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Effects of silicon on the penetration and reproduction events of *Meloidogyne exigua* on coffee roots

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Abstract

Considering that the root-knot nematode ***Meloidogyne exigua*** has caused great yield losses to coffee production in Brazil, this study aimed to determine whether the penetration and the reproduction events of this nematode on the roots of plants from two coffee cultivars with different levels of basal resistance to this nematode could be affected by silicon (Si). Coffee plants from the cultivars Catuaí and IAPAR 59, which are susceptible and resistant, respectively, to ***M. exigua***, were grown in pots containing Si-deficient soil that was amended with either calcium silicate (+Si) or calcium carbonate (–Si). The Si concentration on the root tissue significantly increased by 159 and 97% for the +Si plants from the cultivars Catuaí and IAPAR 59, respectively, compared to the –Si plants of these cultivars. The population of ***M. exigua***, the number of galls and the number of eggs were significantly reduced on the roots of the +Si plants of the cultivars Catuaí and IAPAR 59 compared to the –Si plants of these cultivars. It was concluded that the development and reproduction events of ***M. exigua*** were negatively impacted on the roots of coffee plants supplied with Si.

Key words: *Coffea arabica*, host resistance, plant nutrition, root-knot nematode.

Meloidogyne exigua Goeldi is the most widely distributed nematode species in the areas where coffee (*Coffea arabica*) is grown in Brazil (Campos & Villain, 2005). In the Minas Gerais State, the main coffee producer in Brazil, it is estimated that *M. exigua* is present in almost all municipalities where coffee is grown and in more than 50% of the coffee-producing areas in the other Brazilian states (Castro et al., 2008). On coffee plantations in Rio de Janeiro State, yield losses caused by *M. exigua* reached 45% (Barbosa et al., 2004).

The use of nematicides has been avoided both in small and large areas due to their high cost and the risk that they bring to the applicator and also to the environment. Cultivars that are resistant to *M. exigua* are still scarce in the market, and the continued use of the available resistant cultivars has contributed to overcoming the host resistance because this genetic inheritance is race-specific. In light of these concerns, silicon (Si) shows promising as an environmentally friendly choice. The Si has the potential to decrease the intensity of important diseases in several crops, especially cereals and some dicots (Datnoff et al., 2007). Silva et al. (2010) investigated the effect of Si on coffee resistance to *M. exigua* at the biochemical level and found that the high Si concentration in the roots of plants supplied with Si contributed to reduce the number of galls and the number of eggs due to an increase in the concentration of lignin-thioglycolic acid derivatives and the activities of peroxidases, polyphenoloxidases and phenylalanine-ammonia lyases.

This study aimed to investigate whether the penetration and the reproduction of *M. exigua* on the roots of coffee cultivars with different resistance phenotypes could be affected by Si.

The Brazilian soil type that was used in the experiments was a Si-deficient typical Acrustox red yellow latosol that was collected at the Triângulo Mineiro savannah area with 530 g of clay kg^{-1}; pH in KCl = 4.8; P (Mehlich-1) = 0.5 mg dm^{-3}; K (Mehlich-1) = 13 mg dm^{-3}; Al^{3+}, Ca^{2+}, Mg^{2+} and $H+Al^{3+}$ = 0.1, 0.0, 0.0 and 3.8 $cmol_c dm^{-3}$, respectively; base saturation = 2%; and organic matter = 2.3 dag kg^{-1}. The concentration of available Si (extraction in $CaCl_2$) was 11.8 mg dm^{-3}. Each plastic pot was filled with 1 kg of air-dried, sieved (5 mm) soil. Wollastonite, which was used as the Si source (calcium silicate; Vansil,

EW-20, Ipiranga Chemical Co., São Paulo, Brazil), was composed of 24.2% Si and 31% Ca and it was incorporated into each pot at the rates of 0 and 1.25 g kg^{-1} soil, which corresponded, respectively, to 0 and 0.30 g of elemental Si per pot. Calcium carbonate (40% Ca, Sigma-Aldrich, São Paulo, Brasil) was added at the rate of 0.97 g kg^{-1} of soil to equilibrate the amount of Ca in this treatment with the amount that was present in the pots that received 1.25 g of wollastonite. The amount of Ca among the treatments was fixed at 0.39 g per pot. The soil in each pot was incubated for 60 days with approximately 65% of humidity.

Coffee seeds from the cultivars IAPAR 59 and Catuaí Vermelho IAC 44 (hereafter denominated Catuaí), which are resistant and susceptible to *M. exigua*, respectively, were surface sterilised in 10% (v v^{-1}) NaOCl for 1.5 min, rinsed in sterilised water for 3 min and sowed at the rate of 50 seeds of each cultivar per plastic tray containing washed and sterilised sand. Seedlings in the cotyledonary leaf stage were transplanted at one seedling per pot. The soil in each pot was fertilised before seedling transplanting with 3 g of simple superphosphate, 0.5 g of potassium chloride and 0.22 g of ammonium sulphate per kg of soil. The plants also received 30 mL of Hoagland nutrient solution (Hoagland & Arnon, 1950) every 15 days. Plants were watered as needed. The plants were inoculated with *M. exigua* as previously described by Silva et al. (2010).

The eggs of *M. exigua* were extracted according to the technique that was proposed by Boneti & Ferraz (1981) and incubated with deionised water in a growth chamber at 26 °C based on Baermann's method using a bowl instead of a funnel. Nematodes were discarded after daily collection for four days and were maintained in the refrigerator at 8 °C. Each plant was inoculated with 2000 J2 (4 mL) distributed in three openings that were positioned 1 cm from the collar at an approximately 3-cm depth. Forty-eight hours after inoculation, the plants were removed from the plastic pots and their roots were washed to eliminate any J2 that had not yet penetrated in order to standardise the age of the J2 at two days inside the roots. The penetration and development of *M. exigua* were evaluated at 5, 10 and 15 days after inoculation (dai) in six plants from each cultivar. The nematodes inside the roots were localised by *in situ* staining (Bybd et al., 1983) with some adaptations. Root galling of plants from cvs. Catuaí and IAPAR 59 were counted at 120 dai and the reproduction of *M. exigua* was evaluated by the number of eggs (NE) and the number of galls (NG) per root system according to Boneti & Ferraz (1981). Another group of pots were used for determine the concentration of Si on the root tissue for each treatment.

The plants were removed from the pots at 120 dai. The roots were shaken to remove the bulk of the soil and washed in sterile deionised water. Roots and shoots were dried for 72 h at 65 °C to obtain the dry mass. Roots were ground with a Thomas-Wiley mill to pass through a 40-mesh screen and used to determine the concentration of Si according to the colorimetric analysis proposed by Korndörfer et al. (2004). The root tissue was digested with a nitric-perchloric solution (3:1, v/v) and the Ca concentration was determined by atomic absorption spectrophotometry. A 2×2 factorial experiment, consisting of two Si rates and two coffee cultivars, was arranged in a completely randomised design with six replications. The experiment was repeated once. Each experimental unit consisted of one pot containing one plant. The data from the two experiments were pooled for analysis as indicated by Cochran's test for the homogeneity of variance (Gomez & Gomez, 1984). The data were subjected to an analysis of variance (ANOVA) and the means were compared by a t-test ($p \leq 0.05$) using SAS (SAS Institute, Inc., Cary, NC).

The factors Si rates, coffee cultivars and the interaction Si rates × coffee cultivars were significant only for the concentration of Si on the root tissue (Table 1). The Si concentration on root tissue significantly increased by 159 and 97% for the +Si plants from the cultivars Catuaí and IAPAR 59, respectively, compared to that of the -Si plants of these two cultivars. The Si concentration on the root tissue of the –Si and +Si plants from the cultivar Catuaí was significantly superior by 26 and 65%, respectively, compared to that of –Si and +Si plants from the cultivar IAPAR 59 (Table 1).

Based on the root and the shoot dry weight, the factors Si rates and coffee cultivars and the interaction Si rates × coffee cultivars were significantly. The root dry weight was significantly reduced by 12 and 18% on the roots of the –Si and +Si plants, respectively, from the cultivar IAPAR 59 compared to the roots of the –Si and +Si plants from the cultivar Catuaí. Significant decreases of 15 and 20%, respectively, for the root dry weight occurred in the roots of the -Si plants from the cultivars Catuaí and IAPAR 59 compared to that of the roots of the +Si plants from these two cultivars. Significant reductions of 11 and 17%, respectively, on the roots of -Si and +Si plants from the cultivar IAPAR 59 compared to that of the roots of the –Si and +Si plants from the cultivar Catuaí occurred for the shoot dry weight. The shoot dry weight significantly

Table 1. Silicon (Si) concentration in root tissue of coffee plants from cultivars Catuaí Vermelho IAC 44 (susceptible) and IAPAR 59 (resistant) supplied (+Si) or not (-Si) with silicon at 120 days after inoculation with *Meloidogyne exigua*

Cultivars	Silicon (dag/kg)	
	–Si	+Si
Catuaí	0.44 bA	1.14 aA
IAPAR	0.35 bB	0.69 aB
CV (%)	12.18	

Means within a column followed by the same uppercase letter and means within a row followed by the same lowercase letter are not significantly different (P= 0.05) according to the*t*-test. CV = coefficient of variation.

decreased by 20 and 14%, respectively, in the –Si plants from the cultivars Catuaí and IAPAR 59 compared to that of the roots of the +Si plants from these two cultivars (Table 2).

The factors Si rates and coffee cultivars and the interaction Si rates × coffee cultivars were significant for the population of *M. exigua* on the roots of the plants from the cultivars Catuaí and IAPAR 59 (Table 3). The number of *M. exigua* juveniles inside the roots was significantly reduced by 30 and 25% at 5 dai, by 82 and 77% at 10 dai and by 78 and 77% at 15 dai for the –Si and +Si plants of the cv. IAPAR 59 compared to that of the –Si and +Si plants of the cv. Catuaí. For the cv. Catuaí, the population of *M. exigua* was significantly reduced by 26, 37 and 25% at 5, 10 and 15 ai, respectively, for the +Si plants compared to that of the -Si plants. For the cultivar IAPAR 59, the population of *M. exigua* was significantly reduced by 21% only at 5 dai for the +Si plants compared to that of the -Si plants (Table 3).

The factors Si rates and coffee cultivars and the interaction Si rates × coffee cultivars were significant for the root and shoot dry weights (Table 4). The NG were significantly reduced by 97 and 20%, respectively, on the roots of the +Si and –Si plants from the cv. IAPAR 59 compared to those of the roots of the +Si and –Si plants from the cv. Catuaí. Significant decreases of 99 and 25%, respectively, for the NE occurred in the roots of the +Si and -Si plants from the cv. IAPAR 59 compared to the roots of the +Si and -Si plants from the cv. Catuaí. For the cv. Catuaí, the NG and NE were significantly reduced by 26 and 30%, respectively, for the +Si plants compared to those of the -Si plants (Table 4).

The results of the present study not only support the concept that Si can increase the resistance of several plant species to many root pathogens (Fauteux et al., 2005; Silva et al., 2010), but also provides novel evidence that the penetration and the reproduction of *M. exigua* on the roots of the coffee plants, especially in the most susceptible cultivar, were negatively affected by Si. There was a drastic decrease in penetration of juveniles, and even when there was penetration, the female development was delayed with consequent reduction in both NG and NE indicating to occur resistance to pre and post-penetration of the nematode. The concentration of Si on the roots of plants supplied with Si from both cultivars ranged from 0.4 to 1.2 dag/kg, which

Table 2. Dry weight of roots (DWR) and shoot (DWS) of coffee plants from cultivars Catuaí Vermelho IAC 44 (susceptible) and IAPAR 59 (resistant) supplied (+Si) or not (Si) with silicon at 120 days after inoculation with *Meloidogyne exigua*

Cultivars	DWR (g)		DWS (g)	
	–Si	+Si	–Si	+Si
Catuaí	1.24 bA	1.52 aA	3.24 bA	3.91 aA
IAPAR	1.06 bB	1.21 aB	2.79 bB	3.13 aB
CV (%)	12.55		14.22	

Means followed by the same lowercase number in a line and uppercase letter in a column do not differ among themselves by Tukey's test at 5% probability row. CV = coefficient of variation.

Table 3. Number of J2, J3 and J4 of *Meloidogyne exigua* on the roots of coffee plants from cultivars Catuaí Vermelho IAC 44 (susceptible) and IAPAR 59 (resistant) at 5, 10 and 15 days after inoculation with the nematode

Cultivars	Stages	5 dai		10 dai		15 dai	
		–Si	+Si	–Si	+Si	–Si	+Si
Catuaí	J2	146	108	139	86	21	24
	J3 and J4	0	0	17	12	113	77
	Total	146 aA	108 bA	156 aA	99 bA	134 aA	101 bA
IAPAR	J2	102	81	28	23	13	10
	J3 andJ4	0	0	0	0	16	12
	Total	102 aB	81 bB	28 aB	23 aB	29 aB	22 aB

Means within a column followed by the same uppercase letter or means within a row that were followed by the same lowercase letter are not significantly different (P=0 05) according to the t-test for each evaluation time. J2, J3 and J4 = juveniles at the 2nd, 3rdand 4thstages, respectively. dai = days after inoculation.

Table 4. Number of galls (NG) and number of eggs (NE) of *Meloidogyne exigua* per root system of coffee plants from cultivars Catuaí Vermelho IAC 44 (susceptible) and IAPAR 59 (resistant) that were supplied (+Si) or not (-Si) with silicon at 120 days after inoculation with the nematode

Cultivars	NG		NE	
	–Si	+Si	–Si	+Si
Catuaí	182 aA	135 bA	17,872 aA	12,564 bA
IAPAR	5 aB	4 aB	88 aB	66 aB
CV (%)	19.44		23.76	

For NG and NE, the means within a column followed by the same uppercase letter or means within a row followed by the same lowercase letter are not significantly different (P = 0.05) according to the t-test. CV = coefficient of variation.

is less than what has been reported for rice, known as an Si-accumulator plant with Si concentration on shoots up to 8% (Dallagnol et al., 2009; Datnoff et al., 2007). However, the Si concentration on the roots of coffee plants played a pivotal role, based on the innate physiological capacity of coffee plants to take up this element from the soil solution, in negatively impacting the penetration of the juveniles of *M. exigua* and the further development of galls and the nematode's eggs in addition to improving the plant growth. Carré-Missio et al. (2009) observed an increase of 100% in the Si concentration in the roots of coffee plants that were grown in hydroponic culture containing Si compared to plants that were grown in the absence of this element. The authors found that the coffee plants were not efficient in translocating Si from the roots to the shoots and, therefore, the coffee leaf rust severity did not decrease. The mechanisms behind the variation in the absorption and accumulation of Si in the shoots of many plant species, including *C. arabica*, need to be determined.

The reduction in penetration and reproduction of *M. exigua* on the roots of plants from the susceptible cv. Catuaí indirectly indicates an increase in the basal level of resistance potentiated by Si. By contrast, Si did not have any effect on the penetration and reproduction of *M. exigua* on the roots of plants from cv. IAPAR 59 because of the high level of resistance of this cultivar. Anthony et al. (2005) noted that plants from cultivar IAPAR 59 presents HR-type reaction, involving the *Mex–1* gene, by cellular alterations, occurring the condensation of cytoplasm in feeding sites of J2, an increase in the size of the nucleus and retraction of the plasmatic membrane of the cell wall.

Based on the results of the present study, it can be concluded that the penetration and reproduction events of *M. exigua* were negatively impacted on the roots of coffee plants supplied with Si.

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