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# Potential use of $^{15}N$ stem injection technique to study nitrogen distribution in olive tree under boron foliar application

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**ABSTRACT**

A field experiment was conducted on twenty years old olive trees (c.v. Frantoio) using $^{15}N$ labeled $(NH_4)_2SO_4$ and stem injection technique. The trees were grown at The Nuclear Research Center Experimental Farm, Inshas, El-Qaliubiya Governorate, Egypt. The objective of the experiment is to study the effect of boron foliar application on nitrogen translocation and distribution in olive tree. This attempt is important because our previous findings of boron increases fruit set and yield lack the physiological and biochemical basis of this effect. The results might shed light on the mechanism of boron effect on flowering, fruit set and yield. Results demonstrate that stem injection with $(^{15}NH_4)_2SO_4$ at 50g N/tree rate effectively labeled olive trees. Nitrogen concentration and $^{15}N$ enrichment (%$^{15}N$ a,e.) in leaves and flowers appear to increase in response to boron application. The increase was higher in the top leaves than in the bottom leaves. The maximum increase in $^{15}N$ enrichment was observed at 200 mg.$L^{-1}$ boron application rate for both leaves and flowers. The 200 mg.$L^{-1}$ B treatment is the same concentration that caused the highest flowering, fruit set and yield. The $^{15}N$ distribution was biased toward flowers more than leaves.Although the mechanism by which boron foliar application stimulates fruit production is not yet known, our results indicate that there is a requirement of boron for nitrogen utilization. Thus, we propose that the mechanism could be due to the synthesis of certain nitrogen compounds (nucleic acids, RNA nitrogen base of 'uracil') and protein, which enhance flowering and fruit set processes and yield.

**Keywords:** olive, boron, nitrogen, flower, leaf, stem injection, $(^{15}NH_4)_2SO_4$, $^{15}N$ translocation, $^{15}N$ distribution, %Ndff, %$^{15}Na.e.$

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## INTRODUCTION

Macronutrient mineral status influences the productive stages of young olive trees [1]. Nitrogen and phosphorus concentrations were found to influence flowering intensity and fruit set in olive trees [2-3]. This indicates that nitrogen play a fundamental roles in processes affecting olive-tree productivity. Nitrogen deficiency is a limiting factor for flowering and fruit set in olive fruit trees [4-6]. In shallow hillside soil, N application increased rate of perfect flowers, fruit set and overall productivity [7].

The importance of maintaining an adequate nitrogen status for potential fertilization and fruit set in olive was indicated [8]. Maximum flower intensity was achieved when leaf nitrogen content was between 1.5-1.7%, while fruit load increase to a maximum as leaf N increased from 1.0 to 1.7% and maximum fruit set occurred when leaf N content was 1.6-1.7%. The final number of fruit remaining on individual trees was a function of nitrogen. Fruit number per tree increased drastically when solution nitrogen concentration increased from 5 to 50 ppm [2].

Nitrogen is essential for protein biosynthesis and developing inflorescences have shown to be strong sinks for nitrogen [9] and water-soluble proteins [10]. This indicate that nitrogen availability is important for flowering. Ployamine has been involved in playing an important regulatory role in olive flower induction [11].

Boron proves to enhance the utilization of nitrogen in cotton by increasing the translocation of nitrogen compounds into the boll [12]. The effect of boron on plants could be explained by its effect on RNA metabolism [13]. He also found that RNA content and particularly uracil synthesis decrease under B deficiency. The decrease in nucleic acid content under boron deficiency is a well-documented phenomenon [14].

Boron may be involved in some aspect of the synthesis or utilization of certain nitrogen bases such as uracil [15] and thereby in RNA metabolism and protein synthesis. The growth inhibition under B deficiency was related to the decrease in nucleic acids concentrations [16-17]. Nitrate reductase activity and nitrate assimilation have been shown to increase with increasing B supply [18].

Using $^{15}N$ tracer technique with trees must have a method to adequately label the plant. Several methods have been tested including soil and foliar application [19]. Soil fertilization often results in soil contamination with $^{15}N$ and the residual $^{15}N$ complicates the estimation of reserve use in the second year. Foliar fertilization is neither adequate nor uniform to large trees which requires large amount of solution. Stem injection technique was proposed to be the most suitable method for labeling trees.

Stem injection fertilization of fruit trees has several advantages over soil fertilization [20]. The advantages are: 1) there is no need to use herbicides or pesticides 2) no fertilizer leaching to the ground water 3) maintain clean environment 4) only 5-10% of the recommended soil fertilizers level is sufficient for optimum growth and high yield. Another advantage was mentioned by [19] is that it labels the tree without affecting soil N pools.

The objective of this study is to investigate the effect of boron foliar application on nitrogen translocation and distribution in olive tree using $^{15}N$-labeled $(NH_4)_2SO_4$ and stem injection technique. This information is needed in order to promote our understanding of the biochemical effect of boron on olive tree growth and productivity. Such information might shed light on the mechanism of boron effect on flowering and fruit set.

## MATERIALS AND METHODS

Field experiment was conducted on twenty years old olive trees (c.v. Frantoio) using $^{15}N$ labeled $(NH_4)_2SO_4$ and stem injection technique. The $^{15}N$-labeled ammonium sulfate $[(^{15}NH_4)_2SO_4]$ contains 10.35% a.e., and was applied at 50gN/tree rate. The $^{15}N$ fertilizer was dissolved in 500 ml water. The trunk injection method of the $^{15}N$ fertilizer was used as described by [21]. Boron foliar application was performed at three levels (0, 200, 300 ppm). Since the results showed that January application date has the highest fruit production; this date was chosen to apply $^{15}N$-labeled fertilizer and boron.

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**Stem Injection Technique**

As shown in Figure (1) stem injection technique was performed as follows:

1. A circular bark of about 2-3 cm diameter was removed from the trunk.
2. A pore of about 1 cm diameter was made at the base of the trunk, 15 cm from the ground at 45° angle, and through about 75% of the tree diameter.
3. A hard plastic tube was inserted in the pore and tightened with plastic material.
4. An injection needle was tightly connected to 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.
5. The tank was located 1 m higher than the injection hole. Boron foliar application was performed in January 1$^{st}$ at three levels (0.00, 200, 300 mg.L$^{-1}$). Nitrogen-15 translocation and distribution were determined.

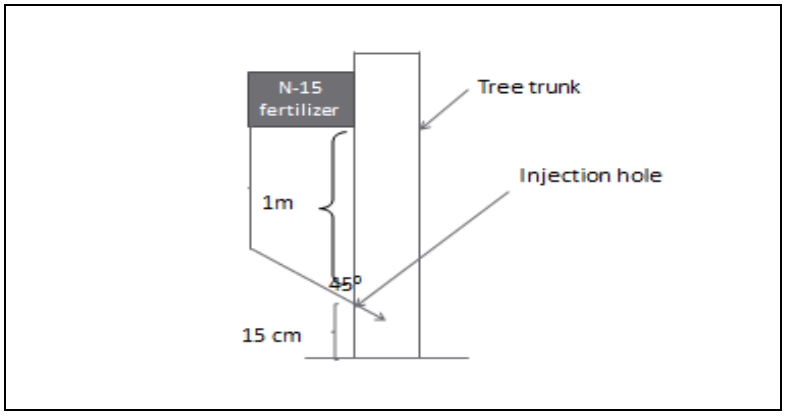


**Fig. (1): Diagram of N-15 Stem Injection Technique**

The leaf samples for $^{15}N$ analysis were collected two month after boron and N-15 application (when the $^{15}N$ solution was taken up by the olive tree). Flowers samples were collected in April. The $^{15}N$ enrichment of the leaf and flower were determined using the Emission Spectrometry N-15 Analyzer (FAN Fisher No. 1-6PC spectrometer).

**Calculation**

The following equation was used to calculate the percent nitrogen derived from fertilizer according to [22]:

$$\%Ndff = \frac{\%^{15}N \text{ a.e. in plant sample}}{\%^{15}N \text{ a.e. in the labeled fertilizer}} \times 100$$

**RESULTS AND DISCUSSION**

Results show that after two month following $(^{15}NH_4)_2SO_4$ and boron application, $^{15}N$ was rapidly distributed throughout the olive trees. This was observed by the $^{15}N$ enrichment in all tested tissues (upper leaf, lower leaf and flowers).

**Translocation of $^{15}N$ tracer**

**Into leaf**

Tracer $^{15}N$ was carried in the transpiration stream to the leaves. Because transpiration is usually higher at the upper young leaves than at the lower older leaves, the $^{15}N$ enrichment (%$^{15}N$ a.e.) was higher in the upper leaves than the lower leaves under all boron treatments. Results presented in Table (1) show the

%$^{15}$N a.e. of the upper leaves to be: 0.039, 0.618, 0.418 for 0.00, 200, 300 mg.L$^{-1}$ boron, respectively. While the %$^{15}$N a.e. of the lower leaves are: 0.029, 0.595, 0.266 for 0.00, 200, 300 mg.L$^{-1}$ boron, respectively.

More nitrogen was translocated into the leaves under boron treatments compared with the control (zero boron). The highest percent of nitrogen derived from $^{15}$N fertilizer are (5.97 and 5.75% for upper and lower leaves, respectively) was recorded after boron application at 200 mg.L$^{-1}$ rate. The lowest percent of nitrogen derived from $^{15}$N fertilizer are (0.38 and 0.28 for upper and lower leaves, respectively) which was recorded at the control treatment (zero boron). While the percent of nitrogen derived from $^{15}$N fertilizer for 300 mg.L$^{-1}$ boron rate treatment was intermediate (4.04 and 2.57 for upper and lower leaves, respectively).

A high leaf %$^{15}$N a.e. was observed under boron treatments. This shows that there is some kind of functional association between boron and nitrogen. It also indicate that boron enhance the utilization of nitrogen in olive leaf by increasing its translocation to the leaf.

There is an increase in total nitrogen percent due to boron treatment in top and bottom leaves. The nitrogen percent of the upper leaves are 0.98%, 1.75% and 1.07% for the control, 200 mg.L$^{-1}$ and 300 mg.L$^{-1}$ boron treatments, respectively. While, the nitrogen percent of the lower leaves are 0.94%, 1.20% and 0.98% for the control, 200 for the control, 200 mg.L$^{-1}$ and 300 mg.L$^{-1}$ boron treatments, respectively.

**Table (1): %$^{15}$N a.e., %Ndff and N concentration (%) of the upper and lower leaves**

| Treatments B (mg.L$^{-1}$) | Upper | | Lower | | %N | |
|---|---|---|---|---|---|---|
| | %$^{15}$N a.e. | %Ndff | %$^{15}$N a.e. | %Ndff | Upper | Lower |
| **0.00 (control)** | 0.039 | 0.38 | 0.029 | 0.28 | 0.98 | 0.94 |
| **200** | 0.618 | 5.97 | 0.595 | 5.75 | 1.75 | 0.98 |
| **300** | 0.418 | 4.04 | 0.266 | 2.57 | 1.07 | 1.20 |

**Into flowers**

Results presented in Table (2) show that nitrogen-15 was translocated to flowers, as flowers show high $^{15}$N enrichment. The %$^{15}$N atom excess in olive flowers increased due to boron application. The %$^{15}$N a.e. values are 0.156, 1.150, 0.902 for the control, 200 and 300 mg.L$^{-1}$ boron treatments, respectively. The maximum %$^{15}$N a. e. value was recorded at 200 mg.L$^{-1}$ boron application rate.

The highest nitrogen percent derived from $^{15}$N fertilizer by flowers was 11.11%. This high value was recorded for 200 mg.L$^{-1}$ boron rate. The lowest nitrogen percent derived from $^{15}$N fertilizer was 1.51% which was recorded for the control treatment (zero boron). While the nitrogen percent derived from $^{15}$N fertilizer for 300 mg.L$^{-1}$ boron rate was intermediate (8.71%).

Total nitrogen percent in flowers generally showed the opposite trends as to those of leaves. Nitrogen percent in flowers decreased as the boron application rate increase. The nitrogen percent in flowers are 1.26%, 1.15% and 1.07% for the control, 200 and 300 mg.L$^{-1}$ boron treatments, respectively.

**Table (2): %$^{15}$N a.e., %Ndff and N concentration (%) in flowers**

| Treatments B (mg.L$^{-1}$) | %$^{15}$N a.e. | %Ndff | %N |
|---|---|---|---|
| **0.00 (control)** | 0.156 | 1.51 | 1.26 |
| **200** | 1.150 | 11.11 | 1.15 |
| **300** | 0.902 | 8.71 | 1.07 |

The 200 mg.L$^{-1}$ boron treatment gave the highest flower density, fruit set and yield and the highest %$^{15}$N a.e. in leaves and flowers. This indicates the requirement of boron for the synthesis of certain nitrogen compounds that enhance the previous processes. This nitrogen compound is probably nucleic acid and in particular the nitrogen base “uracil” (Albert, 1965). In addition, boron has been implicated in nitrogen metabolism [23]. Nucleic acids are required for stimulation of growth [17].

Boron affects nucleic acid, carbohydrate and protein metabolism [24]. Boron is required for the synthesis of the nitrogen bases of RNA and protein. It was found by [16] that boron deficiency inhibits growth due to the reduction in nucleic acids concentration.

**Distribution of $^{15}N$ between leaves and flowers**

Regarding the distribution of $^{15}N$ in olive tree between leaves and flowers in olive tree, results indicate that a higher distribution (enrichment) of $^{15}N$ in flowers than in leaves. The $^{15}N$ derived by flowers from the labeled fertilizer was the highest (11.11%) at 200 $mg.L^{-1}$ boron rate. While $^{15}N$ derived from labeled fertilizer was 5.97% and 5.75% for upper and lower leaf, respectively. At 200 $mg.L^{-1}$ boron application rate, flowers enrichment by $^{15}N$ was double that of the leaves enrichment.

Thus, boron alters the distribution of nitrogen towards flowers more than leaves. This indicates the role of boron and nitrogen on reproductive processes that take place in flowers and subsequently in fruit set.

## CONCLUSION

The tracer-$^{15}N$ results suggest that boron may have a role in nucleic acids synthesis (in particular the nitrogen bases) or utilization. We propose that the mechanism of boron enhancement of fruit production could be "when boron is applied it increases the synthesis of nucleic acids and proteins which stimulate growth, photosynthesis, flowering, fruit set and yield".

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