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ARTICLE

Physiological and biochemical characterization of a susceptible carnation (*Dianthus caryophyllus* L.) cultivar to *Fusarium oxysporum* f. sp. *dianthi* (Fod)

Caracterização fisiológica e bioquímica de um cultivar suscetível de cravo (*Dianthus caryophyllus* L.) à *Fusarium oxysporum* f. sp. *dianthi* (Fod)

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Abstract: The susceptibility of carnations (*Dianthus caryophyllus* L.) to infection by *Fusarium oxysporum* f. sp. *dianthi* (Fod) poses a significant challenge to agricultural production, particularly in Colombia, a country that is a global leader in carnation exportation. This study focused on the physiological and biochemical responses of a highly susceptible carnation cultivar 'Solex', to the presence of Fod to better understand the underlying mechanisms of susceptibility and to enhance early disease detection strategies in carnation crops. At the biochemical level, we analyzed the content of phenolic compounds and flavonoids in roots and stems; and we assessed physiological parameters such as foliar photosynthetic pigment content, stomatal resistance, maximum photochemical efficiency of PSII (F_v/F_m) and leaf temperature. Our research unveiled a marked decrease in metabolite production in the roots of carnation plants inoculated with Fod that was particularly evident at 4 post-inoculation days. Furthermore, we observed an early increase in leaf temperature from 1 post-inoculation day onwards, without changes in stomatal closure dynamics over time. Additionally, we recorded a significative decline in F_v/F_m, photosynthetic pigment content and dry biomass production in Fod-inoculated plants during the symptomatic phase of vascular wilting that contrasted starkly with pathogen-free controls. These findings underscored the intrinsic susceptibility of carnation plants to Fod infection, with significant implications for enhancing plant resistance and developing effective vascular wilting management strategies in crops of this flower.
Keywords: carnation, chlorophyll α fluorescence, flavonoids, leaf temperature, phenolic compounds, stomatal resistance.

Resumo: A susceptibilidade das cravinas (*Dianthus caryophyllus* L.) à infecção por *Fusarium oxysporum* f. sp. *dianthi* (Fod) representa um desafio significativo para a produção agrícola, especialmente na Colômbia, país líder mundial na exportação de cravinas. Este estudo concentrou-se nas respostas fisiológicas e bioquímicas de uma cultivar de cravina altamente suscetível, 'Solex', à presença de Fod, visando compreender melhor os mecanismos subjacentes à suscetibilidade e aprimorar estratégias de detecção precoce de doenças em cultivos de cravina. No nível bioquímico, analisamos o teor de compostos fenólicos e flavonoides em raízes e hastes; e avaliamos parâmetros fisiológicos como conteúdo foliar de pigmentos fotossintéticos, resistência estomática, eficiência fotoquímica máxima de PSII (F_v/F_m) e temperatura foliar. Nossa pesquisa revelou uma diminuição acentuada na produção de metabólitos nas raízes de plantas de cravina inoculadas com Fod, especialmente evidente aos 4 dias pós-inoculação. Além disso, observamos um aumento precoce na temperatura foliar a partir do primeiro dia pós-inoculação, sem alterações na dinâmica de fechamento estomático ao longo do tempo. Adicionalmente, registramos uma significativa redução em F_v/F_m, conteúdo de pigmentos fotossintéticos e produção de biomassa seca em plantas inoculadas com Fod durante a fase sintomática do murchamento vascular, contrastando fortemente com os controles livres de patógenos. Esses achados destacaram a suscetibilidade intrínseca das plantas de cravina à infecção por Fod, com implicações significativas para o aprimoramento da resistência das plantas e o desenvolvimento de estratégias eficazes de manejo do murchamento vascular em cultivos desta flor.
Palavras-chave: cravo, compostos fenólicos, flavonoides, fluorescência da clorofila α, resistência estomática, temperatura foliar.

Introduction

Vascular wilting, caused by the fungus *Fusarium oxysporum* f. sp. *dianthi* (Fod), is perhaps the most destructive disease affecting carnations, and represents a significant economic threat, potentially resulting in complete crop loss (Romero-Rincón et al., 2021). The disease manifests itself through vascular bundle blockage and features symptomatic traits such as chlorosis, wilting of basal leaves, stunted growth, lateral plant inclination, and eventually death (Poli et al., 2013).

The early detection of the disease is a challenge for carnation producers considering that symptomatic expression only occurs late in the degenerative process. Progression of vascular wilting unfolds in two stages: an asymptomatic phase, where the pathogen remains invisible, and a symptomatic phase, characterized by obvious signs and symptoms of the disease. During the asymptomatic stage, hemibiotrophic vascular pathogens like many *Fusarium* species (Wang et al., 2022), are recognized and they begin to thrive within the host tissues during the biotrophic phase. During colonization, they target susceptible tissue for growth. The duration between inoculation and the manifestation of symptomatic expression is called the incubation period (Marín-Ortiz et al., 2019). These stages may exhibit varied timelines depending on the pathosystem.

The response of carnation cultivars to the Fod pathogen has been studied biochemically, molecularly, and histologically, revealing distinctive features. In susceptible cultivars, hyphae of the pathogen accumulate in the vascular bundles at later stages (18 dpi) without a visible defense response at an ultrastructural level (Higuera and Ebrahim-Nesbat, 1999). Resistant cultivars exhibit the production of antifungal compounds, phenolic derivatives, along with other glycosylated flavonoids (Romero-Rincón et al., 2021; Martínez-González et al., 2022); and some are recognized as resistance biomarkers. Recent findings indicate that in a Fod-susceptible cultivar with early post-inoculation the levels of compounds derived from anthranilate, benzoic acid, and glycosylated flavonols increase due to the application of an inert extract prepared from the pathogen (Santos-Rodríguez et al., 2021). In research on the carnation-Fod interaction, resistance to the disease is typically emphasized for developing resistant varieties. However, there is a lack of research providing biochemical markers or early indicators of susceptibility to Fod. Susceptible plants possess the ability to activate their defenses if they encounter the pathogen and this leads to susceptibility responses that transform the plants from independent biological entities into components of a pathosystem. Such responses are becoming more significant, with an increasing focus on

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describing genes that are susceptible (S-genes) (García-Ruiz et al., 2021; Gorshkov and Tsers, 2021).

In plant physiology research, a susceptibility response has been found when studying the combined stress of carnations with Fod and the nematode *Meloidogyne incognita*. This reveals that the presence of these pathogens leads to a reduction in chlorophyll content (Meena et al., 2016). However, at the physiological level studies have been based on the search for cultivars whose responses result in the best management of the disease - resistance responses. For example, banana plants infected with *Fusarium oxysporum* f. sp. *cubense*, increase leaf temperature, with a reduction in stomatal conductance (gs) and transpiration rate (E), contributing to more effective management of water loss (Dong et al., 2012). In the wheat – *Fusarium graminearum* pathosystem, an increase in temperature appears in the spikelets of infected plants, along with a reduction in F_v/F_m values in that specific area of the plant (Francesconi et al., 2021). These studies enable the discovery of responses in the pathosystems that could serve for the early diagnosis of the disease.

In this study, our objective was to evaluate the impact of Fod-inoculation on the highly susceptible carnation cultivar 'Solex'. We examined its physiological and biochemical responses and revealed potential relations between the observed biochemical changes and dysregulation of the shikimate pathway associated with less production of phenols and flavonoids in the asymptomatic stage of disease. This research serves as a foundation for the development of future "susceptibility markers" in this pathosystem. Furthermore, our physiological characterization complements the biochemical findings, providing insights into infection and susceptibility traits that can be assessed non-destructively in crops. These responses, observed during the asymptomatic stage of vascular wilting, hold promise for early disease detection.

Materials and methods

Adaptation of experimental conditions

We cultivated the plants in a controlled greenhouse environment with monitored conditions, including an average temperature of 20 °C, relative humidity of 65.8%, and a photoperiod of 12/12 hours. Light conditions were maintained at an average irradiance of 5 µmol photons m^{-2} s^{-1}, as determined by a portable meteorological station (COLTEIN Ltda., Bogotá, Colombia) equipped with dataloggers (COLTEIN Ltda., Bogotá, and Hobo U12-006, Onset Computer Corporation, Bourne, MA, USA). Temperature and relative humidity were recorded using THR 102 sensors (USA), and photosynthetically active radiation (PAR) was measured with LI 190 B sensors (LI-COR Inc., Lincoln, NE, USA).

Plant material

Certified carnation cuttings were provided by Florval QFC S.A.S, Gachancipá, Cundinamarca, Colombia. These cuttings, of clonal origin and confirmed free of all pathogens, were planted in a sterile mixture of soil and vermiculite (75:25) to ensure a Fod-free environment. Sterilization was carried out in two cycles using an autoclave at 121ºC, 144.8 KPa, with each cycle lasting 30 minutes. The plants were maintained under optimal field conditions with adequate irrigation and nutrient conditions following the recommendations of the growers. The plants underwent a two-week acclimatization period before inoculation.

Inoculum source and controlled inoculation

The Fod isolate was previously characterized at the molecular level by the Plant Metabolic Activities research group of UNAL - Bogotá campus, utilizing conventional PCR with species- and race-specific primers, as detailed in previous research (Pérez-Mora et al., 2021; Vanegas-Cano et al., 2022a).

The *Fusarium oxysporum* f. sp. *dianthi* (Fod) race 2 inoculum, which is the most recurrent and highly pathogenic in the Sabana de Bogotá area, Colombia (Arbeláez et al., 1993), was sourced from carnation plants exhibiting typical vascular wilting symptoms, provided by the grower. The isolate was characterized at the molecular level using conventional PCR with species and race-specific primers proposed by Kamel et al., 2003 and Chiocchetti et al., 1999 as described previously (Pérez-Mora et al., 2021; Vanegas-Cano et al., 2022a). It was classified within the 8 pathotypes (or races) previously described by Garibaldi et al., 1983, as mentioned by (Devappa and Archith, 2019). The Fod isolate virulence was reactivated by introducing it to carnation plants of the susceptible cultivar 'Solex' and subsequently reisolating it from stems planted on PDA (Sigma) (Pérez-Mora et al., 2024a, 2024b). The fungal material for subsequent inoculation was cultured in Czapec Dox Brott (SIGMA) liquid medium at a constant temperature of 25 °C for five days at 200 rpm. Conidial presence was confirmed under an optical microscope at 400 magnifications (GEMMY, Taiwan, Republic of China). The medium was then filtered under aseptic conditions. Subsequently, a group of plants was inoculated with Fod by root immersion at a concentration of $1x10^6$ conidia mL^{-1}. The same root immersion process was applied to control plants (without Fod) using sterile distilled water, as previously described (Pérez-Mora et al., 2024a, 2024b). The plants were kept under low light conditions to facilitate pathogen development.

Disease evaluation

We evaluated the incidence of the disease during 63 days by counting the number of diseased plants with symptoms of vascular wilting over time (Poli et al., 2013) and including the total number of plants per treatment. The relationship was expressed as a percentage, as shown in Equation 1.

Equation 1

$$\% \text{ Incidence} = (\text{plants with external symptoms of vascular wilting/treatment plants}) * 100$$

We evaluated the severity of each treatment during 63 days as the area under the curve of the progress of the disease (AUDPC), considering the severity scale proposed by (Pérez-Mora et al., 2021). The severity scale was measured according to Equation 2 in the plants from three replicates per treatment. Each replicate contained 10 plants, where "t" was the time of each reading measured in weeks, "y" was the estimated severity according to the severity scale, and "n" was the number of readings. (Pérez-Mora et al., 2024a).

Equation 2

$$AUDPC = \sum_{i=1}^{n} \left(\frac{S_i + S_{i+1}}{2} \right) * (t_{i+1} - t_i)$$

Determination of physiological parameters

Stomatal resistance and leaf temperature

Variables relating to stomatal resistance (s m^{-1}) and leaf temperature (°C) were determined using a steady-state SC-1 leaf porometer (LP 3538 DECAGON DEVICES, Washington, USA). We measured six plants per treatment, focusing on the fully expanded (physiologically mature) leaf in the middle third of the plant. Measurements were taken on both, the adaxial (upper) and abaxial (lower) surfaces of the leaf, as we found that it is amphistomatic. We measured the leaves between 7:00 and 9:00 am on 1, 7, 14, 17, 21, 26, 28, 35, 42, 49, 56, and 63 post-inoculation days (dpi), see Fig. 1. These data were based on a prior *in vivo* gas exchange assay on various leaves, revealing the highest exchange rate in the middle third of the canopy. Additionally, the optimal times for tracking infection progression were identified in that experiment (data not shown).

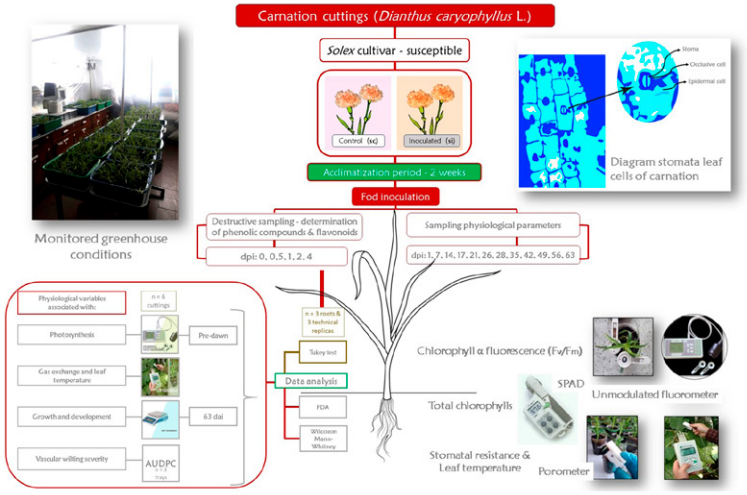

Fig. 1. Experimental design and monitored greenhouse conditions of the *in vivo* Carnation-Fod interaction assay.

Parameters associated with photosynthesis

Determination of chlorophyll fluorescence.

The maximum photochemical efficiency of PSII (F_v/F_m) was at 1 and 49 dpi in pre-dawn in the third fully expanded leaf physiologically mature in five plants per treatment (n = 5), adapted to darkness throughout the night using a Pocket PEA non-modulated fluorometer (HANSATECH INSTRUMENTS, UK).

Quantification of chlorophylls.

Leaves of different intensities of green color were selected and their SPAD units were estimated. We collected leaf discs (diameters 0.5 cm) from the same locations as the SPAD measurement. Pigments were extracted with dimethyl sulfoxide according to the Wellburn method with modifications (Lozano-Montaña et al., 2021). We performed absorbance readings at 665.1 nm, 432.6 nm, 649.1 nm, and 461 nm with a Smart Spec 3000 spectrophotometer (BIORAD, USA). A calibration curve was constructed to relate the chlorophyll content of different coloring shades leaves vs SPAD units. During the experiment, we estimated chlorophyll relative content with the Minolta SPAD 502 using the third leaf fully expanded in five plants per treatment. The SPAD data were interpolated in the calibration curve to calculate the chlorophyll content. We carried out the measurements on the same days and on the same leaves in which stomatal resistance and leaf temperature were measured.

Morphological parameters – dry biomass.

To determine dry biomass, eight complete plants were taken per treatment at 63 dpi. These were dried in an oven at 60 °C during 48 h (Toro-Tobon et al., 2022).

Extraction and determination of phenolic compounds (PCs) and flavonoids (FCs) in carnation root and stem in interaction with Fod

We evaluated the PCs and FCs on 0, 0.5, 1, 2, and 4 days post-inoculation (dpi). We weighed twenty milligrams (dry weight) of roots or stems, macerated with liquid nitrogen, and combined with 1 mL of 80% methanol in an Eppendorf tube. After a 15-minutes ultrasound treatment (42 Hz and 100 W), the mixture underwent centrifugation (12,000 g, 15 min, 4 °C), and we stored the resulting supernatant at 80 °C for further analysis (Pérez-Mora et al., 2021, 2024a, 2024b).

We determined the total phenolic content using the Folin-Ciocalteu method. The reaction mix included 50 µL of methanol extracts, 100 µL of Folin-Ciocalteu reagent, and 100 µL of distilled-deionized H_2O. After 5 minutes, we added 200 µL of 7 % Na_2CO_3 and 200 µL of distilled-deionized water. Following a 1-hour incubation at room temperature (approximately 20 °C), we measured the absorbance at 764 nm using a Thermo Genesys 10 UV spectrophotometer (Madison, Wisconsin, USA). Gallic acid (Sigma®) served as the standard for a calibration curve, and the total phenol content was expressed as mg of gallic acid equivalents per g of dry roots (Pérez-Mora et al., 2021, 2024a, 2024b).

We assessed the flavonoid content using the colorimetric method. The reaction mixture comprised 100 µL of methanol extract, 30 µL of 5% $NaNO_2$ solution and 100 µL of deionized water. After 5 minutes, we added 60 µL of 10 % $AlCl_3$ solution. Following a 6-minute incubation at room temperature, we added 100 µL of 2 M NaOH and measured the absorbance at 510 nm. We employed a calibration curve with catechin (Sigma®) as the standard expressing the results as mg equivalent of catechin per g of dry roots (Pérez-Mora et al., 2021, 2024a, 2024b).

Experimental design and data analysis

For the arrangement of the cuttings in the greenhouse and the selection of the sampling plants, we used a completely randomized factorial scheme. To make comparisons between the control and inoculated treatments with the data of the physiological variables leaf temperature, stomatal resistance and chlorophyll content over time and to determine the presence of significant differences, we used a non-parametric functional ANOVA - Functional Data Analysis (ANOVA-FDA) with a statistical test that compare the means of each of the groups (associate points in time). This converted the specific data into curves that are measured over time to build the test statistic and the *p*-value from non-parametric re-sampling methods (Bootstrap) according to Górecki and Smaga (2019). The test used was the L^2b test - L^2-norm-based Bootstrap that is adequate when using small samples (n = 6). *P* - values less than 0.05 reject the null hypothesis of equality of mean curves, for which there are statistically significant differences. The package used is available on line: https://cran.r-project.org/web/packages/fdANOVA/index.html. We carried out these analyses and graphs using the R – STUDIO package, 1.3.1093 version. To determine differences between physiological variables F_v/F_m and dry biomass, as well as for the incidence percentage and AUDPC values, we analyzed the data using the non-parametric Wilcoxon test (Mann-Whitney U) with a significance value ≤ 0.05, comparing the medians per treatments-dpi (Table 1). To establish the relationships between the physiological variables we used the Spearman correlation using the statistical package in R Studio. We performed principal component analyses (PCA) with Minitab® Software version 17.

Table 1. Statistical analysis of physiological variables studied in the Carnation-Fod interaction, susceptible cultivar 'Solex'.

Total chlorophyll content (mg g^{-1} chlorophylls)								
Cultivar Solex / dpi (time)	**42**	***p*-value**	**49**	***p*-value**	**56**	***p*-value**	**63**	***p*-value**
Without Fod (control)	0.0101 ± 0.0008	0.015	0.0098 ± 0.0011	0.017	0.0096 ± 0.0014	0.026	0.0096 ± 0.0015	0.022
With Fod (inoculated)	0.0085 ± 0.0010		0.0071 ± 0.0033		0.0066 ± 0.0031		0.0066 ± 0.0032	
Percentage relative to control	15.4		27.7		31.1		31.6	
% Incidence								
Cultivar Solex / dpi (time)	**15**	***p*-value**	**30**	***p*-value**	**45**	***p*-value**	**63**	***p*-value**
Without Fod (control)	0.00 ± 0.00	0.005	0.00 ± 0.00	0.002	0.00 ± 0.00	<0.0001	0.00 ± 0.00	<0.0001
With Fod (inoculated)	47.0 ± 5.29		76.7 ± 5.60		98.0 ± 1.00		98.0 ± 1.00	
F_v/F_m								
Cultivar Solex	**dpi 49**				***p*-value**			
Without Fod (control)	0.81 ± 0.01				0.008			
With Fod (inoculated)	0.76 ± 0.06							
Dry biomass (g)								
Cultivar Solex	**dpi 63**				***p*-value**			
Without Fod (control)	0.620 ± 0.13				0.0009			
With Fod (inoculated)	0.410 ± 0.09							
AUDPC								
Cultivar Solex	**dpi 63**				***p*-value**			
Without Fod (control)	1.60 ± 0.3				0.033			
With Fod (inoculated)	8.10 ± 1.7							

To make comparisons between the control and inoculated treatments with the data of the biochemical variables on early times and to determine the presence of significant differences, we performed the experiments using three biological replicates for each treatment, each of which composed of at least 10 healthy rooted carnation cuttings. Data were reported as the mean plus or minus standard deviation with analysis of variance (one-way ANOVA), and significant differences between means (Tukey's test), p-value ≤ 0.05.

Results

Incidence and severity of the disease

At 30 dpi, approximately 80% of the plants inoculated with Fod presented significant levels of incidence when compared to the controls (Fig. 2A). Subsequently, at 63 dpi all inoculated plants showed signs and symptoms of vascular wilting. The calculation of the AUDPC at 63 dpi (Fig. 2B) showed values of 1.6 for the control plants without Fod (cultivar 'Solex') and 8.1 for the inoculated plants.

Physiological response of carnation to Fod in the highly susceptible cultivar - 'Solex'

The dry biomass measured at 63 dpi decreased significantly by 33.6% in the plants inoculated with Fod compared to their control (Fig. 2C). The functional ANOVA analysis, with p-values close to zero indicated that over the course of 63 days leaf temperature tended to be higher in inoculated plants compared to control plants (Fig. 2E). The stomatal resistance values showed no effect of inoculation (Fig. 2F); although the general trend was towards greater stomatal resistance to water vapor diffusion in the inoculated plants compared to the control plants. Parameters associated with photosynthesis decrease due to the effect of vascular wilting was caused by Fod. The maximum photochemical efficiency of PSII (F_v/F_m) in the inoculated plants (Fig. 2D) showed significant differences between treatments towards the end of the experiment (dpi 49. Control plants F_v/F_m = 0.81 and inoculated plants F_v/F_m = 0.76) indicated mild symptoms of photoinhibition. The results of the functional ANOVA analysis carried out for the total chlorophyll content (Fig. 2G) showed that the inoculated carnation plants decreased the total chlorophyll content compared to the control, mainly from 26 dpi. At 63 dpi the percentage decrease was 31.6 % (Table 1). Fig. 3C and 3D illustrates the impact of Fod-inoculation on the growth and development of 'Solex' cultivar plants, showcasing the effect of vascular wilting caused by the pathogen.

At 63 days post-inoculation (dpi), we conducted a Spearman correlation analysis (α = 0.05) to assess the relationship between various physiological parameters in carnation plants during Fod inoculation. Notably, we observed negative correlations between stomatal resistance and both leaf temperature and dry biomass, as depicted in Fig. 4A. These correlations were essential to elucidate the dynamics of susceptibility in these plants, as discussed below. When performing the principal components analysis for the physiological data at 63 dpi (Fig. 4B), we found that the first two components explained 74.6% of the variance. The first component explained 50 % of the variance and classified the treatments associating stomatal resistance and leaf temperature in the negative part of the axis, because of inoculation with Fod. The biplot showed the discriminatory effect of leaf temperature, associated with the inoculated plants.

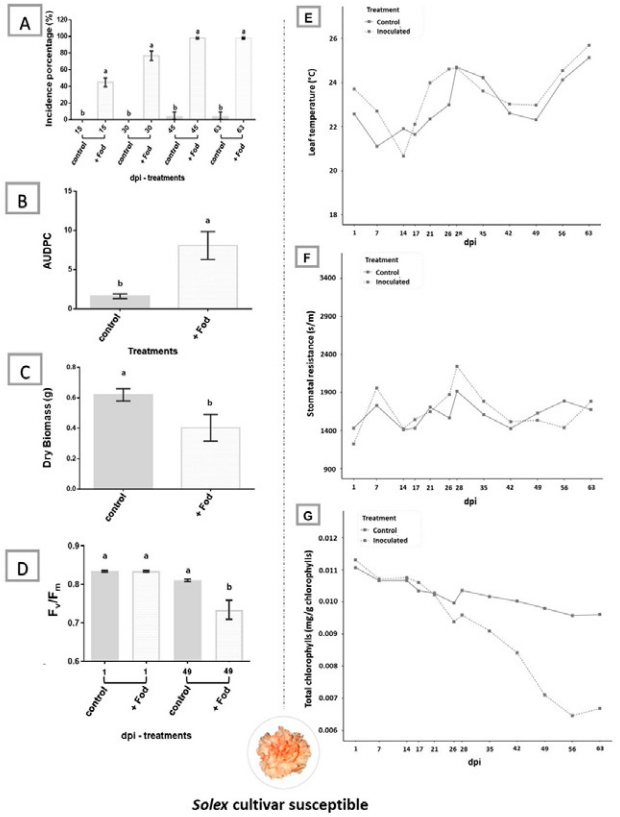

Fig. 2. Carnation plants cultivar 'Solex' susceptible to Fod. (**A**) Percentage incidence disease; (**B**) Area under the disease progression curve (AUDPC) at 63 days; (**C**) Dry biomass at dpi 63; (**D**) Maximum photochemical efficiency of PSII (F_v/F_m) – dpi 49; (**E**) Leaf temperature (°c); (**F**) Stomatal resistance (s m^{-1}); (**G**) Total chlorophyll content (mg g $chlorophyll^{-1}$). For items A, B, C and D, the data was analyzed using the Wilcoxon Mann-Whitney test, *p*-value ≤ 0.05; and the medians between treatments indicated with an asterisk (*) were significantly different, the bars indicating the standard deviation. For AUDPC there were n = 3 replicates per treatment and 10 plants per replicate. For dry biomass there were n = 8 plants per treatment. For F_v/F_m there were n = 5 treatment-dpi plants. Items E, F and G were inoculated with Fod (dashed line) and the control – without Fod (continuous line). The data was analyzed using a non-parametric functional ANOVA - Functional Data Analysis (FDA), L^2b test - L^2-norm-based bootstrap test between curves-treatment, *p*-value tests ≈ 0.00; 0.14 and 0.027, respectively, n=6.

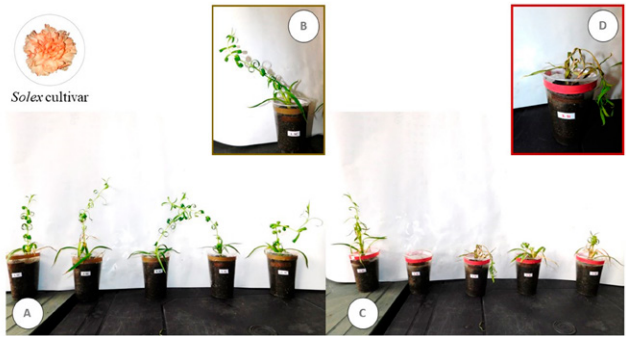

Fig. 3. Photograph of carnation plants cultivar 'Solex' susceptible to Fod at 63 dpi. (**A**) Control plants - without Fod. (**B**) Detail of control plant development. (**C**) Plants inoculated with Fod. (**D**) Detail of plant development with vascular wilting.

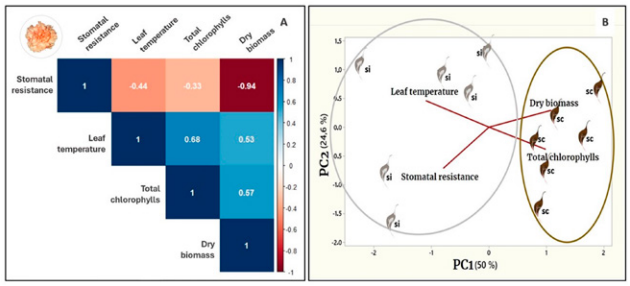

Fig. 4. Carnation plants cultivar 'Solex' at 63 dpi. (**A**) Spearman correlation in plants inoculated with Fod. (**B**) Biplot diagram of principal component analysis, control treatment (sc) circle in brown, treatment inoculated with Fod (si) circle in gray. PC (principal component).

Fod treatment does not increase the accumulation of metabolites in sensitive carnation cultivar 'Solex'

The content of phenolic compounds (PCs) in the roots generally remained constant when comparing the control with the Fod-inoculated treatment with only a decrease in content at 4 dpi caused by the pathogen (Fig. 5A). In the stem, the comparison against the control showed an increase at 0.5 dpi, with no significant difference at 1 and 2 dpi, and a decrease at 4 days after the challenge with the pathogen (Fig. 5B). The flavonoid content (FCs) at the root level showed an increase at 0.5 dpi and a decrease at 4 days after the challenge with the pathogen (Fig. 5C). Meanwhile, we found no significant differences at the stem level between the control and the Fod-inoculated treatment (Fig. 5D).

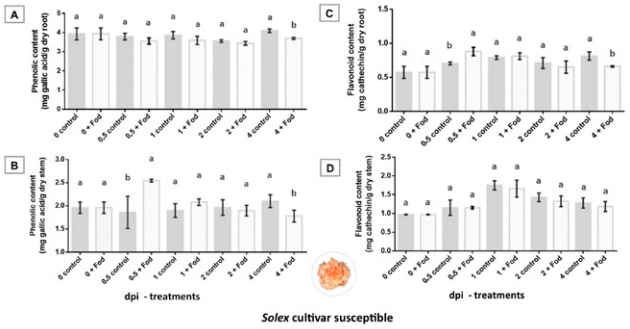

Fig. 5. Phenolic compounds and flavonoids in plants cultivar 'Solex' susceptible to Fod. (**A**) Phenolics content in root; (**B**) Phenolics content in stem; (**C**) Flavonoids content in root; (**D**) Flavonoids content in stem. The average between treatments is indicated with lowercase letters, different letters show differences between treatments at the same sampling time, the bars indicate the standard deviation. The data were analyzed using the one-way ANOVA, *p* - value ≤ 0.05, n = 3 replicates per treatment.

Discussion

In this research we determined the early increase in leaf temperature in 'Solex' cultivar carnation plants subjected to Fod (dpi 1; Fig. 2). This swift induction aligns with the asymptomatic stage of vascular wilting and persistent throughout the infection period. These findings are important because vascular wilt in carnation is not visually evident at the onset of the disease and consistent with observations in other plant-pathogen interaction models involving *Fusarium* species, highlighting the possibility of using leaf temperature variations as diagnostic cues (Calderón et al., 2013; Wang et al., 2015; Francesconi et al., 2021). Some research relating showed that the decrease in stomatal conductance, transpiration, and chlorophyll α fluorescence depended on the severity of the disease (Dong et al., 2012; Calderón et al., 2013). In the case of carnation, over the assessed time frame stomatal resistance did not increase; nevertheless, indications of wilting became apparent after dpi 14 (Fig. 2A). In cucumbers infected by *Fusarium oxysporum* f. sp.

cucumerinum, stomatal closure and the regulation of plant water loss is governed by the cellular impairment disease-induced rather than by stomatal activity *per se* (Wang et al., 2015). In this respect, the Spearman's correlation analysis dpi 63 (Fig. 4A) enabled the identification of these associations, particularly concerning stomatal resistance, revealing an inverse correlation with leaf temperature. This implies that in the 'Solex' carnation cultivars that do not exhibit stomatal regulation upon Fod infection, this phenomenon likely related with leaf cooling, as stomatal aperture facilitates this process at the expense of water regulation under drought stress conditions disease-induced. In plants of cucumber resistants' wilting for *Fusarium*, there is an inverse relationship between stomatal conductance and leaf temperature, like a regulatory mechanism between leaf cooling and stomatal water loss (Wang et al., 2012). The negative correlation observed at symptomatic stages of vascular wilting in carnations may indicate water loss, encompassing both stomatal and cellular damage as the disease progresses, thus delineating a susceptible response of the plant to the infection. Additional research is necessary to confirm this. The initiation of this thermal response aids the plant in detecting the pathogen that is initially localized in the root, thereby triggering a "distant alert" mechanism to elevate leaf temperature, thus priming the plant to confront impending colonization.

We proposed that in 'Solex' cultivar this induction is attributed to hormonal pathways. The involvement of methyl jasmonate (MeJa) in response to Fod is clear with its swift accumulation in root symplasts at dpi 1 and subsequently at dpi 14, without concurrent accumulation of salicylic acid (SA) or its methyl ester (MeSA) (Vanegas-Cano et al., 2022b). Jasmonic acid (JA) and methyl jasmonate (MeJa) typically accumulate in plant tissues following attacks by necrotrophic pathogens, and their signaling pathways usually exhibit antagonism toward those mediated by SA (Wilson et al., 2023). However, considering the pathosystem studied, the interaction of these two hormonal signaling pathways may synergize, offering diverse forms of protection against *Fusarium oxysporum* (Di et al., 2016). In the carnation-Fod pathosystem, this synergy is shown to be associated with resistance (Vanegas-Cano et al., 2022a; 2022b). Although Fod is considered a hemibiotrophic pathogen, and 1 dpi, it may still be in a biotrophic state in the root. We suggest that the accumulation of MeJa, may be involved in the elevation of leaf temperature in carnation, given its potential for inducing a systemic response without activating acquired systemic resistance (Zhu et al., 2014). It is possible that the phenomenon is influenced by the production of other types of long-distance signaling molecules. The data obtained in this study suggest that measuring leaf temperature can serve as a potential physiological marker of pathogen presence from the asymptomatic stage of the carnation-Fod interaction.

Regarding the symptomatic stage of vascular wilting in plants of the highly susceptible cultivar 'Solex', the parameters associated with growth, biomass production, and F_v/F_m were significantly affected by inoculation with Fod. In these plants, phenotypic manifestations of vascular wilting were found towards the end of the experiment with evident signs as follow: chlorosis, yellowing of the plant from the base to the apex, delay in the appearance of new leaves, and delay in growth (Fig. 2C and 3D). All these responses are related to the energy costs of activating defenses with a decrease in metabolism related to plant growth (Kumudini et al., 2018). It cannot be ruled out that this effect is due to the modulation and regulation by growth hormones signaling pathways like auxins, gibberellins, and brassinosteroids with the defense hormones such as jasmonic acid (JA & MeJa) (Vanegas-Cano et al., 2022a; 2022b; Rasool, 2021).

In this research chlorophyll α fluorescence was utilized to assess the maximum photochemical efficiency of PSII towards the conclusion of the experiment. Plants of the 'Solex' cultivar exhibited a detrimental impact of inoculation on PSII status at dpi 49, compared to control plants without Fod (Fig, 2D, Table 1). This physiological parameter has been associated as an indicator for identifying plants experiencing stressful conditions, and in terrestrial plants, typical values for F_v/F_m are approximately 0.83 (Badr and Brüggemann, 2020). Comparable responses have been documented in prior studies involving interactions between other plants and *Fusarium oxysporum* as well as other vascular pathogens that show a reduction in parameters linked to photosynthesis (Villarreal-Navarrete et al., 2017; Chávez-Arias et al., 2019). Additionally, in 'Solex' carnation plants inoculated with Fod, there was a reduction in the content of photosynthetic pigments, particularly chlorophylls, these are crucial for light absorption within the chloroplasts of the leaves (Marín-Ortíz et al., 2019). Similar responses have been documented previously in the carnation-Fod interaction (Meena et al., 2016) and in other plant species, where chlorosis symptoms have been linked to decreased photosynthesis resulting from the disease-progression (Dong et al., 2014).

At the biochemical level, phenolic compounds are integral to plant-pathogen interactions. Increased baseline levels are associated with antimicrobial activity, antioxidant effects, defense signaling, and cell wall reinforcement (Ardila et al., 2013; Pérez-Mora et al., 2021; Romero-Rincón et al., 2021; Santos-Rodríguez et al., 2021). This variability affects resistance or susceptibility to Fod-wilting in nine carnation cultivars (Ardila et al., 2013). These changes in the content of phenolic compounds (PCs) and flavonoids (FCs) represent a primary approach to understanding mechanisms associated with resistant or susceptible defense responses. During the *in vivo* assay at early stages, 'Solex' plants inoculated with Fod exhibited significant variations in PCs and FCs at different levels. Given that the root serves as the initial point of contact between the pathogen and the plant, higher contents are observed in this tissue compared to the stem, consistent with previous findings (Santos-Rodríguez et al., 2021). In resistant carnation cultivars 'Golem', PCs and FCs accumulate in response to Fod-inoculation, particularly at dpi 4 (Romero-Rincón et al., 2021).

In this study, for the susceptible cultivar 'Solex', a decrease in the content of PCs and FCs at the root level and PCs at the stem level was observed at the same time of the analysis that could be associated with susceptibility. At this level, the early biochemical responses described in this study, along with those reported in previous studies on the carnation-Fod interaction for susceptible cultivars (Romero-Rincón et al., 2021; Santos-Rodríguez et al., 2021; Vanegas-Cano et al., 2022a, 2022b), provide evidence to assert that the presence of the pathogen influences the dysregulation of the synthesis pathway of compounds derived from chorismate and shikimate, among which the synthesis of SA becomes relevant (Vanegas-Cano et al., 2022a; 2022b). Although the exact mechanism used by the pathogen to deactivate it is not known, it is possibly a characteristic of carnation susceptibility associated with S-genes. These genes may encode negative regulators of immune signaling to keep defenses at bay, especially in biotrophic organisms where the decrease in SA levels is of paramount importance to activate colonization (Gorshkov and Tsers, 2021). In addition to this, the activation of the jasmonate-derived signaling pathway in 'Solex', as previously described (Vanegas-Cano et al., 2022a, 2022b), may favor the development of the pathogen at these early stages.

Conclusions

This study focused on understanding the susceptibility of carnations to *Fusarium oxysporum* infection leading to vascular wilting. Upon inoculation with Fod, 'Solex' showed an early increase in leaf temperature, indicating a potential diagnostic marker for infection. Stomatal resistance did not escalate significantly over time, but signs of wilting became evident after dpi 14, suggesting an inverse correlation between leaf temperature and stomatal resistance, that the Spearman analysis revealed, indicating a susceptibility response to infection. In this cultivar, significant Fod effects on growth, biomass production, and chlorophyll α fluorescence were observed, indicative of energy costs associated with defense activation. At the biochemical level, phenolic compounds play a crucial role in this plant-pathogen interaction, influencing resistance or susceptibility to wilting Fod. The dysregulation of the synthesis pathway of compounds derived from chorismate and shikimate such as phenols, flavonoids, including the synthesis of salicylic acid (SA), is suggested as a characteristic of susceptibility to carnations associated possibly with S-genes. These findings contribute to understanding carnation susceptibility to Fod infection, and they offer insights for developing prevention and early detection strategies.

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Author Contribution.

EBC: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft and writing – review & editing. **WHPM**: data curation, formal analysis, investigation, methodology, writing – original draft and writing – review & editing. **LMM**: investigation, methodology, visualization, funding acquisition, supervision and validation. **HDA**: investigation, methodology, visualization, project administration, resources, funding acquisition, supervision, and validation. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be available on request.

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