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Research Article

Effect of Temperature on the Mycelium Growth and Sporulation of Four Pathotypes of *Pyricularia oryzae* in Taiwan

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Abstract

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Climate change-related variations in temperature and precipitation may impact the ecology of certain plant pathogens and vectors that are sensitive to changes in humidity and temperature, which could, in turn, affect the incidence and temporal and spatial distribution of plant diseases. Previous research has shown that the rice blast fungus in Taiwan could be divided into 5 pathotype clusters based on virulence to Lijiangxintuanheigu monogenic lines (LTH MLs), with differences in habitat temperatures between these clusters. In this work, we investigated the effect of temperature on mycelial growth and sporulation of different pathotype clusters by analyzing 5 isolates from each of the 4 major clusters. Our findings indicated that the temperature range of 24–32℃ was optimal for the growth and reproduction of rice blast fungus in Taiwan. Among these temperatures, 28℃ was the most favorable. Conditions where the temperature fell below 20℃ or rose above 36℃ significantly restricted the growth and reproduction of the rice blast fungus. All isolates survived, albeit almost completely stopped growing at 36℃. Overall, clusters L4 and L5 exhibited better growth and reproduction at 32℃ compared to 24℃. Conversely, L1 and L2 showed superior growth and reproduction at 24℃ rather than 32℃. L4 had a significantly higher reproductive capacity than the other 3 clusters at 24–32℃. Because of this outcome and the consistent rise in average temperature since 2019, it was believed that temperature was the main cause of L4 and L5 becoming the prevalent clusters in Taiwan. This study clearly illustrated the impact of temperature changes on the epidemic spread of rice blast fungus in Taiwan. It also offered crucial guidance for developing disease-resistant plans for potential climate change scenarios.

Key words: Climate change, Mycelial growth rate, *Pyricularia oryzae*, Sporulation, Temperature.

INTRODUCTION

Climate change might be the greatest threat to conventional agriculture production in Taiwan. As the global temperature increases, the frequency of extreme weather also increases (Mirza 2003), which affects not only crop yields (Song *et al.* 2021) but also host-pathogen interactions (Anderson *et al.* 2004; Viswanath *et al.* 2017). In particular, global temperature increases and water availability changes are expected to cause changes in the suitable areas for crops, which could lead to changes in the distribution of specific crop pathogens (Velásquez *et al.*

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2018). Temperature, precipitation, and relative humidity (RH) all impact the life cycles of many plant pathogens. Thus, extreme weather events may affect their growth patterns. The distribution of pathogens and endemic diseases is changed as a result (Coakley *et al.* 1999; Harvell *et al.* 2002; Mina & Sinha 2008; Yáñez-López *et al.* 2012; Gautam *et al.* 2013; Bevitori & Ghini 2014). For instance, the first record of a wheat blast outside of South America was discovered in Bangladesh, Asia, in 2016 (Montes *et al.* 2022). The disease is predicted to progressively spread to warm, humid regions surrounding Bangladesh in the future, based on the pathogen's (*Magnaporthe oryzae* pathotype Triticum; MoT) climate adaptation (Mottaleb *et al.* 2018; Montes *et al.* 2022). However, the impact of climate change on plant pathosystems may differ depending on the location (Elad & Pertot 2014).

The host, pathogen, and environmental factors interact in a triangle to form the three most important components of any given plant disease (Velásquez *et al.* 2018). Environmental changes may impact the coevolution of hosts and pathogens (Elad & Pertot 2014). The emergence of a novel and highly pathogenic strain may complicate management approaches (Strange & Scott 2005; Garrett *et al.* 2016). For instance, rising CO_2 concentrations will make wheat more susceptible and *Fusarium graminearum* more virulent (Váry *et al.* 2015). At temperatures above 32℃, *Agrobacterium tumefaciens* loses its ability to infect Kalanchoe due to decreased virulence gene expression (Jin *et al.* 1993). *Cercospora zeae-maydis*, the corn gray leaf spot pathogen, exhibited reduced sporulation and hyphae growth rates when field conditions exceeded 32℃ and 95% RH, thereby reducing the severity of the disease (Paul & Munkvold 2005). However, few studies have looked into the effects of climate change on pathogen population dynamics and changes in disease incidence patterns (Viswanath *et al.* 2017).

Rice, a globally important food crop, is primarily cultivated in tropical and temperate climate regions between 53°N and 40°S latitudes (Koo *et al.* 2013). The warm and humid climate of the cultivation areas favors rice growth and serves as a breeding ground for many pests and diseases. The rice blast pathogen (*Pyricularia oryzae* Cavara) causes severe economic loss in many rice cultivation regions (Ou 1985; Skamnioti & Gurr 2009; Hajano *et al.* 2011). *P. oryzae* thrives at 17–28℃ with high RH, preferring sporulation conditions of 25–29℃, RH > 89%, and leaf dew duration greater than 4 h (Greer & Webster 2001; Viswanath *et al.* 2017; Shahriar *et al.* 2020). Computer model simulations show that temperature is a critical factor affecting pathogen growth, sporulation, and disease progress of the rice blast (Viswanath *et al.* 2017). However, no field experiment has been conducted to prove this assumption. The effects of global temperature change on *P. oryzae* may vary by geographical region. Rising temperatures in cooler subtropical regions increase the likelihood of rice blast epidemics. Nevertheless, a drop in temperature raises the possibility of rice blast epidemics in those warm, humid tropical regions because the present temperatures are already above the disease's ideal range (Luo *et al.* 1998; Ghini *et al.* 2008; Viswanath *et al.* 2017). Field surveys in China since 1970 have confirmed the prediction model proposed by Viswanath *et al.* (2017). Higher spring and early summer temperatures cause a decrease in wheat strip rust disease but an increase in rice blast and wheat Fusarium head blight (Coakley *et al.* 1999). Though the computer model suggests that climate change influences the temporal and spatial distribution of plant diseases, quantitative data on the precise impact of climate change on a specific plant pathogen is still lacking (Bevitori & Ghini 2014).

We found that the five pathotype clusters of *P. oryzae* in Taiwan have distinct habitat temperatures based on the population surveillance of rice blast fungus between 2014 and 2021 (Chen *et al.* 2023b). Interestingly, since 2019, the number of L4 cluster isolates viru-

lent to *Pik* alleles (*Pik*, *Pik-p*, *Pik-m*, *Pik-h*, *Pi1*, *Pi7(t)*) has increased more than 1.7 times compared to previous years. Given that 2019 and 2020 saw exceptionally warm winters in Taiwan, we were curious to know if recent changes in *P. oryzae* field populations in Taiwan were influenced by variations in temperature. In this study, twenty *P. oryzae* isolates belonging to four pathotype clusters were tested for their optimal temperatures for growth and sporulation. The findings demonstrate the distinct temperature preferences of various pathotype cluster isolates. Understanding the factors influencing the growth and sporulation of *P. oryzae* may have important implications for disease management, taking into consideration climate changes and the availability of host-esistant genes.

MATERIALS AND METHODS

P. oryzae isolates

A total of 1,749 *P. oryzae* isolates were gathered from rice fields in Taiwan between 2014 and 2021 (Chen *et al.* 2023b). These isolates were pure cultures originating from a single spore culture (Hayashi *et al.* 2009). Based on their virulence towards Lijiangxintuanheigu monogenic lines (LTH MLs) (S Table 1) (Kobayashi *et al.* 2007), these isolates were categorized into five pathotype clusters (L1–L5). Five isolates from each of the L1, L2, L4, and L5 clusters were randomly chosen for the following experiments (Table 1). Because the L3 cluster is a very small percentage of the population overall, it was excluded from this experiment.

Table 1. Pathotype clusters of 20 *Pyricularia oryzae* isolates used in this study (Chen *et al.* 2023b)

Pathotype cluster	Target gene[z]	Isolate[y]	Collection date	Location
L1	*Pish*	DAL2a3-1605	May, 2016	Dalin, Chiayi
		HL5a2-1606	Jun, 2016	Houli, Taichung
		CT2a1(1)-1803	Mar, 2018	Caotun, Nantou
		MN1a2-1803	Mar, 2018	Meinong, Kaohsiung
		MN6a3-1908	Aug, 2019	Meinong, Kaohsiung
L2	*Pita*	JX2a1-1405	May, 2014	Jiaoxi, Yilan
		SHC1a3-1504	Apr, 2015	Qingshui, Taichung
		LN2b3-1605	May, 2016	Linnei, Yunlin
		MN4a2-1804	Apr, 2018	Meinong, Kaohsiung
		LY3a1-1805	May, 2018	Luye, Taitung
L4	*Pik*, *Pik-p*, *Pik-m*, *Pik-h*, *Pi1*, *Pi7(t)*, *Pish*, *Pita*, *Pi19(t)*	EM1a1-1903	Mar, 2019	Emei, Hsinchu
		LJ3a2-1903	Mar, 2019	Liujia, Tainan
		SX3a1-1905	May, 2019	Sanxing, Yilan
		HW2a1-2003	Mar, 2020	Huwei, Yunlin
		LL1a1-2004	Apr, 2020	Linluo, Pingtung
L5	*Piz-t*	KD2a1-1901	Jan, 2019	Kanding, Pingtung
		GS3a1-1903	Mar, 2019	Guanshan, Taitung
		GOGU3a2-1905	May, 2019	Gongguan, Miaoli
		SF5a3-1905	May, 2019	Shoufeng, Hualien
		TL3a1-1905	May, 2019	Tongluo, Miaoli

[z] The target gene means the pathotype cluster compatible (virulent) to these genes.
[y] The number after the "-" represents the year and month of collection.

Mycelial growth test

Fungal isolates from stock cultures were revived on a 9 cm potato dextrose agar (PDA) plate (Bacto, Mt Pritchard, Australia). After the mycelium had completely covered the plate, a 5 mm mycelium disc was taken from the edge and placed in the center of a new PDA plate. Plates were incubated at 12, 16, 20, 24, 28, 32, 36, and 40℃ for 5 d in darkness. The average daily growth rate of the fungal colony was computed by measuring its diameters. All mycelial disc culture plates subjected to temperature treatments were subsequently incubated at room temperature for another two wk to monitor pathogen viability and growth potential. After the two-week observation period, cultures were examined under a microscope. The absence of visible hyphal growth was used as the criterion to confirm pathogen mortality. The experiment was conducted twice, with three replicates of each treatment.

Sporulation test

Fungal isolates were revived on a V-8 juice agar (each liter containing V-8 juice 100 mL, $CaCO_3$ 0.2 g, agar 17 g, pH 6.5) plate and then augmented with the liquid spawn method by Chen *et al.* (2021b). The mycelial pellets were homogenized with the prune broth medium (each liter containing prune 15 g, starch 2.5 g, yeast extract 0.5 g, pH 6.5) using a homogenizer (Oster Blender 6640-022, Oster, Chicago, IL, USA). One mL of the resulting suspension was spread on 5.5 cm OFA plates (oat flour 10 g L^{-1}, agar 20 g L^{-1}), and the excess moisture was air-dried. The plates were then incubated for 4 d at 12, 16, 20, 24, 28, 32, 36, and 40℃. All chambers were set with 12 h under blacklight blue (PULSAR, F40T8/BLB) and 12 h darkness. Spores were collected by adding 5 mL of 0.02% Tween 80 and gently scrapping with a glass slide. The quantity of spores was measured using a hemocytometer (Hausser Scientific, Horsham, PA, USA) after spore suspensions were filtered through a layer of Miracloth (EMD Millipore, Billerica, MA, USA). The experiment was conducted twice, with four replicates per treatment in each trial.

Statistical analyses

In the mycelial growth test and sporulation test, the experiments aimed to evaluate the growth responses of isolates under the effects of different variable sources, including the isolates, temperature treatments, and their interactions. Statistical analysis was conducted through an analysis of variance (ANOVA) for these three factors. The 20 isolates were derived from four distinct pathotype clusters, allowing the factor of pathotype clusters to be further partitioned into variations between clusters and within-cluster isolates. When significant differences were detected in the ANOVA for any factor, Fisher's protected least significant difference (PLSD) test was applied for pairwise comparisons among treatment combinations. Differences were considered significant at the 5% level and were denoted using distinct letters. Statistical analysis was performed by the R package, Agricolae (Version 3.6.0) (de Mendiburu 2015) via ANOVA followed by Fisher's least square difference (LSD) test at a $P < 0.05$ significance level. The data was displayed as the mean ± standard deviation derived from a minimum of three duplicates.

RESULTS

Determination of optimal growth temperatures for four pathotype clusters

The blast isolates were categorized into four major pathotype clusters based on their pathogenic reactions (Table 1). L4 cluster showed pathogenic to rice cultivars carrying the *Pik* alleles (*Pik*, *Pik-p*, *Pik-m*, *Pik-h*, *Pi1*, and *Pi7*), *Pish*, *Pita*, and *Pi19(t)*. L1 cluster showed pathogenic to those carrying the *Pish* gene and L5 cluster showed pathogenic to the rice cultivars carrying the *Piz-t* gene (Chen *et al.* 2023b). ANOVA was performed on the data to compare how temperature affected the mycelial growth rates of various

pathotype cluster isolates of the rice blast fungus in Taiwan. The 20 isolates were derived from four distinct pathotype clusters, allowing the factor of pathotype clusters to be further partitioned into variations between clusters and within-cluster isolates. The findings (Table 2) demonstrated that the two primary factors of temperature and isolate had a significant impact ($P < 0.001$) on the mycelial growth rate of the rice blast fungus, but the primary factor of pathotype clusters did not show a significant difference ($P > 0.05$). Additionally, significant interactions were observed ($P < 0.001$) between temperature and pathotype cluster and between temperature and isolate. It suggests that distinct pathotype clusters and isolates each had a favored temperature for mycelium growth. Among these factors, temperature had the greatest impact on the mycelial growth rate.

The results showed that 28℃ was the optimal mycelium growth temperature for *P. oryzae*, with an average rate of 2.93 mm day^{-1}. Mycelium growth rates at 32 and 24℃ were 2.56 mm and 2.48 mm day^{-1}, respectively (Fig. 1). Most isolates did not grow but remained viable when incubated at 36℃. All test isolates stopped growing and some even perished at 40℃. The isolate KD2al-1901 had the highest growth rate (an average of 1.49 mm day^{-1} under all temperature treatments), and isolate GOGU3a2-1905 was the slowest (an average of 1.19 mm day^{-1}) (Table 3).

The mycelial growth of four pathotype clusters at different temperatures revealed that 28℃ was the optimal temperature for each cluster. The L5 cluster demonstrated a notably greater growth rate at 32℃ in comparison to 24℃, while the other three pathotype clusters did not exhibit any notable variations when cultivated at either 24 or 32℃ (S Table 2).

Effect of temperature on the sporulation of *P. oryzae* pathotypes

The 20 isolates were derived from four distinct pathotype clusters, allowing the factor

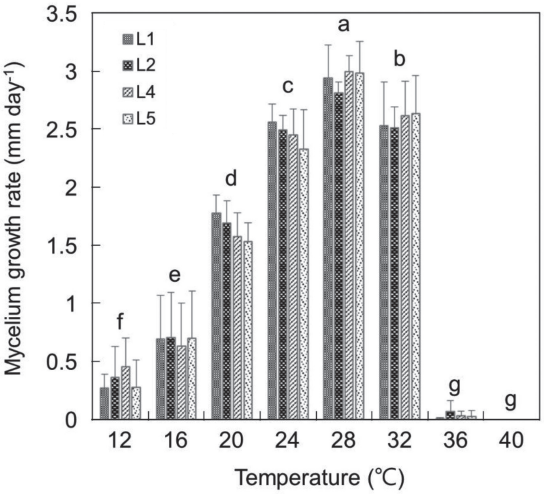

Fig. 1. The average daily mycelium growth rate (mm day^{-1}) of four pathotype clusters of rice blast fungus cultured on potato dextrose agar medium under different temperatures. Error bars represent the standard errors of the mean values of 5 isolates within the cluster. The same lowercase letters above the gray bars indicate that the mean values are not significantly different at $P < 0.05$ among the temperature subgroup.

Table 2. Effects of different incubation temperatures on the mycelium growth rate (mm day^{-1}) of 20 *Pyricularia oryzae* isolates based on analysis of variance (ANOVA).

Treatment	*df*	*MS*	*F*
Temperature	7	88.64	590.933***
Isolate	19		
Among pathotype	(3)	0.05	0.278
Within isolate	(16)	0.18	17.999***
Temperature × pathotype	21	0.07	6.731***
Temperature × isolate	112	0.15	15.129***
Error	320	0.01	

*, **, and *** indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$. The mean value of each isolate represents three replicates.

Table 3. Average daily mycelium growth rate (mm day^{-1}) of 20 isolates of *Pyricularia oryzae* in four pathotype clusters.

Isolate	Pathotype cluster	Mycelium growth rate (mm day^{-1})
DAL2a3-1605	L1	1.37[z] cdef[y]
HL5a2-1606		1.46 ab
CT2a1(1)-1803		1.28 hij
MN1a2-1803		1.38 cde
MN6a3-1908		1.24 ijk
Subgroup mean of L1		1.346
JX2a1-1405	L2	1.35 defg
SHC1a3-1504		1.41 bc
LN2b3-1605		1.24 ijk
MN4a2-1804		1.36 cdef
LY3a1-1805		1.29 ghi
Subgroup mean of L2		1.33
EM1a1-1903	L4	1.41 bcd
LJ3a2-1903		1.44 ab
SX3a1-1905		1.33 efgh
HW2a1-2003		1.36 cdef
LL1a1-2004		1.25 ij
Subgroup mean of L4		1.358
KD2a1-1901	L5	1.49 a
GS3a1-1903		1.33 efgh
GOGU3a2-1905		1.19 k
SF5a3-1905		1.23 jk
TL3a1-1905		1.31 fgh
Subgroup mean of L5	1.31	

[z] The mean value of each isolate represents four replicates.
[y] Values are expressed as the mean of each isolate, and means within a column followed by the same letter(s) are not significantly different at the 5% level as determined by Fisher's protected least significant difference (PLSD) test.

of pathotype clusters to be further partitioned into variations between clusters and within-cluster isolates. The effects of different incubation temperatures on the sporulation of the *P. oryzae* isolates tested by ANOVA showed significant differences ($P < 0.001$) (Table 4). There were also significant differences between isolates but not in pathotypes. Significant differences were observed in the interactions between temperature and pathotype and temperature and isolate as well. The findings showed that distinct pathotypes and isolates had preferred sporulation temperatures.

For *P. oryzae*, the optimal sporulation temperature was 28℃, with an average of 16.6 × 10^5 conidia plate^{-1} produced. The average sporulation at 24℃ and 32℃ was 13.8 × 10^5 conidia plate^{-1} and 12.7 × 10^5 conidia plate^{-1}, respectively. All isolates failed to sporulate when incubated at 40℃ (Fig. 2). Isolate SX3a1-1905 exhibited the highest sporulation of all the tested isolates, with an average of 17.9 × 10^5 conidia plate^{-1} calculated from all the sporulation data at all temperatures (Table 5). Isolate LN2b3-1605 had the lowest sporulation, with an average of 0.2 × 10^5 conidia plate^{-1}. Across pathotypes, the optimal sporulation temperature for the L4 cluster was 28℃, resulting in an average

Table 4. Analysis of variance (ANOVA) result of the effects of different incubation temperatures on the sporulation of 20 rice blast isolates in four pathotype clusters.

Treatment	*df*	*MS*	*F*
Temperature	7	3.842e + 13	16.96***
Isolate	19		
Among pathotype	(3)	1.743e + 13	2.02
Within Isolate	(16)	8.630e + 12	79.76***
Temperature × pathotype	21	4.947e + 12	45.73***
Temperature × isolate	112	2.265e + 12	20.94***
Error	480	1.082e + 11	

*, **, and *** indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$. The mean value of each isolate represents four replicates.

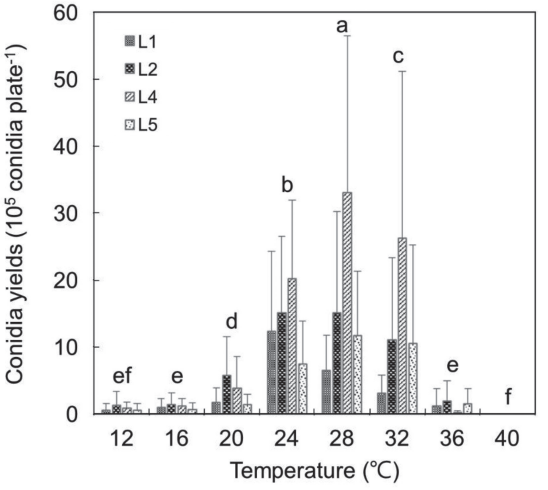

Fig. 2. The average conidia yields (conidia plate^{-1}) of four rice blast fungus pathotype clusters cultured on the OFA medium under different temperatures. Error bars represent the standard errors of the mean values of 5 isolates within the cluster. The same lowercase letters above the gray bars indicate that the mean values are not significantly different at $P < 0.05$ among the temperature subgroup.

of 33.1 × 10^5 conidia plate^{-1}. At 32℃, the L4 cluster generated 26.2 × 10^5 conidia plate^{-1}. For the L1 cluster, 24℃ was the ideal sporulation temperature that sporulated an average of 15.1 × 10^5 conidia plate^{-1}. The optimal sporulation temperature for L5 cluster isolates was 28–32℃ at which an average of 10.5–11.7 × 10^5 conidia plate^{-1} was produced. The L2 cluster generated the highest number of conidia at 24–28℃ with an average of 15.1 × 10^5 conidia plate^{-1} (S Table 2).

DISCUSSION

Global warming alters not only the optimal crop cultivation regions but also the growth and distribution of plant pathogens and pests (Velásquez *et al.* 2018). Taiwan experienced a relatively warm winter in 2019, with average temperatures of 2–4℃ higher from January and February than previous decades (Chen *et al.* 2023a). This abnormally high temperature caused the rice blast to occur earlier, in mid-March rather than April (Chen *et al.* 2021a). As more isolates of the L4 cluster were collected from the field yearly between 2018 and 2020, the L4 cluster of *P. oryzae* began to predominate. In contrast, between 2014 and 2018, the percentages of L1 and L2 cluster isolates declined yearly (Chen *et al.* 2023b). The disease triangle showed that both the host and environment can influence the evolution and adaptation of pathogens. Since the Tainan 11 cultivar was the dominant cultivated rice in Taiwan from 2014 to 2021 with no notable changes, it is hypothesized that the dramatic changes in rice blast fungus pathotypes over the last eight years were related to temperature variations.

According to our results, temperature and isolation, were the two main factors affecting the mycelium growth and sporulation of *P. oryzae*. However, the effect of the pathotype was not substantial. This suggests that the mycelial growth rate and sporulation of the rice

Table 5. Average spore yields of twenty rice blast isolates in four pathotype clusters

Isolate	Pathotype cluster	No. of conidia per plate	
DAL2a3-1605	L1	128,063[z]	ij[y]
HL5a2-1606		838,000	d
CT2a1(1)-1803		222,938	ghi
MN1a2-1803		307,125	fgh
MN6a3-1908		154,531	hij
Subgroup mean of L1		330,131	
JX2a1-1405	L2	502,188	e
SHC1a3-1504		693,813	d
LN2b3-1605		20,094	j
MN4a2-1804		1,599,063	b
LY3a1-1805		411,656	ef
Subgroup mean of L2		645,363	
EM1a1-1903	L4	1,697,219	ab
LJ3a2-1903		1,001,688	c
SX3a1-1905		1,788,219	a
HW2a1-2003		812,813	d
LL1a1-2004		49,469	j
Subgroup mean of L4		1,069,882	
KD2a1-1901	L5	329,750	fg
GS3a1-1903		1,049,094	c
GOGU3a2-1905		418,969	ef
SF5a3-1905		267,438	fghi
TL3a1-1905		37,000	j
Subgroup mean of L5		420,450	

[z] The mean value of each isolate represents four replicates.
[y] Values are expressed as the mean of each isolate, and means within a column followed by the same letter(s) are not significantly different at the 5% level as determined by Fisher's protected least significant difference (PLSD) test.

blast fungus vary with temperature and differ between isolates. Nonetheless, due to individual differences in mycelial growth rate and sporulation of isolates within each cluster, the differences among the clusters were insignificant. Furthermore, the interactions of temperature with pathotype and temperature with isolate also had a notable impact on the mycelial growth rate and sporulation of the rice blast fungus. This indicates that the mycelial growth rate and sporulation of the isolates and pathotypes vary with temperature changes.

Our findings indicated that 24–32℃ is the ideal temperature range for the growth and reproduction of the rice blast fungus in Taiwan. The growth and reproduction of the fungus are severely limited when the temperature drops below 20℃ or rises above 36℃. The rice blast fungus in Taiwan grew most favorably at 28℃, achieving mycelial growth of 2.93 mm day^{-1} and sporulation of 16.67×10^5 conidia $plate^{-1}$. While sporulation was higher at 24℃ (13.8×10^5 conidia $plate^{-1}$) than at 32℃ (12.7×10^5 conidia $plate^{-1}$), mycelial growth rate was faster at 32℃ (2.57 mm day^{-1}) than at 24℃ (2.46 mm day^{-1}). Furthermore, Taiwan rice blast fungus tolerated temperatures up to 36℃, although growth and reproduction were inhibited in these conditions. The average high temperature on Taiwan's flatlands during the first rice crop season, which runs from

January to June, ranges between 20.9℃ and 31.1℃. During the second crop season, from July through November, temperatures typically range from 22.3 to 32.3℃ (data source: https://www.cwa.gov.tw/V8/C/C/Statistics/monthlymean.html). The findings of this experiment, which showed that temperatures above 32℃ were unfavorable for the growth and reproduction of rice blast fungus, precisely explained why rice blast disease occurred more frequently in Taiwan during the relatively cooler first crop season and less frequently during the warmer second season.

For all pathotype clusters, the ideal temperature for mycelial growth was 28℃. Clusters L4 (2.62 mm day^{-1}) and L5 (2.63 mm day^{-1}) exhibited faster mycelial growth rates at 32℃ than do L1 (2.53 mm day^{-1}) and L2 (2.51 mm day^{-1}). In contrast, clusters L1 (2.56 mm day^{-1}) and L2 (2.49 mm day^{-1}) exhibited faster mycelial growth rates at 24℃ than do clusters L4 (2.45 mm day^{-1}) and L5 (2.33 mm day^{-1}). Results showed that mycelial growth and development of clusters L4 and L5 were favored by warmer conditions (32℃), while clusters L1 and L2 were favored by cooler conditions (24℃). The ideal temperatures for sporulation differed slightly between the clusters. The L1 cluster had an ideal temperature of 24℃ (12.4×10^5 conidia $plate^{-1}$); the L2 cluster had an optimal temperature of 24 to 28℃ (both producing 15.1×10^5 conidia $plate^{-1}$); the L4 cluster had an optimal temperature of 28℃ (33.1×10^5 conidia $plate^{-1}$); and the L5 cluster had an optimal temperature of 28 to 32℃ ($11.7–10.5 \times 10^5$ conidia $plate^{-1}$) (S Table 2). The findings indicate that sporulation in clusters L4 and L5 is more advantageous under warmer conditions (32℃), while clusters L1 and L2 benefit from cooler conditions. The highly virulent L4 cluster exhibited significantly higher sporulation levels between 24–32℃ compared to the other three clusters. This indicates that L4 has a greater potential for infection within this temperature range than the other clusters. The L2 cluster was the least virulent of all the clusters, even though it showed more sporulation between 24℃ and 32℃ than the L5 cluster. Consequently, within this temperature range, L2 was not conducive to effective infection and development on the host rice plants. The above results imply that warmer conditions (32℃) will support the survival and growth of clusters L4 and L5. On the other hand, clusters L1 and L2 will grow and develop more readily in colder environments (24℃). The results may explain why L4 cluster was dominant from all *P. oryzae* isolates collected in 2019 and 2020 when the average temperature in Spring were much higher than the previous years.

The L1 and L5 clusters had the widest range of average temperatures in their distribution areas (both 11.9–30.7℃), according to an analysis of temperature data from the habitats of the five pathotype clusters (Chen *et al.* 2023b). In terms of average maximum temperature, cluster L2 had an average of 28.5℃, while clusters L1, L4, and L5 in all distribution areas had an average above 29.3℃. The mean of the lowest temperatures in the distribution areas of clusters L1, L4, and L5 was higher than 22.7℃, while the mean for cluster L2 was 21.9℃. It is inferred that an environmental temperature above 29℃ promotes the survival of clusters L1, L4, and L5, based on the habitat temperature data of pathotype clusters. Conversely, the L2 cluster has a better chance of surviving in an environment with a temperature below 22.6℃. Taking into account the growth and sporulation abilities of pathotype clusters at various temperatures, as well as their adaptability to environmental temperatures, we can reasonably conclude that when the environmental temperature exceeds 29℃, only clusters L4 and L5 will have a competitive advantage, whereas cluster L1 may lose competitiveness due to a significant decline in sporulation ability. Even though the daytime high may be higher than 29℃, if the nighttime low stays at or below 24℃, there is a chance that this will positively impact L1 cluster development. Conversely, the L2 cluster may instead gain a competitive advantage when the environment temperature falls below 23℃. However, it remains unclear what causes such differences in temperature adaptability between the four patho-

type clusters, and further research is needed to clarify this.

Other plant pathogens have also been reported to have different optimal growth temperatures among isolates. For example, *Phytophthora infestans* isolates from Europe and the Mediterranean have different temperature preferences in different stages of the life cycle, such as the latent infection period, sporulation, and sporangia production (Mariette *et al.* 2016). The outbreak of the wheat tripe rust disease in eastern USA and Australia since 2000 is largely due to the increase of a new *Puccinia striiformis* f. sp. *tritici* isolate that is capable of producing more conidia at higher temperatures (Hovmøller *et al*. 2008; Garrett *et al*. 2009; Milus *et al*. 2009). These examples illustrated that temperature plays a selective role in favoring fungal isolates with the highest fitness to become a dominant population in a short time. Global warming favors *P. oryzae* isolates that sporulate and grow faster at high temperatures. We speculate that the L4 cluster might become the predominant pathotype in Taiwan if the same rice cultivars are continually planted.

Under the influence of global warming, temperature rise and water availability changes are expected to be important factors affecting the future distribution areas of crop production, disease vectors, and pathogen populations (Velásquez *et al.* 2018). Among them, the impact of temperature changes on pests and plant diseases is the most critical (Garrett *et al.* 2022). Some studies have pointed out that a higher temperature might suppress the development of rice blast disease in the fields (Bevitori & Ghini 2014). However, in this study, we have found that partial *P. oryzae* could survive at higher temperatures, and these heat-adapted pathogens may become more prevalent in a warmer climate because of their shorter life cycles and sporulation of more conidia. Short life history and high spore yield are conducive to accelerating the evolution of fungal pathogens to produce new virulent strains with better adaptability, thereby changing the pathogen population structure and disease ecology (Velásquez *et al.* 2018). *P. oryzae* had been proven to be a fast-coevolving pathogen with the rice host (Kanzaki *et al.* 2012; Li *et al.* 2019). No experimental evidence has shown that environmental factors could speed up their evolutionary dynamics. Long-term monitoring and data collection are needed to verify the effect of climate changes on plant disease epidemics (Jeger & Pautasso 2008; Garrett *et al.* 2016). This study showed that pathotypes of *P. oryzae* collected from 2014 to 2021 had different optimal temperatures for growth and sporulation. More factors should be evaluated to obtain a better prediction and monitoring model that can help us to make better decisions for disease management in the future.

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S Table 1. Lijiangxintuanheigu monogenic lines (LTH MLs) with different genes for blast resistance

Line	Targeted resistance gene	Donor
IRBLa-A[z]	*Pia*	Aichi Asahi
IRBLa-C		CO39
IRBLi-F5	*Pii*	Fujisaka5
IRBLks-F5	*Pik-s*	Fujisaka5
IRBLks-S		Shin2
IRBLk-Ka	*Pik*	Kanto51
IRBLkp-K60	Pik-p	K60
IRBLkh-K3	*Pik-h*	K3
IRBLz-Fu	*Piz*	Fukunishiki
IRBLz5-CA	*Piz5*	C101A51
IRBLzt-T	*Piz-t*	Toride1
IRBLta-K1	*Pita*	K1
IRBLta-CT2		C105TTP2L9
IRBLta-CP1		C101PKT
IRBLb-B	*Pib*	BL1
IRBLt-K59	*Pit*	K59
IRBLsh-S	*Pish*	Shin2
IRBLsh-B		BL1
IRBL1-CL	*Pi1*	C101LAC
IRBL3-CP4	*Pi3(t)*	C104PKT
IRBL5-M	*Pi5(t)*	Moroberekan
IRBL7-M	*Pi7(t)*	Moroberekan
IRBL9-W	*Pi9*	WHD-1S-75-1-127
IRBL12-M	*Pi12*	Moroberekan
IRBL19-A	*Pi19(t)*	Aichi Asahi
IRBLkm-Ts	*Pik-m*	Tsuyuake
IRBL20-IR24	*Pi20(t)*	IR24
IRBLta2-Pi	*Pita-2*	Pi.No.4
IRBLta2-Re		Reiho
IRBL11-Zh	*Pi11*	Zhaiyeqing8
LTH		

[z] Monogenic lines are designated as IRBL (International Rice Research Institute-bred blast-resistant line) followed by the resistance gene then the abbreviation of the resistant donor variety.

S Table 2. Average daily mycelium growth rate and sporulation of four rice blast pathotype clusters of Taiwan cultured at different temperatures

Temperature	Pathotype cluster	Mycelium growth rate (mm day^{-1})		Sporulation (number of conidia plate^{-1})	
12℃	L1	0.269[z]	n[y]	56,700	j
	L2	0.363	m	129,100	ij
	L4	0.374	m	82,600	j
	L5	0.278	n	50,650	j
16℃	L1	0.691	l	96,900	j
	L2	0.705	kl	139,050	ij
	L4	0.763	k	117,650	ij
	L5	0.701	kl	59,400	j
20℃	L1	1.777	g	171,750	ij
	L2	1.692	h	572,050	fg
	L4	1.618	i	388,000	gh
	L5	1.535	j	137,050	ij
24℃	L1	2.561	cde	1,237,200	e
	L2	2.493	e	1,510,100	d
	L4	2.520	de	2,018,900	c
	L5	2.328	f	746,450	f
28℃	L1	2.941	a	651,350	f
	L2	2.809	b	1,506,850	d
	L4	2.971	a	3,306,750	a
	L5	2.984	a	1,173,050	e
32℃	L1	2.529	de	306,500	hi
	L2	2.513	de	1,110,350	e
	L4	2.579	cd	2,620,100	b
	L5	2.633	c	1,047,150	e
36℃	L1	0.005	o	120,650	ij
	L2	0.068	o	195,400	hij
	L4	0.025	o	25,050	j
	L5	0.024	o	149,850	ij
40℃	L1	0	o	0	j
	L2	0	o	0	j
	L4	0	o	0	j
	L5	0	o	0	j

[z] The mean value of each pathotype subgroup represents five isolates.
[y] Values are expressed as the mean of each pathotype subgroup, and means within a column followed by the same letter(s) are not significantly different at the 5% level determined by Fisher's protected least significant difference (PLSD) test.

溫度對臺灣稻熱病菌 (*Pyricularia oryzae*) 四種病原型菌株生長及產孢之影響

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摘要

陳繹年、吳東鴻、陳美君、陳珮臻。2025。溫度對臺灣稻熱病菌 (*Pyricularia oryzae*) 四種病原型菌株生長及產孢之影響。台灣農業研究 74(2):161–175。

氣候變遷引發的溫度與降雨變化，可能影響某些對濕度和溫度敏感的植物有害生物及其媒介生態，進而改變病害的發生率及其時間和空間分布。先前研究顯示，臺灣稻熱病菌 (*Pyricularia oryzae*) 可根據對麗江新團黑谷單基因系 (Lijiangxintuanheigu monogenic lines; LTH MLs) 的致病力，分為 5 個病原型菌系，且這些菌系在棲地溫度上存在差異。本研究針對 4 個主要病原型菌系，每個菌系選取 5 個分離株，分析溫度對其菌絲生長與孢子形成的影響。結果顯示，24–32℃為適合臺灣稻熱病菌生長與繁殖的溫度範圍，其中 28℃為最適溫度。當溫度低於 20℃或高於 36℃時，稻熱病菌的生長和繁殖受到顯著抑制。所有分離株在 36℃下均能存活，但幾乎完全停止生長。整體而言，L4 和 L5 菌系在 32℃下的生長和繁殖優於 24℃；然 L1 和 L2 菌系相對在 24℃下表現更佳。特別是 L4 菌系在 24–32℃間的繁殖能力顯著高於其他 3 個菌系。根據 4 個主要病原型菌系在不同溫度下的菌絲生長與孢子形成能力，推測自 2019 年以來平均氣溫持續上升，可能導致 L4 和 L5 成為臺灣近年流行菌系的主要原因。本研究顯示溫度變化對臺灣稻熱病菌流行的影響，並為應對氣候變遷提供重要的抗病策略參考。

關鍵詞：氣候變遷、菌絲生長速度、稻瘟病菌、產孢、溫度。

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