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THE PHYSIOLOGICAL EFFECT OF THE PLANT GROWTH REGULATOR 2,4-D ON LEAVES AND FRUIT OF SWEET ORANGE AFFECTED BY CITRUS CANKER

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ABSTRACT:
Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a highly destructive disease that significantly reduces both yield and fruit quality. The pathogen infects young leaf tissues, fruits, and shoots of susceptible citrus plants, often leading to severe defoliation and premature fruit drop. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin used at specific concentrations to mitigate citrus fruit drop. This study aimed to evaluate the in vitro effect of 2,4-D at various concentrations (0, 0.15, 0.30, 0.45, 0.60, and 0.75 mg i.a. L^{-1}) on Xcc colonies, and to assess its impact on the lesion diameter of citrus canker on detached leaves of sweet orange (*Citrus sinensis*). Leaves were sampled 21 days after application (DAA) of 2,4-D at concentrations of 0, 0.20, 0.50, and 0.80 mg i.a. L^{-1}. Additionally, the activity of soluble peroxidase was determined at 2, 7, and 14 DAA. Field trials were conducted to evaluate the development of citrus canker on leaves and the fruit detachment force required to remove fruit from the peduncle under the same concentration gradient. Results indicated that no 2,4-D concentration tested in vitro significantly affected Xcc colony-forming units (CFU). However, the mean lesion diameter on detached leaves and peroxidase activity decreased progressively with increasing concentrations of 2,4-D. While field observations showed a modest reduction in leaf lesion development, no significant impact was observed on the fruit detachment force. We conclude that although 2,4-D demonstrates potential in reducing the severity of citrus canker lesions, it does not significantly mitigate defoliation or fruit drop in field conditions at the tested concentrations.

Keywords: Abscission, diameter of lesions, peroxidase activity, fruit detachment force, phytopathology.

EFEITO FISIOLÓGICO DO REGULADOR DE CRESCIMENTO VEGETAL 2,4-D EM FOLHAS E FRUTOS DE LARANJA DOCE AFETADOS POR CANCRO CÍTRICO

ABSTRACT:
O cancro cítrico, causado pela bactéria *Xanthomonas citri* subsp. *citri* (Xcc), é uma doença destrutiva dos citros que reduz tanto a produtividade quanto a qualidade dos frutos. A bactéria infecta os tecidos foliares jovens, os frutos e os brotos de plantas cítricas suscetíveis, o que pode resultar em desfolha e queda prematura dos frutos. O herbicida 2,4-D (ácido 2,4-diclorofenoxiacético) é um produto sintético capaz de agir como uma auxina e, quando usado em concentração adequada, reduz a queda de frutos cítricos. Este estudo teve como objetivo avaliar o efeito *in vitro* do 2,4-D em diferentes concentrações (0, 0,15, 0,30, 0,45, 0,60 e 0,75 mg i.a. L^{-1}) sobre Xcc em cultura em ágar nutriente e avaliar seu efeito no diâmetro das lesões de cancro cítrico em folhas destacadas de laranja-doce (*Citrus sinensis*) previamente inoculadas com Xcc e amostradas 21 dias após a aplicação (DAA) de 2,4-D nas concentrações de 0, 0,20, 0,50 e 0,80 mg i.a. L^{-1}. A atividade da enzima peroxidase solúvel nas folhas foi determinada aos 2, 7 e 14 DAA. O efeito do 2,4-D (nas concentrações de 0,

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0,20, 0,50 e 0,80 mg i.a. L^{-1}) no desenvolvimento do cancro cítrico nas folhas e na força necessária para remover os frutos do pedúnculo foi testado em campo. Nenhuma concentração de 2,4-D testada *in vitro* afetou o número de UFC de Xcc. O diâmetro médio das lesões nas folhas destacadas e a atividade da peroxidase diminuíram gradualmente com o aumento das concentrações de 2,4-D. Em campo, houve algum efeito do 2,4-D no desenvolvimento do cancro cítrico nas folhas, mas nenhum efeito na força necessária para remover os frutos do pedúnculo. Conclui-se que o 2,4-D tem algum efeito na redução do cancro cítrico, mas não impacta a desfolha ou a queda de frutos em campo nas concentrações testadas neste estudo.

Palavras-chave: Abscisão, diâmetro das lesões, atividade da peroxidase, força de desprendimento do fruto, fitopatologia.

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INTRODUCTION

Citrus canker, caused by the bacterium *Xanthomonas citri* subsp. *citri* (Xcc) (Schaad et al., 2007), is a destructive disease affecting most commercial varieties of sweet orange (*Citrus sinensis* L. Osbeck) (Anthony and Coggins, 1999). The pathogen infects plant tissues through stomata or minor injuries during wind-driven rain events (Bock et al., 2014; Jadhav et al., 2020; Behlau et al., 2021), leading to the development of necrotic lesions on foliage and fruit. Under high disease pressure, significant defoliation, twig dieback, and premature fruit drop can occur, severely impacting orchard productivity (Jadhav et al., 2020).

Although widely recognized as a herbicide in crops such as soybean, 2,4-dichlorophenoxyacetic acid (2,4-D) is also utilized at sub-lethal concentrations as a plant growth regulator, a biphasic dose-response phenomenon known as hormesis. At these reduced auxinic levels, the compound mimics endogenous auxins, promoting beneficial physiological responses such as enhanced root development and improved stress tolerance (Vamshi et al., 2023). In Brazil, the use of 2,4-D as a growth regulator is regulated by MAPA, specifically for its efficacy in modulating plant development and preventing premature organ abscission. This dual functionality underscores the importance of precise dosage and context-specific application to ensure that the compound induces desirable physiological outcomes rather than phytotoxicity.

The application of plant growth regulators (PGRs) in agriculture has expanded significantly in recent decades, as these compounds are known to influence flowering, rooting, vegetative growth, organ abscission, and both the yield and quality of citrus fruit (Stewart et al., 1951, 1952; Hield et al., 1964; Vamshi et al., 2023; Silva-Junior et al., 2026). Specifically, 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin frequently applied as a foliar spray at concentrations ranging from 5 to 20 mg L^{-1} (Stewart et al., 1952; Hield et al., 1964) to prevent pre-harvest fruit drop across most citrus species. The physiological efficacy of 2,4-D lies in its ability to downregulate the activity of cellulase and polygalacturonase, thereby inhibiting the enzymatic degradation of the abscission zone between the calyx (cup) and the fruit (Almeida et al., 2002; Vamshi et al., 2023).

Consequently, 2,4-D exerts physiological effects analogous to endogenous auxins and their synthetic counterparts (Schäfer et al., 2001). As noted by Anthony and Coggins (1999), the use of low-concentration 2,4-D sprays gained widespread adoption across nearly all citrus-producing regions following the validation of its efficacy. Early trials involving 'Navel' and 'Valencia' oranges, as well as grapefruit, also revealed a secondary benefit: a significant increase in fruit size (Hield et al., 1964). While it is well-established that citrus canker induces premature fruit drop (Silva-Junior et al., 2026), the specific interaction between 2,4-D and the host plant under Xcc infection remains poorly understood. Specifically, it is unclear whether 2,4-D mitigates fruit drop solely by downregulating cellulase and polygalacturonase activity at the pedicel-fruit abscission zone (Almeida et al., 2002), or if it also directly influences disease severity. Furthermore, the potential of 2,4-D to reduce the inoculum potential or prevalence of Xcc has not yet been investigated, representing a significant gap in our understanding of disease management and pathogen dispersal.

Given the global economic significance of the citrus industry and the substantial limitations imposed by citrus canker on production and international trade — stemming from reduced yields, compromised fruit quality, and the risk of pathogen dispersal — there is a critical need for management strategies that mitigate disease-related damage. This study aimed to investigate the efficacy of 2,4-D in reducing Xcc inoculum production in vitro and to determine its potential role in minimizing the premature abscission of infected fruit in orchards under high disease pressure.

MATERIAL AND METHODS

Field experiments were conducted between May and July in two commercial six-year-old citrus orchards located near Paranavaí, Paraná state, Brazil. The first site, São Paulo Farm (22º 59'S, 52º36'W; 422 m a.s.l.), was planted with the sweet orange cultivar 'Pêra Rio', while the second site, Sete Lagoas Farm (22°58'S, 52°33'W; 448m a.s.l.), featured 'IAPAR 73'. Both cultivars were grafted onto Rangpur lime (*Citrus limonia* Osbeck) rootstock. The orchards were established in 2008 and maintained according to standard cultural and management practices recommended for commercial citrus production in the region (Behlau et al., 2021; Ali et al., 2023).

For the management of citrus canker, both orchards received standard applications of copper-based bactericides consisting of copper oxychloride (700 g L^{-1}, SC; Cuprital 700, Ascenza Agro) at a dose of 70g a.i.100 L^{-1}, with four annual applications during the spring and summer. To control the citrus leaf miner (*Phyllocnistis citrella*), abamectin 72 g L^{-1}, EC; Abamectin 72 EC Nortox, Nortox S.A.) was applied according to the manufacturer's recommendations. The Xcc inoculum was naturally derived from neighboring infected commercial citrus groves. At the time of 2,4-D application, the initial incidence and severity of citrus canker on leaves were estimated via visual evaluation following the methodology of Bock et al. (2014) and were confirmed to be low (< 1%) in both orchards.

Both experiments were arranged in a randomized complete block design (RCBD) with four treatments and five replicates. Each experimental unit consisted of six trees, with the four central trees used for data collection to avoid edge effects. Plots were separated by nine buffer trees to prevent inter-plot interference. The treatments comprised three concentrations of 2,4-D (0.20, 0.50, and 0.80 mg a.e. L^{-1}) using a commercial dimethylamine salt formulation (806 g L^{-1} SL; Aminol 806, Adama), plus an untreated control (0 mg a.e. L^{-1}). At these sub-lethal concentrations, 2,4-D functions as a plant growth regulator (Vamshi et al., 2023). Calculations were based on the acid equivalent (670 g a.e. L^{-1}), and no surfactants or pH buffers were added to the spray solutions. Treatments were applied at a volume of 4 L per tree using an air-blast sprayer (Arbus 2000 Valência 190EL, Jacto Inc.) calibrated to a constant pressure of 150 PSI and a flow rate of 2000 L ha^{-1}. Applications were performed during the color break stage — the physiological transition of the flavedo from green to orange/yellow (El Otmani et al., 1990). To minimize drift and evaporative losses, spraying was conducted during the morning (07:00 to 10:00 h) under low wind conditions.

Assessments were conducted weekly at 0 (baseline), 7, 14, 21, and 28 days after application (DAA). Disease incidence and severity were evaluated on one representative branch per tree, randomly selected from the middle third of the canopy. Disease incidence was calculated as the percentage of symptomatic leaves relative to the total number of leaves on the sampled branch. Disease severity was estimated as the percentage of leaf area covered by necrotic lesions on each symptomatic leaf, using a validated diagrammatic scale (Braido et al., 2014). To ensure consistency and reduce inter-rater variability, all evaluators were previously calibrated using the aforementioned scale and standardized visual symptoms of citrus canker.

For fruit assessment, four fruits were randomly selected per tree — one from each canopy quadrant. Using a digital caliper (Digimatic 500, Mitutoyo, Japan), the pedicel diameter (mm) and equatorial diameter (mm) were measured. The fruit detachment force (FDF) (kgf) was quantified using a digital dynamometer (PCE-FM 200-ICA, PCE Instruments, UK). Disease incidence on fruit was determined by the proportion of symptomatic individuals, while severity (% area affected) was estimated visually using the diagrammatic scale of Braido et al. (2014). Following field measurements, the sampled fruits were transported to the Laboratory at the Núcleo de Pesquisa em Biotecnologia Aplicada (NBA) (Universidade Estadual de Maringá - UEM, Paraná), where they were bisected along the equatorial plane to measure albedo thickness (mm) using a digital caliper.

To evaluate fruit drop, all abscised fruits beneath the tree canopies were removed at baseline (0 DAA) to ensure a clean experimental area. Subsequently, at 7, 14, 21, and 28 DAA, all newly fallen fruits were collected and evaluated for citrus canker incidence and severity using the methods previously described. The data from each sampling interval were aggregated to determine the cumulative fruit drop per treatment. Incidence on fallen fruit was expressed as the percentage of symptomatic fruits, while severity was recorded as the mean percentage of diseased surface area per fruit.

Immediately following the final field evaluation (28 DAA), 35 fruits were randomly harvested from each plot and transported to Citri Agroindustrial SA (Paranavaí, PR, Brazil) for physicochemical and industrial analysis. The parameters evaluated included the soluble solids content (°Bx), titratable acidity (TA), maturation index (°Bx/TA ratio), juice yield (%), and industrial yield (boxes ton -1 of fruit). Yield per tree was estimated by harvesting and weighing the total fruit from one representative plant per plot, with values expressed in standard 40.8 kg boxes. Separately, to evaluate the effect of 2,4-D on the expansion of canker lesions under controlled conditions, detached leaf assays were performed. Healthy leaves were inoculated with an Xcc suspension, and the resulting

lesion diameters were measured following the established protocol of Gonçalves-Zuliani et al. (2016).

Isolate Xcc 306 (GenBank accession no. NC_003919) was used for all inoculations in this study. The isolate was obtained from the pathogen collection at the Fundo de Defesa da Citricultura (Fundecitrus, Araraquara, SP, Brazil). Xcc 306 is a confirmed *X. citri* subsp. *citri* 'A' pathotype, previously sequenced by the FAPESP Genome Project (Da Silva et al., 2002). Fresh cultures were prepared by transferring a storage suspension (phosphate buffer, 0.075 M, pH 7.0) onto nutrient agar (NA; 3 g beef extract, 5 g peptone, 5 g NaCl, and 15 g agar L^{-1} of distilled water). After incubation for 48 h at 28ºC, an inoculum suspension was prepared in phosphate buffer. The concentration was adjusted to 108 CFU mL^{-1} (OD600 ≈0.3) using a spectrophotometer (Mod. UV5, Mettler Toledo, USA) (Nanami et al., 2025). The suspension was used for inoculation immediately following standardization.

In June, branches featuring fully expanded, asymptomatic leaves were collected from 'Pêra Rio' sweet orange trees at the São Paulo Farm site, 21 DAA for each treatment (0, 0.20, 0.50, and 0.80 mg a.e. L^{-1} of 2,4-D). Only healthy leaves at a uniform stage of maturity, free from insect herbivory or mechanical injury, were selected. To maintain turgidity, samples were stored in sealed plastic bags and kept under high relative humidity during immediate transport to the NBA laboratory. Upon arrival, leaves were surface-sanitized by immersion in a 1% sodium hypochlorite (NaClO) solution for one minute, followed by three successive rinses in sterile distilled water. Finally, the leaves were blotted dry using sterile paper towels under aseptic conditions.

Individual leaves were excised from the branches by cutting through the shoot 4 mm above and below the nodal region, ensuring each leaf remained attached to a segment of the parent branch to maintain physiological functionality and maximize longevity during the assay. Immediately following preparation, each leaf was inoculated with the standardized Xcc suspension (108 CFU mL^{-1}) via the needle-puncture method (0.55 x 20 mm). Six punctures were performed per leaf — three on each side of the midrib — targeting the abaxial surface to facilitate bacterial entry into the mesophyll, as described by Gonçalves-Zuliani et al. (2016).

Each inoculated stem-leaf section was placed individually into a 50 mL conical tube. The basal shoot segment was submerged in a sufficient volume of water to maintain hydration without contacting the leaf blade; water levels were replenished as needed throughout the assay. For each 2,4-D treatment, ten replicate tubes were prepared and arranged in a completely randomized layout within an incubator. The samples were maintained at 28ºC under a 12h photoperiod. After seven days of incubation, the development of canker lesions was quantified by measuring the mean lesion diameter using a digital caliper. To ensure statistical robustness given the seasonal constraints of field sampling (21 DAA), the experiment was performed in parallel independent runs. The final results represent the pooled mean of these two simultaneous trials.

Soluble peroxidase (sPOD) activity was quantified using 'Pêra Rio' sweet orange leaves collected from the São Paulo Farm site at 2, 7, and 14 DAA. Following the selection criteria described previously, leaves were immediately wrapped in aluminum foil, placed in a portable cooler with ice, and transported to the NBA laboratory, where they were stored at -20ºC until analysis. For protein extraction, 1.0 to 1.5 g of leaf tissue was ground to a fine powder using a chilled mortar and pestle [with liquid nitrogen, if applicable]. The macerate was homogenized in 8 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 0.01 g of polyvinylpolypyrrolidone (PVPP). The resulting homogenate was transferred to 2 mL microcentrifuge tubes and centrifuged at 15,000 x g for 30 min at 4ºC. The supernatant was collected and stored in fresh 2 mL tubes at -20ºC for subsequent enzymatic assays.

Soluble peroxidase (sPOD) activity was determined according to the method of Li et al. (2020). The reaction mixture (2.6 mL) consisted of 25 mM sodium phosphate buffer (pH 6.8), 2.58 mM guaiacol, and 10 mM H_2O_2. The enzymatic reaction was initiated by adding 0.4 mL of the enzyme extract, previously diluted (30x) in the extraction buffer. The oxidation of guaiacol was monitored by measuring the increase in absorbance at 470 nm over a 5 min interval using a spectrophotometer (Mod. UV5, Mettler Toledo, USA). Enzyme activity was calculated based on the extinction coefficient of tetraguaiacol (25.5 mM^{-1} cm^{-1}). A blank containing the reaction mixture without the enzyme extract was used to calibrate the equipment. Final sPOD activity

was expressed as μmol of tetraguaiacol min^{-1} g^{-1} of fresh weight (FW).

Isolate Xcc 306 was used to evaluate the direct effect of 2,4-D on the in vitro growth of the pathogen. Bacterial cells were harvested from nutrient agar (NA; 3 g beef extract, 5 g peptone, 5 g NaCl, and 15 g agar L^{-1}) and suspended in phosphate-buffered saline (PBS). The initial suspension was adjusted to a concentration of 108 CFU mL^{-1} (OD 600 ≈ 0.3) as previously described (Nanami et al., 2025). Subsequently, a ten-fold serial dilution series was performed in PBS to achieve a final concentration suitable for the Standard Plate Count method (Gonçalves-Zuliani et al., 2016). This ensured that the resulting colonies on the agar surface were distinct and quantifiable.

The in vitro sensitivity assay was conducted using 9 cm Petri dishes containing 20 mL of NA medium. Each plate received a 50 µL aliquot of 2,4-D solution at concentrations of 0, 15, 30, 45, 60 and 75 mg L^{-1}. These solutions were prepared by diluting the herbicide in sterile distilled water and were spread uniformly across the agar surface to allow for complete absorption. To ensure accuracy, the final 2,4-D concentrations were calculated based on the total volume of the agar medium (20 mL). Following absorption, 50 µL of the standardized Xcc suspension was inoculated onto the surface using the spread-plate technique. Plates were incubated at 28ºC under a 12-h photoperiod for 72h. The experiment was performed twice in a completely randomized design (CRD) with five replicates per treatment. Total colony-forming units (CFU) were enumerated for each plate. Data were subjected to analysis of variance (ANOVA) and mean separation was performed using Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$) via SAS software (SAS Institute, Cary, NC, 2025).

RESULTS

In the detached leaf assays, the diameter of citrus canker lesions exhibited a significant dose-dependent reduction at 7 days post-inoculation (Table 1; Figure 1). On 'Pêra Rio' leaves collected 21 DAA, the average lesion diameter decreased from 2.15 mm in the untreated control to 1.80 mm and 1.35 mm in leaves treated with 0.20 and 0.80 mg a.e L^{-1} of 2,4-D, respectively. Notably, lesions on leaves from trees receiving the highest 2,4-D rate (0.80 mg a.e. L^{-1}) failed to develop the characteristic erumpent, pustular morphology typical of Xcc infection, appearing instead as restricted, flat necrotic spots.

Table 1. Effect of 2,4-D concentration (measured 21 days after application) on the diameter of citrus canker (*Xanthomonas citri* subsp. *citri)* lesions in detached 'Pêra Rio' leaves. Lesion size was evaluated 7 days after wound-inoculation with a bacterial suspension (10^8 CFU mL^{-1}).

Treatments (mg L^{-1})	Diameter of Lesions (mm)
0.00	2.15 a
0.20	1.80 b
0.50	1.58 c
0.80	1.35 d
CV%	**26.84**

*Means followed by the same letters in the same column do not differ statistically from each other at the significance level of 5% by Tukey test.

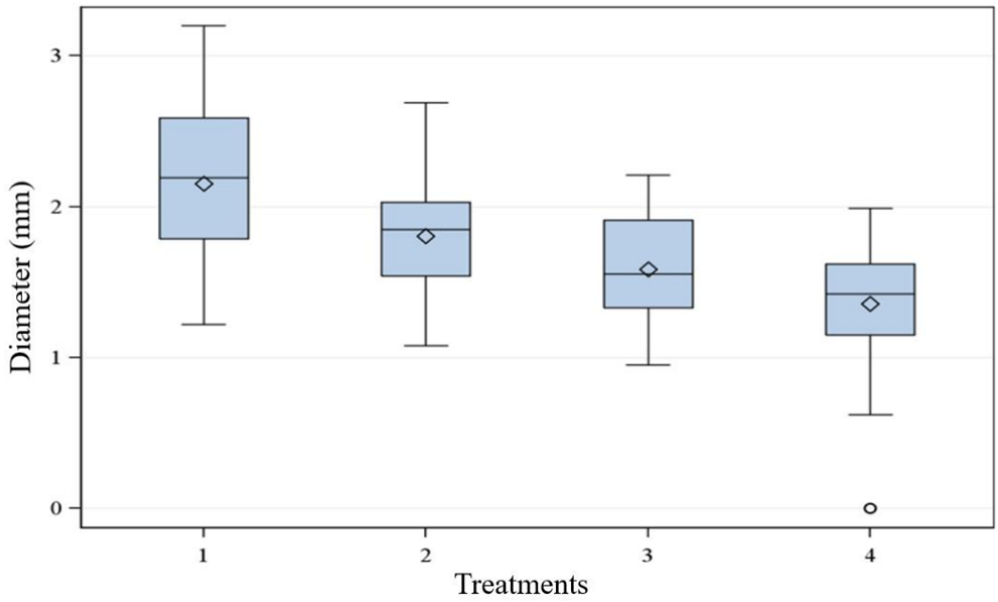

Figure 1. Box plot of citrus canker lesion diameters in 'Pêra Rio' sweet orange leaves 7 days post-inoculation with *Xanthomonas citri* subsp. *citri* 108 CFU mL^{-1}. Data represent the relationship between lesion size and the concentration of 2,4-D spray-applied 21 days prior to the detached leaf assay.

Soluble peroxidase (sPOD) activity in 'Pêra Rio' sweet orange leaves exhibited a significant increase in response to 2,4-D applications across all sampling intervals (2, 7, and 14 DAA; Table 2). While the untreated control maintained an average activity of 2.472 µmol $min^{-1}g$-1 FW, the highest 2,4-D concentration (0.80 mg a.e. L^{-1}) resulted in a marked induction of enzyme activity, reaching 3.131 µmol $min^{-1}g$-1 FW. This up-regulation of sPOD suggests that sub-lethal rates of 2,4-D successfully primed the host's antioxidant and defense-related metabolic pathways.

Table 2. Soluble peroxidase (sPOD) activity (µmol min^{-1} g^{-1} FW) in 'Pêra Rio' sweet orange (*Citrus sinensis*) leaves as a function of 2,4-D concentration.. Values represent the **pooled means** across three sampling intervals (2, 7 and 14 days after application). Study conducted at Fazenda São Paulo, Paranavaí, Paraná, Brazil.

Treatments (mg L^{-1})	**POD (µmol min^{-1} g^{-1})**
0.00	2.472 ab
0.20	3.131 a
0.50	2.719 ab
0.80	2.376 b
CV%	**28,77**

*Means followed by the same letters in the same column do not differ statistically from each other based on Tukey's HSD test (α = 0.05).

Regarding the 'IAPAR 73' variety, 2,4-D concentration had no significant effect on fruit abscission or fruit detachment force (FDF) (Table 3). Morphological parameters were only marginally affected: trees treated with 0.50 mg a.e L^{-1} produced slightly larger fruits (72.40 mm) compared to the control (70.79 mm). Similarly, pedicel diameter was significantly greater in the 0.50 and 0.80 mg a.e L^{-1} treatments (3.46 and 3.44 mm, respectively) relative to the control (3.34 mm). Although albedo thickness was initially greater in the 0.50 mg a.e L^{-1} treatment (5.72 mm), no statistical differences were maintained by the final evaluation. For the 'Pêra Rio' variety, 2,4-D concentration did not significantly influence abscission, FDF, equatorial diameter, or albedo thickness. While a transient increase in pedicel diameter was noted for the 0.50 mg a.e L^{-1} treatment (4.08 mm) compared to the control (3.89 mm), this difference did not persist in the final assessment (Table 3).

Table 3. Physical and morphological fruit parameters of 'IAPAR 73' and 'Pêra Rio' sweet oranges (*Citrus sinensis*) as influenced by 2,4-D concentration. Data represent the pooled means of five assessment dates (May–June) and the final evaluation at 28 days after application (DAA). Variables include fruit abscission (%), fruit detachment force (FDF), equatorial diameter, pedicel diameter, and albedo thickness. Study conducted at Sete Lagoas Farm ('IAPAR 73') and São Paulo Farm ('Pêra Rio') in Paranavaí, PR, Brazil.

Variety	Treatments	Abscission	FDF (kgf)	Diameters (mm)		
'IAPAR 73'	0.00	58.00 a	6.64 a	70.79 b	3.34 b	4.81 b
	0.20	56.00 a	6.65 a	71.86 ab	3.33 b	4.81 b
	0.50	64.00 a	6.81 a	72.40 a	3.46 a	5.72 a
	0.80	54.00 a	6.98 a	71.76 ab	3.44 a	4.99 b
CV%		**35.48**	**31.57**	**6.76**	**16.19**	**27.22**
'IAPAR 73' (28 DAA)	0.00	-	6.55 a	67.74 a	3.50 a	4.78 a
	0.20	-	6.67 a	66.98 a	3.41 a	4.51 a
	0.50	-	6.14 a	68.01 a	3.58 a	5.05 a
	0.80	-	6.88 a	68.37 a	3.44 a	4.82 a
CV%			**27.85**	**7.62**	**17.42**	**21.74**
'Pera Rio'	0.00	27.00 a	6.45 a	70.13 a	3.89 b	4.30 a
	0.20	36.00 a	6.35 a	70.33 a	3.94 ab	4.37 a
	0.50	23.00 a	6.41 a	70.08 a	4.08 a	4.33 a
	0.80	23.00 a	6.51 a	70.93 a	3.96 ab	4.30 a
CV%		**45.83**	**37.68**	**6.26**	**23.36**	**19.10**
'Pera Rio' (25 DAA)	0.00	-	6.16 a	69.26 a	3.72 a	4.29 a
	0.20	-	6.02 a	67.61 a	3.81 a	4.34 a
	0.50	-	5.86 a	67.95 a	3.82 a	4.49 a
	0.80	-	6.27 a	69.32 a	3.85 a	4.22 a
CV%			**36.67**	**6.43**	**14.54**	**22.52**

*Means followed by the same letters in the same column do not differ statistically from each other based on Tukey's HSD test (α = 0.05).

Regarding industrial quality, the maturation index (ºBx/TA ratio), juice yeld (%) amd industrial yield (boxes ton-1) measured at 28 DAA did not differ significantly from the untreated control for either the 'IAPAR 73' or 'Pêra Rio' varieties (Table 4). Furthermore, the in vitro sensitivity assay revealed that 2,4-D concentration had no significant effect on the vegetative growth of Xcc on nutrient agar (Table 5). These results suggest that the suppression of citrus canker symptoms observed in the field and detached leaf assays was not due to direct antibacterial activity of the herbicide, but rather to a host-mediated physiological response.

DISCUSSION

While the use of 2,4-D as a plant growth regulator (PGR) to improve citrus fruit quality and retention is well-documented (Stewart et al., 1951, 1952; Hield et al., 1964), its potential role in modulating citrus canker development has not been previously explored. Our results demonstrate that leaves from 'Pêra Rio' trees treated with 2,4-D developed smaller Xcc lesions with an altered, non-pustular morphology. We hypothesize that sub-lethal concentrations of 2,4-D may have induced localized cell proliferation or a "wound-healing" response, effectively creating a physical barrier that restricted bacterial colonization and delayed symptom progression.

This hypothesis is consistent with the findings of Savita et al. (2015), who observed that low concentrations (1 – 6 mg L^{-1}) of 2,4-D promoted callus induction in citrus explants. Similarly, studies in other species have shown that 2,4-D can trigger rapid cell division and callus proliferation at injury sites (Naqvi et al., 2022). Although microscopic analysis was not conducted in the present study, the observed reduction in lesion size and the lack of erumpent symptoms suggest that 2,4-D-induced hyperplasia may interfere with the typical

development of the hyperplastic pustules characteristic of *X. citri* subsp. *citri*.

Lignin is a fundamental structural component of the vascular plant cell wall, providing a physical barrier against pathogen ingress. Peroxidases (POD) are recognized as key enzymes catalyzing the final step of lignification: the oxidative polymerization of monolignols (such as p-coumaryl, coniferyl, and sinapyl alcohols). As a synthetic auxin, 2,4-D interacts dynamically with cell wall metabolism; while high doses typically induce phytotoxic stress, sub-lethal concentrations can modulate POD activity to influence wall rigidity and elongation. Under the experimental conditions of this study, the observed induction of sPOD suggests a shift toward enhanced lignification, which increases cell wall recalcitrance. This response is consistent with the "dual effect" of 2,4-D noted by Yangyang et al. (2025), where the metabolic outcome — either the promotion or attenuation of lignin deposition — is strictly dependent on the species and the applied concentration. In 'Pêra Rio' leaves, the 2,4-D-mediated increase in sPOD likely fortified the leaf tissue, restricting the expansion of Xcc lesions.

In this study, soluble POD activity in field-collected leaves exhibited a subtle but significant increase (to 3.131 μmol min^{-1} g^{-1}) following 2,4-D application. Given that the field severity of citrus canker remained low (< 1%), the observed enzymatic shift likely reflects a systemic priming effect rather than a reactive response to pathogen - induced stress. This aligns with the findings of Wang et al. (2011), who observed that POD activity is a critical biomarker of the citrus defense response. Interestingly, Wang et al. noted higher POD levels in susceptible varieties during active infection — likely a consequence of the oxidative burst triggered by successful colonization. Conversely, our results suggest that sub-lethal 2,4-D treatments may proactively fortify the host. By accelerating wound healing and callus formation at the inoculation sites, 2,4-D potentially reduces the "pathogen pressure" on the leaf tissue, thereby modulating the total POD demand required to limit Xcc progression.

Contrary to expectations based on established PGR literature, the 2,4-D concentrations applied in this study had negligible effects on fruit abscission, fruit detachment force (FDF), or morphological parameters such as equatorial diameter and pedicel thickness. While it is well-documented that 2,4-D can enhance fruit size and retention (Stewart et al., 1951; Hield et al., 1964), these responses are highly dose-dependent. For example, El-Otmani et al. (1990) utilized 16 mg L^{-1} to effectively control abscission in 'Valencia' oranges, and Anthony and Coggins (1999) suggested that concentrations below 4 mg L^{-1} are generally insufficient to trigger significant changes in fruit retention. This explains why our maximum rate of 0.80 mg a.e. L^{-1} did not deviate significantly from the control regarding these physical parameters. Similarly, our finding that 2,4-D did not alter the maturation index, juice yield, or industrial productivity corroborates the results of Schafer et al. (2001). Collectively, these data suggest that while the applied rates were below the threshold for traditional PGR-mediated fruit retention, they were nonetheless physiologically active enough to modulate host defense enzymes (sPOD) and suppress Xcc lesion development.

Direct antibacterial activity of 2,4-D against *X. citri* subsp. *citri* was not observed in vitro at any of the tested concentrations. This suggests that the suppression of citrus canker symptoms is likely a result of induced host resistance or structural modifications rather than a direct bactericidal or bacteriostatic effect. While higher concentrations — such as those typically used for fruit quality enhancement — might theoretically impact bacterial viability, the sub-lethal rates employed here appear to act solely via plant-mediated pathways. Furthermore, the field experiments encountered exceptionally low disease pressure, with severity remaining below 1% on both leaves and fruit across all plots. This low incidence was likely a synergistic result of established copper-based phytosanitary management and abiotic conditions (high temperatures and low relative humidity) unfavorable for Xcc dissemination. Consequently, these environmental constraints limited the capacity to statistically differentiate the impact of 2,4-D treatments on disease progression under field conditions.

Our findings confirm that the tested 2,4-D concentrations did not compromise agronomic safety, as all industrial and physicochemical parameters remained within commercially acceptable limits. This indicates that the application of sub-lethal 2,4-D rates is compatible with sustainable production frameworks, posing no significant risk to the food chain or the surrounding environment. Ultimately, these results reinforce the technical and environmental viability of integrating these

treatments into citrus management programs on an industrial scale.

CONCLUSION

In conclusion, the sub-lethal concentrations of 2,4-D evaluated in this study did not exhibit direct antibacterial activity against *X. citri* subsp. *citri* (Xcc) in vitro. However, the herbicide successfully modulated the host's physiological response, resulting in a significant reduction in lesion diameter and the development of atypical, non-pustular symptoms. At the applied field rates (up to 0.80 mg a.e. L^{-1}), 2,4-D did not interfere with fruit detachment force (FDF), premature abscission, or industrial quality parameters, confirming its agronomic safety. While low field disease pressure (< 1%) precluded a definitive assessment of field-scale disease suppression, these results highlight the potential of sub-lethal auxin treatments as a novel, host-mediated strategy for integrated citrus canker management.

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