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PHOMOPSIS CAPSICI AND *COLLETOTRICHUM COCCOIDES* INFECTING PEPPER IN MACEDONIA

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Phomopsis capsici and *Colletotrichum coccodes* were found on pepper fruits during a joint expedition carried out in Macedonia. The lesions caused by *P. capsici* often occurred together and resembled slightly those incited by *C. coccodes*. Phomopsis lesions could be differentiated on the basis of pliable leathery condition of the affected tissue and of pycnidium presence while *C. coccodes* produced lesions with regular round shape and abundant acervuli, setae and microsclerotia in colonized fruit tissue. On some fruits *P. capsici* caused single infection but mixed infections of *Phomopsis* and *Colletotrichum* were observed, as well. *C. coccodes* is a soil-borne pathogen that produces long-lasting structures (microsclerotia) in the plant debris. The development of this pathogen on pepper might contribute to the building up of inoculum in the soil which could serve as reservoir for other Solanaceae. To our knowledge, this is the first report of *P. capsici* and *C. coccodes* on pepper in Macedonia.

Key words: *Capsicum annuum, Colletotrichum coccodes,* pepper anthracnose, *Phomopsis capsici*, fruit decay

INTRODUCTION

Last years, *Phomopsis capsici* (Magnaghi) Sacc and several *Colletotrichum* spp. (*Colletotrichum gloeosporioides* (Penz.) Penz. & Saccardo in Penz., *C. acutatum* Simmonds ex Simmonds and *C. coccodes* (Wallr.) S.J. Hughes) occurred on pepper in Bulgaria with increasing frequency (Rodeva et al., 2009a; 2009b; 2009c). In August 2011 a joint expedition was carried out in Macedonia related to the implementation of ERA 226 project. Two new pepper fungal pathogens were found, isolated, described and characterized. The results are presented in this paper.

MATERIAL AND METHODS

Initial isolations from diseased pepper fruits on potato dextrose agar (PDA) revealed the presence of *P. capsici* and *C. coccodes*. Four Macedonian (MK26.1, MK26.2, MK7.1, MK7.2) and one Bulgarian (B8.1) isolates of *C. coccodes* were selected for the investigations. Identification of *Colletotrichum* spp. was performed on the basis of morphological and cultural characteristics (conidial size and morphology, colony morphology and growth

rate, presence or absence of: teleomorph, setae, microsclerotia) (Sutton, 1992; Freeman et al., 1998; Tozze Jr. et al., 2006) and pathogenicity tests. Growth rate and colony appearance were studied on three nutrient media: PDA, 0.2% malt extract agar (MEA) and oatmeal agar (OA), which were inoculated with mycelial discs taken from the edge of growing colonies. For the pathogenicity tests the isolates were grown on PDA. Pin pricked detached pepper fruits were inoculated with agar plugs containing fungal mycelium. Control fruits were inoculated with sterile PDA discs. Tomato and eggplant fruits were additionally inoculated with *C. coccodes* for comparison. Fruits were incubated for 7 days at 25°C under 100% relative humidity. Reisolations were made at the end of the experiments. At least 100 conidia of each isolate were measured on the images with Carnoy program.

Total DNA of investigated *Colletotrichum* isolates was extracted directly from mycelium by DNeasy Plant mini kit (Qiagen, Hilden, Germany). PCR amplifications were performed with both *Colletotrichum*-specific primer set Cc1F1/Cc2R1 and *C. coccodes*-specific nested primers Cc1NF1/Cc2NR1. The gels were visualized by UV transillumination, their electronic images were taken by ImageQuant150 imager (GE Healthcare) and densitometrically analyzed with ImageQuantTL7 software (GE Healthcare) to determine the approximate length of the resulting PCR products.

RESULTS

C. coccodes was isolated mainly from fruits, seeds of heavily infected fruits and occasionally from roots although it could infect stems and leaves. The disease symptoms were observed in the area of Kochani (village Dolni Podlog) on variety Kurtovska kapija and in Strumica (village Bosilovo) also in the postharvest period. Fruit anthracnose appeared first as small, circular, slightly sunken lesions on the surface of ripening fruits (Fig. 1a). Majority of infections were observed on ripe or over-ripe fruits. The spots quickly enlarged in concentric circles, coalesced, become deeply sunken with dark brown border and developed a water-soaked appearance directly beneath the skin (epidermis) of the fruit (Fig. 1b). At first small rounded acervuli containing rose conidial mass were developed on the surface and beneath the lesion (Fig. 1c,e). Later then the fungus formed small, dark survival structures called sclerotia (Fig. 1d).

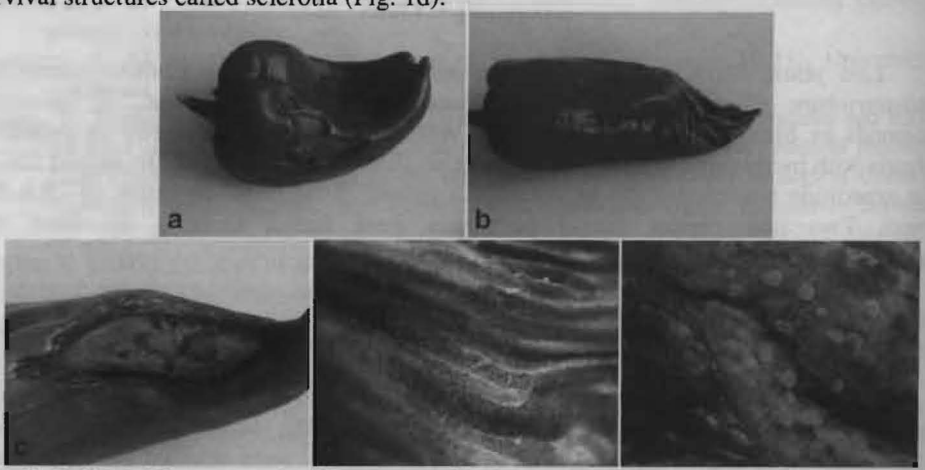

Fig. 1. *Colletotrichum coccodes*: Symptoms of anthracnose on pepper fruits, early infection (a); coalescent lesions (b); young lesions with sporulating acervuli (c); fully developed lesions with microsclerotia (d); sporulating acervuli (e)

The *C. coccodes* colonies were slowly growing. On the ninth day the highest growth rate was recorded on OA (49.5±5.1 mm) and PDA (47.8±4.8 mm) (Fig. 2c,a) and the lowest – on MEA (39.0±7.6 mm) (Fig. 2b). Bulgarian isolate had higher growth rate than Macedonian ones on all nutrient media used in the study. The colony colour was gray with rose nuance mainly in the periphery, where acervuli with conidia developed. With aging a great number of microsclerotia appeared under mycelium.

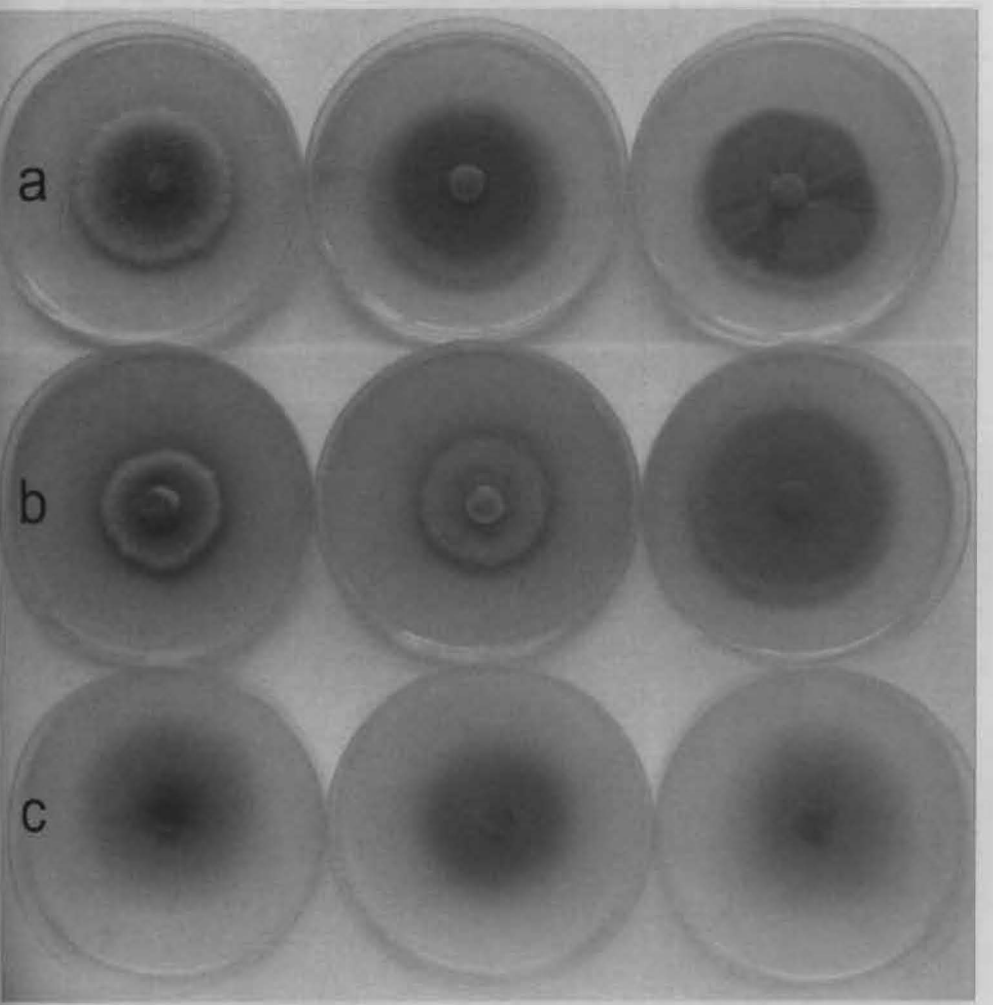

Fig. 2. *Colletotrichum coccodes*: Appearance of 10 days old colonies potato dextrose agar (a), malt extract agar (b) and oatmeal agar (c) (left and middle column Macedonian isolates, right column – Bulgarian isolate)

Conidia were hyaline, straight, cylindrical, aseptate with two to seven oil globules measuring (19.2) 21.3±1.7 (24.6) x (3.1) 4.1±0.4 (4.7) µm (Fig. 3a). Acervuli with setae longer than 100 µm developed (Fig. 3b).

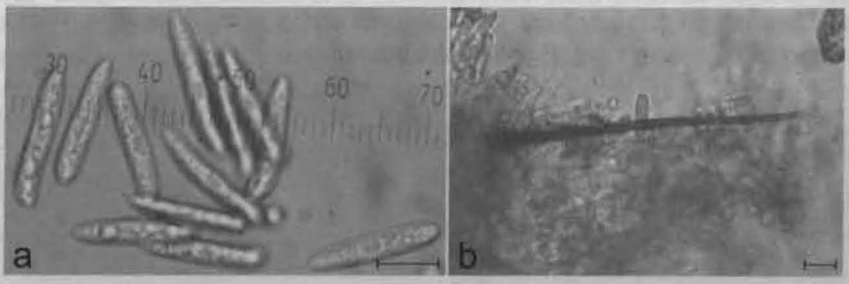

Fig. 3. *Colletotrichum coccodes*: Conidia (a) and acervulus with conidiophores, conidia and setae (b) (Scale bars = 10 µm)

All investigated *C. coccodes* isolates were pathogenic for pepper, tomato and eggplant (Fig. 4a,b,c). Water-soaked circular lesions appeared three days after inoculation (dai) that became soft and slightly sunken. Wet, gelatinous conidial mass from fungal fruiting bodies (acervuli) gradually covered the lesions. About 10 - 14 dai the central lesion part darkened where abundant microsclerotia developed.

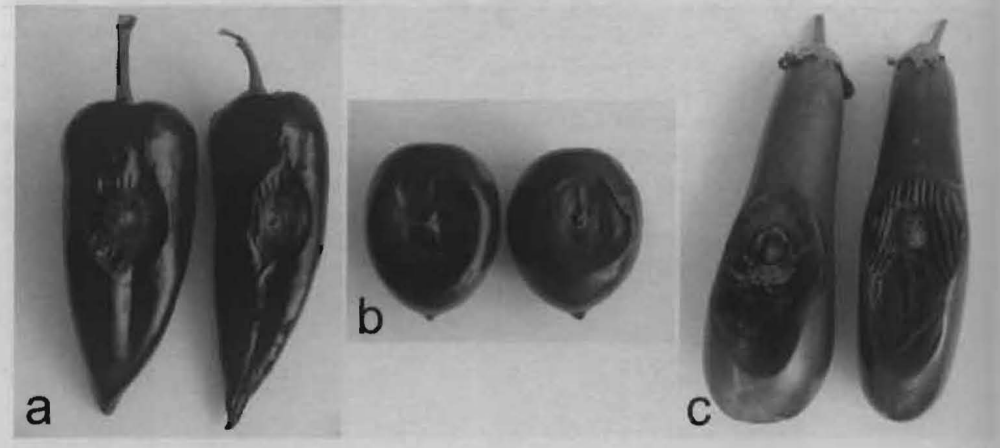

Fig. 4. *Colletotrichum coccodes*: Symptoms on artificially inoculated pepper (a), tomato (b) and eggplant (c) fruits – 14 days after inoculation

PCR amplification with genus-specific primers (Cc1F1/Cc2R1) gave a single band of ~450 bp in all isolates analyzed (*C. coccodes* and *C.* sp.) as expected from the literature (Cullen at al., 2002) (Fig. 5A). However, with the nested primer set Cc1NF1/Cc2NR1, a single specific PCR band of expected size (~350bp) was obtained only in those reactions containing as a template DNA from investigated *C. coccodes* isolates (Fig. 5B).

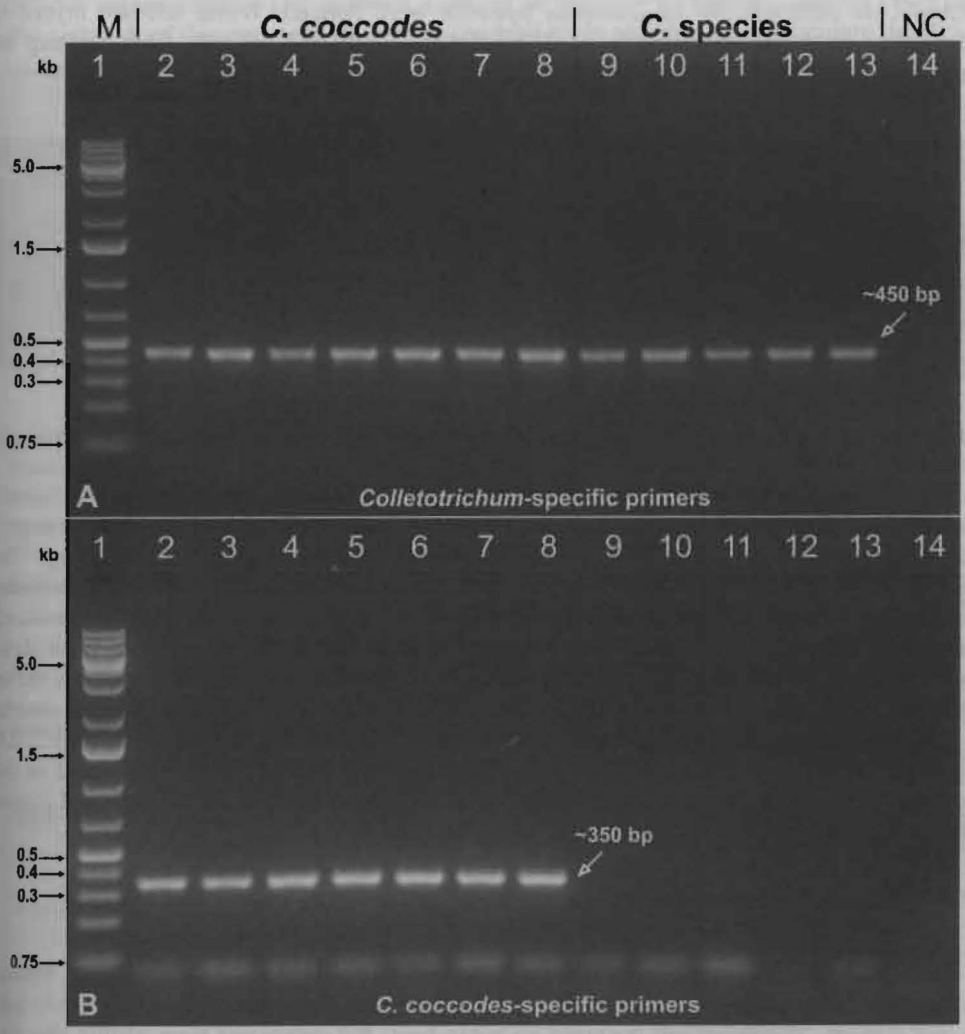

Fig. 5. Molecular identification of different *Colletotrichum* species: Gel (A): PCR amplification with primers Cc1F1/Cc2R1; gel (B): PCR amplification with *C. coccodes*-specific primers Cc1NF1/Cc2NR1; Lanes 2-13 (*C. coccodes* isolates B8.1, B2.1, B40.1a, MK26.1, MK26.2, MK7.1, MK7.2 and *C.* sp. isolates B27, B1.1, B29, S2, S3); lanes 14: Negative controls (mQ water); lanes 1: DNA marker GeneRuler 1kb+ (Fermentas).

P. capsici was found in the village Zubovo, Strumica region, on pepper fruits cv. Zubovska kapija (domestic pepper variety of Kurtovska kapija, which is grown only in this village). Until now *P. capsici* was not recorded on pepper anywhere else in the country. The symptoms of *P. capsici* on the fruits appeared as brown rot extending in wavy rings more rapidly longitudinally than laterally in the tissue (Fig. 6a). Infection progress led to fruit decay. The dead tissue became dry and bleached in the centre where black globose to subglobose subepidermal or erumpent pycnidia were noticed. White felt-like mycelium developed inside the damaged fruits. The fungus was isolated not only from pericarp but also from seeds of diseased pepper fruits.

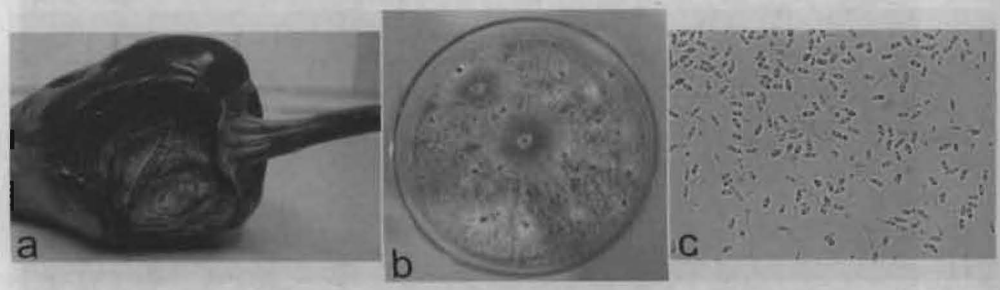

Fig. 6. *Phomopsis capsici*: symptoms (a), colony morphology (b) and alpha and beta conidia (c)

P. capsici developed fast growing colonies on PDA (Fig. 6b). They were initially white, later developing light to dark brown patches and reached the Petri dish borders 7 days after inoculation. Small black pycnidia (150-250 μm) developed after 10-12 days. The extruded conidia were visible as yellowish globose slime. The reverse sides of the colonies were grayish with darker regions coinciding with conidiomata. The isolates produced abundant alpha conidia – unicellular, straight, ovoid to oblong-fusoid, hyaline, biguttulate, with averrage dimensions 6.8 x 2.9 μm, scarce beta conidia which are unicellular, curved or hamate, eguttulate, with averrage dimensions 29.8 x 1.8 μm and very rarely gamma conidia – unicellular, straight, paddle shape, multiguttulate, with averrage dimensions 11.0 x 2.9 μm (Fig. 6c). The first ones only are viable and infective. No perithecia were found on the over wintered diseased pepper fruits or in culture. Artificial inoculations of detached pepper fruits led to successful infection ten day after inoculation.

DISCUSSION

Anthracnose of pepper caused by *C. coccodes* appeared to be a devastating disease of ripe fruits causing severe damages to both field and post harvest levels in warm and rainy seasons. The infections occurred on green fruits but symptoms were visible after the ripening. During the season the pathogen was spread from infected to healthy fruits with conidia splashed by rain, overhead irrigation, or by picking fruit from wet plants. The lasting structures called sclerotia could survive in soil for up to three years and cause infections either directly or by producing secondary spores. The lesions caused by *P. capsici* often occurred together and resembled slightly those resulting from infection by *C. coccodes*. Phomopsis lesions could be differentiated on the basis of pliable leathery condition of the affected tissue and of pycnidium presence while *C. coccodes* produced lesions with regular round shape and abundant microsclerotia in colonized fruit tissue. On

some fruits *P. capsici* caused single infection but mixed infections of *Phomopsis* and *Colletotrichum* were observed, as well.

To our knowledge, this is the first report of *C. coccodes* and *P. capsici* on pepper in Macedonia. Recently, *C. coccodes* has been reported in Bosnia and Herzegovina (Trkulja et al., 2008). *C. coccodes* is an important soil-borne pathogen that produces long-lasting structures (microsclerotia) in the dying plant parts, with host range in Solanaceae that includes pepper, tomato and eggplant, causing anthracnose and potato, causing black-dot. The outbreak of this pathogen on pepper can lead to an enrichment of inoculum density in the soil serving as an important source of inoculum for other solanaceous crops.

ACKNOWLEDGEMENTS

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