



Article

Influence of Culture Media and Environmental Factors (Water Potential and Temperature) on Mycelial Growth of *Phytophthium vexans* (de Bary), the Causal Agent of Dieback Disease in Apple Trees

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Abstract: This study aimed at evaluating the effects of culture media and environmental factors (temperature and water potential (Ψ_w)) on the growth of the pathogenic fungus *Phytophthium vexans* (de Bary) associated with root rot and dieback disease in apple trees. Tomato agar, potato dextrose agar (PDA), and soybean agar were the most favourable for rapid mycelial growth, with optimum growth recorded for PDA medium. To determine the environmental conditions that promoted the development of this phytopathogen, the effects of temperature (5–30 °C), water potential (Ψ_w) (−15.54; −0.67 MPa) (0.89–0.995 a_w), and their interaction were evaluated on the in vitro radial growth rates of the five isolates of *P. vexans* and on their latency phase (time period prior to growth). The results of this study showed that temperature, water potential, and their interaction had significant effects ($p < 0.001$) on the radial growth rates and latency phases of all tested *P. vexans* isolates. All isolates were able to grow throughout the temperature range (5 to 30 °C), with the maximum radial growth rate being observed at the highest temperatures, 25–30 °C. Growth was seen to be faster at −0.67 MPa (0.995 a_w) at 25 °C and 30 °C. No growth was observed at $\Psi_w < -5.44$ MPa (0.96 a_w), regardless of the temperature. It was found that the length of the latency phase depended significantly on both environmental factors. The longest latency phases (5 days on average) were recorded at a temperature of 5 °C and Ψ_w of −0.67 MPa (0.995 a_w) and −2.69 MPa (0.98 a_w), while the shortest latency phases were observed at a temperature of 30 °C and a Ψ_w of −0.67 MPa (0.995 a_w), with an average of 0.2 days. The findings from this study could help to understand the impact of these environmental factors on the occurrence of diseases caused by *P. vexans* and more likely to design a reliable preventive control strategy based on the avoidance of conditions that play in favour of the phytopathogen.

Keywords: *Phytophthium vexans*; root rot; temperature; water potential; latency phase; radial growth rate



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1. Introduction

The apple tree (*Malus domestica* Borkh) is one of the most cultivated fruit species worldwide. It is the subject of a major trade trend [1]. Apple accounts for the third largest fruit production after citrus fruits and banana [2]. The annual world apple production reached 60 million tons in the late 2000s, 62.1 million tons in 2005, 64.2 million tons in 2007, and 87 million tons in 2021 [3]. In Morocco, the apple tree is the species of rosaceous fruit that occupies the largest surface area [1]. Currently, orchards planted with apples are estimated at 52,000 ha, with an annual production of 700,000 tons and an average yield of about 16 T/ha. Middle Atlas, Rif, Saïs, Haouz, and Moulouya are the main apple-growing

areas, since more than 56% of the total area planted with apple trees is located in these regions [4].

In recent years, root rot symptoms and dieback have been observed in apple trees in the main growing areas across the country, causing serious damage to the commercial apple plantations [5]. Ubiquitous microorganisms in the soil and especially those belonging to the Pythiaceae family are considered the main causative agents of root rot and dieback diseases on apples [6]. These soilborne pathogens are divided into several genera and species, the most devastating of which are species belonging to the *Pythium* and *Phytophthora* genera [7–9]. The vast majority of known oomycete pathogens belong to the subgroup of Peronosporales/Peronosporaceae, which includes the two important genera *Phytophthora* and *Pythium*, and other genera, such as *Phytopythium*, *Brema*, and *Peronospora* [10,11]. In the 1980s, *Phytophthora* was recognized as the most widespread oomycete species causing crown rot and root rot diseases in apple trees, whereas in the late 1990s, *Pythium* was the pathogen responsible for apple replant disease, causing symptoms similar to those of *Phytophthora* species [12–14]. In the 2000s, a new aggressive *Phytophthora*-*Pythium* intermediate genus was isolated and identified as *Phytopythium vexans* (de Bary) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque [15–17]. This latter is involved in root rot and apple replant disease [5,16].

Root rot is a disease of plants affecting the root system caused by soil pathogens, which most often are fungi. The diseased plants are characterized by slow growth and exhibit symptoms such as wilting and yellowing leaves, browning roots, sagging brown crown canker, and quick decline if the pathogenic strain is highly aggressive [18,19]. The infection of the host is favoured by wounds inflicted at the level of the crown or on the surface of the secondary root system, which causes the disintegration of the plant organs responsible for water absorption and nutrient uptake. Oomycete pathogens such as *Phytophthora* spp., *Pythium*, and *P. vexans* can survive long in the soil and on diseased plants [17,20]. These pathogens can also grow under conditions of persistent moisture such as frequent watering or excessive irrigation and at temperatures ranging from 15 to 16 °C even in poorly oxygenated soil [21,22]. Their inoculum densities increase more rapidly if there is excess of nitrogen fertilizer and an environmental temperature included between 25 and 28 °C [23,24]. They gradually invade the entire vascular tissue from the root tissue and spread over the soil using mycelia and swimming zoospores through watercourses, watering systems, certain farming practices, and the transport of agricultural products and using contaminated tools from contaminated substrates [5,24,25]. The optimal growth temperature for oomycete pathogens is situated between 15 and 30 °C [23,24]. *Phytophthora* species grow at temperatures ranging from 13 to 15 °C, but their optimal growth temperature is around 25 °C [26], while *Pythium* species become more aggressive at temperatures below 18 °C when substrates are full of moisture; thus, *Pythium* rot occurs more in very wet soils with either little or poor drainage and excessive irrigation during rainy and cool periods (15 to 20 °C) [27]. However, most infections due to *Phytopythium* species occur at a high temperature; the optimum temperature for growth of most *Phytopythium* species is about 30 °C [28,29].

In recent decades, *P. vexans* has become an increasingly major threat to fruit production, in particular apples, as reported in several studies in different parts of the world [30–32]. Root rot disease caused by *P. vexans* is induced by environmental factors such as temperature, soil moisture, nitrogen and phosphorus contents, pH rate, and light intensity. Several studies were conducted to assess the impact of environmental factors on the growth, sporulation, and germination of oomycete species such as *Phytophthora* and *Pythium* [24,33,34]. Among these factors, the temperature and the water potential were shown to be the most important environmental factors limiting the growth of these pathogens [24,35]. The impact of these factors on the mycelial growth of *Phytopythium* species is still undetermined. Therefore, this study aimed at investigating the in vitro effects of the key environmental factors of water potential (a_w) and temperature and their interactions on the radial growth and lag time (time prior growth) of *P. vexans*, the causal agent of dieback in apple trees.

2. Materials and Methods

2.1. Preparation of Oomycete Isolates

Five *P. vexans* isolates were used in this study: *P. vexans* I5 (MW815830), E4 (MW815816), M2 (MW815829), S2 (MW815837), and A7 (MW815815). These oomycete pathogens were previously isolated from the soil of the root system of diseased apple trees in five different regions [18]. These pathogens were subcultured and maintained in potato dextrose agar (PDA; Biokar-Diagnostics, Beauvais, France) medium at 4 °C for no more than 6 months. For long-term use, they were stored in 25% glycerol at −80 °C.

2.2. Influence of Culture Media on *Phytophthium vexans* Radial Growth

The impact of culture media on the mycelial growth of *P. vexans* species was evaluated. Mycelial plugs (5 mm in diameter) of each isolate were taken from the edge of freshly growing, seven-day-old cultures and placed at the centre of each medium (PDA, tomato agar, and soybean agar) in triplicate for each isolate. The colony diameters were measured daily until the Petri dishes were completely covered, and the radial growth rates (mm/day) were obtained by plotting the radius of colonies against time [36].

2.3. Effect of Temperature on *Phytophthium vexans* Radial Growth

To determine the effect of temperature on the mycelial radial growth of *P. vexans*, each isolate was grown in PDA medium for seven days in the dark at 25 °C. Mycelial plugs (5 mm in diameter) were cut from the leading edge of freshly growing colonies in PDA medium and then placed at the centre of Petri dishes containing 20 ml of PDA medium. All plates were then incubated for up to 25 days at 5, 10, 15, 20, 25, and 30 °C with three replicates per temperature treatment.

2.4. Combined Effect of Temperature and Water Potential (Ψ_w) on Radial Growth and Lag Time

To assess the effects of water potential (Ψ_w) and temperature on mycelial radial growth of *P. vexans* isolates, the basic medium used for this experiment was PDA with an a_w of 0.995 ($\Psi_w = -0.67$ MPa; 0.995 a_w). The water potential of this medium was modified by adding an increasing amount of glycerol to reach the Ψ_w contents of −15.54 (0.89 a_w), −12.58 (0.91 a_w), −9.68 (0.93 a_w), −5.44 (0.96 a_w), −2.69 (0.98 a_w), and −0.67 (0.995 a_w) at 30, 25, 20, 15, 10, and 5 °C [37]. The final media were autoclaved at 120 °C for 20 minutes. Mycelial plugs (5 mm in diameter) were cut from the leading edge of 10-day-old colonies growing in PDA and then placed at the centre of Petri dishes containing a test medium. The Petri dishes were then sealed with Parafilm to prevent water loss and incubated for up to 25 days at 5, 10, 15, 20, 25, and 30 °C. At each incubation temperature, Petri plates were arranged in a completely randomized design with three replicates for each combination of Ψ_w –temperature [38].

2.5. Radial Growth and Lag Time Assessment

Following the second day of incubation, Petri plates were examined daily without being opened for up to 20 days, and the averaged diameter of each growing mycelial colony was measured in two perpendicular directions using a digital slide stand [39]. Radial growth rates (μ , mm/day) were calculated for each temperature– Ψ_w combination using linear regression from the linear phase of the growth curve. Simultaneously, the maximum lag time (λ) before growth, the time required (days) for a colony to become visible for each isolate at each temperature– Ψ_w combination, was also recorded [40,41].

2.6. Statistical Analysis

The effect of temperature, water potential (a_w), and their interactions on the radial growth rate of oomycete pathogen *P. vexans* were determined using the general linear model procedure (GLM) of SPSS Statistical Software (version 20; IBM SPSS Statistics 20). When the effect was found to be statistically significant, Tukey's multiple range test was used for mean separation at $p \leq 0.05$.

3. Results

3.1. Influence of Culture Media on Radial Mycelial Growth

The medium significantly affected the mycelial growth of *P. vexans* (Figure 1). The obtained results showed that the PDA medium was the best culture medium for the development of the pathogenic fungus *P. vexans*. The tomato agar medium did not favour a good growth of *P. vexans* in our case, with variability in the types of colonies of several species of *P. vexans* in the different media tested, while the lowest growth was obtained with soybean agar medium. Moreover, the abundance of sporangia was the most observed in the PDA medium compared with the other media.

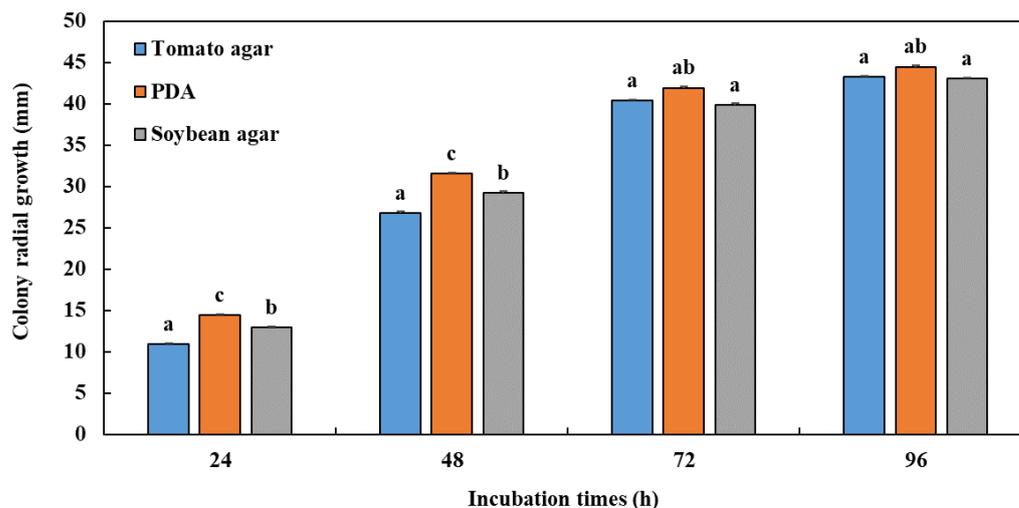


Figure 1. Colony radial growth (mm) of *Phytopythium* species as affected by different culture media (tomato agar, PDA medium, and soybean agar) after an incubation period of 96 h at 25 °C. For each incubation time period, treatments having the same letter were not significantly different according to Tukey's test ($p \leq 0.05$). Error bars represent the standard errors ($n = 3$).

3.2. Effects of Temperature and Water Potential (Ψ_w) on Mycelial Radial Growth Rate

The effect of temperature was evaluated in the range of 5 to 30 °C. The results obtained in this study asserted a highly significant effect of temperature ($p \leq 0.0001$) on the radial growth (μ) of the five *P. vexans* isolates (Table 1, Figure 2). Temperature had a significant effect on radial growth. All *P. vexans* isolates were able to grow in temperatures ranging from 5 to 30 °C with significant differences ($p \leq 0.05$). Isolate *P. vexans* E4 had the highest growth rate compared with the other isolates at 15 °C, with a radial growth rate of 5.1 ± 0.04 mm/day, while *P. vexans* isolate I5 had the highest growth rate compared with the other isolates at 25 °C and 30 °C, with radial growth rates of 5.99 ± 0.09 mm/day and 4.93 ± 0.22 mm/day, respectively. All isolates displayed the lowest growth rate at 5 °C and 10 °C, while their maximum growth rate was observed at 25 °C.

Table 1. Variance analysis results of the effects of temperature and water potential (a_w) on radial growth of *Phytopythium vexans* isolates.

Source	DF	Mean Square	F-Value	Pr > F
Species	4	0.698	50.554	0.000
Temperature	5	26.855	1944.162	0.000
a_w	2	465.160	33,675.175	0.000
Species \times Temperature	20	0.530	38.377	0.000
Species \times a_w	8	0.507	36.670	0.000
Temperature \times a_w	10	8.816	638.267	0.000
Species \times Temperature \times a_w	40	0.316	22.856	0.000

DF, degree of freedom; Pr, probability.

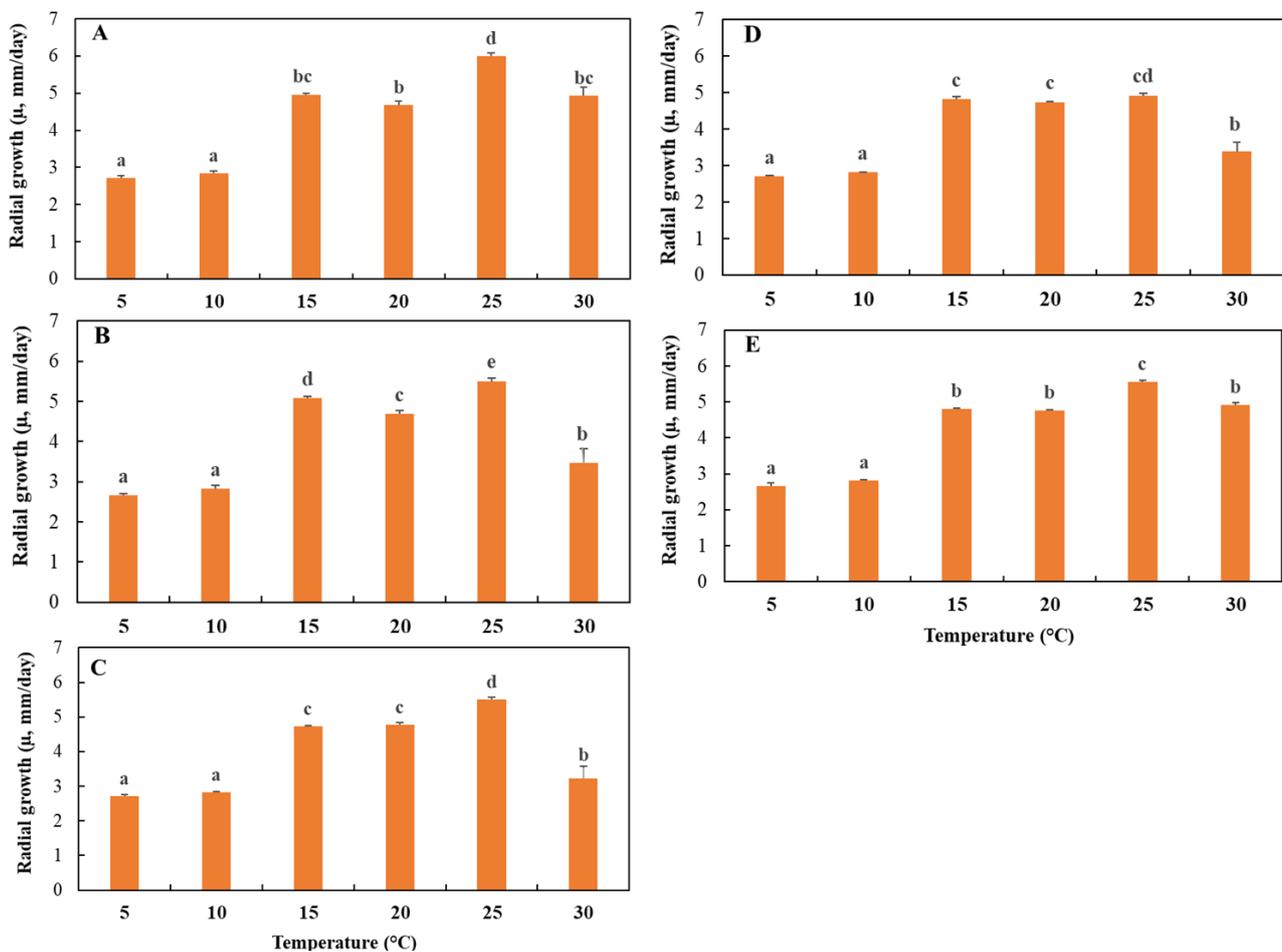


Figure 2. Radial mycelial growth (mm/day) of *Phytophthium vexans* isolates ((A), I5 (Imouzzer); (B), E4 (El Hajeb); (C), M2 (Meknes); (D), S2 (Sefrou); and (E), A7 (Azrou)) versus temperature (°C). For each temperature, treatments having the same letter were not significantly different according to Tukey's test ($p \leq 0.05$). Error bars represent the standard errors ($n = 3$).

As for temperature, the results obtained showed a highly significant effect of water potential Ψ_w ($p \leq 0.0001$) on the mycelial growth of *P. vexans* isolates (Tables 1 and 2). The growth of *P. vexans* isolates was shown to increase with the increase in water availability and temperature. The growth of all isolates was faster at a Ψ_w of -0.67 MPa ($0.995 a_w$) at 25 and 30 °C. In contrast, no growth was detected at Ψ_w of -5.44 MPa ($0.96 a_w$), -9.68 MPa ($0.93 a_w$), -12.58 MPa ($0.91 a_w$), and -15.54 MPa ($0.89 a_w$), regardless of the isolate. *P. vexans* isolates A7 and S2 recorded the maximum radial growth rate at -2.69 MPa ($0.98 a_w$) and at temperatures of 25° and 30 °C, while isolates E4 and M2 registered the highest radial growth at both Ψ_w of -0.67 MPa ($0.995 a_w$) and -2.69 MPa ($0.98 a_w$) at 25 °C. Exceptionally, isolate I5 reached its maximum radial growth only at a Ψ_w of -0.67 MPa ($0.995 a_w$) at 25 °C.

Using Tukey's test for mean separation, the three factors studied, temperature, isolates, and water potential, were grouped into different homogeneous groups. The species level provided three different groups, with the first group consisting of *P. vexans* isolates S2, E4, and M2, while isolate I5 formed the second group, and A7, the third group. In addition, the mean separation for temperatures provided six different groups. Each temperature corresponded to a distinct group in which the highest radial growth rates occurred in a decreasing order at 25, 30, 15, 10, and 5 °C. Regarding the water potential levels, the statistical analysis provided three different groups; each level corresponded to a distinct cluster with the maximum radial growth rates observed at Ψ_w -0.67 MPa ($0.995 a_w$), followed respectively by -2.69 MPa ($0.98 a_w$) and 5.44 ($0.96 a_w$) (Figure 3).

Table 2. Values of radial growth rate (μ) of different studied isolates (*Phytophthium vexans* I5, E4, M2, S2, and A7) at different temperatures (5–30 °C) and water potentials (0.67; 9.68 MPa) (n = 3).

T	Ψ_w	I5	E4	M2	S2	A7
5	0.995	2.72 ± 0.05 ^a	2.66 ± 0.04 ^b	2.70 ± 0.06 ^c	2.70 ± 0.03 ^c	2.66 ± 0.08 ^b
	0.98	2.40 ± 0.02 ^c	2.25 ± 0.24 ^c	2.39 ± 0.06 ^b	2.39 ± 0.03 ^{cd}	2.40 ± 0.02 ^{bc}
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
10	0.995	2.84 ± 0.05 ^d	2.83 ± 0.07 ^b	2.83 ± 0.03 ^c	2.87 ± 0.01 ^d	2.81 ± 0.02 ^{bcd}
	0.98	1.98 ± 0.07 ^b	1.97 ± 0.05 ^c	3.26 ± 0.04 ^d	1.98 ± 0.03 ^b	3.21 ± 0.01 ^{cd}
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
15	0.995	4.95 ± 0.04 ^e	5.09 ± 0.04 ^e	4.73 ± 0.02 ^f	4.83 ± 0.05 ^d	4.81 ± 0.01 ^f
	0.98	3.21 ± 0.07 ^h	3.87 ± 0.19 ^g	3.19 ± 0.10 ^d	2.96 ± 0.03 ^g	3.02 ± 0.05 ^{cd}
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
20	0.995	4.67 ± 0.12 ^g	4.69 ± 0.08 ^f	4.77 ± 0.06 ^f	4.74 ± 0.01 ^g	4.76 ± 0.02 ^f
	0.98	4.23 ± 0.06 ^f	4.21 ± 0.05 ^e	4.19 ± 0.03 ^e	4.18 ± 0.02 ^f	4.17 ± 0.01 ^e
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
25	0.995	5.99 ± 0.09 ⁱ	5.51 ± 0.07 ^h	5.51 ± 0.07 ^h	4.92 ± 0.07 ^g	5.55 ± 0.08 ^g
	0.98	5.34 ± 0.13 ^j	5.35 ± 0.09 ^{gh}	5.35 ± 0.09 ^h	6.17 ± 0.37 ^f	6.01 ± 0.54 ^{gh}
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
30	0.995	4.93 ± 0.23 ^h	3.47 ± 0.35 ^e	3.22 ± 0.36 ^d	3.38 ± 0.25 ^e	4.92 ± 0.07 ^f
	0.98	4.23 ± 0.06 ^h	4.08 ± 0.13 ^d	4.10 ± 0.11 ^e	4.28 ± 0.12 ^f	6.17 ± 0.37 ^h
	0.96	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

The data represent means ± standard deviations (SDs). Treatments in the same column having different letters were significantly different according to Tukey’s test ($p \leq 0.05$).

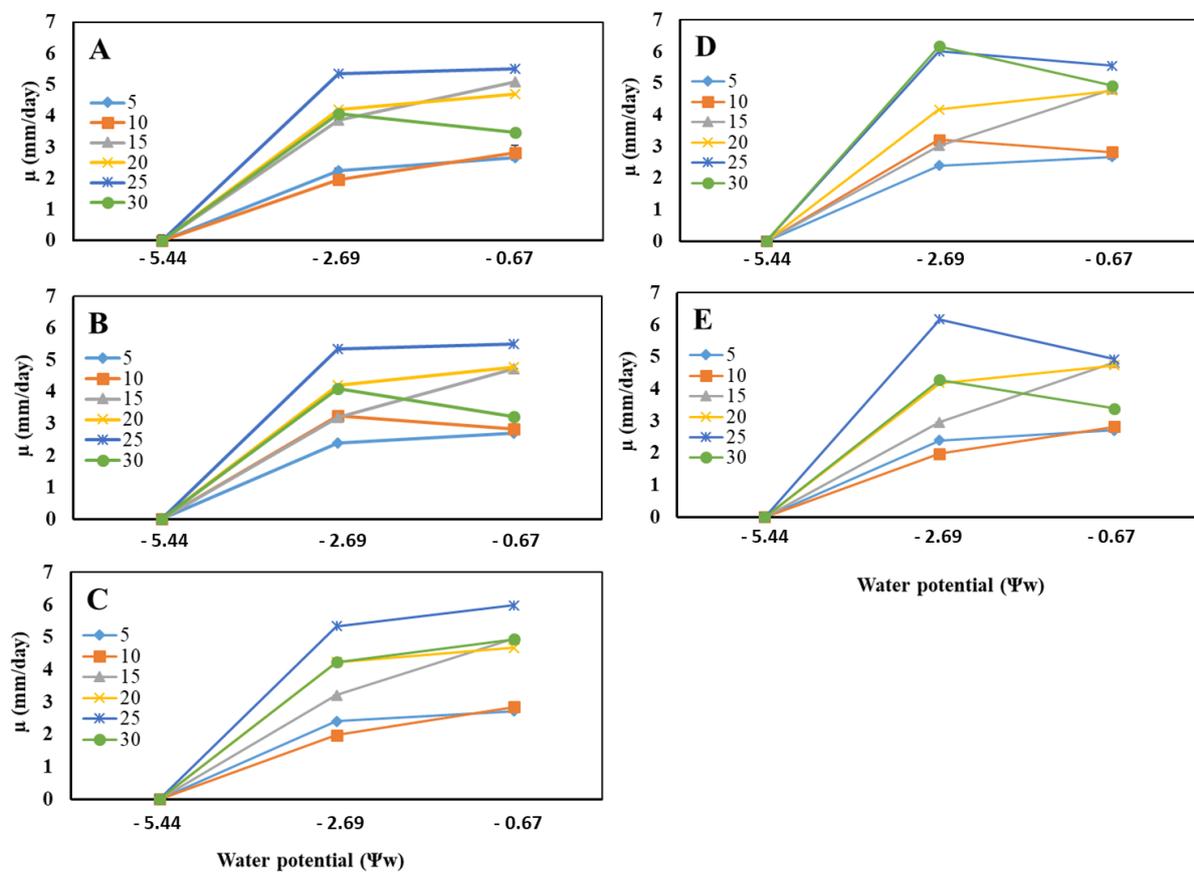


Figure 3. Effect of water potential Ψ_w on the radial mycelial growth rate of *Phytophthium vexans* isolate E4 ((A) El Hajeb), M2 ((B) Meknes), I5 ((C) Imouzzer), A7 ((D) Azrou), and S2 ((E) Sefrou) (n = 3) at different incubation temperatures of 5, 10, 15, 20, 25, and 30 °C.

3.3. Effects of Temperature and Water Potential (Ψ_w) on Lag Times

The effects of temperature and water potential Ψ_w on lag times of *P. vexans* are shown in Table 3. Both key environmental factors, temperature and water potential, had significant effects on the lag time, i.e., the time required to start growth. For all *P. vexans* isolates, the lag time was considerably decreased with the increases in the temperature and water potential of the medium (Table 3). For all *P. vexans* isolates, no growth was observed at water potential Ψ_w of -5.44 MPa ($0.96 a_w$) during the 25 days of the incubation periods regardless of temperature. The longest lag times were recorded at a temperature of 5 °C and Ψ_w of -0.67 MPa ($0.995 a_w$) and -2.69 MPa ($0.98 a_w$) with an estimated averaged time period of 5 days. Conversely, the shortest lag times were recorded at a temperature of 30 °C and a Ψ_w of -0.67 MPa ($0.995 a_w$), with an estimated averaged time period of 0.2 days (Table 3).

Table 3. Water potential effects on the required time (λ) to form a visible colony (in days) of the four *Phytophthium vexans* isolates (I5 (Imouzzer), E4 (El Hajeb), M2 (Meknès), S2 (Sefrou), and A7 (Azrou)) at different temperatures (n = 3).

Temperature	Ψ_w	I5 $\lambda \pm SD$	E4 $\lambda \pm SD$	M2 $\lambda \pm SD$	S2 $\lambda \pm SD$	A7 $\lambda \pm SD$
5	0.995	5.158 ± 0.17^h	4.99 ± 0.09^g	5.01 ± 0.14^f	5.10 ± 0.077^i	5.01 ± 0.20^g
	0.98	5.56 ± 0.04^i	5.44 ± 0.13^h	5.53 ± 0.05^f	5.53 ± 0.04^j	5.54 ± 0.04^h
	0.96	>20 ^a				
10	0.995	2.55 ± 0.17^f	2.54 ± 0.19^e	2.45 ± 0.11^d	2.48 ± 0.10^g	2.40 ± 0.06^e
	0.98	3.13 ± 0.23^g	3.02 ± 0.13^f	3.17 ± 0.05^e	3.11 ± 0.16^h	3.10 ± 0.03^f
	0.96	>20 ^a				
15	0.995	1.23 ± 0.06^d	1.08 ± 0.04^c	1.66 ± 0.01^c	1.52 ± 0.06^e	1.53 ± 0.02^d
	0.98	1.86 ± 0.27^e	1.07 ± 0.03^c	1.59 ± 0.45^c	1.98 ± 0.23^f	1.53 ± 0.07^d
	0.96	>20 ^a				
20	0.995	1.23 ± 0.01^d	1.22 ± 0.06^c	1.22 ± 0.17^c	1.04 ± 0.03^c	1.01 ± 0.02^c
	0.98	1.80 ± 0.51^e	1.83 ± 0.08^d	1.58 ± 0.40^c	1.50 ± 0.43^d	1.69 ± 0.03^d
	0.96	>20 ^a				
25	0.995	0.90 ± 0.03^{cd}	1.08 ± 0.14^c	1.11 ± 0.03^{bc}	1.51 ± 0.18^c	1.05 ± 0.06^c
	0.98	1.15 ± 0.02^d	1.32 ± 0.32^d	1.55 ± 0.32^c	1.10 ± 0.06^{cd}	1.14 ± 0.03^c
	0.96	>20 ^a				
30	0.995	0.23 ± 0.03^{ab}	0.18 ± 0.021^b	0.11 ± 0.025^a	0.12 ± 0.02^{ab}	0.38 ± 0.39^b
	0.98	0.60 ± 0.29^{bc}	0.57 ± 0.14^c	0.66 ± 0.02^b	0.50 ± 0.17^b	0.42 ± 0.14^b
	0.96	>20 ^a				

The data represent means \pm standard deviations (SDs). Treatments in the same column having different letters were significantly different according to Turkey's test ($p \leq 0.05$).

4. Discussion

This study highlighted the influence of culture media and environmental factors such as temperature and water potential on the mycelial radial growth of *P. vexans*, an oomycete pathogen that causes dieback in apple and pear trees in Morocco. In the literature, there are no reports dealing with the impact of these key environmental factors on the growth of *Phytophthium* species. Therefore, our study is the first investigation evaluating the growth of this pathogen in different temperature and water potential ranges, which might help to determine the range of each factor that plays in favour of the pathogen. The outcome of this study can help to understand the epidemiology of the pathogen and design an integrated control strategy for the managing the disease based on the avoidance and exclusion of the pathogen by optimizing the watering systems in apple plantations. It is known that the presence of water in the soil is crucial for the infection and development of root rot diseases in plants as well as their rapid spread, in particular oomycete pathogens [33,42]. To spread and infect plants, oomycete pathogens need free water, because water can help to release

zoospores from sporangia and stimulate the germination of oospores, possibly by creating favourable conditions [43]. In addition, free water must be available in the soil to facilitate the mobility of flagellated zoospores [44].

Culturing *P. vexans* and producing its infective sporangia for plant inoculation have been a challenge for pathological bioassays on the disease caused by these oomycete pathogens. Therefore, having an appropriate medium for the rapid growth of the mycelium of this pathogen and the production of its sporangia is the first step in passing the pathogenicity test. In our study, all isolates grew on all three media tested; however, the PDA medium was the most favourable for the rapid radial growth of the mycelium of *P. vexans*. In addition, the highest abundance of sporangia was observed in PDA medium, followed respectively by Tomato agar and Soybean agar, and this was correlated with the appearance of yellow pigmentation. Several studies investigated the influence of culture media on the growth and sporulation of oomycete pathogens [45,46], but only one study was interested in *Phytophthium* sp. [47]. In this study, the culture medium containing sweet cassava root was found to be the most suitable for mycelial development and pathogen sporulation.

Our results indicated that the environmental factors studied had a significant impact on the radial growth and lag time periods of all *P. vexans* isolates. It was shown that all isolates were able to grow in the range of temperatures used (5 to 30 °C). These results were in agreement with previous findings that underlined that *P. vexans* and *Pythium irregulare* Buisman displayed growth at temperatures ranging from 10 to 35 °C and from 5 to 40 °C, respectively [48]. Similar results were reported for *Cylindrocarpan* isolates and underlined that fungal isolates had the ability to grow at temperatures between 5 and 30 °C [49]. In addition, the maximum radial growth rate of *Cylindrocarpan* isolates was observed at temperatures between 25 °C and 30 °C, which was consistent with the results of our study, according to which *P. vexans* isolates displayed the maximum growth at temperatures ranging from 25 °C to 30 °C. Interestingly, most studies on oomycetes evidenced similar ranges of optimum growth temperature, e.g., 28 °C for *Pythium insidiosum* de Cock, Mendoza, Padhye, Ajello & Kaufman [50]; 25–30 °C for *P. irregulare* [48]; 30 °C for *Pythium aphanidermatum* (Edson) Fitz. [51]; and 25 °C for *Phytophthora clandestina* Taylor, Pascoe & Greenhalgh [52]. Moreover, the genus *Cylindrocarpan* showed a slight lower optimum growth temperature, between 21.9 and 24.5 °C [49].

The water potential is considered as one of the important factors limiting the growth of phytopathogenic fungi [53–55]. In this study, the growth of *P. vexans* was shown to increase with the increases in water availability and temperature. Surprisingly, no growth of *Ph. vexans* was observed at Ψ_w of -5.44 MPa ($0.96 a_w$), -9.68 MPa ($0.93 a_w$), -12.58 MPa ($0.91 a_w$), and -15.54 MPa ($0.89 a_w$), regardless of the temperature. In a similar study, there was no growth at water potentials below -1.2 MPa and at -4.8 MPa for *P. clandestina* and *Phytophthora cinnamomi* Rands, respectively [52,56]. Furthermore, the growth of all *Phytophthium* isolates was faster at -0.67 MPa ($0.995 a_w$) at 25 °C and 30 °C, which was in line with previous findings on *P. clandestina* according to which this oomycete species registered its maximum growth at 0 MPa and at 25 °C, while for other *Phytophthora* species, the maximum growth rate was found at Ψ_w -1 MPa and -1.5 MPa with an optimum growth at -1.08 MPa in *P. cinnamomi* [56,57]. In addition, the growth of *Phytophthium* was significantly reduced at a Ψ_w of -2.69 MPa ($0.98 a_w$). The growth of *P. cinnamomi* was significantly reduced at Ψ_w of -3.03 MPa and -3.79 MPa, while the lowest growth of *Cylindrocarpan* was observed at -5 MPa. Interestingly, the growth of *P. clandestina* and *Phytophthora parasitica* Breda de Haan decreased with the decrease in the water potential, but it persisted up to -5 MPa and showed to be strongly reduced at a Ψ_w of -1.2 MPa at 20 and 25 °C [52,58,59].

To the best of our knowledge, there are very few reports on the influence of environmental factors on the latency phase of oomycetes. The results of the effects of temperature and water potential on the latency time (time period prior growth) underlined that each of these two factors had a significant effect ($p \leq 0.0001$). In all isolates, the latency phase

decreased considerably with the increase in the water potential, regardless of the temperature. At a water potential of -5.44 MPa ($0.96 a_w$) and at all temperatures, no growth was recorded for a growth period of up to 25 days. The longest latency phases were recorded at a temperature of 5 °C and Ψ_w of -0.67 MPa ($0.995 a_w$) and -2.69 MPa ($0.98 a_w$), with a five-day average. Furthermore, the shortest latency phases were recorded at a temperature of 30 °C and at a Ψ_w of -0.67 MPa ($0.995 a_w$), with an average of 0.2 days. These results were in agreement with previous findings on different oomycete and fungal pathogens [49,60].

It was suggested that variations in the soil water content may determine the form of inoculum involved in root rot infection. The formation and motility of zoospores in many oomycete species depend on high soil moisture [61–63], while mycelial growth occurs at relatively low water potentials [25,56,64]. Therefore, preventing oomycete diseases is mainly based on the parafait knowledge of the disease cycle of the pathogen and its epidemiology. Knowing how these pathogens spread makes it easier to prevent their occurrence by draining the soil and avoiding excess watering, because once these microorganisms are present in soil, it is difficult to eradicate them.

5. Conclusions

Our findings evidenced that temperature, water potential, and their interaction had significant impacts on the growth of this phytopathogen. The growth rates were shown decreased with the increases in the temperature and water potential, and no growth was observed at $\Psi_w \leq -5.44$ MPa ($0.96 a_w$), regardless of the temperature. These results can be very useful in preventing the onset and spread of this pathogen. Therefore, good soil drainage and good water flow might help to prevent soil saturation, but important consideration must be given to installing a subsurface drainage and well-used drip irrigation, which prevent the increase in soil moisture and limit the spreading of the pathogen.

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