

Size, Nuclear Number, and Aggressiveness of *Botrytis cinerea* Spores Produced on Media of Varied Glucose Concentrations

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ABSTRACT

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Spores of *Botrytis cinerea* were produced at 15 C on a defined medium containing 20, 150, or 300 g/L of glucose. Increasing the glucose in the growth medium resulted in an increase in the volume and nuclear number

Additional key words: inoculum, rose.

of spores and increased their aggressiveness when inoculated on rose flowers. The effect of increasing the nuclear number on aggressiveness could not be separated from the effect of increased spore volume.

Cultures of the same isolate of *Botrytis* growing on various media differ in the form and size of conidia (4). The size of conidia is dependent on the pH, C:N ratio, nutrients of the culture medium, the age of the culture, and relative humidity of the air in the culture

dish (3). When *B. squamosa* Walker is compared to *B. cinerea* Pers., spore size may contribute in part to variation in lesion expansion, i.e., the *B. squamosa* spores do not require exogenous nutrient for germination and produce expanding lesions on onion, whereas smaller spores of *B. cinerea* form superficial flecks that do not expand (1). We showed that isolates of *Monilinia fructicola* (Wint.) Honey, causal agent of brown rot of stone fruit, grown at 15 C produces spores that are larger, contain more nuclei per spore (5), and are more aggressive than spores produced at 25 C (8,9). An

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isolate of *B. cinerea* from a rose flower produced conidia that increased in volume with an increase of glucose concentration in potato-dextrose agar. This paper reports on the size and nuclear number of spores of *B. cinerea* and their aggressiveness to flowers of rose (*Rosa hybrida* L.) when produced at 15 C on a defined medium containing various concentrations of glucose.

MATERIALS AND METHODS

Isolate. Stock cultures of *B. cinerea* isolated from a rose flower were started each week from single conidia placed on potato-dextrose agar and maintained as stock cultures in incubators under alternating (12 hr) light at 25 C and dark at 15 C. Conidia were taken from 2-wk-old stock cultures, and single germinated conidia were used to start cultures in a plate of the test medium.

Media. To avoid reaction between the glucose and other components of the medium, glucose was autoclaved separately. Two flasks were prepared for each concentration of sugar used in a test. One flask contained a measured amount of sugar and water made to total 500 ml. The second flask contained 1 g of K_2HPO_4 , 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 1.7 g of asparagine, 20 g of agar, and water made to total 500 ml. The flasks were autoclaved for 15 min at 15 psi, mixed, and poured into petri dishes (9 cm diameter, 20 ml per dish). Final concentrations of 20, 150, and 300 g/L of glucose were prepared.

Tests. For each of five tests, the fungus was grown in four dishes of each sugar concentration in constant darkness for 21 days. The dishes were arranged in random blocks within an incubator held at 15 C; each test was considered a completely randomized block. To collect spores, the culture was covered with cold deionized water and rubbed with a glass rod, and the spore suspension was filtered through four layers of gauze. The suspension was then centrifuged, and the spores resuspended in deionized water and then counted and sized. About 100,000 of these spores per sugar concentration per test were counted and sized in 2% NaCl with an Electro Zone

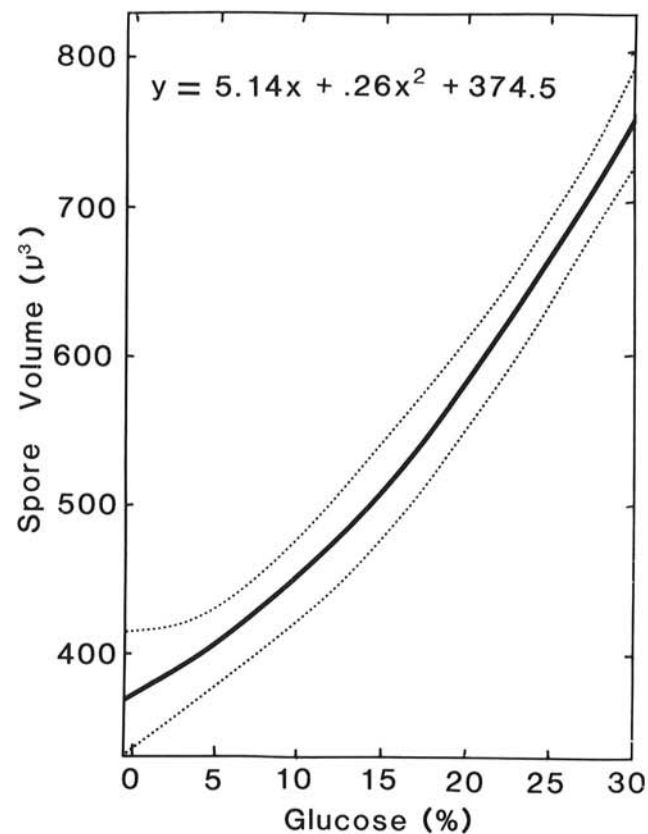


Fig. 1. Estimates of the effect of increasing glucose on the estimated volume of spores produced on a defined medium at 15 C. The dotted lines show the 95% confidence interval of the estimate.

Celloscope (Particle Data, Inc., Elmhurst, IL) (5,8). Some washed spores were used to inoculate rose flowers, others were placed on water agar and examined for germination after 24 hr at 20 C, or they were killed and fixed in Carnoy's fluid to be stained later in acid-Giemsa and examined to determine the average nuclear number (5). The nuclear number was determined in four samples of 50 spores from each glucose concentration for each of the five tests.

Inoculation. Spores were applied by placing a rose flower in a spray of spore suspension from an atomizer supplied with 25 psi air. The spray was directed to each flower from a distance of 76 mm for 5 sec. The inoculum was diluted to a uniform density for each sugar concentration used in a test; however, the density was not the same for each test. Fifteen flowers were sprayed with water containing no spores, or spores grown on 2, 15, or 30% glucose and washed once by centrifugation as described above. We used freshly harvested red rose flowers with 35-cm stems, cultivar Royalty (four tests) or Samantha (one test) to evaluate the aggressiveness of the

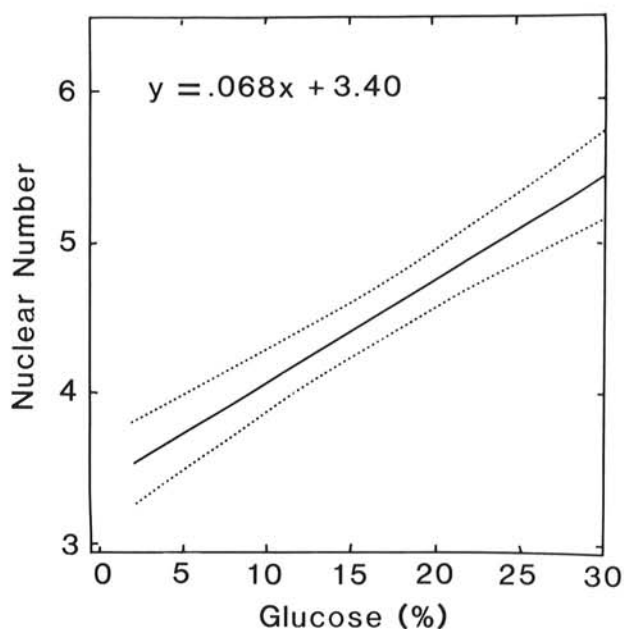


Fig. 2. Effect of increasing glucose on the estimated nuclear number of spores produced on a defined medium at 15 C. The dotted lines show the 95% confidence interval of the estimate.

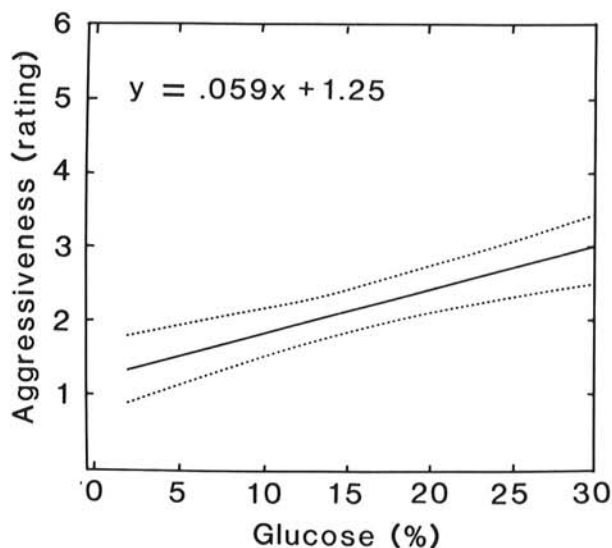


Fig. 3. Effect of increasing glucose on the estimated aggressiveness rating of spores produced on a defined medium at 15 C. The dotted lines show the 95% confidence interval of the estimate.

TABLE 1. Spore volume, nuclear number and aggressiveness of spores of *Botrytis cinerea* when grown on 2, 15 and 30% glucose at 15 C on a defined medium^a

Glucose concentration (%)	Volume (μm^3)	Nuclear number (nuclei/spore)	Aggressiveness (rating) ^b
2	386	3.5	1.5
15	510	4.5	1.9
30	762	5.4	3.1
Least significant difference $P = 0.05$	56	0.5	0.7

^a Each value represents the means of five experiments.

^b The average rating of the uninoculated water control was subtracted from the average of the other treatments to estimate the aggressiveness of the spores applied to the flowers.

spores. The inoculated roses were placed in a vase containing water and held in a controlled temperature chamber at 13 C, 96% RH for 4 days then evaluated by rating each flower as follows: 0 = no lesions, 2 = 1-5 lesions, 4 = 6-10 lesions, 6 = 11-30 lesions, 8 = more than 30 lesions and less than 75% of flower with rot symptoms, and 10 = more than 75% of the flower with rot symptoms. The average rating of the water control was subtracted from the average of the other treatments to estimate the aggressiveness of the spores applied to the flowers.

The results were analyzed using a general linear model analysis of variance with sugar as an independent variable and spore volume, nuclear number of the spore, and the measure of aggressiveness as dependent variables. A simple correlation coefficient was computed for spore volume vs. nuclear number.

RESULTS

Increased glucose content in the growth medium resulted in a significant increase in the volume, nuclear number, and aggressiveness of the spores produced on the medium. This effect of glucose was linear for nuclear number and aggressiveness rating and quadratic for spore volume (Figs. 1-3). There was a significant positive correlation (coefficient = 0.84) between spore volume and the nuclear number of the spores. Spore germination after 24 hr ranged between 96 and 99% and did not differ significantly between treatments.

The volume of the spores increased from an average of $386 \mu\text{m}^3$ when produced on 2% glucose to $762 \mu\text{m}^3$ on 30% glucose. Volumes were consistent among the five tests (Table 1). Number of nuclei per spore and aggressiveness rating significantly increased from 3.5 to 5.4 and 1.5 to 3.1, respectively, when the glucose concentration was increased from 2 to 30% (Table 1). Differences in nuclear number and aggressiveness were significant among the five tests, but there was no interaction between the test blocks and treatment, thus, the differences were consistent in all tests.

DISCUSSION

The results of these tests show that the aggressive or virulent qualities of *Botrytis* inoculum may be changed by the glucose environment in which it is grown. Differences between the five tests in the aggressiveness rating may be due to the response of the differing inoculum densities used in each test, but some

unrecognized factor may affect the nuclear number. Similar unexplained differences in the nuclear number of *Botrytis* spores were found in tests reported by Vanev (11). The observation of a consistent increase of the nuclear number with volume agrees with Hansen (2) and Lauber (3), but the differences between tests in nuclear number suggest variation because of other factors and may corroborate the report of Menzinger (6), who observed many isolates and found no consistent relationship between spore volume and nuclear number.

The effect of glucose on inoculum potential of *B. cinerea* spores reported here may be due to an indirect effect of glucose on spore size or nuclear number. Spore volume or nuclear number provided a reasonable estimate of the aggressiveness. However, because of their close correlation, the effects of increasing nuclear number could not be separated from the effects of increasing spore volume. Similar effects of glucose in the growth medium and an interaction with temperature have been reported for spore size and aggressiveness of *M. fructicola* (10). In *Botrytis* or *Monilinia*, the increase of spore size may show that the spore contains more available endogenous nutrients but other changes in the conidia, such as the increase in nuclear number or in the type of the nutrients stored in the conidia, may also direct or induce changes in pathogenic activity (1,7,12). Media containing 15-30% glucose exert very negative water potentials, and this effect could influence spore size. However, although glucose was found to influence spore size and nuclear number of the spores of *M. fructicola*, similar mannose concentrations did not, and this indicates that a reduced water activity may not explain the effect of glucose on the spores (*unpublished results*).

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