INFLUENCE OF VINEGAR AND CHELATED IRON FIELD SPRAYS ON MINERAL NUTRIENTS AND FRUIT QUALITY OF GRAPES (CV. 'THOMPSON SEEDLESS')

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This experiment was designed to study leaf mineral concentrations and fruit quality of 'Thompson Seedless' grapevines grown in high pH soils under field spray of vinegar, chelated iron or their mixture. We hypothesized that vinegar spray is as good as or better than chelated iron spray in controlling iron chlorosis and improving fruit quality of grapes grown in high pH soils. The first paper published from this research reported, that yield and iron content of this variety improved by vinegar spray. In this manuscript we report that other leaf nutrients except Mg and K were not affected by the field spray treatments of grapevines. Concentration of Mg and K were highest in grapevines grown in high calcareous soils (control) and lowest in grapevines sprayed with mixed vinegar and chelated iron. Vinegar spray also improved fruit quality before and after storage with no adverse effect on appearance, taste and flavor. Berries of grapevines that were sprayed with vinegar had higher levels of total soluble solids and lower levels of titratable acidity before and after 50 days of storage. The main benefit of field vinegar spray was the significant reduction of fungal decay on grape berries after 50 days of storage at 1 °C. But the mixture of vinegar and chelated iron did not reduce fungal decay after storage.
Keywords: acetic acid, chlorosis, fungal decay, calcareous soils, macro-elements, micro-elements

Einfluss von Essig- und Eisenchelatspritzungen auf Mineralstoffe und Fruchtqualität von Trauben der Sorte 'Thompson Seedless'. In diesem Experiment sollten die Gehalte an Mineralstoffen in den Blättern und die Fruchtqualität von Trauben der Sorte 'Thompson Seedless' auf Böden mit hohem pH-Wert untersucht werden, die mit Essig, Eisenchelat oder einer Mischung daraus behandelt wurden. Es wurde angenommen, dass Essigspritzungen bei der Bekämpfung von Eisenchlorose und der Verbesserung der Fruchtqualität von Reben, die auf Böden mit hohem pH-Wert angebaut werden, mindestens genauso gut sind wie Eisenchelatspritzungen. In der ersten Veröffentlichung zu dieser Studie wurde berichtet, dass Ertrag und Eisengehalt dieser Sorte durch Essigspritzungen verbessert wurden. In der vorliegenden Arbeit wird berichtet, dass andere Blattnährstoffe außer Mg und K durch die Spritzungen nicht beeinflusst wurden. Die höchste Konzentration an Mg und K hatten Weinstöcke, die auf kalkreichen Böden (Kontrolle) angebaut wurden, und die niedrigste Konzentration hatten Weinstöcke, die mit einem Gemisch aus Essig und Eisenchelat gespritzt wurden. Essig verbesserte auch die Fruchtqualität vor und nach der Lagerung, ohne das Aussehen, den Geschmack und das Aroma zu beeinträchtigen. Mit Essig gespritzte Beeren wiesen vor und nach 50 Tagen Lagerung einen höheren Gehalt an löslichen Feststoffen und einen geringeren Gehalt an titrierbaren Säuren auf. Der Hauptvorteil der Essigspritzungen war die signifikante Verringerung des Pilzbefalls von Beeren nach 50 Tagen Lagerung bei 1 °C. Die Mischung aus Essig und Eisenchelat reduzierte jedoch nicht den Pilzbefall nach der Lagerung.
Schlagwörter: Essigsäure, Chlorose, Pilzbefall, kalkhaltige Böden, Makroelemente, Mikroelemente

Grapes are grown in a wide range of soil types. However, they are very sensitive to high pH soils with high contents of calcium carbonate. A large part of farm lands in the world are calcareous high pH soils. Although not suitable for grapes, there is a large acreage of grapes in this type of soils (Assimakopoulou et al., 2016; Mengel and Geurtzen, 1986). High bicarbonate content of calcareous soil is the main cause of 'lime-induced chlorosis'. Grapes suffer from leaf chlorosis in these high pH soils and nutrient uptake, yield and fruit quality are adversely affected (Brown et al., 1958; Chen and Barak, 1982; Bienfait, 1988; Brown and Cummins, 1989; Tardaguila et al., 1995; Bavaresco et al., 1999; Tagliavini and Rombola, 2001; Bavaresco and Poni, 2003; Bavaresco et al., 2005; Assimakopoulou et al., 2016).

Iron uptake is severely reduced by high pH as well as the uptake of nitrogen, phosphorus, calcium, and magnesium (Assimakopoulou et al., 2016; Bavaresco and Poni, 2003). The uptake reduction is mostly due to the element being unavailable for uptake rather than being insufficient (Bavaresco and Poni, 2003). Photosynthesis is then reduced by mineral deficiency as several sinks compete for the limited photosynthates and dramatic sink competition occurs which will affect yield and fruit quality (Bavaresco et al., 2003).

Adding fertilizers to the soil does not solve the problem, as the fertilizers convert to the unavailable form for plant uptake and deposit in the soil. The solution to this problem is usually soil application of the chelated form of the nutrient element or field spray (Tagliavini et al., 1995 and 2000; Rombola et al., 2000; Bybordi and Shabanov, 2010). Both of these methods have their pros and cons. Soil application of chelated iron is very costly due to the high price of the fertilizer (García-Laviña et al., 2002). However, field spray is not always effective (Crane et al., 2008).

Grapes nutrient uptake have been studied by several researchers (Assimakopoulou et al., 2016; Amiri and Fallahi, 2007; Bavaresco et al., 2003; Christensen, 1969). Nutrient uptake and ionic composition could be affected by soil conditions, varieties, rootstocks and field sprays. Plant tissue analysis is still the best analytical method to determine the nutritional status of grapevines (Christensen, 1969). Nutrient statuses of vineyards are not well studied in many parts of Iran including Arak. Vineyard soils have low organic matter (<1 %) and are primarily calcareous with a high pH above 7.8. Vines often suffer from mineral deficiency due to lime-induced chlorosis and high bicarbonate content of irrigation waters (Amiri and Fallahi, 2007; Bybordi and Shabanov, 2010).

We hypothesized that a weak acid (vinegar) can reduce leaf chlorosis, increase mineral uptake and yield and improve fruit quality, therefore, this experiment was designed to study the effect of vinegar, chelated iron spray and their mixture on grapes (cv. 'Thompson Seedless'). In the first published report of this research (Hoseinabadi et al., 2018) we reported the changes in yield, vegetative growth and leaf iron concentration. In this paper, we report other leaf macro- and micro-element concentrations and fruit quality of grapes cv. 'Thompson Seedless' before and after storage for 50 days at 1 °C.

MATERIALS AND METHODS

The experimental vineyard and growing conditions were described in detail by Hoseinabadi et al. (2018). A four-years-old trellised vineyard of cv. 'Thompson Seedless' was chosen at a grower's field located at Khandab, Arak, Iran during 2008 and 2009 and the data were averaged over two years. Water and soil were tested before the experiment (Table 1 and 2). Local precipitation is about 300 mm limited to December to March and does not meet plant water requirements during the growing season. The water requirement of the vineyard was calculated by the Ministry of Agriculture as being about 6160 m^3/ha. The required water was applied to the vines between different phenological stages (bud burst, fruit set, fruit elongation, veraison, harvest) by drip irrigation. There were six vines in each experimental plot and a guard row between rows and two vines on the row between treatments to reduce the marginal effects. The experiment had four replicates in a randomized complete block design. The four field spray treatments were I) grape vinegar at 0.8 % (pH 2.0), II) iron chelate at 0.2 % (pH 4.2), III) mixture of grape vinegar and iron chelate (0.8 % and 0.2 %, respectively, pH 2.8) and IV) distilled water as control. Iron chelate (Fe-EDTA; Tradecorp, Madrid, Spain) had 13.2 % active ingredients and grape vinegar had 4.3 % acidity.

Grape vines were fully covered during spray until run-off was observed. The vines were sprayed six times during growing season: 1) before bud break on April 1st; 2) after petal fall on June 4th; 3) small berries on June 12th; 4) two weeks later on June 26th; 5) 4 weeks later on July 10th; and 6) 6 weeks later which coincided with the beginning of veraison on July 24th.

Leaf blades are recommended for diagnosing nutrient deficiency in grapes (Schreiner and Scagel, 2017) and there is a year-to-year variation in leaf nitrate and potassium concentrations as well as a variation within a year. Nitrate, phosphorus and potassium changes rapidly at bloom time and are most stable 3 to 4 weeks after bloom (Christensen, 1969). Shaulis (1961) suggested 8 to 10 weeks after bloom for leaf petiole potassium analysis. Leaf samples were collected three weeks after bloom for macro- and micro-element analysis. About 10 to 20 young and fully expanded leaves (usually the sixth leaf from the tip on an actively growing shoot; Christensen (2005)) were sampled one week after the third field spray on June, 19th, for macro- (N, P, K, Mg and Ca) and micro-elements (Zn, Cu and Mn) analysis.

Leaves were rinsed gently with distilled water for 10 to 15 sec, dried in oven at 70 °C for 48 h, ground and used for leaf macro-elements (except N) analysis. One gram of dried leaves was dry-ashed in a furnace at 500 °C and extracted by HCl. The concentration of P was determined by vanado-molybdophosphate yellow color method, Ca, Mg, Mn and Zn by atomic absorption spectrometry and B by azomethin-H, K by flame photometry in the dry digest method (Assimakopoulou and Tsougrianis, 2012; Tokalioğlu et al., 2004; Assimakopoulou et al., 2016; Sharaf El-Deen and Ashour, 2009). Total nitrogen was measured with wet digestion method using sulfuric and salicylic acids and hydrogen peroxide by Kjeldhal method (Amin and Flowers, 2004; Sáez-Plaza et al., 2013).

Cluster and berry length and width in the widest part were measured by a ruler in cm and mm, respectively. Total soluble solids (TSS), titratable acidity (TA) and grape juice pH were measured right after harvest and after storage (Iranzo et al., 1984).

To test the effect of field sprays on berry appearance, taste and flavor, a random cluster from each treatment was stored at 5 °C overnight. Then, five berries from each treatment were washed with running tap water, drained, patted dry with paper towels, and served to panelists. Panelists tasted the berries for any changes compared to the control.

Fruits were stored at 1 °C and 85 % relative humidity for 50 days. For each treatment 12 aluminum foil containers with lid were filled with about 400 g of fruit in each container. After 50 days of storage the weight of disease-free berries was measured and percentage of fungal decay calculated based on the initial weight (Zahavi et al., 2000).

The data of stored fruit quality were analyzed as a completely randomized design. All data were analyzed using analysis of variance (ANOVA, SAS Institute Inc. version 9.2). Means were compared using Duncan Multiple Range Test at $p \leq 0.05\%$.

RESULTS AND DISCUSSION

SOIL AND WATER MINERAL NUTRIENTS

Water and soil analysis report of the experimental vineyard is presented in Table 1 and 2. The soil has bicarbonate ion at 189.1 mg/l and a pH of 8.2 to 8.4 at three soil levels measured from the soil saturated paste (Kalra, 1995). The high pH (≥ 8.2) created iron chlorosis which has been discussed in Hosseinabadi et al. (2018). None of the elements were in quantities low enough in the soil to limit production (Bonomelli and Ruiz, 2010).

LEAF MINERAL NUTRIENTS

The leaf nutrient concentration of P, Ca, total N, Mn, Zn, and Cu was not significantly affected by the treatments (Fig. 1A to 1C). However, concentrations of Mg and K were the lowest in leaves sprayed with vinegar mixed with iron chelate. In alkaline soils with pH higher than 7, such as the one in this experiment (pH 8.2) the available form of Mg increases, which increases its absorption (Romero, 2013; Schreiner and Scagel, 2017). Bavaresco et al. (2003) also found that calcareous soil induced higher leaf Mg concentrations in comparison with non-calcareous soil, as well as Assimakopoulou et al. (2011), who also reported higher leaf Mg concentrations in Prunus rootstocks under high bicarbonate levels.

It has also been shown that in grapes grown in calcareous soils, increased Fe^{2+} concentration is concurrent with increased Mg in leaves (Belkhodja et al., 1998; Martin et al., 2007). We have shown in the first paper of this work that Fe^{2+} increased in grapes treated with vinegar, in the same way it may have increased leaf Mg content in our experiment, which confirms the previous results (Belkhodja et al., 1998; Martin et al., 2007).

If leaves are considered as representative to assess mineral nutrition of a given plant, the results show that lime-stress conditions impaired K and Mg concentrations in leaves (cv. 'Thompson Seedless'), while Bavaresco and Poni (2003) only reported K reduction in leaves and P and K in other grapevine tissues (cv. 'Aurora').

It should be noted that K uptake can antagonize Mg

Table 1: Water analysis results of the irrigation water for the four-year-old experimental vineyard located at Khandab, Arak, Iran (2008)

Measured element	Unit	Amount
Electrical Conductivity	µmho/cm	1294
pH	meq/l	7.5
CO^{3-}	meq/l	0
Cl^{-}	meq/l	3.2
SO_4^{2-}	meq/l	9.7
Na^{+}	meq/l	3
Ca^{2+}, Mg^{2+}	meq/l	13.1
NO^{3-}	meq/l	-
Sodium Adsorption Ratio	meq/l	12

Table 2: Soil analysis results for the four-year-old experimental vineyard located at Khandab, Arak, Iran

Measured element	Unit	Soil depth (cm)		
		0-30	30-60	60-90
Saturation Percentage	%	36	37	37
Electrical Conductivity	(dS/m)	1.5	1.9	2
pH of saturated paste		8.2	8.2	8.4
P (ava)	ppm	11.1	10.5	7.3
K (ava)	ppm	381	269	164
Ca (ava)	ppm	131	130	130
Fe (ava)	ppm	4.6	5.5	4.4
Mn (ava)	ppm	9.6	10.5	8.3
Zn (ava)	ppm	0.6	0.7	0.8
Cu (ava)	ppm	0.6	0.9	0.7
Total N	%	0.04	0.04	0.03
Total Neutralizing Value	%	53.3	53.3	18.3
Organic carbon	%	0.43	0.36	0.32
Clay	%	18.6	15.2	13.7
Silt	%	46	44.7	42.8
Sand	%	35.4	40.1	43.5
Texture		Loam	Loam	Loam

Note: ava is the abbreviation for available.

uptake and excessive application of one of these elements may result in deficiency of the other one (Amiri and Fallahi, 2007). However, the inhibitory effect of K is confined to the efficiency range of Mg supplied. If the Mg level in plant tissue does not fall below the critical threshold, the increasing K application does not reduce yield (Bybordi and Shabanov, 2010). Potassium and magnesium concentrations both increased under vinegar spray and were not affected by other treatments.

The concentration of macro-elements (% d/w) in leaves of cv. 'Thompson Seedless' differs in reports, which could be due to differences in the environmental and experimental conditions, analytical methods and clonal variations. Nitrogen was found from 1.6 to 5 (Bonomelli and Ruiz; 2010; Kalbhor et al., 2017; Abou-Zaid and Eissa, 2019; Christensen, 1984; Araujo and Williams, 1988), phosphorus from 0.14 to 0.98, potassium from 0.5 to 2.9, calcium from 1.1 to 4.5 and magnesium from 0.4 to 2.2 % (Christensen, 1969; Bavaresco et al., 2003; Fisaraskis et al., 2004; Bonomelli and Ruiz, 2010; Kalbhor et al., 2017; Abou-Zaid and Eissa, 2019; Christensen, 1984; Nikolaou et al., 2000; Vijaya and Rao, 2015). The concentration of elements in grape leaves in this experiment was within the range mentioned. Total nitrogen was about 2.5 % and higher than what several references mentioned (Bonomelli and Ruiz, 2010; Kalbhor et al., 2017; Abou-Zaid and Eissa, 2019) but in the range of other reports (Christensen, 1984; Araujo and Williams, 1988). Nitrogen is very mobile inside the plant and leaf nitrogen concentration can be very variable depending on the time of the year and the location of the leaf on the vine (Christensen, 1969). Also, calcium was in the range of 5 % and higher than 4.5 % reported by Fisaraskis et al. (2004). This could be due to the high calcium contents of the soils and irrigation water in this area which are usually loaded with calcium bicarbonate as indicated in the soil and water analysis report (Table 1 and 2).

There is not much literature available on leaf micro-element concentrations. In the reports available (Vijaya and Rao, 2015; Kalbhor et al., 2017; Christensen, 1984; Belkhodja et al., 1998; Nikolaou et al., 2000; Bavaresco and Poni, 2003; Bybordi and Shabanov, 2010), the concentration of micro-elements in leaves of cv. 'Thompson Seedless' varies for Fe 61 to 400, for Mn 15 to 400 for B 25 to 44 and for Zn 28 to 200 ppm on average. Christensen (1969) sampled leaves one month after bloom on June, 26th, we sampled the leaves almost at the same time as Christensen (1969). The concentration of Fe, Mn, and Zn in the leaves in our experiment (Fig. 1D) is two to three times higher (260, 200, 64 ppm on average, respectively) than what was reported by Christensen (1969), but in line with one report (Kalbhor et al., 2017). This indicates differences in the soil, climatic conditions and clonal variations and reveals that chlorosis observed in our experiment is solely related to deficiency of active iron (Fe^{2+}), not other micro-elements.

FRUIT QUALITY

No differences were observed in appearance, taste and flavor of field spray treatments compared to control. Also no statistical differences were observed between length and width of grape clusters and grape berries (data not shown). In the control treatment, the width and length of clusters were 14 and 10.5 cm and the berries 14.1 and 11.8 mm, respectively. Marzouk and Kassem (2011) reported that preharvest spray of ascorbic acid increased berry width and Bonomelli and Ruiz (2010) reported that the equatorial diameters of cv. 'Thompson Seedless' were 17 to 20 mm. The diameter of berries in our experiment is a little smaller, reflecting the clonal or environmental variations.

TSS AND TA BEFORE STORAGE

Total soluble solids were higher in vinegar-sprayed plants compared to control or other treatments. This could be due to the higher chlorophyll content (vinegar-sprayed 0.012 vs. control 0.006 mg/ml reported in Hosseinabadi et al. (2018)), therefore producing higher assimilates as was reported before (Hosseinabadi et al. (2018)). pH of fruit juices was not affected by treatments. The titratable acidity (TA) of grape berries was higher in control than with other treatments. Higher TA indicates more acidity in berries which is not desirable. Therefore, all treatments except control could reduce TA and improve fruit quality (Fig. 2A to C).

Contrary to our results, Marzouk and Kassem (2011) reported that preharvest spray of ascorbic acid did not affect TSS and TA of grape cv. 'Thompson Seedless', explaining the different physiological role of ascorbic and acetic acids or differences in environmental and experimental conditions. Ascorbic acid exists in cell compartments such as cell wall and acts as an enzyme factor to control cell growth, division and expansion (Smirnoff and Wheeler, 2000).

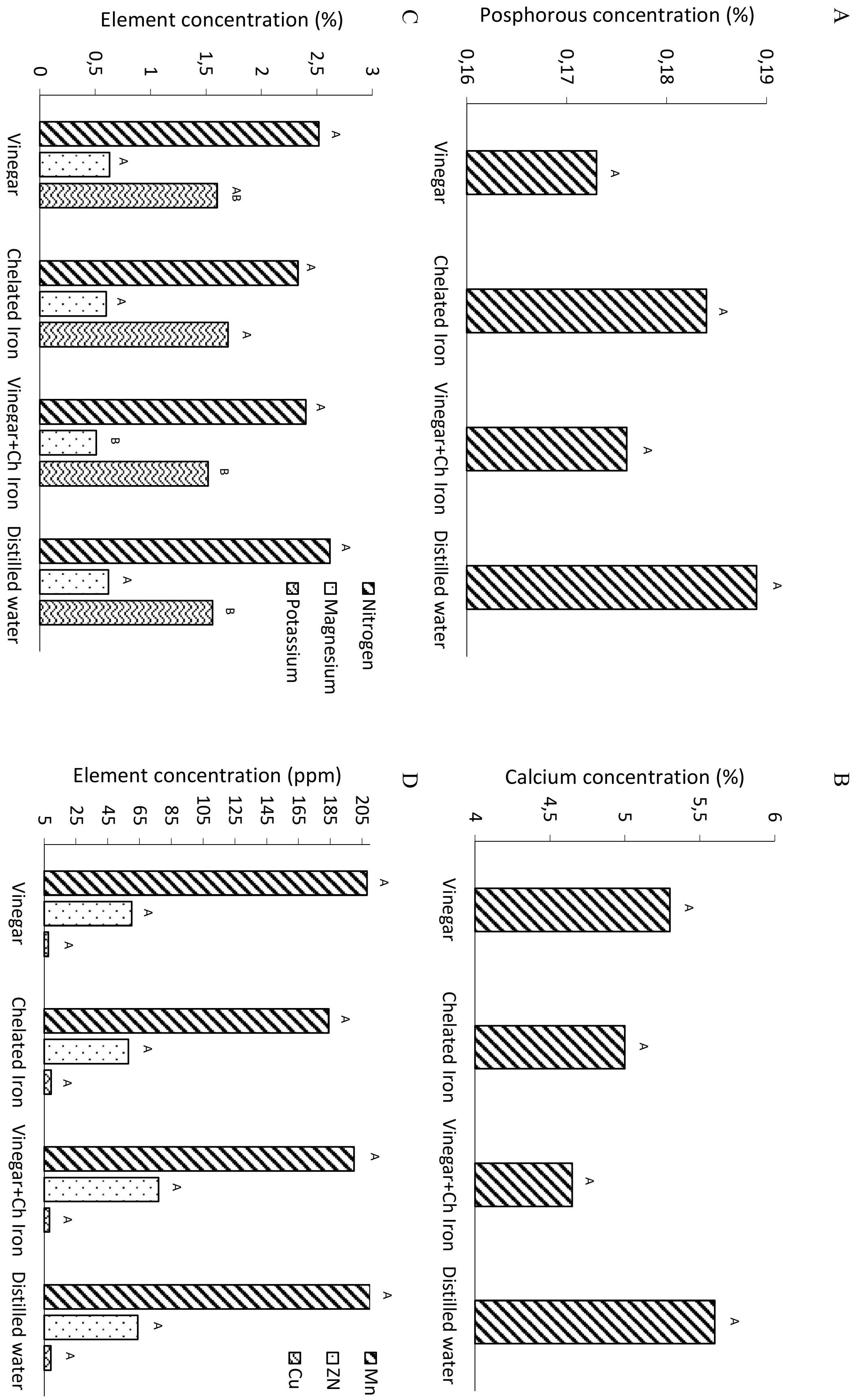

Fig. 1: Concentration of macro- and micro-elements in grape leaves cv. 'Thompson Seedless' sampled June, 29th, one week after the third spray with vinegar (8 ppm), chelated iron (Ch Iron, 2 ppm), mixture of vinegar and chelated iron (8 and 2 ppm, respectively) and water (control); bars with the same letters are not significantly different at $p \leq 0.05$.

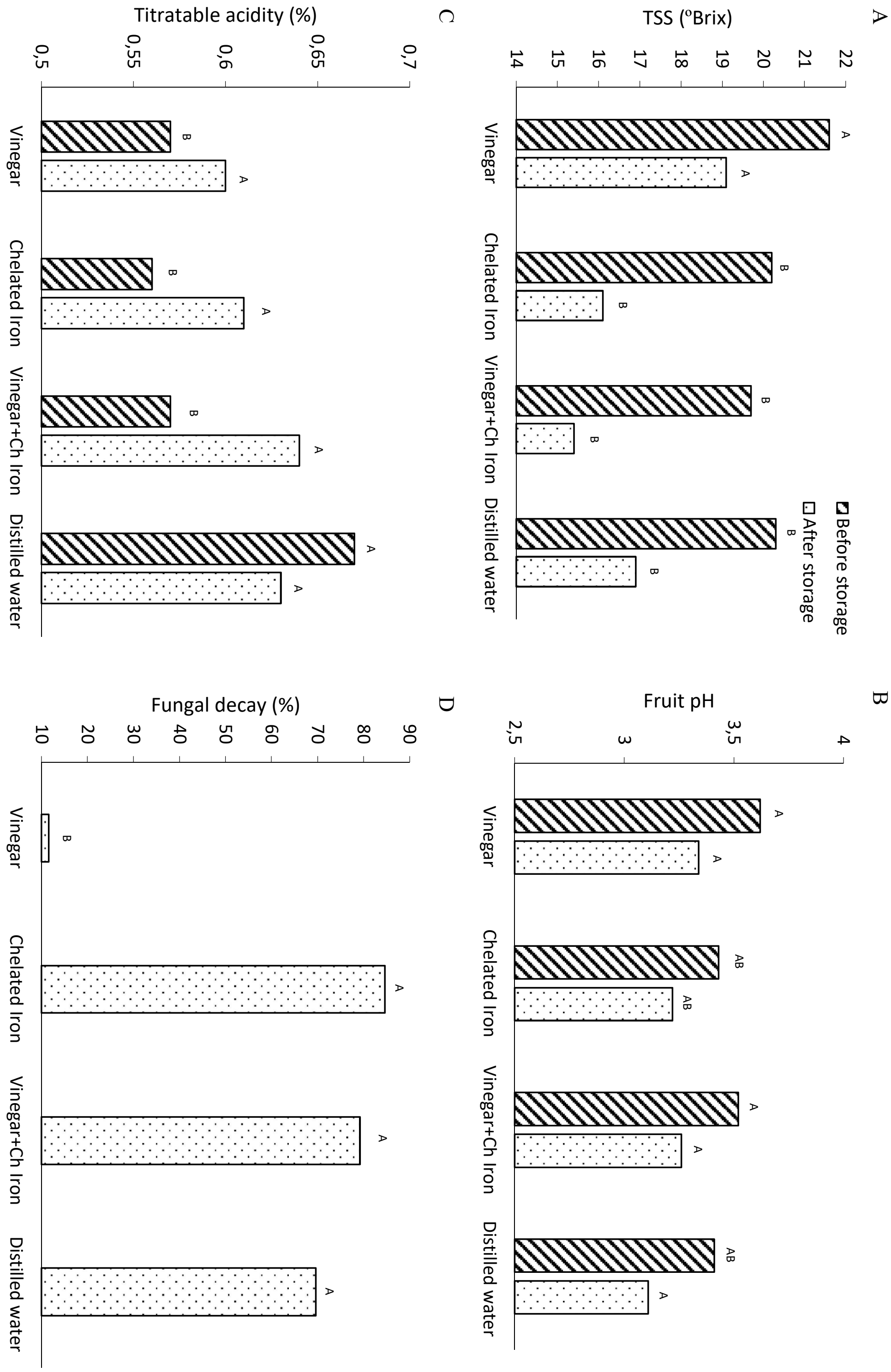

Fig. 2: Fruit quality of grapes cv. 'Thompson Seedless' right after harvest and after storage for 50 days at 1 ºC and 95 % relative humidity; the grapevines were sprayed six times during the growing season with vinegar (8 ppm), chelated iron (Ch Iron, 2 ppm), mixture of vinegar and chelated iron (8 and 2 ppm, respectively) and water (control). The six spraying dates were April, 1st, June, 4th, 12th and 26th, July, 10th and 24th. Bars with the same letters are not significantly different at $p \leq 0.05$.

TSS AND TA AFTER STORAGE

In general, TSS and pH decreased and TA increased during storage. Total soluble solids were higher in vinegar-sprayed berries than with other treatments. After 50 days of storage at 1 °C, TSS was higher in vinegar-sprayed vines, but the pH and TA was not different between treatments (Fig. 2A to C). TSS was reported between 15.8 and 20 (°Brix) in cv. 'Thompson Seedless' (Bonomelli and Ruiz, 2010) depending on the maturity at harvest (Burger et al., 2005), which is very similar to what we found in harvested grapes in this experiment. Muñoz-Robredo et al. (2011) reported the TSS of 15 (°Brix) and TA of 2 (%) in 'Thompson Seedless' grapes grown in Valparaiso, Chile, whereas the TA reported by Burger et al. (2005) is lower and between 0.4 and 0.6 (°Brix) depending on maturity. In our experiment the TA was around 0.6 and very close to the report by Burger et al. (2005). The higher TA reported by Muñoz-Robredo et al. (2011) could be due to clonal variation or climate, as the climate in Valparaiso, Chile, is temperate, whereas in Western Cape, South Africa (Burger et al., 2005) it is moderate and Mediterranean with low summer rainfall. We could not find any report on the influence of the application of vinegar or chelated iron on TSS or TA of this variety.

FUNGAL DECAY

The main benefit of vinegar field spray was the reduction of grape fungal decay after storage. Percentage of fungal decay significantly reduced by vinegar spray on the grapevines at $p \leq 1$ %. However, fungal decay in the mixture of vinegar and chelated iron were not significantly different from chelated iron or control (Fig. 2D).

Vinegar has been used as a sanitizer to control bacterial and fungal contamination in the food industry for a long time (Yagnik et al., 2018; Entani et al., 1998; Kurimoto, 1981). There are also reports that postharvest decay of several fruits was decreased by organic acids. Acetic, peracetic, sorbic, formic and propionic acids reduced decay of table grape, sweet cherry, pear, apple and citrus. (Sholberg et al., 1996; Mari et al., 2004; Sholberg, 1998; Palou et al., 2002).

The inhibitory effect of acetic acid, which is the main ingredient of vinegar on micro-organisms is not only due to its acidic pH, but mostly because it penetrates the microbial cell and exerts its toxic effect (Tripathi and Dubey, 2004). Beside the fungicidal effect of acetic acid in direct contact, acetic acid fumigation also controlled grape spoilage for up to two months in modified atmosphere packaging at 0 °C (Tripathi and Dubey, 2004). Vinegar vapor was also effective at ambient temperatures as well as at refrigerated storage on table grapes (*Vitis vinifera* L.) for control of *Botrytis cinerea* and *Penicillium* sp. (Sholberg et al., 2000). Vinegar was also very effective in reducing the number of conidia on the fruit surfaces of 'McIntosh' apples (Sholberg et al., 2000).

These evidences suggest that field spray of vinegar on grape vines most probably reduces the fungal inocula in the field, therefore, reducing the fungal decay after 50 days of storage in our experiment. However, it is not clear why mixture of vinegar and chelated iron does not show fungicidal activity. The low pH of the vinegar (2.0) could be a primary mechanism for its fungicidal properties and when it is mixed with chelated iron (pH 2.8), the pH increases, making vinegar less effective against pathogens.

CONCLUSION

In general, vinegar application is promising in controlling fruit fungal decay as a field spray or other methods suggested by researchers such as fumigation during storage. There are many advantages of vinegar application. It is a natural product with no or little hazard to humans or environment. Its field application can correct iron and mineral deficiency, improve yield and fruit quality, (e. g. TSS) especially in areas with high pH soils (Hosseinabadi et al., 2018). It is inexpensive compared to other fungicidal compounds and can be used in low concentrations or as a fumigant during storage without the need for extra handling of fruits that may cause physical damage to the produce.

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