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First Record of *Eutypella parasitica* on Maples in Urban Area in Croatia

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ABSTRACT

Background and Purpose: *Eutypella parasitica*, a plant pathogenic fungus attacking maples (*Acer* spp.) was detected for the first time in Croatia in 2007. From 2007 to 2014, it was found only in forests, on several trees in Hum na Sutli, near Slovenian border. In 2015, the presence of *Eutypella parasitica* was monitored for the first time in urban areas.

Materials and Methods: Within the official survey programme, 23 visual surveys were conducted and 24 samples were collected and analysed for the presence of fungi. *E. parasitica* was found in Bundek Park in Zagreb. Typical symptoms of Eutypella canker were detected on two field maples (*Acer campestre*) and two boxelder maples (*Acer negundo*). Twelve isolates were collected from symptomatic trees.

Conclusions: Eleven out of twelve isolates from four trees were confirmed as *E. parasitica* by species-specific polymerase chain reaction. This is the first report of *E. parasitica* in Zagreb, the first record of *E. parasitica* in Croatia outside forests, as well as a record of a new host species in Croatia, boxelder maple (*A. negundo*). Introduction pathway of *E. parasitica* in Zagreb remains unknown.

Keywords: Eutypella canker, survey, *Acer campestre*, *Acer negundo*

INTRODUCTION

Eutypella canker is a disease of maples (*Acer* spp.) caused by the fungus *Eutypella parasitica* R. W. Davidson & R. C. Lorenz [1]. The disease is present in North America and it was not known to occur in Europe until 2005, when it was recorded in Ljubljana, Slovenia [2]. It was subsequently discovered in Austria in forests in 2006 on sycamores (*Acer pseudoplatanus* L.) [3]. In Croatia, *E. parasitica* was found for the first time in 2007 on field maples (*Acer campestre* L.) in Prišlin (Krapina-Zagorje County), near Slovenian border [4]. In 2016, the fungus was reported in Germany [5] and Hungary [6].

Novak Agbaba *et al.* [7] stated that *E. parasitica* is not a quarantine plant pathogen within the European Union, and the damaging potential of this alien fungal species in Europe is largely unknown [7]. Further spread of *E. parasitica* in Croatian forests could damage populations of different valuable maple species, causing economic loss [7]. According to the risk analysis by Ogris *et al.* [8], Croatia is considered to be a state where the risk of *E. parasitica* spread is high. These were the main reasons why *E. parasitica* was an object of official survey programmes, conducted in forests from 2011 to 2015. The review of the current situation of *E. parasitica* in Croatia showed that only two out of 2029 maple trees inspected during four years of survey were found infected with *E. parasitica*, both located in forests around Hum na Sutli [7].

In 2015, the presence of *E. parasitica* was monitored for the first time in urban areas, with the continuation of monitoring activities in forests. The main aim of the monitoring programme in urban areas was to contribute to the knowledge on the presence and distribution of this alien plant pathogen in Croatia.

MATERIALS AND METHODS

Twenty-three visual surveys of maple trees were carried out from the middle of May to the end of October in 2015. Surveys were carried out in parks, alleys, streets and public green areas, where maple trees were visually inspected for

the presence of Euytpella canker symptoms. Sycamores, field maples, boxelder maples (*Acer negundo* L.), Norway maples (*Acer platanoides* L.), silver maples (*Acer saccharinum* L.) and Tatarian maples (*Acer tataricum* L.) were the tree species examined. One survey was carried out on maple trees in Poreč (12 trees examined), Virovitica (52 trees examined), Koprivnica (43 trees examined), Desinec (20 trees examined), Osijek (70 trees examined), Beli Manastir (21 trees examined), Novska (24 trees examined), Čakovec (39 trees examined), Varaždin (59 trees examined) and Metković (11 trees examined). Eleven surveys were conducted in different periods in nine districts of the City of Zagreb, with a total of 187 maple trees examined.

Trees were examined for the presence of cankers with similar symptoms to Eutypella canker. When cankers on the main trunk were discovered, bark at the edge of the canker was removed or peeled off to check for the eventual presence of white mycelium fans beneath. The surface of cankers was examined with magnifying lens to determine the eventual presence of black protruding pertihecial necks. Fragments of wood from the edge of necrotic areas or wood fragments with pieces of bark from the cankers were collected for laboratory analysis. If white mycelial mats beneath the cankers were noted, their fragments were cut out and collected as the additional sub-samples. Multiple sub-samples were taken from each tree showing symptoms, and one tree was considered as one sample. Twenty-four samples were collected, 17 from the City of Zagreb, two from Osijek and one each from Desinec, Beli Manastir, Novska, Čakovec and Varaždin.

Laboratory analyses were carried out at the laboratory for mycology of the Institute for Plant Protection, Zagreb. Wood fragments were cut into chips (approximately 5 x 5 mm), surface-sterilized for one minute in 1% NaOCl, rinsed with sterile water, inoculated on potato-dextrose agar (PDA) and incubated at 22°C in darkness. Isolation from mycelial mats was performed by peeling small pieces of mycelia with a needle and placing them directly on PDA. If developed after incubation, sterile white mycelial colonies were sub-cultured in pure cultures from the edge of the advancing growth.

Among the fungi isolated from samples, 12 isolates from four samples resembled descriptions of *E. parasitica* in pure cultures [1, 7, 9]. Polymerase chain reaction (PCR)-based method and species-specific primer pair (EpR/EpF) developed by Piškur *et al.* [10] were used for the identification of isolates. Total DNA from the cultures was extracted from the mycelium ground in liquid nitrogen using Extract-N-Amp® Plant PCR Kit (Sigma-Aldrich) according to the manufacturer's instructions. PCR mixture and PCR conditions were similar to Piškur *et al.* [10]. Isolates which yielded amplification fragments of 341 bp were identified as *E. parasitica*.

All isolates confirmed as *E. parasitica* are stored at the Institute for Plant Protection in Zagreb.

RESULTS AND DISCUSSION

Declining maple trees were noted on all locations surveyed, but trees with cankers on the main trunk were recorded only in the City of Zagreb, Osijek, Desinec, Beli Manastir, Novska, Čakovec and Varaždin. Typical symptoms of Eutypella canker were observed on two field maple trees and on two boxelder maple trees in Bundek Park in Zagreb. On other locations, cankers were different from those described to be caused by *E. parasitica*, although bark cracking and callus formation were visible on trees affected. However, no characteristic white mycelial mats were found beneath the bark of such trees. Fungi belonging to Botryosphaeriaceae family were isolated from such samples.

In Bundek Park, "cobra neck" symptom with large swollen old canker 45 cm wide and 120 cm long was visible on the trunk of one field maple tree (Figure 1), while distorted trunk growth around the canker (25×58 cm) was evident on the other symptomatic field maple. Similar cankers were noted on two boxelder maples, typically sunken, swollen and also evident on the main trunk (Figure 2), but notably smaller (30×38 cm and 26×25 cm). Mats of white mycelium were visible after removing bark layers at the edge of the cankers (Figure 3). The fungus was readily isolated from both mycelial mats placed directly on PDA, or from the wood tissue chips cut from the edge of the advancing mycelial mats. Twelve isolates were collected from the trees with typical Eutypella canker symptoms. Their colonies on PDA were white and sterile, and all 12 cultures were morphologically similar. Among 12 isolates, 11 yielded 341-bp fragments reported to be specific for *E. parasitica* [10]. Nine isolates originated from field maples, while the remaining two were isolated from boxelder maples.

E. parasitica was isolated from and confirmed in four trees with typical symptoms, but the number of infected maple trees in Bundek Park may be higher. Bark cracking and possible initial phases of canker development were observed

FIGURE 1. Large canker on a field maple tree

FIGURE 2. *Eutypella canker* on a boxelder maple tree

FIGURE 3. White mycelial fans beneath the bark of field maple

on other field maples, sycamores and Tatarian maples within the park. Such trees were not sampled.

Introduction pathway of *E. parasitica* in Zagreb is unknown, but it may be independent of its introduction to the forests around Hum na Sutli. Considering the slow development of Eutypella canker symptoms [7] and the appearance of very large canker on one infected field maple in Bundek Park, the disease has probably been present in the park for decades, as speculated by Cech *et al.* [5], who described the situation with Eutypella canker of maples in Germany. From the initial focus, after *E. parasitica* perithecia develop on an infected tree, the disease may continue to spread to nearby susceptible hosts.

Finding of *E. parasitica* in urban area reflects the risk of introduction of alien, new forest pests and pathogens into urban areas, from where these harmful organisms could spread to forests causing long-term losses. Cases of Asian longhorn beetle (*Anoplophora chinensis*), redneck longhorn beetle (*Aromia bungii*) or thousand cankers disease (*Geosmithia morbida*) are clearly showing such risks [11-13]. In different European countries, all these quarantine and non-quarantine alien harmful organisms were found in gardens in urban areas [11-13] where they can establish their initial populations if efficient phytosanitary measures are not taken. Alien forest pests and pathogens can be introduced from plants imported from all over the world and distributed as "ornamental" or "horticultural" woody plants. The analysis of 123 invasive forest pathogens in Europe has shown that 38% of these organisms have been found on ornamental trees in parks and gardens, while 36% have been found in forests [14]. Trade was indicated to be among the most common pathways of the introduction and diffusion of invasive forest pathogens [14]. Early detection of an invasive forest pathogen and the investigation of pathways for its introduction and spread seem to be the only measures for finding a strategy to prevent this kind of biological invasions [14].

CONCLUSIONS

Finding of *E. parasitica* in Zagreb is the first record of this fungus in an urban area in Croatia. Boxelder maple is a new host species recorded for *E. parasitica* in Croatia, beside previously reported field maple and sycamore [6].

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