https://doi.org/10.1590/1678-992X-2022-0100

ISSN 1678-992X

SCIENTIA AGRICOLA

Research article

Pathogenicity of *Fusarium oxysporum* f. sp. *lactucae* in lettuce cultivars at different temperatures

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Edited by: José Belasque Junior

Received June 01, 2022
Accepted October 01, 2023

ABSTRACT: Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae* Race 1 was first detected in lettuce in the Region of Murcia (Spain) in 2017, in plantations in the northwestern area, where the crop is grown in the summer. In order to establish control strategies against the disease, studies have been carried out under controlled conditions with influence of temperature (25 °C and 28 °C), aggressiveness of isolates (four isolates from Race 1), and behavior of commercial cultivars (three susceptible cultivars and four not susceptible). In our study, all susceptible cultivars died at both temperatures for all the isolates, while the temperature of 25 °C was lethal for only one plant of a non-susceptible cultivar. The plants of non-susceptible cultivars that did not die presented index symptoms within a range from 1.7 to 3.7 according to cultivars and isolates; however, at 28 °C, all plants of the non-susceptible cultivars were infected, with 30 % to 100 % of plants dead. The results suggest that temperature affects the disease and that, in highly contaminated areas where the lettuce crop is grown in hot periods, management of the disease requires strategies complementary to using non-susceptible or resistant cultivars.
Keywords: soil fungi, disease, horticultural crops

Introduction

Lettuce (*Lactuca sativa* L.) is one of the leading horticultural crops in Spain with more than one million tons produced in 35,168 ha in 2020. In the Region of Murcia, the lettuce crop is located in areas of the Valle del Guadalentín, 6,257 ha; Campo de Cartagena, 4,164 ha; Altiplano, 1,216; Vega del Segura, 664 ha; and in the Northwest, 568 ha, which produced 430,459 t (CARM, 2021). Cropping begins in Campo de Cartagena, Vega del Segura, and Valle del Guadalentín in mid-Sept, stretching until the end of Mar and until Nov in the Altiplano. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lactucae* (Matuo and Motohashi,1967) is considered the most important soil-borne pathogen of lettuce (Farr and Rossman, 2020; Matheron and Gullino, 2012). The disease was first identified in Japan in 1955 (Matuo and Motohashi, 1967) and after in California (USA) (Hubbard and Gerik, 1993), Taiwan (Li et al., 2014), Iran (Millani et al., 1999), Italy (Garibaldi et al., 2002), Brazil (Ventura and Costa, 2008; Cabral et al., 2014), Argentina (Malbrán et al., 2014), Belgium (Claerbout et al., 2018), the Netherlands (Gilardi et al., 2017a), France (Gilardi et al., 2017b), Egypt (El-Sayed et al., 2018), England, Ireland (Taylor et al., 2019), Florida (Murray et al., 2020) and Norway in 2021 (Herrero et al., 2021). The disease causes leaf yellowing and wilting of developed plants, vascular obstruction, and, finally, plant death. Race 1 is the most commonly found in Asia, Europe, and the Americas (Farr and Rossman, 2020). Races 2 and 3 have only been cited in Japan and Taiwan (Fujinaga et al., 2001, 2003; Li et al., 2014), and Race 4 has recently emerged in several European countries (Claerbout et al., 2018; Gilardi et al., 2019; Taylor et al., 2019). *Fusarium* wilt was first identified in Spain in 2017 in samples of plants from crops in the Murcia region (Guerrero et al., 2020). Temperature is an essential factor for the disease development. *In vitro* growth range of *Fusarium* was between 8-32 °C, with the optimum at 28 °C Hubbard and Gerik (1993). The hypothesis that Race 4 can infect at lower temperatures than Race 1 (Gilardi et al., 2021) endangers lettuce-growing areas nationwide if Race 4 moves into other areas. However, differences have been observed in the effects on cultivars against the disease in crop areas where Race 1 is prevalent. Host genetic resistance is one of the most crucial control strategies (Matheron et al., 2005; Gilardi et al., 2014; Gordon and Koike, 2015). Thus, the objective of this study was to determine the effect of *Fusarium oxysporum* f. sp. *lactucae* in different cultivars of lettuce commonly grown in south-eastern Spain, under controlled conditions, at two temperature levels and inoculation methods, against Race 1 isolates obtained from crops in the Region of Murcia.

Materials and Methods

The trials were carried out using four *F. oxysporum* f. sp. *lactucae* Race 1 isolates, with susceptible and non-susceptible cultivars, inoculated at two temperatures and two inoculation methods. At first, three cultivars were compared (one susceptible and two non-susceptible) at two temperatures (25 °C and 28 °C), and after, the behavior of seven cultivars (three susceptible and four non-susceptible) was assessed at 28 °C.

Inoculum production

The four isolates used were obtained from infected plants harvested in a commercial field in the region of Murcia, northwestern Spain, at latitude 38°05′58.6″ N, 1°52′05.4″ W, 0 m altitude, between May and Aug 2018 and 2019. Isolates A1 and A3 from the Duende cultivar (Rijk Zwaan) and isolates A2 and A4 from the Sena cultivar (Rijk Zwaan). Portions of roots and plant stems with symptoms were sown in Komada medium (Komada, 1975) and incubated at 25 °C for 4 to 6 d. The colonies formed were replicated in potato dextrose agar medium (Difco S.A.). A micellar solution with a concentration of 10^6 CFU mL^{-1} was obtained by grinding the content of one dish in 100 mL of distilled sterile water and subsequent adjustment by dilution and direct count using a Neubauer counting chamber. *Fusarium* colonies were transferred to PDA and Spezieller Nahrstoffarmer agar (SNA) media (Garibaldi et al., 2004b) for morphological identification. They were identified as *Fusarium oxysporum* based on the morphology on SNA (Leslie and Summerell, 2006). Macroconidia were straight to slightly curved, with one or two septum and spores measuring 19.1 × 4.1 µm (n = 30) or two septa and spores measuring 22.8 × 4.9 µm (n = 30). Microconidia were borne on short monophialides in false heads, ovoid to reniform, and were 11.5 × 3.3 µm (n = 30). Chlamydospores were mostly single, terminal, and intercalary, measuring 10.8 µm (n = 30). The translation elongation factor-1α (EF-1α) gene of ten representative isolates was sequenced using EF-1/EF-2 primer pairs (O'Donnell et al., 1998). All EF-1α sequences were identical; one (isolate Fm1) was deposited in GenBank (accession n° MN379455). BLASTn comparison showed 100 % similarity with the EF-1α sequence of *F. oxysporum* f. sp. *lactucae* (KY009874). A comparison of this sequence in the *Fusarium* ID database (http://fusarium.mycobank.org/) exhibited identical homology. Pathogenicity of the isolates was determined by inoculation in plants of susceptible cultivars Odessa (Clausse- Tèzier Ibérica) and Chiquina (Syngenta Seeds). Disinfected seeds were sown into each plastic pot (one per pot, 500 mL capacity). The pots were filled with a steam-sterilized mixture containing a 3:1 mixture of peat/perlite). Plants with 3-4 leaves were inoculated by immersing the roots in a suspension of spores at 10^6 CFU mL^{-1}. Pots with inoculated plants were kept in a controlled-temperature room (25 °C, relative humidity 60-90 %, 14 h light conditions). Isolates of plants with symptoms were re-isolated, meaning that the isolates were of *F. oxysporum* f. sp. *lactucae*. For the identification of races, we used specific primers Hani3′ and Hanilatt3rev (Pasquali et al., 2005), which produced a 183-bp product specific for *Fusarium oxysporum* f. sp. *lactucae* Race 1, and specific primers for Race 4, FPUF and FPUR (Gilardi et al., 2017a).

The inoculum was prepared in 9 cm-diameter Petri dishes with PDA medium in an incubator at 25 °C until the colonies covered the medium surface. The spores were gathered by washing the medium surface with the fungus developed using sterile distilled water. Conidial concentration was determined using hemocytometry to a concentration of 10^6 spores mL^{-1}.

Three cultivars were tested at 25 °C: 'Metalia' (susceptible, Iceberg, Nunhems); 'Saula' (non-susceptible, Iceberg, Enza Zaden); and 'LI079' (non-susceptible, Iceberg, Meridiem Seeds). Seven cultivars were tested at 28 °C: MS 86 (non-susceptible, Iceberg, Meridiem Seeds); LI079, Menfus (non-susceptible, Iceberg, Nunhems); Nun216 (non-susceptible, Iceberg, Nunhems); Metalia (Iceberg); Chiquina (susceptible, Romaine, Syngenta Seeds); and Odessa (susceptible, Romaine, Clause Tézier Ibérica SA). Susceptible and non-susceptible cultivars were inoculated by irrigation and immersion at both temperatures, ten plants of each cultivar. At 25 °C, plants were inoculated with isolates A1 and A2 and at 28 °C with isolates A1, A2, A3, and A4.

Seeds were disinfected by submersion in a solution of sodium hypochlorite 2 % for 20 min, then washed with distilled water and sown in plastic pots (one per pot, 500mL). The pots were filled with a steam-sterilized mixture containing a 3:1 mixture of peat/perlite. Pots were kept in a controlled temperature room (25 °C, relative humidity 60-90 %, conditions and 14 h light photoperiod). The plants were watered three times a week, and fertilizer was added once a week with N-P-K dissolution with micronutrients. After disinfection, 50 seeds of each cultivar were sown in a PDA medium and kept in an incubator at 25-28 °C for ten days.

The four isolates were inoculated in lettuce plants under controlled conditions following the protocol by Pasquali et al. (2005). Fifteen-day-old plants were inoculated by irrigation and immersing their roots with 5 mL of conidia suspensions (10^6 CFU mL^{-1}). For the inoculation by immersion, the roots were kept in conidial suspension for 30 min.

Plants were irrigated with 5 mL of the conidia suspension, or the roots were immersed in the spore suspensions for 30 min. The inoculated plants (10 per isolate, cultivar, and treatment) were kept in controlled light and temperature conditions at 25 °C ± 0.2 and 28 °C ± 0.44 °C and 14 h photoperiod in growth chambers. The control comprised 10 plants for each cultivar using sterile water by irrigation and immersion. Disease progress was measured using the same scale as Pasquali et al. (2005), every five days, from 30 days after inoculation and transplant, using a scale of symptoms from 0 to 5, where 0 = no symptoms; 1 = slight stunting, no yellowing; 2 = minor wilt and stunting, yellowing (10 to 30 % leaves); 3 = moderate wilt, stunted, yellowing (30 to 60 % leaves); 4 = severe wilt, stunted, yellowing (60 to 90 % leaves); and 5 = dead (no green leaves).

Data analysis

The data analysis was performed using the STATGRAPHICS software package. The ANOVA compared the percentage of dead plants, transforming the data with arcsine of $\sqrt{x+1}$ and the LSD test to compare the means. The symptom indices were compared with the ANOVA and the LSD test for the differences between the means.

Results

Effect of temperature on disease severity

Fungal colonies were not found in the disinfected seeds sown in Petri dishes with a PDA medium. The plants not inoculated (control) remained healthy throughout the corresponding trial periods at both temperatures. At 25 °C, all plants of cultivar Metalia (susceptible) died when they were inoculated by immersion with the two isolates (A1 and A2). They presented the highest symptoms index with no differences between isolates, but none of the non-susceptible cultivars died, although they had symptoms when inoculated by immersion (Table 1). Most Metalia plants died when inoculated by irrigation than when inoculated by immersion, with no differences in the symptom index. All plants of the non-susceptible cultivars survived, except for LI079 when inoculated with isolate A2 by irrigation of the substrate. On the other hand, symptom indices were lower in A1 for other non-susceptible cultivars, except for Saula with immersion (Table 1). The fungus was isolated in all plants with symptoms, with no asymptomatic infections detected when the reisolates were performed on healthy (symptomless) plants at the trial end.

At 28 °C, the three cultivars presented higher disease incidence and severity than at 25 °C, with similar results for both isolates. A smaller numbers of dead plants and lower severity were observed for LI079 inoculated by immersion of the roots, compared with the other two cultivars for both inoculation methods (Table 1).

All inoculated plants became infected, regardless of the cultivar, isolate, and inoculation method. A high proportion of plants of the non-susceptible cultivars died, with the highest symptom index observed for plants inoculated at 28 °C (Table 1). All plants of the three susceptible cultivars (Odessa, Chiquina, and Metalia) died after inoculation at 28 °C with the four isolates, both by root immersion and irrigation of the substrate, presenting the maximum symptom index (Table 2). At 28 °C, most cultivars died (70-100 %), both susceptible and non-susceptible, with all isolates and both methods, except for L1079, which had lower mortality with isolates A1 (50-70 %) and A2 (30-70 %) (Tables 1 and 2). At 25 °C, most susceptible cultivars died (80-100 %), both susceptible (Metalia) and non-susceptible cultivar Saula, but the non-susceptible cultivar L1079 only died 10 % with isolate A2 by irrigation (Table 1).

At 25 °C, the first symptoms appeared two days later when the inoculation was by substrate irrigation in the susceptible cultivars and four days later in the non-susceptible cultivars (Table 3). In the non-susceptible cultivars, the first symptoms appeared ten or more days later than in the susceptible cultivars. At 28 °C, the first symptoms appeared ten or more days later and at the same time for both inoculation methods, with the non-susceptible cultivars taking two days longer for symptoms to appear than in the susceptible cultivars (Table 3), regardless of the isolate, and faster than at 25 °C.

Table 1 – Reaction of three commercial lettuce cultivars to two isolates of *Fusarium oxysporum* f. sp. *lactucae* Race 1 inoculated by irrigation or immersion of the roots at 25 °C and 28 °C, expressed as dead plants (%) and severity.

Cultivars	Isolate	Inoculation	Dead plants(%)[1]		Severity[2]	
			25 °C	28 °C	25 °C	28 °C
Metalia (susceptible)	A1A2	Irrigation	80.0 b/NS 90.0 b/NS	100.0 d/NS 100.0 d/NS	4.4 (0.13) d/NS 4.9 (0.10) d/NS	5.0 (0.0) f/NS 5.0 (0.0) f/NS
Saula (non-susceptible)	A1A2		0.0 a/A 0.0 a/A	80.0 cd/B 90.0 cd/B	2.5 (0.34) ab/A 3.7 (0.21) c/A	4.8 (0.36) def/B 4.9 (0.19) ef/B
LI079 (non-susceptible)	A1A2		0.0 a/A 10.0 a/A	80.0 cd/B 70.0 bc/B	2.3 (0.30) ab/A 3.1 (0.27) bc/A	4.8 (0.19) def/B 4.7 (0.38) cde/B
Metalia (susceptible)	A1A2	Immersion	100.0 b/NS 100.0 b/NS	100.0 d/NS 100.0 d/NS	5.0 (0.0) d/NS 5.0 (0.0) d/NS	5.0 (0.0) g/NS 5.0 (0.0) g/NS
Saula (non-susceptible)	A1A2		0.0 a/A 0.0 a/A	70.0 bc/B 80.0 cd/B	3.4 (0.22) bc/A 3.1 (0.29) bc/A	4.7 (0.17) cde/B 4.8 (0.36) def/B
LI079 (non-susceptible)	A1A2		0.0 a/A 0.0 a/A	50.0 ab/B 30.0 a/B	1.7 (0.54) a/A 3.5 (0.30) bc/NS	4.4 (0.74) b/B 4.0 (0.70) a/NS

[1]Means values (n = 10) followed by a different letter in each column were different according to Fisher's LSD ($p \leq 0.05$). The uppercase letters after "/" are the differences between temperatures within the same row for dead plants and severity symptoms. NS = means not statistical differences. Severity evaluated based on the symptom index: 0 = no symptoms; 1 = slight stunting, no yellowing; 2 = minor wilt and stunting, yellowing (10 to 30 % leaves); 3 = moderate wilt, stunted, yellowing (30 to 60 % leaves); 4 = severe wilt, stunted, yellowing (60 to 90 % leaves); and 5 = dead (no green leaves).

Table 2 – Reaction of seven commercial lettuce cultivars to four isolates of *Fusarium oxysporum* f. sp. *lactucae* Race 1 inoculated by irrigation or immersion of the roots at 28 °C, expressed as dead plants (%) and severity.

Cultivars	Isolate	Irrigation		Immersion	
		Dead plants (%)[1]	Severity[2]	Dead plants (%)[1]	Severity[2]
MS 86 (non-susceptible)	A1	100.0 d	4.9 (0.19) cd	90.0 cd	4.9 (0.19) de
	A2	90.0 cd	4.9 (0.19) cd	90.0 cd	4.9 (0.19) de
	A3	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A4	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
LI079 (non-susceptible)	A1	70.0 abc	4.7 (0.55) abc	60.0 ab	4.5 (0.74) ab
	A2	80.0 bcd	4.8 (0.38) bcf	70.0 abc	4.7 (0.55) bcd
	A3	100.0 d	5.0 (0.0) d	80.0 bc	4.8 (0.38) cde
	A4	100.0 d	5.0 (0.0) d	80.0 bcd	4.7 (0.19) bcd
Num 216 (non-susceptible)	A1	90.0 cd	4.9 (0.19) cd	70.0 abc	4.6 (0.51) abc
	A2	90.0 cd	4.9 (0.19) cd	90.0 cd	4.9 (0.19) de
	A3	100.0 d	5.0 (0.0) d	90.0 cd	4.9 (0.19) de
	A4	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
Menfus (non-susceptible)	A1	70.0 abc	5.0 (0.0) d	80.0 bcd	4.8 (0.36) cde
	A2	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A3	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A4	100.0 d	5.0 (0.0) d	80.0 bcd	4.8 (0.38) cde
Odessa (susceptible)	A1	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A2	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A3	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A4	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
Chiquina (susceptible)	A1	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A2	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A3	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A4	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
Metalia (susceptible)	A1	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A2	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A3	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e
	A4	100.0 d	5.0 (0.0) d	100.0 d	5.0 (0.0) e

[1]Means values (n = 10) followed by a different letter in each column were different according to Fisher's LSD ($p \leq 0.05$). [2]Severity evaluated based on the symptom index: 0 = no symptoms; 1 = slight stunting, no yellowing; 2 = minor wilt and stunting, yellowing (10 to 30 % leaves); 3 = moderate wilt, stunted, yellowing (30 to 60 % leaves); 4 = severe wilt, stunted, yellowing (60 to 90 % leaves); and 5 = dead (no green leaves).

Table 3 – Time (days) after inoculation for the first disease symptoms to appear.

Cultivar	Isolate	Inoculation by immersion		Inoculation by irrigation	
		25 °C	28 °C	25 °C	28 °C
Metalia	A1 and A2	12	7	14	7
Saula	A1 and A2	22	9	26	9
MS86	A1 and A2	22	9	26	9

Discussion

In the present study, we demonstrated that, at 28 °C, Fusarium wilt develops faster in susceptible and non-susceptible cultivars, regardless of the Race 1 isolates tested or the inoculation method. More inoculated plants died at that temperature than at 25 °C. Hubbard and Gerik (1993) determined the isolate pathogenicity from Californian lettuce crops and the behavior of cultivars when inoculated plants were kept at 25 °C, having determined that 28 °C was the optimal temperature for the micellar growth of the fungus.

The temperature effect was more substantial on cultivars considered non-susceptible than on the susceptible ones. At both temperatures, the isolates caused the death of most susceptible cultivar plants, while the non-susceptible cultivar plants only died at 28 °C. Differences were found in the aggressiveness between Race 1 and Race 4 isolates, depending on the cultivars used when inoculated at temperatures below 25 °C. Race 4 isolates were more aggressive at 20 °C or below, producing symptoms and causing plant death at 11 °C, a temperature at which Race 1 isolates were not pathogenic, according to Gilardi et al. (2021). The authors found variations in disease severity between isolates of both races in one of the three susceptible cultivars at temperatures below 16 °C.

The differences in the symptom index in isolates from Spain are like those highlighted by Gilardi et al. (2021) with European isolates in some cultivars. Differences were not very pronounced when inoculated with isolates of Race 4. Similar results were obtained by Scott et al. (2010a) in trials carried out at temperatures

like those of commercial crops, using isolates of Race 1. The authors found differences in symptoms, which were more frequent at hot temperatures than in cold periods. Disease incidence was related to the season that the crop is established, as it was lower if established in autumn than in the spring or summer (Matheron et al., 2005).

The isolates used in our trial were obtained from adult lettuces from an area where lettuce is harvested in the summer, with high temperatures, and where disease incidence causes plant losses of over 60 %. The origin of the isolates may explain the high mortality in susceptible cultivars and high aggressiveness at 28 °C on non-susceptible cultivars. For Californian isolates, Scott et al. (2010b) noted that symptom severity in the Salinas cultivar, in crop conditions of hot periods (32-23 °C day/night), was higher than in warm periods (28-20 °C) and more than the double than in cool periods (23-18 °C). Hubbard and Gerik (1993) considered this cultivar as having low susceptibility by when inoculated with an isolate of the same origin as Scott et al. (2010a) used.

The behavior of inoculated cultivars at 28 °C was homogeneous for susceptible cultivars and varied for non-susceptible ones, depending on the isolate and inoculation method. For L1079, variations were observed when inoculated with isolates A1 and A2 by immersion and only for A1 when inoculated by irrigation. For Num216, differences were only observed in inoculation by immersion with A1 and for Menfus when inoculated by irrigation with isolate A1.

In inoculation by immersion, we found results for non-susceptible cultivars similar to those obtained by several authors who used the same inoculation method. We found similar disease severity when inoculating cultivars Lollo Rossa or Red Rossa at three temperature ranges (32-23 °C day/night; 28-20 °C and 23-18 °C), as they have low susceptibility when compared to the cultivars Salinas, Kahuna or Early Queen Scott et al. (2012). Our results for susceptible cultivars at 28 °C were like those of Gilardi et al. (2021) for cultivars Revisa, Romana Verde, and Descartes inoculated at 30 °C.

Disease severity seems to be related to the cultivar characteristics, to isolate aggressiveness, and to inoculation conditions for the same race of inoculum virulence and density (Garibaldi et al., 2004b; Scott et al., 2010b; Gilardi et al., 2021; Cabral and Reis, 2013; Cabral et al., 2019; Murray et al., 2021). Inconsistency between disease development and the susceptibility of the cultivars for trials carried out in different periods (Oct, Nov, Dec) or temperature ranges was observed by Cabral et al. (2014). The characteristics of resistance or non-susceptibility do not seem to vary between groups or cultivar types (Romaine, Lollo, Iceberg, Batavia, etc) (Garibaldi et al., 2004b; McCreight et al., 2005; Scott et al., 2010b; Michelmore et al., 2017; Cabral et al., 2019; Seki et al., 2020; Slinski et al., 2020). In our study, differences were also found regarding symptom severity among non-susceptible cultivars, despite all belonging the Iceberg group.

Management of Fusarium wilt in lettuce in areas where the crop is grown in hot periods requires the introduction of integrated strategies (Matheron and Gullino, 2012). The use of non-susceptible cultivars seems compromised by the high soil temperatures during the summer. Isolates of Race 4 are more aggressive at lower temperatures than those of Race 1 (Gilardi et al., 2019).

Using pathogen-free seeds is an essential preventive measure to avoid infestation of nurseries and cultivated areas (Garibaldi et al., 2004a). The introduction and development of the crop in cool temperature periods is an effective way of mitigating the damage caused by the disease (Matheron and Porchas, 2010), based on the aggressiveness levels shown by isolates of Race 1 both in susceptible and non-susceptible cultivars. The inoculum reduction in the soil in pre-planting has shown to be effective in controlling the disease, given the variability in the behavior of the cultivars. Soil solarization has shown to be effective in controlling Fusarium wilt in the state of Arizona (USA) (Matheron and Porchas, 2010). With animal manure, or agrifood by-products used in the biodisinfestation, we have achieved significant reductions in disease incidence.

Isolates from the region of Murcia in northwestern Spain have shown to be more aggressive for both susceptible and non-susceptible cultivars at 28 °C, in relation to their geographical origin and the period when the lettuce crop is grown. Most inoculated plants died at 28 °C, suggesting that integrated strategies should be established in warmer areas of lettuce cultivation. Pathogen-free seeds or nursery plants, long-term crop rotation, and inoculum reduction in pre-planting are all complementary practices to non-susceptible cultivars adapted to warmer growing conditions to reduce disease development.

Acknowledgments

This work was supported by the FEDER1420-31 project and PO FEDER 2021-2027. Development of sustainable models of agricultural, livestock, and aquaculture production. Maintenance of the sustainability of lettuce cultivation in the Region of Murcia against the recently introduced pathogen *Fusarium oxysporum f.* sp. *lactucae*

Authors' Contributions

Conceptualization: Guerrero MM. **Data curation:** Guerrero MM, Martínez V, Lacasa CM, Martinez MC. **Formal analysis:** Guerrero MM, Martínez V, Lacasa CM. **Funding acquisition:** Guerrero MM, Monserrat A. **Investigation:** Guerrero MM. **Data curation:** Guerrero MM, Martínez V, Lacasa CM. **Software:** Guerrero MM**. Project administration:** Guerrero MM, Monserrat A. **Resources:** Guerrero MM, Martínez V, Lacasa CM. **Visualization:** Guerrero MM. **Writing-original draft:** Guerrero MM. **Writing-review & editing:** Guerrero MM.

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