

## Effects of Osmotic Potential and Temperature on Growth of Two Pathogens of Figs and a Biocontrol Agent

K. V. Subbarao, T. J. Michailides, and D. P. Morgan

First author: Department of Plant Pathology, University of California, Davis, U.S. Agricultural Research Station, 1636 E. Alisal Street, Salinas 93905; second and third authors: Department of Plant Pathology, University of California, Davis, Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier 93648.

Supported in part by a grant from the California Fig Institute.

We thank J. P. Geaghan for suggestions on data analysis, A. H. C. van Bruggen for providing the SC-10A thermocouple psychrometer, J. M. Duniway for measuring solute potential on figs, K. Herman and W. Butler for providing experimental sites in their orchards, and R. Gonzalez for assistance.

Accepted for publication 15 September 1993.

### ABSTRACT

Subbarao, K. V., Michailides, T. J., and Morgan, D. P. 1993. Effects of osmotic potential and temperature on growth of two pathogens of figs and a biocontrol agent. *Phytopathology* 83:1454-1459.

Growth of *Aspergillus niger* and growth and sporulation of *Fusarium moniliforme*, causing smut and endosepsis, respectively, in figs, were evaluated at 10 osmotic potentials ranging from  $-0.46$  to  $-4.46$  MPa at 15, 20, 25, 30, and 35 C. *Paecilomyces lilacinus*, a fungus that naturally occurs in the cavities of figs, was evaluated under similar conditions. Experiments also were conducted in the field to study the optimal time for spraying *P. lilacinus* as a biocontrol agent. The growth of all three fungi was affected more by temperature than by osmotic potential. The optimum temperature for linear growth of both *A. niger* and *P. lilacinus* was 30 C. At optimal temperatures, decreasing osmotic potentials had the least effect on the growth of *A. niger* and the most effect on *P. lilacinus*. The growth of *F. moniliforme* was reduced significantly at 35 C by high osmotic potentials ( $>-3.12$  MPa) and at 15 C by low osmotic potentials ( $<-1.79$  MPa). Based on their ability to grow in hot, dry

environments, the three fungi can be ranked *A. niger*  $>$  *F. moniliforme*  $>$  *P. lilacinus*. Regardless of osmotic potential, low temperatures (15 and 20 C) limited the growth of *A. niger* and explained, in part, why it does not frequently occur in caprifigs. Regardless of temperature, decreasing osmotic potentials limited the growth of *P. lilacinus*, and thus, among the three fungi tested, it had the least ability to utilize available water, yet for *P. lilacinus* to be effective as a biocontrol agent, adequate moisture is required. Spraying a spore suspension of *P. lilacinus* on caprifigs in mid-March suppressed incidence of endosepsis in fruits by 50% in comparison with unsprayed trees; the same spray treatments did not reduce disease on Calimyrna fig trees because of low-moisture conditions. During the caprifig growing season, temperatures did not exceed 30 C, and there were frequent rains to provide adequate moisture for *P. lilacinus*.

Endosepsis, caused by *Fusarium moniliforme* Sheldon, and "smut", incited by *Aspergillus niger* V. Tiegh., are two important diseases of figs (*Ficus carica* L.) that cause significant losses each year (6). Endosepsis, named for the internal rot of figs, begins as brownish areas within the fig cavity when the fruit begins to ripen. When the majority of the florets in the fig cavity are infected, the disease manifests as water-soaked lesions around the ostiole and progresses toward the stalk. "Smut" is not a true smut, so the name is a misnomer. It refers to the black powdery appearance of dried figs. Endosepsis is common on both inedible caprifigs (male figs) and edible Calimyrna figs, whereas "smut" is common only on Calimyrna figs. On Calimyrna figs, endosepsis appears much earlier than "smut." The most important environmental factors influencing growth and colonization by *Fusarium* spp. are the availability of water and temperature (12). In contrast, *A. niger* is less dependent on water and more dependent on temperature (7). Knowledge of the interaction of temperature and osmotic potential is important in understanding the ecology of the two diseases. *Paecilomyces lilacinus* (Thom) Samson, a natural inhabitant in the cavities of figs, has shown promise as a biocontrol agent of both endosepsis and "smut" (14). It is important, therefore, to identify the environmental conditions that maximize the benefits of using a biocontrol agent.

Figs are of two types: persistent and caducous. The caducous figs are a gynodioecious, insect-pollinated crop with distinct male (caprifig) and edible female figs. The caprifigs serve the dual function of providing the insect pollinator hibernating site and pollen for Calimyrna figs. Details of fig pollination and production have been described previously (25). The pollinator insect, *Blastophaga psenes* L., carries both pollen and fungal propagules into the female fig cavity (2). Therefore, the key to managing endosepsis

in the edible crop is to obtain disease-free male figs. Until recently, disease-free male figs were obtained with fungicides (14,21,25). However, the long-term use of fungicides (including benomyl) has resulted in decreased vigor of pollinator insects and has increased the incidence of *F. moniliforme* isolates resistant to benomyl, resulting in failure to control endosepsis (15). The recent search for alternative methods has shown that it is possible to obtain disease-free male figs for pollination of Calimyrna figs by carefully choosing disease-free figs in the previous crop of male figs (14). The use of *P. lilacinus* as a biocontrol agent to manage endosepsis and "smut" also has shown promise (14).

The simplest method of measuring fungal growth is by determining increases in linear dimensions on an agar medium. Although colony radial growth does not take into account changes in hyphal density or specific growth rate (26), it is usually a reliable parameter to measure the effect of an environmental variable on the growth of fungi. Studies on the effect of osmotic potential on vegetative growth of various fungi show that fungi clearly differ in their ability to absorb water from their environment (1,9,11,12,22,31,32), and with decreasing water potential, fewer species are able to grow and reproduce (4). Decreasing osmotic potential reduces growth of *F. moniliforme* because of increased respiration by the fungus: The energy is diverted away from growth for use in maintaining turgor of hyphal cells (31,32).

The effect of osmotic potential in combination with temperature also has been studied with other plant pathogens (1,9,11,22). Some pathogens not at optimal water potential are unable to overcome host barriers and attain their full pathogenic potential (4). Such information is not available for *A. niger*, *F. moniliforme*, or *P. lilacinus*. The major component of water potential in plant tissues is osmotic (20); therefore, we evaluated its effect on the growth of the three fungi in combination with temperature. The objectives of this study were to evaluate growth and sporulation of these three fungi at various osmotic potential  $\times$  temperature

combinations and identify the fig crop for spraying with *P. lilacinus* for biocontrol of endosepsis. Preliminary results have been published (23).

## MATERIALS AND METHODS

**Isolates.** Single-spore cultures of *F. moniliforme* (F-28) and *P. lilacinus* (4B11), both originally isolated from cultivated caprifigs, and of *A. niger* (AN1), isolated from Calimyrna figs, were evaluated for growth and sporulation at various osmotic potential  $\times$  temperature combinations. The fungi were maintained on silica gel stored at 4–6 C (27). Inocula of all three fungi for seeding osmotically adjusted media were produced on acidified potato-dextrose agar (APDA). For *F. moniliforme*, the inoculum consisted of 4-mm-diameter disks cut from the advancing margin of an actively growing colony. Because of the hydrophobicity of *A. niger* and *P. lilacinus* spores, spore suspensions of each fungus were prepared in 0.1% agar amended with four drops of Tween-80 per liter. Inoculum for these two fungi consisted of a 5- $\mu$ l spore suspension that, after being dispensed on the agar plate, also occupied an area 4 mm in diameter.

**Treatments.** The effects of osmotic potential and temperature on growth of *A. niger*, *F. moniliforme*, and *P. lilacinus* were determined on PDA amended with various concentrations of KCl to provide 10 osmotic potentials ranging from -0.46 to -4.46 MPa (30). Actual osmotic potentials achieved were verified at the end of the experiment with a thermocouple psychrometer (Model SC-10A, Decagon Devices, Inc., Pullman, WA). Twenty milliliters of the appropriate medium was poured into 9-cm-diameter petri dishes left at room temperature ( $23 \pm 1$  C) for 5 days to allow evaporation of any free moisture. The dishes then were seeded centrally by either transferring a 4-mm-diameter mycelial disk of *F. moniliforme* or dispensing 5  $\mu$ l of a spore suspension of *A. niger* or *P. lilacinus*. Because media prepared in different batches are subject to variations in osmotic potentials (33), dishes sufficient to repeat the experiment twice were prepared in one batch. Five dishes of each fungus were inoculated as described above for each temperature  $\times$  osmotic-potential combination. The experimental design was a factorial arrangement of the three fungi and 10 osmotic potentials within each temperature. All dishes of a given temperature  $\times$  osmotic potential  $\times$  fungus combination were placed together in a plastic container, sealed, and incubated in the dark at 15, 20, 25, 30, and 35 C. Colony diameters were measured as soon as the fastest growing colony of each fungus in any temperature  $\times$  osmotic-potential combination had reached the edge of the dish. The experiment was repeated once. Sporulation was determined for *F. moniliforme* and *P. lilacinus* by rinsing each culture with 10 ml of distilled water, scraping the spores with a rubber spatula, and counting them with a hemacytometer. Sporulation was not determined for *A. niger*.

Variance analysis was used to evaluate the effects of experiment (block), temperature (main plot), fungi and osmotic-potential combinations (subplot), and interactions with SAS (19). Growth rates of each fungus at the different temperature  $\times$  osmotic-potential combinations were calculated by dividing the respective colony diameters by the number of days of incubation. Contour maps of growth rates at different temperature  $\times$  osmotic-potential combinations were generated for each fungal species.

**Measurement of fruit water potential.** Because figs are irrigated during the summer and sugar content increases as fruits mature, changes in fruit water potential are expected. Surface drip at the base of each tree once in 2 wk is the primary means of irrigating figs. To record the seasonal changes in maturing fruit, water potentials were measured with a pressure chamber (18) weekly during August and September of 1991 and July and August of 1992. Ten fruits were cut along the stalk and tightly wrapped in a plastic bag to prevent evaporation before and during measurement of balance pressure (28). During 1991, fruit water-potential measurements were made between 1500–1700, which is usually the hottest time of the day in the San Joaquin Valley in California. During 1992, however, water potentials were deter-

mined between 1100–1300. Solute potential of Calimyrna figs as compared to NaCl standards was determined on 12 fresh fruits with a Wescor model 5500 vapor pressure osmometer (Wescor, Inc., Logan, UT).

***P. lilacinus*-sprayed caprifig and Calimyrna figs.** The optimum time for spraying *P. lilacinus* to control endosepsis and "smut" was evaluated by spraying both the caprifig and Calimyrna crops. Ten-day-old cultures of *P. lilacinus* were flooded with 10 ml of sterile-distilled water (with four drops of Tween-80 per liter of water), and the cultures were scraped with a rubber spatula. The spore suspension was strained through cheesecloth and diluted to  $10^7$  conidia per milliliter. The spore suspension was sprayed to runoff on four replicate Calimyrna trees on 7 and 12 June 1990 and 1991, respectively, and on caprifig trees on 22 and 24 March 1991 and 1992, respectively, with a knapsack-type power duster and mist blower (model DM-9, Echo Inc., Lake Zurich, IL). Four unsprayed trees in each crop served as the control treatment. One-hundred Calimyrna figs from each replication of treatments done during 1990 were harvested on 21 September. Similarly, caprifigs from treatments done during 1991 were harvested on 24 May. In experiments on Calimyrna during 1991 and on caprifigs during 1992, however, 3 wk after spraying (after the completion of caprification) 20 figs per replication per treatment were collected randomly at weekly intervals, transported to the laboratory in an ice chest, and the presence or absence of *Fusarium* and other fungi in the fig cavity was determined. Figs from both caprifig and Calimyrna crops were sampled until maturity. Each season, fruits from each replicated tree were surface disinfested with 1.6% sodium hypochlorite solution (household bleach containing 5.25% NaOCl) for 3 min and allowed to air-dry. The fruits were then split in half longitudinally with a sterile knife and placed with cut surface facing up over waxed wire screens (20  $\times$  29.5 cm) in separate, clear plastic containers (23.5  $\times$  32  $\times$  10 cm). APDA prepared earlier was cooled to 45 C by maintaining it in a water bath and dispensed into each fig cavity with a sterile pipette as described previously (13). After 5 days of incubation on laboratory benches ( $24 \pm 1$  C), microflora in the fig cavity were identified to the genus level, and their incidences were determined for each treatment. The number of fig halves with *Fusarium* spp. and *A. niger* were expressed as percentages of all figs sampled for each treatment. Analysis of variance was used to determine treatment differences, and means were compared by Fisher's protected LSD (19). Means of incidence of *F. moniliforme* and the corresponding standard errors for each treatment were plotted against the sampling dates for the caprifig or Calimyrna crop.

## RESULTS

**Growth.** Because colony diameters of all three fungi were consistent in the two experiments, pooled results are reported. Colony diameter of each fungus was significantly different in the different

TABLE 1. Variance analysis for colony diameter of *Aspergillus niger*, *Fusarium moniliforme*, and *Paecilomyces lilacinus* at different osmotic potential  $\times$  temperature combinations<sup>a</sup>

Source of variation	df	Mean square	F	P > F
Model	299	3,322.55	1,246.11	0.0000
Experiment (Expt)	1	2,522.02	2.13	0.2181
Temperature (Temp)	4	141,855.41	119.89	0.0002
Error <sub>a</sub>	4	1,183.21		
Osmotic potential (OP)	9	7,537.52	48.29	0.0001
Fungi	2	31,014.82	198.69	0.0001
Temp $\times$ OP	36	591.01	3.79	0.0001
Temp $\times$ Fungi	8	24,858.72	159.25	0.0001
OP $\times$ Fungi	18	1,123.69	7.2	0.0001
Temp $\times$ OP $\times$ Fungi	72	359.60	2.30	0.0001
Error <sub>b</sub>	145	156.09		
Error <sub>c</sub>	1,200	2.67		
Total	1,499			

<sup>a</sup> The coefficient of variation for the experiment was 3.3%.

temperature  $\times$  osmotic-potential combinations and was affected more by temperature than by osmotic potential. The two-way interactions between temperature  $\times$  fungi and osmotic potential  $\times$  fungi were significant as well as the temperature  $\times$  osmotic potential  $\times$  fungus interaction (Table 1). At optimum temperatures, *A. niger* (30 C) cultures reached the edge of the dishes in 4 days, *F. moniliforme* (25 C) cultures reached the edge of the dishes in 9 days, and *P. lilacinus* (30 C) cultures reached the edge of the dishes in 13 days.

In *A. niger*, there was a homogeneous growth pattern at all osmotic potential  $\times$  temperature combinations. At 15 C, *A. niger* had a lag phase (no fungal growth occurred during this period) of 2 days at all osmotic-potential values, but at all other temperatures, there was no lag phase. Growth of *A. niger* at 15 C was negligible and remained nearly constant over the range of osmotic potentials tested. Growth response at 20 and 25 C was very similar at all osmotic-potential values, except that higher growth occurred at 25 C. Maximal growth occurred at 30 and 35 C, and little growth reduction occurred in response to decreasing osmotic potentials (Fig. 1A).

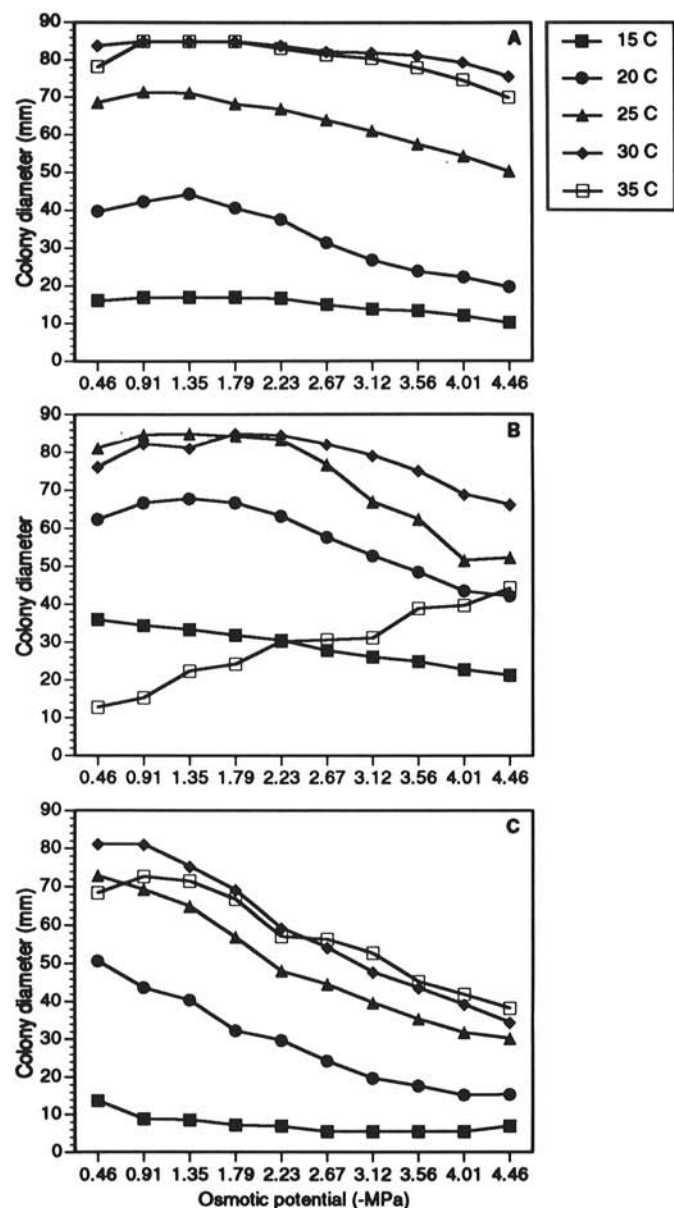


Fig. 1. Mean colony diameters of A, *Aspergillus niger*, B, *Fusarium moniliforme*, and C, *Paecilomyces lilacinus* after 5, 9, and 13 days of incubation, respectively, at different temperature  $\times$  osmotic-potential combinations.

In *F. moniliforme*, there was no lag phase at any of the temperature  $\times$  osmotic-potential combinations. At osmotic potentials between  $-0.46$  and  $-2.67$  MPa, the growth pattern was homogeneous but changed to a feathery pattern (8) at  $<-2.67$  MPa. Growth declined linearly with decreasing osmotic potential at 15 C, increased linearly (between osmotic potentials  $-0.46$  and  $1.79$  MPa) with increasing temperature up to 25 C, and then declined (Fig. 1B). At 20 C, with each incremental decrease in osmotic potential from  $-1.35$  MPa, there was a significant corresponding decrease in growth. Maximal growth occurred at 25 C and was nearly identical at osmotic potentials between  $-0.46$  and  $-2.23$  MPa. At 30 C, a reduction in growth occurred at  $\leq -3.56$  MPa. Least growth occurred at 35 C at most osmotic

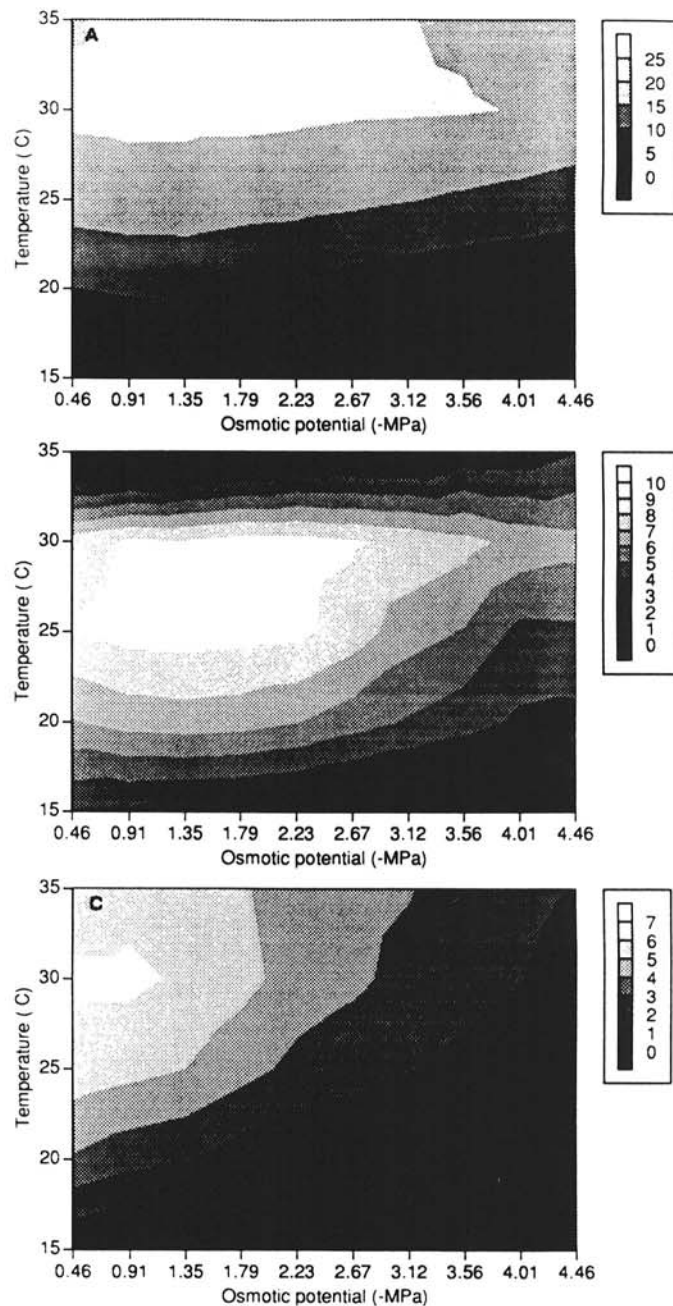


Fig. 2. Contour maps of growth rates of A, *Aspergillus niger*; B, *Fusarium moniliforme*; and C, *Paecilomyces lilacinus* at different temperature  $\times$  osmotic-potential combinations. Colony diameters were measured after 5, 9, and 13 days for *A. niger*, *F. moniliforme*, and *P. lilacinus*, respectively. The different shades in the contour maps of of each fungus represent the growth rates (millimeters per day) of the corresponding fungus. Numbers in the upper-right corner boxes are growth rates (millimeters per day) for the corresponding fungus.

potentials; however, growth increased with decreasing potentials  $< -2.23$  MPa (Fig. 1B), and the promotion of growth at  $-4.46$  MPa was about five times that of  $-0.46$  MPa.

In *P. lilacinus*, there was a 1-day lag period at all temperature  $\times$  osmotic-potential combinations. At all treatment combinations, the growth pattern was homogeneous. Although the osmotic potentials did not decrease growth at 15 C, growth was least at this temperature. Growth decreased linearly with decreasing osmotic potentials at 20 C and was reduced to half at  $-2.67$  MPa. At 25, 30, and 35 C, the growth response was identical, with greater reductions in growth for every incremental decrease in osmotic potentials (Fig. 1C). At all temperatures, maximal growth occurred at the highest osmotic potential used (Fig. 1C).

Among the three fungi tested, growth rates at different osmotic potential  $\times$  temperature combinations were minimal in *P. lilacinus* ( $6 \text{ mm}^{-\text{day}}$ ), moderate in *F. moniliforme* ( $10 \text{ mm}^{-\text{day}}$ ), and maximal in *A. niger* ( $25 \text{ mm}^{-\text{day}}$ ) (Fig. 2). Maximal growth rates occurred in *A. niger* and were maintained at 30–35 C over nearly the entire range of osmotic potentials tested. Growth rates increased with the combination of increasing temperature and decreasing osmotic potentials (Fig. 2A). *F. moniliforme* exhibited the greatest variation in growth rates at the different temperature  $\times$  osmotic-potential combinations. Maximal growth rates occurred at 25–30 C and at osmotic potentials between  $-0.91$  and  $-2.67$  MPa. Growth rates increased linearly with the temperature and remained nearly uniform with decreasing osmotic potentials of up to  $-3.56$  MPa (Fig. 2B). In *P. lilacinus*, maximal growth rates occurred at 30 C and at osmotic potentials  $< -1.35$  MPa. Maximal growth rates within each temperature occurred over a restricted range of osmotic potentials (Fig. 2C).

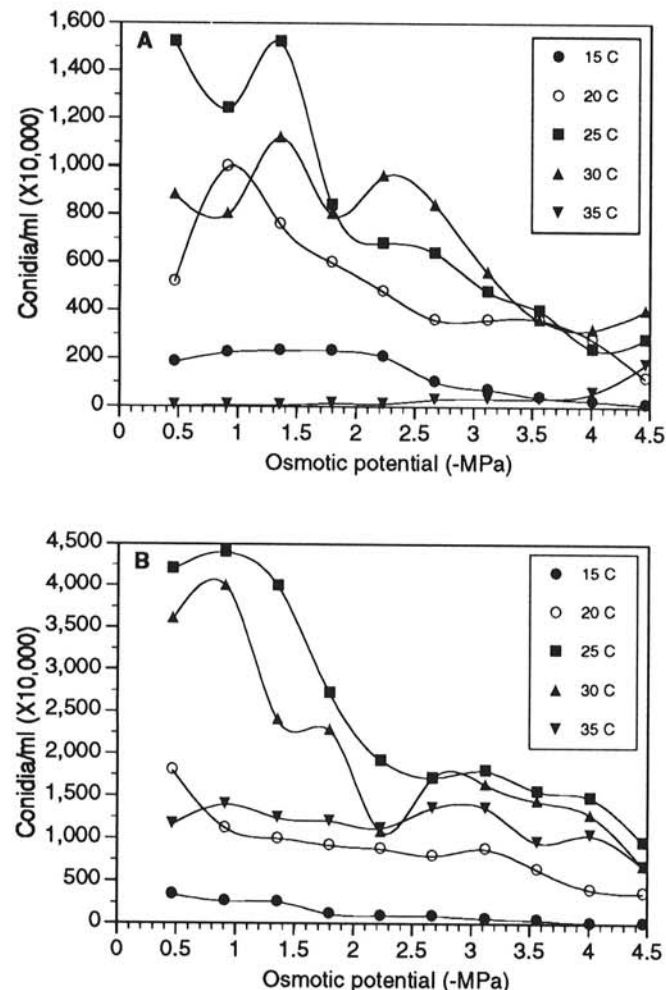


Fig. 3. Sporulation of A, *Fusarium moniliforme* and B, *Paecilomyces lilacinus* after 9 and 13 days growth, respectively, at different temperature  $\times$  osmotic-potential combinations.

**Sporulation.** In *F. moniliforme*, sporulation at different temperature  $\times$  osmotic-potential combinations showed trends similar to those with linear mycelial growth (Fig. 3A). In *P. lilacinus*, trends in sporulation at the temperature  $\times$  osmotic-potential combinations tested differed from linear mycelial growth. Although linear growth was significantly higher at 30 and 35 C in all treatment combinations, maximum sporulation occurred at 25 C (Fig. 3B). At all osmotic potentials, sporulation at 15 C was significantly lower than at 35 C. At all temperatures, sporulation significantly decreased with decreasing osmotic potentials (Fig. 3B). Except at 15 C, *P. lilacinus* sporulated more than did *F. moniliforme* at all treatment combinations.

**Fruit water potential.** Water potential in the fruits fluctuated depending on the growth stage of the fruit and orchard irrigation schedule. However, once irrigations were cut off (days 242 and 210 during 1991 and 1992, respectively) to hasten fig maturity, water potential fluctuated from only  $-1.5$  to  $-1.7$  MPa during 1991 (Fig. 4A) and from  $-0.65$  to  $-1.0$  MPa during 1992 (Fig. 4B). Although fruit water potentials in 1992 were higher than in 1991, their ranges during both years were within the osmotic potentials used in laboratory experiments (Fig. 4). The solute potential of Calimyrna fruits varied between  $-1.67$  and  $-3.33$  MPa with a mean of  $-2.33$  MPa.

***P. lilacinus*-sprayed caprifig and Calimyrna fig trees.** In 1990, mature fruit collected from Calimyrna trees sprayed with *P. lilacinus* showed 13% incidence of *F. moniliforme* compared to 8% incidence in fruit collected from unsprayed trees, but this

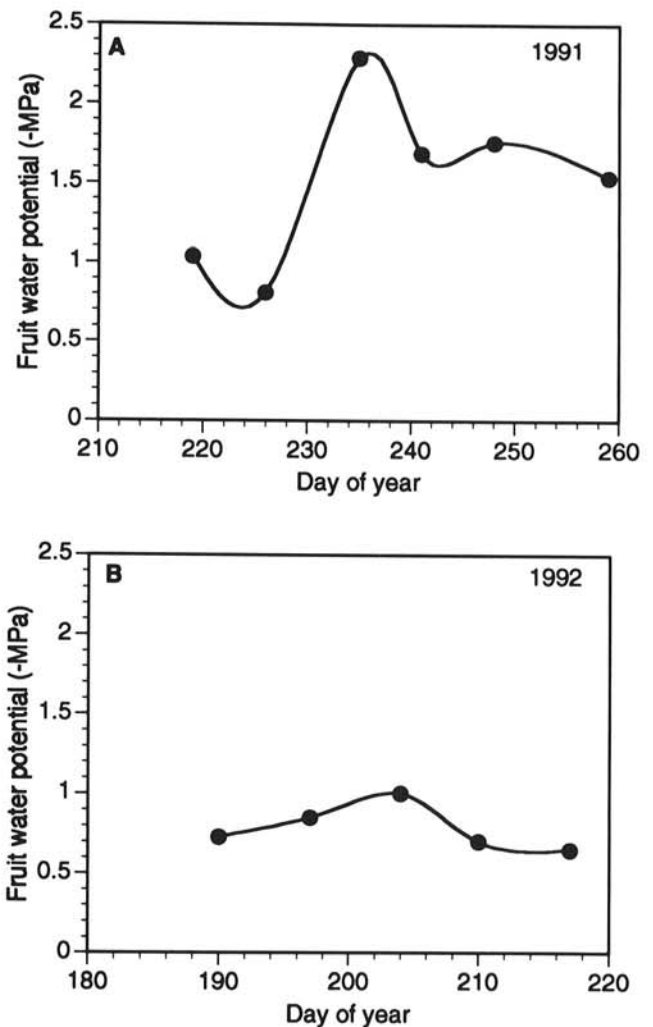


Fig. 4. Fruit water potentials (megaPascals) of Calimyrna figs during the A, 1991 and B, 1992 seasons. Each point is the mean of measurements on 10 fruits. Measurements were made using the pressure chamber technique (18). Solute potential of fresh Calimyrna fruits also were determined with a vapor pressure osmometer.

difference was not significant. In addition, in 1991, for most sampling dates, the percentage of Calimyrna figs from unsprayed trees infested with *F. moniliforme* did not differ significantly from that of figs collected from sprayed Calimyrna trees (Fig. 5A). In contrast, spraying caprifig trees in 1991 and 1992 with *P. lilacinus* significantly reduced the incidence of *F. moniliforme* in figs. In 1991, caprifigs from *P. lilacinus*-sprayed trees had 19% *F. moniliforme*, and those from unsprayed trees had 41% (LSD = 8.5,  $P \leq 0.05$ ). The percentage of caprifigs infested with *F. moniliforme* fluctuated in both sprayed and unsprayed trees in 1992 (Fig. 5B). At maturity, however, significantly lower numbers of caprifigs from the sprayed trees had *F. moniliforme* compared to the caprifigs from unsprayed trees (Fig. 5B). In addition to *F. moniliforme*, other common microflora in the fig cavity were *Serratia* spp., *Alternaria alternata*, *Cladosporium* spp., and some yeasts. The total microflora in the cavities of figs from sprayed trees was less than half of that from unsprayed trees.

## DISCUSSION

The three fungi, *A. niger*, *F. moniliforme*, and *P. lilacinus*, responded differently to the various osmotic potential  $\times$  temperature combinations. Based on their ability to grow in hot, dry environments, the three fungi can be ranked *A. niger*  $>$  *F.*

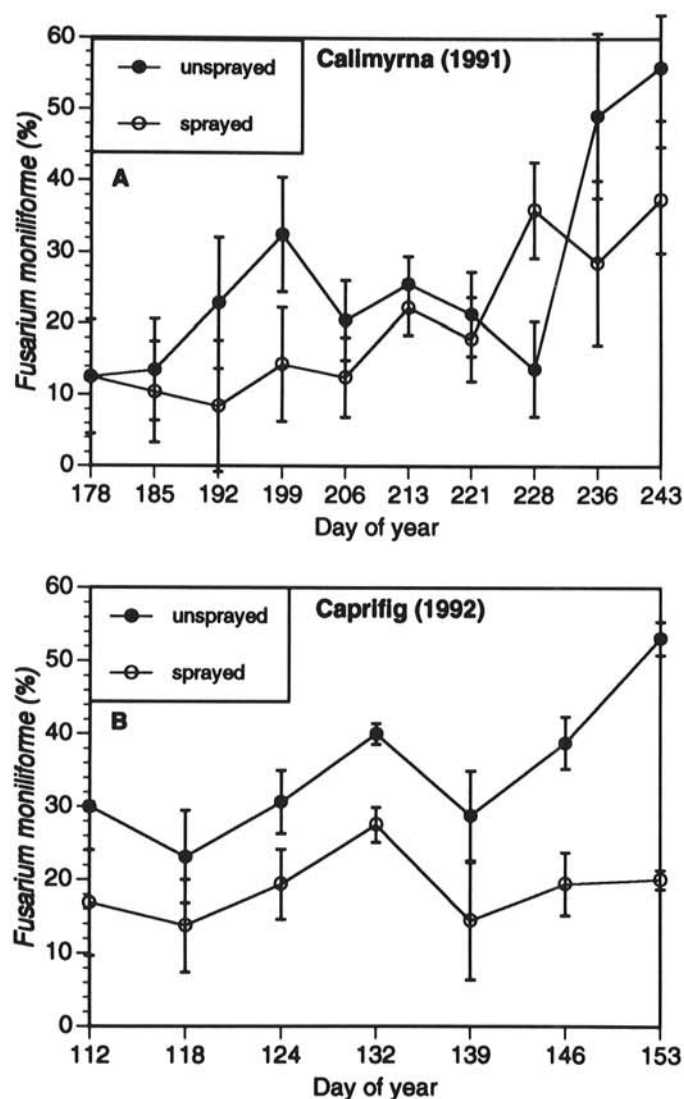


Fig. 5. Percentage of A, Calimyrna and B, caprifig fruits infested with *Fusarium moniliforme* in trees sprayed with *Paecilomyces lilacinus* and fruits from unsprayed trees sampled periodically after the spray. Each point is the mean of 80 recorded fruits. Vertical bars are the corresponding standard errors of the means.

*moniliforme*  $>$  *P. lilacinus*. Under hot, dry conditions, *A. niger* had the most ability to be pathogenic, *P. lilacinus* had the least ability to be an effective biocontrol agent, and *F. moniliforme* had the ability to be pathogenic under a wide range of conditions. The growth of all three fungi was affected more by temperature than by the osmotic potentials employed in our study. The range of osmotic potentials employed in our study represent the range that occurs in figs grown in the San Joaquin Valley in California.

The habitat, distribution, and activity of fungi are a function of their ability to grow at different temperature  $\times$  osmotic-potential combinations. *A. niger* spores are primarily soilborne and are carried to the surface of figs along with dust (16) and to the cavity by beetles that visit figs after sugar accumulates (17). *A. niger* is not an aggressive pathogen and does not infect unwounded figs during earlier stages of fruit growth and development (14). *A. niger* rarely occurs in caprifig crops. On the edible Calimyrna, it occurs just prior to maturity. The inability of *A. niger* to cause "smut" in caprifigs may be due, in part, to the curtailment of its growth at low temperatures. Highest growth rates for *A. niger* occurred at 30 and 35 C across most of the osmotic potentials employed in our study. These conditions probably are optimal also for the expression of its pathogenic potential. Such conditions are very rare during the caprifig crop season but common during the latter part of the Calimyrna crop season, partially explaining why "smut" does not occur on caprifigs or during the earlier stages of Calimyrna fig growth. Growth of *A. niger* also is favored by increasing concentrations of glucose and fructose, the two major sugars in Calimyrna figs (24). The absence of these sugars in caprifigs also may prevent "smut" from developing on this crop.

In contrast to *A. niger*, *F. moniliforme* can be pathogenic under very diverse conditions (32). Its growth in our study was restricted by 35 C at high osmotic potentials and by 15 C at low osmotic potentials. Active growth was maintained at all other temperature  $\times$  osmotic-potential combinations. Highest growth rates occurred at 25 and 30 C over a wide range of osmotic potentials. Woods and Duniway (32) characterized *F. moniliforme* as a xerophilic fungus, and our observations are consistent with their characterization. This finding explains, in part, why *F. moniliforme* is a pathogen on both caprifigs and Calimyrna figs. Furthermore, *F. moniliforme* is transmitted into the cavities of figs during the inevitable entry of the pollinator insect (2). Inoculation with *F. moniliforme* takes place during the very early stages of fig growth (2); therefore, the fungus can exhibit its pathogenic potential when optimal conditions develop during the course of fig growth. One factor that delays active growth of *F. moniliforme* within the fig cavity is the abundance of latex in figs during early stages of growth; latex reduces conidial germination and growth rates of *F. moniliforme* (25). At 35 C, *F. moniliforme* growth was higher at lower osmotic potentials ( $<-2.23$  MPa) than at higher osmotic potentials ( $>-2.23$  MPa). The reasons for this reversal of growth at lower osmotic potentials are unclear, but a similar phenomenon has been observed in other fungi (25).

The morphological switch to the feathery patterns in some mycelial fungi at low osmotic potentials has been attributed to their ability to adapt to desiccating conditions (8). The switch in some mycelial fungi, including *F. solani*, also may be the result of increased  $K^+$  uptake from the medium (5). In our study, KCl was used as the osmoticum to achieve different osmotic potentials in the medium. The switch to feathery patterns at osmotic potentials  $<-2.23$  MPa in *F. moniliforme* also may be the result of increased uptake of  $K^+$  from the medium and may indicate its ability to adapt to desiccating conditions.

The highest growth rates of *P. lilacinus* occurred at 30 C on a very narrow range of osmotic potentials employed in our study. The growth of *P. lilacinus* at low osmotic potentials, even at optimum temperature, was lower compared to that of *F. moniliforme*. Based on these results, *P. lilacinus* can be effective as a biocontrol agent only under conditions with adequate moisture. Because these conditions seldom occur during the Calimyrna growing season, *P. lilacinus* will not be effective as a biocontrol agent of *F. moniliforme* and *A. niger* in Calimyrna figs. In this

study, spraying *P. lilacinus* spore suspension on Calimyrna trees did not suppress the incidence of endosepsis and "smut" compared with unsprayed trees. In contrast, spraying *P. lilacinus* spore suspension on caprifig trees significantly suppressed the incidence of endosepsis in caprifig fruits. During the caprifig growing season, temperature rarely exceeds 30 C, and moderate temperatures are accompanied by frequent spring rains, creating optimal conditions for *P. lilacinus*. Therefore, in a disease-management scheme for figs, *P. lilacinus* is expected to perform well as a biocontrol agent on caprifigs but not on Calimyrna figs.

*P. lilacinus* occurs naturally in the cavities of both caprifigs and Calimyrna figs (14). Spraying *P. lilacinus* spore suspension only augments the natural population for effective management of endosepsis in figs. The fungus also has been extensively used in nematode biocontrol (10,29). The mechanisms by which *P. lilacinus* sprayed on caprifigs lowered the incidence of endosepsis need thorough investigation. Although rarely implicated in human diseases, *P. lilacinus* can cause opportunistic mycotic infections such as, hyalohyphomycosis, endophthalmitis, and sinusitis in immuno-compromised patients (3). Therefore, caution should be exercised and further studies undertaken before the use of *P. lilacinus* to manage endosepsis becomes a routine practice.

#### LITERATURE CITED

- Brownell, K. H., and Schneider, R. W. 1985. Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of *Fusarium oxysporum* in synthetic media and soil. *Phytopathology* 75:53-57.
- Caldis, P. D. 1927. Etiology and transmission of endosepsis (internal rot) of the fruit of the fig. *Hilgardia* 2:287-328.
- Castro, L. G. M., Salebian, A., and Sotto, M. N. 1990. Hyalohyphomycosis by *Paecilomyces lilacinus* in a renal transplant patient and a review of human *Paecilomyces* species infections. *J. Med. Vet. Mycol.* 28:15-26.
- Cook, R. J., and Papendick, R. I. 1972. Influence of water potential of soils and plants on root disease. *Annu. Rev. Phytopathol.* 10:345-374.
- Das, J. 1991. Influence of potassium in the agar medium on the growth pattern of the filamentous fungus *Fusarium solani*. *Appl. Environ. Microbiol.* 57:3033-3036.
- Ferguson, L., Michailides, T. J., and Shorey, H. H. 1990. The California fig industry. *Hortic. Rev.* 12:409-490.
- Griffin, D. M. 1972. Ecology of soil fungi. Chapman and Hall, London.
- Griffith, G. S., and Boddy, L. 1991. Fungal decomposition of attached angiosperm twigs. III. Effect of water potential and temperature on fungal growth, survival and decay of wood. *New Phytol.* 117:259-269.
- Ioannou, N., Schneider, R. W., Grogan, R. G., and Duniway, J. M. 1977. Effect of water potential and temperature on growth, sporulation, and production of microsclerotia by *Verticillium dahliae*. *Phytopathology* 67:637-644.
- Jatala, P., Kaltenbach, R., Bocangel, M., Devaux, A. J., and Campos, R. 1980. Field application of *Paecilomyces lilacinus* for controlling *Meloidogyne incognita* on potatoes. (Abstr.) *J. Nematol.* 12:226-227.
- Kackley, K. E., Grybauskas, A. P., and Dernoeden, P. H. 1990. Growth of *Magnaporthe poae* and *Gaeumannomyces in crustans* as affected by temperature-osmotic potential interactions. *Phytopathology* 80:646-650.
- Magan, N., and Lacey, J. 1984. Water relations of some *Fusarium* species from infected wheat ears and grain. *Trans. Br. Mycol. Soc.* 83:281-285.
- Michailides, T. J., Morgan, D. P., and Klamm, R. Comparison of three methods for determining fig endosepsis caused by *Fusarium moniliforme* and other molds in caprifigs and Calimyrna figs. *Plant Dis.* In press.
- Michailides, T. J., Morgan, D. P., Wang, J., and Beglinger, C. 1991. Sanitation practices and biocontrol approaches for control of fig endosepsis and smut of Calimyrna figs. *Annu. Fig Res. Rep. Crop Year 1990.* California Fig Institute, Fresno. 18 pp.
- Michailides, T. J., Ogawa, J. M., and Ferguson, L. 1987. Investigations on the correlation of fig endosepsis on Calimyrna fig with caprifig infestations by *Fusarium moniliforme*. *Annu. Fig Res. Rep. Crop Year 1987.* California Fig Institute, Fresno. 25 pp.
- Michailides, T. J., Subbarao, K. V., and Morgan, D. P. 1992. Effects of different cultural practices on the epidemiology of fig smut in Calimyrna orchards. *Annu. Fig Res. Rep. Crop Year 1991.* California Fig Institute, Fresno. 14 pp.
- Phillip, E. H., Smith, E. H., and Smith, R. 1925. Fig smut. *Univ. Calif. Agric. Exp. Stn. Bull.* 387. Berkeley. 40 pp.
- Ritchie, G. A. 1975. The pressure chamber as an instrument for ecological research. *Adv. Ecol. Res.* 9:165-254.
- SAS Institute. 1988. SAS User's Guide: Statistics. Version 6.03 ed. SAS Institute Inc., Cary, NC.
- Slatyer, R. O. 1967. Plant water relationships. Academic Press, New York.
- Smith, R. E., and Hansen, H. N. 1935. Directions for control of endosepsis in figs. 1934-35. *Univ. Calif. Agr. Exp. Stn.* 4 pp.
- Stapper, M. F., Lyda, S. D., and Jordan, W. R. 1984. Temperature  $\times$  water potential interactions on growth and sclerotial germination of *Phymatotrichum omnivorum*. *Phytopathology* 74:509-513.
- Subbarao, K. V., and Michailides, T. J. 1992. Effects of osmotic potential  $\times$  temperature interactions on growth and sporulation of *Fusarium moniliforme*, *Aspergillus niger*, and *Paecilomyces lilacinus*. *Annu. Fig Res. Rep. Crop Year 1991.* California Fig Institute, Fresno. 11 pp.
- Subbarao, K. V., and Michailides, T. J. 1992. Effects of carbon sources on growth of *Fusarium moniliforme*, *Aspergillus niger*, and *Paecilomyces lilacinus*, and growth and relative susceptibilities of caprifigs and Calimyrna figs to infection. *Annu. Fig Res. Rep. Crop Year 1991.* California Fig Institute, Fresno. 17 pp.
- Subbarao, K. V., and Michailides, T. J. 1993. Virulence of *Fusarium* species causing fig endosepsis in cultivated and wild caprifigs. *Phytopathology* 83:527-533.
- Trinci, A. P. J. 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *J. Gen. Microbiol.* 57:11-24.
- Trollope, D. R. 1975. The preservation of bacteria and fungi on anhydrous silica gel: An assessment of survival over four years (skim-milk). *J. Appl. Bacteriol.* 38:115-120.
- Turner, N. C., and Long, M. J. 1980. Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. *Aust. J. Plant Physiol.* 7:527-537.
- Vicente, N. E., Acosta, N., and Sanchez, L. A. 1989. Nematicides and *Paecilomyces lilacinus* in nematode control in watermelon. *J. Agric. Univ. Puerto Rico* 73:75-77.
- Wiebe, H. H., Campbell, G. S., Gardner, W. H., Rawlins, S. L., Cary, J. W., and Brown, R. W. 1971. Measurement of plant and soil water status. *Utah Agric. Exp. Stn. Bull.* 484. 71 pp.
- Wilson, J. M., and Griffin, D. M. 1975. Respiration and radial growth of soil fungi at two osmotic potentials. *Soil Biol. Biochem.* 7:269-274.
- Woods, D. M., and Duniway, J. M. 1986. Some effects of water potential on growth, turgor, and respiration of *Phytophthora cryptogea* and *Fusarium moniliforme*. *Phytopathology* 76:1248-1254.