RAMULARIA LEAF SPOT AND BOLL ROT ARE AFFECTED DIFFERENTLY BY ORGANIC AND INORGANIC NITROGEN FERTILIZATION IN COTTON PLANTS

Oscar J. PALMA-ZAMBRANO[1], Freddy ZAMBRANO-GAVILANES[1,2], Diego PORTALANZA[3], Felipe R. GARCÉS-FIALLOS[1,2]

[1] Universidad Técnica de Manabí, Facultad de Ingeniería Agronómica, Campus Experimental La Teodomira, Km 13, Santa Ana, Manabí.
[2] Instituto de Posgrado, Universidad Técnica de Manabí, Portoviejo, Ecuador.
[3] Universidade Federal de Santa Maria, Departamento de Física, Grupo de Pesquisas Climáticas, Av. Roraima, 1000, Santa Maria (RS), Brasil.

Corresponding author:
Felipe R. Garcés-Fiallos
felipe.garces@utm.edu.ec

Authors' ORCID
Freddy Zambrano-Gavilanes: https://orcid.org/0000-0003-0004-9122
Diego Portalanza: https://orcid.org/0000-0001-5275-0741
Felipe Garcés-Fiallos: https://orcid.org/0000-0002-1795-4439

Acknowledgments: We would like to express gratitude to Project + Cotton, the Brazilian Government, through the Brazilian Cooperation Agency, the Ministry of Foreign Affairs (ABC/MRE), the Food and Agriculture Organization of the United Nations (FAO), and partner countries, for providing materials for the present investigation.

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 the 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 fieldwork, data collection, and discussion of the manuscript, revision, editing, and discussion of the manuscript. In addition, all authors approve the final version of the manuscript.

Ethics approval: Not applicable.

Competing interests: The authors declare no conflicts of interest.

Abstract
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 over time, starting in the reproductive stage B1 (first visible flower bud). A randomized complete block design with a 4x4 factorial arrangement was used (fertilizers x dose), totaling 16 treatments with four replications. The disease progress was analyzed with the nonlinear Logistic and Gompertz models, obtaining the epidemiological parameters amount of initial disease (*Y0*) and progress rate (*r*). 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 could represent BR. Only the RLS epidemiological parameters were affected differently in this experiment compared to BR disease. The possible role of organic and inorganic nitrogen fertilization in the RLS and BR management is discussed.

Keywords: Disease progress. *Gossypium hirsutum* L. Logistic and Gompertz models. Nitrogen dose. Nitrogen fertilization.

1. Introduction

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 Guayas and Manabí, the sown area was affected by climatic and economic factors and lack of certified seeds (García et al. 2019). Between the end of the last century and the last decade in the country, cotton fiber production (t) and productive area (ha) decreased 21 and 29 times, respectively, with Manabí province representing 79% of national total production (Vivero 2017). Experimentally, some cotton varieties have been studied in this province, such as Coker and DP-ACALA 90 (Cañarte-Bermúdez et al. 2020).

One of the most important tasks in cotton cultivation management is fertilization (Teixeira et al. 2008). Nitrogen (N) is the most required nutrient by the plant, as it is a limiting factor for growth and a fundamental element for crop production (Bondada and Oosterhuis 2001). Although cotton plants respond positively to N incorporation, this response can be affected by genotype, soil type, 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 synthetic origin nitrogen sources 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, atmosphere pollution, soil acidification, eutrophication, and biodiversity reduction may be observed (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 differences in pathogens' strategies 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 root condition, allowing an adequate plants growth and capsule quality, making them more potent against diseases attacks (Mur et al. 2016; Chen et al. 2018; 2019; 2020). Rich N sources are known to negatively affect various plant pathogens (Blachinski et al. 1996; Artavia et al. 2010; Veromann et al. 2013). This element also affects physical defenses and antimicrobial phytoalexin production but has positive effects on defense-related enzymes and proteins that induce 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*). Nevertheless, in this context, understanding how pathogens with different infection strategies respond to N levels is of fundamental importance (Mur et al. 2016).

On the Ecuadorian coast, the cotton crop is attacked by diseases such as damping off (*Rhizoctonia solani*), cotton anthracnose (*Colletotrichum gossypii*), and Boll rot (*Diplodia gossypina*) (Sión et al. 1992). Other like Ramularia leaf spot (*Ramulariopsis pseudoglycines*) present in neighboring countries (Aquino et al. 2008) and not yet reported in Ecuador, can negatively affect *Gossypium* genus plants foliar area and fiber yield (Ascari et al. 2016; da Silva et al. 2019). Despite the importance of these crop diseases, there is little bibliography about them affecting Ecuadorian cotton. Even studies evaluating the effect of organic fertilizers on cotton cultivation are scarce. Thus, the work aimed to study cotton crop (variety DP ACALA 90) aerial tissue diseases interaction between organic fertilization (bovine manure, chicken manure, and physic nut seed cake), and synthetic (rich in N) fertilization (urea).

2. Materials and methods

Study area and Field experiment

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 (01° 09´ 51 S, 80° 23' 24'' W, 60 m altitude) (INAMHI 2015).

The DP ACALA 90 variety was sown under field conditions, at 0.40 m between plants, 1 m between rows, and 2 m between blocks, on an area where cotton was grown in the previous cycle. The soil type classified as clay loam, according to the soil taxonomy USDA (Soil Survey Staff, 2014) observed in Table 1, was 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. Rainfall and maximum and minimum air temperatures (T max and T min), recorded in Lodana, Manabí, Ecuador during the experiment are shown in Fig.1.

The total experimental area was2688 m² (48 m x 56 m), where each experimental plot constituted 36 m² (6 m x 6 m) with a useful area of 20.8 m² (5.20 m x 4 m). Fertilizers used as N sources were Bovine manure, *Jatropha curcas* seedcake, Poultry manure, and urea (the latter used as a control). 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 flowering stage (50 DAE), completing the exact dosage 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 showed in table 2.

Disease assessment

The intensity was assessed for RLS (*R. pseudoglycines*) and BR (*Diplodia* sp.), at the beginning of the reproductive phase (B1) (76 DAE) (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 measured by counting each of the symptomatic organs (leaves and capsules) present in plants, employing a visual observation, and transforming the average value of each stratum into a percentage. The severity was only estimated for RLS, using the diagrammatic scale proposed by Aquino et al. (2008).

Experimental design and statistical analyses

A randomized complete block design with a 4x4 factorial arrangement was used (fertilizers x dose), totaling 16 treatments with four replications. After verifying the normality and homoscedasticity of the values obtained, variance analysis was performed. For data comparison Tukey test (p < 0.05) was used.

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 executed 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).

3. Results

The factorial analysis showed a significant interaction between fertilizers (factor A) and dose (factor B) only for RLS incidence (P < 0.0194). Differences between doses were only observed for boll rot incidence (P < 0.02519) (Table 3).

In the RLS incidence interaction analysis (Fig. 2), no response pattern was observed for each fertilizer's doses. However, 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 a RLS incidence between 16 and 21% which was lower compared to the other treatments. In general, our results indicated that regardless of the dose used, plants fertilized with bovine manure had the highest RLS incidence (33%) compared to the rest of the fertilizers used.

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

No correlation was observed between incidence, severity and, intensity, with probabilities between 0.002 and 0.892 (data not shown).

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. 4A). 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. 4B).

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. 5).

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 were significant (Table 4). A lower amount of initial disease(Y_0) for RLS was found using the Logistic model, 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 disease progress rate (*r*: 0.012) in plants fertilized with urea was observed, compared to the rest (0.016). This same parameter for the severity variable, no difference was observed between fertilizers. Finally, 150 (0.076) and 200 (0.028) ^{1}doses kg N ha^{-}, negatively affected RLS Y_0 and positively the *r* compared to the other doses.

For BR incidence (%), none of the non-linear models used well fitted the parameters (Table 5). Furthermore, both Y_0 and the *r* for 100 kg N ha-1 dose could not be calculated.

4. Discussion

Currently, countless studies have shown the positive effect generated by N-rich fertilizers in cotton plants. For example, applications between 55 and 240 N kg ha^{-1} increase the canopy photosynthesis and leaves weight, thus inducing a higher number of nodes and capsules, hundred seeds weight, 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 N fertilization in cotton plant diseases. In our 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.

Cotton crop sowed in the previous cycle seems to have allowed a sufficient source for RLS or BR inoculum to appear in vegetative stages, but with more intensity in 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 is susceptible to the *Cotton leafroll dwarf virus* (CLDV) transmitted by aphid *Aphis gossypii*, and to Bacterial blight caused by *Xanthomonas citri* pv. *malvacearum*. For the first time and in field conditions, we showed that this genotype also is susceptible to RLS and BR diseases.

Although nutrients can affect the tolerance or resistance of plants 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 N fertilizer and dosage. N-rich sources can reduce spore germination and mycelial growth of *Alternaria macrospora* in *in vitro* conditions and r lesions diameter and Alternariosis percentage under controlled conditions, but none of the parameters under field conditions (Blachinski et al. 1996). 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 the 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 different ways in plants against pathogens (Sun et al., 2020). In this sense, we can infer that both some external factor and an effect at a cellular level of each fertilizer may have influenced the observed behavior in our work.

Regardless of the dose used, the plants fertilized with bovine manure presented a higher incidence of RLS. We were even able to verify that plants fertilized with 100 kg N ha^{-1} of *J. curcas* seedcake and 100 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 large amount of K compared to Bovine manure and urea. In this manner, K could have affected RLS. Indeed, K-rich fertilizers can reduce 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 dosage 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 physiological and biochemical processes in leaves (Chen et al. 2018), not knowing the diseases' answer. Meanwhile, in other crops as rapeseed (*Brassica napus* L.), an increase in the availability of N can produce emissions of acetic acid (a volatile antifungal), which 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 absorbed by the plant and used to reduce the pathogen infection that causes BR.

None of the diseases was significantly correlated, not even between the incidence and severity of the RLS, which means that this disease responded differently to fertilizers and doses. Two important points need to be discussed here: 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, but the two last diseases only may be 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, 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 type of research (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 adjusted to BR. For the epidemiological components using the Logistic model, the least Y_0 for incidence and severity of RLS in plants fertilized with *J. curcas* seedcake y poultry manure was observed, when compared to the rest of the fertilizers. In general, this result coincided with the progress curves. However, the *r* for RLS incidence was only in plants fertilized with urea, compared to the rest of N sources. Although the increase or reduction of disease due to N 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 vegetable species. For example, the progress of Rice blast in rice crop, regardless of the N 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.

Doses 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 result coincided with the progress curves, where the plants fertilized with higher doses reached a lower final proportion of the disease. It is known that increasing 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. Nonetheless, N mechanism of action of 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).

Though no biological component was analyzed in this study, organic fertilization with *J. curcas* seedcake y poultry manure in the cotton crop could be a long-term sustainable practice by increasing the abundance of soil beneficial microorganisms in the, being an effective method to inhibit soil-borne diseases (Lin et al. 2019; Tao et al. 2020). Organic fertilizers can increase the rhizosphere population's size, i.e. antagonists and pathogens and 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 (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, in the cotton crop, the inorganic fertilizer could be partially replaced by an organic source, or as a complement.

5. Conclusions

An interaction between nitrogen sources and dose was observed in RLS incidence. 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 the Gompertz to RLS, but no model was adapted to BR. Less amount of initial disease (Y_0) for incidence and severity of RLS was found in plants fertilized with *Jatropha curcas* seedcake and poultry manure, but lower progress rate (*r*) for RLS incidence in plants fertilized with urea, compared to the rest of N sources. 150 and 200 kg N ha^{-1} doses negatively affected the RLS Y_0 and positively the *r* in cotton plants, respectively, compared to other doses.

References

ALVES, K. and DEL PONTE, E. *Analysis and Simulation of Plant Disease Progress Curves*. R package version 0.2.0. 2020.
AQUINO, L.A., BERGER, P.G., RODRIGUES, F.Á., ZAMBOLIN, L., HERNÁNDEZ, J.F. and MATTOS, L. Elaboração e validação de escala diagramática para quantificação da mancha de ramularia do algodoeiro. *Summa Phytopathologica*. 2008, **34**(4), 361-363. http://dx.doi.org/10.1590/S0100-54052008000400012
ARTAVIA, S., URIBE, L., SABORÍO, F., ARAUZ, L. and CASTRO, L. Efecto de la aplicación de abonos orgánicos en la supresión de *Pythium myriotylum* en plantas de tiquisque (*Xanthosoma sagittifolium*). *Agronomía Costarricense*. 2010, **34**(1), 17-29.
ASCARI, J.P., MENDES, I.R.N., DA SILVA, V.C. and DE ARAÚJO, D.V. Ramularia leaf spot severity and effects on cotton leaf area and yield. *Pesquisa Agropecuaria Tropical*. 2016; **46**(4), 434-441. https://doi.org/10.1590/1983-40632016v4642781
BERGAMIN FILHO A. *Análise temporal de epidemias*. In: Volume 1. Princípios y conceitos. 5ta. Edição. AMORIM, L., REZENDE, J.A.R., BERGAMIN FILHO, A. Agronômica Ceres, Ouro Fino, MG. 2018; pp. 520-530.
BERGER RD. Comparison of the Gompertz and Logistic equations to describe plant disease progress. *Phytopathology*. 1981. **71**, 716-719. https://doi.org/10.1094/phyto-71-716
BLACHINSKI, D., SHTIENBERG, D., DINOOR, A., KAFKAFI, U., SUJKOWSKI, L.S., ZITTER, T.A. and FRY, W.E. Influence of foliar application of nitrogen and potassium on Alternaria diseases in potato, tomato and cotton. *Phytoparasitica*. 1996, **24**(4), 281-292. https://doi.org/10.1007/BF02981411
BONDADA, B.R. and OOSTERHUIS, D.M. Canopy photosynthesis, specific leaf weight, and yield components of cotton under varying nitrogen supply. *Journal of Plant Nutrition*, 2001, **24**(3), 469-477. https://doi.org/dx.doi.org/10.1081/PLN-100104973
CAÑARTE-BERMÚDEZ, E., SOTELO-PROAÑO, R. and NAVARRETE-CEDEÑO, B. Generación de tecnologías para incrementar la productividad del algodón *Gossypium hirsutum* L. en Manabí, Ecuador. *Revista Ciencia UNEMI*. 2020, **13**(33), 85-95.
CHEN, J., LIU, L., WANG, Z., SUN, H., ZHANG, Y., BAI, Z., SONG, S., LU, Z. and LI, C. Nitrogen fertilization effects on physiology of the cotton boll–leaf system. *Agronomy*. 2019, **9**(6), 271. https://doi.org/10.3390/agronomy9060271
CHEN, J., LIU, L., WANG, Z., ZHANG. Y., SUN, H., SONG, S., BAI, Z., LU, Z. and LI, C. Nitrogen fertilization increases root growth and coordinates the root-shoot relationship in cotton. *Frontiers in Plant Science*. 2020, **11**, 880. https://doi.org/10.3389/fpls.2020.00880
FANG, D.D. *General Description of Cotton*. In: FANG, D. (Eds) Cotton Fiber: Physics, Chemistry and Biology. Springer, Cham. 2018. https://doi.org/10.1007/978-3-030-00871-0_1
FRENCH, A.D. and KIM, H.J. *Cotton Fiber Structure*. In: Fang D. (Eds) Cotton Fiber: Physics, Chemistry and Biology. Springer, Cham. 2018. https://doi.org/10.1007/978-3-030-00871-0_2
GARCÍA, F., SUAREZ-DUQUE, D. and RODRIGUEZ, W. Importancia social del cultivo de algodón en la agricultura familiar campesina de Guayas y Manabí en Ecuador. Congresso Brasileiro do Algodão. 2019. 172. Recuperado el 16 de Diciembre de 2020, de: https://www.researchgate.net/publication/339827693_IMPORTANCIA_SOCIAL_DEL_CULTIVO_DEL_ALGODON_EN_LA_AGRICULTURA_FAMILIAR_CAMPESINA_DE_GUAYAS_Y_MANABI_EN_ECUADOR

HOFFLAND, E., JEGER, M.J. and VAN BEUSICHEM, M.L. Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. *Plant and Soil*. 2000, **218**, 239-247. https://doi.org/10.1023/A:1014960507981
HUANG, J., LI, H. and YUAN, H. Effect of organic amendments on Verticillium wilt of cotton. *Crop Protection*. 2006, **25**, 1167-1173. https://doi.org/10.1016/j.cropro.2006.02.014
HUBER, D.M. and GRAHAM, R.D. *The role of nutrition in crop resistance and tolerance to disease*. In: RENGEL, Z. (Ed.), Mineral nutrition of crops fundamental mechanisms and implications, Food Product Press, New York. 1999; pp. 205–226.
LIN, W., LIN, M., ZHOU, H., WU, H., LI, Z. and LIN, W. The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *PLoS ONE*. 2019, **14**(5), e0217018. https://doi.org/10.1371/journal.pone.0217018
LIU, X., LYU, S., SUN, D., BRADSHAW, C.J.A. and ZHOU, S. Species decline under nitrogen fertilization increases community-level competence of fungal diseases. Proceedings of the Royal Society B. 2017, **284**, 20162621. http://dx.doi.org/10.1098/rspb.2016.2621
LONG, D.H., LEE, F.N. and TEBEEST, D.O. Effect of nitrogen fertilization on disease progress of rice blast on susceptible and resistant cultivars. *Plant Disease*. 2000, **84**, 403-409. https://doi.org/10.1094/PDIS.2000.84.4.403
MUR, L.A.J., SIMPSON, C., KUMARI, A., GUPTA, A.K. and GUPTA, K.J. Moving nitrogen to the centre of plant defense against pathogens. *Annals of Botany*. 2016, **119**(5): 703-709, https://doi.org/10.1093/aob/mcw179
PALOMO, G.A., GAYTÁN-MASCORRO, A., ESPINOZA-BANDA, A., MARTÍNEZ-AGÜERO, H.J. and JASSO-CANTÚ, D. Dosis de nitrógeno y número de riegos en el rendimiento y calidad de la semilla de algodón. *Revista Fitotecnia Mexicana*. 2003, **26**(2), 95-99.
RStudio Team. RStudio (1.0.136). *Integrated Development for R. RStudio*. 2017.
SAUDE, C., MCDONALD, M. and WESTERVELD, S. Nitrogen and fungicide applications for the management of fungal blights of carrot, *HortScience*. 2014, **49**(5), 608-614. https://doi.org/10.21273/HORTSCI.49.5.608
SCUDLARK, J.R., JENNINGS, J.A., ROADMAN, M.J., SAVIDGE, K.B. and ULLMAN, W.J. Atmospheric nitrogen inputs to the Delaware inland bays: the role of ammonia. *Environmental Pollution*. 2005, **135**(3), 433–443. https://doi.org/10.1016/j.envpol.2004.11.017
SINGH, C., JOSRA, R.C. AND KATTI, G.V. Soil and foliar application of nitrogen to rainfed cotton. *Indian Journal of Agronomy*. 1970, **55**(3), 269-271.
SIÓN, F., CASTRO, L., ARROYAVE, J. and TORO, J. *Manual del cultivo del algodón*. Manabi-Portoviejo: Instituto Nacional de Investigaciones Agropecuarias del Ecuador. 1992. Recuperado el 20 de Octubre de 2020, de https://repositorio.iniap.gob.ec/bitstream/41000/1190/1/iniap-Bolet%c3%adn%20divulgativo%20No.%20111.pdf
SUASSUNA ND, MORELLO CL, DA SILVA FILHO JL, PEDROSA MB, PERINA FJ, MAGALHÃES FOC, SOFIATTI V, LAMAS FM. BRS 372 and BRS 416: high-yielding cotton cultivars with multiple disease resistance. *Crop Breeding and Applied Biotechnology*. 2020; **20**(1): e27242016. http://dx.doi.org/10.1590/1984-70332020v20n1c6
USDA. *Soil Survey Staff. Keys to Soil Taxonomy*. In: 12th. USDA-NRCS, Washington, DC, 2014; pp. 37–40.

TAO, R., LIANG, Y., WAKELIN, S.A. AND CHU, G. Supplementing chemical fertilizer with an organic component increases soil biological function and quality. *Applied Soil Ecology*. 2015, **96**, 42-51. https://doi.org/10.1016/j.apsoil.2015.07.009

TAO, R., HU, B. and CHU, G. Impacts of organic fertilization with a drip irrigation system on bacterial and fungal communities in cotton field. *Agricultural Systems*. 2020, **182**, 102820. https://doi.org/10.1016/j.agsy.2020.102820

TEIXEIRA, I., KIKUTI, H. and BORÉM, A. Crescimento e produtividade de algodoeiro submetido a cloreto de mepiquat e doses de nitrogênio. *Bragantia*. 2008, **67**(4): 891-897. https://doi.org/10.1590/S0006-87052008000400011

TJØRVE, K.M.C. and TJØRVE, E. The use of Gompertz models in growth analyses, and new Gompertz-model approach: An addition to the Unified-Richards family. *PLoS ONE*. 2017, **12**(6), e0178691. https://doi.org/10.1371/journal.pone.0178691

VEROMANN, E., TOOME, M., KÄNNASTE, A., KAASIK, R., COPOLOVICI, L., FLINK, J., KOVÁCS, G., NARITS, L., LUIK, A. and NIINEMETS, U. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. *Crop Protection*. 2013, **43**, 79-88. https://doi.org/10.1016/j.cropro.2012.09.001

VIVERO J. Proyecto + Algodón Ecuador. Manabí-Ecuador: Organización de las Naiones Unidas para la Alimentacion y la Agricultura. 2017. Obtenido de http://www.fao.org/3/I9123ES/i9123es.pdf

ZHENG, J., KILASARA, M.M., MMARI, W.N. and FUNAKAWA, S. Ammonia volatilization following urea application at maize fields in the East African highlands with different soil properties. Biol Fertil Soils 2018; 54: 411–422. https://doi.org/10.1007/s00374-018-1270-0

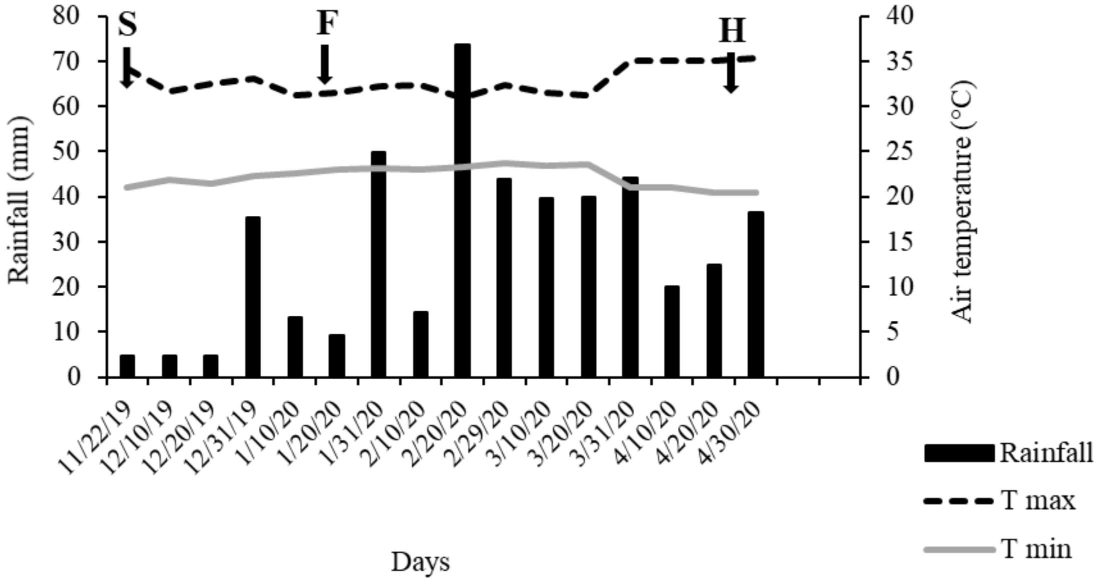

Figure 1. Rainfall and maximum and minimum air temperatures (T max and T min) of Lodana, Manabí, referring to the experimental period (November 22, 2019 to April 30, 2020) with indication of sowing (S), beginning of flowering (F) and harvest (C).

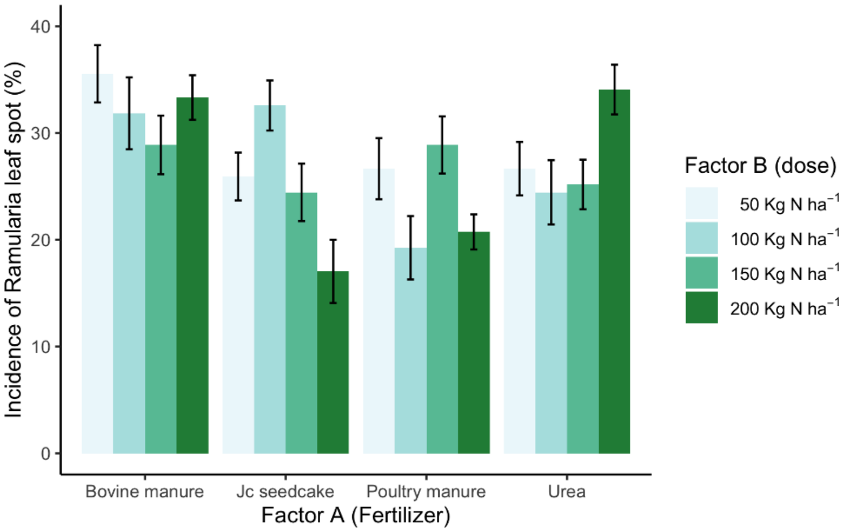

Figure 2. Factorial interaction (fertilizers x dose) for incidence (%) of Ramularia leaf spot, on cotton plants under organic fertilization (bovine manure, *Jatropha curcas* seed cake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 y 200 kg N ha^{-1}) for each source.

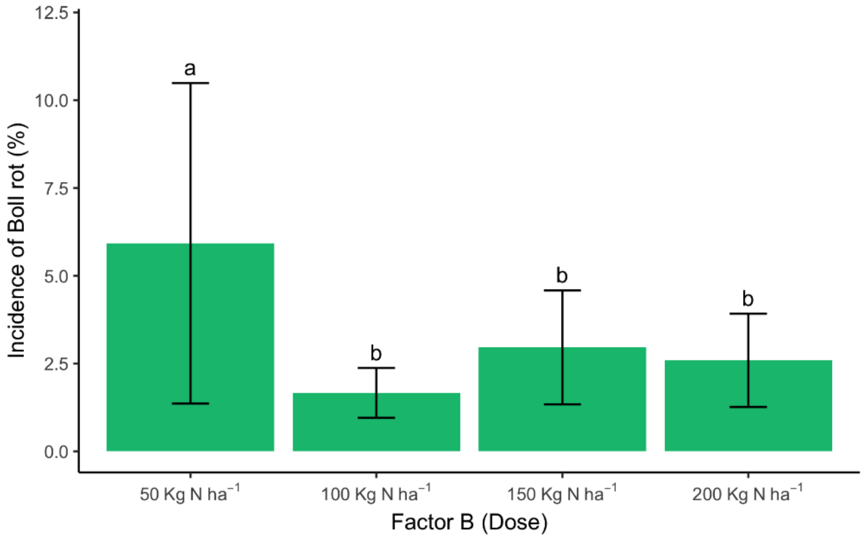

Figure 3. Incidence values (%) de Boll rot in cotton plants, grouped by fertilizer (bovine manure, *Jatropha curcas* seed cake, and poultry manure), using four doses (50, 100, 150 y 200 kg N ha^{-1}) for each source. Lowercase letters indicate a significant difference by Tukey's test ($p < 0.05$).

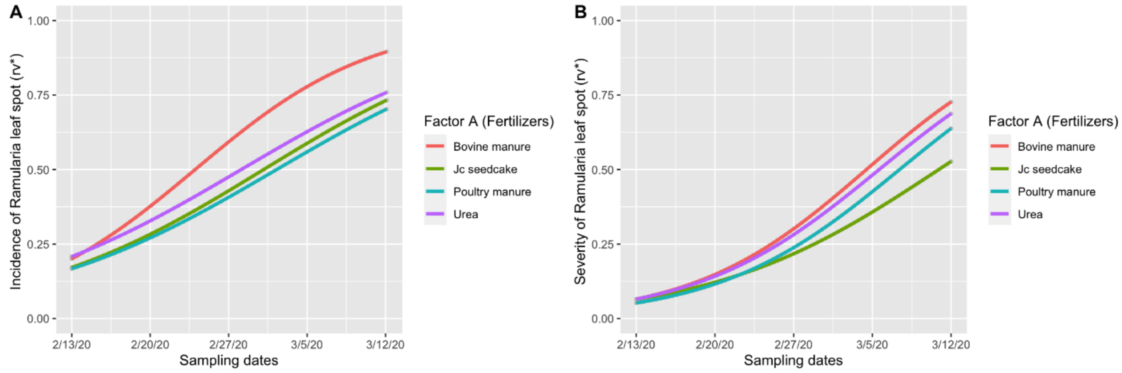

Figure 4. Temporal progress of incidence (A) and severity (B) of Ramularia leaf spot in cotton plants under organic nitrogen fertilization (bovine manure, *Jatropha curcas* seed cake, and poultry manure) and synthetic (urea), using a Logistic model.

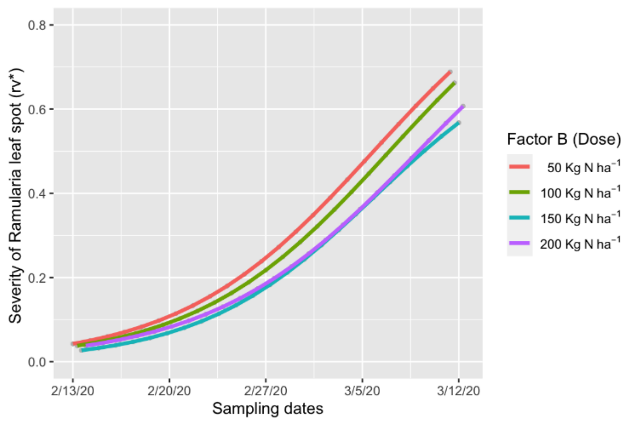

Figure 5. Temporal progression of the severity of Ramularia leaf spot in cotton plants under organic and synthetic nitrogen fertilization, using 50, 100, 150, and 200 kg N ha^{-1}, using a Logistics model.

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

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

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	0.06998

[1] GL: degrees of freedom

[2] F: Fisher's calculated value

* statistical significance $p < 0.05$

Table 4. Amount of initial disease (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 – valor	Y_0	r	R_2	P – valor
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 ($P < 0.05$).

Table 5. Amount of initial disease (Y_0) and rate of disease progression (r) obtained from the quantification of incidence (%) of Boll rot, 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 – valor	Y_0	r	R_2	P – valor
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.