Scientia Horticulturae xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Foliar nutrient applications to 'Wonderful' pomegranate (*Punica granatum* L.). II. Effects on leaf nutrient status and fruit split, yield and size

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ARTICLE INFO

Keywords:
Foliar fertilizer
Fruit cracking
Magnesium sulfate
Leaf nutrient status
Potassium nitrate
Zinc sulfate

ABSTRACT

Though pomegranates are beginning to be farmed more intensively, little is known about pomegranate leaf nutrient concentrations or the effects of foliar nutrient applications on those concentrations, including for one of the industry's standard cultivars, Wonderful. Foliar nutrient applications could potentially be used in commercial pomegranate production, not only to improve plant nutrient status, but also to prevent physiological disorders, such as pomegranate fruit split, the most important physiological disorder in pomegranate production. Pomegranate trees at two 'Wonderful' commercial orchards were treated with foliar applications of zinc sulfate ($ZnSO_4$; 0.3%, 0.4%, and 0.5%), magnesium sulfate ($MgSO_4$; 1%, 2%, and 3%), potassium nitrate (KNO_3; 1%, 2%, and 3%), or deionized (DI) water (control). Leaf mineral nutrient concentrations were determined and fruit were analyzed for fruit split incidence, yield, fruit number per tree, fruit diameter, fruit mass, aril mass, and mass of 100 arils. Leaf Zn concentrations were significantly higher in response to all foliar $ZnSO_4$ treatments. Foliar applications of KNO_3 resulted in significant increases in leaf N and K concentrations and foliar $MgSO_4$ resulted in a significant increase in leaf S concentration. All foliar fertilizer types resulted in significantly lower fruit split incidence at one of the study sites. The foliar fertilizer treatments did not have a significant effect on fruit number per tree, fruit diameter and mass, mass of all arils in fruit, or mass of 100 arils. The results suggest that foliar $ZnSO_4$, $MgSO_4$, and KNO_3 could be used to improve pomegranate nutrient availability and to decrease pomegranate fruit split incidence without negatively impacting fruit yield or size of 'Wonderful' pomegranate.

1. Introduction

Current worldwide production of pomegranate is not known precisely but previously has been estimated at approximately 3 million $t \cdot yr^{-1}$ with 300,000 ha in production (Hernández et al., 2012); the total value of the crop is unknown. The cultivar Wonderful is produced in many pomegranate growing regions and is the industry standard for the United States and Israel (Holland and Bar-Ya'akov, 2008). 'Wonderful' is a vigorous plant with the capacity to produce heavy yields (Levin, 2006) of large fruit with a dark-red exocarp and juice that fit market parameters for fresh market and juice concentrate commercial applications (Stover and Mercure, 2007).

Most pomegranate growers apply inorganic fertilizer by broadcast application (Glozer and Ferguson, 2008) or fertigation (Blumenfeld et al., 2000). Though the commercial fruit tree industry makes widespread use of foliar nutrient applications to correct nutrient deficiencies, increase yield and correct or prevent physiological disorders, there is little published evidence of pomegranate growers using foliar nutrient applications, except for $ZnSO_4$ applications to correct Zn deficiencies (Glozer and Ferguson, 2008; Stover and Mercure, 2007). Experimentally, significant increases in leaf Zn concentration have been reported in response to foliar applications of $ZnSO_4$ (Hasani et al., 2012; Khorsandi et al., 2009) and chelated nano-Zn and nano-boron (B) fertilizers applied alone or in combination (Davarpanah et al., 2016). Increases in leaf Mn concentration (Hasani et al., 2012) and leaf B concentration (Davarpanah et al., 2016) have also been reported in response to foliar applications of $MnSO_4$ and chelated nano-B, respectively. Foliar application of Zn has been shown to improve marketable yield of pomegranate, even in trees not displaying Zn deficiency symptoms (Afria et al., 1999; Davarpanah et al., 2016; Khorsandi et al., 2009). Davarpanah et al. (2016) also reported that foliar applications of chelated nano-B resulted in a significant increase in yield as compared the control. However, none of these studies were conducted on 'Wonderful' pomegranate and little else is known about the effects of foliar nutrient applications on pomegranate leaf nutrient status or yield.

Foliar nutrient applications have been used experimentally to mitigate pomegranate fruit split, also referred to as cracking (Yilmaz and Özgüven, 2009). Fruit split is the rupturing of the rind and is the

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https://doi.org/10.1016/j.scienta.2018.07.015
Received 29 April 2018; Received in revised form 9 July 2018; Accepted 13 July 2018

physiological disorder responsible for the greatest losses of pomegranate fresh market yields (Blumenfeld et al., 2000). Fruit split typically occurs during the final stages of fruit development (El-Rhman, 2010), although some pomegranate cultivars have a tendency to split before fruit maturity (Holland et al., 2009). However, the causes of fruit split of pomegranate are not well understood. Factors affecting the incidence of fruit split include timing of flower development (Glozer and Ferguson, 2008), cultivar (Hepaksoy et al., 2000; Lefeng et al., 2010; Levin, 2006), soil water content (Holland et al., 2009), water use efficiency (Hepaksoy et al., 2000), and fruit size and shape (Saei et al., 2014). Though flower removal (Singh and Kingsly, 2007), plant growth regulator applications (El-Khawaga, 2007; Yilmaz and Özgüven, 2009), antitranspirant application (Bacha and Ibrahim, 1979), and controlled irrigation (El-Rhman, 2010) have been tested as possible strategies to prevent fruit split of pomegranate, a commercially acceptable treatment has not yet been identified.

Foliar applications of 1% zinc sulfate ($ZnSO_4$) significantly decreased fruit split incidence of 'Manfaluty' pomegranate and when used in combination with controlled irrigation, decreased fruit split incidence by nearly 50% as compared to the control (El-Rhman, 2010). Foliar applications of 1% potassium nitrate (KNO_3), 1% magnesium sulfate ($MgSO_4$), 0.002% boric acid (H_3BO_3), or 0.005% H_3BO_3 resulted in significantly lower incidence of fruit split of 'Kandhari' and 'Beedana' pomegranate (Singh et al., 1993), with each treatment reducing the mean fruit split rate by more than 50% as compared to the control. The authors hypothesized that the foliar nutrient applications increased the elasticity and cell wall permeability of the rind, thus reducing the likelihood of fruit split. Davarpanah et al. (2016) reported that Zn and B foliar applications sprayed simultaneously or separately have no effect on fruit split of 'Ardestani' pomegranate. There appear to be no published studies of the use of foliar nutrient applications to decrease fruit split of 'Wonderful' pomegranate fruit. Therefore, the objectives of this study were to assess the effects of foliar applications of $ZnSO_4$, $MgSO_4$, or KNO_3 on 'Wonderful' pomegranate leaf nutrient concentrations and fruit split, yield and size.

2. Materials and methods

2.1. Plant material and experimental design

The study was conducted using 9-year-old bearing 'Wonderful' pomegranate trees at 2 commercial orchards in Kern County, CA, USA (site 1: latitude: 35°04′09.20″N, longitude: 119°18′47.74″W; site 2: latitude: 35°40′33.55″N, longitude: 119°55′16.72″W). The sites had well-drained, deep loam soils (Cerini loam at site 1; Kimberlina fine sandy at site 2). A randomized complete block design with 25 blocks and whole-tree experimental units was utilized. Data trees were selected at each site for uniform health, size, and vigor. Treatments consisted of foliar applications of $ZnSO_4$ (0.3%, 0.4%, and 0.5%), $MgSO_4$ (1%, 2%, and 3%), KNO_3 (1%, 2%, and 3%), and deionized (DI) water (control) for a total of 250 data trees at each site. All solutions were formulated in DI water with 0.50% adjuvant. Treatments were applied using a calibrated professional backpack sprayer (SP1, SP Systems International, Incorporated, Santa Monica, CA, USA) at early fruit set (July, when fruit were green to breaker stage with an equatorial diameter of approximately 50 mm) and late fruit set (August, when fruit were red with an equatorial diameter of approximately 70 mm), except for $ZnSO_4$ treatments, which were applied only at early fruit set. During treatment applications, neighboring data trees were shielded from any possible drift by cardboard panels.

2.2. Measured variables

To determine leaf nutrient concentrations, samples of 50–70 fully expanded leaves per study tree were collected approximately two weeks after the last treatment applications (late August). Up to two leaves per shoot were collected from shoots 1.5 m to 1.8 m above soil level on branches without developing fruit. Leaves were washed in a solution of DI water and phosphate-free soap, rinsed with DI water, and oven-dried to a constant mass. Mineral nutrient analysis was conducted by Precision Agri Lab Inc. (Madera, CA, USA). To determine N concentrations, the P-2.20 method of Gavlak et al. (2003) was conducted using a Leco Elemental Analyzer (Leco 528; Leco Corp., St. Joseph, MI, USA) with the following modification: perchloric acid ($HClO_4$) was used instead of hydrogen peroxide (H_2O_2) to oxidize plant matter. To determine phosphorus (P), K, S, calcium (Ca), Mg, sodium (Na), B, Zn, manganese (Mn), iron (Fe), and copper (Cu) concentrations, the P-4.20 method (Gavlak et al., 2003) was used with the following modifications to the extraction and heating protocol and the method of detection and quantification: 700 ± 250 mg of sample was predigested in 8 ml of nitric acid (HNO_3) for a minimum of 60 min, heated at 120 °C for 60 min, cooled, dissolved in 4 ml of H_2O_2, heated at 110 °C for 30 min, cooled, and filtered before samples were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Model 4300, PerkinElmer Corp., Waltham, MA, USA).

At harvest (22 October and 4 November at sites 1 and 2, respectively), the number of split and unsplit pomegranates was determined for each data tree and the total fruit mass and mass of unsplit fruit from each treatment tree was determined using a field scale. Both sites were strip harvested but harvest data were not collected from immature green fruit. A subsample of 10 unsplit fruit per treatment tree was selected randomly at harvest and stored at 5–8 °C in a refrigerated produce cooler for up to 5 d until the mass and equatorial diameter were measured. An additional random sample of up to five unsplit fruit per data tree was collected at harvest and stored at 5–8 °C for up to 19 d until arils could be extracted to determine total aril mass and the mass of 100 randomly selected arils per fruit.

2.3. Statistical analyses

For each variable, fertilizer treatments were compared using a mixed effects analysis of variance (ANOVA) with fertilizer concentration nested in fertilizer type as fixed effects and experimental blocks as random effects. Models for each site were fit separately. Partial F-tests to examine all terms with common factors of interest were used prior to tests for individual model terms to reduce Type I error. When ANOVA indicated significant differences, post-hoc comparisons were run utilizing Tukey's honestly significant difference (HSD) with an experimentwise error rate of $\alpha = 0.05$. Results for analyses are expressed as least squares mean (LSM). Relationships between percent fruit split and leaf nutrient concentrations, mean fruit diameter, or mean fruit mass and between leaf nutrient concentrations and yield were analyzed using multiple linear regression ($\alpha = 0.05$) without accounting for the applied treatments. Prior to regression analysis, a square root transformation of percent fruit split values was used to satisfy normality and homogeneity of variance conditions. All statistical tests were performed using JMP, Version 10 statistical software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Leaf nutrient concentrations

Significant treatment effects were detected for leaf N, K, S, Cu, Mn, and Zn concentrations (Tables 1 and 2). Foliar KNO_3 applications resulted in significantly higher leaf N concentration (site 1 only, $P < 0.001$; Table 1), with significantly higher leaf N concentrations in response to 2% and 3% KNO_3. At both sites, leaf K concentration was highest in response to the foliar KNO_3 treatments ($P < 0.001$; Table 1), with 2% and 3% KNO_3 resulting in significantly higher leaf K concentrations than the control at site 1 and all foliar KNO_3 treatments resulting in significantly higher leaf K concentrations than the control