

ORIGINAL ARTICLE OPEN ACCESS

Late Leaf Rust Monocyclic Components and Primary Metabolism of Raspberry Plants Facing Water Deficit

Lucas Henrique Santos Barbosa¹  | Lilian Amorim²  | Rafael Vasconcelos Ribeiro³ | Ulla Neumann⁴ | Ton Timmers⁴ | Tonni Grube Andersen⁵  | Marcel Bellato Spósito⁶  | Beatriz Appezzato-da-Glória¹

¹Department of Biological Science, “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, Brazil | ²Department of Plant Pathology and Nematology, “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, Brazil | ³Department of Plant Biology, University of Campinas, Campinas, Brazil | ⁴Central Microscopy, Max Planck Institute for Plant Breeding Research, Cologne, Germany | ⁵Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany | ⁶Department of Crop Science, “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, Brazil

Correspondence: Lucas Henrique Santos Barbosa (barbosa.lhs@gmail.com)

Received: 25 April 2025 | **Revised:** 14 July 2025 | **Accepted:** 10 August 2025

Funding: We gratefully acknowledge the São Paulo Research Foundation (FAPESP, Brazil) for financial support (Grant 2019/13191-5) and for fellowships awarded to Lucas Henrique Santos Barbosa (Grants 2022/00888-0 and 2024/02834-0). Lucas Henrique Santos Barbosa also thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 for the fellowship granted. Lilian Amorim, Rafael Vasconcelos Ribeiro, Marcel Bellato Spósito and Beatriz Appezzato-da-Glória acknowledge the National Council for Scientific and Technological Development (CNPq, Brazil) for fellowships granted (Grants 301689/2022-9, 304295/2022-1, 303897/2021-0 and 302361/2022-7, respectively).

Keywords: *Aculeastrum americanum* | climate change | epidemiology | leaf gas exchange | *Rubus idaeus*

ABSTRACT

Drought reduces host resistance and allows the emergence of pathogens that benefit from changes in plant physiology under water stress. However, for pathogens that penetrate only via the stomata, like *Aculeastrum americanum*, stomatal closure due to water stress can prevent infection. Here, we test the hypothesis that raspberry (*Rubus idaeus*) late leaf rust severity is intensified when water deficit is imposed after penetration of *A. americanum* in leaves. We perform epidemiological and quantitative analyses of *A. americanum* colonisation, as well as measurements of leaf gas exchange, on healthy and inoculated leaves of *R. idaeus* ‘Heritage’. Plants were either grown under well-watered conditions throughout the experimental period (control) or submitted to water withholding 2 days after inoculation (water deficit). Our results revealed that the incubation and latent periods, urediniospore production and lesion density did not differ between the two water regimes. However, water deficit significantly reduced rust severity, *A. americanum* colonisation and leaf gas exchange in both healthy and inoculated plants. Comparing the biotic and abiotic stresses, late leaf rust has a greater impact on leaf CO₂ assimilation than water deficit. In addition, there was a synergism between leaf rust and water deficit, causing further decreases in leaf gas exchange of raspberry plants. Based on these findings, we conclude that the occurrence of water deficit after the onset of pathogen infection reduces rust severity by limiting its colonisation capacity, a consequence of low photoassimilate supply to the pathogen due to impaired photosynthetic activity.

1 | Introduction

Climate change, characterised by shifts in rainfall patterns and rising global temperatures, has significant implications for agriculture (Ortiz-Bobea et al. 2021). It is associated with crop yield losses exceeding 50% in various agricultural regions

(Zhao et al. 2017) and reductions in crop quality (Hýsek et al. 2017). In addition to these impacts, climate change is expected to affect pathogen virulence and disease development (Nnadi and Carter 2021; Velásquez et al. 2018). Thus, as environmental conditions change, plant diseases may behave differently, increasing the risk of epidemic outbreaks

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Plant Pathology* published by John Wiley & Sons Ltd on behalf of British Society for Plant Pathology.

driven either by changes in plant resistance or in the evolution of pathogens (Choudhary and Senthil-Kumar 2024; Singh et al. 2023).

As a specific climatic phenomenon, drought can either aggravate or attenuate the occurrence and severity of plant diseases (Choudhary and Senthil-Kumar 2024; Lahlali et al. 2024), which can lead to substantial consequences for agriculture (Lahlali et al. 2024). Low water availability allows the emergence of pathogens because of changes in the plant physiology under water stress (Singh et al. 2023). Physiological changes include stomatal closure, restricted CO₂ diffusion in the mesophyll and reduced photosynthetic rate (Abid et al. 2018; Anjum et al. 2011, 2016; Khelvandi et al. 2021; Li et al. 2020; Percival et al. 1998; Ribeiro and Spósito 2022). Reduced stomatal conductance limits water loss but could also restrict pathogen entry (Lim et al. 2015; Ribeiro and Spósito 2022). Lower CO₂ diffusion and photochemical activity reduce carbon assimilation, which may constrain the synthesis of metabolites essential for plant defence (Werner et al. 2020). Furthermore, drought-induced hormonal shifts and defence signalling reconfiguration can suppress plant resistance (Choudhary and Senthil-Kumar 2022). Normally, the association between abiotic and biotic stresses elicits a distinct plant response compared to that induced by a single stress factor (Rasmussen et al. 2013).

Decreased host resistance caused by drought was observed in the pathosystems *Oryza sativa*–*Magnaporthe oryzae* (Bidzinski et al. 2016) and *Picea abies*–*Endoconidiophora polonica* (Krokene et al. 2023). For diseases caused by pathogens that penetrate only via the stomata, such as many rusts (Grimmer et al. 2012) caused by obligate biotrophic fungi (Duplessis et al. 2021), a direct connection to stomatal closure has been demonstrated in the pathosystems *Apocynum venetum*–*Melampsora apocyni* (Gao et al. 2018) and *Rubus idaeus* (raspberry)–*Aculeastrum americanum* (Ribeiro and Spósito 2022). In these studies, the pathogens were inoculated when the hosts were already under water deficit, which made it difficult for the pathogens to penetrate through the stomata, leading to a reduction in rust severity. If the water deficit had occurred after the pathogen had already penetrated, the combined stresses have the potential to cause severe damage, as reported for brown rust (*Puccinia recondita*) and stripe rust (*Puccinia striiformis*) in *Triticum aestivum* (Choudhary and Senthil-Kumar 2024).

Based on these observations, our hypothesis is that if the onset of water deficit occurs after *A. americanum* infection in raspberry leaves, the disease will be intensified. *A. americanum* is native to North America (Luffman and Buszard 1989), widespread across several countries and was recently reported in New Zealand (Hofer et al. 2025) and Slovenia (Schroers et al. 2025). In Brazil, late leaf rust is considered the most important raspberry disease (Figueiredo et al. 2003). Climate change is expected to increase the frequency of droughts (Lahlali et al. 2024), which may impact plant–pathogen relationships at different stages, requiring a thorough understanding of its effects. Here, we evaluated the late rust monocyclic components and the primary metabolism of raspberry plants under water deficit initiated after pathogen infection.

2 | Materials and Methods

2.1 | Plant Material

Raspberry seedlings of *R. idaeus* ‘Heritage’ were transplanted to 7 L pots, containing 6 L of sterilised substrate (clay soil and sand, 1:2) with granulometry of 10% clay and 70% sand, and kept in a greenhouse where air temperature was 25°C ± 5°C and air relative humidity ranged from 60% to 90%. The pots were watered daily with approximately 300 mL of water and fertilised weekly with 50 mL of liquid fertiliser containing NPK (8:3:8) and micro-nutrients (Forth Jardim).

2.2 | Inoculum and Inoculation of *A. americanum*

The *A. americanum* monopustular isolate used in the experiments was genetically characterised (Ribeiro and Spósito 2022) and the inoculum was maintained in Heritage raspberry plants under greenhouse conditions described in Section 2.1. Ten days after inoculating raspberry leaves for inoculum maintenance, the urediniospores were removed from the abaxial surface of the leaves by brushing with 50 mL of sterile distilled water (SDW). Inoculations were performed by spraying a suspension of 5 × 10⁴ urediniospores/mL with 0.05% Tween 20 on both leaf surfaces with a spray nozzle (NS 19/26; Lenz) coupled to a portable electric atomising sprayer at 1 bar (MA 2057; Marconi) until dew point. Control plants were sprayed only with SDW containing 0.05% Tween 20. After inoculation, all the plants were kept in a dark room for 24 h under > 90% relative humidity and 23°C ± 2°C. After this period, the plants were moved back to the greenhouse. The viability of inoculum was measured in vitro by assessing the percentage of urediniospore germination. Three Petri dishes, each containing three droplets of the urediniospore suspension, were kept under the same conditions as the plants after inoculation. After 24 h, percentage germination was assessed by counting 100 urediniospores per droplet; urediniospores with germ tubes equal to or longer than their diameter were considered germinated. The mean germination percentage of the three droplets per dish was used as the replicate value.

2.3 | Combination of Biotic and Abiotic Stress

Water conditions for the raspberry plants were managed based on substrate water holding capacity (available water capacity, AWC), as done by Ribeiro and Spósito (2022). AWC was calculated as the difference between the pot mass when the substrate is water-saturated and when it is fully dry (Pereira et al. 2010). For treatments without water limitation, 60% AWC was used, while for the treatment with water limitation, 30% AWC was applied.

The experiments consisted of four treatments, combining water regimes (60% and 30% AWC) and disease conditions (healthy and inoculated). Here, healthy plants maintained at 60% AWC served as the control treatment. Each treatment included five raspberry plants. The experimental design was randomised, and the experiment was performed twice (referred to as Experiment 1 and 2 throughout). All plants were maintained

at 60% AWC for 1 week. After this period, half of the plants (10 plants in total; five per water regime) were inoculated with *A. americanum* suspension as described in Section 2.2. For water deficit treatments, 48 h after inoculation, inoculated and healthy plants were kept without watering until the pots reached 30% AWC, which took about 5–6 days. Irrigation was carried out to maintain plants under 60% and 30% AWC by weighing the pots daily throughout the 22 and 23 days of Experiments 1 and 2, respectively.

2.4 | Monocyclic Components of Raspberry Late Rust

The monocyclic components were evaluated on raspberry plants inoculated with *A. americanum*: (i) incubation period—the time interval from inoculation to the appearance of 50% of the total number of lesions; (ii) latent period—the time interval from inoculation to the appearance of 50% of the total number of sporulating lesions; (iii) lesion density—number of lesions per cm²; (iv) disease severity—the proportion of diseased tissue on the leaf blade; and (v) urediniospore production per lesion.

For the lesion density, three 1 cm² squares were randomly marked on the central leaflet of two leaves from the middle third of the stem per plant and the lesions within these areas were counted using a hand-held magnifying glass. Disease severity was estimated on all inoculated leaves of each plant using the diagrammatic scale developed for late rust in raspberry plants (Dias, Ribeiro, et al. 2023). Both components were assessed 3 days per week, with the first assessment conducted on the day symptoms appeared, and subsequent evaluations typically performed on alternate days. The monomolecular model (1) was fitted to the lesion density (number of lesions per cm²/100) and disease severity (proportion of diseased tissue) curves over time (Bergamin Filho 2018):

$$y = y_{\max} - (y_{\max} - b_1) \exp(-rt) \quad (1)$$

where y represents the rust intensity, $y_{\max}$ represents the asymptote, b_1 is a parameter related to the initial amount of disease, r represents the rate of disease increase (per day) and t is the time (days after inoculation). The model was fitted to the data using Statistica v. 7 software (StatSoft Inc.). Regression parameters were compared by Student's t test ($p < 0.05$).

At the end of the experiments and to analyse urediniospore production, three 1 cm² samples from each inoculated plant were collected and transferred to a Falcon tube (15 mL) containing 2 mL distilled water and 0.2 mL lactoglycerol. The suspension containing the samples was homogenised in a tube shaker (AP-59; Phoenix) for 1 min to remove the urediniospores, after which the discs were removed from the suspension. The spore concentration was determined using a Neubauer chamber, and urediniospore production was calculated as the ratio between the concentration of urediniospores in the suspension and the number of lesions on the samples. Results were compared by Student's t test ($p \leq 0.05$).

2.5 | Effect of Water Stress on the Colonisation of *A. americanum* in Raspberry Plants

To investigate the effect of water deficit on pathogen colonisation, an additional experiment was carried out with three plants per inoculated treatment from both water regimes (60% and 30% AWC). At 7 and 14 days after inoculation (DAI), five leaf samples (0.8 cm²) were collected per plant and fixed in 0.15% (wt/vol) trichloroacetic acid in ethanol/chloroform 4:1 (vol/vol). The fixation/destaining solution was renewed regularly over a period of 1 week until the samples were completely cleared. Then, the fixation/destaining solution was replaced with a sterile 25% glycerol solution in distilled water. The leaf tissue fragments were transferred to phosphate-buffered saline (PBS) pH 7.4. Samples were treated in 1 M KOH solution at 37°C for 1 h, followed by washing in PBS pH 7.4 containing 0.1% Triton X-100. Afterwards, the samples were vacuum-infiltrated in a staining solution (20 µg/mL WGA-Alexa Fluor 488, 50 µg/mL propidium iodide, 20 µg/mL bovine serum albumen [BSA] and 0.1% Triton X-100 in PBS pH 7.4) and incubated overnight at 4°C. The stained samples were washed in fresh PBS pH 7.4 containing 0.1% Triton X-100 and finally placed in PBS pH 7.4 containing 25% glycerol. Confocal laser scanning microscopy was performed using an LSM 980 system (Zeiss). Image acquisition settings for the two fluorophores were as follows: excitation 488 nm, emission 499–542 nm for Alexa Fluor, excitation 561 nm, emission 605–649 nm for propidium iodide.

The following two characteristics were analysed: total area of colonised mesophyll tissues (%) and lesion area per colonisation (mm²). For each variable, five samples were analysed per plant of each inoculated treatment ($n = 15$). Measurements were performed using the Fiji software (Schindelin et al. 2012). The results of the colonisation analyses were statistically evaluated using a generalised linear model (GLM) and treatments were compared by Tukey's test using the SAS software (Statistical Analysis System, v. 9.4, SAS Institute).

2.6 | Leaf Gas Exchange and Photochemical Activity

Leaf CO₂ assimilation (A), stomatal conductance (g_s), transpiration (E), intercellular CO₂ concentration (C_i), instantaneous carboxylation efficiency (CE, A/C_i) and effective quantum efficiency of photosystem II (Φ_{PSII}) were measured on the central leaflet of a leaf from the middle third of the stem per plant of each treatment. For these analyses, the evaluations were taken with a portable infrared gas analyser (model Li-6400XT; LI-COR) equipped with a multiphase flash fluorometer (LI-6800-01A; LI-COR). The first measurement was performed 48 h after inoculation and at 7, 14 and 21 days after inoculation. Measurements were performed between 08:30 and 11:00 under air CO₂ concentration of 400 µmol/mol, photosynthetic active radiation of 1000 µmol m⁻² s⁻¹ and natural fluctuation of air temperature and relative humidity. The results were subjected to analysis of variance (ANOVA) and treatments were compared by Tukey's test ($p \leq 0.05$), using the R program (R Core Team 2021).

2.7 | Estimates of Virtual Lesions on Raspberry Leaves

Disease severity was quantified in the same areas where leaf gas exchange was measured through digital images processed in ImageJ software (Schneider et al. 2012). Rust severity was correlated with the relative leaf CO₂ assimilation of raspberry leaves using the model Bastiaans (1991):

$$P_x / P_o = (1 - x)^\beta \quad (2)$$

where P_x refers to the leaf CO₂ assimilation (A) of the leaf with rust severity x , P_o is the average A of healthy leaves, and β is a parameter of the equation. Estimated β values > 1 indicate that the pathogen reduces A on asymptomatic areas of diseased leaves. The higher the β values, the higher the effect of disease severity on the green leaf area adjacent to the lesion; that is, the higher the size of the virtual lesion (Nogueira Júnior et al. 2017). The model was fitted to the data with nonlinear regression using Statistica v. 7 software (StatSoft Inc.). Regressions obtained from each AWC were compared by Student's t test ($p \leq 0.05$).

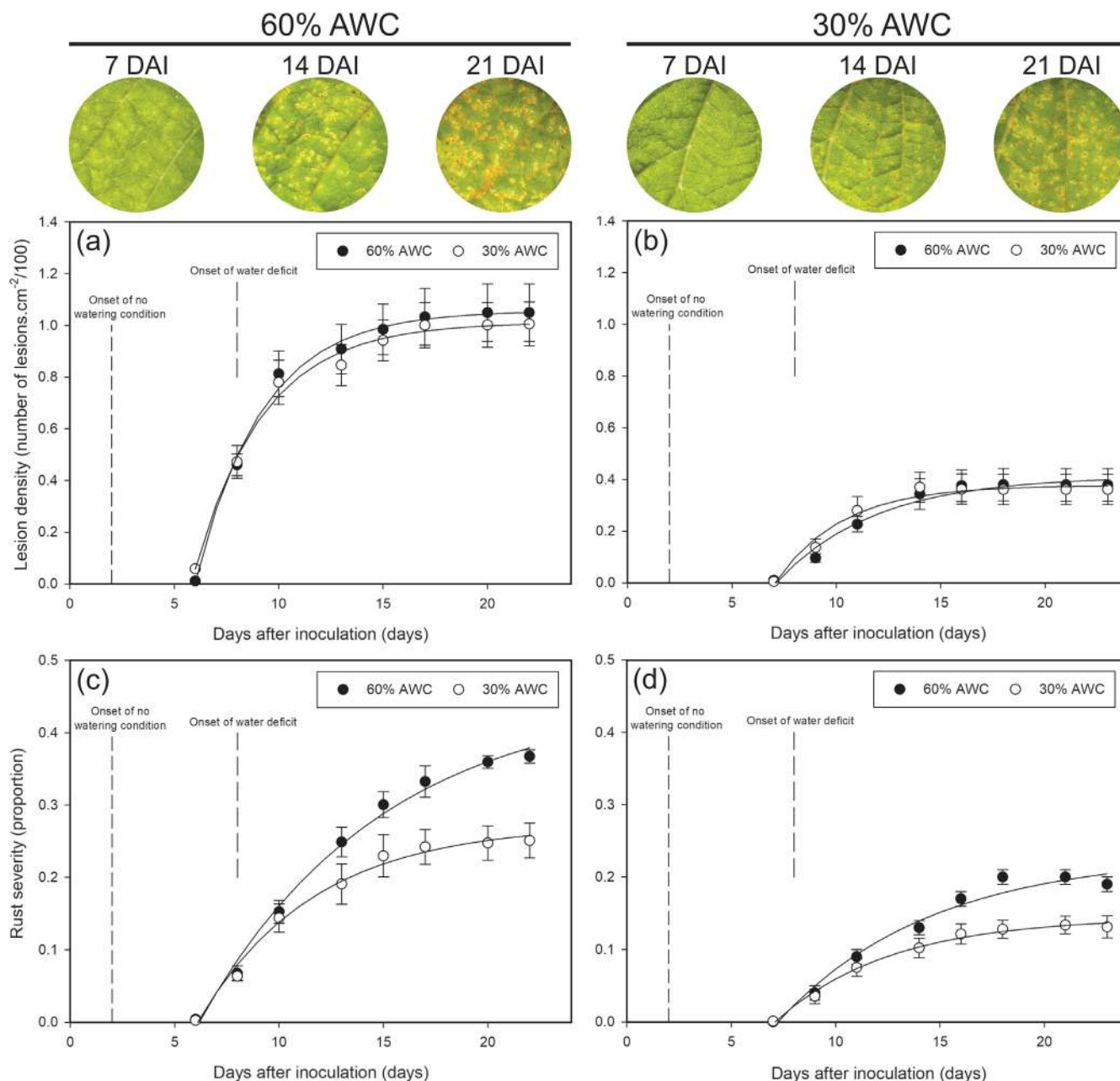


FIGURE 1 | Progress curves of raspberry late rust (*Aculeastrum americanum*) kept under well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) conditions. Lesion density (number of lesions per cm²/100) in Experiment 1 (a) and 2 (b). Rust severity (proportion) in Experiment 1 (c) and 2 (d). Vertical lines represent the mean standard error ($n = 5$). Fitting lines represent the monomolecular model $y = y_{\max} - (y_{\max} - b_1) \exp(-rt)$, where y represents the lesion density or rust severity, $y_{\max}$ represents the asymptote, b_1 is a parameter related to the initial amount of disease, r represents the rate of disease increase (per day) and t is the time (days after inoculation). Leaf discs (2 cm²) above the figure illustrate disease progress on the abaxial leaf surface at 60% (above a) and 30% (above b) AWC from the first experiment at 7, 14 and 21 days after inoculation (DAI; from left to right).

TABLE 1 | Estimated parameters ($\pm$ SE, $n=5$) and coefficients of determination (R^2) of a monomolecular model fitted to the proportion of lesion density and rust severity of *Aculeastrum americanum* in raspberry leaves under two water regimes: well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) conditions.

Experiment	Variable	AWC (%)	$y_{\max}$	b_1	r	R^2
1	Lesion density	60	1.06 $\pm$ 0.02 a	6.56 $\pm$ 1.17 a	0.31 $\pm$ 0.03 a	0.99
	Lesion density	30	1.01 $\pm$ 0.02 a	5.87 $\pm$ 1.20 a	0.30 $\pm$ 0.03 a	0.99
	Rust severity	60	0.45 $\pm$ 0.04 a	2.03 $\pm$ 0.27 a	0.11 $\pm$ 0.02 b	0.99
	Rust severity	30	0.27 $\pm$ 0.01 b	3.01 $\pm$ 0.53 a	0.18 $\pm$ 0.02 a	0.99
2	Lesion density	60	0.41 $\pm$ 0.03 a	4.56 $\pm$ 1.63 a	0.21 $\pm$ 0.05 a	0.97
	Lesion density	30	0.38 $\pm$ 0.02 a	9.30 $\pm$ 4.00 a	0.32 $\pm$ 0.06 a	0.98
	Rust severity	60	0.23 $\pm$ 0.02 a	2.69 $\pm$ 0.74 a	0.14 $\pm$ 0.03 a	0.98
	Rust severity	30	0.14 $\pm$ 0.01 b	3.65 $\pm$ 0.65 a	0.18 $\pm$ 0.02 a	0.99

Note: Different letters in columns represent significant differences between plant-available water capacities (AWC) by Student's t test ($p < 0.05$), for a given variable. Estimated parameters obtained with the monomolecular model $y = y_{\max} - (y_{\max} - b_1) \exp(-rt)$, where y represents the lesion density or rust severity, $y_{\max}$ represents the asymptote, b_1 is a parameter related to the initial amount of disease, r represents the rate of disease increase (day^{-1}) and t is the time (days after inoculation).

3 | Results

3.1 | Monocyclic Components of Raspberry Late Rust as Affected by Water Deficit

The viability of *A. americanum* inoculum varied between 60% and 49% in vitro in Experiments 1 and 2, respectively. The incubation and latent periods coincided, as lesion appearance and sporulation occurred simultaneously, exclusively on the abaxial leaf surface. The latent period was 8 days in Experiment 1, regardless of the water regime. In Experiment 2, it was 10 days at 60% AWC and 9 days at 30% AWC.

The lesion densities of *A. americanum* (minimum, mean and maximum, respectively) were 70.8, 104.9 and 129.3 lesions per cm^2 at 60% AWC and 70.0, 100.5 and 116.8 lesions per cm^2 at 30% AWC by 22 days after inoculation (DAI) in Experiment 1. In the second experiment, the lesion densities were 25.3, 37.8 and 57.8 lesions per cm^2 at 60% AWC and 18.7, 36.1 and 51.5 lesions per cm^2 at 30% AWC (Figure 1a,b). In both conditions, the monomolecular model showed a good fit to the lesion density curves of *A. americanum*, and no differences were observed in fitting parameters (Figure 1a,b; Table 1).

Rust severity differed between water regimes at the end of both experiments (Figure 1c,d). In the first experiment, the minimum, mean and maximum severity values were 35%, 37% and 40% in plants under 60% AWC, respectively. In plants under 30% AWC, the rust severity was lower, varying between 17% (minimum) and 30% (maximum). In the second experiment, the rust severity varied between 17% and 21% at 60% AWC and between 8% and 17% at 30% AWC (Figure 1d). In both water conditions, the monomolecular model showed a good fit to the progress of disease severity, and the estimated values for the asymptote ($y_{\max}$) were approximately 65% higher in well-watered plants than in water-stressed plants (Figure 1c,d; Table 1). The production of *A. americanum* urediniospores per lesion in raspberry leaves did not differ significantly between water regimes at the end of the experiments (Table 2).

TABLE 2 | Urediniospore production ($\pm$ SE, $n=5$) per lesion of *Aculeastrum americanum* in raspberry leaves under water regimes: well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) conditions.

AWC (%)	Experiment	
	1	2
60	3160 $\pm$ 280 a	2670 $\pm$ 610 a
30	2640 $\pm$ 200 a	1680 $\pm$ 360 a

Note: Same letters in columns represent not significant differences between plant-available water capacities (AWC) by Student's t test ($p < 0.05$).

3.2 | Effect of Water Stress on the Colonisation of *A. americanum* in Raspberry Plants

The colonisation of *A. americanum* on raspberry leaves differed between water regimes at 7 and 14 DAI (Figure 2). The total area of colonised mesophyll tissues by *A. americanum* was significantly higher in plants at 60% AWC compared to plants at 30% AWC. However, no significant differences were observed in the total area of colonisation between 7 and 14 DAI within each AWC condition (Figure 2e). The area per colonisation point was also higher in well-watered plants, around two times larger than the one found in plants under water deficit. The area per colonisation point also increased over the experimental period, with higher values noticed at 14 DAI in both watering conditions (Figure 2f).

3.3 | Leaf Gas Exchange and Photochemical Activity as Affected by Late Rust and Water Deficit

On average, the highest leaf CO_2 assimilation rate (A) was found in well-watered and healthy plants throughout Experiment 1 (Figure 3). Under water deficit, A values decreased over time in healthy plants, while a drastic decline was observed in infected plants starting from 7 DAI (Figure 3a).

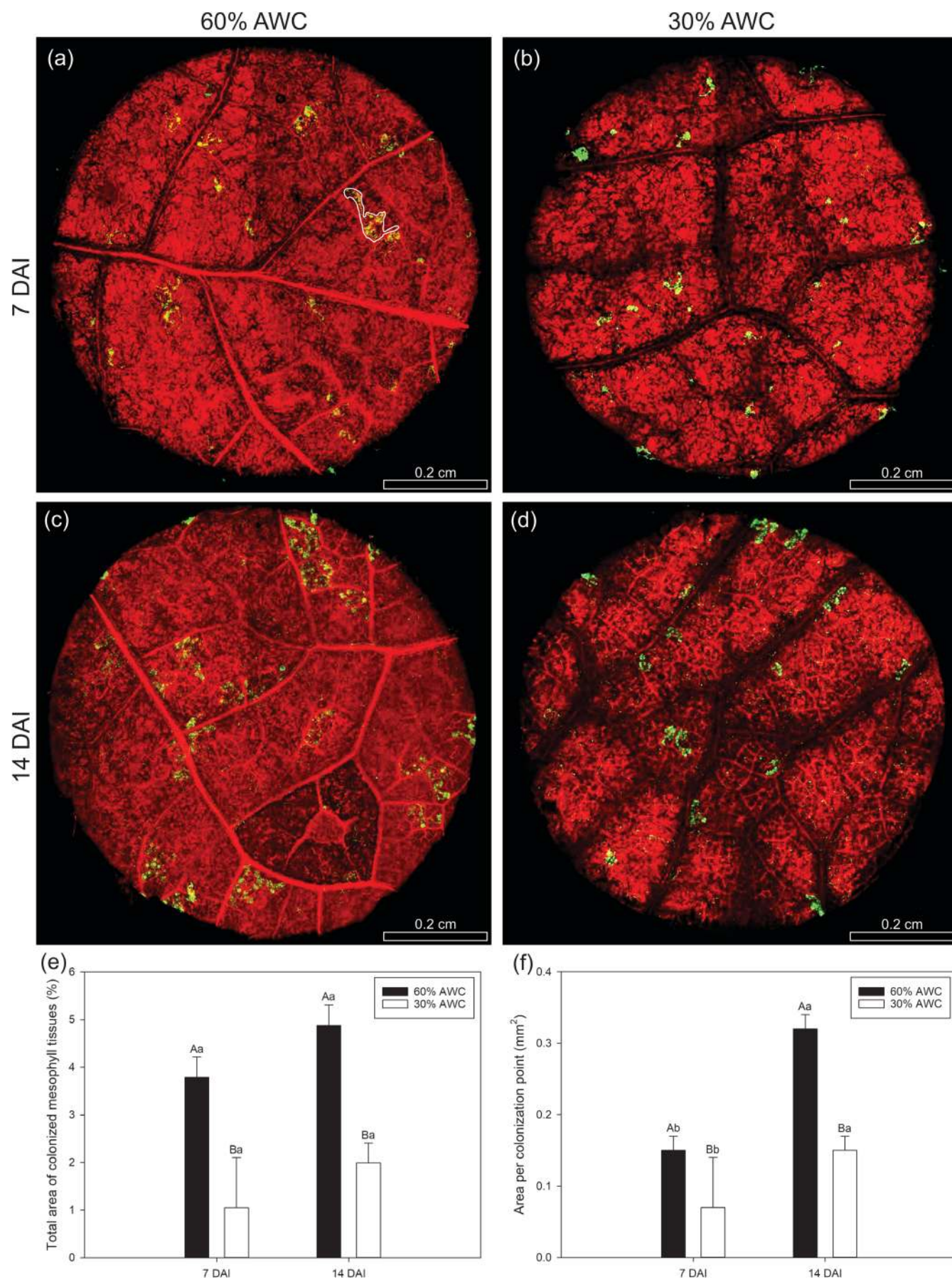


FIGURE 2 | Legend on next page.

FIGURE 2 | Progress of *Aculeastrum americanum* colonisation in mesophyll tissue of raspberry leaves from well-watered (60% available water capacity [AWC], a, c) and water deficit (30% AWC, b, d) plants. Area of colonised mesophyll tissue at 7 (a, b) and 14 (c, d) days after inoculation (DAI). Area of a lesion is delimited (outlined) in (a), and a neighbouring colonisation point separated by a vein can also be observed. False colours in channel overlay images (a–d) represent WGA Alexa Fluor 488 signal (green) and propidium iodide signal (red). Quantification of colonisation, showing total area of colonised mesophyll tissues (e) and area per colonisation point (f). Significant differences (ANOVA followed by Tukey's post hoc test, $p < 0.05$) are indicated by letters in (e, f). Uppercase letters compare the parameters between AWC in the same DAI and lowercase letters compare the parameters between DAI of each AWC.

The well-watered and healthy plants had A values 32% higher than those of healthy plants under water deficit. Infection with *A. americanum* significantly reduced A values in similar ways under both water conditions (Figure 3a). Although the results were similar between Experiments 1 and 2, a significant impact of infection and water condition on A was noticed only from 14 DAI in Experiment 2. Again, the highest A values were noticed in well-watered and healthy plants, while the lowest ones were measured in infected plants, regardless of the water condition (Figure 3b).

Regarding the stomatal conductance (g_s), the pattern of response was similar in plants under water deficit and infection, with the highest g_s found in healthy and well-watered plants (Figure 3c,d). In Experiment 2, differences among treatments became more apparent towards the end of the experimental period. In general, the transpiration (E) followed the same trend as g_s and again, there was a clear difference between the two groups of healthy and well-watered plants versus water-stressed and infected plants (Figure 3e,f).

Infection with *A. americanum* and water conditions affected the effective quantum efficiency of photosystem II (ϕ_{PSII}) only in Experiment 1 (Figure 4a,b), when late rust severity was higher than 30% (Figure 1; Table 1). The highest ϕ_{PSII} was measured in healthy and well-watered plants throughout the experimental period (Figure 4a). After 21 DAI, infected plants showed the lowest ϕ_{PSII} , while healthy and water-stressed plants presented intermediate values (Figure 4a). The instantaneous carboxylation efficiency (CE) was significantly reduced by infection throughout the experimental periods (Figure 4c,d). At the end of Experiment 1, infected plants showed lower CE values than healthy plants in both water regimes and water deficit did not impact CE in healthy plants (Figure 4c). Such responses were also found in Experiment 2, but with lower intensity as compared to the first experiment (Figure 4c,d).

3.4 | Estimates of Virtual Lesions on Raspberry Leaves

The β values estimated by the Bastiaans model did not change due to water regime and varied between 5.14 and 5.64 (Figure 5). According to the model and regardless of the water regime, leaves with rust severities varying between 5% and 50% showed reductions in A of 23%–25% and 97%–98%, respectively (Figure 5). P_x/P_o values close to zero were observed with rust severities between 61% (well-watered plants) and 64% (water deficit plants).

4 | Discussion

It has been demonstrated that water deficit in plants hinders infection by rusts that only penetrate through the stomata (Gao et al. 2018; Ribeiro and Spósito 2022), as plants close their stomata to reduce water loss. This study focused on understanding the effect of water deficit initiated after *A. americanum* infection on the late rust monocyclic components and primary metabolism of raspberry plants. As differences in infection efficiency, driven by factors such as the number of spores deposited on the leaf surface, host status and abiotic conditions, can lead to different lesion densities (Pariaud et al. 2009), our study revealed that the incubation period, latent period, lesion density, and urediniospore production per pustule did not differ between the water conditions. Thus, our data suggest that penetration of the pathogen into the leaves was similar in well-watered and water-stressed plants, with water deficit initiated 48 h after inoculation.

In fact, water deficit resulted in reduced disease severity, which is in accordance with Ribeiro and Spósito (2022). In both scenarios when water deficit occurred either before (Ribeiro and Spósito 2022) or after inoculation (our study), there was low late rust severity in raspberry plants, suggesting that climate change would directly influence pathogen aggressiveness and disease progression (Afzal et al. 2022; Gautam et al. 2013; McElrone et al. 2005; Singh et al. 2023). The monomolecular model fitted to the progress of disease severity revealed that the asymptote (y_{max}) was approximately 65% higher in well-watered than in water-stressed plants. Such lower y_{max} under water deficit is related to the impaired colonisation of *A. americanum*. Accordingly, the area of colonised mesophyll tissues by *A. americanum* and area per colonisation point were significantly lower in plants under water deficit as compared to well-watered ones. This difference is probably due to *A. americanum* being a biotrophic fungus, which needs the plant photosynthetic performance for success in colonisation (Singh et al. 2023).

Water deficit is known to reduce leaf gas exchange (Percival et al. 1998; Ribeiro and Spósito 2022), photochemical activity (Yan et al. 2023) and immunity mechanisms (Choudhary and Senthil-Kumar 2022), changing the susceptibility or resistance to pathogen attack (Choudhary and Senthil-Kumar 2024; Pandey et al. 2024). Notably, significant changes in leaf gas exchange and photochemistry were observed throughout the two experiments. Specifically, decreases in A , g_s and E in healthy plants under water deficit corroborate previous studies in *R. idaeus* (Morales et al. 2013; Percival et al. 1998; Ribeiro and Spósito 2022). As the lowest A values were found in infected plants grown either in well-watered or water-stressed conditions, one may argue that late rust infection in raspberries has a greater impact on A than

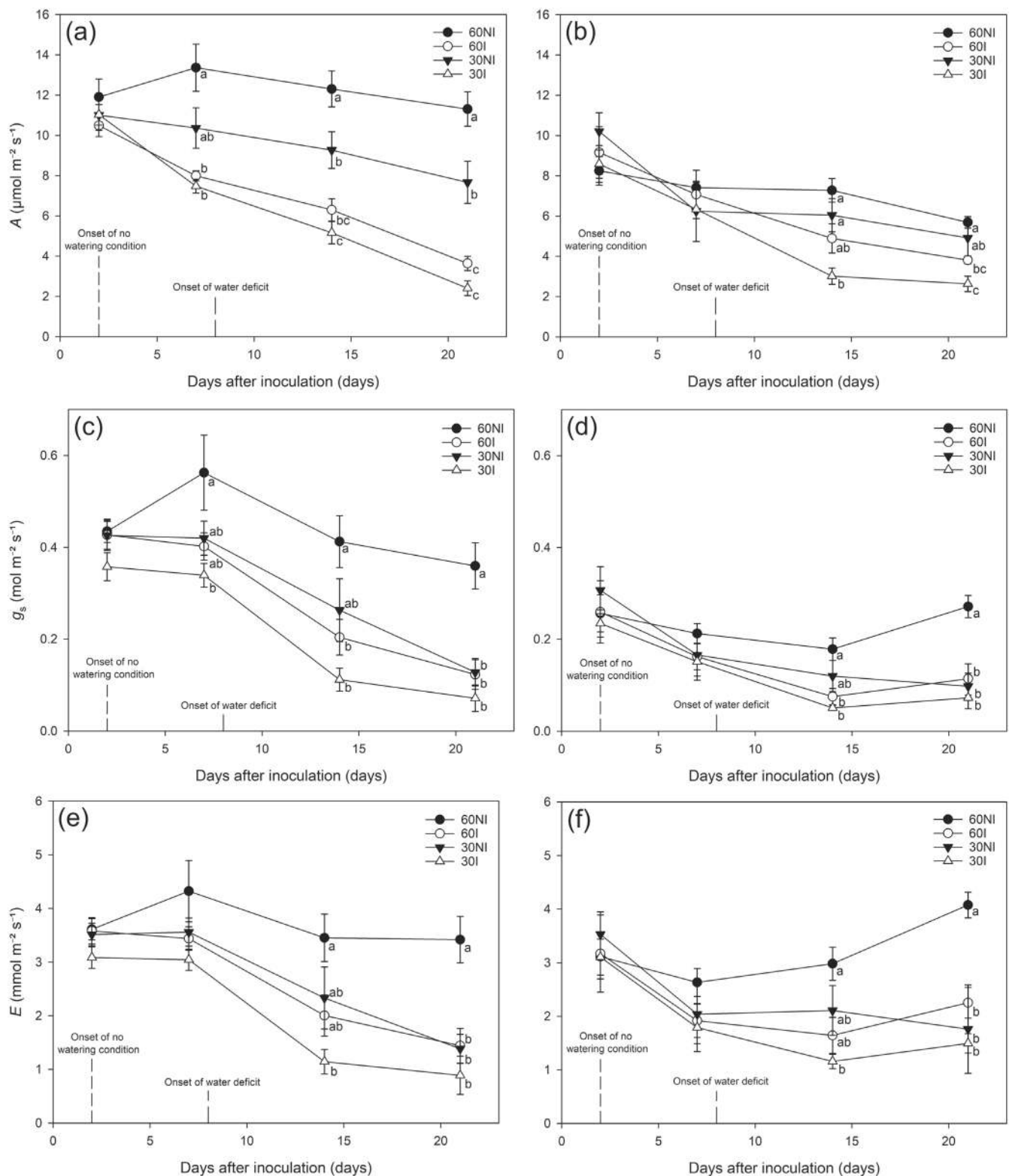


FIGURE 3 | Leaf CO₂ assimilation (A; a, b), stomatal conductance (g_s; c, d) and transpiration (E; e, f) in Experiments 1 (a, c, e) and 2 (b, d, f) of leaves of well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) plants, non-inoculated (NI) and inoculated (I) with *Aculeastrum americanum*. Data points represent the mean values at each time and bars represent the mean standard error ($n=5$). Different letters denote significant differences at 5% probability by the Tukey test between treatments.

water deficit. As there was no significant change in C_i , which is regulated by g_s , such drastic reduction in photosynthesis of inoculated plants was caused by decreases in ϕ_{PSII} and CE. Reduced photochemical activity in photosynthesis is a common response

to rust diseases, such as found in grapevine leaves infected by *Neophytopella tropicalis* (Rasera et al. 2023) and guava leaves infected by *Austropuccinia psidii* (Gonçalves et al. 2022). Here, we cannot rule out a potential diffusive limitation of photosynthesis

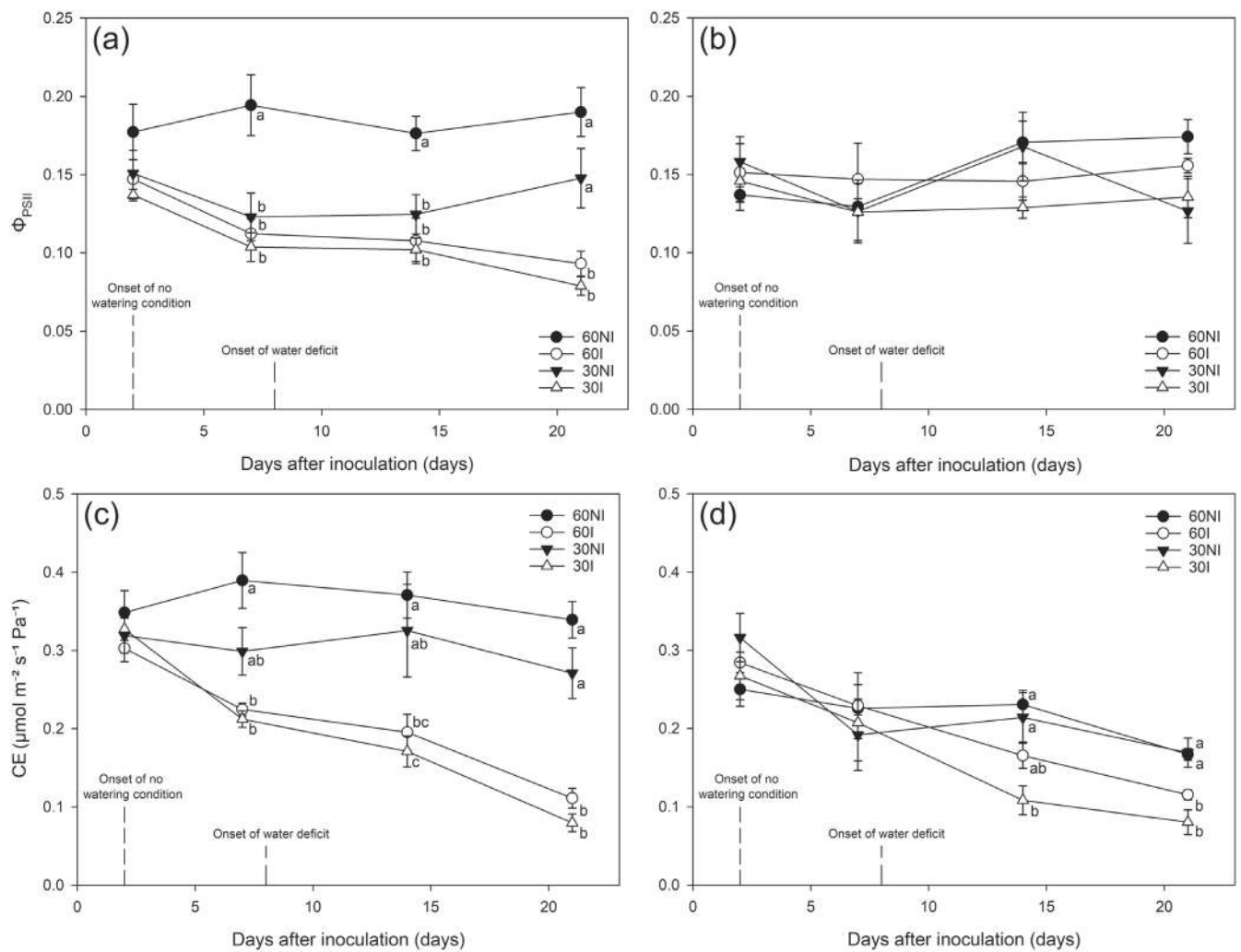


FIGURE 4 | Effective quantum efficiency of photosystem II (Φ_{PSII} ; a, b) and instantaneous carboxylation efficiency (CE; c, d) in Experiments 1 (a, c) and 2 (b, d) of leaves of well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) plants, non-inoculated (NI) and inoculated (I) with *Aculeastrum americanum*. Data points represent the mean values at each time and bars represent the mean standard error ($n=5$). Different letters denote significant differences at 5% probability by the Tukey test between treatments.

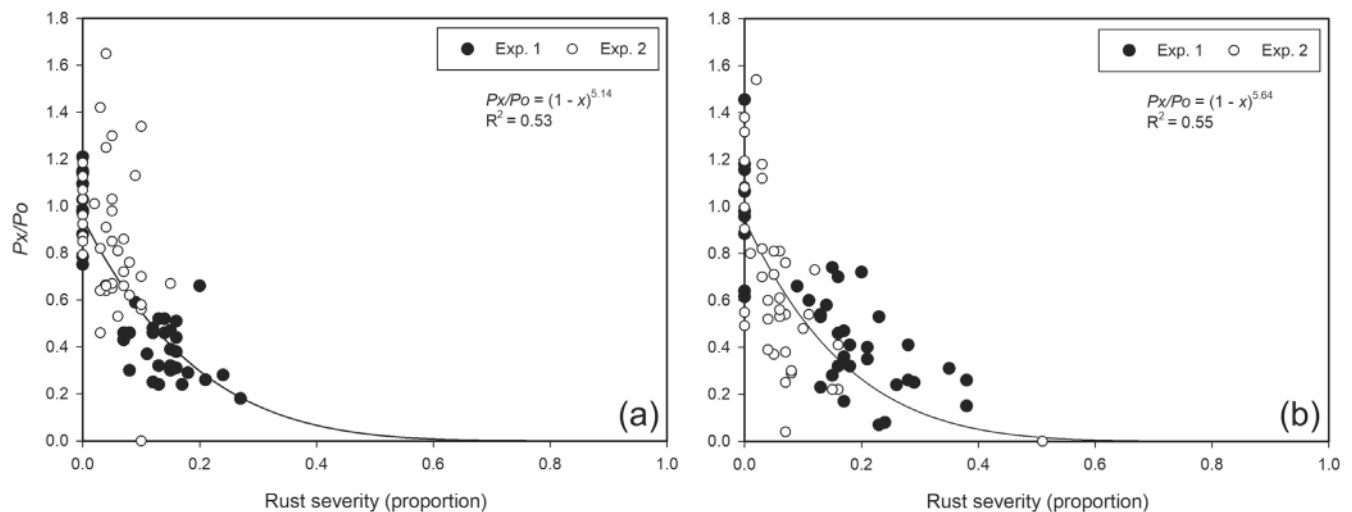


FIGURE 5 | Relationship between the relative net photosynthesis (P_x/P_o) and rust severity (*Aculeastrum americanum*) in raspberry cv. Heritage kept at 60% (a) and 30% available water capacity (AWC) (b). The line represents the model $P_x/P_o = (1 - x)^\beta$. According to Student's t test there was no statistical difference between β values at 60% and 30% AWC. $n=80$ in a; $n=79$ in b.

induced by pathogen infection. While CO₂ diffusion from atmosphere to substomatal chambers is regulated by g_s , the diffusion from this point until the carboxylation sites inside chloroplasts is modulated by mesophyll conductance—a variable not explored in this study.

In inoculated plants, the obstruction of the intercellular spaces of the mesophyll by hyphae of *A. americanum*, especially in the substomatal chambers where uredinial primordia are formed (Dias, Spósito, et al. 2023), compromises the mesophyll CO₂ diffusion, as observed in wheat leaf rust (Carretero et al. 2011), grapevine leaf rust (Navarro et al. 2019; Nogueira Júnior et al. 2017; Rasera et al. 2023), poplar leaf rust (Gortari et al. 2018) and guava leaf rust (Gonçalves et al. 2022, 2023). Such reduced diffusion would decrease the CO₂ inside chloroplasts without any change in C_i , as noticed here. Alternatively, maintenance of C_i levels even with reduced g_s could be a consequence of increased leaf respiration induced by the pathogen or even by pathogen respiration itself (Ray 2024). In poplar leaves, rust disease caused a significant increase in respiration (Gortari et al. 2018), and such an increase was related to the positive regulation of the host's metabolic response to the pathogenic challenge.

The infection significantly reduced the instantaneous carboxylation efficiency (CE) throughout the experimental period. An exponential reduction in CE with increasing rust severity has been previously reported in grapevine leaves infected by *Phakopsora euvtitis* (Navarro et al. 2019) and guava leaves infected by *A. psidii* (Gonçalves et al. 2022), suggesting biochemical constraints on photosynthesis. Low RuBisCO activity and abundance were reported in *Arabidopsis thaliana* leaves infected by *Albugo candida*, white rust of Brassicaceae (Tang et al. 1996). The reduction in the RuBisCO abundance may be due to the lower availability of nitrogen (N) for its synthesis, because N can be allocated to the plant defence responses (Schultz et al. 2013; Sun et al. 2020; Wang et al. 2019). Moreover, RuBisCO fragmentation can also occur, as reported in the pathosystem *T. aestivum*–*P. striiformis* f. sp. *tritici* (Li et al. 2011).

In addition to reducing photosynthesis of the diseased leaf area, the pathogen can also affect the green leaf area surrounding the lesion (Bastiaans 1991). Some studies with abiotic stresses initiated before rust infection revealed a reduction in virtual lesions, with decreases in β values of raspberry plants under water deficit (Ribeiro and Spósito 2022) and grapevine plants under warmer conditions (Rasera et al. 2023). Here, β values were similar in both water regimes, probably because the water deficit only started 48 h after inoculation. The values in this study are higher in comparison to bean rust (2.1; Bassanezi et al. 2001), wheat leaf rust (0.8–1.0; Robert et al. 2005), Asian soybean leaf rust (2.1–2.5; Kumudini et al. 2010) and guava leaf rust (2.13; Gonçalves et al. 2022), being similar only to grapevine leaf rust (4.99–5.78; Navarro et al. 2019; Nogueira Júnior et al. 2017; Rasera et al. 2023). The combination of biotic (infection) and abiotic (water deficit) stresses resulted in a severe reduction of A and led to a distinct plant response compared to that induced by a single stress factor (Choudhary and Senthil-Kumar 2024; Rasmussen et al. 2013). However, this was not the case for biotic stress alone. When comparing infected and well-watered plants with those infected and water-stressed,

the physiological responses were similar in both experiments. The lack of difference between the combined stress conditions and the biotic stress alone was due to the lower disease severity in plants under water deficit. Similar findings were reported for another pathosystem: in Asian grapevine leaf rust (*N. tropicalis*), the combination of abiotic and biotic stresses on the leaf gas exchange and photochemistry of *Vitis labrusca* was mitigated by low disease severity under warmer environments (Rasera et al. 2023).

We conclude that the occurrence of abiotic stress after the onset of infection reduced disease severity by reducing the colonisation capacity of *A. americanum*. Such loss of the pathogen's ability to colonise the leaf tissues of plants under water deficit was associated with decreases in leaf gas exchange and photochemical activity, which impact the nutrient availability for the fungus. We have shown that the onset of abiotic stress in relation to the onset of infection is decisive in reducing the rust severity, not only through stomatal closure, but also in mitigating the effects of the stress combination on photosynthesis in the green areas surrounding the lesions. Water deficit is an abiotic stress linked to climate change and further studies dealing with how other stressful environmental conditions affect the monocyclic components and primary metabolism of infected plants are needed to understand the consequences of climatic events after plant disease infection.

Author Contributions

Lucas Henrique Santos Barbosa: conceptualization, methodology, investigation, experimental work, formal analysis, visualization, writing – original draft, writing – review and editing. **Lilian Amorim:** conceptualization, funding acquisition, methodology, formal analysis, resources, supervision, writing – review and editing. **Rafael Vasconcelos Ribeiro:** methodology, formal analysis, writing – review and editing. **Ulla Neumann:** methodology, formal analysis, resources, visualization, supervision, writing – review and editing. **Ton Timmers:** methodology, formal analysis, resources, visualization, supervision. **Tonni Grube Andersen:** methodology, formal analysis, resources, visualization, supervision, writing – review and editing. **Marcel Bellato Spósito:** conceptualization, funding acquisition, methodology, formal analysis, resources, supervision, writing – review and editing. **Beatriz Appezzato-da-Glória:** conceptualization, funding acquisition, methodology, resources, supervision, writing – review and editing.

Acknowledgements

We gratefully acknowledge the São Paulo Research Foundation (FAPESP, Brazil) for financial support (Grant 2019/13191-5) and for fellowships awarded to Lucas Henrique Santos Barbosa (Grants 2022/00888-0 and 2024/02834-0). Lucas Henrique Santos Barbosa also thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 for the fellowship granted. Lilian Amorim, Rafael Vasconcelos Ribeiro, Marcel Bellato Spósito and Beatriz Appezzato-da-Glória acknowledge the National Council for Scientific and Technological Development (CNPq, Brazil) for fellowships granted (Grants 301689/2022-9, 304295/2022-1, 303897/2021-0 and 302361/2022-7, respectively). We are grateful to Sílvia Lourenço for her technical support. Lucas Henrique Santos Barbosa also thanks the Max Planck Institute for Plant Breeding Research (MPIPZ) and the Central Microscopy Facility (CeMic) in Cologne, Germany, for providing access to their infrastructure during his research internship as a visiting PhD student. The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Abid, M., S. Ali, L. K. Qi, et al. 2018. "Physiological and Biochemical Changes During Drought and Recovery Periods at Tillering and Jointing Stages in Wheat (*Triticum aestivum* L.)." *Scientific Reports* 8: 4615.
- Afzal, A., M. Ijaz, S. Ashraf, et al. 2022. "Determination of Relationship of Environmental Factors With Stripe Rust in Wheat." *Plant Protection* 6: 151–159.
- Anjum, S. A., M. Tanveer, U. Ashraf, et al. 2016. "Effect of Progressive Drought Stress on Growth, Leaf Gas Exchange, and Antioxidant Production in Two Maize Cultivars." *Environmental Science and Pollution Research* 23: 17132–17141.
- Anjum, S. A., X. Xie, L. C. Wang, M. F. Saleem, C. Man, and W. Lei. 2011. "Morphological, Physiological and Biochemical Responses of Plants to Drought Stress." *African Journal of Agricultural Research* 6: 2026–2032.
- Bassanezi, R. B., L. Amorim, A. B. Filho, B. Hau, and R. D. Berger. 2001. "Accounting for Photosynthetic Efficiency of Bean Leaves With Rust, Angular Leaf Spot and Anthracnose to Assess Crop Damage." *Plant Pathology* 50: 443–452.
- Bastiaans, L. 1991. "Ratio Between Virtual and Visual Lesion Size as a Measure to Describe Reduction in Leaf Photosynthesis of Rice due to Leaf Blast." *Phytopathology* 81: 611.
- Bergamin Filho, A. 2018. "Análise Temporal de Epidemias." In *Manual de Fitopatologia: Princípios e Conceitos*, edited by L. Amorim, J. A. M. Rezende, and A. Bergamin Filho, 519–530. Agronômica Ceres.
- Bidzinski, P., E. Ballini, A. Ducasse, et al. 2016. "Transcriptional Basis of Drought-Induced Susceptibility to the Rice Blast Fungus *Magnaporthe oryzae*." *Frontiers in Plant Science* 7: 1558.
- Carretero, R., M. O. Bancal, and D. J. Miralles. 2011. "Effect of Leaf Rust (*Puccinia triticina*) on Photosynthesis and Related Processes of Leaves in Wheat Crops Grown at Two Contrasting Sites and With Different Nitrogen Levels." *European Journal of Agronomy* 35: 237–246.
- Choudhary, A., and M. Senthil-Kumar. 2022. "Drought Attenuates Plant Defence Against Bacterial Pathogens by Suppressing the Expression of *CBP60g/SARD1* During Combined Stress." *Plant, Cell and Environment* 45: 1127–1145.
- Choudhary, A., and M. Senthil-Kumar. 2024. "Drought: A Context-Dependent Damper and Aggravator of Plant Diseases." *Plant, Cell and Environment* 47: 2109–2126.
- Dias, M. G., R. R. Ribeiro, C. M. Barbosa, J. M. I. de Jesus, and M. B. Spósito. 2023. "Diagrammatic Scale for Improved Late Leaf Rust Severity Assessments in Raspberry Leaves." *Canadian Journal of Plant Pathology* 45: 140–147.
- Dias, M. G., M. B. Spósito, M. A. Tessmer, and B. Appezzato-da-Glória. 2023. "Investigating Biochemical and Histopathological Responses Between Raspberries and *Aculeastrum americanum*." *Journal of Fungi* 9: 337.
- Duplessis, S., C. Lorrain, B. Petre, M. Figueroa, P. N. Dodds, and M. C. Aime. 2021. "Host Adaptation and Virulence in Heteroecious Rust Fungi." *Annual Review of Phytopathology* 59: 403–422.
- Figueiredo, M. B., E. M. C. Nogueira, J. T. Ferrari, C. C. Aparecido, and J. F. Hennen. 2003. "Ocorrência de Ferrugem no estado de São Paulo." *Arquivos do Instituto Biológico de São Paulo* 70: 199–201.
- Gao, P., T. Y. Duan, M. J. Christensen, et al. 2018. "The Occurrence of Rust Disease, and Biochemical and Physiological Responses on *Apocynum venetum* Plants Grown at Four Soil Water Contents, Following Inoculation With *Melampsora apocyni*." *European Journal of Plant Pathology* 150: 549–563.
- Gautam, H. R., M. L. Bhardwaj, and R. Kumar. 2013. "Climate Change and Its Impact on Plant Diseases." *Current Science* 105: 1685–1691.
- Gonçalves, M. P., L. Amorim, R. V. Ribeiro, M. K. Misaki Soares, and B. Appezzato-da-Glória. 2023. "*Austropuccinia psidii* Infection Changes Stomatal Dynamics and Leaf Gas Exchange of Guava Leaves: A Physio-Histopathological Assessment." *Physiological and Molecular Plant Pathology* 128: 102159.
- Gonçalves, M. P., A. F. Nogueira Júnior, R. V. Ribeiro, and L. Amorim. 2022. "Monocyclic Components and Photosynthetic Damage Caused by Myrtle Rust in Guava Leaves." *Phytopathology* 112: 1467–1475.
- Gortari, F., J. J. Guiamet, and C. Graciano. 2018. "Plant–Pathogen Interactions: Leaf Physiology Alterations in Poplars Infected With Rust (*Melampsora medusae*)." *Tree Physiology* 38: 925–935.
- Grimmer, M. K., F. M. John, and N. D. Paveley. 2012. "Foliar Pathogenesis and Plant Water Relations: A Review." *Journal of Experimental Botany* 63: 4321–4331.
- Hofer, K., R. Griffin, and M. Toome. 2025. "First Report of Late Leaf Rust on Raspberry Caused by *Aculeastrum americanum* in New Zealand." *New Disease Reports* 51: e70013.
- Hýsek, J., R. Vavera, and P. Růžek. 2017. "Influence of Temperature, Precipitation, and Cultivar Characteristics on Changes in the Spectrum of Pathogenic Fungi in Winter Wheat." *International Journal of Biometeorology* 61: 967–975.
- Khalvandi, M., A. Siosemardeh, E. Roohi, and S. Keramati. 2021. "Salicylic Acid Alleviated the Effect of Drought Stress on Photosynthetic Characteristics and Leaf Protein Pattern in Winter Wheat." *Heliyon* 7: e05908.
- Krokene, P., I. Børja, E. Carneros, et al. 2023. "Effects of Combined Drought and Pathogen Stress on Growth, Resistance and Gene Expression in Young Norway Spruce Trees." *Tree Physiology* 43: 1603–1618.
- Kumudini, S., C. V. Godoy, B. Kennedy, et al. 2010. "Role of Host-Plant Resistance and Disease Development Stage on Leaf Photosynthetic Competence of Soybean Rust Infected Leaves." *Crop Science* 50: 2533–2542.
- Lahlali, R., M. Taoussi, S.-E. Laasli, et al. 2024. "Effects of Climate Change on Plant Pathogens and Host–Pathogen Interactions." *Crop and Environment* 3: 159–170.
- Li, H., G. Wei, J. Xu, L. Huang, and Z. Kang. 2011. "Identification of Wheat Proteins With Altered Expression Levels in Leaves Infected by the Stripe Rust Pathogen." *Acta Physiologiae Plantarum* 33: 2423–2435.
- Li, P., Y. Zhu, X. Song, and F. Song. 2020. "Negative Effects of Long-Term Moderate Salinity and Short-Term Drought Stress on the Photosynthetic Performance of Hybrid *Pennisetum*." *Plant Physiology and Biochemistry* 155: 93–104.
- Lim, C., W. Baek, J. Jung, J.-H. Kim, and S. Lee. 2015. "Function of ABA in Stomatal Defense Against Biotic and Drought Stresses." *International Journal of Molecular Sciences* 16: 15251–15270.
- Luffman, M., and D. Buszard. 1989. "Susceptibility of Primocanes of Six Red Raspberry Cultivars to Late Yellow Rust (*Pucciniastrum americanum* (Farl.) Arth.)." *Canadian Plant Disease Survey* 69: 117–119.
- McElrone, A. J., C. D. Reid, K. A. Hoyer, E. Hart, and R. B. Jackson. 2005. "Elevated CO₂ Reduces Disease Incidence and Severity of a Red Maple Fungal Pathogen via Changes in Host Physiology and Leaf Chemistry." *Global Change Biology* 11: 1828–1836.

- Morales, C. G., M. T. Pino, and A. del Pozo. 2013. "Phenological and Physiological Responses to Drought Stress and Subsequent Rehydration Cycles in Two Raspberry Cultivars." *Scientia Horticulturae* 162: 234–241.
- Navarro, B. L., A. F. Nogueira Júnior, R. V. Ribeiro, and M. B. Spósito. 2019. "Photosynthetic Damage Caused by Grapevine Rust (*Phakopsora euvtis*) in *Vitis vinifera* and *Vitis labrusca*." *Australasian Plant Pathology* 48: 509–518.
- Nnadi, N. E., and D. A. Carter. 2021. "Climate Change and the Emergence of Fungal Pathogens." *PLoS Pathogens* 17: e1009503.
- Nogueira Júnior, A. F., R. V. Ribeiro, B. Appezzato-da-Glória, M. K. M. Soares, J. B. Rasera, and L. Amorim. 2017. "*Phakopsora euvtis* Causes Unusual Damage to Leaves and Modifies Carbohydrate Metabolism in Grapevine." *Frontiers in Plant Science* 8: 1675.
- Ortiz-Bobea, A., T. R. Ault, C. M. Carrillo, R. G. Chambers, and D. B. Lobell. 2021. "Anthropogenic Climate Change Has Slowed Global Agricultural Productivity Growth." *Nature Climate Change* 11: 306–312.
- Pandey, P., M. Patil, P. Priya, and M. Senthil-Kumar. 2024. "When Two Negatives Make a Positive: The Favorable Impact of the Combination of Abiotic Stress and Pathogen Infection on Plants." *Journal of Experimental Botany* 75: 674–688.
- Pariaud, B., V. Ravigné, F. Halkett, H. Goyeau, J. Carlier, and C. Lannou. 2009. "Aggressiveness and Its Role in the Adaptation of Plant Pathogens." *Plant Pathology* 58: 409–424.
- Percival, D. C., J. T. A. Proctor, and J. P. Privé. 1998. "Gas Exchange, Stem Water Potential and Leaf Orientation of *Rubus idaeus* L. Are Influenced by Drought Stress." *Journal of Horticultural Science and Biotechnology* 73: 831–840.
- Pereira, L. S., J. A. d. J. Valero, M. R. P. Buendia, and J. M. T. Martín-Benito. 2010. *El Riego y Sus Tecnologías*. CREA-UCLM.
- R Core Team. 2021. "R: A Language and Environment for Statistical Computing Vienna, Austria R Foundation for Statistical Computing."
- Rasera, J. B., B. Appezzato-da-Glória, R. V. Ribeiro, A. F. Nogueira-Júnior, and L. Amorim. 2023. "Damage on Grapevine cv. Niagara Rosada Leaves Caused by the Combined Effect of Temperature and Asian Grapevine Leaf Rust (*Neophytophthora tropicalis*)." *Plant Pathology* 72: 1406–1415.
- Rasmussen, S., P. Barah, M. C. Suarez-Rodriguez, et al. 2013. "Transcriptome Responses to Combinations of Stresses in *Arabidopsis*." *Plant Physiology* 161: 1783–1794.
- Ray, R. V. 2024. "Effects of Pathogens and Disease on Plant Physiology." In *Agrios' Plant Pathology*, edited by R. P. Oliver, 63–92. Elsevier.
- Ribeiro, R. R., and M. B. Spósito. 2022. "Interference of Late Rust Associated With Water Deficit in the Primary Metabolism of Raspberries." *European Journal of Plant Pathology* 163: 279–292.
- Robert, C., M. Bancal, B. Ney, and C. Lannou. 2005. "Wheat Leaf Photosynthesis Loss due to Leaf Rust, With Respect to Lesion Development and Leaf Nitrogen Status." *New Phytologist* 165: 227–241.
- Schindelin, J., I. Arganda-Carreras, E. Frise, et al. 2012. "Fiji: An Open-Source Platform for Biological-Image Analysis." *Nature Methods* 9: 676–682.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. "NIH Image to ImageJ: 25 Years of Image Analysis." *Nature Methods* 9: 671–675.
- Schroers, H. J., U. Prislan, V. Kocjan, W. Maier, and J. Zajc. 2025. "First Report of *Aculeastrum (Pucciniastrum) americanum* Causing Late Leaf Rust on Raspberry in Slovenia." *Plant Disease*. <https://doi.org/10.1094/PDIS-02-25-0294-PDN>.
- Schultz, J. C., H. M. Appel, A. P. Ferrieri, and T. M. Arnold. 2013. "Flexible Resource Allocation During Plant Defense Responses." *Frontiers in Plant Science* 4: 324.
- Singh, B. K., M. Delgado-Baquerizo, E. Egidi, et al. 2023. "Climate Change Impacts on Plant Pathogens, Food Security and Paths Forward." *Nature Reviews Microbiology* 21: 640–656.
- Sun, Y., M. Wang, L. A. J. Mur, Q. Shen, and S. Guo. 2020. "Unravelling the Roles of Nitrogen Nutrition in Plant Disease Defences." *International Journal of Molecular Sciences* 21: 572.
- Tang, X., S. A. Rolfe, and J. D. Scholes. 1996. "The Effect of *Albugo candida* (White Blister Rust) on the Photosynthetic and Carbohydrate Metabolism of Leaves of *Arabidopsis thaliana*." *Plant, Cell & Environment* 19: 967–975.
- Velásquez, A. C., C. D. M. Castroverde, and S. Y. He. 2018. "Plant-Pathogen Warfare Under Changing Climate Conditions." *Current Biology* 28: R619–R634.
- Wang, M., Z. Gu, R. Wang, et al. 2019. "Plant Primary Metabolism Regulated by Nitrogen Contributes to Plant-Pathogen Interactions." *Plant and Cell Physiology* 60: 329–342.
- Werner, C., L. Fasbender, K. M. Romek, A. M. Yáñez-Serrano, and J. Kreuzwieser. 2020. "Heat Waves Change Plant Carbon Allocation Among Primary and Secondary Metabolism Altering CO₂ Assimilation, Respiration, and VOC Emissions." *Frontiers in Plant Science* 11: 1242.
- Yan, H., X. Liu, H. Ding, Z. Dai, X. Niu, and L. Zhao. 2023. "Hormonal Balance, Photosynthesis, and Redox Reactions in the Leaves of *Caragana korshinskii* Kom. Under Water Deficit." *Plants* 12: 2076.
- Zhao, C., B. Liu, S. Piao, et al. 2017. "Temperature Increase Reduces Global Yields of Major Crops in Four Independent Estimates." *Proceedings of the National Academy of Sciences of the United States of America* 114: 9326–9331.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Intercellular CO₂ concentration (C_i; a, b) in Experiments 1 (a) and 2 (b) of leaves of well-watered (60% available water capacity [AWC]) and water deficit (30% AWC) plants, non-inoculated (NI) and inoculated (I) with *Aculeastrum americanum*. Full circles represent data from 60% AWC non-inoculated, empty circles represent data from 60% AWC inoculated, full triangle represents data 30% AWC non-inoculated and empty triangle represent data from 30% AWC inoculated. Data points represent the mean values at each time and bars represent the mean standard error ($n=5$). No significant differences were observed between treatments at 5% probability by the Tukey test.