

Article

Resistance of three grapevine cultivars to Grape Erineum Mite, *Colomerus vitis* (Acari: Eriophyidae), in field conditions

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Abstract

The Grape Erineum Mite (GEM), *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae), is a serious pest of vine (*Vitis vinifera* L.) in western Iran. Host plant resistance has not been explored as a method for its Integrated Mite Management. In this study, GEM-vine interactions and the biochemical features potentially related to vine resistance/sensitiveness have been investigated on Fakhri, Gazne and White Thompson seedless cultivated in field conditions in western Iran. The lowest and highest mite densities were found on leaves of White Thompson Seedless and Gazne, respectively. As expected, reduction of healthy leaf area, increase of leaf weight, shortening of shoots and erineum formation were more relevant on the most infested Gazne; White Thompson Seedless appeared to be affected by GEM infestation. The diameter of grape berries and their weight did not differ significantly between infested and control plants on all cultivars. Mite density appeared to be negatively related to the sugar content only for Gazne grape. The amount of the leaf waxes was highest in White Thompson Seedless on which the lowest mite density, the lowest percentage of erineum and largest healthy leaf areas were detected. Carbohydrate content of leaves was the lowest on the least mite-infested Thomson seedless and the highest on the most infested Gazne, while phenols decreased in Gazne after mite infestation.

Key words: Plant resistance, Erineum Mite, growth features, biochemical characters.

Introduction

Vine, *Vitis vinifera* L., is one of the most important crops in the world and is commercially produced in the western regions of Iran (Tafazoli *et al.* 1992).

Several insect and mite pests target vine during its growth period. Among them, *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae) affects vine production and growth in various countries with different levels of damage (Duso & de Lillo 1996; Avgin & Bahadiroğlu 2004; Bernard *et al.* 2005; Walton *et al.* 2007; Duso *et al.* 2010; Tomoioaga & Comsa 2010). *Colomerus vitis* was considered to comprise three morphologically similar, but biologically distinct strains: a bud-dwelling, a leaf gall-inducing erineum/blister (GEM), and a localized leaf curl strain (*cf.* Duso & de Lillo 1996). GEM feeds on the under-surface of leaves and elicits very obvious blisters on their

upper surface and white, later brown, hairy growths within the raised blisters on their lower surface. Bud mite may cause malformed leaves, aborted or damaged bunches, and even tip and bud death. Finally, the leaf curl strain was associated with leaf abnormalities and stunted and scarred shoots. Carew *et al.* (2004) distinguished the bud mite from the GEM as two distinct species based on the results of the analysis of the first internal transcribed spacer (ITS1) regions. GEM has wide distribution in vineyards of western Iran and it can cause damages on susceptible cultivars (Gholami *et al.* 2005a; Khanjani & Hadad Irani-Nejad 2009). To date, only its population dynamics were studied in the western part of the country (Gholami *et al.* 2005b).

Due to the hazards and adverse consequences caused by the use of chemical pesticides, more eco-friendly control methods required to be investigated. The selection and cultivation of crops more resistant to pests is one of the approaches persuaded. Resistant plants can efficiently oppose mite density increase (Delaney & Macedo 2001; Hochwender *et al.* 2005), affect body size and other phenotypic characteristics of the mites (Skoracka *et al.* 2002), such as eriophyid fecundity, which is influenced by quantity and quality of food sources (Bergh & Weiss 1993). Biochemical, physiological and morphological plant characters can act singly and synergically in order to reduce pest impact. As consequence, susceptibility of a host plant depends also by its enrichment in nutrients, especially carbohydrates and proteins (Sadasivam & Manickam 1992; Dhaliwal & Dilawari 1993).

Host-plants resistance to eriophyoid mites has been reported in some species such as black currant (Kiyazev 2006; Laugale 2007), coconut (Shalini *et al.* 2007), olive (Chatti *et al.* 2007; Mohiseni *et al.* 2011), wheat (Li *et al.* 2007) and in classical weed control (Smith *et al.* 2010).

Little information is available on vine resistance to eriophyids and, particularly, against GEM (Gholami *et al.* 2005a). Therefore, the present studies were carried out on a few of vine cultivars in order to identify plant features which could be involved in influencing resistance/susceptibility of Iranian-native vine to GEM in field conditions, and could be useful for a provisional and preliminary screening.

Material and methods

Research sites

The resistance of the different vine cultivars to GEM was recorded in experimental orchards during early April to late September 2010. These orchards were located in the Hamedan vicinity (Hamedan, Iran; 34°48'N, 48°28'E, 1830 m asl). Three common cultivars namely Fakhri, White Thompson seedless and Gazne were selected, monitored and sampled. These were grown in three separate 20 year old homogeneous orchards. They were separated from each other by a distance of at least 100 meters. The three plots of Gazne, Fakhri and White Thompson seedless cultivars, consisted of 250, 300 and 325 vines, respectively, planted at 1.5 × 2 m each. No fertilizer or acaricides were used during the experiments.

Evaluation of leaf area, weight and damage

Leaves were collected from two types of vines: Control vines without erinea and infested vines which had at least one to as many as 50–200 erinea on leaf surfaces. Leaves were collected from branches of previous season's growth of each vine in late summer during the mid-ripening growth stage of the berries (growth stage 35) according

to Eichhorn and Lorenz (1984) method. In each orchard, twenty vines were randomly chosen and five leaves from each them were collected per five tendrils, being the third one on the shoot from below. Leaf areas were measured by a planimeter (Kuiezumi AJP. Model).

Population density

Population densities were calculated by counting the mites presence on leaves coming from each of three cultivars. Leaf collection was carried out after the onset of budding of vines at growth stage 09 and in late summer during the mid-ripening growth stage of berries, growth stage 35 (Eichhorn & Lorenz 1984). The population density of each sample was calculated by washing and sieving method (de Lillo 2001; Monfreda *et al.* 2008). Twenty vines were randomly chosen from each plot, then 5 shoots and 5 leaves per vine were picked from them in each plot; therefore, each unit sample consisted of 100 shoots and leaves. Mites were, then, extracted from each pool, counted, slide-mounted according to Amrine and Manson (1996).

Shoot length and internode distance

Twenty vines were initially selected randomly from each plot and, then, a shoot was collected for each of the first five tendrils on the branches of the previous season's growth of the vine plants and measured from basis to apex in spring at growth stage 09 (Eichhorn & Lorenz 1984).

Crop rating

The vines under study were completely stripped of grapes at harvest and weight and diameter of berries were measured by a precision balance (readability of 0.001 g) and calliper respectively (Gholami *et al.* 2005a). Grape juice was extracted by crashing them and the sugar content of the juice was measured using a refract meter (0-32 Model) (Hluchý & Pospíšil 1992).

Leaf epicuticular waxes

Mid- and terminal-shoot leaves of each Control plant per each cultivar were randomly selected. This plant material was washed in distilled water and dried on filter paper. The cuticular waxes were extracted by immersing chopped fresh leaves in pure chloroform for 60 seconds (100 g leaf/100 ml chloroform). After removing the leaf material, the organic solvent was left to evaporate at room temperature under a fume hood and the wax residues were measured with a precision balance (readability of 0.001 g) according to Casado and Heredia (1999). Values in epicuticular wax equivalents were recorded using units of mg/100 g of fresh leaf weight.

Total soluble carbohydrates in leaves

Leaves were randomly selected among mid and terminal-shoot ones of each Control plant per each cultivar. A colorimetric method was applied to determine the total soluble carbohydrates according to Irigoyen *et al.* (1992). About 0.5 g of fresh leaves was treated with 0.1 ml of ethanol (83%). Then, the alcoholic extract was added to 3 ml of a fresh anthron solution (150 mg anthron + 100 ml sulfuric acid at 72%) and placed in a boiling water bath for 10 minutes. After cooling, the absorbance was measured at 625 nm by a UV-visible spectrophotometer (Carry model 100, Bio UV-Visible, USA) and compared to a curve of calibration. A calibration curve was obtained measuring the

absorbance of a series of standard solutions at 20, 40, 60, 80, 100 and 120 mg of glucose/l. Values in soluble carbohydrate equivalents were recorded using units of mg/g based on the following formula.

$$\frac{\text{mg}}{\text{g}} = \frac{\text{distillate (15 ml)}}{\text{leaf (0.5 g)}} \times \frac{\text{observed value}}{1000}$$

Total phenols in leaves

A colorimetric method was applied for determining the total phenolic content according to Singleton and Rossi (1965). Leaves were randomly selected from mid and terminal-shoot ones of infested and Control plants per each cultivar. About 0.5 g of fresh leaf lamina was powdered with methanol 85% on a pounder. The suspension was filtered on a filter paper, and 0.3 ml of it was taken and mixed with 1.5 ml of diluted Folin Ciocalteu reagent (1:10 with distilled water). After 8 minutes at room temperature, 1.2 ml of Na₂CO₃ 7% were added and the solution was placed on a Burrill shaker and treated for 90 minutes at room temperature and darkness. Sample absorbance was measured at 765 nm by a UV-visible spectrophotometer (Carry model 100, Bio UV-Visible, USA) and total phenols were compared to a curve of calibration and expressed as mg of gallic acid equivalents per 100 g of fresh leaf. A calibration curve was obtained measuring the absorbance of a series of standards solutions at 0, 4, 8, 16, 24 and 48 mg of gallic acid/100 ml.

Data analyses

All experiments excepting those regarding biochemical features were carried out according to a Complete Block random Design (PROC GLM, SAS Institute 2003). Data of biochemical features were carried out according to a complete random design with five replicates per each cultivar. Data of plant growth features were analyzed with t-test with 100 replicates per each cultivar using SPSS 13.0 (SPSS 2004). Standard error of differences (SE) was calculated for each treatment. Means were separated using the Duncan's multiple range tests at $P < 0.05$. All results, excepting those regarding plant growth features, were subjected to a one-way Analysis of Variance (ANOVA) using Proc GLM procedure of SAS (2003), after checking normal distribution and equal variance of data.

Results

Erinea leaf patches occurred in all assayed cultivars. Gazne had the highest percentage of affected leaves. There were differences in size of selected vegetative growth features in infested vs. Control vine cultivars (Table 1). In particular, the mites/symptoms had a more pronounced effect on the area and weight of the vine leaves, while influence on the internode length was not significant. Infested leaves were significantly smaller than control ones except for White Thompson seedless whose leaves did not show a significant reduction in the area of infested plants vs the control ones. In contrast, infested leaves of Gazne and Fakhri showed the highest decrease of leaf area in comparison with their controls. Similarly, infested leaves were always heavier than those collected from control plants and significant differences were observed on Fakhri and Gazne (Table 1). Gazne cultivar displayed the highest reduction of leaf weight in consequence of mite infestation.

Table 1. Differences in size of selected vegetative growth features in infested vs un-infested vine cultivars.

Cultivars	Leaf area (cm ²)	Leaf weight (g)	Internodes distance (cm)	Shoot length (cm)
Gazne (infested)	56.36±1.20b	2.32±0.01a	2.67±0.06a	36.5±1.50b
Gazne control	75.99±1.30a	1.63±0.02b	2.80±0.08a	58 ±1.70a
WTs (infested)	29.34±1.91a	0.72±0.07a	2.43±0.04a	51.7±1.21a
WTs control	35.64±1.74a	0.59±0.05a	2.81±0.02a	63.68±1.38a
Fakhri (infested)	50.49±1.69b	1.45±0.02a	3.25±0.08a	28.78±0.21b
Fakhri control	65.30±1.28a	1.03±0.02b	3.33±0.04a	50±0.21a

All measurements regard means (±SE). Within columns, means for infested vs. control plants of the same cultivar (e.g., infested Gazne and control Gazne) followed by different letters are significantly different (P <0.05, t-test).

WTs: White Thompson seedless

Shoot and internode lengths were slightly longer on the control plants than on infested ones. Significant differences between GEM infested and control plants were ascertained only for the length of the shoots on Fakhri and Gazne (Table 1). Even though the diameter of grape berries and their weight were lower in infested vines than in control one, no significant difference were detected (Table 2). The mean sugar content of leaves was less than controls but no significant difference were observed on infested plants in comparison with the Control ones in Fakhri and White Thompson seedless cultivars, on the contrary of infested Gazne cultivars (Table 2).

Table 2. Pair wise comparison between means of grape qualities, in three grape vine cultivars infested by GEM (for exp. infested Thompson compared to control Thompson).

Cultivars	Berry weight (g)	Berry diameter (cm)	Sugar (Brix)
Gazne (infested)	1.69±0.04a	12.79±0.32a	23.98±0.38b
Gazne control	2.04±0.10a	13.74±0.29a	25.67±0.54a
WTs (infested)	1.52±0.04a	11.54±0.14a	16.62±0.51a
WTs control	1.68±0.06a	12.25±0.22a	16.70±0.62a
Fakhri (infested)	2.12±0.07a	13.93±0.28a	14.17±0.38a
Fakhri control	2.29±0.04a	14.15±0.25a	15.72±0.28a

Means (±SE) are shown, those data in the same column, accompanied by similar alphabet letters, does not have a significantly difference among them (P <0.05, t-test).

WTs: White Thompson seedless

Densities of GEM population were largely and significantly dependent on the vine cultivars. The lowest mean number of juveniles and adults was recorded on White Thompson seedless while the most severe mite infestations were detected on Gazne at early spring (growth stage 09) and late summer (growth stage 035) (Table 3).

Table 3. Mite density (mean number juveniles and adults ±SE per leaf) on the assayed cultivars.

Vine cultivars	Gazne	White Thompson Seedless	Fakhri
GEM population growth stage 09	534.17±3.51a	49.11±3.72c	145.44±3.38b
GEM Population growth stage 035	1720.3±2.81a	85.5±3.23c	467.20±1.27b

Means in a rows followed by different letters are significantly different (P <0.05, Duncan's test).

White Thompson seedless leaves contained the significantly highest amount of epicuticular waxes and no significant differences were observed on leaf wax amount among Gazne and Fakhri (Table 4). Gazne displayed the highest amount of total carbohydrates contents of the leaves (Table 4). The amount of phenolic compounds in control leaves were largely similar among the assayed cultivars and no significant differences were observed among them (Table 4). When infested by GEM, phenol content of leaves was significantly lowest in Gazne (Table 4).

Table 4. Selected biochemical compounds (\pm SE) in leaves of assayed vine cultivars content means.

Cultivars	Waxes (mg/100 g)	Carbohydrates (mg/g)	Phenols in control vines (mg/ 100g gallic acid)	Phenols in infested vines (mg/100 g gallic acid)
WTs	0.13 \pm 0.03a	220.30 \pm 2.73c	0.12 \pm 0.02a	0.12 \pm 0.01a
Fakhri	0.11 \pm 0.01b	243.97 \pm 1.89b	0.12 \pm 0.03a	0.12 \pm 0.02a
Gazne	0.11 \pm 0.01b	295.42 \pm 4.60a	0.12 \pm 0.02a	0.10 \pm 0.02b

Within columns, means followed by different letters are significantly different (P <0.05, Duncan's test).

WTs: White Thompson seedless

Discussion and conclusions

Reduction of leaf surface, leaf weight increase, shoot shortening and erineum formation seemed to be strictly related to the mite density and were more relevant on Gazne and Fakhri cultivars, while White Thompson seedless seemed to be scarcely influenced by GEM infestation. On the contrary, internode length was very scarcely affected by *C. vitis* and, consequently, the strain should definitely belong to the erineum formation and have little effect on internod length (Duso & de Lillo 1996). Several other possible explanations were proposed in literature to explain the shortening of the canes, including boron deficiency, low carbohydrate reserves, and damage from thrips or other arthropods like bud and rust mites, and it can be induced by high activity of the vine mites on the nodes (cfr. Duso and de Lillo, 1996). In general, plant feeding (distortive and non-distortive) effects are the result of the Eriophyoid cell piercing and injection of saliva into the protoplasm whose fine pathway in re-directing the plant physiology and cell development has not been clearly established yet (Oldfield 1996; de Lillo & Monfreda 2004; Monfreda & Spagnuolo 2004; Petanović & Kielkiewicz 2010). Leaf erineum mites, like GEM, are actually well known to induce cell re-differentiation, which alters size and chemical content of the infested organs (Petanović & Kielkiewicz 2010).

Current observations pointed out that grape berry weight was not significantly different between infested and control plants in all selected cultivars, and this is consistent with the results of Hluchý and Pospíšil (1991), and Králíková (1990) investigations on other cultivars in other areas.

The current study showed that sugar content of the grape juice of the Gazne cultivar was lower than that of its control and this is in accordance with Avgin and Bahadiroğu (2004) who stated that GEM was able to affect the sugar content and have an impact to economic value of the yield. Similarly, infestations of apple rust mite decreased the weight of fresh fruits of Jonagold but did not affect the Golden delicious (Spieser *et al.* 1999).

The effects induced by the different cultivars on specific biological parameters of GEM (*i.e.*, oviposition rate, life cycle duration, longevity, etc.) could express differences in host acceptance and they should be investigated for a wider consideration

of host resistance/sensitiveness, extending the observations for more successive generations in order to take in count of possible physiological adaptations of the mite to the cultivars in the long-term period (McIntyre & Whitham 2003). A few previous studies demonstrated that quality of the food sources can influence the fitness (survival rates, developmental duration, egg laying and other parameters) of a few Eriophyoids of economic interest (Kozłowski & Boczek 1987; Bergh & Weiss 1993; Kozłowski 1995; Easterbrook & Palmer 1996; Gonçalves *et al.* 1998; Wang *et al.* 2008).

Based on the results of the current research, the amount of leaf waxes was clearly the highest in White Thompson seedless cultivars, which hosted the lowest mite density, showed the least percentage of erinea induction on infested leaves and the largest leaf area (in the two planned samplings). These data are in accordance with those by Howe and Schaller (2008) who stated that epicuticular waxes of the plant surfaces could affect the feeding behaviour of herbivores and physically prevent their movement across the leaf surface. So, wax compounds could interfere with mite feeding and, consequently, affect their survival rates, developmental duration, egg production and other biological parameters. In short, wax layer can decrease in some way the phytophagous life performances and it might be a valuable indicator for screening resistant cultivars. This can find relevance taking in count that Eriophyoids pierce the cuticle and the epidermal cell walls by means of their short stylets, combining this action along with the injection of saliva into the cells, or, as few researchers believe, stylet piercing follows the digestion of the epidermal cell surface components by means of mite salivary secretion (Schmeits & Sassen 1978; Thomsen 1988; Hoy 2011). The interference of the wax layer with cell piercing and, actually, the thickness of the leaf cuticle have been already related to the resistance of the pigeonpea, *Cajanus cajan* (L.) Millsp., to *Aceria cajani* Channabasavanna due to the obstacles found in stylet penetration up to cell protoplasts (Reddy *et al.* 1995).

Total soluble carbohydrates and phenols content were highly correlated to the mite impact on the assayed cultivars. In particular, the mite appeared to affect more leaves on cultivars with higher carbohydrate content. Actually, carbohydrate amount in control leaves was the highest on the largely infested Gazne while phenols decreased in those of Gazne after mite infestation.

Similarly, lower contents of reducing sugars and water-soluble sugars were observed also in shoots of tea varieties resistant to *Acaphylla theae* (Watt) and this was supposed to have antibiosis influence (Ning *et al.* 1996). It is well known that carbohydrates along with proteins are highly required as nutrients by herbivore arthropods (Bernays & Chapman 1994), that the plant susceptibility might be related to the richness of some cues, mainly carbohydrates and proteins (Sadasivam & Manickam 1992; Dhaliwal & Dilawari 1993) and that changes in their content in the hosts plants can have relevant implications on the success of the phytophagous organisms (Shi & Tomczyk 2001).

The mechanisms that Eriophyoids apply to induce modifications in plant phenol profile are still unclear and, also, the studies on other biochemical responses of the host plants to the Eriophyoid feeding are scarce (Royalty & Perring 1996; Shi & Tomczyk 2001; Petanović & Kielkiewicz 2010). However, the increase of phenols after mite infestation was recorded in olives fruits infested by *Aculus olearius* Castagnoli and *Aceria oleae* (Nalepa) (Cetin *et al.* 2011), in lemon buds infested by *Aceria sheldoni* (Ewing) (Ishaaya & Sternlicht 1969), in leaves of blackberry cultivars infested by

Epitrimerus gibbosus (Nalepa) (Shi & Tomczyk 2001), but its fine relationship with resistance of plants to the mite species has not always confirmed.

The present study showed differences in responses to GEM attack of susceptible and resistant vine cultivars. Our results suggested that biochemical characters of investigated vine cultivars (total soluble carbohydrates, total phenol compounds and wax content in the leaves) could be used as marker traits to select vine for resistance to GEM. Finally, the current investigation showed that the impact of erineum strain of *Colomerus vitis* on vine yield was not very significant, although the Gazne cultivar showed the higher susceptibility than other ones.

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مقاومت سه رقم انگور به کنه نمدی برگ مو، *Colomerus vitis* (Acari: Eriophyidae) در شرایط مزرع‌ای

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چکیده

کنه نمدی برگ مو (*Colomerus Vitis* (Pagenstecher) (Acari: Eriophyidae) یکی از عوامل محدودکننده تولید انگور در غرب ایران است. مقاومت گیاهی به عنوان یک روش برای مدیریت تلفیقی این آفت مورد بررسی قرار نگرفته است. بنابراین مقاومت سه رقم انگور رایج در غرب ایران به کنه نمدی برگ مو به منظور تعیین پتانسیل کاربرد ارقام مقاوم گیاه در برنامه‌های مدیریت این آفت مورد بررسی قرار گرفت. در این مطالعه، ارتباط متقابل میان ویژگی‌های بیوشیمیایی و ریخت‌شناسی سه رقم انگور شامل فخری، گزنه و کشمش بی‌دانه سفید و مقاومت آن‌ها در شرایط مزرع‌ای در غرب ایران مورد ارزیابی واقع شد. مشاهدات نشان دادند که کشمش بی‌دانه و گزنه به ترتیب کم‌ترین و بیش‌ترین تراکم کنه را بر روی برگ‌ها داشتند. همان‌طور که انتظار می‌رفت، کاهش سطح برگ، افزایش وزن برگ، کاهش طول شاخه و تشکیل نمد ارتباط تنگاتنگی با میزان آلودگی با کنه داشت.

در حالی که خصوصیات ریخت‌شناسی کشمش بی‌دانه سفید به ندرت تحت تأثیر آلودگی کنه نمدی قرار گرفت، رقم گزنه بیشترین علائم را نشان داد. تحلیل‌های آماری نشان دادند که قطر و وزن حبه‌های انگور آلوده به کنه نمدی و شاهد تفاوت معنی‌داری با یکدیگر نداشتند. نتایج نشان دادند که این کنه می‌تواند میزان قند رقم‌های حساس انگور، همانند رقم گزنه را کاهش دهد. بیشترین میزان موم برگ بر روی رقم کشمش بی‌دانه سفید مشاهده شد که کمترین تعداد کنه، کمترین تعداد نمد و بیشترین سطح برگ را داشت. کمترین میزان ترکیبات کربوهیدراتی در رقم کشمش بی‌دانه و بیشترین میزان آن در رقم گزنه که بیشترین آلودگی را به خود اختصاص داده بود مشاهده شد، در حالی که میزان ترکیبات فنولی در رقم گزنه پس از آلودگی به این آفت کاهش یافت.

واژگان کلیدی: مقاومت گیاهان، کنه نمدی، ویژگی‌های رشدی، خصوصیات بیوشیمیایی.

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