VOL II

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

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2024

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APRESENTAÇÃO

O campo das Ciências Agrárias e Ambientais desempenha um papel fundamental na compreensão e solução dos desafios contemporâneos relacionados à produção de alimentos, à conservação ambiental e ao bem-estar animal. Em um mundo em constante transformação, questões como a sustentabilidade dos agroecossistemas, o manejo eficiente dos recursos naturais e a saúde pública se tornam cada vez mais relevantes. É com este espírito que apresentamos o volume II da coletânea "Estudos em Ciências Agrárias e Ambientais", que reúne pesquisas de autores de diversas partes do mundo, cada um contribuindo com sua perspectiva e expertise únicos.

Os quinze artigos que compõem este volume abordam uma variedade de tópicos, refletindo a riqueza e a diversidade das Ciências Agrárias. Desde práticas conservacionistas que buscam melhorar e manter agroecossistemas, até investigações sobre o uso de fitohormonas e fertilização na produção vegetal, o uso de tecnologias de processamento de madeira e a promoção do bagre armado - cada estudo traz à tona questões cruciais que impactam tanto a produção agrícola quanto a saúde ambiental.

Neste volume, também exploramos a crescente relevância dos produtos agrícolas locais, especialmente em tempos desafiadores como os que vivemos, marcados pela pandemia da COVID-19. A importância de circuitos curtos de proximidade se torna evidente, promovendo não apenas a segurança alimentar, mas também a resiliência das comunidades.

Além disso, as contribuições da veterinária destacam a importância do cuidado animal e da saúde pública, ilustrando a interconexão entre os seres humanos, os animais e o meio ambiente.

Esperamos que esta coletânea não apenas informe, mas também inspire debates e colaborações futuras entre pesquisadores, profissionais e estudantes da área. Juntos, podemos avançar em direção a um futuro mais sustentável e equilibrado, em que conhecimento pesquisa sejam os pilares para soluções efetivas.

Agradecemos a todos os autores e colaboradores que tornaram este trabalho possível. É nossa esperança que os estudos aqui apresentados contribuam para um entendimento mais profundo das questões agrárias e ambientais, e que possam servir de base para novas investigações e práticas inovadoras.

Eduardo Eugênio Spers

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SEVERITY OF 'WOOD POCKET' PHYSIOPATHY IN SELECTED PERSIAN LIME PLANTS OF DIFFERENT GENERATIONS

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ABSTRACT: As an export product, Persian lime faces various challenges that threaten its sustainability, especially phytosanitary issues caused by fungal, bacterial, and viral pathogens that affect citrus crops in general. In addition, Persian lemon is particularly impacted by a genetic condition known as "wood pocket" which significantly weakens the tree, often leading to its death. The selection of specific plant lines helps mitigate the progression of this condition. This study aimed to evaluate the evolution of "wood pocket" across three generations of inherited and selected plants. Evaluations were conducted in three Persian lime orchards located in Michoacán, Mexico. The selection criteria were based on observations from a progenitor orchard, Selection 1 orchard, and Selection 2 orchard. In each orchard, 80 trees were randomly selected and monitored using a four-level symptomatology scale. Collected data were transformed into percentage values using the arcsine square root transformation and processed through an analysis of variance. The results revealed significant differences between the three orchards. The progenitor orchard showed the highest incidence (37.5%) at severity level 2. In Selection 1 orchard, level 1 dominated with 36.56%. In Selection 2 orchard, 91.56% of the plants exhibited minimal or imperceptible symptoms (level 1). Across generations, the incidence of levels 2, 3, and 4 decreased, while level 1

increased, indicating the potential to reduce the impact of “wood pocket” physiopathy through plant selection.
KEYWORDS: *Citrus latifolia*. Persian lime. Tahiti lime. Sectorial spot.

SEVERIDAD DE LA FISIOPATÍA ‘WOOD POCKET’ EN PLANTAS SELECCIONADAS DE LIMÓN PERSA DE DIFERENTES GENERACIONES

RESUMEN: Como producto de exportación y ante el riesgo de impactar negativamente, el limón persa enfrenta diversos problemas que constantemente presionan su permanencia, destacan los fitosanitarios migrantes de origen patógenos, cuyos agentes fúngicos, bacteriales y virosos afectan en lo general a los cítricos. Sin embargo, en limón persa, además, una condición genética llamada “wood pocket” lo afecta gravemente. Esta condición debilita rápidamente al árbol, hasta su muerte, por lo que la restitución de plantas seleccionadas mitiga su progreso. El objetivo fue evaluar la evolución de “wood pocket” en plantas heredadas y seleccionadas en tres ciclos. La evaluación se llevó a cabo en tres huertas limón persa de la zona productora de Michoacán, México. El criterio de selección, derivó de una huerta progenitora, una huerta de selección 1 y una huerta de selección 2. En cada huerta se monitorearon 80 árboles por cada línea de cuatro al azar. En ellos, se buscó sintomatología basada en una escala diseñada de cuatro niveles. Los datos obtenidos fueron porcentuales y transformados al arcoseno de la raíz cuadrada de la proporción, y procesados por análisis de varianza. El análisis detectó diferencias para las tres huertas observadas. Según características sintomáticas, en la huerta progenitora, el nivel 2 tuvo mayor incidencia (37.5%) de plantas afectadas. En la huerta selección 2, el nivel 1 sobresalió con 36.56%. En la huerta selección 3, el nivel 1 se obtuvo en el 91.56% de plantas con síntomas imperceptibles. Al comparar el resultado entre huertas, los niveles 2, 3 y 4 tendieron a disminuir, en cambio el nivel 1 tendió a incrementar. Así, es posible acotar la incidencia de plantas afectadas por la fisiopatía de “wood pocket” a través de selección de plantas.
PALABRAS CLAVE: *Citrus latifolia*. Lima persa. Limón Tahití. Mancha sectorial.

1 INTRODUCTION

According to the FAO, in 2021, Mexico ranked second worldwide in harvested citrus area, with 195,619 hectares, only behind India, which reported 327,000 hectares. In terms of production, Mexico yielded 2,983,802 tons, compared to 3,548,000 tons in India. However, regarding yield per hectare, Mexico ranked 39th, with an average of 15.25 tons per hectare (FAOSTAT, 2023). Mexican lime represents 70% of the national citrus production, while Persian lime accounts for the remaining 30%, meeting both national and international market demands. Mexico is one of the world’s leading citrus producers and exporters, with the United States as the primary importer, followed by Japan (Ruiz *et al*., 2017). In 2022, the most productive states were Veracruz (27.7%), Michoacán (27.6%), and Colima (10%) (SIAP-SADER, 2023).

Persian lime (*Citrus latifolia*), a round, seedless hybrid fruit, is larger and sweeter than a typical lime. Its characteristic acidic juice makes it ideal for beverages, nectars, and food preparation. It is also a rich source of vitamin C, providing 64% of the daily recommended value in a 100-gram serving. Additionally, Persian lime contains numerous phytochemicals, such as polyphenols, terpenes, and tannins, and has significant concentrations of citric acid. Its composition is 88% water, 10% carbohydrates, and less than 1% fats and proteins.

Due to its economic significance (Almaguer-Vargas *et al*., 2011), Persian lime is constantly under pressure from various phytosanitary challenges, including pathogens of fungal, bacterial, and viral origin (Hernández-Mora *et al*., 2023). Moreover, a genetically-based abiotic disease known as "sectorial spot" or more commonly, "wood pocket" severely affects this citrus variety, particularly in warm climates. In the field, the symptoms of "wood pocket" are often mistaken for those of Huanglongbing (HLB) disease in citrus (Villegas and Mora, 2011). The interval between the onset of symptoms and tree death can be as short as one month, though it often takes longer. Some trees exhibit symptoms while others remain symptom-free, and it is possible to find both healthy and affected fruits on the same plant. The symptoms include leaf mottling, branch cracks, and spots on fruits, all of which weaken the tree until it dies.

Although most known Persian lime clones are susceptible to "wood pocket" the physiopathy manifests only in hot, dry regions. In other climates, the plant behaves normally. Selecting specific plants helps mitigate the progression of the physiopathy. Therefore, this study aimed to evaluate the evolution of "wood pocket" in inherited and selected plants over three cycles.

2 MATERIALS AND METHODS

The evaluation was conducted in Persian lime orchards belonging to a cooperating farmer in Felipe Carrillo Puerto, also known as "La Ruana," in the municipality of Buenavista, Michoacán, Mexico. The area is located at an average altitude of 400 meters above sea level. The region's climate is classified as Bs_1, corresponding to the least dry of the dry climates (García, 2004). Vegetation consists of deciduous tropical forest species, and the soil type is pellic Vertisol (INEGI, 2016).

Three orchards were selected to explore the presence of "wood pocket" symptoms. The first orchard, known as the progenitor, was approximately nine years old. From this orchard, the second orchard was derived, referred to as Selection 1 (first generation), which was about six years old. Similarly, the third orchard, known as Selection 2 (second generation), was derived from the previous one.

Visual monitoring was carried out to identify the characteristic symptoms of “wood pocket” in each orchard. A four-level severity scale (Table 1) was designed to assess the observable symptoms in the sampled Persian lime plants.

Table 1. Scale used to evaluate the level of impact (severity) of symptoms related to “wood pocket” physiopathy in Persian lime.

Level	Extended visual characteristics	Short description
1	Imperceptible symptoms	No visible symptoms
2	Leaf wrinkling on branches and/or noticeably dry branches	Some dry branches
3	Dry branches, sectorial leaf mottling, and vertical spots on fruits	Strong symptoms
4	Significant leaf loss, exposed or dry branches, and tree decline	Severely affected

In each orchard, visual exploration was performed on 80 productive plants across four random lines (320 plants per orchard). The severity of “wood pocket” was measured using the four-class scale. The data were transformed using the arcsine square root of the proportion and analyzed through a randomized block design and factorial analysis. The statistical software used was SAS 9.2 (2019).

3 RESULTS AND DISCUSSION

Table 2 presents the mean values obtained from the variance analysis. Since the data were transformed to percentages to detect accurate differences in symptom severity levels, the results reflect these transformations. In the progenitor orchard, severity level 2, which corresponds to “some dry branches,” was the most prevalent, affecting a significant number of plants. Meanwhile, in Selection 1 orchard, severity level 1, meaning “no visible symptoms,” was observed most frequently. However, statistically, severity levels 1, 2, and 3 were not significantly different from each other. In Selection 2 orchard, severity level 1 was clearly the most common, indicating a reduction in symptom progression across generations (Table 2).

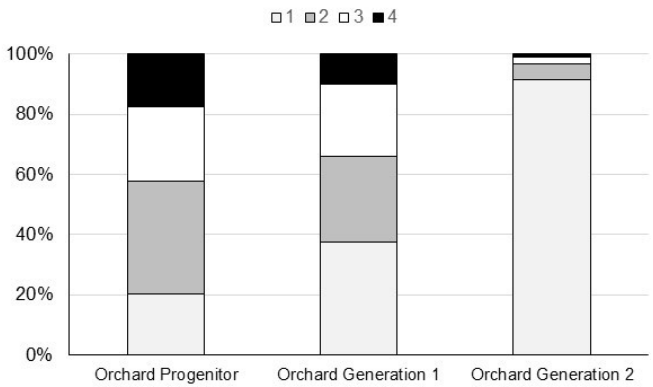
Additionally, non-transformed severity data are shown in Figure 1, highlighting the large proportion of plants in Selection 2 orchard with level 1 symptoms (“no visible symptoms”). This suggests a noticeable decrease in the occurrence of “wood pocket” over time.

Table 2. Variance analysis conducted in progressive orchards for the presence of “wood pocket” physiopathy in Persian lime.

Symptomatic level	Orchard†		
	Progenitor*	Selection 1	Selection 2
1	0.46 b	0.64 a	1.27 a
2	0.65 a	0.55 ab	0.23 b
3	0.51 b	0.50 b	0.14 bc
4	0.43 b	0.31 c	0.06 c
C.V.	8.30	12.79	12.42
P	0.0002	0.0003	0.0001

†Data previously transformed to arcsine of the square root of the ratio; *Means followed by the same letter within columns do not differ statistically (Tukey, 0.05).

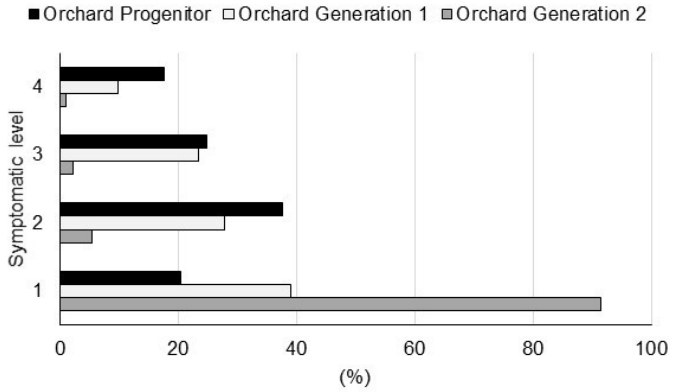
Figure 1. Percentage distribution derived from the statistical analysis carried out in the orchards for the presence of “wood pocket” physiopathy in Persian lime.



The comparison of the three generations (Figure 2) shows that the progenitor orchard reached its highest symptomatic incidence (37.5%) at severity level 2. In contrast, severity level 4 (most severe) affected only 17.5% of the plants in this orchard. In Selection 1 orchard (first generation), severity level 1 was the most frequent (36.56%), while severity level 4 affected only 9.68% of plants. In Selection 2 orchard (second generation), 91.56% of plants showed no visible symptoms (severity level 1), while only 0.93% of plants were categorized as severity level 4.

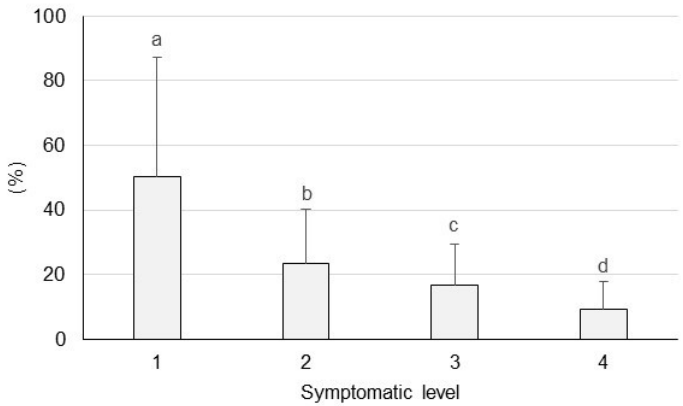
In general, severity levels 2, 3, and 4 tended to decrease across generations, while severity level 1 increased (Figure 2). This trend suggests that selecting specific plants can effectively reduce the impact of “wood pocket.”

Figure 2. Presence of “wood pocket” physiopathy in Persian lime: comparative range analysis of the three orchards.



Further analysis was conducted to explore the interaction between orchard (H) and symptom level (N) as independent factors. Significant statistical differences were detected for the H × N interaction. When analyzed independently, the orchard factor (H) did not show significant differences, but the symptom level factor (N) did, as shown in Figure 3. This analysis confirmed that severity level 1 represented the highest proportion of plants, followed by the other severity levels. Thus, although “wood pocket” intensity decreases, it remains present in varying degrees across orchards.

Figure 3. Separate analysis of variance for the symptomatic level (N) factor of “wood pocket” physiopathy in Persian lime.



On the other hand, the analysis of variance performed on the combined treatments (H/N) for the incidence of “wood pocket” revealed statistical differences. The highest number of healthy plants was observed in the H3/N1 combination. In the remaining combinations, “wood pocket” was still present, though its incidence tended to decrease with each generation (Table 3).

Table 3. Combined analysis of variance for the effect of orchard (H) and symptomatic level (N) factors on "wood pocket" physiopathy in Persian lime.

Progenitor Orchard †		Selection 1 Orchard		Selection 2 Orchard	
HI/N1	0.47 ed	H2/N1	0.67 b	H3/N1	1.27 a
H1/N2	0.66 cb	H2/N2	0.55 cd	H3/N2	0.23 gf
H1/N3	0.52 ed	H2/N3	0.50 ed	H3/N3	0.14 gh
H1/N4	0.43 e	H2/N4	0.31 f	H3/N4	0.68 h

†Data were previously transformed using the arcsine square root of the proportion; means followed by the same letter within columns do not differ statistically (Tukey, 0.05); C.V. 9.51; Significance **.

The economic potential of Persian lime has been prematurely overshadowed by the emergence of this phytopathy, which defoliates plants, dries branches, stains fruits, and ultimately leads to the death of affected trees. It is crucial to identify the early symptoms to mitigate the effects of stress and other factors that trigger the manifestation of "wood pocket" (Ríos-Rojas *et al*., 2018).

Although the specific physiology associated with the response of plants exposed to high temperatures and the incidence and severity levels of "wood pocket" remains unknown (Rodríguez *et al*., 2020), an unidentified genetic component causes varying reactions among plants of the same clone. In an affected plantation, severely impacted trees may coexist alongside others that appear healthy. The time between the onset of symptoms and plant death has not yet been clearly established; it can range from a few weeks to several months, and not all individuals in a population exhibit the same symptoms.

4 CONCLUSIONS

The severity levels of "wood pocket" symptoms varied between orchards, gradually decreasing over generations. As the incidence of affected plants declined, the proportion of plants at severity level 1 increased to 81%, 77%, and 53% for levels 4, 3, and 2, respectively. This indicates that selecting specific plant lines helps control the impact of "wood pocket."

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