

Description of the relationship between trunk disease expression and meteorological conditions, irrigation and physiological response in Chardonnay grapevines

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ABSTRACT

Esca disease and *Botryosphaeria* dieback are currently considered as serious grapevine diseases which affect vineyard health and induce economic losses. Both of these trunk diseases (GTDs) are caused by a complex of pathogens, and foliar expression is influenced by several factors, including environmental factors such as water stress. To manage water stress in some vine areas, culture practice based on irrigation systems for limiting water stress have been developed; however, little knowledge of the influence of such systems on GTD emergence is currently available. The present paper addresses the impact of irrigation systems and climatic factors (rainfall and temperature) on the expression of GTDs, specifically esca and *Botryosphaeria* dieback. A field experiment on Chardonnay in North East Spain, a vine growing area where drought is present and which is managed by an irrigation system, was therefore carried out during a 3-year period. The water stress impact on GTD expression was evaluated by measuring the GTD incidence and analysing different physiological parameters at different phenological stages, including principal component analysis and gene expression. The main finding of this study was the significant roles of vine transpiration and water availability, which depend on irrigation volume and rainfall amount; together, they may explain the erratic symptom expression in plants infected by GTD fungi depending on the year. All these parameters are discussed to better understand the relationship between GTD expression, irrigation system and climatic factors.

KEYWORDS

irrigation, Chardonnay, grapevine trunk diseases, physiology, rainfall, vineyard,

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INTRODUCTION

Grapevine (*Vitis vinifera*) in vineyards is subject to multiple stresses, either biotic or abiotic, and frequently in combination. Depending on their incidence, they can negatively impact vine health by affecting its growth and physiology, and in turn yield, thus inducing economical losses. In terms of biotic stress, grapevine trunk diseases, namely GTDs, have been described as serious diseases causing a decline in vine from the nursery (Gramaje and Di Marco, 2015; Gramaje *et al.*, 2018) to the vineyard (Mondello *et al.*, 2018). Among GTDs, Esca disease, *Botryosphaeria* dieback, Eutypa dieback, Black foot and Phomopsis cane and leaf spot are considered to be the most serious diseases (Guerin-Dubrana *et al.*, 2019). The spread and incidence of GTDs depends on several factors, such as environmental conditions, plant characteristics including the cultivar, age, rootstock or clone, and cultural practices (for review Songy *et al.*, 2019, Fischer and Peighami-Ashnaei, 2019). Esca disease and *Botryosphaeria* dieback are widespread worldwide, especially in Europe, including in Spain (De la Fuente *et al.*, 2016; Guerin-Dubrana *et al.*, 2019). These diseases are derived from a complex of fungi, the main ones being *Phaeoconiella chlamydospora* and *Phaeoacremonium minimum*, as well as *Fomitiporia mediterranea* which causes white rot, and other wood-rooting basidiomycetes for Esca disease (Mugnai *et al.*, 1999) and *Botryosphaeria* species for *Botryosphaeria* dieback (Úrbez-Torres, 2011). GTD fungi are wood pathogens which are never found in foliar organs. They gradually colonise the woody tissues of vine, resulting in different types of inner necrosis depending on the GTD pathogen. After an undetermined period of latency, their presence can result in the appearance of different types of foliar and berry symptoms depending on the disease (Mugnai *et al.*, 1999; Mondello *et al.*, 2018).

As previously reported, the intensity of GTD foliar symptoms is closely related to the cultivar; for example, a lower expression has been found in Chardonnay than in Cabernet-Sauvignon (Songy *et al.*, 2019). Expression has also been found to be correlated with wine areas and cultural practices, including the pruning system: there was less expression in Chardonnay in the Champagne area than in Chardonnay cultivated in Burgundy (Grosman and Doublet, 2012). A wine area is defined by several factors, such as soil, climate and specific cultural practices more or less adapted by the winegrowers. With regards to the climate,

we know that temperature and water influence both plant development and the growth and aggressiveness of pathogens, and that any changes can result in a higher expression of foliar symptoms (for review Songy *et al.*, 2019). The responses of vines to either single or combined abiotic stresses are relatively well-described in the literature. These responses have been found to result in greater morphological and physiological damage; for example, a decrease in both weight and leaf area (Edwards and Pascoe, 2001), an alteration in the photosynthetic apparatus (leading to a decline in photosynthesis and carbohydrate synthesis, as well as ROS (reactive oxygen species) production), and the activation of antioxidant mechanisms (Zandalinas *et al.*, 2017). In the context of climate change and considering the importance of heat and water stress, some countries have adapted by, for example, developing irrigation systems to counteract drought. To date, few studies have focused on the relationship between cultural practices using an irrigation system and GTD expression (Songy *et al.*, 2019).

This paper addresses the impact of a culture practice (irrigation to limit water stress) in combination with climatic factors (temperature and rainfall) on the expression of grapevine trunk diseases, especially esca and *Botryosphaeria* dieback. The study was carried out during a three year period in field conditions, in a vineyard located in Spain (cv Chardonnay, Mediterranean climate), where drought was dealt with by using an irrigation system. Different physiological parameters were analysed at different phenological stages, as well as gene expression. All the data is discussed to better evaluate the relationship between GTD expression-irrigation system-climatic factors.

MATERIALS AND METHODS

1. Experimental vineyard plot characteristics and management

The field experiment was conducted in a commercial vineyard located in Barbastró, in the Appellation of Origin “Somontano”, North-East Spain (41° 59' 44.83" N; 0° 8' 9.82" E). This region is characterised by an inland dry Mediterranean climate with hot summers and cold winters, due to the influence of the Pyrenees mountains. The average cumulative rainfall per year is 465 mm, and the mean temperatures in the coldest and hottest months, January and July, are 4.6 °C and 24.4 °C respectively. A summary of basic historical meteorological data from 1944 to 2011 of the Barbastró area is presented in Table 1.

TABLE 1. Historic monthly average temperature and rainfall in the Barbastro area close to the experimental plot.

Historic series	Variable	Units	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
1944-2011	Mean Temperature	°C	4.6	6.6	10.0	12.6	16.9	21.2	24.4	23.9	20.2	15.1	9.0	5.3	14.1
1944-2011	Max. mean Temperature	°C	9.5	12.7	16.7	19.4	23.8	28.5	32.2	31.4	27.0	21.3	14.4	9.9	20.6
1944-2011	Min. mean Temperature	°C	-0.3	0.5	3.3	5.8	10.0	13.8	16.6	16.4	13.3	9.02	3.6	0.7	7.7
1974-2010	Mean cumulative Rainfall	mm	33.2	24.7	31.2	49.8	53.0	39.6	20.7	29.6	52.3	50.1	42.3	39.0	465.4

The Chardonnay (clone R8) plot was planted in 2006 on a SO4 rootstock, at a plantation density of 3030 plants per ha. The vines were Royat cordon trained on a plot with a total surface of 5.1 ha and 4.6 % slope. A drop irrigation system was installed on the plot. The soil had a silt-loam texture with 25.1 % carbonates and a slightly basic pH, in accordance with geological characteristics of the region, mainly including limestone soils. Organic matter level was 1.7 %, and no significant deficiency in macro- or micro-nutrients was detected in the physicochemical analysis provided by a certified chemical analytics laboratory.

In 2015, two groups of twelve plants were selected for study in the following three growing years (2016, 2017 and 2018), depending on their expression of esca disease symptoms, and they were categorised as being either symptomatic (S) or asymptomatic (A). All the vines with symptoms were identified inside a subplot of 1 ha, and twelve of them were then selected randomly. Six asymptomatic vines were also randomly selected within the same subplot to configure the experimental set up.

In 2016, the whole vineyard was irrigated under the same conditions for both A and S plants. In 2017 and 2018, in order to observe the effect of irrigation on the measured variables, the 12 selected plants from each category were further grouped according to irrigation practice: one group comprised six plants which were irrigated (I), and the other group comprised six plants which were not irrigated (N). The absence of irrigation was achieved by blocking the irrigation drippers of three consecutive vines; the central vine was used for variable observation and the first and third vines were buffers.

In 2018, one of the asymptomatic plants (NA-plant 2) showed foliar disease symptoms at pre-harvest sampling (the methodology for which is described in the following sections); it was therefore necessary to exclude the plant with the inconsistent symptom expression from every subsequent statistical analysis. The final number of vines per condition was therefore as follows: NA = 5, NS = 6, IA = 6 and IS = 6.

The irrigation regime consisted of frequent applications of water volumes by drop irrigation ranging from 3.1 L m⁻² to 9.3 L m⁻² from mid-May to the beginning of September, depending on the water requirements as advised by the vineyard manager. A complete list of irrigation dates and volumes is provided in Table 1S. During the three year period, meteorological data were collected at hourly intervals by a public weather station located at a distance of 2 km from the studied plot. Additional variables were also calculated: number of 30 °C-Heat days per season, number of 35 °C-heat days per season and number of heat events per season (a heat event was defined as being at least three consecutive days with mean Max temperatures of over 30 °C).

2. Measurements of physiological variables and assessment of GTD foliar symptoms

The physiological variables of the grapevine were measured on leaves at key phenological stages during the season: pea-sized berries (BBCH 75), pre-veraison (BBCH 83) and pre-harvest (BBCH 87) according to the Meier scale (Meier, 2001). The specific dates of the measurements are reported in Table 2. The variables considered in this study are: midday stem water potential f (Ψ_{mds} ; Mpa), stomatal conductance (gs; mol m⁻²/s), transpiration

rate (E ; $\text{mmol m}^{-2}/\text{s}$) and photosynthetic rate (A_N ; $\mu\text{mol}/\text{m}^2/\text{s}$). Measurements were made at 09:00 (solar time), when photosynthesis was maximal. Water potential was measured on one leaf per plant using a Scholander pressure chamber and by observing a sap film on the leaf stem coming out from the leaf in the chamber. The remaining variables, A_N , g_s and E , were measured on two leaves (taking the leaf in opposite position to the first bunch) per plant using an infrared gas analyser device (IRGA; LCI-500 model; ADC BioScientific Ltd., Herts, UK). The mean values of these variables per plant were considered as the experimental unit for subsequent analysis.

The foliar symptom monitoring was carried out twice during each pre-veraison and pre-harvest visit to the vineyard plot in each of the three survey years. Since the literature reports that it is difficult to distinguish the foliar symptoms of esca disease from those of *Botryosphaeria* dieback at the end of the vegetative period (Larignon *et al.*, 2001; Lecomte *et al.*, 2012; Surico *et al.*, 2006), we decided to consider them all as GTD symptoms. Only the chronic symptoms characterised by yellow-orange spots on the leaf margins and the blade were taken into account.

3. Identification of some GTD pathogens in woody samples

In 2018, three woody samples were collected from each plant monitored in the study on the day of pre-harvest sampling. Samples were taken from three different canes (current-year wood) by cutting with pruning scissors close to the cane base, simulating a pruning operation. A portion approximately 3 cm long from the base of the cane was then placed in a plastic bag. In the laboratory, we followed the protocol described by Martín and Cobos (2007) with some modifications: woody

samples were cleaned by removing bark with a scalpel, and then their surface was sterilised by being sequentially soaked in a 2 % hypochlorite solution for 1 min and then soaked twice in a row in sterilised deionised water for 1 min at a time. After drying in a laminar flow hood, each wood sample was cut into smaller cubes of approximately 0.5 cm using sterilised scissors. Six of these cubes were then placed on one Petri dish containing Potato Dextrose Agar medium with chloramphenicol (PDA; Biokar Diagnostics; Allonne, France). The resulting three plates of each of the 23 studied plants (5 plants in NA and 6 plants in IS, IA and NS) were incubated at 25 °C for 15 to 40 days depending on the pathogenic fungus. The development of mycelia was monitored every three days, in order to visually identify mycelia of causal GTD pathogens, via their classic culture morphology. Samples of mycelia growing around a wood cube were taken with a sterile inoculating loop and processed for positive identification through DNA extraction and nested PCR. DNA extraction of mycelium was carried out using the Redextract-N-Amp- Plant PCR KIT (Sigma-Aldrich; Merck Life Science S.L.U., Madrid, Spain), following the manufacturer's protocol. Pathogen determination was carried out using a nested-PCR, in which a first PCR reaction was undertaken to amplify the fungal ITS region, for the later detection of specific target species. The PCR product of the first reaction was then used as a DNA template for three different PCR reactions, specific to three GTD pathogen groups, according to previously published protocols with slight modifications in some cases: *Phaeoacremonium* spp. (Aroca and Raposo, 2007), *P. chlamydospora* (Tegli *et al.*, 2000) and *Botryosphaeriaceae* family (Spagnolo *et al.*, 2011). Descriptive information on the primers and the specific PCR programmes used are summarised in Table 3.

TABLE 2. Dates of key phenological stages and sampling times during the three years of field experiment.

Phenological stages	2016	2017	2018
Full flowering (BBCH 65) – T1	30/05/2016	24/05/2017	25/05/2018
<i>Sampling time - Pea size (BBCH 75)</i>		T1 + 15d	T1+26d
<i>Sampling time - Bunch closure (BBCH 83)</i>		T2-7d	T2-7d
Veraison (BBCH 85) – T2	31/07/2016	18/07/2017	01/08/2018
<i>Sampling time - 1 week before harvest (BBCH 89)</i>		T3-7d	T3-7d
Harvest (BBCH 89) – T3	07/09/2016	02/09/2017	04/09/2018

d = days, T1 = full flowering, T2 = veraison and T3 = harvest.

TABLE 3. Primers and PCR programmes used for detecting GTD fungi.

Target	Primer name	Sequence 5' – 3'	Thermocycler programme		
			Initialisation	Denaturation/Annealing/Elongation	Final elongation
Fungal DNA	ITS4	TCCTCCGCTTATTGATATGC	94 °C – 5'	94 °C – 45'' / 52 °C – 30'' / 72 °C – 90'' / (x30)	72 °C – 6'
	ITS5	GGAAGTAAAGTCGTAACAAGG			
<i>P. chlamydospora</i>	Pch1	CTCCAACCCCTTTGTTTATC	95 °C – 3'	95 °C – 60'' / 62 °C – 45'' / 72 °C – 120'' (x30)	72 °C – 5'
	Pch2	TGAAAAGTTGATATGGACCC			
<i>Phaeoacremonium</i> sp.	Pm1	CTCCAAAACCCCTTTGTGAACAT	94 °C – 5'	94 °C – 30'' / 57 °C – 30'' / 72 °C – 50'' (x30)	72 °C – 7'
	Pm2	CGAGCCCCGCCACTGACTT			
<i>Botryosphaeriaceae</i>	BoitsA	GACCATCAACTCCAGTCAG	94 °C – 5'	94 °C – 45'' / 61 °C – 30'' / 72 °C – 30'' (x35)	72 °C – 7'
	BoitsB	AAAGTTCAGAAAGGTTTCGTCCGG			

The PCR reaction was carried out in a thermocycler (T100 model; BioRad, Hercules, CA, USA), and the mix composition of each reaction consisted of 3 µl dNTP [10 mM], 2.5 µl 10 x buffer, 1.25 µl MgCl₂, 0.25 µl of Taq Polymerase (BioTaq DNA Pol; Bioline Meridian Bioscience; London, UK) and 0.6 µl of each forward or reverse primer, adjusted to a final volume of 25 µl. Finally, 2 µl of the final PCR products were loaded, mixed with 2 µl of loading dye on a 1 % agarose gel with TBE 1x. Gel electrophoresis was run at 90 mV for 30 min. A pathogen group in the sample was considered as being present when a light band was observed in a UV photography apparatus (Gel Doc™ XR+ model; BioRad; Hercules, CA, USA) at the specific amplicon size in bp. The positive detection of GTD pathogens in one of the incubated woody cubes was considered to indicate their presence in the woody cane sample.

4. Leaf sampling for gene expression analysis

To decipher the relationship between GTD pressure and the irrigation system on grapevine physiology, leaves were collected and subjected to transcriptomic analyses. Leaf samples for RNA extraction were collected at 3 phenological key stages - flowering, pea-sized and before harvest - in 4 vine growing conditions: no-watering and asymptomatic (NA), no-watering and symptomatic (NS), watering and asymptomatic (IA), and watering and symptomatic (IS). Samples were collected in liquid nitrogen, stored at - 80 °C, and then treated to obtain a fine powder according to the protocol reported by Spagnolo *et al.* (2017). Six leaves from the 6 selected plants for the physiological study were used per condition, and the experiment was repeated in both 2017 and 2018. On symptomatic vines (S), the collected leaves were selected based on expression of light to moderate visible GTD-disease symptoms.

4.1 RNA extraction

Total RNA was isolated from 3 x 50 mg of leaf powder using the PureLink Plant RNA Purification Reagent (Invitrogen, Cergy Pontoise, France) according to Spagnolo *et al.* (2017). The manufacturer's protocol was followed until the phase of separation with the chloroform: isoamyl alcohol (24:1). Next, 0.5 vol of ethanol (96-100 %) was added to the cleared lysate. This new solution containing the total RNA was purified using the RNeasy Plant mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of RNA was checked by agarose gel

electrophoresis, and the quantity was determined by measuring the absorbance at 260 nm.

4.2 Real-time RT-PCR analysis of gene expression

Reverse transcription was performed on 150 ng of total RNA using the Verso cDNA synthesis kit (Thermo Fischer Scientific Inc.). Real-time PCR was performed with Absolute Blue QPCR SYBR Green (Thermo Fischer Scientific Inc.) using a CFX96 thermocycler system (Bio-Rad, Hercules, CA, USA). The thermal profile was: 95 °C for 15 s

(denaturation) and 60 °C for 1 min (annealing/extension) for 40 cycles. Melting curve assays were performed from 65–95 °C at 0.5 °C/s. Melting peaks were visualised to check the specificity of each amplification. The results are expressed as the values of relative expression ($\Delta\Delta C_t$) and correspond to the mean of six independent experiments; they were expressed relative to the control corresponding to a fixed value of 1. Control sample conditions consisted of watering and asymptomatic vines (IA). The expression of the genes was considered significantly up- or down-regulated when changes in their

TABLE 4. Primers of genes analysed by real-time reverse-transcription polymerase chain reaction.

Function	Genes	Primer sequences	Genbank or TC TIGR* accession number
<i>Housekeeping genes</i>	<i>EF1</i> (EF1- α elongation factor)	5'-GAACTGGGTGCTTGATAGGC-3' 5'-AACCAAAATATCCGGAGTAAAAGA-3'	GU585871
	<i>60SRP</i> (60S ribosomal protein L18)	5'-ATCTACCTCAAGCTCCTAGTC-3' 5'-CAATCTTGCTCCTTTCCT-3'	XM_002270599
<i>Phenylpropanoid metabolism</i>	<i>STS</i> (stilbene synthase)	5'-AGGAAGCAGCATTGAAGGCTC-3' 5'-TGCACCAGGCATTTCTACACC-3'	X76892
	<i>CHI</i> (chalcone isomerase)	5'-GCAGAAGCCAAAGCCATTGA-3' 5'-GCCGATGATGGACTCCAGTAC-3'	XM_002282072
<i>Detoxification</i>	<i>cyAPX</i> (cytoplasm ascorbate peroxidase)	5'-AAGGTCGCTTGCCTAATGCA-3' 5'-TGGCACCTGCCAGAGTATG-3'	EU280159
	<i>GST1</i> (glutathione-S-transferase)	5'-TGCATGGAGGAGGAGTTCGT-3' 5'-CAAGGCTATATCCCCATTTTCTTC-3'	AY156048
<i>Defence protein</i>	<i>GLUC</i> (β -1.3 glucanase)	5'-TCAATGGCTGCAATGGTGC-3' 5'-CGGTTCGATGTTGCGAGATTTA-3'	DQ267748
	<i>PR6</i> (serine-protease inhibitor 6)	5'-AGGGAACAATCGTTACCCAAG-3' 5'-CCGATGGTAGGGACACTGAT-3'	AY156047
<i>Stress tolerance</i>	<i>PIP2.2</i> (aquaporin plasma membrane intrinsic protein 2-2)	5'-GAAAACACAAAGGGGTTC-3' 5'-GGTTCAGTCTCCATTGCACATG-3'	XM_002271336
	<i>HSP70</i> (heat shock proteins 70)	5'-CTACAGCAGGAGACACCCAC-3' 5'-AGACACTTCTCAACGGGCTC-3'	XM_002263563.3
	<i>TIP1</i> (putative aquaporin)	5'-GTTGTTGTCTCAACCCATTTC-3' 5'-ATCACCAACCTCATTTCATATGC-3'	AF271661
<i>Photosynthesis</i>	<i>RbcL</i> (large subunit of Rubisco)	5'-AATTTTTCTCCACGGCGATA-3' 5'-ATCTGCGCCCGCTTTATA-3'	TC57584
	<i>SBP</i> (sedoheptulose-1.7-bisphosphatase)	5'-TGCCACCAGCTCCTATTTGA-3' 5'-TCAACTGGGCCTCCCATGT-3'	XM_002263013.3
<i>Signalling ABA</i>	<i>NCED1</i> (9-cis-epoxycarotenoid dioxygenase 1)	5'-TGCAGAGGACGAGAGTGTA-3' 5'-AGCTAGACCAAAAGCTACGA-3'	XM_019216859.1

* see <http://www.jcvi.org/cms/research/projects/tdb/overview/>

TABLE 5. Summary of meteorological and irrigation conditions during the assay.

Season	Season stage	Temperature			Mean RH ^a	Cumulative rainfall	Number Heat days (> 30 °C) ^b	Number Heat days (> 35 °C) ^c	Number of Heat events ^d	Irrigation (l m ⁻²)
		Mean	Mean Max	Mean min						
2016	21 March to flowering	13.03	19.33	6.98	68.00	137.00				13.95
	Flowering to Veraison	22.03	28.37	15.13	56.24	16.20				105.40
	Veraison to harvest	23.38	29.87	17.14	57.74	0.00				93.00
	Overall	18.82	25.17	12.39		153.20	43	2	9	212.35
2017	21 March to flowering	12.99	19.98	5.70	67.05	138.00				
	Flowering to Veraison	22.09	29.60	13.85	60.54	84.20				75.95
	Veraison to harvest	23.81	31.58	15.90	60.13	5.80				82.15
	Overall	19.42	26.82	11.59		228.00	63	12	7	158.10
2018	21 March to flowering	13.05	19.59	6.50	73.13	218.50				
	Flowering to Veraison	22.24	29.94	14.31	63.79	139.60				52.70
	Veraison to harvest	24.01	31.96	16.48	64.02	138.80				43.40
	Overall	19.05	26.34	11.76		499.70	69	8	6	96.10

^a RH: Relative Humidity

^b Number of days during the growing season with absolute max temperature higher than 30.0 °C

^c Number of days during the growing season with absolute max temperature higher than 35.0 °C

^d Number of heat events (two or more consecutive days with absolute max temperature higher than 30.0 °C) during the growing season

expression were > 2-fold or < 0.5-fold respectively. The specific primers for the 12 targeted genes which are related to defence response, phenylpropanoid pathways, detoxification, stress tolerance, photosynthesis and signalling are listed in Table 4 and were selected according to a previous study on GTDs (Fontaine *et al.*, 2016; Spagnolo *et al.*, 2017).

5. Statistical analysis

Data analysis of physiological and epidemiological variables was performed using JMP15 (SAS Institute Inc., Cary, NC, USA) for all data sets. The effect of physiological variables was analysed using a Generalised Linear Model (normal distribution; identity link function). Mean separations within a factor were performed by applying orthogonal contrasts. Principal Components Analysis (PCA) was performed on the results of all the variables in each studied plant, including: means of meteorological variables from flowering to harvest, means of meteorological variables in three periods (21 March to flowering date, flowering to veraison, veraison to harvest), number of 30 °C-Heat days per season, number of 35 °C-heat days per season, number of heat events per season (considered as at least three consecutive days with mean Max temperatures

over 30 °C), irrigation volume, effective water (irrigation volume plus cumulated rainfall during the season), means of four physiological variables in two sampling times (- Ψ_{mds} , E, gs and A_{N} ; pre-veraison and pre-harvest samplings), and presence of GTD foliar symptoms at pre-harvest sampling. For molecular analysis, the relative expression of gene in leaves of irrigated symptomatic (IS), non-irrigated asymptomatic (NA) or non-irrigated symptomatic (NS) grapevine is expressed compared to the control irrigated and asymptomatic plants. A Kruskal-Wallis' test was carried out for the different conditions on one gene at a time ($p < 0.05$).

RESULTS

1. Meteorological conditions and irrigation practices during the field trials

The compiled meteorological data described typical Mediterranean climate conditions with mean temperatures of over 22 °C from flowering to harvest and low rainfall during the summer months (Table 5). The observed values are in accordance with the historic data of the region (Table 1), which show similar mean and max temperatures during the summer period and 143 mm of precipitation between May and August.

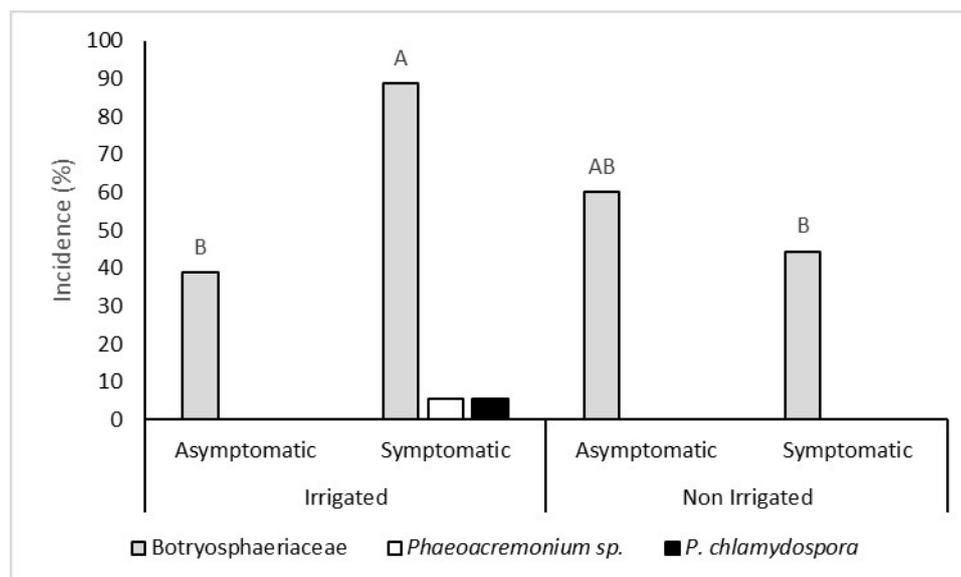


FIGURE 1. Incidence of three major pathogens causing GTDs in wood samples taken from both asymptomatic and symptomatic vines present in the 2018 field experiment.

Grey bars = incidence of *Botryosphaeriaceae* family fungi; white bars = incidence of *Phaeoacremonium* sp.; black bars = incidence of *Phaeomoniella chlamydospora*. Values linked by the same letter, per pathogenic fungus, are not significantly different according to the orthogonal contrasts.

Spring conditions (21 March to flowering) in the three seasons were similar in terms of temperature and rainfall, although precipitation was highest during this period in 2018 (Table 5). However, during the grapevine productive period, from flowering through to harvest, temperatures were the lowest in 2016, with one degree less in Mean Max temperatures (28.4 °C before veraison and 29.9 °C before harvest in 2016, compared to 29.6 °C and 31.6 °C in 2017 and 29.9 °C and 31.9 °C in 2018). In addition to the aforementioned warmer conditions in 2017 and 2018, a higher occurrence of heat days was observed, with at least twenty more 30 °C-Heat days and six more 35 °C-heat days than in 2016 (Table 5). Nonetheless, heat periods in 2016 were more concentrated in time, since the number of heat events, considered as at least three consecutive days with absolute Max temperatures over 30 °C, was nine in 2016 compared to seven and six in 2017 and 2018 respectively.

In terms of cumulated rainfall, each season was different (Table 5): the 2016 season was very dry, with only 16.2 mm of rainfall between flowering and harvest; and the 2017 season also had very low amounts of rainfall during the same period, although it cumulated 90 mm. Conversely, 2018 was an atypical rainy year (499 mm compared to 143 mm during the same period in the historic series), with an even distribution of precipitation between the beginning of spring and harvest. These tendencies are also confirmed by the relative

humidity values in 2018, which were three points higher than in 2017, and a further three points higher than in 2016 (Table 5).

The effective water received by the plants - considered as the cumulative rainfall from 21 March to harvest day, plus the total irrigation volume during the season - was 365.5 L m⁻², 386.1 L m⁻² and 595.8 L m⁻², in 2016, 2017 and 2018 respectively (Table 5). Thus, the irrigation programme tended to balance the differences in yearly cumulative rainfall, despite the overall water supply in 2018 being at least 50 % higher than that in either of the other two years.

2. Detection of three major GTD pathogens in woody tissues

The results of the molecular identification of mycelia developed from woody samples is summarised in Figure 1, as the incidence (%) of *Phaeoacremonium* sp., *P. chlamydospora* and the *Botryosphaeriaceae* family in the analysed vine canes (three per plant). The most frequent taxonomic group detected was *Botryosphaeriaceae*, being present in samples from all conditions tested, ranging from 39 % (IA) to 89 % (IS). Incidence in IS was much higher than in the other treatments, with significant differences in the comparison with IA and NS treatments. The pathogenic fungi *Phaeoacremonium* sp. and *P. chlamydospora* were only detected in IS plants, with low incidences of 5.5 % for both taxonomic groups.

TABLE 6. Incidence of Esca foliar symptoms on the studied plants.

		2016	2017	2018
Irrigated	Symptomatic	100 %	17 %	0 %
	Asymptomatic	0 %	0 %	0 %
Non irrigated	Symptomatic	ND	100 %	17 %
	Asymptomatic	ND	0 %	0 %

Plants were classified according to irrigation and symptom appearance in the season previous to the field experiment as IS (irrigated symptomatic), IA (irrigated asymptomatic), NS (non-irrigated symptomatic) and NA (non-irrigated asymptomatic). An evaluation of GTD symptoms was carried out one week before the commercial harvest of the grapes. Six plants per category were observed (n = 6), except for NA (n = 5), and further expressed as a percentage. ND: not determined.

3. Disease incidence of GTDs on the studied plants

The visual evaluation of GTD foliar symptoms, carried out at pre-harvest sampling time, showed different symptom expression depending on the growing season (Table 6). In 2016, when all the vines were irrigated, the 12 plants considered as symptomatic in 2015 again showed foliar symptoms of the diseases. The same plants were then separated into two groups - irrigated and non-irrigated - in 2017 and 2018. In 2017, disease symptoms were expressed by one vine out of six (17 %) of the irrigated plants (IS) previously considered as being symptomatic. The lack of irrigation supply strongly increased the symptom expression, with 100 % of the NS targeted plants (NS) developing GTD symptoms. In 2018, only NS plants presented foliar disease symptoms (17 %).

4. Some physiological responses to GTDs of symptomatic and asymptomatic vines with or without irrigation

4.1 Water potential and photosynthetic activity is year- and sampling time- dependent

The results of the two or three physiological measurements per year, performed at key phenological stages during the three seasons, are summarised in Table 8. No differences were found in water potential, which was expressed as a negative value ($-\Psi_{\text{mds}}$), between symptomatic (S) and asymptomatic (A) plants in 2016, when all the plants were equally irrigated. However, there were significant differences between all the physiological variables, indicating a lower physiological activity in S plants. In the 2017 season, the irrigation effect provided significant differences in Ψ_{mds} in the pre-veraison sampling. At pre-harvest, NS plants had significantly lower Ψ_{mds} than both IS and IA plants. Significant

differences in E were observed at the pea-sized berry stage and at pre-veraison, indicating low E in irrigated plants (particularly in IS). In the last assessment at pre-harvest date, E was similar in all plants, whereas we observed significant differences between S and A plants in terms of gs and A_N . The irrigated plants showed higher values, but the difference was only significant within symptomatic ones. Interestingly, the NS value of $4.87/\mu\text{mol}/\text{m}^2/\text{s}$ is considered to be extremely low. The Ψ_{mds} values in 2018 were significantly lower in non-irrigated compared to irrigated plants at pea-sized stage and at pre-veraison, but not at pre-harvest. No significant differences were observed for other physiological variables in any sample, although the lowest gs and A_N values were generally found in NS plants.

A further overall analysis of the data was carried out by performing a GLM analysis of the four physiological variables, including the factors: 'Year' of study, 'Sampling time' in different phenological stages, supply of 'Irrigation' and 'Symptomatic' condition of the plant. First grade interactions between factors were also included in the models (Tables 2S to 5S). The results of the analysis indicated that 'Year' and 'Sampling time' had a strong influence on the four physiological variables ($p < 0.05$), with the exception of 'Year' on A_N . The plants tended to reduce their water potential and physiological activity across the season, and the lowest values ($p < 0.05$) of each variable were always observed in the pre-harvest sampling. The 'irrigation' effect was very significant on Ψ_{mds} , gs and A_N , involving significantly higher values for these three variables in irrigated plants compared to non-irrigated plants. Irrigation did not modify the transpiration rate (E). The classification of plants as symptomatic or asymptomatic had a weak influence on the physiological variables. The only remarkable effect was observed on the transpiration rate (E), with a nearly significant

TABLE 7. Results of physiological measurements made on grapevine leaves at key phenological stages during field trials.

		$-\Psi_{\text{mds}}$ (Mpa)	E (mmol/m ² /s)	gs (mol/m ² /s)	A_N (μmol/m ² /s)
		28 July – Pre-veraison (BBCH 83)^a			
2016	IS	-0.84 ± 0.05 a	4.32 ± 0.23 b	0.17 ± 0.01 b	11.39 ± 0.57 b
	IA	-0.81 ± 0.06 a	5.16 ± 0.22 a	0.24 ± 0.02 a	13.85 ± 0.56 a
		30 August – Pre-harvest (BBCH 87)			
	IS	-0.72 ± 0.06 a	2.62 ± 0.21 a	0.11 ± 0.01 b	7.70 ± 0.53 b
	IA	-0.63 ± 0.01 a	3.31 ± 0.35 a	0.16 ± 0.03 a	9.46 ± 1.18 a
		7 June – Pea-sized berries (BBCH 75)			
	IS	-0.38 ± 0.04 a	3.41 ± 0.11 b	0.18 ± 0.03 a	12.98 ± 1.32 a
	IA	-0.40 ± 0.05 a	3.80 ± 0.19 b	0.19 ± 0.02 a	13.03 ± 0.81 a
	NS	-0.39 ± 0.03 a	4.42 ± 0.34 a	0.19 ± 0.02 a	13.35 ± 1.29 a
	NA	-0.44 ± 0.03 a	4.71 ± 0.34 a	0.21 ± 0.02 a	13.33 ± 1.08 a
		12 July – Pre-veraison (BBCH 83)			
2017	IS	-0.68 ± 0.05 a	7.00 ± 0.50 b	0.42 ± 0.06 a	15.89 ± 0.98 a
	IA	-0.64 ± 0.04 a	7.83 ± 0.32 ab	0.45 ± 0.04 a	16.17 ± 0.72 a
	NS	-0.81 ± 0.07 b	8.75 ± 0.28 a	0.38 ± 0.03 a	14.57 ± 0.70 a
	NA	-0.84 ± 0.08 b	8.52 ± 0.68 a	0.36 ± 0.06 a	13.92 ± 0.80 a
		23 August – Pre-harvest (BBCH 87)			
	IS	-0.98 ± 0.12 a	4.13 ± 0.46 a	0.16 ± 0.03 a	10.02 ± 1.35 a
	IA	-1.06 ± 0.10 a	4.09 ± 0.67 a	0.15 ± 0.02 ab	9.10 ± 1.03 ab
	NS	-1.40 ± 0.04 b	2.49 ± 0.25 a	0.06 ± 0.01 c	4.87 ± 0.43 c
	NA	-1.20 ± 0.10 ab	3.91 ± 0.52 a	0.10 ± 0.02 b	7.56 ± 0.83 b

		$-\Psi_{\text{mds}}$ (Mpa)	E (mmol/m ² /s)	g_s (mol/m ² /s)	A_N ($\mu\text{mol/m}^2/\text{s}$)
19 June - Pea-sized berries (BBCH 75)					
	IS	-0.48 ± 0.01 a	5.95 ± 0.35 a	0.42 ± 0.06 a	15.62 ± 0.75 a
	IA	-0.50 ± 0.03 a	6.25 ± 0.27 a	0.39 ± 0.07 a	15.30 ± 0.74 a
	NS	-0.53 ± 0.01 b	6.74 ± 0.32 a	0.32 ± 0.02 a	15.39 ± 0.67 a
	NA	-0.54 ± 0.02 b	6.65 ± 0.34 a	0.30 ± 0.03 a	14.57 ± 0.82 a
26 July -Pre-veraison (BBCH 83)					
	IS	-0.88 ± 0.07 a	5.88 ± 0.59 a	0.18 ± 0.02 a	9.39 ± 0.79 a
2018	IA	-0.96 ± 0.04 a	6.87 ± 0.55 a	0.26 ± 0.09 a	9.74 ± 0.70 a
	NS	-1.13 ± 0.05 b	5.99 ± 0.89 a	0.15 ± 0.03 a	8.86 ± 1.03 a
	NA	-1.13 ± 0.10 b	5.11 ± 0.79 a	0.11 ± 0.02 a	6.91 ± 1.01 a
29 August - Pre-harvest (BBCH 87)					
	IS	-0.77 ± 0.09 a	4.72 ± 0.52 a	0.24 ± 0.03 a	10.48 ± 0.72 a
	IA	-0.74 ± 0.08 a	4.46 ± 0.30 a	0.20 ± 0.02 a	9.21 ± 0.90 a
	NS	-1.08 ± 0.14 a	4.64 ± 0.43 a	0.16 ± 0.02 a	8.18 ± 0.71 a
	NA	-1.08 ± 0.17 a	5.13 ± 0.52 a	0.18 ± 0.03 a	9.69 ± 0.75 a

^a Sampling time

The plants were classified according to irrigation and symptom appearance in the season previous to the field experiment as *IS* (irrigated symptomatic), *IA* (irrigated asymptomatic), *NS* (non-irrigated symptomatic) and *NA* (non-irrigated asymptomatic).

Leaf water potential (Ψ_{mds}), Stomatal conductance (g_s), Transpiration rate (E) and Photosynthetic rate (A_N), were measured at 09:00 (solar time). Means were calculated on two repeated measurements on 6 plants per category, except for *NA* ($n = 5$). Values linked by the same letter in different plant categories, per sampling time, are not significantly different according to orthogonal contrasts.

effect ($p = 0.051$), indicating higher transpiration in asymptomatic plants compared to symptomatic ones.

4.2 Correlation between foliar symptom emergence and meteorological conditions

A PCA analysis was performed to determine the relationship between the recorded variables describing meteorological conditions, irrigation supply, and physiological response of the plants, with a special focus on the correlation with foliar symptom appearance. The observed incidence of foliar symptoms in each plant over the seasons was included in the analysis, instead of the *a priori* classification done in 2016 for the experimental set-up. This variable may allow us to observe the particular effect of variables on the expression

of symptoms observed in the field. In order to optimise the model, we first tested the inclusion of different meteorological and physiological variables, finding the highest percentage of explicability of components when we excluded pre-veraison data. The final list of variables included the model and their partial contribution is reported in supplementary data (Table 6S).

The PCA showed that the first two axes accounted for 77.70 % of total variability (Prin1: 55.4 %, Prin2: 22.3 %; Figure 2). The first component was mainly related to temperature variables, whereas the second axis included the four physiological variables as major contributors (Table 6S). In both components, the appearance of symptoms was found to have a low contribution (2.29 % and 2.42 % in Prin1 and Prin2 respectively).

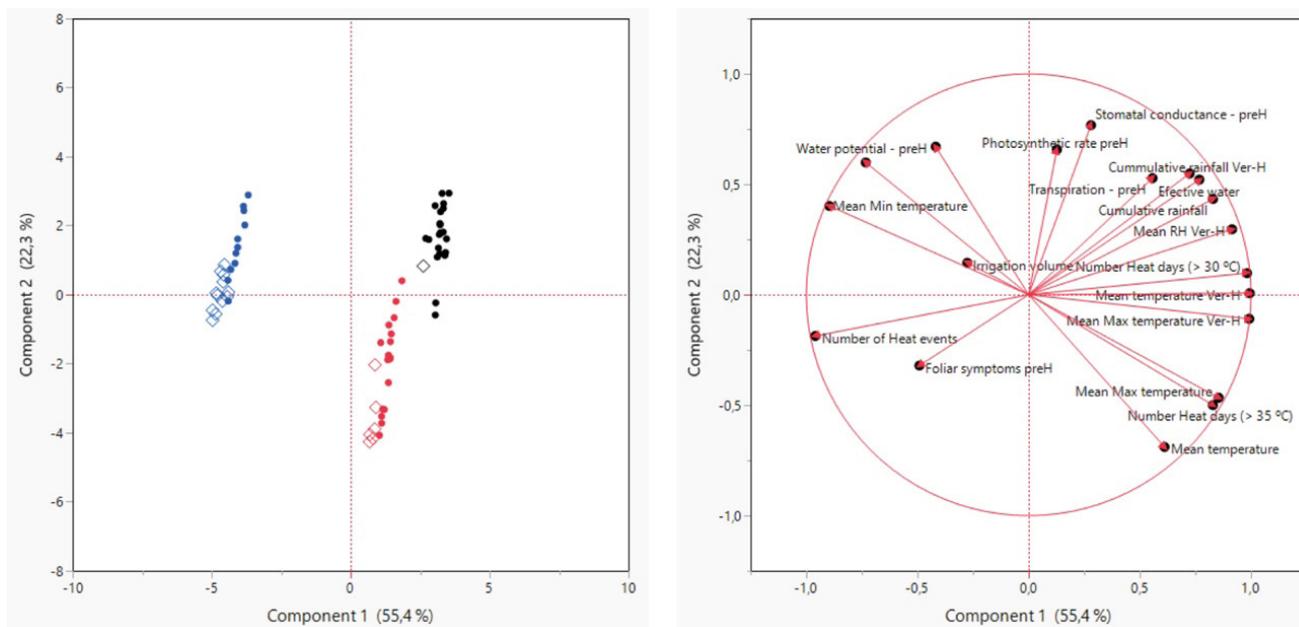


FIGURE 2. Principal Component Analysis of the physiological, meteorological and epidemiological variables measured during the three-year field experiment.

A PCA analysis was performed on the results of all the variables in each studied plant in each year (n = 69).

Diamond marks represent symptomatic plants and solid-point marks represent asymptomatic plants. Blue dots = 2016, red dots = 2017 and black dots = 2018.

Despite this fact, it should be noted that symptom appearance showed the highest correlation (inverse) with water availability (‘Cumulative rainfall’ and ‘Effective water’ variables) and transpiration rate (E), indicating a higher incidence of symptoms with lower water availability and lower transpiration in symptomatic plants. Interestingly, temperature variables were less correlated with symptoms, except for the number of heat events.

In Figure 2, we observed a clear distribution of samples according to the year, which determines the influence of the meteorological conditions in the analysis. Therefore, the relationships between physiological and meteorological variables (Figure 2 vector plot) may also have been influenced by the season effect, thus affecting the results. Consequently, we performed a separate analysis for each season only including physiological data (Figures 1SA, 1SB and 1SC), to achieve a finer analysis of the relationship between foliar symptoms and plant activity. The PCA in 2016, when all plants were irrigated, showed that symptom development was associated with lower values of E, g_s and A_N , whereas there was a very low correlation between water potential and symptoms, or any of the other variables. In 2017, with similar conditions and with non-irrigated

plants in the experimental setup, the water potential had a higher correlation with symptom appearance, which was always inversely correlated to the four physiological variables. Lastly, in 2018, with milder meteorological conditions and the lowest incidence of foliar symptoms, the partial contribution of symptom appearance in the PCA was very low and did not correlate with any physiological variable measured.

4.3 Alteration of some gene expression in the leaves at veraison only

The expression of the 12 targeted genes involved in different functions, especially stress tolerance and photosynthesis, was studied (Tables 8 and 7S). Since no modification of the expression for *STS*, *TIP1*, *GST1*, *RbcL* and *SBP* was observed at the 3 phenological stages in 2017 (Table 7S), we decided to not to carry out an analysis on them in 2018. Moreover, neither significant induction nor repression was reported for any of the studied genes at both flowering and pea-size in the years 2017 and 2018 (Table 7S), and data before veraison in 2017 were only described (Table 8). Before veraison, a significant induction of *Gluc* and *PR6* was observed in IS (1.81 ± 0.50 and 1.48 ± 0.68 respectively) and NS (2.43 ± 0.78 and 2.78 ± 0.52 respectively), and of *cyAPX* in NA (1.34 ± 0.29)

TABLE 8. Relative gene expression in leaves before harvest in 2017.

Function	Genes	Irrigated Symptomatic (IS)	Non-irrigated Asymptomatic (NA)	Non-irrigated Symptomatic (NS)
<i>Phenylpropanoid metabolism</i>	<i>CHI</i>	-0.25±0.14 _a	0.44±0.05 _b	-0.02±0.21 _{ab}
	<i>STS</i>	-0.23±0.29 _a	-0.28±0.22 _a	-0.38±0.47 _a
<i>Defence proteins</i>	<i>Gluc</i>	1.81±0.50_{ab}	0.07±0.27 _a	2.43±0.78_b
	<i>PR6</i>	1.48±0.68_{ab}	-0.31±0.47 _a	2.78±0.52_b
	<i>PIP2.2</i>	-0.19±0.10 _a	0.73±0.18 _b	0.55±0.13 _b
<i>Stress tolerance</i>	<i>HSP70</i>	-0.19±0.21 _a	0.53±0.16 _a	0.43±0.19 _{ab}
	<i>TIP1</i>	-0.20±0.15 _a	0.02±0.17 _a	-0.12±0.13 _a
<i>Detoxification</i>	<i>cyAPX</i>	0.19±0.39 _a	1.34±0.29_a	1.42±0.15_a
	<i>GST1</i>	0.05±0.29 _a	-0.43±0.25 _a	-0.07±0.36 _a
<i>Signalling ABA</i>	<i>NCEDI</i>	0.11±0.39 _a	0.30±0.05 _{ab}	0.90±0.17 _b
<i>Photosynthesis</i>	<i>RbcL</i>	-0.07±0.12 _a	0.24±0.12 _a	-0.51±0.24 _a
	<i>SBP</i>	0.01±0.12 _a	-0.08±0.07 _a	-0.44±0.15 _a

Values (mean of 3 technical replicates ± standard error) represent the expression level in reported condition relative to the control; i.e., leaves from irrigated and asymptomatic plant (IA). The relative expressions are log₂ transformed. The relative expression of a given gene was considered up- or down-regulated when the value of relative expression was > 1 or < -1 respectively compared to the control. Genes significantly down- or over-expressed compared to the control appear in light or dark grey respectively. Letters indicate significant differences between the different conditions for one gene at a time at $p < 0.05$ using the Kruskal-Wallis test.

and NS (1.42 ± 0.15) compared to the relative control, and the irrigated and asymptomatic plants.

DISCUSSION

In the present study, a set of experiments was carried out in order to investigate the influence of meteorological factors on GTD symptoms development, as well as the relationship between symptom appearance and the physiological and defence responses of grapevine.

1. Pathogens related to GTDs were identified from symptomatic and asymptomatic grapevines

The studied grapevine was 10-year-old Chardonnay with low-moderate global GTD incidence and severity, mainly caused by *Botryosphaeria* species infections according to our results (Figure 1), although other fungi were also isolated from woody samples. In agreement with other studies carried out in European vineyard countries and on other cultivars (Bruez *et al.*, 2014; Bruez *et al.*, 2020), GTD pathogens were also detected in asymptomatic vines (Figure 1). These data confirm that most of the vines in the field were

infected and that the foliar symptom expression is strongly linked to environmental factors, and is also directly correlated with the physiological status of the plants (Fontaine *et al.*, 2016; Claverie *et al.*, 2020).

2. A vintage-dependent effect on foliar symptom expression related to the meteorological conditions

The work was carried out in a plot characterised by typical viticultural practices of very dry winegrowing regions, in which irrigation is used to achieve higher grape quality at harvest. Meteorological data during the study confirmed very low precipitation levels (between 350 L/m² and 600 L/m² during the growing season) similar to the historical data (Table 1), and a maximum irrigation volume of 212 L/m² in the driest year (Table 5). The 2018 season was found to be warmer and wetter than the other two seasons. The Ψ_{mds} values were significantly lower for non-irrigated vines compared to irrigated ones at pre-harvest in 2017 only, and at both pea-sized stage and pre-veraison in 2018 (Table 7). More differences were reported in 2017 than in 2018 for the other variables (E, gs, A_N). The use

of irrigation tended to generally compensate for the water deficit, but the effective water received in 2018, whether by irrigated or non-irrigated plants, was much higher than that received during the 2016 and 2017 seasons. As a consequence, symptom appearance was higher in 2016 and 2017 than in 2018 (Table 6), indicating that the influence of the meteorological conditions was relevant and year-dependent (Figure 2). Moreover, irrigation also had a remarkable effect on symptom development, since symptom expression was always higher in non-irrigated plants, although the same plants had previously shown symptoms in 2016, when all plants had been irrigated (Table 6). Thus, the effective water received by the plant seemed to have played a major role in symptom development in our study; this is supported by the PCA analysis, which showed that, out of all the meteorological variables, effective water had the highest correlation with symptom occurrence (Tables 2S to 5S, Figure 1S). Similar year-dependent GTD foliar symptom expression is reported in the literature (Mugnai *et al.*, 1999; Fischer and Peighami-Ashnaei, 2019; Moret *et al.*, 2020). In general, drought has been frequently considered as a pre-disposing factor for woody plant declines in forest trees (Desprez-Loustau *et al.*, 2006; Piou *et al.*, 2006) and grapevine (Surico *et al.*, 2006; Claverie *et al.*, 2020), because the reduced water availability limits photosynthesis and thus the carbon allocation useful for growth and defence response. The results of our 2016 experiment also indicate a weak correlation between GTD expression and the lower values for the photosynthesis variables (A_N , gs, E, Table 7, Figure 2).

Temperature was also found to be inversely correlated to symptom appearance in the PCA, indicating a reduction in symptom expression with higher temperatures (Figure 2). This may have occurred because the highest temperatures were observed during the wettest season, 2018, when symptom expression was reduced. Interestingly, the occurrence of very high temperatures (max temperatures over 30 °C or 35 °C) were also negatively correlated with symptoms, whereas the occurrence of heat events was positively correlated with symptom appearance. The effect of temperature in our experiment is thus unclear compared to the influence shown by the effective water. Qiu *et al.* (2016) reported the effects of temperature and water stress on the virulence of *Botryosphaeriaceae* spp., which caused dieback of grapevines, and they proposed a distribution of the four *Botryosphaeriaceae* species in Australia

based on their cardinal temperatures. Nevertheless, no data on the foliar symptom incidence were given in the study from Qiu *et al.* (2016). In addition, based on the literature focusing on the relationship between GTD incidence and the temperature (for review Songy *et al.*, 2019), Claverie *et al.* (2020) integrated the latter into the drivers in the model of GTDs. The frequency of the heat events in both spring and summer also needs to be taken into account in the GTD symptom incidence, as suggested by Larignon *et al.* (2009). Finally, few studies have focused on the combination of a heat and water stress on GTD expression, probably due to the complexity of the model (for review Songy *et al.*, 2019; Claverie *et al.*, 2020).

3. The potential efficient effect of irrigation practices on foliar symptom expression

In our study, 2017 data show that the irrigated vines expressed less GTD foliar symptoms compared to the non-irrigated ones (Table 6). The results of 2018 could not be taken into account since the high level of rainfall probably meant there were no water deficits in both tested conditions, irrigated and non-irrigated, as confirmed by no significant differences having been found between the physiological variables measured in either condition (Table 7). The few studies carried out on deficit irrigation practices have relatively contrasting conclusions on GTD incidence: Sosnowski *et al.* (2016) reported that deficit irrigation practices are not likely to contribute to increased prevalence of GTDs in Australian vineyards; conversely, Van Niekerk *et al.* (2011) observed that the length of lesion due to *Botryosphaeriaceae* species declines linearly as the irrigation volume increases. What is certain is that deficit irrigation practices in vineyards may be damaging to grapevine physiology and, as a consequence, to plant responses (Edwards *et al.*, 2011) to GTD pathogen attacks.

4. Foliar symptom appearance is associated with lower physiological activity in diseased plants without after-effects in the following seasons

In the multivariate study, which included symptom incidence at the single plant scale, higher symptom expression was associated with lower values of physiological variables, particularly E (Figure 1S). Transpiration allows plants to adapt to water loss and leaf temperature, and both transpiration and stomatal conductance (gs) have generally been found to be reduced in response to water deficit in grapevine

(Lanari *et al.*, 2015); i.e. in non-irrigated conditions. In a recent study comparing the physiology of Esca symptomatic and asymptomatic plants, Ouali *et al.* (2019) also found significantly higher transpiration in asymptomatic vines, although other parameters such as water potential did not show any relevant differences. Overall, our results indicate poorer physiological and photosynthetic activity during the season in plants expressing symptoms at harvest, the transpiration rate being the most affected, which is consistent with the findings reported in current literature (for review Fontaine *et al.*, 2016). These general trends were also observed when comparing S and A plants at the three phenological stages, but these differences were not always significant.

As a result of the influence of meteorological conditions and the consequent seasonal effect, the highest foliar symptom incidence was observed in both 2016 and 2017; not many of the same plants showed symptoms again in the following seasons. Interestingly, according to the results of the GLM test performed on the 2017 and 2018 data (Tables 2S to 5S), the general physiology of the plants that expressed GTD foliar symptoms in 2016, represented by the IS and NS categories, was not altered in the following two years. Only the transpiration rate (E; Table 3S) was shown to be influenced by the expression of symptoms, supporting the findings of the quantitative analysis. In the same way, no relevant differences between A and S plants were revealed by the analysis of the expression of targeted genes at the three sensitive phenological stages (flowering, pea-sized, pre-veraison). Altogether, these observations suggest that the erratic expression of foliar symptoms seems to be year-dependent (i.e., influenced by meteorological conditions) without a strong impact on the following year, as reported by Calzarano *et al.* (2018) and Moret *et al.* (2020).

CONCLUSION

In conclusion, the results of our evaluation of the influence of meteorological conditions and irrigation on the appearance of GTD foliar symptoms, and of the related implications for vine physiology, indicate that meteorological conditions are the major driving factor for symptom development. Water availability was also found to have a significant role, depending on the irrigation volume. Limiting water stress by irrigating may be a way of reducing GTD symptom expression during dry years; however, in order to support this argument, it would be necessary to collect and analyse data from more years of experiments.

The driving effect of meteorological conditions may explain the erratic symptom expression in plants infected by GTD fungi, which depends on the year, as previously reported by Mugnai *et al.* (1999) and Calzarano *et al.* (2018). Lower physiological activity was found in the plants expressing foliar symptoms; this was more evident in the case of transpiration E, whereas the impact was lower for other variables, such as water potential or targeted gene defence expression. Overall, our study provides insights into the complexity of symptom expression in GTDs with a complex etiology, highlighting several key factors to be studied in further research on the relationship between wood infection and its physiological consequences, as recently reported in Claverie *et al.* (2020). In dry years, an irrigation system could be useful for limiting GTD emergence when applied early in the season; i.e., from before flowering to bunch closure, which is the period with the highest symptom expression.

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