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Phytophthora Root Rot in Rangpur Lime Cultivated in Soil Managed with Ecological and Conventional Mower

Caio Barbosa Prates [1], Eduardo Feichtenberger [2], Rodrigo Martinelli [3], Biana Pelissari Gadanhoto [3], Fernando Trevizan Devite [3], Patrícia Marluci da Conceição [4] and Fernando Alves de Azevedo [3,*]

[1] Syngenta Proteção de Cultivos Ltda, Avenida Nações Unidas, 18001-2º Andar, São Paulo 04795-900, SP, Brazil; caio.prates@syngenta.com
[2] Laboratório Regional de Sorocaba, Instituto Biológico (IB), Rua Antônio Gomes Morgado, 340, Sorocaba 18013-440, SP, Brazil; e.feichtenberger@gmail.com
[3] Centro de Citricultura Sylvio Moreira/Instituto Agronômico (CCSM/IAC), Cordeirópolis 13490-970, SP, Brazil; martinelli@ccsm.br (R.M.); devite_fernando@hotmail.com (F.T.D.)
[4] Departamento de Desenvolvimento Rural, Universidade Federal de São Carlos (UFSCar), Araras 13600-970, SP, Brazil; patricia.conceicao@ufscar.br
* Correspondence: fernando@ccsm.br

Abstract: Root rot and gummosis caused by *Phytophthora* spp. are considered one of the most important citrus diseases in Brazil. Symptoms of *Phytophthora* spp. infection in citrus plants are associated with lesions on the bark at the base of the plant, roots, and even lower branches. Inappropriately performed cultural practices, such as excessive irrigation and practices that result in reduced aeration and soil drainage, favor the disease. The objective of this study was to evaluate the effect of *Urochloa ruziziensis* mulching provided by an ecological mower on the severity of *Phytophthora nicotianae* root rot and the development of citrus plants and their root system. The experiments were carried out under controlled conditions on Rangpur lime seedlings kept in pots containing soil from 'Hamlin' orange orchards with the use of an ecological mower or a conventional mower in the management of inter-row vegetation for six years. *Urochloa ruziziensis* mulching (9 t ha^{-1}) was added or not to the pots. The inoculation of *P. nicotianae* in the roots occurred through infection of the soil with a solution containing mycelium and sporangia of the pathogen. A 2 × 2 × 2 factorial scheme was used, with the following factors A: soil type, B: inoculation of *P. nicotianae* and C: mulching of *U. ruziziensis*. The attributes of plant development, such as root growth, average number of leaves, leaves and dry mass of shoots and roots, in addition to assessments of the severity of disease in the roots, were evaluated. The mulch of *U. ruziziensis* did not promote an increase in root rot. The soil resulting from this management promoted the better development of Rangpur lime plants. Therefore, vegetation management of inter-row with an ecological mower is not associated with increased symptoms of *Phytophthora* root rot.

Keywords: *Citrus*; mulching; *Urochloa ruzizienses*

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1. Introduction

Brazil is renowned as the world's leading producer of sweet oranges, and the Brazilian citrus industry accounts for three-quarters of global exports of frozen concentrated orange juice [1]. Over the past three decades, both production and exports have gradually increased [2]. Despite the high level of technology employed in Brazilian citriculture throughout its history, the industry has periodically encountered various challenges along the production chain, which is a concern for producers. The citrus culture in Brazil, particularly in São Paulo, faces issues related to politics, economics, and phytosanitary matters.

Among these challenges, diseases are the primary biotic factors that pose a threat to citrus crops [3]. One of the most significant diseases is root rot caused by *Phytophthora* spp., an oomycete pathogen [4], where this disease is responsible for substantial losses, particularly in the state of São Paulo. *Phytophthora* root rot primarily affects new orchards and its significance increased after the emergence of the citrus tristeza virus (CTV). This virus rendered the use of the sour orange rootstock (*Citrus aurantium*), which was dominant in the country until the 1940s and moderately resistant to *Phytophthora*, impractical [5–7].

Phytophthora spp. can produce resistant structures, making their elimination from the soil virtually impossible once introduced to an area. This poses challenges for control measures. The pathogen can also be present in water, making it one of the most devastating diseases in citrus cultivation [8,9]. High temperatures and soil moisture favor the pathogen's development, along with improper cultural practices that cause plant damage, allowing for the pathogen to enter [10]. These practices include reduced soil aeration, poor drainage, excessive irrigation, and the choice of scion and rootstock variety [11]. *Phytophthora* species can induce root rot, leading to shoot death and, ultimately, plant mortality. Root rot symptoms are characterized by lesions on the bark or roots near ground level [12,13].

To prevent disease, the development and identification of tolerant rootstocks is one of the main strategies for control. Hence, understanding the level of resistance of potential rootstocks is one of the selection criteria for breeding programs [14,15]. Furthermore, the adoption of improved management techniques is necessary. One notable technique widely employed in citrus groves is the use of cover crops such as *Urochloa brizantha*, *U. decumbens*, and *U. ruziziensis*, in combination with ecological mowing. This approach produces on-site mulch, which suppresses weeds, enhances microbial activity, and increases productivity [13]. Considering these aspects, the objective of this study was to evaluate the mulching effect of *U. ruziziensis*, resulting from long-term use of ecological mowing in citrus orchards, and its impact on the severity of *Phytophthora* root rot in citrus.

2. Materials and Methods

2.1. Citrus Plants with Soil Inoculated with Phytophtora nicotianae

To evaluate the development of citrus plants in soils experimentally infected with *P. nicotianae*, the LRS 13/18 isolate from the collection of *Phytophthora* spp. cultures at the Micoteca "Dr. Victoria Rossetti" in the Instituto Biológico (IB) was used. The experiment was conducted for 152 days. Soil samples were collected from two areas: one with a six-year history of inter-row management using an ecological mower (Figure 1A), and another with a history of conventional management (Figure 1B). Both areas had *U. ruziziensis* present in the inter-row and were cultivated with Hamlin orange grafted on Rangpur lime, with a spacing of 6.5 × 2.0 m. The experiment took place at the Centro de Citricultura Sylvio Moreira, Instituto Agronômico (CCSM/IAC) in Cordeirópolis, Sao Paulo State, Brazil. Soil samples were collected at a depth of 0–20 cm from two points directed towards the planting line. Simultaneously, soil samples were sent for chemical analysis to the Soil Fertility Laboratory of the Soil Center and Environmental Resources of the IAC (Figure 2).

Regarding soil chemical parameters, organic matter (O.M.), pH, sum of bases (S.B.), cation exchange capacity (C.E.C.), and base saturation (V%) were similar for both types of soil. Nutrients such as phosphorus (P), calcium (Ca), and magnesium (Mg) also showed similar levels between the two soil types. However, the soil managed with *Urochloa ruziziensis* showed an increase in potassium (K) levels (Figure 2), which was within the adequate range for citrus production [16].

The experiment involved three-year-old Rangpur lime plants transplanted into plastic bags (with 7 L) containing 5 L of each type of soil (conventional or ecological mower management) to establish the root system before inoculating the soil with *P. nicotianae*. Fifty days after transplanting, when the seedlings were well-established, the soil was inoculated with a 20 mL solution containing distilled water and *P. nicotianae* mycelium obtained from the surface of pathogen colonies in carrot culture medium agar, as described by Feichtenberger et al. [7], with some adaptations.

Figure 1. Soil collection process: (**A**) collected from an orchard with a history of inter-row management with an ecological mower: red arrow indicates amount of straw removed before soil sampling and (**B**) from an area with conventional management without mulching (Cordeirópolis, Sao Paulo State, Brazil, 2020).

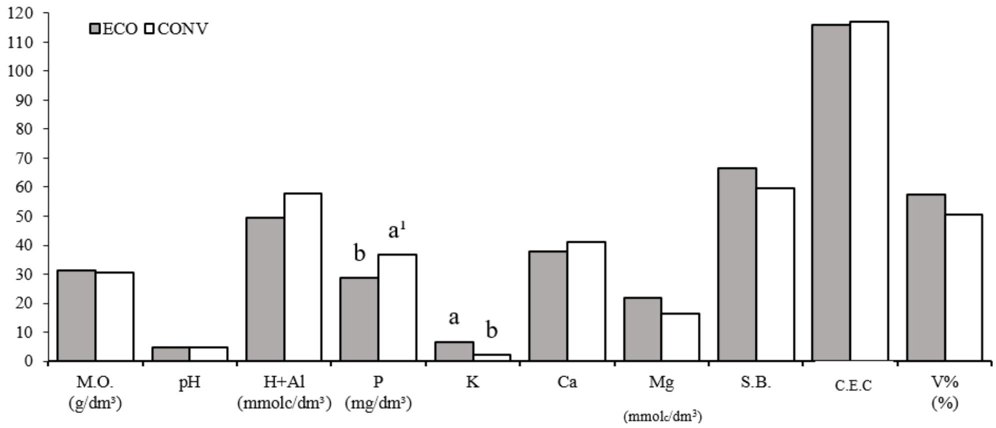

Figure 2. Organic matter (M.O.), pH, hydrogen + aluminum (H + Al), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sum of bases (S.B.), cation exchange capacity (C.E.C.) and base saturation (V%) in soils collected with a six-year history of inter-row management with an ecological mower (ECO) and soils with a conventional management history, that is, without the use of a mower ecological (CONV). [1] Averages shown by the different letter, in each parameter, show significant differences between them (*t*-test, $p = 0.05$).

After preparing the solution, the sporangia were counted using a Neubauer chamber, and the solution had a concentration of 6.3×10^5 sporangia mL^{-1}. To enhance the spread of the spore suspension and promote plant root injury to facilitate pathogen infection, four holes were made in the soil around each plant, reaching a depth of 10 cm using a glass rod. Then, 5 mL of the *P. nicotianae* mycelium solution was added to each hole. The same procedure was performed on the control plants, but without the addition of the inoculum. On the same date, in treatments simulating the mulching effect provided by the ecological mower, 15 g (equivalent to 9 t ha^{-1}) of dry *U. ruziziensis* phytomass was incorporated into the soil. This phytomass consisted of stems and leaves collected from an experimental area at the Federal University of São Carlos (UFSCar, Araras/SP) where *U. ruziziensis* was cultivated between citrus rows for five years. Subsequently, the phytomass was dried for 72 h in an oven at 65 °C. After inoculation, Rangpur lime seedlings were kept in a controlled environment for 62 days at a temperature of 25 ± 2 °C, relative air humidity around 80 ± 10% and irrigation every two days with a total application of 150 mL H_2O.

The experiment followed a randomized block design in a 2 × 2 × 2 factorial arrangement. The first factor (A) consisted of soils collected from two managements: ecological and conventional mowing (with *U. ruziziensis* in inter-rows). The second factor (B) represented the presence or absence of *P. nicotianae* in the soil, while the third factor (C) included two types of soil cover: with *U. ruziziensis* mulching and without mulching (Table 1). The experiment was conducted with six replications, with each experimental unit consisting of one plant.

Table 1. Treatments at a factorial level of 2 × 2 × 2 for the Rangpur lime plant development experiment in soils from areas with different mowing management, infected or not with *Phytophthora nicotianae* and with or without mulching of *Urochoa ruziziensis*.

Factor A: Soil Type	**Factor B: Inoculation with *P. nicotianae***	**Factor C: Mulching de *U. ruziziensis***
Managed soil EM *	uninoculated	wthout mulching
Managed soil EM	uninoculated	with mulching
Managed soil EM	Inoculated	wthout mulching
Managed soil EM	Inoculated	with mulching
Managed soil CM	uninoculated	wthout mulching
Managed soil CM	Uninoculated	with mulching
Managed soil CM	Inoculated	wthout mulching
Managed soil CM	Inoculated	with mulching

* EM = ecological mowing; CM = conventional mowing.

2.2. Plant Development and Root System in Soils Infected or Not with P. nicotianae

Sixty-two days after inoculation, several variables were evaluated in the aerial part of the plants. These included plant growths, measured as the difference in plant height (cm) after inoculation compared to the previous evaluation. Plant height was measured from the ground to the youngest leaf using a ruler. Additionally, the growth in stem diameter (cm) was recorded by comparing the stem diameter after inoculation with the previous measurement. The average number of leaves (NL) was determined by counting all the leaves on the plant.

To assess the root system, the plants were removed from the plastic bags and were carefully washed with running tap water. The root volume (mL) was determined by measuring the water displacement in a test tube before and after inserting the roots. Disease severity was evaluated by three people using a 5-point scale (1: no growth reduction; 5: strong growth reduction) based on visual observation of the root system and the qualitative features of the aerial plant organs. The dry mass of the root system (g) was measured by individually placing the taproot and rootlets in paper bags, drying them in an oven at 55 °C for 72 h, and weighing them on an analytical balance.

The obtained data were subjected to analysis of variance, and the means were compared using *t*-test at a significance level of 5%. The statistical analysis was performed using the SISVAR 5.3 program [17], and further post-hoc analyses were conducted when significant interaction effects were observed.

3. Results

3.1. Plant Development

There was no significant interaction observed between the tested factors for plant height growth, plant diameter growth, shoot dry mass and number of leaves per plant. The only significant influence observed was the effect of soil type on plant height growth, plant diameter growth, and shoot dry mass. Additionally, *P. nicotianae* infection in the soil had an impact on the number of leaves (Figure 3D). On the other hand, the presence or absence of *U. ruziziensis* mulching on the soil did not affect any of these characteristics.

Plant height growth values ranged from 0 to 14 cm, and the soil managed with an ecological mower showed a higher growth rate ($p \leq 0.05$) compared to the conventional soil without the use of an ecological mower (Figure 3A). Similarly, the stem diameter growth values of plants from the soil managed with an ecological mower were significantly higher ($p \leq 0.05$) compared to the soil managed with a conventional mower (Figure 3B). The range of values for shoot dry mass of Rangpur lime plants (MSPA) was 1.5 to 5.0 g, and once again, plants treated with soil from the ecological mower management showed higher values compared to those grown in conventional soil (Figure 3C).

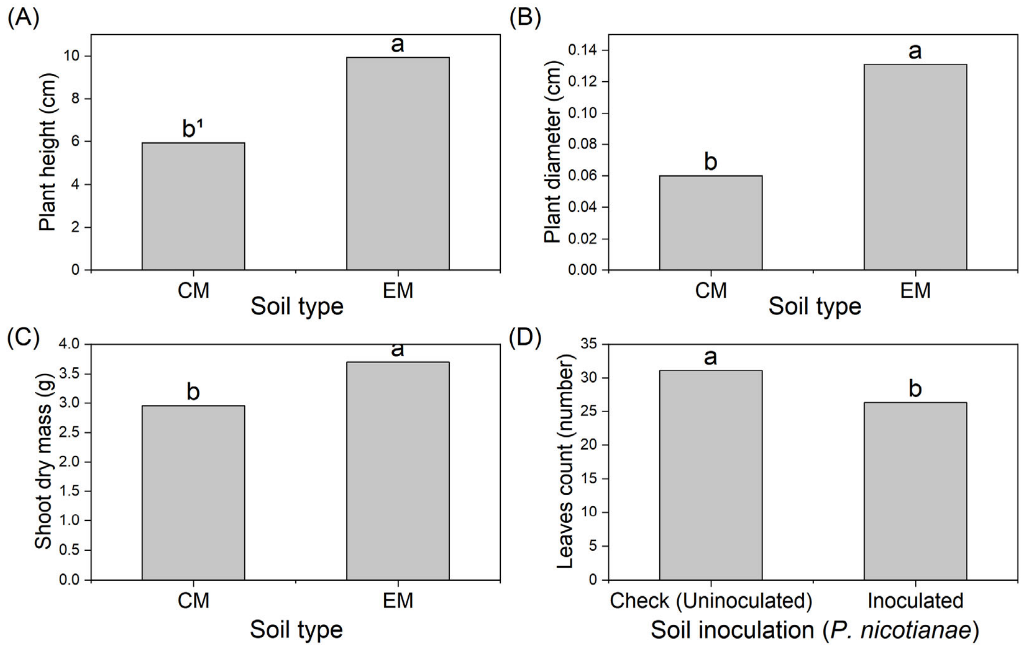

Figure 3. Rangpur lime plant height growth (**A**), plant diameter growth (**B**), aerial part dry mass (**C**) and number of leaves (**D**), after treatments with different types and soil cover and soil infection with *Phytophthora nicotianae* (Cordeirópolis, Sao Paulo State, Brazil, 2020). [1] Averages followed by the same letter, within each variable, do not show significant differences between them (*t*-test, $p = 0.05$). EM = ecological mowing; CM = conventional mowing.

Regarding the number of leaves (NF), which ranged from 15 to 42 leaves per plant, there was a significant difference ($p \leq 0.05$) attributed to the infection factor, with plants grown in soil without *P. nicotianae* showing a higher number of leaves compared to plants in the infected soil treatment (Figure 3D).

The observed differences in plant height growth (cm), stem diameter growth (cm), and number of leaves (NL) can be explained by the benefits derived from the long-term use of the ecological mower with *Urochloa ruziziensis* mulching as a cover crop. A previous study [18] confirmed, through an 8-year experiment, that the proper management of cover crops in a Tahiti acid lime orchard, with biomass deposition between citrus rows using ecological mower equipment, increased the basal respiration of the soil and carbon content in the biomass, increased CO_2 levels, enhanced microbial abundance, and greater root colonization by mycorrhizal fungi compared to conventional mowing. This management approach, similar to no-tillage, contributes to the vegetative and root development of the plants, as observed in the present study (Figure 4), and has also been found to be productive in mature citrus plants, as described by Arantes et al. [18].

3.2. Root System

There was no significant interaction observed between the tested factors for the attributes of root volume (RV), visual assessment of *P. nicotianae* severity in Rangpur lime plant roots (SR), and root dry mass. The only influence detected was related to soil type, affecting root volume and root disease severity attributes. Similarly, *P. nicotianae* infection in the soil had an impact on these attributes, as well as root dry mass. However, the presence or absence of *U. ruziziensis* mulching beneath the soil did not affect any of these characteristics.

Figure 4. Rangpur lime plants submitted to soil derived from management with ecological mower (**A**–**D**) and conventional mower (**E**–**H**); without (**A**,**B**,**E**,**F**) and with inoculation of *Phytophthora nicotianae* (**C**,**D**,**G**,**H**); without (**A**,**C**,**E**,**G**); with mulching of *Urochloa ruziziensis* (**B**,**D**,**F**,**H**), 62 days after installation of the test. Cordeirópolis, Sao Paulo State, Brazil, 2020.

Root volume (RV) values ranged from 2 to 11 mL, with the soil managed using an ecological mower resulting in significantly greater root volumes ($p \leq 0.05$) for Rangpur lime plants compared to conventional soil (Figure 5A). Plants grown in soil without *P. nicotianae* infection also exhibited larger root volumes ($p \leq 0.01$) compared to those grown in infected soil (Figure 5B).

Disease severity was evaluated by three people using a 5-point scale (1: no growth reduction; 5: strong growth reduction) based on a visual observation of the root system, and qualitative features of the aerial plant organs. There were significant differences ($p \leq 0.01$) between treatments regarding the type of soil-clearing management, with conventional management showing the highest visual severity values compared to the management with an ecological mower (Figure 5C). Likewise, plants with *P. nicotianae* in the soil exhibited the highest severity values ($p \leq 0.01$) compared to plants without *P. nicotianae* (Figure 5D). The dry mass data of citrus plant roots (DMR) ranged from 0.6 to 2.8 g, and there was a significant difference ($p \leq 0.05$) between treatments with and without *P. nicotianae* infecion in the soil (Figure 5E). Plants in non-infected soil had higher values of root dry mass compared to plants in inoculated soil.

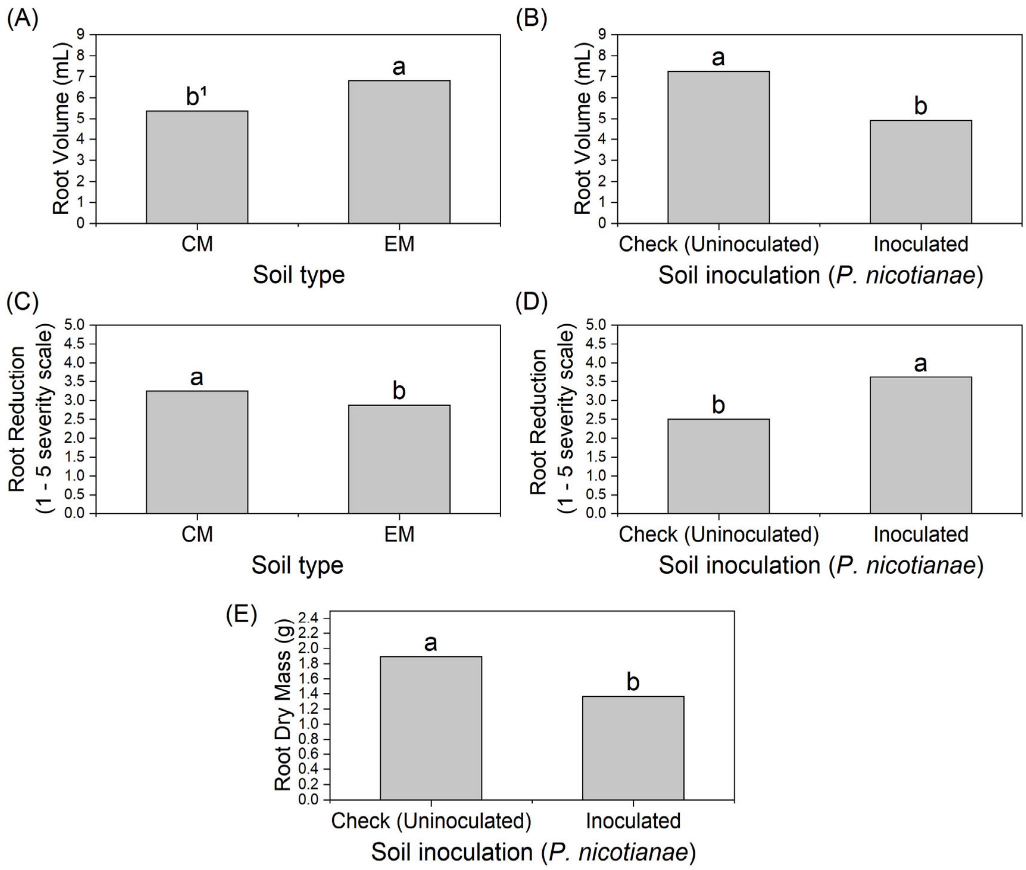

Figure 5. Root volume (**A**,**B**), root reduction - severtiy (**C**,**D**) and root dry mass (**E**) in Rangpur lime plants, after treatments with different types of soil cover and soil infection with *Phytophthora nicotianae* (Cordeirópolis, Sao Paulo State, Brazil, 2020) [1] Averages followed by the same letter do not show significant differences between them (*t*-test, $p = 0.05$).EM = ecological mowing; CM = conventional mowing.

4. Discussion

The results obtained for these attributes evaluated in this work demonstrate that the mulching of *Urochloa ruziziensis*, resulting from the long-term use of the ecological mower, did not promote the development of root rot in Rangpur lime plants. Similar studies conducted on other crops, such as avocado and papaya, have also shown that mulching can reduce the impact of root rot caused by *Phytophthora* spp., especially when applied in the early stages of orchard establishment or when the disease is not advanced. For example, the 'Ashburner system', which focuses on improved drainage and mulching, has been successfully used to control *Phytophthora* root rot in avocado trees [19]. In other studies, soil management with an ecological mower has led to a reduction in potential acidity (H + Al) and an increase in base saturation (V%), which has positively influenced avocado root development.

Several studies including Dixon et al. [20], Downer et al. [21], Faber et al. [22], Gregory and Rajkumar [23], and Mavuso [24] have confirmed, through their own results, the positive effects of mulch application on root development in avocado plants. These studies have shown increased root density and improved root systems in plants treated with mulch.

According to San Martin Matheis et al. [25], an increase in vegetation mass under the citrus canopy facilitates the release of nutrients through the decomposition process of the interlayer vegetation. This is particularly pronounced when using a lateral ecological mower, which crushes the vegetation and promotes decomposition.

The results presented by Silva et al. [26] demonstrate that various types of mulch, such as cassava husk, sugarcane bagasse, bean husk, and kale leaf, can reduce the incidence of root rot caused by *P. nicotianae* in citrus seedlings by more than 75%. Mulch acts as a physical barrier against pathogens and promotes the development of antagonistic microbiota. Additionally, citrus seedlings grown with mulch exhibited improved parameters, such as aerial height and root length. These mulch materials provide nutrients to the plants, promote the growth of the superficial root system, and reduce temperature fluctuations, thereby maintaining soil moisture and decreasing nutrient leaching.

In this study, in addition to the presented characteristics, the soil managed with an ecological mower exhibited a higher nutrient content, and particularly higher levels of potassium (K) and magnesium (Mg), which may have contributed to the observed increase in plant growth as well as suppression of the pathogen *P. nicotianae*. Appiah et al. [27] observed that high concentrations of K^+ ions reduced the swimming speed of *P. palmivora* zoospores. In the same study, the authors treated these cells with potassium ionophores, affecting swimming speed and orientation. Therefore, potassium ions play a key role in regulating zoospore behavior [28]. Similarly, Halsall et al. [29] found that K^+ and Mg^+ interacted to inhibit sporangial production in *P. cinnamomi* and *P. drechsleri* isolates.

In other crops, such as avocado orchards, the application of natural soil coverings aims to control diseases such as *Phytophthora cinnamomi* by improving soil structure, aeration, porosity, fertility, and reducing soil temperature fluctuations [19,20,25]. It also minimizes weed competition [30] and enhances the density of the root system and population of antagonistic microorganisms [31]. All these effects of using natural soil coverings, combined with proper nutrient supply and the assimilation of photosynthetic products, contribute to fruit production and growth [8,11].

The results obtained in this study support the absence of increased disease severity in citrus roots and highlight the benefits derived from the use of *Urochloa ruziziensis* mulching, which contribute to improved soil management. Therefore, by implementing integrated disease management strategies, effective control can be implemented.

5. Conclusions

The application of *Urochloa ruziziensis* mulching on Rangpur lime seedlings did not contribute to an increase in root rot caused by *Phytophthora nicotianae*. However, the soil managed with *Urochloa ruziziensis* exhibited significant improvements in organic matter content, the presence of antagonistic microflora, and the availability of essential nutrients such as potassium (K). These favorable soil conditions ultimately facilitated enhanced plant development.

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Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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