EFFECT OF NUTRIENT STATUS, pH, TEMPERATURE AND WATER POTENTIAL ON GERMINATION AND GROWTH OF *RHIZOPUS STOLONIFER* AND *GILBERTELLA PERSICARIA*

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SUMMARY

Understanding the impact of environmental factors favouring natural niches of plant pathogens is a key component toward designing efficient management strategies. *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. and *Gilbertella persicaria* (E.D. Eddy) Hesseltine are two economically important pathogens that are commonly associated in simultaneous infections of many stone and pome fruits. The ability of *R. stolonifer* and *G. pesicaria* to assimilate diverse carbon (C) and nitrogen (N) sources and the effect of pH and the interaction temperature/water potential (Ψ) on their conidial germination and growth were investigated. The fungi were grown on a liquid medium containing D-glucose, sucrose, fructose, maltose, or raffinose as C sources and urea, NH_4NO_3, $NaNO_2$, glycine, or glutamine as N sources. The biomass production of *R. stolonifer* was the greatest when sucrose and fructose were used as C sources, respectively, whereas biomass production of *G. persicaria* was greater on fructose and glucose than on other sources. Glutamine and sodium nitrite ($NaNO_2$) used as N sources yielded the highest biomass for *R. stolonifer* and *G. persicaria*, respectively. Sporangiospore germination and mycelial growth of *R. stolonifer* and *G. persicaria* were optimal between pH 3 and 10 and were totally inhibited at pH ≤2.5. The two fungi were affected by the interaction of temperature and Ψ. Both *R. stolonifer* and *G. persicaria* were sensitive to low temperatures (<10°C) and were completely inhibited at Ψ levels of -17 and -18.3, respectively. The nutrients utilization, pH, and Ψ x temperature profiles for sporangiospore germination and mycelial growth were to some extent similar between the two species corroborating that *R. stolonifer* and *G. persicaria* occupy similar ecological niches. Identifying ecophysiological determinants of these commercially important pathogens will contribute to our understanding of their physiology and epidemiology and will aid in developing appropriate models for disease prediction.

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INTRODUCTION

Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. and *Gilbertella persicaria* (E.D. Eddy) Hesseltine are two major pre- and post-harvest pathogens of stone fruits worldwide. In addition, *R. stolonifer* is an important pathogen of pome fruits, berry fruits, and sweet potatoes (Splittstoesser, 1979) and frequently contaminates food products as well as animal feeds. For instance, *R. stolonifer* and other *Rhizopus* spp. are amongst the most common fungi that develop in high moisture grain such as sorghum, barley and wheat products (Christensen and Sauer, 1982; Rabie and Lüben, 1984) and are also involved in the spoilage of meat and meat products (Jay, 1979). On the other hand, *G. persicaria* is mainly pathogenic to plants and has a potential to cause economic loss to apricot and peach (Ogawa and English, 1991; Ginting *et al.*, 1996), pear (Mehrota, 1964a), apple (Mehrota *et al.*, 1971) and tomato (Mehrota, 1964b).

On stone fruits, primary infections occur in the orchards generally a few weeks prior to harvest and during storage. Inocula of the two fungi originate from organic material on the orchard floor or in the soil (Ogawa *et al.*, 1995; Ginting *et al.*, 1996). Both fungi can infect ripe fruits of the same tree simultaneously, sporulate readily, and release large amounts of air-borne spores to contaminate healthy fruits on the same or neighboring trees. Insects feeding on sporulating fruits can serve as vectors for dissemination of *Rhizopus* and *Gilbertella* spp. to healthy fruit on the trees (Ogawa *et al.*, 1995). It is not completely clear which pathogen is more common; however, *G. persicaria* has been more commonly reported than *R. stolonifer* in South Carolina and California (Ginting *et al.*, 1996; Michailides *et al.*, 1997). Distinguishing the two species can be challenging as they both produce abundant black sporangiospores, which could be easily confused with infections caused by *Mucor* spp.

Fruits are particularly susceptible to fungal spoilage because of their high content in sugars and other nutri-

ents, low pH, and richness in available water. Furthermore, fruits are subjected to multiple changes in soluble solid and volatile compounds, flesh firmness, and water content during their growth in the field and during storage. Such conditions may differentially favor fungal infections including those caused by *R. stolonifer* and *G. persicaria*. In most fruits, sugar metabolism is primordial for fruit development and organoleptic qualities with sucrose, glucose, and fructose being respectively the main sugars in peaches (Genard *et al.*, 2003). While glucose and fructose are accumulated at constant rate throughout development and ripening (Souty *et al.*, 1998), sucrose, which is the main sugar in peach, accumulates rapidly in the last few days of maturation on the tree (Layne and Bassi, 2008). The utilization of sugars as carbon sources has been investigated in several ectomycorrhizal fungi (Hampp *et al.*, 1995; Martin *et al.*, 1998; Grelet *et al.*, 2005; Deveau *et al.*, 2008), ascomycetous yeasts (Leandro *et al.*, 2009), and some filamentous fungi such *Aspergillus* spp. and *Neurospora crassa* (Forment *et al.*, 2006; Xie *et al.*, 2004). However, the potential impact of change in sugar content during fruit growth, ripening and storage on disease development is underinvestigated in many fungal pathogens such as *R. stolonifer* and *G. persicaria*. Many fungal species can utilize low molecular-weight compounds such as mono- and disaccharides but not large molecules composed of these same small subunits. In addition, one fungal species may have the ability to assimilate one particular sugar for germination of its sporangiospores but not for mycelial growth and sporulation (Agnihotri, 1969). The determination of sugar utilization as carbon sources could help in determining niche overlap indices (Wilson and Lindow, 1994) which will determine ecological similarity between different fungi that occupy similar niches.

Organic acid metabolism is another crucial determinant of fruit quality. In peaches, malate and citrate are the two major organic anions (Layne and Bassi, 2008) and account for most of the titratable acidity. Their accumulation occurs mainly in early stages of fruit growth and progressively decreases during fruit ripening inducing consequently, a decrease in fruit acidity. The pH is a well known growth regulator in many fungi and its effect on fungal growth has been extensively studied for several fungal species *in vitro*. For sugar metabolism, however, the impact of acidity change during fruit growth and storage on Rhizopus and Gilbertella rots development is unknown.

Environmental factors such as temperature, water potential, and their interaction affect the competitiveness and co-existence of spoilage fungi in fruits and other food ecosystems. The effect of temperature on sporangiospore germination, sporulation, or disease development has been investigated in *R. stolonifer* (Miller *et al.*, 1959; Smith and McClure, 1960; Pierson, 1965; Dennis and Blijham, 1980) but to a lesser degree in *G. persicaria* (Ginting *et al.*, 1996; Michailides *et al.*, 1997). However, there is a paucity of knowledge on the impact of water potential, interaction between temperature and water potential, and other conditions such as pH and carbon and nitrogen sources availability on competitiveness and pathogenicity of the two aforementioned species. Water content in fresh fruit may not be a limiting factor for fungal growth; however the extent to which these pathogens can survive and assimilate sugars and other nutrients from the soil, cankers, or mummies on which they overwinter may determine their ability to germinate, sporulate, ensure the continuity of their developmental cycles, and will define their ecological niches. Control of Rhizopus rot relies mainly on prophylactic measures to reduce inoculums and pre- and postharvest fungicide applications (Northover and Zhou, 2002), whereas no efficient chemical control strategies currently exist for Gilbertella rot management. Hence, understanding the development of these species on different substrates and under different environmental conditions may constitute a basis for developing efficient and sustainable disease methods for their control. The objectives of this study were to evaluate the ability of *R. stolonifer* and *G. persicaria* to utilize a range of carbon and nitrogen sources and to investigate the effect of pH, water potential, temperature, and their interaction on sporangiospore germination and mycelial growth.

MATERIALS AND METHODS

Origin, maintenance, and inoculum production from fungal isolates. Two *R. stolonifer* isolates (Rh.by2 and Rh.by5) and two *G. persicaria* isolates (Gp.pch1 and Gp.my1) were used in this study. *G. persicaria* isolates were obtained from decaying peach fruit collected from two commercial orchards in Pickens and Edgefield counties, South Carolina (USA), while the *R. stolonifer* isolates were obtained from the USDA Research Station, Byron, Georgia. The isolates were characterized at a molecular level based on the internal transcribed spacer ITS1 and ITS4 regions (White *et al.*, 1990). Isolates were stored at -80°C on silica gel (grade 40; Sigma-Aldrich, USA) and new cultures were grown from this stock on potato dextrose agar (PDA) medium for each experiment. Sporangiospores from *R. stolonifer* and *G. persicaria* were obtained from 4-day-old PDA cultures. Sporangiospores from PDA cultures were washed in 15 ml of sterile distilled water containing 0.05% Tween 80, filtered through cheesecloth and the concentration of the suspension was determined using a haemocytometer.

Media. The basal medium (BM) used to assess the utilization of carbon and nitrogen sources contained (g l^{-1}): K_2HPO_4 (1.0); $MgSO_4.7H_2O$ (0.2); NaCl (0.07); $ZnSO_4.7H_2O$ (0.04); ferric EDTA (0.01) (Grelet *et al.*,

2005). Glutamine was added to the BM at 1% (w/v) to assess the effect of each carbon source separately, whereas D-glucose at the same concentration was added to the BM when testing for nitrogen sources. The medium was adjusted to pH 5.5 and autoclaved for 20 min. The effect of pH, water activity, and temperature on sporangiospore germination and mycelial growth was determined on PDA.

Effect of carbon and nitrogen sources on biomass production. For each isolate, three plugs (6 mm diameter) were taken from the advancing margins of 4-day-old cultures and aseptically transferred into a 250 ml Erlenmeyer flask containing 50 ml of sterilized BM with 1% of the desired nitrogen or carbon source. Fructose, D-glucose, maltose, raffinose and sucrose were used as carbon (C) sources. Glutamine, glycine, $NaNO_2$, NH_4NO_3, peptone and urea were used as nitrogen (N) sources. All C and N sources were added to the BM prior to autoclaving. Three-replicate flasks were inoculated for each C or N source and for each isolate and the test was conducted twice. A control flask containing the BM without a C or N source was inoculated for each isolate. Flasks were sealed with aluminum foil and plastic film and incubated at 22°C in dark for 25 days. Fungal mycelium was harvested by filtration through miracloth membrane, washed twice with distilled water and transferred to pre-weighed filter papers, and dried at 75°C for 24 h. Biomass was estimated by subtracting the weight of fungal biomass of the control (without C or N source) from the dried biomass of the treatment.

Effect of water potential and temperature on sporangiospore germination and mycelial growth. The water potential (Ψ) of the PDA medium was modified with the ionic solute NaCl and the non-ionic solute D-glucose to Ψ levels of -1.4, -3.1, -6.0, -9.8, -14, -17, and -18.3 equivalent to 0.996, 0.983, 0.966, 0.936, 0.908, 0.893 and 0.887 a_w levels. Calculated amounts of the two solutes were separately mixed with PDA, autoclaved and poured into 9 cm diameter Petri dishes. The a_w of the medium was measured using a 2080RP instrument (Digitron, UK) at 20°C with an accuracy of 0.01 after pouring the medium into the plates. The a_w values were converted to Ψ values using the formula: $\Psi = RT/V \ln a_w$, where R is the gas constant for water (462 kPa K^{-1}), T is the Kelvin temperature, and V the partial molal volume of water (Wiebe, 1981). To test the effect of Ψ and temperature on sporangiospore germination, a 100 µl spore suspension ($2x10^5$ sporangiospores ml^{-1}) volume from each isolate was spread evenly onto solidified PDA at different Ψ levels using a sterilized glass rod. Petri dishes were placed in sterile plastic bags, incubated at 2, 5, 10, 20 and 30°C and germination was determined 24 and 48 h later as described previously (Amiri *et al.*, 2009). A sporangiospore was considered 'germinated' when it had produced a germ tube two times longer than its diameter. Two replicate Petri dishes per Ψ value were assessed for each isolate and the test was conducted twice.

To test the effect of Ψ and temperature interaction on mycelial growth of test fungi, plates containing Ψ modified-PDA were inoculated with mycelial plugs (6 mm in diameter) taken from the advancing margins of 3- to 4-day-old colonies of each isolate. Each plate was inoculated with three plugs, sealed with parafilm, placed in plastic bags to minimize evaporation, and incubated at 2, 5, 10, 20, and 30°C in the dark. Radial mycelial growth was measured after 2, 4, 8, 16, and 32 days. Three replicate-plates were inoculated for each isolate/temperature combination and the experiment was conducted twice.

Effect of pH on germination and mycelial growth. The pH of PDA medium was modified to values ranging from 2 to 6 and 6 to 10 by adding borate phosphate (0.1 M boric acid; 0.2 M $Na_2HPO_4.7H_2O$) and Tris-HCl (0.2 M Tris-hydroxymethyl-aminomethane; 0.2 M HCl) buffers, respectively (Yamanaka, 2003). The effect of pH on sporangiospore germination and mycelial growth was examined as explained above for the Ψ test. Two replicate Petri dishes per pH value and isolate combination were used for the sporangiospore germination tests and three plates per pH value and isolate combination were inoculated for the mycelial growth assay. The entire experiment was conducted twice.

Statistical analysis. Data from the two isolates from each species across two experimental runs were not significantly different ($P = 0.05$) based on a separate ANOVA; consequently, data from the two isolates were combined for analysis and means were separated using the least significant difference test (LSD) at $P \leq 0.05$. Data of the effect of C and N sources on biomass production were subjected to analysis of variance. Conidial germination and mycelial growth rates were analyzed by a general linear model (GLM). All statistical tests were performed using SigmaStat software (Version 3.00, SPSS, USA).

RESULTS

Effect of different carbon and nitrogen sources on biomass production. Biomass production was significantly ($P = 0.05$) higher for both fungi on the BM amended with any carbon or nitrogen source compared to the control (Table 1). Biomass production by *R. stolonifer* was always higher than biomass of *G. persicaria* on all C sources. *R. stolonifer* yielded higher biomass on sucrose followed by fructose and D-glucose. The trisaccharide raffinose supported *R. stolonifer* growth the least. Biomass yield by *G. persicaria* was nu-

merically greater on fructose and D-glucose, however, no significant difference was observed between the five different sugars (Table 1).

For nitrogen sources, *G. persicaria* yielded higher biomass than *R. stolonifer* on all sources except glutamine. The greatest biomass production by *R. stolonifer* and *G. persicaria* were on glutamine and nitrite ($NaNO_2$), respectively. No significant differences were observed between N sources for *G. persicaria*, whereas significance difference ($P = 0.03$) was observed between glutamine and the four other N sources for *R. stolonifer* (Table 1).

Effect of pH on germination and mycelial growth. Germination of *R. stolonifer* and *G. persicaria* was completely inhibited at pH ≤2.5. The two species fully germinated at pH values between 3 and 10 (Fig. 1A). Growth of both species was inhibited at pH 2 and significantly reduced (< 5 mm) at pH 2.5 (Fig. 1B). Optimal mycelial growth for *R. stolonifer* and *G. persicaria* was observed between pH value ranges 3 to 8 and 3 to 9, respectively. Growth declined by more than 50% at pH 10 after 96 h of incubation at 22°C (Fig. 1B).

Effect of water potential on germination and mycelial growth. Germination of *R. stolonifer* and *G. persicaria* was unaffected up to Ψ level -9.8 MPa and was reduced by about 50 and 60% for *R. stolonifer* and *G. persicaria*, respectively, at -14 MPa (Fig. 2). Germination was completely inhibited at -17 and -18.3 MPa, for *G. persicaria* and *R. stolonifer*, respectively (not shown).

Growth of *R. stolonifer* and *G. persicaria* was comparable and was not affected at Ψ levels up to -6 MPa although growth trend was slower at -6 MPa compared to -1.4 MPa (Fig. 3) and -3.1 MPa (not shown). Mycelial

Table 1. Effect of different carbon and nitrogen sources on biomass production by *Rhizopus stolonifer* and *Gilbertella persicaria.*

			Biomass (mg)[a]±SD	
			Fungal species	
Source		Class[b]	*R. stolonifer*	*G. persicaria*
Carbon				
	Control (no C)		250 ± 2 a	230 ± 1 a
	Raffinose	T	365 ± 5 b	350 ± 6 b
	Maltose	D	387 ± 9 c	355 ± 6 b
	D-glucose	M	398 ± 3 c	359 ± 2 b
	Fructose	M	403 ± 4 c	363 ± 3 b
	Sucrose	D	420 ± 8 d	351 ± 2 b
Nitrogen				
	Control (no N)		210 ± 7 a	231 ± 4 a
	Urea	I	348 ± 4 b	358 ± 7 b
	$NaNO_2$	I/Nit	348 ± 5 b	362 ± 1 b
	NH_4NO_3	I/Am	351 ± 2 b	360 ± 2 b
	Glycine	O	357 ± 4 b	360 ± 3 b
	Glutamine	O	373 ± 9 c	360 ± 7 b

[a] Data are the mean of twelve replicates from two isolates per species across two experimental runs. SD indicates standard deviations of the mean. Values followed by the same letters are not significantly different at $P \leq 0.05$ according to LSD Fisher's test.

[b] M, D and T indicate mono, di and trisaccharide, respectively. I and O indicate inorganic and organic, respectively, whereas Am and Nit indicate Ammonia and Nitrite, respectively.

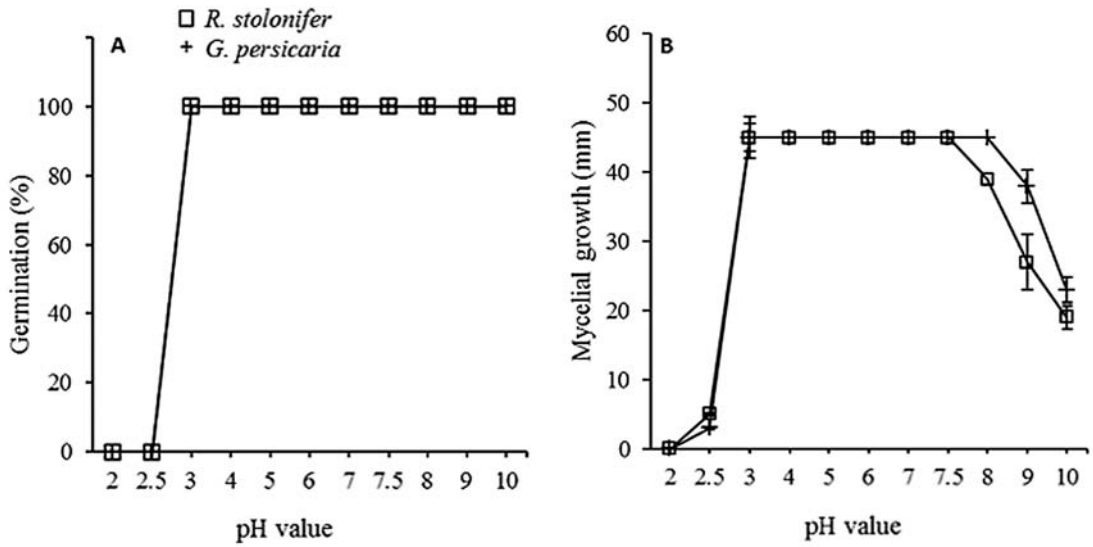

Fig. 1. Effect of pH on germination (A) and mycelial growth (B) of *Rhizopus stolonifer*, and *Gilbertella persicaria* after 24 h for germination and 96 h for mycelial growth at 22°C. Data points are the mean of 12 and 18 replicates for germination and mycelial growth, respectively, across two experimental runs. Vertical bars indicate the standard deviations.

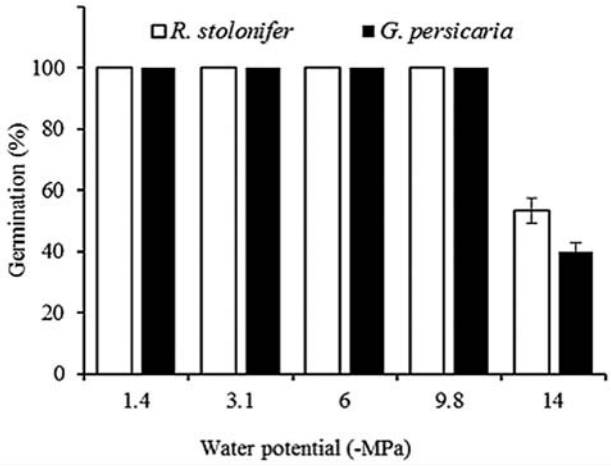

Fig. 2. Effect of water potential on sporangiospore germination of *Rhizopus stolonifer* and *Gilbertella persicaria* after 48 h at 20°C. Data are the mean of 18 replicates from two isolates per species across two experimental runs. Vertical bars indicate the standard deviations.

growth was drastically reduced at Ψ level of -9.8 MPa and inhibited at lower Ψ levels. The mycelial growth of both fungi tested was affected by the nature of solute added to PDA to generate different Ψ levels. Based on GLM analysis, the growth was significantly reduced with NaCl compared to glucose at Ψ levels ≤ -9 MPa (Fig. 4).

Effect of water potential and temperature interaction on germination and mycelial growth. Overall, sporangiospore germination of *R. stolonifer* and *G. persicaria* was inhibited in a temperature range of 5 to 10°C regardless of the Ψ level (Fig. 5). Both species germinated at a 100% rate at Ψ levels up to -6 MPa at 20°C. At this temperature, germination of *stolonifer* and *G. persicaria* was reduced to 53 and 39%, respectively, at -14 MPa. Germination of *G. persicaria* decreased sharply to 35% at 30°C and -9.8 MPa, whereas both species where completely inhibited at -14 MPa.

Neither *R. stolonifer* nor *G. persicaria* grew at temperatures below 10°C regardless of the Ψ level (Fig. 5). Optimal growth for both species was observed at 20°C and Ψ levels up to -6 MPa. Mycelial growth of both species decreased at 30°C for all Ψ ranges and was completely inhibited by a -14 MPa/30°C combination.

DISCUSSION

Fungi have a variety of requirements for organic energy, space, water, other nutrients and oxygen for growth and reproduction. It is primordial to consider that fungal contamination in plants and foods is not a result of one single, but communities of genera and species that compete for nutrients and other require-

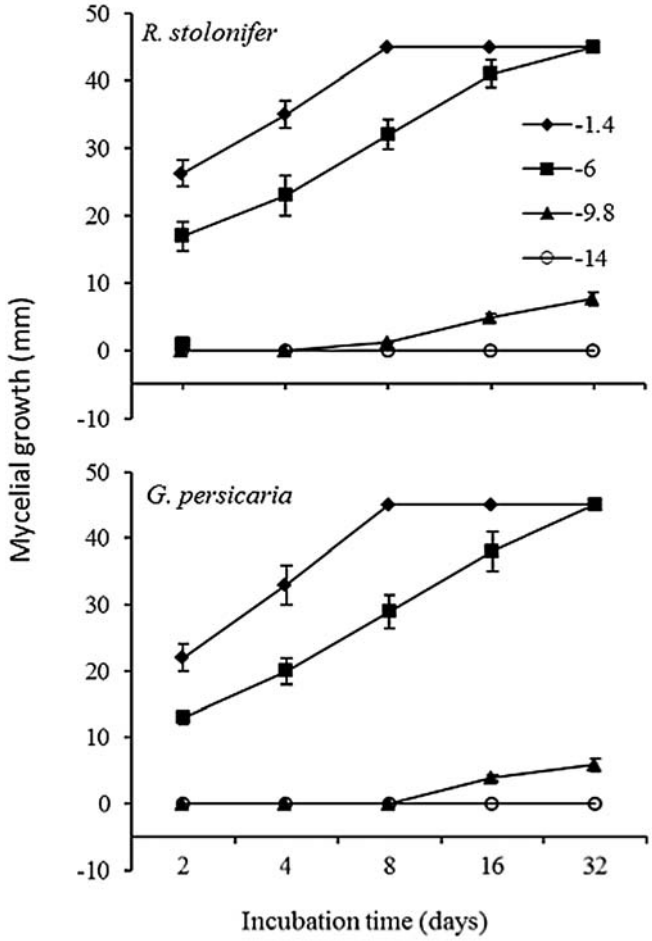

Fig. 3. Temporal effects of water potential on mycelial growth of *Rhizopus stolonifer* and *Gilbertella persicaria* at 20°C. Data are the mean of 18 replicates across two experimental runs. Vertical bars indicate the standard deviations.

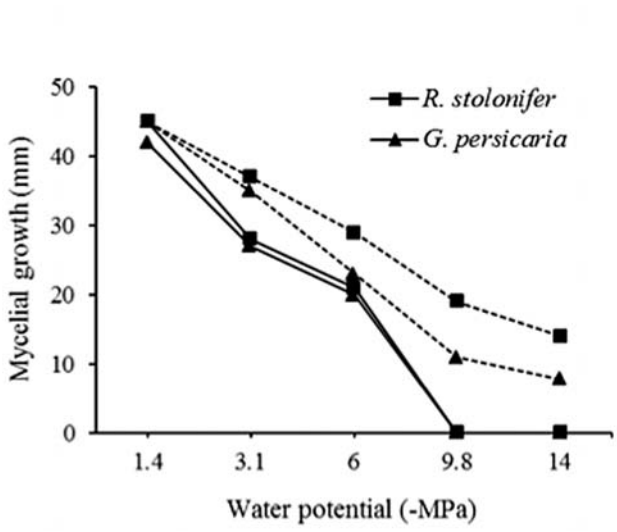

Fig. 4. Mycelial growth of *Rhizopus stolonifer* and *Gilbertella persicaria* on potato dextrose agar with different Ø values obtained with sodium chloride (solid line) and glucose (dashed lines) at 20°C.

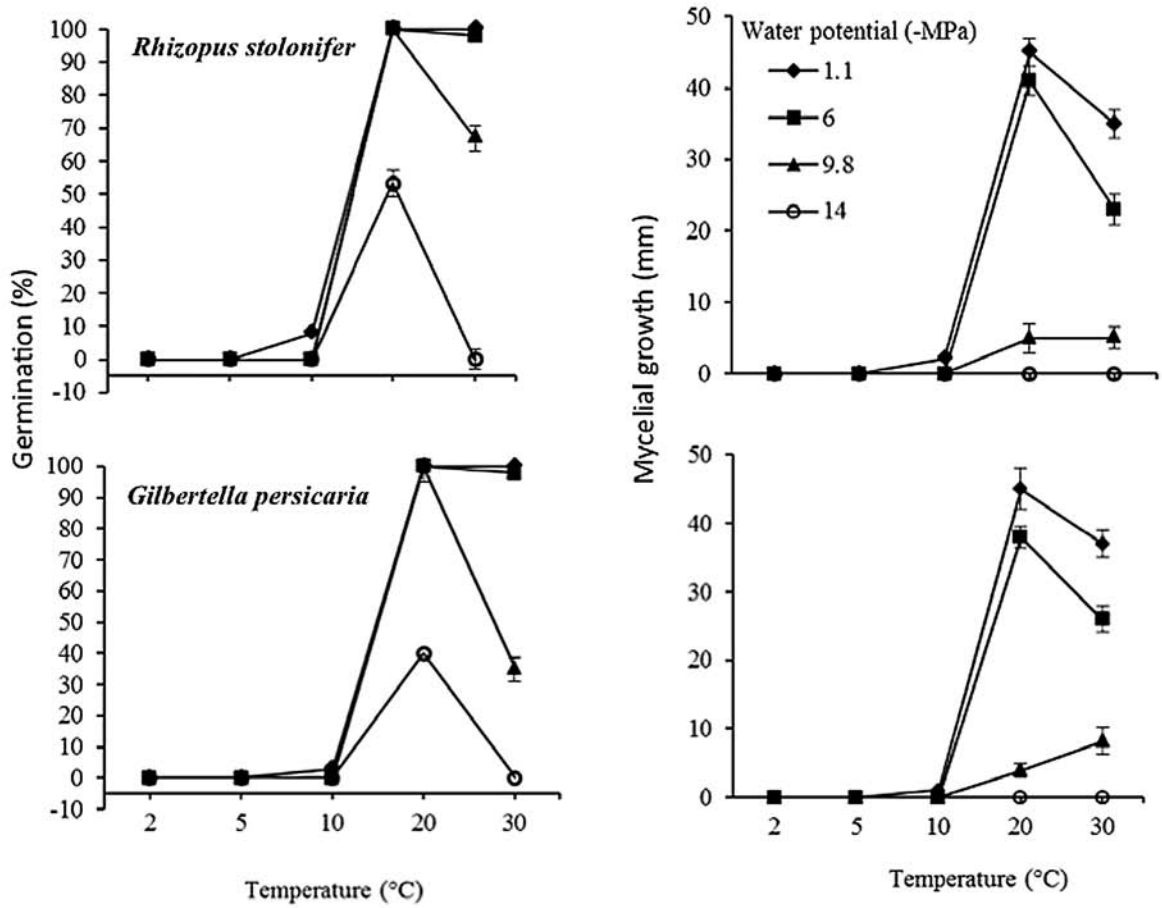

Fig. 5. Effect of water potential and temperature on sporangiospore germination (after 48 h) and mycelial growth (after 16 days) of *Rhizopus stolonifer* and *Gilbertella persicaria*. Data are the mean of 18 replicates across two experimental runs. Vertical bars are the standard deviations.

ments. To ensure the continuity of its developmental cycle, a fungus must reduce the effect of potential competitors or utilize effective competitive mechanisms.

Although *R. stolonifer* has a wider host range than *G. persicaria*, these two pathogens are commonly observed or recovered at similar proportions from infected or symptomless stone fruits (Ginting *et al.*, 1996; Michailides *et al.*, 1997). The present study is the first attempt to evaluate and compare the ability of these species to utilize different C and N sources and to grow under different environmental conditions. *R. stolonifer* and *G. persicaria* were able to grow on a basal medium (BM) with only a C (glucose) or an N (glutamine) source. Nevertheless, all C and N sources supplemented into the BM had a significant positive effect on biomass production. Out of five C sources tested, *R. stolonifer* grew best on two important sugars of fruits, i.e. the disaccharide sucrose composed of two units of glucose and fructose and the monosaccharide fructose. Overall, biomass production of *G. persicaria* was slighter compared to *R. stolonifer*, and was preferentially higher on monosaccharides. This may denote a wider ecological niche for *R. stolonifer* over *G. persicaria*, although parameters other than nutrient utilization may define competitiveness. Our results also show a pattern in sugar utilization that may help in understanding the pathogenicity of these species on stone fruits. In fact, *R. stolonifer* used better sucrose over fructose and glucose contrary to *G. persicaria*. Sucrose, fructose and glucose in proportion of about 3:1:1 are the mains sugars in peaches (Genard *et al.*, 2003; Vizzoto *et al.*, 2002). Glucose and fructose accumulate throughout fruit development but their ratio decreases during maturation (Layne and Bassi, 2008), whereas sucrose accumulates at a higher rate (>50% of total dry weight) during the last few days of maturation on the tree (Souty *et al.*, 1998; Bregoli *et al.*, 2002; Vizzoto *et al.*, 2002). It is therefore plausible to hypothesize that *G. persicaria* may be more pathogenic on fruit at early stages of development whereas *R. stolonifer* infects better ripen fruit richer in sucrose. This may also explain the fact that *G. persicaria* is more frequently encountered than *R. stolonifer* on fruits sampled from orchards in South Carolina and California (Ginting *et al.*, 1996; Michailides *et al.*, 1997). Compared to the other sugars tested, the trisaccharide raffinose was the least used sugar by *G. persicaria* and more

particularly by *R. stolonifer*. This molecule is composed by galactose, fructose, and glucose and is found in many vegetables and fruits. Its weak utilization might be related to the fact that the two fungi lack α-galactosidase activity that can hydrolyze raffinose to D-galactose and sucrose.

Based on their ability to assimilate different N sources, fungi have been divided into two classes: those able to assimilate molecular nitrogen (N_2) and those which grow preferably on inorganic and organic nitrogen sources (nitrate, nitrite, or ammonium and organic N compounds) (Lilly, 1965). While N_2 utilization has not been clearly shown in fungi, the majority of fungi can efficiently grow on substrates supplied with either an inorganic or an organic N source. Based on our results, *R. stolonifer* and *G. persicaria* belong to the second class of fungi being able to assimilate all N sources, although *R. stolonifer* showed a clear preference for organic N compounds. Both fungi produced maximum biomass on glutamine (glutamic acid), which was reported to be a suitable N source for many microorganisms such as *Alternaria tenuis*, *Aspergillus niger*, and *Trichoderma* spp. (Singh and Tandon, 1970; Apsmo *et al.*, 2005; Rossi-Rodrigues *et al.*, 2009). A likely explanation for this preference may be related to the primordial role of glutamine in the nitrogen utilization cycle in fungi (Garraway and Evans, 1984). Seabi *et al.* (1999) found that *R. stolonifer* can grow adequately on glutamatic acid but not on ammonia (NH_4Cl), nitrate (KNO_3), or nitrite ($NaNO_2$) amended to 0.1 g l^{-1} in a solid medium. The same authors, however, found that other *Rhizopus* species such as *R. oryzae* were able to grow on NH_4Cl and KNO_3 but not on $NaNO_2$.

In our study, N sources were added at a final concentration of 10 g l^{-1} to a liquid medium and our findings are more consistent with those of Inui *et al.* (1965) who reported that *Rhizopus* species were unable to utilize 21 g l^{-1} nitrate in a liquid medium as well. Although both fungi were able to germinate and grow at pH value up to 10 in this study, it has been reported that nitrite compounds may cause an increase of pH in the medium and inhibit the growth of some mycetes (Agnihotri, 1968). Furthermore, ammonium ($NH4^+$), a relatively poor N source for the fungi tested, has been linked to the degradation of DNA and proteins in fungi (Pinon, 1977; Goller *et al.*, 1998). To our best knowledge, there are no reports in the literature on the ability of *G. persicaria* to use C or N sources, however in this study, *G. persicaria* used more efficiently different N sources including nitrite than *R. stolonifer*. This capacity may favor the overwintering of *G. persicaria* over *R. stolonifer* in soils and mummified fruits and would place *G. persicaria* among the succession of microorganisms more suitable for nitrogen mineralization of organic matter.

Fungi generally are known to grow well at acidic conditions similar to those found in fruit at commercial maturity. Environmental H^+ concentration (pH) is thought to have little direct effect on fungal metabolism due to the buffering system in hyphae and conidia (Dix and Webster, 1995). However, such conditions may influence their enzymatic activity and therefore their ability to colonize and infect their hosts (Enokibara *et al.*, 1993; Aleandri *et al.*, 2007). In consistence with previous reports, *R. stolonifer* and *G. persicaria* were only inhibited in very acidic conditions (pH ≤ 2.5) but not affected at alkaline pH although mycelial growth was reduced at pH higher than 9. For example, zygospore production by *G. persicaria* on PDA medium decreased at pH below 4 but not at pH values up to 10 (Michailides *et al.*, 1997). Mycelial growth of *R. stolonifer* was optimal at pH 7 and was reduced at 4≤ pH ≥ 9 in a liquid medium (Adisa, 1983). Under normal condition (temperature, water availability), it is unikely that pH will be a limiting factor for the growth or sporangiospore germination of these species. However, during the growth of stone fruits, pH can decrease sometimes below 3.25 before harvest due to a high content in malate and citrate anions, especially in high-acid cultivars (Kakiuchi *et al.*, 1981; Moing *et al.*, 1998).

While the effect of temperature on growth of *R. stolonifer* has been studied extensively, there is paucity in studies regarding *G. persicaria* and the effect of water potential and temperature interaction on both fungi. *R. stolonifer* and *G. persicaria* were sensitive to low temperatures since no germination or mycelial growth was observed below 10°C regardless of the Ψ level, whereas optimum germination and growth were observed between 15 and 30°C at Ψ levels up to -6 MPa. Previous studies reported a temperature range between 5 and 25°C for optimal germination and growth of *R. stolonifer* (Miller *et al.*, 1959; Pierson, 1965; Adisa, 1983). Zygospore production by *G. persicaria* was inhibited at temperatures ≤15°C and significantly reduced at 25°C compared to 20°C (Michailides *et al.*, 1997) and symptoms of *G. persicaria* developed on peaches incubated at 21-24°C (Ginting *et al.*, 1996). Our results and other reports suggest that cooling the fruit immediately after harvest to temperatures below 4°C is one way to reduce postharvest disease infections due to these two pathogens. However, once the fruit are exposed again to room temperature, the risk might be elevated since these species have been show to keep their viability after long period of chilling (Smith and McClure, 1960; Denis and Blijham, 1980).

The minimum Ψ observed for germination for *R. stolonifer* and *G. persicaria* was -14 MPa at 20°C. Other studies have reported that *R. stolonifer* has germinated at Ψ values as low as -8 to -10 MPa (Homer and Anagnostopolous, 1973) and -26 MPa (0.84 a_w) (Hocking and Miscamble, 1995) at 25°C. The minimum Ψ observed for mycelial growth was -9.8 MPa, lower compared to germination which is in consistence with many

other fungi (Wheeler *et al.*, 1988), whereas other studies reported similar effect of water potential on germination and mycelial growth (Hocking *et al.*, 1994).

The ability of both species to grow was affected by the nature of the solute used to modify the water potential of the medium. Growth was more affected, although significant differences were not observed, by an ionic solute (NaCl) compared to a non-ionic solute (glucose) which was intended to mimic changes of water potential in soil or in fruit, respectively. This may provide some insights on the ability of these two species to survive in soils where NaCl or other ionic solutes may be a limiting factor or on fruit where changes in concentrations of glucose and other non-ionic compounds, as discussed above, could differentially affect the persistence of the pathogens under different conditions.

Overall, we have shown that *G. persicaria* and *R. stolonifer* can persist in dry conditions since they are able to germinate and grow at very narrow stress water ranges. These findings support general field observations since *R. stolonifer* and *G. persicaria* are commonly encountered at comparable frequencies, especially during dry years in the southeast (Ginting *et al.*, 1996). This study has defined, for the first time, the optimal conditions for sporangiospore germination and growth of *R. stolonifer* and *G. persicaria* and their ability to assimilate different nutritional sources. Our results suggest a similarity in ecological niches occupied by these two members of mucorales. However, definitive conclusions on the nutritional needs of these species cannot be made at this point because of the relatively low number of isolates and compounds tested; still, our data constitute a base line for further studies involving a wider range of C and N sources for a better understanding of specific nutritional need for each of these fungi. Our investigation on the effect of pH, temperature and potential water on *R. stolonifer* and *G. persicaria* will also be useful for a better understanding of their ecology and epidemiology, in developing appropriate models for disease prediction of each pathogen, and in developing more efficient control strategies.

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