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Research article

Interaction of *Neophysopella tropicalis* and Cabernet Sauvignon at two different temperatures

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ABSTRACT: Viticulture is one of the most produced fruit crops in the world; therefore, challenges of cropping systems are very relevant. Altered environments pose an obstacle; in addition, climate change influences plant-pathogen relationships and their risks. This work described the interaction of *Neophysopella tropicalis* (Ono) × 'Cabernet Sauvignon' grapevine at 25 and 30 °C, from an epidemiological and anatomical perspective. Rust severity showed no differences in plants kept at 25 and 30 °C; however, a significantly higher number of rust pustules was observed at 30 °C. The accumulation of phenolic compounds in healthy leaves at 25 °C was denser and more homogeneous than in the other treatments. The phenol pattern alteration observed in healthy leaves at 30 °C is possibly related to the larger number of lesions observed at this temperature.

Keywords: *Phakopsora euvitis*, *Vitis vinifera*, anatomy, histopathology, severity

Introduction

Grape is one of the most produced fruits worldwide, reaching over 74 million tons in 2022 (FAO, 2023). *Vitis vinifera* (L.) is the most popular species of grapevine among producers and consumers, especially for winemaking (Bowers and Meredith, 1997), and 'Cabernet Sauvignon' is known as one of the best grapes for fine red wine production (Robinson, 1994). Like every crop, grapevine production is influenced by environmental conditions, and climate change can be a challenge for farmers, as reported by the Intergovernmental Panel on Climate Change (IPCC, 2022).

Asian grapevine leaf rust (AGLR) is a fungal disease, caused by *Neophysopella tropicalis*, that attacks especially fully expanded leaves and causes pustules in the abaxial surface (Santos et al., 2021). Besides reducing the photosynthetic leaf area, pathogen infection can damage the photosynthetic apparatus, reducing plant yield and crop carbohydrate storage (Nogueira Júnior et al., 2017).

Both AGLR and high temperatures can induce the formation of gerontoplasts in grapevine leaves, with the replacement of starch by lipids in chloroplasts (Buttrose et al., 1971; Rasera et al., 2019). The photosynthetic activity and crop yield have shown a reduction in grapevines kept for long periods at temperatures above 30 °C (Hale and Buttrose, 1974). Besides, high temperatures may also reduce or inhibit metabolic processes, such as phenolic compound synthesis (Mori et al., 2005, 2007).

Histopathological studies can be a helpful tool to interpret epidemiological results due to the visual analysis of the structure of the plant and chemical tests (Marques et al., 2017). This research aimed to understand the effect of high temperature on the infection by *N. tropicalis* in the 'Cabernet Sauvignon' grapevine from an epidemiological and anatomical perspective. We hypothesize is that a temperature increase from 25 to 30 °C raises 'Cabernet Sauvignon' susceptibility to *N. tropicalis* and consequently causes high leaf damage.

Materials and Methods

Plant material and inoculation

Grapevines (*V. vinifera*) 'Cabernet Sauvignon' grafted on '1103 Paulsen' [*Vitis berlandieri* Planch. × *Vitis rupestris* Scheele] rootstocks were grown in pots (7 L) containing sterilized substrate (clay soil, manure, and sand, 1:1:1) and kept in a greenhouse (22°42′27.295″ S, 47°37′51.380″ W, 542 m), where the air temperature was 25 ± 5 °C. After the bud break, the vines remained with a single stem and each pot received 200 mL of water daily. The plants were fertilized monthly with 20 g of NPK (10:10:10) fertilizer.

All treatments resulting from the combination of temperature (25 and 30 °C) and disease (control and inoculated plants) comprised five vines (experimental units) with five leaves (observational units) each. The experimental design was completely randomized and performed twice (from 01 to 28 Sept 2020 and from 20 Oct to 16 Nov 2020).

The leaves of each vine had more than 20 days after sprouting and were inoculated on the abaxial surface with *Neophysopella tropicalis* AGLR064 (Genebank accession number MK290816.1). Urediniospores from 7-day pustules were removed by brushing from the abaxial leaf surface with 20 mL of sterile distilled water. A suspension adjusted to 10^5 urediniospores mL^{-1} was obtained by using a Neubauer chamber. Inoculations were performed

by spraying the urediniospore suspension over 'Cabernet Sauvignon' leaves with a compressor (Ferrari Mega Air CFA 7.6/24l) until run-off. For the control group, the leaves of each plant were sprayed on the abaxial surface with water. Inoculated and control vines were kept in a dark room for 24 h under 100 % relative humidity at the respective experimental temperature (25 or 30 °C).

The experiment was performed in two growth chambers (Model E-7, Conviron), with a photoperiod of 12 h and photosynthetic active radiation (PAR) of 400 µmol m^{-2} s^{-1}, and temperature of 25 ± 1.5 °C or 30 ± 1.5 °C.

Monocyclic components of grapevine rust

A 2-cm^2 leaf area was delimited and marked to evaluate the monocyclic components. The relative lesion density (relationship between the number of pustules per cm^2 and the highest observed number of pustules per cm^2) and rust severity (proportion of leaf damaged area) were assessed weekly. Leaf surfaces were photographed with a 64 MP smartphone camera until 30 days after inoculation (DAI), while the relative lesion density and rust severity were estimated with image analysis Quant® software.

Model fitting to temporal dynamics of relative lesion density (proportion) and rust severity (proportion) was performed using 'epifitter', the R package for the analysis, while simulation of plant disease progress curves developed by Alves and Del Ponte (2021).

Histopathological analysis

For the light microscopy analyses, samples of 1 cm^2 from the first fully expanded leaves (from the plant base) were collected 14 DAI or water spraying and fixed in Karnovsky solution (Karnovsky, 1965; modified with phosphate buffer pH 7.2). The samples were taken to a vacuum pump to remove the air from the tissues and then dehydrated in an ethanol series (from 10 to 100 %, in ten steps). After dehydration, the samples were embedded in hydroxy-ethyl-methacrylate (Leica Historesin®). The blocks were sectioned at 10 µm in a rotary microtome (Leica RM 2045). Then, the sections were stained with toluidine blue (O'Brien et al., 1964) and analyzed under a Leica DMLD microscope. Images were captured using a Leica DC 300F camera.

Results

Latent periods were 11 and 10 days at 25 and 30 °C, respectively, in both experiments. Rust severity in the last assessment ranged from 0.19 to 0.43 at 25 °C and from 0.24 to 0.57 at 30 °C (Figure 1A-E). The estimated asymptotes (*ymax*) of the grapevine rust severity curves were similar in both treatments (Table 1). The maximum lesion density ranged from 0.10 to 0.16 at 25 °C and from 0.23 to 0.28 at 30 °C at 30 DAI (Table 1, Figure 1F), and the asymptote estimate of the grapevine rust lesion density curves differed between treatments (Table 1).

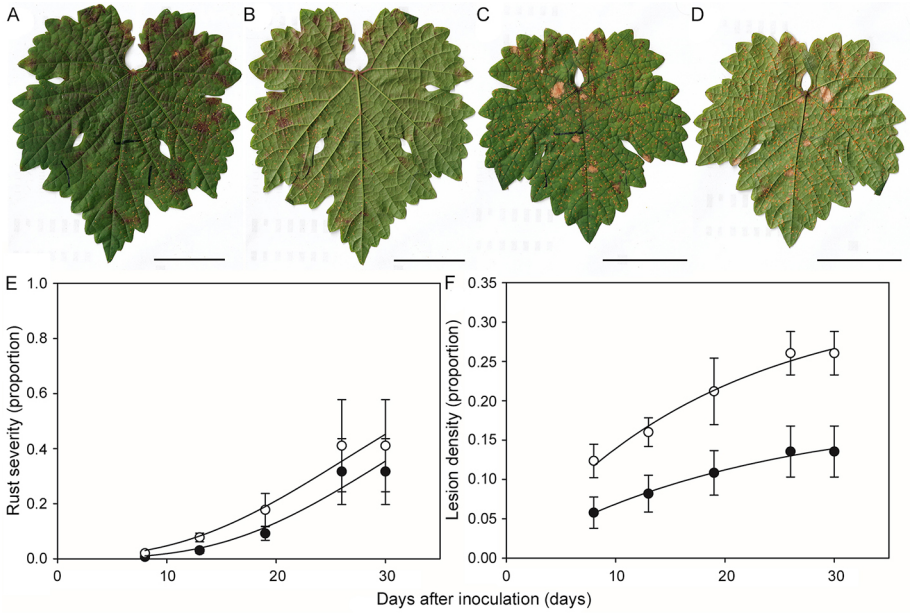

Figure 1 – Images of adaxial (A, C) and abaxial (B, D) leaf surfaces of 'Cabernet Sauvignon' vines kept at 25 (A, B) and 30 °C (C, D) at 30 days after inoculation with *Neophysopella tropicalis* and disease severity (E) and relative lesion density (F) over time at 25 °C (closed circles) and 30 °C (open circles). The images correspond to the maximum rust severity observed in chambers before leaf drop. In E, F, the lines represent the logistic model. All analyses were performed with pooled data from two experiments. Data points represent the mean values (± standard error) at each sampling day over two experiments, with fifteen replications. Scale bar corresponds to 4 cm.

The estimated rate of progress for lesion density and rust severity curves were similar at both temperatures (Table 1, Figure 1E and F).

The histopathological analyses showed no structural differences in the leaf blade of 'Cabernet Sauvignon' kept at 25 and 30 °C (Figure 2A-D). However, the phenolic content of the palisade and spongy parenchyma cells of the healthy material kept at 25 °C was denser and more homogeneous (Figure 2A) in relation to the other treatments (Figure 2B-D). The phenolic compounds of healthy vines kept at 30 °C were present in several small spherical droplets (Figure 2B).

Discussion

'Cabernet Sauvignon' grapevines are highly susceptible to AGLR, based on the number of pustules as a classification factor (Hennessy et al. 2007). Here, our findings agree with the authors observations, but susceptibility to AGLR also increases at higher temperatures.

In our study, increasing temperature in healthy plants (30 °C) led to visual changes in the phenolic compound characteristics, which were present in several small spherical droplets. The continuous temperature of 30 °C at day and night can inhibit or reduce the biosynthesis of phenolic compounds, such as anthocyanin, in the skin of berries of 'Cabernet Sauvignon' (Mori et al., 2005). The reduction of the phenolic content due to the temperature increase can be attributed to several factors, such as a decrease in the transcript levels of the anthocyanin biosynthetic genes, as well as chemical and enzymatic degradation (Mori et al., 2007). Phenol accumulation also occurred in the five soybean cultivars analyzed for resistance against *Phakopsora pachyrhizi* Syd. & P. Syd. (soybean rust pathogen), with higher content in resistant cultivars (Lygin et al., 2009).

The change in phenolic compounds due to the temperature increase probably reduced the host defense and allowed a higher infection at 30 °C, which caused the statistical difference between temperatures for lesion density, with a higher number of pustules at 30 °C.

Table 1 – Estimated parameters (± standard error) and coefficients of determination (R^2) of a logistic model fitted to the proportion of rust severity and lesion density of *Neophysopella tropicalis* in 'Cabernet Sauvignon' grapevine leaves at 25 °C and 30 °C.

Variable	Temperature	ymax	b1	r	R^2
Rust severity	25 °C	1 (± 2) a	0.0004 (± 0.003) a	0.07 (± 0.1) a	0.95
	30 °C	1 (± 1) a	0.0025 (± 0.008) a	0.07 (± 0.06) a	0.97
Lesion density	25 °C	0.18 (± 0.02) a	0 (± 0.003) a	0.046 (± 0.08) a	0.99
	30 °C	0.33 (± 0.03) b	0.0009 (± 0.008) a	0.054 (± 0.09) a	0.99

Estimated parameters obtained with the Gompertz model $y_t = ymax(\exp(-(-\ln(b1))\exp(-rt)))$, where y_t corresponds to the rust severity (proportion), *ymax* to the asymptote, *b1* to the initial inoculum, *r* to the proportional progress rate of the rust severity, and *t* to time (in days after inoculation). Estimated parameters obtained with the monomolecular model $y_t = ymax - (ymax - b1)\exp(-rt)$, where y_t corresponds to the lesion density (proportion), *ymax* to the asymptote, *b1* to the initial inoculum, *r* to the proportional progress rate of the lesion density, and *t* to time (in days after inoculation).

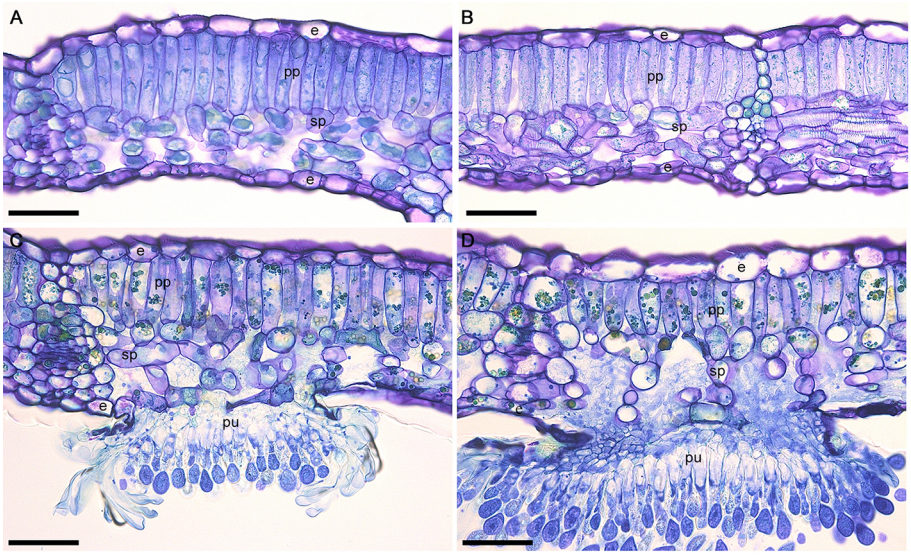

Figure 2 – Photomicrographs of 'Cabernet Sauvignon' grapevine leaves cross-sectioned and stained with toluidine blue. A = healthy leaf kept at 25 °C for 14 days; B = healthy leaf kept at 30 °C for 14 days; C = leaves were kept at 25 °C for 14 days after inoculation with *Neophysopella tropicalis*; D = leaves were kept at 30 °C for 14 days after inoculation with *N. tropicalis*. Phenolic compounds are stained in green. Note the differences in patterns of phenolic compounds accumulation. e = epidermis; sp = spongy parenchyma; pp = palisade parenchyma; pu = pustule. Scale bar corresponds to 50 µM.

In this work, we analyzed the effect of temperature increase on AGLR on *V. vinifera* 'Cabernet Sauvignon' leaves. From an epidemiological perspective, rust severity showed no differences between 25 and 30 °C; however, a statistically larger number of pustules was observed at 30 °C. The histopathological analysis showed changes in the phenolic compounds patterns of accumulation in the parenchyma of healthy leaves submitted to a higher temperature. The phenol alteration is possibly related to the larger number of lesions at 30 °C.

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Authors' Contributions

Conceptualization: Rasera JB, Appezzato-da-Glória B, Amorim L. **Data curation**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Formal analysis**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Investigation**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Methodology**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Software**: Rasera JB. **Validation**: Rasera JB. **Resources**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Visualization**: Rasera JB, Appezzato-da-Glória B, Amorim L. **Project administration**: Appezzato-da-Glória B, Amorim L. **Funding acquisition**: Appezzato-da-Glória B, Amorim L. **Supervision**: Appezzato-da-Glória B, Amorim L. **Writing-original draft**: Rasera JB. **Writing-review & editing**: Rasera JB, Appezzato-da-Glória B, Amorim L.

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