

## Critical Crop Load Threshold in Nutrition and Biennial Bearing of Apple Trees

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### ABSTRACT

Crop load regulation is vital for achieving excellence in orchards, particularly in terms of consistent yields and high-quality fruit. It also has a direct impact on tree nutrition. The objective of this study was to investigate the relationship between crop load and tree nutrition using segment linear regression models. The focus was on identifying any breakpoints in this relationship and exploring the connection between leaf nutrient contents and fruit quality characteristics. Additionally, the study aimed to determine the critical crop load level that influences biennial bearing. The research was conducted in a high-density 'Golden Delicious'/M.9 apple orchard located in the Lake Region of Turkey over three consecutive years (2013-2015). Twenty-four different crop load levels were examined to assess the impact of the number of fruits on leaf nutrient contents. The critical threshold levels were determined as follows: potassium [0.91 kg cm<sup>-2</sup> Trunk Cross-Sectional Area (TCSA)], phosphorus (0.96 kg cm<sup>-2</sup> TCSA), magnesium (0.97 kg cm<sup>-2</sup> TCSA), manganese (0.99 kg cm<sup>-2</sup> TCSA), zinc (1.0 kg cm<sup>-2</sup> TCSA), and iron (1.15 kg cm<sup>-2</sup> TCSA). This suggests that a crop load ranging from 3.71 to 4.69 fruit/cm<sup>2</sup> TCSA could be considered critical depending on the specific nutrient in tree nutrition. The results revealed significant negative correlations between leaf mineral contents and overall fruit quality characteristics. Moreover, the critical crop load threshold for biennial bearing (0.77 kg cm<sup>-2</sup> TCSA) was found to be lower than the nutrient threshold. Building on previous studies, this research significantly contributes by clarifying the critical crop load level at which a sudden change occurs in macro- and micro-nutrients, as well as biennial bearing.

**Keywords:** Breakpoint, Fruit load, *Malus × domestica* Borkh, Piecewise linear regression.

### INTRODUCTION

Crop Load (CL) refers to the amount of fruit remaining per unit of Trunk Cross-Sectional Area (TCSA) on a tree (Robinson, 2008). It has long been recognized as one of the most crucial practices in horticulture due to its direct impact on biennial bearing in fruit crops (Yang *et al.*, 2021). Biennial bearing is a phenomenon characterized by alternating heavy and light fruit production years, occurring in various perennial fruit species and leading to economic losses (Monselise and Goldschmidt, 1982). Studies have shown that controlling CL can mitigate biennial bearing (Elgar *et al.*, 1999; Suo *et*

*al.*, 2016).

Furthermore, CL significantly influences both the quality and quantity of fruit, which ultimately determine the overall crop value (Robinson *et al.*, 2013). Several physiological processes, including tree water status and maximum daily trunk diameter, are affected by CL (Atay *et al.*, 2021). High CL creates stress that depletes the tree's carbohydrate reserves necessary for growth (Goldschmidt, 1999). Consequently, there is competition for resources between CL and vegetative sinks (Anthony *et al.*, 2019). Controlling CL effectively regulates the distribution of carbon in plants (Ding *et al.*, 2017).

When persimmon trees were defruited, the

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root accounted for 44 and 35% of the total increase in nitrogen and potassium, respectively (Choi *et al.*, 2010). CL can affect the nutrient contents of both leaves and fruit (Neilsen *et al.*, 2015). However, the effect of CL on leaf macronutrients can vary depending on the fruit species (Wünsche and Ferguson, 2005). Previous studies (Smith, 1962; Hansen, 1971; Hansen, 1973; Wünsche and Ferguson, 2005; Samuoleine *et al.*, 2016; Ding *et al.*, 2017; Reig *et al.*, 2018; Anthony *et al.*, 2019; Meszaros *et al.*, 2021; Ngao *et al.*, 2021; Sidhu *et al.*, 2022) indicate that the impact of CL on leaf nutrients is not consistently apparent. In fact, studies comparing different CL levels often involve limited categories that fail to encompass moderate and extreme loads. Therefore, the true interaction between CL and plant nutrition remains incompletely understood, and there is still no consensus on how CL influences nutrition in various fruit species.

It is worth noting that Turkey is the world's second-largest producer of apples (FAO, 2023). 'Golden Delicious' accounts for approximately 24% of apple production in Turkey and is the second most popular cultivar after 'Red Delicious' (TURKSTAT, 2023). 'Golden Delicious' has been observed to be susceptible to biennial bearing in the study area (Atay *et al.*, 2013; Atay, 2017).

The primary objective of the present study was to investigate the relationship between CL and leaf nutrients in 'Golden Delicious'/M.9 apple using Segmented Linear Regression, a statistical approach that is not widely utilized. Segmented Linear Regression determines if there is a breakpoint (if any) in the relationship between CL and leaf nutrients, providing further clarity on the effect of CL on plant nutrition and discussing the phloem mobility of nutrients. Additionally, the study aimed to assess the correlation between leaf nutrient contents and fruit quality characteristics. Lastly, it aimed to identify the critical CL level that influences biennial bearing.

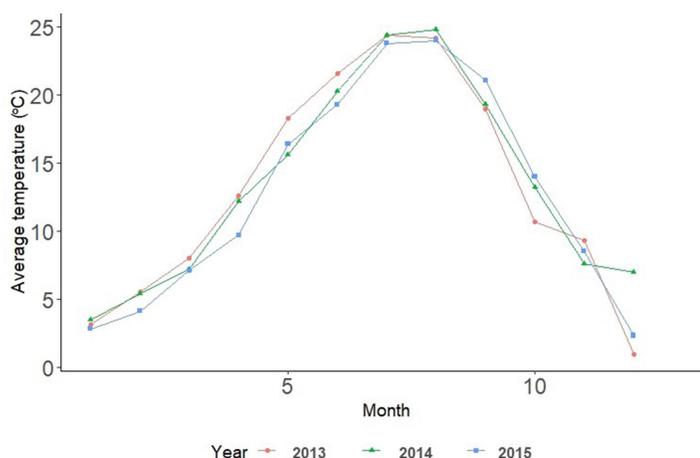
## MATERIALS AND METHODS

This study was conducted over a span of three consecutive years (2013-2015) in a mature orchard of 'Golden Delicious'/M.9 apple located in the Lake Region of Turkey (latitude 37° 48' 52.16" N, longitude 30° 52' 39.66" E, elevation 920 m). The region has a characteristic Mediterranean climate, with hot summers and mild winters. The annual average temperature is around 13°C (Figure 1). The orchard was established in 2005 with a planting density of 2857 trees per hectare, utilizing a 3.5×1.0 m layout. Trees were trained to the vertical axis. The soil in the orchard has a clay-loam texture (Table 1).

The orchard was drip-irrigated with evapotranspiration-based full irrigation at 4-day intervals. Fertilization was carried out based on soil analysis. No organic compounds or foliar fertilizers were applied to the orchard during the study period. After the physiological fruitlet drop, trees were manually thinned following local practices to achieve the desired commercial fruit quality.

In the last two years of the study (2014 and 2015), only yield values were recorded to examine the relationship between CL and biennial bearing. Following the commercial hand thinning in 2013, a total of 24 trees representing a range of minimum, maximum, and intermediate crop loads were selected from the trial orchard (i.e., one tree per CL level). These selected CL levels encompassed a broad range from 0.38 to 1.84 kg cm<sup>-2</sup> TCSA (= 1.55 to 7.51 fruit cm<sup>-2</sup> TCSA). The experimental trees were labeled to ensure consistent data collection from the same trees for subsequent investigations, such as leaf sampling and fruit picking.

The fruit from each tree were harvested manually in a single picking event and weighed. To assess the fruit quantity relative to the wood mass, the yield efficiency, referred to as CL in this study, was calculated as kg cm<sup>-2</sup> TCSA. Tree trunk diameters were measured 15 cm above the budding point, and TCSA was determined



**Figure 1.** The monthly average temperature at the research area over three years (2013-2015).

**Table 1.** Soil properties of the study orchard (0-30 cm).

Soil property and unit (in relation to dry matter)	Value
Saturation (%)	71
Sand (%)	18
Silt (%)	39
Clay (%)	43
EC (mS/cm)	0.16
pH (1:2.5)	7.51
Total lime (%)	8.3
Organic matter (Smith Weldon) (%)	3.9
Phosphorus (Olsen ICP) (ppm)	42
Potassium (Ammonium Acetate-ICP) (ppm)	353
Calcium (Ammonium Acetate-ICP) (ppm)	4564
Magnesium (Ammonium Acetate-ICP) (ppm)	570
Sodium (Ammonium Acetate-ICP) (ppm)	25.17
Iron (DTPA-ICP) (ppm)	23.53
Copper (DTPA-ICP) (ppm)	17.05
Manganese (DTPA-ICP) (ppm)	14.91
Zinc (DTPA-ICP) (ppm)	1.75

using the formula:  $TCSA = \pi (\text{Trunk diameter}/2)^2$ .

Based on the recommendation by Nielsen and Nielsen (2003) that apple leaf samples should be collected 110-125 days after full bloom to minimize annual variation in nutrient content, leaf sampling in this study was conducted 110 days after full bloom. Each sample for each CL level consisted of approximately 40 mid-shoot leaves gathered

from four directions of the trees. Initially, the leaves were rinsed sequentially with tap water, 1N HCl, and distilled water. Subsequently, the leaf samples were placed on absorbent papers at room temperature for 1 hour to allow water drainage. Then, they were dried in an oven (Model FN 500, Nuve, Ankara, Turkey) at 45°C for 24 hours, followed by 70°C until stable weights were obtained. The dried samples were then



ground for macro- and micro-nutrient analyses. The total Nitrogen (N) content was determined using the Kjeldahl method with a distillation unit (Gerhardt, Königswinter, Germany). The contents of Phosphorus (P), potassium (K), Calcium (Ca), Magnesium (Mg), iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), and Boron (B) were determined using an inductively coupled plasma spectrometer (Perkin-Elmer, Optima 2100 DV Optical Emission Spectrometer, Shelton, CT 06 484, USA). The nutrient contents in leaf tissues were reported on a dry mass basis.

At each CL level (i.e., each experimental tree), a randomly selected sample of ten fruits was picked and assessed for fruit quality characteristics. Fruit weight was measured using a digital scale sensitive to 0.01 g. Fruit flesh firmness ( $\text{kg cm}^{-2}$ ) was measured on two opposite sides of the fruit using a handheld penetrometer (Model FT 011, Effegi, Italy) fitted with an 11.1 mm probe. Soluble Solids Content (SSC) was measured in fruit juice using a digital refractometer (HANNA, HI 96801, USA). Titratable acidity was determined by titrating 10 ml of fruit juice with 0.1 M NaOH to pH 8.15, using a digital buret (Isolab Digitrate, UK), and malic acid content was calculated. The pH of the fruit was measured using a digital pH meter (HANNA, HI 2211, USA). Fruit skin colour values ( $L^*$ ,  $a^*$ ,  $b^*$ , hue, and C) were recorded using a colourimeter (Minolta CR-400, Japan). The CIELAB colour space, also known as  $L^*$ ,  $a^*$ ,  $b^*$ , represents colours using three values ( $L^*$  for Lightness,  $a^*$  and  $b^*$  for unique human vision colors- red, green, blue and yellow), while the derived CIELCh or CIEHLC space uses polar coordinates (Hue for Hue angle and C for saturation) instead of Cartesian coordinates, without altering the Lightness ( $L^*$ ) of CIELAB ([https://en.wikipedia.org/wiki/CIELAB\\_color\\_space](https://en.wikipedia.org/wiki/CIELAB_color_space), 1976).

Segmented Linear Regression (SLR), also known as piecewise regression, was performed to investigate the presence of a

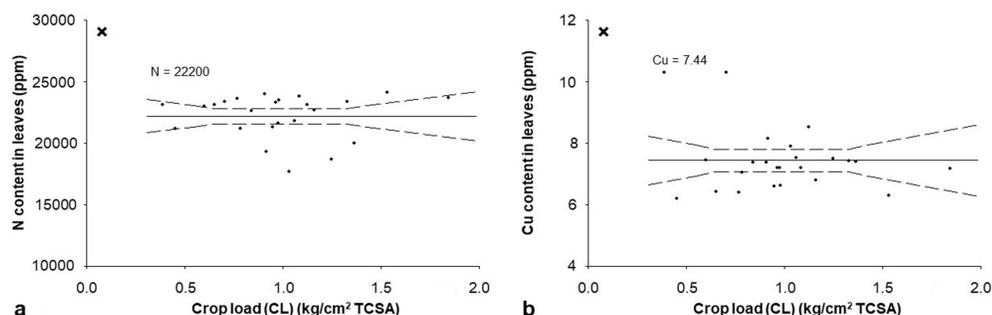
breakpoint, indicating a sudden change or threshold, in the relationship between CL and the nutrients. The SegReg software (Oosterbaan, 2011) was used for this analysis. If a breakpoint exists in the data, SegReg identifies its location and significance, and provides regression functions for the segments before and after the breakpoint (Domkin *et al.*, 2016). SegReg offers seven function types (0-6) that maximize the coefficient of determination ( $R^2$ ) (Oosterbaan, 2011). Type-0 represents a horizontal line with no significant relationship or breakpoint, Type-1 is a linear regression (positive or negative) without a breakpoint, Type-2 connects two segments at a breakpoint, each with a positive or negative slope, Type-3 is a horizontal line until the breakpoint followed by a positive or negative sloping line, Type-4 is a positive or negative sloping line until the breakpoint followed by a horizontal line, Type-5 is a step function with two horizontal lines having significantly different means, and Type-6 consists of two disconnected segments with, at least, one of them sloping towards or away from the breakpoint (Korosi *et al.*, 2008; Grimstead, 2017). Pairwise correlation analyses were performed to investigate the relationship between fruit characteristics and CL using the 'Performance Analytics' package in R.

## RESULTS AND DISCUSSION

### Type-0 Relationship

In order to understand the impact of CL on leaf nutrient contents, the levels of macronutrients and micronutrients were analyzed for each CL level in the leaves of 'Golden Delicious'/M.9 trees. The relationship between CL and N (Figure 2-a) as well as Cu (Figure 2b) showed a Type-0 relationship, indicating no significant association, as represented by a horizontal line.

In apple orchards, the leaves of fruiting trees generally have higher N levels



**Figure 2.** Leaf (a) Nitrogen (N) and (b) Copper (Cu) content relationships with Crop Load (CL). The solid line represents the fitted segmented regression line. Dashed lines represent the 90% confidence interval. ×: Segmented Linear Regression (SLR) is insignificant at  $P < 0.05$ .

compared to non-fruiting trees (Wünsche and Ferguson, 2005). Urban *et al.* (2004) observed a significant 8% increase in N content in the leaves of high CL treatment compared to mango's light CL treatment. Anthony *et al.* (2019) found that N content in the leaves of 'WA38'/M.9 Nic29' apple increased with increasing CL. However, Ding *et al.* (2017) reported contrasting results, showing a decrease in N allocation to leaves in 'Red Fuji'/M.26(interstock)/Malus hupehensis Rehd) apples as CL increased. In 'Nadorcott' mandarin, Stander *et al.* (2018) found that "off" trees had higher leaf N content compared to "on" trees. Regarding leaf Cu content in 'Catherine'/Nemaguard peach trees, Blanco *et al.* (1995) indicated that it did not show any alteration with increasing CL.

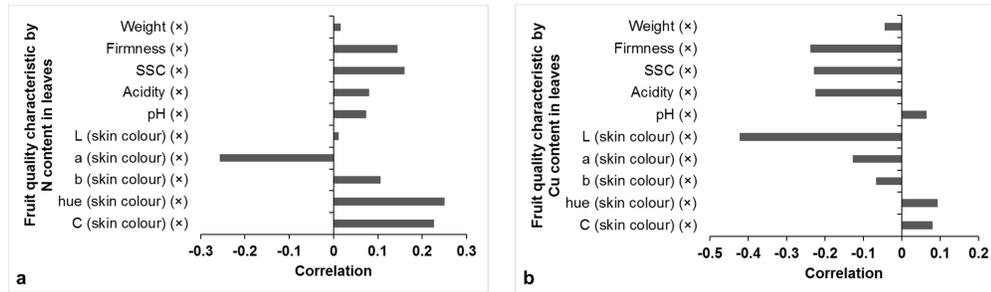
In addition to the different genetic backgrounds, the physiological variations observed among these studies are likely attributed to differences in CL levels, which potentially influence the uptake of N from the soil through the xylem, as suggested previously (Meszaros *et al.* 2021; Sidhu *et al.* 2022). Similarly, the non-significant findings regarding Cu may be attributed to the application of Bordeaux mixture, a copper sulphate solution commonly used in orchards. Under the conditions of the current study, it is likely that the availability of both minerals was sufficient. The leaf contents ranged from 17,696 to 24,136 ppm for N

and from 6.2 to 10.3 ppm for Cu. Thus, the targeted leaf contents of N and Cu in mature apple trees typically fall within the range of 17,000-25,000 ppm and 5-12 ppm, respectively (Nielsen and Nielsen, 2003).

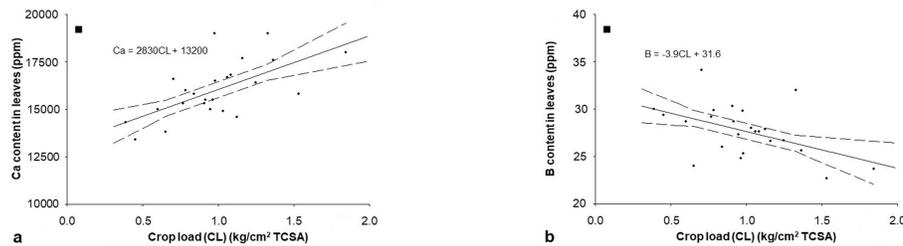
Correlation analyses revealed that the content of Nitrogen (N) (Figure 3-a) and Copper (Cu) (Figure 3-b) in the leaves did not significantly impact fruit quality. However, it is worth noting that leaf N content generally has significant effects on fruit size and composition, with an increase in leaf N content leading to enhanced fruit size (Dris *et al.*, 1999). Additionally, N content in leaves plays a considerable role in the acidity of apple fruit (Jivan and Sala, 2014). The influence of CL on N accumulation in fruit remains unclear (Verdenal *et al.*, 2020).

### Type-1 Relationship

A Type-1 function was determined for Ca, showing a positive linear regression (Figure 4-a), while B exhibited a negative linear regression (Figure 4-b). Some studies (e.g., Anthony *et al.*, 2019; Sidhu *et al.*, 2022) reported no significant relationships between CL and Ca content in apple leaves. However, consistent with the findings of the present study, previous research on high cropping apple trees (Wünsche and Ferguson, 2005; Meszaros *et al.*, 2021), mandarin trees (Stander *et al.*, 2018), and



**Figure 3.** Correlations between (a) Nitrogen (N) and (b) Copper (Cu) content in leaves and fruit quality characteristics. ×: Correlation is insignificant at  $P < 0.05$ .



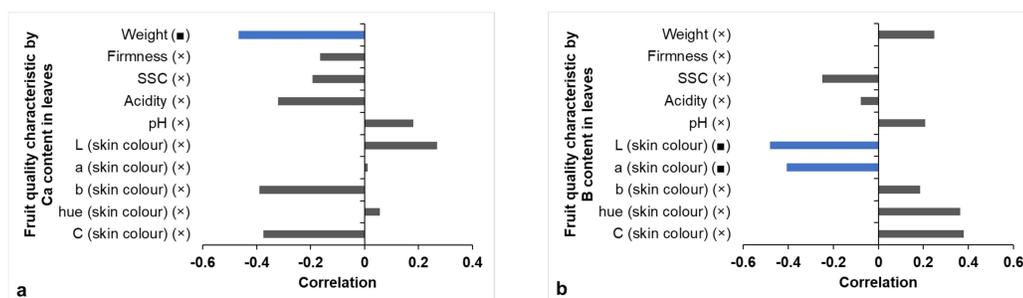
**Figure 4.** Leaf (a) Calcium (Ca) and (b) Boron (B) content relationships with Crop Load (CL). The solid line represents the fitted segmented regression line. Dashed lines represent the 90% confidence interval. ■: Segmented Linear Regression (SLR) is significant at  $P < 0.05$ .

pistachio trees (Gündeşli *et al.*, 2021) demonstrated higher Ca content compared to trees with low CL. On the other hand, CL did not have a significant impact on the leaf B content in apples (Sidhu *et al.*, 2022).

Since Ca is a relatively phloem-immobile nutrient, it is not transported from leaves to fruits (Vilhena *et al.*, 2022). The accumulation of Ca in fruits primarily relies on transpirational water flow (Nestby and Retamales, 2020). In line with the increase in CL, the uptake of Ca from the soil may have increased due to higher transpiration rates. Consequently, the Ca content in leaves tends to increase linearly with increasing CL, as Ca remains in the leaves and is not transported to the fruit. B is typically considered phloem-immobile in some fruit trees (Mousavi and Motesharezadeh, 2020). However, in the case of *Malus*, which is a phloem-mobile genus (Brown and Hu, 1996), the linear decrease in leaf B content as CL increases suggests that B is transported from the leaves to the fruit.

The Ca content in leaves exhibited a negative correlation with fruit weight ( $P < 0.05$ ) (Figure 5-a). This negative correlation

could be influenced by the ratio of other nutrients to Ca, considering the presence of antagonisms and synergism between nutrients. Since Ca is phloem immobile, its relationship with fruit weight may be influenced by the interplay with other nutrients. The B content in leaves showed a negative correlation with the  $L^*$  and  $a^*$  skin colour values of the fruit (Figure 5-b).  $L^*$  (luminance) ranges from black (0) to white (100) (Alcobendas *et al.*, 2012). In this study, an increase in leaf B content led to a decrease in the  $L^*$  value of the fruit, resulting in a darker skin colour. The  $a^*$  value represents the redness (positive) or greenness (negative) of the fruit. With increasing leaf B content, more negative  $a^*$  values were observed in the fruit, indicating a greener skin colour. Both Ca and B in leaves contribute to shoot growth and cell wall structure (Wu *et al.*, 2021; Chen *et al.*, 2022). The efficient synthesis of Ca and B, particularly during the shoot growth period coinciding with the cell division phase, may stimulate shoot growth. Carbohydrates are utilized during the cell division period for shoot growth (Naschitz *et al.*, 2010). The



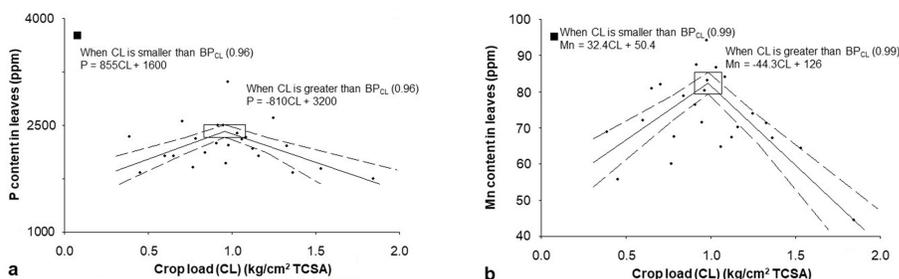
**Figure 5.** Correlations between (a) Calcium (Ca) and (b) Boron (B) content in leaves and fruit quality characteristics. The '×' symbol indicates that the correlation is not significant, while the '■' symbol represents a significant correlation at  $P < 0.05$ .

decrease in fruit size and poor colour development observed in this study may be related to the carbohydrate mechanisms associated with Ca and B.

### Type-2 Relationship

Phosphorus (P) showed a Type-2 relationship with CL, exhibiting two significant trends. The CL breakpoint was observed at  $0.96 \text{ kg cm}^{-2}$  TCSA, where the increasing trend ( $0.38\text{-}0.96 \text{ kg cm}^{-2}$  TCSA of CL) shifted to a decreasing trend ( $0.97\text{-}1.84 \text{ kg cm}^{-2}$  TCSA of CL) (Figure 6-a). Also, Mn displayed a Type-2 relationship, with a CL breakpoint of  $0.99 \text{ kg cm}^{-2}$  TCSA. Up to this threshold of CL (from  $0.38$  to  $0.99 \text{ kg cm}^{-2}$  TCSA), Mn content increased, while the other connected segment showed a negative slope (Figure 6-b). Previous studies have reported higher P content in the

leaves of 'On' trees in pistachio trees, while mandarin trees showed higher P values in 'Off' trees (Gündeşli *et al.*, 2021; Stander *et al.*, 2018). The effect of CL on P content in apple trees has not been clearly established in studies (Hansen, 1971; Samuoline *et al.*, 2016; Anthony *et al.*, 2019; Sidhu *et al.*, 2022). Similarly, the impact of CL on leaf Mn content has been found to be statistically insignificant in certain studies (Anthony *et al.*, 2019; Sidhu *et al.*, 2022), whereas contrasting results have been reported in others (Blanco *et al.*, 1995; Atay, 2016; Gündeşli *et al.*, 2021). In our study, the effect of CL on leaf P and Mn content, which exhibited, respectively, initially positive and then negative trends, has been clarified. The presence of fruit stimulates photosynthesis and respiration through stomatal opening (Silber *et al.*, 2013), potentially leading to increased influx of xylem-transported P and Mn from the soil to



**Figure 6.** Leaf (a) Phosphorus (P) and (b) Manganese (Mn) content relationships with Crop Load (CL). The solid line represents the fitted segmented regression line. Dashed lines represent the 90% confidence interval. The threshold or breakpoint is indicated by the breakpoint box. BP: Breakpoint. ■: Segmented Linear Regression (SLR) is significant at  $P < 0.05$ .

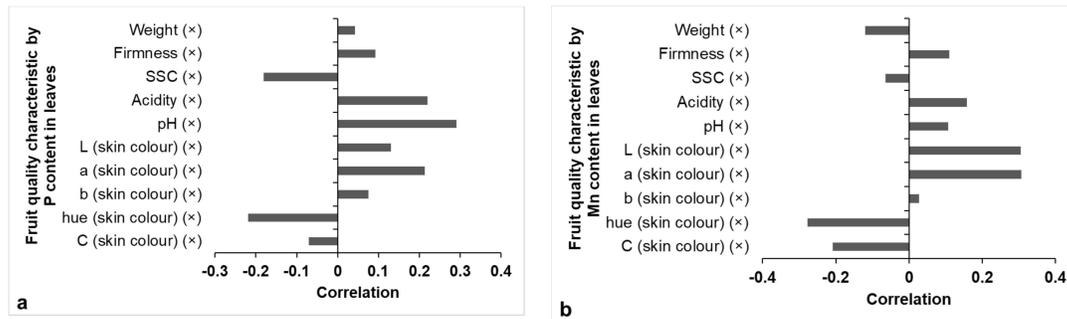


Figure 7. Correlations between (a) Phosphorus (P) and (b) Manganese (Mn) content in leaves and fruit quality characteristics. x: Correlation is insignificant at P< 0.05.

the leaves until the CL breakpoint. However, competition for these minerals between the fruit and leaves may begin after the CL breakpoint (approximately 1.00 kg cm<sup>-2</sup> TCSA). In apples, around 6-10 weeks after flowering, the fruit becomes disconnected from the xylem, and water, carbohydrates, and nutrients can move to the fruit from the leaves through the phloem (Drazeta *et al.*, 2004). Consequently, P exhibits high mobility (Epstein and Bloom, 2005), while Mn shows relatively low mobility (Parent *et al.*, 2020) in the phloem, from older tissues to actively growing parts of the plant. P (Figure 7-a) and Mn (Figure 7-b) content in leaves did not significantly affect any fruit quality characteristics.

### Type-3 Relationship

Figure 8 shows the relationships between (a) K and (b) Zn content in leaves with CL. The function type observed for K and Zn is Type-3, characterized by a flat line until the breakpoint, followed by a negative sloping line when CL exceeds the breakpoint. In apples, the K content in leaves has been observed to decrease linearly with increasing CL (Atay, 2016). This decrease can be attributed to the competition for K between the leaves, which cannot compete with the fruit as a large sink for K (Hansen, 1971; Neilsen and Neilsen, 2003). The present study aligns with previous research (Wünsche and Ferguson, 2005; Stander *et*

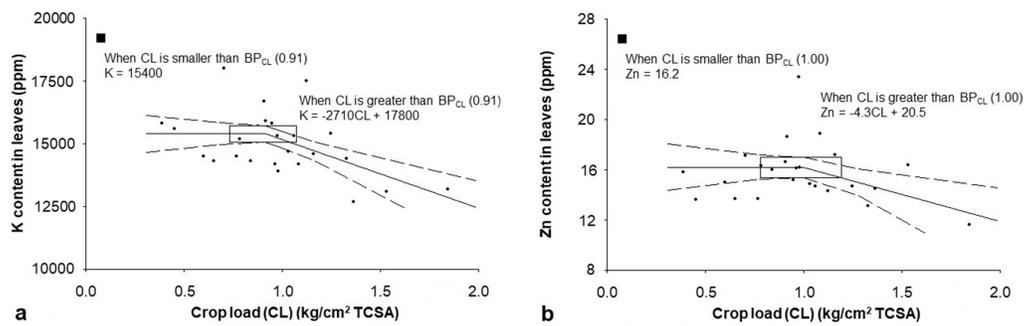


Figure 8. Leaf (a) potassium (K) and (b) Zinc (Zn) content relationships with Crop Load (CL). The solid line represents the fitted segmented regression line. Dashed lines represent the 90% confidence interval. The threshold or breakpoint is indicated by the breakpoint box. BP: Breakpoint. ■: Segmented Linear Regression (SLR) is significant at P< 0.05

*al.*, 2018; Anthony *et al.*, 2019) in demonstrating that increasing CL leads to a reduction in leaf K content. The effect of CL on leaf Zn content has generally been considered insignificant in previous studies (Blanco *et al.*, 1995; Anthony *et al.*, 2019). However, this study, along with others, reveals that the effect of CL on leaf K and Zn content remains constant up to the breakpoint, after which the content decreases as CL increases.

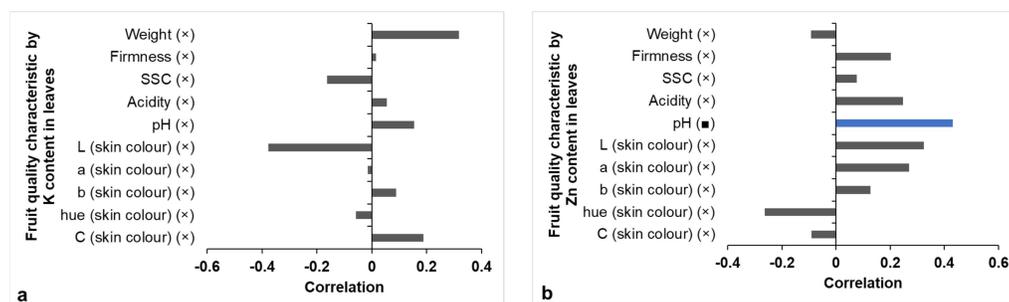
Potassium (K) began to move from the leaves to the fruit when the crop load exceeded  $0.91 \text{ kg cm}^{-2}$  TCSA. The CL breakpoint for Zn was observed to be  $1.00 \text{ kg cm}^{-2}$  TCSA. This suggests that K exhibits high mobility in the phloem (Toselli *et al.*, 2020). On the other hand, Zn is considered a variable mobile nutrient in the phloem, and its efficiency can be enhanced in plants with a rich Zn supply (Etesami and Jeong, 2020). Therefore, it can be concluded that, after the CL breakpoint, both K and Zn in the leaves are transported to the fruit via the phloem.

Leaf K did not correlate with any of the fruit quality characteristics (Figure 9-a). On the other hand, leaf Zn content displayed a positive correlation with fruit pH (Figure 9-b). The total Zn content in the orchard soils was approximately 80 ppm (Nielsen and Nielsen, 2003). However, in the specific orchard where the current study was conducted, the soil Zn content was very low, measuring only 1.75 ppm (See Table 1). This indicates a potential risk of Zn

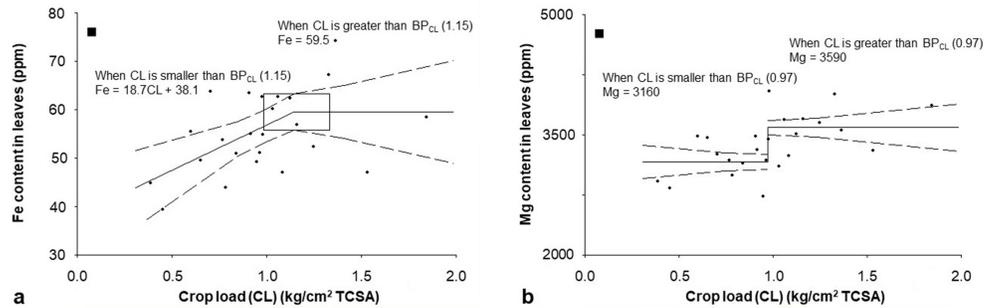
deficiency at the tree level in the orchard. The influence of CL on leaf assimilation is dependent on the availability of water and minerals in the soil (Yang *et al.*, 2021). Zn deficiency may occur if the leaf Zn content falls below 14 ppm (Nielsen and Nielsen, 2003). In this study, the leaf Zn content in the experimental trees was generally low, and some trees even recorded values below the threshold of 14 ppm. It is possible that the Zn in leaves interacted with other elements, leading to an increase in fruit pH.

### Type-4 and Type-5 Relationships

Significant findings were observed for Fe and Mg in relation to CL. Figure 10 (a) illustrates a Type-4 function for Fe, characterized by a sloping line followed by a flat line. Fe content increased in parallel with CL until reaching the CL breakpoint of  $1.15 \text{ kg cm}^{-2}$  TCSA. Beyond the breakpoint, Fe exhibited a constant value of 59.5 ppm between 1.15 and  $1.84 \text{ kg cm}^{-2}$  TCSA of CL. In Figure 10 (b), Mg demonstrated a step function (Type-5) in relation to CL, with a CL breakpoint of  $0.97 \text{ kg cm}^{-2}$  TCSA. Below the breakpoint, Mg content was smaller compared to higher CL levels, with values of 3160 ppm for the left side ( $0.38\text{-}0.97 \text{ kg cm}^{-2}$  TCSA of CL) and 3,590 ppm for the right side ( $0.98\text{-}1.84 \text{ kg cm}^{-2}$  TCSA of CL). Previous studies (e.g., Blanco *et al.*,



**Figure 9.** Correlations between (a) potassium (K) and (b) Zinc (Zn) content in leaves and fruit quality characteristics. The 'x' symbol indicates that the correlation is not significant, while the '■' symbol represents a significant correlation at  $P < 0.05$ .



**Figure 10.** Leaf (a) iron (Fe) and (b) Magnesium (Mg) content relationships with Crop Load (CL). The solid line represents the fitted segmented regression line. Dashed lines represent the 90% confidence interval. The threshold or breakpoint, if it exists, is indicated by the breakpoint box. BP: Breakpoint. ■: Segmented Linear Regression (SLR) is significant at  $P < 0.05$ .

1995; Samuoliene *et al.*, 2016; Anthony *et al.*, 2019) generally indicated that the effect of CL on leaf Fe content was insignificant. However, some studies (e.g., Gündeşli *et al.*, 2021) reported an increase in leaf Fe content with increasing CL. Regarding leaf Mg content, some studies on apple (Samuoliene *et al.*, 2016; Anthony *et al.*, 2019; Sidhu *et al.*, 2022) found no significant effect of CL, while others (Wünsche and Ferguson, 2005) showed that leaves of high cropping trees generally had higher Mg content than trees with low CL. Consistent with the present study, research on pistachio (Gündeşli *et al.*, 2021) and mandarin (Stander *et al.*, 2018) trees also indicated an increase in leaf Mg content with increasing CL. Notably, the present study revealed the critical threshold for CL-related Fe and Mg content in leaves.

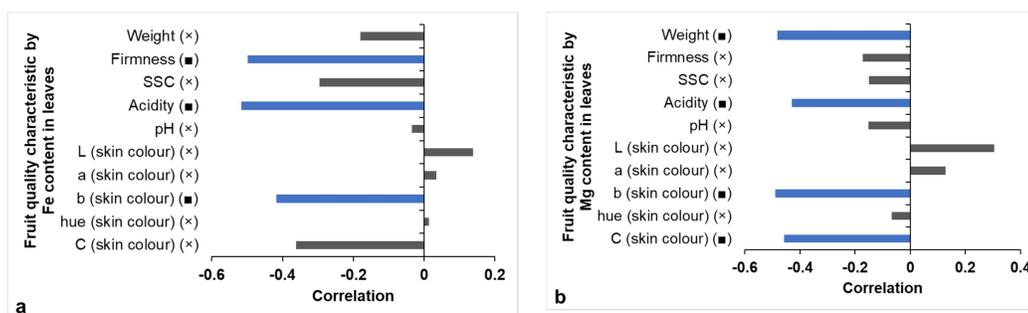
Physiologically, the leaf Fe content, like P and Mn (Type-2 function), may have increased parallel with transpiration streams up to the CL breakpoint. After this point, we can deduce a relationship between leaf and fruit that is in balance and does not turn into fierce competition.

Leaf Fe content correlated negatively with firmness, acidity, and  $b^*$  value (Figure 11-a), whereas leaf Mg content correlated with weight, acidity,  $b^*$  value, and C value (Figure 11-b). The increase in Fe and Mg content transported to the leaves from the root system may have caused a deficiency of these elements in the fruit. Thus, while Fe is

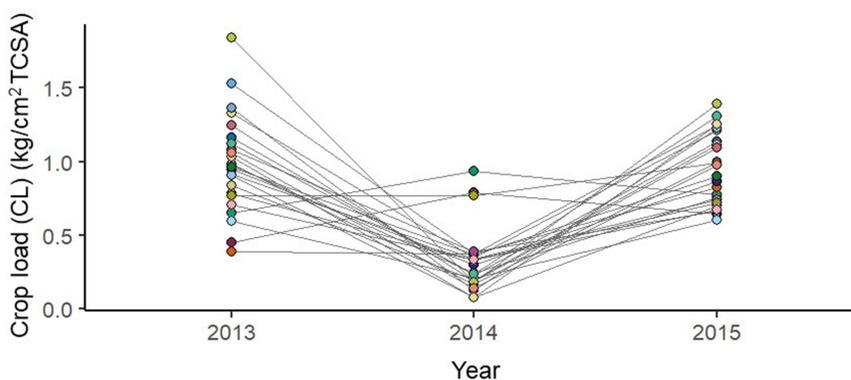
not highly mobile in the phloem, Mg exhibits high mobility from older tissues to actively growing parts of the plant (Epstein and Bloom, 2005). Despite the different phloem mobility of these nutrients, they had similar effects on fruit quality under the current study conditions. As a result, the fruit may have been disadvantaged in terms of the aforementioned quality characteristics. Additionally, Fe and Mg may have stimulated photosynthesis and shoot growth, leading to shading within the tree. Branch shading can negatively affect fruit development and, ultimately, fruit quality.

### Biennial Bearing Relationship

Although the results are inconsistent when considering all twenty-four experimental trees, the yield for the following year (i.e., 2014) either remained the same or increased in trees with a CL of  $0.77 \text{ kg cm}^{-2} \text{ TCSA}$  and below in 2013. In the current orchard conditions in 2013 (data not shown), a CL of  $0.77 \text{ kg cm}^{-2} \text{ TCSA}$  corresponded to  $3.14 \text{ fruit cm}^{-2} \text{ TCSA}$ . Consequently, the average fruit weight at the 2013 harvest was determined to be 245 g (data not shown). Trees that had relatively low CL values in 2013 exhibited relatively high CL values in 2014. Interestingly, only two trees with CL values above  $0.77 \text{ kg cm}^{-2} \text{ TCSA}$  in 2014



**Figure 11.** Correlations between (a) iron (Fe) and (b) Magnesium (Mg) content in leaves and fruit quality characteristics. The '×' symbol indicates that the correlation is not significant, while the '■' symbol represents a significant correlation at  $P < 0.05$ .



**Figure 12.** Crop load of twenty-four experimental trees over three consecutive years from 2013 to 2015.

showed lower CL values in 2015 (Figure 12). CL should be managed to diminish the occurrence of biennial bearing (Elgar *et al.*, 1999). Thus, controlling CL can effectively reduce fluctuations in apple production, leading to appropriate productivity (Suo *et al.*, 2016). Biennial bearing can be avoided if CL is strictly limited to 4, 5, or 6 fruit  $\text{cm}^{-2}$  TCSA, depending on the cultivar, until mid-June (Robinson, 2008). Although genotype is the critical determinant of biennial bearing tendency in apples, CL, rootstock, hormones (mainly gibberellins) associated with seed development, and environmental factors can contribute to biennial bearing (Pellerin *et al.*, 2011; Atay *et al.*, 2013).

## CONCLUSIONS

To conclude, this study revealed abrupt changes at a critical threshold level of CL in

nutrient contents in leaves, as indicated by Type-2 (P and Mn), Type-3 (K and Zn), Type-4 (Fe), and Type-5 (Mg) relationships. Interestingly, the critical CL threshold for biennial bearing was found to be even lower than the nutrient threshold.

## ACKNOWLEDGEMENTS

We would like to express our gratitude to our colleagues at the Fruit Research Institute (Egirdir, Isparta), our previous workplace, for their valuable support.

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## آستانه باردهی بحرانی در تغذیه و باردهی متناوب (دوساله) درختان سیب

## ۱. آتای، و آ.ن. آتای

## چکیده

تنظیم باردهی محصول برای دستیابی به کیفیت برتر در باغ‌ها، به ویژه از نظر ثبات عملکرد و میوه با کیفیت بالا، از اهمیت بالایی برخوردار است. این کار همچنین تأثیر مستقیمی بر تغذیه درختان دارد. هدف این پژوهش بررسی رابطه بین باردهی درخت‌ها و تغذیه آنها با استفاده از مدل‌های رگرسیون خطی قطعه‌ای (segment linear regression) بود. تمرکز بررسی بر شناسایی هر نقطه گسست در این رابطه و بررسی ارتباط بین محتویات مواد مغذی برگ و ویژگی‌های کیفیت میوه بود. افزون بر این، هدف این پژوهش تعیین سطح بار-دهی بحرانی محصول بود که بر باردهی متناوب تأثیر می‌گذارد. این پژوهش در یک باغ سیب 'Golden Delicious'/M.9 با تراکم بالا واقع در منطقه دریاچه ترکیه طی سه سال متوالی (۲۰۱۳-۲۰۱۵) انجام شد. بیست و چهار سطح مختلف باردهی محصول برای ارزیابی تأثیر تعداد میوه بر محتویات مواد مغذی برگ بررسی شد. سطوح آستانه بحرانی به شرح زیر تعیین شد: پتاسیم (۰.۹۱ کیلوگرم بر سانتی‌متر مربع مقطع تنه (TCSA)، فسفر (۰.۹۶ کیلوگرم بر سانتی‌متر مربع (TCSA)، منیزیم (۰.۹۷ کیلوگرم بر سانتی‌متر مربع (TCSA)، منگنز (۰.۹۹ کیلوگرم بر سانتی‌متر مربع (TCSA)، روی (۱.۰ کیلوگرم بر سانتی‌متر مربع (TCSA) و آهن (۱.۱۵ کیلوگرم بر سانتی‌متر مربع (TCSA). این نشان می‌دهد که، بسته به ماده مغذی خاص در تغذیه درخت، باردهی محصول از ۳.۷۱ تا ۴.۶۹ میوه بر سانتی‌متر مربع TCSA می‌تواند حیاتی در نظر گرفته شود. نتایج، همبستگی منفی معنی‌داری بین محتوای مواد معدنی برگ و خصوصیات کیفی میوه نشان داد. افزون بر این، آستانه باردهی بحرانی محصول برای باردهی دوساله (biennial bearing) (۰.۷۷ کیلوگرم بر سانتی‌متر مربع (TCSA) کمتر از آستانه مواد مغذی بود. بر پایه بررسی‌های قبلی، تحقیق حاضر با مشخص کردن سطح باردهی بحرانی، که در آن طی تغییر ناگهانی در مواد مغذی پرمصرف و کم‌مصرف، و همچنین باردهی دوساله رخ می‌دهد، به طور قابل توجهی کمک می‌کند.