# Morphotypes of *Botrytis cinerea* Pers.: Fr. in the Conditions of Belarus

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**Abstract**

Studies in all three agroclimatic regions of Belarus have allowed us to obtain more then 100 isolates of *B. cinerea* causing gray mold of many plants. The isolates were shown to belong to the three cultural-morphological groups such as mycelial, sporulating and sclerotial. Mycelial isolates (48.5%) concerned by some researchers as the high pathogenic group prevail over other two groups. Nutritive substrate was proved not to determine the formation of strictly definite morphotype. The rate of radial growth of *B. cinerea* colonies reveals no correlation with morphological peculiarities of these. Strict regularities in the forming of spores and sclerotia were not established when characterizing isolates from different cultural groups what indicates the presence of transitional forms between detected ones. Thus, population of *B. cinerea* in Belarus conditions is morpho-physiologically diverse.

**Keywords**: fungi; *Botrytis cinerea* Pers.: Fr.; pathogen; populations; isolates; morphotype; mycelial; sporulating; sclerotial; radial growth

## INTRODUCTION

The isolates of *B. cinerea* causing grey rot of the various wild plant species and crops have the specific traits (air mycelium, sclerotia, sporulation), which permit to define the different morphotypes with high significance.

For example, VASIN (1966) has differentiated three morphotypes: sporulating, mycelial, and sclerotial. Sporulating group includes forms with flat and pressed mycelium consisting only of conidiafores with a great deal of conidia. Mycelial group includes forms with high developed, unflat air mycelium which sporulates slightly and does not form the spores in some area of mycelium. Forming of individual sclerotia have been observed in this case. Sclerotial group includes forms which are intirely lack of air mycelium. In this case, numerous charcoal sclerotia are spreaded evenly and disorderly on the surface of medium. Conidiafores with conidia formed by substrate mycelium have found out in some places.

LESOVOI *et al.* (1982) has differentiated two groups of *B. cinerea* isolates on the basis of cultural and morphological characters, such as sporulating and sclerotic types. The first group is characterized by the presence of abundant air mycelium with mass conidial sporification, and single rare sclerotia were founded on the colony surface. Isolates of sclerotic group have skinny surface of colonies with numerous flat sclerotia of irregular form appearing either in the center or edge of the colony. Sometimes small air mycelium with conidial sporification can be found on the colony borders.

CHIKIN and LICHACHEV (1997) has described six main types of *B. cinerea* isolates collected from the different plant species. These cultural features do not saved during cultivation on the native plant materials because the degree of the specific traits expression of *B. cinerea* isolates depends from the cultivation conditions. But nevertheless it is possible to differentiate that or the other morphotype in *B. cinerea* population. Thus, VASIN (1966) has noted the prevailing of mycelial form in pathogen populations, which is the most pathogenic. CHIKIN and LICHACHEV (1997) has shown that mycelial-sporulating isolates are predominate in *B. cinerea* populations. According to LICHACHEV (2000), correlation in the development of sporification sclerotia and air mycelium can serve the trend of fungus life strategy in phytocenoses. This is particularly important for the Belarus territory, where such investigations were not yet carried out.

## MATERIALS AND METHODS

Samples isolation for the obtaining of pure fungi cultures and their identification have performed in three agroclimatic regions of Belarus: Northern moderately warm humid, Central warm moderately humid, and Southern warm unstable humid. Isolation of fungi into pure cultures have conducted according to VASIN (1966). Isolates were cultivated in agar potato-glucose medium at 22°C according to BILAI (1982). Identification of isolates was performed as described by VASIN (1966). Radial rate of colony growth was calculated using the formula by BILAI (1982):

$$K_r = (r - r_0)/\Delta t$$

where: $r$ – colony radius after cultivating
$r_0$ – colony radius at the initial moment
$\Delta t$ – time of cultivation

Intensity of sporification was determined using the formula by BILAI (1982):

$$I = LN/SV$$

where: $L$ – water quantity for the washing off the spores (ml)
$N$ – average quantity of spores in the small square of Fux-Rosental box (piece)
$S$ – the area of the cut out fragment of sporifying surface ($cm^2$)
$V$ – volume of the small square of Fux-Rosental box (ml)

## RESULTS AND DISCUSSION

We has isolated 101 samples of *B. cinerea* during our investigation, including those from Northern moderately warm humid agroclimatical region (80%), Central warm moderately humid agroclimatical region, Southern warm unstably humid agroclimatical region – 5% of isolates (Table 1).

The fungal samples were selected from the diseased plants belonging to following families: *Liliaceae* (25.8% of general quantity of isolates), *Brassicaceae* (17.5%), *Solanaceae* (30.9%), *Cucurbitaceae* (14.1%), *Apiaceae* (5.2%), *Fabaceae* and *Asteraceae* (3.1% for either), *Caryophyllaceae*, *Paeoniaceae*, and *Araceae* (1% for each family).

We has distinguished the next morphotypes during analysing of the isolates morphological traits: mycelial, sporulating and sclerotic (Figure 1). Mycelial isolates are dominated among others (48.5%). Some researches refer such isolate morphotypes to highly pathogenic.

*B. cinerea* isolates, which were selected from the same plant species, were put to different morphotypes. From the other hand, the isolates of the same morphotype were selected from the different plants genera and families (Table 2). Thus, the nutritive substrate does not determine a formation of this or other morphotypes.

The rate of radial growth of 46 investigated *B. cinerea* colonies in most cases differed in the same and different types of morphotype (Table 2). This index does not correlate with morphological traits of fungus colonies. The divergence of isolates in the rate of radial growth of fungus colonies has permitted to suggest the different way of their mutual relations with other components of mycosinusia, including the interaction with potential antagonists. The type of interaction can vary from the fungistatic alimentary antagonism to the territorial antagonism in depends from the fungus growth intensity.

Table 1. Distribution of isolated samples of *B. cinerea* to morphotypes in Belarus conditions

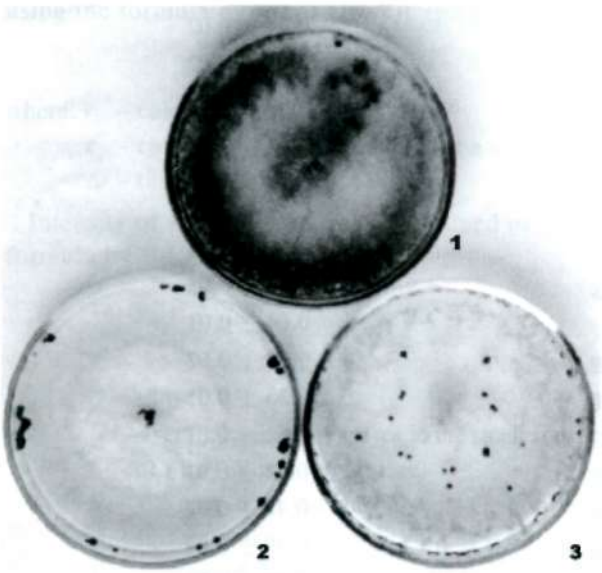
| Agroclimatic region | Isolates | Morphotype | | | All isolates |
|---|---|---|---|---|---|
| | | sporulating | mycelial | sclerotial | |
| Northern moderately warm humid | quantity | 32 | 32 | 10 | 74 |
| | % | 31.7 | 31.7 | 9.9 | 73.5 |
| Central warm moderately humid | quantity | 1 | 17 | 4 | 22 |
| | % | 0.9 | 16.8 | 3.9 | 21.6 |
| Southern warm unstably humid | quantity | 0 | 0 | 5 | 5 |
| | % | 0 | 0 | 4.9 | 4.9 |
| All isolates | quantity | 33 | 49 | 19 | 101 |
| | % | 32.7 | 48.5 | 18.8 | 100.0 |

Table 2. Comparative characterization of *B. cinerea* isolates

| No. of isolate | Source of isolation | Morphotype | Average radial growth rate ($K_r$) (mm/h) |
|---|---|---|---|
| 32 | *Allium* cepa | mycelial | 0.42 ± 0.01 |
| 36 | the same | mycelial | 0.48 ± 0.02 |
| 31 | the same | sclerotial | 0.49 ± 0.03 |
| 18 | the same | sclerotial | 0.50 ± 0.02 |
| 19 | the same | mycelial | 0.51 ± 0.01 |
| 28 | the same | mycelial | 0.51 ± 0.03 |
| 24 | the same | mycelial | 0.53 ± 0.01 |
| 92 | *Alokasia* sp. | sporulating | 0.48 ± 0.02 |
| 9 | *Brassica oleracea* | mycelial | 0.31 ± 0.01 |
| 1 | the same | mycelial | 0.44 ± 0.02 |
| 4 | the same | sclerotial | 0.44 ± 0.02 |
| 14 | the same | sclerotial | 0.45 ± 0.03 |
| 7 | the same | mycelial | 0.47 ± 0.01 |
| 2 | the same | mycelial | 0.49 ± 0.01 |
| 8 | the same | mycelial | 0.49 ± 0.01 |
| 5 | the same | sclerotial | 0.49 ± 0.01 |
| 13 | the same | sporulating | 0.55 ± 0.03 |
| 15 | the same | mycelial | 0.64 ± 0.01 |
| 78 | *Capsicum annuum* | sporulating | 0.33 ± 0.02 |
| 82 | the same | sporulating | 0.33 ± 0.01 |
| 85 | the same | sporulating | 0.34 ± 0.01 |
| 74 | the same | sporulating | 0.41 ± 0.01 |
| 73 | the same | sporulating | 0.42 ± 0.02 |
| 80 | the same | sporulating | 0.49 ± 0.03 |
| 72 | *Cucurbita pepo* | mycelial | 0.48 ± 0.02 |
| 64 | the same | mycelial | 0.49 ± 0.01 |
| 71 | the same | sclerotial | 0.51 ± 0.01 |
| 68 | the same | sporulating | 0.52 ± 0.02 |
| 62 | the same | mycelial | 0.53 ± 0.03 |
| 69 | the same | sclerotial | 0.53 ± 0.05 |
| 45 | *Daucus sativus* | mycelial | 0.33 ± 0.02 |
| 43 | the same | sclerotial | 0.49 ± 0.03 |
| 44 | the same | mycelial | 0.49 ± 0.01 |
| 46 | the same | sclerotial | 0.49 ± 0.01 |
| 90 | *Faseolus vulgaris* | sclerotial | 0.50 ± 0.01 |
| 89 | *Lactuca sativa* | mycelial | 0.53 ± 0.03 |
| 52 | *Lycopersicum esculentum* | sporulating | 0.35 ± 0.02 |
| 48 | the same | mycelial | 0.36 ± 0.05 |
| 49 | the same | sporulating | 0.39 ± 0.01 |
| 51 | the same | sporulating | 0.39 ± 0.01 |
| 57 | the same | sporulating | 0.42 ± 0.01 |
| 50 | the same | sporulating | 0.51 ± 0.01 |
| 91 | *Melandrium album* | sporulating | 0.39 ± 0.03 |
| 95 | *Paeonia* sp. | sclerotial | 0.47 ± 0.01 |
| 93 | *Tagetes* sp. | sclerotial | 0.52 ± 0.02 |
| 94 | the same | mycelial | 0.55 ± 0.01 |

Table 3. Characterization of some isolates of *B. cinerea* by sporo- and sclerotiaformation

| No. of isolate | Morphotype | Source of isolation | Intensity of sporulation, (number of spores/m$^2$) × 10$^4$ | Number of sclerotia per Petri dish | Sclerotia distribution |
|---|---|---|---|---|---|
| 1 | mycelial | *Brassica oleracea* | 1.33 ± 0.46 | 62.6 ± 8.28 | cyclic |
| 2 | mycelial | the same | 0.25 ± 0.08 | 71.6 ± 3.54 | cyclic |
| 4 | sclerotial | the same | 0.66 ± 0.06 | 62.8 ± 3.36 | chaotic |
| 5 | sclerotial | the same | 4.73 ± 0.55 | 34.0 ± 2.12 | chaotic |
| 6 | sclerotial | *Daucus sativus* | 4.81 ± 0.63 | 37.2 ± 2.51 | chaotic |




1 – sporulating, 2 – mycelial, 3 – sclerotial

Figure 1. Morphotypes of *B. cinerea*

We have found out that isolates of mycelial morphotype differ one from another more then 5 times in their intensity of sporulation (Table 3). The similar difference in this characteristic (more then 7 times) was also observed for the sclerotic. At the same time, sclerotic isolates from 2.6 to 3.6 times surpassed mycelial ones in the intensity of sporulation.

The results of our study have not shown the strict regularities in the process of spore and sclerotia formation during evaluation of isolates of different cultural groups, which demonstrate the presence of transitional forms between detected ones. These observations are consistent with the LICHACHEV'S (2000) results.

Thus, *B. cinerea* population is non-uniform by morpho-physiological traits. The divergence of the population in the formation of spores, mycelium and sclerotia is a result of adaptation of *B. cinerea* to changeable environmental conditions.

The morphotypes with ability of rapid reproduction in absence of environmental stress as well as the morphotypes which able to be resistant to increasing environmental stress and thus surviving as sclerotia are presented in Belarus conditions. The fact that mycelial forms are predominate and highly pathogenic among all selected isolates should be taken into account in evaluating of plants for resistance to botrytiosis and elaborating of the control strategies for crops grey rot.

The heterogeneity of *B. cinerea* population in growth rate should be considered for the elaboration of mechanisms of regulation of the number of natural groups of the phytopathogen.

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