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Liming and biofungicide for the control of clubroot in cauliflower[1]

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ABSTRACT

Clubroot, caused by *Plasmodiophora brassicae*, is a disease that limits the cauliflower cultivation and is difficult to control. This study aimed to evaluate the efficiency of liming combined with the use of *Trichoderma harzianum*-based biofungicide for the control of clubroot in cauliflower. In a field experiment, the use of the biofungicide in combination with the application of calcined limestone doses (0 Mg ha^{-1}, 1.0 Mg ha^{-1}, 2.0 Mg ha^{-1} and 4.0 Mg ha^{-1}) was evaluated. Subsequently, in a greenhouse, the biofungicide combined with liming with quicklime (2.54 Mg ha^{-1}) was tested, and cyazofamid and water were tested as controls. The disease severity and attributes related to root and plant development were analyzed. In the field experiment, the healthy root volume and fresh weight, total root dry weight and inflorescence fresh weight and diameter were all significantly increased, while the diseased root volume, in response to the limestone doses, was reduced. The biofungicide reduced the root growth and inflorescence fresh weight. In the greenhouse, liming increased the healthy root volume and fresh weight, as well as total root dry weight, and reduced the disease severity. No significant difference was observed between the biofungicide and the control (water), which were inferior to cyazofamid. The biofungicide was not efficient in controlling the disease and did not favour the growth of cauliflower plants, either alone or combined with liming. Liming reduced the disease severity and increased the cauliflower root growth and yield.

KEYWORDS: *Brassica oleracea* var. *botrytis*; *Plasmodiophora brassicae*; *Trichoderma harzianum*; cyazofamid.

RESUMO

Calagem e biofungicida para o controle de hérnia das crucíferas em couve-flor

A hérnia das crucíferas, causada por *Plasmodiophora brassicae*, é uma doença de difícil controle, sendo limitante ao cultivo de couve-flor. Objetivou-se avaliar a eficiência da calagem, combinada ao uso de biofungicida à base de *Trichoderma harzianum*, no controle de hérnia das crucíferas em couve-flor. Em ensaio de campo, avaliou-se o biofungicida associado à aplicação de doses (0 Mg ha^{-1}; 1,0 Mg ha^{-1}; 2,0 Mg ha^{-1} e 4,0 Mg ha^{-1}) de calcário calcinado. Posteriormente, em casa-de-vegetação, avaliou-se o biofungicida combinado à calagem com cal virgem (2,54 Mg ha^{-1}), além das testemunhas ciazofamida e água. Foram analisados a severidade da doença e os atributos relacionados ao desenvolvimento da raiz e da planta. No experimento a campo, houve incrementos significativos no volume e massa fresca de raiz sadia, massa seca total de raiz, massa fresca e diâmetro das inflorescências e redução no volume de raízes doentes, em resposta às doses de calcário. O biofungicida reduziu o crescimento das raízes e a massa fresca das inflorescências. Em casa-de-vegetação, a calagem incrementou o volume e massa fresca de raízes sadias e a massa seca total de raiz, bem como reduziu a severidade da doença. Não foi observada diferença significativa entre o biofungicida e a testemunha (água), que mostraram-se inferiores à ciazofamida. O biofungicida não foi eficiente no controle da doença e não favoreceu o crescimento das plantas de couve-flor, seja isoladamente ou combinado à calagem. A calagem reduziu a severidade da doença e aumentou o crescimento radicular e a produtividade de couve-flor.

PALAVRAS-CHAVE: *Brassica oleracea* var. *botrytis*; *Plasmodiophora brassicae*; *Trichoderma harzianum*; ciazofamida.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.), a temperate species, is a vegetable of great economic and social importance. Currently, due to genetic improvement, cauliflower cultivation has been possible in tropical regions and in the mid-season and summer in higher altitudes (May et al. 2007, Filgueira 2008).

One of the main problems to be overcome in several regions is the generalized distribution of *Plasmodiophora brassicae* Woronin, the causal agent of clubroot. This disease has caused severe losses in the production and quality of cauliflower and other

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brassicas in Brazil (Bhering et al. 2017) and in the world (Diederichsen et al. 2016).

The biotrophic pathogen *P. brassicae* is a common soil protozoan (Niwa et al. 2008, Peng et al. 2011). It has a complex life cycle divided into two phases: in the soil, where it survives in the form of robust resting spores (Dixon 2009), and in plants, infecting the roots and causing cell hyperplasia and hypertrophy with the consequent formation of galls. Depending on the intensity of the disease, a reduction in the active root system and impairment of water and nutrient absorption occurs, which can lead to symptoms such as wilting, underdevelopment and even plant death (Penalber 2009). Disease management must involve preventive measures that reduce soil inoculum levels, such as crop rotation with non-host species (Donald & Porter 2009).

The soil pH and calcium content contribute independently to disease reduction (Dixon 2009); however, the suppressive effects exerted by each of these factors are difficult to differentiate (Niwa et al. 2008). Increasing soil pH may inhibit resting spore germination (Niwa et al. 2008) and hinder root infection and colonisation (Webster & Dixon 1991). In turn, increased Ca^{2+} levels may negatively affect spore germination and the motility of primary zoospores and increase plant resistance to primary infection (Dixon 2014).

Liming, besides helping to reduce losses caused by clubroot (Donald & Porter 2009), can increase cauliflower yield, as cauliflower is highly demanding of soil fertility and poorly tolerant to acidity and toxic Al^{+3} (May et al. 2007, Bhering et al. 2017). Moreover, the elevation of pH with liming may favour an increase in soil biological activity (Campos et al. 2013), which may be important in suppressing clubroot (Penalber 2009).

Beyond liming, disease management strategies are limited (Donald & Porter 2009). In Brazil, no cauliflower cultivars are resistant to clubroot, and only one active principle is registered in the Brazilian Ministry of Agriculture for its chemical control: cyazofamid. This molecule was registered recently and acts on resting spore germination (Agrofit 2017). Thus, cauliflower producers, such as those in Serra Fluminense, a region with serious problems with clubroot (Bhering et al. 2017), have been using biofungicide based on *Trichoderma* spp., together with other control measures, and obtaining variable results.

The biological control with biofungicides based on *Trichoderma* spp. has been recommended for many diseases caused by different plant pathogens, such as *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Fusarium* spp. (Pomella & Ribeiro 2009). These biofungicides act through different mechanisms, such as antibiosis (antibiotics, toxins and enzymes that affect fungal development), parasitism and competition for space and substrate (Bettiol et al. 2012). However, little is known about their effect on cauliflower crops and their efficiency in reducing losses caused by clubroot.

Most studies on clubroot control (Penalber 2009, Peng et al. 2011) are based on the reduction of disease intensity without considering other aspects, such as the increase in root volume, especially of healthy roots, or do not evaluate combinations of different forms of control. Thus, this study aimed to evaluate the use of a *Trichoderma harzianum*-based biofungicide combined with liming in reducing the severity of clubroot in cauliflower and their effects on root and inflorescence attributes under field conditions, as well as the effect of liming combined with the biofungicide and cyazofamid on disease symptoms and progress and on root attributes in cauliflower under greenhouse conditions.

MATERIAL AND METHODS

Two experiments were conducted: one in a field with a history of occurrence of clubroot, located in Nova Friburgo, Rio de Janeiro state, Brazil (-22º28'42"S and -42º63'61"W), from September to December 2015, and another in a greenhouse on the same property, from July to October 2016. Meteorological data from the respective periods were collected from the National Institute of Meteorology (Brasil 2016).

In the field experiment, the effect of four doses of agricultural limestone [0 Mg ha^{-1}, 1.0 Mg ha^{-1}, 2.0 Mg ha^{-1} and 4.0 Mg ha^{-1} of calcined limestone with relative total neutralizing power (RTNP) = 104.5 %], combined or not with the commercial *T. harzianum*-based biofungicide (ESALQ 1306 strain; 2 x 10^9 conidia mL^{-1}), formulated as an emulsifiable concentrate, was evaluated. The experiment followed a randomized complete block design with four replications, in a 4 x 2 factorial scheme (four limestone doses and an application or not of biofungicide). The soil of the experimental

field was classified as Haplic Cambisol and presented (0-20 cm depth), prior to the application of the treatments, the values of pH (H_2O) = 5.29; Ca = 5.45 $cmol_c$ dm^{-3}; Mg = 1.20 $cmol_c$ dm^{-3}; Al = 0.30 $cmol_c$ dm^{-3}; K = 236.0 mg dm^{-3}; P = 56.0 mg dm^{-3}; C = 2.15 %; and V = 42.5 %.

The limestone was applied by broadcasting and incorporated with a rotary spader. The commercial biofungicide was applied by watering trays with seedlings (1 mL 500 mL^{-1} of water), one week before and at the date of transplanting and in the field at 20 days after transplanting (DAT) (800 mL in 1,300 L ha^{-1} of water), by spraying the soil around the plants with the help of a backpack sprayer.

Before the plants were transplanted, soil samples were collected (0-20 cm depth) for determination of the pH (H_2O) and Ca^{+2}, Mg^{+2} and Al^{+3} levels, using absorption spectrometry (Donagemma et al. 2011), and for quantification of the number of resting spores of *P. brassicae*, according to the modified technique of Murakami et al. (2000). The number of spores found was 2.97 x 10^{-1} units g^{-1} of soil, a density that is considered medium-to-high and conducive to the development of clubroot galls (Penalber 2009).

Thirty-day-old seedlings of the Barcelona cultivar were transplanted to pits measuring 20 cm x 20 cm x 15 cm and spaced 0.60 m x 0.60 m apart. Simultaneously, 0.035 Mg ha^{-1} of N in the form of ammonium sulfate, 0.100 Mg ha^{-1} of P_2O_5 in the form of single superphosphate and 0.040 Mg ha^{-1} of K_2O in the form of potassium chloride were applied.

Irrigation was performed by sprinkling in 3-day irrigation shifts to complement rainfall. Two topdressings were applied at 20 and 85 DAT, with an application of 0.085 Mg ha^{-1} of N (as ammonium sulfate) and 0.075 Mg ha^{-1} of K_2O (as potassium chloride) accompanying both applications. At 57 DAT, 1.400 Mg ha^{-1} of commercial organomineral compost (containing 15 % of organic carbon, 1 % of N, C/N ratio of 18 and pH of 6.5) were applied. Foliar application of boric acid (2 g L^{-1}) was also performed.

Each plot contained an area of 20 m^2 and 56 plants, and the 10 central plants were considered useful. The harvest was performed at 104 DAT, when the inflorescences were fully developed and flower buds were still firm and closed (May et al. 2007). The inflorescences were transported to the laboratory, where the fresh weight was determined on a bench scale, and the transverse diameter was determined using a ruler attached to an adjustable wooden support.

The roots were also collected, with the aid of a straight blade, washed and then evaluated individually for disease severity, based on a scale composed of seven levels of severity: 0 %, 8 %, 20 %, 42 %, 68 %, 87 % and 95 % of roots with galls. This scale was developed and validated in previous trials (data not shown) based on 151 samples of properly washed cauliflower roots, which were photographed and measured as the volume of healthy and infected roots, and the ratio of infected to total root volume was calculated. The maximum and minimum values observed were used as the scale end values, whereas the intermediate levels were defined mathematically, following a logarithmic increment and respecting the Weber-Fechner law of perception (Horsfall & Barratt 1945).

Subsequently, the roots were segmented into healthy (free of symptoms) and diseased (with presence of galls) fractions. The volumes of the healthy and galled root fractions were determined by measuring the water displacement in a graduated cylinder. The fresh weight of healthy and galled roots was also obtained by weighing on a precision scale. The root samples, including the healthy and galled fractions, were dried in an oven at 70 ºC to constant weight, to obtain the total root dry weight.

In the greenhouse, the use of liming combined with three types of control was evaluated: *T. harzianum*-based biofungicide, cyazofamid-based fungicide (400 g L^{-1}) and water. The experimental design was a randomized block with four replications, in a 2 x 3 factorial scheme (with or without the use of liming and three types of control).

Eight-litre pots, filled with soil collected from the surface layer of the control plots (0 Mg ha^{-1} of limestone) of the previous experiment, were used. The soil was homogenized in a concrete mixer, with five pots being established per plot. Samples of the homogenized soil were collected for chemical analysis and for spore counts of *P. brassicae.* The soil used contained 4.5 x 10^7 spores g^{-1}; pH (H_2O) = 4.90; Ca = 4.80 $cmol_c$ dm^{-3}; Mg = 1.40 $cmol_c$ dm^{-3}; Na = 0.038 $cmol_c$ dm^{-3}; Al = 0.50 $cmol_c$ dm^{-3}; H + Al = 12.80 $cmol_c$ dm^{-3}; CEC = 19.6 $cmol_c$ dm^{-3}; K = 236.0 mg dm^{-3}; P = 56.0 mg dm^{-3}; C = 1.64 %; OM = 2.83 %; and V = 25 %.

Subsequently, to determine the amount of corrective to be applied, a neutralization curve was constructed by incubating the soil with increasing

doses of quicklime - CaO (RTNP = 125 %), in which the pH was evaluated until stabilization. The soil was maintained at 70 % of field capacity. The data allowed the generation of the following equation: pH = 5.532 + 0.3799(dose), R^2 = 94.46 %, with values expressed as Mg ha^{-1}. The proposed pH was 6.5, ideal for cauliflower cultivation (May et al. 2007, Guerra et al. 2013), and was reached with the dose equivalent to 2.54 Mg ha^{-1} of CaO RTNP 125 %. Quicklime was used as a corrective because it reacts quickly in the soil (Campos et al. 2013) and may be used by the producers in the region of the study.

The same product from the previous experiment was used as the biofungicide and applied on seedlings on the day of transplanting via irrigation (1 mL 500 mL^{-1} of water per tray) and in the soil around the plants, at 7 and 17 DAT (800 mL in 1,080 L ha^{-1} of water). The fungicide was applied by irrigation (2 mL 500 mL^{-1} of water per tray) and in two additional applications in the pots (1.5 L in 1,080 L ha^{-1} of water), at the same time as the biofungicide applications. Water was used as an absolute control, applied at the same times.

The corrective was homogenized into the soil with the use of a concrete mixer and soon after was distributed into the pots, which were kept moist (with 70 % of field capacity) until transplanting. On this occasion, 30 days after the application, soil samples were collected for fertility evaluation, according to the previous experiment. Subsequently, a 30-day-old seedling of the Bola de Neve cultivar was transplanted in each pot, totalling five plants per plot and 120 useful plants. The choice of the cultivars used in both experiments was made according to the options available and commonly used by the producers of the region for the respective growing seasons.

Base fertilization and two additional topdressings were performed at 35 and 75 DAT, and foliar fertilization with boric acid was applied at 75 DAT (2 g L^{-1}). The doses and fertilizers used were the same as those used in the previous experiment.

The disease severity was quantified using a scale with seven levels of severity in one plant per plot at 30, 50, 70, 90 and 100 DAT. After the severity data had been obtained, values for the area under the disease progress curve (AUDPC) were calculated (Shaner & Finney 1977), as it follows:

$$AUDPC = \sum_{i=1}^{n} \left[\frac{(Y_{i+n1} + Y_i)}{2} \right] [X_{i+1} + X_i]$$

where Y_i = clubroot disease severity in that evaluation; X_i = time (days) in the i*th* evaluation; and n = total number of evaluations.

In the last evaluation, performed at 100 DAT, the volume and fresh weight of the healthy and diseased root and the total dry weight of the root were also evaluated, with the same methodologies being used as described for the field experiment.

The data obtained in the two experiments were subjected to analysis of variance (Anova), and the means were compared using the Tukey test ($p \leq 0.05$). Additionally, for the first experiment, regression analysis (linear or quadratic) was performed as a function of the limestone doses, when these were significant. The statistical software Sisvar (Ferreira 2011), version 5.6, was used.

RESULTS AND DISCUSSION

During the field experiment, mean temperatures of 15-20 ºC, with occasional peaks of 32 ºC, relative humidity above 75 % and light and frequent rainfall (mean of 1.95 mm day^{-1}) in the first 75 days, followed by an intense rainfall in the last 30 days of the cycle (mean of 8.0 mm day^{-1}), were recorded (Brasil 2016).

Significant differences were observed in the pH values and exchangeable Ca^{+2} contents and a reduction in the Mg^{+2} and Al^{+3} levels was observed as a function of the corrective doses (Figure 1). The effects of the corrective doses on pH, Ca and Mg contents were described by simple linear equations (Figure 1), whereas the Al^{+3} contents were best fit by a quadratic equation. The reduction in the Mg^{+2} content may have been due to its displacement by the mass action of Ca^{+2} added via application of the calcined limestone (Campos et al. 2013) (Figure 1).

Liming improved the soil attributes associated with acidity, increasing the pH and reducing the Al^{+3} content. The pH value obtained at the dose of 4.0 Mg ha^{-1} agrees with the one recommended for cauliflower (6.0-6.8) (Filgueira 2008), whereas the levels of toxic Al^{3+} were low in all doses tested (Sousa et al. 2007).

Clubroot occurred in all treatments, but the biofungicide had a significant effect on healthy root volume [with *Trichoderma*: 24.45 mL; without *Trichoderma*: 32.24 mL; coefficient of variation (CV): 23.10 %], diseased root volume (with *Trichoderma*: 6.95 mL; without *Trichoderma*: 10.95 mL; CV: 16.50 %) and diseased root fresh weight (with

Trichoderma: 7.28 g; without *Trichoderma*: 12.00 g; CV: 17.19 %). Despite the lower volume and weight of galled roots in the treatment with *Trichoderma*, a confirmation that the biofungicide helped to control the disease was not possible, because it also reduced the volume of healthy roots.

Increasing the dose of limestone led to significant increases in healthy root volume and fresh weight and total root dry weight, as well as a reduction in the galled root volume (Figure 2). No significant differences were observed among treatments when the disease severity was estimated using the scale composed of seven levels of severity. The values found for this variable were between 24.92 % and 39.68 %.

The inflorescence development, expressed as both fresh weight accumulation and mean diameter (Figure 2), was significantly and positively influenced by the increase in limestone doses. This effect was probably due to pH increase, neutralization of Al^{+3} and increase in Ca^{+2} availability in the soil (Figure 2), favouring the development of healthy roots and a reduction in galled roots.

In the greenhouse experiment, a significant effect of liming was observed on the pH (H_2O) value (with liming: 5.43; without liming: 5.10; CV: 1.92 %) and levels of Ca^{+2} (with liming: 5.54 $cmol_c$ dm^{-3}; without liming: 4.31 $cmol_c$ dm^{-3}; CV: 7.24 %), Mg^{+2} (with liming: 3.38 $cmol_c$ dm^{-3}; without liming: 2.18 $cmol_c$ dm^{-3}; CV: 25.37 %) and Al^{+3} (with liming: 0.08 $cmol_c$ dm^{-3}; without liming: 0.27 $cmol_c$ dm^{-3}; CV: 4.69 %). However, despite differing significantly, the pH value obtained in the treatment with limestone is lower than that recommended for cauliflower (May et al. 2007). This result is probably due to the high buffering power of the soil used. The Al^{3+} contents in both treatments can be considered non-phytotoxic to cauliflower (Sousa et al. 2007).

At the date of the last collection (100 DAT), cauliflower plants had not yet bloomed, and thus,

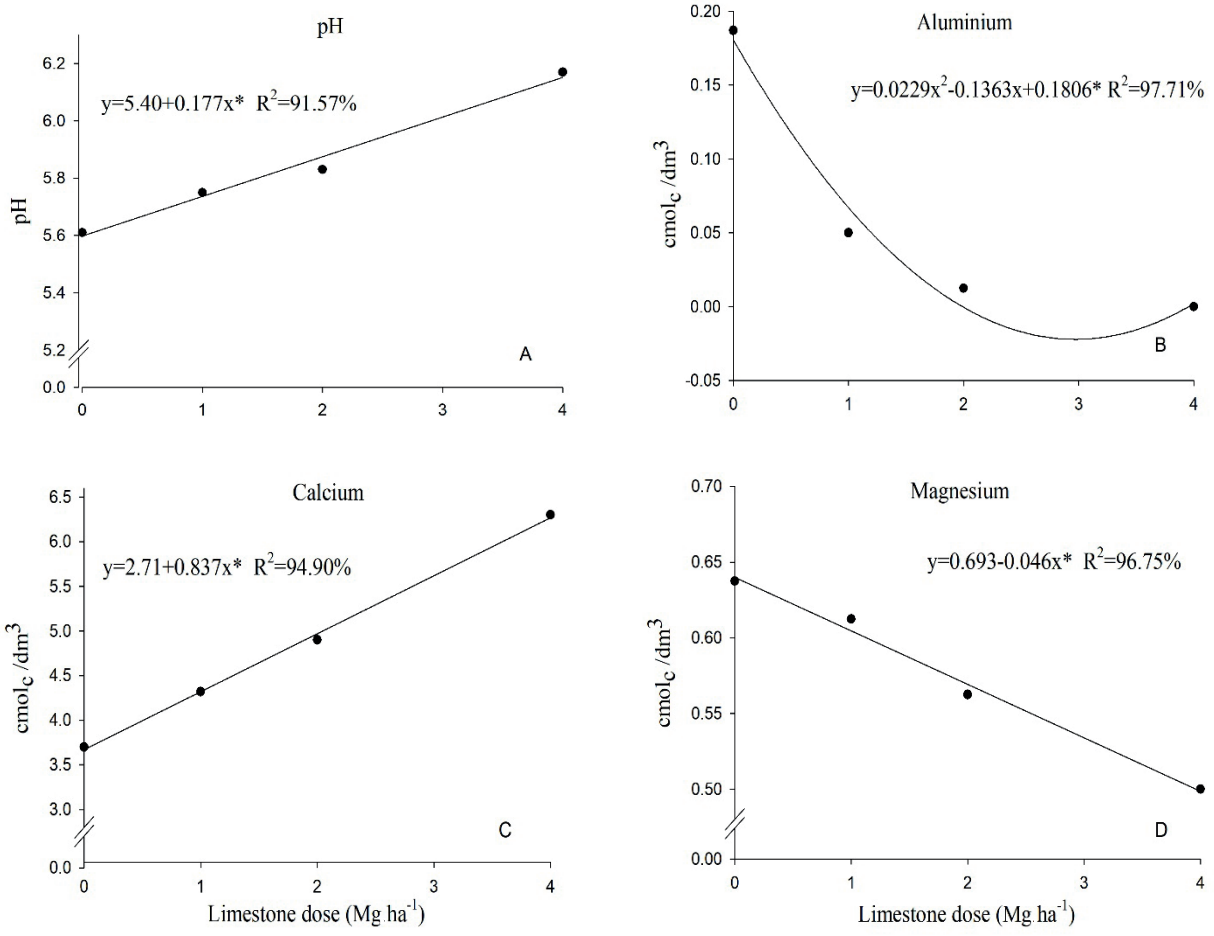

Figure 1. pH values (A), Al^{+3} (B), Ca^{+2} (C) and Mg^{+2} (D) contents, as a function of calcined limestone doses. * Significant ($p \leq 0.05$).

production data could not be obtained. The delayed blooming is because of the Bola de Neve cultivar used, which requires cold temperatures to bloom (May et al. 2007), and during the experiment atypical periods occurred, with high temperature peaks (30 ºC) and a predominance of average temperatures (15 ºC) (Brasil 2016).

Significant differences were observed between the control types, regarding disease progress over time, which is represented by the AUDPC variable

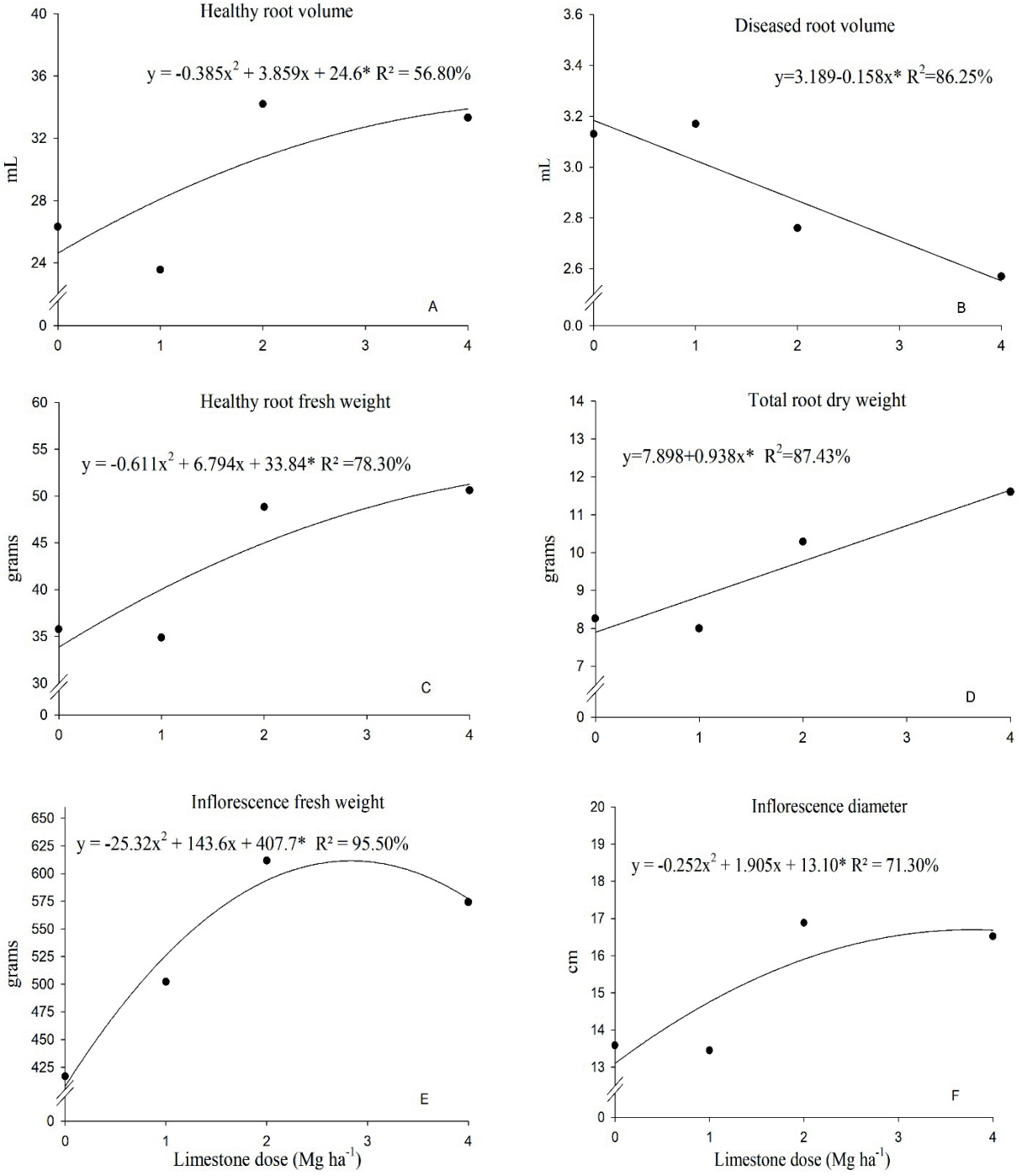

Figure 2. Volume of healthy (A) and galled (B) roots caused by *Plasmodiophora brassicae*, healthy root fresh weight (C), total root dry weight (D), inflorescence fresh weight (E) and diameter (F), as a function of calcined limestone doses. * Significant ($p \leq 0.05$).

(Table 1). In general, less severe symptoms of clubroot disease were observed in the treatment with cyazofamid, which resulted in significantly lower AUDPC values (Table 1). The use of biofungicide did not reduce the gall fresh weight or the AUDPC values, as the values were similar to those of the control (water). The diseased root volume was significantly lower in plants treated with cyazofamid, if compared to the control, and statistically equal to the root volumes observed with the biofungicide, which presented intermediate values (Table 1).

Liming led to a rise in pH and, although these values were below the recommended values for cauliflower, they generated significant increases in healthy root volume (with liming: 26.44 mL; without liming: 12.41 mL; CV: 16.11 %) and total root dry weight (with liming: 4.77 mL; without liming: 2.44 mL; CV: 22.97 %).

A significant effect was observed in the interaction between liming and the types of control on healthy root fresh weight and disease severity at 100 DAT (Table 2). Liming favoured the formation of healthy roots in both combinations and reduced the final disease severity in the treatments with biofungicide and control (water) (Table 2).

The difference in healthy root fresh weight among the control types was only observed in the plots without liming, as a higher mean was observed in the treatment with cyazofamid, when compared to the others (Table 2). In this situation, the treatment with *T. harzianum* was inferior to the control (water). In turn, the effect of the treatments on disease severity evaluated at 100 DAT was significantly more pronounced in the non-liming plots, which had higher disease development, except in the treatment with cyazofamid, which presented low disease severity, regardless of liming (Table 2). The treatments with cyazofamid and *T. harzianum*, when combined with liming, did not differ and led to lower disease severity. The treatment with cyazofamid without liming presented the lowest disease severity, differing from the others.

These findings, concerning disease severity and root development, especially the healthy root fresh weight, indicate that the use of the *T. harzianum*-based product did not aid in the control of clubroot in cauliflower nor in plant root development. However, the cyazofamid-based chemical product, although not conducive to root development, reduced the disease severity and seems to be a promising measure. Liming, in turn, led to an increase in the healthy root fraction expressed by the healthy root fresh weight and volume, in addition to favouring a reduction in disease severity.

The effect of liming probably derives from both its beneficial action on the neutralization of Al^{+3} and its phytotoxic effect on root development (Sousa et al. 2007, Bhering et al. 2017), as well as from its positive effect on the increase in pH and Ca^{+2} levels, which negatively affect the germination of *P. brassicae* spores and root infection by the

Table 1. Effect of the treatments cyazofamid (1.5 L ha^{-1}), *Trichoderma harzianum* (800 mL ha^{-1}) and control (water) on the volume and fresh weight of galled roots and area under the disease progress curve (AUDPC) values.

Treatment	Galled root volume (mL)[1]	Galled root fresh weight (g)[1]	AUDPC[1]
Cyazofamid	9.25 b*	5.60 b	294.43 b
T. harzianum	15.75 ab	13.73 a	1,809.84 a
Control	18.20 a	13.87 a	1,588.34 a
CV (%)	25.77	22.97	33.63

* Means followed by the same letter in the column do not differ significantly according to the Tukey test ($p \leq 0.05$). [1] Data transformed using $\sqrt{x + 1}$.

Table 2. Effect of the interaction between liming (2.54 Mg ha^{-1} of CaO), combined or not with cyazofamid (1.5 L ha^{-1}), *Trichoderma harzianum* (800 mL ha^{-1}) and control (water), for the control of clubroot, caused by *Plasmodiophora brassicae*, on healthy root fresh weight and disease severity, at 100 days after transplanting (DAT) seedlings, under greenhouse conditions.

Control type	Healthy root fresh weight (g)[1]		Severity at 100 DAT (%)[1]	
	With liming	Without liming	With liming	Without liming
Cyazofamid	36.27 Aa*	17.29 Ba	11.66 Ab	12.66 Ab
T. harzianum	31.85 Aa	3.49 Bb	25.00 Aab	73.33 Ba
Control	22.7 Aa	13.25 Bab	38.33 Aa	67.00 Ba
CV (%)	18.55		23.00	

* For each variable, means followed by the same uppercase letter in the row and lowercase letter in the column do not differ significantly according to the Tukey test ($p \leq 0.05$). [1] Data transformed using $\sqrt{x + 1}$.

pathogen (Webster & Dixon 1991, Niwa et al. 2008). In addition to the previously mentioned benefits, liming may also increase the soil biological activity (Sousa et al. 2007, Campos et al. 2013) and favour the occurrence of possible antagonists (Penalber 2009).

The practice of liming, however, despite the positive effects of disease reduction and improvements in plant development, is neglected in the study region. The low efficiency of the biofungicide in the biological control of the disease is not uncommon (Peng et al. 2011) and is due to different factors, such as environmental and soil microflora conditions and pathogen spore density and viability, among others, as shown in the present study. The low efficiency of *T. harzianum* and positive effect of a cyazofamid-based product in the control of clubroot are reported by Peng et al. (2011) in canola, although these authors also showed a promising effect of other antagonists such as *Bacillus subtilis* and *Gliocladium catenulatum*.

The low efficiency of the biological clubroot control may also be related to the reduced period of exposure of the pathogen to the antagonistic agents, from the germination of the resting spore to infection of the root hairs. According to Blum (2006), the more internal the pathogen is during the infection and colonization process, the less vulnerable it will be to the action of antagonists. In addition, many beneficial microorganisms commonly found in the rhizosphere and root cortex of most cultivated plants do not form associations with *Brassica* spp. roots (Usuki & Narisawa 2007). This characteristic may limit the use of traditional rhizosphere-colonizing microorganisms with potential for clubroot management (Gossen et al. 2013).

The chemical control, in turn, because it is not subject to these interactions, tends to present more stable results. However, the use of the same active principle in the control of a phytopathogen over time may result in loss of efficiency in the medium and long-term, due to the selection of variants resistant to the active principle used (Vale et al. 2004). Additionally, the use of chemicals may lead to problems of human and environmental contamination, in addition to the occurrence of residues in food (Soares 2010).

For the control of clubroot, as the disease is caused by a common soil pathogen, the adoption of integrated and preventive measures is suggested, including the use of healthy and quality seedlings, crop rotation with non-host plants, in addition to soil acidity correction, and elevation of calcium levels in the soil. Furthermore, liming is important for cauliflower, because it is a high soil fertility-demanding crop, and it is more suitable for soils with pH ranging from 6.0 to 6.8, and poorly tolerant to Al^{3+} phytotoxicity (May et al. 2007). In the present study, liming was found to be beneficial in reducing the severity of clubroot and favouring plant root development, compensating for root losses due to the disease.

CONCLUSIONS

1. The biofungicide is not efficient in controlling clubroot and does not favour cauliflower root and inflorescence development, either alone or in combination with liming;
2. Liming increases the volume and weight of healthy roots and the fresh weight of inflorescences and reduces the volume of galled roots;
3. The fungicide cyazofamid reduces the progress and severity of clubroot independently of liming.

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