



Use of limestone and agricultural gypsum in cauliflower crop management and clubroot control in mountain farming

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ABSTRACT. The effects of the dose and application method of limestone - broadcast or in furrow - and of agricultural gypsum on soil fertility, the control of clubroot, and cauliflower development in mountain farming areas were evaluated. Initially, four doses of broadcast limestone (0.0, 1.0, 2.0, and 4.0 Mg ha⁻¹) and two cauliflower cultivars (Sharon and Piracicaba Precoce) were analyzed. A second experiment evaluated limestone (4.0 Mg ha⁻¹) application treatments: broadcast and in furrow, broadcast limestone + gypsum (3.0 + 1.0 Mg ha⁻¹), and broadcast gypsum (1.0 Mg ha⁻¹). Soil fertility was improved, and significant increases were observed in the total and healthy root volume with increasing doses of limestone. With 4.0 Mg ha⁻¹, a 58 and 85% increase in yield was observed in Sharon and Piracicaba, respectively, compared to the control. Treatments with limestone and limestone + gypsum, regardless of the application method, elevated pH ($\geq 10\%$), base saturation (V%) ($\geq 37\%$), and calcium (Ca) contents ($\geq 100\%$), and reduced the levels of aluminum ions (Al³⁺) ($\geq 60\%$) and clubroot severity ($\geq 64\%$) and favored biomass accumulation ($\geq 27\%$) and yield ($\geq 9.2\%$). The application of limestone in the furrow yielded results similar to the broadcast application.

Keywords: *Brassica oleracea* var. *botrytis*; *Plasmodiophora brassicae*; liming; slope; application method.

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Introduction

The municipality of Nova Friburgo, in the state of Rio de Janeiro, is one of the largest cauliflower (*Brassica oleracea* var. *botrytis*) producers in Brazil (IBGE, 2006). Its cultivation in the region is mainly carried out by family farmers in areas with high slopes and acid soils and under intensive management. The local topography and climate, as well as the agricultural practices adopted, make this a model of mountain farming in a tropical region. Additionally, regular and intensive planting of cauliflower for more than 30 years in the region; intensive soil tillage, including plowing and harrowing along the slope; and the use of community machinery and implements have resulted in the widespread dissemination and distribution of clubroot (Bhering, Carmo, Matos, Lima, & Amaral Sobrinho, 2017), which has limited the cultivation of the species on some farms. A similar situation has been observed in several other regions of Brazil and the world for *Brassica* spp. cultivation (Dixon, 2009a).

Clubroot is caused by *Plasmodiophora brassicae* Woronin, a common soil protozoan that is an obligatory parasite specific to *Brassicaceae* species (Dixon, 2014). In the absence of host plants, *P. brassicae* survives in the soil for several years in the form of resting spores (Dixon, 2009b). The pathogen infects the root system of plants, where it causes the development of galls or tumors from cellular hyperplasia and hypertrophy. Consequently, water and nutrient absorption is reduced, which can lead to underdevelopment, reduced production potential, and even death of the plant (Dixon, 2009a). The losses are further increased when a high density of resting spores occur in the soil ($> 10^6$ units g⁻¹ of soil) (Narisawa, Shimura, Usuku, Fujuhara, & Hashiba, 2005) under favorable environmental conditions. The germination of resting spores, infection, and root colonization are favored by acidic or partially acidic soils (pH_(H₂O) < 6.2), humidity, low calcium (Ca) levels, and intermediate temperatures of 20 to 25°C (Dixon, 2009b; Donald & Porter, 2009; Dixon, 2014; Gossen, Deora, Peng, Hwang, & McDonald, 2014). The losses caused by the disease can be further increased by aluminum ion (Al³⁺) phytotoxicity (Bhering et al., 2017).

Considering the existing limitations for the chemical and genetic control of the disease (Donald & Porter, 2009; Gossen et al., 2015), management practices are the most important strategy for reducing the losses caused by the disease. Among these, soil acidity correction is the most important, followed by basic strategies for successful crop and disease management (Dixon, 2009b; Donald & Porter, 2009; Gossen et al., 2014; Bhering et al., 2017; Santos, Amaral Sobrinho, Costa, Diniz, & Carmo, 2017). Cauliflower is a demanding crop in terms of phytotechnical, phytosanitary, and soil fertility management [$\text{pH}_{(\text{H}_2\text{O})} = 6.0$ to 6.8 , $V = 80\%$, and $\text{Ca} + \text{magnesium (Mg)}$ content $\geq 3.0 \text{ cmol}_c \text{ dm}^{-3}$] (May et al., 2007; Guerra, Leal, & Ferreira, 2013) as well as the required environmental conditions, especially temperature and humidity. Cauliflower has also been reported as being intolerant to Al^{3+} , despite the lack of studies on this specific topic (May et al., 2007).

Despite the recognized importance of soil acidity correction for cauliflower nutrition and clubroot control, this strategy is often neglected (Bhering et al., 2017), possibly due to lack of knowledge or the practical difficulties experienced in steep areas. The broadcast application of limestone, followed by uniform incorporation, favors the reaction of the soil amendment (Sousa, Miranda, & Oliveira, 2007; Campos et al., 2013) and, consequently, the management of the disease (Donald & Porter, 2009). However, in steeper areas, especially in regions with high precipitation, the traditional form of incorporation - by plowing and harrowing - may favor water erosion. That is, alternative forms of application and the incorporation of amendments compatible with the conditions of tropical mountain farming and able to aid in the management of clubroot need to be investigated and validated.

Limestone, which is composed of Ca and/or magnesium carbonate, is the most used amendment for the neutralization of soil acidity, for the reduction of toxic Al^{3+} contents, and as a source of Ca^{2+} and/or Mg^{2+} for plants (Sousa et al., 2007, Campos et al., 2013). The efficiency of this technique for soil acidity correction and clubroot control, however, depends on characteristics such as the total relative neutralizing power (TRNP) (Campos et al., 2013), neutralizing value, particle size and distribution, soil quantity, moisture and texture, and variations in the period between application and planting (Donald & Porter, 2009).

Agricultural gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), in turn, has also been recommended to improve soil chemical conditions for root development (Sousa et al., 2007). As this salt is more water soluble than limestone, under certain conditions, it can mobilize Ca^{2+} and Al^{3+} to the subsoil through formation of an ion pair with sulfate (Sousa et al., 2007), where it reduces the toxic effect of Al^{3+} on the roots and reduces the subsurface Ca^{2+} deficiency (Lopes & Guilherme, 2007). These two effects could favor the growth of roots in deeper soil layers and, consequently, increase the efficiency of water and nutrient use (Sousa et al., 2007) and reduce root losses from the disease and the negative impacts of these losses on production. However, the effect of gypsum on cauliflower and clubroot management likely is more attributable to the supply of Ca^{2+} than to the pH correction/elevation (Donald & Porter, 2009).

Therefore, two field experiments were carried out on the cauliflower crop. In the first experiment, the objective was to determine the best limestone dose based on the improvements in the soil conditions, clubroot control, and development of the cauliflower plants. In the second experiment, the objective was to evaluate the efficiency of the application method of limestone - broadcast or in furrow - and the use of agricultural gypsum on soil fertility, disease control, and cauliflower development.

Material and methods

Two experiments were carried out under field conditions in an area of family farms, in the municipality of Nova Friburgo, state of Rio de Janeiro, Brazil ($22^\circ 19' 45''\text{S}$ – $22^\circ 23' 45''\text{S}$ and $42^\circ 35' 05''\text{W}$ – $42^\circ 40' 10''\text{W}$). The studied mountain farming areas have been cultivated with cauliflower for more than 30 years and exhibited clubroot occurrence.

Prior to treatment application and soil preparation, composite soil samples (exploratory analysis) were collected from the two study areas for fertility analysis at a depth of 0–20 cm in the first and 0–20 and 20–40 cm in the second. The soil was analyzed according to Donagema, Campos, Calderano, Teixeira, and Viana (2011); no physical soil analyses were performed. Weather data for the respective periods were obtained from the Instituto Nacional de Meteorologia (INMET, 2017).

First experiment

The first experiment was conducted from September 2014 to March 2015 following the last planting of *Brassica* spp. in the area one year prior. The soil analysis of the area showed the following values: $\text{pH}_{(\text{H}_2\text{O})} =$

5.29, Al = 0.30 cmol_c dm⁻³, hydrogen (H) + Al = 9.65 cmol_c dm⁻³, Ca = 5.45 cmol_c dm⁻³, Mg = 1.20 cmol_c dm⁻³, potassium (K) = 0.34 cmol_c dm⁻³, cation exchange capacity (CEC) = 16.71 cmol_c dm⁻³, C = 2.15%, base saturation (V%) = 42.5%, and phosphorus (P) = 144.5 mg L⁻¹.

Three doses of partially calcined limestone (TRNP 104.5%) (1.0, 2.0, and 4.0 Mg ha⁻¹), the control (no limestone), and two cauliflower cultivars [Sharon^H (Sakata) and Piracicaba Precoce (TopSeed)] were evaluated. Based on the neutralization curve, the limestone dose required to achieve a pH of 6.5, which been recommended for cauliflower (May et al., 2007) and clubroot management (Dixon, 2009b), was determined. To obtain the curve, soil samples from the experimental area (200 g) were incubated with increasing doses of limestone. The pH_(H₂O) was measured at 48-hour intervals until stabilization was reached 30 days after the start of the incubation. Using the obtained data, the dose was estimated [pH = 5.28 + 0.302 (dose), R² = 0.96], and the amount of limestone required to reach pH 6.5 was calculated to be 4.0 Mg ha⁻¹. The other doses were obtained by sequentially dividing of this dose.

The respective limestone doses were applied by broadcasting followed by incorporation with a rotary hoe at a depth of 20 cm. After 70 days, the soil samples of the respective plots were collected at 0 - 20 m depth for fertility analysis (Donagema et al., 2011) and for counts of the number of *P. brassicae* spores (Bhering et al., 2017). In December, 34-day-old seedlings of the respective cultivars were transplanted into 20 × 20 × 15 cm furrows, spaced 60 × 60 cm apart. The furrows were previously fertilized with ammonium sulfate (35 kg ha⁻¹ of N), single superphosphate (100 kg ha⁻¹ of P₂O₅), and potassium chloride (40 kg ha⁻¹ of K₂O). The amount of nutrients to be applied was defined based on soil fertility analysis and the crop recommendations prescribed in the “Manual de Calagem e Adubação para o Estado do Rio de Janeiro” (Freire et al., 2013; Guerra et al., 2013).

A randomized complete block design was used in a 4 × 2 factorial arrangement, with four replications, totaling 32 plots of 16 m² and 64 plants.

Then, standard crop management practices for the region, which comprise two weeding rounds, sprinkler irrigation, and three top-dressing fertilizations, were applied. In the first and third fertilizations, at 20 and 60 days after transplanting (DAT), ammonium sulfate (85 kg ha⁻¹ of N) and potassium chloride (75 kg ha⁻¹ of K₂O) were applied as top-dressing. In the second fertilization, approximately 120 g of poultry litter were applied at 40 DAT, and the plants were sprayed with boric acid solution (2 g L⁻¹).

Throughout the cycle, until the beginning of the harvest, all dead, underdeveloped, withered, and nonflowering plants were counted, as were the normal plants, which had fully expanded leaves and well-formed inflorescences. From 53 to 86 DAT, the inflorescences were harvested at commercial maturity, i.e., when well developed and with flower buds still attached (May et al., 2007). In each plot, the number of plants with normal or at-commercial-maturity inflorescences (NI) was counted, and ten plants were sampled for analysis. Initially, the shoot was harvested from each plant by cutting it close to the base of the stem. The roots were then harvested with the aid of a spade to maintain maximum integrity and washed in running water.

The leaf (LFW), stem (SFW), inflorescence (IFW), and shoot (ShFW) fresh weights were determined by their sum, and the inflorescence longitudinal diameter (ID) was also calculated. The IFW and NI data per plot were used to estimate the yield (Mg ha⁻¹). Root development (volume and fresh weight) and clubroot severity were also quantified. The clubroot severity was estimated with the aid of a scale composed of seven scores (0%, 8%, 20%, 42%, 68%, 87%, and 95% of roots with galls) (Santos et al., 2017). The volumes of the healthy roots (HRV) and diseased roots (DRV) were calculated based on the water displacement method, and the total volume (TRV) was calculated by the sum of the HRV and the DRV (Bhering et al., 2017). The fresh weights of the healthy roots (HRFW), diseased roots (DRFW), and total roots (TRFW) were also determined. The percentages of the diseased roots were calculated using the root volume and the fresh weight data, and these are expressed as volume (PDRV = DRV.TRV⁻¹.100) and fresh weight (PDRFW = DRFW.TRFW⁻¹.100).

Second experiment

The second experiment was conducted from June to December 2015 following the last planting of *Brassica* spp. in the area five years prior. The soil analysis of the area showed the following values at a depth of 0 - 20 cm: pH_(H₂O) = 4.58, Al = 1.00 cmol_c dm⁻³, H + Al = 9.08 cmol_c dm⁻³, Ca = 1.60 cmol_c dm⁻³, Mg = 1.90 cmol_c dm⁻³, K = 0.82 cmol_c dm⁻³, CEC = 13.47 cmol_c dm⁻³, C = 2.06%, V% = 37.27%, and P = 68.64 mg L⁻¹. The values at a depth of 20 - 40 cm were the following: pH_(H₂O) = 4.87, Al = 1.00 cmol_c dm⁻³, H + Al = 10.23 cmol_c

dm^{-3} , $\text{Ca} = 1.10 \text{ cmol}_c \text{ dm}^{-3}$, $\text{Mg} = 2.50 \text{ cmol}_c \text{ dm}^{-3}$, $\text{K} = 0.73 \text{ cmol}_c \text{ dm}^{-3}$, $\text{CEC} = 14.63 \text{ cmol}_c \text{ dm}^{-3}$, $\text{C} = 0.98\%$, $\text{V}\% = 38.21\%$, and $\text{P} = 65.68 \text{ mg L}^{-1}$.

This second experiment was designed based on the results from the first experiment and the difficulty of incorporating large amounts of limestone under the local conditions, i.e., areas with steep slopes. The objective was to test methods of corrective application that are more practical and require less soil movement. We defined 4.0 Mg ha^{-1} as the maximum amount of product (limestone or limestone + gypsum) to be incorporated. The gypsum was added as a treatment to test its possible effect of improving the chemical conditions in the subsurface soil layer (Sousa et al., 2007) and increasing cauliflower root development that would compensate for root losses due to *P. brassicae* infection (Bhering et al., 2017). The gypsum dose was defined based on the soil characteristics in the subsurface layer (clay, CEC, V% and Al^{+3}) and the criteria established by Sousa, Lobato, and Rein (2005).

Five treatments were evaluated, consisting of the combination of two limestone application methods and the use or not of gypsum: 1) limestone applied by broadcast, 4.0 Mg ha^{-1} ; 2) limestone applied in furrows, 4.0 Mg ha^{-1} ; 3) limestone and gypsum, applied by broadcasting, 3.0 and 1.0 Mg ha^{-1} , respectively; 4) gypsum applied by broadcasting, 1.0 Mg ha^{-1} ; and 5) control. Calcined limestone with 104.5% TRNP was used. The limestone and/or gypsum were applied by broadcasting, with the aid of a rotary hoe, at 15-cm depth. In furrows, limestone was applied in a radius of approximately 10 to 15 cm and incorporated to a depth of 15 cm using a hoe.

At 83 days after application of the treatments, composite soil samples were collected, at depths of 0 - 20 cm and 20 - 40 cm, for analysis of the fertility and for a count the number of resting spores of *P. brassicae* (0 - 20 cm) (Bhering et al., 2017). The composite soil samples from each plot were obtained by combining six simple samples. In the in-furrow application treatment, the samples were collected on the edge of the furrows (with a radius of approximately 15 cm).

In September, 90 days after treatment application, 30-day-old seedlings of the Barcelona^H (Seminis) cauliflower cultivar were transplanted spaced $60 \times 60 \text{ cm}$ apart. At the time of transplanting, the furrows were fertilized as described in the previous experiment.

The standard crop management practices were applied, which consisted of two weeding rounds, sprinkler irrigation and three top-dressing fertilizations at 20, 57, and 85 DAT. At 20 and 85 DAT, fertilization was performed as described in the previous experiment. At 57 DAT, 1.4 Mg ha^{-1} of organic compound [15% of organic C, 1% of nitrogen (N)] were applied, and the plants were sprayed with boric acid solution (2 g L^{-1}).

A randomized block design with five treatments and four replications was used, totaling 20 plots of 13.5 m^2 and 40 plants each. The 12 central plants were used for data collection.

The plants, shoot, and roots were harvested at 100 and 106 DAT, and the evaluations were performed according to the methodology described for the previous experiment. The evaluations determined disease severity (Santos et al., 2017) and root development by measuring the volume and weight of the healthy and diseased roots [HRV, DRV, TRV, HRFW, DRFW, TRFW, PDRV, PDRFW, and total root dry weight (TRDW)] and IFW and ID. Yield (Mg ha^{-1}) was estimated based on the IFW. In addition, leaf, stem, and inflorescence samples were dried, and the respective dry weights (LDW, SDW, and IDW) and the total plant dry weight (TPDW) were determined by summing the dry weights of the different parts.

Statistical analysis

Data from the first experiment were subjected to analysis of variance (ANOVA) and when significant, linear regression analysis ($p < 0.05$) as a function of the dose. Data from the second experiment were subjected to ANOVA and, when significant, to Tukey's test ($p < 0.05$) for a comparison of the means. SISVAR software was used for the analyses (Ferreira, 2011).

Results and discussion

Effect of limestone doses

Frequent and well-distributed rainfall was recorded in the period between the application of the soil amendment and transplanting (314.6 mm) and during the crop cycle (458.8 mm), with 120 mm falling in the first 10 DAT (INMET, 2017). The average temperature during the crop cycle was 15 to 20°C and was slightly higher (17 to 22°C) during the first 10 DAT (INMET, 2017). These conditions, especially in the first 10 DAT, and the high density of *P. brassicae* spores—above $10^8 \text{ spores g soil}^{-1}$ —were favorable to clubroot infection

and development (Narisawa et al., 2005; Gossen et al., 2013; Gossen et al., 2014). The first symptoms of the disease were observed at approximately 30 DAT—plants withered in the hottest hours of the day and were underdeveloped—in all plots. The diagnosis was confirmed by the observation of root galls.

A significant effect ($p < 0.05$) of the limestone doses was observed on the Ca, Mg, P, and K contents; on the sum of the bases (SB) and V%; and on the attributes related to soil acidity, namely, the pH, H + Al, and Al^{3+} content and saturation in the soil. A linear increase was observed for the Ca and Mg contents in the soil with an increasing limestone dose ($\text{Ca cmol}_c \text{ dm}^{-3} = 4.08 + 1.22x$; $R^2 = 0.91$) and ($\text{Mg cmol}_c \text{ dm}^{-3} = -0.10x^2 + 0.46x + 0.76$; $R^2 = 0.76$). However, even in the control treatment, the Ca + Mg contents were high ($4.07 \text{ cmol}_c \text{ dm}^{-3}$) and approached the level recommended for the crop: $3.0 \text{ cmol}_c \text{ dm}^{-3}$ (Guerra et al., 2013). With increasing limestone doses, a similar increase occurred in the pH, with a maximum of 6.7 (Figure 1A), and in V%, which reached a maximum value of 68.1% at the dose of 4.0 g ha^{-1} (Figure 1B); this value is less than that recommended for the crop, which is 80% (May et al., 2007). Furthermore, a linear reduction of potential acidity (Figure 1C) was also observed. Doses of 2.0 and 4.0 g ha^{-1} of limestone resulted in adjustment of the soil pH to values favorable to the cauliflower crop - higher than 6.0 (May et al., 2007) - and unfavorable to clubroot (Figure 1A). The minimum pH recommended in the management of clubroot is 5.7 (Webster & Dixon, 1991; Donald & Porter, 2009; Dixon, 2014; Gossen et al., 2014).

The Al^{3+} content and saturation were significantly reduced from $0.20 \text{ cmol}_c \text{ dm}^{-3}$ to $0.0 \text{ cmol}_c \text{ dm}^{-3}$ and from 5.7% to 0.0%, respectively, with the addition of 1.0 Mg ha^{-1} of limestone due to the pH values being elevated above 5.5 (Figure 1A), as described by Sousa et al. (2007). The original levels of Al^{3+} in the experimental area were below $0.3 \text{ cmol}_c \text{ dm}^{-3}$, the amount considered critical for most crops (Freire et al., 2013), and were neutralized with the addition of the lowest dose of limestone to the soil: 1.0 Mg ha^{-1} . The improvement in the chemical conditions of the soil provided an increase in the availability of P, with a linear increase in assimilable P as a function of the limestone doses (Figure 1D).

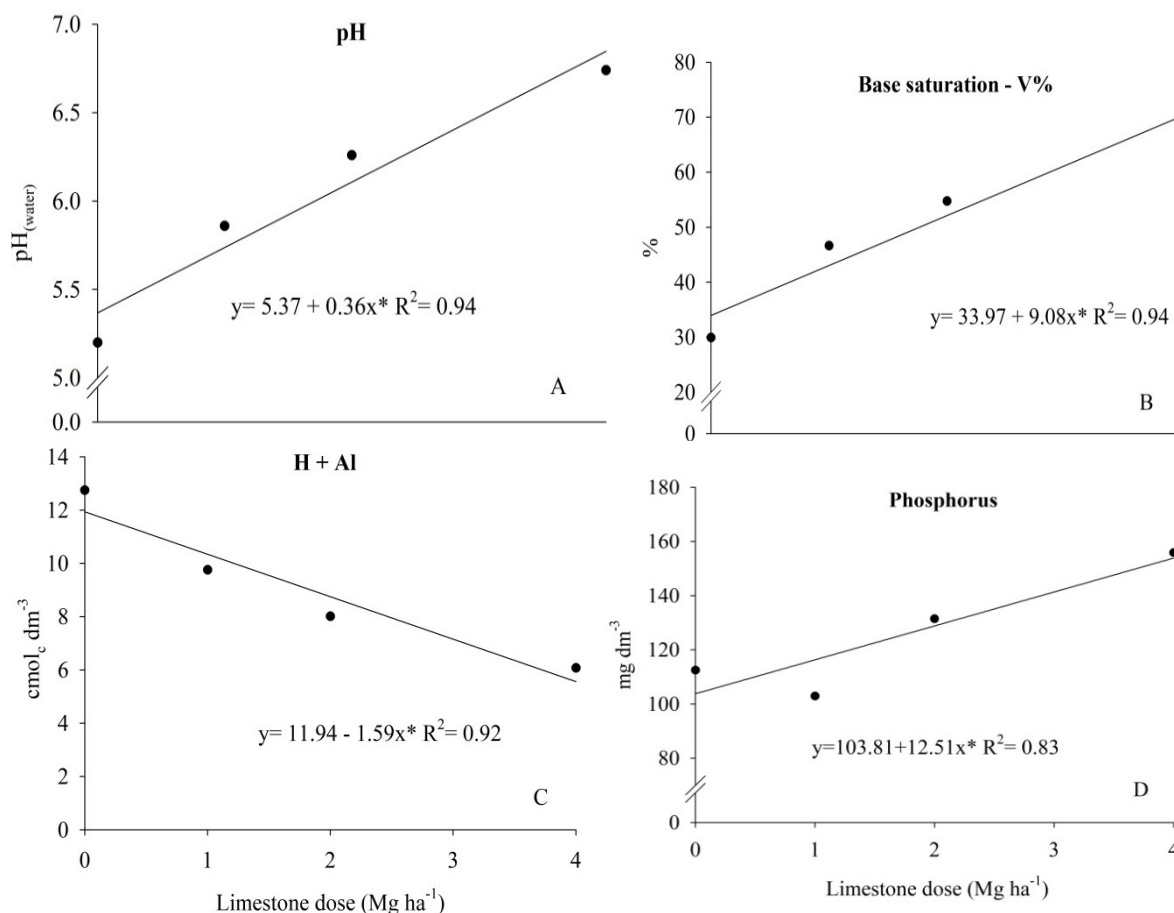


Figure 1. Values of $\text{pH}_{(\text{H}_2\text{O})}$ (A), base saturation (V%) (B), potential acidity (H + Al) (C), and phosphorus content (D) as a function of the limestone doses—partially calcined limestone with TRNP of 104.5%—70 days after application and incorporation into the soil. Nova Friburgo, Rio Janeiro State, Brazil, 2015. * indicates significance ($p < 0.05$).

The improvement of soil chemical conditions, namely, the elevation of pH, reduction of potential acidity, increases in V% and P availability (Figure 1A, B, C, and D), and the neutralization of Al^{3+} , favored root development with a linear increase observed in TRFW, TRV, and HRV of the roots (Figure 2A and B). However, a significant interaction ($p < 0.05$) was observed between the limestone dose and cultivar on the TRFW, with a stronger response from Sharon than from Piracicaba Precoce (Figure 2A), and a simple effect of dose and cultivar ($p < 0.05$) on the volume, with higher mean volume of total and healthy roots in Sharon (79.84 and 74.37 mL, respectively) compared to Piracicaba Precoce (58.38 and 53.93 mL, respectively). However, no significant effect of dose or cultivar was observed on DRV, HRFW, DRFW, and, consequently, the severity of clubroot according to the scale or percentage of diseased root calculated based on the volume (PVRH) or the fresh weight (PMFRH). That is, the effect of limestone doses on the cauliflower production and on disease management is due to the favorable root development and the greater weight and volume of the healthy roots, which could compensate for the loss of active roots due to infection.

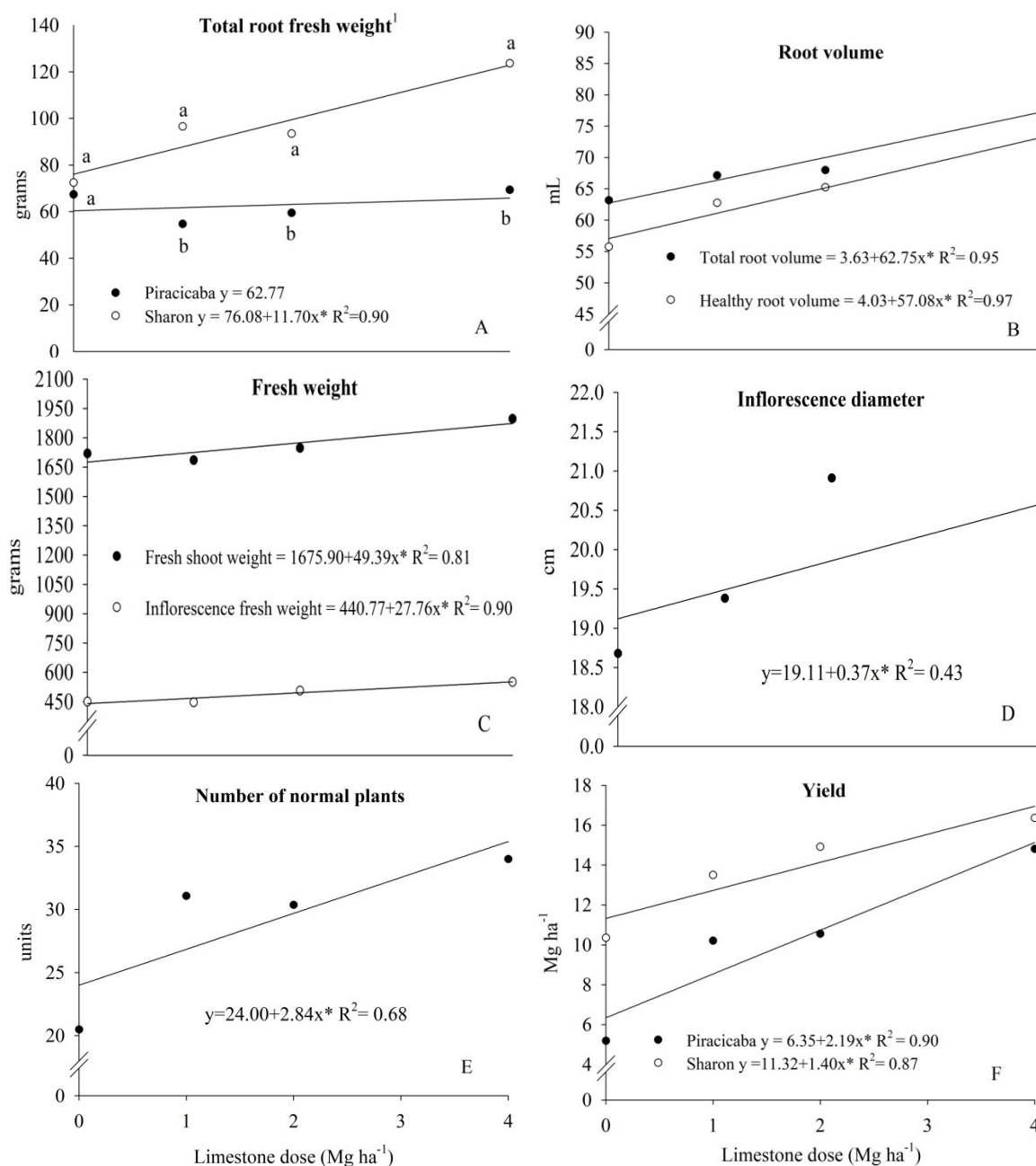


Figure 2. Mean values of total fresh root weight (A), total and healthy root volume (B), shoot and inflorescence fresh weight (C), inflorescence longitudinal diameter (D), number of normal plants (E), and estimated yield (F) of cauliflower as a function of doses of partially calcined limestone with TRNP = 104.5% applied to the soil. Nova Friburgo, Rio Janeiro State, Brazil, 2015. *indicates significance ($p < 0.05$). ¹In Figure 2A, the means of each cultivar followed by the same letter at each dose did not differ significantly from each other by Tukey's test ($p < 0.05$). In Figure 2B, C, D, and E, the data represent the means of the two cultivars.

A positive relation between the increase in pH and neutralization of Al^{3+} with root development, with a consequent reduction of losses from clubroot, was observed by Bhering et al. (2017) in a survey conducted in approximately 16 cauliflower production areas in Nova Friburgo, Rio de Janeiro State, Brazil. The positive effect of increasing doses of limestone on cauliflower (Figure 2) may also be characterized by an increase in the percentage of normal and apparently healthy plants (fully expanded leaves and inflorescences) recorded at the beginning of the harvest, regardless of the cultivar. Approximately 32.0% of the plants with normal inflorescences were identified in the control treatment, without liming, and 48.5, 47.4, and 53.1% were identified in the treatments with 1.0, 2.0, and 4.0 g ha^{-1} of limestone, respectively. Most of the dead or underdeveloped plants had galls and an underdeveloped root system (Figure 2E).

The improvement of soil fertility (Figure 1) and root development (Figure 2A and B) with increasing limestone doses significantly favored shoot development and consequently showed a linear increase in IFW and ID (Figure 2C and D) regardless of the cultivar. The Sharon cultivar was characterized by a significantly ($p < 0.05$) higher accumulation of LFW, SFW, and IFW (1,270.4, 277.6, and 509.2 g, respectively) compared to Piracicaba Precoce (850.9, 151.3, and 469.46 g). The gain in mass and diameter resulted in a gain in quality, as longitudinal diameter is an important attribute in the commercial classification of cauliflower (May et al., 2007). The increases in inflorescence weight and diameter and in the number of plants with normal inflorescences resulted in a linear and significant increase in yield (Figure 2F), with increases of approximately 10.36 to 16.37 Mg ha^{-1} in Sharon and of 5.19 to 14.81 Mg ha^{-1} in Piracicaba at a dose of 4.0 Mg ha^{-1} of limestone compared to the control. These values represent a gain of approximately 58.01 and 85.35% in the cultivars, respectively.

Effect of the application of limestone and the addition of gypsum

A significant effect ($p < 0.05$) of the treatment was observed on the $\text{pH}_{(\text{H}_2\text{O})}$, Ca^{2+} , K^+ , and Al^{3+} , the potential acidity ($\text{H} + \text{Al}$), SB, aluminum saturation (m), and V% at a soil depth of 0 - 20 cm and on the $\text{pH}_{(\text{H}_2\text{O})}$, Ca^{2+} , Al^{3+} , SB, CEC, aluminum saturation (m), and V% at a depth of 20 - 40 cm (Table 1).

The three treatments with limestone, applied in the furrow, by broadcasting, and by broadcasting combined with gypsum, significantly increased $\text{pH}_{(\text{H}_2\text{O})}$ from 5.02 to 5.47, 5.66, and 5.40, respectively, in the 0 - 20 cm layer. The dose applied, however, was not enough to reach the ideal pH recommended for cauliflower, which is 6.0 to 6.8 (May et al., 2007) (Table 1). The lower efficiency of the limestone treatment in this trial compared to the first, even using the same dose, was probably due to the lower initial pH value and the meteorological conditions. During the period from the application of limestone in June to the collection of soil samples in September, dry and cold days predominated. In this experiment, only 90 mm of rainfall were recorded during the limestone reaction period, but in the previous experiment, approximately 315 mm of rainfall were recorded from the application of limestone in September until the collection of the soil samples in December. Compared to the control, a significant increase occurred in the V%, from 22.39 to 39.82, 46.80, and 40.69%, and a significant reduction occurred in the Al^{3+} content from 0.92 $\text{cmol}_c \text{ dm}^{-3}$ of soil to 0.32, 0.12, and 0.36 $\text{cmol}_c \text{ dm}^{-3}$ in the treatments with limestone applied in the furrow, by broadcasting, and by broadcasting combined with gypsum, respectively. The values obtained were below the recommended values for the crop: $V = 80\%$ (May et al., 2007; Guerra et al., 2013) and Al^{3+} content $< 0.30 \text{ cmol}_c \text{ dm}^{-3}$ (Freire et al., 2013).

Despite being elevated with the addition of limestone, the $\text{pH}_{(\text{H}_2\text{O})}$ remained below 5.15 in the 20 - 40 cm layer, with a significant difference only observed between the broadcast limestone application and gypsum treatments. At this depth, the Al^{3+} content decreased from 1.02 to 0.63, 0.53, and 0.66 in the treatments with limestone applied in the furrow, by broadcasting, and by broadcasting in combination with gypsum, respectively, compared to the control (Table 1). However, these levels remained very high for the crop (Freire et al., 2013). Gypsum application did not significantly reduce the Al^{3+} content compared to the control in the soil layers at both depths, and only the broadcast limestone treatment significantly reduced the potential acidity ($\text{H} + \text{Al}$) in the 0 - 20-cm layer and the Al^{3+} content in the 20 - 40-cm layer (Table 1). Although the three limestone treatments significantly increased the Ca^{2+} content and SB, the original contents aligned with the recommended range for the crop (Guerra et al., 2013).

Table 1. Effects of limestone, the application method, and agricultural gypsum on soil fertility at depths of 0 - 20 cm and 20 - 40 cm, evaluated at 90 days after treatment application. Nova Friburgo, Rio Janeiro State, Brazil, 2015.

Treatments	Depth: 0 - 20 cm												
	pH (_{H₂O})	Ca	Mg	Al ¹	H + Al	Na	SB	CEC	OM	P	K	m ¹	V
	cmol _c dm ³				g dm ³		mg dm ³				%		
Control	5.02 b	2.07 b	0.37 a	0.92 a	10.35 a	0.035 a	3.06 b	13.41 a	43.85 a	34.50 a	226.00 a	26.5 a	22.39 b
Limestone in furrow	5.47 a	4.68 a	0.37 a	0.32 bc	8.31 ab	0.037 a	5.57 a	13.89 a	44.84 a	36.12 a	187.00 b	8.00 b	39.82 a
Broadcast limestone	5.66 a	5.47 a	0.30 a	0.12 c	7.17 b	0.043 a	6.34 a	13.51 a	44.04 a	36.25 a	205.37 ab	2.51 b	46.80 a
Limestone + gypsum	5.40 ab	5.00 a	0.36 a	0.36 bc	8.75 ab	0.038 a	5.85 a	14.60 a	46.67 a	38.12 a	178.25 b	7.69 b	40.69 a
Gypsum	5.03 b	2.86 b	0.37 a	0.86 ab	10.22 a	0.038 a	3.81 b	14.04 a	44.84 a	40.87 a	212.25 ab	20.68 a	27.13 b
CV (%)	4.89	25.64	25.13	12.50	15.91	25.78	21.73	9.37	10.80	24.17	12.63	39.32	20.11
Treatments	Depth: 20 - 40 cm												
	pH (_{H₂O})	Ca	Mg	Al ¹	H + Al	Na	SB	CEC	OM	P	K	m ¹	V
	cmol _c dm ³				g dm ³		mg dm ³				%		
Control	4.93 ab	1.67 b	0.28 a	1.02 a	10.73 a	0.023 a	2.26 b	13.00 b	40.47 a	32.37 a	109.62 a	36.35 a	17.05 b
Limestone in furrow	5.15 ab	2.90 ab	0.30 a	0.63 ab	10.60 a	0.023 a	3.46 ab	14.06 ab	41.84 a	32.75 a	94.37 a	18.65 ab	24.56 ab
Broadcast limestone	5.26 a	3.23 a	0.28 a	0.53 b	9.95 a	0.025 a	3.83 ab	13.78 ab	40.92 a	33.75 a	112.17 a	14.78 b	27.43 ab
Limestone + gypsum	5.08 ab	3.50 a	0.36 a	0.66 ab	10.7 a	0.033 a	4.15 a	14.85 a	43.83 a	33.75 a	102.37 a	15.82 b	28.26 a
Gypsum	4.91 b	2.21 ab	0.35 a	1.06 a	11.53 a	0.031 a	2.88 ab	14.42 ab	43.29 a	37.37 a	113.12 a	31.06 ab	19.69 ab
CV (%)	4.62	36.82	40.13	11.59	10.50	33.24	33.82	7.87	10.32	36.85	35.62	34.19	30.92

^{*}Means followed by the same letter in a column did not differ significantly from each other by Tukey's test ($p < 0.05$). ¹- $\sqrt{x+1}$ transformed data. pH: in water; Ca: exchangeable calcium; Mg: exchangeable magnesium; Al: exchangeable aluminum; H + Al: potential acidity; Na: exchangeable sodium; SB: sum of bases; CEC: cation exchange capacity at pH = 7.0; OM: organic matter; P: assimilable phosphorus; K: exchangeable potassium; m: aluminum saturation; V: base saturation.

Applying gypsum alone promoted a slight increase in the Ca²⁺ content and in V% at a depth of 20 - 40 cm, but the mean values did not differ significantly from the mean values of the control. The application of limestone combined with gypsum significantly increased the CEC in the subsurface layer (20 - 40 cm) (Table 1), probably due to the dissolution of the gypsum and the mobilization of the Ca²⁺ to the lower soil layers (Sousa et al., 2007). In general, the treatments did not affect the organic matter (OM) and the Mg, P, and K contents, except in the treatments with broadcast limestone, applied alone or in combination with gypsum. Compared to the control, these treatments promoted a reduction in the K content in the 0 - 20-cm layer (Table 1).

Although the treatments did not raise the soil pH to levels considered adequate for the management of clubroot (pH_{H₂O} > 6.2) and crop production (pH_{H₂O} 6.0 to 6.8), they promoted a reduction in the severity of the disease, which was estimated based on the scale and PDRFW and increased the accumulation of TPDW and yield. However, they did not affect the volume and the fresh weight of the healthy, total, and diseased roots or PDRV. The severity of the disease, estimated with aid of the scale, was lower in all treatments compared to the control, being significantly lower in the treatment with broadcast limestone combined with gypsum (0.65%) compared to the control (9.49%). This result is due to the significant reduction in the percentage of diseased root fresh weight observed in the treatments with limestone by broadcasting, by broadcasting combined with gypsum, and with gypsum (Table 2).

Table 2. Effect of limestone and its application method and of the use of gypsum on the severity of clubroot, percentage of diseased root weight fresh (PDRFW), total plant dry weight (TPDW), inflorescence fresh weight (IFW), and estimated yield in Barcelona cauliflower plant cultivars, in an experiment conducted from September to December 2015. Nova Friburgo, Rio Janeiro State, Brazil.

Treatments	Severity (%) ¹	PDRFW (%) ¹	TPDW (g)	IFW (g)	Yield (Mg ha ⁻¹)
Control	9.49 a	7.07 a	61.85 b	326.02 b	9.03 b
Limestone in furrow	3.38 ab	2.22 ab	87.92 a	440.97 a	12.24 a
Broadcast limestone	2.26 ab	1.07 b	85.47 a	427.32 a	11.86 a
Limestone + gypsum	0.65 b	0.56 b	78.54 ab	355.02 ab	9.86 ab
Gypsum	2.28 ab	1.26 b	83.73 ab	380.95 ab	10.58 ab
CV (%)	39.3	33.75	13.04	13.73	13.73

^{*}Means followed by the same letter in a column did not differ significantly from each other by Tukey's test ($p < 0.05$). ¹- $\sqrt{x+1}$ transformed data.

In general, although the conditions were favorable to the disease, with intermediate temperatures of 15°C to 20°C, high precipitation (rainfall > 400 mm), and acidic soil ($\text{pH}_{\text{H}_2\text{O}} < 5.7$), the clubroot severity was low compared to that recorded in the previous experiment and to reports by Bhering et al. (2017) and Santos et al. (2017). This low severity may have made a comparison of the effects of the treatments on the disease control difficult. The low intensity of the disease is probably due to the lower spore density of *P. brassicae* in the soil (1.90×10^7 to $2.33 \times 10^7 \text{ g}^{-1}$ soil units) compared to the first experiment (above 10^8 soil g^{-1}), its low viability due to a long period (more than 5 years) without the cultivation of *Brassica* spp. in the area, and adverse weather conditions, i.e., the long dry period in the initial phase of the crop cycle. This spore density is considered intermediate (Narisawa et al., 2005; Ruaro, Lima Neto, & Motta, 2010) and is related to an intermediate to high probability of the occurrence of clubroot in Chinese cabbage (*Brassica rapa* var. *pekinensis*). However, the viability of resting spores of *P. brassicae* decrease over time without cultivation of *Brassica* spp., and a minimum interval of 2 years between cultivation of host species is recommended as an important strategy for disease management (Peng et al., 2015). The last cultivation of *Brassica* spp. in the study area occurred approximately five years prior, while the duration was only one year in the first experiment.

In general, however, the mild improvement of the soil chemical conditions, such as the increases in the $\text{pH}_{(\text{H}_2\text{O})}$, Ca^{2+} , and V%; the decrease in the Al^{3+} and H + Al (Table 1); and the reduction in the disease severity provided a significant increase in the accumulation of TPDW and IFW and, consequently, the yield (Table 2). The higher accumulation of TPDW, IFW, and yield were obtained in the treatments with limestone applied in the furrow and by broadcasting but were not significantly different from treatments with limestone combined with gypsum and only gypsum (Table 2). These last two treatments, which contained gypsum, did not differ significantly from the control (Table 2). No significant effect was observed for the treatments on the dry weight of the different organs of the plant, only on their sum.

Despite the higher yield in the treatments with limestone applied in the furrow and by broadcasting, 12.24 and 11.86 Mg ha^{-1} , respectively, this result was lower than the highest yield observed in the first experiment (16.37 Mg ha^{-1}). Additionally, the yield was lower than that reported by Morais Junior, Cardoso, Leão, and Peixoto (2012) (26 to 34 Mg ha^{-1}) and by Santos et al. (2017) (11.57 to 16.99 Mg ha^{-1}) and higher than that reported by Torres et al. (2015) (5.3 to 8.2 Mg ha^{-1}), all in summer crops and with different cultivars. This low yield is probably due to the adverse climatic conditions along the crop cycle: high but poorly distributed rainfall, totaling 404 mm; with 260 mm concentrated in the last 30 days and accompanied by a rising temperature that peaked at 32°C (INMET, 2017); and with inadequate soil fertility conditions, as discussed above. These conditions affected the commercial quality of the inflorescences, contributing to lower diameter and fresh weight and consequently to a low yield. The period of high rainfall also affected the harvesting and evaluation processes and probably the accuracy of the evaluation of the effect of the treatments on the development of the plants.

Because cauliflower has a low tolerance to Al^{3+} (May et al., 2007), the high levels of Al^{3+} in the soil (Table 1) and the consequent chemical impedance to root development (Sousa et al., 2007) also negatively affected the plant development and production. Although no specific studies exist on the effect of the different soil levels of Al^{3+} on the cauliflower plants, this toxic element negatively affects cauliflower production and potentiates the damages caused by clubroot due to the reduction of the active root system (Bhering et al., 2017). In the present experiment, only the treatment with limestone applied by broadcasting satisfactorily reduced the Al^{3+} contents in the soil surface layer to values below $0.3 \text{ cmol}_c \text{ dm}^{-3}$ ($0.12 \text{ cmol}_c \text{ dm}^{-3}$), identified as the critical or high threshold value (Freire et al., 2013) although treatment with limestone in the furrow yielded a value ($0.32 \text{ cmol}_c \text{ dm}^{-3}$) close to that of the threshold. In the 0 - 40 cm layer, the contents of the Al^{3+} were greater than $0.53 \text{ cmol}_c \text{ dm}^{-3}$, that is, very high (Table 1).

Under the experimental conditions adopted in this study, although the pH value was below that recommended for the crop (May et al., 2007), the application of limestone, even when incorporated only in the planting furrow, led to a reduction in the soil acidity and improvement of its fertility and allowed gains, such as a reduction in the clubroot severity and greater biomass accumulation of the plant and the inflorescences.

In the conditions of the region under study, with a predominance of acidic soils, high levels of toxic Al^{3+} , and the wide distribution of *P. brassicae*, liming is an essential practice for improved crop performance, since the crop requires high soil fertility (May et al., 2007), as well as for the reduction of losses from clubroot, favored by acid soils (Donald & Porter, 2009; Dixon, 2009b; Gossen et al., 2014). However, this is a neglected practice due to the lack of information and difficulties imposed by the climate and terrain as well

as the high rainfall and steep slopes (greater than 21%) and altitude (above 1300 m). Under these conditions, the application of limestone using the traditionally recommended method, broadcasting followed by incorporation with disc harrow or rotary hoe, is prohibitive, either due to operational difficulty or because it worsens erosion problems. Due to the operational ease, most of the time, the soil preparation and the planting furrows follow the direction of the slope. The methods of acidity correction most compatible with this terrain, climate, and management characteristics need to be tested. In this way, the application in furrows and adequate incorporation can represent a viable alternative. However, adjustments such as the use of amendments with greater reactivity in the soil, such as quicklime or hydrated lime (Sousa et al., 2007), and better dose and uniformity in the incorporation in the furrows should be considered. In addition, the reaction period of the amendments to the soil is important to obtain satisfactory results (Sousa et al., 2007).

Conclusion

Under the experimental conditions, liming, particularly a dose of 4.0 Mg ha⁻¹, provided improvements in the soil chemical conditions for the crop and reduced the occurrence of clubroot.

Correction of the soil acidity favors cauliflower plant and root growth and reduces root loss due to *P. brassicae* infection.

Different cultivars may respond differently to soil acidity correction.

Both cultivars responded positively to the application of limestone, but Sharon proved to be more productive and more tolerant of acidity.

The application of limestone, whether by broadcasting or in furrow, provides better soil chemical conditions as well as higher yield and biomass accumulation in the plants.

The application of limestone in the furrow, followed by incorporation with a hoe, can be recommended for the cauliflower crop in steep areas that do not support soil tillage.

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References

- Bhering, A. S., Carmo, M. G. F., Matos, T. S., Lima, E. S. A., & Amaral Sobrinho, N. M. B. (2017). Soil factors related to the severity of Clubroot in Rio de Janeiro, Brazil. *Plant Disease*, 101(8), 1345-1353. DOI: 10.1094/PDIS-07-16-1024-SR
- Campos, D. V. B., Freire, L. R., Zonta, E., Eira, P. A., Duque, F. F., De-Polli, H., ... Anjos, L. H. C. A. (2013). Adubos e corretivos. In L. R. Freire (Ed.), *Manual de calagem e adubação do Estado do Rio de Janeiro* (p. 107-128). Seropédica, RJ: Editora Universidade Rural.
- Dixon, G. E. (2014). Clubroot (*Plasmodiophora brassicae* Woronin) – an agricultural and biological challenge worldwide. *Canadian Journal of Plant Pathology*, 36(1), 5-18. DOI: 10.1080/07060661.2013.875487
- Dixon, G. R. (2009a). The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. *Journal of Plant Growth Regulation*, 28(3), 194-202. DOI: 10.1007/s00344-009-9090-y
- Dixon, G. R. (2009b). *Plasmodiophora brassicae* in its environment. *Journal of Plant Growth Regulation*, 28(3), 212-228. DOI: 10.1007/s00344-009-9098-3
- Donagema, G. K., Campos, D. B., Calderano, S. B., Teixeira, W. G., & Viana, J. M. (2011). *Manual de métodos de análise de solo*. Rio de Janeiro, RJ: Embrapa Solos.
- Donald, C., & Porter, I. (2009). Integrated control of clubroot. *Journal Plant of Growth Regulation*, 28(3), 289-303. DOI: 10.1007/s00344-009-9094-7
- Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, 35(6), 1039-104. DOI: 10.1590/S1413-70542011000600001

- Freire, L. R., Campos, D. V. B., Anjos, L. H. C., Zonta, E., Pereira, M.G., Bloise, R. M., ... Eira, P. A. (2013). Análise química de amostras de terra. In L. R. Freire (Ed.), *Manual de calagem e adubação do Estado do Rio de Janeiro* (p. 107-128). Seropédica, RJ: Editora Universidade Rural.
- Gossen, B. D., Deora, A., Peng, G., Hwang, S. E., & McDonald, M. R. (2014). Effect of environmental parameters on clubroot development and the risk of pathogen spread. *Canadian Journal of Plant Pathology*, *36*(1), 37-48. DOI: 10.1080/07060661.2013.859635
- Gossen, B. D., Kasinathan, H., Cao, T., Manolii, V. P., Strelkov, S. E., Hwang, S. F., & McDonald, M. R. (2013). Interaction of pH and temperature affect infection and symptom development of *Plasmodiophora brassicae* in canola. *Canadian Journal of Plant Pathology*, *35*(3), 294-303, 2013. DOI: 10.1080/07060661.2013.804882
- Gossen, B. D., Strelkov, S. E., Manolii, V. P., Rennie, D. C., Cao, T., Hwang, S. F., ... & McDonald, M. R. (2015). Spread of *Plasmodiophora brassicae* on canola in Canada, 2003–2014: Old pathogen, new home. *Canadian Journal of Plant Pathology*, *37*(4), 403-413. DOI: 10.1080/07060661.2015.1105871
- Guerra, J. G. M., Leal, M. A. A., & Ferreira, M. B. C. (2013). Recomendações de adubos, corretivos e de manejo da matéria orgânica para as principais culturas do Estado do Rio de Janeiro: Brócolos, couve, couve-flor e repolho. In Freire L. R. (Ed.), *Manual de calagem e adubação do Estado do Rio de Janeiro* (p. 107-128). Seropédica, RJ: Editora Universidade Rural.
- Instituto Brasileiro de Geografia e Estatística [IBGE]. (2006). *Censo agropecuário*. Retrieved on Jan. 25, 2018 from <https://sidra.ibge.gov.br/Tabela/818#resultado>
- Instituto Nacional de Meteorologia [INMET]. (2017). *Estações automáticas*. 2017. Retrieved on Jan. 5, 2017 from <http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas>
- Lopes, A. S., & Guilherme, L. R. G. (2007). Fertilidade do solo e produtividade agrícola. In R. F. Novais, V. H. Alvarez, N. F. Barros, R. L. F. Fontes, R. B. Cantarutti, & J. C. L. Neves (Eds.), *Fertilidade do solo* (p. 2-64). Viçosa, MG: Sociedade Brasileira de Ciência do solo.
- May, A., Tivelli, S., Vargas, P., Samra, A. G., Sacconi, L. V., & Pinheiro, M. Q. (2007). *A cultura da couve-flor*. Campinas, SP: Instituto Agrônômico.
- Morais Junior, P. O., Cardoso, A. F., Leão, E. F., & Peixoto, N. (2012). Desempenho de cultivares de couve-flor de verão em Ipameri. *Ciência Rural*, *42*(11), 1923-1928. DOI: 10.1590/S0103-84782012005000085
- Narisawa, K., Shimura, M., Usuku, F., Fujuhara, S., & Hashiba, T. (2005). Effects of pathogen density, soil moisture, and soil pH on biological control of clubroot in Chinese cabbage by *Heteroconium chaetospira*. *Plant Disease*, *89*(3), 285-290. DOI: 10.1094/PD-89-0285
- Peng, G., Pageau, D., Strelkov, S. E., Gossen, B. D., Hwang, S. F., & Lahlali, R. (2015). A > 2-year crop rotation reduces resting spores of *Plasmodiophora brassicae* in soil and the impact of clubroot on canola. *European Journal of Agronomy*, *70*, 78-84. DOI: 10.1016/j.eja.2015.07.007
- Ruaro, L., Lima Neto, V.C., & Motta, A.C.V. (2010). Efeito do pH do solo em diferentes níveis de inóculo no controle de *Plasmodiophora brassicae*. *Summa Phytopathologica*, *36*(1), 16-20. DOI: 10.1590/S0100-54052010000100002
- Santos, C. A.; Amaral Sobrinho, N. M. B., Costa, E. S. P., Diniz, C. S., & Carmo, M. G. F. (2017). Liming and biofungicide for the control of clubroot in cauliflower. *Pesquisa Agropecuária Tropical*, *47*(3), 303-311. DOI: 10.1590/1983-40632016v4746936
- Sousa, D. M. G., Lobato, E. & Rein, T. A. (2005). *Uso de gesso agrícola nos solos de cerrado* (Circular Técnica, 32). Planaltina, DF: Embrapa-Cerrados.
- Sousa, D. M. G., Miranda, L. N., & Oliveira, S. A. (2007). Acidez do solo e sua correção. In R. F. Novais, V. H. Alvarez, N. F. Barros, R. L. F. Fontes, R. B. Cantarutti, & J. C. L. Neves (Eds.), *Fertilidade do solo* (p. 205-274). Viçosa, MG: Sociedade Brasileira de Ciência do Solo.
- Torres, J. L. R., Araújo, A. S., Barreto, A. C., Silva Neto, O. F., Silva, V. R., & Vieira, D. M. S. (2015). Desenvolvimento e produtividade de couve-flor e repolho influenciados por tipos de cobertura do solo. *Horticultura Brasileira*, *33*(4), 510-514. DOI: 10.1590/S0102-053620150000400017
- Webster, M. A., & Dixon, G. R. (1991). Calcium, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae*. *Mycological Research*, *95*(1), 64-73. DOI: 10.1016/S0953-7562(09)81362-2