African Journal of Biotechnology Vol. 10(57), pp. 12195-12201, 28 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2038
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effects of bud loading levels and nitrogen doses on yield, physical and chemical properties of brined grape-leaves

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Accepted 19 August, 2011

The aim of this study was to investigate the effects of several bud loading levels in winter pruning and nitrogen doses on yield and physical and chemical properties of fresh vine-leaves of grape cultivar "Narince". Vines trained with bilateral cordon system was pruned to yield 35000 to 53000 buds/ha (16 or 24 buds/vine) above second bud in 2005 to 2006 growing season. Four nitrogen doses (0, 70, 140, 210 kg N /ha) were applied, in a completely randomized block with four replications. Brined-grape leaves were collected four times during growing season. Total fresh leaf yield (g/vine; kg/ha), number of leaves in 100 g samples, leaf area for physical properties; dry matter, ash, pH and titratable acidity for chemical properties were determined. Bud loading levels and N applications had significant effect (P<0.05) on fresh leaf yield. Fresh leaf yield varied from 2233 to 2978 kg/ha in 2005 and 3356 to 4221 kg/ha in 2006, respectively. Mean leaf area decreased with increasing of N doses and bud loading levels. Number of leaves in 100 g samples ranged from 33.3 to 38.9. Pruning and nitrogen doses had no effect on percent dry matter and ash ratio. Bud loading increased with pruning and N applications resulted in higher leaf yield which had no effect on total soluble solid (TSS), dry matter, ash ratio and decreased total leaf area. The results indicate that 140 kg N/ha and 40000 to 45000 buds/ha loading levels as vine growth is recommended.

Keywords: Brined-grape leaves, nitrogen, bud loading level, narince grape cultivar, Turkey.

INTRODUCTION

Turkey is one of the most important grape-growing countries in the world, with a viticultural area of about 479 024 ha, and a production of 4 264 720 tons (FAO Statistics, 2010). The grape in Turkey is consumed at various types: 53.6% in the fresh, 34% in raisins, 10% for crushed grapes (traditional foods made from grape juice) and 3% for wine (Agaoglu et al., 1988; Celik et al., 2010).

Grape leaves are used to make stuffed leaves (Dolma) and forcemeat which are well known meals in Middle East countries. Grapes are cultivated predominantly for fruit but the leaves are also consumed by canning or pickling as by-products (Ağaoğlu et al., 1988). Aside from the leaves traditional utilization, in the last decade, they have become an industrial product with an increasing demand and agricultural potential (Unver et al., 2007). The grape variety affects the quality of pickled grape leaves. Thick and hairy leaves with lobes are not preferred by consumers. The leaves of 'Narince' and 'Sultani Cekirdeksiz' cultivars are widely used for canned food products (Gokturk et al., 1997). Grape leaves contain several vitamins and minerals. Fibres and fruit acids in grapes play vital roles in cleaning flood functions of digestive system and kidney (Baysal, 1993; Celik et al., 1998; Nehir et al., 1997). The trace element contents in stuffed grape leaves collected from Turkish markets were: 5,33 µg/g Cu, 9,22 µg/g Zn,15,6 µg/g Mn, 74,9 µg/g Fe, 0,09 µg/g Se, 1,48 µg/g Al, 0,32 µg/g Cr, 0,58 µg/g Ni and 0,68 µg/g Co (Tuzen and Soylak, 2007).

Tokat province is one of the most important production centres of stuffed grape leaves in Turkey (Agaoglu et al., 1988; Cangi et al., 2005). Over 12 000 tons of brined

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grape leaves were produced in 2009 (Anonymous, 2010). 'Narince' is the most popular grape cultivar of Tokat province. Stuffed grape leaves production has made an important economic contribution to the region for three decades.

Pruning is the most important cultural practice in the management of grapevines. Grapevines require annual pruning to sustain production. Two basic types of pruning are cane and spur pruning. Selective spur pruning is commonly practiced for *Vitis vinifera*. In this system, canes are pruned to spurs, a section of one-year-old wood consisting of one to five nodes depending on the species and cultivars. Balanced pruning is used regardless of the type of pruning and training system. An average mature grapevine may have 200 to 300 buds capable of producing fruit. Pruning methods have been developed to balance the fruit productivity and vegetative growth to give maximum yield without reducing vine vigour or maturity. Pruning formulae have been developed for many cultivars based on their productivity (Kliewer and Weaver, 1971; Winkler et al., 1974; Szoeke et al., 1987; Mikhailov and Mikhailova, 1988). An increase in the severity of the pruning will increase the vigour of the individual shoots at the expense of total growth and crop (Winkler et al., 1974; Weaver., 1976; Celik et al., 1998).

Nutrients involved in development of grapevines, photosynthetic functioning and metobolic pathways are required in certain quantities to ensure healthy growth and performance. Nitrogen had a major effect on vine development and grape composition. High N increased vine vigour, yield and vegetative growth. Concerning to the functions in a plant, nitrogen is the primary nutrient to achieve maximum yield. Nitrogen is an essential constituent of metabolically active compounds such as proteins and enzymes. Nitrogen supply is easily manipulated and has the greatest impact on shoot growth, leaf function, reproductive development and grape composition (Treeby et al., 2000). When nitrogen is a limiting factor for growth, the rate and extent of protein synthesis are depressed (Abdel, 2008). Many studies were conducted on grapevines cultivars and indicated that vine yield was positively affected by the nitrogen fertilizer (Abdel-Al., 1967; Spayd, et al, 2000; Xia and Cheng., 2004). However, Gao and Cahoon (1991), Licina (1999), Keller et al. (1999) and Gay-Eynard (2000) reported that nitrogen application did not improve the yield. Ahmed (1991) applied 139.5 g N/vine to achieve the maximum leaf area of 'Thompson seedless' vines. Salem et al (2004) found that increasing nitrogen rates from 150 to 200 kg/ha resulted in an obvious increase in 'Thompson seedless'. The leaf area of 'White Muscat' grapevines was the greatest with (224 cm^2) the highest N levels of 160 kg/ha (Gay-Eynard, 2000).

The effect of pruning severity, crop loading and N fertilization on grapes growing has been extensively investigated (Kliewer and Weaver, 1971; Peacock, 1991; Neilsen et al., 2010). Effect of pruning and fertilization applications on fresh leaf properties and the properties and yield of brined-grape leaves fertilizer have not been adequately investigated. The purposes of this study were to evaluate the bud loading levels in winter pruning and nitrogen doses on fresh yield and quality of grapevine leaves.

MATERIALS AND METHODS

Experimental vineyard

The study was conducted in 2005 and 2006 growing season at a ten-year old *V. vinifera L.* cultivar 'Narince' grafted onto R 99 in Tokat province, located in the middle Black Sea region of Turkey (40° 40.8' N, 36° 36.0' W). Some of the ampeleographic characters of'Narince' grape cultivar (*V. vinifera*) are shown in Figure 1. The row and vine spacings were 3.0 and 1.5 m, respectively. Vines were trained as bilateral cordon onto single-curtain (wire) trellis system. Soil samples were collected from 0 to 30 and 30 to 60 cm depths using an auger to determine soil texture (Bouyoucus, 1951), pH (Jackson, 1962), $CaCO_3$ (Caglar, 1949), organic matter (Walkley, 1947), salt (Richards, 1954) and plant available P (Olsen et al., 1954). The soil was loamy in texture and pH was 8.35. Lime and organic matter contents ranged from 15.97 to 19.0% and from 2.44 to 1.31%, respectively. The electrical conductivity was 0.06 ds/m and phosphate (P) content was 1630 to 1339 kg P_2O_5/da (Table 1).

Pruning and nitrogen fertilization strategy

The treatments were: two bud levels [16 and 24 buds per vine (about 35 000±200 and 53 000±200 buds/ha)] with two buds per spur. These were combined with three N levels [70, 140, 210 kg N/ha (NH_4 NO_3, 33**%** N)]. The rates of N were split into equal amounts in February (pruning date) and May (berry-set) (Celik et al., 1998). During the first N application, P was applied as 250 kg ha^{-1} as triple superphosphate.

Plant sampling and analysis

Blades from leaves opposite to basal clusters were collected at veraison. Total N was determined using the micro-Kjeldahl method (Bremner, 1965), P was determined after drying with HNO_3 (AOAC, 1990), K and Mg were measured by atomic absorption spectrometry and Ca was determined by flame photometry.

Leaf characteristics

The best quality of brined-grape leaves were harvested from the third to fifth leaf stage (up to two thirds of mature leaves) from the apex. In trial vineyard, brined-grape leaves were picked four times; between 20 May (pre-blossom) to 15 July (veraison). Total grape fresh leaf yield (FLY), number of leaves/100g, mean leaf area (MLA) from physical properties; dry matter, ash, pH and titratable acidity (TA) were also determined. Fresh leaf yields were calculated by summing the amount of leaves pickled at each pickling.

The pH of fresh grape leaves was determined according to the methods of the Association of Official Analytical Chemists (1984). TA was measured by titration with 0.1 N NaOH. Dry matter (DM) was determined by drying 2 g of each representative leaf sample. Ash content was determined by the incineration of 2 g of each sample in a muffle furnace at 600°C for 2 h (Dokuzlu, 2004).

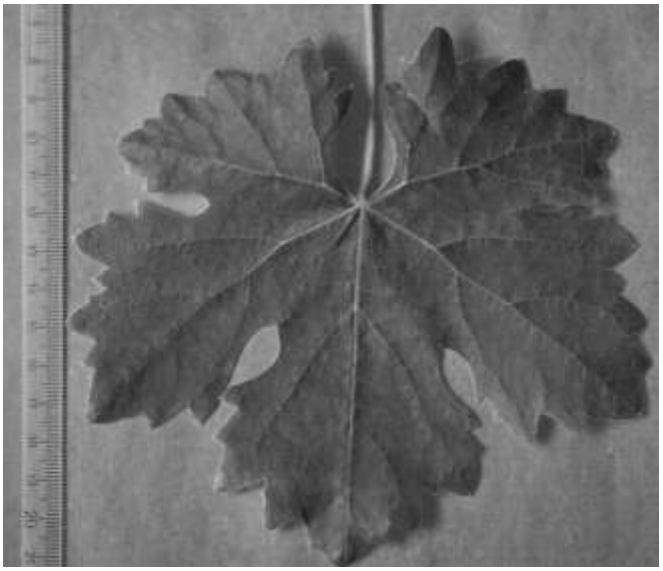

Figure 1. Some of ampeleographic characters determinated for 'Narince' grape cultivar (*V. vinifera*) (Kara, 1990). Colour of young leaf (4-6. leaf), Bronze speckled green; shape of mature leaf, pentagonal; mature leaf area, 232.67 cm^2; colour of mature leaf, dark green (PL-XXIX-421); bunch weight, 227.65 g; berry weight, 3.34 g.

Table 1. Soil properties of experimental vineyard inTokat Province,Turkey.

Soil property	Sampling depth	
	0 to 30 cm	30 to 60 cm
Texture	Loamy	Clay-loamy
Lime (%)	15.97	19
Organic matter (%)	2.44	1.31
Phosphorus (P_2O_5, kg /da)	16.30	13.39
pH	8.35	8.35
EC (ds/m)	0.06	0.06

Experimental design

The study was a completely randomized block design, with four replications and four vines per treatment replicate. Data were evaluated using analysis of variance with the MSTAT software package and the means were compared using the Duncan's multiple range test.

RESULTS AND DISCUSSION

The effects of bud levels and N application on fresh leaf yield are presented in Table 2. Increasing bud levels and N per vine induced an increase in FLY. A difference occurred in the FLY between treatments in the first year ($P< 0.05$); however, there were no differences between treatments in year 2 (Table 2). FLY increased from 2233 to 2978 kg/ha in 2005 to 3356 to 4221 kg/ha in 2006. The highest FLY in the first season was obtained with 210 kg N/ha as 1360 g/vine (2978 kg/ha FLY) and 24 buds/vine.

Many investigators have explained the effect of N on vine vigour, yield and vegetative growth. The effects of N fertilization on shoot growth, leaf number and leaf quality in the grapevine have also been reported by others (Alleweldt, 1963; Kliewer and Cook, 1974; Delas et al., 1991; Abdel Mohamed, 2008). Celik et al. (1995) reported that the shoot growth rate and shoot length of 'Narince' were affected by N levels. Studies on several grapevine cultivars indicated that vine yield was increased by increasing level of N fertilizers (Celik et al., 1995; Xia and Cheng., 2004). The results from this study are consistent with prior literature.

The N fertilizer application had a strong effect on vegetative growth, and these treatments increased FLY compared to the control. MLA also decreased with increasing fresh leaf yield and bud levels (Table 3). The mature leaf area of 'Narince' grape cultivar was reported

Table 2. Effect of bud levels and different nitrogen doses on the fresh leaf yield of Narince grapes, Tokat Province, Turkey.

Year	Nitrogen doses (kg/ha)	Fresh leaf yield* (kg/ha)		
		16 buds/vine	24 buds/vine	Mean
2005	0	2233,0^{NS}	2813.1	2530.0 ^{b}
	70	2366.7	2879.5	2623.1^{ab}
	140	2886.2	2956.1	2921.1^{ab}
	210	2937.2	2978.3	2957.7^{a}
	Mean	2605.7^{b}	2906.7^{a}	
2006	0	3356.4 ^{NS}	3807.9	3582.1 ^{NS}
	70	3616.6	3959.1	3787.8
	140	4221.5	4090.3	4155.9
	210	3954.4	3853.2	3903.8
	Mean	3787.2 ^{NS}	3927.6	

*Values within columns (N levels; lowercase superscripts) or the bottom row of each season (bud level; uppercase superscripts) marked with different superscripts are significantly different (P< 0.05); otherwise nonsignificant.

Table 3. Effect of bud levels and different nitrogen doses on MLA and the number of 'Narince' grape leaves per 100 g in Tokat Province, Turkey.

Year	Nitrogen (kg/ha)	Mean leaf area* (cm^2)			Number of leaf/100 g*		
		16 buds/vine	24 buds/vine	Mean	16 buds/vine	24 buds/vine	Mean
2005	0	151.93	145.55	148.74	34.76	36.34	35.55
	70	144.55	151.06	147.80	37.86	35.85	36.85
	140	151.88	136.98	144.43	36.70	36.75	36.73
	210	137.19	135.55	136.37	36.38	38.53	37.45
	Mean	146.39	142.29		36.42	36.87	
2006	0	153.48	146.97	150.23	35.36	33.76	34.53
	70	146.14	152.60	149.37	36.02	34.16	35.10
	140	153.32	138.41	145.87	38.12	34.68	36.40
	210	138.68	136.91	137.80	36.39	33.37	34.88
	Mean	147.91	143.72		36.46^{A}	33.10^{B}	

*Values within columns (N levels; lowercase superscripts) or the bottom row of each season (bud level; uppercase superscripts) marked with different superscripts are significantly different (P< 0.05); otherwise nonsignificant.

previously as 232.67 cm^2 (Kara, 1990). The best quality brined-grape leaves are typically harvested from the third, fourth and fifth leaves (up to two thirds of mature leaves) from the shoot apex. One third of 232.67 cm^2 is 154 cm^2. The effects of bud levels and N treatments on MLA were not different for both years. MLA values were lowered with increased N doses; however MLA values were adequate for desired brined-grape leaf properties. The MLA changed from 135.5 to 151.8 cm^2 in the first year and from 136.9 to 153.5 cm^2 in the second year (Table 3). These results are contradictory to the results of N fertilization of different grape cultivars reported by Krusteva-Kastova and Kartarcv (1977), Maatouk et al. (1988), Ahmed (1991) and Gay-Eynard (2000).

The number of leaves per 100 g sample of both years was not different. The number of leaves in the 100 g samples ranged from 34.7 to 38.9 for the first year and 33.3 to 38.1 for the second year (Table 3). However, the leaves per 100 g samples were reported as 40 to 49 for 'Sultani' (Ic and Denli, 1997), 34.0 to 36.6 for 'Sultani' (Özcan et al., 2004), 19.5 to 40.3 for 'Sultani', 'Alphonse' and 'Erenkoy' cultivars (Basoglu et al., 1996) and 28 to 41 for 'Hacıtespihi', 'Karaerik', 'Kabayufga' and 'Agrazaki' cultivars (Sat et al., 1997). Unver at al. (2007) found mean weights of fresh leaves for 'Eksikara', 'Hesapali' and 'Siyah Pekmezlik' as 2.55, 2.36 and 1.98 g, respectively. The weight of fresh leaves for 'Sultani' was determined as 2.05 g (Ic and Denli, 1997).

The effects of pruning and fertilizer rates on percent dry matter and ash ratios were not different. The percent dry matter of the fresh leaves ranged from 26.01 to 27.31% (Table 4). Different N doses and bud levels resulted in similar dry matter contents. Ash ratios varied between years and treatments. Ash ratio of fresh leaves ranged from 1.54 to 1.85% for the first year and 1.72 to 1.97% for the second year (Table 4). The fresh leaves of 'Eksikara'

Table 4. Effects of bud loading levels and different nitrogen doses on the dry matter and ash ratio of 'Narince' grape leaves, Tokat Province. Turkey.

Year	Nitrogen (kg/ha)	Dry matter (%)*			Ash (%)*		
		16 buds/vine	24 buds/vine	Mean	16 buds/vine	24 buds/vine	Mean
2005	0	27.35	25.62	26.48	1.70	1.54	1.62
	70	25.95	27.29	26.62	1.62	1.58	1.60
	140	25.52	26.50	26.01	1.57	1.57	1.57
	210	27.51	25.47	26.49	1.85	1.64	1.75
	Mean	26.58	26.22		1.68	1.58	
2006	0	27.39	25.32	26.35	1.73	1.75	1.74
	70	26.59	26.12	26.35	1.96	1.97	1.96
	140	26.42	27.44	26.93	1.73	1.85	1.79
	210	27.71	26.92	27.31	1.72	1.86	1.80
	Mean	27.03	26.45		1.78	1.86	

*Values within columns (N levels; lowercase superscripts) or the bottom row of each season (bud level; uppercase superscripts) marked with different superscripts are significantly different (P< 0.05); otherwise nonsignificant.

Table 5. Effects of bud levels and different nitrogen doses on the pH and titratable acidity of 'Narince' grape leaves in Tokat Province, Turkey.

Year	Nitrogen (kg/ha)	pH*			Titratable acidity*(%)		
		16 buds/vine	24 buds/vine	Mean	16 buds/vine	24 buds/vine	Mean
2005	0	3.72	3.66	3.69	1.29 ^{c}	1.40 ^{bc}	1.34
	70	3.72	3.66	3.69	1.37 ^{bc}	1.30 ^{c}	1.33
	140	3.69	3.68	3.69	1.36 ^{bc}	1.50 ^{b}	1.43
	210	3.68	3.72	3.70	1.42 ^{a}	1.27 ^{c}	1.34
	Mean	3.70	3.68		1.36	1.37	
2006	0	3.61	3.61	3.61	1.62	1.62	1.62
	70	3.59	3.52	3.56	1.60	1.68	1.64
	140	3.59	3.57	3.58	1.66	1.71	1.69
	210	3.63	3.58	3.61	1.48	1.74	1.61
	Mean	3.60	3.57		1.59	1.69	

*Values within columns (N levels; lowercase superscripts) or the bottom row of each season (bud level; uppercase superscripts) marked with different superscripts are significantly different (P< 0.05); otherwise nonsignificant.

had the highest dry matter (31.69%), 'Siyah Pekmezlik' (27.89%) followed by 'Hesapali' (25. 36%) (Unver et al., 2007). Sat et al. (2002) reported that dry matter of 'Hacıtespihi', 'Karaerik', 'Kabayufga' and 'Agrazaki' grape cultivars were 17.75, 18.2, 15.0 and 13.76%, respectively. The percent dry matter and ash ratio ranged, respectively, from 22.86 to 24.89% and 1.65 to 1.68% for 'Sultani' grape cultivar (Ic and Denli, 1997). The dry matter content was the lowest (21.41 %) for 'Kabayufga' cultivar while it was the highest (24.72%) in 'Karaerik'. The highest (2. 11%) ash ratio was obtained in 'Hacıtesbihi' while the lowest (1. 52%) was obtained in 'Karaerik' (Sat et al., 2002). Dry matter and ash ratio for 'Alfons', 'Erenkoy Beyazı' and 'Sultani' cultivars were found as 25.01, 25.51, 26.54% and 1.52, 1.81, 1.66%, respectively (Basoglu, 1996).

There were no differences among treatments for pH, but differences were obtained for TA (bud loading x nitrogen combinations). With increasing bud levels per vine, TA values were also increased (Table 5). The mean pH values of the N applications were similar while the TA varied among years. pH values ranged from 3.69 to 3.70 for the first year and 3.52 to 3.61 for the second year, respectively. The pH values obtained in this study were higher than those reported by others. The geographic area and the season might have an influence on chemical composition of vine by-product (Romero et al., 2000). TA values were higher in the first year (1.33 to 1.43%) as compared to that of the second year (1.61 to 1.69%) (Table 5). The pH values of 'Hacıtespihi', 'Karaerik', 'Kabayufga' and 'Agrazaki' grape cultivars were reported as 3.39, 4.43, 3.31 and 3.43, respectively (Sat et al., 2002). The TA of 'Hacıtespihi', 'Karaerik', 'Kabayufga' and 'Agrazaki' grape cultivars were 1.88,

Table 6. The effects of N, P, K, Ca and Mg contents of leaves on different bud loading levels and different nitrogen doses in Tokat Province, Turkey.

Bud level	Nitrogen (kg/ha)	N		P		K		Ca		Mg	
		2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
16	0	4.12	5.30	0.05	0.14	0.99	0.97	2.56	2.55	0.23	0.22
	70	4.00	5.11	0.08	0.12	1.10	0.92	2.58	2.72	0.23	0.24
	140	3.82	5.11	.0.06	0.14	1.12	0.92	2.52	2.59	0.23	0.22
	210	4.12	4.70	0.08	0.15	1.17	0.86	2.80	3.11	0.23	0.23
24	0	4.12	4.70	0.12	0.15	1.08	0.94	2.30	3.08	0.22	0.22
	70	3.90	5.30	0.16	0.15	1.23	1.38	2.62	3.00	0.23	0.23
	140	4.30	4.88	0.16	0.15	1.39	1.03	2.66	2.90	0.23	0.22
	210	4.06	4.88	0.11	0.15	1.14	1.16	2.25	2.54	0.23	0.22

1.73, 1.96 and 1.78%, respectively (Sat et al., 2002).

The N, K and Ca contents were adequate and Mg was low in both years, whereas P was inadequate in the first year (Table 5). N, P, Ca and Mg contents in grape leaves were not affected by N treatments, but K content increased with higher N doses in the second year (Table 6). Christensen et al. (1978) stated that foliar nutrient con-tents at veraison stage ranged from 2.2 to 4.0% for N, 0.15 to 0.30% for P, 0.8 to 1.6% for K, 1.8 to 3.2% for Ca and 0.3 to 0.6% for Mg. Bud load was reported as the major determinant of leaf N, K and Mg contents at bloom and P and Ca at ripening (Szoeke et al., 1987). Training systems had an influence on N and Mg contents during fruit ripening, P during bloom and ripening and K and Ca during bloom (Mikhailov and Mikhailova, 1988). Bud load did not affect macroelement content in leaves during bloom. Interactions between training system and bud load affected the N content during bloom as well as that of K and Ca during ripening. Licina (1999) in 'Cabernet Sauvignon' grapevine and Gay-Eynard (2000) in 'White Muscat' cultivar reported similar results on effect of N applications on leaf N content. In contrast, some researchers reported that increasing N rates caused an increase in N content of petioles (Martin et al., 2004; Salem et al., 2004; Abdel, 2008).

Conclusions

Fresh leaf yield increased with bud levels and N applications. The results underline the important role of nitrogen application and bud levels on fresh leaf yield and leaf quality for brined-grape leaf production. Application of 140 kg N/ha and 40000 to 45000 buds/ha levels are recommended to obtain high quality leaves for marketing.

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