Special Report

Soil Factors Related to the Severity of Clubroot in Rio de Janeiro, Brazil

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Abstract

Soil pH and calcium levels are determining factors in the success or failure of managing clubroot during the cultivation of *Brassica* spp. The aim of the present study was to evaluate the influence of soil attributes in tropical regions on the development of roots and clubroot and the accumulation of biomass and nutrients in cauliflower. One hundred and fifty-one samples of soil and plants were collected from 16 family farms that have a history of more than 50 years of regular cauliflower cultivation in Nova Friburgo, Rio de Janeiro, Brazil. Chemical and physical analyses were performed on the soil samples, and the severity of clubroot and the accumulation of biomass and

macronutrients in individual plants and plant tissues. Clustering and main principal component analyses were performed on the data. The disease occurred on all farms, but with different intensities. A direct relationship was observed for the soil attributes (acidity and exchangeable aluminum content in particular) with the percentage of roots with clubroot and with the accumulation of biomass and macronutrients in the different plant organs. To reduce losses from clubroot in weathered soils, practices should aim to reduce the pathogen's inoculum potential and improve the physical and chemical conditions of the soil, which would favor root development of the plants.

Clubroot disease is caused by *Plasmodiophora brassicae* (Wor.), and occurs widely in humid areas with routine cultivation of *Brassica* spp., whether in temperate or tropical high-altitude areas (Dixon 2009a). In Brazil, its occurrence has been recorded in the states of Rio Grande do Sul and São Paulo (Dixon 2009a; Ruaro et al. 2009), and it is widely observed in the mountainous region of the state of Rio de Janeiro (Grisel and Assis 2012).

The incidence and severity of clubroot worsens with increased frequency and intensification of brassica vegetable cultivation, such as cauliflower (*Brassica oleracea* L. var. *botrytis* L.), bok choy (*B. pekinensis* L.), broccoli (*B. oleracea* L. var. *italica* Plenck), and canola (*B. napus* L.) (Dixon 2009a; Gossen et al. 2014). The life cycle of *P. brassicae* is complex and can be divided into two phases: i) germination of the resting spores under stimulation from root exudates of host species, release of the primary zoospores, and subsequent infection of the root hairs; and ii) colonization and proliferation of the pathogen in the cortex of the roots and subsequent formation of root galls (Niwa et al. 2008). As the disease progresses, there is a reduction in the effective root system of the plant and, consequently, a reduction in the water and nutrient absorption process, which leads to the appearance of secondary symptoms, such as wilting during the hottest hours of the day, stunting, and reduced biomass production and commercial quality of the vegetables (Gossen et al. 2013). With the decay and decomposition of the infected roots comes the release of resting spores that survive in the soil for long periods, even in the absence of a host (Wallenhammar 1996).

The severity of disease and subsequent losses are related to the pathogen population (e.g., inoculum density and distribution), host plant (level of susceptibility and life cycle), and to physical (texture, humidity, and temperature), chemical (pH and levels of Ca, N, and B), and biological (macro- and microflora) factors of the soil (Dixon 2009b; Donald and Porter 2009; Gossen et al. 2014; Sharma et al. 2011). The losses caused by clubroot in susceptible crops are

increased by sequential planting of host species, frequent soil turning, movement of machinery and implements, and regular irrigation, which increase pathogen survival and dissemination and subsequent plant infection. These losses, however, are difficult to quantify and may vary according to plant species, the timing of initial infection, and the age of the plant (Dixon 2009b; Gossen et al. 2014). Disease quantification is complex and is done using descriptive (Wallenhammar et al. 2000) or numeric scales (Takahashi et al. 2005).

There is consensus in the literature that pH and Ca levels in the soil are determining factors in the management of clubroot because they affect the longevity and germination of resting spores and the infection and colonization of the root hairs (Dixon 2014). The effects of pH and Ca on clubroot are independent and complementary, but the effect of pH dominates because pH also regulates Ca solubility in the soil (Dixon 2009b; Niwa et al. 2007). Increasing the pH to values equal to or greater than 7.2 is recommended for controlling clubroot. However, failures with this method have been reported and these have been attributed to the high inoculum potential in the soil and issues such as the method used for applying and incorporating limestone, its neutralization power and granulometry, and the moisture and texture of the soil (Donald and Porter 2009). High levels of organic matter (OM) in the soil have also been associated with suppression of and reduction in losses due to clubroot; however, there are conflicting reports (Dixon 2014; Gossen et al. 2013).

Generally, studies on clubroot have focused on the pathogen's behavior and its interaction with the host plant but have not addressed the morphological, physiological, and nutritional characteristics of the plant itself. Cauliflower typically presents root growth to a depth of 85 to 115 cm. This plants horizontal root growth is approximately three times as extensive as its vertical growth, with intense branching and root hair production in the upper soil layers. Branching and root hair production increase with increasing N supply (Thorup-Kristensen and Boogaard 1998). Cauliflower has a high water demand and is irrigated from planting until harvest to maintain moisture close to field capacity. In terms of soil fertility, cauliflower responds best when grown in soils with a pH between 6.0 and 6.8, with a base saturation of 80%, and with high levels of macro- and micronutrients (May et al. 2007). The availability of micronutrients for plants in soils with pH equal to or greater than 7.2 is reduced and can lead to deficiency symptoms in the plant (Meurer 2007). Moreover, acidic

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soils with a pH of less than 5.5 favor the disease, reduce the availability of Ca, K, Mg, and P, and increase the concentration of Al^{+3} , which is phytotoxic (Meurer 2007).

The present study was conducted in the municipality of Nova Friburgo, located in the mountainous region of the state of Rio de Janeiro, Brazil. This region has high natural vulnerability to water erosion, due to its rugged topography, and a predominance of family farmers who are organized in associations and share machinery and implements. Acidic soils are also common. These conditions favor the dispersion of *P. brassicae* and clubroot development. The farmers intensively cultivate vegetables year round due to the area's favorable climate (mean temperatures of 18.4°C and precipitation of 1,372 mm per year) (Climate-Data 2016). Previous soil fertility surveys performed in the region at more than 400 different cauliflower and tomato cropping sites point to recurrent failures to correctly manage soil fertility, including not conducting soil acidity amelioration, excessive and unbalanced use of chemical fertilizers, and the use of large amounts of fresh poultry manure (i.e., uncomposted). Despite these shortcomings, the municipality is the largest producer of cauliflower in Brazil (Grisel and Assis 2012; IBGE 2006).

This study aimed to evaluate the influence of soil attributes on the development of roots and clubroot in cauliflower and on the accumulation of biomass and nutrients in the plants.

Materials and Methods

The study was conducted on 16 family farms that have a history of more than 50 years of regular cauliflower cultivation in the communities of Serra Nova, Serra Velha, and Rio Grande in the municipality of Nova Friburgo, Rio de Janeiro state (RJ), Brazil (22°17'14"S, 42°32'01"W). Initially, a survey of the farms was performed to determine the history of soil usage, soil characteristics, and management practices adopted. The selected farms have steep slopes and intensive soil preparation is routinely performed via plowing and harrowing using machines and tools shared by the community of growers. Soil conservation practices have not been adopted. On all 16 farms, large amounts of chemical fertilizers were applied at planting and as a top-dressing. Planting fertilizations were done with simple and triple superphosphate, urea, and KCl. In general, top-dressing fertilizations were done with uncomposted poultry manure (approximately 30 Mg/ha), applied about 4 weeks after planting, and with urea plus KCl, applied about 6 and 9 weeks after planting. Foliar fertilization with micronutrients were also made. Fertilization is usually not based on results of soil analyses and liming is not performed on most of the farms.

All plantings used one of the two standard plant spacings (0.60 × 0.60 m or 0.70 × 0.70 m), giving a mean population of 27,777 and 20,408 plants/ha, respectively. Sampling was performed in August/September 2013 (the winter harvest) when the plants, cvs. Bola de Neve or Teresópolis Gigante, were nearly ready to be harvested, approximately 80 to 90 days after transplanting.

In each farm, about 8 to 10 whole plants and the soil around these plants were harvested. In total, 151 samples of soil and 151 plant samples were obtained in the study region. Samples were harvested every 20 m in a zig-zag pattern traversing from the highest point to the lowest of the terrain. Plants were selected at the commercial harvesting point, that is, with fully developed inflorescences and flower buds attached.

For the harvest of the aerial part of the plants, the flush cut was performed at the base of the plant, separating the aerial part from the root. The aerial part of each plant was divided into leaves, stems, and inflorescence, and the fresh weight of each organ was determined.

In order to maintain the integrity of the roots, samples were collected together with the soil in undisturbed soil samples (centered on each root). These samples were collected with the aid of a metal cylinder 25 cm in diameter and 30 cm deep. The samples of soil plus the roots contained in the cylinders were carefully removed, labeled, and transported to the laboratory. The volumes containing the soil and roots were broken up, and the roots were carefully separated, washed to remove the remaining soil, and then analyzed.

The fresh weight of root was determined. Subsequently, the healthy roots and roots with galls were separated. Their fresh weights were measured, and their volumes were determined by measuring the volume of water displaced by galls and by the healthy roots in a graduated beaker. The percentages of roots with clubroot (PVCR) and percentages of healthy roots (PVHR) were calculated by using the ratio of the root volumes with clubroot (VCR) and the healthy root volumes (VHR), respectively, to the total root volume (VTR).

To determine the dry weight, the different organs of the plants (stems, leaves, inflorescence, and roots) were dried in a forced-air oven at 60°C until a constant weight was reached (about 96 h). Dried organs were then ground in an IKA model A-11 analytical mill, and 1.0 g portions were taken to determine the Ca, Mg, K, and Al content of each organ of each plant. These samples were digested by wet processing in a MARS Xpress Digestor (CEM brand) using the SW-846 3051A method (USEPA 2007) with 10 ml of HNO_3 (VETEC 65%) (1,600 W power for 16 min at 180°C). After digestion, the total concentrations of Ca, Mg, and Al were quantified in the extracts using a Perkin Elmer OPTIMA 3000 plasma emission spectrometer (ICP-OES) with detection limits of 0.021, 0.027, and 0.089 mg/kg and quantification limits of 0.07, 0.09, and 0.30 mg/kg, respectively. The content of K in the extracts was measured in a Digimed DM-62 flame photometer (Digimed Analytical Instruments, São Paulo, Brazil) with a detection limit of 0.5 mg/kg and a quantification limit of 1.0 mg/kg. The detection limit of the method was calculated as three times the standard deviation of the blank sample of all the analyses (10 repetitions). The certified sample "SRM 1573a - Tomato Leaves," certified by the National Institute of Standards and Technology (NIST 1995), was the reference material used as an analytical standard. This sample had the following concentrations: 50.5 ± 0.9 g/kg of Ca (93% recovery), 27.0 ± 0.5 g/kg of K (92% recovery), and 598 ± 12.0 mg/kg of Al (93% recovery). The results obtained from the certified reference materials were within the ranges accepted by NIST as normal for plant samples. The nitrogen content was determined from 1.0 g samples dried directly by combustion, using the Dumas method in RapidN Cube equipment.

Samples from the undisturbed soil cores were collected and frozen to count the number of resting spores of *P. brassicae* using the modified method of Murakami et al. (2000). Moreover, 1 g soil samples were suspended in 50 ml of distilled and autoclaved water and stirred for 5 min. The pH of the suspension was then adjusted to 10.0 using 1 M NaOH, and the suspension was then subjected to another 5 min cycle of stirring. At the conclusion of the cycle, the pH of the suspension was readjusted to 9.0 with 1 M NaOH, followed by another 5 min cycle of stirring. The sample was sieved in a filter formed by four layers of muslin cloth, and the filtered sample was then centrifuged at 2,000 rpm for 10 min (New Instruments Benchtop Centrifuge, model NI1814). The residue was washed with distilled and deionized water, centrifuged three more times at 2,000 rpm for 10 min, and finally resuspended in 50 ml of distilled water. To facilitate visualization of the spores, 90 µl of cotton blue was added to 400 µl aliquots of the suspension. The number of resting spores was counted in three samples from each suspension with the aid of a hemacytometer and visualization at 100× magnification under an optical microscope.

For the assessment of soil attributes, the soil samples were processed (air-dried and passed through a sieve with a 2.0 mm diameter mesh), and chemical analyses were performed to determine the levels of organic matter (OM), pH (H_2O), assimilable P and exchangeable K, Ca, Mg, and Al (Al^{+3}), the potential acidity ($H+Al$), the cation exchange capacity (CEC) at pH 7.0, in accordance with the methodology of EMBRAPA (Donagemma et al. 2011), and the levels of B in accordance with the azomethine-H methodology (de Abreu et al. 1994, modified). For the extraction of B, 10 g aliquots of soil plus 20 ml of extraction solution at 1.25 M ($BaCl_2 \cdot 2H_2O$) were heated for 8 min using a microwave at 700 W. Aliquots of 2 ml were taken from each extract, and 2 ml of azomethine-H solution and 2 ml of buffer solution were added, followed by a spectrophotometer reading at 420 nm. For validation of the Ca, Mg, and K levels in the soil samples, the "NIST SRM 2709a - San Joaquin Soil" certified sample was used, which had the following concentrations: Ca (19.1 ± 0.9 g/kg

and 95% recovery), Mg (14.6 ± 0.2 g/kg and 95% recovery), K (21.1 ± 0.6 g/kg and 96% recovery), and Al^{+3} (75.7 ± 0.16 mg/kg and 91% recovery). The results of the analyses were within the ranges accepted by NIST (1995) as normal for soil samples.

The data for the soil attributes, development of the cauliflower plants, and intensity of the clubroot (Table 1) were subjected to multivariate analysis of the main components, clustering, and cross-validation in the discriminant analysis using R software (R Core Team, version 3.2) and descriptive analysis using Microsoft Excel.

To assess the relationships between soil attributes, development of the cauliflower plants, and intensity of the clubroot, principal component analysis (PCA) was performed using the prcomp function of R software (R Core Team, version 3.2). The Pearson correlation between the response variables was measured before carrying out the

PCA. To minimize the autocorrelation effect, the highly correlated variables were removed from the analysis. Among the variables with autocorrelation, we selected those that had higher coefficients of correlation with variables related to clubroot intensity.

To identify samples with similar characteristics, cluster analysis was performed according to the soil attributes. The Euclidean distance was used as a measure of similarity, while Ward's hierarchical method was used as a clustering algorithm. This minimized the sum of squares (SSQ) within the groups such that more homogeneous groups were formed in each clustering step (Hair et al. 2005). For this, the soil attributes were used as clustering variables with standardized data for the mean 0 and variance 1. To interpret and combine the groups, a link distance greater than 1.25 times the standard deviation of the link distance between all the observations was adopted as the cutoff point

Table 1. Variables of soil attributes, intensity of clubroot attributes, and development of cauliflower plant attributes used in multivariate and descriptive analysis

Variables	Abbreviation
Soil attributes	
Soil pH in water	pH _(H2O)
Levels of macronutrients: total N, assimilable P and exchangeable K, Ca and Mg	N, P, K, Ca, and Mg
Potential acidity	H+Al
Exchangeable aluminum	Al ⁺ ³
Clay	Clay
Organic matter	OM
Extractable boron	B
Cation exchange capacity at pH 7.0	CEC
Clubroot intensity attributes	
Concentration of resting spores of <i>P. brassicae</i> in soil	SPO
Clubroot severity estimated with scale ^a	SEV
Volume of healthy roots	VHR
Volume of roots with clubroot	VCR
Total root volume	VTR
Percentage of healthy roots – (VHR/VTR) × 100	PVHR
Percentage of roots with clubroot – (VCR/VTR) × 100	PVCR
Plant development attributes	
Plant biomass accumulation	
Total fresh and dry weight of cauliflower plants	TFW and TDW
Dry weight of stem	SDW
Dry weight of inflorescence	IDW
Dry weight of root	RDW
Dry weight of leaf	LDW
Macronutrients and aluminum accumulation in plant and plant organs	
N, K, Ca and Mg accumulated in cauliflower plants	NAT, KAT, CaAT, and MgAT
N, K, Ca and Mg accumulated in the stem	NAS, KAS, CaAS, and MgAS
N, K, Ca and Mg accumulated in the leaf	NAL, KAL, CaAL, and MgAL
N, K, Ca and Mg accumulated in the inflorescence	NAI, KAI, CaAI, and MgAI
N, K, Ca and Mg accumulated in the root	NAR, KAR, CaAR, and MgAR
Aluminum accumulated in the root	AlAR
Principal component analysis	PCA

^a Numeric scales of Takahashi et al. (2005).

Table 2. Mean, standard deviation (SD), and minimum and maximum values of the severity of clubroot, expressed by the percentages, in volume, of healthy roots and roots with clubroot; number of spores of *Plasmodiophora brassicae* in the soil; total dry weight; and the dry weights for the inflorescence, leaves, stems, and roots of cauliflower plants in the two groups of plants formed by cluster analysis: Group 1 and Group 2 (G1 and G2)

	Root volume (%)		Spores g/soil (×10 ⁶)	Dry weight (g)				
	Healthy	Clubroot		Total	Inflorescence	Leaf	Stem	Root
Group 1 ^a								
Mean	42.99	57.01	7.8	315.84	76.56	181.71	32.77	29.27
SD	25.44	25.44	3.5	90.33	22.13	82.62	10.69	15.88
Minimum	5.00	7.69	1.5	128.23	41.92	44.93	10.96	12.24
Maximum	92.31	95.00	29.0	697.00	149.46	576.11	64.08	119.17
Group 2 ^a								
Mean	25.01	74.99	13.0	232.24	54.86	134.77	23.12	24.59
SD	10.77	10.77	8.5	79.80	18.55	68.67	6.49	9.23
Minimum	7.14	43.28	1.2	110.55	10.07	44.93	7.92	9.00
Maximum	56.72	92.86	39.0	385.75	85.13	275.24	32.21	52.95

^a Groups 1 and 2 formed by cluster analysis according to the soil attributes.

(Milligan and Cooper 1985). For selection of the correct number of groups, cross validation was performed in the discriminant analysis using the same discriminating variables that were used in the clustering and assuming equality of the covariance matrix and the classification probabilities to be equal among groups. Once the groups were

identified, the respective parameters for the position (mean) and amplitude (standard deviation, minimum, and maximum) of the different variables related to the soil attributes, plant development, and intensity of the clubroot were calculated. These data are presented graphically and in tables.

Table 3. Mean, standard deviation (SD), and minimum and maximum values of soil attributes in the two groups of plants formed by cluster analysis, Group 1 and Group 2 (G1 and G2), of the areas sampled to study clubroot in cauliflower^a

	pH	K	P	Ca	Mg	H+Al	Al	CEC	Clay	OM	B	N
Group 1												
Mean	5.27	250.51	214.72	4.48	0.91	6.61	0.35	12.75	369.96	31.75	0.75	2.00
SD	0.51	114.79	165.71	1.88	0.31	2.54	0.44	3.04	84.26	8.08	0.60	0.66
Minimum	4.20	38.00	42.00	0.90	0.20	0.70	0.00	4.99	119.00	9.65	0.20	0.90
Maximum	6.40	714.00	638.00	10.90	1.70	13.60	2.10	22.08	570.00	58.44	3.60	3.60
Group 2												
Mean	4.98	231.52	202.09	3.89	0.73	7.11	0.61	12.40	358.11	29.47	1.02	1.68
SD	0.36	134.36	137.80	2.27	0.31	1.41	0.58	2.85	60.99	5.86	0.95	0.56
Minimum	4.00	76.00	42.00	1.30	0.30	4.00	0.00	7.42	187.00	12.59	0.02	0.00
Maximum	5.70	869.00	638.00	10.80	1.40	9.90	1.80	20.25	475.00	40.51	4.40	2.80

^a pH = pH in water; K = exchangeable potassium (mg/kg); P = assimilable phosphorus (mg/kg); Ca = exchangeable calcium (cmolc/kg); Mg = exchangeable magnesium (cmolc/kg); H+Al = potential acidity (cmolc/kg); Al = exchangeable aluminum (cmolc/kg); CEC at a pH of 7.0 (cmolc/kg); Clay = clay (g/kg); OM = organic matter (g/kg); B = extractable boron (mg/dm³); and N = total nitrogen (g/kg). Groups 1 and 2 formed by cluster analysis according to the soil attributes.

Fig. 1. Principal component analysis (biplot graph) relative to the study of clubroot in cauliflower. **A**, Soil attributes $\times$ severity of the clubroot; **B**, severity of the clubroot $\times$ accumulation of Ca and Mg in the plant and Al in the root $\times$ biomass production of the plant; **C**, soil attributes $\times$ severity of the clubroot and concentration of spores in the soil; and **D**, number of spores $\times$ root attributes. Al = exchangeable aluminum; pH in water; Ca = exchangeable calcium; TDW = total dry biomass of the plant; PVCRC = percentage of roots with clubroot; PVHR = percentage of healthy roots; AlAR = aluminum accumulated in the root; CaAP and MgAP = accumulated calcium and magnesium in the plant; SPO = concentration of spores of *P. brassicae* in the soil; Clay = clay; VTR = total root volume; VHR = healthy root volume; and VCR = root volume with clubroot.

Results

Plants with clubroot were found on all of the 16 family farms at varying intensities. Better correlations between the production of biomass by plants and measurements of clubroot severity were obtained with the volume data (PVCr; $r = -0.26$) compared with the severity estimated based on the scale (SEV; $r = -0.010$). PVCr ranged from 7.69 to 95.0%, while SPO ranged from 1.5×10^6 to 39.0×10^6 spores of *P. brassicae* per g of soil (Table 2).

There was a large variation among the soil samples for their attributes, especially those associated with soil acidity (pH, K, Ca, H + Al, and Al^{+3}), detectable by their minimum and maximum values (Table 3). Great variation was also observed among the plant samples for the accumulation of dry matter in the inflorescences, leaves, stems, roots, and total (Table 2). The accumulation of Al in the roots also varied significantly according to the sample, from 0.0002 to 0.1844 g/root.

The relationship among the soil attributes, the development of the cauliflower plants, and the intensity of the clubroot was identified through PCA. Components 1 and 2 (PC1+PC2) of the PCA between pH, Ca, and Al^{+3} in soil and PVHR, PVCr, and TDW explain 66.8% of the observed variance (Fig. 1A). Components 1 and 2 (PC1+PC2) between CaAT, MgAT, AlAR, and TDW and PVCr and PVHR, explain 67.6% of the observed variance (Fig. 1B). Components 1 and 2 (PC1+PC2) between pH, Ca, and clay in soil and SPO, PVCr, and PVHR explain 60.0% of the observed variance (Fig. 1C). Components 1 and 2 (PC1+PC2) between SPO, PVCr, and PVHR and VTR, VHR, and VCR explain 84.8% of the observed variance (Fig. 1D). The TDW was directly related to the pH and level of exchangeable bases, Ca and Mg, in the soil and inversely related to Al^{+3} (Fig. 1A). The PVCr exhibited a direct relationship with the AlAR and an inverse relationship with the CaAT and MgAT (Fig. 1B). The SPO also exhibited a close relationship with the PVCr, an inverse relationship with the pH and exchangeable Ca of the soil (Fig. 1C), an inverse relationship with the VHR, and a direct relationship with the VCR (Fig. 1D).

In the cluster analysis, when using a link distance of 0.03 (equivalent to 1.25 times the standard deviation of the distance) as the cut-off point in the dendrogram (Milligan and Cooper 1985), eight groups were identified (Fig. 2). This result was subjected to cross-validation in a discriminant analysis for evaluating the overall error rate of the distribution of the samples in the groups. Due to the lower rate for classification errors (5.95%), only two groups were formed: Group 1 (G1) and Group 2 (G2), consisting of 107 and 44 samples, respectively (Fig. 2).

These two groups (G1 and G2) showed very distinct characteristics in relation to soil attributes, with consequent effects on the intensity of the clubroot and the accumulation of biomass in the plant, as well as macronutrients in the different organs of the plants and Al in the roots (Fig. 3). In comparison with G2, G1 was characterized by higher values for pH, exchangeable cations (Ca, Mg, and K), total N, and OM in the soil, as well as lower values for Al^{+3} and H+Al (Fig. 3A, Table 3), greater VHR and PVHR and lower SPO in the soil (Fig. 3B, Table 2), greater TDW, RDW, SDW, LDW, and IDW (Fig. 3C, Table 2), greater accumulation of Ca, Mg, K, and N in the different organs of the plants, and less accumulation of Al in the roots (Fig. 3D, Table 3). G2 stood out due to low soil pH values and high Al^{+3} content (Fig. 3A, Table 2), greater VTR and VCR, higher SPO (Fig. 3B, Table 2), lower biomass accumulation in all the plant organs (Fig. 3C, Table 2), less accumulation of all nutrients, and, in particular, greater accumulation of Al in the roots (Fig. 3D, Fig. 4). The macronutrients most accumulated by the plants were, in order, N, Ca, Mg, and K. Accumulation occurred predominantly in the leaves. Al was only detected in the roots (Fig. 4).

Discussion

The study was located in a tropical climate zone in the state of Rio de Janeiro, Brazil. This area has been producing cauliflower for over 50 years, where recurrent problems with clubroot and its management occur. In this present study, we evaluated the effect of soil attributes on the root system, the severity of the disease, and the

Fig. 2. Dendrogram related to the cluster analysis of the soil and cauliflower samples based on the mean soil characteristics established by the Ward method and Euclidean distance.

accumulation of biomass and nutrients in cauliflower plants. A direct relationship between soil acidity and Al^{+3} content with reduction in the VHR and an increase in the VCR were observed. A direct effect these variables on the accumulation of biomass and macronutrients in the plants was also observed.

The pH values recorded on these 16 farms ranged from very acidic (4.0) to appropriate (6.4) for the development of cauliflower, whose ideal range is between 6.0 and 6.8 (May et al. 2007). These values are lower than the value ($pH \geq 7.2$) reported as being suitable for the control of clubroot (Donald and Porter 2009; Gossen et al. 2014). The low pH resulted in conditions favorable for the development of clubroot as well as the increased availability of Al^{+3} in the soil. Al^{+3} causes a thickening, shortening, and reduction in branching of the roots and a drastic reduction in the growth of the aerial portion of plants (Clune and Copeland 1999; Echart and Cavalli-Molina 2001; Meurer 2007). PCA clearly showed that the variations in the development of clubroot, expressed by the percentage of healthy roots (PVHR), roots with clubroot (PVCR), and plant biomass production (TDW), were best explained by the strong negative relationship between soil pH and levels of Al^{+3} (Fig. 1A).

Under the conditions studied, there are probably two factors acting directly on the reduction of the root system and the water and nutrient

absorption efficiency of the plants: a chemical impediment caused by the phytotoxicity of Al^{+3} and the biological impediment due to infection and colonization of roots by *P. brassicae*. However, it is believed that the reduced development of the root system was partially offset by the application, in all 16 farms, of high doses of CaH_2PO_4 (simple and triple superphosphate) at the time of planting and the addition of N and K as a topdressing. In general, the levels of P and exchangeable Ca and K in the soil for all 16 farms are also higher than the levels recommended for the crop (Guerra et al. 2013; May et al. 2007). Thus, the production of cauliflower has been made possible by maintaining high levels of nutrients in the soil (Table 3). It is also believed that because of the high inoculum density ($>10^6$ spores/g of soil) and low to medium pH values on some of the farms, production was made possible by the low temperatures recorded during the crop cycle (10 to 15°C).

However, given that cauliflower is predominantly grown in temperate regions (FAO 2006), where slightly weathered soils with pH values ranging from neutral to alkaline are predominant, reports on limitations of its cultivation because of Al^{+3} are not common. These results are clearly supported by the inverse and very close relationships observed among the amount of Al accumulated in the roots, the accumulation of Ca and Mg in the plants, and the total biomass

Fig. 3. Standardized mean of (A) soil attributes; (B) intensity of the clubroot; (C) biomass of the root, inflorescence, stem, leaf, and total of cauliflower plants; and (D) N, K, Mg, and Ca accumulated in the inflorescence, stem, leaf, and root, as well as the aluminum accumulated in the cauliflower root in the two groups (G1 and G2) formed via cluster analysis. Clay = clay; CEC = cation exchange capacity at pH 7.0; OM = organic matter of the soil; B = extractable boron; N = total nitrogen; H+Al = potential acidity; Al = exchangeable aluminum; Mg = magnesium; Ca = calcium; K = potassium; P = phosphorus; pH = pH in water; SPO = concentration of spores of *P. brassicae* in the soil; PVCR = percentage of roots with clubroot; PVHR = percentage of healthy roots; VHR = volume of healthy roots; VCR = volume of roots with clubroot; VTR = total root volume; RDW = dried weight of root; IDW = dried weight of inflorescence; SDW = dried weight of stem; LDW = dried weight of leaf; TDW = total dried weight; AlAR = aluminum accumulated in the root; KAT = total accumulated potassium; KAR = potassium accumulated in the root; KAI = potassium accumulated in the inflorescence; MgAT = total accumulated magnesium; MgAR = magnesium accumulated in the root; MgAI = magnesium accumulated in the inflorescence; CaAT = total accumulated calcium; CaAR = calcium accumulated in the root; and CaAI = calcium accumulated in the inflorescence.

production (TDW) (Fig. 1B). The data obtained do not enable separation of the effects caused by the disease from those caused by the phytotoxicity of Al^{+3} (Fig. 1A and B), and it is also not possible to determine a combined effect, thus indicating the need for further investigations. Although there are no previous reports for cauliflower, Al concentrations higher than 15 mM/liter (equivalent to half the critical level in weathered soils) have been observed to strongly inhibit root growth in canola (*B. napus*) (Clune and Copeland 1999).

The result of the cluster analysis (Figs. 2 and 3) shows that the cauliflower population sampled split into two distinct groups (G1 and G2) in relation to the characteristics discussed above. The soil samples in G1 had, on average, lower concentrations of H+Al and

exchangeable Al, higher pH values, and higher concentrations of exchangeable Ca, Mg, K, and total N in comparison with those of G2 (Table 3, Fig. 3A). Of these attributes, the most significant differences were related to pH and exchangeable Al. The mean values of pH and exchangeable Al observed in G1 were 5.27 and 0.35 cmol/kg, respectively, while in G2, they were 4.98 and 0.6 cmol/kg, respectively. These pH values represent an increase of 100% in the levels of Al^{+3} in the soils of G2 compared with G1, reinforcing the hypothesis that Al^{+3} is a limiting factor for the production of cauliflower (Table 3). Similarly, G2 plants had a higher concentration of spores (13×10^6 spores/g of soil) and greater PVC (75%) and VCR (45.61 ml) compared with G1 plants (7.8×10^6

Fig. 4. For groups G1 and G2: mean amounts of (A) calcium, (B) magnesium, (C) potassium, and (D) nitrogen accumulated in the roots, inflorescences, stems, and leaves, as well as the total amounts; (E) aluminum accumulated in the roots of cauliflower plants.

spores/g of soil, PVCR = 57%, and VCR = 29.02 ml) (Table 2, Fig. 3B). In G1, under more favorable soil conditions, lower mean inoculum density (SPO), higher pH, lower levels of Al^{+3} (Table 3, Fig. 3A), and greater production of dry weight were also recorded in all of the plant organs in comparison with G2 (Table 2, Fig. 3C). The greater production of total dry biomass and inflorescences in G1 (316 g/plant and 77 g/inflorescence, respectively) compared with that of G2 (232 g/plant and 55 g/inflorescence, respectively) represents a mean gain of 36% in total biomass and 5.9 t/ha of cauliflower or an additional production of inflorescences equal to 40%.

These results were confirmed by the higher amounts of Ca, Mg, K, and N accumulated in the roots, leaves, and inflorescences of the plants of G1 in relation to those of G2 (Fig. 4A to D). However, the amount of Al accumulated in the roots was approximately 25% higher in the plants of G2 compared with those of G1 (Fig. 4E).

The lower root Ca levels observed for G2 plants may have affected root hair penetration and infection by *P. brassicae* and other postinfection events. Ca levels have been reported to affect *P. brassicae* spore germination, root hair penetration and infection, and the maturation rate of the remaining pathogen stages (Dixon 2009b), whereas Al^{3+} may inhibit a wide variety of essential metabolic processes regulated by Ca (Echart and Cavalli-Molina 2001).

Young et al. (1991) reported that soils suppressing clubroot were 3 to 15 times richer in Ca and Mg than soils favorable to clubroot. It should be noted, however, that the levels of exchangeable Ca, Mg, and K, as well as assimilable P, in the soils of both groups G1 and G2 were high and not limited to the production of cauliflower. Furthermore, the levels of Ca, Mg, and N accumulated in the different plant organs were in accordance with the levels described for the crop (Takeishi et al. 2009). Boron, although often associated with reduced severity of clubroot and increased crop yield (Dixon 2009b; Ruaro et al. 2009), did not show a relationship with a reduction in the disease in the present study. Boron levels were higher in samples with higher disease severity, in contrast to previous reports (Dixon 1996; Gossen et al. 2014; Webster and Dixon 1991). However, G1 and G2 had high and very similar mean B concentrations (Table 3).

One component relevant to the proper development of the plant and reduction in losses from clubroot may be related to the plant's ability to produce new roots and compensate for those infected by the pathogen. The limitation on the development of the plants, especially in G2, was probably due to the greater development of galls and the losses in the water and nutrient absorption capacity of the roots. These losses were compounded by the inhibition of the formation and development of new roots due to the low pH and high Al^{+3} content in the soil. The volume and, in particular, the percentage of healthy roots had an inverse relationship with the increased volume and percentage of roots with clubroot and direct relationships with the increase in pH and, consequently, the reduction in H+Al content in the soil (Figs. 1A and 3B). In other words, in the study of the relationship between clubroot and the development and production of the plant, the quantification of the healthy roots produced by the plant is as important as the quantification of the severity of roots affected by clubroot. Another important aspect to consider is the tendency of the cauliflower roots to have much greater horizontal than vertical growth (Thorup-Kristensen and Boogaard 1998), exploring a wide range of the soil's superficial layers (0 to 20 cm of deep) in which there are also higher concentrations of resting spores of *P. brassicae* (Dixon 2009b). In the soils typical of the region (latosols and acrisols), the Al^{+3} content increases with soil depth and inhibits vertical root growth (chemical impediment) (Meurer 2007), thus restricting the roots to the surface layers in which there is a higher probability of contact between roots and spores and, consequently, occurrence of infections.

Increased levels of soil OM have been reported to decrease the severity of clubroot, although conflicting results have been obtained (Gossen et al. 2013). However, in the present study, high levels of soil OM were observed (9.65 to 58.44 g/kg soil), with similar mean values for the two groups.

The results of this study confirm the close relationship between soil attributes (particularly acidity) and the severity of clubroot and

losses in cauliflower. However, in addition to the pH, for tropical soils, one more variable can be added: Al^{+3} content. By chemically inhibiting root development, Al^{+3} may compromise the production and elongation of new roots that could compensate the loss of active roots caused by clubroot. The effects of Al^{+3} on roots might also contribute to the invasion and primary infection of root hairs by *P. brassicae*. In other words, depending on the extent to which the acidity of the soil increases, the availability of Al^{+3} increases and restricts root development, thus increasing the potential for losses from the disease. Additionally, the predominant soils in the region have a high buffering capacity, which hinders the changes in pH up to the levels suggested for management of clubroot (≥ 7.2). This difficulty is aggravated in land with steep slopes, which limits the uniform incorporation of the limestone that is essential for avoiding outbreaks and failures in the management of the disease.

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Literature Cited

- Clune, T. S., and Copeland, L. 1999. Effects of aluminium on canola roots. *Plant Soil* 216:27-33.
- Climate-Data. 2016. Clima: Nova Friburgo. <http://pt.climate-data.org/location/33678/>. Accessed on June 14, 2016.
- de Abreu, C. A., Abreu, M. F., Van Raij, B., Bataglia, O. C., and de Andrade, J. C. 1994. Extraction of boron from soil by microwave heating for ICP OES determination. *Commun. Soil Sci. Plant Anal.* 25:3321-3333.
- Dixon, G. E. 1996. Repression of the morphogenesis of *Plasmodiophora brassicae* Wor. by boron - a review. *Acta Hort.* 407:393-402.
- Dixon, G. E. 2009a. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. *J. Plant Growth Regul.* 28:194-202.
- Dixon, G. E. 2009b. *Plasmodiophora brassicae* in its environment. *J. Plant Growth Regul.* 28:212-228.
- Dixon, G. E. 2014. Clubroot (*Plasmodiophora brassicae* Woronin) - an agricultural and biological challenge worldwide. *Can. J. Plant Pathol.* 36:5-18.
- Donagemma, G. K., Campos, D. V. B. de, Calderano, S. B., Teixeira, W. G., and Viana, J. H. M. 2011. Manual e métodos de análise de solo. 2nd ed. Embrapa solos, Rio de Janeiro, RJ, Brazil.
- Donald, C., and Porter, I. 2009. Integrated control of clubroot. *J. Plant Growth Regul.* 28:289-303.
- Echart, C. L., and Cavalli-Molina, S. 2001. Fitotoxicidade do alumínio: efeitos, mecanismo de tolerância e seu controle genético. *Cienc. Rural* 31:531-541.
- FAO. 2006. Economics and Statistics. Food and Agriculture Organization of The United Nations. Online: <http://www.fao.org>.
- Gossen, B. D., Deora, A., Peng, G., Hwang, S. F., and McDonald, M. R. 2014. Effect of environmental parameters on clubroot development and the risk of pathogen spread. *Can. J. Plant Pathol.* 36:37-48.
- Gossen, B. D., McDonald, M. R., Hwang, S. F., Strelkov, S. E., and Peng, G. 2013. A comparison of clubroot development and management on canola and Brassica vegetables. *Can. J. Plant Pathol.* 35:175-191.
- Grisel, P. N., and Assis, R. L. 2012. Adoção de práticas agrícolas sustentáveis: estudo de caso de um sistema de produção hortícola familiar em ambiente de montanha. *Cad. Ciênc. Tecnol.* 29:133-158.
- Guerra, J. G. M., Leal, M. A. de A., and Ferreira, M. B. C. 2013. Recomendações de adubos e corretivos e de manejo da matéria orgânica para as principais culturas do Estado do Rio de Janeiro. Pages 303-304 in: Manual de calagem e adubação do Estado do Rio de Janeiro. L. R. Freire, F. C. Balieiro, E. Zonta, L. H. C. dos Anjos, M. G. Pereira, E. Lima, J. G. M. Guerra, M. B. C. Ferreira, M. A. de A. Leal, D. V. B. de Campos, and J. C. Polidoro, eds. Editora Universidade Rural, Seropédica, Brazil.
- Hair, J. F., Anderson, R. E., Tatham, R. L., and Black, W. C. 2005. Análise multivariada de dados, 5th Ed. Bookman, Porto Alegre, RS, Brazil.
- IBGE. 2006. Produção Agrícola Municipal (PAM). Instituto Brasileiro de Geografia e Estatística (IBGE). Online: <https://sidra.ibge.gov.br/home/pms/brasil>.
- May, A., Tivelli, S. W., Vargas, P. F., Samra, A. G., Sacconi, L. V., and Pinheiro, M. Q. 2007. A cultura da couve-flor. Boletim Técnico IAC, 200. Instituto Agronômico, Campinas, SP, Brazil.
- Meurer, E. J. 2007. Fatores que influenciam o crescimento e o desenvolvimento das plantas. Pages 65-90 in: Fertilidade do solo. R. F. Novais, V., V. H. Alvarez, N. F. de Barros, R. L. F. Fontes, R. B. Cantarutti, and J. C. L. Neves, eds. SBCS, Viçosa, Brazil.
- Milligan, G. W., and Cooper, M. C. 1985. An examination of procedures for determining the number of cluster in a data set. *Psychometrika* 50:159-179.
- Murakami, H., Tsushima, S., Akimoto, T., and Shishido, Y. 2000. Effects of growing leafy daikon (*Raphanus sativus*) on populations of *Plasmodiophora brassicae* (clubroot). *Plant Pathol.* 49:584-589.

- NIST. 1995. Standard Reference Materials 1573a Tomato Leaves. National Institute of Standards and Technology (NIST), Gaithersburg, MD.
- Niwa, R., Kumei, T., Nomura, Y., Yoshida, S., Osaki, M., and Ezawa, T. 2007. Increase in soil pH due to Ca-rich organic matter application causes suppression of the clubroot disease of crucifers. *Soil Biol. Biochem.* 39: 778-785.
- Niwa, R., Nomura, Y., Osaki, M., and Ezawa, T. 2008. Suppression of clubroot disease under neutral pH caused by inhibition of spore germination of *Plasmodiophora brassicae* in the rhizosphere. *Plant Pathol.* 57:445-452.
- Ruaro, L., Lima Neto, V. C., and Ribeiro Júnior, P. J. 2009. Influência do boro, de fontes de nitrogênio e do pH do solo no controle de hérnia das crucíferas causada por *Plasmodiophora brassicae*. *Trop. Plant Pathol.* 34:231-238.
- Sharma, K., Gossen, B. D., and McDonald, M. R. 2011. Effect of temperature on cortical infection by *Plasmodiophora brassicae* and clubroot severity. *Phytopathology* 101:1424-1432.
- Takahashi, L. M., Cebrian, I. T., and Souza, N. L. 2005. Inoculação de *Plasmodiophora brassicae* agente causal da "hérnia das crucíferas". *Summa Phytopathol.* 31:16-18.
- Takeishi, J., Cecílio Filho, A. B., and Oliveira, P. R. 2009. Crescimento e acúmulo de nutrientes em couve-flor 'Verona'. *Bioscience* 25:1-10.
- Thorup-Kristensen, K., and Boogaard, R. 1998. Temporal and spatial root development of cauliflower (*Brassica oleracea* L. var. *botrytis* L.). *Plant Soil* 201:37-47.
- USEPA. 2007. Method 3051 A Microwave assisted acid digestion of sediments, sludges, soils, and oils. United States Environmental Protection Agency, Washington, DC.
- Wallenhammar, A. C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oil seed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathol.* 45:710-719.
- Wallenhammar, A. C., Johnson, L., and Gerhardson, B. 2000. Agronomic performance of partly clubroot-resistant spring oilseed turnip rape lines. *J. Phytopathol.* 148:495-499.
- Webster, M. A., and Dixon, G. R. 1991. Boron, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae*. *Mycol. Res.* 95:74-79.
- Young, C. C., Cheng, K. T., and Waller, G. R. 1991. Phenolic compounds in conducive and suppressive soils on clubroot disease of crucifers. *Soil Biol. Biochem.* 23:1183-1189.