Volume, Nuclear Number, and Aggressiveness of Conidia of *Monilinia fructicola* Produced on Media of Varied Glucose Concentrations at 15 and 25 C

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ABSTRACT

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Conidia of two isolates of *Monilinia fructicola* were produced at 15 and 25 C on potato-dextrose agar made with 20, 150, or 300 g of glucose per liter. At 15 C on agar containing glucose at 20 g/L, both isolates produced conidia that had greater volume, nuclear number, germination after 5 hr, and aggressiveness (measured as lesion development) than conidia produced at 25 C. Increasing glucose concentrations significantly influenced the aggressiveness of one isolate and the spore volume, nuclear number, and germination after 5 hr of both isolates. When the isolates were grown on agar containing glucose at 300 g/L, no significant differences resulting from temperature occurred in the germination after 5 hr and aggressiveness of both isolates and in the spore volume and nuclear number of one of the isolates. The aggressiveness of both isolates was more positively correlated with spore volume and nuclear number than with germination after 5 or 24 hr. Of the factors studied, the volume of the spore was the most useful estimate of the potential aggressiveness of conidia of *M. fructicola*.

Additional keywords: brown rot, inoculum, peach.

Monilinia fructicola (Wint.) Honey, the causal agent of brown rot of stone fruit, produces larger and more aggressive conidia, containing more nuclei per conidium, when grown at 15 C than when grown at 25 C on potato-dextrose agar (PDA) (5–7). The term *aggressiveness* is used to describe environmental effects during spore ontogeny; aggressiveness is measured by lesion development. In addition to temperature, the substrate influences spore volume and aggressiveness (3–5,7). We found that glucose or sucrose concentrations higher than 20 g/L in the growth substrate influence spore volume (8). Sugars could be important factors influencing the aggressiveness of conidia. The object of this paper was to determine effects of adding glucose at 20, 150, and 300 g/L to PDA on conidia produced by two isolates of *M. fructicola*. The conidia were produced at 15 or 25 C. The volume, nuclear number, and germination of the conidia were evaluated in relation to the aggressiveness of the conidia. An abstract of this work has been published (7).

MATERIALS AND METHODS

Isolates. Stock cultures of *M. fructicola*—ATCC 32670 (A) and ATCC 44557 (B), isolates previously shown to produce larger, more aggressive conidia at 15 C than at 25 C (4–6)—were started each week from single germinated conidia placed on PDA and maintained in incubators under alternating 12-hr periods of light at 25 C and dark at 15 C. Conidia were taken from these stock cultures when 2 wk old, and single germinated conidia used to start growth in a plate of a test medium.

Media. To avoid reaction between the glucose and other components of the PDA, the glucose was autoclaved separately. Two flasks were prepared for each concentration of sugar used in a test; one flask contained a measured amount of glucose and water, and the second flask potato broth and agar. The flasks were autoclaved for 15 min at 15 psi, mixed, and poured into petri dishes (9 cm in diameter, with 20 ml per dish). Final concentrations of 20, 150, and 300 g of glucose per liter were prepared.

Tests. For each isolate, three tests measuring spore volume, nuclear number, and aggressiveness and three tests measuring only

spore germination were conducted. In all tests, the fungus was grown in four to 10 dishes of each medium in constant darkness for 14 days. The dishes were arranged in random blocks within an incubator at 15 or 25 C. To collect conidia, the culture was covered with cold deionized water and rubbed with a glass rod, and the spore suspension filtered through four layers of gauze. The suspension was then centrifuged, and the conidia were resuspended in deionized water and then evaluated as follows.

Spore volume and nuclear number. About 100,000 conidia per treatment per test were counted and sized by volume in 2% NaCl with an Electro Zone Celloscope (Particle Data, Inc., Elmhurst, IL) (5). Some washed conidia were killed and fixed in Carnoy's fluid to be stained later in acid Giemsa and examined to determine the average nuclear number (4). The nuclear number was determined in each test from four samples of 50 conidia from each treatment.

Spore aggressiveness. Spore aggressiveness was determined by measuring the diameter of the lesion formed at an inoculation site

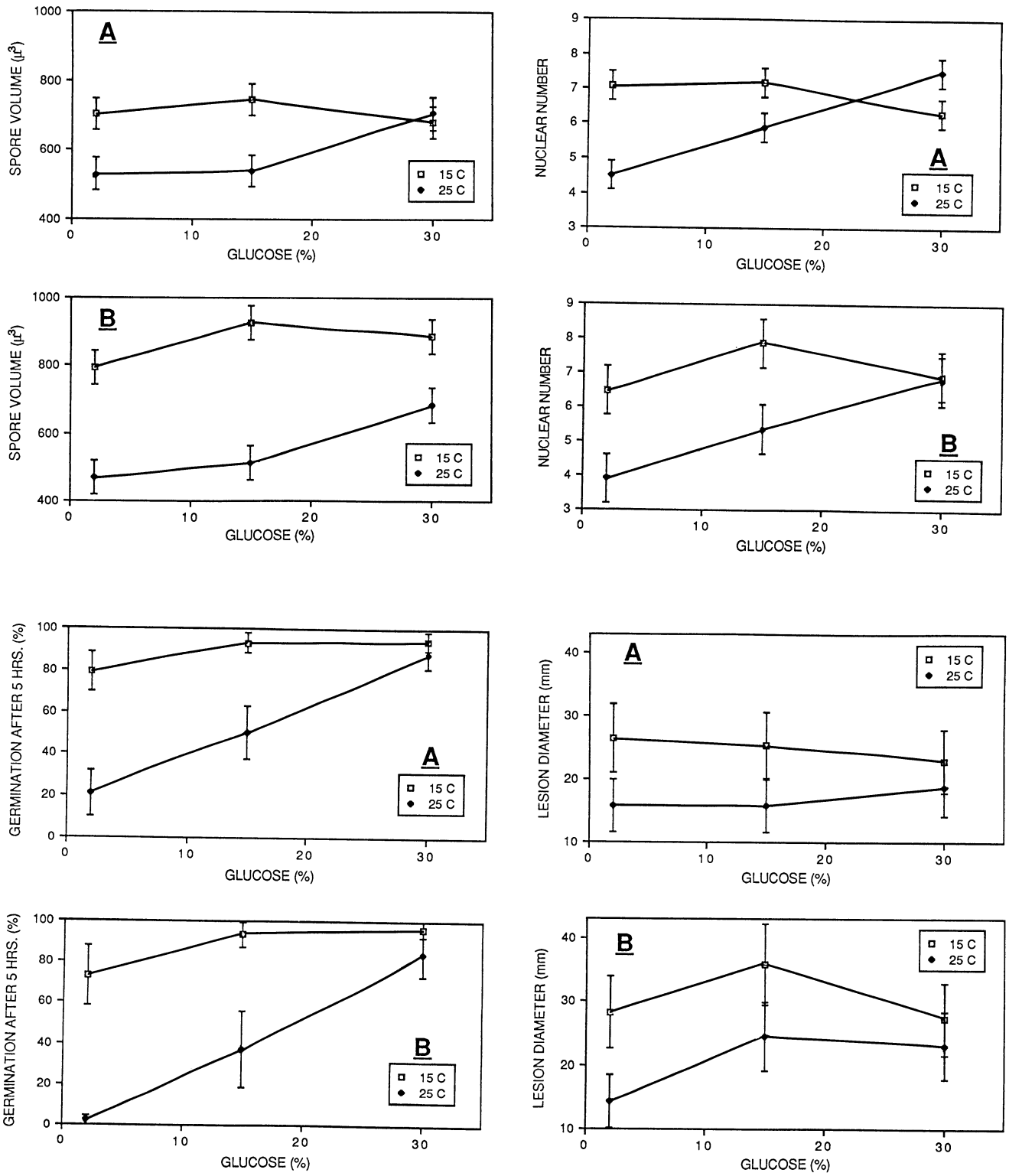

Fig. 1. Spore volume, nuclear number, germination after 5 hr, and the lesion diameter induced by conidia of two isolates of *Monilinia fructicola* (A and B) produced on potato-dextrose agar containing 20, 150, and 300 g of glucose per liter, at 15 and 25 C. For each variable, the mean of three tests with the 95% confidence interval is indicated by a vertical bar.

on peach fruit. Suspensions of conidia, washed once as indicated above, were diluted to 350 conidia per 0.03 ml of distilled water, and 0.03 ml was placed onto a puncture wound 2 mm in diameter and 2 mm deep, as described previously (6). Diameters of the lesions formed at the inoculation site were measured after the fruit was held 4 days at 21 C. In each test, aggressiveness was measured in four replicates of each treatment (10 fruit per replicate). The peach cultivars Fairtime and O'Henry were used with both isolates. The cultivar Carnival was also used with isolate A, and Autumn Gem with isolate B.

Spore germination. Washed conidia collected from each treatment as described above were placed onto water agar plates. The plates were held 5 or 24 hr at 20 C. Germination was determined by microscopically examining conidia from four separate plates (100 conidia per plate) from each treatment. A spore was considered germinated when a germ tube was longer than the length of the spore.

Analysis of data. An analysis of variance of the dependent variables measured for each isolate was conducted on the sample means from tests. Transformation of the data to stabilize the variance was necessary for the mean values of lesion diameter and germination. The mean lesion diameters were transformed to the square root of the value, and the mean percent germination to the arcsine of the square root of the proportion. Confidence limits (95%) were calculated for the treatment means from each analysis. A simple correlation coefficient was calculated for the nontransformed means from both isolates for spore volume, nuclear number, germination after 5 and 24 hr, and lesion diameter.

RESULTS

Temperature and glucose. Conidia of both isolates produced at 15 C on PDA containing glucose at 20 g/L had greater volume, nuclear number, germination after 5 hr, and aggressiveness than conidia produced at 25 C (Fig. 1). Increasing glucose concentration significantly influenced the spore volume, nuclear number, germination after 5 hr, and aggressiveness of isolate B. There was a significant interaction between temperatures and glucose concentrations for all these factors except aggressiveness. The effect of temperature for measurements of volume, nuclear number, germination after 5 hr, and aggressiveness was reduced when the fungus was grown on PDA containing glucose at 300 g/L. Germination after 24 hr was significantly reduced ($P = 0.05$), from 98.0% at 15 C to 92.9% at 25 C for isolate A and from 97.5% at 15 C to 89.7% at 25 C for isolate B. Germination of the conidia after 24 hr was not affected by the glucose concentration (data not shown).

Correlation. Aggressiveness was most highly correlated with spore volume, followed by nuclear number and germination after 5 and 24 hr (Table 1). Volume was significantly and positively correlated with nuclear number and germination, but we did not find any factor to clearly be the best indicator of aggressiveness. The highest correlation found was between nuclear number and germination after 5 hr. Increasing the number of nuclei per spore may improve the ability of conidia to germinate quickly.

DISCUSSION

Lesion expansion in ripe fruits may depend on the rate of growth of the fungus on nutrients supplied by the fruit. This conclusion was derived from the observation that the rate of growth of the fungus on synthetic media is significantly correlated with the rate of lesion expansion in ripe peach fruit (2). Although germination after 5 hr was correlated with lesion development, it did not have the highest correlation in our study, and other factors should be considered important in estimating aggressiveness.

Perhaps a large spore incites a larger lesion than a small spore because it contains more of specific kinds of endogenous materials. Differences in the aggressiveness of conidia were found to be similar whether the conidia were placed onto sound fruit or punctured fruit (5). Material contained in the spore influenced the infection.

The effect of temperature on the spore volume, nuclear number, germination, and aggressiveness could be eliminated by producing the conidia on PDA containing glucose at 300 g/L rather than 20 or 150 g/L, except for the spore volume of isolate B. For isolate B significantly larger conidia were produced at 15 C than at 25 C on plates containing glucose at 300 g/L. The temperature effect may be a simple increase of usable nutrient. This does not explain the tendency for the largest and most aggressive conidia to be produced on plates containing glucose at 150 g/L, not 300 g/L. The analysis by correlations of the volume, nuclear number, or germination of the conidia did not reveal any factor that was always indicative of aggressiveness. These results are similar to those we found using *Botrytis cinerea* Pers., in which volume and nuclear number were correlated with the aggressiveness of conidia with which rose flowers were inoculated (8). Spore volume seems to be a useful estimate of aggressiveness, but it is likely that the glucose that caused a change in volume also influences nutrients such as iron (1,9), which determine the rate or degree of spore aggressiveness. The nutrition of the spore could influence the pathogen's abilities to grow or the host's abilities to recognize the pathogen and react to the infection (1,9).

Some factor or factors that affected aggressiveness were altered by temperature and tended to increase with the volume of the spore. The volume of the spore provides an estimate of the potential aggressiveness of conidia produced by an isolate of *M. fructicola*. For example, when all treatments were averaged, isolate A had a volume of 651 μm^3 and produced a lesion 20.9 mm in diameter; B was 712 μm^3 and produced a lesion 25.5 mm in diameter. Additional studies using many strains from different sources would be useful to determine whether spore volume can be used to estimate the virulence of strains. Endogenous factors of the spore associated with lesion size and development should also be considered in such investigations.

TABLE 1. Correlation coefficients between the spore volume, nuclear number, germination after 5 and 24 hr, and aggressiveness of isolates A and B of *Monilinia fructicola*, calculated from nontransformed means from all tests[a]

	Aggressiveness	Volume	Nuclear number	Germination (5 hr)
Volume	0.844**			
Nuclear number	0.723**	0.844**		
Germination (5 hr)	0.673*	0.839**	0.928**	
Germination (24 hr)	0.638*	0.748**	0.576	0.684*

[a]** Significant at $P = 0.01$; * significant at $P = 0.05$.

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