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ARTICLE

Effect of Alternate Bearing Phenomenon and Boron Foliar Application on Nitrogen-15 Uptake, Translocation and Distribution in Mango Tree (cv. Zebda)

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ABSTRACT

The objectives of this investigation are to study nitrogen uptake, translocation, accumulation and distribution in mango tree organs using labeled nitrogen (^{15}N) and to understand the mechanism of boron action in increasing fruit yield in the off-year. A field experiment was conducted using fifteen-year-old mango trees (cv. Zebda) grown at Al Malak Valley Farm, El-Sharkeya Governorate-Egypt. Treatments included the application of $(^{15}NH_4)_2SO_4$, "in the on-year", at a rate of 50 g nitrogen/tree through the stem injection technique. While boron was sprayed on the same trees "in the off-year" at the following rates: 0.0 (control), 250 and 500 mg·L^{-1}. The authors hypothesize that boron and nitrogen act synergistically to increase mango fruit yield in the off-year. Results indicated that the highest ^{15}N uptake and accumulation in the on and off-years was observed in the upper (young leaves). When boron was applied at 250 mg·L^{-1}, in the off-year, the upper (young leaves) recorded the highest ^{15}N uptake and accumulation (%^{15}Ndff = 13.93) relative to the other two leaf categories and those of the on-year. In the on-year fruit accumulated higher ^{15}N than leaf or bud. In the off-year, bud exhibited the highest ^{15}N accumulation without boron application, while leaves exhibited the highest ^{15}N with boron application. The highest %^{15}Ndff in all tree organs was observed at 250 mg·L^{-1} boron rate. Boron increased nitrogen uptake, translocation and accumulation in mango tree organs. A synergistic relationship was observed between boron and nitrogen which led to an increase in fruit yield in the off-year.

Keywords: Mango; ^{15}N distribution; ^{15}N-stem injection technique; ^{15}N translocation; ^{15}N uptake, ^{15}N accumulation; On and off-year; Synergistic relationship

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1. Introduction

Mango (*Mangifera indica* L.) is one of the most popular fruit crops in Egypt and is of great economic importance. Irregularity of flowering in mango, which varies in time and intensity from year to year, is a common phenomenon [1]. Mango has many problems related to fruit set, yield and quality due to the imbalance of nutrient supply [2]. Mango is an extremely alternate bearing species and varieties vary in their alternation degree. The "Zebda" is classified as being a highly alternate bearing cultivar with low productivity due to this phenomenon. Strategies to enhance yield in the off-year or to develop more consistent fruiting in the on- and off-years would improve grower returns.

Alternate-bearing trees are those trees that do not bear a regular crop year after year, but rather a heavy yield followed by an extremely light one and vice versa. There are several theories of the alternate bearing phenomenon. The alternation phenomenon is caused by environmental triggers and endogenous factors, which lead to a shift in the balance between vegetative and reproductive growth [3]. The C/N ratio was reported to influence fruit set and consequently yield, rather than flowering [4-6]. Regulatory roles in various phases of the alternate bearing cycle have been related to plant hormones [7], mineral nutrients [8] and carbohydrates [9,10].

Boron has a crucial function in improving flower fertility and fruit set [11]. This is mainly due to the role of boron in increasing pollen grains germination and pollen tube elongation which leads to higher fruit set and yield [12,13] and/or to an increase in the flavonoid content of the pollen [14]. Boron increases the initial and final fruit set in almonds [15] . Boron deficiency can result in a reduction of fruit quality and yield potential in mangoes [16]. The increase in fruit set and yield in mango due to boron foliar application was emphasized by several researchers [17-24]. Thus, boron may help to solve the alternate bearing phenomena by promoting flowering and fruit set in the off-year.

Application of nitrogen fertilizer at the proper rate and time may reduce irregular bearing in mangoes. Significant increase in blooming occurred in the off-year and flushing in the on-year when mango trees received 3% urea [25]. Nitrogen increases leaf chlorophyll levels and photosynthesis, thus promoting shoot growth and flowering. Spraying "Langra" mango trees with urea at 2% decreased flowering percentage in the "on-year" considerably [26]. Sprayed 15-year-old mango trees with urea solution (0.8%) increased panicle length and number of male and perfect flowers [27].

Nitrogen and boron when applied together may be involved in the alleviation of alternate bearing. Boron in combination with urea significantly increased the flowering and fruiting of mango "Fazli" particularly at a 0.4% rate [28]. Along the same line, several researchers reported a positive interaction "synergistic" between boron and nitrogen. It was reported by Miley et al. [29] that boron enhanced the utilization of nitrogen by increasing the translocation of nitrogen compounds into the cotton boll. Davis et al. [30] found that in field culture, boron application (foliar and/or soil) resulted in an increase in boron and nitrogen uptake and tissue concentration by tomato plants.

The synergistic effects of B and N may be a consequence of enhanced nitrate assimilation and nitrate reductase activity as shown in Tobacco plants grown with differential boron application [31]. The effect of nitrogen and boron fertilizer on the alternate bearing cycle in Pistachio trees was investigated by Weinbaum et al. [32]. Their results showed that the on-year trees have greater reproductive demand for N and carbohydrates, reduced accumulation of C and N reserves and reduced recovery of applied labeled-N fertilizer than the off-year trees.

The allocation pattern of ^{15}N in leaves of different ages was studied by Biddulph [35] who stated that the different ^{15}N distributions found in leaves of different ages are probably due to the fact that the leaves differ in their basic metabolism according to age. In young leaves, the predominant process is the synthesis of new protoplasm, which results in growth towards maturity. In older, mature leaves, photosynthesis is the dominant function, and little growth takes place.

Burr and Takahashi [36] demonstrated the uneven distribution of the atomic percentage of ^{15}N in the tissue of different origins and ages with the highest ^{15}N accumulation in leaves that were at the stage of maximum activity. They added that nitrogen flows to places where there is metabolic demand and not to those where there is a nutritional vacuum. It was suggested that the redistribution of nitrogen in the plant is the result of competition between meristems and other tissues of different metabolic activity [37].

The distribution of ^{15}N in plants might give valuable information about the extent and rate of dynamic exchange (breakdown vs. resynthesis) of cellular proteins in tissues of various ages, as well as from different plant organs [38]. The distribution of ^{15}N-labeled fertilizer applied to Pecan trees was studied by Kraimer et al. [39]. They found that early spring growth, flowering and embryo development used fertilizer-N applied the previous year as well as that applied during the current year.

El-Motaium et al. [40] found that using the stem injection technique in olive trees, ^{15}N a.e. in leaves and flowers appear to increase in response to boron foliar application. The distribution of ^{15}N between the leaves and flowers of the olive tree indicated a higher enrichment of ^{15}N in the flowers than in the leaves. The maximum increase in %Ndff was observed at 200 $mg \cdot L^{-1}$ boron application rate for both leaves and flowers. This boron dose (200 $mg \cdot L^{-1}$) coincides with the highest values for flowering; fruit set and yield [41]. They suggested that boron is likely required for the synthesis of certain nitrogen compounds. This might be nucleic acid particularly the nitrogen base "uracil" [42] that enhances flowering and fruit set processes. Boron has been shown to increase flowering and fruiting by increasing pollen grains germination and pollen tube elongation [43,44,12] which can lead to higher fruit set and yield.

Several methods have been tested for using the ^{15}N tracer technique with trees including soil and foliar application [33]. Soil fertilization often results in soil contamination with ^{15}N and the residual ^{15}N complicates the estimation of reserve use in the second season. Foliar fertilization is neither adequate nor uniform to large trees which requires a large amount of solution. The stem injection technique was proposed to be the most suitable method for labeling trees as it is a non-destructive technique. Injection fertilization of fruit trees has several advantages over soil fertilization [34]. These advantages are: There is no fertilizer loss to the groundwater, only 5-10% of the label N applied to the soil is needed, and weed control is not needed since weed roots do not compete with tree roots for nutrient uptake. In addition, it labels the tree without affecting the soil N pools.

Although biennial bearing has been reported to be genetically controlled, the physiological factors governing such bearing habit have not been clearly understood [45,46]. Therefore, the objective of this research is to study the effect of the on and off years and boron foliar spray on nitrogen uptake, translocation and accumulation in mango trees in an attempt to shed light on the physiological and biochemical basis of boron's role in increasing flowering, fruit set and yield in the off-year.

2. Materials and methods

Fifteen years old mango (*Mangiferaindica*) trees (cv. Zebda) grown at Al Malak Valley Farm, El-Sharkeya Governorate-Egypt (30-51° North; 32-53° East) were used in this field experiment. The trees were grown in sandy soil, "Typic Torripsamments" [47] with total N = 0.06%, total B = 19.4 $mg \cdot kg^{-1}$, available B = 0.51 $mg \cdot kg^{-1}$) under drip irrigation system.

2.1 Preparation of labeled nitrogen fertilizer (N-15)

Labeled ammonium sulfate fertilizer was applied to mango trees at 50 g N/tree rate once in January of the on-year. The fertilizer was dissolved in 500 mL of deionized water. The labeled fertilizer, $(^{15}NH_4)_2SO_4$, is enriched with 10.35% ^{15}N atom excess. The $(^{15}NH_4)_2SO_4$ solution was loaded into the trees' xylem vessels through the trunk using the stem injection technique.

2.2 Stem injection technique

The injection of $(^{15}NH_4)_2SO_4$ solution was conducted according to the following steps and as shown in **Figure 1**:

- A circular disk of about 2-3 cm diameter was removed from the bark at the base of the trunk;
- In the middle of the removed disk a pore of about 1 cm in diameter was made at the base of the trunk, 15 cm from the ground at a 45° angle, and through about 75% of the tree diameter;
- A hard plastic tube was inserted in the pore and tightened with plastic material;
- An injection needle was tightly connected to a 500 mL tank (reservoir) containing the N-15 fertilizer solution (50 g ^{15}N) + $CuSO_4$(0.588 g) to avoid introducing pathogens into the tree;
- The tank was located 1 m higher than the injection hole.

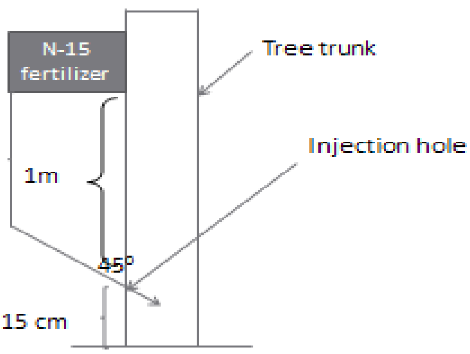

Figure 1. Diagram of N-15 stem injection technique.

The only source of N-15 in this experiment is the injection solution in the on-year. While, in the off-year, the reserved and recycled ^{15}N within the tree was the source of the labeled nitrogen. Our results show that the N-15 enriched [$(^{15}NH_4)_2SO_4$] at 50 g N/tree rate was sufficient to label the mango trees and to detect the ^{15}N in all the tree organs in both the on and off years.

Boron was sprayed as boric acid (H_3BO_3) in the same trees in January of the following year (off-year), using tractor mounted sprayer at the following rates: 0.0 (control), 250 and 500 $mg \cdot L^{-1}$. Tween 20 was added to the foliar solution at a 1 mL/L rate. Each tree was sprayed with 15 litres of the boron solution to achieve runoff.

2.3 Leaves and fruit sampling

Leaf and bud samples of the on-year were collected in March, two months from the initiation of the ^{15}N injection following the depletion of the injection solution. At the commencement of the off-year, leaves were selected from the same relative position to leaves of the on-year. Leaf and bud samples were collected in February, one month after the boron spray. Leaves were collected in three categories according to their position on the branch and age, upper leaf (3-month-old), middle leaf (6-month-old) and lower leaf (10-month-old). In the on-year and off-year fruits were collected in August. All samples were dried at 70 °C then ground up to very fine powder.

Leaf, bud and fruit were analyzed for $\%^{15}N$ atom excess and total nitrogen content. The percentage of ^{15}N atom excess was determined using the Emission Spectrometry N-15 Analyzer (FAN Fisher No. 1-6PC Spectrometer). Total nitrogen was determined using the C/N Analyzer (Elementar, Germany).

2.4 Calculation

The following equation was used to calculate nitrogen percentage in the plant organs derived from fertilizer (%Ndff) according to Zapata [48]:

$$\%N\,dff = (\%\,15N\ a.\,e.\ in\ plant\ sample\ /\ \%\,15N\ a.\,e.\ in\ the\ labeled\ fertilizer) \times 100$$

2.5 Soil analysis

Soil physical and chemical properties

Soil samples were collected from the soil profile at (0-25 and 25-50 cm) depth to determine the following characteristics, results are shown in **Table 1**:

- pH and EC using pH meter and conductivity meter, respectively (Cole Parmer, USA);
- Calcium carbonate ($CaCO_3$) using Calcimeter;
- Bulk density (BD) according to Mckenzie et al. [49];
- Organic matter (OM) using the Walkley-Black procedure, according to Chapman and Pratt [50];

- Texture class, according to Dewis and Freitas [51].

Soil nutrients analysis

Soil available boron was extracted by the hot water extract method according to Johnson and Fixen [52]. Soil total boron was extracted by (5 mL HNO_3 + 2 mL HF + 2 mL HCL acids) using the Microwave Digestion System (Milestone, Italy). Boron was determined by the Azomethine dye method according to Johnson and Fixen [52]. Soil total nitrogen was determined using the C/N Analyzer (Elementar, Germany). Soil phosphorus was determined using the vanadate-molybdate method [53] and measured using the UV-VIS Spectrophotometer (Shimadzu, Japan) at 430 nm. Soil potassium was determined using the Flame-Atomic Absorption Spectrometry (Shimadzu 6800, Japan).

2.6 Flowering, fruit set and yield analysis

Flower percentage

Counting the number of flowers in a mango tree is complex, because of the huge number of flowers in addition to the difficulty of distinguishing between male flowers and Hermaphrodite flowers. Therefore, an indication of flowering percentage was used. Twenty-one-year-old branches were chosen for every tree. Flower percentage was calculated at the end of April using the following equation:

Flower percentage = (*the number of flowering branches / the total number of labeled brances*) *x* 100

Final fruit set percentage

The final fruit set was determined as the number of retained fruits per panicle at harvest relative to the initial fruit set according to Shaban [54] and calculated using the following equation:

Final fruit set = [(*the number of retained fruit per panicle at harvest / the number of initial fruit set*)] *x* 100

Yield

Fruits were collected at the maturity stage. Each individual tree was harvested manually and fruit weight was estimated. The average weight for each treatment was calculated. Yield was expressed as kg fruit/tree.

2.7 Experimental design and statistical analysis

Treatments were arranged in a completely randomized block design with three replicates for each treatment and nine trees per block. Statistical analysis was performed using the MSTAT microcomputer program. The significant means were compared using LSD at 5% probability according to Snedecor and Cochran [55].

Table 1. Soil physical, chemical and nutrient characteristics.

Depth	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class	pH	EC (dS/m)	Organic matter (%)	TCC (%)	BD (g/cm^3)	N (%)	Boron ($mg\cdot kg^{-1}$)	
												Total	Available
0-25	67.9	28.9	1.88	1.25	Sandy	7.56	0.34	1.55	13.0	1.64	0.07	19.7	0.53
25-50	63.5	33.1	2.05	1.36	Sandy	7.81	0.30	1.00	15.0	1.95	0.05	19.1	0.50

3. Results and discussion

The fate of the labeled nitrogen (^{15}N) in the mango tree organs is shown in **Tables 2-5**. Results are expressed as an average of two successive seasons.

3.1 ^{15}N uptake, translocation, distribution and total nitrogen percentage in mango tree organs during the "on-year"

Results in **Table 2** show that the %Ndff values are 7.41 for the upper leaves, 6.10 for the middle leaves and 5.23 for the lower leaves. The buds showed a %Ndff value of 6.28 while fruits had the highest translocated ^{15}N enrichment of 12.08% and 8.99% for peel and pulp, respectively. The ^{15}N distribution pattern was highest in fruit but lowest in leaf.

Results in **Table 2** show that the descending order of %^{15}Ndff is as follows: Fruit is 12.08 peel and 8.99 pulp, upper leaves is 7.41, buds is 6.28, medium leaves is 6.10 and lower leaves is 5.23. Total nitrogen percent was highest in the old lower leaves (1.24%) then it decreased with decreasing the leaf age to reach 1.19% for the middle leaves and 1.05% for the upper leaves. The labeled N in the fruit of the on-year represents 84% of the total labeled N accumulated in leaf + bud.

The current study shows that in the on-year, the difference in %^{15}Ndff between the different leaves categories (young, medium, old) is due to their position and age. The high %^{15}Ndff shown by the upper young leaves could be due to they were at their maximum activity stage. The on-year trees showed higher ^{15}N enrichment in the reproductive organ (fruit) than in the vegetative organ (leaf). This finding supports the idea of Burr and Takahashi [36] who stated that nitrogen flows to places where there is high metabolic demand (activity). Fruits in the on-year represent the organ of high metabolic demand (preferential sink).

In the on-year, the distribution pattern of the labeled (^{15}N), was not uniform among mango tree organs. Higher ^{15}N enrichment was observed in young leaves than the buds which indicates that young leaves have higher metabolic demand for nitrogen than buds. Fruit was the dominant sink for nitrogen. The results agree with Weinbaum et al. [32]. The uneven distribution of ^{15}N could be due to the slow translocation and redistribution of mobile nitrogen in the tree [38]. It also indicates that the stationary state of the breakdown-resynthesis turnover of nitrogen is still not reached [38]. Our results agree with Wallace et al., [56] who found that the uneven distribution of ^{15}N was observed in plant parts after 60 days of ^{15}N-labeled $(NH_4)_2SO_4$ application.

3.2 Influence of boron on ^{15}N accumulation in mango tree organs during the "off-year"

In the absence of boron application (zero boron), the greatest ^{15}N translocation occurred to the upper leaves (%^{15}Ndff = 5.24) compared with the middle ((%^{15}Ndff = 4.34) or the lower ((%^{15}Ndff = 3.86) leaves. With boron application at (250 mg·L^{-1}) the %^{15}Ndff increased by almost three fold in all leaf categories compared with the control (**Table 3**). The upper leaf (13.93) still maintained the highest %^{15}Ndff values compared with the middle (12.95) and lower leaf (10.86). Boron treatment at 500 mg·L^{-1} rate showed lower values for %^{15}Ndff than those at 250 mg·L^{-1} rate for all leaf categories. The %^{15}Ndff for 500 mg·L^{-1} treatment values are (5.63, 5.66, 5.00) for the upper, medium and lower leaves, respectively. The %^{15}Ndff tends to decrease with increasing leaf age. The high %^{15}Ndff in the young leaves could be due to leaves being at their maximum activity and transpiration stage [36].

In our previous work [40] we found that using the stem injection technique in olive (cv. Frantoio) tree, the maximum increase in %^{15}Ndff was observed at 200 mg·L^{-1} boron rate for both leaves and flowers. In the current study, we found that boron has resulted in more translocation of nitrogen to the upper leaves of mango trees particularly at 250 mg·L^{-1} rate. Our results agree with Miley et al. [29] and El-Motaium et al. [40] who found that boron enhances the utilization of nitrogen. Following boron application leaf became the dominant sink for nitrogen in the off-year.

The ^{15}N is translocated to the bud and fruit as shown by the %^{15}Ndff values. Boron application resulted in ^{15}N translocation to the buds as %^{15}Ndff values for buds are 6.37, 11.09, and 8.71 for 0.00, 250, 500 mg·L^{-1} boron application rate, respectively (**Table 4**). The maximum %^{15}Ndff value was obtained at 250 mg·L^{-1} boron treatment. The ^{15}N was also translocated to the fruit, peel and pulp (**Table 5**) with %^{15}Ndff values of 0.734 and 0.628 for peel and pulp, respectively. The labeled N in the leaf of the off-year represents 190% of the total labeled N accumulated in fruit + bud.

3.3 Influence of boron on ^{15}N internal cycling in mango tree organs in the "off-year"

Boron affects the distribution of nitrogen between the tree organs (leaf, bud and fruit). When boron was applied at 250 mg·L^{-1} rate higher %^{15}Ndff and %N was observed in the leaf than in the bud or the fruit. The descending order of %^{15}Ndff in mango tree organs at 250 mg·L^{-1} boron application rate is as follows: average of leaf categories (12.58) > bud

(11.09) > fruit peel (0.734) > fruit pulp (0.628). Boron applied at 500 mg·L^{-1} rate shows lower %^{15}Ndff values than those at 250 mg·L^{-1} rate.

The total nitrogen percent of the upper leaves increased as the boron application rate increased as follows: 1.10, 1.36, 1.56 for 0.00, 250, 500 mg·L^{-1} boron treatments, respectively. A similar trend was observed in the other leaf categories. Fruit peel contains higher total nitrogen percent than the pulp and was greatest at 500 mg·L^{-1} boron.

The application of boron at 250 mg·L^{-1} in the off-year has modified ^{15}N internal accumulation among mango tree organs. More nitrogen (^{15}N) accumulated in the leaf than in the bud or fruit, as shown by the high %^{15}Ndff values. There is a tendency for an increase in total nitrogen percent in all the tree organs (leaf, bud and fruit) as the boron application rate increases. This may be due to boron enhancement of nitrogen uptake and tissue concentration [30].

Nason and McElory [57] found that boron is involved in nitrogen metabolism, in particular the synthesis of the nitrogen base "uracil" in nucleic acid, RNA [42]. In addition, boron is involved in nucleic acid and protein metabolism [11]. Nucleic acids are required for the stimulation of growth [58], protein synthesis, photosynthesis, fruit set and yield.

Nitrogen flows to places where there is high metabolic demand [36] and boron is involved in nitrogen and hormone metabolism [58]. In the off-year the leaf represents an organ of high metabolic demand; therefore, more ^{15}N-labelled nitrogen flows to the leaf under boron application. Thus, we suggest that the high accumulation of nitrogen in the leaf followed by boron application has stimulated plant growth and formation of the flowering hormone, upon its translocation to the growing points it stimulates flower induction, fruit set and yield. Our suggestion is in the same line with the early literature of Reece et al. [59] who emphasized leaf effects and hormonal factors in flower formation.

Table 2. Leaf, bud and fruit %^{15}N a.e., %Ndff and %N in the on-year.

Upper leaf			**Medium leaf**			**Lower leaf**		
%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N
0.767	7.41	1.05	0.631	6.10	1.19	0.541	5.23	1.24
Bud %^{15}N a.e.			Bud %Ndff			Bud %N		
0.65			6.28			1.23		

Fruit peel			Fruit pulp		
%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N
1.25	12.08	1.07	0.93	8.99	1.17

Table 3. Leaf %^{15}N a.e., %Ndff and %N in the off-year.

Boron (mg·L^{-1})	**Upper leaf**			**Medium leaf**			**Lower leaf**		
	%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N
0.0 (control)	0.542	5.24	1.10	0.449	4.34	1.02	0.400	3.86	1.00
250	1.442	13.93	1.36	1.340	12.95	1.13	1.124	10.86	1.28
500	0.583	5.63	1.56	0.586	5.66	1.52	0.517	5.00	1.44
LSD (0.05)	*0.255*	*2.292*	*0.064*	*0.022*	*0.794*	*0.080*	*0.032*	*0.299*	*0.082*

Table 4. Bud %^{15}N a.e., %Ndff and %N in the off-year.

Boron (mg·L^{-1})	**%^{15}N a.e.**	**%^{15}Ndff**	**%N**
(control)	0.659	6.37	1.0
250	1.148	11.09	1.18
500	0.902	8.71	1.33
LSD (0.05)	0.*012*	0.*408*	*0.102*

Table 5. Fruit %^{15}N a.e., %Ndff and %N in the off-year.

Boron (mg·L^{-1})	**Fruit peel**			**Fruit pulp**		
	%^{15}N a.e.	%^{15}Ndff	%N	%^{15}N a.e.	%^{15}Ndff	%N
0.0 (control)	0.045	0.435	1.19	0.029	0.280	1.08
250	0.076	0.734	1.25	0.065	0.628	1.17
500	0.056	0.541	1.31	0.045	0.435	1.24
LSD (0.05)	*0.0044*	*0.0023*	*0.035*	0.*0021*	*0.0013*	*0.044*

3.4 Comparison between "on and off-year"

The on-year trees exhibited a greater %^{15}Ndff than the off-year trees likely as a result of N dilution with growth. The buds accumulated almost similar percent of (%^{15}Ndff = 6.28) in the on-year and in the off-year (%^{15}Ndff = 6.37), at zero boron rate (**Tables 2 and 4**). With boron application, at 250 mg·L^{-1} rate in the off year, the buds accumulated higher ^{15}N (%^{15}Ndff = 11.09) than the buds of the on-year (%^{15}Ndff = 6.28).

Results in **Tables 2 and 3** show that on year, upper, middle and lower leaves contain higher (%^{15}Ndff) than those of the off year, at zero boron rate (control treatment). The on-year upper leaf contains %^{15}Ndff = 7.41 while the off-year contains %^{15}Ndff = 5.24. The descending order of %^{15}Ndff was the same in the on and off years (upper leaves) > (middle leaves) > (lower leaves). However, the off-year showed lower values than the on-year. This indicates that the accumulation and utilization of N maintained the same trend in both years [on-year (2 months post N-15 injection) and off-year (14 months post N-15 injection)].

Comparing the accumulation of ^{15}N in the on and off years, there is a greater accumulation of nitrogen in fruits of the on year than of those of the off-year. This could be due to the greater reproductive demand for nitrogen in the on year than in the off year. Our results agree with Weinbaum et al. [32]. Boron application in the off-year at 250 mg·L^{-1} resulted in twice as much ^{15}N accumulation in the leaf than that of the on-year.

3.5 Effect of boron foliar spray in the off-year on yield, flowering and fruit set

In the on-year, fruit yield averaged 67.4 kg/tree while in the off-year yield in the control (zero B) trees was 9 kg/tree. Boron foliar application at 250 mg·L^{-1} rate in the off-year caused a significant increase in fruit yield (33.5 kg/tree) relative to the control (9.0 kg/tree). The percent increase in mango fruit yield due to boron was 372% relative to the control and alternate bearing was alleviated by 37% [24]. This indicates a synergistic relationship between B and N.

Flower and final fruit set percentage significantly increased in response to boron foliar application (**Table 6**). The highest flower percentage and final fruit set percentage relative to the control was observed at 250 mg·L^{-1} boron rate. Our results agree with Hegazi et al. [41] who found that boron rate of (200 mg·L^{-1}) coincides with the highest flower percentage, fruit set and yield in olive cv. Frantoio. Along the same line, Negi et al. [2] found an increase in mango (cv. Dashehari) fruit set as a result of boron foliar application at 200 ppm. Boron function in increasing nucleic acid and hormone synthesis [11] could stimulate growth, flowering, fruit set and yield in the off-year.

Table 6. Effect of boron application rates on mango tree yield, flowering and final fruit set in the off-year.

B concentration ($mg·L^{-1}$)	**Yield (kg/tree)**	**Flowering (%)**	**Final fruit set (%)**
0.00	9.0	18.6	27.0
250	33.5	33.0	87.0
500	24.0	21.5	41.0
LSD (0.05)	*0.79*	*0.79*	*8.8*

4. Conclusions

We concluded that the highest uptake and translocation of ^{15}N in the on and off years was achieved by the upper (young leaves) compared with the middle and lower leaves. In the on-year, fruits maintained the highest %Ndff. While in the "off-year", buds maintained the highest $\%^{15}Ndff$ without boron application. However, boron application at 250 $mg·L^{-1}$ rate in the off-year resulted in higher ^{15}N enrichment in the leaf, bud and fruit.

In the "on-year" the distribution pattern of the labeled nitrogen (^{15}N) tended to show more enrichment in the fruit than in the other organs (leaf, bud). This result indicates that fruit has a greater reproductive demand for nitrogen than the other tree's organs. In the "off-year", the distribution pattern of the labeled nitrogen (^{15}N) tends to exhibit more enrichment in the bud, without boron application (control treatment). However, after boron application, at 250 $mg·L^{-1}$ rate, the distribution pattern of ^{15}N was altered in favor of the leaf.

Foliar boron application in the off-year enhanced nitrogen translocation and accumulation in all tree organs (leaf, bud, fruit). This indicates a functional association between boron and nitrogen. There is evidence of a synergistic relationship (positive interaction) between boron and nitrogen (boron facilitates N uptake, translocation and accumulation). This was achieved at a boron rate of (250 $mg·L^{-1}$) which coincides with the highest flowering, fruit set and yield. The results prove that our hypothesis is true.

Author Contributions

First author contribution: Develop the research idea and objective, execution of the experiments, conduct the statistical analysis and writing of the manuscript.

Second author contribution: Provide help in the development of the research concept and review the manuscript.

Third author contribution: Provide help in the development of the research concept and review the manuscript.

Fourth author contribution: Provide help in conducting the experimental part of the research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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