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CHARACTERIZATION OF *BOTRYTIS CINEREA* ISOLATES FROM SMALL FRUITS AND GRAPEVINE IN SERBIA

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Abstract — Twenty-six single-spore isolates of *Botrytis cinerea* from blackberry, raspberry, strawberry, and grapevine were investigated using transposable elements, morphological characterization, and sensitivity to fungicides. Both transposable elements, Flipper and Boty, were detected among isolates from all the hosts. Six *vacuma* (without transposable elements) and seven *transposa* (containing both elements) isolates were found to be present in sympatry in Serbia. Isolates containing only the Boty element were detected. Eight morphological types of colonies on PDA and MA media were observed, confirming the great phenotypic variability of *B. cinerea*. Sensitivity to fungicides was various, depending on both the fungicide and the isolate.

Key words: Gray mold, disease, small fruits, transposable elements, *Boty, Flipper, vacuma, transposa*

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INTRODUCTION

Botrytis cinerea Pers. Fr., the anamorph of *Botryotinia fuckeliana*, is a pathogen that causes gray mold on a wide variety of plants worldwide. It is one of the main factors that influence berry production in Serbia (Nikolic et al., 2008). Yield losses in commercial fields exceed 50%, especially during periods of rainy, wet weather right before harvest. In addition, the fungus causes significant losses during shipping and marketing (Ellis and Grove, 1982; Nikolic et al., 2008).

An important biological feature is that the process of infection by *B. cinerea* is often associated with prior colonization of dead or dying plant debris as a nutrient-providing saprotrophic base (Jervis, 1980). It can also establish latent infections, which may lead to destructive postharvest gray mold (Baraldi et al., 2002). Many studies have reported that this fungus can exhibit great phenotypic diversity (Grindle, 1979; Di Lena et al., 1981; Chardonnet et al., 2000; Yourman et al., 2001). Somatic variability among isolates is often attributed to the multinucleate and heterokaryotic nature of hyphae or conidia and the aneuploid state of nuclei (Hansen and Smith, 1932; Buttner et al., 1994). Recent studies on French and Chilean populations of *B. cinerea* provided a new finding that this species is composed of two sympatric species, *transposa* and *vacuma*. *Transposa* was characterized by the presence of two transposable elements, *Boty* and *Flipper*, whereas strains of *vacuma* had neither (Giraud et al., 1997, 1999; Munoz et al., 2002). In addition, a certain degree of host specialization seems to exist in the pathogen (Giraud et al., 1997). Another study has shown that there is significant genetic differentiation among isolates collected from different host plants in France, and that the level of fungicide resistance differs significantly in *transposa* and *vacuma* (Giraud et al., 1999). Moreover, some *vacuma* isolates were naturally resistant to the hydroxyanilide fungicide fenhexamid (Albertini et al., 2002; Fournier et al., 2002; Leroux et al., 2002), a recently introduced fungicide with a high preventive activity against gray mold on various crops. Since the management of gray mold control involves chemical and biological methods,

use of organic systems, and protection programs based on disease monitoring and prediction, information on genetic structure of the pathogen population could help us to develop effective strategies for its control. Accordingly, the aims of this study were: a) to determine whether two sympatric species, *transposa* and *vacuma*, are present in pathogen populations on several economically important crop species in Serbia; b) to characterize some biological features of the isolates from different hosts and from both subpopulations, viz., morphology of the colony, growth rate, sporulation, and sclerotial production on different growth media; and c) to evaluate sensitivity of the isolates to the fungicides which are frequently used for *B. cinerea* control.

MATERIALS AND METHODS

Chemicals

The following fungicides were used: vinclozolin (Ronilan WG, 500 g/kg, BASF), benomyl (Benfungin WP, 500 g/kg, Galenika-Fitofarmacija), thiophanate-methyl (tested formulation, 700 g/kg, Agromarket), fenhexamid (Teldor SC, 500 g/l, Bayer CropScience), pyrimethanil (Mythos SC, 300 g/l, Aventis CropScience), and cyprodinil (Chorus 75-WG, 750 g/kg, BASF).

Isolates

All the isolates were derived from decaying fruits after incubation of the plant material in moist chambers for seven days at 20°C. Isolates were then purified by monospore isolation and assigned an uppercase letter indicating the host from which they were collected: S – strawberry, R – raspberry, B – blackberry, and G – grapevine, followed by a number. Control strains 632, 412, and 397 were kindly provided by E. Fournier from INRA Centre de Versailles, France, whereas strains 5144 and 5145 were obtained from the DSMZ Collection of microorganisms from Germany.

Pathogenicity test

The pathogenicity test was performed as described by Vignutelli et al. (2002) with slight modifications. Apples (cv. Golden Delicious) were divided in half, surface sterilized with ethanol (70%), and cut-injured with a cork borer at three positions on each half. Mycelial disks were placed beneath circularly cut parts. Sterile PDA disks were used as a control. The isolates were considered pathogenic if fruits showed soft rotting after two to four days of incubation at 20°C in the dark.

Maintenance

The isolates were cultured on potato-dextrose-agar medium (PDA) at 20°C and stored on slants at 4°C.

Identification

The isolates were identified on the basis of pathogenic characteristics, morphology of the colony, and microscopic observations of conidiophores and conidia.

Mycelial growth in vitro

The *in vitro* growth rate was determined by transferring mycelial plugs (10 mm in diameter) from the edge of 4-day-old colonies on PDA and malt-extract-agar medium (MA). Growth was recorded after 3-day incubation at 20°C by measuring two diameters of each colony at right angles. Three replicates per isolate per medium were used and the experiment was repeated twice.

Morphological characterization

The isolates were grown on PDA and MA media for three weeks. Afterwords, phenotypic observations were performed macroscopically, taking into account mycelial aspect, sporulation, and sclerotial production. Eight different morphological types were recognized: four mycelial (M1, M2, M3, and M4) and four sclerotial (S1, S2, S3, and S4) (Martinez et al., 2003). The isolates were distributed into these classes. The average number of sclerotia per isolate per plate was also recorded on both media.

DNA isolation

We obtained DNA directly by scraping mycelia with a pipette tip from 4-day-old culture on PDA. The mycelia was transferred into 50 µl of PrepMan Ultra Sample Preparation Reagent (Applied Biosystems,

CA, USA), vortexed briefly, incubated for 30 min at 56°C and then for 10 min at 100°C, and stored at - 20°C until use (Harrington and Wingfield, 1995). The DNA quality from each isolate was confirmed to be suitable for the polymerase chain reaction (PCR) by generation of a single band with universal primers ITS1 and ITS4 (White et al., 1990).

Molecular identification

Identification of the pathogen was confirmed by the PCR reaction using primers C_{729+} and C_{729-} (Rigotti et al., 2002), which amplify a 0.7-kb product. The primers were synthesized by Fermentas (Lithuania). The reactions were conducted in a final volume of 25 µl containing: 1 X Master mix (Fermentas, Lithuania) (0.625 U Taq polymerase, 2 mM $MgCl_2$, 0.2 mmoles of each of the dNTPs), 1 µl of primers (20 µM), and 1 µl of fungal DNA. Reactions were performed in Eppendorf Master Cycler programmed as follows: 1 cycle at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 37°C for 30 s, 72°C for 1 min, and one cycle at 72°C for 10 min. Separation of PCR products was accomplished on a 1% agarose gel in TBE buffer (86 mM Tris, 89 mM boric acid, 2 mM EDTA) and observed by UV illumination after staining with ethidium bromide.

Detection of transposable elements 'Flipper' and 'Boty'

Flipper – a 1872-bp class II element (Levis et al., 1997) – and *Boty* – a 6-kb retrotransposon (Diolez et al., 1995) – have been identified in *B. cinerea*. Isolates were tested for the presence of transposable elements using the duplex PCR scheme described by Munoz et al. (2002). The primers used for detection of the *Flipper* element (F300: 5'-GCA CAA AAC CTA CAG AAG A-3' and F1550: 5'-ATT CGT TTC TTG GAC TGT A-3') amplify a 1250-bp product that corresponds to the major part of the *Flipper* element (GenBank association no. U74294). The primers used to detect the *Boty* element (LTR98: 5'-AGC CTG TAG AAT CAC CAA CG-3' and LTR28: 5'-CGG TAT TTC TGG TTG GCA-3') amplify a 648-bp product that spans the region corresponding to the long terminal repeat (LTR) of *Boty* (GenBank association no. X81790). The primers were synthesized by Fermentas (Lithuania). A single PCR reaction was performed to detect both transposable elements because of the difference in size of the amplified products. The reactions were conducted in a final volume of 25 µl containing: 1 X Master mix (Fermentas, Lithuania) (0.625 U Taq polymerase, 2mM $MgCl_2$, 0.2 mmoles of each of the dNTPs), 1 µl of each primer (20 µM), and 1 µl of fungal DNA. The thermal cycler was programmed as follows: 4 cycles of 3 min at 95°C, 1 min at 60°C, and 2 min at 72°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C, and 90 s at 72°C. A final 5 min of incubation was carried out at 72°C. Amplified products were separated on 1.5% agarose gel in TBE and stained with ethidium bromide. Strains 397 (*transposa*) and 412 (*vacuma*) were used as positive and negative control, respectively. Samples that gave negative reactions for the presence of transposable elements were reexamined in separate reactions. The presence of the *Boty* element was tested using another pair of primers (BotyF4: 5'-CAG CTG CAG TAT ACT GGG GGA-3' and BotyR4: 5'-GGT GCT CAA AGT GTT ACG GGA G-3'), which amplify a 510-bp product (Ma and Michailides, 2005). Samples that were negative for the presence of *Flipper* were rechecked using the same pair of primers (F300 and F1550) in a separate reaction. The reaction mixture was the same as that used in the duplex PCR, while the thermal cycler was programmed as follows: an initial preheat for 3 min at 95°C, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 60°C for primers F300 and F1550 or 68°C for BotyF4 and BotyR4 for 40 s, extension at 72°C for 1 min, and termination with final extension at 72°C for 10 min. Products were separated on 1% agarose gel and stained with ethidium bromide. All negative PCR reactions were performed three times.

Sensitivity of isolates to fungicides

Sensitivity of isolates was determined in radial growth experiments on PDA medium amended with discriminating concentrations of fungicides (1 and 5 mg/l of benomyl, tiophanat-methyl, vinclozolin, and fenhexamid or 2.5 and 5 mg/l of pyrimethanil and cyprodinil), allowing growth of resistant but fully inhibiting growth of sensitive isolates (Stehman and De Waard, 1996). Fungicides were suspended in sterile destilled water and added to autoclaved media

that had cooled to 50°C. Petri dishes were inoculated with inverted mycelial plugs (10 mm) cut at the edge of 4-day-old colonies on PDA, and incubated for 48 h at 20°C. The experiment was conducted in four replicates and repeated twice. Strains 5144 – sensitive to benzimidazols (benomyl and tiophanat-methyl) and dicarboximides (vinclozolin) – and 5145 – resistant to benzimidazols and dicarboximides – were used as control strains in the experiment. Isolates that did not grow at the lower concentration of a fungicide were designated as sensitive, those that were able to grow at the higher concetration were considered as resistant, while those that grew at the lower but not the higher concentration were classified as weakly resistant.

RESULTS

Disease symptoms

The most common disease symptoms found in investigated hosts were decay, necrotic lesions, and flecking or rotting of fruits. Infecton of small fruits occurred primarily at the flower stage of fruit development, remained quiescent until the fruits matured, and then developed rapidly, causing large brown lesions, typically at the stem end of the ripening fruit, accompained by profuse sporulation of the pathogen (Figs. 1 and 2).

Fig. 1. *Botrytis cinerea*: gray mold on naturally infected strawberry fruit.

Fig. 2. *Botrytis cinerea:* gray mold on naturally infected raspberry fruits.

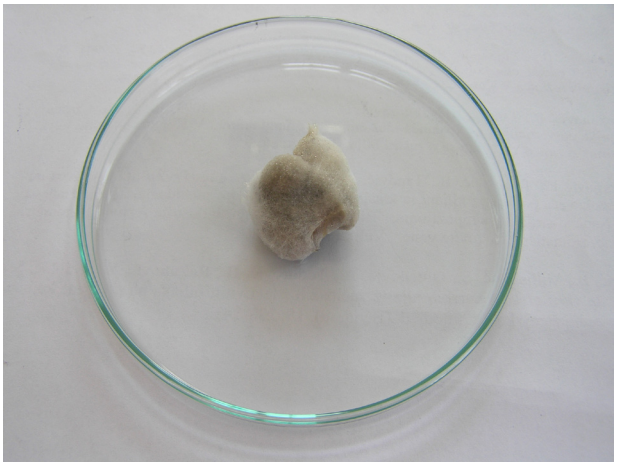

Fig. 3. *Botrytis cinerea:* thick and woolly mycelium on raspberry fruit after 7-day incubation in moist chamber at 20°C.

After 7-day incubation of diseased fruits in a moist chamber at 20°C, thick and woolly mycelium was developed (Fig. 3). In total, 26 isolates were derived by transferring the mycelium onto PDA medium (Table 1).

Table 1. List of *B. cinerea* isolates collected from different host plants in Serbia and their classification as *vacuma, transposa*, and *Boty*. [1]Isolates without transposable elements, [2]Isolates containing only the *Boty* transposable element, [3]Isolates containing both the *Boty* and *Flipper* transposable elements.

Host	Number of isolates	Type of isolates		
		vacuma[1]	*Boty*[2]	*transposa*[3]
Blackberry	8	1	6	1
Raspberry	7	1	3	3
Strawberry	7	4	2	1
Grapevine	4	0	2	2
Total	26	6	13	7

Fig. 4. *Botrytis cinerea:* soft rot of artificially inoculated apple fruit after 4-day incubation at 20oC (left) and control (right).

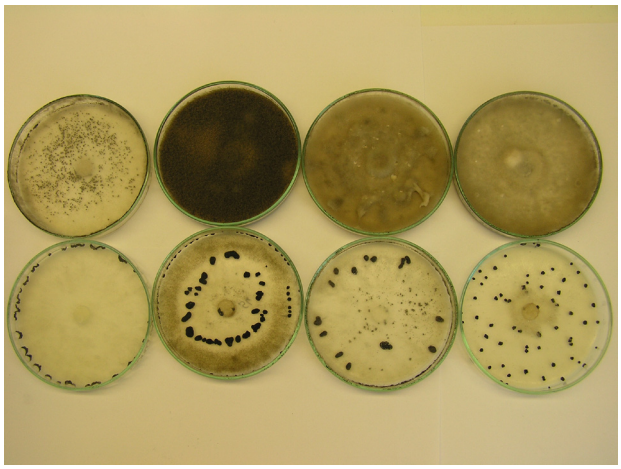

Fig. 5. *Botrytis cinerea:* morphological types of colonies: mycelial (upper row - left to right: M1 - short mycelium without sporulation, M2 - aerial mycelium with sporulation, M3 - mycelial masses, and M4 - thick and woolly mycelium) and sclerotial (row below - left to right: S1- sclerotia on the edge of Petri dish, S2 - sclerotia large and in circle, S3 - sclerotia large, placed irregularly, and S4 - sclerotia small and scattered).

Pathogenicity tests

All tested isolates caused soft rotting of apple fruits after two to four days of incubation (Fig. 4). The pathogen was succesfully reisolated from inoculated fruits so that the pathogenicity of isolates was confirmed.

Morphological characterization

Colonies of *B. cinerea* on PDA and MA media were

Table 2. Phenotypic classification of *B. cinerea* strains on PDA and MA media.

Phenotype	Mycelial type „M“				Sclerotyal type „S“			
	M1	M2	M3	M4	S1	S2	S3	S4
Mycelium	Short	Aerial	Mycelial masses	Thick and woolly	Rather short	Rather short	Rather short	Rather short
Sporulation	-	+	±	-	±	±	±	-
Sclerotia	-	-	-	-	On the edge of Petri dish	Often large, in circle	Often large, placed irregularly	Numerous, small and scattered
Number of isolates on PDA medium	3	0	1	7	6	-	6	3
Number of isolates on MA medium	4	1	-	8	-	-	13	-
Control strains on PDA medium	-	1	-	-	-	3	1	-
Control strains on MA medium	-	2	-	-	1	1	1	-

classified visually into eight morphological types (Fig. 5). Two main morphological types, without marked differences in sporulation, were identified: mycelial (characterized by the absence of sclerotia) and sclerotial (including colonies with numerous sclerotia). The isolates were mostly of the sclerotial type on PDA medium (61.3%), while the number of isolates of the sclerotial and mycelial type on MA medium was almost equal (16 sclerotial and 15 mycelial). Sporulation on PDA medium was recorded in 6.4% of isolates, whereas 36.7% of isolates produced conidia on MA medium.

The frequency distributions into morphological classes on both media are presented in Table 2.

Mycelial growth rate, sclerotia formation, and sporulation

The isolates showed significant differences in mycelial growth rate at 20°C on both media (Table 3). A statistically significant difference was found in the number of sclerotia per plate (Table 4).

The growth rate was also influenced significantly by the type of medium: faster growth and more sclerotia were recorded on MA medium (Figs. 6 and 7). The growth rate on PDA medium varied from 7.3 to 26.7 mm/day, while the lowest growth rate on MA medium was 10.1 mm/day. Moreover, only two isolates had a growth rate higher than 25 mm/day on PDA, whereas on MA medium 20 isolates had a growth rate higher than 25 mm/day. The number of sclerotia also varied depending on the isolate and media, and ranged from 0 on both media to 70.7 on PDA or 114 on MA medium, respectively. A strong correlation was found between the growth rate of isolates on PDA and MA media (r_{growth} = 0.88), as well as between the number of sclerotia on both media ($r_{scler.}$ = 0.86).

Template DNA

All of the isolates amplified an expected product of approximately 600 bp, using universal primers ITS1 and ITS4. It was thereby confirmed that the quality and concentration of template DNA was suitable for further PCR reactions (Fig. 8).

Molecular identification

Based on pathogenic characteristics, morphology of the colony, and microscopic observations of conidiophores and conidia, the pathogen was identified as *Botrytis cinerea*, the anamorph of *Botryotinia fuckeliana*. In order to confirm identity of the pathogen, a PCR reaction was conducted, using primer pair $C_{729+/-}$. A PCR product with expected size of 0.7 kb, specific for *B. cinerea*, was detected (Fig. 9) in 29 of 31 isolates tested (control strains are included). Two isolates, one from grapevine and control strain 632,

Table 3. Analysis of variance of the daily mycelial growth rate of *B. cinerea* on PDA and MA media. [1]ANOVA -Two-factor without replication.

ANOVA[1]						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Media	1370.52	1	1370.52	74.24652	1.3E-09	4.170877
Isolates	8909.444	30	296.9815	16.08866	1.14E-11	1.840872
Error	553.7715	30	18.45905			
Total	10833.74	61				

Table 4. Analysis of variance of the number of sclerotia of *B. cinerea* on PDA and MA media. [1]ANOVA -Two-factor without replication.

ANOVA[1]						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Media	2256.065	1	2256.065	9.45375	0.004463	4.170877
Isolates	52090.97	30	1736.366	7.276019	2.48E-07	1.840872
Error	7159.269	30	238.6423			
Total	61506.31	61				

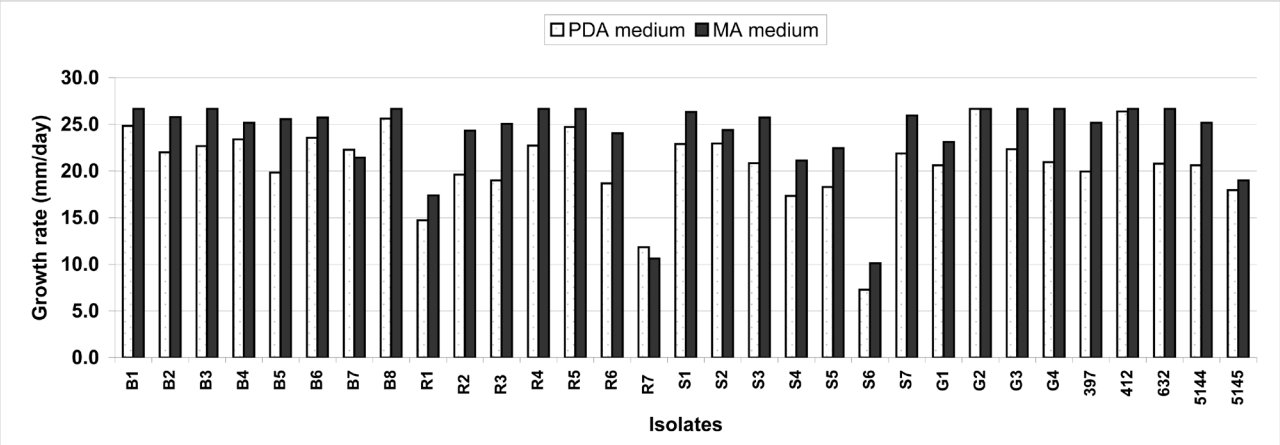

Fig. 6. Growth rate of isolates of *Botrytis cinerea* on PDA and MA media.

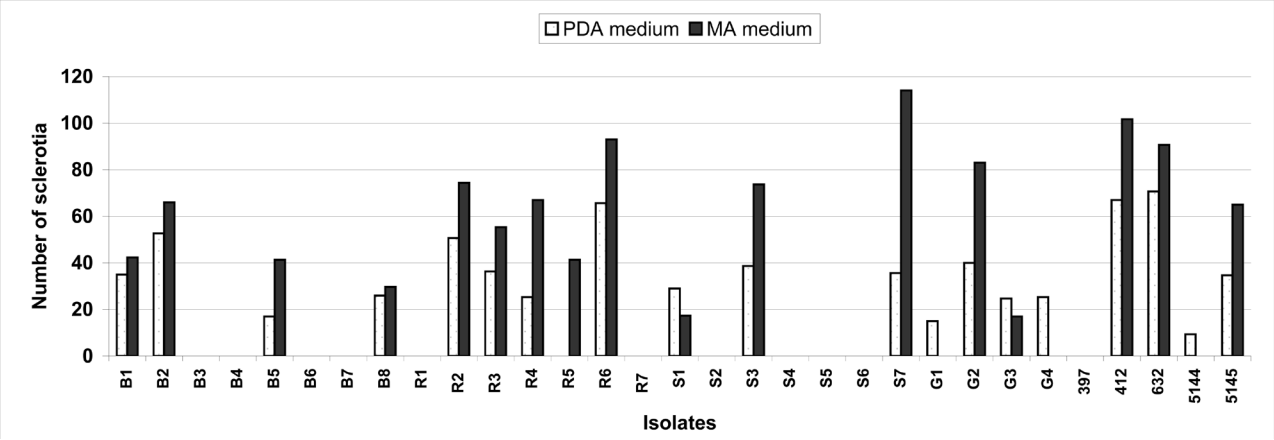

Fig. 7. Number of sclerotia formed by isolates *B. cinerea* on PDA and MA media.

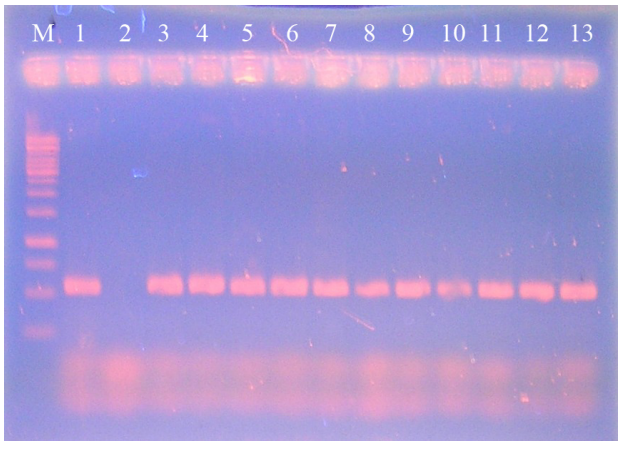

Fig. 8. Amplification of PCR products of approximately 600 bp with DNA of the following *Botrytis cinerea* isolates: positive control strain 397 (line 1); negative control (line 2); isolates B1, B2, B3, B4, B5, B6, B7, B8, R1, R2, R3, R4 (lines 3 to 14, respectively); M = molecular weight marker 1 kb ladder.

did not amplify the expected product.

Detection of transposable elements 'Flipper' and 'Boty'

The presence or absence of two transposable elements (*Flipper* and *Boty*) was tested in every strain using the PCR reaction. Strains from the *transposa* subpopulation contained both transposable elements, whereas strains of the *vacuma* subpopulation had neither. However, a third type of isolates having only the *Boty* transposable element was found in our samples. The resulting molecular determination of elements is presented in Table 1. Using duplex PCR, the *Flipper* transposable element was detected in six samples, while none of the isolates amplified the expected 648-bp product corresponding to the *Boty* element (Fig. 10). In additional separate PCR reac-

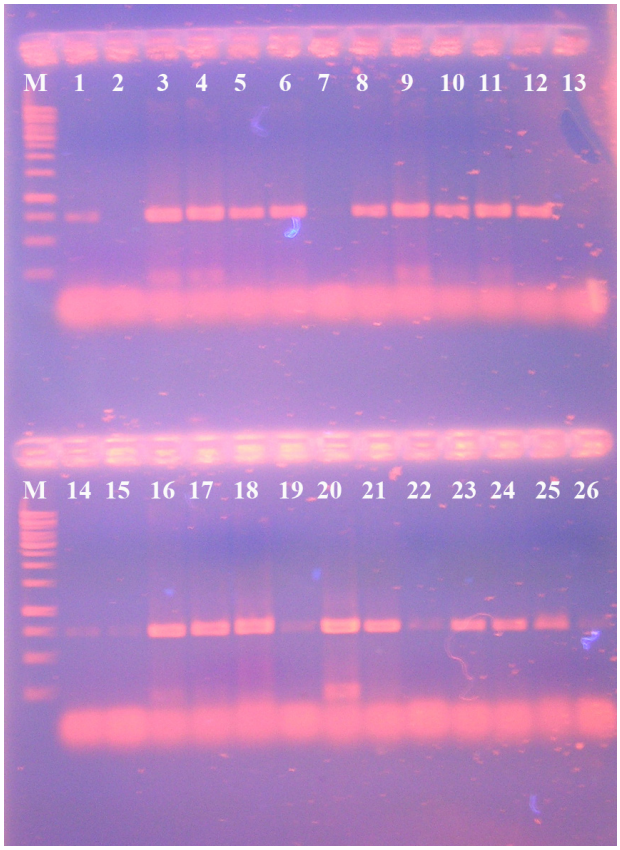

Fig. 9. Amplification of PCR products of 0.7 kbp with DNA of the following *Botrytis cinerea* isolates: positive control strain 397 (line 1); negative control (line 2); isolates B1, B2, B3, B4, 632, B5, B6, B7, B8, R1, G1, G2, G3, G4, S1, S2, S3, S4, S5, S6, S7, R2, R3, and R4 (lines 3 to 26, respectively); M = molecular weight marker 1 kb ladder.

tions, the *Flipper* element was detected in one isolate using the same pair of primers, whereas the *Boty* element was found in 20 isolates using primers that amplify a 510-bp product (Fig. 11).

Sensitivity of isolates to fungicides

Qualitative tests for fungicide resistance were carried out for the total number of isolates using discriminatory concentrations. In all of the experiments, mycelial growth of the control strain 5144 (sensitive to benzimidazols and dicarboximides) was completely inhibited on PDA medium amended with benomyl, thiophanate-methyl, or vinclozolin at 1 mg/l, while the resistant control strain 5145 was able to grow at 5 mg/l. The number of isolates in each of the sensitivity-phenotypes is presented in Table 5. All isolates were highly sensitive to vinclozolin;

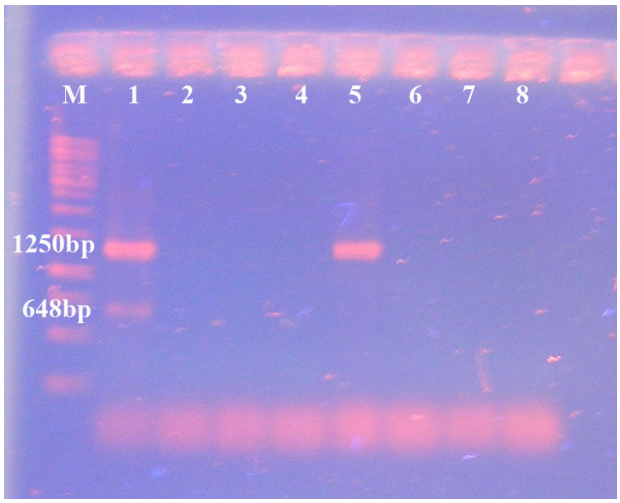

Fig. 10. Amplification products of 1250 and 648 bp obtained by duplex PCR with DNA from the following *Botrytis cinerea* strains: positive *transposa* control strain 397 (line 1); negative *vacuma* control strains 412 and 632 (lines 2 and 3, respectively); isolates B1, R1, S1, S2, and S4, (lines 4 to 8, respectively); M = molecular weight marker 1 kb ladder.

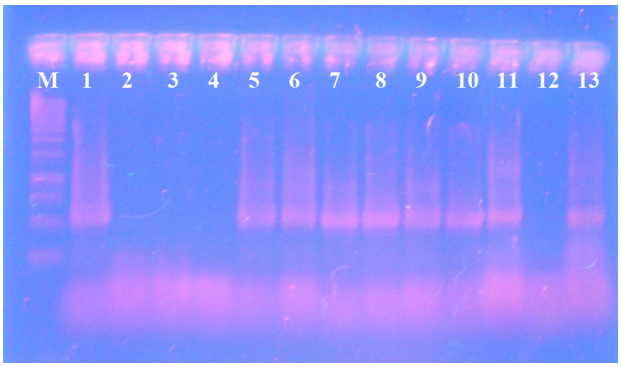

Fig. 11. Amplification of PCR products of 510 bp with DNA of the following *Botrytis cinerea* isolates: positive *transposa* control strain 397 (line 1); negative vacuma control strains 412 and 632 (lines 2 and 3, respectively); isolates B4, G1, G2, G3, G4, R1, R2, R3, S1, S3 (lines 4 to 13, respectively); M = molecular weight marker 1 kb ladder.

none of the isolates were classified as low-resistant to vinclozolin. On the other hand, sensitivity to the other fungicides varied, depending on the isolate. Of all 26 isolates tested, nine isolates were low resistant or resistant to benomyl. Pyrimethanil and cyprodinil were less toxic to the isolates than the other fungicides used in the experiment; 10 of 26 isolates were able to grow at 5 mg/l of either pyrimethanil- or cyprodinil-amended PDA medium. Under the present experimental conditions, two isolates were low- and nine were resistant to fludioxonil. The B3

Table 5. Number of *B. cinerea* isolates collected from small fruit crops and grapevine in each of three fungicide-sensitivity phenotypes. (1) Phenotypes were: Sensitive – S - radial growth fully inhibited at 1 mg/l (or 2.5 mg/l of pyrimethanil or cyprodinil), Low-resistant –LR - radial growth inhibited by 5 mg/l or Resistant – R - radial growth not inhibited by 5 mg/l, (2) Number of isolates.

Host	Number of isolates	Fungicide	Sensitivity phenotype[1,2]		
			S	LR	R
Blackberry	8	Benomil	6	1	1
		Thiophanate-methyl	5	2	1
		Vinclozolin	8	0	0
		Pyrimethanil	2	0	6
		Cyprodinil	2	0	6
		Fenhexamid	4	1	3
Raspberry	7	Benomil	4	1	2
		Thiophanate-methyl	4	2	1
		Vinclozolin	7	0	0
		Pyrimethanil	3	1	3
		Cyprodinil	3	1	3
		Fenhexamid	4	0	3
Strawberry	7	Benomil	5	0	2
		Thiophanate-methyl	5	0	2
		Vinclozolin	7	0	0
		Pyrimethanil	5	1	1
		Cyprodinil	5	1	1
		Fenhexamid	4	1	2
Grapevine	4	Benomil	3	0	1
		Thiophanate-methyl	3	0	1
		Vinclozolin	4	0	0
		Pyrimethanil	4	0	0
		Cyprodinil	4	0	0
		Fenhexamid	3	0	1

isolate from blackberry showed the lowest level of sensitivity; it was resistant to five of six fungicides used in the experiment.

DISCUSSION AND CONCLUSIONS

Great phenotypic diversity of *Botrytis cinerea* isolates from different host plants was found in this study. Eight morphological types of colonies on PDA and MA media were detected, similar to those observed by Martinez et al. (2003). The number of isolates that produce sclerotia on PDA medium was higher than on MA medium. On the contrary, the number of sclerotia per isolate was much higher on MA compared to PDA medium. In addition, sporulation was observed more frequently on MA medium.

Two isolates, one from grapevine and control strain 632, did not amplify the expected product of 0.7 kb, specific for *B.cinerea*, so the identity of the isolates was not confirmed molecularly. In view of the fact that these two isolates are typical representatives of the species *B. cinerea* (in regard to pathogenicity, colony morphology, and shape of conidiophores and conidia), further investigation is needed to explain the lack of this species-specific amplification.

Both transposable elements, *Flipper* and *Boty*, were found in isolates derived from all host plants included in the study. The two groups *transposa* and *vacuma* are therefore sympatric in Serbia and thus not restricted to the region of Champagne (Giraud et al., 1997), Chile (Munoz et al., 2002), and California (Ma and Michailides, 2005). *Transposa* and *vacuma* subpopulations were also detected in grapevine in Croatia (Topolovec-Pintaric et al., 2004). In this study, isolates containing only the *Boty* element were

also detected among isolates from all hosts. Such strains were previously found in France (Giraud et al., 1999) and Chile (Munoz et al., 2002), but their frequency was low. Under the present experimental conditions, this subgroup was dominant. More studies are needed to determine if this means that *Boty* is invading the *vacuma* group, or these strains belong to another subpopulation of *B. cinerea*. The activity of transposable elements may influence genomic regions that are involved in vegetative growth. Integration of transposable elements in fungal chromosomes has been shown to influence DNA sequence and gene expression (Daboussi, 1997). The presence and activity of transposable elements may negatively influence fungal fitness (McDonald, 1993). A previous study (Martinez, 2003) showed that the *vacuma* subpopulation was characterized by a faster mycelial growth rate than the *transposa* subpopulation. In the present study, isolates from the *vacuma* group did not exhibit a higher growth rate, neither were they less sensitive to fludioxonil as described previously (Albertini et al., 2002; Fournier et al., 2002; Leroux et al., 2002). Further evaluation of the fitness and fungicide sensitivity of *B. cinerea* subpopulations with and without transposable elements will help to clarify this point.

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КАРАКТЕРИСТИКЕ ИЗОЛАТА *BOTRYTIS CINEREA* ИЗ ЈАГОДАСТИХ ВОЋАКА И ВИНОВЕ ЛОЗЕ ПОРЕКЛОМ ИЗ СРБИЈЕ

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У раду су проучене морфолошке карактеристике, присуство транспозона у геному и осетљивост на фунгициде 26 моноспоријалних изолата *Botrytis cinerea*, изолованих из купине, малине, јагоде и винове лозе. Присуство оба транспозона, *Flipper* и *Boty*, утврђено је у изолатима из свих домаћина. Детектовано је шест *vacuma* (не садрже транспозоне) и седам *transposa* (садрже оба транспозона) изолата, као и 13 изолата који садрже само транспозон *Boty*. Запажено је осам морфолошких типова колонија на PDA и MA подлози, што потврђује познату високу варијабилност врсте *B. cinerea*. Осетљивост на фунгициде је била различита, зависно од фунгицида и изолата.