

## Infection by *Xanthomonas campestris* pv. *viticola* under temperature increase and carbon dioxide concentrations

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### Abstract

The experiments were carried out under controlled conditions to evaluate the impact of increased temperature and concentration of carbon dioxide on infection of *Xanthomonas campestris* pv *viticola*, the causal agent of bacterial canker in Vine seedlings. It proceeded the evaluation of the following epidemiological components: incubation period (PI), severity (SEV) and using that data were calculated the area under the disease progress curve (AUDPC). It used grape seedlings (Italia, Crimson Seedless, Sugaone and Selection 8) inoculated with bacterial suspension ( $10^8$  CFU mL<sup>-1</sup>). The experimental design was completely randomized, factorial arrangement 4x4 (cultivar x temperature) and 4x2 (cultivar x carbon dioxide concentration) which was carried out twice. The data were subjected to variance analysis. Increasing temperature reduced bacterium's incubation period with significant differences between genotypes. For Selection 8 and Crimson temperature increase caused enhancement on severity and AUDPC. For Seleção 8 the incubation period (PI) was extended from 7.93 to 30.18 days when the concentration changed from 390 to 770  $\mu\text{mol/mol}$ . The increased CO<sub>2</sub> concentration reduced AACPSD and SEV for Sugaone and Selection 8. The results show that the temperature and carbon dioxide (CO<sub>2</sub>) concentration of the air may have different effects on bacterial canker of grapevine.

**Keywords:** bacterial canker, climate change, *Vitis vinifera*

### Introduction

Canker is the major disease of bacterial origin in grape vine (*Vitis* sp.), whose causal agent is *Xanthomonas campestris* pv *viticola* (Nayudu) Dye. This disease has already been identified in São Paulo (Rodrigues Neto et al., 2011), Ceará, Roraima, Piauí, Bahia and Pernambuco (Malavolta Jr et al., 2008) states, while in São Francisco Valley (Brazil) it is of great importance, because it is under conditions of temperature between 25 and 30 °C and high humidity, and ultimately reduced the grape production (Lima et al., 1999). According to Lima & Ferreira (2012), the symptoms of this disease varies according to the tolerance of each cultivar and the environmental

conditions.

The human activities, especially in the last 150 years, have contributed to the elevation in the world temperature due to the ever increasing concentration of greenhouse gases. The concentration of carbon dioxide (CO<sub>2</sub>) has increased by approximately 40% since 1750, with an estimated increase of 730 to 1020  $\mu\text{mol/mol}$  for the A2 scenario (worst case). As a consequence of increasing greenhouse gases amount, the average temperature has risen by 0.6 °C, with an estimated increase of 1.4 °C to 5.8 °C until 2100 (IPCC 2007, IPCC 2013).

It is known that the environment affects directly all developmental stages of both the

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pathogen and the host plant, and thus the climate change may interfere with the geographic and temporal distribution of diseases (Ghini et al., 2012). According to Beriam et al. (2011), species of plant-pathogenic bacteria may react in different ways to environmental changes, which can have deleterious or beneficial effects, or even not cause changes in the life cycle and pathogenicity of plant bacteria, though they may change the physiology and resistance of the host plant. However, few studies have been carried out to effectively simulate the effect of climate change on pathogens (Garrett et al., 2006) and host plants to develop measures of mitigation and adaptation for the disease management (Chakraborty & Newton, 2011).

The objective of this study was to evaluate the effect of temperature increase and concentration of atmospheric carbon dioxide on infection by *Xanthomonas campestris* pv. *viticola* in four grape vine cultivars.

#### Material and Methods

The first experiment was conducted in the facilities of the Department of Technologies and Social Sciences (DTCS) of Universidade do Estado da Bahia - UNEB, Campus III, located in Juazeiro/BA, Brazil (geographical coordinates 9° 24' S and 40° 30' W) during the first semester of 2012, in laboratory and greenhouse conditions. The second experiment consisted of evaluations performed at the Climate Change Sector of Embrapa Tropical Semi-arid, located in Petrolina/PE, Brazil (9° 09' S and 40° 22' W) during the second semester of 2012.

The trials were conducted with grape vine (*Vitis vinifera*) seedlings with four different materials, as follows: three cultivars (Italia, Crimson seedless and Sugraone) and a selection from the Active Germplasm Bank of Embrapa Grape and Wine (Seleção 8), cultivated in polyethylene bags (0.22 × 0.14 m), using rooted cuttings of grape vines from commercial areas, propagated via stem cuttings. The substrate was prepared using organic compost + sand (1:1).

Before and after inoculation, the seedlings were placed in a moist chamber for 24 hours. The pre-treatment aimed to promote stomatal opening, facilitating the pathogen

penetration, whereas the post-treatment was performed to ensure the water congestion in the adjacent tissues, which is crucial to the infectious process (Romeiro, 2005).

For inoculation, the methodology proposed by Nascimento et al. (2006) was adopted, in which double gauze swabs moistened with 0.5 mL of the bacterial suspension were gently rubbed on the leaf surfaces. The bacterial suspension, prepared in sterilized distilled water was obtained from the isolate *Xanthomonas campestris* pv. *viticola* (Xcv3) derived from the collection of bacterial cultures of the Plant Pathology Laboratory of UNEB, collected from a commercial area of production of fine table grapes in the irrigated area of Irrigated Perimeter Senador Nilo Coelho, in Petrolina/PE (Brazil), cultivated with Red Globe grape cultivar. Using a photocolormeter at 570 nm, the suspension was adjusted to 10<sup>8</sup> CFU mL<sup>-1</sup> (A<sub>570</sub>= 0.4) concentration.

#### Experiment I - Effect of temperature

After the treatment in moist chamber, the plants were inoculated and subjected to different temperatures in a B.O.D. (Biological Oxygen Demand) growth chamber, 12-h photoperiod, during 48 h. Inoculation occurred at 45 days after the seedlings were planted, using five leaves per plant, totaling 20 leaves per genotype. Subsequently, all seedlings were kept in a greenhouse at 26 to 30 °C with relative humidity above 70% until the end of the evaluations.

#### Experiment II - Effect of CO<sub>2</sub> concentration

The treatments were distributed in a 4 × 4 factorial arrangement with 20 replicates, as follows: four temperatures (26.0, 28.0, 29.1 and 31.8 °C) and four genotypes [Sugraone, Italia, Crimson Seedless and Seleção 8 (Embrapa Grape and Wine)]. The temperature values adopted corresponded to the local average temperature with an addition of 2, 3.1 and 5.8 °C, respectively referring to the future scenarios B1, B2 and A1F estimated by the IPCC (2007). The experiment was conducted twice.

Four leaves were inoculated per plant for the experiment with CO<sub>2</sub>, totaling 16 leaves

per cultivar. After the inoculation, the seedlings were subjected to the concentrations of 390 and 770  $\mu\text{mol/mol}$   $\text{CO}_2$  in growth chambers at 29 °C and relative humidity above 70%. After 48 h, the plants were transferred to a greenhouse under humidity above 70% and average temperature 29 °C, and evaluated daily until the end of the experiment.

The experimental design was completely randomized, in a 4 × 2 factorial arrangement represented by four genotypes (Sugraone, Italia, Crimson Seedless and Seleção 8 (Embrapa Grape and Wine) and two concentrations of  $\text{CO}_2$  (390 and 770  $\mu\text{mol/mol}$ ). The experiment was performed twice.

For the two experiments, all plants were evaluated for five weeks, recording the following epidemiologic components: a) period of incubation (PI), corresponding to the number of days between inoculation and appearance of the first symptoms; and b) severity of the disease (SEV), determined using a diagrammatic scale (Nascimento et al., 2005), which showed severity levels of the diseased leaf area varying from 2

to 91%. The evaluations were performed every seven days, for five consecutive weeks.

From the data obtained during the evaluations of severity, it was possible to determine the area under the disease progress curve (AUDPC), calculated by the expression:  $\text{AUDPC} = \sum(y_i + y_{i+1})/2 \cdot dt_i$ , where  $y_i$  and  $y_{i+1}$  correspond to the severity values found in two consecutive assessments, and  $dt_i$  is the interval (in days) between the assessments (Shaner & Finney, 1977).

The obtained data were subjected to variance analysis using the Assistat (7.6 Beta) statistical software and the means were grouped by the Scott-Knott test ( $p \leq 5\%$ ).

## Results and Discussion

### Experiment I – Effect of temperature

The symptoms of bacterial canker were observed in all cultivars, for all the tested temperatures, with the period of incubation (PI) varying according to genotype and temperature (Table 1).

**Table 1.** Period of incubation (PI) (days) of bacterial canker in seedlings of grape cultivars under different temperatures. Juazeiro/BA, Brazil.

Temperatures (°C)	Grape cultivar/ PI			
	Sugraone	Crimson	Seleção 8	Italia
26.0	13.00 aA	13.00 aA	12.70 aA	12.50 aA
28.0	15.00 aA	9.00 bB	14.70 aA	10.75 aB
29.1	9.90 bBC	9.30 bC	12.65 aA	12.20 aAB
31.8	8.85 bA	5.15 cB	6.80 bAB	7.85 bA
CV(%) = 27.14				

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Scott-Knott test at 5% probability.

The temperature increase promoted a reduction in PI for the tested cultivars. In the seedlings of Sugraone, Crimson, Seleção 8 and Italia cultivars, the increase of 5.8 °C reduced the PI from 13, 13, 12.7 and 12.5 to 8.85, 5.15, 6.80 and 7.85 days, respectively. This increase in the local average temperature (26 °C) favored the establishment of the disease, i.e., the time necessary for the appearance of the first symptoms. Seleção 8 and cv. Italia plants showed a decrease in PI when subjected to the temperature of 31.8 °C only, which indicates greater tolerance to temperature increase without the occurrence of benefits for the establishment of the pathogen (Table 1).

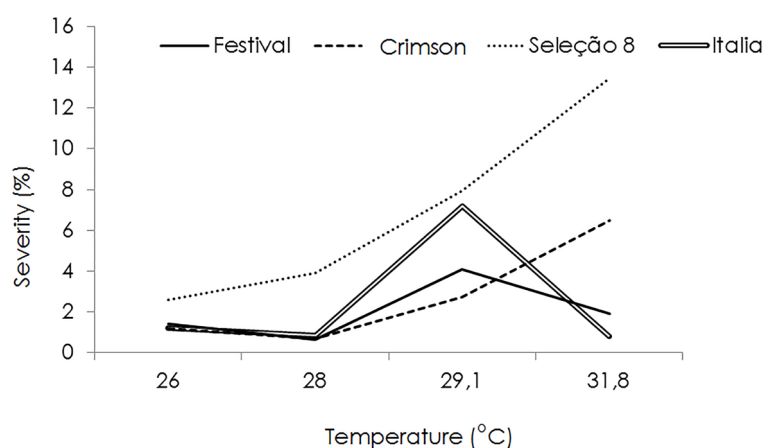
Data obtained by Ghini et al. (2011) also showed a tendency towards a reduction in the incubation of coffee rust (*Hemileia vastatrix*) when simulated temperature increase.

When compared with each other, under the average temperature of 26 °C, the cultivars do not presented significant differences to the number of days between inoculation and appearance of the first symptoms. No significant difference were observed in PI for cv. Sugraone and Seleção 8 either at 28 °C, elapsing 15 and 14.7 days for the appearance of the symptoms, respectively. In seedlings exposed to 29.1 °C, the shortest incubation period was observed for the Sugraone and Crimson cultivars, with 9.9 and 9.3 days,

respectively (Table 1). Differences in incubation period reflect in differences in the growth rate of the pathogen in the host, and consequently in the disease progress rate (Parlevliet, 1979). Thus, the short incubation period observed due to the temperature elevation is a factor that contributes to the occurrence of epidemics. Moreover, it was observed that for every cultivar evaluated there was a difference in the period of incubation. According to Parlevliet (1979), PI is a component of resistance, and this variation in the time for appearance of the symptoms may be related to

differences in the genotypic composition of the biological materials.

Significant differences were observed ( $P=0.05$ ) between the treatments for the variables severity and AUDPC (Figure 1; Table 2). The canker severity in the grape vine varied from 0.5 to 13.5% at the different temperatures evaluated. The highest values were observed when the seedlings were exposed to temperatures of 29.1 and 31.8 °C. For Seleção 8 and Crimson, the temperature increase promoted an increase on disease severity (Figure 1).



**Figure 1.** Severity of bacterial canker in grape vine seedlings under different temperatures. Juazeiro/BA, Brazil.

**Table 2.** Area under the disease progress curve (AUDPC) (bacterial canker) in seedlings of grape cultivars under different temperatures. Juazeiro/BA, Brazil

Temperatures (°C)	Grape cultivars / AUDPC			
	Sugraone	Crimson	Seleção 8	Italia
26.0	13.60 bA	25.20 cA	49.70 cA	37.30 bA
28.0	19.00 bB	19.60 cB	69.50 cA	25.40 bB
29.1	81.30 aB	74.00 bB	113.80 bA	134.80 aA
31.8	43.00 bB	148.00 aA	182.70 aA	17.80 bB

CV(%) = 54.32

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Scott-Knott test at 5% probability.

The temperature increase also showed significant effects on infection by *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria* on pepper, identified as one of the most important environmental factors in the outbreak of disease (Shin & Yun, 2010).

The analysis of AUDPC also showed differences in susceptibility among the cultivars evaluated at the different temperatures. The worst scenario, with a temperature increase of 5.8 °C, corresponding to 31.8 °C, provided a greater value of AUDPC for cv. Crimson and Seleção 8, with 148 and 182.70, respectively. Sugraone and

Italia cultivars presented higher values of AUDPC (81.3 and 134.8) when under the temperature of 29.1 °C only (Table 2).

In citrus, the temperature increase also provoked an increase in the percentage of leaf area diseased by *X. axonopodis* pv. *citri* (Dalla Pria et al., 2006), thus in disagreement with Marcuzzo et al. (2009), who found a reduction in the development of bacterial spot of tomato plant caused by *Xanthomonas* spp. at temperatures above 25 °C.

*Experiment II - Effect of CO<sub>2</sub> concentration*

The symptoms of bacterial canker were observed in all grape vine varieties in the experiment in the carbon-dioxide-enriched environment. Only Seleção 8 showed a significant difference ( $P=0.05$ ) for the incubation period at different CO<sub>2</sub> concentrations. The incubation period for the other cultivars varied from 7 to 12 days, with no difference ( $P=0.05$ ) in the different treatments. For Seleção 8, the period of incubation was 30.18 days (Table 3).

If considering that Seleção 8 takes approximately 122 days between pruning and fruit maturation, in case of plant infection after pruning, a PI of 30 days corresponds to 25% of the total time for cultivation, representing a reduction in the epidemics. Climate change will alter the current scenario of phytosanitary problems. According to Ghini et al. (2007), the economic, social and environmental impacts caused can be positive, negative or neutral, as climate change could decrease, increase or have no effect on the different phytosanitary problems in each region. In eucalypt, Santos et al. (2013) found that the increase of CO<sub>2</sub> concentration reduced the incubation period of *Ceratocystis fimbriata*.

The increased concentration of CO<sub>2</sub> reduced the severity of bacterial canker for cv. Sagraone and Seleção 8. Cultivars Italia and Crimson did not present a significant difference regarding the CO<sub>2</sub> increase. In the seedlings of grape vine submitted to 390  $\mu\text{mol/mol}$  CO<sub>2</sub>, a significant difference was observed for severity among the grape cultivars ( $P=0.05$ ) (Table 4). The same was found for the seedlings subjected to 770  $\mu\text{mol/mol}$  CO<sub>2</sub>. Sagraone and Seleção 8 cultivars showed the lowest percentages of SEV of the disease, not differing from each other (Table 4).

For the CO<sub>2</sub> concentration of 390  $\mu\text{mol/mol}$ , Italia and Crimson Seedless cultivars presented a lower AUDPC. At the concentration of 770  $\mu\text{mol/mol}$ , cv. Sagraone and Seleção 8 presented a significant reduction of AUDPC from 210.25 to 90.75 and 310.50 to 110.25, respectively (Table 5).

With the results obtained in this study, it can be inferred that the grape vine cultivars have different responses to CO<sub>2</sub> concentration increase. Nowadays it is known that natural surface openings and wounds are passive ports for bacterial entry. Stomata regulate gas exchange between the plant and environment,

**Table 3.** Period of incubation (PI) (days) of bacterial canker in seedlings of grape cultivars subjected to two levels of CO<sub>2</sub>. Petrolina/PE, Brazil

Concentration of CO <sub>2</sub> ( $\mu\text{mol/mol}$ )	Grape cultivars / PI			
	Italia	Crimson	Sagraone	Seleção 8
390	12.68 aA	9.87 aA	7.25 aA	7.93 aA
770	9.75 aB	9.12 aB	9.25 aB	30.18 bA

CV (%) = 21.18

Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by the Scott-Knott test at 5% probability.

**Table 4.** Severity (%) of bacterial canker in seedlings of grape cultivars subjected to 390 and 770  $\mu\text{mol/mol}$  of CO<sub>2</sub>. Petrolina/PE, Brazil

Concentrations of CO <sub>2</sub> ( $\mu\text{mol/mol}$ )	Varieties / Severity			
	Italia	Crimson	Sagraone	Seleção 8
390	6.81 aC	5.37 aC	9.31 aB	13.25 aA
770	6.12 aA	5.25 aA	3.31 bB	2.06 bB

CV (%) = 49.04

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Scott-Knott test at 5% probability.

**Table 5.** Area under the disease progress curve (AUDPC) of bacterial canker inoculated in seedlings of grape cultivars subjected to 390 and 770  $\mu\text{mol/mol}$  CO<sub>2</sub>. Petrolina/PE, Brazil

Concentrations of CO <sub>2</sub> ( $\mu\text{mol/mol}$ )	Grape cultivars / AUDPC			
	Italia	Crimson	Sagraone	Seleção 8
390	154.75 aBC	132.50 aC	210.25 aB	310.50 aA
770	137.50 aA	116.50 aA	90.75 bA	110.25 bA

CV (%) = 38.38

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Scott-Knott test at 5% probability.

and water loss control by changing the stomatal pore size. This stomatal movement is affected by several environmental stimulus, such as CO<sub>2</sub> concentration and other one (Melotto et al., 2008).

Thus, climate change may alter the physiology and resistance of the host plant and modify the stages and rates of pathogens development (Yáñez-López et al., 2012). The reduction in the stomatal opening may have played a part in the reduction of the disease for cultivar Sugaone and Seleção 8, by diminishing the specific area for pathogen penetration. These pieces of information are important for grape vine varieties choice to be cultivated in the future climate scenarios.

In studies on the plant-pathogen interaction involving elevations in the CO<sub>2</sub> levels, Lake & Wade (2009) observed that resistant varieties of *Arabidopsis thaliana* treated with concentrations of 800 µmol/molCO<sub>2</sub> presented a greater number of stomata, becoming more sensitive to mildew (*Erysiphe cichoracearum* de Candolle). Aiming to evaluate the effects of air-CO<sub>2</sub> concentration increase on leaf-spot caused by *Cylindrocladium candelabrum* in *Eucalyptus urophylla*, Silva & Ghini (2014) concluded that the high concentration of CO<sub>2</sub> decreased the disease incidence and severity.

The same was observed by Eastburn et al. (2010) for mildew of soybean (*Peronospora manshurica* (Naum. Syd.). However, Lessin & Ghini (2009) evaluated the severity of mildew (*Microsphaera diffusa* Cooke & Peck) in different soy varieties and observed that CO<sub>2</sub> concentration increase resulted in enhanced disease severity.

Few research studies have been conducted aiming to determine the effects of CO<sub>2</sub> air concentration increase on occurrence of bacterial disease. Developments should occur to elucidate how different genotypes react to the climate change scenarios, because, as observed in this study, the grape vine cultivars show different responses to infection by *X. campestris* pv *viticola*.

## Conclusions

The results obtained in this study demonstrated that temperature variation and carbon dioxide concentration of the air have different effects and consequently have different responses to increase the bacterial canker of grapevine.

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