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Journal

Mycologia, 100(6)

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Publication Date

2008-10-14

Peer reviewed

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C1 *Mycologia*, 100(6), 2008, pp. 902–910. DOI:
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Nonlinear colony extension of *Sclerotinia minor* and *S. sclerotiorum*

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Abstract: Fungal colonies initially extend exponentially and reach a constant linear extension rate determined solely by their growth in the peripheral zone. However the radial extension rates of *Sclerotinia sclerotiorum* and *S. minor* accelerate over time on PDA. Experiments were conducted to analyze the variable extension rat[illegible] the two *Sclerotinia* species and compare ~~tham~~ with those [illegible]*Verticillium dahliae* and *Cladosporium* sp. In addition the effects of starter disk size, disk position in the parent colony, the age of the parent colony, the concentration of potato dextrose broth and of incubation temperature also were determined. While the growth of *Cladosporium* sp. and *V. dahliae* followed established linear trends, the radial extension of *S. sclerotiorum* and *S. minor* colonies continuously accelerated over time until they reached the edge of the (150 mm diam) Petri dish. A polynomial model fitted the radial extension of colonies of *Sclerotinia* spp. Furthermore the accelerating colony extension rate was partly due to increasing colony radius. The rates of extension from mycelial disks transferred from the parental colony were positively correlated with the radius of the mycelial disks transferred. The rates of extension also were dependent on where the transferred disks were taken from parent colonies and the age and radius of the parent colony. On potato dextrose agar medium the extension rates of colonies of *S. sclerotiorum* and *S. minor* also were affected by broth concentration and temperature. With increasing nutrient concentration colony extension rates increased and were highest at 25 C. This study revealed a novel pattern of radial growth for *Sclerotinia* spp. that diverged from the established growth patterns of fungal colonies. Knowledge of the differences in growth behavior may be exploited in the laboratory studies on fungal competition and hyperparasitism and potentially in disease control strategies.

Key words:

INTRODUCTION

Filamentous fungi exist ubiquitously in nature and play an important role in the global carbon cycle as decomposers. They are considered more adapted than unicellular bacteria or yeast for growth on solid substrates because hyphal extension offers additional avenues for new nutrient exploration. Growth kinetics of filamentous fungi has been extensively studied (Trinci 1978, Mckerracher and Heath 1987, Prosser and Tough 1991, Prosser 1993, Gooday 1995, Harold 1997, Pazouki and Panda 2000, Boswell et al 2003, Papagianni 2004). During typical cell division in filamentous fungi, genetic material is replicated and segregated and a septum formed between the mother and daughter cells facilitating signal relay and material transport between multiple cells. A typical fungal colony originates from a single spore or a mycelial fragment. Growth, taking the form of cell elongation, is localized at the tips of germ tubes or hyphae, where new cell wall is continuously assembled. Individual hyphae generate new tips via branching and form an interconnection via hyphal fusion. Eventually this leads to a colony of interwoven filamentous hyphae called mycelium. While mycelium grows at its apex, metabolism remains vigorous behind the apex. Nutrients can be absorbed along the length of the branches and transported along the mycelium network, evidenced by translocation of radioactive isotopes (such as P and C) from the center to the edge of fungal colonies (Olsson and Gray 1998, Tlalka et al 2002) and by the continuous decrease (lower concentration at the center than along the edge) in glucose conce[illegible]ion in the medium at the colony center ~~3–6 d~~ (Robson et al 1987). Proteins and other macromolecules are produced and organelles and cytoskeleton are assembled along the hyphal trunk (Mckerracher and Heath 1987, Harold 1997, Suelmann et al 1997). These materials are transported continuously to hyphal tips to aid further growth.

On agar-solidified medium under favorable conditions a germ tube from a fungal spore extends exponentially and eventually reaching a rate at which extension is limited by the rate of nutrient transport from distal regions. Subsequently excess energy aids new branching and thereby new growing tips.

Accepted for publication xx xxx 2008.
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Branching therefore is highly favored on rich media and inhibited on poor media (Prosser and Tough 1991). During the initial linear phase of fungal colony extension after the log phase both the total mycelial length and the total number of branches increase exponentially at the same specific rate $a \cdot \exp(\mu t)$ and $c \cdot \exp(\mu t)$, where μ is equivalent to the specific growth rate of the fungus under the same conditions in liquid medium (Trinci 1971, 1974). The hyphal growth unit (Caldwell and Trinci 1973, Robinson and Smith 1979) calculated from the ratio of total mycelial length to total number of hyphal tips ($G = a/c$) is constant and represents the average hyphal volume associated with each branch. As the density of hyphal tips increases collision occurs among tips at the center of a mycelial colony due to restricted space (Ferret et al 1999) and hyphal growth enters a new phase. During this phase only a mycelial ring with a constant width (ω) called peripheral growth zone with width is actively involved in colony expansion. The rate of radial expansion is determined solely by the growth in the peripheral growth zone and is related to the specific growth rate (μ) in liquid culture (Trinci 1971, 1974), $R_c = \mu\omega \cdot t$. Assuming that the biomass is uniformly distributed throughout the colony, the biomass of the fungal colony grows quadratically, proportional to the square of the time t from the beginning of the linear phase. A constant colony extension rate has been reported for a variety of fungi, including *Aspergillus nidulans* (Trinci 1969, 1971, 1974), *Gibberella fujikuroi* and *Aspergillus oryzae* (Ferret et al 1999), *Trichoderma viride* and *Penicillium rogueforti* (Gervais et al 1988), *Fusarium oxysporum* (Steinberg et al 1999) and *Sclerotium rolfsii* (Farina et al 1997).

Sclerotinia minor and *S. sclerotiorum* are important plant pathogens that cause severe yield losses on a variety of economically important crops worldwide (Purdy 1979, Boland and Hall 1994, Melzer et al 1997). A common characteristic of these fungi is the formation of sclerotia, which survive many years and germinate carpogenically to produce apothecia or myceliogenically to produce hyphae. On potato dextrose agar (PDA) plates both species produce a flat colony consisting of multicellular vegetative hyphae. The colony extends rapidly without producing spores. Instead sclerotia are produced after hyphae reach the edge of the solid medium. Contrary to the widely documented phenomenon of colony extension among fungi at a constant rate, preliminary observations revealed that growth of *S. minor* and *S. sclerotiorum* on PDA accelerated over time. The objectives of this work were to determine whether colony extension rates of these two fungi are constant and if not to determine potential reasons. Isolates of *Verticillium dahliae* and *Cladosporium spp.* also were included for comparison.

MATERIALS AND METHODS

Isolates used.—Two isolates of *S. minor*, Bm005 collected from infected lettuce in Salinas, California, and Sc2 collected from infected peanut in Oklahoma (provided by J.P. Damicone) were used in all experiments. The isolates of *S. sclerotiorum* used were BS001 collected from lettuce in Salinas and BS014 collected from cauliflower in Santa Maria, California (provided by F. Laemmlen). Sclerotia of *S. minor* and *S. sclerotiorum* were produced on autoclaved potato pieces in a flask inoculated with 2–3 agar disks from the growing margins of the colony of each isolate. These cultures were incubated at room temperature (20 ± 3 C) 3 wk, and the sclerotia of each isolate was harvested, cleaned, dried and stored at room temperature. The sclerotia were used to generate fresh colonies on PDA before use in all experiments. One isolate of *Cladosporium* sp. was collected from air in Salinas and maintained on PDA. Two isolates of *Verticillium dahliae*, Ls16 and Ls17, were collected from infected lettuce and maintained on NP10 medium (Sorensen et al 1991) before use in Experiment I.

Experiment I: temporal colony growth patterns in S. sclerotiorum, S. minor, V. dahliae *and* Cladosporium sp.—Two isolates each of *S. minor*, *S. sclerotiorum* and one isolate of *Cladosporium* sp. were grown on PDA. *V. dahliae* was grown on NP10. When colonies covered about half of the 100 mm plates, 4.5 mm diam mycelium disks were taken at 5 mm from the colony edge and each transferred upside down to the center of a new PDA (or NP10 for *V. dahliae*) plate (150 mm diam Petri dish for *S. minor* and *S. sclerotiorum* due to their rapid growth and 100 mm diam Petri dish for those that grow slowly). Half of the plates were incubated at 15 C and the other half at 20 C. For each treatment and isolate 4–5 replicate plates were included. Colony diameter was measured for each plate at intervals of 12 h for *S. minor* and *S. sclerotiorum* but 2–4 d for *Cladosporium* sp. and *V. dahliae* due to slower growth of their colonies. This was determined based on the rate of growth of each fungus. The temporal trend was analyzed by regression of the radial growth against incubation duration using a linear and a polynomial model for each isolate-temperature combination. When the response of two isolates of a species was not significantly different one regression model was fitted for the species-temperature combination. In all regression models the intercepts were set as zero in SAS (version 9.1, SAS Institute, Cary, North Carolina). For *S. minor* and *S. sclerotiorum* at each temperature 12 h radial growth also was calculated every 12 h and plotted against the radius of the colony at the beginning of the 12 h period. A logarithmic model was fitted for the relationship between 12 h radial growth and the colony radius at the beginning for each species and temperature combination using a nonlinear regression procedure in SAS.

Experiment II: effects of original disk size on colony growth.—The results of Experiment I indicated a nonlinear radial

growth for all colonies of *S. minor* and *S. sclerotiorum* and a positive correlation between the colony radius and the radial growth of the colony during 12 h intervals. To determine whether nonlinear growth was an artifact of the disk size instead of the changes in the colony over time as reported in *Neurospora crassa* (Mclean and Prosser 1987, Steele and Trinci 1975) and *Botrytis cinerea* (Zhu and Gooday 1992), experiments were designed to determine the relationship between radial growth rate of colony and radius of mycelium disks transferred. When colonies of *S. minor* and *S. sclerotiorum* covered about one-half of the 100 mm plates, mycelial disks 3, 4.5, 6, 8, 9.5, 11, 12, 13.5, 15 and 16 mm diam were taken from a spot equidistant from the colony center. The minimum distance from the extending edge to the edge of the largest disks was at least 5 mm. Each disk then was transferred to the center of a new PDA plate. For Experiment IIA the disk was upside down with the surface with fungal growth touching the medium. For Experiment IIB the disk was placed upside up in a predug well of the same diameter as the disk. For each isolate and disk size combination three plates were included as replications. The plates were incubated at 20 C, and the (maximum) diameter of the colony was measured for each plate after 24 h incubation. A logarithmic model was fitted to the relationship between the 24 h radial growth and the original radius of the disk transferred using SAS.

Experiment III: effects of disk status (position in the parent colony and radius of the parent colony) on the growth rate of the daughter colony.—The growth rate of the daughter colonies that originated from disks taken from a parent colony at the same time was used as an indicator of growth potential of the disks. The two isolates of *S. minor* and *S. sclerotiorum* were cultured at 20 C for 2–3 d. When the colonies reached about 80 mm diam but before they reached the edge of the 100 mm diam Petri dish, 4.5 mm diam mycelium disks were taken from the leading edge and placed at the center of two 150 mm diam PDA plates for each isolate. After incubation at 20 C for 24, 36, 48, 60 and 72 h, colony radius in each plate was measured and a 3 mm diam disk was taken at 10, 15, 20, 25, 30, 35, 40, 45 and 50 mm from the center, depending on the radius of the colony at the time, and transferred (upside down) to the center of a new PDA plate. The new plates were incubated at 20 C, and the diameter of the new (daughter) colony from each disk was measured after 24 h incubation. Analysis of variance was performed in SAS to test whether the effects of the parent colony age, the disk's original position and their interaction were significant on the extension rate of the daughter colony. Similarly, disks were taken at 10 mm from disk centers to the leading edge of parent colonies in different radial directions in a separate experiment, and the 24 h radial growth of each daughter colony was measured. Analysis of variance was performed in SAS to determine whether the effects of the parent colony size was significant on the growth of the daughter colony, and then a second order polynomial model was fitted with nonlinear regression procedure in SAS.

Experiment IV: effects of temperature and nutrient concentration on growth rate of S. minor *and* S. sclerotiorum.—Two experiments were conducted to evaluate the effects of incubation temperature and nutrient concentration on the growth of *S. minor* and *S. sclerotiorum*. In the first experiment 4.5 mm diam mycelial disks were transferred from the leading edge of the colonies of two isolates from each species onto new PDA plates. The plates were incubated at 5, 10, 15, 20, 25 and 30 C. The diameter of the colony on each plate was measured after 24 h incubation. Analysis of variance was conducted to test whether the effects of temperature on the radial growth of colonies of *S. sclerotiorum* and *S. minor* were significant.

In the second experiment PDA plates with different concentrations of potato dextrose broth were prepared. The concentrations of potato dextrose broth in the media were 0, 6, 12, 18 and 24 g/L. The agar concentration (15 g/L) was constant. About 10 mm from the leading edge of *S. minor* and *S. sclerotiorum* colonies on PDA, 4.5 mm diam mycelial disks were taken and transferred onto the plates with different concentrations of potato dextrose broth. Half of the plates were incubated at 15 C and another half at 20 C. The diameter of the colony on each plate was measured every 12 h. The experiment was conducted three times. The same polynomial model, $r = at^2 + bt$, was fitted to each temperature × species × medium combination and each replication using nonlinear regression procedure in SAS. Analysis of variance was conducted to test the effects of incubation temperature, concentration of potato dextrose broth in the medium and their interactions on the estimated parameters a and b for each species.

RESULTS

Temporal colony growth patterns of colonies of S. sclerotiorum, S. minor, V. dahliae *and* Cladosporium sp.—Radial growth of *Cladosporium* sp. was the slowest; 0.867 mm d^{-1} at 15 C and 1.428 mm d^{-1} at 20 C (FIG. 1A). The two *V. dahliae* isolates were intermediate, with faster growth at 20 C (1.645 and 1.932 mm d^{-1} respectively for isolates Ls16 and Ls17) than at 15 C (0.977 and 1.142 mm d^{-1} respectively for isolates Ls16 and Ls17) (FIG. 1B). The growth rates were much greater for *S. minor* (>6.5 and 7.1 mm d^{-1} at 15 and 20 C respectively) and *S. sclerotiorum* (>8.2 and 11.1 mm d^{-1} at 15 and 20 C respectively) (FIG. 2A). The growth of *Cladosporium* sp. and *V. dahliae* followed a linear trend at both 15 and 20 C (FIG. 1A, B), and there was no significant trend for the 2 d radial growth of their colonies over time (data not shown). However the temporal growth of the two *Sclerotinia* species was nonlinear (FIG. 2A) and could be fitted better by a second-order polynomial model ($y = a \cdot t^2 + b \cdot t$) than a linear model (data not shown). The 12 h growth rates of *Sclerotinia* colonies, which were derived from the same dataset, increased with colony radius and fitted a logarithmic model with R^2 of 0.85–0.97 (FIG. 2B).

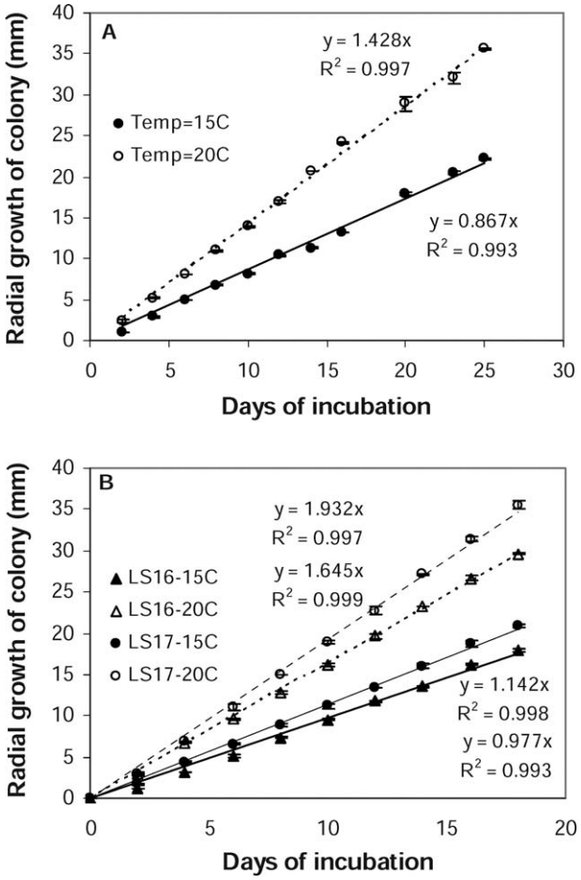

FIG. 1. Radial growth of an isolate of *Cladosporium* spp. on PDA (A), and two isolates of *Verticillium dahliae* (Ls16 and Ls17) on NP10 (B) incubated at 15 and 20 C. The linear regression lines are forced through the origin.

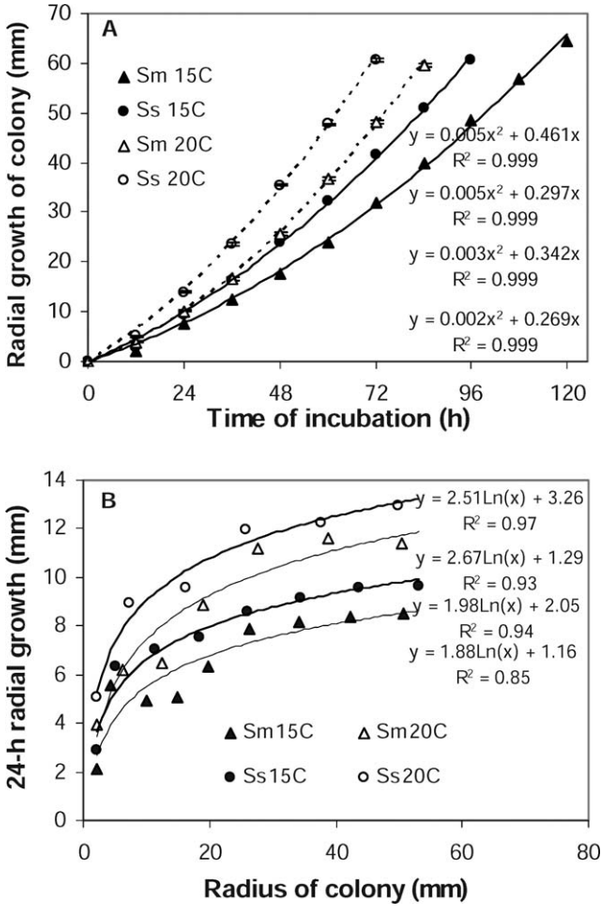

FIG. 2. Radial growth of *Sclerotinia sclerotiorum* (Ss) and *S. minor* (Sm) on PDA incubated at 15 and 20 C (A); and the derived relationship between the colony radius and the next 12 h radial growth (B).

Effects of original disk size on colony growth.—As with the temporal growth trends in Experiment I, 24 h radial growth of *S. minor* and *S. sclerotiorum* colonies increased with the radius of the mycelium disk transferred (FIG. 3A). This relationship in both species was explained by a logarithmic model (FIG. 3A). The rate parameters estimated (FIG. 3A) were smaller than those derived from the first experiment (FIG. 2B), indicating slower growth at similar disk sizes. This suggested that cutting and transferring disks upside down results in growth lag of the daughter colony. We therefore designed experiment IIB (FIG. 3B), in which growth of *S. sclerotiorum* was faster than in Experiment IIA (FIG. 3A) but still slower than in the Experiment I (FIG. 2B). The poor connection between the well walls and the disks placed inside and damage to hyphal tips during cutting and transferring might have contributed to the growth lag. This also was confirmed by the uneven and slow growth of *S. minor* along the crack between the disks and the well walls (data not shown).

Effects of the disk position and age of the parent colony on growth rate of the daughter colony.—The growth potential of any disk from a parent colony, as suggested by the growth rate of the daughter colonies that originated from the disks taken at the same time from the parent colony, changed with the position of the disk (distance from the disk center to the parent colony center) (FIG. 4A, B). As the position of the disk changed from colony edge to the center the growth potential increased first. This growth was more prominent for *S. minor* (FIG. 4A) than for *S. sclerotiorum* (FIG. 4B) and more considerable for a large colony than a small colony; it then decreased and increased again for *S. sclerotiorum* (FIG. 4B) but continued to decline for *S. minor* (FIG. 4A) when the position approached the center.

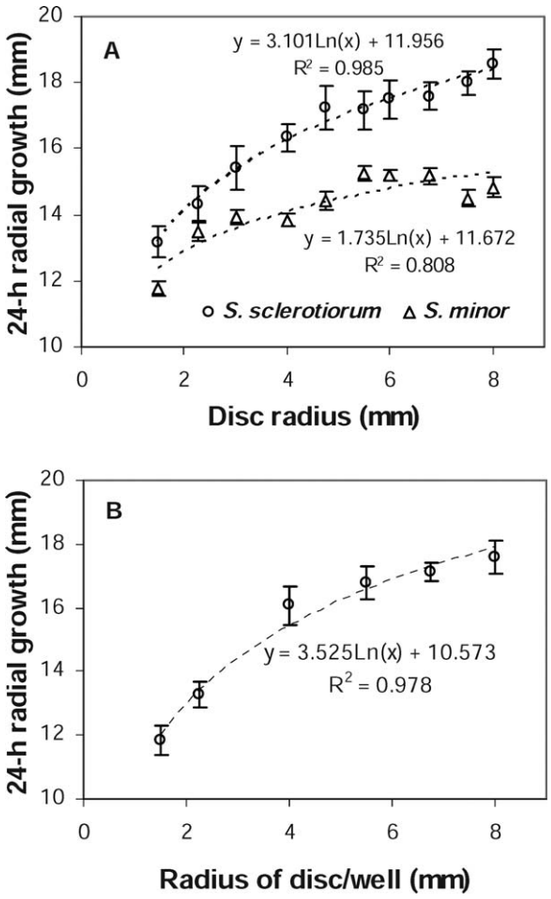

FIG. 3. Relationship between the 24 h radial growth of *Sclerotinia sclerotiorum* and *S. minor* on PDA and the radius of the transferred disk when the disk was placed upside down on the surface (A) and upside up in a predug well (B). Both parent and daughter colonies were incubated at 20 C.

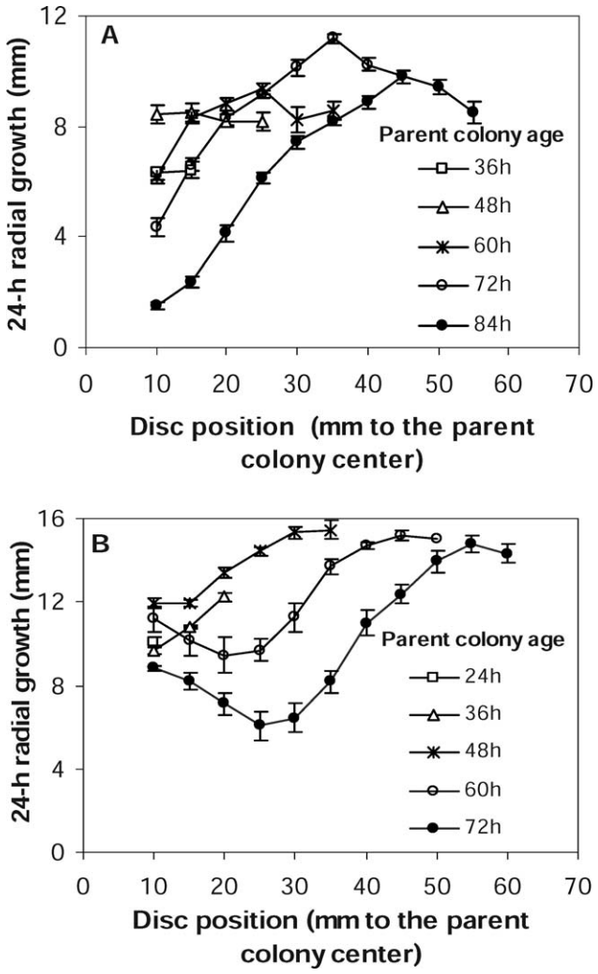

FIG. 4. Effects of the disk position (distance to the colony center) in the parent colony and the age of the parent colony on the growth of the daughter colonies of *S. minor* (A) and *S. sclerotiorum* (B). The diameters of the transferred disks were 3 mm, and both parent and daughter colonies were incubated at 20 C.

When growth potentials of disks transferred at different times and from the same distance to the center of a parent colony were compared, for example at 15 mm from the center, the growth potential increased first and then decreased with colony age for both *Sclerotinia* species (FIG. 4A, B). For disks taken at 10 mm from the leading edge, growth rate increased first as the parent colony extended to 30–40 mm in radius and then remained nearly constant or declined slightly as the parent colony extended further (FIG. 5).

Effects of temperature and nutrient concentration on growth rate of S. minor *and* S. sclerotiorum.—The growth rate of both species of *Sclerotinia* increased as the incubation temperature increased 5–25 C, peaked at 25 C, and then declined at 30 C (FIG. 6). Generally the growth of *S. sclerotiorum* colonies were faster than *S. minor* colonies. The difference between the growth rates of the two species was greatest at 25 C but was small at 5 C and 30 C (FIG. 6).

For both species, regardless of the temperature, growth rates increased with the concentration of potato dextrose broth in the medium (data not shown). This was reflected in the higher values of parameters a and b in the second order polynomial models ($r = at^2 + bt$) (FIG. 7A, B). As the concentration of potato dextrose broth in the PDA medium increased from 0 g/L to 24 g/L, parameter a increased from near 0 to 0.0013–0.0054 (0.0054 for *S. sclerotiorum* at 20 C) (FIG. 7A). Regardless of species the estimated parameter a was significantly affected by temperature, the concentration of potato

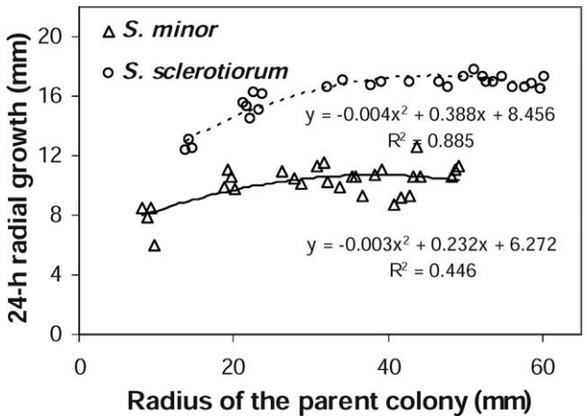

FIG. 5. Effect of the radius of the parent colony on the radial growth of daughter colonies of *Sclerotinia sclerotiorum* and *S. minor*. The daughter colonies started from 3 mm diam disks that were transferred from a single parent colony and from spots 10 mm inward from the colony edge. Both parent and daughter colonies were incubated at 20 C.

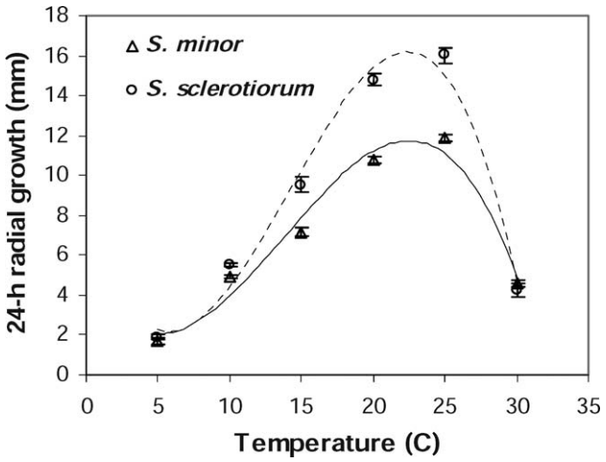

FIG. 6. Effects of incubation temperature on growth rate of *Sclerotinia minor* and *Sclerotinia sclerotiorum* on PDA. All colonies started from 4.5 mm diam disks that were transferred from a single parent colony and from spots 10 mm inward from the colony edge. The 24 h radial growth was measured as the increase in radius during the first 24 h of incubation.

dextrose broth in the medium and their interaction (TABLE I). For both species parameter *a*, which is an indicator of nonlinearity, was near zero for media with ~~at~~ 0 g/L potato dextrose broth at both 15 and 20 C. Parameter *a* increased with the concentration of potato dextrose broth, more so at 20 C than at 15 C (FIG. 7A). Parameter b, which can be considered an indicator of the growth rate in the initial stages, was significantly affected by both incubation temperature and the concentration of potato dextrose broth in the medium, but interactions were not as significant as for parameter *a* (TABLE I). The higher value of parameter *b* at higher concentrations of potato dextrose broth followed similar trends for both species at 20 C and 15 C (FIG. 7B).

DISCUSSION

The temporal growth of *S. sclerotiorum* and *S. minor* followed a nonlinear pattern unlike that of *V. dahliae*, *Cladosporium* sp. and other fungi (Trinci 1969, 1971, 1974; Gervais et al 1988; Farina et al 1997; Ferret et al 1999; Steinberg et al 1999). The radial growth rate of colonies of *V. dahliae* or *Cladosporium* sp. remained nearly constant over time and could be estimated from the slope of the linear regression line as in previous studies (Trinci 1969, 1971, 1974; Gervais et al 1988; Farina et al 1997; Ferret et al 1999; Steinberg et al 1999). The extension of *S. sclerotiorum* and *S. minor* colonies however was nonlinear and did not fit the linear regression model. Radial growth rate of colonies of these two fungi increased with time until the colonies reached the edge of the 150 mm diam Petri dishes.

The nonlinear growth of *S. minor* and *S. sclerotiorum* perhaps results from the unique characteristics of the two species. Unlike the colonies of *Verticillium* sp., *Cladosporium* sp. and many other fungi, the two *Sclerotinia* species do not produce spores or sclerotia before nutrients become limited. Although the mycelium density increases early (within about 5 mm wide peripheral zone at extending edge), it remains nearly stagnant during the rest of the colony extension (Wu and Subbarao unpubl). Both the diameter of hyphal tips and the tip density (d) along the colony edge remained nearly constant over time while tip extension was negligible away from the edge (Wu and Subbarao unpubl). Therefore as the colony radius increased, assuming all hyphae remain active in uptake of nutrients, the areas of mycelia involved in nutrient absorption increased proportionally to the square of the radius ($=c \cdot \pi r^2$), while the number of extending tips increased proportionally to the radius ($d \cdot 2\pi r$). If all hyphae function equally well (not limited by the physiological status, nutrient supply or the distance to the extending tips) in supporting growth and all the energy gained is used in tip extension, the radial growth rate will increase almost linearly with the colony radius because average volume to support one hyphal tip to growth will be $[cr/(2d)]$. However the absorption by a unit area of mycelium can hardly remain constant over time because of the differential abilities of newly formed and old hyphae to absorb and transport nutrients as evidenced by the effects of

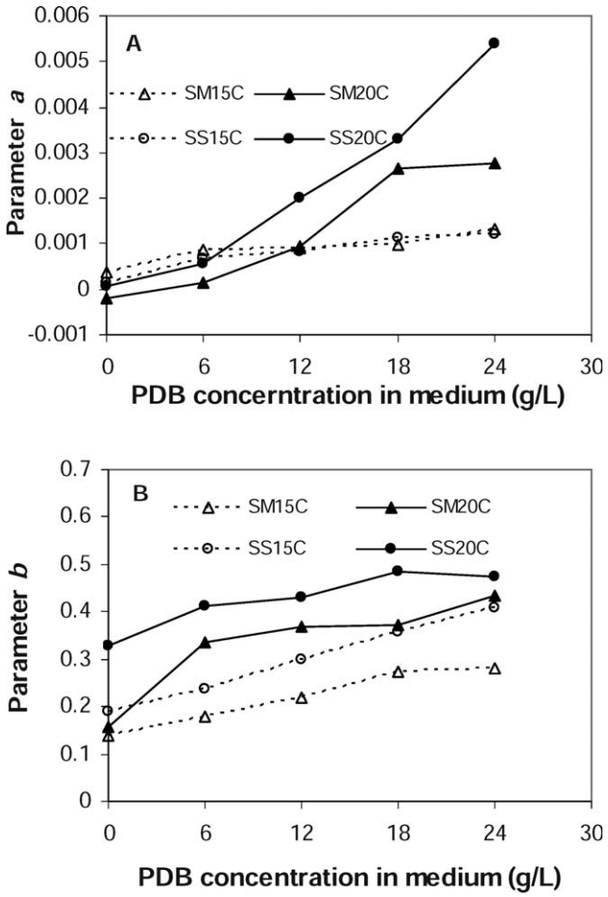

FIG. 7. Effects of nutrient concentration on parameters *a* (A) and *b* (B) in a polynomial model ($r = at^2 + bt$) that were fitted to radial growth (colony radius *r* in millimeters) of *Sclerotinia sclerotiorum* and *S. minor* over incubation duration *t* (in hours). All colonies started from 4.5 mm diam disks that were transferred from a single parent colony and from spots 10 mm from the colony edge.

disk position and parent colony age on the extension rate of daughter colonies in this study and the available nutrients varying from the center to the edge of a colony (Robson et al 1987). The cost of transportation also might increase with the distance to the extending tips. Therefore the relationship between the radial growth rate of a disk and the radius of the disk was not linear but logarithmic in this study. The increase in radial growth was rapid when the radius was small but slowed dramatically with further increase in the radius.

This positive correlation between radial growth rate and the original radius of the mycelial disk was observed for all sizes of disks tested (the greatest diameter of the disk was 16 mm), implying a >8 mm wide ''peripheral growth zone''. However using the method proposed by Trinci (1971) the ''peripheral growth zone'' was measured at only about 5 mm wide (Wu and Subbarao unpubl), which appears to underestimate the width of ''peripheral growth zone'' in both *S. minor* and *S. sclerotiorum*. Mycelium of these *Sclerotinia* species is a complex network of hyphae, and materials required for hyphal extension can be transported in any direction inside the hyphae except within a narrow peripheral zone at the edge of the colony, where the hyphal density is low and the network is not well developed. We believe that the width measured with Trinci's method is actually the width at the colony edges where the hyphal networks are not well developed and therefore the lateral transportation of materials is not significant. This also was supported by the increasing hyphal density inward from the colony edges observed on *Rhizopus stolonifer* by Trinci (1974) and on *S. minor* and *S. sclerotiorum* in this study. This is also why the theoretical specific growth rate in the ''peripheral growth zone'' was highly correlated with the specific growth rate in submerged culture (Trinci 1971). When a network of hyphae is not well developed the interference among hyphal branches is limited, and thus the growth of hyphae more closely resembles the growth in liquid cultures with unlimited nutrient supply.

Measuring the width of peripheral growth zone by transferring different sizes of disks from an extending parent colony however is limited to those fungi with a narrow peripheral growth zone because it is not easy to transfer a large disk, the growth potential will vary significantly within the disk if it is too large and the oxygen supply will be significantly lower in the middle of the disk when the disk is placed upside down on a new medium. Although placing the disks in a predug well in the agar as described in this study can partially solve the oxygen supply problem, it sometimes resulted in a new problem: Mycelium of the disk failed to cross the small gap between the disk and the well wall. It also is impossible to avoid the cutting damage from carving out agar disks. In contrast the minimum radius for a colony to reach a constant extension rate (estimated from the temporal growth curve as presented in this study) reflects the true growth potential of the colony at different stages. Although this method is more time consuming and the results are affected by nutrient concentration in medium and other factors as demonstrated in this study, it provided a better estimation for the width of the ''peripheral growth zone''.

As observed in other fungi (Steele and Trinci 1975, Trinci 1969) the radial growth rate of a colony of *S. minor* and *S. sclerotiorum* was affected by incubation temperature. The radial growth rate of *S. sclerotiorum* was more rapid than that of *S. minor* at 20 and 25 C,

TABLE I. Analysis of variance on the effects of incubation temperature and concentration of potato dextrose broth (PDB) in the potato dextrose agar medium on estimated parameters *a* and *b* of the second order polynomial regression model (radius = $at^2 + bt$) for the temporal radial extension of colonies of *S. minor* and *S. sclerotiorum*[a]

Factors	Effects on parameter *a*[b]		Effects on parameter *b*[b]	
	S. minor	*S. sclerotiorum*	*S. minor*	*S. sclerotiorum*
Temperature	0.004	<0.0001	<0.0001	<0.0001
PDB	<0.0001	<0.0001	<0.0001	<0.0001
PDB × Temperature	<0.0001	<0.0001	0.0336	0.3712

[a] All colonies were started from 4.5-mm-diameter discs that were transferred from a single parent colony, and all discs were taken 10 mm away from the leading edge of the parent colony.

[b] A value smaller than 0.05 indicates significant effects of the factor on the parameter.

but nearly equal at 5–15 C. This might be a result of different geographical distribution of the two fungi and their adaptation to local environments during evolution. *S. sclerotiorum* is adapted to environs over a wide temperature range, while *S. minor* is adapted to a narrow range of regions with cool weather (Purdy 1979, Ekins et al 2002).

The growth rate of a colony of *S. minor* and *S. sclerotiorum* also was affected by the nutrient concentration, consistent with Trinci (1969). The radial growth w[illegible] low nutrient concentration. More interesting, the radial growth of *Sclerotinia* colony was almost linear at low nutrient concentration, with parameter *a* not significantly different from 0, suggesting a narrow ''peripheral growth zone'' had been reached soon after onset of incubation. This also suggested that hyphae at the center of a colony might contribute less to the radial growth because nutrient concentration is lower at the colony center than at the extending edge and nutrient gradients are formed in the medium beneath the extending fungal colony (Robson et al 1987). The effect of nutrient concentration also might have contributed to the difference in growth of disks transferred from different positions of a colony and at different times.

Based on the literature (Trinci 1978, Prosser and Tough 1991, Gooday 1995, Papagianni 2004, Pazouki and Panda 2000) and our results obtained with the two *Sclerotinia* spp., a hypothesis can be constructed that the growth rate of a colony depends on the balance of energy acquired by absorption of nutrients from the medium and consumption of energy for regular physiological processes, material transportation, branching and hyphal tip extension. Contribution of any hyphal cell to the colony extension depends on the energy balance in the cell and the transport cost for sending the supporting materials to the extending tips. Nutrient absorption and energy consumption processes in a fungal cell are temperature/nutrient dependent and affected by the physiological status of the hyphal cells. In addition, transportation of nutrients to elongating hyphal tips is also distance dependent in that the transportation will consume more energy as the distance to the extending tips increases. Although the hypothesis of ''peripheral growth zone'' can still hold the width of the zone varies with temperature and nutrients in the medium and is determined more by the nutrient gradients due to depletion and the cost for transportation, but less by the maximum transport speed because transport[illegible] of required materials such as nuclei were greater than the radial extension rate of the fungal colony (<600 μm min^{-1} by Ross [1976] and 40 μm min^{-1} by Suelmann et al [1997]). Contributions of the hyphal cells within this zone to the radial extensi[illegible]lso might vary with their positions.

In summary, this study revealed deviations from established patterns of the kinetics of fungal colony growth. The radial extension rates of *S. sclerotiorum* and *S. minor* accelerate over time. Knowledge of the differences in growth behavior of these fungi may be exploited in the laborato[illegible]udies on fungal competition and hyperparasitism, and potentially in disease control strategies.

ACKNOWLEDGEMENT

We thank Dr Steve Klosterman at USDA-ARS, Salinas, California, for his presubmission review of the manuscript.

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