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Reaction of biofortified lettuce genotypes to different strains of *Xanthomonas campestris* pv. *vitians*

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ABSTRACT
Lettuce bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians* is an aggressive disease that is difficult to control. So far there are no reports of the reaction of biofortified lettuce genotypes to different isolates of the bacteria. Thus, the objective was to evaluate the aggressiveness of *X. campestris* pv. *vitians*, as well as the reaction of biofortified lettuce genotypes to bacterial spot. Two experiments were performed in two distinct seasons (winter and summer), in greenhouse at the Vegetable Experimental Station of the Federal University of Uberlândia (UFU). The experimental design in both experiments was a randomized block design, in a factor scheme of 5 × 4 (five genotypes and four strains), with four repetitions. Were evaluated the severity and the area under the disease progress curve. In general, the biofortified lettuce 'Uberlândia 10000' was more resistant to most bacterial strains in the summer cultivation, and in the winter period UFU 'Crespa 206'. The commercial cultivar Robusta was the most susceptible to the strains during both seasons. The UFU E125 strain was the most aggressive for most genotypes in both seasons.

Keywords: bacterial leaf spot; *Lactuca sativa* L.; pathogenicity.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in the world. In addition to the benefits provided to human health, such as the prevention of diseases related to oxidative stress (ROCHA; REED, 2014), lettuce is also an important source of carotenoid precursors of vitamin A (CASSETARI et al., 2015; MACIEL et al., 2019; OLIVEIRA et al., 2019). It is a biofortified food that can be used as an alternative to reduce the risk of deficiency (SILVEIRA et al., 2019). Despite the nutritional potential of lettuce, there has been a significant increase in disease incidence in the vegetable, making its cultivation unsustainable.

Among the diseases that affect lettuce, bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians* (Brown) Dye stands out. This disease is economically important worldwide (LU; RAID, 2013) and has been reported in Brazil in the Midwest, South, and Southeast, as well as in the states of São Paulo, Rio de Janeiro, Federal District and Minas Gerais (MALAVOLTA JÚNIOR et al., 2008; TEBALDI et al., 2015).

Xanthomonas campestris pv. *vitians* is a potentially destructive pathogen, favored by high humid conditions, and is capable of spreading rapidly in a greenhouse through irrigation water (WANG et al., 2015), especially in greenhouses (TEBALDI et al., 2015). Typical symptoms of bacterial leaf spot include water soaked, translucent, and angular brown lesions, which turn black and eventually thin and dry out. In severe outbreaks, the lesions may coalesce and expand

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along the leaf vein, forming necrotic patches. Thus, the infected lettuce is of lower quality and yield and may present higher post-harvest losses.

Current management strategies are related to cultivation practices, which consist of avoiding fields with a history of the disease, since the pathogen may survive in remnants of previous harvests or of cultivation being carried out during periods of the year when environmental conditions do not favor the onset of bacterial leaf spot. The application of bactericides can reduce the disease severity when applied preventively; however, their use can significantly increase the costs of crop production (HAYES et al., 2013).

The most sustainable, effective, and reliable control measure against bacteria and other disease-causing pathogens would, therefore, be the development of resistant cultivars, while considering the aggressiveness of the strains to be inoculated into the hosts. In Brazil, studies of resistance in biofortified lettuce are available only from studies carried out with downy mildew (JACINTO et al., 2019) and nematodes (SOUSA et al., 2019). To date, there are no studies investigating the reaction of biofortified lettuce genotypes to *X. campestris* pv. *vitians* nor on the behavior of different bacterial strains.

The present study aimed to evaluate the aggressiveness of *X. campestris* pv. *vitians* strains and to determine the reaction of biofortified lettuce genotypes to the bacteria.

MATERIAL AND METHODS

The research was conducted at the Laboratory of Plant Bacteriology, Institute of Agricultural Sciences, Federal University of Uberlândia (UFU), in Uberlândia, state of Minas Gerais, as well as in a greenhouse at the Experimental Station of Vegetables, UFU, Campus Monte Carmelo, Minas Gerais (18°42'43.19"S, 47°29'55.8"W; 873 m altitude).

Two experiments were performed in two distinct seasons, characterized as winter and summer. The first experiment took place between May and August 2017 and the second between December 2017 and March 2018. The experimental design in both experiments was a randomized block design, in a factor scheme of 5 × 4 (five genotypes and four strains), with 4 repetitions of 3 plants per pot.

On May 31, 2017 (winter season) and January 15, 2018 (summer season) commercial cultivars Mimosa, Robusta, and UFU MCMinibiofort, as well as the UFU Crespa 206 and Uberlândia 10000, were sown in 200-cell trays containing a commercial substrate based on coconut fiber. The three genotypes 'Uberlândia 10000', UFU 'Crespa 206' and UFU 'MCMinibiofort' were obtained from the UFU's Biofortified Lettuce Breeding Program.

Seedlings were transplanted 35 days after sowing to 5 L pots. The pots were conditioned in an arch-type greenhouse, with dimensions 5 × 6 m and a ceiling height of 3.5 m, covered with transparent, UV-protected polyethylene film 150 × 10–6 m thick, as well as side curtains of white antiaphid screen. The maximum and the minimum temperatures (°C) inside the greenhouse were recorded using a digital thermometer (Fig. 1). Cultivation treatments were performed as recommended for the crop (FILGUEIRA, 2008).

To evaluate the reaction of biofortified lettuce genotypes were used different strains of *X. campestris* pv. *vitians*. The *X. campestris* pv. *vitians* strains UFU E125, UFU F27, UFU F35, and UFU G127 preserved and maintained in the work collection of the Laboratory of Plant Bacteriology, were grown using culture medium 523 that contains, per liter: 10 g sucrose, 8 g casein hydrolysate, 4 g yeast extract, 2 g K_2HPO_4, 0.3 g $MgSO_4 \cdot 7H_2O$, and 15 g agar (pH 6.9) (KADO; HESKETT, 1970) at 28 °C. After 48 h, the bacterial suspension was prepared in sterile distilled water and adjusted to an optical density of 550 [$OD_{550} = 0.1$ (1×10^8 CFU mL^{-1})] using a spectrophotometer.

Seven days after transplantation, plants were inoculated by spraying the bacterial suspension at ~1×10^8 CFU·mL^{-1}, and kept in a humid chamber for 24 h before and after inoculation (MARCUZZO et al., 2009).

The severity of the disease was evaluated at 3, 6, 9, 12, 15, 18, and 21 days after inoculation (DAI) using a 0–5 scoring scale as described by HAYES et al. (2013), where 0 = no disease; 1 = less than 10 lesions of less than 3 mm in diameter; 2 = individual disease lesions greater than 3 mm in diameter or more than 10 lesions; 3 = large coalesced lesions covering less than 20% of leaf area; 4 = lesions covering 20% to 50% of leaf area; 5 = lesions covering greater than 50% of leaf area.

The area under the disease progress curve (AUDPC) was calculated using the formula shown in Eq. 1 (CAMPBELL; MADDEN, 1990):

$$\text{AUDPC} = \sum\left[\left(\frac{Y_i + Y_{i+1}}{2}\right)(t_{i+1} - t_i)\right] \quad (1)$$

where: Y is the intensity of the disease (score assigned according to the diagram scale used), t is the time (interval between assessments in days), and i is the number of assessments in time.

The AUDPC data were subjected to analysis of variance for individual analysis, and the means were compared by Scott–Knott test at 5% significance. The data obtained in the experiments were statistically analyzed by sowing season as well as within the same season. Furthermore, a joint analysis of the two seasons was carried out to determine the following treatment interactions: (genotype × strain) × sowing seasons, while considering the effect of the treatments and sites as fixed. The means were compared using the Tukey's test at 5% probability with help of the Genes program (CRUZ, 2016). All strains were compared one with another by differentiating within each genotype that was established with the AUDPC data, and means compared using the Scheffé's test at 5% probability.

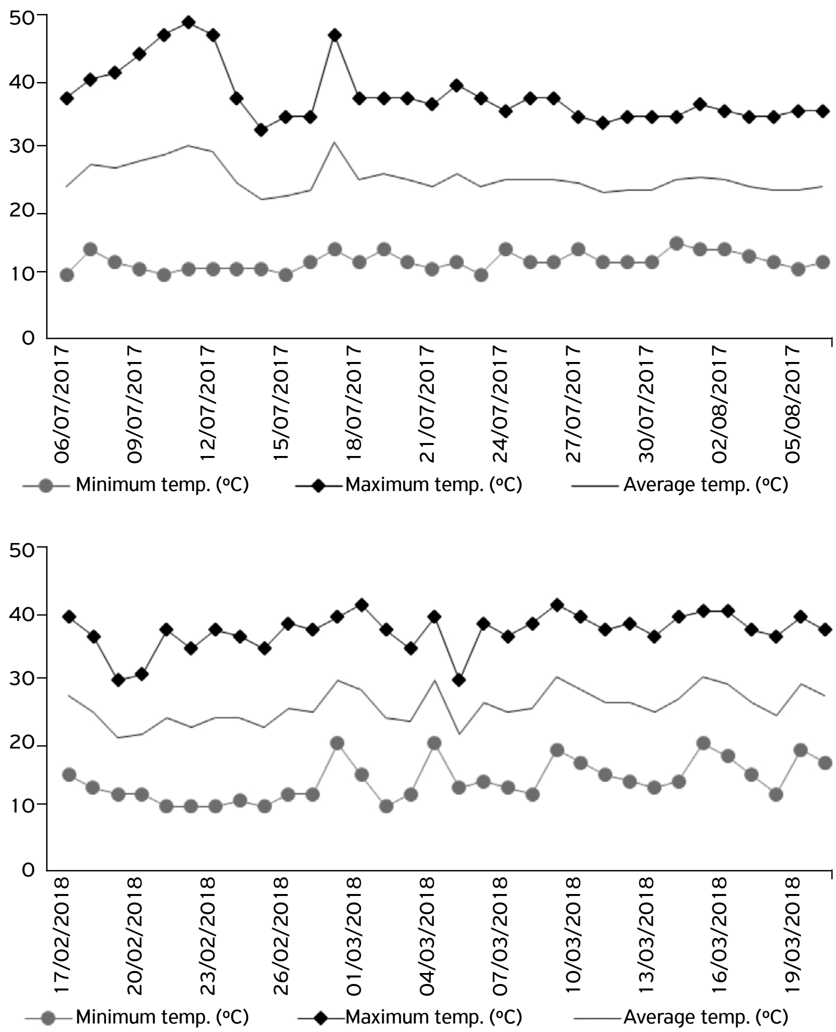

Figure 1. Minimum, average, and maximum temperatures (°C) recorded between transplantation and assessment of disease severity in the greenhouse at Experimental Station of Vegetables, UFU, Campus Monte Carmelo, Minas Gerais; (a) in the first experiment in winter, in 2017; (b) in the second experiment in summer, in 2018.

RESULTS AND DISCUSSION

A summary of the analysis of variance with the mean square, as well as the respective mean values and variation coefficients for the AUDPC were obtained (Table 1).

It was observed that the interaction between genotypes and strains was influenced only in winter, where a significant effect was recorded. Joint analysis showed that the season influenced treatments, due to the significant results, obtained for each evaluated characteristic.

Table 1. Individual and joint variance analyses for the area under the disease progress curve of lettuce bacterial leaf spot to different genotypes and different strains, carried out in two seasons.

Source of variation	DF	Mean square (winter 2017)	Mean square (summer 2018)
Genotypes (G)	4	564.268*	198.769*
Strains (S)	3	2902.283*	23.648**
Strains × Genotypes	12	239.460*	6.362ns
Block	3	67.483**	36.759**
Residue	57	20.66	6.75
Average		21.77	21.41
CV (%)		20.88	12.14
Source of variation	**DF**	**Joint analysis**	
Treatments (G×S)	19	421.73*	
Seasons	1	2.15^{ns}	
Treatments × seasons	19	357.46*	
Block	3	89.65	
Residue	114	13.64	
Average	21.52		
CV (%)		17.15	

CV: coefficient of variation. **and *, significant at the level of 1% and 5%, respectively. ns: not significant (p ≥ 0.05) by the F test. DF: degree of freedom.

The aggressiveness of the strains was individually presented for each genotype at different seasons (Fig. 2).

All *X. campestris* pv. *vitians* strains induced typical leaf spot symptoms. The mean period for the onset of symptoms was between 6 and 9 DAI with the pathogen. In their study, NICOLAS et al. (2019) observed the symptoms of bacterial leaf spot in lettuce 14 DAI.

Summer cultivation resulted in a later onset of symptoms, when compared to with winter cultivation. During the winter period, the maximum temperature ranged from 38 to 50 °C, potentially creating a greater disease severity, while summer the maximum temperatures did not exceed 40 °C (Fig. 1). It is important to mention that in this winter the high temperature was atypical.

Temperature is a fundamental factor in the process of infection by *X. campestris* pv. *vitians*. The optimum temperature for the development of bacterial strains in lettuce was determined to be 22.7 °C based on growth chamber studies (ROBINSON et al., 2006). In contrast, TOUSSAINT (1999) also found the optimal temperature for in vitro growth of *X. campestris* pv. *vitians* to be approximately 28 °C. These data show that environmental conditions directly influence the infiltration of pathogens in the hosts, whereby the disease becomes dependent on favorable climatic conditions for its appearance. For disease to occur depends on the susceptibility of the host, aggressiveness of the pathogens and favorable environmental conditions.

UFU 'Crespa 206' cultivated in winter (Fig. 2a) exhibited relatively slower disease development at approximately 12 DAI. The UFU E125 strain showed greater aggressiveness shortly after 6 days, with a faster rate of disease development compared to others, over time. The UFU F27, UFU F35 and UFU G127 strains presented a slow development of the disease over time, with similar maximum severity. Conversely, summer-cultivated UFU 'Crespa 206' (Fig. 2b) showed higher disease progression after 12 days, with a similar final degree of maximum severity.

The winter-cultivated 'Mimosa' (Fig. 2c) also displayed faster disease development over the first 12 days following inoculation with UFU E125, UFU F27, and UFU F35 strains. These strains exhibited similar behaviors throughout the evaluations, except for UFU F27, which did not display a drastic increase in disease severity over time. Strains used in the summer cultivation (Fig. 2d) presented similar aggressiveness for the duration of the experiment, except for the E125 strain, which behaved differently from the others 12 DAI.

Inoculated winter-cultivated cultivar Robusta (Fig. 2e) displayed a significant increase in disease severity, especially from 6 to 10 DAI, except for the UFU F27 strain, which only displayed a higher degree of aggressiveness after 18 days. Although there was no significant difference in aggressiveness among the UFU E125, UFU F35, and UFU G127 strains, the UFU G127 strain was more expressive than the others after 12 days. Similarly, the G127 UFU strain used during summer (Fig. 2f) behaved differently from the others, but only after 18 DAI, while the rest of the strains behaved in a similar way during the entire disease progression.

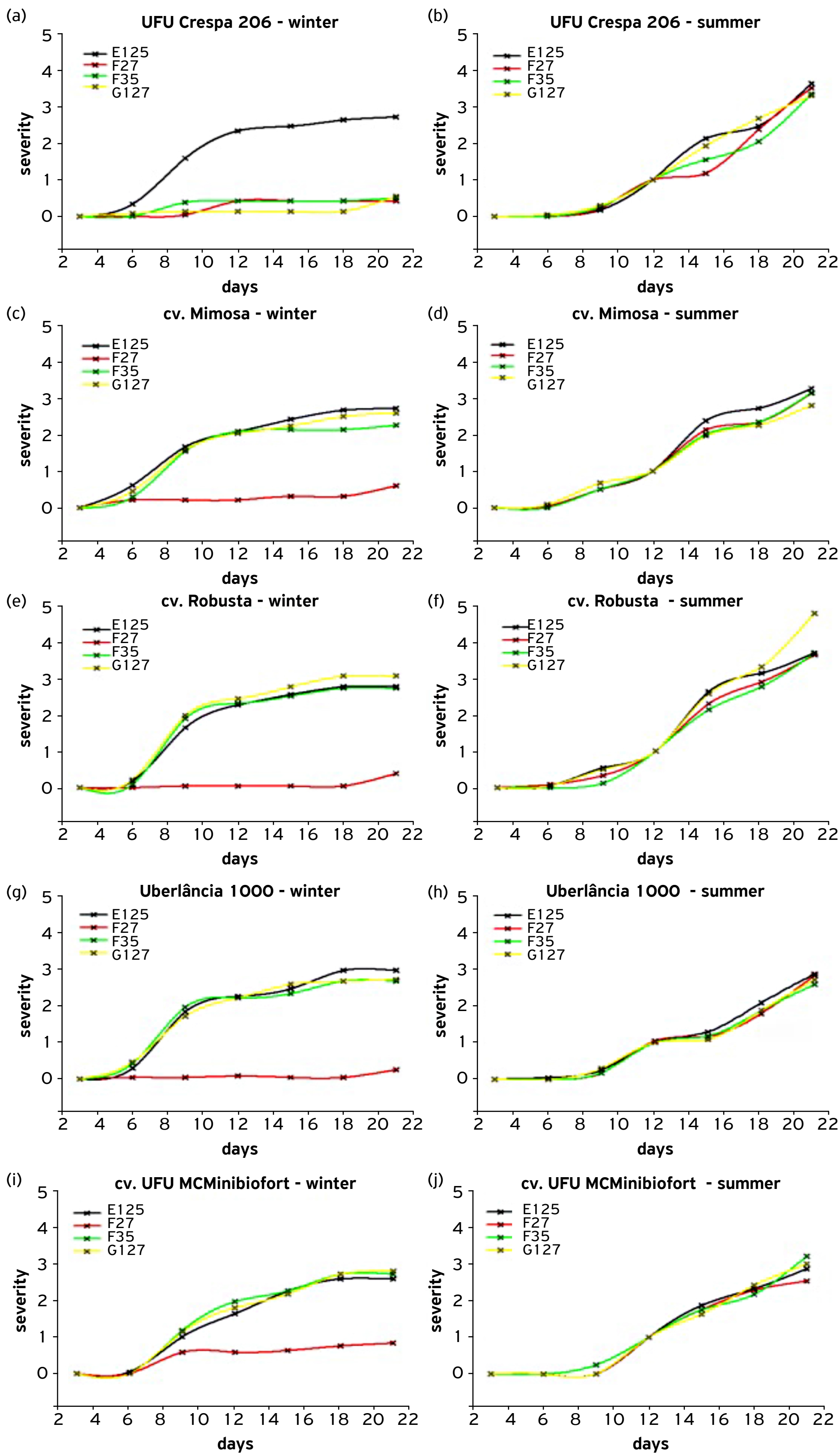

Figure 2. Lettuce bacterial leaf spot progress curve of the different genotypes, different strains in two seasons: UFU 'Crespa 206' (a, b); 'Mimosa' (c, d); 'Robusta' (e, f); 'Uberlândia 10000' (g, h); and UFU 'MCMinibiofort' (i, j). The x-axis data refer to the number of DAI in which the assessments were made.

Winter-cultivated 'Uberlândia 10000' (Fig. 2g) also showed a significant increase in the disease for most of the strains, from 6 to 10 DAI, except for UFU F27, which did not exhibit progression in the disease until 18 DAI. Referring to experiments carried out in summer (Fig. 2h), all strains presented a similar expression of the disease over time.

The UFU E125, UFU F35 and UFU G127 strains also behaved similarly in the winter-cultivated cultivar MCMinibiofort (Fig. 2i) during the days following inoculation. However, inoculation with the F27 strain resulted in an expression of symptoms after 6 days, but with no significant increase in the development of the disease. The strains behaved in a similar way in the summer experiment (Fig. 2j), with no significant difference in disease severity.

Regarding the AUDPC, lettuce genotypes presented significant differences in both seasons (Table 2) regardless of the strain used.

Table 2. Area under the disease progress curve (AUDPC) of lettuce bacterial leaf spot in different genotypes using different strains, in two seasons.

Genotypes	Strains	AUDPC (winter)*	AUDPC (summer)*
UFU 'Crespa 206'	UFU E125	32.06 a	22.66 b
	UFU F27	4.50 b	19.49 a
	UFU F35	5.63 b	19.48 a
	UFU G127	2.56 b	22.66 a
'Mimosa'	UFU E125	32.31 a	24.61 b
	UFU F27	4.63 b	22.65 a
	UFU F35	27.88 a	22.18 a
	UFU G127	30.19 a	22.03 b
'Robusta'	UFU E125	32.13 a	27.47 a
	UFU F27	1.06 b	25.17 a
	UFU F35	32.44 a	23.47 b
	UFU G127	35.69 a	29.27 a
'Uberlândia 10000'	UFU E125	33.81 a	18.27 b
	UFU F27	1.13 b	16.71 a
	UFU F35	32.75 a	16.49 b
	UFU G127	32.94 a	16.79 b
UFU 'MCMinibiofort'	UFU E125	26.38 a	19.92 a
	UFU F27	8.88 b	18.93 a
	UFU F35	28.31 a	20.30 b
		27.56 a	19.62 b
Genotypes		**General Average***	
UFU 'Crespa 206'		11.18 B	21.07 C
'Mimosa'		23.75 A	22.87 B
'Robusta'		25.33 A	26.35 A
'Uberlândia 10000'		25.25 A	17.07 D
UFU 'MCMinibiofort'		22.78 A	19.69 C

*Averages followed by the same lowercase letters in the line and uppercase letters in the column do not differ from each other by the Tukey and Scott–Knott tests, respectively, at the 5% significance level.

In winter, UFU 'Crespa 206' proved to be more resistant when compared to the others, with an average disease progression rate of up to 126.56% lower than that of the other genotypes. The cultivars Robusta, Uberlândia 10000, Mimosa, and MCMinibiofort exhibited similar behaviors, with no significant differences among their average disease progression rates.

The highest variation was obtained among the genotypes cultivated in summer, thus forming the largest number of groups for each evaluated characteristic, resulting in a total of four different groups. 'Uberlândia 10000' presented the highest resistance, a value 54.36% lower than that presented by 'Robusta', which itself displayed greater susceptibility to the pathogen when compared to the others. UFU 'Crespa 206' and 'MCMinibiofort' did not differ from each other, with averages 25.06 and 33.82% lower than the most susceptible cultivar, respectively. 'Mimosa' was also intermediate, with an average value 15.21% lower than 'Robusta'.

Significant differences were observed between the cultivation seasons with respect to the tested strains, in all evaluated genotypes. The AUDPC averages were lower in summer than in winter, regardless of genotype. UFU E125, UFU F35, and UFU G127 strains, in general, showed a disadvantage in summer. UFU F27 was the only strain that exhibited a lower degree of aggressiveness in winter.

The UFU E125 strain in 'Robusta' and 'MCMinibiofort' displayed similar behavior, both during summer and winter. By contrast, pathogen aggressiveness in 'Crespa 206', 'Mimosa' and 'Uberlândia 10000' presented mean values of 41.48, 31.28, and 85.05%, respectively, which were lower in summer than in winter. The inverse was observed in all genotypes when using the UFU F27 strain, which was less favored in winter. In summer, the average was up to 5.44 times higher (in 'Robusta') than in winter.

The UFU F35 strain in 'Mimosa' proved to be indifferent to the seasons, while summer averages were 38.21, 98.60, and 39.46% in 'Robusta', 'Uberlândia 10000', and 'MCMinibiofort', respectively, thereby being simultaneously lower than in winter. The pathogen showed a disadvantage in winter only in UFU 'Crespa 206', while the summer average was 3.46 times higher.

Similarly, UFU G127 also produced lower averages in winter-cultivated UFU 'Crespa 206', while presenting an average 8.85 times higher in summer. However, the pathogen was indifferent to the seasons in 'Robusta', with statistically equal averages. It presented mean values 37.04, 96.18, and 40.46% lower in summer than in winter in 'Mimosa', 'Uberlândia 10000', and 'MCMinibiofort', respectively.

The contrasts used in the comparison of the treatment mean values using the Scheffé's test (Table 3) were as follows: comparison of UFU E125 strain versus the other strains (C1), a comparison of UFUF27 strain versus the others (C2), comparison of UFUF35 strain versus the others (C3), and comparison of UFUG127 strain versus the others (C4).

Table 3. Coefficients and estimation of contrasts with their respective significance.

	Genotypes	Contrast of interest of UFU strains	Estimation of contrasts	
			Winter	Summer
C1	UFU 'Crespa 206'	[(E125) – (F27 + F35 + G127)/3]	27.83**	2.12^{ns}
C2		[(F27) – (E125 + F35 + G127)/3]	–8.92**	–2.11^{ns}
C3		[(F35) – (E125 + F27 + G127)/3]	–7.41**	–2.12^{ns}
C4		[(G127) – (E125 + F27 + F35)/3]	–11.50**	2.12^{ns}
C1	'Mimosa'	[(E125) – (F27 + F35 + G127)/3]	11.41**	2.33^{ns}
C2		[(F27) – (E125 + F35 + G127)/3]	–25.49**	–0.29^{ns}
C3		[(F35) – (E125 + F27 + G127)/3]	5.50*	–0.92 ^{ns}
C4		[(G127) – (E125 + F27 + F35)/3]	8.58**	–1.12^{ns}
C1	'Robusta'	[(E125) – (F27 + F35 + G127)/3]	9.06**	1.50^{ns}
C2		[(F27) – (E125 + F35 + G127)/3]	–32.36**	–1.56^{ns}
C3		[(F35) – (E125 + F27 + G127)/3]	9.48**	–3.84*
C4		[(G127) – (E125 + F27 + F35)/3]	13.81**	3.90*
C1	'Uberlândia 10000'	[(E125) – (F27+ F35+ G127)/3]	11.53**	1.61^{ns}
C2		[(F27) – (E125+ F35+ G127)/3]	–32.03**	–0.47^{ns}
C3		[(F35) – (E125+ F27+ G127)/3]	10.12**	–0.76^{ns}
C4		[(G127) – (E125+ F27+ F35)/3]	10.37**	–0.37^{ns}
C1	UFU 'MCMinibiofort'	[(E125) – (F27+ F35+ G127)/3]	4.79^{ns}	0.30^{ns}
C2		[(F27) – (E125+ F35+ G127)/3]	–18,54**	–1.02^{ns}
C3		[(F35) – (E125+ F27+ G127)/3]	7,37**	0.81^{ns}
C4		[(G127) – (E125+ F27+ F35)/3]	6,37*	–0.09^{ns}

**and *: significant at 1% and 5%, respectively. ^{ns}: not significant, by the Scheffé's test.

No significant effect was recorded in the summer-cultivated genotypes, except cultivar Robusta, where significant differences were observed between UFU F35 and UFU G127, with the UFU G127 strain showing a percentage variation greater than 19.81% in relation to UFU F35.

Most genotypes cultivated during winter exhibited differences between the strains. UFU E125 strain was more aggressive in UFU 'Crespa 206', 'Mimosa' and 'Uberlândia 10000', as compared to the others. Furthermore, UFU E125 was 12.52 times higher for AUDPC than the less aggressive strain (G127) in UFU 'Crespa 206'. For 'Mimosa' and 'Uberlândia 10000', UFU E125 was 6.98 and 29.92 times higher for AUDPC, respectively, than that of the UFU F27 strain, thereby presenting the lowest mean aggressiveness, with a respective differences of 27.68 and 32.69%.

The UFU G127 strain differed from the others in the cultivar Robusta, unlike in UFU Crespa 206, by being more aggressive with a difference of 34.63% when compared to the UFU F27, which presented 33.66 times less aggressiveness.

In 'MCMinibiofort', the UFU E125 strain showed no significant difference when compared to other strains, while UFU F35 showed a maximum average 68.63% higher than UFU F27, which again showed the lowest mean.

The goal of breeding bacterial leaf spot-resistant lettuce cultivars is to develop cultivars with high-level resistance that can protect lettuce from damage by *X. campestris* pv. *vitians* strains that are highly aggressiveness. The aggressiveness diversity of *X. campestris* pv. *vitians* strains and variation of host responses among lettuce genotypes are very important epidemiological aspects for understanding and controlling bacterial leaf spot of lettuce outbreaks (WANG et al., 2015).

The genotypes obtained in the UFU's Biofortified Lettuce Breeding program were different from those of commercial cultivars. 'Uberlândia 10000' proved to be more resistant to the pathogen when cultivated in summer, while higher pathogen resistance was observed in UFU Crespa 206 when cultivated in winter. In general, the summer season was unfavorable for disease development. During the winter period, the maximum temperature ranged from 38 to 50 °C, potentially creating a greater disease severity, while summer the maximum temperatures did not exceed 40 °C. It is important to mention that in this winter the high temperature was atypical. In the winter cultivation, the highest aggressiveness levels were observed when using the UFU E125 strain in the UFU Crespa 206 and cultivars Mimosa and Uberlândia 10000, while the lowest aggressive levels were found in the F27 strain, except for UFU Crespa 206.

CONCLUSIONS

The biofortified lettuce 'Uberlândia 10000' was more resistant to most bacterial strains in the summer cultivation, and in the winter period UFU 'Crespa 206'. The commercial cultivar Robusta was the most susceptible to the strains during both seasons. The UFU E125 strain was the most aggressive for most genotypes in both seasons.

AUTHORS' CONTRIBUTIONS

Conceptualization: Maciel, G.M.; Tebaldi, N.D.; Oliveira, B.S. **Funding acquisition:** Maciel, G.M. Methodology: Maciel, G.M.; Tebaldi, N.D.; Siquieroli, A.C.S.; Oliveira, B.S. **Project administration:** Maciel, G.M.; Oliveira, B.S.; Tebaldi, N.D.; Clemente, A.A.; Ribeiro, A.L.A. **Writing – review & editing:** Maciel, G.M.; Oliveira, B.S.; Tebaldi, N.D.; Finzi, R.R.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable

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