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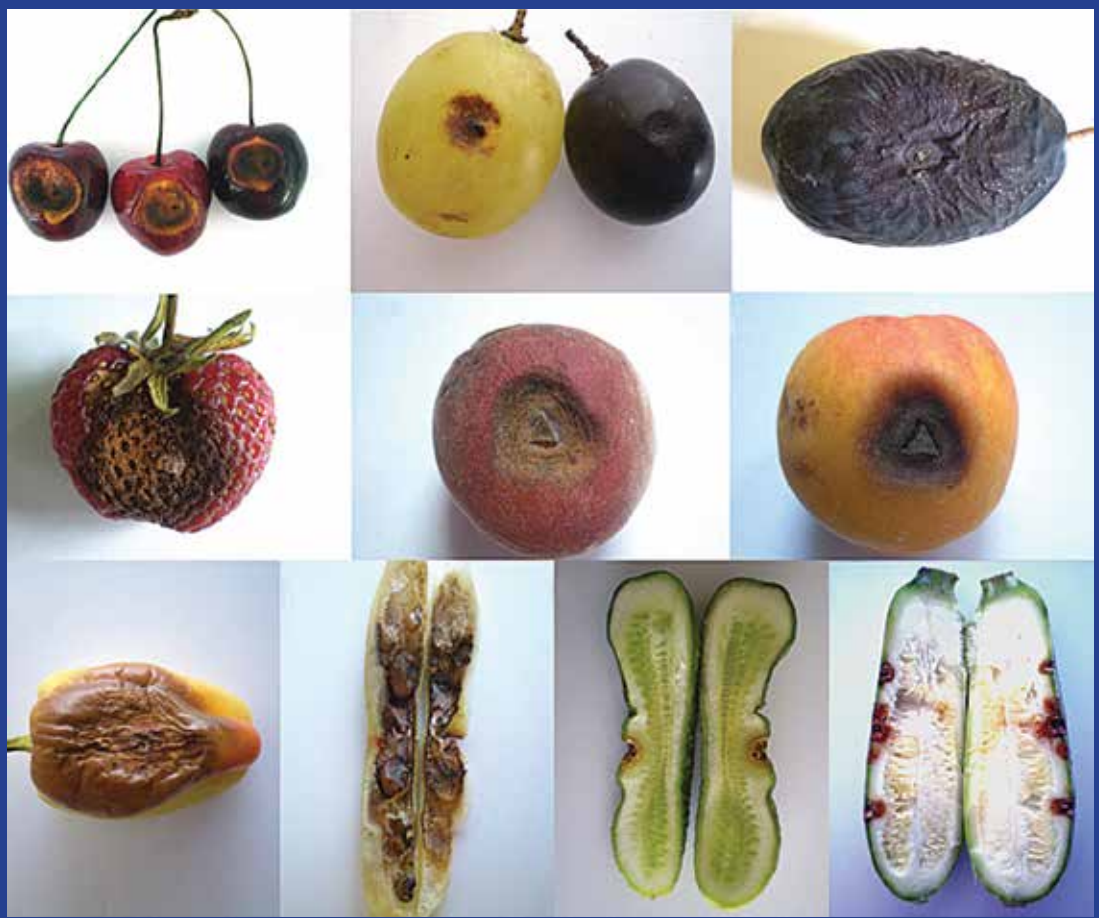
PLANT PROTECTION



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INSTITUTE FOR PLANT PROTECTION AND ENVIRONMENT - BELGRADE

ZAŠTITA BILJA
PLANT PROTECTION

Zaštita bilja | Vol. 63 (1) | № 279 (str 1-55) | Beograd, 2012.

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Simptomi antraknoze na veštacki inokulisanim plodovima trešnje, grožda, šljive, jagode, breskve, kajsije, paprike, boranije, krastavca i tikvice.

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PATOGENOST IZOLATA *COLLETOTRICHUM* SPP. – PROUZROKOVAČA ANTRAKNOZE

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REZIME

U radu su prikazane patogene karakteristike 20 izolata *Colletotrichum* spp. poreklom sa plodova kruške, jabuke, višnje i paradajza, kao i referentnih sojeva *C. acutatum* (CBS 294.67) i *C. gloeosporioides* (CBS 516.97). U proučavanja kruga domaćina izolata *Colletotrichum* spp. uključeno je 17 biljnih vrsta. Devet dana nakon veštačkih inokulacija svi testirani izolati prouzrokovali su antraknozne lezije na plodu jabuke, kruške, kajsije, trešnje, višnje, šljive, jagode, grožđa, paradajza, paprike, plavog patlidžana, krastavca, tikvice i boranije. Izolati *Colletotrichum* spp. poreklom sa istog domaćina pokazuju izvesne razlike u stepenu agresivnosti, što se može tumačiti genetskom varijabilnošću populacija. Rezultati jednofaktorijalne analize varijanse ukazuju na statistički značajne razlike u patogenosti izolata *Colletotrichum* spp. na nezrelim i zrelim plodovima kruške, jabuke, višnje i paradajza. Poređenjem stepena nekroze utvrđena je slaba do umerena osetljivost nezrelih plodova. Zreli plodovi su manifestovali jaku osetljivost prema svim testiranim izolatima *Colletotrichum* spp.

Ključne reči: antraknoza, *Colletotrichum* spp., patogenost, nezreo i zreo plod

UVOD

Gljive roda *Colletotrichum* – prouzrokovači antraknoze su kosmopolitske i izrazito agresivne vrste, prisutne gotovo na svim meridijanima. Kao patogeni voćaka, povrtarskih, ratarskih, industrijskih, krmnih, ukrasnih, lekovitih, šumskih i biljaka korovske flore, mogu prouzrokovati značajne ekonomske gubitke (Bailey et al., 1992). U uslovima subtropske i tropske klime simptomi se manifestuju u toku vegetacije, a u umerenoj klimatskoj zoni je češći slučaj ostvarivanja latentnih infekcija koje nakon berbe plodova i tokom naedekvatnih uslova skladištenja kulminiraju pojavom nekroze i truleži ploda.

Osim rasprostranjenosti na velikom broju vrsta iz botanički udaljenih familija, za gljive roda *Colletotrichum* je karakteristična i nespecifičnost prema biljkama domaćinima. Mogućnost unakrsnih infekcija, kao i činjenica da u velikom broju slučajeva više različitih vrsta istovremeno parazitira istog domaćina umnogome otežava proces pravilne identifikacije i diferencijacije patogena ovog roda (Freeman et al., 1998).

U Srbiji je tokom poslednjih godina utvrđena antraknoza ploda kruške, jabuke, višnje i paradajza. Tipični simptomi na plodovima su nekrotične, tamne, ulegnute, kružne lezije, koje se vremenom povećavaju i u okviru kojih dolazi do formiranja acervula - reproduktivnih organa gljiva *Colletotrichum* spp. Antraknoza je progresivna i u većini slučajeva dovodi do potpune truleži ploda (Živković, 2011).

S obzirom na ekonomski značaj gljiva *Colletotrichum*, kao i činjenice da su dve dominantne vrste ovog roda *C. acutatum* i *C. gloeosporioides* izrazito polifagni patogeni, osnovni ciljevi rada su: definisati krug domaćina, utvrditi infekcioni potencijal izolata sa ovog područja i ispitati dali na pojavu simptoma antraknoze osim ekoloških faktora utiče i fiziološka zrelost ploda.

MATERIJAL I METODE

Primenom standardnih fitopatoloških metoda iz plodova sa karakterističnim simptomima antraknoze dobijen je veliki broj izolata *Colletotrichum* spp. Za proučavanja patogenih osobina odabrano je 6 izolata sa ploda kruške (KC-6, KC-9, KC-12, KC-21, KC-23 i KC-82); 4 sa ploda jabuke (JC-4, JC-5, JC-6 i JC-7); 4 sa ploda višnje (VC-3, VC-5, VC-7 i VC-9); 6 sa ploda paradajza (PC-1, PC-2, PC-3, PC-4, PC-5 i PC-6), kao i 2 referentna soja: *C. gloeosporioides* (CBS 516.97) i *C. acutatum* (CBS 294.67) iz kolekcije Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, Holandija.

Proučavanje kruga domaćina

Proučavanje kruga domaćina izolata *Colletotrichum* spp. obavljeno je veštačkim inokulacijama plodova voća i povrća koji se u našoj zemlji intenzivno gaje, čuvaju u skladištima i prodaju na tržištu. U ispitivanja je uključeno 17 biljnih vrsta iz sledećih familija: Rosaceae: *Malus domestica*, jabuka (sorta zlatni delišes), *Pyrus communis*, kruška (sorta viljamova), *Prunus persica*, breskva (sorta redhaven), *Prunus armeniaca*, kajsija (sorta novosodska rodna), *Prunus cerasus*, višnja (sorta oblačinska), *Prunus avium*, trešnja (sorta burlat), *Prunus domestica*, šljiva (sorta stanley), *Fragaria vesca*, jagoda (sorta madeleine); Viticeae: *Vitis vinifera*, grožđe (sorte muskat hamburg i gročanka); Solanaceae: *Lycopersicon esculentum*, paradajz (sorta saint pierre), *Capsicum anuum*, paprika (sorta župska rana), *Solanum melongena*, plavi patlidžan (sorta jubilej), *Solanum tuberosum*, krompir (sorta riviera); Cucurbitaceae: *Cucumis sativus*, krastavac (sorta pariski kornišon), *Cucurbita pepo*, tikvica (sorta beogradska); Fabaceae: *Phaseolus vulgaris*, boranija (sorta darina), Alliaceae: *Allium*

cepa, crni luk (sorta holandski žuti).

Zdravi plodovi su površinski sterilisani potapanjem u 70% etanol u trajanju od 1 min, potom u 0,5% rastvor NaOCl 20 min, nakon čega su isprani u destilovanoj vodi i ostavljeni na filter papir da se osuše (Smith and Black, 1990). Inokulacije plodova su obavljene prema metodi Jones et al. (1996). Kontrolni plodovi su tretirani na isti način, ali su za njihovu inokulaciju korišćeni fragmenti podloge, bez micelije gljiva. Inokulisani plodovi su postavljeni u vlažne komore tokom 48 h, a nakon toga izneti i ostavljeni na sobnoj temperaturi. Ogled je postavljen u 3 ponavljanja, a devetog dana su izvršena merenja prečnika antraknoznih lezija prema sledećoj skali: – bez pojave nekroze; + nekroza vrlo slabog intenziteta (na krupnim plodovima Ø do 10 mm, na sitnim plodovima Ø do 5 mm); ++ nekroza slabog intenziteta (na krupnim plodovima Ø 10–20 mm, na sitnim plodovima Ø 5–10 mm); +++ nekroza srednjeg intenziteta (na krupnim plodovima Ø 20–30 mm, na sitnim plodovima Ø 10–15 mm); ++++ nekroza jakog intenziteta (na krupnim plodovima Ø >30 mm, na sitnim plodovima Ø>15 mm).

Ispitivanje uticaja zrelosti ploda na osetljivost prema izolatima *Colletotrichum* spp.

Za ispitivanja uticaja zrelosti ploda na osetljivost prema izolatima *Colletotrichum* spp. korišćeni su zdravi nezreli i zreli plodovi kruške (sorta viljamova), jabuke (sorta zlatni delišes), višnje (sorta oblačinska) i paradajza (sorta saint pierre). Plodovi su inokulisani prema prethodno navedenoj metodi Jones et al. (1996). Ogled je postavljen u tri ponavljanja, a očitavanje rezultata, odnosno merenje prečnika nekroze obavljeno je nakon devet dana.

Intenzitet sporulacije na formiranim nekrotičnim površinama ocenjen je dve nedelje nakon veštačkih inokulacija, prema sledećoj skali: – bez pojave sporulacije; + vrlo slaba sporulacija (pojava konidijalne mase neposredno oko mesta inokulacije); ++ slaba sporulacija (do 1/4 nekrotične površine pokriveno konidijalnom masom); +++ umerena sporulacija (do 1/2 nekrotične površine pokriveno konidijalnom masom); ++++ jaka sporulacija (> od 1/2 nekrotične površine pokriveno konidijalnom masom); +++++ vrlo jaka sporulacija (potpuna pokrivenost nekrotične površine konidijalnom masom).

Statistička obrada rezultata

Patogenost izolata *Colletotrichum* spp. na inokulisanim nezrelim i zrelim plodovima analizirana je preko osnovnih pokazatelja deskriptivne statistike (aritmetičke sredine, standardne devijacije i standardne greške aritmetičke sredine) i grafički predstavljena u vidu box-plotova. U slučaju homogenih podataka u uzorcima ($C_v \leq 30\%$) i homogenih varijansi uzoraka primenjen je parametarski model analize varijanse (ANOVE), dok je za uzorke sa varijabilnim vrednostima ($C_v > 30\%$) i heterogenim varijansama primenjen Kruskal Wallisov model ANOVE. Provera adekvatnosti ovih modela za konkretnu analizu sprovedena je na osnovu vrednosti koeficijenata varijacije (C_v) i Leveneovog testa za homogenost varijansi. Statistička obrada rezultata obavljena je upotrebom paketa STATISTICA v. 6 (StatSoft, Inc.).

REZULTATI

Proučavanje kruga domaćina

Rezultati proučavanja kruga domaćina ukazuju na izrazitu polifagnost izolata *Colletotrichum* spp. (Tabela 1).

Devet dana nakon obavljenih veštačkih inokulacija izolati sa ploda kruške, KC-6, KC-9 i KC-12, ispoljavaju nekrozu jakog intenziteta i obilno formiranje reproduktivnih organa na plo-

dovima breskve i kajsije. Na jabuci, krušci, višnji, trešnji, jagodi i grožđu ovi izolati manifestuju patogenost srednje jačine i masovnu produkciju acervula, a slabu nekrozu i fruktifikaciju na plodovima šljive, paradajza i paprike. Na krastavcima (Sl. 1I), tikvicama i mahunama boranije konstatovana je veoma slaba nekroza, neposredno oko mesta inokulacije i bez formiranih reproduktivnih organa. Najjači stepen nekroze i formiranje velikih antraknoznih površina, kao i karakterističnu smežuranost plodova većine testiranih biljnih kultura manifestuju izolati KC-21, KC-23 i KC-82 (Sl. 1E;1G). Do intenzivnog obrazovanja acervula i masovnog oslobađanja konidija u vidu žutonandžastog matriksa dolazi nakon 7 dana. Na mahunama boranije ovi izolati prouzrokuju jaku unutrašnju nekrozu i dezorganizaciju tkiva koja se progresivno širi i nakon dve nedelje u potpunosti zahvata mahunu i seme boranije (Sl.1H). Na plodovima krastavca inokulisanim izolatima KC-21, KC-23 i KC-82, konstatovana je slaba, a na tikvicama veoma slaba nekroza, bez sporulacije patogena. Ovim izolatima je po tipu simptoma i ispoljenoj patogenosti najsličniji referentni soj *C. acutatum* (CBS 294.67), (Sl. 1.D).

Izolati sa ploda jabuke, JC-4, JC-5, JC-6 i JC-7 ispoljavaju umerenu patogenost na inokulisanim plodovima kruške, šljive (Sl. 1C) i grožđa (Sl. 1B). Veoma jaku nekrozu i obilno formiranje reproduktivnih organa navedeni izolati manifestuju na jabuci, breskvi i kajsiji (Sl. 1F). Nekroza slabog i veoma slabog intenziteta uočena je na paradajzu i paprici, krastavcima, tikvicama i boraniji. Izolati poreklom sa ploda jabuke na plodovima povrtarskih kultura ne formiraju acervule i njima je po ispoljenoj agresivnosti najsličniji referentni soj *C. gloeosporioides* (CBS 516.97). Izolat JC-4 je u poređenju sa ostalim izolatima ove grupe, nešto agresivniji prema plodovima višnje, trešnje i jagode.

Izolati sa višnje, VC-3, VC-5, VC-7 i VC-9 su prilično ujednačene patogenosti. Na većini testiranih kultura izazivaju nekrozu srednjeg ili slabog intenziteta. Izrazite antraknozne pege i formiranje acervula konstatovani su jedino na plodovima višnje i trešnje (Sl. 1A). Jedva primetne nekrotične promene bez sporulacije patogena, zabeležene su na boraniji.

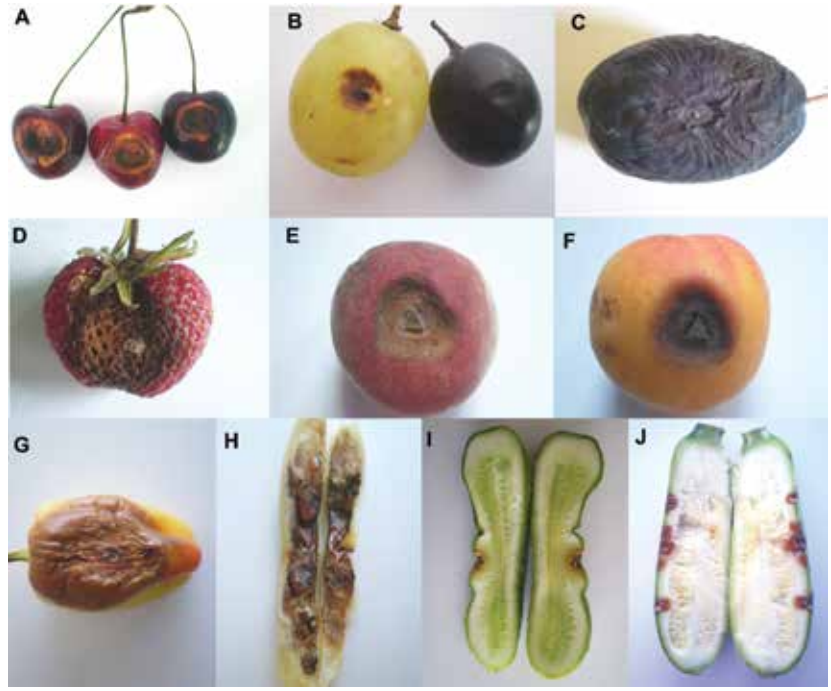
Najjači stepen antraknoze i masovnu fruktifikaciju izolati sa ploda paradajza, PC-1, PC-2, PC-3, PC-4, PC-5 i PC-6 manifestuju na jabuci, jagodi i paradajzu. Nekroza nešto manjeg intenziteta zabeležena je na inokulisanim plodovima paprike. Na mahunama boranije ovi patogeni izazivaju nekrotične promene slabog, a na krastavcima i tikvicama (Sl. 1J) veoma slabog intenziteta. Od svih ispitivanih izolata *Colletotrichum* spp., jedino izolati poreklom sa paradajza izazivaju veoma slabu nekrozu plavog patlidžana. Na mestu inokulacije gotovo da nisu uočeni vidljivi simptomi antraknoze, ali je na preseku konstatovana blaga nekroza tkiva koja se širila ka unutrašnjosti ploda.

Kohovi postulati su zadovoljeni reizolacijama sa inukulisanih plodova. Nijedan od ispitivanih izolata *Colletotrichum* spp., uključujući i referentne sojeve *C. acutatum* i *C. gloeosporioides*, ne prouzrokuje antraknozu krtola krompira i lukovica crnog luka.

Devet dana nakon veštačkih inokulacija svi izolati *Colletotrichum* spp., kao i referentni *C. acutatum* (CBS 294.67) i *C. gloeosporioides* (CBS 516.97), na nezrelim i zrelim plodovima jabuke, kruške, višnje i paradajza prouzrokuju nekrozu i sporulaciju različitog intenziteta. Na kontrolnim plodovima nije utvrđena pojave nekroze.

Tabela 1. Krug domaćina i patogenost izolata *Colletotrichum* spp.
Table 1. Host of range and pathogenicity of isolates of *Colletotrichum* spp.

Domaćin Host	Izolat Isolate																					
	KC-6	KC-9	KC-12	KC-21	KC-23	KC-82	JC-4	JC-5	JC-6	JC-7	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	VC-3	VC-5	VC-7	VC-9	CBS 294.67	CBS 516.97
jabuka	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	++ ++	++ ++
kruška	+++	+++	+++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	++ ++
breskva	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	++ ++
kajsija	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	++ ++
višnja	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
trešnja	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
šljiva	++	++	++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
jagoda	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	++ ++	+++
grožđe	+++	+++	+++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++ ++	+++
paradajz	++	++	++	++ ++	++ ++	++ ++	++	++	++	++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+++	+++	+++	+++	++ ++	+++
paprika	++	++	++	++ ++	++ ++	++ ++	++	++	++	++	+++	+++	+++	+++	+++	+++	++	++	++	++	++ ++	++
pl.patl.	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–
krompir	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
krastav.	+	+	+	++	++	++	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+
tikvica	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
boranija	+	+	+	++	++	++	++	+	+	+	++	++	++	++	++	++	+	+	+	+	+	+
crni luk	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–



Sl. 1. Simptomi antraknoze na inokulisanim plodovima: A. trešnja (izolat VC-5); B. grožđe (izolat JC-7); C. šljiva (izolat JC-5); D. jagoda (*C. acutatum* CBS 294.67); E. breskva (izolat KC-21); F. kajsija (izolat JC-4); G. paprika (izolat KC-23); H. boranija (izolat KC-21); I. krastavac (izolat KC-12); J. tikvica (izolat PC-3).

Fig. 1. Anthracnose symptoms on inoculated fruits of: A. sweet cherry (isolate VC-5); B. grape (isolate JC-7); C. plum (isolate JC-5); D. strawberry (*C. acutatum* CBS 294.67); E. peach (isolate KC-21); F. apricot (isolate JC-4); G. pepper (isolate KC-23); H. green beans (isolate KC-21); I. cucumber (isolate KC-12); J. zucchini (isolate PC-3).

Uticaj zrelosti ploda kruške na osetljivost prema izolatima *Colletotrichum* spp.

Na nezrelim i zrelim plodovima kruške najjaču nekrozu manifestuju izolati KC-21, KC-23 i KC-82. Na nezrelim plodovima, svi ostali ispitivani izolati *Colletotrichum* spp. prouzrokuju nekrotični proces neposredno oko mesta inokulacije (Sl. 2 i 3).

Izuzimajući izolate KC-21, KC-23 i KC-82, koji obilno formiraju plodonosna tela u vidu koncentrično raspoređenih prstenova, sporulacija ostalih patogena na inokulisanim nezrelim plodovima nije utvrđena. Na antraknoznim površinama zrelih inokulisanih plodova, svi ispitivani izolati *Colletotrichum* spp. u manjem ili većem stepenu produkuju acervule iz kojih se oslobađa žutonarandžasti matriks (Tabela 2).

Na osnovu Leveneovog testa utvrđeno je da su varijanse ispitivanih izolata homogene (za zelene plodove $F=1,527$, $p=0,112$; za zrele plodove $F=1,222$, $p=0,277$), pa je primenjen parametarski model ANOVE. Rezultati jednofaktorijalne analize varijanse pokazuju da postoje statistički značajne razlike u pogledu patogenosti ispoljene na nezrelim i zrelim plodovima kruške (za zelene plodove: $F=102,134$; $p<0,001$; za zrele plodove $F=806,989$; $p<0,001$).

Uticaj zrelosti ploda jabuke na osetljivost prema izolatima *Colletotrichum* spp.

Merenjem prečnika nekroze nezrelih i zrelih plodova jabuke devet dana nakon veštačkih inokulacija, utvrđeno je da su najagresivniji izolati sa jabuke, JC-5, JC-6 i JC-7. Najmanji stepen antraknoznih promena na inokulisanim nezrelim i zrelim plodovima manifestuju izolati poreklom sa ploda višnje (Sl. 4 i 5).

Na nekrotičnim površinama veštački inokulisanih nezrelih plodova jabuke, jedino izolati KC-6, KC-9 i KC-12 ne formiraju reproduktivne organe. Svi ostali patogeni, uključujući i referentne sojeve CBS 294.67 i CBS 516.97, dve nedelje nakon postavljanja eksperimenta manifestuju vrlo slabu ili slabu fruktifikaciju. Obrazovani reproduktivni organi su sitni, tamno mrke boje i koncentrično raspoređeni oko

Tabela 2. Sporulacija izolata *Colletotrichum* spp. na nezrelim i zrelim plodovima kruške, jabuke, višnje i paradajza.
Table 2. Sporulation of isolates of *Colletotrichum* spp. on immature and mature fruits of pear, apple, sour cherry and tomato.

Izolat Isolate	Sporulacija Sporulation							
	kruška pear		jabuka apple		višnja sour cherry		paradajz tomato	
	NZ* IF*	ZP** MF**	NZ IF	ZP MF	NZ IF	ZP MF	NZ IF	ZP MF
KC-6	–	++++	–	++	++	++	+	+++
KC-9	–	++++	–	++	++	++	+	+++
KC-12	–	++++	–	++	++	++	+	+++
KC-21	++++	+++++	++	+++	+++	++++	+	+++
KC-23	++++	+++++	++	+++	+++	++++	+	+++
KC-82	++++	+++++	++	+++	+++	++++	+	+++
JC-4	–	++++	++	+++	++	+++	+	++
JC-5	–	++++	++	+++	++	+++	+	++
JC-6	–	++++	++	+++	++	+++	+	++
JC-7	–	++++	++	+++	++	+++	+	++
PC-1	–	++	++	++	+	+++	+	++++
PC-2	–	++	++	++	+	+++	+	++++
PC-3	–	++	++	++	+	+++	+	++++
PC-4	–	+++	+	++	+	+++	+	++++
PC-5	–	+++	+	++	+	+++	+	++++
PC-6	–	+++	+	++	+	+++	+	++++
VC-3	–	+++	+	+++	++++	++++	–	++
VC-5	–	+++	+	+++	++++	++++	–	++
VC-7	–	+++	+	+++	++++	++++	–	++
VC-9	–	+++	+	+++	++++	++++	–	++
CBS 294.67	–	+++++	+	++	+++	+++	+	++
CBS 516.97	–	+++	+	++	++++	++++	+	++

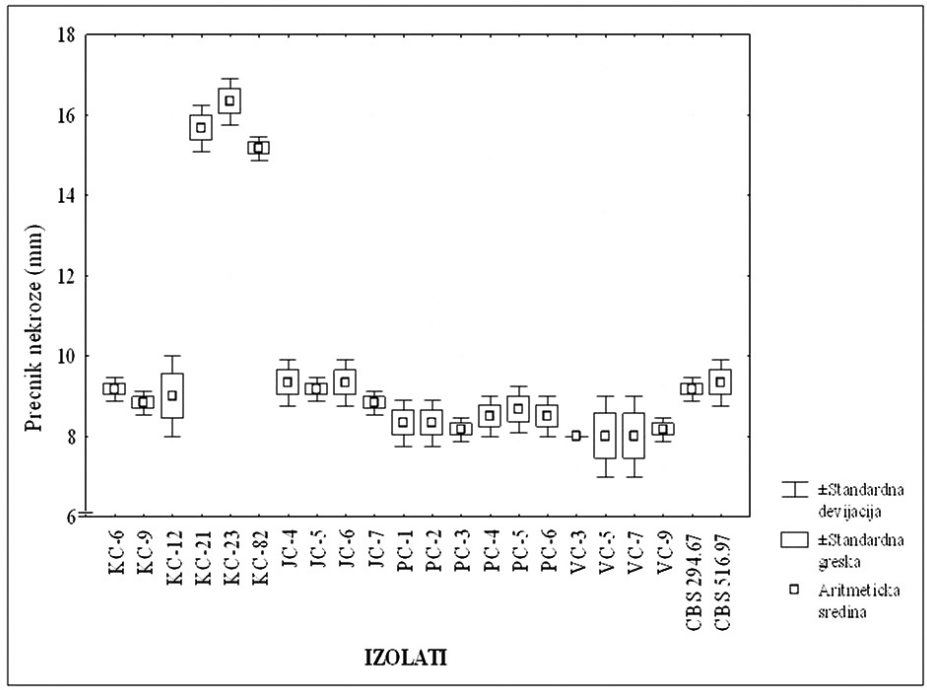
*NP - nezreo plod; *IF - immature fruit
**ZP - zreo plod; **MF - mature fruit

mesta inokulacije. Na zrelim plodovima jabuke izolati KC-21, KC-23 i KC-82, kao i svi izolati sa ploda jabuke i višnje ispoljavaju fruktifikaciju umerenog intenziteta. Ostali ispitivani patogeni u znatnu slabijem stepenu produkuju plodonosna tela (Tabela 2). Na antraknoznim pegama formirani acervuli su crne boje i većih dimenzija, a mogu biti nepravilno razbacani ili raspoređeni u vidu koncentričnih prstenova. U većini slučajeva iz plodonosnih tela dolazi do oslobađanja konidija u obliku narandžaste želatinozne mase.

Leveneovim testom je utvrđeno da varijanse izolata *Colletotrichum* spp. u eksperimentu provere patogenosti na nezrelim plodovima jabuke nisu homogene (F=3,166, p=0,00006), pa je primenjen Kruskal-Wallisov neparametarski tip ANOVE. Potvrđeno je postojanje statistički vrlo značajnih razlika u patogenosti svih ispitivanih kultura gljiva (H=61,593). U ogledu provere patogenosti na plodovima zrele jabuke Leveneovim testom konstatovana je homogenost ispitivanih varijansi (F=1,444, p=0,145), pa je stoga za analizu korišćen parametarski model analize varijanse i utvrđene statistički vrlo značajne razlike u stepenu agresivnosti izola-

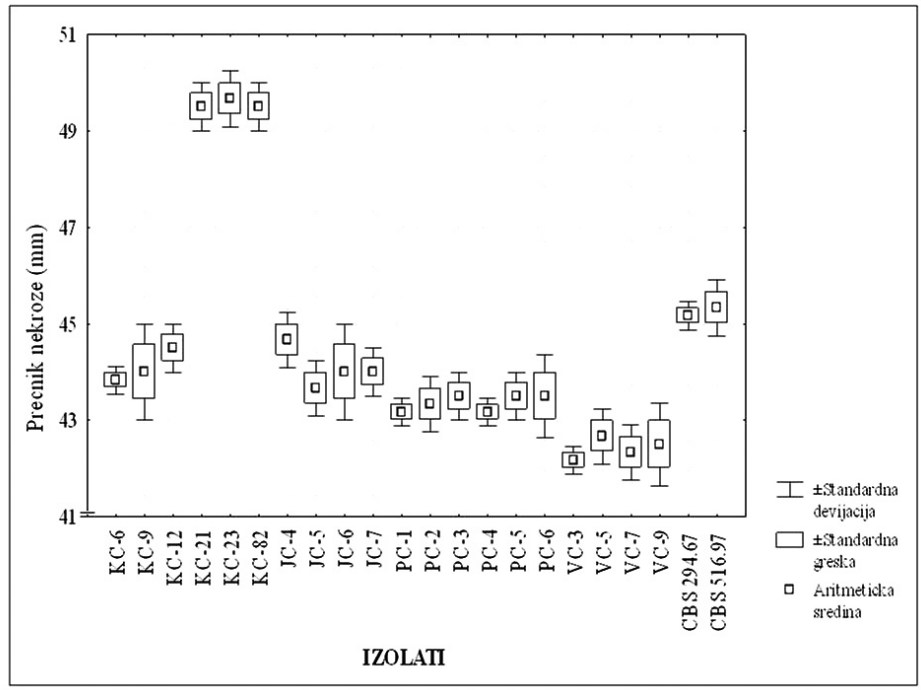
Sl. 2. Patogenost izolata *Colletotrichum* spp. na nezrelim plodovima kruške.

Fig. 2. Pathogenicity of isolates of *Colletotrichum* spp. on immature pear fruits.



Sl. 3. Patogenost izolata *Colletotrichum* spp. na zrelim plodovima kruške.

Fig. 3. Pathogenicity of isolates of *Colletotrichum* spp. on mature pear fruits.



ta *Colletotrichum* spp. u odnosu na kontrolu ($F=992{,}366$, $p<0{,}001$).

Uticaj zrelosti ploda višnje na osetljivost prema izolatima *Colletotrichum* spp.

Na veštački inokulisanim nezrelim i zrelim plodovima višnje najjaču patogenost manifestuju izolati poreklom sa ovog domaćina, ali i referentni soj *C. gloeosporioides*. Najmanji prečnik nekroze u oba testa provere patogenosti, prouzrokuju izolati *Colletotrichum* spp. sa ploda jabuke (Sl. 6 i 7).

Izolati VC-3, VC-5, VC-7, VC-9 i referentni soj CBS 516.97, najobilne formiraju plodonosna tela na površini antraknoznih pega nezrelih i zrelih plodova višnje. Patogene kulture poreklom sa paradajza najmanji stepen fruktifikacije ispoljavaju na nezrelim, a izolati KC-6, KC-9 i KC-12 na zrelim inokulisanim plodovima višnje (Tabela 2). Acervuli na antraknoznim površinama su sitni, crne boje i u većini slučajeva koncentrično raspoređeni. Iz plodonosnih struktura formiranih dolazi do oslobađanja žutonarandžaste mase konidija.

Na osnovu Leveneovog testa konstatovana je nehomogenost ispitivanih varijansi u oba testa patogenosti (test na nezrelim plodovima: F=3,069, p=0,001; test na zrelim plodovima: F=2,999, p=0,001). Rezultati Kruskal-Wallisovog model ANOVE ukazuju na postojanje statistički značajnih razlika u pogledu patogenosti izolata *Colletotrichum* spp. na inokulisanim nezrelim i zrelim plodovima višnje (test na nezrelim plodovima: H=65,311; test na zrelim plodovima: H=65,106).

Uticaj zrelosti ploda paradajza na osetljivost prema izolatima *Colletotrichum* spp.

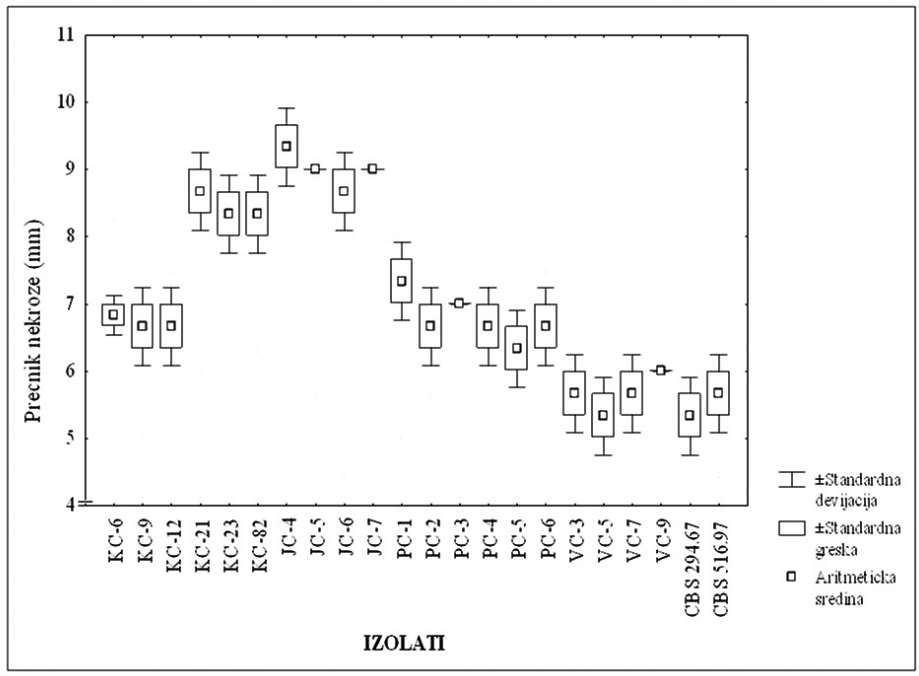
Na nezrelim plodovima paradajza najveću patogenost ispoljava izolat KC-12, a na veštački inokulisanim zrelim plodovima referentni soj *C. gloeosporioides* (CBS 516.97). Najmanji prečnik nekroze u oba testa provere patogenosti manifestuje izolat VC-3 (Sl. 8 i 9).

Na veštački inokulisanim nezrelim plodovima paradajza izolati poreklom sa višnje ne obrazuju acervule, a ostali ispitivani patogeni *Colletotrichum* spp. manifestuju veoma slabu fruktifikaciju. Na nezrelim plodovima ove povrtarske kulture, izolati sa paradajza intenzivno formiraju plodonosna tela, dok je nešto slabija produkcija acervula konstatovana na plodovima inokulisanim izolatima sa kruške. Na antraknoznim površinama zrelih plodova paradajza, izolati poreklom sa jabuke i višnje slabo fruktificiraju (Tabela 2).

U oba eksperimenta provere patogenosti izolata *Colletotrichum* spp., Leveneovim testom je utvrđena homogenost ispitivanih varijansi, (test na nezrelim plodovima: F=1,455, p=0,145;

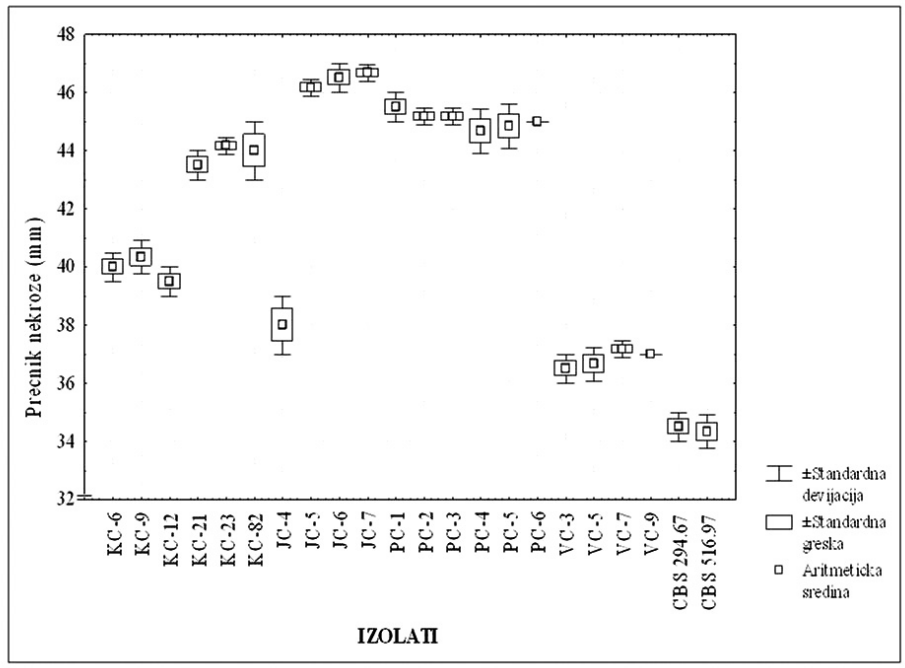
Sl. 4. Patogenost izolata *Colletotrichum* spp. na nezrelim plodovima jabuke.

Fig. 4. Pathogenicity of isolates of *Colletotrichum* spp. on immature apple fruits.



Sl. 5. Patogenost izolata *Colletotrichum* spp. na zrelim plodovima jabuke.

Fig. 5. Pathogenicity of isolates of *Colletotrichum* spp. on mature apple fruits.

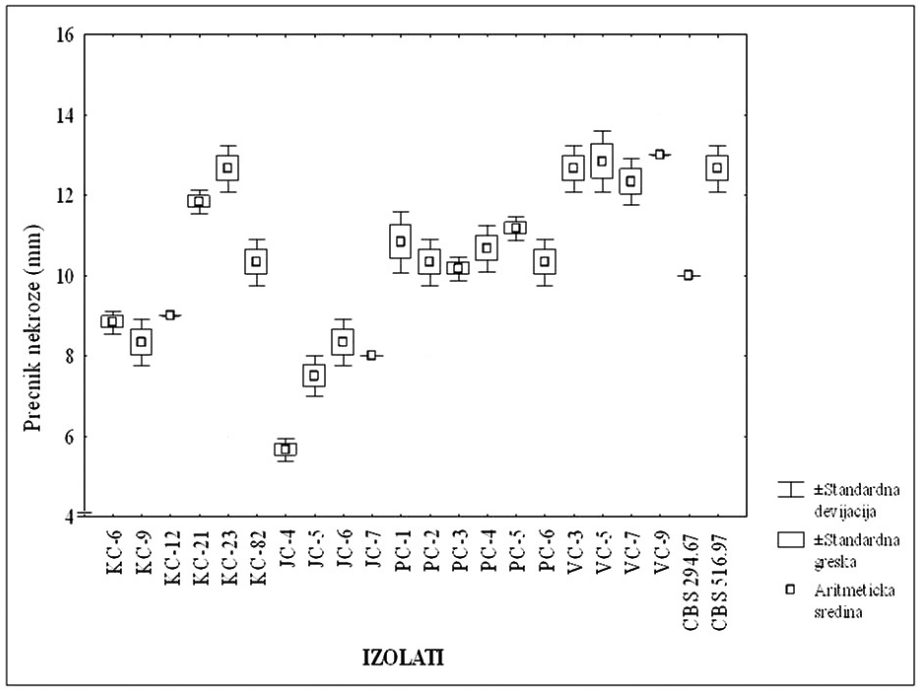


test na zrelim plodovima: F=1,226, p=0,274), pa je za statističku analizu primenjen parametarski model ANOVE. Rezultati jednofaktorijalne analize varijanse pokazuju da postoje statistički značajne razlike u pogledu patogenosti ispoljene na nezrelim i zrelim plodovima paradajza (za zelene plodove: F=183,249, p<0,001; za zrele plodove F=731,826, p<0,001).

DISKUSIJA

Zbog izrazite polifagnosti gljiva roda *Colletotrichum* od velikog je značaja utvrditi njihov potencijal za unakrsne infekcije, odnosno odrediti krug biljaka domaćina. Veštačkim inokulacijama je utvrđeno da izolati *Colletotrichum* spp. poreklom sa ploda kruške, jabuke, višnje i paradajza, od 17 testiranih biljnih vrsta ne prouzrokuje jedino antraknozu krtola krompira i lukovica crnog luka. Na ostalim inokulisanim plodovima, konstatovana je nekroza i fruktifikacija patogena različitog intenziteta, što ukazuje na visok stepen polifagnosti i mogućnost ostvarivanja unakrsnih infekcija. U najvećem broju slučajeva izolati poreklom sa istog domaćina manifestuju ujednačen intenzitet nekroze na plodovima testiranih kultura, a izvesna odstupanja se mogu tumačiti genetskom varijabilnošću ispitivanih populacija.

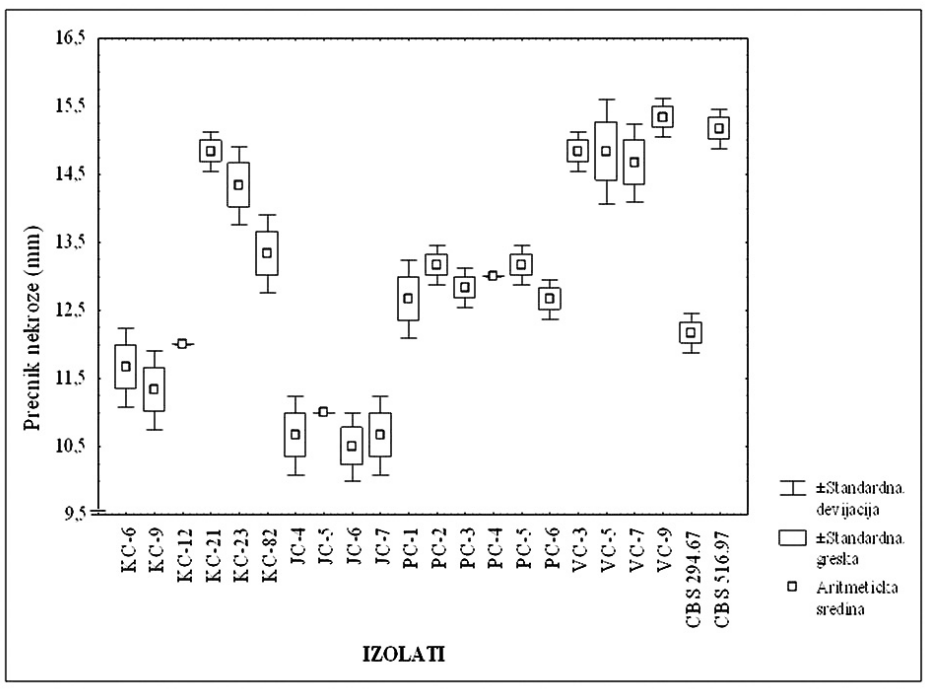
Širok krug biljaka domaćina za izolate *C. acutatum* i *C. gloeosporioides*, dobijenih sa plodova raznog tropskog, subtropskog i kontinentalnog voća, ustanovili su u svojim istraživanjima Adaskaveg and Hartin (1997) i Freeman et al. (1998). Unakrsnim inokulacijama dokazana je polifagnost patogena, bez obzira na njihovo poreklo, tj. udaljenu botaničku pripadnost biljaka sa kojih su izolovani. Slične rezultate navode i Bompeix et al. (1988). Ovi autori konstatuju da *C. acutatum* i *C. gloeosporioides* sa antraknoznih plodova jabuke, višnje, trešnje, badema i kruške nisu usko specijalizovani, odnosno da su sve kombinacije unakrsnih veštačkih inokulacija imale pozitivan ishod. Nasuprot navedenim rezultatima, Dyco and Mordue (1979) govore o izvesnoj specijalizovanosti izolata *C. acutatum* poreklom sa leguminoza, paradajza i bora. Unakrsnim inokulacijama utvrđen je slab infekcioni potencijal proučavanih izolata prema kulturama koje nisu



Sl. 6. Patogenost izolata *Colletotrichum* spp. na nezrelim plodovima višnje.

Fig. 6. Pathogenicity of isolates of *Colletotrichum* spp. on immature sour cherry fruits.

njihovi prirodni domaćini. Prilikom određivanja spektra domaćina izolata *C. gloeosporioides* poreklom sa jabuke, paprike i grožđa, Ahn et al. (2003) su utvrdili postojanje *forma specialis*, koje u većini slučajeva patogenost manifestuju samo na plodovima primarnih biljaka domaćina. S obzirom na izrazito komplikovani taksonomski status vrsta *C. acutatum* i *C. gloeosporioides*, Jonston (2000) ističe da nema opštih pravila koja se tiču odnosa patogen - domaćin, a Freeman et al. (1998) naglašavaju

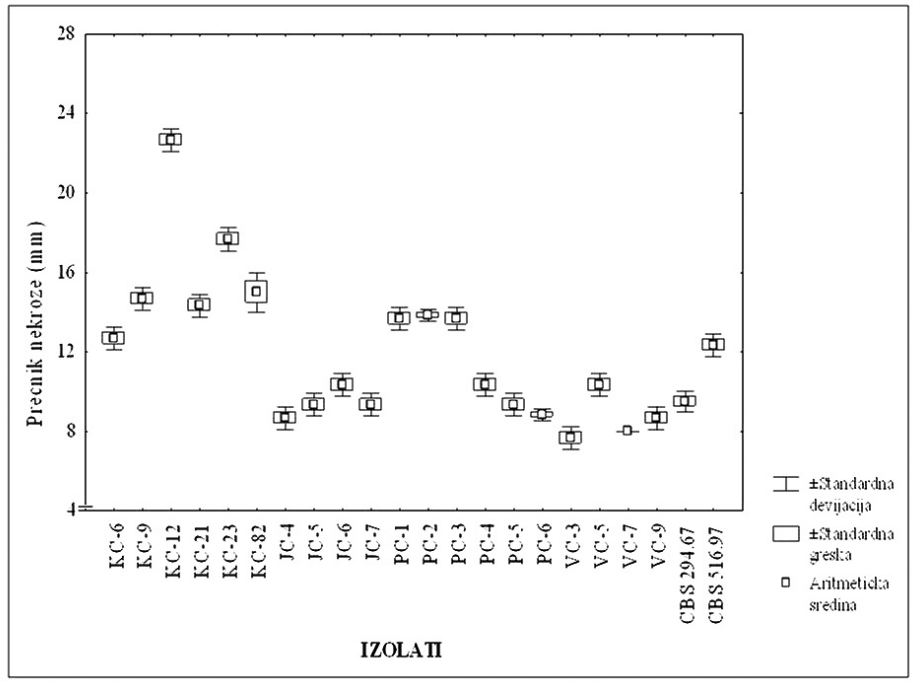


Sl. 7. Patogenost izolata *Colletotrichum* spp. na zrelim plodovima višnje.

Fig. 7. Pathogenicity of isolates of *Colletotrichum* spp. on mature sour cherry fruits.

Sl. 8. Patogenost izolata *Colletotrichum* spp. na nezrelim plodovima paradajza.

Fig. 8. Pathogenicity of isolates of *Colletotrichum* spp. on immature tomato fruits.

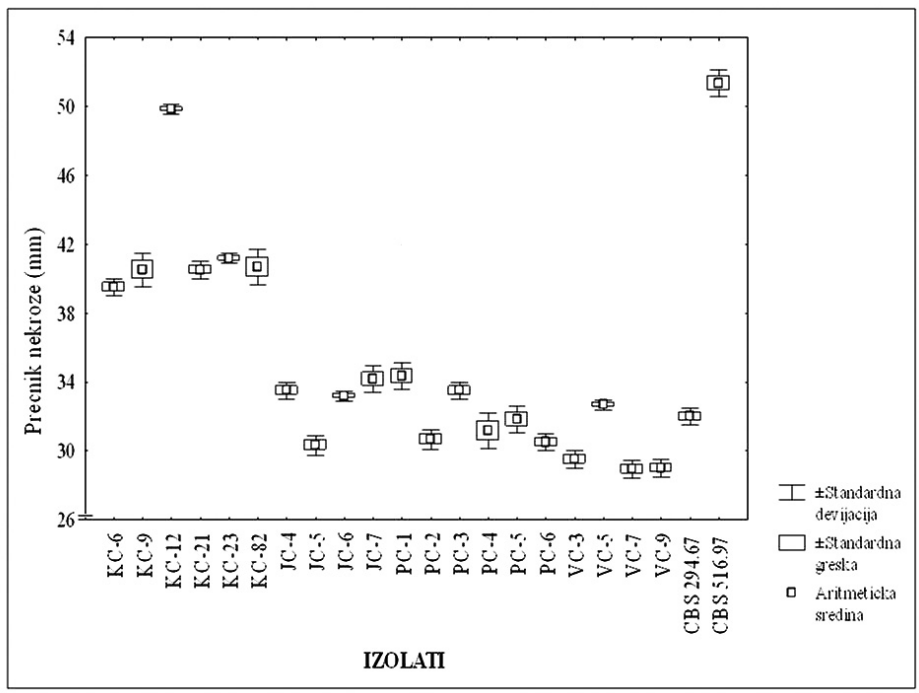


da diferencijacija vrsta zasnovana na poreklu izolata nije pouzdan kriterijum, naročito kada su u pitanju polifagne i kosmopolitske vrste. Autori takođe navode da su varijacije u intenzitetu patogenosti unutar *C. acutatum* i *C. gloeosporioides* često praćene i fenotipskim varijacijama, pa se može govoriti o daljem diferenciranju ovih vrsta u odgovarajuće *forme specialis*.

Simptomi antraknoze na plodovima voća i povrtarskih kultura se uglavnom manifestuju u kasnim fazama vegetacije na već sazrelim plodovima, ali je veštačkim inokulacijama u polju utvrđe-

Sl. 9. Patogenost izolata *Colletotrichum* spp. na zrelim plodovima paradajza.

Fig. 9. Pathogenicity of isolates of *Colletotrichum* spp. on mature tomato fruits.



na osetljivost i nezrelih plodova prema patogenima *Colletotrichum* spp. (Shane and Sutton, 1981; Noe and Starkey, 1982; Zaitlin et al., 2000). Navedeni autori konstatuju da vrste roda *Colletotrichum* mogu inficirati plodove u ranim fazama vegetacije, ukoliko postoji dovoljno inokuluma i povoljni metereološki uslovi. Na osnovu poljskih i ogleda sprovedenih u laboratoriji, Trkulja (2004) takođe konstatuje da *C. acutatum* i *C. gloeosporioides* mogu inficirati jabuke u svim fenofazama njihovog razvoja, ali da je stepen ispoljene antraknoze najjači na zrelim plodovima. Rezultati našeg eksperimenta potvrđuju prethodnu tezu, jer je nakon statističke analize utvrđeno da postoje značajne razlike u pogledu patogenosti koje izolati *Colletotrichum* spp. manifestuju na nezrelim i zrelim plodovima kruške, jabuke, višnje i paradajza. Komparacijom dobijenih vrednosti konstatovana je slaba ili umerena osetljivost nezrelih i istovremeno jaka osetljivost zrelih plodova navedenih biljnih vrsta prema testiranim patogenima.

Osim uslova spoljne sredine koji imaju važnu ulogu u formiranju i disperziji konidija, kasna pojava simptoma antraknoze u prirodnim uslovima se može dovesti u vezu i sa razlikom u veličini nezrelih i zrelih plodova, odnosno sa povećanjem potencijalne infekcione površine (Shane and Sutton, 1981). Razvoj gljive u nesazrelim plodovima i zelenim biljnim organima ograničen je i usled nedostatka hranljivih i energetskih izvora (Wharton and Uribeondo, 2004). Sitterly and Shay (1960) ističu da je jedan od najznačajnih faktora veće osetljivosti zrelih plodova voća i povrća povećanje sadržaja saharoze i drugih šećera u procesu zrenja. Ova energetska jedinjenja su osim za biljku domaćina, osnovni preduslov opstanka i razvoja patogena *Colletotrichum* spp.

ZAHVALNICA

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PATHOGENICITY OF ISOLATES OF *COLLETOTRICHUM* SPP. – THE CAUSAL AGENTS OF ANTHRACNOSE

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SUMMARY

The pathogenic characteristics of 20 isolates of *Colletotrichum* spp. originating from pear, apple, sour cherry and tomato fruits, as well as reference strains of *C. acutatum* (CBS 294.67) and *C. gloeosporioides* (CBS 516.97) are presented in this paper. In the studies of host range of isolates of *Colletotrichum* spp. were included 17 plant species. Nine days after artificial inoculation all tested isolates were caused anthracnose lesion on fruits of apple, pear, peach, apricot, sour cherry, sweet cherry, plum, strawberry, grape, tomato, pepper, eggplant, cucumber, zucchini, and green beans. Isolates of *Colletotrichum* spp. originating from the same host showed some differences in the degree of aggressiveness, which can be interpreted as the genetic variability of populations. The results of one-way analysis of variance indicate that the pathogenicity of isolates of *Colletotrichum* spp. is statistically significantly different on immature and mature fruits of pear, apple, sour cherry and tomato. Low to moderate sensitivity of immature fruits was confirmed by comparison of the degree of necrosis. Mature fruits were manifested a strong sensitivity to all tested isolates of *Colletotrichum* spp.

Key words: anthracnose, *Colletotrichum* spp., pathogenicity, immature and mature fruit

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EFFECT OF RACE 3 OF *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* ON SOME TOMATO CULTIVARS

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SUMMARY

Aim of this study is to determine the impact of race 3 of fusarium wilt on some tomato cultivars if it occurs in Serbia. For this purpose eleven tomato cultivars were inoculated with this pathogen: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1 by applying classic method of inoculation by submersing the injured root in fungi suspension. Disease was assessed 30 days after inoculation using an ordinal scale range from 1 – 5. After assessment, Nada F_1 and 129-Sprin had lowest average disease rating (2,9), and marked as tolerant. All the other cultivars were consider susceptible with ADR values higher than 3,0 in the following order: Narvik (3,6), Šampion F_1 (3,8), M-7 (3,9), Z. Jubilej F_1 (4,1), C. Jasmin (4,2), Danubius F_1 (4,4), Balkan F_1 (4,5), Marko F_1 and M-10 (4,6). Based on these results we can conclude that if the race 3 of *Fusarium oxysporum f. sp. lycopersici* occur in Serbia it could seriously jeopardize tomato production.

Key words: race 3, *Fusarium oxysporum* f. sp. *lycopersici*, resistance, tomato, breeding

INTRODUCTION

Cultivated tomato (*Lycopersicon esculentum* Mill.) is one of the world's most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products (Giovanni et al., 2004). World volume of production has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of the tomato. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production (Tanyolaç and Akkale, 2010).

Fusarium wilt of tomato (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) is one of the most impor-

tant and widespread diseases of tomato. It is a soil-borne fungus specilized for colonization of tomato. It produces chlamidospores that remain viable for long period of time, and because of that it was first described by Massee (1895) as the „sleepy disease“ (*cit*. Huang-Cheng and Lindhout, 1997). This pathogen has three identified races, till now, and races 1 and 2 have a world wide distribution, whereas race 3 has a more limited geographical range (Reis et al., 2005). As the most effective mean for control of this pathogen methyl-bromide was used. But because of the harmful effects of this substance on the ozone layer it was banned for use (Gullino et al., 2002). Since than, methyl-bromide does not have adequate replacements (Bell, 2000; Ioannou, 2000; Ivanović and Ivanović, 2007).

Due to the inefficiency of fungicides and other conventional control methods, considerable breeding efforts have been directed toward the development of resistant tomato cultivars. Three major resistance loci have been genetically characterized in *Lycopersicon* species and all of them have been incorporated into comercial cultivars (Reis et al., 2004). Resistance genes conferring resistance to Fol race 1 (*I* gene) have been identified and mapped to chromosomes 11 (Bohn and Tucker, 1939; Paddock, 1950) and 7 (Sarfatti et al., 1991). The *I-2* gene, conferring resistance to Fol race 2, lies within a cluster of seven similar genes on the long arm of chromosome 11 (Laterrot, 1976; Segal et al., 1992). Gene *I-3* provides resistance against Fol races 1, 2 and 3 and was mapped to chromosome 7 (Bournival et al., 1989, 1990; Scott and Jones, 1989). Due to limitted geographical distribution of race 3 resistant genes are also limitted mostly to those regions.

Taking in consider facts that race 3 of Fol is very agressive and that it has not been proven to be present in Serbia, it would be of great importants to be prepare for its eventual occurence. The aim of this research is to investigate effect of race 3 of Fol on some of the tomato cultivars in order to simulate what would happen if this race 3 appear in Serbia and what would be the consequences.

MATERIAL AND METHODS

Isolate of pathogen was provided by Dr Bart Lievens, Sciencia Terrae Research Institute, Belgium. Pathogen is being kept in phytopathogen fungi collection on PDA at 4°C in refrigerator until further use.

Tomato cultivars from the Institute for Vegetable Crops, Smederevska Palanka: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1 have been inoculated.

For the purpose of inoculation pathogen has been grown on PDA and kept for 15 days at 24°C in thermostat. After this period the suspension has been made by rinsing of mycelia with distilled water through sterile gauze (5x5cm). The concentration of suspension of 10^8 conidia/ml has been determined by hematocytometer (Đorđević et al., 2012).

Seeds were sown in styrofoam trays with 103 cells, filled with sterile substrate. When the plant had four true leaves completely developed they have been removed from containers and the root was washed in order to be cleaned from substrate. The apical sector of root system, about 2 cm of it, was removed with scissors (Gale et al., 2003; Reis and Boiteux, 2007). After that, ten plants from each group have been submerged in pathogen suspension for 6 minutes. Control was ten plants submerged in distilled water also for 6 minutes. After that period plants were planted in pots of 19cm diameter in sterile substrate and kept in glass house. Disease was assessed 30 days after inoculation using an modified ordinal scale (1 – 5) by Reis and Boiteux (2007) where 1

Table 1. Reaction of tested tomato cultivars and hybrids to race 3 of *Fusarium oxysporum f.sp. lycopersici*

Genotype	Category*	Duncan's Multiple range test **	Susceptibility***
129 - sprin	2,9	h	T
Balkan F_1	4,5	a	S
Crveni Jasmin	4,2	a	S
Danubius F_1	4,4	a	S
M - 7	3,9	d e	S
M - 10	4,6	a	S
Marko F_1	4,6	a	S
Nada F_1	2,9	h	T
Narvik	3,6	d e f g	S
Šampion F_1	3,8	d e f	S
Zlatni jubilej F_1	4,1	d	S

* Average of 10 plants. Plants were evaluated using an ordinal scale ranging from 1-no symptoms to 5-dead plants
** Values with different letters are significantly different according to Duncans Multiple Range test for level of significance P=0,01
*** Varieties with disease ratings between 1,0 – 2,0 were consider resistant (R), with disease ratings between 2,1 – 3,0 were considered tolerant (T) and higher than 3,1 were considered susceptible (S)

= plant free of symptoms; 2 = plant without wilt symptoms but present conspicuous vascular browning; 3 = plant showing vascular browning with wilting symptoms or with chlorosis; 4 = severe wilting associated with the presence of foliar necrosis and chlorosis, and 5 = dead plant. Cultivars with average disease ratings (ADR) in range of 1,0 – 2,0 were consider resistant (R), from 2,1 – 3,0 were consider as tolerant (T) and cultivars with average disease ratings higher than 3,1 were considered susceptible (S).

Experiment has been set in totally random design with two replications. Data was proceeded in MATLAB Ver. 7.0 by applying variance analysis and differences were compared using Duncan Multi Range test for the level of significance 0,01.

RESULTS AND DISCUSSION

About 7-10 days after inoculation on all of tested cultivars and hybrids first symptoms occured, expressed as wilting in the wormest part of the day. As the time passed plants expressed more severe wilting with occurence of chlorosis, defoliation of lower leaves and even death. After the final evaluation Nada F_1 and 129 – sprin expressed highest level of tolerance, among tested cultivars, with ADR value 2,9. Based on this value they were marked as tolerant (T). All of the other cultivars and hybrids were highly susceptible with ADR higher than 3,0. Highest value of ADR (4,6) had Marko F_1 and M-10 (Table 1.).

Occurence of symptoms of wilt were not as severe as it would be expected. In fact symptoms were at first moderate intesity and as the time passed simtoms were more intensive and resulted with high procent of dead plants. Even Balkan F_1, Marko F_1 and M-10 that had highest values of ADR initially did not express intensive wilt symptoms but at the end of research majority of plants were dead. Nada F_1 and 129-sprin expressed moderate symptoms of wilt and chlorisis of leaves but cross section showed necrotic changes of xylem.

Most of the cultivars and hybrids in our research reacted as susceptible toward race 3,

except Nada F_1 and 129-sprin that expressed symptoms characteristic for fusarium wilt but marked as tolerant. Cultivar and hybrids that expressed susceptibility toward this race are most likely laking of the *I-3* gene. The *I-3* gene conferring resistance to race 3 was discovered in *L. pennellii* accessions PI414773 (McGrath et al., 1987) and LA716 (Scott and Jones, 1989). At first *I-3* gene from LA716 was found to confer resistance to race 1 and 2 (Bournival et al., 1990) but in recent findings of Scott et al. (2004) indicate that this gene does not confer resistance to race 1 and 2 but other genes *I-1* and *I-2* previously reported by Sarfatti et al. (1991). Nada F_1 and 129-sprin may have *Tfw* gene for tolerance to race 3 of fusarium wilt and confers limited resistance to all thre races (Bournival et al., 1989; 1990). This assumption will be tested using molecular methods in further reseach. The severity of infection of tomato plants by race 3 of FOL is in accordance with results of Scott et al. (2004) as well as with Reis et al. (2004) that inoculated 94 different tomato cultivars with race 3 and observed on 64 cultivars high level of susceptibility with the same pattern of symptom develpment.

Our results are expected due to the fact that resistant cultivars are mostly located in regions of the world with reported race 3 (Reis et al., 2005; Scott et al., 2004). Larger number of cultivars, especialy ones located among „wild" population, should be tested in order to find *I-3* or *Tfw* genes. This will be a subject for further research.

CONCLUSION

Due to the high mobility of people and goods it is very easy to introduce this and other pathogens especially on seeds. Based on the results of our experiment race 3 of *Fusarium oxysporum* f. sp. *lycopersici* might became economycally important disease if introduced in our region since race 3-resistant cultivars are not yet available. Further reseach should be performed and large population of domestic cultivars of tomato should be examined in order to find gene or genes that confer resistance to this race, and introgress them into breeding programs of cultivated tomato. This will allow the anticipation of potential problem that will at some point in future occure.

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UTICAJ RASE 3 *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* NA POJEDINE KULTIVARE PARADAJZA

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REZIME

Cilj ovog istraživanja je da se utvrdi kakav bi bio uticaj rase 3 fuzarioznog uvenuća paradajza na pojedine kultivare ukoliko bi se ova rasa pojavila u Srbiji. U te svrhe inokulisano je jedanaest kultivara ovim patogenom i to: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1, primenom klasične metode inokulacije umakanjem povređenog korena u suspenziju gljiva. Nakon 30 dana rađena je procena pojave oboljenja upotrebom skale od 1 – 5. Nakon evaluacije, Nada F_1 i 129-Sprin su imali najnižu vrednost ADR-a (prosečni nivo oboljenja) (2,9), i obeleženi su kao tolerantni. Svi ostali kultivari smatrani su osetljivim sa vrednostima ADR višim od 3,0, po sledećem rasporedu: Narvik (3,6), Šampion F_1 (3,8), M-7 (3,9), Z. Jubilej F_1 (4,1), C. Jasmin (4,2), Danubius F_1 (4,4), Balkan F_1 (4,5), Marko F_1 and M-10 (4,6). Na osnovu ovih rezultata možemo zaključiti da ako bi se rasa 3 *Fusarium oxysporum* f. sp. *lycopersici* pojavila u Srbiji mogla bi značajno da ugrozi proizvodnju paradajza.

Ključne reči*:* rasa 3, *Fusarium oxysporum* f. sp. *lycopersici*, otpornost, paradajz, selekcija

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EFFECT OF SINGLE RESISTANCE GENES TO *PUCCINIA TRITICINA* ON DEGRADATION OF FUNGICIDES WITH SULFUR

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SUMMARY

Five for wheat leaf rust resistance genes (Lr) declared as race specific and nonspecific Lr 22b in near isogenic lines, were compared according to leaf growth of seedlings grown at approximately 20°C eight days after treatment by fungicides characterized by linked sulphur. By Lr 16 enzymatic degradation of S=C-S bond in Dithane M 70 growth was decreased for 20% while influence on N-C-S in Folpan was 3%. Degradation of last mentioned bond in Folpan was similar accelerated by Lr 29 causing reduced growth for 17%. Hydrolitic effect according to Lr 22b was 1 and 2%. Specific genes Lr 2a and Lr 15 were low influential to the both fungicides while Lr 1 effect was similar only to Dithane M 70 in different doses. Four groups of Lr genes were strongly indicated. The sulphur containing target of leaf rust resistance genes enzymatic product and specificity was confirmed as well as activity without eliciting structures from fungi. Achieved results suggested that secondary residua degradation of Dithane M 70 and Folpan when were introduced in plant could be shortened by adequate combinations of Lr genes and fungicides. Consequence was predicted to be minute disturbance of grain filling according to much lower proposed dose of the fungicides necessary for fungi sufficient restriction. By application of most effective genes could be facciliated restriction of the parasite in optimal time for development without residua.

Key words: *Puccinia triticina*, Lr genes, fungicide

INTRODUCTION

The leaf rust was declared as most often disease of wheat (Jerković, 1995; Rijsdiks and Zadoks, 1976) causing the grain yield damages in semiarid region of approximately 3.5% for each of 10% of last two leaves coverage (Jerković, 2008). The Genes responsible for leaf rust resistance (Lr) were divided in two groups specific and nonspecific according to effect on parasite population parts (Nelson, 1978). Until nowadays it was founded 63 of them but number of declared as same effective was increased (Kolmer, et al., 2010). Negative effects of their introduc-

tion in the same genotype on regional grain yield were recognized (The et al., 1988). Contrary, resistance genes activation was also explained as elicited by structures from fungi (De Witt, 1993). Becouse of specific resistance overcome process the regional stydies of protection by fungicides were parallel (Boskovic et al., 1986).

Enzymatic conformity of the genes for resistance was not found until knowadays. Specific ones Lr 1, Lr 15 and Lr 19 were same influential on wheat seed protein degradation while lower but not same effective were Lr16, Lr 37 and Lr 29 (Jerković and Prijić, 2012). Stabilizing of proteins in seed was achieved by enzymes as were protein disulphide isomerase's (PDI) (d'Aloisio et al., 2010). For the explanation of specific resistance genes influence, suggested was contrary effect to similar targets. The 1080 amino acids in product of Lr 21 weighted more approximately twice in comparation with ordinary protease (Huang et al., 2005) confirming the endoprotese behind. Transfer of units heavier than 100 kd through the wheat cell wall was not possible (Lasaro et al., 1975). The increased accumulation of sulphur and nitrogene was related to haustorium mother cell (Chong and Harder, 1982), stage before the fungi entrance to the host cell. Such, responsible enzymes were clasified as endoprotease as well as functional in drought conditions.

Resting time of systemic fungicides of at least thirty days (Osborne and Stein, 2009) was not adequate to most efficient application approximately twenty days before disappearing of green leaf area at the beginning of June characterized by less than 100 mm of rainfall (Jerković, 1997; Jerković and Prijić, 2011). Period of two weeks was proposed for mankozeb degradation in region with approximately 160 mm of rainfall in June (Škerbot, 2011).

Across presented results, it was hypothesized that specific leaf rust resistance genes will be effective on degradation of fungicides containing the linked sulphur with consequenties on wheat growth. Practical aim was to accelerate the fungicide residua degradation in more arid regions and facilitate the protection in optimal period for parasite development.

MATERIAL AND METHODS

Six lines created by introducing the leaf rust resistance genes in the variety Thatcher (Lr lines) by backcrossing declared as race specific according to negative influence on adult next plant parts growth also as opposite inflental nonspecific one Lr 22b (Jerković and Prijić, 2012) were grown in the greenhouse at air temperature of approximately 20°C in three replications. The density in pots (2 dl) filed by soil was about one plant per 25 mm^2. The near equal length of the first leafs close to 9 cm six days after germinating was achieved by removing of not adequate plants present because of later germination. Rest was treated with solution contained five grams of fungicides Dithane M 70 (Zinc ion coordination product with Mn ethylene-1,2-bisdithiocarbamate polymer) and Folpan or N-(trichloromethylthio) phthalimide in 0.5 l of water. Once per three next days the 1.5 dl of the solution was applied to the 1 m^2 of the area partially covered by different genotypes using hand sprayer. Watering of the soil was stopped before treatments in order to facilitate the leaf rust resistance genes effects predicted to be in water deficiency. Also, two hours after the treatment by fungicide the area was permanent daily treated by same amount of water with aim to insure the fungicides entrance. Control plants were previous treated equally but further simultaneous only with water. Estimation was eight days after first treatment. Presented was average first leaf length (FLL) and total leaf length (TLL)

of each of the genotypes. Values when first and total leaf lengths were divided were pronounced SLGR, SLGRD when Dithane M 70 and SLGRF when Folpan was applied and averaged. In the second experiment the procedure was partially the same. The difference was in applied Dithane M 70 amount of 1 gram in 0.5 l of water as well as control plants were in the same pots. During the fungicide application they were protected by cover. The TLL correlation between treatments with Dithane M 70 was calculated.

RESULTS AND DISCUSSION

The lowest decreasing effect on growth after treatment by fungicides was recognized at Lr 22b near isogenic line as was expected by previous Lr genes clasification. Leaf growth of other lines were generaly disturbed in same direction but different according to applied fungicide. The large differences indicated the residua presence in proposed time. Five times higher dose of Dithane 70 approximately doubled the effect on growth. Slight chlorosis was also observed when fungicides were applied. Unproportional effect of dose was explainable by multiple suspension application and short entrance period. The second leaf length was more affected proving the presence of Lr 22b simultaneous with specific genes (Tab 1 a and b).

The correlation coefficient between total leaf lengths of the by different fungicide amount treated genotypes was 0.93. Degradation of chlorophyll when Ph value was decreased below 6,5 was extensive recognized. Speculated ratio of the fungicides degradation influence on wheat growth reduction was 4: 2.5 according four sulphur atoms able to create sylphydril groups or other acidic consequences in oxsidative stress conditions from Dithane M 70 and sulphur one and three chlorine in Folpan when

Table 1: a and b. Effects of larger (a) and slighter (b) Dithane M 70 amount and Folpan on growth of seedlings.

a.

	FLL	TLL	FLL	TLL	FLL	TLL	Growth reduction by TLL		SLGRD SLGRF SGLR		
	Dithane M 70		Folpan		Control		Dithane M 70	Folpan	Dithane M 70	Folpan	Control
Lr 1	10.0	18.9	9.5	19.5	8.7	19.7	4%	1%	0.53	0.49	0.46
Lr 2[a]	9.8	19.1	10.1	19.5	10.3	20.6	7%	5%	0.51	0.52	0.50
Lr 15	10.9	20.3	10.1	20,6	10.5	21.7	6%	5%	0.54	0.49	0.48
Lr 16	10.8	16.6	10.9	19.9	10.1	20.7	20%	3%	0.65	0.55	0.48
Lr22b	10.2	21.4	11.2	21.6	10,2	21.8	2%	1%	0.48	0.52	0.47
Lr 29	10.7	19.5	11,8	17,9	10.2	21.6	9%	17%	0.55	0.65	0.47
Aver.	**10.3**	**19.3**	**10.5**	**19.8**	**10.2**	**21.0**	**8%**	**5%**			
b.											
Lr 1	12.3	19.5			11.3	20.4	3%		0.62		0.55
Lr 2[a]	12.9	21.4			13.3	22.2	4%		0.60		0.60
Lr 15	12.7	18.7			13.0	19.2	4%		0.68		0.68
Lr 16	12.6	18.3			12.2	19.5	7%		0.68		0.62
Lr22b	9.7	20.9			9.7	21.2	1%		0.46		0.46
Lr 29	12.2	18.9			12.6	20.0	5%		0.65		0.63
Aver.	**11.7**	**19.6**			**12.0**	**20.4**	**4%**				

focused were Mg salts. Average growth reduction caused by two fungicides degradation of 5% and 8% was accidently convenient to predicted ratio. Result of Lr 22b line was near declaring hydrolytic effect as well as was of low effective Lr 2a and Lr 15 ones. By comparing of Lr 16 with highest influence on Dithane M 70 degradation consequented by 20% TLL reduction and Lr 29 similar effective to Folpan (17%), most evidently was suggested that previous pathway had to be followed by recovering process linked to sulphur containing consequences. The pathway for the Mg 2+ kation release from sulphate was across sulphate adenylyl transferase (Knowlevs et al., 1980; Michaels et al., 1970; Rosenthal and Leustek, 1995). Such pathway for $MgCl_2$ was not reported as well as not indicated via presented results. Beside, Zn and very likely Mn predicted to be at first liberated from Dithane M 70 could be embedded to phytin but photosynthetic function of the product was only speculated (Hirai et al., 2011). The accelerated disconnection of at first present one and later consequential formed another double covalent CS linkage was at first sight transparent as reasons for adequacy of Lr 16 and low influence on Folpan. N-C-S bound character of Folpan in proposed surrounding appeared to be the most adequate target for Lr 29. The molecular weight of targeted disulphide bond was same as was in Dithane M 70 but distribution and surrounding was different. C-S link was described as 40% stronger than S-S, when protein degradation was described (Masson et al., 1986). Influence of Lr 29 on protein degradation was opposite of those on Folpan, most evidently confirming specificity of the gene effect. Difference between presented results of Lr 1 and Lr 15 contrary to similar effect on wheat seed proteins also supported explanation of genes specificity across differences in energetic level and approach ability defined trough the molecular weight of for the effect necessary element surrounding influential on strength of its linkages. By last mentioned genes involvement in the trial covered was the strongest effect on possible protein degradation. Such outcome was not recognized at seedlings grown on proposed temperature.

CONCLUSION

Parasite free effect of leaf rust resistance genes was confirmed again but this time across accelerated degradation of substances not synthesized in plant proving that proteinic nearby structure was not necessary for the effect. Sulphur surrounding appeared to be crutial for Lr genes specification. At least four different genes for specific resistance were defined across vice verse to boath or highly dissimilar effects to same fungicide.

The consequences of interaction with fungicides in practice were predicted to be decreased of those achieved in presented trial because of lower dose proposed to reduce parasites. Discovered could be usefull for parasite reduction in dry regions. Also, the method could be also applied for to parasitic population not efective Lr genes detection.

The amount of different structures created on the base of most common organic elements linkages with sulphur adequate to be degraded by particular Lr gene also appeared to be the explanation of prolonged latency period transformation to lower reaction type dependent of parasite isolate.

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EFEKAT POJEDINAČNIG GENA ZA OTPORNOST PREMA *PUCCINIA TRITICINA* NA DEGRADACIJU FUNGICIDA SA SUMPOROM

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REZIME

Sumporne veze su bile istaknute kao moguća mesta dejstva enzima odgovornih za specifičnu otpornosti prema prouzrokovaču lisne rđe (Lr geni), degradaciju proteina pšenice i usporen rast sledećih organa. Pet izogenih linija linija sa specifičnim i jedna sa nespecifičnim genom za otpornost su bile upoređene na osnovu dužina prvog i drugog lista u stadijumu sejanaca osam dana nakon tretmana kontaknim fungicidima koji sadrže vezan sumpor pri različitim koncentracijama. Umanjeni rast je bio potpuno koreliran s većom dozom Dithane M 70 (r=0.93). Lr 16 je najefikasnije razdvajao S=C-S veze u Dithane M 70 i reducirao rast za 20%, dok je uticaj preko Folpana bio svega 3%. Degradacija N-C-S veze u Folpanu preko Lr-a 29 gena bila je najbrža, što je zaključeno preko 17% redukovanog rasta. Pomenuti gen razgradio je i Dithane M 70, 9 ili 5% u zavisnosti od doze. Najviše su se razlikovali rezultati linije nosioca gena za nespecifičnu otpornost Lr 22b (1 i 2%). Efekat ostalih gena bio je intermedijaran 4-7%. Specifični geni Lr 2a i Lr 15 su bili od slabijeg uticaja na oba preparata od prvopomenutih gena dok Lr 1 nije uticao na razgradnju Folpana više od Lr 22b. Četiri grupe Lr gena su bile diferencirane, te funkcija produkata Lr gena bez elicitinog dejstva istih iz gljive dokazana. Praktično, uz prisustvo odgovarajućih gena propisana karenca za Dithane M 70 u semiaridnom regionu od dve nedelje može biti skraćena i odgovarajuća sušnijem regionu u Junu, te primeni na početku bez rezidua.

Ključne reči: *Puccinia triticina*, Lr geni, fungicidi

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FITOPLAZMOZE LUCERKE (*Medicago sativa* L.) U CENTRALNOJ SRBIJI

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REZIME

Simptomi kržljavosti, proliferacije, filodija, žutila i crvenila lucerke uočeni su prvi put u četiri lokaliteta u Centralnoj Srbiji (Tuleš, Sićevo, Ljubava i Belanovice) tokom 2008 godine. Godišnji indeks porasta zaraze kretao se od 2 do 6, a smanjenje prinosa zelene mase između 30–50%. Primenom RFLP i analize sekvenci 16S rDNK, identifikovano je prisustvo fitoplazmi u uzorcima lucerke koje pripadaju 16SrIII-B i 16SrXII-A grupama. Fitoplazma Stolbur tipa bila je dominantna i dokazana je u 80% uzoraka simptomatičnih biljaka.

Ključne reči: lucerka, fitoplazma, 16SrIII-B fitoplazma, Stolbur, rasprostranjenost

UVOD

Lucerka (*Medicago sativa* L.) je najvažnija leguminozna biljka u Srbiji. Gaji se zbog visokog prinosa kvalitetnog sena. Značajna je kultura i sa aspekta očuvanja strukture zemljišta i njegove plodnosti. Površine pod lucerkom u Srbiji menjaju se iz godine u godinu, ali se kreću oko 200 000 ha (Djukić, 2005). U našim uslovima ostvaruje dva otkosa.

Simptomi kržljavosti, proliferacije, filodija, žutila i crvenila lucerke uočeni su u nekoliko lokaliteta u Centralnoj Srbiji tokom 2008. godine (Starović i sar., 2012). Na prikupljenim uzorcima utvrđeno je prisustvo Stolbur fitoplazme i fitoplazme žutila deteline ("Clover yellows edge"-SYE) (Starović i sar., 2012).

Brojni su podaci o fitoplazmozama lucerki u svetskoj literaturi. Najzastupljeniji simptom "veštičinih metli", povezan je, u zavisnosti od geografske pozicije, sa različitim grupama fitoplazmi: sa fitoplazmom filodija pasulja ("faba bean phyllody" – FBP) (Marcone et al. 1997; Khan et al. 2002), proliferacije de-

teline ("clover proliferation" - CP) (Wang and Hiruki 2001), zvezdastog žutila ("aster yellows" - AY) (Valiunas et al. 2000), Australijskog žutila lucerke (ALuY) (Pilkington et al. 2003), *Candidatus* Phytoplasma astera u Boliviji (Jones et al. 2005) i fitoplazmom lucerke u Argentini (Conci et al. 2005). Prisustvo stolbur fitoplazme potvrđeno je na lucerki u Italiji (Marzachi i sar., 2000) u Indiji (Suryanarayana et al. 1996) i u SAD (Peters et al. 1999). Takođe, lucerka je rezervoar fitoplazme žutila repice (Wang and Hiruki 2001).

MATERIAL I METODE

TERENSKA ISPITIVANJA

Prikupljanje materijala

Po deset uzoraka lucerke koje su ispoljavale simptome i jedan uzorak biljaka bez simptoma sakupljeno je za PCR analizu u lokalitetima Tuleš, Sićevo i Ljubava (Tab. 1) tokom 2008 i 2009. godine (ukupno 66 uzoraka), a tokom 2010. u lokalitetu Belanovice.

Stepen pojave oboljenja

U lucerištima uočeni su po prvi put fitoplazmozni simptomi u lokalitetima Tuleš, Sićevo, Ljubava i Belanovice u leto 2008 godine. Procenat zaraženih biljaka ocenjivan je sva četiri lokaliteta tokom 2008-2010. Drveni ram površine ¼ m^2 bacan je 10 puta po dijagonali u svakoj parceli i izračunavan je procenat obolelih biljaka i godišnji indeks porasta zaraze u dve uzastopne godine.

Procena prinosa zelene mase

Prinos zelene mase drugog otkosa određen je na svakoj parceli merenjem pokošene nadzemne mase direktno na parceli i preračunata na t ha^{-1} (Katić i sar., 2011) tokom 2010. godine.

Detekcija i identifikacija fitoplazmi

Nukleinske kiseline su ekstrahovane iz lišća lucerke po metodi koju su opisali Angelini i saradnici (2001). Sukcesivnim korišćenjem para prajmera P1/16S-Sr i R16F2n/R2 (Lee et al. 2006) u umetnutom (nested) PCR detektovano je prisustvo fitoplazmi u simptomatičnm uzorcima lucerke. Ekstrahovana DNA iz 8 asimptomatičnih biljaka korišćena je kao negativna kontrola. PCR amplifikacije su izvedene prema Gundersen i Lee (1996) korišćenjem DreamTaqGreen PCR Master Mix (Fermentas, Lithuania) u aparatu Eppendorf Master Cycler Personal, Germany.

Polimorfizam dužine restrikcionih fragmenata- Restriction Fragment Length Polymorphism (RFLP) R16F2n/R2 amplikona dobijen je pojedinačnim digestijama restrikcionim endonukleazama: *Alu*I i *Tru*I. Dobijeni RFLP profili upoređivani su sa referentnim profilima Stol, AY, FD-C i CYE fitoplazmi. *Hpa*II i *Hha*I restrikcione endonukleaze dodatno su korišćene pri analizi amplikona koji su pokazali sličnosti sa CYE fitoplazmom posle digestije sa *Alu*I i *Tru*I , a njihovi profili su dodatno upoređeni sa PoiBI i CYE fitoplazmama. Produkti amplifikacije razdvajani su elektroforezom na 1.2 % agaroznim gelovima, a RFLP produkti na 2.3% agaroznim gelovima, obojeni etidijum bromidom i vizuelizovani pomoću UV transiluminatora.

REZULTATI I DISKUSIJA

Simptomi oboljenja

Prvi simptomi u polju uočeni su tokom juna u vidu proliferacije izdanaka sa skraćenim internodijama, filodija i sitnog atipičnog lišća (Sl.1), zatim žutila i crvenila vršnih liski (Sl. 2). Obolele biljke ne donose cvet ili je on sasušen (Sl.3). Stabljike zaraženih biljaka mogu ispoljiti i simptom spljoštenosti.

Slika 1. Početni simptomi – proliferacija izdanaka, skraćene internodije, filodije i sitno atipično lišće

Figure 1. Early symptoms of phytoplasma disease (shoot prolification, short internods, phyllody and small atypical leaves)

Slika 2. Simptomi žutila i crvenila lišća

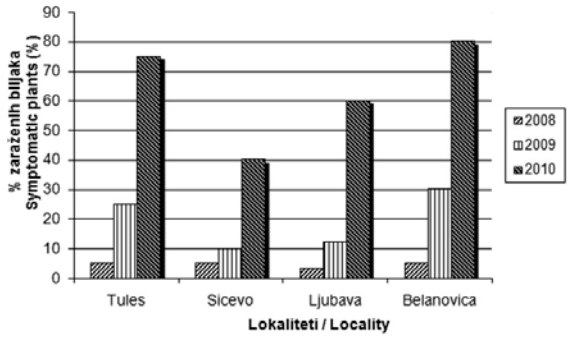
Figure 2. Advance symptoms: yellowing and reddening of the leaves

Slika 3. Obolele biljke ne donose cvet

Figure 3. Diseased plants failing to flower

Stepen pojave oboljenja

Tokom prve godine istraživanja procenat zaraženih biljaka kretao se između 3 (Ljubava) - 5 % (Belanovice i Tuleš). Sledeće godine taj procenat je bio i do 6 puta veći (Belanovice). U trećoj godini procenat zaraženih biljaka iznosio je 75% u Tulešu, 40 % Sićevu, 60% Ljubavi i 80 % u Belanovici (Graf. 1). Godišnji indeks porasta zaraze kretao se od 2 do 6. U lokalitetu Belanovice konstatovano je značajno prisustvo viline kosice (*Cuscuta*) (Sl. 4), čime se može objasniti najviši godišnji indeks porasta zarze.



Grafik 1. Procenat zaraze fitoplazmama u četiri lokaliteta u tri uzastopne godine 2008-2010.

Graf. 1 Disease incidence in four localities in Serbia over 3 years in Serbia

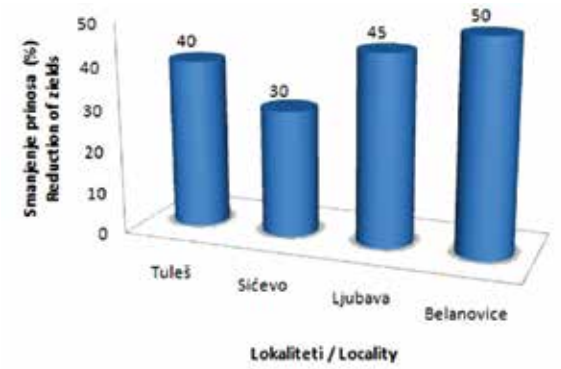
Slika 4. Vilina kosica - vector fitoplazmi u lucerki

Figure 4. *Cuscuta* - phytoplasma vector in alfalfa

Prinos zelene mase

Prinos zelene mase lucerke u drugom otkosu u trećoj godini istraživanja (2010) bio je 3.6, 4.2, 3.3 i 3 t ha^{-1} u lokalitetima Tuleš, Sićevo, Ljubava i Belanovice, dok je prinos zdrave lucerke iznosio 6 t ha^{-1}. Ovi rezultati ukazuju da je zaraza

fitoplazmama uslovila smanjenje prinosa zelene mase lucerke od 30% (Sićevo) do 50% (Belanovice) (Graf. 2). Zbog izuzetno visokog procenta zaraženih biljaka i značajnog smanjenja prinosa u sva četiri lokaliteta, dalje gajenje lucerke postalo je neprofitabilno, zbog čega je ona preorana tokom 2010. godine u lokalitetu Tuleš.



Grafik 2. Procenat smanjenja prinosa lucerke u četiri lokaliteta u 2010 godini

Graf. 2. Reduction of yields in four localities

Detekcija i identifikacija fitoplazmi

Umnožavanje prajmerima P1/16S-Sr rezultiralo je amplikonom od 1.5 kb koji obuhvata veliki deo 16S rDNK (Tab. 1) i to kod 27 od 60 biljaka sa simptomima. Ovaj amplikon je detektovan kod svih uzoraka sa simptomima (8) u lokalitetu Belanovica. Posle nested PCR-a, fragmenti od 1.2 kb detektovani su kod 51 biljke sa simptomima. Kod biljaka bez simptoma nije bilo amplifikovanih produkata. RFLP analiza- delovanje restrikcionih endonukleaza *Alu*I i *Tru*I na amplikone od 1.2 kb, rezultovala je dobijanjem 2 tipa profila. Kod 48 pozitivnih uzoraka RFLP profil bio je identičan Stol fitoplazmi koja pripada podgrupi 16SrXII-A, a samo 3 uzorka su imala profil identičan CYE - clover yellow edge fitoplazmi koja pripada 16SrIII fitoplazmama. Pripadnost 16SrIII-B subgrupi dokazana je dodatnom restrikcionom analizom pomoću *Hpa*II i *Hha*I enzima koji daju različite profile za podgrupe B (CYE) i H (poinsettia branch-inducing –PoiBI) u okviru 16SrIII grupe, što se na osnovu delovanja restrikcionih endonukleaza *Alu*I i *Tru*I nije moglo ustanoviti.

U uzorcima lucerke iz različitih regiona Italije prvi put je detektovana Stolbur fitoplazma

Tabela 1. Detekcija fitoplazmi u uzorcima lucerke sa i bez simptoma kolekcionisanim u 2008 (L1-L44) i 2009 (L45-L88) direktnom, nested i PCR-RFLP analizama
Table 1. Phytoplasma detection from symptomatic and asymptomatic (as) alfalfa collected in 2008 (L1-L33) and 2009 (L34-L66) by direct-, nested- and PCR-RFLP analyses

Lokalitet Locality	Broj PCR testiranih uzoraka PCR tested sample no	Broj P1/16S-Sr pozitivnih uzoraka/P1/16S-Sr positive samples no	Broj R16F2n/R2 pozitivnih uzoraka R16F2n R2 positive samples no	RFLP tip odgovarajuće grupe RFLP type corresponding to group	
				16SrIII-B	16SrXII-A
Tuleš	L1-L10	5	9	-	9
	L11 (bs)	0	0	-	-
	L34-L43	8	10	3 (L41)	7 (L42)
	L44 (bs)	0	0	-	-
Sićevo	L12-L21	2	7	-	7
	L22 (bs)	0	0		-
	L45-L54	3	8		8
	L55 (bs)	0	0		-
Ljubava	L23-L32	3	7	-	7
	L33 (bs)	0	0		-
	L56-L65	6	10		10
	L66 (bs)	0	0		-
Ukupno (% simptomatičnih uzoraka) **Total** (% of symptomatic samples)	**66 (60=100%)**	**27 (45%)**	**51 (85%)**	**3 (5%)**	**48 (80%)**

(Marzachi et al., 2000). Analizom 16S rDNA sekvenci reprezentativnih izolata L41 i L42 iz Tuleša potvrđeno je prisustvo 16SrIII-B i 16SrXII-A podgrupa fitoplazmi (Starović i sar., 2012). Pored lucerke, u Srbiji je Stolbur fitoplazma detektovana na različitim biljnim vrstama koje imaju veliki ekonomski značaj: vinova loza (Duduk et al. 2004; Kuzmanović et al. 2003; 2008; Josic et al. 2006), kukuruz (Duduk and Bertaccini, 2006), šargarepa (Duduk et al. 2008a), a u novije vreme i kod značajnih lekovitih biljaka poput kupine (Kuzmanovic et al. 2011), ehinacee (Pavlovic et al. 2011), kantariona (Pavlovic et al, 2012), bokvice (Josic et al. 2012), saponarije (Josic et al., 2012). Istraživanja elongacionog faktora *tuf* gena u uzorcima lucerke u kojima je detektovana 16SrXII-A fitoplazma ukazala su na prisustvo TufAY/*Hpa*II produkata koji odgovaraju TufAY-b tipu (Starović i sar., 2012), koji je u Srbiji dokazan i na kukuruzu (Duduk i Bertaccini, 2006), celeru (Ivanovic i sar., 2011) i kelju (Trkulja i sar., 2011).

Samo je 5% testiranih uzoraka lucerke sa ispoljenim simptomima fitoplazmi, bilo zaraženo 16SrIII-B fitoplazmom i to u lokalitetu Tuleš. Fitoplazma iz 16SrIII-B podgrupe je već ranije identifikovana na *Cirsium arvense* u više od 10 lokaliteta širom Srbije (Rančić i sar., 2005) i na krušci (Duduk i sar., 2008b). Duduk i saradnici (2008a) su utvrdili prisustvo fitoplazme iz16SrIII-B podgrupe, po prvi put, u insektu *Psammotettix notatus* (Melichar), skakvcu iz podfamilije *Deltocephalinae*, nađene na polju mrkve. Na gajenoj *T. pratense* oboleloj od patuljave bolesti u više lokaliteta u Češkoj Republici, pored rabdovirusa utvrđeno je i pet različitih podgrupa fitoplazmi: 16SrI-B, 16SrI-C, 16SrIII-B i 16SrX-A, na *T. repens* 16SrI-C i *T. hybridum* dve podgrupe fitoplazmi: 16SrI-C i 16SrIII-B (Franova et al. 2004).

Visok procenat (66) detektovanih fitoplazmi u 60 simptomatičnih biljaka sugeriše da one mogu biti prouzrokovač ovog oboljenja lucerke u ispitivanim lokalitetima. Ne ispoljavaju se razlike u simptomima između biljaka zaraženih fitoplazmom Stolbur (16SrXII-A) i fitoplazmom Clover yellow edge (16SrIII-B). Simptomi na lucerki ne mogu biti dijanostički, jer ne manifestuju razlike kod dokazanih grupa fitoplazmi. Ovo je već ranije pokazano na primeru mešane zaraze Stolbur i Aster Yellows fitoplazmom na paradajzu, koji ispoljavaju identične simptome (Vellios i Lioliopoulou, 2007). Davis i Sinclair (1998) ukazuju da različite fitoplazme mogu ispoljiti identične simptome na određenim biljnim vrstama, kao i da vrlo bliske fitoplazme mogu ispoljiti različite simptome na istoj biljnoj vrsti.

Ispoljeno smanjenje prinosa zelene mase lucerke zaražene fitoplazmama u jednom otkosu koje se kretalo između 30-50% je vrlo značajno. Za utvrđivanje štetnosti fitoplazmi na lucerki neophodno je izračunati i prosečno godišnje smanjenje prinosa u dva otkosa, kao i prinos i kvalitet suve materije.

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PHYTOPLASMA DISEASES OF ALFALFA (*Medicago sativa* L.) IN CENTRAL SERBIA

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SUMMARY

The symptoms of stunding, phylody, yellowing and reddening of alfalfa were observed for the first time in the four localities in Central Serbia: Tuleš, Sićevo, Ljubava and Belanovice, during 2008. Annual index of infection increase was between 2-6, while the reduction of the green mass was between 30-50%. Application of RFLP and sequence analysis 16S rDNA identified the presence of the phytoplasm in the samples of alfalfa belonging to 16SrIII-B and 16SrXII-A groups. Solbur type phytoplasm was the dominant type found in the 80% of plants exhibiting the symptoms.

Key words: alfalfa, phytoplasma, 16SrIII-B phytoplasma, Stolbur, distribution

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MORFOLOŠKE I ODGAJIVAČKE KARAKTERISTIKE IZOLATA *CERCOSPORA BETICOLA*

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REZIME

Utvrđivanje morfoloških i odgajivačkih karakteristika izolata *C. beticola* izvedeno je na KDA, MEA i CDA podlozi. Utvrđivanje morfoloških karakteristika obuhvatilo je praćenje porasta micelije, teksture, boje, ivične zone i boje naličja micelije. Porast izolata na različitim temperaturama praćen je na KDA podlozi na temperaturama 10°C, 15°C, 20°C, 25°C, 30°C, 35°C i 40°C. Utvrđena je značajna varijabilnost porasta izolata na KDA, MEA i CDA podlozi, a variabilnost postoji i u porastu različitih izolata na istoj podlozi. Izolati su ispoljili i variranje u boji i širini ivične zone tokom porasta na različitim podlogama. Tekstura micelije bila je pamučasta na svim podlogama, dok je boja sa naličja bila mrka do tamno-maslinasta. Na temperaturama 10°C i 40°C izolati *C. beticola* nisu razvijali miceliju, a najintenzivniji porast je ostvaren na 25°C.

Ključne reči: *C. beticola*, porast micelije, morfologija, temperature

UVOD

Pegavost lista šećerne repe koju izaziva fitopatogena gljiva *Cercospora beticola* Sacc. je najznačajnija bolest šećerne repe u svetu. Prvi put je bolest pegavosti lista šećene repe opisana krajem 19. veka (Saccardo, 1876). Rasprostranjena je u gotovo svim zemljama gde se gaji šećerna repa: SAD, Nemačka, Holandija, Grčka, Italija (Windels et al., 1998, Wolf and Werreet, 2002, Vereijssen et al., 2007, Karaoglanidis et al., 2002, Moretti et al., 2004). U našoj zemlji prvi podaci o štetnosti *C. beticola* datiraju iz 50-tih godina prošlog veka (Marić, 1956).

Ranija istraživanja ukazuju na visoku morfološku varijabilnost izolata *C. beticola* pri porastu na različitim hranljivim podlogama (Moretti et al. 2004). Neki autori utvrdili su razlike u patogenosti i virulentnosti kod izolata koji se na osnovu morfoloških karakteristika mogu svrstati u različite grupe (Canova, 1959; Brilova, 1987). Whitney i Lewellen (1976) su ustanovili da

različite fiziološke rase *C. beticola* na različitim podlogama razvijaju konidiofore i konidije koje se značajno razlikuju. Uticaj temperature na porast izolata može imati veliki značaj kako za karakterizaciju izolata unutar jedne vrste, tako i za identifikaciju vrsta koje su morfološki veoma slične (Groenewald et al., 2005).

Cilj ovog rada bio je utvrđivanje morfoloških i odgajivačkih karakteristika izolata *C. beticola* poreklom sa šećerne repe i cvekle, sa više lokaliteta na teritoriji Srbije.

MATERIJAL I METODE

Uzorkovanje i izolacija

Tokom trogodišnjeg perioda (2007–2011) sa nekoliko lokaliteta na teritoriji Srbije obavljeno je prikupljanje listova šećerne repe i cvekle sa karakterističnim simptomima lisne pegavosti (Tabela 1). Uzorci su pakovani u papirne kese i u ručnom frižideru transportovani do laboratorije Odseka za bolesti bilja u Institutu za zaštitu bilja i životnu sredinu u Beogradu, gde su dalje obrađivani u cilju izolacije patogena. Listovi sa vidljivim pegama pregledani su pod binokularom u cilju utvrđivanja prisustva konidija *C. beticola*. Kod listova bez vidljivih konidija, u cilju podsticanja sporulacije vršeno je njihovo ispiranja sterilnom destilovanom vodom. Nakon ispiranja listovi su odlagani u termostat na temperaturu od 25°C, bez svetlosti, u trajanju od 24 sata. Za dobijanje monosporijalnih izolata iz jedne pege sa jednog lista uzimane su pojedinačne konidije i prenošene na podlogu od krompir-dekstroznog agara (KDA). Inkubacija kolonija obavljena je u termostatu u trajanju od 2-3 dana na temperaturi 25°C. Formirana micelija presejana je na novu KDA podlogu i gajena tokom 14 dana na temperaturi od 25°C nakon čega su izolatima dodeljene šifre i korišteni su za dalja ispitivanja.

Utvrđivanje morfoloških i odgajivačkih karakteristika izolata *C. beticola*

Proučavanje morfoloških i odgajivačkih karakteristika izolata *C. beticola* poreklom sa šećerne repe i cvekle, obuhvatilo je: praćenje porasta micelije na različitim hranljivim podlogama; teksture i boje kolonija; porast micelije na različitim temperaturama i ispitivanje uticaja sastava hranljivih podloga na sporulaciju patogena.

Za testiranje su korištene tri hranljive podloge: podloga od krompir-dekstroznog aga-

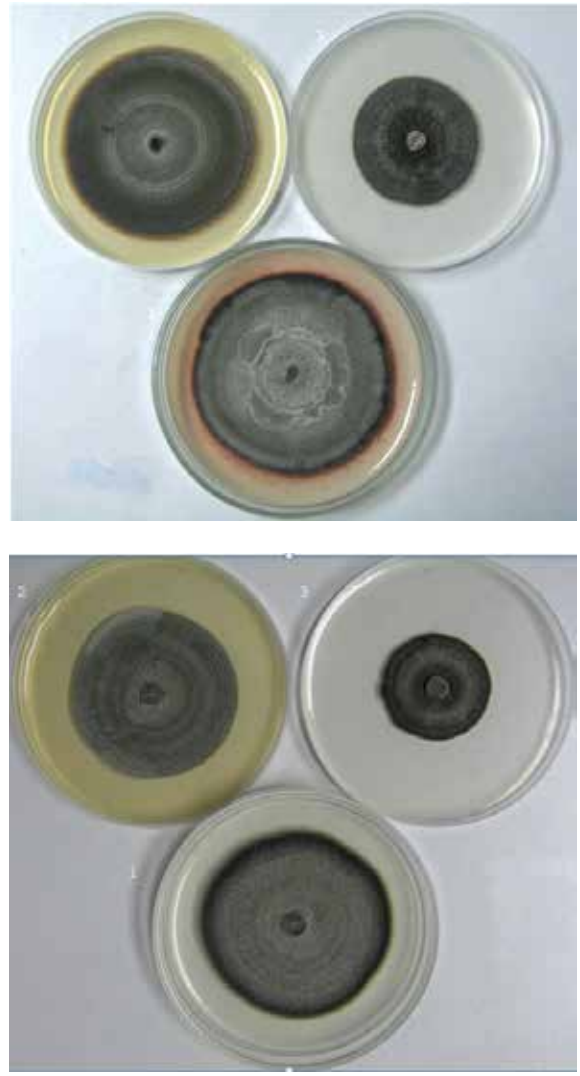
Tabela 1. Šifre izolata C. beticola sa podacima o biljci domaćinu, geografskom poreklu i godini izolacije
Table 1. Codes of C. beticola isolates with information on plant hosts, geographical origin and year of isolation

Šifra izolata	Biljka domaćin	Poreklo izolata	Godina
Q2	šećerna repa	Srem, Šid	2010
Q3	šećerna repa	Srem, Šid	2010
Q6	šećerna repa	Srem, Šid	2010
Q11	šećerna repa	Srem, Šid	2010
PSD10	šećerna repa	Južni Banat, Kovačica	2011
PSD16	šećerna repa	Južni Banat, Kovačica	2011
STO93	šećerna repa	Južni Banat, Kovin	2011
STO942	šećerna repa	Južni Banat, Kovin	2011
RO98	šećerna repa	Srem, Ruma	2009
RO91	šećerna repa	Srem, Ruma	2009
G2	cvekla	Moravica, Guča	2010
NEGA2	cvekla	Bor, Negotin	2007
NEG3	cvekla	Bor, Negotin	2007

ra (KDA); podloga od malca (MEA) i Čapekova kisela podloga (CDA) (Dhingra and Sinclair, 1986). Nakon pripreme i sterilizaacije po 20 ml podloge razliveno je u Petri kutije (90 mm). Zasejavanje isečaka micelije na podloge je izvršeno nanošenjem fragmenata za svaki izolat (Ø 5 mm) iz kultura starih 14 dana, u centar nove Petri kutije. Izolati *C. beticola* su potom inkubirani u termostatu na temperaturi od 25°C. Porast izolata praćen je nakon 7 i 14 dana merenjem prečnika kolonije. Pored porasta micelije ocenjivana je tekstura micelije, boja, širina ivične zone i boja micelije sa naličja. Procena sporulacije patogena na različitim podlogama (KDA, MEA, CDA) izvršena je struganjem površinskog sloja micelije sterilnom kopljastom iglom iz centralnog dela kolonije starosti 14 dana i prebrojavanjem konidija pomoću hemocitometra.

Ispitivanje uticaja temperature na porast *C. beticola*

Ispitivanje uticaja temperature na porast izolata *C. beticola*, vršeno je na temperaturama 10ºC, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC i 40 ºC, gajenjem izolata na KDA podlozi u trajanju od 7 dana.



Slika 1. Izgled izolata *C.beticola* poreklom sa šećerne repe i cvekle nakon porasta na KDA (1), MEA (2) i CDA (3) podlozi u trajanju od 14 dana

Figure 1. Appearance of *C.beticola* isolates originating from sugar beet and beetroot after growth on PDA (1), MEA (2) and CDA (3) medium for 14 days

REZULTATI

Morfološke i odgajivačke karakteristike izolata *C. beticola*

Izolati *C. beticola* gajeni na tri različite podloge, prvi put su mereni 7 dana nakon zasejavanja. Najveći porast micelije izolati su pokazali na KDA podlozi (25.8 mm) (Tabela 2), nešto manji porast na MEA podlozi (21.1 mm) (Tabela 3), dok je najmanji porast micelije izmeren na CDA podlozi (13.6 mm) (Tabela 4). Na KDA podlozi izolati pokazuju varijabilnu brzinu rasta, od najvećeg 30.2 mm utvrđenog za izolat Q6, do najmanjeg porasta koji je izmeren za izolat RO98 koji je iznosio 21.7 mm. Na MEA izolat Q6 je imao najintenzivniji porast (26.8 mm), dok je izolat NEG3 najslabije rastao (17.2 mm). Na CDA najintenzivniji rast pokazao je izolat STO942 (16.3 mm), dok je najmanji rast pokazao izolat NEG3 (10.2 mm). Porast izolata *C. beticola* izmeren je i posle 14 dana. Izolati su najintenzivnije rasli na KDA hranljivoj podlozi (50.4 mm) (Tabela 2), nešto manje na MEA podlozi (44.7 mm) (Tabela 3), dok je najmanji rast izmeren na CDA podlozi (29.3 mm) (Tabela 4) (Slika 1). Posle 14 dana na

KDA najveći porast imao je izolat Q6 (62.2 mm), a najmanji porast, izolat G2 (37.5 mm). Na MEA najintenzivnije je rastao izolat RO98 (53.2 mm), dok je izolat G2 pokazao najslabiji rast (37.2 mm). Na CDA najbolje je rastao izolat PSD10 (34.0 mm), a najmanje izolat Q3 (21.5 mm).

Rezultati proučavanja morfoloških karakteristika izolata na KDA podlozi ukazuju da izolati formiraju pamučastu miceliju bele, sive, svetlo-maslinaste i tamno-maslinaste boje, ravnih i pravilnih ivica. Širina ivične linije značajno varira između izolata, od 1 mm za izolat PSD10, do 10 mm za izolat RO98. Izolati se značajno razlikuju u boji ivične linije koja varira i može biti bela, crvena, mrka ili braon. Na naličju Petri kutije kolonije svih izolata su formirale miceliju mrke boje. Na KDA podlozi izolati *C. beticola* nisu sporulisali (Tabela 2).

Izolati na MEA podlozi formiraju pamučastu kompaktnu miceliju bele, svetlo sive, svetlo-maslinaste i tamno-maslinaste boje, pravilnih ili blago talasastih ivica. Širina ivične linije kod ispitivanih izolata na MEA podlozi manje varira u odnosu na variranje ivične linije na KDA podlozi, i kreće se u intervalu 1 mm do 3 mm. Većina izolata ima belu boju ivične linije, dok je za izolat Q3 tamno maslinasta. Sa naličja kolonije izolata formiraju miceliju mrke do tamno-maslinaste boje. Nijedan izolat nije ostvario sporulaciju na podlozi od malca (Tabela 3).

Izolati na CDA formiraju pamučastu miceliju, znatno tamnije boje nego na KDA i MEA, blago režnjevitih ili nepravilnih ivica. Boja micelije je dosta ujednačena svetlo-maslinasta do tamno-maslinasta. Širina ivične linije izolata na CDA podlozi varira od 1 mm, do najviše 8 mm za izolat STO93. Utvrđeno je značajno variranje u boji ivične linije koja može biti bela svetlo-maslinasta , tamno-maslinasta i crvena. Sa naličja kolonije su mrke do tamno-maslinaste boje. Izolati *C. beticola* na CDA podlozi nisu sporulisali (Tabela 4).

Uticaj temperature na porast micelije *C. beticola*

Ispitivanja uticaja temperature na morfo-

Tabela 2. Morfološke karakteristike izolata C. beticola na KDA podlozi
Table 2. Morphological characteristics of C. beticola isolates on PDA medium

Šifra Izolata	Porast (7 dana)	Porast (14 dana)	Sporulacija	[a]Tekstura micelije	[b]Boja	Ivična linija (mm)	Boja ivične linije	Boja naličja
Q2	23.5±0.50	53.2±1.04	[c]nu	Pk	B	5	Br	M
Q3	24.3±0.29	48.3±1.10	nu	Pk	Sm	3	B	M
Q6	30.2±0.76	62.2±1.04	nu	Pk	Ss	5	Br	M
Q11	28.3±0.29	43.2±0.76	nu	Pk	Sm	3	B	M
PSD10	25.3±1.53	49.2±1.04	nu	Pk	Sm	1	C	M
PSD16	25.3±1.15	49.7±1.15	nu	Pk	Sm	2	B	M
STO93	26.0±0.50	52.5±0.87	nu	Pk	Sm	5	B	M
STO942	26.5±0.50	53.2±0.76	nu	Pk	Sm	2	B	M
RO91	25.7±0.76	48.2±0.76	nu	Pk	Sm	2	B	M
RO98	21.7±0.58	55.5±0.50	nu	Pk	Sm	10	M	M
G2	25.5±0.50	37.5±0.50	nu	Pk	Ss	3	B	M
NEGA2	26.7±0.58	49.5±1.32	nu	Pk	Tm	2	Br	M
NEG3	24.7±0.76	46.8±0.76	nu	Pk	Ss	3	Br	M
[d]Ms	**25.8±2.09**	**50.4±5.45**	-	-	-	-	-	-

[a] Tekstura micelije: Pk – pamucasta kompaktna
[b] Boja: B – bela, Br – braon, C – crvena, M – mrka, Sm – svetlo-maslinasta, Tm – tamno-maslinasta, Ss – svetlo siva, [c]nu – nije utvrđena, [d] Srednja vrednost

Tabela 3. Morfološke karakteristike izolata C. beticola na MEA podlozi
Table 3. Morphological characteristics of C. beticola isolates on MEA medium

Šifra Izolata	Porast (7 dana)	Porast (14 dana)	Sporulacija	[a]Tekstura micelije	[b]Boja	Ivična linija (mm)	Boja ivične linije	Boja naličja
Q2	18.0±0.50	39.3±0.76	cnu	Pk	B	1	B	M
Q3	18.8±0.75	43.2±1.04	nu	Pk	B	3	Tm	Tm
Q6	26.8±0.29	52.3±0.76	nu	Pk	B	1	B	M
Q11	21.0±1.00	42.2±0.76	nu	Pk	B	1	B	M
PSD10	22.0±1.00	44.0±0.50	nu	Pk	B	3	B	M
PSD16	22.2±0.76	44.8±1.04	nu	Pk	Sm	2	B	M
STO93	25.7±0.76	43.0±1.50	nu	Pk	Ss	1	B	M
STO942	25.8±0.29	52,2±0.76	nu	Pk	Sm	2	B	M
RO91	20.0±0.50	40.8±0.76	nu	Pk	B	3	B	M
RO98	19.0±0.50	53.2±0.76	nu	Pk	Sm	3	B	M
G2	17.7±0.76	37.2±0.29	nu	Pk	Sm	3	B	Tm
NEGA2	18.8±0.76	44.2±0.76	nu	Pk	Tm	3	B	M
NEG3	17.2±0.29	40.0±1.00	nu	Pk	Sm	3	B	M
[d]Ms	**21.1±3.18**	**44.7±5.06**	-	-	-	-		-

[a] Tekstura micelije: Pk – pamucasta kompaktna
[b] Boja: B – bela, Br – braon, C – crvena, M – mrka, Sm – svetlo-maslinasta, Tm – tamno-maslinasta, Ss – svetlo siva, [c]nu – nije utvrđena, [d] Srednja vrednost

loške karakteristike i rast micelije na PDA podlozi pokazuje da nijedan od testiranih izolata ne može da raste na temperaturama 10°C i 40°C. Opseg temperatura na kojima je zabeležen rast izolata nalazi se u opsegu od 15°C do 35°C. Temperature 15°C i 35°C su znatno nepovoljnije za rast i formiraju se kolonije slabe bujnosti. Optimalna temperatura za porast izolata *C. beticola* je 25°C (Tabela 5).

Tabela 4. Morfološke karakteristike izolata C. beticola na CDA podlozi
Table 4. Morphological characteristics of C. beticola isolates on CDA medium

Šifra Izolata	Porast (7 dana)	Porast (14 dana)	Sporulacija	[a]Tekstura micelije	[b]Boja	Ivična linija (mm)	Boja ivične linije	Boja naličja
Q2	11.5±0.50	23.3±1.04	[c]nu	Pk	Sm	1	Tm	Tm
Q3	12.1±0.29	21.5±1.00	nu	Pk	Sm	1	Sm	Tm
Q6	13.3±0.58	22.3±0.76	nu	Pk	Tm	2	B	M-Tm
Q11	11.5±0.50	27.3±0.76	nu	Pk	Sm	1	B	M
PSD10	15.3±0.58	34.0±0.50	nu	Pk	Sm	3	C	M
PSD16	16.2±1.04	31.2±0.76	nu	Pk	Sm	3	B	M
STO93	15.3±0.76	31.0±1.80	nu	Pk	Sm	8	C	M
STO942	16.3±0.76	30.2±1.04	nu	Pk	Tm	1	B	M
RO91	14.3±0.29	33.0±0.50	nu	Pk	Sm	1	B	M
RO98	13.8±0.58	33.2±1.26	nu	Pk	Sm	3	B	M-Tm
G2	10.8±0.58	23.3±0.76	nu	Pk	Tm	2	B	Tm
NEGA2	11.7±0.58	32.8±0.76	nu	Pk	Tm	2	B	M
NEG3	10.2±0.29	30.7±1.15	nu	Pk	Tm	2	B	M
[d]Ms	13.6±2.20	29.3±4.56	-	-	-	-		-

[a] Tekstura micelije: Pk – pamucasta kompaktna
[b] Boja: B – bela, Br – braon, C – crvena, M – mrka, Sm – svetlo-maslinasta, Tm – tamno-maslinasta, Ss – svetlo siva, [c]nu – nije utvrđena, [d] Srednja vrednost

Tabela 5. Uticaj temperature na porast izolata C. beticola
Table 5. The influence of temperature on the mycelial growth of C. beticola isolates

Šifra izolata	Srednja vrednost±Standardna devijacija						
	10°C	15°C	20°C	25°C	30°C	35°C	40°C
Q2	0.00±0.00	8.5±0.40	18.7±0.25	25.5±0.40	20.8±0.72	9.9±0.26	0.00±0.00
Q3	0.00±0.00	8.2±0.30	18.3±0.32	25.4±0.40	21.3±0.29	9.2±0.31	0.00±0.00
Q6	0.00±0.00	8.6±0.17	19.0±0.26	28.5±0.45	22.2±0.58	9.8±0.30	0.00±0.00
E1	0.00±0.00	8.2±0.15	20.4±0.38	25.1±0.56	22.6±0.38	10.0±0.32	0.00±0.00
PSD10	0.00±0.00	8.1±0.36	17.7±0.45	25.0±0.67	19.1±0.35	10.2±0.40	0.00±0.00
PSD16	0.00±0.00	8.8±0.20	18.0±0.26	25.3±0.10	19.2±0.30	10.3±0.26	0.00±0.00
STO93	0.00±0.00	9.0±0.60	19.3±0.32	25.7±0.56	21.3±0.51	10.0±0.46	0.00±0.00
STO942	0.00±0.00	9.5±0.42	19.3±0.49	24.6±0.35	20.5±0.35	10.5±0.23	0.00±0.00
RO98	0.00±0.00	10.2±0.21	19.3±0.46	25.0±0.40	20.8±0.64	10.2±0.15	0.00±0.00
RO91	0.00±0.00	7.8±0.38	18.6±0.35	24.2±0.38	21.7±0.56	11.0±0.21	0.00±0.00
G2	0.00±0.00	9.5±0.36	19.7±0.11	24.6±0.15	21.3±0.11	10.7±0.26	0.00±0.00
NEGA2	0.00±0.00	8.2±0.68	16.9±0.25	24.7±0.78	18.7±0.29	10.6±0.30	0.00±0.00
NEG3	0.00±0.00	8.2±0.25	18.4±0.25	25.1±0.15	20.0±0.50	10.6±0.30	0.00±0.00
Srednja vrednost	**0.00±0.00**	**8.2±1.96**	**18.8±0.84**	**25.1±0.99**	**20.8±1.26**	**10.1±0.46**	**0.00±0.00**

DISKUSIJA

Cercospora beticola je najznačajniji patogen šećerne repe i cvekle koji dovodi do sušenja listova i značajnog smanjenja veličine korena i sadržaja šećera. Istraživanja morfološke varijabilnosti i odgajivačkih karakteristika na različitim mikološkim podlogama daju veliki doprinos za dalju karakterizaciju i proučavanje patogenih osobina gljive.

Proučavanjem morfoloških i odgajivačkih karakteristika izolata *C. beticola* Ruppel i sar. (1972) utvrdili su značajnu morfološku varijabilnost izolata *C. beticola* koji imaju široko geografsko poreklo. Tokom ovog istraživanja, uočena je razlika u morfologiji i brzini porasta micelije kod izolata *C. beticola* poreklom sa šećerne repe i cvekle, sa nekoliko lokaliteta iz Srbije, gajenih na tri (KDA, MEA, CDA) različite mikološke podloge. Istraživanje Groenewald i sar. (2005) ukazuje da različite vrste iz roda Cercospora imaju različit porast na istoj hranljivoj podlozi i konstatuju da se ova osobenost može uzeti u obzir kao karakter za identifikaciju vrste. Izolati C. *beticola* u našem istraživanju ispoljili su visoka variranja u porastu na istoj hranljivoj podlozi. Najveći porast izolati *C. beticola* ostvarili su na KDA podlozi zatim na MEA, dok je najslabiji porast bio na CDA podlozi. Ovi rezultati su u skladu sa istraživanjem Groenewald i sar. (2005) koji su ustanovili najintenzivniji rast izolata *C. beticola* na KDA, a nešto manji na MEA podlozi.

Izolati *C. beticola* ispoljili su variranja u boji micelije, na KDA i MEA podlozi preovladavala je svetla boja dok je izolat NEGA2 imao micelju tamno-maslinaste boje. Na CDA podlozi boja micelije je bila znatno tamnija. Širina ivične linije značajno je varirala na KDA (1mm - 10mm) i CDA podlozi (1mm - 8mm), dok je na MEA bila u intervalu 1mm – 3mm. Boja ivične linije varirala je na podlogama KDA i CDA dok je na MEA podlozi bila ujednačena. Slična istraživanja morfološke karakterizacije izveli su Jenns i sar. (1989) i ustanovili visoku morfološku varijabilnost izolata *C. beticola*. Porema istraživanju Moretti i sar. (2004) izolati *C. beticola* izolovani iz jedne pege mogu ispoljiti visok stepen morfološke varijabilnosti.

Iako se grupisanje izolata nije moglo ost-

variti na osnovu karakteristika koje su izolati *C. beticola* pokazali na testiranim hranljivim podlogama, dve forme micelija na KDA medijumu, bela i siva, prema nekim autorima (Canova, 1959; Brillova, 1987) su u direktnoj vezi sa patogenošću. Naime, izolati koji na KDA hranljivoj podlozi obrazuju miceliju tamne boje, odlikuju se većom patogenošću. Naša istraživanja su ukazala na razliku u morfologiji izolata *C. beticola* koja može imati značaj za dalja testiranja patogenosti izolata.

Ispitivanje porasta micelija na različitim temperaturama na KDA podlozi pokazalo je da je optimalna temperatura za rast vrste *C. beticola* oko 25°C, a da su temperature 10°C i 40°C nepovoljne za razvoj micelije. Ranijim istraživanjem na MEA podlozi utvrđeno je da je optimalana temperatura za rast micelije *C. beticola* na 27°C, dok su kardinalne tačke porasta 12°C i 33°C (Groenewald et al. 2005).

ZAHVALNICA

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MORPHOLOGICAL AND BREEDING CHARACTERISTICS OF *CERCOSPORA BETICOLA* ISOLATES

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SUMMARY

Determination of breeding and morphological characteristics of *C. beticola* isolates was performed on PDA, MEA i CDA mediums. Determination of morphological characteristics were evaluated by monitoring mycelial growth, texture, color, edge and color of beneath of mycelium. Isolates growth on different temperatures was monitored on PDA medium on 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. There was a significant increase in variability of the isolates on PDA, MEA and CDA mediums, but there is a variability in the growth of different isolates of the same medium too. Isolates expressed the variation in color and the width of the edge zone during growth on different substrates. The mycelium texture was cottony on all surfaces, while the beneath was brown to dark-olive colour. At temperatures of 10°C and 40°C isolates of *C. beticola* did not develop mycelium, and the highest increase was recorded at 25°C.

Key words: *C. beticola*, mycelial growth, morphology, temperature

UPUTSTVO AUTORIMA

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Naslov poglavlja u radu (prvi nivo naslova) pisati centrirano, velikim slovima, boldovano.

Podnaslov (drugi nivo naslova) pisati centrirano, prvo slovo veliko, ostala slova mala, boldovano, sa jednim redom razmaka od teksta na koji se odnosi.

Podnaslov (treći nivo naslova) pisati od početka reda, prvo slovo veliko, ostala slova mala. Pisati zakošeno (italik), sa jednim redom razmaka od teksta na koji se odnosi.

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TABELE I GRAFIKONI – Tabele i grafikoni se daju na posebnim stranicama. U rukopisu označiti mesto za tabele i grafikone. Naslove tabela i grafikona obavezno dati prvo na srpskom, pa na engleskom jeziku, a ako je rukopis na engleskom, onda prvo navesti engleski, pa srpski tekst naslova.

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A manuscript, double-spaced printed, contains: chapter, title, the name of an author and addresses, abstract, key words, text (including the chapters: introduction, material and methods, results, discussion, acknowledgements, references and summary followed by key words), tables and graphs, photographs and drawings.

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