

PIETRO HUNGER MICHERI

**DUNITE IN ANNUAL CROPS: PHYSIOLOGICAL CHANGES, NUTRITIONAL
STATUS IN SOYBEAN AND MAIZE YIELD**

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STATUS IN SOYBEAN AND MAIZE YIELD**

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Supervisor: Prof. Dr. Carlos Alexandre
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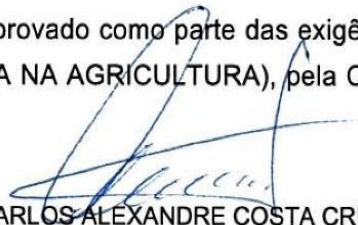
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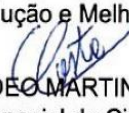
AUTOR: PIETRO HUNGER MICHERI

ORIENTADOR: CARLOS ALEXANDRE COSTA CRUSCIOL

Aprovado como parte das exigências para obtenção do Título de Mestre em AGRONOMIA (ENERGIA NA AGRICULTURA), pela Comissão Examinadora:


Prof. Dr. CARLOS ALEXANDRE COSTA CRUSCIOL
Produção e Melhoramento Vegetal / Faculdade de Ciências Agrônômicas de Botucatu


Prof.ª Dr.ª PATRÍCIA PEREIRA DIAS
Pós-Doutoranda - Produção e Melhoramento Vegetal / Faculdade de Ciências Agrônômicas - UNESP


Prof. Dr. CLAUDIO HIDEO MARTINS DA COSTA
Unidade Acadêmica Especial de Ciências Agrárias / Universidade Federal de Goiás

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RESUMO

A crescente demanda por fertilizantes concomitante ao aumento dos preços desse insumo e a escassez de suas fontes levam a incessante busca por fontes alternativas de nutrientes para as plantas. Os afloramentos rochosos no Brasil coincidem com as localidades de áreas produtivas, tornando o acesso fácil dos produtores rurais à essas fontes, uma vez que o maior custo do pó de rocha está relacionado ao frete. Portanto, uma das alternativas estudadas é o uso de pó de dunito, rico em magnésio em culturas anuais, como soja e milho. Estas são uma das principais culturas do Brasil em área de produção. O objetivo desse estudo foi avaliar os efeitos de doses de pó de dunito no teor foliar de Magnésio (Mg) e Silício (Si), teores foliares de açúcares redutores, sacarose e amido, e atributos químicos de solo em dois tipos de solo. Os tratamentos consistiram em cinco doses de pó de dunito (0, 42, 208, 542 and 1542 mg kg⁻¹) no solo argiloso e (0, 150, 238, 411 and 933 mg kg⁻¹) no solo arenoso, respectivamente. Em ambos os solos, os teores de Mg e Si foliar, os açúcares redutores e glicose nas folhas, assim como pH, Mg, e Si do solo e os componentes de produção aumentaram com o aumento das doses de dunito. A melhor nutrição de Mg fornece menor teor de amido foliar e consequentemente melhor partição de metabolitos na planta, levando ao melhor desenvolvimento, enchimento e produção de grãos de soja.

Palavras-chave: *Glycine max*, *Zea mays*, fertilização de magnésio, metabólitos, silício.

ABSTRACT

The growing demand for fertilizers, coupled with the increase in fertilizer prices and the scarcity of fertilizer sources, lead to the incessant search for alternative sources of nutrients for the plants. The rocky outcrops in Brazil coincide with the localities of agriculture productive areas, making access easy for rural producers to these sources, since the higher cost of rock dust is related to the transport. Therefore, one of the alternatives studied is the use of dunite powder, rich in magnesium in annual crops such as soybean and corn. These are one of the main crops of Brazil in production area. The aim of this study was to evaluate the effect of dunite rates on Magnesium (Mg), Silicon (Si), foliar contents of reducing sugars, sucrose, and starch, soil chemical attributes and soybean yield in two soil types. The treatments consisted in the five rates of Dunite (0, 42, 208, 542 and 1542 mg kg⁻¹) in a clayey soil, and five rates of Dunite (0, 150, 238, 411 and 933 mg kg⁻¹) in a sandy soil. In both soils, the Mg and Si leaves concentration, foliar reducing sugars, and glucose, as well as soil pH, Mg, and Si, and the yield components increased with input rates. The best Mg nutrition provides lower foliar starch levels, consequently, the best partition of metabolites to plant leads to better development, filling and yield of soybeans.

Keywords: Glycine max, Zea mays, magnesium fertilization, metabolites, silicon.

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GENERAL INTRODUCTION

Brazil has the largest harvest area in maize and soybeans. Maize production is 257 million of tons, lower than United States, cultivated in 17.5 million of hectares. Soybean produces 117 million of tons in 36.1 million of hectares (2018/2019 harvest; USDA, 2019). Cereals, legumes and oilseeds 2019 production had 1.6% increase in Brazil. Mato Grosso is the major Brazilian state in grains production (IBGE, 2019).

Fertilizers consumption increased in 2019 due to higher producer's capitalization (IBGE, 2019). Despite the increase in grain production with higher fertilizer amounts applied, the nutrients are not complete used by plants, some of rapid release, as K and N could contaminate water table, and the investment is worthless. Moreover, the fixation process of P in the soil retain in Al and Fe oxides, and become useless as well (LOPES, 1998; FAGAN et al., 2016).

Then, some sources with slower release have been studied. One of them is the rock for crops, consisting in add milled rock to the soil as conditioner, proportioning benefits to the soil and, consequently, for crops (STRAATEN, 2006; MANNING et al. 2017).

The benefits can include physical, chemical, and biological changes in the soil, such as increase in soil attributes, pH, Mg, K, and Si, and against plants disease (HARLEY; GILKES, 2000; MELO et al., 2012; LI; DONG, 2013, DANIEL et al., 2013; BRANDÃO et al., 2014; RAMOS et al., 2015; MANNING et al., 2017).

Since many "annual" crops' harvests are used the same area in Brazil, despite the profit increasing, the investments had increased as well. If there are three harvests per year, the production costs have to be multiplied by 3, then a slowly release and inexpensive nutrients source is necessary to cover all harvests.

Despite the Magnesium function in plants have been elucidated, its function from non-synthetic sources still being understood (MAYLAND, 1990; SHAUL, 2002).

The Dunite is a mining slag resulting from the process of nickel extraction from Serpentine rocks called nickel slag with large amounts of magnesium and silicon. In the extraction process the rock is melted in electric furnaces at a temperature of 1800oC, forming at this moment two immiscible liquids: the iron - nickel alloy and the magnesium silicate.

Iron - nickel alloy is a denser liquid that concentrates at the bottom of electric furnaces, causing magnesium silicate to concentrate at the top of tanks.

At the moment of removal of Magnesium Silicate from the tanks, it is at a temperature of 1700oC and receives a water jet for solidification and then taken to decantation tanks. At the moment when the mineral receives the water jet, it prevents the solidification from forming magnesium and silicon crystals, thus giving the mineral reactivity characteristics in soils in a timely manner to be an excellent source of fertilizer.

Thereby, this study has the following hypothesis: Dunite application can improve the Mg contents in the soil, and plants, its application can improve the yields and components of production in annual crops, and despite the short time the dunite rock application can change the plants metabolites, depends on the soil textures.

Thus, this study aimed to evaluate the effect of magnesian fertilization with Dunite on the nutritional, metabolic and grain yield of annual crops, as well as on the soil chemical attributes in two soils with different clay content.

CHAPTER 1

DUNITE IN ANNUAL CULTURES: PHYSIOLOGICAL CHANGES, NUTRITIONAL STATUS AND SOYBEAN YIELD**Abstract**

Brazil is one of the most fertilizers importers in the world. For this reason, the production costs are expensive, then the use of alternative nutrients sources is increasing in agriculture. The aim of this study was to evaluate the effect of dunite rates on Magnesium (Mg), Silicon (Si), foliar contents of reducing sugars, sucrose, and starch, soil chemical attributes and soybean yield in two soil types. The treatments consisted in the five rates of Dunite (0, 42, 208, 542 and 1542 mg kg⁻¹) in a clayey soil, and five rates of Dunite (0, 150, 238, 411 and 933 mg kg⁻¹) in a sandy soil. In both soils, the Mg and Si leaves concentration, foliar reducing sugars, and glucose, as well as soil pH, Mg, and Si, and the yield components increased with input rates. The best Mg nutrition provides lower foliar starch levels, consequently, the best partition of metabolites to plant leads to better development, filling and yield of soybeans.

Key-words: Glycine max, magnesium fertilization, metabolites, silicon.

1.1 INTRODUCTION

The ability of soils to produce the food needed to support a global population expected to exceed 9.0 billion by 2050 is fundamental to sustainable development (Keesstra et al. 2016). In this sense, the soybean crop plays an essential role as one of the most important 'commodities' in Brazil, accounting for approximately 13% of all exports in the country (Moretti et al. 2018). Soil fertility management and crop fertilization are determinant for higher economic yields, and vary for each region, according to each production system (TPS 2013).

Rock dust technology is a mechanism of remineralization of soils for a sustainable tropical agriculture. Replacing the use of chemical fertilizers used in monoculture of large scale, with environmental and economic gains, this technology that has been developed in Brazil and allows restoring the food and economic sovereignty of agricultural countries that are heavily dependent on the import of chemical inputs (Moreira et al. 2006; Theodoro et al. 2012).

In this sense, several efforts have been made in recent years to mitigate the different environmental impacts related to agricultural activities. In particular, the use of conventional synthetic fertilizers, draw significant attention due to the adverse environmental impacts caused by the excessive application of these products (Ramos et al. 2014; Nunes et al. 2014). According to Ramos et al. (2015), since Brazil is one of the main food's suppliers around the world, investigations in the potential of volcanic rock dust as a fertilizer related to nutrients release and concentration and the economic viability is not still elucidated to afford the sustainable development policies for quarry activity and agricultural production.

The volcanic rock dust is composed mainly of SiO_2 , Al_2O_3 , Fe_2O_3 , MnO , MgO , Na_2O , K_2O , and CaO (Ramos et al. 2014; Nunes et al. 2014; Ramos et al.

2015). According to Assis and Dias (2007), Dunite is an igneous rock, which is essentially a peridotite, magmatic or eruptive, consisting mostly of olivine $(\text{MgFe})_2\text{SiO}_4$, containing approximately 40% of Mg oxide (MgO) and 34% of Si oxide (SiO_2).

In soil remineralization, the rock dust application is related to its mineralogical characteristics and its plant-soil-microbiome interaction to increase soil quality (Ramos et al. 2017).

Thus, this study aimed to evaluate the effect of fertilization with Dunite on the nutritional, metabolic and grain yield of soybean, as well as on the soil chemical attributes in two soils with different clay content.

1.2 MATERIAL AND METHODS

A greenhouse experiment carried out in the 2016/2017 cropping season at College of Agricultural Science, Department of Crop Science, Botucatu, São Paulo State, Brazil. The experiment was composed by clayey and sandy texture of Rhodic Haplud soil (Santos et al., 2013). The clayey soil contained 602 g kg^{-1} clay and 281 g kg^{-1} sand and the sandy soil 259 g kg^{-1} clay and 724 g kg^{-1} of sand.

The soil chemical attributes (0.0 - 0.2 m) were determined according to Raji et al. (2001). Clay soil - soil organic matter (SOM) 25.2 g dm^{-3} ; pH in CaCl_2 5.2; phosphorus (P_{resin}) 6.0 mg dm^{-3} , potassium (K^+) $0.7 \text{ mmol}_c \text{ dm}^{-3}$, calcium (Ca^{2+}) $35.0 \text{ mmol}_c \text{ dm}^{-3}$, Mg^{2+} $8.0 \text{ mmol}_c \text{ dm}^{-3}$, potential acidity ($\text{H}^+ + \text{Al}^{3+}$) $29.0 \text{ mmol}_c \text{ dm}^{-3}$, aluminum (Al^{3+}) $0.0 \text{ mmol}_c \text{ dm}^{-3}$, cation exchange capacity (CEC) $73.0 \text{ mmol}_c \text{ dm}^{-3}$, iron (Fe) 20.0 mg dm^{-3} , copper (Cu) 8.4 mg dm^{-3} , manganese (Mn) 15.3 mg dm^{-3} , zinc (Zn) 0.9 mg dm^{-3} , and boron (B) 0.4 mg dm^{-3} and sandy soil - SOM 13.1 g dm^{-3} , pH in CaCl_2 5.4; P_{resin} 3.0 mg dm^{-3} , K^+ $0.5 \text{ mmol}_c \text{ dm}^{-3}$, Ca^{2+} $18.0 \text{ mmol}_c \text{ dm}^{-3}$.

³, Mg^{2+} 2.0 $\text{mmol}_c \text{dm}^{-3}$, $\text{H}^+ + \text{Al}^{3+}$ 18.0 $\text{mmol}_c \text{dm}^{-3}$, Al^{3+} 0.0 $\text{mmol}_c \text{dm}^{-3}$, CEC 38.0 $\text{mmol}_c \text{dm}^{-3}$, Fe 35.0 mg dm^{-3} , Cu 1.6 mg dm^{-3} , Mn 1.9 mg dm^{-3} , Zn 0.3 mg dm^{-3} , and B 0.3 mg dm^{-3} .

Dunite presents the following chemical characteristics: 24% Mg (40% MgO) and 16% Si (34% SiO_2), mainly due to its mineralogical composition (Figure 1).

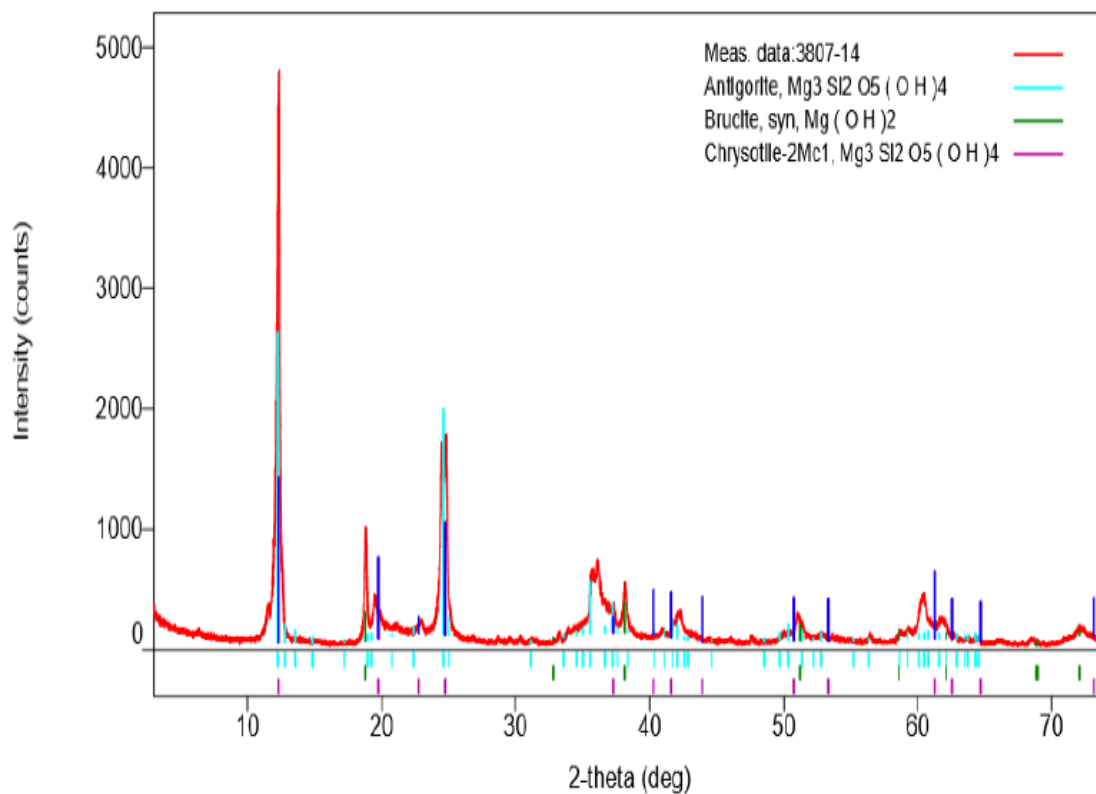


Figure 1. X-ray diffractometry from dunite rock dust mineralogical composition (Source: CR Fertilizers, 2017).

The experimental design was completely randomized, composed of four rates of Dunite and an absolute control, with four replicates, for both soils. The treatments consisted in the application of five rates of Dunite: 0, 42, 208, 542 and 1542 mg kg^{-1} for clayey soils, and five rates of Dunite: 0, 150, 238, 411, and 933 mg kg^{-1} for sandy soil. These application rates were based on the absorption ratio

between Calcium and Magnesium (3x1) in plant nutrition. Pots with 15 L of capacity were used in two experiments.

Nutrients were supplied as 200 mg P kg⁻¹, 150 mg K kg⁻¹, 15.0 mg S kg⁻¹, 5 mg Zn kg⁻¹, 1.5 mg B kg⁻¹, and 0.5 mg Cu kg⁻¹. Phytosanitary treatments were carried out according to the recommendation for the soybean crop (TPS 2013). Soil water content in pots was corrected to -0.01 MPa (field capacity), corresponding to 112 g kg⁻¹, respecting the equilibration period (60- to 100% field capacity). Five seeds (cultivar TMG 7062 RR) were sown per treatment, and three plants were left per pot after the emergence. Four reference pots were weighed every four days, and as needed, distilled sterile water was added to return the water content to field capacity (-0.01 MPa).

In the soybean R₂ growth stage (Fehr et al. 1971), leaves were collected and ground in a Wiley-type mill coupled to a 1.0 mm sieve to then determine the content Mg and Si (Malavolta et al. 1997). The partitioning of carbohydrates by reducing sugars (glucose and fructose), sucrose and starch content, obtained in the dry matter of leaves, was determined according to the methodology described by Nelson (1944).

At the same time of the foliar diagnosis, two plants were collected for analysis. The remaining plant was conducted until the end of the cycle, and from it the mass of 100 grains was determined by means of a precision scale weighing and value correction (13% on a wet basis). The grain yield was expressed as g plant⁻¹. After harvest, a soil sample of each pot was collected and analyzed the content of pH, Mg and Si, according to a methodology of Raij et al. (2001).

Data from each experiment were first submitted to tests of normality and homogeneity of variances for each variable. The data was submitted to the analysis

of variance (ANOVA) and F test ($p \leq 0.05$). A polynomial regression analysis was performed to determine, response curves for measured soybean and soil traits, where significant regression equations with the highest coefficients of determination were selected.

1.3 RESULTS AND DISCUSSION

The increase of Dunite rates in soybean cultivation in both soil types increased the Mg and Si leaf contents linearly ($p \leq 0.05$) (Figure 1A-D).

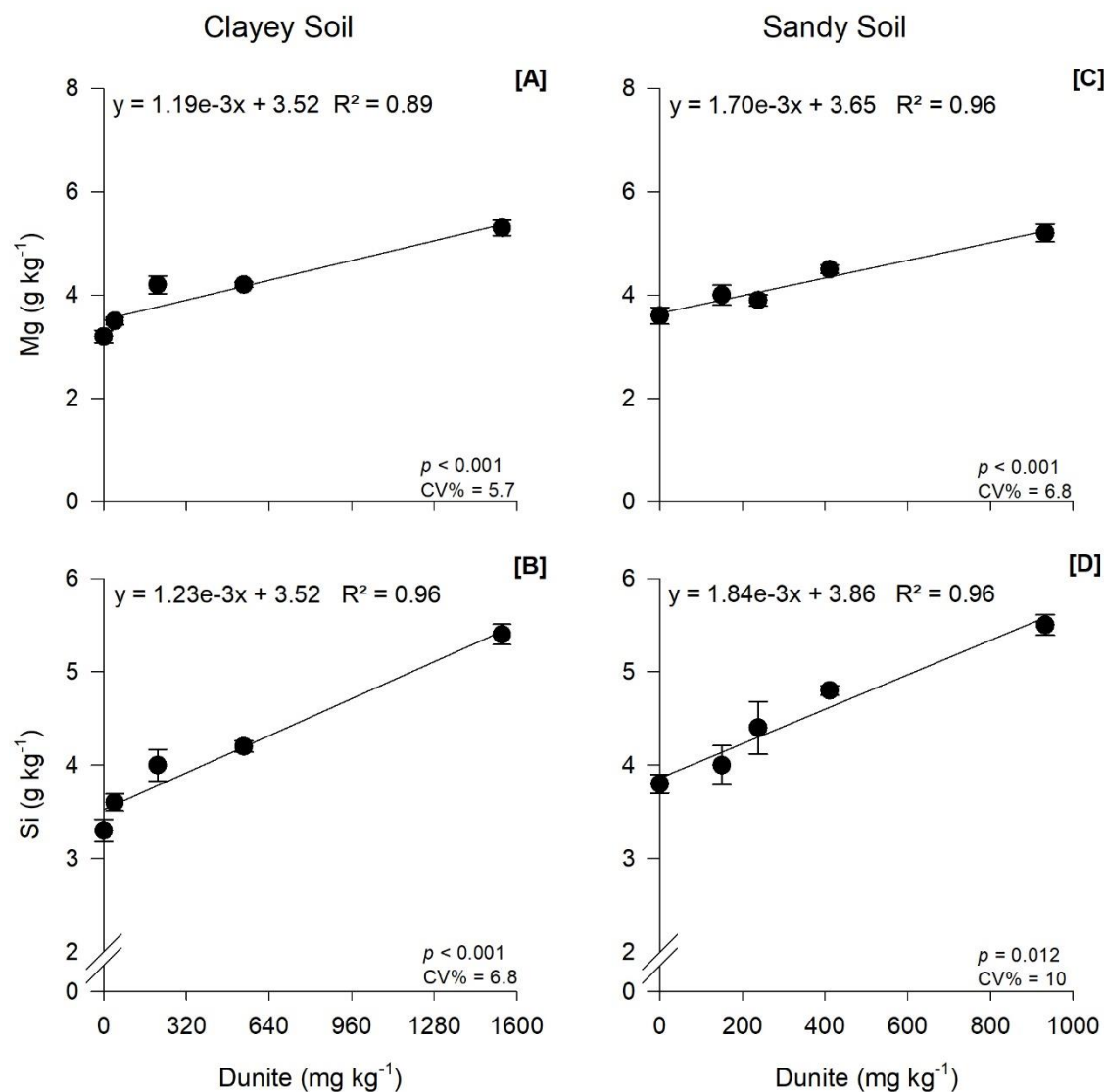


Figure 1. Mg content [A] and Si [B] in soybean leaves grown in clayey soil as a function of Dunite rates. Mg content [C] and Si [D] in soybean leaves grown in sandy soil as a function of Dunite rates.

The leaf contents obtained from these elements at the highest rate of the product were of 5.3 and 5.4 g kg⁻¹ (Figure 1A and B) in the clayey soil culture and 5.2 and 5.6 g kg⁻¹ (Figures 1C and D) in the sandy soil. By passing through the serpentinization procedure, whereby more than 90% of Mg and Si of Dunite undergo temperature variations below 400 °C, a hydration of these minerals occurs, making them more soluble in the system and susceptible for uptake by plants (Fernandes et al., 2010). As for Mg, it is important to emphasize the importance of this nutrient in productive systems, given its functions for plant growth and development (Gransee and Fuhrs, 2013), as well as its relation to a great number of key functions in plants (Cakmak and Yazici, 2010; Canizella et al., 2015). Besides being a main component of the chlorophyll molecule, much of the non-structural Mg is involved in the activation of enzymes, such as rubisco, in the photosynthetic process, in the supply of adenosine triphosphate (ATP) as an energy source and in the transport of photosynthates from source organs to sink organs (Cakmak and Kirkby 2008; Ceylan et al. 2016).

The Si supply, although not considered an essential element, has a secondary importance in several processes, working as a stress relief of biotic and abiotic origin due to the formation of a silica layer on the cell wall (Luyckx et al. 2017), besides having roles in the balance of phytohormones, reducing the endogenous concentration of jasmonic acid (JA) and salicylic acid (SA) and increasing the abscisic acid (ABA) in situations of stressors (Kim 2014). The soybean is as an intermediate plant in terms of Si accumulation in the plant tissue; however, the content of this element, in the form of silicic acid Si(OH)₄, can reach relevant levels, demonstrating that there is an active transport of this element from the roots (Mitani and Ma 2005) by aquaporin channels (Ma et al. 2007).

The carbohydrate metabolism of soybean plants was significantly influenced ($p \leq 0.05$) by the use of Dunite rates, regardless of the soil in which it was cultivated (Figure 2A-F).

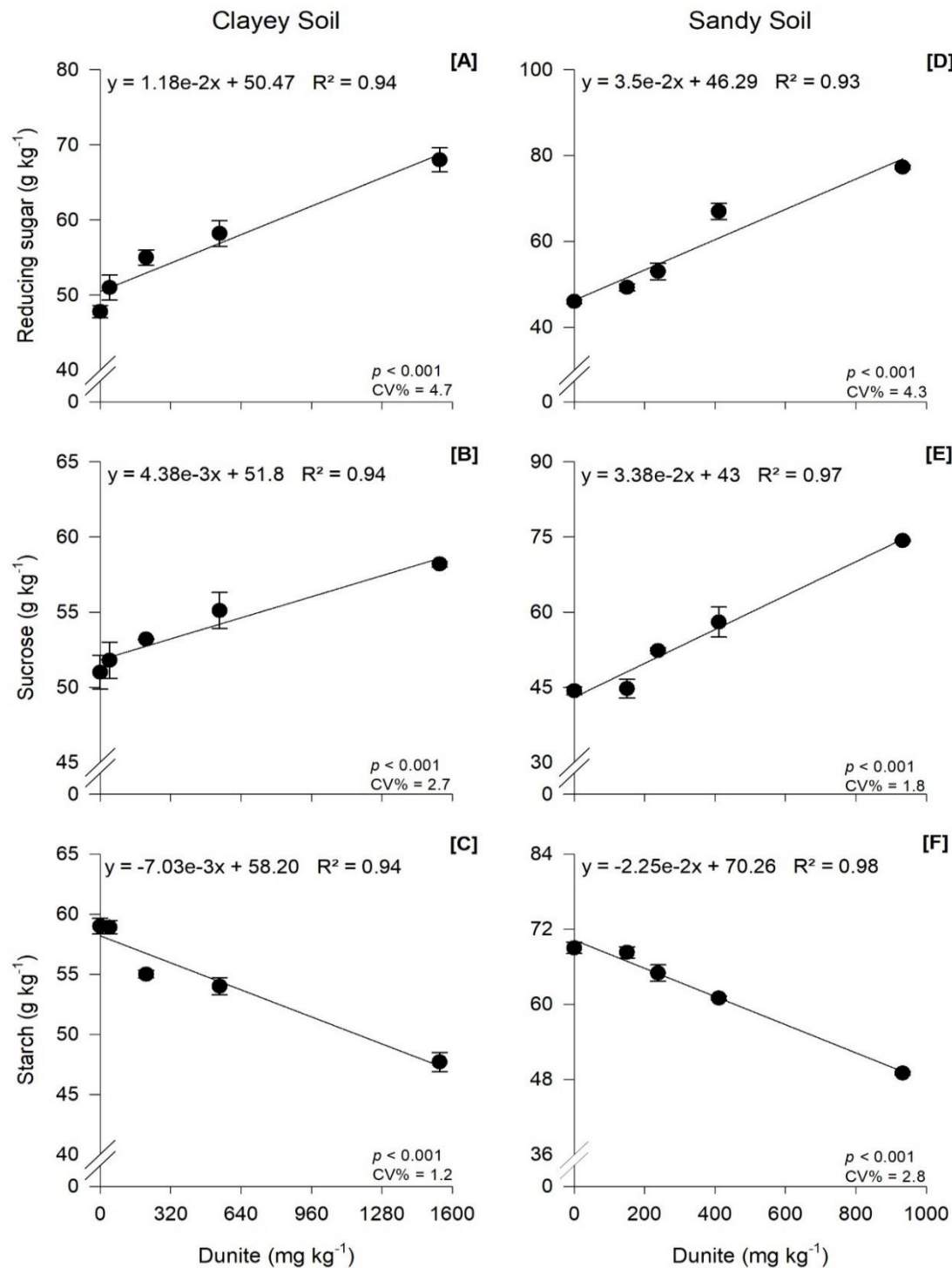


Figure 2. Reducing sugars [A], sucrose [B] and starch [C] in dry weight of soybean leaves grown in clayey soil as a function of Dunite rates. Reducing sugars [D], sucrose [E] and starch [F] in dry weight of soybean leaves grown in sandy soil as a function of Dunite rates.

The leaf contents of reducing sugars and sucrose increased linearly with the increase of rates, reaching values of 68.7 and 58.6 g kg⁻¹ (Figures 2A and B) for cultivation in clayey soil and 78.9 and 74.5 g kg⁻¹ (Figures 2D and E) in the sandy soil. The starch contents of the source leaves were reduced by the use of larger Dunite rates (Figures 2C and F).

The Mg, which is an element that is highly supplied by the solubilization of rock dust (Theodoro and Leonardos 2006; Moreira et al. 2006), acts on several processes that modulate the production and translocation of carbohydrates in plants (White 2012; Ceylan et al. 2016). The transport of carbohydrates over long distances, such as reducing sugars (fructose + glucose) and sucrose, is carried out by the phloem and is strongly affected by the Mg availability (Farhat et al. 2016). The sucrose on the phloem is an active process catalyzed by a proton gradient and H⁺/sucrose cotransporter, established by H⁺-ATPase located on the plasma membrane (Ward et al. 1998). For its proper functioning, the existence of Mg-ATP (Igamberdiev and Kleczkowski 2003) is essential, and for that, an additional Mg is needed. Moreover, the increase in Mg supply may have provided improvements in the H⁺-ATPase activity of the phloem cells, increasing the sucrose concentration for export by triose-P, not allowing its accumulation in the chloroplast of leaves. As a consequence, the enzyme ADP-glucose pyrophosphorylase (AGPase) is not activated and there is no accumulation of starch in the chloroplasts (Sonnewald 2001), demonstrating that the translocation of carbon skeletons to the plant sinks happens efficiently due to the greater Mg availability (Ceylan et al. 2016; Farhat et al. 2016).

The phytotechnical and productive components of the soybean crop increased linearly ($p \leq 0.05$) by using higher Dunite rates in both soils (Figure 3A-F).

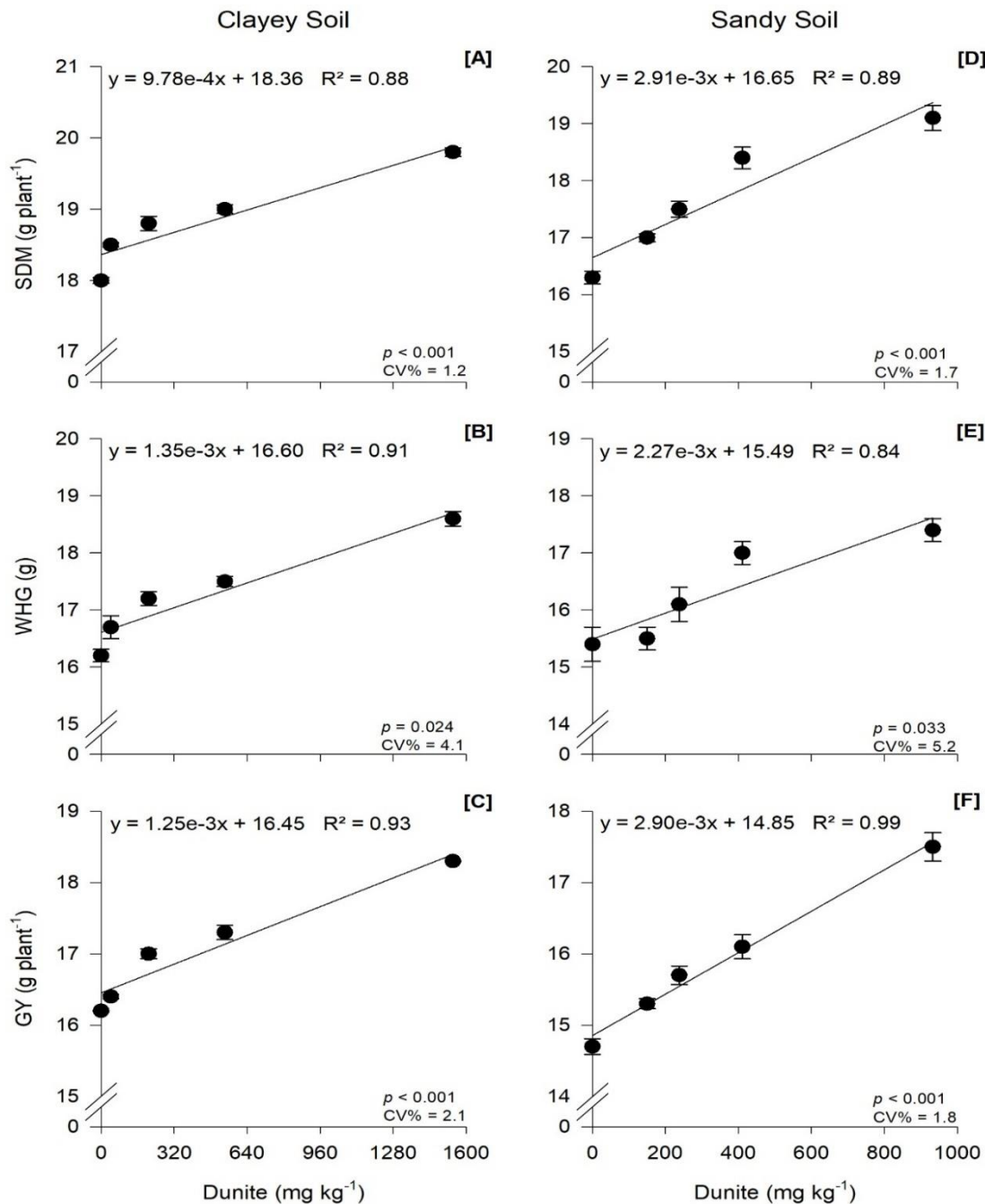


Figure 3. Shoot dry weight - SDM [A], weight of 100 grains - WHG [B] and grain yield - GY [C] in clayey soil as a function of Dunite rates. Shoot dry weight - SDM [D], weight of 100 grains - WHG [E] and grain yield - GY [F] in sandy soil as a function of Dunite rates.

The accumulation of dry weight in the shoot area, mass of 100 grains and yield of soybean grains per plant presented values of 19.9 g plant⁻¹, 18.7 g and

19.4 g, respectively (Figure 3A-C) for soybean cultivation in clayey soil and 19.4 g plant⁻¹, 17.6 g and 17.6 g (Figure 3 D-F) on sandy soil.

The metabolism of carbohydrates and their partitioning among plant organs are factors that determine the productive potential of the crops. The benefits evidenced in this work on the dynamics of the production and destination of the photosynthates provided a greater contribution of dry weight in the soybean crop, besides increasing the grain mass and, consequently, the grain yield per plant.

During the vegetative phase, the photoassimilates are converted into plant growth, especially if there is no nutritional limitation (Tränkner et al. 2018). During grain filling (reproductive period of culture), sugars from photosynthesis are directed to the reproductive organs. The number of fertilized ovules and the final grain weight are determined by the rate of transport and assimilation during this period and also by the duration of the grain filling (Yang and Zhang 2006). Both the direct translocation of assimilates and the redistribution of the reserve pool of assimilates contribute to better indices of these variables (Tränkner et al. 2018).

This way, the reduction of the translocation of carbohydrates to grains, mainly under Mg deficiency, directly affects the productive potential of the crops (Ceylan et al. 2016; Farhat et al. 2016). The Dunite, regardless of the soil in which it was used, provided adequate nutrition to the soybean crop, especially by supplying Mg, as well as Si, an element through which innumerable plant defense processes (physical barriers, improved plant architecture and antioxidant metabolism) are activated (Luyckx et al., 2017; Haynes et al. 2017).

Significant changes ($p \leq 0.05$) in soil chemical attributes were also obtained by the use of Dunite rates in clayey and sandy soils (Figure 4 A-F).

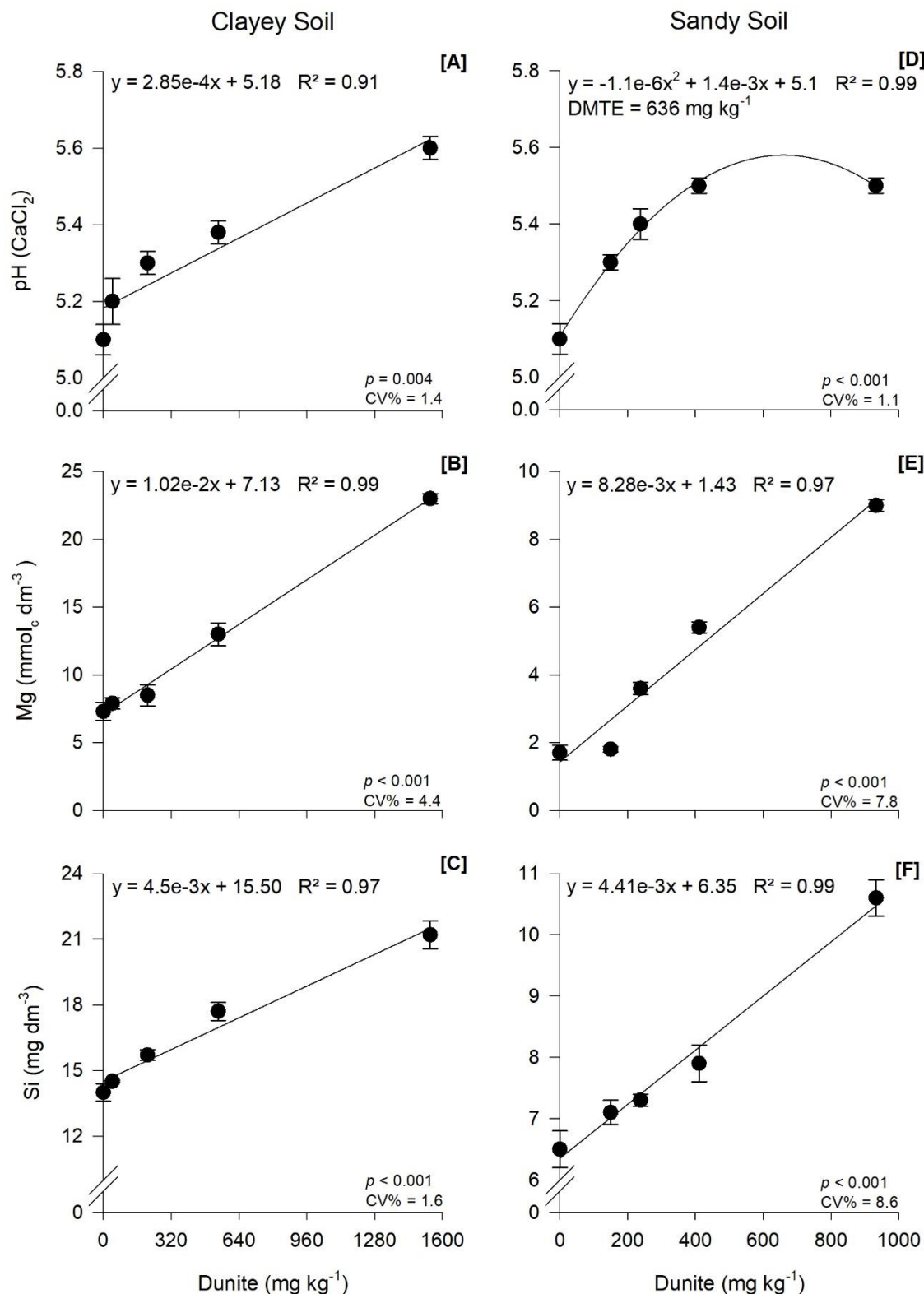


Figure 4. pH [A], Mg [B] and Si [C] contents in clayey soil after soybean cultivation under Dunite rates. pH [D], Mg [E] and Si [F] contents in sandy soil after soybean cultivation under Dunite rates.

The pH of the clayey soil increased linearly until reaching the value of 5.5 (Figure 4A), while for the sandy soil, the means obtained were adjusted to a

quadratic equation, obtaining a rate of maximum technical efficiency (DMTE) of 636 g kg^{-1} of soil and a maximum pH value of 5.5 (Figure 4D). Mg and Si contents of the soil followed the same tendency obtained for the leaf, which increased linearly with the increase of Dunite rates. The values obtained of Mg and Si were $22.9 \text{ mmol}_c \text{ dm}^{-3}$ and 22.4 mg dm^{-3} in the clayey soil (Figures 4B and C), and $9.2 \text{ mmol}_c \text{ dm}^{-3}$ and 10.5 mg dm^{-3} in the sandy soil (Figures 4E and F), respectively.

The soil texture directly influences the Dunite behavior, since in clayey soils, changes in pH values respond linearly to the increment of the Dunite rates, whereas in the sandy soil, due to the low soil buffering, the pH values are difficult to change with the increase of Dunite rates (Barbosa et al. 2008). This increase in pH values is an important characteristic of rock dust, mainly because it contains 24% of MgO and 51% of SiO_2 in its composition, which are materials with high power of neutralization of the active acidity of soil (Castro and Crusciol 2013; Crusciol et al. 2016).

The increase of pH, besides bringing benefits to the chemical attributes, it is also beneficial to the Mg and Si contents, besides increasing the cation-exchange capacity (CEC) and the base saturation, and it also has a high potential to stimulate the microbiological activity of the soil, guaranteeing the selection of microorganisms that are beneficial to the system (Stevenson and Cole 1999; Silva et al. 2012).

As for Mg and Si, clayey soil naturally presents higher amounts of these elements when compared to sandy soil, mainly due to the higher cation retention capacity in their soil colloids (Quaggio 2000). On the other hand, it should be noted that the Mg contents increased by 3.2 and 1.5 times when comparing the contents obtained in the absence of Dunite application, while the soil Si contents increased

by 6.4 and 1.7 times under the same conditions for clayey and sandy soil, respectively. This fact indicates that the soil of clayey texture, for having higher retention capacity of water, SOM and consequently higher microbiological activity (Silva et al. 2012), may have presented a greater decomposition of minerals, and besides that, the organic acids extruded by plants may also have helped in this availability of nutrients (Marschner 2012). If we consider that in the sandy soil the availability of nutrients to the system is slower when compared to the clayey soil, this fact can be considered advantageous, since it can meet the demand of the plants for a long period and reduce possible losses by leaching (Silva et al. 2012).

1.4 CONCLUSIONS

Mg and Si foliar concentrations increase with the use of Dunite, as well as the contents of these elements in the soils. The use of Dunite also promotes the correction of soil acidity (pH). In both, clayey and sandy soils, the increment of rates provides higher contents of reducing sugars and sucrose, and lower contents of leaf starch. There is a greater vegetative growth, filling and soybean grains yield with the use of rock dust in a Rhodic Hapludox with different clay levels.

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CHAPTER 2

CAN DUNITE PROMOTE PHYSIOLOGICAL CHANGES, MAGNESIUM NUTRITION AND INCREASED MAIZE GRAIN YIELD?

Abstract

Several efforts have been made in recent years to mitigate the different environmental impacts related to agricultural activities. Rock dust technology is an important soil remineralization mechanism for sustainable tropical agriculture. The objective of this study was to evaluate the effect of dunite rates on magnesium (Mg), silicon (Si), reducing sugars, sucrose and foliar starch, soil chemical attributes and corn yield [*Zea mays* L.] in two soil types. The treatments consisted of five dunite rates (0, 42, 208, 542, and 1542 mg kg⁻¹) in a clayey soil and five dunite rates (0, 150, 238, 411, and 933 mg kg⁻¹) in a sandy soil. In both crops and soils, the content of Mg, Si, leaf reducing sugars, pH, Mg and Si of the soil and productivity components presented a positive response as a function of an increase in the input dose. However, the higher Mg nutrition resulted in lower levels of sucrose and foliar starch. The better plant partitioning of metabolites led to better development, filling and yield of corn grains.

Index terms: *Zea mays*, magnesium fertilization, silicium, volcanic rock powders, natural soil fertilizers.

Resumo

Diversos esforços foram feitos nos últimos anos para mitigar os diferentes impactos ambientais relacionados às atividades agrícolas. A tecnologia de pó de rocha é um importante mecanismo de remineralização do solo para a agricultura tropical sustentável. O objetivo deste trabalho foi avaliar o efeito de doses de dunita sobre o magnésio (Mg), silício (Si), açúcares redutores, sacarose e amido foliar, atributos químicos do solo e produtividade de milho [*Zea mays* L.] em dois tipos de solo. Os tratamentos consistiram de cinco doses de dunita (0, 42, 208, 542 e 1542 mg kg⁻¹) em solo argiloso e cinco doses de dunita (0, 150, 238, 411 e 933 mg kg⁻¹) em solo arenoso. Em ambas as culturas e solos, o teor de Mg, Si, açúcares redutores de folhas, pH, Mg e Si do solo e componentes de produtividade apresentaram resposta positiva em função do aumento da dose ingerida. No entanto, a maior nutrição de Mg resultou em menores níveis de sacarose e amido foliar. A melhor partição de plantas de metabólitos levou a um melhor desenvolvimento, enchimento e rendimento de grãos de milho.

Palavras chave: *Zea mays*, fertilização com magnésio, silício, pó de rocha vulcânica, fertilizantes naturais de solo.

2.1 INTRODUCTION

Brazil is currently one of the world's largest producers of corn, with a production of approximately 97 million tons, and the second largest exporting country (CONAB 2019). The growing demand is due to its wide use in food and feed. Corn agribusiness is the sixth largest income generating activity in the national agricultural economy (CONAB 2019). Soil fertility management and crop fertilization, which determine the highest economic yields, are variable for each region, according to the chemical and granulometric properties of soils. Thus, dunite is a viable option in agriculture because this is a nutrient source for the crop and can be used as a magnesium (Mg) source for several crops (Moretti et al. 2019).

According to Ramos et al. (2015), given that Brazil is one of the largest suppliers of food in the world, study of volcanic rock dust as a potential fertilizer with respect to the content and release of nutrients and to the economic and market viability is still required to provide the development of sustainability policies for mining activity and food production. According to Assis and Dias (2007), dunite is an igneous rock, which is essentially a peridotite, magmatic or eruptive material, consisting mostly of olivine ($(\text{MgFe})_2\text{SiO}_4$, containing approximately 40% Mg oxide (MgO) and 34% Si oxide (SiO_2). The application of rock dust for remineralizing the soil is related to its mineral characteristics and its interaction with the environment in which it will be applied to improve the conditions of soil fertility (Moreira et al. 2006; Ramos et al. 2017).

The role of Mg in several vital functions in plants is well understood. A deficiency of Mg in plants may be caused by soil reserve depletion but can also be induced by low Mg assimilation by the roots due to competitive inhibition caused by an imbalance of some cations, such as calcium (Ca), potassium (K), manganese (Mn), and zinc (Zn) (Crusciol et al. 2016, Kwano et al. 2017; Canizella et al. 2018; Tränkner et al. 2018).

Reduced transport and accumulation of carbohydrates in Mg-deficient leaves cause alterations in photosynthetic carbon metabolism and restrict CO₂ fixation (Farhat et al. 2016; Kwano et al. 2017).

Magnesium is closely linked to the partitioning between the synthesis of starch, transport of triose phosphates in the cytosol and the formation of sucrose, so that low availability of Mg leads to a reduction in cytosolic glucose-fructose bonds (Ceylan et al. 2016). However, the accumulation of carbohydrates in leaves is an evident response that is part of a signaling system that still needs further studies to understand the role of Mg in photoassimilate phloem loading and the accumulation of metabolites in deficient leaves. Thus, this study aimed to evaluate the effect of fertilization with dunite on the nutritional, metabolic and grain yield of corn, as well as on the soil chemical attributes in two soils with different clay contents.

2.2 MATERIAL AND METHODS

A greenhouse experiment was carried out in the 2016-2017 cropping season at the College of Agricultural Science, Department of Crop Science, Botucatu, São Paulo State, Brazil. The soils used in the experiment were two Rhodic Hapludoxes (Santos et al. 2013) with clayey and sandy textures. The clayey soil contained 602 g kg⁻¹ clay and 281 g kg⁻¹ sand, and the sandy soil contained 259 g kg⁻¹ clay and 724 g kg⁻¹ sand. The soil chemical attributes (0.0-0.2 m depth) were determined according to van Raij et al. (2001), as demonstrated in Table 1.

Table 1. Chemical and granulometry characteristics of soils (0–0.2-m depth), Botucatu, São Paulo State, Brazil, 2016/17.

Property	Soils	
	Clayey Soil	Sandy Soil
Chemical properties		
pH (CaCl ₂)	5.2	5.4
P (resin)	6 mg dm ⁻³	3 mg dm ⁻³
S.O.M. [†]	25.2 g dm ⁻³	13.1 g dm ⁻³
Ca ²⁺	35 mmol _c dm ⁻³	18 mmol _c dm ⁻³
Mg ²⁺	8 mmol _c dm ⁻³	2 mmol _c dm ⁻³
K ⁺	0.7 mmol _c dm ⁻³	0.5 mmol _c dm ⁻³
H ⁺ +Al ³⁺	29 mmol _c dm ⁻³	18 mmol _c dm ⁻³
Al ³⁺	0 mmol _c dm ⁻³	0 mmol _c dm ⁻³
S-SO ₄ ²⁻	2.6 mg dm ⁻³	1.5 mg dm ⁻³
Fe ²⁺	20 mg dm ⁻³	35 mg dm ⁻³
Cu ²⁺	8.4 mg dm ⁻³	1.6 mg dm ⁻³
Mn ²⁺	15.3 mg dm ⁻³	1.9 mg dm ⁻³
Zn ²⁺	0.9 mg dm ⁻³	0.3 mg dm ⁻³
B ³⁺	0.4 mg dm ⁻³	0.3 mg dm ⁻³
Si ⁴⁺	13 mg dm ⁻³	7 mg dm ⁻³
CEC [‡]	73 mmol _c dm ⁻³	39 mmol _c dm ⁻³
BS [§]	60%	53%
Granulometry properties		
Clay	602 g kg ⁻¹	259 g kg ⁻¹
Sand	281 g kg ⁻¹	724 g kg ⁻¹
Silt	117 g kg ⁻¹	17 g kg ⁻¹

[†]Soil organic matter; [‡]Cation exchange capacity; [§]Base saturation

Dunite presents the following chemical attributes: 24% Mg (40% MgO) and 16% Si (34% SiO₂). The experimental design was completely randomized, with four replicates for both soils. The treatments consisted of the application of five dunite rates: 0, 42, 208, 542 and 1542 mg kg⁻¹ for clayey soil, and five dunite rates: 0, 150, 238, 411, and 933 mg kg⁻¹ for sandy soil. These application rates were based on the absorption ratio between Calcium and Magnesium (3x1) in plant nutrition. Pots with a 20-L capacity were used in both experiments.

Nutrients were supplied as 200 mg kg⁻¹ – N and P, 150 mg kg⁻¹ – K, 15.0 mg kg⁻¹ – S, 5.0 mg kg⁻¹ – Zn, 1.5 mg kg⁻¹ – B, and 0.5 mg kg⁻¹ – Cu. The soil water

content in pots was corrected to -0.01 MPa (field capacity), corresponding to 112 g kg⁻¹, respecting the equilibration period (60 to 100% field capacity). Five seeds (Dow 2B 587 PW hybrid) were sown per treatment, and three plants were left per pot after emergence. Four reference pots were weighed every four days, and as needed, distilled sterile water was added to return the water content to field capacity (-0.01 MPa).

Leaves were collected and ground in a Wiley-type mill coupled to a 1-mm sieve. Then, the Mg and Si contents were determined (Malavolta et al. 1997). The partitioning of carbohydrates as reducing sugars (glucose and fructose), sucrose and starch content, measured in the dry matter of leaves, was determined according to the methodology of Nelson (1944). The readings were performed using a spectrophotometer at 535 nm. At the same time as the foliar diagnosis, two plants were collected for dry mass analysis. The plants were grown until the end of the cycle, and the mass of 100 grains was determined by means of precision scale weighing, value correction (13% on a wet basis) and the number of grains per ear. The grain yield was expressed as g plant⁻¹. After harvest, a soil sample of each pot was collected, and the pH, Mg and Si contents were analyzed according to van Raij et al. (2001).

All data were initially tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) from the UNIVARIATE procedure of SAS version 9.3 (SAS Institute 2015), and the results indicated that all data were distributed normally ($W \geq 0.90$). The data were submitted to analysis of variance (ANOVA) and F-test ($p \leq 0.05$). A polynomial regression analysis was performed to determine dunite-rate response curves for measured corn and soil traits, where significant regression equations with the highest coefficients of determination were selected.

2.3 RESULTS AND DISCUSSION

The Mg and Si leaf contents of the corn crop increased linearly as a function of the increase in the dunite rates, regardless of soil texture. The maximal contents obtained for these nutrients in corn leaves were 3.4 g kg⁻¹ Mg and 18.3 g kg⁻¹ Si when grown in clayey soil (Figures 1A and B) and 3.9 g kg⁻¹ Mg and 15.3 g kg⁻¹ Si when grown in sandy soil (Figures 1C and D). Dunite is a mineral material that undergoes heating at temperatures close to 400°C, a process known as serpentinization. As a result, approximately 90% of the magnesium and silica minerals present are hydrated, thus increasing their solubility in the system, making them more easily absorbed by the plant root system (Fernandes et al. 2010).

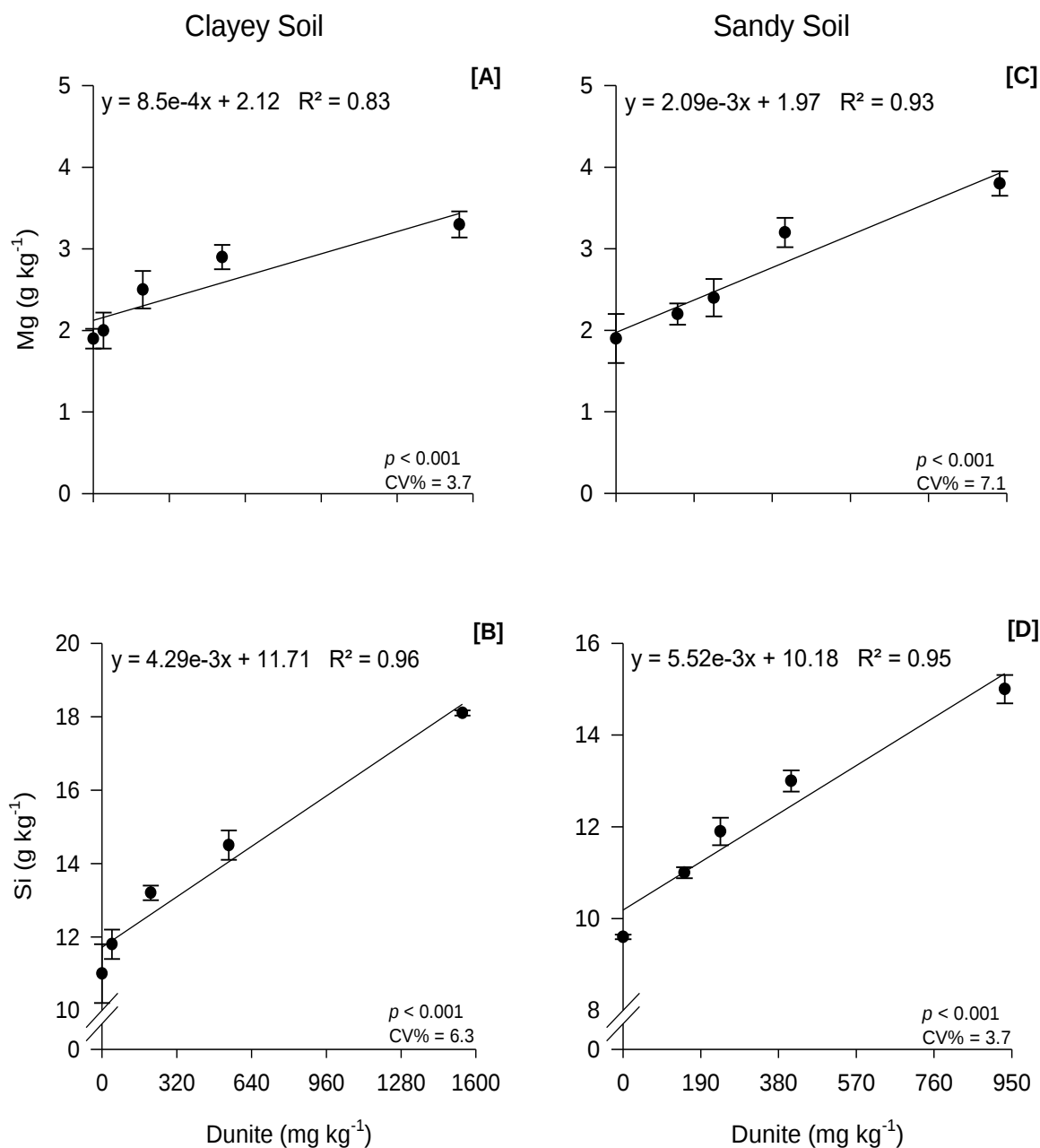


Figure 1. Mg [A] and Si [B] contents in corn leaves grown in clayey soil as a function of the dunite rate. Mg [C] and Si [D] contents in corn leaves grown in sandy soil as a function of the dunite rate in Botucatu, São Paulo State, Brazil, 2016/17.

Mg is the most abundant divalent cation in the plant cytosol. It has a fundamental role in plants and several other organisms due to its ability to interact with nucleophilic binders (Cakmak and Kirkby 2008), as well as being the central atom of the chlorophyll molecule and being involved in numerous photosynthetic processes (Ceylan et al.

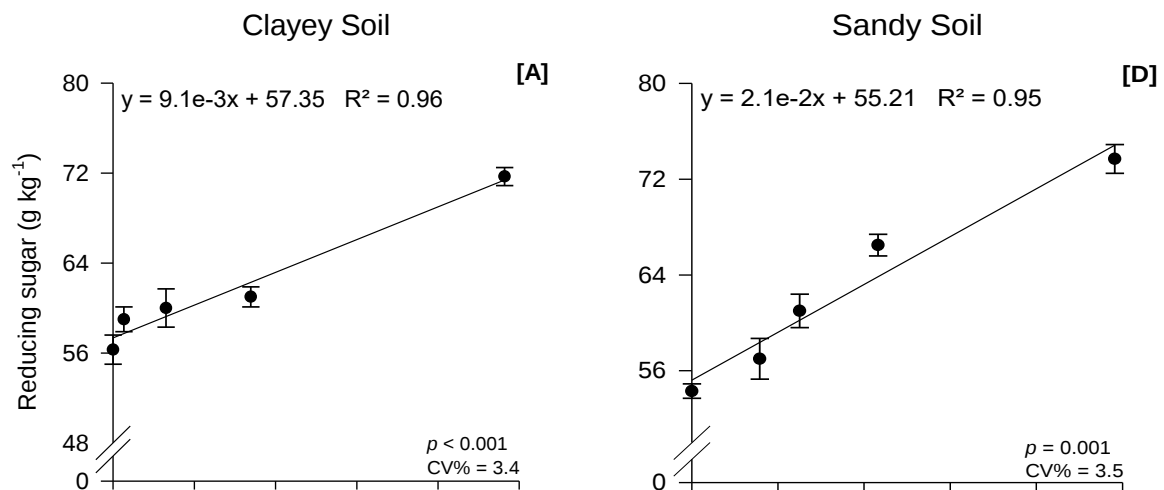
2016). It is known to be the largest enzymatic activator, of enzymes such as RNA polymerases, ATPases, protein kinases, phosphatases, glutathione synthetase and carboxylases (Tränkner et al. 2018). Small variations in the concentrations of this element in chloroplasts are sufficient to alter the activity of several important enzymes of plant metabolism (Wang et al. 2012). Considering the bottleneck of the Mg supply for agricultural systems, dunite is an important source of this element. Furthermore, due to its behavior similar to magnesium silicate, it has a high potential of supplying Si to the soil.

In general, most grass plants (e.g., corn, wheat, triticale, ryegrass, sorghum, rye and barley) are known as Si-accumulating species, although root uptake is inferior to rice (Nikolic et al. 2007). The first Si transporters were studied in rice plants (Ma et al. 2007). They are responsible for infiltration (Lsi1) and silicon efflux (Lsi2). These same carriers were also identified in barley and corn (Chiba et al. 2009, Mitani et al. 2009). In the case of corn, the ZmLsi1 transporters are located in the epidermal, hypodermic and cortical cells, and their expression levels are not influenced by the Si supply (Chiba et al. 2009).

In contrast, the efflux transporters (ZmLsi2) are more numerous in the mature root zone (> 10 mm) than in the root tips (Mitani et al. 2009). These efflux transporters are located only in the root endoderm (Ma et al. 2011). In the plant, Si is transported in the form of $\text{Si}(\text{OH})_4$ silicic acid, and its supply to these types of plants is of paramount importance because it is related to numerous processes of defense against biotic and abiotic factors (Luyckx et al. 2017). Its accumulation in the cell wall has the potential to reduce insect pest attack efficiency and to reduce plant transpiration, especially under conditions of low water availability. In addition to its physical effects, Si participates in the metabolic processes that control salicylic acid, jasmonic and

abscissic acid, which are important in the stress-fighting process of plants (Kim et al. 2014).

With the increase in dunite rates, there was a linear increase in reducing sugars and a linear decrease in sucrose and starch contents in the leaves of corn grown in clayey soil (Figure 2). The photosassililated contents at the highest dose of rock powder were 71.4 and 74.8 g kg⁻¹ for reducing sugars (Figures 2A and D), 42.5 and 42.8 g kg⁻¹ for sucrose contents (Figures 2B and E) and 43.3 and 41.9 g kg⁻¹ for starch contents (Figures 2C and F) for corn grown in clayey and sandy soil, respectively. Moretti et al. (2019) reported similar result with the addition of dunite in soybean.



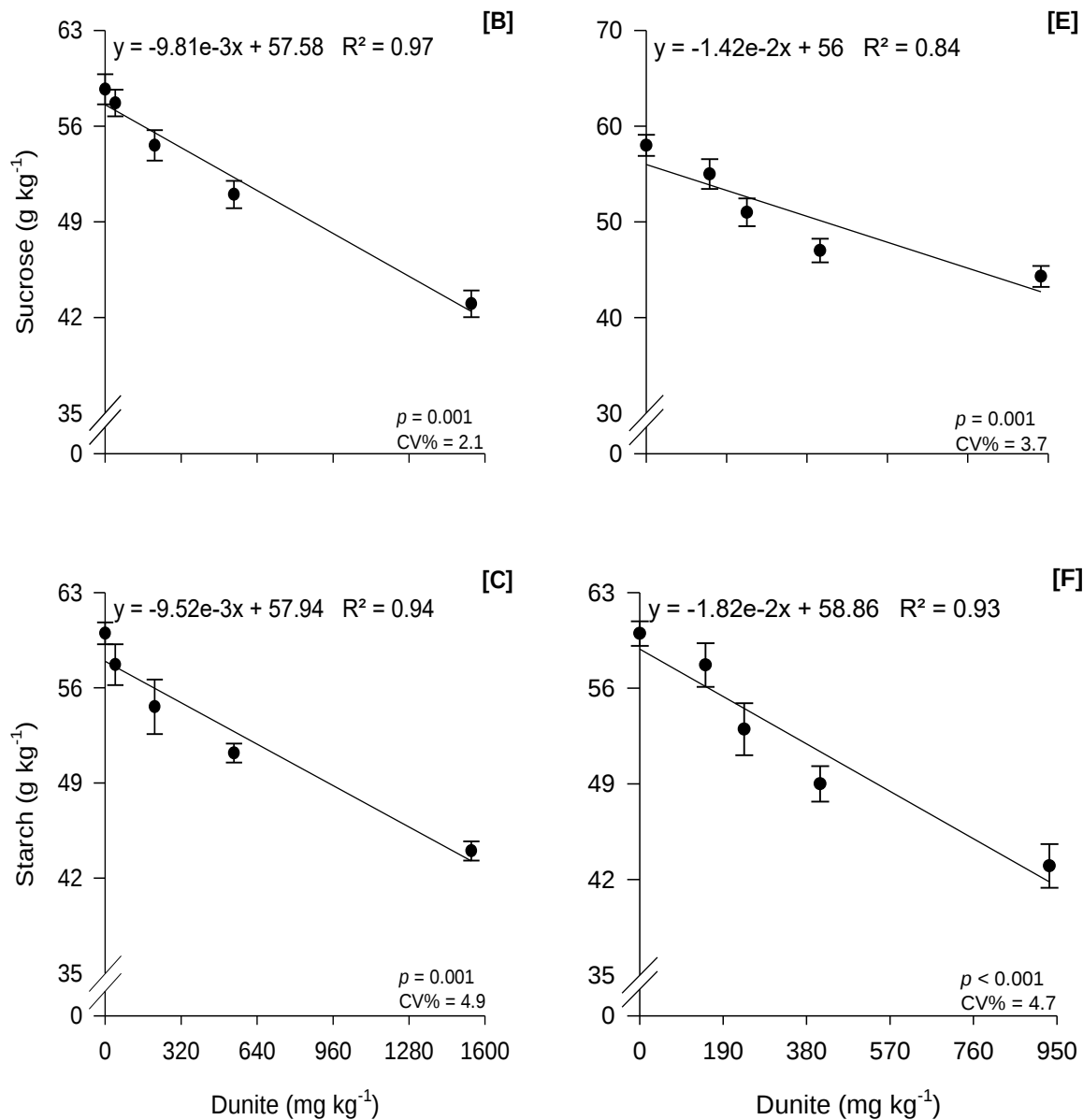


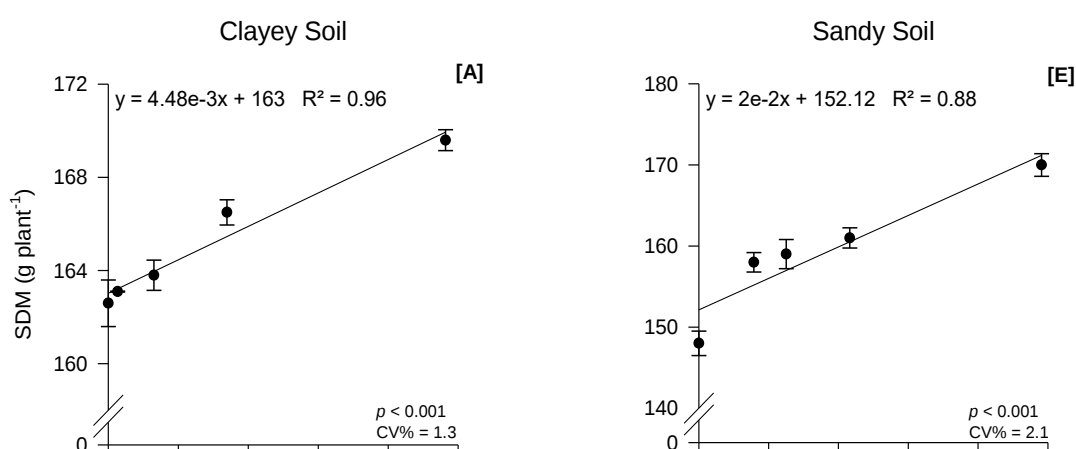
Figure 2. Reducing sugars [A], sucrose [B] and starch [C] in dry leaves of corn grown in a clayey soil as a function of the dunite rate. Reducing sugars [D], sucrose [E] and starch [F] in dry leaves of corn grown in a sandy soil as a function of the dunite rate in Botucatu, São Paulo State, Brazil, 2016/17.

The supply of Mg provided by the solubilization of rock dust strongly influences several processes that modulate the long-distance production and translocation of carbohydrates from the source organs to the plant sink organs (White and Broadley 2009, Ceylan et al. 2016). The production of reducing sugars (fructose + glucose) and

their subsequent conversion into sucrose and other more complex carbohydrates are important steps that guarantee plant development. Sucrose is the main form of transport of carbon skeletons in plants and is carried out via the phloem using a proton gradient and an H⁺/sucrose co-transporter established by a plasma membrane H⁺ - ATPase, which provides energy for the loading process and maintains sucrose transport in phloem cells (Farhat et al. 2016).

The results indicate that the corn plant under increasing doses of dunite, mainly due to the greater availability of Mg, increased the production of reducing sugars, indicating full photosynthetic activity and carbon fixation, whereas decreases in the amount of sucrose indicate that the transport of photoassimilates to the sink organs occurred more efficiently as the levels of rock dust increased. Similarly, the starch formed in the chloroplasts is consumed overnight as the carbon skeleton source for the respiration process, with sucrose as a by-product. This is then transported to the plant sinks and/or is used metabolically (Cakmak and Kirkby 2008, Ceylan et al. 2016).

The corn shoot dry matter (SDM) increased linearly as result of the increase in the dunite rates, regardless of the soil texture, reaching 170 g in the clay soil and 171 g in the sandy textured soil (Figures 3A and E).



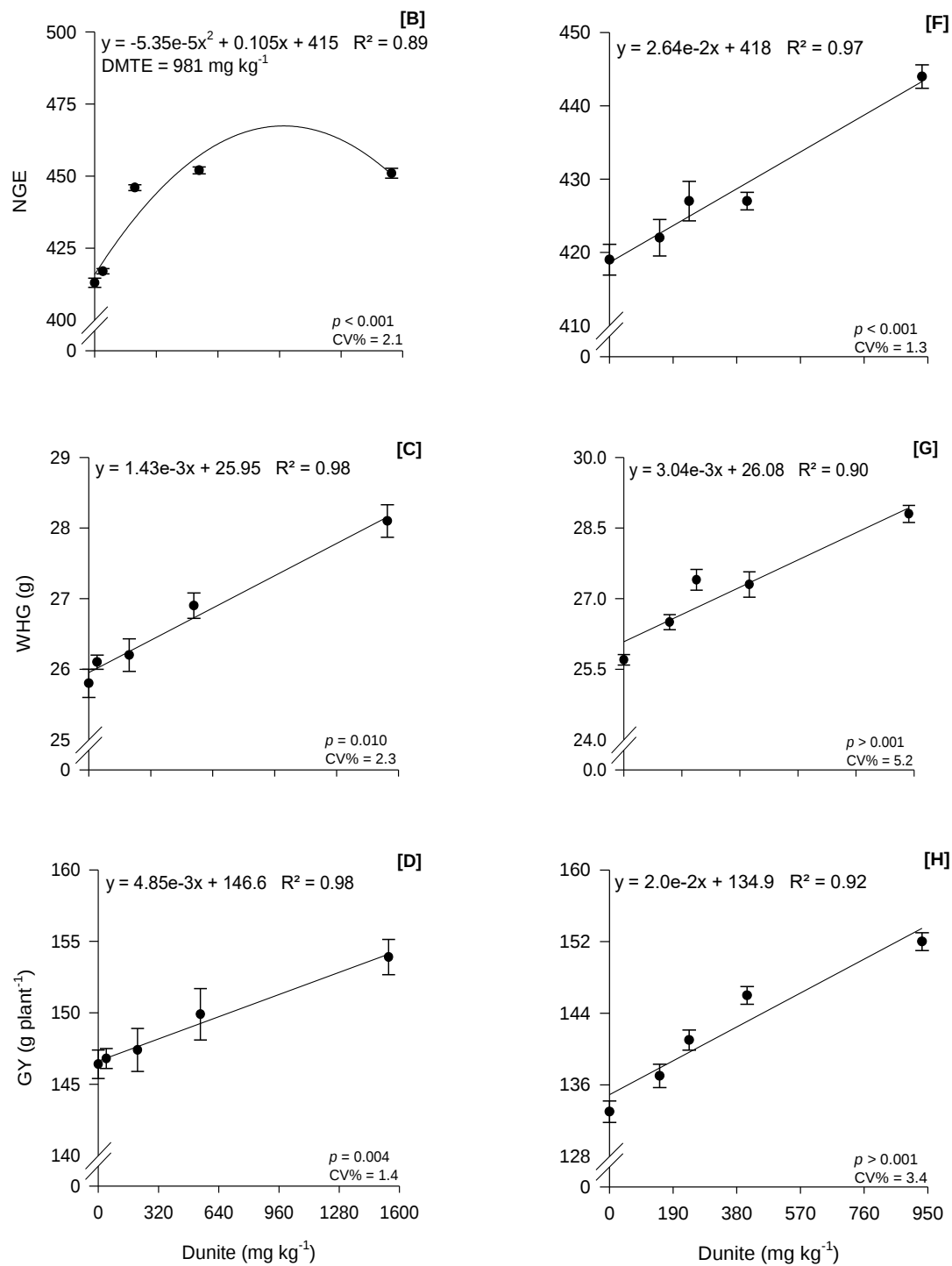


Figure 3. Shoot dry matter – SDM [A], number of grains per ear - NGE [B], weight of 100 grains – WHG [C] and grain yield – GY [D] as a function of the dunite rate in clayey soil. Shoot dry matter – SDM [E], number of grains per ear - NGE [F], weight of 100 grains – WHG [G] and grain yield – GY [H] as a function of the dunite rate in sandy soil in Botucatu, São Paulo State, Brazil, 2016/17.

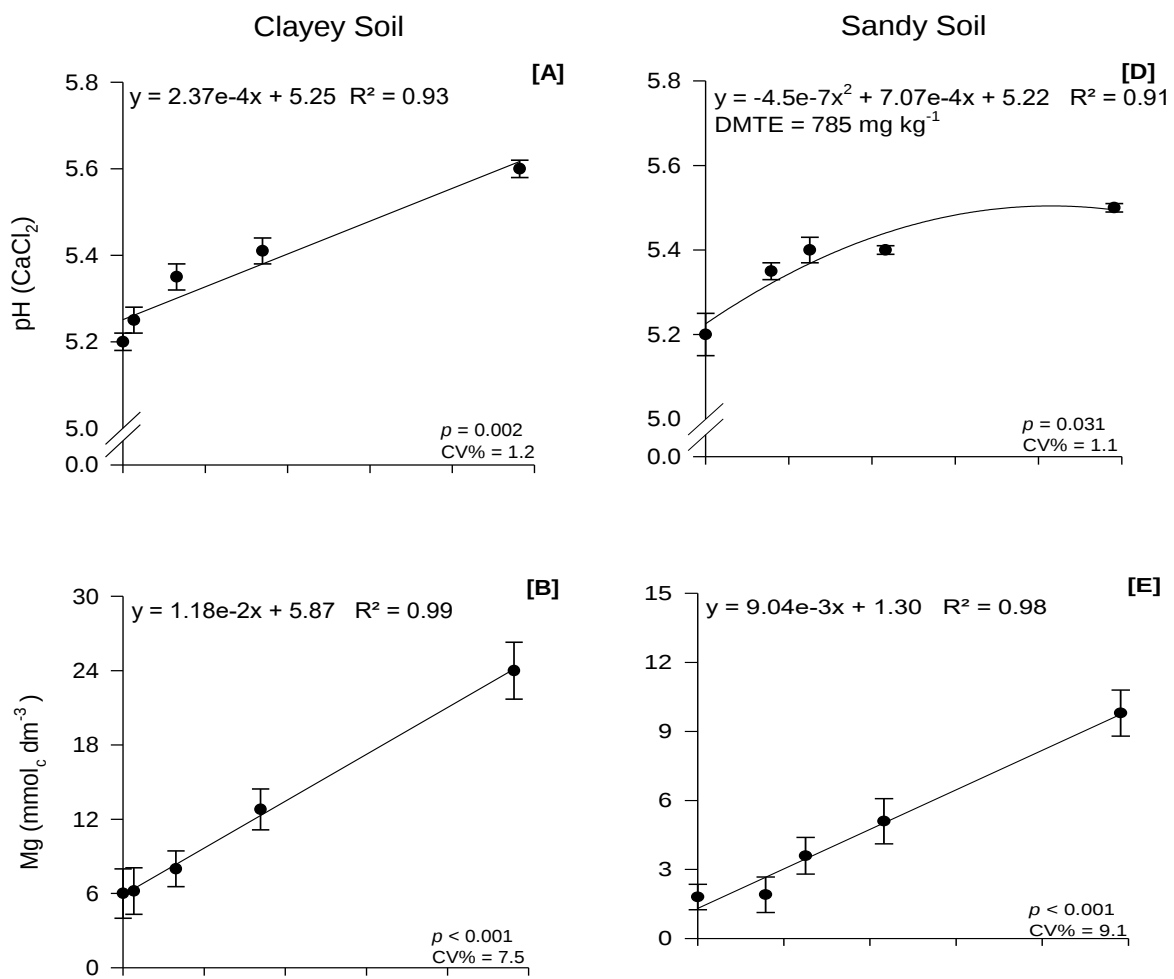
The number of grains per spike showed a quadratic behavior as a function of the increase in the dunite rate in the clayey soil, with a dose of maximum technical

efficiency (DMTE) of 981 kg ha⁻¹ of rock dust (Figure 3B) and a mean of 466 grains per ear, while under cultivation in sandy soil, the averages adjusted to a linear equation increased, with a maximum value of 442 grains per spike (Figure 3F). The mass of 100 grains and grain yield per corn plant increased linearly with increasing dunite rates, with respective values of 28.2 g and 154 g plant⁻¹ when grown in clay soil (Figures 3C and D) and 28.9 g and 153 g plant⁻¹ when grown in sandy soil (Figures 3G and H).

The biomass accumulation of corn plants presented the same trend as the nutritional and photosynthetic metabolism. Cell division and expansion depend directly on the full metabolic functioning of the plant, which is influenced by photosynthetic nutrition and performance. All processes governing photosynthesis, the activation of enzymes linked to carbon fixation, and partitioning of photoassimilates are strongly modulated by the Mg supply (Ceylan et al. 2016). In this way, energy conversion occurs in SDM accumulation and grain filling. The direct translocation of photoassimilates and the redistribution of the reserve pool to the drainage organs contribute to grain filling (Ceylan et al. 2016). The reserve tissue of cereal grains is amyloiferous; thus, the poor translocation of carbohydrates to grains implies a reduction in the final weight (Wang et al. 2012). Si may also have contributed to the results obtained. Si is a strong agent in the composition of the cell wall, providing mechanical support to the plant structures (Ma et al. 2007). Its supply guarantees better leaf architecture, favoring light interception, and contributing to greater photosynthetic activity (Haynes et al. 2017).

The application of the dunite altered some soil chemical attributes, increasing the pH of the clayey soil after corn cultivation, raising the pH to 5.6 at the highest dose of rock dust (Figure 4A), whereas when applied to sandy soil, the response was quadratic for this same attribute, with DMTE of 785 kg ha⁻¹ and a pH value of 5.5 (Figure 4B). In the same way that the Mg and Si contents increased linearly by

increasing the dose of rock dust, the content of these elements increased linearly in the soil. The respective contents of Mg and Si at the highest dunite rate were 24.1 mmol_c dm⁻³ and 21.31 mg dm⁻³ in the clayey soil (Figures 4B and C) and 9.7 mmol_c dm⁻³ and 10.3 mg dm⁻³ in the sandy soil (Figures 4E and F).



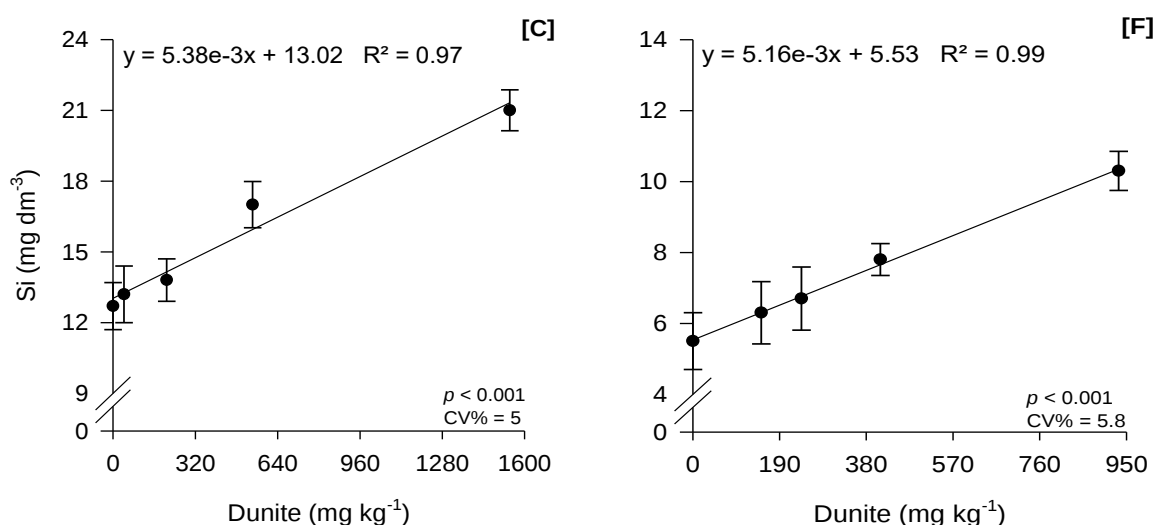


Figure 4. pH [A], Mg [B] and Si [C] contents in clayey soil after corn cultivation under different dunite rates. pH [D], Mg [E] and Si [F] contents in sandy soil after corn cultivation under different dunite rates in Botucatu, São Paulo State, Brazil, 2016/17.

Dunite is composed of magnesium silicate, a neutralizing species capable of reducing the active acidity of the soil. The rock powder consists of 34% SiO₂ and 40% MgO, both with a neutralization capacity relative to CaCO₃ of 1.0 and 2.5 times, respectively (Alcarde 2005). This material is another important source of soil acidity correction, in addition to providing Mg and Si to the system (Castro and Crusciol 2013).

The levels of these soil elements increased in the same proportion as the dunite levels used. It is of great importance to emphasize that the texture of the soil directly influences the changes in pH depending on the dose. Soil sandy soils have a relatively low buffering power (Barbosa et al. 2008), which explains the absence of responses to the higher dunite rates in this type of soil, while in the clay soil, the pH change response was linear with an increase in the dunite rates. As for the Mg and Si contents obtained in the two soil types, it is evident that the clayey soil naturally has a higher capacity of cation retention in its colloids. In contrast, the values found in the sandy soil did not limit the development of the crop, presenting data similar to those obtained

when corn was grown in the clay soil. This fact indicates that the process of serpentinization that occurs during dunite processing is highly efficient and is able to make these nutrients available in less fertile soils.

In general, it is necessary that the minerals present in the rock powder be weathered to become available in the soil and absorbed by the plants, a fact that is potentially greater in soils with a more clayey texture. More clayey soils present higher water retention capacity, soil organic matter and, consequently, higher soil microbiological activity, which are highly connected with the intemperization of soil minerals (Silva et al. 2012). Thus, sandy soils would be less prone to successful application of rock dust products. However, in the present study, the dunite was not limiting in the soil with a sandy texture and was able to supply the plants with Mg and Si.

2.4 CONCLUSIONS

Rock dust technology is a mechanism of remineralization of soils for a sustainable tropical agriculture. There was an increase in the Mg and Si contents in the plant and soil and acidity correction (pH) with the application of dunite to clayey and sandy soils. The increased rates resulted in a higher content of reducing sugars and lower levels of sucrose and foliar starch and thus better partitioning of metabolites for the corn crop. Dunite resulted in greater vegetative growth, filling and yield of corn kernels in soil with a clayey and sandy texture.

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FINAL CONSIDERATIONS

Dunite application have been being paradoxical issue since Hensel's reports. Its function as source of nutrients to plants has being questioned due to many studies unworked, but this product depends on many factors, hardly to taken all into account.

Each mineral compound has a different reaction faced to all these factors, for this reason is very difficult to get common spots among the studies.

This is the reason of the importance of study a particular a Dunite rock, mainly its interaction with plants and soil.

Since the dunite improved soil attributes and provided better grain yields in two different soil textures, then we can presume the high potential to employ dunite as conditioner and fertilizer in the soil.

Further researches can cover plant morphology, microbiological index of soil quality, rock weathering, and total chemical elements, other crops, and a field experiment.

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