Research Square

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Ramularia leaf spot and Boll rot are affected differently by organic and inorganic nitrogen fertilization in cotton plants

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Abstract

Background: In this work, the interaction among nitrogen fertilization using bovine manure, poultry manure, *Jatropha curcas* seed cake and urea, and the diseases Ramularia leaf spot (RLS) and Boll rot (BR), caused by *Ramulariopsis pseudoglycines* and *Diplodia gossypina*, respectively, on cotton plants (*Gossypium hirsutum* L.), was studied under field conditions. The intensity (incidence and severity in percentage) of RLS and the incidence (%) of BR were evaluated six times, starting in the reproductive stage B1 (first visible flower bud). A randomized complete block experimental design with a 4x4 factorial arrangement (fertilizers x dose), totaling 16 treatments were used. The disease progress was analyzed with the nonlinear Logistic and Gomperts models, obtaining the initial disease's epidemiological parameters (*Y0*) and progress rate (*r*).

Results: Cotton plants fertilized with 100 kg N ha^{-1} of *J. curcas* seed cake and poultry manure with 100 and 200 kg N ha^{-1} showed an incidence between 16 and 21% of RLS. In contrast, plants fertilized with bovine manure presented the highest incidence of ramularia leaf spot (33%). Regarding factor B (dose), cotton plants fertilized with 50 kg N ha^{-1}, showed a higher percentage of BR incidence, being different from those fertilized with the other doses. In the analysis of the interaction corresponding to the BR incidence, no response pattern was found in the doses for each fertilizer. No correlation was observed between the three health variables analyzed, finding probabilities between 0.002 and 0.892. In the temporal progress of RLS incidence, it was shown that fertilization with *J. curcas* seedcake and poultry manure was lower than the rest. In severity, the plants were fertilized by *J. curcas* seedcake concerning the rest. The progression curve of RLS severity temporarily increased similarly, observing that plants fertilized with higher doses reached a lower final proportion of the disease.

Regarding the modeling of epidemics using the nonlinear Logistic and Gompertz models, the first model better represented the RLS, except for factor B, where none of the curves was significant. In the Logistic model, a lower amount of initial disease was found (*Y0*) of RLS, both in incidence (0.259) as in severity (0.081), in plants fertilized with *J. curcas* seed cake and poultry manure, compared to the rest. For the case of BR incidence, none of the nonlinear models used could be represented.

Conclusions: Plants fertilized with 50 kg N ha^{-1}, presented an incidence twice higher than those obtained with other fertilizers. The Logistic model better fits RLS, but no model was adapted to BR. Only the RLS epidemiological parameters were affected differently in this experiment compared to BR disease.

Introduction

The cotton (*Gossypium hirsutum* L.) is currently one of the main crops worldwide (Fang 2018), producing the fiber constituted by complex structures almost exclusively by cellulose molecules and by D-glucose residues (French and Kim 2018). Although this species had significant participation in Ecuador's agricultural sector between 1970–1990, mainly in the provinces of Guayas and Manabí, the planted area was reduced due to climatic and economic factors and lack of certified seed (García et al. 2019). Between the end of the last century and the last decade in the country, the production of fiber (t) and the productive area (ha) of cotton decreased 21 and 29 times, respectively, with the province of Manabí representing 79% of the national production total (Vivero 2017). Experimentally, in this province some varieties of cotton have been studied, such as Coker and DP-ACALA 90 (Cañarte-Bermúdez et al. 2020).

One of the most important tasks in cotton cultivation management is plant nutrition (Teixeira et al. 2008). Nitrogen (N) is the nutrient most required by the plant, as it is a limiting factor for growth and a fundamental element for crop production (Bondada and Oosterhuis 2001). Although cotton responds positively to N incorporation, this response can be influenced by the variety, type of soil, and humidity conditions during its development (Singh 1970). N application has a significant impact on physiological parameters, cotton growth, boll development, fluff yield, and fiber quality (Bondada and Oosterhuis 2001; Teixeira et al. 2008).

Although the use of nitrogen sources of synthetic origin mainly based on ammonia (NH_3) raises crop yields, this can be detrimental to an agricultural system. The loss of N as NH_3 causes a hostile situation between using this resource and eco-environmental conservation, driving even a low efficiency of this type of fertilizer when applied to a crop (Zheng et al. 2018). In addition to this problem, there is a pollution of the atmosphere, soil acidification, eutrophication, and a decrease in biodiversity (Scudlark et al. 2005). As an alternative to this type of management, more environmentally friendly practices emerge, such as using organic nitrogenous sources rich in ammonia (NH_4).

Inorganic and organic fertilizers could exert suppressive effects on plant pathogens, the type of nitrogen source, and the plant-pathogen interaction being able to influence. Indeed, N applications in a crop can increase or decrease plants' resistance to pathogens, showing that there are differences in pathogens' strategy to infect plant tissues (Mur et al. 2016). Likewise, these fertilizers can introduce biocontrol agents to the soil, providing food for its establishment and activity (Artavia et al. 2010), improving the condition of the root, allowing an adequate growth of the plants and quality of capsules, making them more potent against the attack of diseases (Mur et al. 2016; Chen et al. 2018; 2019; 2020). Rich N sources are known to have a negative effect on various plant pathogens (Blachinski et al. 1996; Artavia et al. 2010; Veromann et al. 2013). N has an adverse impact on physical defenses and antimicrobial phytoalexin production but has positive effects on defense-related enzymes and proteins that affect local protection and systemic resistance (Sun et al. 2000). However, these sources' effect on diseases that attack foliar tissues in cotton crops is unknown, especially in their epidemiological parameters as an initial disease (*Yo*) and progress rate (*r*). Either way, understanding how pathogens with different infection strategies respond to N levels is of fundamental importance in this context (Mur et al. 2016).

On the Ecuadorian coast, the cotton crop is attacked by diseases such as damping off (*Rhizoctonia solani*), anthracnose of cotton (*Colletotrichum gossypii*), and Boll rot (*Diplodia gossypina*) (Sión et al. 1992). Others like Ramularia leaf spot (*Ramulariopsis pseudoglycines*) present in neighboring countries (Aquino et al. 2008) and not yet reported in Ecuador, it can negatively affect the foliar area and fiber yield in plants of the genus *Gossypium* (Ascari et al. 2016; da Silva et al. 2019). Despite the importance of these diseases in cultivation, I have studied little or nothing. Even studies evaluating the effect of organic fertilizers on cotton cultivation are scarce, and even more so in this country. Thus, in this work, the interaction between organic fertilization (bovine manure, chicken manure, and physic nut seed cake) and synthetic fertilization (urea) rich in N was studied, with aerial tissue diseases in cotton crop var. DP ACALA 90.

Materials And Methods

Description of the experimental areas

This research was carried out between November/2019 and April/2020, at the La Teodomira experimental campus, belonging to the Faculty of Agronomic Engineering, Technical University of Manabí (UTM), Ecuador, located at the geographic coordinates of 01 ° 09´ 51 South latitude and 80° 23' 24" west longitude, at a latitude of 60 meters above sea level (INAMHI 2015).

The DP ACALA 90 variety was planted under field conditions, at a distance between plants of 0.40 cm, 1 m between rows, and 2 m between blocks, on a field where cotton was previously established.

The soil was classified as clay loam, according to the soil taxonomy USDA (Soil Survey Staff, 2014) observed in Table 1, were obtained by soil analysis in the Laboratory of soils, plant tissues, and water of the National Institute of Agricultural Research (INIAP), Tropical Experimental Station “Pichilingue”.

Table 1
Physical characteristics (soil type and pH: hydrogen ionic potential) and chemical (OM: organic matter, N: Nitrogen, P: Phosphorus, K, Potassium, Ca: Calcium, Mg: Magnesium, H: Hydrogen, Mn: Manganese, Co: Cobalt, and Z: Zinc).

Soil	pH	MO	N	P	K	Ca	Mg	H	Mn	Co	Z
		%	%	mg kg^{-1}		cmol kg^{-1}		mg kg^{-1}			
Clay loam	7.5	0.90	0.04	17.4	1.06	15.25	5.27	26.7	5.55	2.19	< 2.60

The rainfall and maximum and minimum air temperatures (T max and T min), recorded in Lodana, Manabí, Ecuador during the research period, are shown in Fig. 1.

The test area was constituted by 2688 m² (48 m x 56 m), where each experimental plot measured 36 m² (6 m x 6 m). The useful area of each plot was 20.8 m² (5.20 m x 4 m). Nitrogen sources as Bovine manure, *Jatropha curcas* seedcake, Poultry manure and urea (the latter used as a control), were used in the present study as fertilizers.

The first fertilization was done 20 days after emergence (dae), placing half the dose that corresponded to it in each treatment, the second fertilization was carried out at the time of flowering (50 dae), completing the exact doses of nitrogen from its sources (50, 100, 150, 200 kg of N ha^{-1}). The concentrations of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), and Magnesium (Mg) of each organic fertilizer are shown in Table 2.

Table 2
Concentrations (%) of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), and Magnesium (Mg) of organic fertilizers Bovine manure, Seedcake, and Poultry manure

Fertilizers	Concentrations (%)				
	N	P	K	Ca	Mg
Bovine manure	3.9	0.6	1.6	1.4	0.5
Jatropha curcas seedcake	2.1	0.9	2.6	0.9	0.6
Poultry manure	3.0	0.7	2.3	2.7	0.6

Disease Assessment

The intensity was assessed RLS (*R. pseudoglycines*) and BR (*Diplodia* sp.), at the beginning (76 dae) reproductive phase B1 (first visible flower bud), (Marur and Ruano 2004), in the lower, middle, and upper strata (a plant organ for each) of four cotton plants from the useful plot, totaling six evaluations over time. The incidence was quantified by counting each of the symptomatic organs (leaves and capsules) present in plants, employing a visual evaluation of the symptoms, and transforming the average value of the three strata in percentage. The severity was only estimated for ramularia leaf spot, using the diagrammatic scale proposed by Aquino et al. (2008).

Experimental Design And Statistical Analyses

A randomized complete block experimental design with a 4x4 factorial arrangement was used (fertilizers x dose), totaling 16 treatments with four replications. Four fertilizers as Bovine manure, *Jatropha curcas* seedcake, Poultry manure and urea (the latter used as a control) and four dose 50, 100, 150, 200 kg of N ha^{-1} were evaluated.

After verifying the normality and homoscedasticity of the values obtained in the last evaluation, an analysis of variance was performed. For comparison data test was used Tukey test ($p < 0.05$). Also, Pearson analysis was performed to investigate the correlation among all variables evaluated.

For plant disease progress analysis, was estimated the primary inoculum ($Y_{0)}$ and the progress rate (r) using the Logistic (Eq. 1) and Gompertz (Eq. 2) model equations (Berger 1981; Tjørve and Tjørve 2017);

y = 1(1 + exp (-a + rt)) (1)

where y = disease proportion ($0 < y < 1$), a = logit (y_0), r = rate, and t = time

y = exp (-B*exp (-kt)) (2)

where B is a position parameter, k = rate, and t = time.

A comparison of means was performed only when the probability for all the values of a factor was significant, using the Tukey test ($p < 0.05$). Progress curves were plotted using the epifitter package (Alves and del Ponte 2020). All analyses were performed with Rstudio (RStudio Team 2017).

Results

The factor analysis showed a significant interaction between fertilizers (factor A) and dose (factor B), both for the incidence of RLS ($p < 0.0194$) and BR ($p < 0.00998$). Differences were only observed between the doses used for the incidence of boll rot ($p < 0.02519$) (Table 3).

Table 3
Results of two-way analysis of variance for average incidence and severity of Ramularia leaf spot and incidence of boll rot.

Variable	Factors	GL[1]	F[2]	*p*-valor
Ramularia leaf spot (*Ramulariopsis pseudoglycines*)				
Incidence	Fertilizers	3	1.67050	0.17420
	Dose	3	0.62240	0.60121
	Interaction	9	2.25960	0.01941*
Severity	Fertilizers	3	0.45900	0.71110
	Dose	3	0.36110	0.78120
	Interaction	9	0.71340	0.69650
Boll rot (*Diplodia* sp.)				
Incidence	Fertilizers	3	1.08210	0.35747
	Dose	3	3.16910	0.02519*
	Interaction	9	2.48790	0.00998**

[1] GL: degrees of freedom

[2] F: Fisher's calculated value

* statistical significance $p < 0.05$

** statistical significance $p < 0.01$

In the analysis of the interaction corresponding to RLS incidence (Fig. 2), no response pattern was observed in each fertilizer's doses. However, it was found that cotton plants fertilized with 100 kg N ha^{-1} of *J. curcas* seedcake and 100 and 200 kg N ha^{-1} of poultry manure showed an incidence between 16 and 21% of RLS, which was lower compared to the other treatments. In general, it was evidenced that regardless of the dose used, plants fertilized with bovine manure had the highest incidence of ramularia leaf spot (33%) compared to those fertilized with the rest of fertilizers.

Regarding factor B (dose), cotton plants fertilized with 50 kg N ha^{-1}, presented a higher percentage of incidence of BR, being different from those fertilized with the other doses (Fig. 3).

In the analysis of the interaction corresponding to incidence of BR (Fig. 4), no response pattern was found in the doses for each fertilizer. It is shown that BR in cotton plants fertilized with 100 kg N ha^{-1} of bovine manure and urea with 100 and 200 kg N ha^{-1} of *J. curcas* seedcake, being similar to the lower incidence (1 %) obtained with 150 kg N ha^{-1} of bovine manure, 100 and 150 kg N ha^{-1} of *J. curcas* seedcake, and 200 kg N ha^{-1} of poultry manure. Except for bovine manure, a similar or higher incidence was observed (7%) of BR in plants fertilized with 50 kg N ha^{-1} than the rest doses within the three remaining fertilizers.

No correlation was observed between the three health variables analyzed, finding probabilities between 0.002 and 0.892 (data not revealed).

The RLS incidence progress curve in cotton plants fertilized with *J. curcas* seedcake and poultry manure started lower than the rest. However, the curve that presented a higher proportion of final disease was in plants fertilized with Bovine manure (Fig. 5A). Although all the curves had similar starting points in the case of RLS severity, the endpoint of the disease found in plants fertilized by *J. curcas* seedcake was the smallest compared to the rest (Fig. 5B).

The RLS severity (B) progress curve temporarily increased similarly, observing that plants fertilized with higher doses reached a lower final proportion of the disease (Fig. 6).

Concerning the modeling of epidemics using the nonlinear Logistics and Gompertz, the first model better represented the RLS, except factor B, where none of the curves was significant (Table 4). Using the Logistic model, a lower amount of initial disease was found (Y_0) of RLS, both in the incidence (0.259) as in severity (0.081), in plants fertilized with *J. curcas* seed cake, and poultry manure, compared to the rest. On the other hand, a lower rate of disease progress (r) (0.012) was found in plants fertilized with urea, compared to the rest (0.016). This same parameter for the severity variable, no difference was observed between fertilizers. Finally, doses of 150 (0.076) and 200 (0.028) kg N ha^{-1}, negatively affected the Y_0 and positively the r of RLS in cotton plants, respectively, compared to the rest of the doses.

Table 4
Initial disease amount (Y_0) and rate of disease progression (r) obtained from the quantification of incidence and severity (%) of Ramularia leaf spot, over time in cotton plants under organic fertilization (bovine manure, *Jatropha curcas* seed cake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 and 200 kg N ha^{-1}) for each source. From the analysis obtained for the models Logistic and Gompertz, the coefficient of determination was obtained (R_2) and the probability (p).

Fertilizer	Logistic				Gompertz			
	Y_0	R	R_2	P	Y_0	r	R_2	p
Incidence (%) of Ramularia leaf spot								
Factor A (Fertilizers)								
Bovine manure	0.354 a[£¥]	0.015 a	0.34	0.00001	0.867	0.040	0.39	0.07078
J. curcas seedcake	0.263 b	0.016 a	0.31	0.00004	0.615	0.074	0.37	0.03309
Poultry manure	0.254 b	0.016 a	0.32	0.00003	0.583	0.079	0.39	0.02156
Urea	0.327 a	0.012 b	0.29	0.00032	0.648	0.064	0.28	0.09145
Factor B (Dose)								
50 kg N ha^{-1}	0.487	0.000	0.00	0.59825	0.533	0.511	0.05	0.40871
100 kg N ha^{-1}	0.485	0.000	0.00	0.98839	0.502	0.951	0.06	0.47845
150 kg N ha^{-1}	0.429	0.004	0.02	0.31305	0.506	0.333	0.06	0.34263
200 kg N ha^{-1}	0.473	0.000	0.00	0.99526	0.469	0.073	0.00	0.95881
Severity (%) of Ramularia leaf spot								
Factor A (Fertilizers)								
Bovine manure	0.099 a	0.026 [ns]	0.59	0.00000	13.600	0.007	0.59	0.00737
J. curcas seedcake	0.077 b	0.022	0.42	0.00000	0.652	0.028	0.45	0.09016
Poultry manure	0.074 b	0.027	0.58	0.00000	8.870	0.008	0.58	0.46556
Urea	0.102 a	0.024	0.54	0.00000	1.158	0.022	0.56	0.08271
Factor B (Dose)								
50 kg N ha^{-1}	0.113 a	0.023 b	0.57	0.00000	0.804	0.032	0.61	0.03292
100 kg N ha^{-1}	0.096 ab	0.024 b	0.54	0.00000	1.266	0.019	0.56	0.11966
150 kg N ha^{-1}	0.074 b	0.028 a	0.60	0.00000	3.330	0.012	0.60	0.01596
200 kg N ha^{-1}	0.078 b	0.027 a	0.48	0.00000	11.020	0.007	0.48	0.57280

[£] An analysis of variance with its respective comparison of means was performed only when the probability for all the factor values was significant ($p < 0.05$).

[¥] Lowercase letters in the column indicate the difference between means by Tukey's test ($p < 0.05$).

[ns] There is no difference between column means by Tukey's test ($p < 0.05$).

None of the models used nonlinear representation in the case of incidence (%) BR (Table 5). Including both the Y_0 as *r* in the dose for 100 kg, N ha^{-1} could not be calculated.

Table 5
Initial disease amount (Y_0) and rate of disease progression (*r*) obtained from the quantification of incidence (%) of BR, over time in cotton plants under organic fertilization (bovine manure, *Jatropha curcas* seedcake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 and 200 kg N ha^{-1}) for each source. From the analysis obtained for the models Logistic and Gompertz, the coefficient of determination was obtained (R_2) and the probability (*p*).

Fertilizer	Logistic				Gompertz			
	Y_0	r	R_2	p	Y_0	r	R_2	p
Incidence (%) of boll rot								
Factor A (Fertilizers)								
Bovine manure	0.026	0.040	0.09	0.16337	0.706	0.026	0.09	0.73157
J. curcas seedcake	0.022	0.040	0.10	0.19940	0.218	0.114	0.12	0.51944
Poultry manure	0.136	0.015	0.02	0.56079	0.271	0.114	0.05	0.40132
Urea	0.052	0.033	0.19	0.08442	0.340	0.095	0.22	0.27887
Factor B (Dose)								
50 kg N ha^{-1}	0.083	0.033	0.23	0.26119	0.829	0.030	0.25	0.38284
100 kg N ha^{-1}	0.049	0.013	0.01	0.62627	*	*	*	*
150 kg N ha^{-1}	0.025	0.045	0.22	0.10295	5.900	0.001	0.23	0.77601
200 kg N ha^{-1}	0.052	0.024	0.03	0.51379	0.188	0.088	0.05	0.58102

* The data did not allow us to calculate the epidemiological parameters studied in the present work.

Discussion

Currently, countless studies are showing the positive effect generated by fertilizers rich in N in cotton plants. For example, applications between 55 and 240 N kg ha^{-1} increase the photosynthesis of the canopy and the weight of leaves, thus inducing a greater number of nodes, a number of capsules, weight of one hundred seeds, and fiber production (Bondada and Oosterhuis, 2001; Teixeira et al. 2008; Chen et al., 2018; 2019; 2020). However, little is known about the consequence of nitrogen fertilization in cotton plant diseases. Thus, in this work carried out under field conditions, we present for the first time the performance of Ramularia leaf spot (RLS) and Boll rot (BR) to the application with four sources and increasing doses of N in cotton plants variety DP ACALA 90.

The cotton cultivation established in the previous cycle seems to have allowed a sufficient inoculum source for RLS or BR to appear in vegetative stages of plants of the variety DP ACALA 90 but with more intensity than the first disease. Indeed, RLS can accumulate a primary inoculum available in the early stages in future cotton cycles (da Silva et al. 2019). On the other hand, the genotype used seems to be susceptible to both diseases, and according to Suassuna et al. (2020), the *Cotton leafroll dwarf virus* (CLDV) transmitted by aphid *Aphis gossypii*, and bacterial blight caused by *Xanthomonas citri* pv. *malvacearum*.

Although nutrients can affect disease tolerance or plant resistance to pathogens (Mur et al. 2016; Artavia et al., 2010; Veromann et al. 2013), we observed that regardless of the disease, they responded differently to each nitrogen fertilizer and dose. Blachinski et al. (1996) mention that sources rich in N can reduce spore germination and mycelial growth of *Alternaria macrospora* in conditions *in vitro*, o the diameter of the lesions and the percentage of Alternariosis under controlled conditions, but no parameters under field conditions. However, the same authors mention that this effect was generated only by some nitrogenous sources, such as potassium nitrate (KNO_3), which negatively affects the diameter of lesions but not the severity. Also, applications of different forms of N, such as ammonia (NO_3) and ammonium (NH_4), can act biochemically, physiologically, and molecularly in a different way in plants against pathogens (Sun et al., 2020). In this way, it can be inferred that both some external factor and an effect at the cellular level of each fertilizer in cotton plants may have influenced the behavior observed in our work.

Regardless of the dose used, the plants fertilized with bovine manure presented a higher incidence of RLS. It was even verified that plants fertilized with 100 kg N ha^{-1} of *J. curcas* seedcake and100 and 200 kg N ha^{-1} of poultry manure showed a lower incidence of this disease than the other treatments. According to the laboratory analysis carried out on the fertilizer, it has 33 and 47% more N than Poultry manure and *J. curcas* seedcake, respectively. Conversely, this pair of fertilizers have a greater amount of K compared to Bovine manure and urea. In this manner, K could have affected RLS. Indeed, K reduces bacterial blight incidence (*Xanthomonas citri* pv. *malvacearum*) in cotton, decreasing its effect when the plants reach the optimum growth level (Huber and Graham, 1999).

The BR incidence was two times lower in cotton plants fertilized with doses between 100 and 200 kg N ha^{-1}, compared to the initial dose of 50 kg N ha^{-1}. In cotton, N higher than 200 kg ha-1 can increase root growth, especially in shallow soil layers, and increase physiological and biochemical processes in leaves (Chen et al. 2018), not knowing the diseases' answer. It is known that the increase in the availability of N in crops such as rapeseed (*Brassica napus* L.) produces emissions of acetic acid (a volatile antifungal) that could reduce levels of dark spot disease (*Alternaria brassicae*) (Veromann et al. 2013). In this way, perhaps some compound or substance produced by the fertilizers could be being absorbed by the plant and used to reduce the infection of the pathogen that causes BR.

In the interaction between BR factors, in general, in all fertilizers, it was observed that lower doses significantly reduce the disease, except for bovine manure. Conversely, the application of increasing or high doses of N (between 20 and 168 kg ha^{-1}) in rice crops and watermelon can significantly increase the incidence and severity of diseases that attack airborne organs, but only in varieties susceptible to rice cultivation (Long et al. 2000; Santos et al. 2009). However, the negative or positive effect of fertilizers on diseases depends on the experimental conditions and nitrogen source type. For example, infection of Fusarium head blight measured by DNA and mycotoxin content in barley grains is negatively affected by nitrogen fertilization (40–140 kg N ha^{-1}) with Calcium and Ammonium nitrate, only under controlled conditions (Hofer et al. 2016). On the other hand, the response of diseases to N in a crop depends substantially on the plant species, the type of soil, the fertilizer source, the limitations of agri-environmental conditions, and the management of crops general (Antille and Moody 2021). In any case, the response found by us in BR demonstrates the importance of this result under field conditions and using a susceptible cotton genotype.

None of the diseases was significantly correlated, not even between the incidence and severity of RLS, which means that both diseases responded differently to fertilizers and doses. Two important points need to be addressed in this discussion: each pathogen's particularity and the tissue it affects, and the nitrogen source and dose used. In this regard, the susceptibility of tomato plants to Fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*), Bacterial speck (*Pseudomonas syringae* pv *tomato*), and Powdery mildew (*Oidium lycopersicum*) are dependent on the supply of N, only the last two being affected (Hoffland et al. 2000). Also, both the amount of nitrogen added (direct effect) and pathogen competition (indirect effect) play an important role in the plant-pathogen interaction (Liu et al. 2017). This supports the hypothesis that disease response would depend on the nutritional source and the response of each pathogen, or both.

Although the Logistic model is the most used to describe the epidemic progress, the Gompertz model is also among the most used in this research type (Berger 1981; Bergamin Filho 2018; Tjørve and Tjørve 2017). In our work, the Logistic model fits better than Gompertz to RLS, but no model was adapted to BR. To our best knowledge, this is the first study to analyze the progress of RLS and BR in a cotton crop.

With respect to the epidemiological components using the Logistic model, a lower amount of Y_0 for incidence and severity of RLS in plants fertilized with *J. curcas* seedcake y poultry manure it was found, in comparison to the rest of the fertilizers, coinciding in general with the progress curves. However, the *r* for RLS incidence was only lower in plants fertilized with urea, compared to the rest of the nitrogen sources. Although the increase or reduction of disease due to nitrogen fertilization (NH_4, NO_3, or another source), forms of N can act biochemically, physiologically, and molecularly in a differentiated way in plants (Sun et al. 2020). However, this may vary in other species. For example, the progress of Rice blast in rice cultivation, regardless of the nitrogen source, followed a unimodal curve, so that the incidence of the disease and the total area of lesions per plant reached a maximum of about half of the season, subsequently gradually decreasing (Long et al. 2000). In this way, it may be that each nitrogen source is affecting some physiological process in cotton plants, resulting in that differentiated effect on the progress of RLS.

Dose of 150 and 200 kg N ha^{-1} negatively affected the Y_0 and positively the *r* of RLS in cotton plants, respectively, compared to the rest of the doses. This coincided with the progress curves, where it was observed that plants fertilized with higher doses reached a lower final proportion of the disease. It is known that increasing doses of N can negatively affect the area under the disease progress curve (AUDPC) and severity index of Alternaria leaf blight (*Alternaria dauci*) and Cercospora leaf spot (*Cercospora carotae*) on carrot plants (Saude et al. 2014). Perhaps these differences are due to the behavior of each disease and crop. Either way, the mode of action of N to reduce disease pressure is not yet fully understood. One hypothesis would be that N's additional application promotes the growth of new leaves, which would temporarily reduce the severity indices of RLS in cotton plants (Saude et al. 2014).

Although biological components were not evaluated in this study, organic fertilization with *J. curcas* seedcake y poultry manure in cotton cultivation could be a long-term sustainable practice by increasing the abundance of beneficial microorganisms in the soil, being an effective method specially to inhibit soil-borne diseases (Lin et al. 2019; Tao et al. 2020). Organic fertilizers can increase the rhizosphere population's size, which include antagonists and pathogens and/or functional groups of rhizospheric fungi and actinomycetes, protecting cotton plants from especially soil-borne pathogens (Huang et al. 2006). Even this type of fertilizer can reduce the content of heavy metals, i.e., Cd, Pb, and As in the soil, compared to inorganic fertilizers (Lin et al. 2019). Although more studies are needed to investigate disease responses to nitrogen fertilization, work like ours shows that this option is viable. Perhaps, the inorganic fertilizer could be partially replaced by organic compost or both supplemented in cotton crops.

Conclusions

An interaction between nitrogen sources and dose was observed in the incidence of Ramularia leaf spot (RLS) y Boll rot (BR). The BR incidence was two times lower in cotton plants fertilized with doses between 100 and 200 kg N ha^{-1}. Each of the diseases responded differently to fertilizers and doses. The logistic model fits better than Gompertz to RLS, but no model was adapted to BR. Less initial illness (Y_0) for incidence and severity of RLS was found in plants fertilized with *Jatropha curcas* seedcake and poultry manure, but a rate of progress (*r*) lower for the incidence of RLS in plants fertilized with urea, compared to the rest of nitrogen sources. Dose of 150 and 200 kg N ha^{-1} negatively affected the Y_0 and positively the *r* of RLS in cotton plants, respectively, compared to other doses.

Declarations

Availability of data and materials

No other data related to this study is available at this time.

Ethics approval and consent to participate

Not applicable.

Consent for publication

We accept publication.

Competing interests

The authors declare no conflicts of interest.

Funding

Not applicable.

Authors' contributions

O.P.Z., conducted field and laboratory work, collected and analyzed data, and contributed to the writing, review, editing, and discussion of the manuscript. F.Z.G., contributed to discussion of the manuscript and organized the structure and translation. D.P., contributed to the data analysis and discussion of the manuscript. F.R.G.F., supported the field work, data collection and discussion of the manuscript, revision, editing and discussion of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

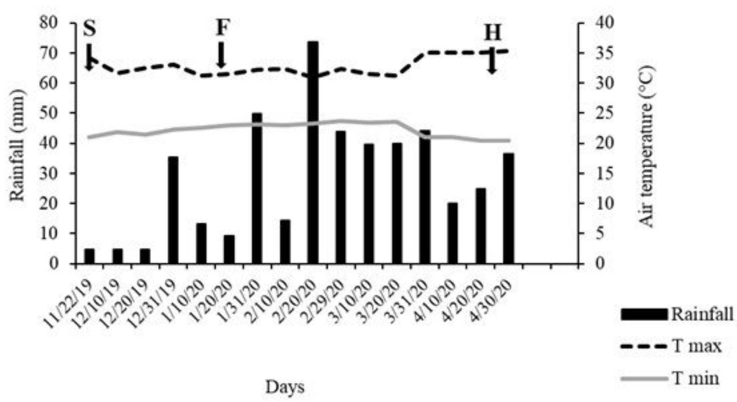

Figure 1

Rainfall and maximum and minimum air temperatures (T max and T min) of Lodana, Manabí, referring to the experimental period (November 22, 2019 to April 30, 2020) with indication of sowing (S), beginning of flowering (F) and harvest (C).

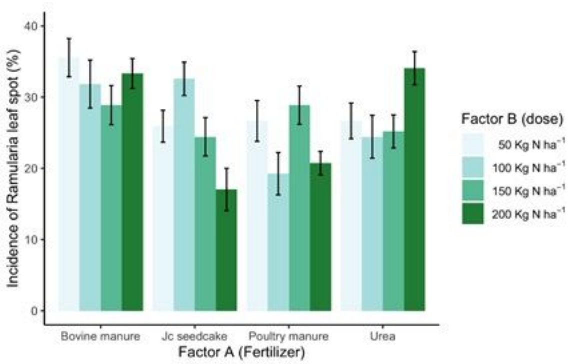

Figure 2

Factorial interaction (fertilizers x dose) for incidence (%) of Ramularia leaf spot, on cotton plants under organic fertilization (bovine manure, Jatropha curcas seed cake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 y 200 kg N ha-1) for each source.

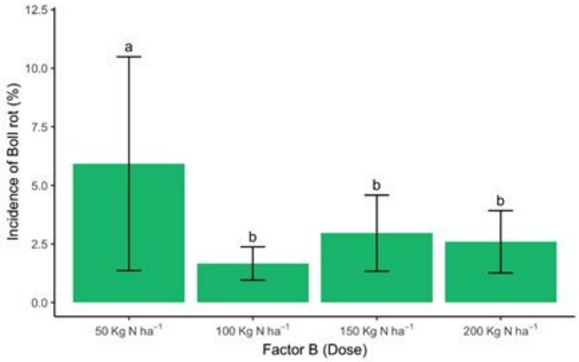

Figure 3

Incidence values (%) de Boll rot in cotton plants, grouped by fertilizer (bovine manure, Jatropha curcas seed cake, and poultry manure), using four doses (50, 100, 150 y 200 kg N ha-1) for each source. Lowercase letters indicate a significant difference by Tukey's test ($p < 0.05$).

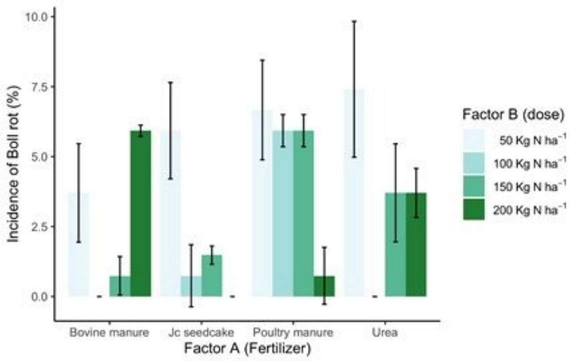

Figure 4

Factorial interaction (fertilizers x dose) for incidence (%) of Boll rot, on cotton plants under organic fertilization (bovine manure, Jatropha curcas seed cake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 and 200 kg N ha-1) for each source.

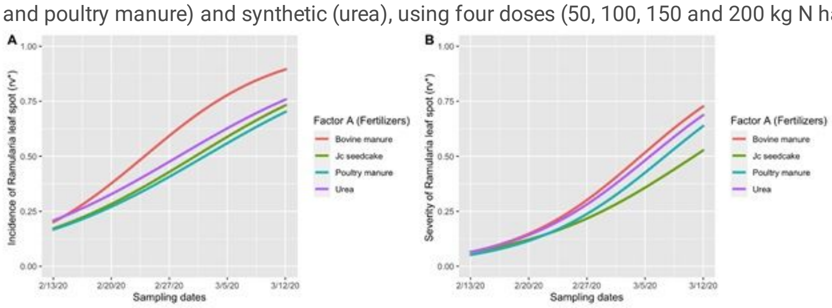

Figure 5

Temporal progress of incidence (A) and severity (B) of Ramularia leaf spot in cotton plants under organic nitrogen fertilization (bovine manure, Jatropha curcas seed cake, and poultry manure) and synthetic (urea), using a Logistic model.

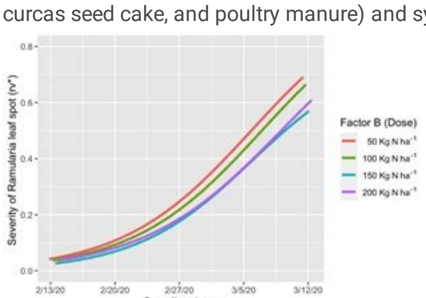

Figure 6

Temporal progression of the severity of Ramularia leaf spot in cotton plants under organic and synthetic nitrogen fertilization, using 50, 100, 150, and 200 kg N ha-1, using a Logistics model.