BOTRYTIS BLIGHT OF BLUEBERRY IN BOSNIA AND HERZEGOVINA*

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Abstract

In June 2022, blight symptoms of blueberry plants (cv. Bluecrop) were observed in a blueberry plantation in Bihać, Bosnia and Herzegovina. Blackish-brown young shoots, leaves, flower parts and fruits, and brownish mycelia and conidia were observed. Approximately 5% of the plants were affected. The causal fungus was identified as *Botrytis cinerea* based on cultural, morphological, and molecular characteristics. Pathogenicity studies were performed in a greenhouse with 2-year-old blueberry plants (cv. Bluecrop). This is the first report of *Botrytis cinerea* causing the blight disease of blueberries in Bosnia and Herzegovina.

Key words: *Botrytis cinerea, Botrytis blight, Blueberry, Bosnia and Herzegovina*

INTRODUCTION

Blueberry species, which belong to the *Vaccinium* genus of the *Ericaceae* family, are a type of fruit adapted to the temperate climate zone. There are three different types of blueberries that are economically cultivated: highbush form (*Vaccinium corymbosum*), lowbush form (*Vaccinium angustifolium*) and rabbit eye (*Vaccinium ashei*). Blueberries like organic matter-rich and acidic soils. Blueberries, which have a high antioxidant content, are used for many different purposes. These are: as fresh fruit, in the fruit juice industry, in the pharmaceutical industry, in milk and dairy products technology, in dried fruit technology, in fruit bread, buns, cakes, puddings and pastries, in the spice industry, in fruit salads, in jam, marmalade and canning industry, in tea, diet menus, in wine making and in the production of plant handles (stalks) (Çelik, 2005).

The commercial production of highbush blueberry in Bosnia and Herzegovina has been expanded in recent years. However, the limiting factor in producing plantation highbush blueberry is the soil, since this plant can only be grown on acidic soils. As an alternative, growing in bags or pot containers can be possible. Despite certain shortcomings, blueberry cultivation in containers, compared to the current cultivation method, is still much more efficient, both from a technological and an economic point of view.

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The area of the city of Bihać has a moderately continental climate. It is characterized by warm summers and cold winters. Absolute summer temperatures can reach 40 °C, while winter temperatures can drop to -35 °C (Kurtović *et al*., 2013). The annual average precipitation is 1327/m², and the average monthly temperature is 10.8 ˚C.
In June 2022, blight symptoms of highbush blueberry (*Vaccinium corymbosum*) plants (cv. Bluecrop) grown in container bags were observed in a blueberry plantation in Bihać, Bosnia and Herzegovina. Blackish-brown young shoots, leaves, flower parts, and fruits and brownish mycelia and conidia were observed. Approximately 5% of the plants were affected. This study was undertaken to identify the causal agent of the disease.

MATERIALS AND METHODS

The highbush blueberry plantation surveyed was established in 2018, in Bihać (Ružica locality), Bosnia and Herzegovina, with an area of 0.65 ha. One-year-old seedlings in containers were planted in geotextile bags with a volume of 40 liters. The substrate used for planting is especially intended for blueberry, which included a mixture of white and brown peat with a pH value of 3.5 to 4.8, coconut fibers, perlite, NPK, and microelements. Natural spring water was used for irrigation with a drip system.
In the surveyed highbush blueberry plantation, disease symptoms were observed on plants. Diseased shoots, leaves, flower parts and fruits were brought to the laboratory and small pieces (0.5-1 cm) were surface sterilized with 1% NaOCl for 1 min and they were placed on Potato Dextrose Agar (PDA) plates. After fungal development in PDA, mycelia, conidiophore, conidia and sclerotia were examined under a light microscope and a stereomicroscope.
For molecular characterization of *Botrytis cinerea* isolate, mycelium grown in Potato dextrose agar (PDA) medium for 10 days at 23 ± 1 °C was scraped with a sterile spatula and disrupted with extraction buffer, and DNA was extracted with a DNA extraction kit (QIAGEN). Polymerase chain reaction (PCR) analysis, was carried out according to Rigotti et al., (2006). PCR amplification with primers BC108+(5'-ACCCGCACCTAATTCGTCAAC-3') and BC563–(5' GGGTCTTCGATACGGGAGAA-3') was performed. One cycle at 95 °C for 3 min, followed by 34 cycles at 94 °C for 20 s, 59 °C for 20 s and 72 °C for 30 s and final extension at 72 °C for 3 min were carried out. Additionally, PCR analysis was conducted on the internal transcribed spacer (ITS) region (ITS1-ITS4) in accordance with the methodology proposed by Behr et al., (2013). The initial denaturation was conducted at 98 °C for a period of two minutes. Subsequently, 30 cycles were performed, each comprising denaturation at 98 °C for 15 seconds, annealing at 64 °C for 20 seconds, and elongation at 72 °C for 20 seconds. Finally, a final elongation step was carried out at 72 °C for 10 minutes. The PCR product was electrophoresed on a 1% agarose gel containing ethidium bromide at a concentration of 0.1 µg ml^{-1} in 0.5 x Tris-borate-EDTA (TBE) at 100 V for 3 hours. The gel was photographed under UV light (Quantum ST4, Montreal Biotech, Canada) and the DNA fragment size in the gel was

compared with the GeneRuler 100 bp DNA ladder Plus (MBI Fermentas, USA). For fulfilling Koch's postulates, pathogenicity studies were performed in a greenhouse with 2-year-old blueberry plants (cv Bluecrop). 1 x 10^5 conidia ml^{-1} were sprayed onto shoots and leaves and covered with plastic bags for 3 days. The greenhouse temperature ranged between 17±2/24±2 °C night/day. Control plants were sprayed with sterile water only.

RESULTS AND DISCUSSION

Blackish-brown symptoms on young blueberry shoots, leaves, flower parts, and fruits, and brownish mycelia and conidia were observed (Figure 1).

Figure 1. Disease symptoms observed in a highbush blueberry plantation in Bihać, Bosnia and Herzegovina

On Potato Dextrose Agar medium, brownish gray mycelia, black sclerotia (2-4 mm in diameter, n=30), and long and branched conidiophores were observed. Hyaline and ovate conidia were one-celled. Conidial measurements were (7.5) 7.95 (10) x (8.75) 13.06 (16.25) µm (n=30). The causal fungus was identified as *Botrytis cinerea* based on morphological characteristics (Ellis, 1971; Caruso & Ramsdell, 1995). PCR amplification with primers BC108+ (5'-ACCCGCACCTAATTCGTCAAC-3') and BC563–(5' GGGTCTTCGATACGGGAGAA-3') was performed and a 480 bp DNA fragment was observed (Rigotti *et al.,* 2006). After amplification of the complete ITS rDNA of the *B. cinerea* isolate using ITS1-ITS4 primers a band was observed at 576 kb (Behr et al., 2013). ITS1-ITS4 sequence results showed 99.18% sequence similarity with other ITS sequences of *B. cinerea* in GenBank (Accession numbers: KX061437.1, KY828219.1, KY114879.1). The sequence results were submitted to NCBI (Accession No: PQ686329). These morphological and molecular studies confirmed the fungus as *Botrytis cinerea* Pers.:Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel.

Rigotti *et al.* (2002) used the species-specific primers C729+/– (5'-AGCTCGAGAGAGATCTCTGA-3'; 5'-CTGCAATGTTCTGCGTGGAA-3') to detect *Botrytis cinerea* isolates. However, these primers were not sufficient to molecularly identify some of the *Botrytis cinerea* isolates obtained from different host plants. Therefore, they designed two new primers, BC108+ and BC563−. These new primers amplified a DNA fragment of 480 bp for the main group of 26 *Botrytis cinerea* strains and 360 bp for another group of 13 strains of *Botrytis cinerea*. Other closely related species such as *B. allii* and *B. fabae* from the genus *Botrytis* could not be amplified with these primers, confirming the specificity of the BC108+ and BC563− primer for *B. cinerea* and identifying it as a precision molecular tool for detecting this fungus in host plants (Rigotti *et al*., 2006). In our study, a DNA fragment was observed at 480 bp in accordance with Rigotti *et al*. (2006).

Fourteen days after inoculation of 2-year old blueberry plants (cv Bluecrop) in the greenhouse, small blackenings were observed in shoots (2 mm) and leaves (5 mm). The disease progressed with time (Figure 2). *Botrytis cinerea* was reisolated from these shoots and leaves. Control plants showed no symptoms.

These morphological, molecular and pathogenicity studies confirmed the *Botrytis cinerea* as the causal agent of blueberry blight disease observed in Bihać. This is the first report of *Botrytis cinerea* causing the blight disease of blueberries in Bosnia and Herzegovina.

Figure 2. Under greenhouse conditions, *Botrytis cinerea* showed pathogenicity to highbush blueberry leaves and shoots

Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is an ascomycetous polyphagous pathogen and affects a number important fiber, protein, oil, and horticultural crops in temperate and subtropical regions. It is also an important pathogen under storage conditions (Williamson *et al*., 2007). Foster *et al*. (2024) reported *Botrytis cinerea* causing disease in blueberries in South Africa and Kwon *et al*. (2011) reported *B. cinerea* from Korea as a postharvest pathogen. Dil *et al*. (2013) reported a *Botrytis* sp. affecting blueberries grown in Rize province of Türkiye. They reported that Botrytis leaf spot was the most common disease of blueberries in Rize, Türkiye.

CONCLUSIONS

The commercial production of highbush blueberry in Bosnia and Herzegovina has been expanded in recent years. However, diseases can hamper the blueberry production. *Botrytis cinerea* can cause disease in over 200 mainly dicotyledonous plant species and is also a significant pathogen under storage conditions (Williamson *et al*., 2007). This study is the first to demonstrate that *Botrytis cinerea* caused disease in blueberries grown in Bihać, Bosnia and Herzegovina. It is important to implement control measures for this significant pathogen.

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SIVA TRULEŽ BOROVNICE U BOSNI I HERCEGOVINI

Rezime

U junu 2022. godine uočeni su simptomi prisustva gljivice kod mladih biljaka borovnice (cv. Bluecrop) na plantaži borovnice u Bihaću, Bosna i Hercegovina. Uočeni su crnosmeđe obojeni mladi izdanci, listovi, dijelovi cvjetova i plodovi, te smeđkaste micelije i konidije. Približno 5% biljaka je bilo zaraženo. Uzročnik je identificiran kao *Botrytis cinerea* na osnovu kulturnih, morfoloških i molekularnih karakteristika. Istraživanja patogenosti vršena su u stakleniku na dvogodišnjim biljkama borovnice (cv. Bluecrop). Ovo je prvi izvještaj o uzrokovanju sive truleži (*Botrytis cinerea*) borovnice u Bosni i Hercegovini.

Ključne riječi: *Botrytis cinerea, siva trulež, borovnica, Bosna i Hercegovina*