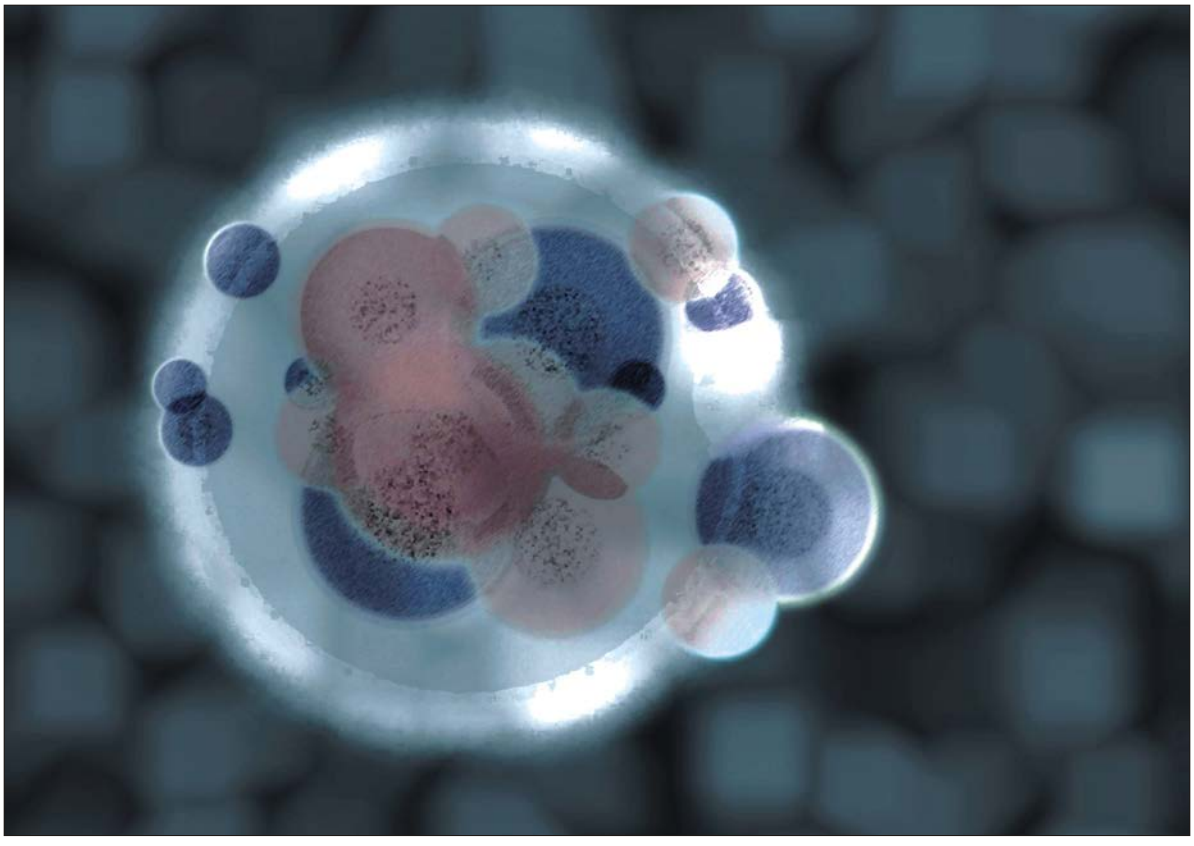

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Responses of Grapevines to Two-Spotted Spider Mite Mediated Biotic Stress

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Abstract: The effect of feeding damage by two-spotted spider mite (*Tetranychus urticae* Koch.) on leaf-level physiological characteristics of grapevines was investigated. Uniform plants (*Vitis vinifera* L., cvs. Muskule and Sultana) with unbranched solitary shoot having about five fully expended mature leaves were artificially infested with the mites (100 mites per leaf). After seven days of infestation, grapevine cultivars significantly differed in their support to mite density. Muskule showed sensitive characteristics by supporting a higher population density (240.75 mites per leaf) than Sultana (192.59 mites per leaf). Hence, the percentage of electrolyte leakage and cell membrane injury was much higher in the infested leaves of Muskule as compared to Sultana. In addition, mite feeding induced lipid peroxidation and protein degradation was observed only in Muskule. The relative chlorophyll content and photosynthetic activity in infested leaves of each cultivar was significantly lower than that of uninfected ones. Although, transpiration was not significantly altered in the infested leaves of grapevine, mite feeding caused a significant reduction in the leaf water content of both cultivars. There was a significant decrease in the levels of soluble sugar of the infested leaves in both cultivars as compared to the uninfected ones. When comparing the two cultivars, mite damage concerning above parameters was more striking in the leaves of Muskule than that of Sultana ones. Thus, proline, as a sensitive indicator signaling biotic stress intensity, accumulated in the infested leaves of both cultivars, especially in Muskule. Although, no change was observed in Ca, Mg, Mn and Fe concentrations, Na concentration of infested leaves significantly rose in both cultivars. In addition, mite attack significantly increased K, Zn and Cu uptake only in the leaves of Muskule plants.

Key words: Biotic stress, *Tetranychus urticae* Koch., *Vitis vinifera* L.

INTRODUCTION

The increase in the use of nitrogen and potassium fertilizers and non-selective pesticides in viticulture promoted outbreaks of spider mites, which were previously known as occasional grapevine pests (Rilling, 1989). Together with the European red mite, *Panonychus ulmi* Koch., the two-spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae), is considered to be the most important pest of grapevines in Europe (Candolfi *et al*., 1992a). It is also the major spider mite pest of grapevines in dry summer regions of Europe (Schruft, 1985), being especially significant in Aegean Region of Turkey (Altincag and Akten, 1993).

T. urticae over-winter as mated adult females and can be observed on fallen foliage, winter weeds and under the bark of vines during the winter and early spring months (Schruft, 1985). In Europe, grapevines are not colonized until summer and infestation can generally occurs with the drying out of the undergrowth caused by the weather (Pringle *et al*., 1986). When two-spotted spider mites start to feed on the undersurface of grapevine leaves, the mesophyll tissue collapses and a small chlorotic spot forms at each feeding site. Continued feeding causes a stippled-bleached effect and later, the leaves turn yellow, gray or bronze. If bronzing and defoliation occurs early enough in the season, a negative effect on fruit ripening may occur as feeding may interfere with the normal photosynthetic process of the leaves. Fruit clusters may also be attacked, resulting in dark spots on the skin (Schruft, 1985; Pringle *et al*., 1986; Flaherty and Wilson, 1988; Prischman *et al*., 2002).

Tetranychus urticae infestation represents potential biotic stress to its host plant and adversely affects many physiological and biochemical processes, thereby reducing growth and yield in grapevines (Pringle *et al*., 1986; Prischmann *et al*., 2002). These reactions to mite infestation can be understood only on the basis of the

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mutual interactions between mites and their host plants. Although, much information on the changes in the host plant after herbivore feeding has now been accumulated, the mechanism of the mutual interactions between pest and host plant is not sufficiently well known (Tomczyk and Kropczynska, 1985). Accordingly, there is only scarce information on the impact of two-spotted spider mites on grapevine physiology. Reduction in the net CO_2 assimilation, transpiration as well as stomatal and mesophyll conductance as a result of two-spotted spider mite feeding has been shown in grapevines (Candolfi *et al*., 1992b). Recently, Van den Boom *et al*. (2004) reported that *T. urticae* infested grapevine leaves released several novel compounds as semiochemicals (infochemicals) that dominated the blend. One of the major component of these compounds was an unusual acyclic C_{11} homoterpene, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), which was also emitted by many other species following herbivore damage. In absolute amounts, the compound was produced about 450 times more abundantly relative to uninfected grapevines.

However, these results are not enough to adequately understand the physiological responses of grapevines to feeding damage by the two-spotted spider mite. A thorough understanding of pest ecology and host plant physiology is necessary to understand host-pest relationships and host resistance mechanisms. This is especially important for elaborating integrated pest management strategies in agriculture and reducing yield loss. Losses due to insect herbivores, estimated at 10-20% for major crops, are a significant factor in limiting food production (Ferry *et al*., 2004).

The objective of the present study was to investigate the effect of two-spotted spider mite infestation on leaf-level physiological characteristics of grapevines.

MATERIALS AND METHODS

The study was conducted at the University of Uludag during March-November 2008. One-year-old rooted cuttings of Sultana and Muskule (*Vitis vinifera* L.) were planted in 2.5 L pots containing a mixture of soil, sand, sphagnum peat and farmyard manure (2:1:1:1 v/v). Plants were grown in a climatic room on a 16 h light (27±1°C, 350±50 μmol/m^2/sec)/8 h dark (18±1°C) cycle supplemented with white lamps. Plants were irrigated every third day with tap water and fertilized fifteen day intervals with a commercial water-soluble fertilizer containing macro and micro nutrients (Rocket 20-20-20 NPK, Turkey).

Uniform plants with newly developed unbranched solitary shoot (approximately 0.5-0.6 m long with about five fully expended mature leaves) having about equal leaf area were selected. Three days prior to the experiment selected plants were transferred to a climate room (at 25±1°C, a 16/8 h light regime with 500 μmol/m^2/sec and 60±5% relative humidity) and placed in two compartments in order to disconnect infested and uninfected plants.

The plants were artificially infested with *T. urticae*. The mites were reared on bean plants (*Phaseolus vulgaris*) under the same climatic conditions in a separate growth chamber. The plants were infested with 100 mobile mites (deutonymphs and adult females) per leaf that is 500 mites per plant. Mobile mites were transferred with a soft-bristle paintbrush from the bean plants onto grapevine leaf discs. The infestation was performed by placing these discs on each expanded grapevine leaves. Uninfected plants served as the control.

Mite populations were allowed to increase in numbers naturally. The measurements were performed seven days later when the visual symptoms of chlorosis appeared markedly on the infested leaves. Randomly selected ten leaves (attached to the plants) of each replicate in each treatment were labeled and net photosynthesis rate and transpiration were measured between 11:00 and 13:00 h using a photosynthetic rate analyzer (LI-6400, LiCor, USA). The relative chlorophyll content was determined in the same set of leaves with a portable leaf chlorophyll meter (SPAD 502, Minolta Co. Ltd., Japan). Another 15 randomly selected leaves were harvested from each replicate in each treatment in order to determine *T. urticae* population density. The mites on both upper and lower surfaces of each leaf were swept into a test tube using a soft-bristle brush. The number of mobile mites was counted using a dissecting stereo microscope with a magnification of 10 times and a hand counter for a final estimate of mites per leaf. Non-infested leaves were also inspected.

The solute leakage of grapevine leaves was assessed to study the extent of membrane damage due to two-spotted spider mite feeding. The electrolyte leakage was determined as described by Masood *et al*. (2006) with some modification. Briefly, leaf samples (500 mg) were cut into pieces of 5 mm length and placed in test tubes containing 20 mL distilled deionized water. The tubes were incubated in a water bath at 32°C for 2 h and the initial electrical conductivity of the medium (EC_1) was measured. The samples were autoclaved at 121°C for 20 min to release all electrolytes, cooled to 25°C and the final electrical conductivity (EC_2) was measured. The electrolyte leakage was calculated as EC_1/EC_2 and expressed as a percentage. Cell membrane stability was computed on the same samples used for electrolyte leakage and as

indicated by Shibli *et al*. (2007). Stability was expressed as:

$$\text{Injury (\%)} = 1-(1-E_1/E_2)/(1-E^*_1/E^*_2)$$

where, E* is the electrical conductivity of the control sample.

The degree of membrane damage was also indicated by the degree of lipid peroxidation. Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1 g of leaf fresh weight according to Madhava Rao and Sresty (2000). MDA is a product of lipid peroxidation by the thiobarbituric acid reaction. The concentration of MDA was calculated from the absorbance at 532 nm (correction was made by subtracting the absorbance at 600 nm for non-specific turbidity) by using extinction coefficient of 155 mM^{-1} cm^{-1}.

A modification of the leaf water content may have consequences on plant development and thus may be an indicator of mite injury-induced biotic stress. The water content of leaves was calculated from the final fresh and dry mass of leaves. Dry matter of leaves was obtained by heating at 70°C to a constant weight.

For protein, proline and soluble sugar analyses, triplicate samples of leaf tissue were frozen immediately in liquid nitrogen and stored at -20°C until they were analyzed. Protein was assayed using the Bradford Assay (Bradford, 1976) with bovine albumin serum as a standard. Proline determination was carried out according to the method of Bates *et al*. (1973) using 0.5 g frozen leaf samples. Soluble sugar were extracted and determined according to the methods described by Saladin *et al*. (2003) and results were expressed in mg glucose equivalents $(g\ DW)^{-1}$.

To determine the mineral composition, previously dried and ground material was dry ashed at 540°C for 6 h. Ion extraction was achieved in 65% HNO_3. A Unicam (Model 929 AA) flame atomic absorption instrument was used for the determination of Cu, Zn, Mn, Fe, Mg, Na, Ca and K in sample digests.

In order to eliminate surface contamination, all the leaves sampled for above chemical analyses were carefully washed with tap water and rinsed in deionized water.

The experiments were set up in a completely randomized design. Each treatment included three replicates (with 6 plants in each replicate). Analysis of variance was performed on the data and significant differences among treatment means calculated by LSD test at $p<0.05$.

RESULTS

The numbers of two-spotted spider mite mobile forms significantly elevated in the stressed plants of both cultivars during the 7 day experimental period (Table 1). However, the increase in the mite density was significantly differed in terms of cultivars. The initial population of 100 mites rose to 240.75 mites per leaf in Muskule while it was remained limited by 192.59 mites per leaf in Sultana.

Electrolyte leakage (membrane permeability) was significantly increased with mite feeding and it was accompanied by a drastic augmentation in cell membrane injury (Table 1). However, the percentage of solute leakage and membrane injury was much higher in Muskule as compared to Sultana.

After 7 days of mite infestation, in Sultana, the MDA content increased slightly, but not significantly ($p>0.05$). In contrast with the results obtained from Sultana, mite feeding led to a significant increase in MDA content in the leaves of Muskule.

Leaf-sucking mite feeding induced membrane damage is accompanied by metabolic disturbances in the leaves of grapevines. The relative chlorophyll contents in the leaves of both cultivars reduced after attacked by two-spotted spider mites (Table 1). However, the reduction in chlorophyll content of attacked leaves relative to their controls was more striking in Muskule than Sultana vines.

Table 1: Changes in the mite population, membrane damage, lipid peroxidation, chlorophyll content, photosynthetic rate and transpiration in grapevines infested with two-spotted spider mite

Grapevine cultivars	*T. urticae* infestation	Total mite No./leaf	Electrolyte leakage (%)	Cell membrane injury (%)	MDA content (nmol g^{-1})	Relative chlorophyll content (SPAD)	Photosynthetic rates (mmol CO_2 m^{-2} sec^{-1})	Transpiration (mmol m^{-2} sec^{-1})
Muskule	Infested	240.75a*	54.67a	21.87a	36.82a	27.62c	22.75c	2.17
	Uninfected	0.00c	41.51d	0.00c	21.42b	37.27a	30.76a	2.21
Sultana	Infested	192.59b	48.82b	8.27b	25.63b	26.10c	24.54c	1.88
	Uninfected	0.00c	45.09c	0.00c	21.94b	32.66b	27.22b	2.03
ANOVA								
Cultivar (A)		**	**	**	**	**	ns	**
Infestation (B)		**	**	**	**	**	**	ns
A×B		**	**	**	**	**	**	ns

*Values not associated with the same letter(s) are significantly different ($p<0.05$); **Significant at 0.05 level; ns: Not significant

Table 2: The effect of *T. urticae* feeding on leaf water content, total soluble protein, sugar and proline contents in the leaves of grapevine

Grapevine cultivars	*T. urticae* infestation	Leaf water content (%)	Protein (mg μL^{-1})	Proline (μmol g^{-1} f.w.)	Soluble sugar (mg g^{-1} d.w.)
Muskule	Infested	68.36c	0.138c	53.66a	56.16
	Uninfected	75.92a	0.165b	8.04c	59.84
Sultana	Infested	71.97b	0.180a	51.47a	56.42
	Uninfected	76.33a	0.164b	12.26b	61.87
ANOVA					
Cultivar (A)		**	**	ns	ns
Infestation (B)		**	ns	**	**
A×B		**	**	**	ns

*Values not associated with the same letter(s) are significantly different ($p<0.05$); **Significant at 0.05 level; ns: Not significant

In plants attacked with two-spotted spider mites, there was a significant decrease in photosynthesis of both grapevine cultivars. However, when compare to infested and uninfected leaves in each cultivar, more severe reduction in photosynthetic rate was observed in Muskule grapevine cultivar. On the other hand, *T. urticae* feeding for 7 days did not significantly alter transpiration in the leaves of both grapevine cultivars (Table 1).

Infestation of grapevine leaves with two-spotted spider mites which feed on the lower epidermis with piercing-sucking mouthparts caused a significant reduction in their water contents (Table 2). There was no significant difference between the cultivars in terms of water content of uninfected leaves. However, mite feeding induced water loss was more severe in the leaves of Muskule than in Sultana ones.

Regarding the protein content, grapevine cultivars showed different tendency in their response to two-spotted spider mite attack (Table 2). Mite feeding had a positive effect on protein content in the leaves of Sultana grapevine cultivar whereas, it induced significant protein degradation in the leaves of Muskule plants.

There was a significant decrease in the level of soluble sugar of the infested leaves in both cultivars as compared with uninfected ones (Table 2). However, mite infestation caused soluble sugar reduction was not affected by genotypic difference in grapevines. In contrast, 7 days mite attack stimulated an accumulation of proline in the leaves of both cultivars (Table 2).

At the nutrient level, Ca content showed an increase in the leaves of Muskule and a decrease in Sultana after seven days of infestation, which were not statistically significant (Table 3). The K content of leaves in infested plants of Muskule rose significantly, whereas they remained fairly constant in Sultana compared to the control (Table 3). However, mite feeding encouraged an accumulation of Na ions in the leaves of both grapevine cultivars (Table 3). Similarly, infested groups of both cultivars accumulated more Zn and Cu ions in their leaves compare to uninfected ones (Table 4). In fact, the positive effect of mite infestation on Zn and Cu accumulation was statistically significant only in Muskule. Moreover, infestation of grapevine leaves with two-spotted spider mites did not significantly influence Mg, Mn and Fe contents (Table 3, 4). It is worthy to note that Mg, Mn and Fe contents of leaves significantly varied by cultivars in such a way that Sultana leaves had higher ion concentrations than the leaves of Muskule vines.

Table 3: The effect of *T. urticae* feeding on Ca, K, Mg and Na contents (mg g^{-1} d.w.) in the leaves of grapevine

Grapevine cultivars	*T. urticae* infestation	Ca	K	Mg	Na
Muskule	Infested	41.80ab	18.87b	3.59	1.23
	Uninfected	37.49b	16.11c	3.74	1.09
Sultana	Infested	40.69ab	19.46ab	3.89	1.41
	Uninfected	45.62a	20.50a	3.98	1.15
ANOVA					
Cultivar (A)		ns	**	**	ns
Infestation (B)		ns	ns	ns	**
A×B		**	**	ns	ns

*Values not associated with the same letter(s) are significantly different ($p<0.05$); **Significant at 0.05 level; ns: Not significant

Table 4: The effect of *T. urticae* feeding on Mn, Zn, Fe and Cu contents (mg kg^{-1} d.w.) in the leaves of grapevine

Grapevine cultivars	*T. urticae* infestation	Mn	Zn	Fe	Cu
Muskule	Infested	215.37	151.78a	111.84	9.18a
	Uninfected	212.93	131.65c	111.60	6.44c
Sultana	Infested	234.73	146.95ab	131.05	7.68b
	Uninfected	258.98	141.65b	128.25	7.16bc
ANOVA					
Cultivar (A)		**	ns	**	ns
Infestation (B)		ns	**	ns	**
A×B		ns	**	ns	**

*Values not associated with the same letter(s) are significantly different ($p<0.05$); **Significant at 0.05 level; ns: Not significant

DISCUSSION

It is evident from the results that *T. urticae* infestation resulted in a significant increase in the mite density feeding on the infested leaves of both grapevine cultivars (Table 1). Muskule and Sultana plants, initially infested with 100 mites per leaf, were existed 2.41 and 1.93 times higher mite population per leaf than at the beginning, respectively. *Tetranychus urticae* mites are vigorous, multiplying both sexually and asexually and completing their life cycle within 6-12 days on the grapevine leaves (Walsh, 2001; Sekhar *et al*., 2008). According to WenJuan and DaHan (2006), the double

population time of *T. urticae* reared (at 21-28°C) on grapevine leaves were 3.35 days, in the laboratory. However, grapevine cultivars significantly differed in their support to mite density. Muskule showed sensitive characteristics by supporting a higher population density than Sultana. Earlier studies indicated that increases or decreases in population density of tetranychid mites are associated with the tissue quality of host plant, which does not depend only on the level of primary plant metabolites, but also on the quantity and nature of secondary metabolites (Lege *et al*., 1995; Van den Boom *et al*., 2003).

Tissue relative electrolyte leakage and cell membrane stability has long been used to assess the development of membrane damage in crop plants induced by various abiotic stress conditions. Moreover, this study has also shown that *T. urticae* induced biotic stress significantly increased the membrane damage in both grapevine cultivars (Table 1). However, the percentage of solute leakage and cell membrane injury was significantly higher in Muskule as compared to Sultana. In crop plants, these two parameters have been used as criteria to distinguish stress tolerant and susceptible cultivars. In addition, the higher solute leakage with more membrane injury utilized to specify the lower tolerance to certain abiotic stress (Masood *et al*., 2006; Shibli *et al*., 2007). Therefore, Muskule could be classified as a sensitive grapevine cultivar to two-spotted spider mite feeding due to higher solute leakage and membrane injury.

It is well known that biotic stress can cause oxidative stress due to the over production of Reactive Oxygen Species (ROS) (Tomczyk, 2001; Mithöfer *et al*., 2004; Leitner *et al*., 2005; Khattab, 2007). These cytotoxic ROS can seriously disrupt normal metabolism through peroxidating lipids, denaturing proteins and nucleic acids. Lipid peroxidation causes degradation and impairment of structural components. Thus, free radical-induced peroxidation of lipid membranes is both a reflection and a measure of stress-induced cellular damage (Jain *et al*., 2006). It is clear from the results that *T. urticae* feeding induced alterations in the lipid peroxidation is the sign of genotypic difference of grapevine (Table 1). Thus, mite feeding induced lipid peroxidation was observed only in Muskule. Insignificant MDA increases found in the infested leaves of Sultana suggests a better protection from *T. urticae* stimulated oxidative stress.

Several studies have reported that chlorosis is the most obvious injury symptom of leaves damaged by two-spotted spider mites and is indicative of chlorophyll loss (Landeros *et al*., 2004; Reddall *et al*., 2004). In the present study, after 7 days of mite feeding, chlorophyll content of both cultivars dropped very drastically (Table 1). However, the level of reduction in chlorophyll content caused by the mites was more striking in Muskule. This genotypic difference might have been resulted from the different mite load of grapevine cultivars reached at the end of the study. In fact, Landeros *et al*. (2004) reported that the decrease in chlorophyll content of rose plants was correlated with the *T. urticae* population density. On the other hand, the decrease in the chlorophyll content may be due to the mechanical damage to the chloroplast during the infestation of mites and to the effect of reactive oxygen species mediated lipid peroxidation on chlorophyll pigments (Tomczyk and Kropczynska, 1985; Khattab, 2007).

As observed in the chlorophyll content, *T. urticae* mediated biotic stress significantly decreased photosynthetic rate of both grapevine cultivars (Table 1). However, the inhibiting effect of mite feeding on photosynthetic rate was more marked in Muskule than in Sultana plants. The data indicated that higher mite density coincided with a striking decrease in the photosynthetic rate of Muskule as observed in chlorophyll content. In addition, transpiration was slightly but not significantly lower in the infested leaves of each cultivar than in uninfected ones (Table 1). A reduction in the rate of photosynthesis and transpiration is a primary response to spider mite infestation and it has been reported many plants including grapevine (Candolfi *et al*., 1992b), rose (Landeros *et al*., 2004), cotton (Reddall *et al*., 2004) and strawberry (Klamkowski *et al*., 2006). Earlier studies indicated that the decrease in assimilation of CO_2 was proportional to mite population density and duration of feeding (Candolfi *et al*., 1992b) and was also correlated to the chlorophyll content of leaves (Tomczyk, 2001; Landeros *et al*., 2004). According to Candolfi *et al*. (1992b), the decrease in the net assimilation rate of grapevine by mite feeding was limited both by the CO_2 entry through the stomata and by its transport through the mesophyll cells and fixation in the chloroplast. Moreover, Klamkowski *et al*. (2006) stated that mechanical destruction of the leaf tissue by spider mites could also affect photosynthesis. The number of photosynthetically active leaf cells that are punctured and emptied per mite was calculated as 100 min^{-1}. In gut content studies of two-spotted mites, it was observed that there were only thylakoid granules inside their digestive tract following feeding. The thylakoid grana on which *T. urticae* focus their feeding are the key photosynthetic engines in plant cells (Walsh, 2001).

Although, transpiration was slightly lower in infested grapevine leaves, leaf water content was significantly reduced by mite feeding (Table 2). The results obtained from both grapevine cultivars corroborate similar

findings in *T. urticae* infested leaves of peppermint plants (De Angelis *et al*., 1982). Furthermore, De Angelis *et al*. (1982) showed that feeding injury by the two-spotted spider mite significantly increased nigh-time (cuticular) transpiration in affected leaves of peppermint and this resulted day-time water stress. The increase in the rate of dark respiration also observed in *T. urticae* infested leaves of chrysanthemum, bean and cucumber compare to uninfected leaves of these plants (Tomczyk, 2001). Water deficiency probably seems from disruption of leaf epidermal cell and cuticle by the probing action of the mite chelicerae during feeding (De Angelis *et al*., 1982). According to Walsh (2001), another possibility of water deficiency could be a big drain from the filter-feeding of two-spotted spider mites on the lower epidermis with piercing-sucking mouthparts. In essence, spider mites filter feed the water and nutritious contents from leaf cells. At the microscopic level, significant quantities (relative to mite size) of plant fluid pass through the digestive tract of spider mites during feeding. This volume estimated as 1.2×10^{-2} μL/mite/h. This quantity represents roughly 50% of the mass of an adult female spider mite. It has been noticed that water loss increases with increasing mite damage (Tomczyk and Kropczynska, 1985). In the current study, Muskule had lower water content than Sultana in response to higher mite density.

The leaf-sucking mite feeding continually controls and/or modifies the metabolic substances levels of the surrounding tissues. This is supported in the current work where the soluble protein contents of leaves significantly changed by two-spotted spider mites (Table 2). However, the effect of *T. urticae* feeding on the protein content of grapevine varied depending on the cultivars. In Sultana mite infestation raised soluble protein content of leaves while it induced significant protein degradation in the leaves of Muskule plants. Soluble protein content, as one of the oxidative stress indicators, was reported to decrease in plants subjected to water stress (Sharma and Dubey, 2005). In the current study, Muskule had significantly lower water content than Sultana in response to higher mite density in addition to mite feeding induced lipid peroxidation. These results suggest that protein degradation in the infested leaves of Muskule might be due to the *T. urticae* induced water and oxidative stress.

After 7 days of mite infestation, the levels of soluble sugar of leaves were lower in both grapevine cultivars (Table 2). This effect of mite feeding on soluble sugar content was also reported in chrysanthemum, bean and cucumber plants (Tomczyk, 2001). Such effect might be due to the drain of assimilates towards the mites and/or decrease in their photosynthesis induced by mites.

On the other hand, free proline content in infested leaves of both grapevine cultivars was greater than that of uninfected ones (Table 2). Similar response has been reported in tomato leaves in response to carmine spider mites (Kielkiewicz, 2005). However, the level of proline accumulation in response to two-spotted spider mite varied depending on the cultivar. Mite infestation caused an increase in proline contents by 6.7 and 4.2 fold in the leaves of Muskule and Sultana, respectively. Furthermore, the mite density, membrane damage and leaf water content was closely related to the proline accumulation in the infested leaves of grapevine cultivars. The rapid increase in mite population on the leaves of Muskule with higher membrane injury and lower water content might be explained as the result of the predisposition of this cultivar, indicated by the high proline content. However, a slower increase in *T. urticae* population density, lacking lipid peroxidation with less membrane damage and water deficiency accompanied with lower proline content in the leaves of Sultana plants. Therefore, the concentration of proline in T. *urticae* infested leaves of grapevine might be considered a highly sensitive indicator signaling biotic stress intensity.

Tetranychus urticae feeding for 7 days did not significantly alter Ca, Mg, Mn and Fe contents of leaves in both grapevine cultivars (Table 3, 4). However, mite feeding induced Na accumulation was observed in the leaves of each cultivar (Table 3). In addition, mite attack significantly raised K, Zn and Cu uptake only in the leaves of Muskule plants (Table 3, 4). To date, the data on the relationship between mite feeding and changes in the mineral composition of leaf tissue are scarce. In the current study, accumulated inorganic ions could be contributing to osmotic adjustment in the leaves of grapevine due to *T. urticae* induced water stress. The role of ion accumulation in osmotic adjustment was also observed in drought-stressed grapevine (Patakas *et al*., 2002). On the other hand, Poschenrieder *et al*. (2006) has recently been suggested that plants absorb high concentrations of metals such as Cu and Zn from the substrate as a self-defense mechanism against herbivores. On a molecular basis, metal defense against biotic stress seems to imply common and/or complementary pathways of signal perception, signal transduction and metabolism. However, more studies are needed to shed light on this hypothesis.

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