

Exploring Silicon Dose Effects On Disease And Cadmium Control In CCN-51 Cacao (*Theobroma Cacao*) Via Image Processing

Favio Eduardo Herrera Eguez¹, Carolina Giselle Herrera Eguez², Wilson Andrés Ramírez Montesdeoca³, Luis Emilio Beltrán Muñoz⁴

¹Escuela Superior Politécnica de Chimborazo, fherrerae@uteq.edu.ec, <https://orcid.org/0000-0003-1376-423X>,

²Escuela Superior Politécnica de Chimborazo, <https://orcid.org/0000-0003-2632-7426>

³Universidad Técnica Estatal de Quevedo. Los Ríos, Ecuador, <https://orcid.org/0009-0008-5785-6789>

⁴Universidad Técnica Estatal de Quevedo. Los Ríos, Ecuador, <https://orcid.org/0000-0003-4829-2252>

Abstract

The CCN-51 variety of cocoa (*Theobroma cacao*) stands out as one of the most profitable crops in Ecuador. However, its production is threatened by diseases such as moniliasis (*Moniliophthora roreri*), witches' broom (*M. perniciosa*), and black pod (*Phytophthora* sp.), which adversely affect its yield. In this context, the inclusion of silicon in the phytosanitary practices of CCN-51 cocoa emerges as a viable alternative to improve the crop's phytosanitary condition. The main objective of this research was to assess the impact of different silicon doses on the phytosanitary management of cocoa using image processing. The trials were conducted in a 15-year-old CCN-51 cocoa plantation, employing a completely randomized block design (CRBD) that included the following treatments: T1 (N-P-K), T2 (100 kg/ha of SiO₂), T3 (200 kg/ha of SiO₂), and T4 (300 kg/ha of SiO₂). Two silicon applications were performed during the trial. Agronomic variables, including yield, were examined. Additionally, the severity of moniliasis, witches' broom, and black pod was assessed using visual scales and the Leaf Doctor software. At the conclusion of the study, it was determined that the application of silicon at a dose of 100 kg/ha (T2) had a positive impact on the health of CCN-51 cocoa pods, exhibiting the highest number of healthy pods. This treatment showed lower incidence of moniliasis and black pod, although no significant differences were observed in the incidence of witches' broom. Furthermore, this treatment demonstrated a higher productive response, with a yield of 2.19 kg in the evaluated plot, and a better Benefit/Cost Ratio (1.12) compared to other treatments. Additionally, the use of silicon can reduce cadmium levels in cacao crops.

Keywords: moniliasis, phytophthora, cadmium, Leaf Doctor.

INTRODUCTION

Cocoa is a tropical fruit, and its cultivation is found in the Coastal and Amazon regions. In Ecuador, the production is found in the provinces of Los Ríos, Guayas, Manabí, and Sucumbios (Guerrero, 2014; PROECUADOR, 2013). In the country, two types of cocoa are cultivated: CCN-51 Cocoa and the so-called Nacional Cocoa. The Nacional Cocoa is a Fine Aroma Cocoa known as 'Arriba' since colonial times. (Guerrero, 2014; PROECUADOR, 2013). CCN-51 is an Ecuadorian-origin cocoa cloned on June 22, 2005. Differences exist between CCN-51 and Nacional cocoa, from production to export. CCN-51 is utilized by mass-market chocolate industries, while Nacional cocoa is preferred by countries seeking higher quality (ANECACAO, 2010). CCN-51 is preferred due to its resistance to diseases like moniliasis (*Moniliophthora roreri*), witches' broom (*M. perniciosa*), and black pod (*Phytophthora* sp.) compared to Nacional Cocoa.

Moniliasis is caused by the basidiomycete fungus *Moniliophthora roreri*. Its conidia function as sexual spores, serving roles in reproduction, dissemination, and resistance. The mycelium forms a pseudoestroma, 2 to 3 mm thick, on fruit lesions and in the environment, covered by a dense layer of cream-colored powdery spores. The mycelium is hyaline, branched, with septa exhibiting typical dolipores of basidiomycetes (Sánchez-Mora *et al.*, 2015). The life cycle begins with the survival and resistance of the pathogen in harvest residues or cobs contaminated by the fungus. Subsequently,

conidia spread through various factors such as wind and water. Spores move involuntarily during cultural and harvesting practices, leading to new infections. Once conidia land on a healthy fruit, they germinate in the presence of water or perish due to excess sunlight. Upon germination, they can penetrate directly into the fruit's peel (Phillips-Mora, 2006). Infection progresses to central tissues, including seeds, initiating necrosis development from the inside to the epidermis. Externally, it appears as very small circular oily spots that evolve into irregular yellow and brown lesions or spots. The process from infection to spot appearance lasts approximately 60 ± 10 days. Between 3 and 4 days, white mycelium develops on the lesions, followed by the appearance of spores, imparting a cream to brown color (Jaimes and Aranzazu, 2010). Symptoms manifest in the fruit depending on climatic conditions. The symptom is detected when a brown spot, also known as "chocolate spot," appears on the fruit (Bustos, 2017; Jaimes and Aranzazu, 2010; Phillips-Mora, 2006). In very young fruit (less than a month), chlorotic protrusions form with some deformation; before reaching half its normal size, the fruit undergoes necrosis. The almonds become a soft and watery mass (Sánchez-Mora *et al.*, 2015). Fruit infection occurs after 3 months, and they may remain asymptomatic or show limited wounds, indentations, surrounded by areas of premature ripening. Subsequently, a reddish-brown necrosis appears when internal tissues show necrosis (Sánchez-Mora *et al.*, 2015).

Frostry pod (*Phytophthora* sp.) causes significant damage to fruits (as well as various parts of the plant) (Phillips-Mora and Cerda, 2009). According to the Colombian Agricultural Institute (ICA, 2012), seven pathogenic species have been identified in cocoa plantations: *P. palmivora*, *P. megakarya*, *P. capsici*, *P. citrophthora*, *P. nicotianae* var. *Parasitica*, *P. megasperma*, and *P. arecae*. The life cycle of *Phytophthora* is dependent on environmental conditions. The cycle begins when the sporangium emerges under optimal humidity and temperature conditions (15° - 38° C), releasing zoospores. Zoospores are mobile structures with a short lifespan and two flagella, serving two fundamental roles: transmitting the pathogen from one host to another and guiding the pathogen to the infection site or host (Jaimes and Aranzazu, 2010). The disease develops rapidly during colder seasons. Once the fruit is infected, symptoms appear within five days as dark brown spots anywhere on the cob. After a few days, the brown or coffee-colored spot covers the entire fruit, and white mycelium begins to form. After several days of colonization and maturation, it can produce more spores and infect new fruits (Phillips-Mora and Cerda, 2009). *Phytophthora* sp. goes through various phases during the disease cycle, including mycelium, sporangia, zoospores, and chlamydospores formation. The primary inoculum (in various parts of the plant) forms sporangia that germinate in humid conditions to establish infection. The pathogen's resistance persists in the soil in the form of chlamydospores. These can remain for nine months in the case of *P. palmivora* and 18 months in *P. megakarya* (Sánchez-Mora *et al.*, 2015). The disease is diagnosed based on features and symptoms such as the chocolate-brown color on the cob. Over time, this color extends and completely covers all fruits. The pathogen invades the internal tissue, causing discoloration and rotting of the almonds, which remain undamaged for several days until they eventually decay (Jaimes and Aranzazu, 2010; Phillips-Mora and Cerda, 2009; Sánchez-Mora *et al.*, 2015). Infection also occurs in seedlings through young leaves and stems resembling burning. Additionally, *P. palmivora* and *P. megakarya* infect the stem, floral cushions, and suckers, leading to the formation of cankers as they sometimes remain hidden in the bark (Sánchez-Mora *et al.*, 2015). *Phytophthora* sp. causes cankers on the trunks of affected plants, developing into a brown necrotic area on the bark around the trunk. When the fungus encircles the trunk, it causes the total death of the crop (ICA, 2012; Jaimes and Aranzazu, 2010). In the roots, necrosis occurs, giving the appearance of a brown-colored spot. Invading the root perimeter, the root dries up, affecting the communicating vessels, which cease to absorb nutrients and water, leading to the death of the tree (ICA, 2012).

Witches' broom (*Monilophthora perniciosa*) occurs in various parts of the cultivation, such as shoots, floral cushions, cobs, or grains (Osorio-Solano *et al.*, 2012). This pathogen exhibits morphologically variable and dependent mycelium. The sexual spores or basidiospores are white, formed on basidia in inseparable lamellae, and have a short life. As the brooms dry, the saprophytic phase begins, forming thinner hyphae. Basidiocarps and spores develop during alternating dry and humid periods, and

structures called chlamydospores are also formed (Jaimes and Aranzazu, 2010). The National Health, Safety, and Agri-Food Quality Service (SENASICA, 2013) states that infection development occurs when the tissue is active. *M. pernicioso* is characterized by two phases. The first attacks young tissues, causing hypertrophy and hyperplasia, living as an obligate intracellular parasite. The second phase occurs when the tissue dies, and the fungus grows as a saprophyte. In fruits, the incubation time of the fungus lasts approximately six weeks. From its appearance, the bark and cob's pith begin to grow due to hormonal imbalance. Tissues with the witch's broom symptom result from the loss of apical dominance, forming axillary shoots that give it the well-known appearance. These remain green when the infected tissues appear but only for a short period before drying up and turning brown. When the tissue dies, the mycelium fragments forming chlamydospores. After four weeks of incubation, basidiocarps form (Sánchez-Mora *et al.*, 2015; SENASICA, 2013). *M. pernicioso* causes various symptoms in the plant, depending on the affected part and its developmental stage (ICA, 2012; Jaimes and Aranzazu, 2010; SENASICA, 2013). The symptoms are most noticeable in areas where they develop, such as branches, floral cushions, and fruits. Broom formations on branches develop forming internodes. When this disease attacks floral cushions, cobs with vegetative shoots do not emerge. This pathogen survives in the dry season in brooms and fruits adhering to the tree and becomes reactivated when the rains arrive in the form of basidiocarps (ICA, 2012). The disease also originates from infected seeds, causing hypertrophy at the base of the hypocotyl, seedling weakening, leaf chlorosis, very thin and parchment-like leaves, brittle leaves, and leaf necrosis. In fruits, the symptoms vary depending on the age and phenotype of the fruit. They exhibit irregular and elongated dark-colored necrosis. Most fruits show premature ripening, with yellow areas on green cobs (Sánchez-Mora *et al.*, 2015).

The control of diseases relies on chemical controls which tend to be detrimental to the environment. Recently silicon has been adopted in several crops as an alternative to reduce the disease. Silicon (Si) stands as the Earth's second most prevalent element, comprising 28% of the terrestrial crust (Quiroga, 2016). Its ability to form various SiO₂ structures holds agricultural promise, particularly in crops like rice and sugarcane (Álvarez and Osorio, 2014; SEPHU, 2009). Photosynthetic organisms absorb silicon in a soluble form, contributing to growth, strength, and defense mechanisms (Quero, 2009). Silicon influences plant growth, especially under stress conditions (Snyder *et al.*, 2001). Fontão de Lima's research (2010) reinforces silicon's role in enhancing crop resilience to environmental, soil, and biological challenges. Silicon enhances resistance to both biotic and abiotic stress (SEPHU, 2009). Studies confirm silicon's potential to boost pest and disease resistance, showcasing inhibitory effects on pathogen growth in avocados (Bekker *et al.*, 2007). Once integrated into cell walls, silicon remains immobile, fortifying cells against diseases. Foliar silicon application at the initial signs of disease presents significant benefits (Mejisulfatos, 2010). There are abundant sources of silicon. There are several examples like diatomite, calcium silicate, sodium/potassium/magnesium silicates, orthosilicic acid, and hydrated silica dioxide. (RedAgricola, 2017). This comprehensive understanding of silicon's diverse applications underscores its potential significance in agricultural practices.

There are several methods to evaluate diseases in cacao. Traditional visual assessment scales have limitations due to evaluator fatigue and potential variability in data interpretation (Nutter Jr., 1993). The image processing software Leaf Doctor reduces researcher bias in the Leaf Doctor software (Pethybridge and Nelson, 2015). The cellphone app is aimed to quantitatively assessing plant diseases, utilizes color images to distinguish between healthy and diseased tissues, providing a percentage of disease severity. The user taps a leaf area in a photograph, activating a circular magnifying lens to specify a distinct healthy tissue color. The algorithm evaluates each pixel's color, determining its proximity to specified healthy colors and assigning a healthy or diseased status. The Leaf Doctor software allows flexibility by accommodating up to eight colors for "healthy" tissue, improving accuracy. Moreover, the software facilitates the observation of disease severity in the presence or absence of different disease colors through sampling (Barbedo, 2014). This innovative method not only overcomes the drawbacks of traditional assessments but also enhances precision and efficiency in plant disease evaluations.

The elevated concentration of cadmium (Cd) in cocoa beans above the critical level (0.5 mg/kg) has caused serious concerns due to its association with chocolate consumption and its impact on human health. The accumulation of Cd in cocoa beans in Ecuador has been linked to soil contamination. From this perspective, it is crucial to seek mitigation strategies for this heavy metal (Rodríguez-Serrano *et al.*, 2018). Previous research states that 2 L/ha of potassium silicate can mitigate cadmium levels in cocoa plantations up to 72% (Estrada López, 2019; Gonzales Sobrados E.C., 2022).

The aim of this study is to assess the Leaf Doctor software's effectiveness in evaluating cacao diseases over a two-year period. To control the disease, various doses of silicon were applied to a 12-year-old cacao crop. We evaluated several agronomically relevant variables and conducted an economic analysis based on market prices to determine the profitability of using silicon as a tool. Finally, foliar and soil analysis were taken to compare cadmium levels in the system.

MATERIALS AND METHODS

Location

The research was conducted at the Experimental Farm "La María" of the Technical State University of Quevedo, located at Km 7 on the Quevedo - El Empalme road. The geographical coordinates of the site are 79° 27' West longitude and 01° 06' South latitude, with an altitude of 67 meters above sea level.

Experimental design

For the statistical design of the research, a randomized complete block design (RCBD) was employed, consisting of four treatments: control, 100 kg/ha SiO₂, 200 kg/ha SiO₂ and 300 kg/ha SiO₂ with three replications for each treatment (Sornoza, 2018). The research comprised a total of 12 experimental units, each containing 6 plants spaced 3 meters apart. The experimental units were rectangular in shape, measuring 4.25 meters in length and 9 meters in width, resulting in an area of 38.25 square meters per unit (Castillo, 2018). To compare the efficiency of the program a correlation analysis was used from the data retrieved by Leaf Doctor (Pethybridge and Nelson, 2015) and the disease scale for *Moniliophthora roreri* and *Phytophthora* sp. in cacao (Arteaga *et al.*, 2019; Pilaloe *et al.*, 2021).

Data was collected every week including several agronomic variables of interest such as number of pods, harvested pods, discarded pods, healthy pods, and diseased pods. Additionally, the count comprises healthy cherelles, diseased cherelles, the weight of cocoa beans in the pulp (grams), the weight of dried cocoa beans (grams), the weight of 100 seeds in the pulp (pounds), and the weight of 100 dried seeds (pounds) (Beltrán, 2021). The data was processed using the statistical software InfoStat (Di Rienzo *et al.*, 2010), and each evaluated treatment will undergo ANOVA tests and Tukey's multiple range tests with a significance level of 0.05%.

Agronomic Management

Prior to initiating the research project, controls were conducted for diseases such as Witches' Broom (*M. perniciosa*), employing Bordeaux mixture at a dosage of 50 g per 10 liters of water. Simultaneously, mechanical weed control was carried out using a brush cutter. Cultural practices involved sanitary pruning every 15 days, manually removing diseased pods due to Moniliasis (*M. roreri*) and Black Pod (*P. palmivora*), as well as pruning to control Witches' Broom (*M. perniciosa*). Additionally, weed control was performed every month across all treatments. Conventional and silicon fertilization was carried out twice, with N-P-K doses based on the minimum requirements for cocoa cultivation during production (Crespo and Crespo, 1997; Sornoza, 2018). The application dose was divided, with 60% in the first application and 40% in the second application, conducted four months into the research. Silicon and N-P-K were applied in a crown shape around plants at 45 cm from the stem, applied twice during the study. Fertilizer quantity for each treatment was measured and dosed using a balance. Weed control and pruning involved manual weed control with a machete and maintenance pruning with pruning shears. This included removing dry, diseased, or torn branches, parasitic plants, and trimming lateral branches that intersected with neighboring trees to achieve a balanced structure, improving crop radiation. Evaluation of treatments commenced at the beginning of the research, with weekly

measurements of variables. Healthy and diseased cherelles and pods were counted, distinguishing them by disease type (Moniliasis, Black Pod, and Witches' Broom). Subsequently, healthy pods were tallied.

Cadmium Evaluation

Prior and after the investigation a soil and tissue (leaves) sample were collected. For the determination of cadmium levels in cocoa tissues (leaves), the methodology used by Chavez *et al.* (2015) was followed. Samples of leaves were collected from a random selection of 6 plants. Between 15 and 20 medium-aged leaves were taken from each tree, considering leaves 3, 4, and 5 from each branch. The collected leaves were then washed with deionized water and rinsed three times with the same water to remove particles that could affect the analyses. Three repetitions per sample were considered, resulting in a total of 12 sub-samples for the experiment. For the laboratory analysis of cadmium, an acid digestion of the plant material was performed. Prior to digestion, 0.4 g of each sample (leaf) was weighed and placed in 100 ml capacity test tubes. Then, 8 ml of perchloric nitric acid ($\text{HNO}_3\text{-HClO}_4$) was added, along with an anti-foam tablet to each sample, to prevent complete evaporation during digestion and to ensure a sufficient volume for subsequent analysis. To determine the change in soil nutrient levels the samples obtained from each treatment were sent to the Soil and Water Management Laboratory at the "Pichilingue" Experimental Station of INIAP, located in Quevedo, for the analysis of the remaining chemical properties. The analyses conducted at the INIAP laboratory included: organic matter by the potassium dichromate method, sulfur (S) by the calcium phosphate method, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) by the modified Olsen method, and boron (B) by the curcumin method (USDA-NRCS, 2014).

RESULTS

Visual and Leaf Doctor Correlation disease rating evaluation for *Moniliophthora roreri*

To determine the damage of the *M. roreri* disease on cocoa pods, a correlation analysis was carried out between the visual evaluation and the Leaf Doctor program. There is a positive correlation with the R factor or Pearson factor which was 0.92 (Figure 1).

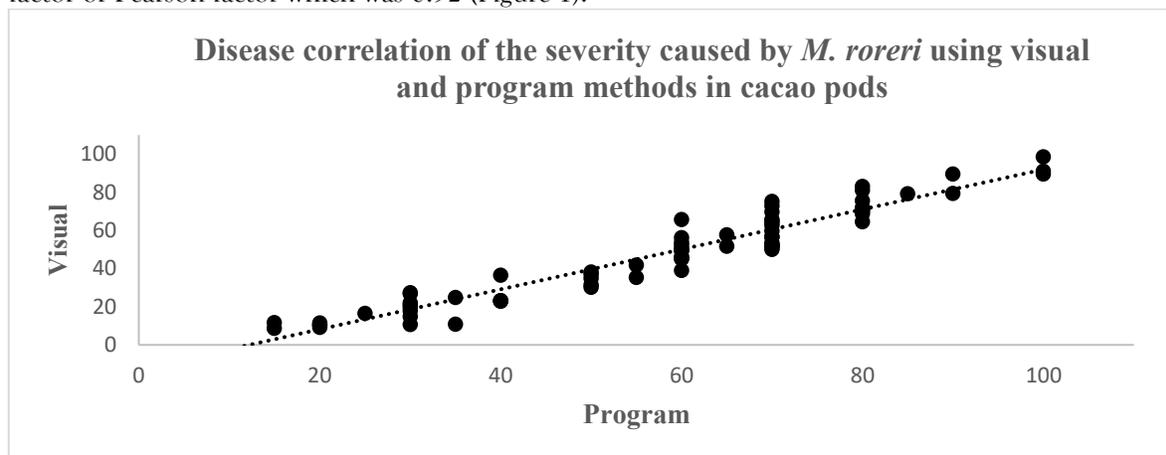


Figure 1. Correlation analysis between two methods of damage assessment in cocoa pods (*Theobroma cacao*) using visual damage assessment scales and the Leaf Doctor software for the disease caused by *Moniliophthora roreri*. $R^2 = 0.92$.

Comparison between disease rating methods for *Moniliophthora roreri*

To determine *M. roreri* severity, cacao pods from each treatment were harvested for 10 weeks. Identification of the pathogen was made using morphological characterization (Maridueña-Zabala *et al.*, 2016). One to four photos were taken to rate the complete cacao pods. After the analysis of variance, the disease rating was not improved with more than one picture per cocoa pod. The program provided a more accurate rating with less error and lower means without changing the statistical output. According to the analysis of variance (Tukey $P < 0.05$), the treatment that had the greatest visual damage

was treatment 1 (T1) with 10.20% damage and the least damage was in treatment 2 (T2) with 5.23%, while that treatment 3 and 4 had equal results using visual scales. Through the evaluation of the Leaf Doctor program, it shows us that treatment 1 (T1) obtained 10.93% damage, followed by treatment 2 (T2) with a lower percentage of 5.61% damage, as seen in Figure 2.

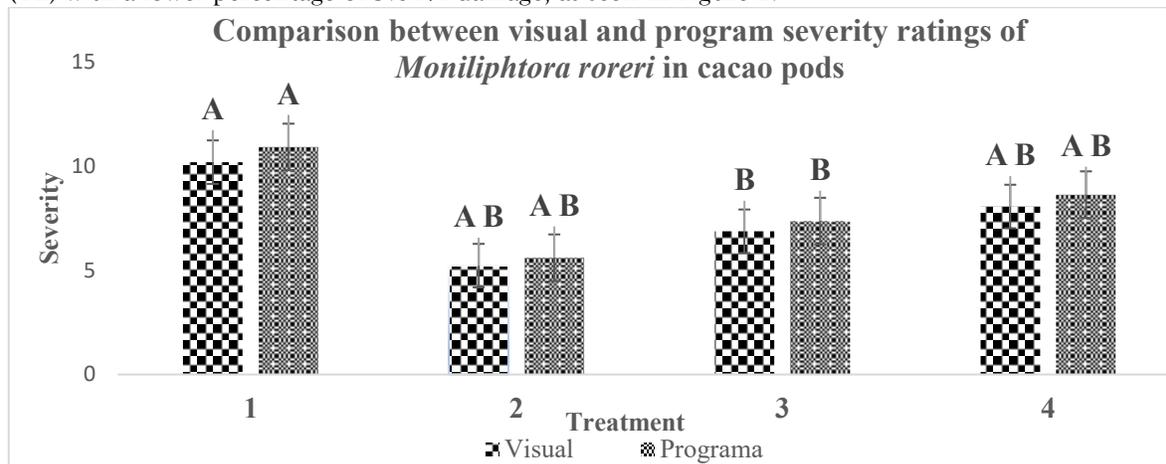


Figure 2. Comparison of the severity of *Monilophthora roreri* using visual assessment scales and Leaf Doctor software. Error bars indicate standard error. Different letters indicate significant differences between the averages presented by each treatment (Tukey $p < 0.05$).

Visual and Leaf Doctor Correlation disease rating evaluation for *Phytophthora* sp.

To determine the damage of the *Phytophthora* sp. disease, a correlation analysis was carried out between the visual evaluation and the Leaf Doctor program. In Figure 3 shows that there is a positive correlation between the two methods. The R factor or Pearson factor was 0.93.

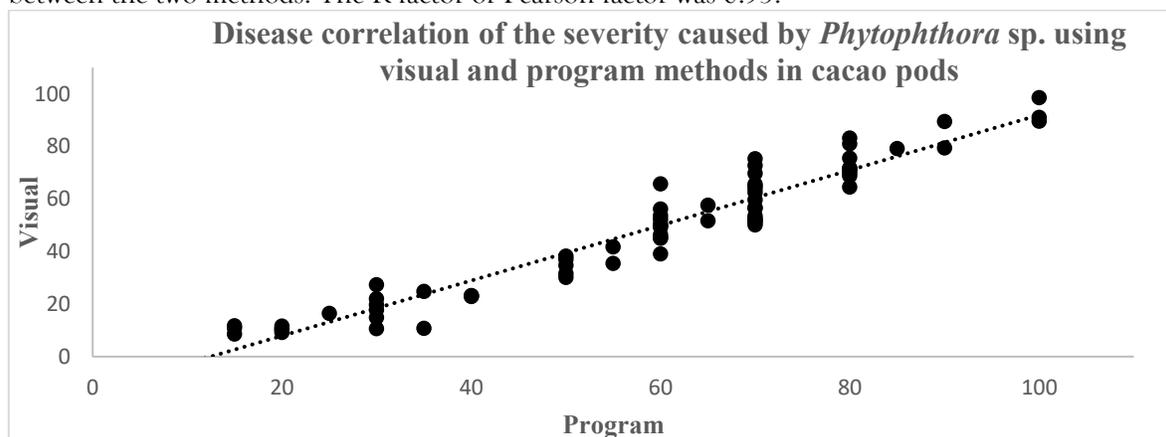


Figure 3. Correlation analysis between two methods of damage assessment in cocoa pods (*Theobroma cacao*) using visual damage assessment scales and the Leaf Doctor software for the disease caused by *Phytophthora* sp. $R^2 = 0.93$.

Comparison between disease rating methods for *Phytophthora* sp.

To determine *Phytophthora* sp. severity, cacao pods from each treatment were harvested for ten weeks. *Phytophthora* was recovered from the cacao pods using morphological identification (Kellam and Zentmyer, 1986). One to four photos were taken to rate the complete cacao pods. According to the analysis of variance, it shows us that in treatments T1, T2, T3 and T4 there was no significant difference since they obtained the same results as seen in Figure 4.

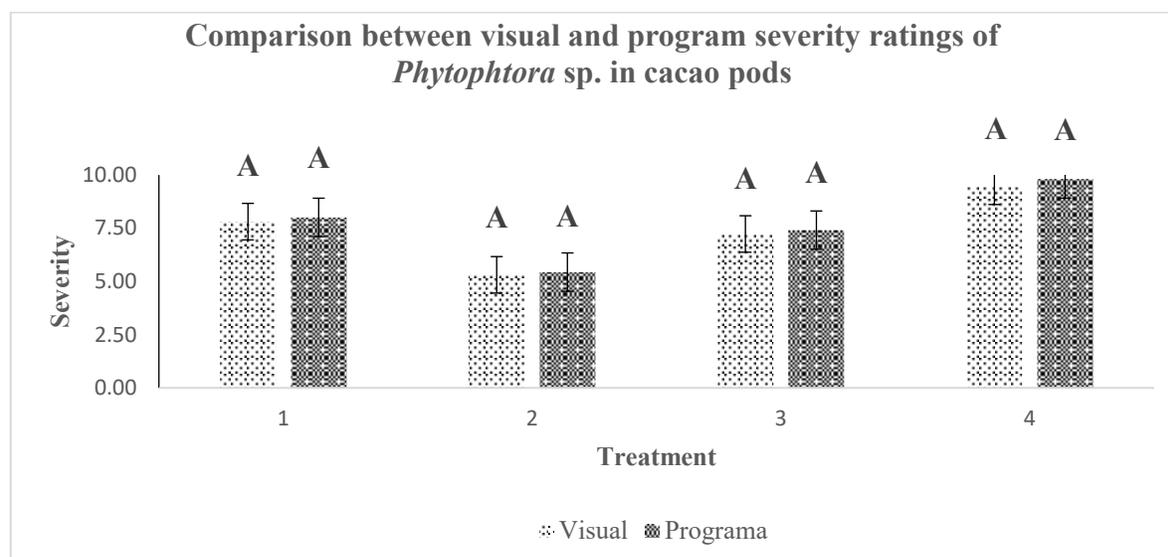


Figure 4. Comparison of the severity of *Phytophthora* sp. using visual assessment scales and Leaf Doctor software. Error bars indicate standard error. Different letters indicate significant differences between the averages presented by each treatment (Tukey $p < 0.05$).

To determine the damage of *M. perniciosa*, it was rated by visual evaluation. Pictures were not taken since the disease does not allow image processing due to the irregularity of the affected tissue. A count of each treatment was carried out for ten weeks. According to the analysis of variance, it shows us that in treatments T1, T2, T3 and T4 there was no significant difference (data not shown).

Agronomic variables

All data for the following variables were collected weekly over a 14-week period, except for harvested pods, which were recorded biweekly. Total number of cacao pods, healthy cacao pods and infected cherelles were statistically different in treatment 2. The rest of agronomic variables (Number of infected cacao pods, number of healthy cherelles, fresh and dry bean weight and the weight of 100 fresh and dry beans were not significantly different (Table 1).

Table 1. Analysis of the agronomic variables of interest in cocoa under the application of silicon. Different letters indicate significant differences between the averages presented by each treatment (Tukey $p < 0.05$).

Variable	T1 (control)	T2 100 kg/ha SiO ₂	T3 200 kg/ha SiO ₂	T4 300 kg/ha SiO ₂
Total number of cacao pods	5.09 b	12.18 a	6.13 b	5.38 b
Number of infected cacao pods	1.00 a	1.17 a	1.00 a	1.00 a
Number of healthy cacao pods	5.03 b	11.56 a	5.93 b	5.14 b
Number of healthy cherelles	68.43 a	69.20 a	69.12 a	62.92 a
Number of infected cherelles	7.05 a	4.94 b	7.42 a	7.36 a
Fresh beans weight (g)	648.33 a	642.26 a	650.00 a	669.79 a
Dry beans weight (g)	357.67 a	385.91 a	370.95 a	347.18 a
100 fresh weight beans (lb)	274.20 a	301.39 a	298.26 a	284.35 a
100 dry weight beans (lb)	357.67 a	285.95 a	270.95 a	347.18 a

Cost benefit analysis

Treatment 2 (100 kg/ha SiO₂) was economically profitable compared to the rest of the treatments due to the increase of total and healthy cacao pods. It reported an 12% increase of the economic benefits

compared to the control and 4 to 6% greater than treatment 3 and 4 respectively. The price at the moment of the experiment was \$1.05 per pound (ANECACAO, 2021).

Cadmium reduction and soil analysis

A foliar reduction of cadmium was observed when the analysis was compared using Chavez *et al.* (2015) methodology. The higher cadmium reduction level was observed in the 300 kg/ha SiO₂ treatment (Table 2).

Table 2. Cacao leaf tissue analysis for cadmium using doses of silicon (SiO₂)

Treatment	Cadmium mg kg ⁻¹	
	Before	After
Control	1.28	1.16
100 kg/ha SiO ₂	2.03	1.81
200 kg/ha SiO ₂	1.73	1.56
300 kg/ha SiO ₂	1.94	1.58

Major soil elements levels

Soil samples were taken before and after the silicon application to determine major soil nutrient levels. Nitrogen and calcium levels increased after the silicon application. The rest of nutrients remain similar or were reduced after the experiment (Table 3).

Table 3. Major soil elements levels comparison using doses of silicon (SiO₂). B= before silicon treatment; A= after silicon treatment.

Treatment	Major soil elements levels (%)											
	N		P		K		Ca		Mg		S	
	B	A	B	A	B	A	B	A	B	A	B	A
Control	2.0	2.3	0.17	0.18	1.62	1.30	0.89	1.74	0.49	0.42	0.17	0.14
100 kg/ha SiO ₂	2.5	2.2	0.17	0.17	1.27	1.12	0.87	1.98	0.35	0.60	0.11	0.10
200 kg/ha SiO ₂	2.1	3.1	0.17	0.16	1.72	1.14	1.05	1.83	0.43	0.55	0.14	0.15
300 kg/ha SiO ₂	3.4	3.1	0.17	0.17	1.49	0.77	1.04	1.96	0.44	0.61	0.16	0.11

Minor soil elements levels

Soil samples were taken before and after the silicon application to determine minor soil nutrient levels. Zn, Cu, Fe levels were lower after the silicon application in all treatments. Mn levels increased after the treatment application (Table 4).

Table 4. Minor soil elements levels comparison using doses of silicon (SiO₂). B= before silicon treatment; A= after silicon treatment.

Treatment	Minor soil elements levels (ppm)							
	Zn		Cu		Fe		Mn	
	B	A	B	A	B	A	B	A
Control	60	41	15	6	215	148	134	225
100 kg/ha SiO ₂	55	35	16	6	179	148	141	207
200 kg/ha SiO ₂	52	33	16	6	203	151	117	167
300 kg/ha SiO ₂	54	29	15	6	167	147	126	266

DISCUSSION

In this research, we analyzed the capacity of silicon to improve the health and productivity of CCN-51 cocoa cultivation in the province of Los Ríos, canton Mocache. The variables included the incidence of frosty pod rot, black pod disease, and witches' broom; the number and percentage of healthy

cherelles, healthy pods, pod and seed index, and the weight of wet almonds. Additionally, a Benefit-Cost ratio analysis was performed for the evaluated treatments.

Various studies have used visual scales compared to image processing with minimal error margins. For instance, Lectong *et al.* (2016) used visual scales for the severity of *M. royeri* with high efficiency. In this research, comparisons were made using software (Leaf Doctor), which also has high precision and has been used in various previous studies. Pethybridge and Nelson (2015) compared Leaf Doctor with another software (Assess) and determined that disease severity estimates by Leaf Doctor and Assess for the same images were very similar, with significant linear relationships and coefficients of variation below 3%, indicating high precision. The amount of variation in Leaf Doctor estimates explained by Assess was at least 0.94 for diseases with necrotic lesions: bronze leaf spot, downy mildew, gummy stem blight, and the tomato foliar disease complex.

Alheeti *et al.* (2021) compared the reliability of disease severity assessment using two different digital image quantification techniques (ImageJ and Leaf Doctor) and visual evaluations, including ImageJ (IJ), Leaf Doctor (LD), and visual assessments (VA) for lettuce downy mildew and *Cercospora* leaf spot of Swiss chard. This research indicates that LD application is easier, faster, more reliable, and precise for measuring disease severity, regardless of the disease and its severity. In this study, the high precision of Leaf Doctor was confirmed for evaluating frosty pod rot, black pod disease, and witches' broom in cocoa, using pods.

The application of 100 kg/ha of silicon reduces the incidence of frosty pod rot in cocoa pods. These results align with Ariza (2019), who applied calcium silicate to the soil at a level of 200 mg of Si/kg of soil, resulting in the lowest incidence of frosty pod rot (*M. royeri*) at 1.6%, achieving 80% control compared to the untreated control. This indicates the control over frosty pod rot generated by silicon from various sources. In this research, it is possible that other dosages did not have the same effect due to the solubility of silicon in the soil being influenced by factors such as pH, temperature, redox potential, organic matter content, particle size, and chemical composition (Hasing, 2007).

The lowest incidence of black pod disease occurred at the 100 kg/ha silicon dose, similar to frosty pod rot. Castellanos *et al.* (2015) indicate that silicon can enhance cell wall resistance, creating a mechanical barrier to protect the plant from pest and disease attacks, which coincides with the low disease incidence observed in this research. Conversely, Bustos (2017) identified that cocoa is a non-accumulating species of silicon, so silicon-based applications are insufficient to observe short-term results, aligning with the need for multiple silicon applications in this study to observe results. These contrasting results suggest validating the experiment over more harvest periods.

The economic analysis was based on two silicon applications in cocoa cultivation across all treatments. In terms of yield (kg/ha), treatment 2 (100 kg/ha of SiO₂) showed a higher productive response compared to the other treatments. According to Quero (2008), the benefits of higher silicon concentration in the soil and the supply of silicon-rich minerals through fertilization processes provide an economical and profitable solution for agricultural production. Ariza (2019) conducted research in Tingo María, Peru, at 530 meters above sea level in a seven-year-old CCN-51 cocoa plantation, applying a completely randomized block design. The study determined that calcium silicate applied to the soil at a level of 400 mg of Si/kg of soil resulted in a higher yield of 2221 kg/ha of dry cocoa beans, increasing yield by 34.7% compared to the fertilized control. Notably, the doses were lower than in the present research, which may explain the higher results observed in Ariza's younger plantation.

In this study, the treatment with the lowest silicon dose (100 kg/ha) achieved the best yields and Benefit-Cost Ratio, followed by treatment 4 (with the highest silicon dose: 300 kg/ha). These results contradict Caicedo and Chavarriaga (2008), who stated that higher amounts of active soluble silicon present yield better benefits for the soil, plant, and ultimately yield. Álvarez and Osorio (2014) indicate that silicon is dissolved in the soil and absorbed by plants due to mineral weathering, nutrient recycling from litterfall, and the type and age of surrounding vegetation.

Currently, cadmium is a significant problem for Ecuadorian cocoa producers (López-Ulloa *et al.*, 2021). Cadmium in cocoa can cause health issues such as kidney damage and developmental disorders in the

brain, especially in children, due to its low excretion rate. Furthermore, cadmium can bioaccumulate in cocoa beans, leading to the direct transmission of this metal into chocolate and other cocoa-derived products consumed by humans. Consequently, the European Union has established regulations to control cadmium levels in chocolate and its derivatives. The factors influencing cadmium bioavailability include soil pH, the presence of salts such as chloride and sulfate, cation exchange capacity, organic matter, and the types of clay present in the soil. Acidic pH increases cadmium availability, while salinity and the presence of salts can enhance its bioavailability. Additionally, the chemical and physical form of cadmium in the soil and the plants' ability to absorb it are also key factors (Marchive *et al.*, 2021). Recommendations include using mechanical means for weed control, verifying the suitability of herbicides, evaluating land selection and measures to avoid cadmium contamination, applying hygiene procedures during harvest and post-harvest, conducting cadmium analyses in soil, beans, leaves, and water, and utilizing hygiene and waste management protocols during cocoa harvest and post-harvest (López-Ulloa *et al.*, 2021). Some inorganic amendments have been proposed as solutions for cadmium (Carrillo *et al.*, 2021). Inorganic amendments such as lime (CaCO_3), calcite, zeolite, zinc sulfate (ZnSO_4), and other products like $\text{Ca}(\text{OH})_2$ and CaSO_4 can be used to mitigate cadmium presence in the soil. These amendments reduce cadmium mobility and absorption, decreasing its availability and reactivity in the soil. Although there is no perfect solution to eliminate cadmium, these amendments can help reduce its impact. As of writing this document, silicon has not been evaluated as an alternative and could be considered based on the observed results.

This research determined that silicon at 100 kg/ha improves production, is involved in most plant processes, and enhances the plant's resistance to frosty pod rot and black pod disease, which are the most common diseases in this crop. Economically, silicon applications offer advantages in this perennial crop, although it requires periodic applications. However, in Arias's (2020) study, yield showed a highly significant correlation with the number of almonds per pod, the number of healthy pods per plant, and chlorophyll content. The treatment with the highest profitability percentage was the one that received only 26 kg of Si ha⁻¹ year⁻¹, supporting the recommendation for Si use in cocoa cultivation under normal edaphoclimatic conditions (Altitude between 15 to 800 meters above sea level, temperature between 24 to 25°C, precipitation of 1500 to 3000 mm, fertile, deep, loamy soils, and pH of 6.0 to 7.0), achieving comparable yields with those receiving higher N doses (over 121 kg ha⁻¹ year⁻¹). This is consistent with Ariza (2019) and the present research, as both studies found the best profitability results with low silicon doses.

BIBLIOGRAPHY

1. Alheeti, A., Farhan, M., Al-Saad, A., & Theer, R. (2021). Evaluation of the Performance of ImageJ, Leaf Doctor Applications, and Visual Assessments in Measuring Severity of Two Leaf Spot Diseases. *IOP Conference Series: Earth Environmental Science*, 761(1), 1-8.
2. Álvarez, C., & Osorio, W. (2014). Silicio agrónomicamente esencial (No. DC-0483). Colombia: Multisulfatos SAS. Universidad Nacional de Colombia.
3. Álvarez, C., & Osorio, W. (2014). Silicio agrónomicamente esencial (No. DC-0483). Colombia: Multisulfatos SAS. Universidad Nacional de Colombia.
4. ANECACAO. (2010). *Cacao CCN51*. Recuperado el 19 de Febrero de 2020, de ANECACAO: <http://www.anecacao.com/es/quienes-somos/cacaoccn51.html>
5. Ariza, J. C. (2019). Fuentes y niveles de silicio en el incremento del rendimiento del cultivo de cacao (*Theobroma cacao* L.) y reducción en la incidencia de moniliasis (*Moniliophthora roreri* Cif y Par). Tesis. Tingo María, Ecuador: Universidad Nacional Agraria de La Selva.
6. Arteaga S. Eficacia de dos productos a base de *Trichoderma*, en tres dosis de aplicación para el control de la mancha de hierro (*Cercospora coffeicola*) y moniliasis (*Moniliophthora roreri*), en el cantón Francisco de Orellana Prov. Riobamba - Ecuador: Escuela Superior Politécnica de Chimborazo. 2019. Obtenido de <http://dspace.esPOCH.edu.ec/bitstream/123456789/4828/1/13T0820%20.pdf>
7. Barbedo, J. G. (2014). An automatic method to detect and measure leaf diseasesymptoms using digital image processing. *Plant Disease*, 98(12), 1709-1716. doi:10.1094/pdis-03-14-0290-re
8. Bekker, T. F., Labuschagne, N., Aveling, T., & Kaiser, C. (2007). Efficacy of water-soluble potassium silicate against *Phytophthora* root rot of avocado under field conditions. South Africa: University of Pretoria.

9. Beltrán Muñoz, L. E. (2021). Efecto de la aplicación de silicio en el manejo fitosanitario del cultivo de cacao (*Theobroma cacao*) variedad CCN-51.
10. Bustos, G. S. (2017). Efecto de un compuesto a base de silicio, sobre el manejo fitosanitario del cultivo de cacao CCN-51. Santo Domingo, Santo Domingo de los Tsáchilas, Ecuador: Escuela Superior Politécnica del Ejército (ESPE).
11. Carrillo, M., Durango, W., Peña, K. & Albán, C. (2021). Guía 10: Mitigación de cadmio en el suelo mediante enmiendas inorgánicas. Caja de herramientas para la prevención y mitigación de la contaminación de cadmio en la cadena de cacao-Ecuador (1.ª ed., pp. 1-33). Quito, Ecuador.
https://balcon.mag.gob.ec/mag01/magapaldia/Caja%20de%20Herramientas_Cadmio_Cacao/
12. Castellanos, L., De Mello, R., & Silva, C. N. (2015). El Silicio en la resistencia de los cultivos.
13. Castillo Morejón, César Paul (2018). Efecto del silicio en el manejo fitosanitario del cultivo de cacao (*Theobroma cacao* L. Cv. CCN-51) en época lluviosa. Carrera de Ingeniería Agropecuaria. Universidad de las Fuerzas Armadas ESPE. ESPESD. Extensión Santo Domingo.
14. Chavez, E., He, Z. L., Stoffella, P. J., Mylavarapu, R. S., Li, Y. C., Moyano, B., & Baligar,
15. Crespo, E., & Crespo, F. (1997). Cultivo y Beneficio de cacao CCN 51. Quito, Pichincha, Ecuador: Editorial El Conejo. *Cultivos Tropicales*, 36(Supl. 1), 16-24.
16. Di Rienzo, J., Balzarini, M., Gonzalez, L., Casanoves, F., Tablada, M., & Walter Robledo, C. (2010). Infostat: software para análisis estadístico.
17. Estrada López, J. N. (2019). Aplicación de niveles de sílice al suelo, como cascarilla carbonizada de arroz, sobre la disminución de cadmio en el cultivo de cacao (*Theobroma cacao* L.) en Von Humboldt.
18. Gonzales Sobrados, E. C. (2022). Fuentes de silicio en la absorción del cadmio en el cultivo de (cacao) *Theobroma cacao*
19. Guerrero, G. (2014). *Revista Líderes*. Recuperado el 19 de Febrero de 2020, de <https://www.revistalideres.ec/lideres/cacao-ecuatoriano-historia-empezo-siglo.html>
20. Hasing, F. M. (2007). Impacto de las Aplicaciones de un Mineral Bio-activo sobre Parámetros Agronómicos y Fitosanitarios en Plantas de Banano del Grupo Cavendish, Variedad Williams a Nivel de Laboratorio e Invernadero. Tesis. Guayaquil, Guayas, Ecuador: Escuela Superior Politécnica del Litoral.
21. http://www.proecuador.gob.ec/wpcontent/uploads/2013/08/PROEC_AS2013_CACAO.pdf Alfa Revista de Investigación en Ciencias Agronómicas y Veterinaria, 5(15), 70-85. Epub 00 de diciembre de 2021. <https://doi.org/10.33996/revistaalfa.v5i15.129>
22. ICA. (2012). *Manejo fitosanitario del cultivo del cacao (Theobroma cacao L.) - Medidas para la temporada invernal*. Bogotá, Colombia: Instituto Colombiano Agropecuario (ICA).
23. Jaimes, Y., & Aranzazu, F. (2010). *Manejo de las enfermedades del cacao (Theobroma cacao L.) en Colombia, con énfasis en monilia (Moniliophthora rozeri)*. Colombia: Corporación Colombiana de Investigación Agropecuaria (CORPOICA).
24. Kellam, M. K., & Zentmyer, G. A. (1986). Morphological, physiological, ecological, and pathological comparisons of *Phytophthora* species isolated from *Theobroma cacao*. *Phytopathology*, 76(2), 159-164.
25. Lectong, P. F., Chávez, J., España, C., & Vélez, S. (2016). Evaluación de moniliasis (*Moniliophthora rozeri* H.C Evans et al) en cacao en el cantón Bolívar. Tesis. Bolívar, Manabí, Ecuador: Escuela Superior Politécnica Agropecuaria de Manabí.
26. López-Ulloa, M., Garzón, I., Proaño, A. & Herrera, L. (2021). Guía 7: Recomendaciones de Buenas Prácticas Agronómicas para prevenir el riesgo de contaminación con cadmio en la cadena de cacao. Caja de herramientas para la prevención y mitigación de la contaminación de cadmio en la cadena de cacao-Ecuador (1.ª ed., pp. 1-24). Quito, Ecuador.
https://balcon.mag.gob.ec/mag01/magapaldia/Caja%20de%20Herramientas_Cadmio_Cacao/
27. López-Ulloa, M., Jaimez, R. & Orozco, L. (2021). Guía 1: El cadmio en el cultivo de cacao. Caja de herramientas para la prevención y mitigación de la contaminación de cadmio en la cadena de cacao-Ecuador (1.ª ed., pp. 1-20). Quito, Ecuador.
https://balcon.mag.gob.ec/mag01/magapaldia/Caja%20de%20Herramientas_Cadmio_Cacao/
28. Marchive, L., López-Ulloa, M., Chávez, E. & Atkinson, R. (2021). Guía 3: Factores que influyen en la biodisponibilidad de cadmio en suelo y su acumulación por la planta. Caja de herramientas para la prevención y mitigación de la contaminación de cadmio en la cadena de cacao-Ecuador (1.ª ed., pp. 1-24). Quito, Ecuador.
https://balcon.mag.gob.ec/mag01/magapaldia/Caja%20de%20Herramientas_Cadmio_Cacao/
29. Maridueña-Zavala, M. G., Villavicencio-Vásquez, M. E., Cevallos-Cevallos, J. M., & Peralta, E. L. (2016). Molecular and morphological characterization of *Moniliophthora rozeri* isolates from cacao in Ecuador. *Canadian Journal of Plant Pathology*, 38(4), 460-469.
30. Mejisulfatos. (2010). Silicio. El costo oculto de los químicos. Itagüí, Colombia: Mejisulfatos S.A.
31. Nutter Jr, F. W., Gleason, M. L., Jenco, J. H., & Christians, N. C. (1993). Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. *Phytopathology*, 83(8), 806-812.
32. Osorio-Solano, C., Orozco-Castaño, C. A., López-Gartner, G. A., & Rivera-Páez, F. A. (2012). Variabilidad genética de *Moniliophthora perniciosa* (Stahel) Aime y Phillips-Mora, comb. nov. (Agaricales-Marasmiaceae) en variedades de cacao (*Theobroma cacao* L.). *Acta agronómica*, 61(2), 93-101.
33. Pethybridge, S. J., & Nelson, S. C. (2015). Leaf Doctor: A new portable application for quantifying plant disease severity. *Plant disease*, 99(10), 1310-1316.
34. Phillips-Mora, W. (2006). La moniliasis del cacao: un enemigo que podemos y debemos vencer. En *Taller Regional Andino de Aplicación Tecnológica en el Cultivo de Cacao* (págs. 21- 25). Quevedo, Los Ríos, Ecuador: Instituto Interamericano de Cooperación para la Agricultura (IICA).

35. Pilalao David, Wilmer, Pérez Vaca, Dora, Alvarado Aguayo, Allan, & Torres Sánchez-Mora, Sinthya. (2021). Manejo agroecológico de la Moniliasis en el cultivo de cacao (*Theobroma cacao*) mediante la utilización de biofungicidas y podas fitosanitarias en el cantón La Troncal.
36. PROECUADOR. (2013). *ANÁLISIS DEL SECTOR DE CACAO*. Obtenido de
37. Quero, E. (Octubre de 2009). Nutrición con silicio y sus aplicaciones a cultivos a cielo abierto y en agricultura protegida: Un pequeño recorrido por la naturaleza. *Simposio Internacional de Nutrición Vegetal*. Guadalajara,, México.
38. Quiroga, A. M. (2016). Respuesta a las aplicaciones de silicio en el cultivo de pepino (*Cucumis sativus*L) Variedad MODAN, en condiciones de estrés hídrico bajo cubierta en Culiacán, Sinaloa. Bogotá, Colombia: Universidad de Ciencias Aplicadas y Ambientales.
39. RedAgrícola. (Marzo de 2017). *Productos de Silicio: Ayudan a las plantas a superar estrés biótico y abiótico*. Recuperado el 21 de Marzo de 2020, de RedAgrícola: <https://www.redagricola.com/cl/productos-de-silicio-ayudan-las-plantas/>
40. Rodríguez-Serrano, M., Martínez de la Casa, N., Romero-Puertas, M. C., Del Río, L. A., & Sandalio, L. M. (2008). Toxicidad del Cadmio en Plantas. *Revista Ecosistemas*, 17(3), 139-146. <https://doi.org/10.7818/RE.2014.17-3.00>
41. Sánchez-Mora, F. D., Medina-Jara, S. M., Díaz-Coronel, G. T., Ramos-Remache, R. A., Vera-Chang, J. F., Vásquez-Morán, V. F., ... & Onofre-Nodari, R. (2015). Potencial sanitario y productivo de 12 clones de cacao en Ecuador. *Revista fitotecnia mexicana*, 38(3), 265-274.
42. SENASICA. (2013). *Escoba de bruja del cacao (Moniliophthora perniciosa)*. D.F, México: Dirección General de Sanidad Vegetal.
43. SEPHU. (14 de Mayo de 2009). El Silicio (Si) como elemento fertilizante y protector de enfermedades y plagas. *Boletín N° 28*. Zaragoza, España: Sociedad Española de Productos Húmicos S.A.
44. Snyder, G. H. (2001). Methods for silicon analysis in plants, soils, and fertilizers. In *Studies in plant science* (Vol. 8, pp. 185-196). Elsevier.
45. Sornoza Ormaza, John Emilio (2018). Efecto de la aplicación edáfica de silicio, en época lluviosa, en el cultivo de cacao CCN51. Carrera de Ingeniería Agropecuaria. Universidad de las Fuerzas Armadas ESPE. ESPESD. Extensión Santo Domingo.
46. USDA-NRCS. (7 de Febrero de 2014). USDA Natural Resources Conservation Service. Obtenido de: <https://www.nrcs.usda.gov/wps/portal/nrcs/main/national/enespanol/ley2014/>
47. V. C. (2015). Science of the Total Environment Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador, 533, 205-214.