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Silicon Reduces the Incidence of *Venturia oleaginea* (Castagne) Rossman & Crous in Potted Olive Plants

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***Abstract*. Silicon (Si) is the second most abundant element in the Earth's crust. It is a nonessential element for plant growth, but it is considered beneficial because it can prevent biotic and abiotic stresses. Because nothing is known about the effects of Si in the olive, two experiments were performed with young plants of 'Arbequina' and 'Picual' cultivars to evaluate the effect of continuous Si applications on the incidence of olive leaf spot, the main foliar disease affecting this crop. Plants were grown in pots containing a mixture of washed sand and peat. In the first experiment, Si was foliar sprayed (foliar treatment) or applied to the soil through irrigation water (soil treatment) at the concentrations of 0, 2.5, 5, and 10 or 0, 1.25, 2.5, and 5 mg·L^{-1}, respectively. The treatments were arranged in a completely randomized design for each cultivar. In the second experiment, the experimental design was a randomized complete block design in a 2 × 4 factorial arrangement, consisting of two forms of Si application (foliar vs. soil) and four concentrations (0, 5, 10, or 20 mg·L^{-1}). Leaf Si concentration significantly increased with the amount of Si applied. After 5 months of treatments, plants were inoculated with a conidial suspension of the pathogen, and the disease index (DSI) was calculated. Shoot growth only increased in 'Picual' after Si application. The DSI showed a significant reduction in both cultivars treated with Si when compared with control plants, although differences between cultivars were observed.**

Olive leaf spot, incited by the fungus *Venturia oleaginea* (Castagne) Rossman & Crous, is the most important foliar disease of the olive (*Olea europaea* L.). This disease is widespread in many olive-growing regions of the world (Trapero Casas et al., 2017). The disease is also called peacock's eye, and the main symptom in olive trees is circular lesions, sometimes with a chlorotic halo on the upper side of the leaves. Lesions change from a typical black scab to a whitish scab when high temperatures occur (Graniti, 1993; Viruega et al., 2013). Defoliation of the affected trees conduces to a significantly weakening, reducing yield (Graniti, 1993) and affecting organoleptic characteristics of the olive oil (Nigro et al., 2018).

Cooper fungicides have been widely used to control olive leaf spot (Roca et al., 2007). This traditional strategy of applications has resulted in average levels of up to 10 and 12 kg·ha^{-1}·year^{-1} of the copper metal in intensive and superintensive olive orchards, respectively, when applying 1000 L·ha^{-1} with a dose of 2 kg of copper (Romero et al., 2017). Consequently, copper concentrations increase in the receiving soils. The contamination of agricultural soils with inorganic, copper-based, and organic pesticides, including their residues, presents a major environmental and toxicological concern (Vitanovic, 2012). This is due to the probability of food contamination through the soil–root interface (Nazir et al., 2015). The highest concentrations of copper in the European Union due to human activity are found in the Mediterranean countries, mainly from agrochemicals applied in agriculture, indicating a potential problem for food production (Tóth et al., 2016).

Nowadays, Directive 2009/128/EC of the European Union requires the reduction in the amount of copper to be applied per hectare and year in olive orchards (European Commission, 2017; Roca et al., 2017). So, the development of new methods to supplement existing disease control strategies, especially aimed to reduce the number of a.i. which are more dangerous to human and the environment (Emmert and Handelsman, 1999), has become a priority (Ballabio et al., 2018).

An alternative technique for controlling plant diseases is the stimulation of resistance through mineral nutrition (Marschner, 2012) because there are evidences that tolerance or resistance to plant diseases could be affected by the nutritional status of plants (Huber, 1980). Also, there are mineral elements that are not essential for plant growth but are beneficial to some plant species. One of this is Silicon (Si) (Epstein, 1999; Ma, 2004). Si is the second most abundant element in the Earth's crust (Guntzer et al., 2012) following oxygen (Epstein, 2009). In the soil solution, Si occurs mainly as monosilicic acid H_4SiO_4 (Richmond and Sussman, 2003). Plants absorb Si by their roots in the form of monosilicic acid, which is translocated via passive transport to the shoot through the xylem and is deposited as amorphous silica ($SiO_2{\cdot}H_2O$), mainly in the epidermis and in the sheath cells of vascular bundles (Epstein, 1999; Prychid et al., 2003).

It has been reported that Si has favorable effects on the control of pests and diseases (Ma et al., 2001) and on abiotic stresses such as salinity, metal toxicity, drought, radiation damage, nutrient imbalance, high temperature, and freezing (Guntzer et al., 2012). A remarkable effect of Si is the reduction in the incidence of diseases in plants (Debona et al., 2017; Pozza et al., 2015; Wang et al., 2017). This element is capable of increasing plant tolerance to diseases by acting as a physical barrier to pathogenic infection or biochemical and molecular regulatory mechanisms (Ma and Yamaji, 2008; Wang et al., 2017). In addition, Si seems to facilitate the rapid deposition of phenolic compounds or phytoalexins at the site of infection which has been associated with resistance in plants to diseases (Rodrigues et al., 2004; Sun et al., 2010).

Because nothing is known about the effects of Si in the olive, the aim of this study was to evaluate the effect of Si applications on the incidence of olive leaf spot.

Material and Methods

Experimental design and plant measurements. Two experiments were conducted with young olive plants of 'Arbequina' and 'Picual' cultivars growing under shade-house conditions. For this purpose, mist-rooted olive plants were transferred to 1.1-L pots containing a mixture of washed river sand and peat (2:1 by volume). Plants were kept in the shade-house for 1 month for acclimation, and then groups of homogeneous plants were selected to receive the treatments. YaraVita ACTISIL (Bio Minerals N.V., Belgium), whose active compound is choline-stabilized orthosilicic acid, was applied in both experiments as the Si source. Actisil contains a minimum of 0.5% (w/v) Si.

In the first experiment, an aqueous solution of Actisil® at concentrations of 0%, 0.05%, 0.1%, or 0.2% (v/v) (equivalent to 0, 2.5, 5, or 10 mg·L^{-1} of Si, respectively)

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was uniformly sprayed onto leaves until the dripping point (foliar treatment) or applied to the soil through irrigation water at concentrations of 0%, 0.025%, 0.5%, or 0.1% (v/v) (equivalent to 0, 1.25, 2.5, or 5 mg·L^{-1} of Si, respectively). The experiment was arranged in a completely randomized design with three single plant replications per treatment. In the second experiment, plants were arranged in a factorial experiment with two forms of Actisil® application, foliar and soil, and four concentrations 0, 0.1, 0.2, or 0.4% (v/v) (equivalent to 0, 5, 10, or 20 mg·L^{-1} of Si, respectively), and six single plants replications in both cultivars. In both experiments, plants were grown for 2 months, receiving the Si treatments once per week when the product was sprayed and three times per week when it was applied through irrigation water. To fertilize the plants, 2 g·L^{-1} of Hakaphos Verde fertilizer 15–10–15 (Compo, Germany) containing 15% N, 4.4% P, 12.4% K, 1.2% Mg, 12% S, 0.01% B, 0.05% Fe, 0.05% Mn, 0.02% Zn, 0.02% Cu, and 0.001% Mo was applied every month to prevent nutritional deficiencies.

In the first experiment, to determine new shoot growth, the total shoot length was measured monthly until the inoculation with the pathogen.

Silicon determination. In both experiments, 1 week before the inoculation with *V. oleaginea*, fully expanded leaves from each treatment were collected, washed, and dried in an oven at 70 °C until constant weight. Si concentration was determined as described by Kleiber et al. (2015). Briefly, the plant material was digested in nitric acid (33%) under high pressure in a microwave. Si concentration was measured by electrothermal atomic absorption spectrometry with inverse longitudinal Zeeman background correction (Perkin Elmer Analyst 800; Perkin Elmer, Waltham, MA) and pyrocoated graphite tubes with an L'vov platform (Perkin Elmer).

Inoculation and incubation. After 5 months of Si treatments, inoculation was performed in both experiments according to the methodology described by López-Doncel et al. (2000). Olive plants were sprayed with a conidial suspension of the pathogen, obtained from naturally infected leaves with sporulating olive leaf spot lesions, and adjusted to 1×10^5 conidia/mL. The plants were maintained for 48 h in a dark growth chamber at 15 to 17 °C and 100% RH to favor infection (Viruega et al., 2013). Subsequently, the plants were placed in a shade-house until the appearance of symptoms. The evaluation of symptoms was carried out 12 weeks after inoculation. For this purpose, ten fully expanded young leaves of each treatment and replications were selected and dipped in 5% NaOH for 10 min and examined for characteristic black spots. Disease was evaluated attending to the incidence and severity of the disease, defined as the percentage of affected leaves and the percentage of the affected foliar area, respectively, according to a 0 to 8 scale (0 = 0%; 1 = <12.5%; 2 = 12.5% to 25%; 4 = 25% to 50%; 6 = 50% to 75%; and 8 = >75%) (Viruega et al., 2011). The disease index (DSI) was calculated and expressed as percentage proposed by McKinney (1923):

$$\mathrm{IE} = [(\Sigma n_i \cdot \times \cdot i)/(N \cdot \times \cdot S_{\max})] \cdot \times \cdot 100,$$

where i represents severity (0–8), n_i is the number of leaves with a severity of i, N is the total number of inoculated leaves, and $S_{\max}$ is the maximum value of severity (equal to 8).

Statistical analysis. Analyses of variance were performed on the data using the statistical program "Statistix" version 9.0 (Analytical Software, Tallahassee, FL). All percentage values were transformed using the arcsin of the square root before analysis. Where a significant F was observed in the ANOVA, mean separation among treatments was obtained by polynomial contrasts for quantitative factors, or by the F test for qualitative factors.

Results

In the first experiment, leaf Si concentration significantly increased with the amount of Si applied, except for soil application in 'Arbequina' which results showed no significance (Tables 1 and 2). Si did not affect shoot growth in 'Arbequina', but it had a stimulating effect on 'Picual'.

The disease index only decreased significantly when Si was applied through irrigation water in 'Arbequina' (Table 2), but nonsignificant effects were observed when it was foliar applied or applied through the irrigation water in 'Picual'. However, the coefficients of variation obtained in the analysis of the disease index were very high.

In the second experiment, plants were arranged in a factorial experiment to detect some possible interactions between the forms of Si application. A significant interaction between the forms of Si application was observed in 'Arbequina' (Fig. 1). At the highest doses of Si, foliar application was significantly higher than soil

Table 1. Effect of foliar application of Si on leaf Si concentration, vegetative growth, and susceptibility to *Venturia oleaginea* in 'Arbequina' and 'Picual' cultivars (Expt. 1).

Foliar application of Si (mg·L^{-1})	Arbequina: Leaf Si concn (µg·mg^{-1} DM)	Arbequina: Shoot growth (cm)	Arbequina: Disease index[z] (%)	Picual: Leaf Si concn (µg·mg^{-1} DM)	Picual: Shoot growth (cm)	Picual: Disease index[z] (%)
0	4.4	44.6	37.9	6.2	19.1	37.9
2.5	6.2	41.7	32.9	7.9	29.8	32.5
5	6.5	45.2	26.7	10.3	36.9	35.0
10	9.0	38.4	13.8	12.0	27.6	33.3
Significance[y]	L***	NS	NS	L**	L* Q**	NS
CV (%)	7.6	19.6	58.0	14.7	26.7	43.5

[z]The disease index (DI) was calculated as follows: DI = $[(\Sigma n_i \times i)/(N \times S_{\max})] \times 100$, where i represents severity (0 to 8), n_i is the number of leaves with a severity of i, N is the total number of inoculated leaves, and $S_{\max}$ is the maximum value of severity (equal to 8) (McKinney, 1923).
[y]NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. L = linear; Q = quadratic.

Table 2. Effect of soil application of Si on leaf Si concentration, vegetative growth, and susceptibility to *Venturia oleaginea* in 'Arbequina' and 'Picual' cultivars (Expt. 1).

Soil application of Si (mg·L^{-1})	Arbequina: Leaf Si concn (µg·mg^{-1} DM)	Arbequina: Shoot growth (cm)	Arbequina: Disease index (%)[z]	Picual: Leaf Si concn (µg·mg^{-1} DM)	Picual: Shoot growth (cm)	Picual: Disease index (%)[z]
0	3.4	46.9	49.2	4.0	14.7	47.5
1.25	3.7	43.9	25.4	3.6	20.3	42.9
2.5	4.6	48.7	24.2	6.0	34.0	45.0
5	5.4	43.5	14.2	7.2	37.1	55.4
Significance[y]	NS	NS	L***	L**	L***	NS
CV (%)[x]	18.2	24.9	36.5	22.7	40.0	27.9

[z]The disease index (DI) was calculated as follows: DI = $[(\Sigma n_i \times i)/(N \times S_{\max})] \times 100$, where i represents severity (0 to 8), n_i is the number of leaves with a severity of i, N is the total number of inoculated leaves, and $S_{\max}$ is the maximum value of severity (equal to 8) (McKinney, 1923)
[y]NS, **, ***Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively. L = linear.

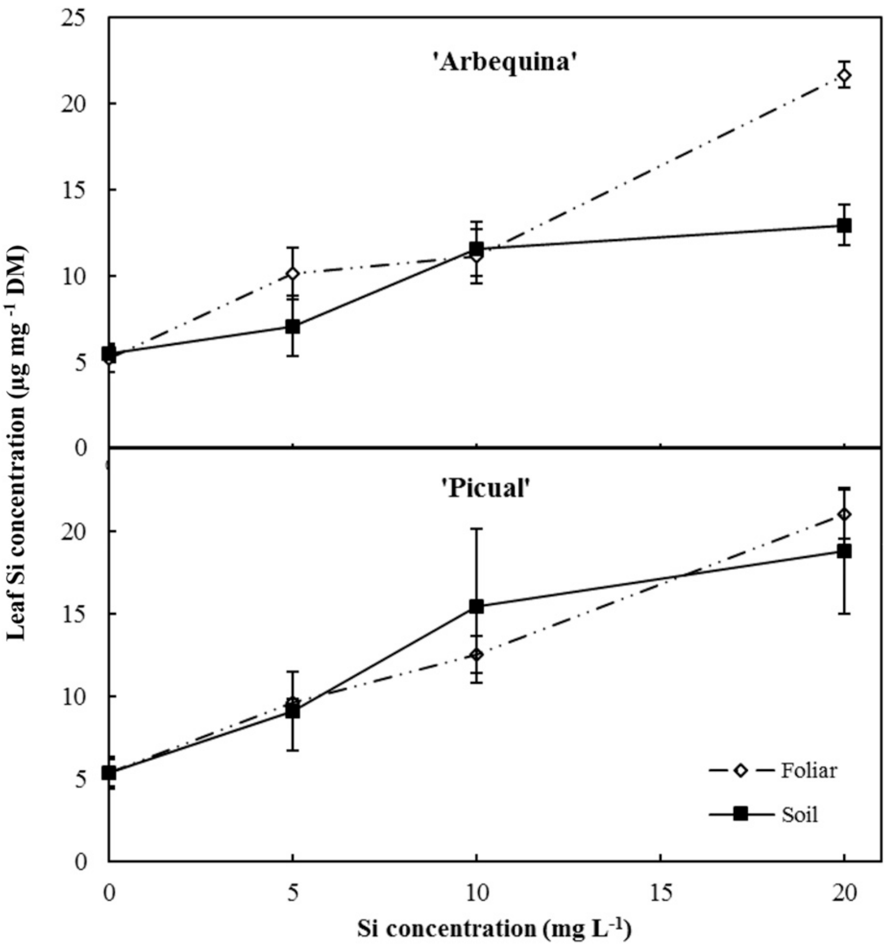

Fig. 1. Leaf Si concentration according to the form and the amount of Si applied in 'Arbequina' and 'Picual'.

Table 3. Effect of the application form (foliar vs. soil) and Si concentration on the leaf disease index in 'Arbequina' and 'Picual' cultivars (Expt. 2).

	Arbequina	Picual
	Disease index (%)[y]	Disease index (%)[y]
Application form[z]		
Foliar	19.4 a	49.1 a
Soil	20.9 a	38.5 a
Si concentration (mg·L⁻¹)		
0	41.7	63.8
5	13.0	42.1
10	13.2	39.2
20	12.8	30.2
Significance[x]	L*** Q*	L***
CV (%)	19.0	43.5

[z]Same letters in the same column indicate nonsignificant differences.
[y]The disease index (DI) was calculated as follows: $DI = [(\Sigma n_i \times i)/(N \times S_{max})] \times 100$, where i represents severity (0 to 8), n_i is the number of leaves with a severity of i, N is the total number of inoculated leaves, and S_{max} is the maximum value of severity (equal to 8) (McKinney, 1923).
[x]*, ***Significant at $P \leq 0.05$ or 0.001, respectively. L = linear; Q = quadratic.

application. These effects were not observed in 'Picual'. In this cultivar, leaf Si concentration increased significantly with the amount of Si applied, but no differences were found between both forms of Si application.

No interactions were found when the disease index was analyzed (Table 3). In both cultivars, nonsignificant differences were obtained between both forms of Si application, but the disease index significantly decreases with the amount of Si applied. This effect was more pronounced in 'Arbequina' (Fig. 2). The data show that there is a clear difference between the Si treatments and the control, but little differences were showed between the amounts of Si applied.

Discussion

Both foliar and soil applications of Si through the irrigation water were effective in increased Si leaf concentration in olive plants, although at the highest amounts of Si applied, foliar application was more effective than soil application. This effect was more marked in 'Arbequina' than in 'Picual'. Savvas and Ntatsi (2015) indicate that usually soil application is more effective in increasing the Si levels in plant tissues than foliar application. But there are also references indicating the effectiveness of foliar application of Si (Pilon et al., 2013; Wang et al., 2015). The results have a particular interest in olive culture because most of the olive orchards are cultivated under rainfed conditions, and foliar sprays are the common form of chemical application.

Silicon is not an essential element for plant growth (Marschner, 2012), so stimulation of growth by Si application could not be expected. However, in our work, the vegetative growth of 'Picual' was stimulated by Si application, regardless of the application method. This effect was not observed in 'Arbequina'. We have no explanation to this result, but Pilon et al. (2013) also found an increase in the leaf area in potato plants after Si application. In papaya plants, there was an effect of Si concentration on growth in height, obtaining an increase of 16.8% on the growth in height in comparison with plants that received no fertilization with Si (Sá et al., 2015). This suggests that Si may stimulate growth depending on the species and cultivar.

Continuous application of Si reduces the incidence of *V. oleaginea*, regardless of the application method. Although in the first experiment, this effect was observed only when Si was applied through irrigation water in 'Arbequina', the increase in the number of plant replications in the second experiment allows a clearer evidence of this effect to be obtained. Even so, the variability was very high, particularly in 'Picual'. The beneficial effect of Si in plants has been explained in two ways. The accumulation and polymerization of Si beneath the cuticle and the cell walls forming a mechanical barrier, was the first proposed hypothesis to explain how this element reduced the severity of plant diseases (Debona et al., 2017; Wang et al., 2017). The mechanical barrier prevents the formation of water film, important for the vital processes of the pathogenesis as the germination and the penetration of fungi in the plant (Pozza et al., 2004). But in addition to acting as a physical barrier against the attack of pathogens, some studies show that the presence of Si seems to facilitate the rapid deposition of phenolic compounds or phytoalexins in the sites of infection, which constitute a defense mechanism (Rodrigues et al., 2004; Sun et al., 2010). Both hypotheses could explain the reduction in the fungus incidence in the olive.

It was evident again the differences between cultivars in response to Si application. The effect was more pronounced in 'Arbequina', a moderate susceptible cultivar, than in 'Picual', a more susceptible one (Moral et al., 2015). This effect was observed regardless of the amount of Si applied.

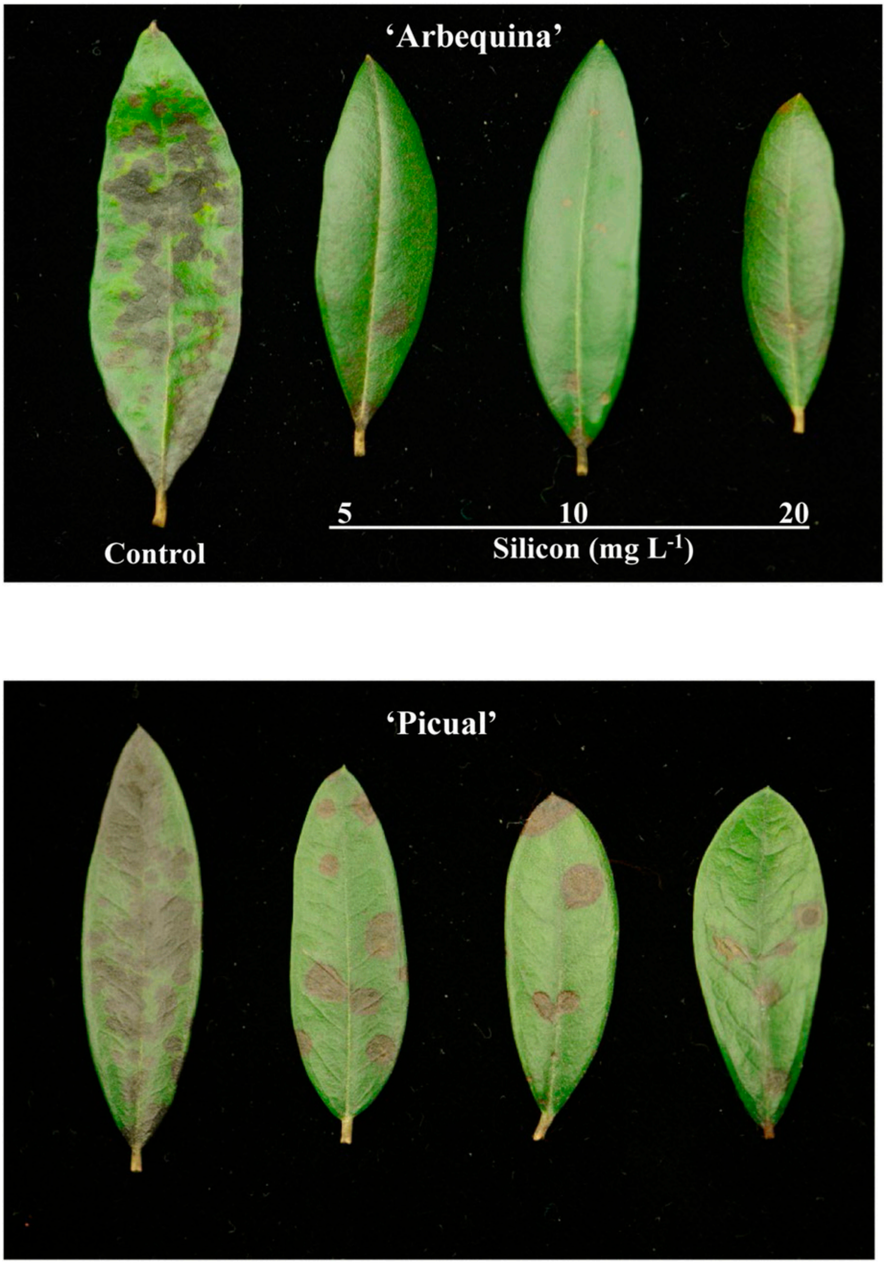

Fig. 2. Olive leaf spot infections in response to Si application in 'Arbequina' and 'Picual' cultivars.

In conclusion, the application of Si, both by foliar sprays or through irrigation water to the soil, increased the concentration of this element in olive leaves. This seems to reduce the incidence of *V. oleaginea*, the most important foliar disease of the olive.

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