezdang Ghalat	Rishbaba	Shahani	
Leaf area	Uninfested	80.42 ± 0.42a	65.59 ± 0.43 cd	54.37 ± 0.86f	78.33 ± 0.74ab	48.12 ± 0.87i	–
	I	80.62 ± 0.94a	65.01 ± 0.56cde	52.33 ± 0.43 fg	77.87 ± 0.41ab	48.99 ± 0.62ghi	0.50δ
	Ii	80.55 ± 0.95a	61.35 ± 0.38e	46.66 ± 0.77ij	66.77 ± 0.64c	48.45 ± 0.31hi	6.75γ
	Iii	77.15 ± 0.69ab	51.87 ± 0.81fgh	41.68 ± 0.50 k	62.60 ± 0.36de	43.12 ± 0.89jk	15.68β
	Iv	76.02 ± 0.72b	46.36 ± 0.94ij	33.99 ± 0.64 l	54.47 ± 0.95f	34.06 ± 0.84 l	26.26α
	Cultivar index	2.25δ	14.35β	19.45α	16.38β	9.05γ	
Leaf weight	Uninfested	1.74 ± 0.03a	1.15 ± 0.03 g	1.00 ± 0.02 h	1.40 ± 0.03d	0.88 ± 0.02i	–
	I	1.72 ± 0.04a	1.19 ± 0.03 fg	1.17 ± 0.03 fg	1.41 ± 0.02d	0.99 ± 0.02 h	4.71γ
	Ii	1.73 ± 0.02a	1.25 ± 0.04ef	1.16 ± 0.04 fg	1.50 ± 0.03bc	0.98 ± 0.02 h	6.75βγ
	Iii	1.76 ± 0.03a	1.23 ± 0.04efg	1.28 ± 0.01e	1.58 ± 0.04b	1.06 ± 0.03 h	10.90αβ
	Iv	1.76 ± 0.01a	1.30 ± 0.02e	1.45 ± 0.02 cd	1.69 ± 0.03a	1.03 ± 0.04 h	14.69α
	Cultivar index	0.27δ	6.80γ	19.34α	8.50β	11.95β	

Within a variable, means followed by the same Latin letters, and indexes with the same Greek letters within a row or within a column, do not differ significantly (factorial ANOVA followed by Tukey's test: $P < 0.05$)

(Table 4). The highest density index for leaf weight was recorded at treatment IV (though not significantly different from the index at treatment III) and the lowest density index at treatment I (though not significantly different from the index at treatment II) (Table 4). The highest and lowest significant cultivar index values were obtained on Sezdang Ghalat and Sahebi Uroomie, respectively. Interaction effects were found between cultivars and mite densities ($F_{24,275} = 4.95$, $P < 0.0001$) (Fig. 2).

Leaves of plants treated with the highest mite density (treatment IV) showed the most and the largest erinea per leaf—this effect was significant for four of the five cultivars, but not for Shahani (Table 5). Treatment means of the number of erinea per leaf clearly separated the treatments, indicating its dependence on mite density. A similar result was obtained for the size (diameter) of the erinea, although no difference was observed between the two lowest mite density levels. The highest and the lowest cultivar means were found on Sezdang Ghalat and Shahani, respectively, both for the number and the size of erinea.

Overall, the percentage of leaves with erinea on plants increased with the level of mite density—the highest percentage of leaves with erinea was found for treatment IV in all cultivars except Rishbaba, where treatments II–IV did not differ (Table 5). Treatment means of percentage of leaves with erinea were clearly separated among the mite density treatment levels. Cultivar mean was lowest for Shahani and Sahebi Uroomie, and highest for Sezdang Ghalat.

Cultivars and mite densities displayed interaction effects for number of erinea per leaf ($F_{19,220} = 18.53$), erineum diameter ($F_{19,220} = 24.41$) and percentage of leaves with erinea ($F_{19,220} = 12.69$, all $P < 0.0001$) (Table 5).

Mite density

The highest mean density of GEM population was assessed on leaves and in buds of plants infested with treatment IV for each cultivar (only exception: on leaves of Khalili Bovanat peak density was recorded for treatment III; Table 6). Overall, the counted mite density—both in buds and on leaves—increased with the initial level of infestation on all cultivars, but not all pairwise comparisons yielded significant differences—e.g., at the three lower levels of initial mite infestation (treatments II–IV), the mite density counted in buds was not significantly different on cultivars Rishaba and Shahani; no

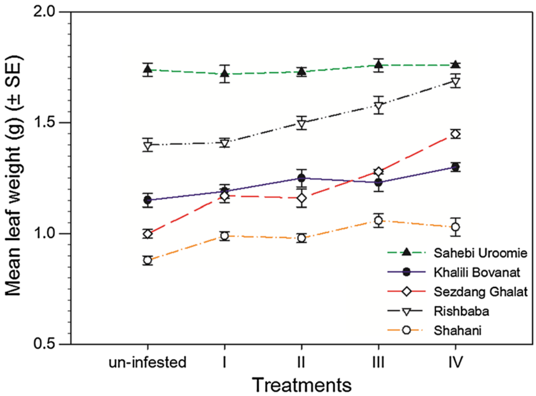

Fig. 2 Mean (± SE) leaf weight (g) of five grapevine cultivars treated with five *Colomerus vitis* mite densities (12 replicates per cv. and mite density)

Table 5 Mean (±SE) number of erinea per leaf, diameter of erinea (cm) and percentage of leaves with erinea in five grapevine cultivars treated with four *Colomerus vitis* mite densities (12 replicates per cv and mite density), and overall means per factor level

Variable	Density treatments	Cultivars					Treatment mean
		Sahebi Uroomie	Khalili Bovanat	Sezdang Ghalat	Rishbaba	Shahani	
Erineum number	I	0.58 ± 0.13 h	7.58 ± 0.29e	8.75 ± 0.21ed	6.00 ± 0.37f	0.70 ± 0.29 h	4.72δ
	II	0.82 ± 0.17 h	7.50 ± 0.32ef	10.75 ± 0.47bc	10.58 ± 0.43bc	1.08 ± 0.13 h	6.14γ
	III	1.83 ± 0.16 h	8.08 ± 0.28ed	11.49 ± 0.19b	11.07 ± 0.32b	1.17 ± 0.17 h	6.73β
	IV	3.91 ± 0.29 g	9.25 ± 0.41 cd	13.41 ± 0.40a	14.25 ± 0.34a	1.17 ± 0.30 h	8.40α
	Cultivar mean	1.79δ	8.10γ	11.10α	10.47β	1.03ε	
Erineum diameter	I	0.09 ± 0.01 h	1.36 ± 0.01ed	1.43 ± 0.02 cd	1.19 ± 0.06f	0.05 ± 0.02 h	0.83γ
	II	0.09 ± 0.02 h	1.16 ± 0.03f	1.54 ± 0.03bc	1.14 ± 0.01f	0.09 ± 0.02 h	0.81γ
	III	0.11 ± 0.02 h	1.26 ± 0.01ef	1.62 ± 0.01b	1.62 ± 0.02b	0.09 ± 0.02 h	0.94β
	IV	0.34 ± 0.01 g	1.34 ± 0.01ed	1.87 ± 0.05a	1.89 ± 0.04a	0.19 ± 0.01 h	1.13α
	Cultivar mean	0.16δ	1.28γ	1.67α	1.46β	0.11δ	
% leaves with erinea	I	3.85 ± 0.21kj	15.22 ± 0.28gh	22.32 ± 0.21ef	22.91 ± 0.24ed	3.12 ± 0.13 k	13.48δ
	II	4.11 ± 0.47kj	15.52 ± 0.37gh	23.86 ± 0.13ed	28.00 ± 0.42bc	3.19 ± 0.40 k	14.94γ
	III	4.95 ± 0.26kj	18.97 ± 0.40 fg	31.25 ± 0.59b	28.26 ± 0.19bc	7.20 ± 0.47ij	18.12β
	IV	9.52 ± 0.21i	26.43 ± 0.12 cd	38.14 ± 0.42a	28.12 ± 0.23bc	13.67 ± 0.55 h	23.17α
	Cultivar mean	5.61δ	19.03γ	28.89α	26.82β	6.79δ	

Within a variable, means followed by the same Latin letters, and overall means with the same Greek letters within a row or within a column, do not differ significantly (factorial ANOVA followed by Tukey's test: $P < 0.05$)

Table 6 Mean (± SE) number of mites counted in buds and leaves with erinea on five grapevine cultivars treated with five *Colomerus vitis* mite densities (12 replicates per cv and mite density), and overall means per factor level

Variable	Density treatments	Cultivars					Treatment mean
		Sahebi Uroomie	Khalili Bovanat	Sezdang Ghalat	Rishbaba	Shahani	
Mites/bud samples	I	4.25 ± 0.25jk	22.00 ± 0.41 h	14.50 ± 1.19i	48.75 ± 1.04e	0.50 ± 0.29 k	18.00δ
	II	3.50 ± 1.00jk	15.50 ± 0.91i	43.75 ± 0.75f	71.25 ± 1.31c	1.00 ± 0.41 k	27.00γ
	III	13.25 ± 0.85i	29.25 ± 0.87 g	64.00 ± 0.63d	73.00 ± 0.82c	2.25 ± 0.48 k	36.35β
	IV	17.50 ± 0.85hi	41.00 ± 0.86f	153.75 ± 1.37a	100.25 ± 1.31b	7.00 ± 0.48j	63.90α
	Cultivar mean	9.62δ	26.94γ	69.00β	73.31α	2.69ε	
Mites/leaf samples with erinea	I	7.42 ± 0.54i	525.83 ± 7.32 h	1125.00 ± 9.99 g	1125.00 ± 7.33 g	12.50 ± 1.68i	446.15δ
	II	12.50 ± 1.68i	533.75 ± 4.06 h	1770.83 ± 7.29c	1482.50 ± 7.29d	15.83 ± 1.72i	763.08γ
	III	13.42 ± 1.09i	1436.67 ± 9.48e	1754.99 ± 15.87c	2051.00 ± 11.41b	16.75 ± 1.04i	1054.56β
	IV	40.00 ± 3.25i	1341.67 ± 10.99f	2982.27 ± 10.82a	2074.49 ± 5.29b	36.67 ± 2.56i	1295.02α
	Cultivar mean	18.33δ	959.48γ	1908.27α	1541.99β	20.44δ	

Within a variable, means followed by the same Latin letters, and overall means with the same Greek letters within a row or within a column, do not differ significantly (factorial ANOVA followed by Tukey's test: $P < 0.05$)

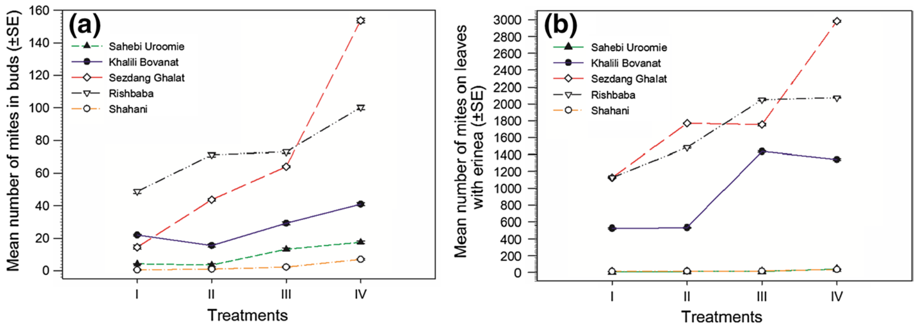

Fig. 3 Mean (± SE) *Colomerus vitis* mite population density **a** in buds with erinea (number of individuals per bud) and **b** on leaves (number of individuals per leaf) of five grapevine cultivars treated with five mite densities (12 replicates per cv. and mite density)

Table 7 Pearson's correlation coefficient among plant responses and levels of *Colomerus vitis* mite infestation. Content of chlorophyll, leaf weight and area refer to data recorded on November 2013, whereas internode and cane lengths refer to data recorded on April 2014

Plant responses	Density treatments			
	I	II	III	IV
Relative content of chlorophyll	0.12 ns	0.20 ns	−0.60**	−0.46**
Internode length	−0.25*	−0.27*	−0.28*	−0.23 ns
Cane length	−0.31*	−0.28*	−0.28*	−0.90**
Leaf area	−0.31*	−0.57**	−0.49**	−0.54**
Leaf weight	−0.08 ns	0.05 ns	0.16 ns	0.53**
Erineum number	0.90**	0.94**	0.97**	0.91*
Erineum diameter	0.74*	0.81*	0.99**	0.95*
% leaves with erinea	0.89*	0.95*	0.96**	0.98**

**$P<0.01$; *$P<0.05$; ns, $P>0.05$

significant difference was observed in the mite population density on leaves of Sahebi Uroomie and Shahani at all four initial mite density levels (Table 6).

Cultivar and treatment means were well separated, emphasizing overall effects of cultivars and initial mite infestation levels. The highest GEM density was recorded in buds of Rishbaba and on leaves of Sezdang Ghalat whereas the lowest density was found in buds of Shahani and on leaves of Sahebi Uroomie and Shahani. An interaction effect was calculated for mite population in buds ($F_{19,220}=3104.18$) and on leaves ($F_{19,220}=740.74$, both $P<0.0001$) (Fig. 3).

Correlations

The correlation analysis (Table 7) indicated that:

- relative content of chlorophyll in leaves did not show any correlation with mite infestation at treatment levels I and II, whereas it was negatively correlated at levels III and IV;
- internode length was negatively related to mite infestation at all four mite density levels, but significantly so at levels I-III;
- cane length was significantly negatively related to mite infestation at all four mite density levels; the relation was clearly the strongest at the highest mite population density (IV);
- leaf area was moderately to strongly negatively related to mite infestation at all four mite density levels;
- leaf weight did not show any correlation with mite infestation at treatment levels I-III, whereas a strong positive correlation was assessed at level IV;
- both, number and size (diameter) of erinea on leaves showed a strong positive correlation with mite infestation at all treatment levels;
- percentage of leaves with erinea was strongly and positively correlated to mite infestation at all four mite density levels.

Discussion

In general, little information is available in literature concerning the feeding effects of eriophyoid mites on plant vegetative traits and cultivars, or their interaction (Petanović and Kielkiewicz 2010). The impact of the erineum strain of *Co. vitis* on morphological and physiological features of grapevine has been scarcely explored through assays in the laboratory or field. Recently, some Iranian cultivars were investigated for tolerance and resistance to GEM (Javadi Khederi et al. 2014a, b, 2018). The current experiment pointed out the effects of the mite on the relative content of leaf chlorophyll, on plant morphological parameters (such as internode and cane length, leaf area and weight) and on alterations on the infested plants induced by the mite (number and size of the erinea, percentage of leaves with erinea). A density-dependent relationship was established between the mite population levels and plant responses. Almost all parameters allowed the separation of Sahebi Uroomie and Shahani from the other cultivars—they were less affected by the mite.

The relative content of chlorophyll (Spad units) in infested leaves was reduced in proportion to the increase of the mite density. The correlation between treatments and Spad units was highly significant at the two highest mite density levels. Chlorophyll content of Shahani and Sahebi Uroomie appeared to be less affected by the infestation and this could be related to the very low mite population density that established on the leaves of these two cultivars during the trial. The lower chlorophyll content of the other assayed cultivars may have diminished the photosynthetic activity and contributed to the reduction of leaf size and cane length. These data appear to be in accordance with the effects induced by *Aculops lycopersici* (Tryon) on tomato (Wu et al. 2006) and with fluorescence yield of apple leaves infested by *Aculus schlechtendali* (Nalepa) (Ioriatti et al. 1997).

Correlations were found to be highly significant between the highest mite density treatments and leaf weight (positive) and size (negative). It seems that GEM induces leaves to become heavier but smaller. Leaves of Sahebi Uroomie appeared to be less affected: based on the cultivar indexes of leaf area and weight, it was calculated that an average reduction of 1 cm^2 of leaf area corresponds to an average increase of only ca. 0.01 g in leaf weight. The same size reduction produces an increase of 0.5–1.3 g for leaves of the other cultivars.

Dividing the cultivar index of the percentage of leaves with erinea by that of the weight increase, it seems that the leaves (with an erineum) of Sahebi Uroomie increase, on average, less than 0.05 g per leaf, Khalili Bovanat and Rishbaba leaves increased about 0.3 g, Sezdang Ghalat about 0.7 g, and Shahani about 1.8 g. Hence, Shahani appears to tolerate the development of mite populations but its plant response on the leaves is much more pronounced than that of other cultivars. Leaf deterioration is economically relevant for Sahebi Uroomie, Khalili Bovanat and Rishbaba, as also their leaves are commonly used for making food according to traditional Iranian cuisine.

No difference was observed on the internode length at the applied density levels within each cultivar. A general shortening of the internodes was evidenced only by the density index and treatments I, II and III were negatively correlated with internode length. Vice versa, the most infested plants produced shorter canes and their length appeared to have a strong negative correlation with treatment IV. Also the cultivar responses were different indicating a substantial effect of the highest mite density on Khalili Bovanat and a very mild effect on Sahebi Uroomie. Shorter canes can be the result of a reduction in the number of nodes and, consequently, of leaves along the canes, possibly indicating abnormal development of the buds. It means that the mites feeding on the growing points directly affected the cane and leaf development during the vegetative growth of the plants (from August to November, in the current trials). But indirect effects may also arise from lower photosynthetic activity, as previously indicated.

The highest mite densities in buds and on leaves with erinea were detected at the highest treatment level in the medium-late (Rishbaba) and late ripening (Sezdang Ghalat) cultivars, rather than in the early and early-medium ones. These data resemble those reported by Dellei and Szendrey (1991). No relationship was found between mite density on the leaves (in November 2013) and in the buds (in April 2014). Denser populations seemed to be much more common in larger buds which were characterized by smooth surfaces and thick outer scales, and are commonly set on the basal part of the canes (Javadi Khederi et al. 2018). There is very little information on this trend. Dennill (1991) studied the distribution of the bud strain and found the highest density in the fourth and sixth bud from the base of the cane of Chenin Blanc, but no data on the crop system were given. However, the author explained a higher density in the basal buds than in the distal ones, relating the colonization of the first ones to their earlier morphological maturation. Unfortunately, data on the morphology of the buds were not recorded systematically and the current observations require experimental confirmation.

The symptoms observed in the present trials and induced by mites coming from typical erinea were like those observed in South Africa by Whitehead et al. (1978). The symptoms showed some similarities with those induced by the bud strain of *Co. vitis* concerning the shortening of shoots, the reduction in size of leaves, distortion, and surface scarification of canes and bud scales (unpubl. observations). Whitehead et al. (1978) reported that the bud strain does not induce typical erinea, but causes hypertrophied cells on the bud epidermis which assume the shape of 'polyps', in combination with the scarification of green bark, shortening of basal internodes, flattening of shoots, death of terminal and dormant buds, development of lateral canes, deformities mainly of the basal leaves, and premature dropping of flower clusters (Smith and Stafford 1948; Kido and Stafford 1955; Bernard et al. 2005). On the other hand, Kido and Stafford (1955) recorded negligible injury to Californian vineyards caused by the erineum strain (GEM). In our current trial, the most severe population densities in buds were detected in treatment IV (i.e., highest mite density applied) for Sezdang Ghalat and Rishaba with a mean density of over 100 individuals per bud. In literature, this density value was associated with primary bud-axis necrosis and development of secondary buds caused by the bud strain on Cabernet Franc at bud swellings, in Australia (Bernard et al. 2005).

In the current trial, no correlation between grape yield and *Co. vitis* density was assessed, probably because of the age of the assayed plants. Previous research did not prove any negative effect by *Co. vitis* feeding on grape yield even though the infestations were indicated to be substantial in spring (Králíková 1990; Hluchý and Pospíšil 1992). More recently, first Avgin and Bahadiroğlu (2004) and later Javadi Khederi et al. (2014b) detected a reduction in weight and diameter of berries, in yield, as well as in grape sugar content.

Summarizing the current data, they confirm the results previously reported by Gholami et al. (2005) and Javadi Khederi et al. (2014a) on the modest populations of GEM and the resulting mild effects on Shahani buds, leaves and canes in comparison with the other grapevine cultivars. Sahebi Uroomie also seems to be less affected by GEM. Biochemical plant defense mechanisms, possibly identifying genetic pathways by which the impact of GEM on grapevine could be reduced, has been recently investigated (Javadi Khederi et al. 2018). Further information on plant susceptibility is found in Dellei and Szendrey (1991) for Hungarian cultivars and Whitehead et al. (1978) for South African cultivars. Evaluating together all data collected in the current experiment, it may be supposed that the mite density applied with treatment III (about 1250–1500 mites per leaf—the applied amount in Table 1 for 5–6 leaves of the infested plant stage) represents the injury threshold to induce detectable effects to the grapevine, consistent with Javadi Khederi et al. (2014b) who noted that *Co. vitis* caused injury with 1720.3 mites per leaf on the most affected Gazne. Experiments should be extended to plants in production, in order to evaluate the mite impact on grape yield quality and quantity. The mite-grapevine interaction requires further evaluation concerning potential biomarkers related to the various plant responses, aimed at identifying the best GEM management procedures, including the choice of cultivars and clones.

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