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Research Paper

Effect of temperature and moisture on soil pathogen *Fusarium solani* of lemon in Adjara, Georgia

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ABSTRACT

The research was conducted during 2022 and 2023 seasons in the agrometeorology and plant protection laboratory and citrus greenhouse of Batumi Shota Rustaveli State University, the aim of which was role of temperature and soil moisture (SM) content on aggressive soil pathogen *F. solani* on lemon, also effects of pathogen density and soil moisture on belowground and aboveground morphological traits. In our study, we could find that the both temperature and soil moisture played a decisive role in influencing the root rot disease scenario. As per the disease susceptibility index (DSI), a combination of high temperature (35°C) and low SM (60%) was found to elicit the highest disease susceptibility in lemon. High pathogen colonization was realized in lemon root tissue at all time-points irrespective of genotype, temperature, and SM. Interestingly, this was in contrast to the DSI where no visible symptoms were recorded in the roots or foliage during the initial time-points. For each time-point, the colonization was slightly higher at 35°C than 25°C, while the same did not vary significantly with respect to SM. Shoot biomass was not affected by either pathogen density or soil moisture. However, the two experimental factors have additive effects on the severity of leaf damage. Leaf damage increased with the density of *F. solani* in the soil, being significantly higher at 60 CFU $g^{-1}$and 120 CFU g^{-1} than in control seedlings. Leaf damage was higher at the two extreme soil moisture levels (15% and 100% WHC) than at the two intermediate levels (40% and 50%). In addition, differential expression studies revealed the involvement of defense-related genes, such as endochitinase and chitinase, in the resistant lemon cultivar Meyer, which contribute to retarding root rot disease progression in lemon. In the early stages of infection, especially with low SM. That can be beneficial for farmers and researchers who involve in Citrus.

Keywords: Climate, Disease, *F. solani,* Temperature, Soil moisture (SM), Disease susceptibility index (DSI)

Citrus are considered the most important economic fruit crops in the Adjara, Georgia and root rot and wilt diseases of citrus transplants primarily caused by *Fusarium* spp. are commonly found in soils of citrus orchards and of nurseries . In Adjara, lemon (*Citrus limon* (L.) Osbeck is the highest cultivated fruit and several pathogens causing diseases result in serious losses in terms of quality and quantity. Root rot of lemon transplants are primarily caused by *Fusarium oxysporum, F. solani, F. proliferatum* and other fungi. Long-term studies have shown that *Fusarium solani* was the predominant species, in citrus nursery and groves of Adjara, followed by *F. oxysporum*; the percentage of *F. solani, F. oxysporum,* and *F.spp.* frequency in Adjara were 63, 24 and 13% respectively (Shainidze, 2020). *F. spp.* in citrus nurseries and groves has been reported in many countries California, Arizona , Florida, Brazil, Greece, South Africa, Canary Islands, Italy, India, Tunisia, Greece and Egypt. (Khanna *et al.,* 2022; Yaseen and D'Onghia, 2010).

In Adjara, *F. solani* is ubiquitous fungus in soil and around citrus tree roots, especially on lemon roots. It was established that trees that are stressed because of environmental factors, for example trees growing under poor drainage conditions, showed

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higher incidence of *F. solani* compared to trees growing under no stress. Low soil moisture associated with lower precipitation and extreme droughts cause a direct abiotic stress on tree species that strongly reduces their performance and that might make them more susceptible to pathogen attack. Moreover, low water potential of the host cortical tissues has been shown to have a negative direct effect on pathogen growth within the host. Therefore, it could happen that the severity of the damages caused by soil-borne pathogens to susceptible hosts in a climate change scenario were lower than expected due to direct negative effects of lower soil moisture on pathogen population growth. Since the damage caused by soil-borne pathogenson tree species is strongly dependent on soil inoculum density (Gómez-Aparicio *et al.,* 2012), its maintenance in the soil under the minimum threshold required for disease expression might indirectly favour performance of seedlings and adults of susceptible species. The coastal zona Adjara, is considered one of the Georgia most susceptible regions to change around the climate. On the one hand, climatic models predict for this region a 1-5ºC increase in mean annual temperature by the end of the by 2050, as well as a 25% annual rainfall reduction and a higher frequency of extreme climatic events such as droughts and floodings. On the other hand, soil pathogenic microbes are causing devastating epidemics throughout the Adjara, particularly those belonging to the genus Fusarium.

The objective of our study was to investigate the role of temperature and soil moisture content (SMC) on the aggressive soil pathogen *F. solani* on lemon, as well as the effect of pathogen density and soil moisture on below- and above-ground morphological traits.

MATERIALS AND METHODS

The experiment was conducted in the 2022 and 2023 seasons in the agrometerology and plant protection laboratory and citrus greenhouse of Batumi Shota Rustaveli State University of Adjara, Georgia, located at latitude of 41°34′29.62 N and longitude of 41°51′30.02 E at an altitude of 23 m.a.s.l. Climate Adjara is well known for its humid climate and prolonged rainy weather, although there is plentiful sunshine during the Spring and Summer months. Average air temperature and moisture ri ng the experiment were 22.4±2.4°C and 87±11.2% (respectively), the soil type where the grenhaus was located was yellow earth.

To study the host–pathogen (lemon × *F. solani*) interaction under simulated environmental conditions, the experiment was undertaken in specialized plant growth chamber (PGC) of Conviron in two scenarios. Temperature and relative humidity were set in two PGC separately to establish the gradients such that one represents a cool scenario (PGC 1) with the peak temperature reaching a maximum of 25°C, whereas the second represents a warm scenario (PGC 2) with the peak temperature reaching a maximum of 35°C (Fig. 1).

The pots used for the study are of small 5-inch sizes; hence, we assume that any differences between the temperatures in the growth chamber and that of the potted soil to be small or negligible. Since the soil temperatures in field conditions are highly variable and difficult to mimic in controlled conditions, we have not accounted it in our study. To study the effect of SMC on infection and disease progression, the treatments under both scenarios were further divided into two sets of 60 and 80% SMC, respectively. The moisture regimes reflected poorly irrigated and well-irrigated field conditions, respectively. Details of the entire experimental setup providing information on the combinations of temperature, SMC, and pathogen used during the study are given in Table 1. The experiment was kept in a completely randomized design (CRD) with all the treatments maintained as triplicates. A threefold number of pots was also kept to facilitate destructive sampling and subsequent analysis.

The disease severity was scored by visual observation using the 1–9 scale (Shainidze, 2020) and then brought under a 1–4 modified scale (Table 2). Modified scale was further used to derive percent disease susceptibility index (DSI) (Padaria *et al.,* 2016) by using the following equation:

$$DSI (\%) = [(4*A4+3*B3+2*C2+1*D1+0*E0)/4N]*100,$$

where: A4 denotes the number of plants recording the score 4, B3 the number of plants scoring 3, and so on; N denotes the total number of plants (A4 + B3 + C2 + D1 + E0) under the particular treatment.

Fig. 1: Specialized plant growth chamber (PGC) of Conviron used for two scenarios (cool and warm)

Fig. 2: Greenhouse equipped with modern technologies

Table 1: Details of experimental setup and summary of observation

Temperature (°C)	SMC (%)	Pathogen remarks/observations		
25	60	Infected soil	1.	1. Low-to-moderate disease susceptibility
			2.	2.Plants under biotic + low soil moisture stress
			3.	3. Considered for varieties endurance studies
	80	Non-infected soil	4.	1. No disease recorded
			5.	2. Plants low soil moisture stress
			6.	3. Not considered for varieties endurance studies
			7.	4. Root tissues at 14 DAS was taken as control
35	60	Infected soil	8.	1. Highest diseases susceptibility
			9.	2. Plants under very biotic + high abiotic stress
			10.	3. Optimum conditions for DRR development
			11.	4. Considered for varieties endurance studies
		Non-infected soil	12.	1. No diseases recorded
			13.	2. Plants under high temperature stress
			14.	3. Not considered for varieties endurance studies
35	80	Infected soil	15.	1. Low-to moderate disease susceptibility
			16.	2. Plants under biotic + high – temperature stress
			17.	3. Considered for varieties endurance studies
		Non-infected soil	18.	1. No diseases recorded
			19.	2. Plants under high temperature stress
			20.	3 Not considered for varieties endurance studies

Table 2: Modified disease severity scale for dry root rot of lemon

Actual scale	Modified scale	Diseases severity
	0	No infection
2-3	1	Very few small lesions on root
4-5	2	Lesions on roots clear but small and new roots free from infection
6-7	3	More lesion on roots; many new roots generally free from lesions
8-9	4	Roots infected and completely discolored

On February, one-year old seedlings of lemon varieties dioscuria and meyer provided by a local nursery were individually planted in pots, free of *F. solani* (Fig. 2). Seedlings were assigned to different experimental groups following a full factorial design with two factors: *F. solani* inoculum density and soil moisture. Each factor had four levels, resulting in 24 experimental treatments and 240 seedlings (4 inoculum densities × 4 moisture levels × 10 replicates). Inoculum consisted of *F. solani* spores in sterile water suspension prepared following. With this substrate we aimed to reproduce the sandy texture and acidic pH typical of subtropical soils (Gómez-Aparicio *et al.,* 2012), as well as its native microbiota. The absence of *F. solani* in the soil was tested following (Romero *et al.,* 2007).

We used 124 non inoculated seedlings as control treatment; low inoculum density, corresponding to a soil inoculated with 100 ml of water suspension with 2.3×103 spores/ml equivalent to 30 colony forming units per gram of dry soil (CFU g^{-1}); medium inoculum density, where each pot received 100 ml of inoculum with 4.6×103 spores/ml equivalent to 58 CFU g^{-1}, near the minimum inoculum for root disease expression in *F. solani* seedlings and high *F. solani* density, where each pot received 102 ml of inoculum with 105 spores/ml equivalent to 122 CFU g^{-1}, enough to cause severe root damage in lemon seedlings.

Four different soil moisture levels were chosen. Saturated soil at 100% water holding capacity (WHC), simulating a wet spring where soils remained saturated most of the time; 60% WHC, simulating the average soil moisture; 40% WHC, simulating a 20% reduction over the previous treatment predicted for 2050 using an ACGCM for the scenario SRES IS92a (Manabe *et al.,* 2004); and 15% WHC, simulating an extremely dry spring (Ávila *et al.,* 2019). Soil moisture was controlled twice per week for every pot, weighting them and watering those which needed it to maintain constant moisture levels. Average volumetric soil water content along the study period was 23.7±0.3%, 12.2±0.1%, 10.2±0.1% and 3.6±0.1% for the 100% WHC, 60% WHC, 40% WHC and 20% WHC treatments, respectively. Pots were randomly distributed within the greenhouse and repositioned monthly to avoid the effect of possible small differences in environmental conditions. The height of all seedlings was measured before the application of the experimental treatments.

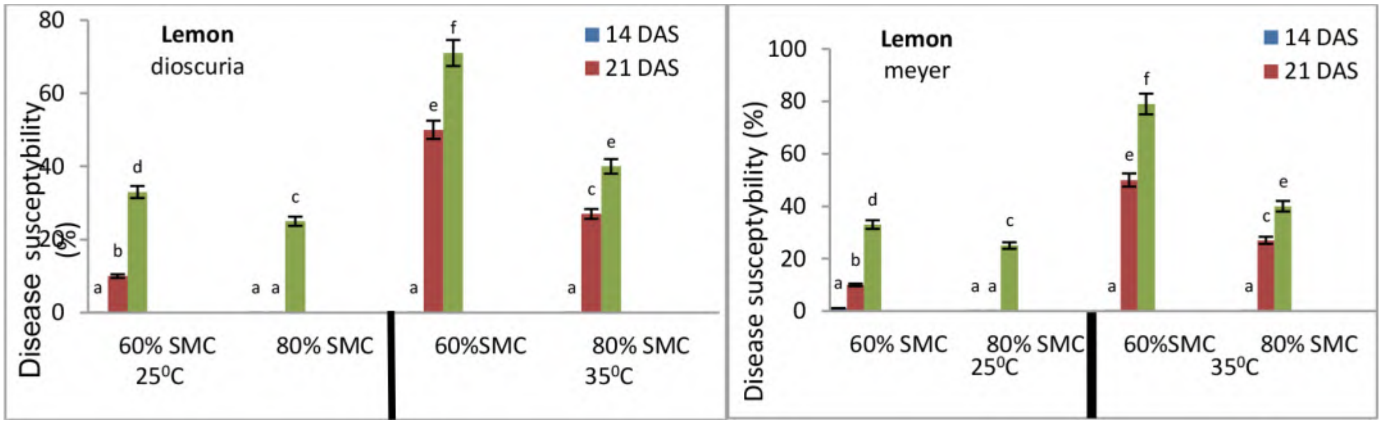

Fig. 3: Disease susceptibility index of *F. solani*-infected lemon roots at 28 days after planting (DAS) under different abiotic stress conditions, $p < 0.05$

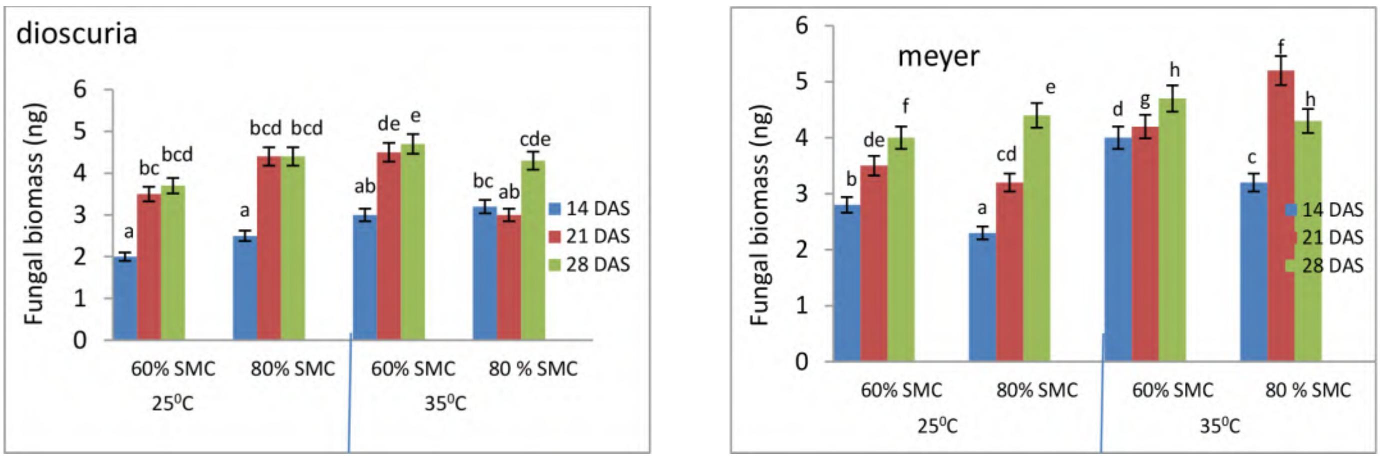

Fig. 4: Pathogen colonization in root tissues of lemon varieties dioscuria and meyer. x-axes show the temperature regime and SMC; y-axes show the fungal biomass (nanogram) in lemon root tissue. Error bars significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Disease susceptibility index under different abiotic conditions

In laboratory conditions, it the factors temperature, soil moisture (SMC), and time-points were found to in influencing disease susceptibility index (DSI) in lemon varieties dioscuria and meyer. The interaction effects of the above factors also similarly proved significant ($p < 0.001$) for both varieties (Table 1). As evident from the graphs (Fig. 3, 4), time-point had the highest influence over the disease susceptibility. Irrespective of varieties, the (DSI) was observed to gradually increase over time with no disease recorded up to 14 DAS and the highest disease susceptibility recorded at 28 DAS. During all time-points, we observed an overall low DSI at 80% SMC; however, DSI was significantly higher in all the time points at 60% SMC. Similarly, the indices from 35°C were much higher when compared to the counterparts at 25°C. A combination of 35°C with 60% SMC produced the highest DSI of 74.3 and 82.4% in dioscuria and meyer, respectively, at 28 DAS.

As we mentioned above, the combination of higher temperature (35°C) and low SMC (60%) was found to elicit the highest disease susceptibility in lemon roots, while either low temperature (25°C) or higher SMC (80%) or a combination of both was found to lower the disease susceptibility and delay disease progression considerably. The increase in DSI over time can be translated to the gradual progression of disease in the root system. The above results corroborate with the findings of Kanchaveli (2018), where the optimum temperature and SMC for DRR incidence in lemon were found to be 35°C and 60%, respectively.

F. solani colonization in root tissues of lemon varieties dioscuria and meyer

The fungal biomass in root tissues of lemon was recorded at 14 DAS. Colonization pattern was found different for both varieties. The fungal biomass was observed to increase gradually throughout the time-points. In variety dioscuria, the peak fungal biomass was observed at 21 DAS and remained non-significant from that observed at 28 DAS. The fungal colonization at 80% SMC was on par with those at 60% SMC throughout the time-points in dioscuria at both the temperatures, unlike in meyer, where at 35°C, the fungal biomass was recorded to be slightly higher for 60% SMC than its 80% SMC counterpart. Also, throughout infection, the colonization was found to be significantly higher at 35°C, than at 25°C. Although the interaction effect of SMC is significant, the interaction results showed that temperature is more important than SMC for fungal colonization in *dioscuria* ($p < 0.001$). For both varieties, the fungal biomass from 35°C was approximately 1.5-fold higher than that from 25°C, at 28 DAS (Fig. 4).

Our studies also revealed that the *F. solani* colonization

Table 3: Results of the statistical analyses (GLMs) performed to test the effect of the experimental treatments and its interaction on the morphological traits of lemon seedlings. Significant differences among treatments (p<0.05) are highlighted in bold, and marginal differences (p<0.10) in italics.

Treatment Shoot biomass		Aboveground traits			Belowground traits		
		Leaf damage	Total root biomass	Fine root biomass	Root mass fraction	Root damage	
Pathogen density (PD)	P-value Deviance df (3)	0.06 15.34	**<0.0001** 1.99	0.93 0.09	*0.08* 0.39	**0.007** 0.03	**<0.0001** 16.36
Soil moisture (SM)	P-value Deviance df (3)	0.33 7.35	**<0.0001** 2.19	**0.0002** 4.14	**<0.0001** 1.89	**0.001** 0.04	0.16 0.95
PD × SM	P-value Deviance df (9)	0.37 21.26	0.27 0.94	**0.012** 4.52	*0.07* 0.89	**0.03** 0.06	**0.0005** 5.4
Initial Height	P-value Deviance df (1)	**0.01** 12.46	0.52 0.03	*0.07* 0.71	0.90 0.17	**0.03** 0.01	0.93 0.01
	Explained deviance	0.15	0.30	0.24	0.29	0.27	0.23

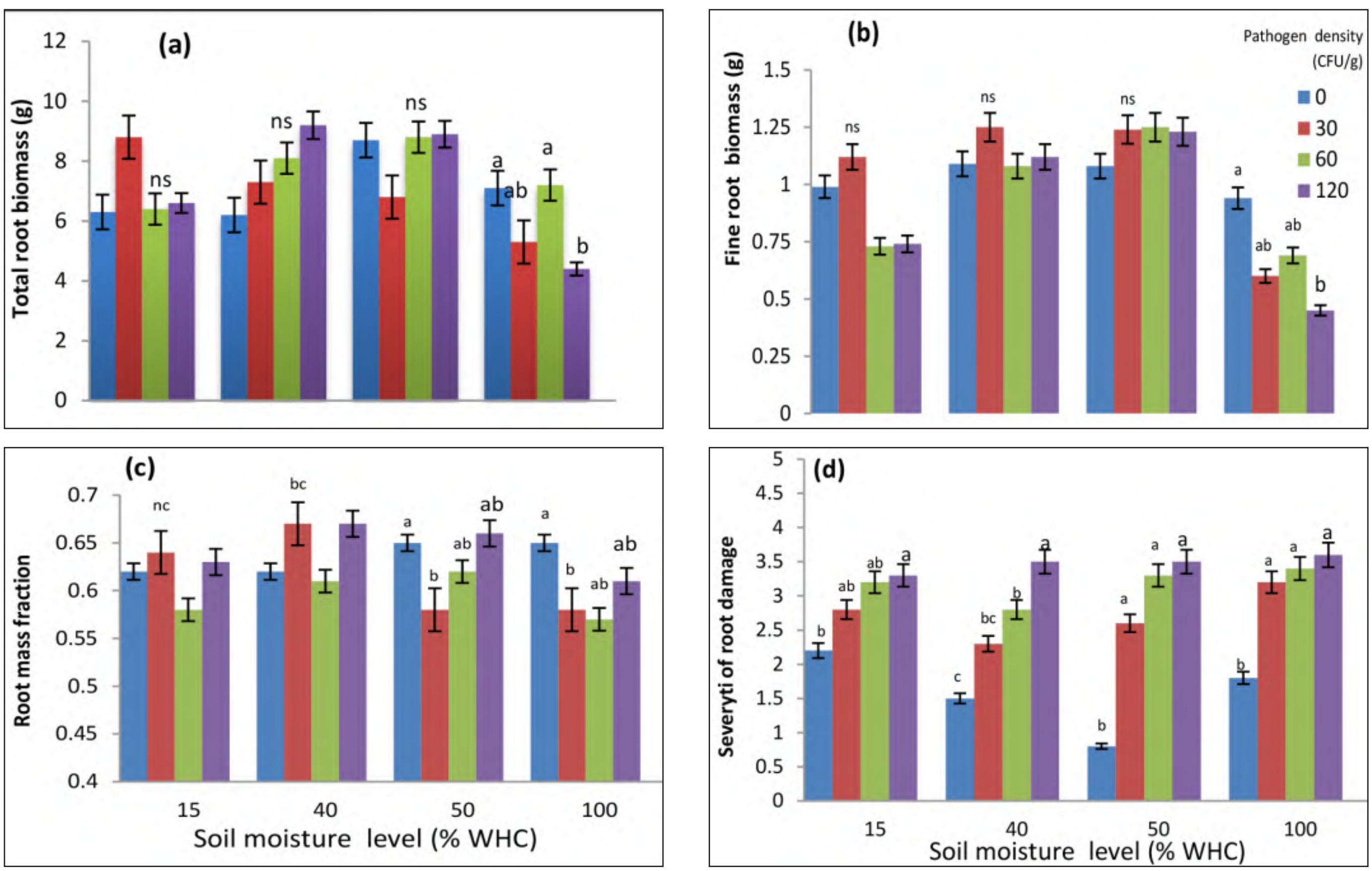

Fig. 5: Interactive effects of pathogen density (measured as number of colonies forming units per gram of dry soil, CFU g^{-1}) and soil moisture (measured as % of water holding capacity, WHC) on belowground morphological traits of lemon meyer seedlings: (a) total root biomass, (b) fine root biomass, (c) root mass fraction and (d) severity of root damage. Different letters show significant differences after Tukey tests. Bars represent mean ± SE (n=10).

pattern of lemon roots in 60% SMC did not vary significantly from that of 80% SMC irrespective of temperature and variety, but contrary to fungal colonization, interestingly, the difference in DSI was significant. No or very low DSI was recorded in the roots and foliage during the initial time-points, although fungal colonization was recorded at 14 DAS, indicating the time taken by the plants

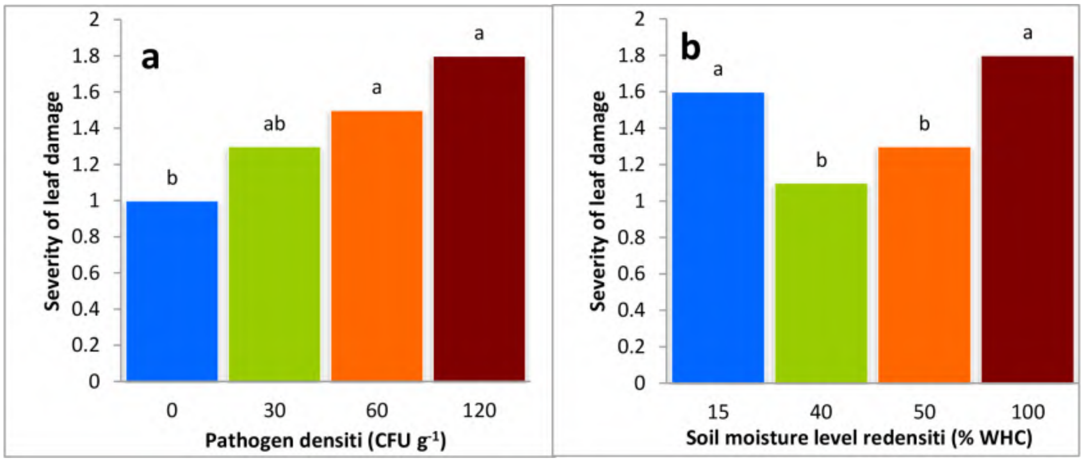

Fig. 6: Effects of pathogen density (measured as number of colonies forming units per gram of dry soil, CFU g^{-1}) and soil moisture (measured as % of water holding capacity, WHC) on the severity of leaf damage of lemon meyer seedlings. Different letters show significant differences after Tukey tests. Bars represent mean **±SE (n=10).**

to express the symptoms despite being colonized by the pathogen. The severity was found to be aggravated during the later stages of growth, which very well correlated with the fungal colonization. The results indicated that higher temperatures may prove encouraging for *F. solani* colonization, whereas the SMC majorly influences the DRR disease severity in lemon. A higher atmospheric temperature invariably leads to elevated soil temperatures, which in turn means higher growth and survivability of infection spores of *F. solani*. Kanchaveli (2018) made a similar observation for another soil-borne lemona disease and reported a shift in the resistance reaction of lemon against *Fusarium oxysporum*, where the plants showing moderate resistance to the pathogen under 25/22°C day/night temperature regime were converted to highly susceptible when the temperature was increased to 28/26°C.

Effects of pathogen density and soil moisture on belowground morphological traits

Soil pathogen density and soil moisture had significant interactive effects on the four belowground variables measured (total root biomass, fine root biomass, root mass fraction and root damage), although for fine root biomass the interaction was only marginally significant (Table 3).

Total and fine root biomass were affected by soil pathogens only at the highest level of soil moisture, being lower at the highest pathogen density (120 CFU g^{-1}) than in control seedlings (Fig. 5 a, b). Root mass fraction (RMF) was affected by soil pathogens only at the two highest levels of soil moisture (50% and 100% WHC). It was highest in control seedlings than in those inoculated with low pathogen density (30 CFU g^{-1}), seedlings inoculated with medium (60 CFU g^{-1}) and high pathogen density (120 CFU g^{-1}) showing intermediate RMF values (Fig. 5c). Root damage was influenced by pathogen density at the four soil moisture levels explored (Fig. 5d). However, the pathogen density required to cause significant higher damage in experimental than control seedlings decreased as soil moisture increased. Thus, at the lowest soil moisture level (15% WHC), only seedlings inoculated with the highest *F. solani* density (120 CFU g^{-1}) showed significantly more root damage than control seedlings. At 40% WHC, significant root damage was detected for seedlings inoculated with 60 CFU g^{-1} and 120 CFU g^{-1}. Finally, at the highest soil moisture levels (50% and 100% WHC), all inoculated seedlings showed more damage than control seedlings independently of inoculum density. *F. solani* was re-isolated from the roots of seedlings growing in infested soil but never from control seedlings.

A main result of our study is that pathogen density and soil moisture had interactive effects on the belowground performance of lemon seedlings, in agreement with our hypothesis. Thus, the severity of the root damage caused by the aggressive soil borne pathogen *F. solani* to lemon seedlings differed substantially among the four soil moisture levels used as surrogates of different climate change scenarios. As predicted, the largest levels of root damage occurred in general at the highest levels of soil moisture (50% and 100% WHC), translating into a somewhat lower RMF in infected seedlings compared to control seedlings. Pathogen effects on total and fine root biomass were detected only at the highest soil moisture level (100% WHC), where highly infected seedlings had on average 22% and 61% lower total and fine root biomass (respectively) than control seedlings (Fig. 5 a,b).

The fact that the largest negative effects of *F. solani* on root biomass occurred under soil water saturation conditions is consistent with previous knowledge that shows high soil water levels to favor the infective inoculum build-up of *F. solani.* High soil moisture and free water favors fruit bodies of fungus production and spore release, and increases spore mobility through the soil to infect roots, allowing for rapid secondary cycles and multiple infections on the host roots (Nemec *et al.,* 1989). However, our results also suggest that the pathogen capacity to cause root damage can be sensitive to moderate reductions in soil moisture, in agreement with previous studies that have shown water restriction to reduce root damage induced by *F. solani* (Polizzi *et al.,* 1992). Thus, whereas in the 100% and 50% WHC scenarios a low density of *F. solani* (30 CFU g^{-1}) was enough to cause significant higher root damage in infected than control lemon seedlings, in the 40% WHC scenario only seedlings infected with moderate and high densities (>60 CFU g^{-1}) differed from control seedlings. This implies that even a slight decrease of soil moisture simulating a 30% reduction in annual precipitation was enough to trigger differences in root damage caused by *F. solani.* Further decreasing the soil water content to 15% WHC increased even further (>120 CFU g^{-1}) the minimum threshold required to cause significant damage to the root system.

This negative relationship between soil moisture and the inoculum threshold required to cause disease is consistent with the more acute decline of lemon species observed in fine textured soils with high water retention compared to well-drained sandy soils (Gómez-Aparicio *et al.,* 2012; Polizzi *et al.,* 1992). From a global change perspective, these results imply that disease trajectories of citrus plantation already invaded by *F. solani* will strongly depend on rainfall regimes and associated variations in soil moisture that might exert a significant control on the growth rates of *F. solani* populations.

Effects of pathogen density and soil moisture on aboveground morphological traits

Shoot biomass was not affected by either pathogen density or soil moisture (Table 3). However, the two experimental factors have additive effects on the severity of leaf damage. Leaf damage increased with the density of *F. solani* in the soil, being significantly higher at 60 CFU g^{-1} and 120 CFU g^{-1} than in control seedlings (Fig. 6a). Leaf damage was higher at the two extreme soil moisture levels (15% and 100% WHC) than at the two intermediate levels (40% and 50%, Fig. 6 b).

Soil moisture did however affect the severity of leaf damage shown by the seedlings at the end of the experiment, although the severity of the damage did not show a linear response to the decrease in soil moisture. On the contrary, leaf damage was maximal at the two extremes of the soil moisture gradient, showing how the lack of water can be as detrimental as its excess (Gómez-Aparicio *et al.,* 2008; Pérez-Ramos and Marañón 2009). Soil saturation can generate hypoxia conditions that translate into a poor root functioning and consequent wilting of some part of the foliage (Sairam *et al.,* 2008). Overall, our results demonstrated that *F. solani* and soil moisture had additive and non-linear effects on aboveground seedling performance that varied in sign and magnitude depending on the trait considered. However, these effects were in general of much lower magnitude than those detected belowground, likely due to a delay between pathogen infection and aboveground symptoms, which suggests that understanding the interactive effects of global change drivers on plant performance requires a close look belowground. Almost the same results were obtained in different studies (Hometa *et al.,* 2019). The obtained results of the studies agree with the results of the studies on the stress phenomena of plants caused by meteorological factors (Shainidze *et al.,* 2024).

CONCLUSION

The study clearly points toward the emerging threat due rot rot in root system lemon and indicates higher temperatures and low soil moisture as key drivers for rot rot expression in lemon. High temperature renders lemon plants susceptible to disease, whereas lowsoil moisture content (SMC) dictates the extent of rotting or severity of disease in the root system. The pathogen colonization of lemon root tissues was pronounced at all the time-points despite the climatic conditions provided, suggesting the ability of F. solani to thrive and grow in a wide range of temperature and moisture conditions.

Results involving experimental seedlings lemon under controlled conditions need to be carefully extrapolated to adult trees under natural conditions, our results strongly suggest that the capacity of F. solani to cause disease is modulated by even small variations in soil moisture, and that a drier climate might imply sub-optimal conditions for root infections, lengthening the time required for disease expression in susceptible hosts. This finding, together with the physiological plasticity shown by lemon to counteract pathogen effects under moderate inoculum abundance, might allow for a slower advance of the root rot caused by F. solani in a drier future. However, it is important to take into account that the consequences of an average drier climate on disease dynamics will be strongly modulated by the effects of the also predicted most frequent extreme climatic events.

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***Authors contribution*:** **O. Shainidze:** Methodology and Data collection, Writing-original draft; **N. Beridze:** Investigation, Data Analysis, Referencing, Critical review; **Sh. Lamparadze**: Data collection and analysis and model development; **Sh. Lominadze:** Investigation, Manuscript writing and data compilation; **L. Ebralidze:** Visualization, Writing-original draft; **M. Turmanidze**: Investigation, Analysis, Investigation, editing; **G. Macharadze**: Investigation, Conceptualization, Visualization, Investigation; **G. Jincharadze:** Data collection and analysis; G. **Datunaishvili:** Investigation, Methodology.

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