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Article

The Occurrence of Clubroot in Colombia and Its Relationship with Climate and Agronomic Practices

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Abstract: Clubroot, caused by *Plasmodiophora brassicae,* is a major disease of cruciferous crops in Colombia. Limited information is available, however, regarding its distribution or epidemiology in this country. The objectives of this study were to determine the occurrence of clubroot in the main regions of Colombia where cruciferous crops are grown, and to examine the relationship between pathogen inoculum density and the likelihood of field infestation with crop management practices and climatic information. In total, 127 fields were surveyed across eight departments, the pathogen inoculum density was estimated, climatic information was obtained, and farmers were surveyed on their crop management practices. More than half (53.7%) of the fields visited were found to be clubroot-infested and pathogen DNA was detected in 91.3% of the surveyed fields. The only department where clubroot symptoms were not observed was Nariño. In infested fields, *P. brassicae* inoculum density varied between 3×10^2 and 1×10^6 resting spores per gram of soil, with the highest inoculum density observed in Norte de Santander. All other departments had comparable spore loads. Inoculum density positively affected the likelihood of infestation of a field, and both spore loads and infestations were positively affected by the average temperature.

Keywords: clubroot; pathogen spread; *Plasmodiophora brassicae*; soilborne disease

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1. Introduction

Clubroot is one of the most important diseases of cruciferous crops worldwide, causing average yield losses of 10% to 15% [1]. The causal agent, *Plasmodiophora brassicae* Woronin, is a soilborne pathogen that infects host roots, resulting in the development of galls that impair the uptake of water and nutrients; as a result, infected plants may wilt and die when symptoms are severe [2]. The clubroot disease cycle consists of three main stages. During primary infection, resting spores in the soil germinate to produce primary zoospores that infect the plant root hairs, forming primary plasmodia. The plasmodia later cleave into zoosporangia that produce secondary zoospores, which are responsible for secondary infection. During this stage, the root cortex is infected, and clubroot symptoms become visible. In the last stage of the disease cycle, resting spores are produced and released back into the soil as the root galls decompose, serving as primary inoculum for future infections [2]. Previous studies have shown that between 1×10^7 and 1×10^{10} resting spores per plant can be produced in a single infection cycle [3–6]. The resting spores of *P. brassicae* are very resilient and can remain viable in the soil for many years, with an average half-life of about 4 years [4,7]. The durability of the resting spores represents a major challenge for clubroot management, since it is very difficult to eradicate the pathogen from a field once it has become established [8].

Clubroot incidence and severity are modulated by host genetics, environmental conditions, pathogen genetics and inoculum potential [9,10]. Clubroot resistance in *Bras-*

sica oleracea L. is genetically complex, mainly recessive, and difficult to use for hybrid development [11]. For this reason, the availability of clubroot-resistant cultivars of vegetable Brassicas is limited. In Colombia, only the cauliflower (*B. oleracea* L. var. *botrytis*) hybrids 'Clapton' (resistant to *P. brassicae* pathotypes 0, 1 and 3 according to Williams differential set (1966); [12]) and 'Clarify' (clubroot tolerant; [13], and the cabbage (*B. oleracea* var. *capitata*) hybrids 'Kilazol' and 'Tekila' (resistant to pathotypes 0, 1 and 3 according to Williams differential set (1966)) [12] are available to farmers. These hybrids were obtained by the introgression of a major clubroot resistance gene from *B. rapa* L. into *B. oleracea* [11].

Numerous environmental conditions affect clubroot development, the most widely studied being temperature, soil moisture, and soil properties including pH and nutrient content. Multiple studies have demonstrated that temperatures between 20 °C and 25 °C are optimal for root hair and cortical infection and favor greater clubroot severity [14–20]. Soil moisture is one of the most important factors affecting clubroot development, with disease incidence and severity increasing with higher moisture [21–24]. Soil pH can also have an important influence on clubroot development, with the disease generally more severe in acidic soils. Various studies have found that clubroot is favoured at pH values between 5.0 and 6.0, reduced at pH $\geq$ 7.0, and eliminated at pH > 8.0 [25–28]. Nevertheless, severe clubroot symptoms can sometimes occur in alkaline soils, particularly under high resting spore loads and favourable moisture and temperature [19,29–31].

On the pathogen side, the inoculum potential, defined as a function of the inoculum density and the effects of the environment upon it, is one of the main factors determining the occurrence and severity of clubroot [32–34]. Different authors have observed that disease incidence and severity in various crops increase with increasing pathogen inoculum density [33–35]. Inoculum densities between 1×10^3 and 1×10^5 resting spores plant^{-1} have been reported as the minimum required to cause disease in susceptible hosts [33,34,36]. Furthermore, an interconnected relationship between pathogen virulence, host resistance and inoculum density has been observed [36].

Given the importance of *P. brassicae* inoculum density in clubroot development, its quantification becomes important for disease management [37]. Multiple methods for the detection and quantification of *P. brassicae* have been developed, allowing the implementation of management practices such as exclusion, where susceptible cruciferous crops are avoided in infested fields [38]. Numerous PCR-based techniques have been developed for the detection and quantification of *P. brassicae*, including conventional PCR, quantitative PCR (qPCR), competitive positive internal control PCR (CPIC-PCR), propidium monoazide PCR (PMA-PCR), droplet digital PCR (ddPCR), and loop-mediated isothermal DNA amplification (LAMP) [39–44]. Independently of the technique, the success and reliability of the quantification methods depend on the sampling quality due to the patchy distribution of the pathogen in most fields [39].

In Latin America, clubroot has been reported in Mexico, Costa Rica, Guatemala, Bolivia, Venezuela, Brazil and Colombia. However, studies reporting the disease incidence, severity and inoculum density in any of those countries are scarce [45]. In Colombia, cruciferous vegetable crops, including broccoli, cabbage, and cauliflower, were grown on over 2600 ha in 2017 [46]. While clubroot can cause yield losses between 42.5% and 74.5% in Colombia [47], to our knowledge, there are no reports either on the distribution of the disease or on the *P. brassicae* inoculum density in the main regions of the country where cruciferous crops are grown. The objectives of this study were to determine clubroot prevalence and pathogen inoculum density in Colombia, and to evaluate their relationship with crop management practices and environmental conditions.

2. Materials and Methods

2.1. Sampling

In total, 127 fields were surveyed for the occurrence of clubroot between January and March of 2017. The survey included the departments of Cundinamarca, Antioquia, Nariño, Boyacá, Norte de Santander, Valle del Cauca, and Cauca, representing the major regions

where cruciferous crops are grown in Colombia (Table 1). The department where clubroot was first reported in Colombia in 1969, Caldas, was also included [48]. The number of surveyed fields in each department was based on the area cropped to cabbage, broccoli and cauliflower in 2016 [46]. A total of 42 municipalities were visited, 18 in Nariño, 7 in Cundinamarca, 6 in Antioquia, 4 in Boyacá, 3 in Valle del Cauca, 2 in Norte de Santander, 1 each in Caldas and Cauca (Figure 1). The municipalities and sampling points were selected based on information provided by local agronomists, who reported them as the most productive areas within each department. All sampling locations and altitudes were georeferenced with a smartphone Moto G (3rd generation) (Motorolla Mobility, Chicago, IL, USA) and the geocoordinates recorded using the mobile application MapIt Spatial [49].

Table 1. Area sown to cruciferous vegetable crops in Colombia in 2016 [46] and number of samples collected from each department in this study.

Department	Cabbage Area (ha)	% National Area	Broccoli Area (ha)	% National Area	Cauliflower Area (ha)	% National Area	Total Area Cruciferous Vegetables (ha)	% National Area	Number of Samples
Antioquia	551.70	38	197.00	45	81.8	17	831.33	35	29
Cundinamarca	318.50	22	311.36	71	100.91	21	731.70	31	35
Nariño	218.70	15	100.80	23	202.5	42	522.38	22	28
Norte de Santander	90.20	6	73.00	17	70	15	233.43	10	9
Valle del Cauca	221.76	15	0	0	0	0	221.91	9	10
Boyacá	87.49	6	27.10	6	2.5	1	117.21	5	10
Caldas	51.90	4	0.00	0	0	0	51.94	2	3
Cauca	4.00	0	23.00	5	10.5	2	37.56	2	3
Colombia total	1445.4	100	436.00	100	479.8	100	2363.16	100	127

Since Cundinamarca, Antioquia, and Nariño represented 88% of the area planted to cruciferous crops in Colombia in 2016 [46], between 28 and 35 samples were collected from each of those departments. The sampling points were located in the municipalities with the most production, and the distribution of points was adjusted to a grid previously designed using Google Earth to cover most of the cultivated area in each region. In Cundinamarca and Antioquia, the average distance between the closest points in the grid was 5 km, while in Nariño it was 10 km (Figure 2).

2.2. Clubroot Prevalence

If a cruciferous crop was growing in a field at the time of the survey, plants of that crop were evaluated directly for the presence of clubroot symptoms. If a different crop was being grown, cruciferous weeds found in the field were assessed for the presence of symptoms. The presence of clubroot was evaluated following a "W" pattern in each field. When cruciferous crops were grown, 20 plants were dug out from the soil and assessed for the presence of root galls, with 10 plants evaluated near the field entrance, and 10 more along the arms of the "W". When a different crop was grown, nine points were assessed along the arms of the "W" for the presence of cruciferous weeds; if they were found to occur, the cruciferous weeds were dug out and evaluated for clubroot symptoms. In either case, once the disease symptoms were observed, sampling was stopped, and the field was designated as clubroot infested. In fields where the farmer confirmed previous observation of the disease symptoms, plants were also evaluated at the patches where the disease had been observed before.

2.3. Soil Samples

A composite soil sample (200 g) was collected from each field. Briefly, nine subsamples were collected at a 20-cm depth along the arms of a "W" transect, placed in a bucket and mixed thoroughly. Two-hundred g of soil, representing a composite field sample was placed in a plastic bag, labelled, and transported to the National University of Colombia for further processing. The remaining soil was placed back in the field. The shovel, boots and implements used during the sampling process were cleaned with a 4% bleach solution to avoid cross-contamination among fields.

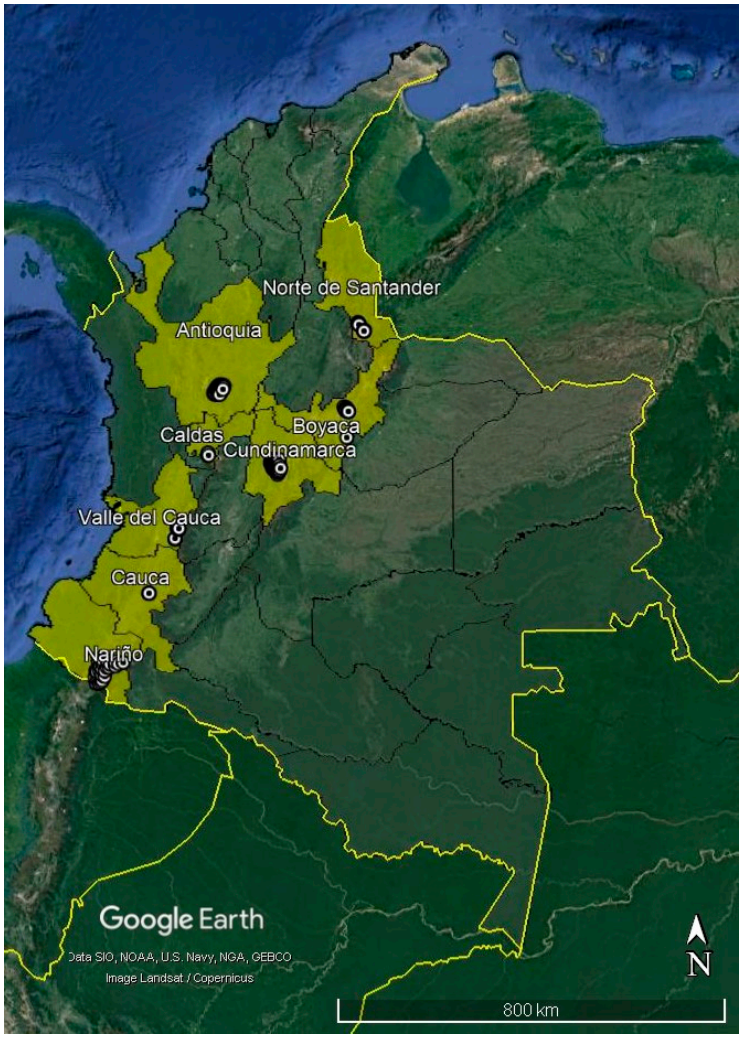

Figure 1. Location of fields surveyed for the presence of *Plasmodiophora brassicae* in Colombia in 2017. The departments where samples were collected are highlighted in light green with white dots indicating the sampled fields each department.

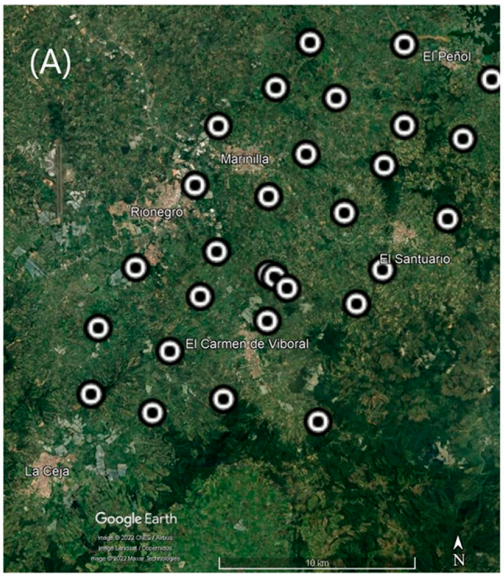

Figure 2. *Cont.*

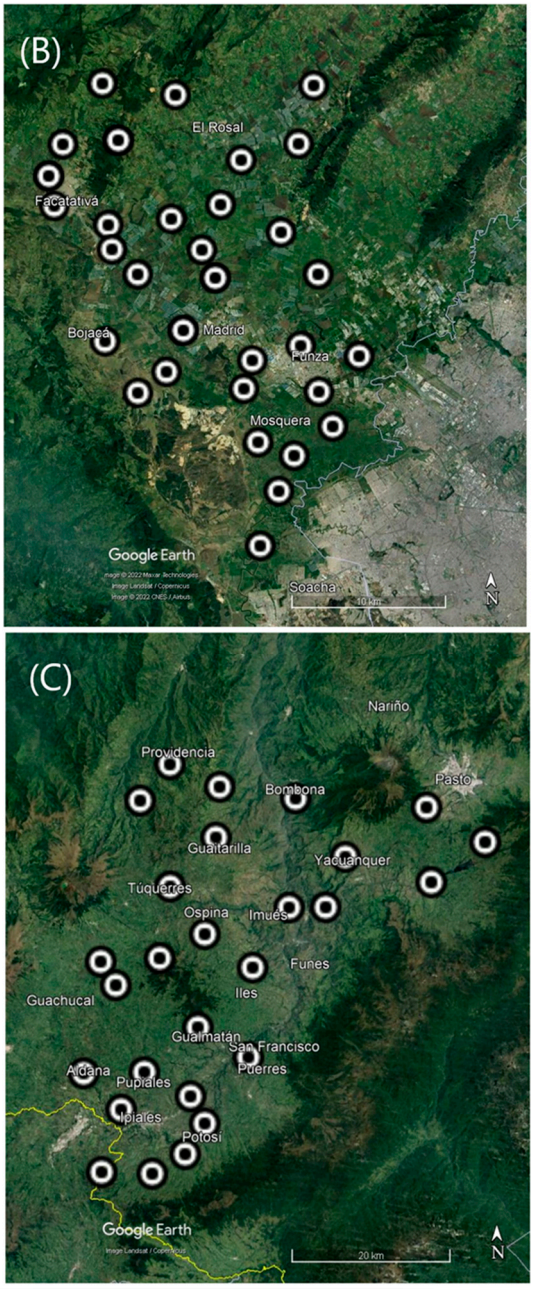

Figure 2. Sampling strategy in the departments of (**A**) Antioquia, (**B**) Cundinamarca, and (**C**) Nariño, Colombia. Each of the white points represents a location where a soil sample was collected for *Plasmodiophora brassicae* detection and quantification by conventional and quantitative PCR, respectively. Cruciferous crops and weeds (if present) were also visually examined for the presence of typical clubroot symptoms.

Once at the National University of Colombia, all soil samples were dried at room temperature and stored at −20 °C until processing. All samples were ground in a mortar with a pestle, which were washed with ethanol between samples, and one 250 mg subsample from each homogenized soil sample was used for DNA extraction as described below.

2.4. Crop Management Information

Information regarding crop management practices and clubroot disease history was obtained by interviewing the farmers in the fields visited. Farmers were asked if they were familiar with clubroot disease, and if not, photographs of typical symptoms were shown, and they were asked again if they had observed it before. On the management strategies

side, farmers were asked how long they had been cultivating the field, the cropped area, the rotation scheme, the cruciferous species and cultivars planted, the propagation strategy, the machinery used and its provenance, the type and application frequency of liming materials and compost, and harvest residue management. In total, 98 farmers were interviewed, since in some cases it was not possible to contact the field owner or tenant.

2.5. Climatic Information

Climatic information was retrieved from the weather station closest to each sampled field. The dataset obtained consisted of the historical normalized data from 1982 to 2010 [50]. Data included average temperature, maximum temperature, minimum temperature, annual precipitation, and number of rainy days per year.

2.6. DNA Extraction and P. brassicae Quantification

Genomic DNA was extracted from 250 mg of each soil sample using a DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions, with the only modification in the protocol being a reduction in the volume of the final elution buffer from 100 µL to 50 µL. The concentration and purity of the DNA were evaluated with a NanoDrop ONE (TermoFisher Scientific, Waltham, MA, USA).

For qPCR analysis, all DNA samples were diluted tenfold except for those where the DNA concentration was <15 ng·µL^{-1}, in which case undiluted DNA was used. All qPCR samples were analyzed in triplicate. Quantification of *P. brassicae* DNA in the soil samples was conducted by qPCR with the primers DR1F and DR1R as per Rennie et al. 2011 [40] in a LightCycler 480 (Roche Diagnostics Corp, Indianapolis, IN, USA). Estimation of the number of resting spores per sample was completed by comparison with a standard curve generated with DNA extracted from known quantities of resting spores [40]. After each qPCR run, a melting point analysis was conducted to identify the amplified product.

2.7. Statistical Analysis

All statistical analyses were conducted using R Studio [51]. Inoculum density was log-transformed and a linear model was fitted to evaluate the differences among departments using the package *nlme* [52]. Geographically weighted regression models were fitted using the *glm* function to assess the effect of management practices on *P. brassicae* inoculum density. Also, assuming a binomial distribution of the response variable, binary logistic geographically weighted regression models were fitted to assess the effect of different management practices and pathogen inoculum densities on the likelihood that clubroot symptoms were observed in a field.

To assess the differences among departments in the climatic variables, linear models were fitted using the *lm* function, and means separation was done by a Tukey's test at 95% using the function *cld* from the package *lsmeans* [53].

3. Results

3.1. Clubroot Infestation

The prevalence of clubroot was established within the departments where most of the cruciferous crops are grown in Colombia. Those fields where disease symptoms were observed in any host plant or where the farmer reported its occurrence in previous cycles of cruciferous crops were regarded as clubroot-infested. Clubroot was present in 53.6% of the sampled fields where cruciferous crops were grown, including 48.8% where the disease was observed directly by the researchers and 4.8% where the farmer reported the disease in previous crop cycles. Clubroot was detected in all departments visited except for Nariño (Table 2).

Table 2. Number of surveyed fields for clubroot presence in Colombia by department.

Department	Number of Surveyed Fields	Fields with Cruciferous Crops at the Time of Visit		Fields Where Resistant Hybrid * Cultivars Were Grown		Fields Where Clubroot Symptoms Were Observed		Fields Where *Plasmodiophora brassicae* DNA Was Detected	
		Number of Fields	%	Number of Fields	%	Number of Fields	% **	Number of Fields	%
Antioquia	29	17	58.6	10	34.5	6	20.7	25	86.2
Cundinamarca	35	19	54.3	1	2.9	10	28.6	31	91.9
Nariño	28	12	42.9	0	0.0	0	0.0	25	89.3
Norte de Santander	9	9	100.0	0	0.0	8	88.9	9	100.0
Valle del Cauca	10	10	100.0	0	0.0	7	70.0	10	100.0
Boyacá	10	9	90.0	0	0.0	5	50.0	10	100.0
Caldas	3	3	100.0	0	0.0	2	66.7	3	100.0
Cauca	3	3	100.0	0	0.0	2	66.7	3	100.0
Total	127	82	64.6	11	8.7	40	48.8	116	91.3

* Hybrid cabbage 'Tekila'. ** Estimated percentage of infestation was based on the number of fields where cruciferous crops were grown.

3.2. Inoculum Density and Relationship with Clubroot Infestation

Inoculum (DNA) of *P. brassicae* was detected in 116 of the 127 sampled fields (91.3%). The only samples testing negative included four from Antioquia, four from Cundinamarca, and three from Nariño. Those fields where the pathogen was detected had inoculum densities between 3.08×10^2 and 1.12×10^6 resting spores per gram of soil. Inoculum density was different among departments (p-value = 0.02). The lowest average inoculum density was found in the department of Boyacá (3.4×10^3 resting spores g^{-1} of soil), followed by Caldas (4.0×10^3 resting spores g^{-1} of soil), Antioquia (4.1×10^3 resting spores g^{-1} of soil), Cundinamarca (4.9×10^3 resting spores g^{-1} of soil), Cauca (5.0×10^3, resting spores g^{-1} of soil), Nariño (7.0×10^3 resting spores g^{-1} of soil) and Valle del Cauca (7.3×10^3 resting spores g^{-1} of soil) (Table 3). The highest inoculum density was found in the department of Norte de Santander (1.1×10^6 resting spores g^{-1} of soil).

Table 3. Average, minimum, and maximum inoculum densities in 127 soil samples collected from the main regions producing cruciferous crops in Colombia in 2017.

Department	Average (Resting Spores g^{-1} of Soil)	Minimum Inoculum Density in Positive Samples (Resting Spores g^{-1} of Soil)	Maximum Inoculum Density in Positive Samples (Resting Spores g^{-1} of Soil)	Number of Samples Negative for *Plasmodiophora brassicae*
Boyacá	3.4×10^3	8.4×10^2	1.0×10^4	0
Caldas	4.0×10^3	2.2×10^3	5.8×10^3	0
Antioquia	4.1×10^3	1.5×10^3	3.6×10^4	4
Cundinamarca	4.9×10^3	3.0×10^2	1.3×10^5	4
Cauca	5.0×10^3	2.9×10^3	6.2×10^3	0
Nariño	7.0×10^3	2.0×10^3	2.1×10^4	3
Valle del Cauca	7.3×10^3	4.0×10^3	2.2×10^4	0
Norte de Santander	1.6×10^5	1.6×10^3	1.1×10^6	0

The binary logistic regression model indicated that the inoculum density positively and significantly predicted the probability of observing clubroot symptoms in an infested field (p-value < 0.001). The model also showed differences among departments with respect to the probability of identifying an infested field (p-value < 0.001); the departments with the lowest odds of finding disease symptoms were Nariño and Antioquia.

3.3. Effect of Management Practices and Weather Conditions on Field Infestation and Inoculum Density

Field infestation by clubroot was affected by *P. brassicae* inoculum density (p-value < 0.001), and previous history of cruciferous cropping in the field (p-value < 0.001). In contrast, pathogen inoculum density was affected only by field infestation (p-value = 0.0074), and marginally affected by the cultivation of resistant cultivars (p-value = 0.05).

Disease symptoms were not observed in any of the 11 fields where resistant 'Tekila' cabbage was grown, Table 2; however, *P. brassicae* DNA was detected in all of the fields with inoculum densities between 1×10^3 and 1×10^4 resting spores per gram of soil (data no presented).

Among the climatic factors, only the average temperature had a significant effect on the likelihood of field infestation (p-value = 0.005) and inoculum density (p-value = 0.008). Both of these variables were positively affected by an increase in the average temperature.

3.4. Climatic Information

Visited fields were located between 1754 and 3163 m above sea level (masl). Average annual precipitation of the visited departments ranges between 765.7 mm and 2524 mm; the departments with the highest precipitation include Norte de Santander and Valle del Cauca, whereas the departments with the lowest precipitation are Antioquia and Boyacá. Mean average temperature in the visited departments was between 13.2 °C and 20.4 °C, statistical differences were found among departments, with Valle del Cauca being the warmest and Antioquia and Nariño the coldest (Table 4).

Table 4. Climatic data for the Colombian departments included in this study. The table presents historical normalized data from 1982 to 2010 [50], including annual precipitation, number of rainy days per year, and average, minimum and maximum temperatures. Data were obtained from the closest weather station to the sampled points in eight departments of Colombia.

	Annual Precipitation (mm)				Rainy Days per Year (Days)				Average Temperature (°C)				Minimum Temperature (°C)				Maximum Temperature (°C)			
Department	**Mean**	**Min**	**Max**		**Mean**	**Min**	**Max**		**Mean**	**Min**	**Max**		**Mean**	**Min**	**Max**		**Mean**	**Min**	**Max**	
Antioquia	775.5	567.5	880.9	a	151	115	174	ab	13.2	11.2	14.4	a	9.9	6.5	16.8	abc	20.7	16.0	25.2	b
Boyacá	793.2	548.6	972.4	a	146	92	176	ab	13.3	11.7	15.6	ab	7.4	6.8	8.9	a	19.1	16.1	22.1	ab
Cundinamarca	1394.0	728.0	2111.0	bc	172	88	236	bc	15.8	11.0	23.6	cb	11.2	5.8	19.1	bc	20.5	16.0	29.4	b
Caldas	765.7	728.0	784.5	ab	116	88	130	a	14.2	14.2	14.2	abc	6.0	6.0	6.0	ab	21.3	21.3	21.3	abc
Cauca	807.7	784.5	819.3	ab	145	130	152	abc	15.1	14.2	15.6	abcd	7.9	6.0	8.9	abc	21.8	21.3	22.1	abc
Nariño	1650.4	826.4	2699.6	c	206	154	279	d	13.6	11.0	17.0	a	10.0	7.1	13.5	abc	19.9	15.5	21.3	a
Norte de Santander	2273.0	2178.0	2606.0	d	204	194	238	cd	18.2	18.2	18.2	cd	12.9	12.9	12.9	cd	23.3	23.3	23.3	bc
Valle del Cauca	2524.0	1964.0	2606.0	d	239	238	241	d	20.4	17.0	24.1	d	15.2	12.5	18.8	d	25.7	22.2	29.5	c

Different letters are different according to Tukey's test at $p < 0.05$.

3.5. Cruciferous Crops in Colombia and Management Practices

Our survey found that cruciferous crops in Colombia are mostly grown in small areas with a national average of 3 ha. At the time of our survey, the most grown cruciferous crops included green cabbage, red cabbage, broccoli, and cauliflower; most of the cultivated varieties were clubroot susceptible, except for the clubroot resistant cabbage 'Tekila' that was mostly grown in Antioquia. In terms of machinery use, 63.5% of the farmers used rented equipment for soil preparation, but none of them washed or disinfected the machinery before preparing the soil, increasing the risk of pathogen spread.

Our survey allowed the identification of four main rotation schemes: (i) fields where cruciferous crops are continuously grown with two cycles per year (1% of the fields); (ii) cruciferous crops are grown once a year (4% of the fields); (iii) cruciferous crops are grown once every two years (32% of the fields), and (iv) cruciferous crops are grown every two years or longer (63.3% of the fields).

4. Discussion

To our knowledge, this is the first clubroot survey ever conducted in Latin America. The survey confirmed clubroot infestation in all departments where cruciferous crops are grown in Colombia. These results expand on previous reports from Jaramillo and Diaz (2006) [54] and Torres (1969) [48], who confirmed the presence of the disease in Cundinamarca, Antioquia and Caldas, and documented for the first time the occurrence of clubroot in Norte de Santander, Cauca, Valle del Cauca, Nariño, and Boyacá.

While no symptoms of clubroot were observed in any of the fields visited in Nariño, *P. brassicae* DNA was detected in multiple soil samples from that department. The inoculum density in samples collected from Nariño ranged from 2×10^3 up to 2×10^4 resting spores per gram of soil. Nevertheless, those inoculum densities where low and towards the lower end of the range required for symptom development, particularly under field conditions [55]. Interestingly, these densities were not different from the levels observed in Antioquia, Boyacá, Cundinamarca, Caldas, Cauca, and Valle del Cauca. These results

suggest that environmental conditions in Nariño are not as conducive for clubroot development; therefore, this department should be studied further because it shows promise for the cultivation of cruciferous crops.

When the environmental conditions in Nariño were analyzed, it was observed that, in addition to having the highest altitude, this department had the highest number of rainy days per year (206 days), and the lowest average temperature (13.6 °C) and maximum average temperature (19.9 °C) among those surveyed. Of these variables, only average temperature was found to affect pathogen inoculum density and the likelihood of field infestation. The low temperatures in Nariño may explain the absence of symptom development under the observed inoculum densities, since studies have demonstrated that temperatures <17 °C cause delays in the onset of symptoms [16–18].

Our study showed that the likelihood of field infestation increased at higher inoculum densities and with a previous history of cruciferous crop cultivation. These results are consistent with earlier research indicating that the continuous cropping of susceptible host species increases disease severity as well as spore loads in the soil [56–59]. Furthermore, despite the apparent longevity of *P. brassicae* resting spores, recent studies suggest that spore numbers can decline by up to 90% following a 2-year break from a host crop; the spore density then stabilizes [57,60], resulting in a Type III survivorship curve [61]. This result confirms that to maintain and/or increase spore densities in the soil at levels sufficient to cause disease, cruciferous crops should be grown regularly in infested fields. Otherwise, spore loads will eventually fall below the level required to cause disease. Work with canola indicates that the cropping of clubroot-resistant varieties will result in much smaller contributions of new spores to the soil, relative to susceptible varieties [4], although these may be enriched for resistance-breaking pathotypes [60].

Hwang et al. (2011) [55] reported that for consistent clubroot symptom development under highly conducive conditions, a minimum inoculum density of 1×10^3 resting spores per gram of soil is required. In our study, clubroot symptoms were observed in fields with spore loads as low as 3×10^2 resting spores per gram of soil. In general, the spore densities in the Colombian samples were lower than those reported from Canada (10^3–10^8 resting spores per gram of soil) [44,62], China (10^4–10^7 resting spore per gram of soil) [42] and Poland (1×10^3–7.7×10^8 resting spores per gram of soil) [63]. These results suggest that environmental conditions in Colombia are more conducive for clubroot development, and thus lower spore loads are required to cause more severe disease symptoms, or that an improved sampling strategy should be designed for future surveys to account for the pathogen patchiness in the field. The ability to detect the clubroot pathogen in pooled samples can diminish due to inoculum dilution effects, for example, if uninfested subsamples are pooled with mildly infested ones [39]. Since ours were composite samples, it is likely that inoculum densities in the infested patches are higher than what was estimated, due to unaccounted variability among soil cores [64].

5. Conclusions

This research indicated a widespread presence of clubroot in the departments producing most of the cruciferous crops in Colombia and provided the first estimates of *P. brassicae* soil resting spore densities in Colombia and Latin America.

Our results showed that at least half of the surveyed fields (53.7%) were clubroot infested. Furthermore, the pathogen DNA was detected in 91.3% of fields, and the estimated inoculum densities ranged between 3×10^2 and 1×10^6 resting spores per gram of soil. Those inoculum densities appear to be lower compared with reports from other countries, indicating either that environmental conditions in Colombia are more conducive for clubroot development, or that an improved sampling strategy should be designed for future surveys to account for the pathogen patchiness in the field. It is clear that *P. brassicae* inoculum is well established in Colombia, and that farmers must consider this when growing crucifers and selecting crop rotations.

Additionally, it should be noted that the pathogen was also detected in some fields where symptoms were not observed or reported. That was the case for the fields surveyed in Nariño, where clubroot symptoms were not observed, suggesting that environmental conditions in this department are not conducive for clubroot development, and therefore it should be further studied for potential production of cruciferous crops.

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