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# Silicon Affects Growth and Nitrogen Uptake of Young Olive Plants

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***Abstract.*** **Nitrogen (N) is commonly used in the fertilization plans for Mediterranean olive orchards, often regardless of the nutritional status of the plant. However, improper use of N fertilizers can reduce soil health and crop yields. Silicon (Si), although not essential for plants, could be an environmentally friendly alternative to some chemical fertilizers. The application of Si seems to positively affect many aspects of N nutrition (uptake, assimilation, and remobilization), but that related to olive is unknown. Therefore, the aim of this work was to study the effect of Si application on N nutrition in the olive. To study these effects, an experiment involving young olive plants of 'Picual' growing mainly under shade house conditions in Córdoba, Southern Spain, was performed. The experiment was arranged in a completely randomized block design with 9 treatments resulting from the combinations of two Si levels, 0 or 20 mg·L$^{-1}$ Si [YaraVita ACTISIL® (YARA, Bio Minerals N.V., Belgium); 0.5% Si], applied through the irrigation water or onto leaves, and different levels of N [0, 100, 400 ppm N, $Ca(NO_3)_2$]. The results indicated that vegetative shoot growth was significantly affected by Si application, and that this effect was more marked when it was applied through the irrigation water. In addition, these plants showed longer and thinner stems. Additionally, Si fertilization increased the N concentration in plants grown under 0 or 100 ppm N. However, no response was observed in plant treated with the highest doses of N, thus showing the interaction effect. The positive effect of Si on N nutrition suggested the use of Si as a sustainable practice for olive orchards to reduce N fertilizers.**

Silicon (Si) is a nonessential element for plant growth (Martos-García et al. 2024), but it is a beneficial element, mainly because of its influence on the tolerance to biotic and abiotic stresses in many plant species (Debona et al. 2017; Ma 2004). After oxygen, Si is the second most abundant element in the earth's crust. Additionally, Si in soils can be found in the solid phase, mainly composed of silica ($SiO_2$) and silicates adsorbed to soil particles and iron (Fe) and aluminum (Al) oxides and hydroxides, or in the liquid phase, mainly in the form of monosilicic acid ($H_4SiO_4$) (Tubana et al. 2016). Monosilicic acid does not dissociate at a pH less than 9, and the uptake of Si from the soil in this form is performed by plants (Epstein 1994; Ma and Takahashi 2002). All plants growing in soil contain Si in their tissues, and the soil type and plant species depend on the Si content (Debona et al. 2017; Tubana et al. 2016). Sandy soils or soils with high contents of Fe and Al oxides usually have a lower content of available Si. Plant species differ greatly in their ability to accumulate Si, with olive considered as a nonaccumulator of Si (<0.5% dry weight) (Debona et al. 2017; Epstein 1994; Tubana et al. 2016).

Several studies have shown that an increase in the Si content in plants is beneficial, particularly when plants are under stress conditions (Debona et al. 2017; Tayade et al. 2022). Nascimento-Silva et al. (2022) observed that the application of Si at 20 mg·L$^{-1}$, both by foliar sprays or to the soil through the irrigation water, effectively increased the Si content in leaves of olive plants. This dose efficiently increased vegetative growth of this species (Martos-García et al. 2024). The positive effect of Si on plant growth has been linked to nutrient uptake (Ali et al. 2020). In fact, some authors have suggested different Si strategies that interact with almost all aspects of nitrogen (N) nutrition, such as uptake, assimilation, and remobilization (Pavlovic et al. 2021). Other studies have shown that an exogenous supply of Si solves or alleviates the negative effect associated with N imbalance (Raza et al. 2023). However, most of the existing studies of the effect of Si on plant nutrition have been focused on herbaceous plants, and limited information regarding Si application on fruit tree crops is available. Additionally, there are some controversies. For example, Si seems to increase the leaf concentrations of N, phosphorus (P), and potassium (K) in mango (Helaly et al. 2017) and apple (Saleem and Joody 2019); however, it seems to decrease the fruit N concentration in nectarine (Quirante-Moya et al. 2022) and leaf xylem sap of young avocado trees (Gross-Urrego et al. 2022). Other studies reported that Si application did not affect the leaf N concentration in 'Fuji' apple tree (Karagiannis et al. 2021) and 'Valencia' orange seedlings (Abo El-Enien et al. 2017).

The effects of Si on olive nutrition are unknown; however, the increase in vegetative growth after Si application has been associated with improved uptake and translocation of K (Martos-García et al. 2024). Therefore, the aim of the present work was to study the effects of Si application on olive N nutrition using plants under three N treatments.

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## Materials and Methods

*Plant material and growth condition.* One-year-old 'Picual' olive plants obtained from a certified nursery were transferred to 2.5-L plastic pots containing washed sand. The young olive plants were placed in a shade house located at the Experimental Farm of Rabanales (University of Cordoba, Southern Spain) between August and mid-November; the temperature range was 15 to 35 °C. In mid-November, they were moved to a controlled growth chamber with relative humidity between 60% and 80%, temperature of 25/22 °C (day/night), and photoperiod of 14 h of light (MZD LED tube, 1500 mm, 20 W, 4000 K, Cool White, Philips, Spain) to avoid decreased growth caused by low winter temperatures.

Before treatments were applied, the plants were acclimated to the shade house conditions for 1 month (August). During this period, they were periodically irrigated with tap water to satisfy the plants' water needs and fertilized once with a Hoagland-type standard nutrient solution without N. The nutrient solution comprised 0.5 M KCl, 0.5 M $MgSO_4$, 0.5 M $KH_2PO_4$, 0.5 M $CaCl_2$, 12.5 μM $H_3BO_3$, 1.0 μM $MnSO_4$, 1.0 μM $ZnSO_4$, 0.25 μM $CuSO_4$, 0.2 μM $(NH_4)_6Mo_7O_{24}$, and 10 μM Fe-ethylenediamine-di-o-hydroxyphenylacetic acid.

*Treatments.* The experiment was arranged in a completely randomized block design with five blocks and 9 treatments resulting from the combination of Si treatment [Si absence, Si foliar dose (20 mg·L$^{-1}$), and Si soil application (dose of 20 mg·L$^{-1}$)] and different levels of nitrogen (0, 100, and 400 ppm N). These treatments were applied weekly.

The experiment lasted 126 d and comprised two stages. During the first stage (Si accumulative stage), we established three groups of 30 plants that received two weekly levels of Si, 0 or 20 mg·L$^{-1}$, applied by foliar sprays or to the soil through the irrigation water to obtain plants with different Si levels. YaraVita ACTISIL® (BioMinerals N.V., Belgium), in which the active compound is choline-stabilized orthosilicic acid, was applied as the Si source. Actisil contains a minimum of 0.5% (w/v) Si. An aqueous solution of Actisil at a concentration of 0.4% (v/v) (equivalent to 20 $mg \cdot L^{-1}$) was uniformly sprayed onto leaves until the dripping point (Si foliar treatment) or applied to the soil through the irrigation water (soil treatment) until the substrate water content was close to field capacity. This stage lasted 52 d. Thereafter, when differences in the leaf Si concentration were observed, the second stage began and each homogenous group from the first stage was split into three groups, which received three weekly levels of N (0, 100, or 400 ppm N) applied through the irrigation water at different times. Additionally, N was provided in the form of calcium nitrate. This stage lasted 74 d. Plants received one or two applications of 100 mL of tap water per week, depending on the water requirements of the plants; to prevent nutritional deficiencies, the nutrient solution described was applied every 4 weeks.

*Measurements.* After the Si treatments were initiated, the total shoot length in each plant was measured weekly. The specific shoot length was determined at the end of the experiment according to the following calculation: total shoot length (cm)/shoot dry weight (g).

Plants were harvested after 126 d. Each plant was separated into leaves, stems, and roots. Each organ was individually rinsed with deionized water and dried in an oven at 70 °C for at least 48 h to determine the dry weight. Then, samples were ground and stored in an oven at 60 °C until the analysis. Next, N was analyzed with a EuroVector EA3000 CHN analyzer using the Dumas procedure (Dumas 1831). Nitrogen uptake efficiency was estimated according to the following formula:

$$NUE = \frac{N\ uptake}{N\ applied} \times 100$$

where N uptake represents the difference between total N in the plant and plant N content in treatments without N application (0 ppm N). N applied refers to N applied as fertilizer (100 and 400 ppm N).

The Si concentration in the leaf was determined. The plant material was digested in nitric acid and hydrofluoric acid using the UltraWave microware system (Milestone, Shelton, CT, USA). Inductively coupled plasma mass spectrometry (NexION 350X; Perkin Elmer, Waltham, MA, USA) was performed to measure elements.

*Statistical analysis.* An analysis of variance of the data was performed using the Statistix 10.0 software package (Analytical Software, Tallahassee, FL, USA). All percentage values were transformed using the arcsine of the square root before the analysis. When a significant F was observed, the mean separation between treatments was obtained by performing a polynomial contrast for quantitative factors (N levels) or Tukey's test for qualitative factors (Si application). In all the analyses, residual plots were generated to identify outliers and confirm that the variance was common and normally distributed.

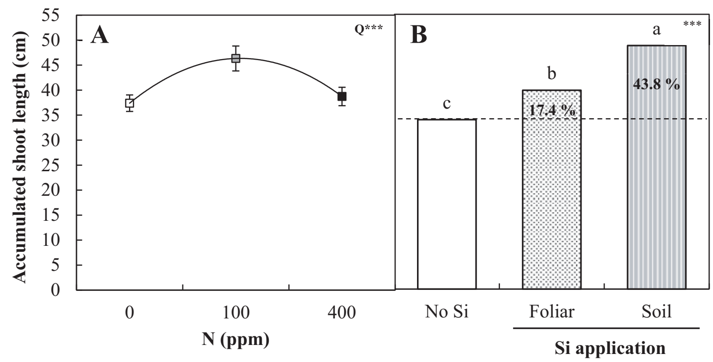


Fig. 1. Accumulated vegetative growth in response to different nitrogen (N) doses (**A**) and silicon (Si) applications (**B**). Different letters indicate significant differences. Q = quadratic. ***$P \leq 0.001$.

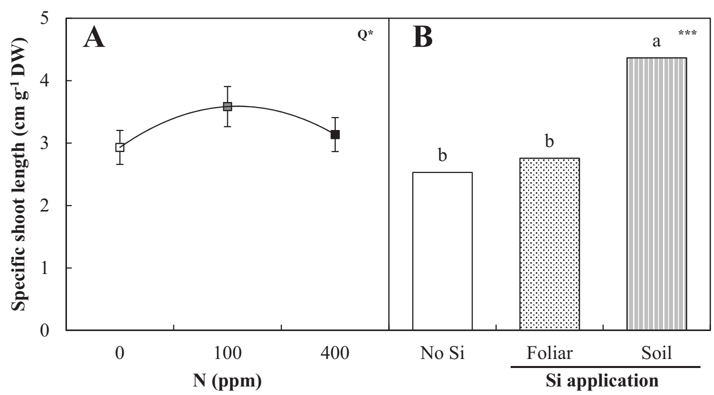


Fig. 2. Specific shoot length in response to different nitrogen (N) doses (**A**) and silicon (Si) applications (**B**). Different letters indicate significant differences. Q = quadratic. ***$P \leq 0.001$.

## Results

The Si application promoted shoot length during the period of Si accumulation independently of the application method (foliar or soil) (data not shown). After 46 d of Si application, plants showed a significant increase in the leaf Si concentration with regard to the control plants (0.064% soil Si, 0.062% foliar Si, and 0.052% control); then, N treatments were initiated. No interaction between N doses and the Si application was observed in the accumulated shoot length at the end of the experiment. However, a significant effect was observed when both factors were studied separately. A quadratic response in response to the N doses was observed (Fig. 1A), with the dose of 100 ppm being the most effective for increasing vegetative growth. Additionally, Si application affects the shoot length (Fig. 1B). Both forms of Si application significantly increased shoot growth compared to that of the control, but the soil application method was more effective. Similar results were observed when a specific shoot length was determined (Fig. 2); however, in this case, foliar application of Si did not result in a shoot length different from that of the control. The Si applied to the soil significantly increased the specific shoot length, indicating that these plants showed thinner stems (Fig. 2B). No interaction between both factors was observed.

The N doses also affect the dry matter accumulation of the different plant organs and the whole plant, and a quadratic response was observed in all cases (Table 1). However, when Si was applied the only response was observed in the root dry weight, which decreased when Si was applied to the soil.

A significant effect of the interaction of the N doses and Si application on the N concentration in the whole plant was observed. This value increased as the N doses increased (Fig. 3). Additionally, Si promoted the N

Table 1. Effects of silicon (Si) applications and nitrogen (N) doses on the dry weight of different plant organs.

| | Dry wt (g) | | | |
|---|---|---|---|---|
| Treatments | Leaf | Stem | Root | Whole plant |
| | N concentration (ppm) | | | |
| 0 | 8.7 | 12.4 | 8.4 | 29.5 |
| 100 | 9.8 | 14.1 | 10.3 | 34.1 |
| 400 | 9.1 | 11.8 | 7.2 | 28.2 |
| Significance[i] | Q** | Q** | Q*** | Q*** |
| | Si application | | | |
| No Si | 8.9 | 13.2 | 10.0 a | 32.1 |
| Foliar | 9.1 | 12.8 | 8.9 a | 30.7 |
| Soil | 9.7 | 12.3 | 7.0 b | 29.0 |
| Significance[i] | NS | NS | *** | NS |
| *CV* (%)[ii] | 12.4 | 17.2 | 19.7 | 13.6 |

[i] NS, **, *** Nonsignificant or significant at $P \leq$ 0.01 or 0.001, respectively.
[ii] Coefficient of variation of the full experiment.
Q = quadratic.
Data are the average of 10 replicates.
Different letters indicate significant differences.

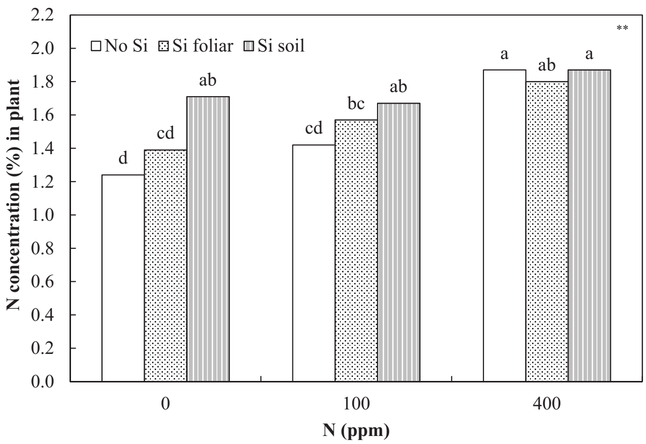


Fig. 3. Interaction between silicon (Si) applications and nitrogen (N) doses on the N concentrations (%) of the whole plant. Different letters indicate significant differences. $^{**}P \leq 0.01$.

concentration when N was applied at 0 or 100 ppm. At the highest dose of N, no differences between the types of Si application and the control without Si were observed, thus showing the interaction effect.

The N uptake efficiency significantly decreased when the N doses were increased (Table 2); however, the Si application showed no effect.

## Discussion

Several studies have shown the positive effects of Si on the uptake, translocation, and availability of nutrients in plants with nutritional imbalances, including those caused by N (Ali et al. 2020). In the olive, as occurs in other crops, N is part of the fertilization program regardless of the nutritional status of the plant. Consequently, this abuse of N had negative effects on the plant and the environment (Fernández-Escobar et al. 2009). Many studies have suggested limiting the use of N in olive fertilization (Fernández-Escobar et al. 2004, 2006, 2008, 2011, 2014a, 2014b; Haberman et al. 2019).

We hypothesized that Si could affect N uptake in the olive, and that Si application could reduce N fertilization in olive orchards. Several studies of other crops have supported this hypothesis (Ali et al. 2020; Haddad et al. 2018; Mabagala et al. 2020; Mali and Aery 2008; Reithmaier et al. 2017; Xu et al. 2020). During our study, the accumulated shoot length and the dry weight of different plant organs exhibited a significant quadratic response to N doses, as was observed previously (Fernández-Escobar et al. 2014a). Additionally, we observed a significant increase in vegetative growth after Si application, in agreement with previous data (Martos-García et al. 2024); however, there was no effect on the dry weight of the plant. This may be attributable to the thinness of the shoots observed. No effects of the interaction between N doses and Si application on plant growth were observed.

Table 2. Effects of silicon (Si) applications and nitrogen (N) doses on N uptake efficiency (NUE).

| Treatments | NUE (%) |
|---|---|
| | N concentration (ppm) |
| 100 | 119.0 a |
| 400 | 49.5 b |
| Significance[i] | * |
| | Si application |
| No Si | 80,917 |
| Foliar | 90,487 |
| Soil | 81,467 |
| Significance[i] | NS |
| *CV* (%)[ii] | 74.02 |

[i] NS, * Nonsignificant or significant at $P \leq 0.05$.
[ii] Coefficient of variation of the full experiment.
Data are the average of 10 replicates.
Different letters indicate significant differences.

The effect of Si on N uptake in olive has not been previously studied; however, in other fruit tree crops, both soil application (Helaly et al. 2017) and foliar application (Saleem and Joody 2019) of Si increased the leaf N concentration. We observed a significant interaction between N doses and Si application. The N concentration increased in the whole plant with the Si application, but only in plants that did not receive N or those that received 100 ppm of N. No effect was observed in plants that received the highest dose of 400 ppm N. These results suggest that Si can increase N uptake by plants under natural conditions, which include reduced N fertilizers. However, no effect of Si application on N uptake efficiency was observed.

In conclusion, although studies of the effect of Si on the olive are scarce, Si reduces the incidence of olive leaf spot (*Venturia oleaginea*) (Martos-García et al. 2024; Nascimento-Silva et al. 2019), which is the most important foliar disease of the olive, and stimulates vegetative growth (Nascimento-Silva et al. 2019, 2022) and K uptake (Martos-García et al. 2024). During this study, Si increased the N uptake in olive. Although it is possible to achieve other positive effects, these results suggest that, in the near future, the application of Si in olive groves could be a sustainable agricultural practice.

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