DOI: 10.22620/agrisci.2025.46.003

Botrytis elliptica - discovery in Bulgaria and characterization

Aneta B. Lyubenova[1,2]

[1]Department of Agrobiotechnology, AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria
[2]Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), Sofia, Bulgaria
Corresponding author: anetalyubenova@abi.bg

Abstract

Lilies are grown for various purposes and rank among the top flower bulbs and cut flowers globally. Botrytis blight impacts lilies in almost all cultivation areas and is regarded as the primary foliar disease affecting *Lilium* spp. *Botrytis elliptica* is considered a limiting factor in the cultivation of ornamental lilies, being one of the most common *Botrytis* species impacting *Lilium* spp, together with *B. cinerea*. In the current study is described the isolation and identification of *B. elliptica* from *Lilium* sp. in Bulgaria. Manifestation of the disease include oblong purple-brown lesions on the leaves and crown rot. Initially, on potato dextrose agar (PDA) *B. elliptica* formed white colonies which turn gray with the aging of the mycelia and black sclerotia appear in older cultures. The ITS region sequences of isolate Krem Pernik are 100% identical to those of *B. elliptica* isolates deposited in the NCBI GenBank. The conidiospores are predominantly ovoid and ellipsoid, smooth, and translucent with average size of 23.95 µm in length and 17.61 µm in width, with a corresponding ratio of 1.37. The established general growth temperatures for *B. elliptica* isolate are as follows: minimal above 5°C, optimal 20°C and maximum below 30°C. The isolate form necrotic lesions on *Lilium* leaves, but not on *Capsicum annum*. The presence of economically important pathogen on *Lilium* is confirmed in Bulgaria, based on morphology, molecular and pathogenicity tests.

Keywords: *Lilium* fire blight, disease symptoms, grow temperatures, pathogenicity

INTRODUCTION

Lilies (*Lilium* spp. and their hybrids) are cultivated for various purposes, including bulb production, as cut floral displays, as potted plants, or for garden planting (Chastagner et al., 2017). The genus *Lilium* belongs to the family *Liliaceae* and its representatives are spread across the cooler and temperate regions of the Northern Hemisphere. Their number is approximately 100 species since the first description of *Lilium* in China almost 2000 years ago. In the last half-century, the lilies have gained prominence as one of the leading flower bulbs and cut flowers around the world, with main producer the Netherlands (Van Tuyl et al., 2018). In Bulgaria, the market demands in recent years have led to expansion of lily cultivation, as well as to a development of a breeding program for creation of Bulgarian varieties (Kaninski et al., 2017). Botrytis blight, commonly referred to as "fire" impacts lilies in almost all cultivation areas and is regarded as the primary foliar disease affecting this plant species. All aboveground sections of the plant, such as leaves, stems, and flowers, can be attacked. The potential of the disease to damage floral tissues in storage makes it of great concern for cultivators during both the pre- and postharvest periods. The occurrence of the pathogen can entirely devastate a lily plantation, stunting bulb growth and making it impossible to market cut flowers or potted varieties. *Botrytis elliptica* (Berkeley) Cooke, 1901 and *Botrytis cinerea* Persoon: Fries are the most common *Botrytis* species to infect lily. *B.*

elliptica is considered a limiting factor in the production of ornamental lilies (Gao et al., 2018). Although the species is viewed as a host specific pathogen, it has been reported on other plant species, like autumn crocus, daylily, dogstooth violet, gladiolus, tuberose and etc.(Chastagner et al., 2017). In contrast, *B. cinerea* has a wide variety of host species and is frequently found on the petals of flowers from both outdoor and indoor cultivated plants (Chastagner et al., 2017). The disease presents a significant economic risk and has been recorded in Argentina, China, Korea, Italy, Japan, Netherlands, Taiwan, United Kingdom, and the United States. The use of fungicides to control *B. elliptica* is a widespread practice around the world; however, it has been noted that this organism can develop resistance to multiple fungicides including benzimidazoles, dicarboximides, diethofencarb, and two sterol biosynthesis inhibitors (Terhem et al., 2015).
The aim of this study is to report the presence of *Botrytis elliptica* in Bulgaria, confirm its identity using molecular techniques, and characterize its morphology, pathogenicity, and general growth temperature range in comparison with *Botrytis cinerea*.

MATERIALS AND METHODS

Isolation

Lilium plants showing symptoms of fire blight were collected from a garden in Pernik, where a disease outbreak was observed in May 2024. The plants were transported to a laboratory, where the isolation of the fungal pathogen took place. The parts of the leaves, bearing the necrotic spots were carefully washed under running water in order to remove all surface particles and spores and dried with filter paper. In the laminar flow box, they were disinfected by immersion in 70% ethanol for about 1 min and in a solution of bleach: sterile distilled water (1:1) for about 30 seconds, followed by two rinses in sterile distilled water for 2 minutes. After the pieces were dried with sterile filter paper, small plant tissue parts from the margin of the necrotic lessons were placed on BD DIFCO™ Potato Dextrose Agar (PDA) in 9 cm Petri dishes. They were incubated at 20°C in dark. When the hyphae of the fungus were visible, they were taken and re-cultured on new agar plates.

Molecular identification

Pure culture of the isolate was obtained by taking only one hyphal tip of the mycelium (Werres, 2015). The aerial mycelium of the isolate was collected, frozen with liquid nitrogen and grinded. The isolation of total DNA was done according to the manufacturer instructions using DNeasy Plant Mini Kit (QIAGEN GmbH). DNA amplification of the ITS region with Primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990) was performed with Illustra PuReTaq Ready-To-Go PCR kit. The PCR reaction was performed as follows: pre-denaturation at 95°C for 120 s, 35 cycles, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 50, final elongation at 72°C for 30 sec and cooling at 4°C. The amplified target DNA was purified with PCR/DNA clean up Purification kit (EURx Poland) and sequenced at Macrogen Europe (www.macrogen-europe.com). The obtained sequences were examined with Finch TV 1.4.0 (Geospiza, Inc., Seattle, WA, USA; www.geospiza.com) and bad quality reads were removed. Sequence similarity searches for the identification of the species were conducted using the basic length alignment search tool (BLAST) of the National Centre for Biotechnology Information (NCBI).

Colony morphology

The isolate was maintained on PDA. Stereo microscope Nikon SMZ745T and a compound microscope ZEISS Axio Imager A2 with a digital camera (AxioCamERs 5S) and a biometric software (AxioVision LE) studies were used to study morphology of the isolates. The sizes and length to breadth ratios of 50

conidiospores, chosen at random were determined at ×400 magnification.

Growth rates and cardinal temperatures

Growth rates and cardinal temperatures of *B. elliptica* isolate Krem Pernik and *B. cinerea* isolate B. c. were determined in order to compare both species. The isolates were incubated at 5, 10, 15, 20, 25, 30, 33 and 35°C (±0.5 °C). For each temperature, sets of three replicate PDA plates (90 mm) per isolate were prepared by placing small agar piece with mycelium in the center of a Petri dish. The Petri dishes were incubated overnight for initiation of the mycelial growth. Two perpendicular lines, intersecting the forming mycelial colony were drawn on the bottom of each Petri and the periphery of the colony was marked, indicating the starting point of the measurements. The cultures were incubated at the corresponding temperatures in dark. After 3–9 days of incubation, the linear mycelial growth along the four radial lines of each Petri dish was measured. The average radial growth rate per day (mm/day) was also calculated (Hall, 1993).

Pathogenicity test

Two parallel experiments for the evaluation of pathogenicity of the *B. elliptica* were performed – with lily and pepper plants. *B. cinerea* isolate B.c. was used as a control. Well-developed leaves of *Lilium* sp. and *Capsicum annuum* L., respectively were used for the tests. Six leaves per each plant were inoculated with agar plugs of *B. eliptica* isolate Krem Pernik and *B. cinerea* as a positive control. *B. cinerea* isolate B. c. was previously isolated from raspberry and identified based on morphology and ITS sequencing. The sequence is deposited in NCBI under accession number PQ345538. Agar plugs from the fresh fungal colony grown on PDA of each isolate was placed on the leaflets, facing its adaxial sides. The leaf surface under the agar plugs was slightly punctured with sterile needle. For negative control, was used clean PDA instead of fungal culture. Inoculated leaves were kept in sterilized 120 mm glass Petri dishes on wet filter paper at daylight and room temperature for 7 days. Images of the necrotic spots of the variants with lilium leaves were taken with the Automatic Colony Counter IUL micro SphereFlash® camera. The images were exported in PNG format and used for the measurement of the necrotic spots with the program ImageJ 1. 54g (Schneider et al., 2012). The obtained data were statistically processed with Excel and the average areas of the necrosis for both *B. elliptica* and *B. cinerea* were calculated.

RESULTS AND DISCUSSION

Disease symptoms

The first manifestation of the disease was the appearance of oblong purple-brown necrotic lesions on the leaves. The centers of these lesions were straw-colored, while the margins remained purple (Fig. 1– a, b). Later on, the spots merged, and under favorable environmental conditions, necrosis gradually covered a large area of plant tissues, including the stems (Fig. 1 – c, d). Crown rot was also observed (Fig. 1 – e). The symptoms are similar to those the described by other authors (Chastagner et al., 2017).

Isolation

After 1-2 days, mycelial growth was observed from all plant pieces in the Petri dishes. Since all the colonies have similar morphology, only one representative – isolated as Krem Pernik – was selected for further studies.

Molecular identification

The results from the NCBI blast search demonstrates that the sequences of the isolate Krem Pernik shared the highest level of similarity, achieving 100% identity with the isolates *Botrytis elliptica* strain lilyBC-2 (GenBank: EU519207), and *Botrytis elliptica* strain LBM137423 (GenBank: KR055047). The sequence has been submitted to NCBI under the accession number PV394098.

Figure 1. Symptoms of fire blight disease on *Lilium* sp. caused by *Botrytis elliptica*: a, b - oblong purple-brown necrotic lesions on the leaves; c – plant with twisted leaves due to water-soaked lesions, leaves, d- necrotic lesions on the stem, e – crown rot

Morphology

Initially, the isolate Krem Pernik forms white colonies on PDA, which turn gray as the mycelium ages (Fig. 2). In cultures older than two weeks, the pathogen forms single, oval or irregularly shaped black sclerotia (Fig. 2 – d).

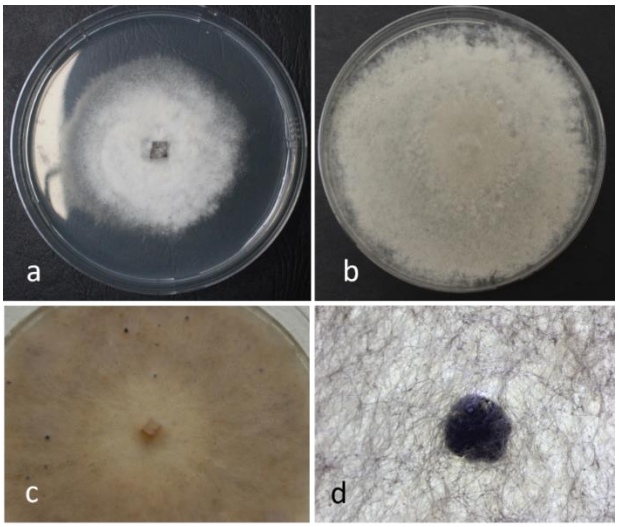

Figure 2. *B. elliptica* colony on PDA after 6 (a) and 14 days (b) at 25°C. Close up of sclerotia of *B. elliptica* formed on PDA in the Perti (c) sclerotium under stereomicroscope (d).

Numerous single-celled spores are observed on the infected plant tissues. They are predominantly ovoid and ellipsoid, smooth, and translucent with average size 23.95 µm in length and 17.61 µm in width, with a corresponding ratio of 1.37 (Fig. 3). These results are in accordance with previous studies (Chang et al., 2001). Apart from the differences in their host preferences, spore size is an additional feature that helps to differentiate *B. elliptica* from *B. cinerea*.

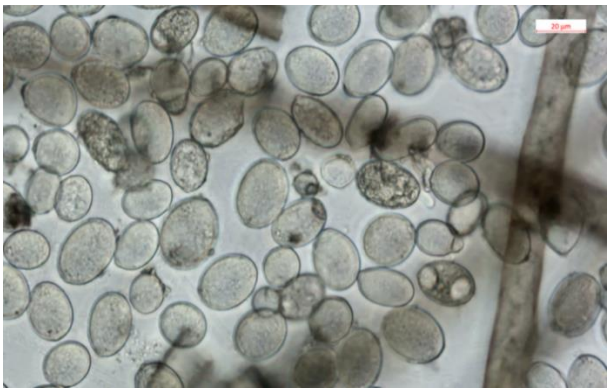

Figure 3. Mycelium and conidiospores of *B. elliptica* isolate Krem Pernik

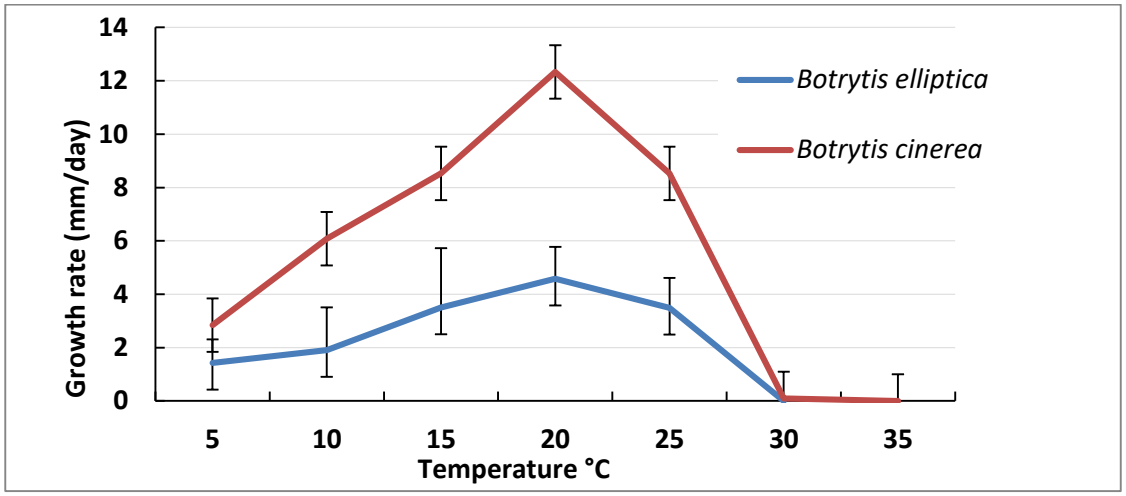

Figure 4. Growth temperature range of *B. elliptica* isolate Krem Pernik. Bars represent standard errors.

The conidial sizes of both species are described by various authors (Kim et al., 2007). *B. cinerea* conidiospores are significantly smaller – between 6.5 -16.3 x 5.5-10µm, while for *B. elliptica* they are between 25 – 43.8 x 16.3-27.8µm.

Growth temperature

The established general growth temperatures for *B. elliptica* isolate Krem Pernik are as follows: minimum above 5°C, optimum 20°C, and maximum below 30°C. For *B cinerea* (isolate B.c.) the minimal growth temperature is also above 5°C, the optimum 20°C and maximum slightly above 35°C. In comparison to *B. cinerea*, *B. elliptica* exhibits a reduced rate of mycelial growth (Fig. 4). These results align with those reported by Kim et al. (2007) for both *Botrytis* spp.

Pathogenicity

Both *Botrytis elliptica* and *B cinerea* form necrotic lesions on *Lilium* leaves. The results were recorded after 6 days. Necrotic regions triggered by *B cinerea* were larger on average compared to those associated with *Botrytis elliptica* (Fig. 5).

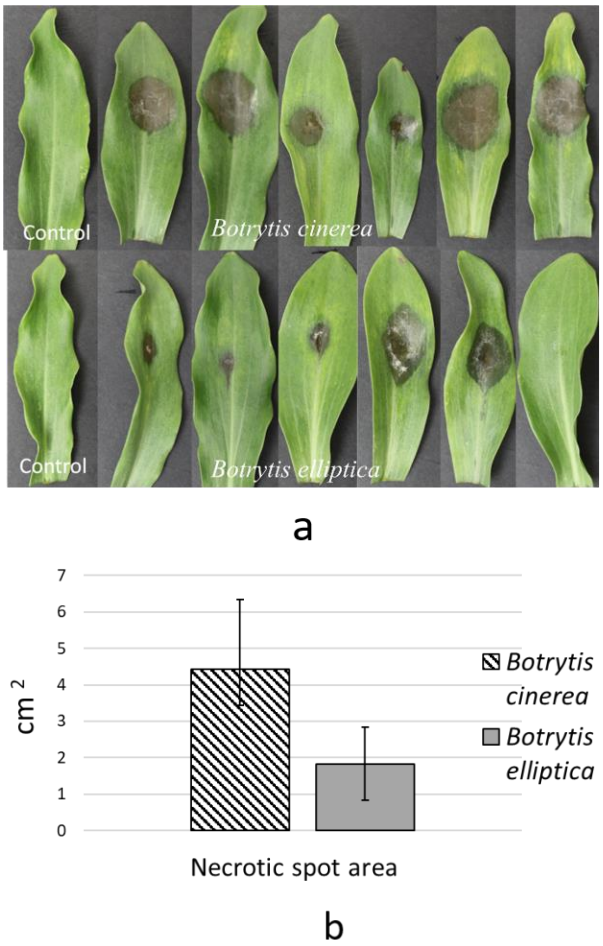

Figure 5. a - Development of necrotic lesions on *Lilium* sp. leaves inoculated with *Botrytis cinerea* B.c. and *Botrytis elliptica* Krem Pernik (a); Average areas of the necrotic spots caused by pathogens (b). Error bars represent the standard deviations of the mean (n=6)

These results suggest a correlation between the necrotic spots area caused by the corresponding pathogen and its growth rate, which is higher in *B. cinerea* in the current experiment. On *C. annuum* leaves, *B. elliptica* produce necrotic lesions similar to those caused by *B. cinerea* (Fig. 6).

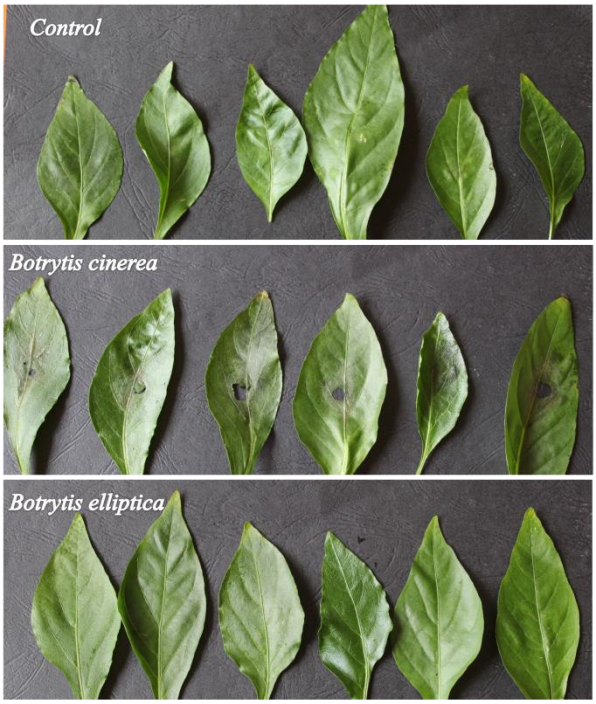

Figure 6. Pathogenicity of *B. cinerea* (B. c.) and *B. elliptica* (Krem Pernik) on *C. annuum* leaves after 4 days

These results indicate the differences in the host specificity of *B. cinerea* and *B. elliptica*.

CONCLUSIONS

For the first time in Bulgaria, the economically important pathogen on Lilium spp., Botrytis elliptica, is confirmed based on morphology, molecular, and pathogenicity studies. As Lilium species hold significant horticultural value in ornamental cultivation, these findings could be useful in plant breeding programs for resistance and for the control of Botrytis blight disease.

ACKNOWLEDGEMENTS

This research is financially supported by the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG) project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU.

REFERENCES

Chang, S.-W., Kim, S.-K., & Hwang, B.-K. (2001). Gray Mold of Day Lily (Hemerocallis fulva L.) Caused by Botrytis elliptica in Korea. *The Plant Pathology Journal*, *17*(5), 305–307.

Chastagner, G. A., van Tuyl, J. M., Verbeek, M., Miller, W. B., & Westerdahl, B. B. (2017). Diseases of Lily. In R. J. McGovern & W. H. Elmer (Eds.), *Handbook of Florists' Crops Diseases* (pp. 1–61). Springer International Publishing. https://doi.org/10.1007/978-3-319-32374-9_45-1

Gao, X., Cui, Q., Cao, Q., Zhao, Y., Liu, Q., He, H., Jia, G., & Zhang, D. (2018). Evaluation of resistance to *Botrytis elliptica* in *Lilium* hybrid cultivars. *Plant Physiology and Biochemistry*, *123*, 392–399. https://doi.org/10.1016/j.plaphy.2017.12.025

Hall, G. (1993). An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. *Mycological Research*, *97*(5), 559–574. https://doi.org/10.1016/S0953-7562(09)81179-9

Kaninski, A., Ivanova, I., & Galeva, A. (2017). Negovan new lily variety. *Bulgarian Journal of Crop Science*. https://cropscience-

bg.org/page/bg/details.php?article_id=405

Kim, W.-K., Park, M.-S., Hahm, S.-S., & Yu, S.-H. (2007). Morphological, Molecular and Pathogenic Characteristics of Botrytis elliptica and Botrytis cinerea Associated with Leaf Blight of Lily. *The Korean Journal of Mycology*, *35*(2), 68–75. https://doi.org/10.4489/KJM.2007.35.2.068

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), Article 7. https://doi.org/10.1038/nmeth.2089

Terhem, R. B., Staats, M., & van Kan, J. A. L. (2015). Mating type and sexual fruiting body of Botrytis elliptica, the causal agent of fire blight in lily. *European Journal of Plant Pathology*, *142*(3), 615–624. https://doi.org/10.1007/s10658-015-0639-2

Van Tuyl, J. M., Arens, P., Shahin, A., Marasek-Ciołakowska, A., Barba-Gonzalez, R., Kim, H. T., & Lim, K.-B. (2018). Lilium. In J. Van Huylenbroeck (Ed.), *Ornamental Crops* (pp. 481–512). Springer International Publishing. https://doi.org/10.1007/978-3-319-90698-0_20

Werres, S. (2015). PROTOCOL 01-09.1: Preparation of hyphal tip Phytophthora cultures. In W. Sabine (Ed.), *Laboratory Protocols for Phytophthora Species* (pp. 1–2). The American Phytopathological Society. https://doi.org/10.1094/9780890544969.01.09.1

White, T., Bruns, T., Lee, S., Taylor, J., Innis, M., Gelfand, D., & Sninsky, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *Pcr Protocols: A Guide to Methods and Applications*, (pp. 315–322), 31.