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# The variability of morphological characters and mycelium growth rate of monoconidial culture of *Botrytis cinerea* Pers.

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Abstract

Variability in size of conidia, formation of sclerotia and mycelial growth were examined in monoconidial cultures of 5 isolates of *Botrytis cinerea* Pers. The influence of two media on the above-mentioned features was also studied. It was found that *Botrytis cinerea* Pers. is relatively homogenous in respect to the size of conidia. Out of the 50 monoconidial cultures tested, only two had conidia somewhat larger than the remaining cultures. Monoconidial cultures derived from the same isolate varied among themselves in the rate of mycelial growth and in the number and size of sclerotia. The kind of medium used influenced mycelial growth and sclerotia formation. Better mycelial growth was observed on PDA medium than on Czapek's medium. On the latter, the fungus produced more sclerotia which were, however, smaller in size than on PDA medium.

## INTRODUCTION

The species *Botrytis cinerea* Pers. (perfect stage *Botryotinia fuckeliana* (de Bary/Whetzel) is very unhomogeneous genetically (H a n s e n and S m i t h, 1932). Many forms, or strains, of this fungus which differ in their morphological characters, growth on artificial media, ability to produce conidia and sclerotia, have been shown to exist (P a u l, 1929; G u p t a, 1960; G r i n d l e, 1979). Some authors are of the opinion that the morphological features of this fungus change depending on its environmental and nutritional conditions. V a n e v (1972) showed that the size of conidia changed depending on whether the fungus grew on artificial medium or its natural host.

In the opinion of other authors, *Botrytis cinerea* Pers. is relatively homogenous in respect to its morphological features (G o r l e n k o and M a n t u r o v s k a y a, 1971; D u b o s and B u l i t, 1973).

In most studies, differences in morphological characters among isolates were examined. Data is lacking on the question if morphological characters within an isolate are identical or not.

The aim of this study was to define the degree of variability of morphological characters and mycelium growth-rates of monoconidial culture within five selected isolates of *Botrytis cinerea* Pers. Also studied was the effect of the type of medium on the size of conidia, mycelial growth and sclerotium formation by this fungus.

## MATERIAL AND METHODS

The study was conducted on five isolates of *Botrytis cinerea* Pers. differing in their origin and pathogenicity to apples. The method used to determine their pathogenicity is described in a previous paper (B r y k, 1985). The following isolates were used:

no. 11 – isolated from peaches – weakly pathogenic to apples,
no. 20 – isolated from raspberries – moderately pathogenic to apples,
no. 28 – isolated from raspberries – moderately pathogenic to apples,
no. 17 – isolated from raspberries – moderately pathogenic to apples,
no. 9 – isolated from tomatoes – highly pathogenic to apples.

Ten monoconidial cultures were obtained from each isolate using the method of "gradual dilution" of inoculum and additional microscopic observations. Each culture was inoculated on two media – potato-agar (PDA, Difco) and Czapek's mineral medium set with agar. The experiment was run in 5 replications, where a replication was one, 10 cm in diameter, Petri dish of each of the media. After 3 days of incubation of the cultures in the dark at 20°C, the growth of the colony was measured by determining their 2 perpendicular diameters. After 14 days, sclerotium formation was evaluated by counting the number of sclerotia on one dish and measuring their lengths and widths. In the microscopic studies, the lengths and widths of the conidia were measured and the length to width ratio (l/w) was calculated, because according to D u b o s and B u l i t (1973), this index is more suitable in studying morphological variability of fungi than the simple dimensions of the spores. One hundred conidia were measured from each monoconidial culture on each medium. The results dealing with growth on two media were analysed statistically using the variance method; averages within each isolate were compared by the Duncan Test.

## RESULTS

The dimensions of conidia from 50 monoconidial cultures of *Botrytis cinerea* Pers. are given in Table 1. The extremes of length and width are given as well as the range of the l/w index for each culture. It was found that the variability in

Table 1

The dimensions of conidia of monoconidial cultures of *Botrytis cinerea* Pers. (μm)

| Culture no. | Potato medium (PDA) | | Czapek's medium | |
|---|---|---|---|---|
| | length × width | ratio of length to width (l/w) | length × width | ratio of length to width (l/w) |
| 1 | 2 | 3 | 4 | 5 |
| 901 | 8.9-11.6 × 7.4-9.9 | 1.1-1.5 | 8.4-11.6 × 6.8-8.4 | 1.1-1.4 |
| 902 | 8.4-11.0 × 7.4-8.9 | 1.1-1.4 | 8.9-11.0 × 6.8-9.5 | 1.1-1.5 |
| 903 | 9.5-12.1 × 7.4-8.9 | 1.2-1.6 | 8.9-11.6 × 7.4-9.5 | 1.1-1.5 |
| 904 | 8.9-11.0 × 6.8-8.9 | 1.1-1.4 | 8.9-11.0 × 7.4-8.9 | 1.1-1.5 |
| 905 | 8.4-11.0 × 6.8-9.5 | 1.1-1.5 | 8.4-11.0 × 6.8-8.4 | 1.1-1.5 |
| 906 | 8.9-12.1 × 7.4-8.9 | 1.1-1.5 | 9.5-11.6 × 7.4-9.9 | 1.1-1.4 |
| 907 | 9.5-12.1 × 7.4-9.5 | 1.1-1.5 | 8.4-11.0 × 7.4-9.5 | 1.1-1.4 |
| 908 | 8.9-11.0 × 7.4-9.5 | 1.1-1.4 | 8.9-11.0 × 6.8-8.4 | 1.1-1.4 |
| 909 | 9.5-12.1 × 7.4-9.5 | 1.1-1.5 | 8.9-11.0 × 7.4-8.9 | 1.1-1.4 |
| 910 | 8.4-11.0 × 7.4-8.9 | 1.1-1.4 | 8.4-11.6 × 6.8-9.5 | 1.1-1.4 |
| 1101 | 8.4-11.1 × 6.8-9.5 | 1.0-1.4 | 8.4-11.1 × 6.3-8.4 | 1.0-1.5 |
| 1102 | 8.4-11.6 × 6.8-9.5 | 1.1-1.4 | 7.9-11.1 × 6.8-9.5 | 1.1-1.4 |
| 1103 | 7.9-11.6 × 6.8-8.9 | 1.1-1.4 | 8.4-11.6 × 6.8-9.9 | 1.1-1.4 |
| 1104 | 7.9-11.1 × 7.4-9.5 | 1.1-1.4 | 7.9-10.5 × 6.8-8.9 | 1.1-1.4 |
| 1105 | 8.4-11.1 × 6.8-9.5 | 1.1-1.4 | 8.4-11.1 × 6.8-9.5 | 1.1-1.4 |
| 1106 | 7.9-11.6 × 6.3-9.5 | 1.1-1.4 | 7.9-11.1 × 6.3-8.9 | 1.1-1.5 |
| 1107 | 7.9-10.5 × 6.8-9.5 | 1.1-1.4 | 7.9-11.1 × 6.3-9.5 | 1.1-1.5 |
| 1108 | 8.4-10.5 × 6.8-9.5 | 1.1-1.3 | 8.4-10.5 × 6.8-8.9 | 1.1-1.5 |
| 1109 | 8.4-11.6 × 6.8-9.5 | 1.1-1.4 | 8.4-10.5 × 6.8-9.9 | 1.0-1.4 |
| 1110 | 8.4-10.5 × 6.8-9.5 | 1.1-1.4 | 8.4-10.5 × 6.8-8.9 | 1.1-1.5 |
| 1701 | 8.4-11.4 × 7.4-8.9 | 1.1-1.4 | 8.4-11.0 × 6.8-9.5 | 1.1-1.4 |
| 1702 | 8.4-10.5 × 7.4-8.9 | 1.1-1.4 | 8.4-10.5 × 6.8-9.5 | 1.1-1.4 |
| 1703 | 7.9-11.0 × 6.8-8.9 | 1.1-1.5 | 7.9-10.5 × 6.8-8.9 | 1.0-1.4 |
| 1704 | 8.4-10.5 × 6.8-8.4 | 1.1-1.4 | 8.4-10.5 × 7.8-9.5 | 1.0-1.3 |
| 1705 | 7.4-10.5 × 7.4-8.4 | 1.0-1.4 | 8.4-11.6 × 7.4-9.9 | 1.0-1.5 |
| 1706 | 8.4-10.5 × 7.4-9.5 | 1.1-1.3 | 8.9-11.6 × 7.9-10.5 | 1.0-1.4 |
| 1707 | 8.4-11.0 × 7.4-8.9 | 1.1-1.4 | 8.4-11.0 × 7.9-9.5 | 1.0-1.3 |
| 1708 | 8.9-11.0 × 7.4-9.5 | 1.1-1.4 | 8.4-10.5 × 7.4-8.9 | 1.0-1.4 |
| 1709 | 8.4-11.6 × 7.4-9.5 | 1.1-1.4 | 8.9-11.6 × 7.4-9.5 | 1.2-1.5 |
| 1710 | 8.4-11.6 × 7.4-9.5 | 1.1-1.5 | 8.4-11.6 × 6.8-8.4 | 1.1-1.5 |
| 2001 | 8.4-10.5 × 7.4-8.9 | 1.1-1.4 | 8.4-10.5 × 7.4-9.5 | 1.1-1.3 |
| 2002 | 7.9-10.5 × 7.4-8.9 | 1.0-1.3 | 8.4-11.1 × 7.9-10.5 | 1.0-1.3 |
| 2003 | 8.4-11.6 × 7.4-9.5 | 1.0-1.3 | 8.9-11.1 × 7.4-8.9 | 1.1-1.4 |
| 2004 | 8.4-11.1 × 7.4-8.9 | 1.1-1.4 | 8.4-11.1 × 7.4-9.5 | 1.0-1.5 |
| 2005 | 8.9-11.6 × 7.4-9.5 | 1.1-1.4 | 8.9-11.6 × 7.4-9.5 | 1.1-1.3 |
| 2006 | 8.4-10.5 × 7.4-8.9 | 1.0-1.3 | 8.4-11.6 × 7.4-9.5 | 1.1-1.5 |
| 2007 | 8.4-11.1 × 7.4-9.5 | 1.0-1.4 | 8.9-12.1 × 7.4-9.5 | 1.0-1.5 |

Table 1 cont.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 2008 | 8.4-10.5 × 6.8-8.9 | 1.0-1.5 | 8.4-11.1 × 8.4-9.5 | 1.0-1.3 |
| 2009 | 8.4-11.1 × 7.4-8.9 | 1.1-1.3 | 8.4-11.1 × 7.4-9.5 | 1.0-1.4 |
| 2010 | 9.9-14.2 × 7.9-11.6 | 1.1-1.4 | 7.9-11.6 × 7.4-10.5 | 1.0-1.4 |
| 2801 | 8.9-13.7 × 7.9-10.5 | 1.0-1.3 | 8.9-11.6 × 7.9-10.5 | 1.1-1.4 |
| 2802 | 8.9-11.1 × 6.8-8.9 | 1.1-1.4 | 9.5-11.6 × 7.9-10.5 | 1.0-1.3 |
| 2803 | 8.4-11.6 × 7.4-9.5 | 1.0-1.4 | 8.4-11.6 × 7.4-9.5 | 1.1-1.4 |
| 2804 | 8.4-10.5 × 6.8-8.4 | 1.1-1.3 | 8.4-11.6 × 7.4-9.5 | 1.1-1.3 |
| 2805 | 7.9-11.1 × 7.4-9.5 | 1.0-1.4 | 8.4-10.5 × 7.4-9.5 | 1.1-1.4 |
| 2806 | 8.9-11.6 × 7.4-8.9 | 1.1-1.4 | 8.4-11.6 × 7.4-9.5 | 1.1-1.4 |
| 2807 | 8.4-11.6 × 7.4-9.5 | 1.1-1.4 | 8.4-11.1 × 7.4-8.9 | 1.1-1.4 |
| 2808 | 8.9-11.6 × 7.4-9.5 | 1.1-1.3 | 8.4-11.1 × 7.4-8.9 | 1.1-1.3 |
| 2809 | 8.9-11.1 × 7.4-8.9 | 1.1-1.4 | 8.4-11.6 × 7.4-8.9 | 1.1-1.4 |
| 2810 | 8.9-12.1 × 7.9-9.5 | 1.1-1.5 | 8.9-11.1 × 7.4-9.5 | 1.0-1.4 |

conidial size, both within the studied isolates and among them, is small. The size range of spores is similar. Only cultures no. 2010 and 2801 on PDA medium formed slightly larger conidia. The dimensions of the spores from culture no. 2010 oscillated in the range from 9.9-14.2 × 7.9-11.6 $\mu$m, and culture no. 2801 – 8.9-13.7 × 7.9-10.5 $\mu$m. The type of medium had no effect on the size of the conidia of the remaining cultures.

The l/w index oscillated between 1.0 and 1.5; only in one case – culture no. 903 – was it 1.2-1.6. The index range of 1.0 to 1.5 shows that both round and elliptic spores were found.

Table 2 presents the colony diameters of the studied fungus cultures after 3 days of incubation. Monoconidial cultures within one isolate differed in their rate of growth on the medium. The least variability in this respect was shown by cultures from isolate no. 20; statistical analysis delineated 2 classes of significance on PDA medium and 3 on Czapek's medium. A decided effect on the rate of mycelium growth by medium type was found. As a rule, cultures grew more rapidly on PDA, only one culture – no. 904 – grew equally well on both media. Variability of culture growth-rate within one isolate was greater on Czapek's medium.

The greatest variability of all of the studied characters was found in the number of sclerotia formed. Table 3 gives the average number of sclerotia on the surface of a Petri dish and the length and width of the most numerous sclerotia in a given monoconidial culture. Culture no. 2802 on PDA medium formed several large sclerotia arranged wreath-like in the center of the dish. The sclerotia of the remaining cultures were round or oval in shape. Monoconidial cultures within one isolate differed from each other in the number of sclerotia formed. The greatest variance was seen within isolate no. 17 on Czapek's medium; the fungus

Table 2

Mycelium growth of monoconidial cultures of *Botrytis cinerea* Pers. depending on the medium type

| Culture no. | Diameter of the mycelium (in mm) on medium | |
|---|---|---|
| | Czapek's | PDA |
| 1 | 2 | 3 |
| 907 | 26.2 a* | 88.5 k |
| 903 | 28.5 a | 63.3 g |
| 909 | 29.0 ab | 80.0 j |
| 902 | 33.0 b | 59.0 f |
| 905 | 33.4 b | 75.1 i |
| 906 | 36.0 bc | 68.5 h |
| 910 | 39.0 c | 76.9 ij |
| 908 | 40.4 cd | 74.9 i |
| 901 | 43.5 d | 74.0 i |
| 904 | 49.8 e | 51.4 e |
| 1110 | 21.1 a | 51.5 e |
| 1105 | 27.4 b | 61.2 f |
| 1109 | 28.4 b | 52.0 ef |
| 1102 | 28.5 b | 56.6 f |
| 1101 | 29.8 bc | 67.6 gh |
| 1104 | 33.9 c | 67.4 gh |
| 1107 | 36.6 cd | 67.8 gh |
| 1106 | 39.5 d | 71.5 h |
| 1108 | 40.8 d | 67.3 g |
| 1103 | 54.2 ef | 66.1 g |
| 1703 | 22.5 a | 57.7 e |
| 1708 | 27.4 ab | 60.8 e |
| 1706 | 27.5 ab | 53.0 de |
| 1704 | 27.9 ab | 57.0 e |
| 1701 | 28.2 b | 58.0 e |
| 1710 | 28.9 bc | 59.4 e |
| 1707 | 30.2 bc | 59.2 e |
| 1709 | 33.8 c | 58.6 e |
| 1705 | 34.5 c | 59.5 e |
| 1702 | 50.4 d | 70.6 f |
| 2010 | 19.7 a | 56.1 d |
| 2007 | 23.1 ab | 58.3 d |
| 2003 | 25.4 b | 59.3 d |
| 2001 | 26.0 b | 58.4 d |
| 2005 | 27.5 bc | 55.0 d |
| 2006 | 28.2 bc | 66.0 e |
| 2002 | 28.3 bc | 59.6 d |

Table 2 cont.

| 1 | 2 | 3 |
|---|---|---|
| 2009 | 28.9 bc | 67.1 e |
| 2004 | 32.1 c | 57.9 d |
| 2008 | 33.7 c | 69.0 e |
| 2801 | 13.5 a | 39.4 d |
| 2803 | 20.6 b | 60.8 f |
| 2804 | 21.0 b | 59.9 ef |
| 2807 | 22.6 bc | 56.0 e |
| 2810 | 24.0 bc | 58.8 ef |
| 2802 | 24.2 bc | 58.0 ef |
| 2809 | 24.6 bc | 67.1 g |
| 2806 | 26.8 c | 58.2 ef |
| 2805 | 27.2 c | 66.5 g |
| 2808 | 30.6 c | 69.5 g |

*The significance of differences was evaluated separately for each 10 monoconidial cultures within one starting isolate.

Table 3

Sclerotium formation by monoconidial cultures of *Botrytis cinerea* Pers. depending on the medium type

| Culture number | Potato medium (PDA) | | Czapek's medium | |
|---|---|---|---|---|
| | no. of sclerotia | size (mm) | no. of sclerotia | size (mm) |
| 1 | 2 | 3 | 4 | 5 |
| 901 | 58 | 3 × 3 | 10 | 3 × 3 |
| 902 | 2 | 4 × 4 | 20 | 2 × 2 |
| 903 | 25 | 3 × 3 | 0 | – |
| 904 | 8 | 3 × 3 | 15 | 3 × 3 |
| 905 | 11 | 3 × 2 | 21 | 1 × 1 |
| 906 | 0 | – | 0 | – |
| 907 | 6 | 2 × 2 | 0 | – |
| 908 | 0 | – | 22 | 3 × 3 |
| 909 | 3 | 3 × 3 | 37 | 1 × 1 |
| 910 | 0 | – | 3 | 2 × 2 |
| 1101 | 23 | 4 × 4 | 54 | 3 × 2 |
| 1102 | 20 | 4 × 3 | 64 | 3 × 3 |
| 1103 | 34 | 4 × 3 | 72 | 3 × 2 |
| 1104 | 31 | 4 × 4 | 85 | 3 × 3 |
| 1105 | 5 | 3 × 2 | 39 | 2 × 2 |
| 1106 | 22 | 4 × 4 | 68 | 3 × 2 |
| 1107 | 16 | 4 × 4 | 93 | 3 × 3 |
| 1108 | 23 | 4 × 3 | 53 | 3 × 3 |

Table 3 cont.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 1109 | 28 | 4 × 3 | 104 | 3 × 2 |
| 1110 | 4 | 3 × 3 | 24 | 3 × 3 |
| 1701 | 26 | 3 × 3 | 190 | 2 × 2 |
| 1702 | 24 | 3 × 2 | 341 | 2 × 2 |
| 1703 | 13 | 2 × 2 | 9 | 1 × 1 |
| 1704 | 2 | 2 × 3 | 11 | 2 × 2 |
| 1705 | 0 | – | 1 | 2 × 2 |
| 1706 | 140 | 2 × 2 | 165 | 2 × 2 |
| 1707 | 129 | 3 × 2 | 148 | 3 × 3 |
| 1708 | 93 | 3 × 3 | 68 | 2 × 2 |
| 1709 | 76 | 3 × 2 | 131 | 2 × 2 |
| 1710 | 1 | 4 × 4 | 45 | 3 × 2 |
| 2001 | 16 | 4 × 3 | 153 | 2 × 2 |
| 2002 | 2 | 3 × 3 | 9 | 2 × 2 |
| 2003 | 19 | 4 × 2 | 13 | 2 × 2 |
| 2004 | 0 | – | 4 | 2 × 2 |
| 2005 | 8 | 4 × 3 | 13 | 3 × 2 |
| 2006 | 4 | 5 × 3 | 32 | 2 × 2 |
| 2007 | 12 | 4 × 3 | 12 | 4 × 3 |
| 2008 | 0 | – | 6 | 1 × 1 |
| 2009 | 3 | 3 × 2 | 21 | 3 × 3 |
| 2010 | 9 | 4 × 3 | 6 | 3 × 2 |
| 2801 | 5 | 4 × 3 | 6 | 4 × 3 |
| 2802 | 1 | 10 × 10 | 3 | 2 × 2 |
| 2803 | 9 | 4 × 3 | 12 | 3 × 3 |
| 2804 | 12 | 4 × 3 | 9 | 4 × 3 |
| 2805 | 4 | 3 × 3 | 27 | 2 × 2 |
| 2806 | 4 | 5 × 3 | 5 | 2 × 2 |
| 2807 | 17 | 4 × 3 | 23 | 4 × 3 |
| 2808 | 4 | 3 × 3 | 36 | 2 × 2 |
| 2809 | 4 | 4 × 3 | 4 | 3 × 3 |
| 2810 | 8 | 5 × 4 | 9 | 4 × 3 |

formed from 1 to 341 sclerotia depending on the monoconidial culture. The medium had a significant effect on sclerotium formation. Generally, on Czapek's medium, more numerous and smaller sclerotia were formed than on the potato medium.

## DISCUSSION

Information on the existence of "morphological strains" of the fungus (P a u l, 1929) as well as on the lack of differences among isolates (G o r l e n-

k o and M a n t u r o v s k a y a, 1971) is to be found in literature. In this study, it has been attempted to determine if variability exists among monoconidial cultures within one isolate. On the basis of observations of 50 monoconidial cultures from 5 isolates of the fungus, it can be stated that variability in the size of conidia is small. Only two cultures (no. 2010 and no. 2801) stood out from the others in respect to the size of their conidia. On the other hand, the monoconidial cultures exhibited a high degree of variability in their mycelium growth-rates on artificial media and in sclerotium formation.

On the basis of the experiments carried out here, the conclusion can be drawn that monoconidial cultures of *Botrytis cinerea* Pers. within one isolate are not homogenous. The reason for this probably is the heterokaryotic nature of *Botrytis cinerea* Pers. (H a n s e n and S m i t h, 1932).

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## Zmienność cech morfologicznych i szybkości wzrostu kultur jednozarodnikowych *Botrytis cinerea* Pers.

S t r e s z c z e n i e

Badano zmienność cech morfologicznych, tworzenie sklerocjów i szybkość wzrostu grzybni kultur jednozarodnikowych w obrębie 5 wybranych izolatów *Botrytis cinerea* Pers. Badano także wpływ pożywki na wymienione cechy grzyba. Stwierdzono, że pod względem wielkości zarodników

konidialnych *Botrytis cinerea* Pers. jest względnie jednorodny. Tylko dwie kultury (na 50 badanych) charakteryzowały się nieco większymi zarodnikami od pozostałych.

Kultury jednozarodnikowe, wywodzące się z jednego izolatu, różniły się szybkością wzrostu na pożywkach oraz tworzeniem sklerocjów. Pożywka ziemniaczana (PDA) była lepsza dla wzrostu większości kultur niż pożywka Czapka. Z kolei na pożywce Czapka kultury tworzyły liczniejsze i drobniejsze sklerocja niż na PDA.