Wilting by *Fusarium oxysporum* Schlthl in masaguaro *(Pseudosamanea guachapele)* (Kunth).

Marchitez por *Fusarium oxysporum* Schlthl en masaguaro (*Pseudosamanea guachapele*) (Kunth).

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Abstract

Introduction. The masaguaro (*Pseudomanea Guachapele*) was used in reforestation by the" Fundación Orinoquia Biodiversa" in Arauca, and it presented an unknown wilting

Objective. Characterize the disease and its causal agent, and evaluate the possibilities of chemical and biological control of the disease.

Methodology: The research was carried out in Campo Alegre Arauquita neighborhood, Arauca, Colombia. The samples were analyzed in the microbiology laboratory of the University of Pamplona. The disease and its causal agent were characterized, the incidence and severity of the disease were determined and the monetary losses considering the dead plants, were estimated. A wilting control experiment using benomyl and *Bacillus subtilis* was carried out, comparing them to a control without treatment. *Fusarium oxysposum* was determined as the causal agent.

Results. *Fusarium oxysporum* was identified as causal agent of the disease. Wilting reached between 13 and 54% of incidence. The economic losses were estimated at more than 19 million pesos / ha.

Conclusion: The preventive and eradicative action of benomyl and only preventive action of *B. subtilis* were verified.

Key words: forest tree, *Bacillus subtilis*, benomyl, fungus, wilting

Resumen.

Introducción: El masaguaro (Pseudomanea Guachapele) fue utilizado en la reforestación por la "Fundación Orinoquia Biodiversa" en Arauca y presentó un marchitamiento desconocido.

Objetivo. Caracterizar la enfermedad y su agente causal, y evaluar el posible control químico y biológico de la enfermedad.

Metodología: La investigación se realizó en el barrio Campo Alegre Arauquita, Arauca, Colombia. Las muestras se analizaron en el laboratorio de microbiología de la Universidad de Pamplona. Se caracterizó la enfermedad y su agente causal, se determinó la incidencia y severidad de la enfermedad y se estimaron las

pérdidas monetarias considerando las plantas muertas. Se realizó un experimento de control de marchitez utilizando benomyl y *Bacillus subtilis*, comparándolos con un control sin tratamiento.
Resultados: *Fusarium oxysporum* fue identificado como agente causal de la enfermedad. El marchitamiento alcanzó entre el 13 y el 54% de incidencia. Las pérdidas económicas se estimaron en más de 19 millones de pesos / ha.
Conclusión: Se verificó la acción preventiva y erradicativa de benomyl y solo acción preventiva de *B. subtilis.*
Key words: árbol forestal, *Bacillus subtilis*, benomyl, hongo, marchitamiento

1. INTRODUCTION

Pseudomanea guachapele (Kunth) Harms [1]. synonymy *Albizia guachapele* (Kunth) Dugand [2] is known in Colombia as masaguaro o iguá but have others common name in América

. It is an arboreal legume of Fabaceae family. Masaguaro is of great importance, so it helps to improve soil fertility and contribute to a more sustainable use of the land. Studies showed that this legume trees increased the availability of N in the soil. For that may result in significant economic and ecological benefits due to the entry of N through biological nitrogen fixation [3] [4].

This tree is typical of the tropical dry forests of the Pacific lowlands of Central America, and grows where temperatures vary from 22 to 32 ºC (annual biotemperature higher than 24 ºC average) and rainfall that fluctuates between 1000-2500 mm. Masaguaro grows up to 1200 meters above sea level but grows better below 800 meters above sea level, in places with a good high-water level. Prefer fertile soils, but tolerate infertile and shallow ones [3].

This plant is found at low elevations in humid, sub-humid and dry areas, often in gallery forests and in particular along water courses. It is fast growing and very abundant in secondary dry forest, tolerates partial shade when young, and somewhat resistant to fire. It is distributed from southeastern Mexico through all of Central America to Ecuador in South America, it can also be found in the Caribbean islands. It shows great ability to adapt to various climatic conditions, so this species could be considered due to its adaptability and resistance as an alternative in reforestation programs [5].

The cultivation of masaguaro requires a sandy and well-drained soil with a good content of organic matter, the soil must be light so that the roots develop optimally and allow it to stay on considerable slopes between 10 and 25%. Soil pH should be in neutral range, 6 to 7 with a light to medium texture. The plant does not tolerate frost or heavy poorly drained soils, is sensitive to winds, which greatly affect its survival, shape and growth [6].

The municipality of Arauquita, Arauca, Colombia has an area of 304,500 ha, of this total only 641 ha are planted with forestry, including 11 ha of masaguaro or iguá (*P. guachapele*) for restoring degraded areas planted by the Fundación Orinoquía Biodiversa (Tame municipality). In August 2018, the presence of a wilt caused by an unknown causal agent was reported, which became a serious concern because it caused plant death in their first stage of development

The present article aims to characterize the wilt in the masaguaro, *Pseudosamanea guachapele* (Kunth) Harms. and its causal agent in Arauquita, Arauca, reporting the results of the control assay carried out, the dynamics of the disease and the economic losses

2. METHODOLOGY

A field research was carried out in Campo Alegre village located in the municipality of Arauquita, in 18-month plantations of masaguaro belonging to the Fundación Orinoquía Biodiversa, consisting of two plots, one with 10.5 ha and the other with 0.5 ha. The geographical location of the central point is at Longitude 71º04'51.30 "W and Latitude 4º35'56.57" N.

Characterization of wilt and the causal agent that affects the plantation of masaguaro in Arauquita

Plants with different symptoms and levels of damage were selected. The collected material was taken to the Laboratory of the University of Pamplona fulfilling the standard protocols. For the isolation and identification of the causal agent of wilt, the samples, both in the field and in the laboratory, were thoroughly reviewed.

The Laboratory worked on the isolation of the microorganisms from each of the samples with symptoms of possible diseases. Pieces of tissue of approximately 5 mm were cut with the apparently healthy half and the other half diseased, which were subjected to a disinfection protocol that consisted of: one minute in distilled water with constant agitation, two minutes in 2% ethanol, washing in distilled water for one minute, two minutes in 2.5% sodium hypochlorite with agitation, and finally the samples were immersed for three minutes in distilled water, followed by drying in absorbent towel. The protocol was run under the influence of a lighter flame to avoid contamination of the samples.

After drying, the samples were taken to the Laminar flow hood/cabinet where the tissue sowing was carried out on PDA culture medium (Papa Dextrose Agar). Four pieces of the diseased tissue of the sample were placed per Petri dish with five repetitions. The Petri dishes were incubated at a constant temperature (25 ◦C), for 10 days. During this time, observations were made on the third, sixth and tenth days, in order to record the changes of the microorganism colonies.

Subsequently, the fungal colonies were purified by sowing mycelium on PDA medium. Once the colonies were purified, they were incubated for 10 days at a constant temperature (25 ◦C), with revisions on the third, sixth and tenth day. Then, they were removed at room temperature, where dishes were observed under a microscope.
For the identification, the macroscopic features of the fungus colonies were considered, such as pigmentation of the media, the size of the colony, growth rate, color, texture, among others. Under microscopy the type of mycelium produced, the presence or absence of fruiting bodies, the shape of the conidia, types of spores and resistance structures were analyzed. For *Fusarium* specie identification "The *Fusarium* laboratory manual" was used [7].

To verify the pathogenicity of each isolated microorganisms according to Koch's postulates, five healthy two-month-old masaguaro seedlings were inoculated, which were kept isolated. Inoculation was performed using a sterile scalpel for each plant. Wounds were made at the base of the seedlings stem and inoculated with the help of a sterile piece of cotton from the microorganisms developed in Petri dishes. To each Petri dish, 5 ml of sterile distilled water was added to achieve a suspension of the reproductive structures of the microorganisms.

Comparison of control alternatives for wilt management in plantations of masaguaro in Arauquita

Once the microorganism was isolated, a demonstrative assay was carried out in the field, using benomyl chemical fungicide and "Bactox" biological fungicide based on *Bacillus subtilis* (Ehrenberg) Cohn and a control without treatment. For each treatment, 15 healthy trees were marked, 15 trees that presented initial symptoms (Grade 1), 15 trees that presented advanced symptoms (degree 2) were used. A scale proposed to evaluate the draught in pea were used [8]. Five applications were made to the 45 plants with benomyl and *B subtilis* every 15 days for two months.

The incidence and severity were evaluated to determine the dynamics of the disease according to treatment. A scale proposed by [8] to evaluate the draught in pea was adapted and used (0**=** heathy plant, **1=** the plant with initial symptom, **2=** the plant with advanced symptom, **3=** dead plant).

Two weeks' time surveys were made in order to determine de incidence of the wilt using the fallowing equation:

$$\text{Incidence }(\%) = \frac{\text{n (diseased plants)}}{\text{N(evaluated plants)}} X100 \qquad \text{Equation 1}$$

Like the severity of the disease, using the fallowing equation.

$$S\ (severity\ \%) = \frac{\Sigma(aXb)}{KN} X100 \qquad \text{Equation 2.}$$

Where: S = severity., a = scale grade, b= Number of plants with one determined grade of the scale.

K= maximum grade of the scale, N= Number of evaluated plants

A statistical analysis of sample proportions at the end of the trial in comparison with the beginning was carried out for the incidence of the disease after five applications every 14 days, for N = 15. STATISTIX 10 package was used with 95% of confidence level [9]. A month after the trial began, it was decided to apply the best product to the rest of the planted area, both in the 10.5 ha plot and 0.5 ha one.

Determination of the disease dynamic in masaguaro cultivation in Arauquita.

In the small lot of 0.5 ha 100 alive plants and in the 10.5 ha lot 200 plants were sampled, selecting them in random way walking the field in a double diagonal, in the center of the plantation, no monitoring plants from the border. This represented 100 plants/field up to 1 ha, and in fields larger than 1 ha, 100 plants/ha plus 10 plants for each increased ha (National Plant Health Center, 2006). During the first sampling, plants were marked keeping them until the end of the investigation. Symptoms of wilt, degree of severity in each plant was registered. The scale before mentioned [8], was used. With the information obtained in each sampling, the percentage of incidence and severity per lot was determined, according to formulas 1 and 2, respectively.

Determination of the economic losses caused by wilt in the cultivation of masaguaro in Arauquita

For the calculation of the economic losses caused by wilt in the cultivation of masaguaro in Arauquita, the dead plants in each plot were counted directly from the georeferencing that the company had of them. The purchase price of each plant certified by ICA, 1500 pesos, and the cost of care, estimated by the Company at 20,000 pesos per plant in the 20 months of planting, were considered.

3. RESULTS

Characterization of wilt and the causal agent that affects the plantation of masaguaro in Arauquita

The initial symptoms of the disease showed an incipient wilt with some yellow leaves on the lower branches of the plant (Fig. 1 a). As the disease progressed, especially in the larger plants, they showed symptoms of a yellow discoloration of the leaves. Whole branches turned yellow and some folded, producing a yellow flag appearance in the crop (Fig. 1b). With the progression of the disease, the plant partially defoliated and all the leaves turned yellow and some with partial necrosis. By giving a cross section to the stem of the diseased plants, the necrotic vascular bundles with a purplish brown coloration could be seen, in some cases in the form of pits and others of bands (Fig. 1 c) Finally, some plants died (Fig. 1 d). Another distinctive feature in diseased plants was a chancre at the base of the stem that presented in corky form as gangrene (Fig.1 e), which was complemented by necrosis of the vascular bundles when making a cross section (Fig. 1 f).

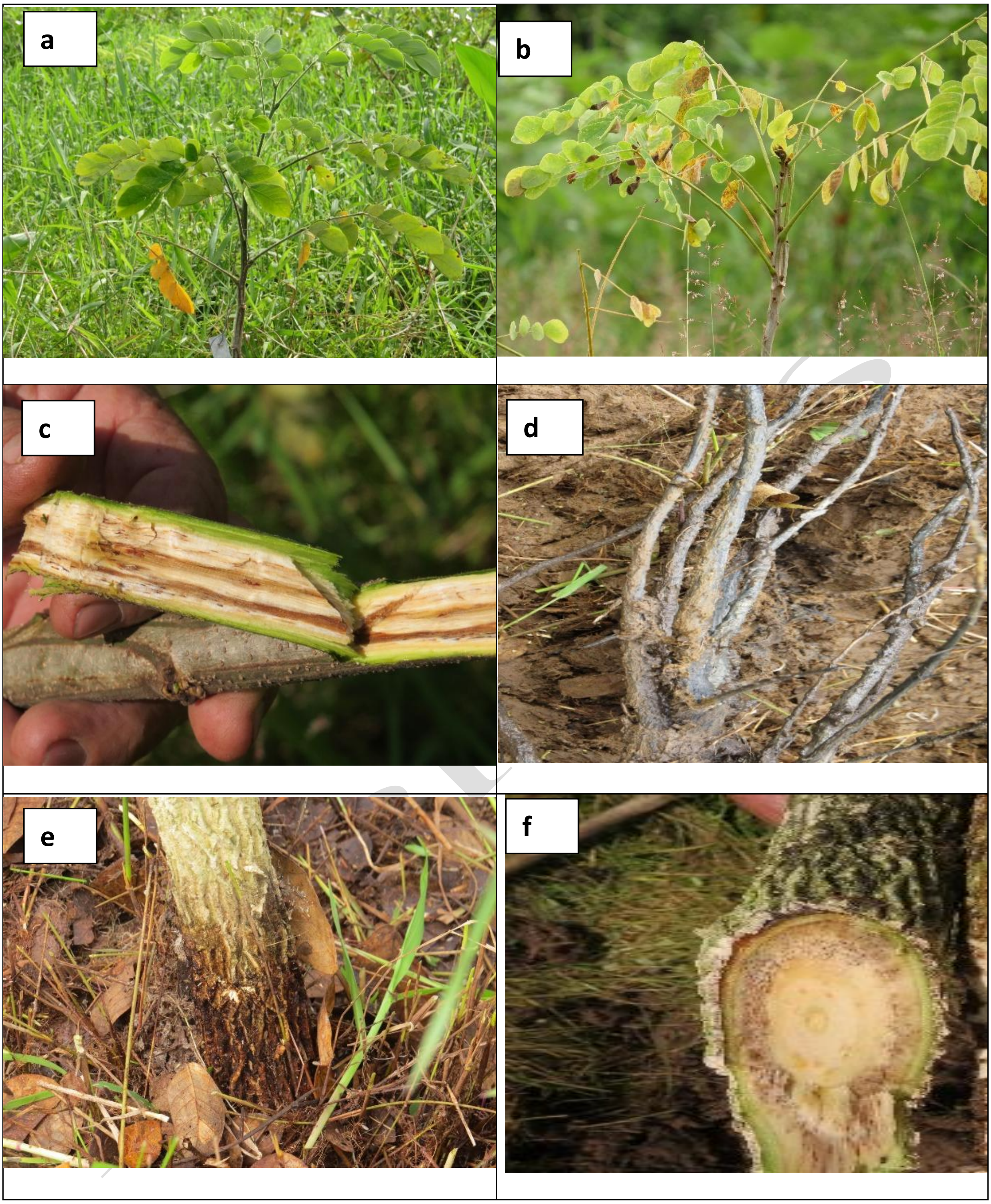


Fig. 1. Dynamics of disease progress a); initial symptoms b); symptoms with fallen branches c); stem necrosis view in longitudinal section d); dead plant e); chancre at the base of the stem f); stem necrosis view in cross section.

In the laboratory, two fungi grow on the sown tissues in PDA medium. In some diseased tissues, a fine whitish mycelium developed that colored salmon-colored the medium from the beginning (Fig. 2 a). For its pigmentation in PDA, and for presenting short septate macroconidia, abundant microconidia and chlamydospores it was identified as *Fusarium oxysporum,* according to the key [7]. Another thin whitish mycelium was also

developed on the diseased tissues, but it did not change the color the PDA medium (Fig. 2 c). A strain of *Fusarium* sp. was purified from this. It was not possible to identify the species due to not pigmenting the medium (Fig. 2 d), and although it did present microconidia and macroconidia, did not develop chlamydospores, nor other structures that could differentiate it morphologically from other *Fusarium* species.

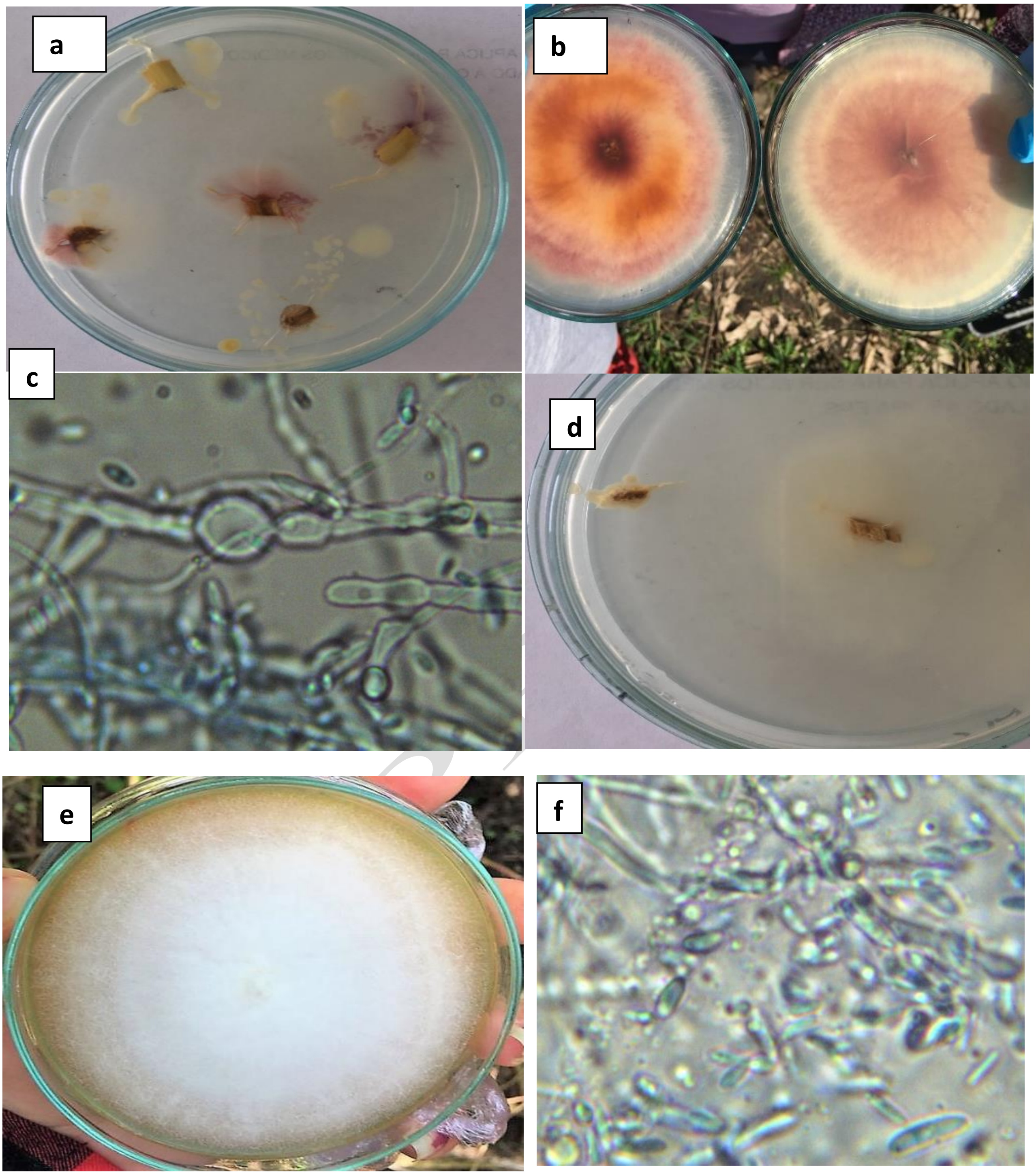


Fig. 2. Mycelium of *Fusarium oxysporum* growing in PDA. a); on diseased tissue b); purified culture c) fungus structures and *Fusarium* sp. growing in PDA. (d); on diseased tissue e); purified culture f) fungus structures.

The seedlings inoculated with *F. oxysporum* presented the disease and after two months, they showed incipient wilt with affected vascular bundles, while those inoculated with *Fusarium* sp. remained healthy. Fungus colonies with the same characteristics of those used in the inoculation that produced salmon coloration in the PDA medium were isolated from the diseased plants, producing macro, meso and microconidia, and chlamydospores. This confirmed to *F. oxysporum* as causal agent of the disease, as Koch's postulates were fulfilled.

Comparison of control alternatives for wilt management in plantations of masaguaro in Arauquita

In the small lot of maraguaro was observed that healthy plants applied wit benomil kept the incidence of the disease at 0% all the time. For the plants treated with grade 1, the incidence decreased from 60% at second sampling time (28 days from the beginning of the trial) to 0% at the fourth sampling. For the plants treated with grade 2, it was not until the third sampling that a reduction of the plants with symptoms was observed, achieving 46.67% of the plants with symptoms al the end of the trial, but with statistical differences P ‹0.01 with the proportion of diseased plants (= 1) at the beginning (Fig. 3 a). Similar situation was observed in the test with benomyl in the large lot where at the end of the assay the healthy plants, not became diseased. Those with symptoms decreased to 6.67 and 46.67% for the plants with grade 1 and 2 respectively, with statistical difference P ‹0.01 with the proportion of diseased plants (= 1) at the starting of the trial (Fig. 3 b).

Healthy plants treated every 15 days with *B. subtilis* remained until the end of the trial without symptoms, those with grade 1 showed 80% with symptoms on the third sampling and on the fourth sampling 46.6%, with statistical differences P ‹0.01 with the proportion of diseased plants at the beginning (= 1), however, 93.33% of those with grade 3 ended with symptoms, with no statistical difference P ‹0.05 with the initial proportion of diseased plants at the beginning of the trial (Figure 3 c). A similar situation was observed with the incidence of the disease in those treated with *B. subtilis* in the 10.5 ha field, the healthy ones ended without symptoms. Those with grade 1, remained with wilt only 53,33% of the plants (difference for P ‹0.01 from de initial proportion), however those of grade 2, all continued diseased, without any treatment effect (Fig. 3 d).

In the small lot the plants with grade 1 and grade 2 that were not treated continued with symptoms, however, the healthy ones began to gradually become diseased until reaching a 46.7% incidence at the end of the trial (Figure 3 e), something similar occurred in the large lot, at the end of the trial 100% of the diseased plants remained with wilt, while the healthy ones began to show symptoms until reaching a 60% incidence (Fig. 3 f).

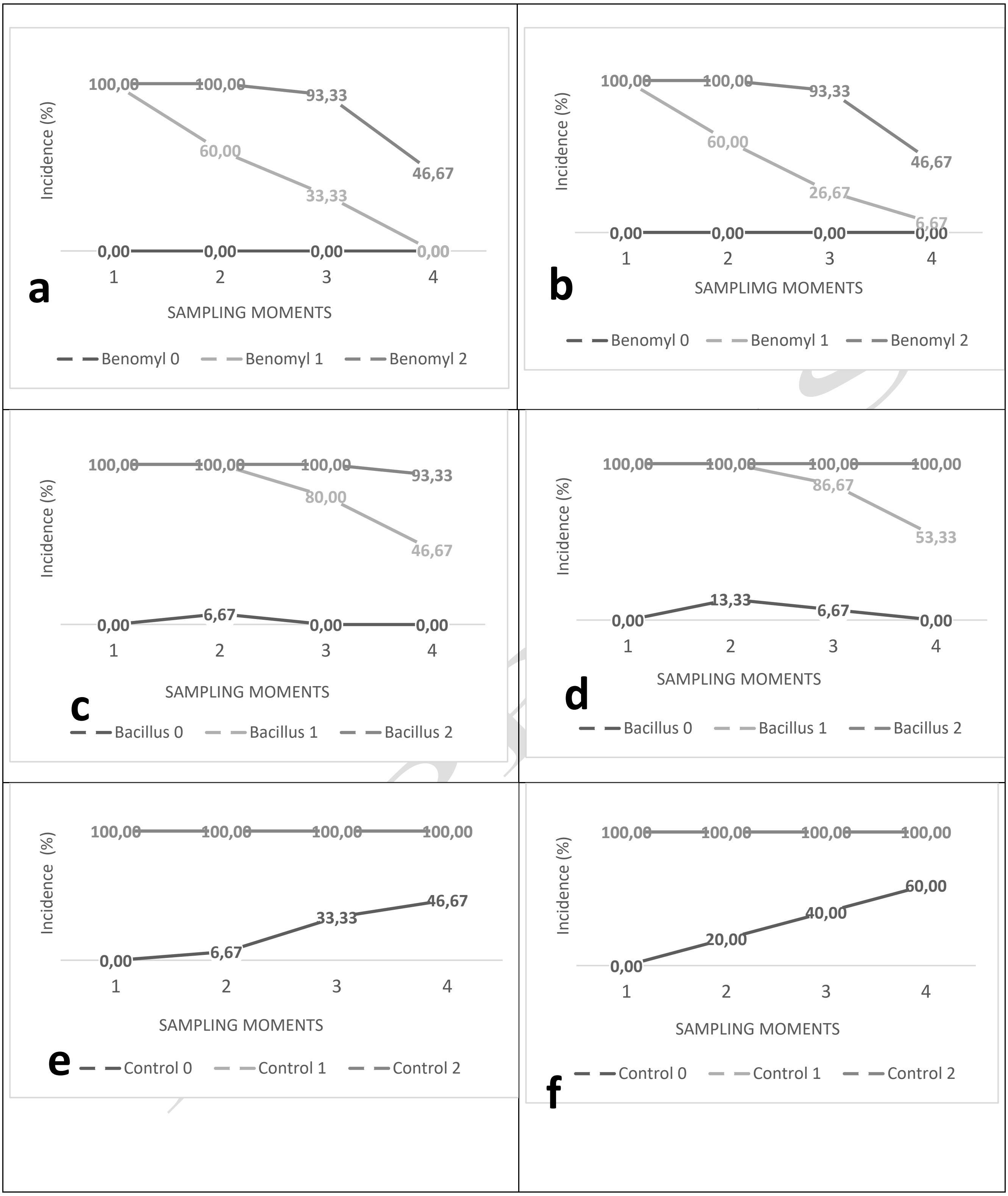
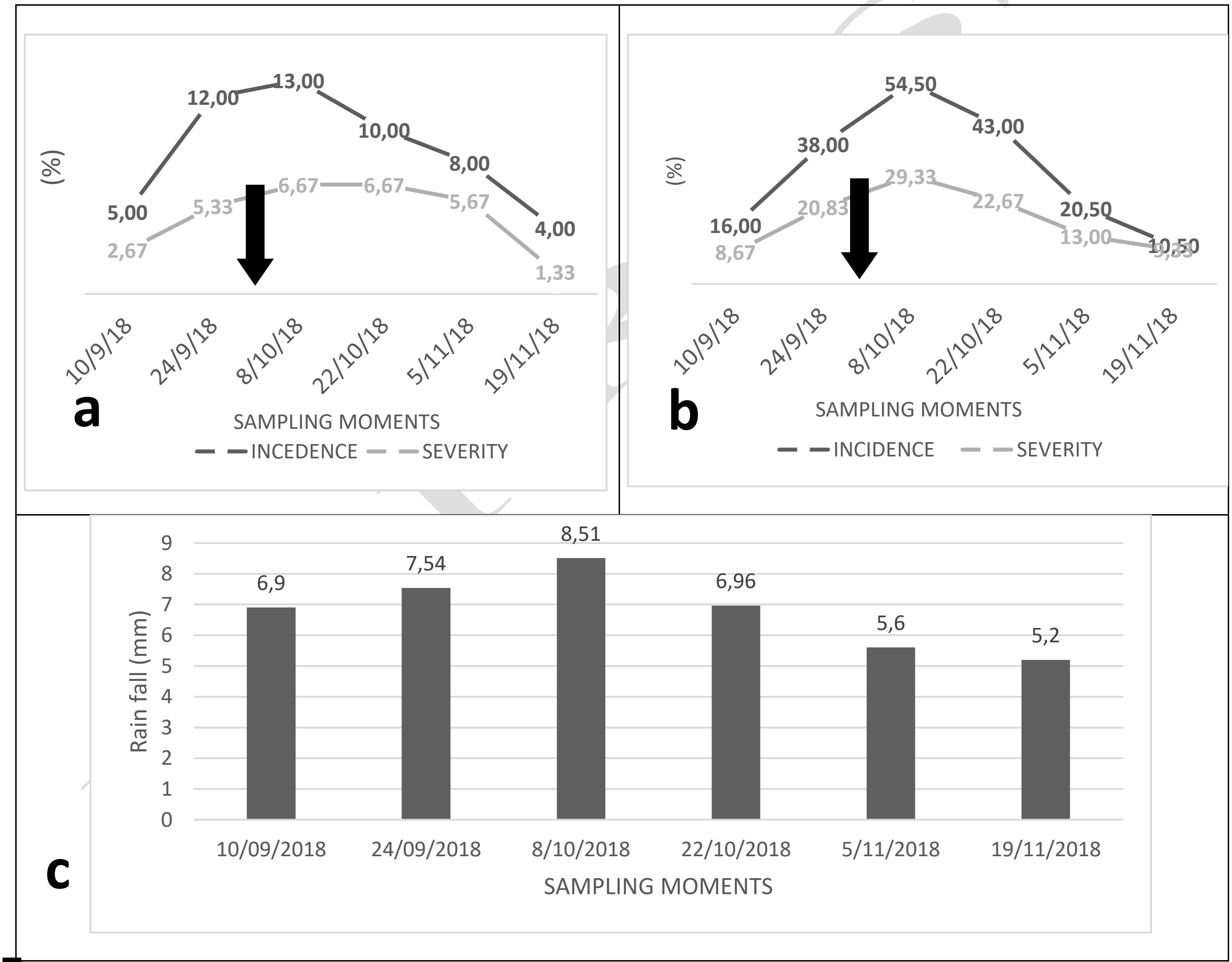


Fig. 3. Dynamics of the incidence of *Fusarium oxysporum* in the different treatments for masaguaro plants using benomyl, *Bacillus subtilis or* for the control, according to the disease severity of the plants: grade zero (0), grade one (1), and grade two (2). Treated with benomyl lot of 0.5 ha (a) and lot 10.5 ha (b), treated with *B. subtilis* 0.5 ha (c) and lot 10.5 ha (d) and untreated, lot 0.5 ha (e) and lot 10.5 ha (f).

In addition, it was possible to see the rapid action of chemical control on wilt, since from the second moment of sampling the levels of incidence and severity decreased markedly. In in biological control it was observed that its efficacy at the time controlling wilt was long term, showing less favorable results compared to chemical control.

The incidence of *Fusarium oxysporum* wilt in general in the two plots of the masaguaro crop varied between 1 and 13% for the 0.5 ha lot and 8 and 54% for the 10.05 ha one. In the 0.5 ha lot, the lowest incidence of the disease was observed (Fig.4a) and the highest level was the 10.5 ha lot (Fig 4b), while the severity in the two lots varied, from 2 up to 6% for the smaller and from 8 up to 29% for the larger one. This was explained due to higher humidity conditions that were created within the larger plot due to its high population of planted, and moreover to this, the plot was placed in a lower area where the soil texture was clay loam.

During this time period, the rainfall, which had been intense in the month of August, remained in the area between 6.9 and 8.51 mm accumulated over 14 days, although it showed a tendency to decrease towards the month of November, diminishing the waterlogging of the ground and favouring the action of benomyl (Fig. 4 c).



Starting with benomyl treatments

Fig. 4. *Fusarium oxysporum* wilt incidence and severity dynamics a;) small lot dynamics b;) large lot dynamics c;) precipitation two weeks before sampling times.

Determination of the economic losses caused by wilt in the cultivation of masaguaro in Arauquita

In total, 919 dead plants were estimated in the entire Orinoquia Biodiversa Foundation massaguar area, representing 7.9% in the large lot of 10.5 ha and 2.4% in the small lot of 0.5 ha in nearly 22 months. The Entity

had an economic loss of more than 19 million pesos, reflecting that *Fusarium* wilt can produce losses of more than 7% of the plantation, which was represented in this case with up to 1.76 million pesos / ha of economic losses (Table 1).

Table 1. Economic losses caused by Fusarium oxysporum wilt in two lots.

Unit	**Área (ha)**	**Planted plants**	**Dead plants**	**Seedling cost**	**Attention cost**	**losses pesos**	**losses /ha**
Large lot	10.5	11666	905	1500	20000	19457500	1853095.24
Small lot	0.5	583	14	1500	20000	301000	602000.00
Total	**11.0**	**12249**	**919**	1500	20000	**19758500**	**1766227.27**

4. DISCUSION

In the walks for the two lots, wilting was observed in different stages almost in a general way in the large lot with 10.5 ha while in the small lot with 0.5 ha there was a tendency towards a dispersed distribution, with the presence of apparently healthy patches. Although the soil was classified as clay loam and there was greater impermeability in the large lot, it was added that there was a higher level of weeding, which favored moisture retention and therefore more favorable condition for the development of the disease. The existing literature was compared but no reports of *F. oxysporum* affecting *P. guachapele* or *A guacgapele* were found.

In Costa Rica, studies were carried out with 12 native forest plants where *P. guachapele* was included, resulting in one of the species that was not established at all in pure plots. This was attributed to the presence of "chlorosis" and the root attack by a rodent (*Orthogeomys* spp.), but it was also one of the lesser surviving plants in mixed plots where after 16 years only isolated trees remained, being reported as pests to different species of cutter ants, as well as the presence of diseases, not to mention which, or what pathogens [10].

In the Tropical Forestry Handbook, *Albizzia guachapele* is listed among the 215 most frequently planted forest species in the world, which is recommended for tropical and subtropical regions of America and Africa, in rainfall areas between 650 and 2000 meters above sea level, but none pests and diseases are reported [2].

A deeper analysis of the literature found a report of *F. oxysporum fs perniciosus* as the cause of vascular wilt of adult mimosa trees *Albizia julibrissin* Durazz in California EEUU [11]. The symptoms in the branches very similar to those observed in the masaguaro. This disease was also reported more recently in Spain [12]. while in China *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg was reported on this plant [13]. On the other hand, other researcher states that *F. oxysporum fs perniciosus* is present in *Albizia* spp. in the US, which suggests continuing studies on hosts range, as well as making a molecular diagnosis and sequencing genes of *F. oxysporum* isolated on masaguaro to achieve its definitive identity [14].

The effect of benomyl against the wilting of the masaguaro in the young plantations of Arauquita corresponds with reported in literature. Benomyl is characterized by being a benzimidazole fungicide that is known to suppress fungal growth in a wide variety of plants. Its mode of action is involved with the inhibition of tubulin polymerization of the microorganism, acting in one point of the fungus metabolism [15]. This product has been reported as very effective for the control of *Fusarium oxysporum* in alfalfa seed [16] and against other diseases caused by this pathogen, such as wilt of the pea [17], Panama's banana disease (*Fusarium oxysporum f. sp. cubense (*E.F. Sm.) W. C. Snyder & H. N. Hansen [18] and vascular wilt in tomato [19], reason why the obtained results agree with the already demonstrated effect of benomyl against *F. oxysporum.*

However, in the case of vascular wilt of adult trees of *A. julibrissin*, has been stated that fungicide treatments would be ineffective, although this tree is mainly used as an ornamental [14]. The way in which the benomyl treatments were carried out, in drench, around the small trees and the existing humidity in the soil, could be factors in favor of the observed effect, since benomyl for the control of *Fusarium oxysporum f. sp. cubense* in banana is recommended to apply after moistening the soil strongly or doing injections to the root system to take advantage of the acropetal movement of the fungicide [18].

It is worth noting the importance of the results with the treatments with *B. subtilis*, which, although it could not eradicate the disease in the plants that were already diseased with grade 1 and 2, but they kept the healthy ones without getting wilt. This biological product constitutes an alternative to benomyl and could be applied in the nursery stage or preventively in young plantations in the early stages of wilting, and reserve the chemical for an extreme case, since the possibility of this fungicide for selecting races *Fusarium* tolerant has been known for years such as *F. subglutinans f. sp. ananas* reported in pineapple [20].

Bacillus subtilis, is one of the most recognized biocontrol plant diseases nowadays, has been reported as a growth promoter and antagonist of a variety of pathogens in vitro, in the greenhouse and in the field. Suppression of *B. subtilis* disease results from multiple mechanisms, including stimulation of plant growth (PGP), antibiosis, competition for space and nutrients, lysis of pathogenic hyphae, and induced systemic resistance (ISR). Most *B. subtilis* isolates exhibit various mechanisms that can affect the "disease triangle" directly, indirectly, or synergistically [21] effect that has been verified in the present investigation, although the mechanisms of action could not be studied.

There are references that when applying *B. subtilis* Y-IVI against *Fusarium oxysporum* in muskmelon, the colonization of the rhizosphere was verified as the main biocontrol mechanism of the antagonist agent to avoid the invasion of pathogens. After that the antibiotics produced by *B. subtilis* contribute to the biological control of muskmelon *Fusarium* wilt [22].

On the other hand, the mechanisms of action of a *B. subtilis* strain against *Fusarium oxysporum* and other coffee fungi were studied, confirming the inhibition of the pathogen and synergistic effects of secondary metabolites, lytic enzymes and siderophores, identifying as the main inhibitory metabolite secondary to harmine (β-carboline alkaloids) [23], Many *Bacillus* species are also known to synthesize γ-PGA (a natural anionic polymer composed of several thousand glutamic acid residues linked via the γ-glutamyl bond), helping them to adapt to hostile conditions by increasing resistance to drought, food shortages and pollution. The functional role that γ-PGA plays in biocontrol against *Fusarium graminearum* Schwabe root rot has been demonstrated, not only is important for the colonization capacity of the antagonist's mobile and plant roots, but it is also essential for the biological control performed. against *Fusarium* root rot [24].

The effect observed by *B. subtilis* as biocontrol of *F. oxysporum* in the present investigation confirms the results of these authors and suggests continuing studies to clarify the mechanisms of action of the antagonist against this *F oxysporum* isolate.

It would be important to test other biological control alternatives such as *Trichoderma harziaanum* Rifai, which has been reported with a biocontrol effect of *Fusarium* sp. in saman seedlings (*Phitecellobium saman* (Jacq.) Merr) legume plant related to masaguaro [25], and on which a biostimulatory effect of this antagonist was also reported [26]. On this occasion, given the high humidity condition of the soil in the study area, it was decided to test *B. subtilis*, since the causal agent had not been confirmed either, and this antagonist could also act against bacterial pathogens.

F. oxysporum wilt caused more than 7% death masaguaro plant in the study area, in plantations lesser than two years, with losses that exceeded 19 million pesos / ha. Losses could be higher if no treatments were performed with benomyl, since the disease reached incidence levels above 50%. In another context it would be possible to reach the total loss of the plantation as was reported in Costa Rica [10].

The results highlighted the need to investigate more about the masaguaro due to the little information on the losses caused by diseases in the large plantations, which are not always placed in compliance with the edaphoclimatic requirements for the species as it have been recommended [6]. More research must be done in order to increase the efficacy or the reforestation action.

5. CONCLUSIONS

Symptoms of wilt in the masaguaro were characterized and it was possible to identify two species of *Fusarium* verifying that *F. oxysporum* was the causal agent of the disease, since the symptoms were reproduced in the inoculated seedling and the pathogen was isolated again. The preventive and eradicative action of benomyl was verified for the control of wilt in the masaguaro, especially in healthy plants and with initial symptoms, and only preventive action of *Bacillus subtilis* against healthy plants. The incidence of *Fusarium oxysporum* wilt was manifested in the two planted plots, but with very different incidence and severity, these variables presenting higher levels in the 10.5 ha plot due puddling and greatest weed in that lot. The economic losses were estimated at more than 19 million Colombian pesos / ha due to dead plants.

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