Зборник Матице српске за природне науке / Proc. Nat. Sci., Matica Srpska Novi Sad, № 113, 83—91, 2007

UDC 634.22:632.26

Svetlana T. Živković[1], *Saša D. Stojanović*[1], *Jelica Balaž*[2], *Veljko P. Gavrilović*[1]

[1] Institute for Plant Protection and Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia
[2] Agriculture Faculty, Department for Plant Protection and Environment, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

CHARACTERISTICS OF *PHOMOPSIS* SP. ISOLATES OF PLUM TREES ORIGIN

ABSTRACT: Twelve isolates of *Phomopsis* sp. were obtained from the branches and the trunk of plums (*Prunus domestica* L.) with decay symptoms in Valjevo, Ljig, Koceljeva and Ub vicinity during 2004—2006. Morphological, pathogenic and growing characteristics were studied. Pathogen caused tissue necrosis of branches around the inoculate seats, and wrinkling and watering of plum fruits. All media were suitable for pathogen development, except prune agar. The best growth of isolates was at medium pH 5,5. The optimal temperature for growth and germination of pycnidiospores was 25°C.

KEY WORDS: development, morphology, pathogenisity, *Phomopsis* sp., plum, *Prunus domestica*

INTRODUCTION

The plum (*Prunus domestica* L.) is the most frequent fruit tree in Serbia. During the middle of the '90s of the 20th century, a new plum disease occurred. It caused wilting and decay of young plum trees in the orchards. It has been first registered in the vicinity of Valjevo on cv. Stenly and Požegača. In some orchards more than 40% of plum trees were diseased. Later on, the disease was spread on the other cultivars, expecially on cv. Čačanska lepotica and Čačanska rodna. This disease occurred also in the other plum growing area. From the samples of diseased plum brunches, Stojanović et al. (2004) isolated fungi belonging to the following genera: *Cytospora*, *Phomopsis*, *Sphaeropsis*, *Seimatosporium* and *Fusarium*. As the obtained isolates showed extensive virulens and aggressivnes to plum branches, the investigations of some characteristics of pathogen isolates were conducted.

MATERIAL AND METHODS

The samples of diseased plum branches were collected in Valjevo, Ljig, Koceljeva and Ub vicinity during 2004—2006. Isolation of pathogen was carried out by cutting the fragments of cca 0.5 cm^2 from the border of diseased and healthy plum tissues. The fragments were surface sterilized with 3% NaOCL for 3 minutes, and transferred on potato dextrose agar (PDA). From numerous isolates obtained, 12 were chosen for further investigations and were designated as SL-1, SL-M, SL-T-1, SL-B-2, SL-Br-2, SL-K-1, SL-K-3, SL-K-4, SL-K-5, SL-K-7, SL-K-8 i SL-U-4.

Morphology of pathogen was studied on infected branches and on nutrient media. A variety of mycological media supported the growth of plum *Phomopsis* cultures, including PDA, Czapek-Dox agar (CA), oatmeal agar (OA), malt extract agar (MA) and prune agar (PA). The cultures were incubated in 90 mm Petri dishes at 25°C in darkness, for 7 days.

Pathogenicity studies were conducted on (1) young green branches of plum cvs. Stenly, Požegača, Čačanska rodna and Čačanska lepotica in the field, (2) one-year-old shoots of cv. Stenly 30 cm in length, which were drived into wet sterile sand in tin containers, and (3) plum fruits of cv. Stenly under laboratory conditions. A V-shaped incision was made at 45°-angle to expose the tissue under bark of green branches and shoots. An agar plug (0,5 cm^2), containing young hyphae from 10-day-old culture of a *Phomopsis* sp., was placed in the incision and wrapped with moist sterilized cotton wool and parafilm (Weingartner and Klos, 1975). Control branches and shoots were wounded in the same way and inoculated with blocks of sterile PDA. The parafilm and cotton wool were removed 10 days after the inoculation, and the disease development was evaluated by measuring the tissue necrosis length after 6 weeks. Plum fruits were washed with tap water, surface sterilized by 70%--ethanol, washed with distilled water, and mycelial fragments were put into wounds made with sterile needle. Control fruits were wounded in the same way and inoculated with blocks of sterile PDA. Inoculated plum fruits were placed in glass dishes (30 cm in diameter) and covered with wet filter paper. The dishes were wrapped in plastic bags for two days and then stored under laboratory conditions (Arsenijević et al.,1995).

The colony growth on PDA and the conidial germination were examined at temperature range from 5° to 35°C, with 5°C intervals, in the darkness. Effect of medium pH on the pathogen development was studied on PDA which was adjusted with 0,1 M NaOH or 0,1 M HCl to pH 3.5, 4.0, 5.5, 4.0, 8.5 and 9.5. Colony diameter was measured 7 days after the inoculation. The conidia in water drops (0,2 ml) on microscope slides were placed in Petri dishes with wet filter paper and incubated for 18 hours, since the high percentage of conidial germination at all temperatures, after the incubation of 24 hours, was found in the preliminary germination tests.

RESULTS

Symptoms. The first symptoms were expressed in the stage of flowering and leaf forming. Leaves were smaller and chlorotic, while later both leaves and flowers became necroted, if the infection was centered around the flower buds and leaf petioles. If the infection started around the vegetative buds, it resulted in young shoots becoming diseased and necroted (Fig. 1 a). On the stem and the main branches necrotic sunken lesions occurred, the bark longitudinally cracked, and cankers were formed (Fig. 1 b). The necrotic lesion expanded rapidly, disrupted the vascular tissues, and caused the shoot to wilt and die (Fig. 1 c).

Fig. 1 — Necrosis of current season's shoot of plum resulting from infection started around vegetative bud (a); Elongate canker and numerous pycnidia on diseased plum branches (b); Brown tissue necrossis under the bark removed from the diseased plum branches (c).

Morphology. Colonies were farinaceous, or woolly to cottony, white or whitish, pale to light brown or pale greyish (Fig. 2 a). Conidiomata was pycnidial, stromatic, dark brown to black, single or aggregated, often botryose in culture, uniloculate, over 500 mm in diameter (Fig. 3 a-d). The a-conidia in all 12 isolates were hyaline, fusiform to ovate, straight, aseptate, and frequently biguttulate, with average size 7,3 x 2,5 μm, and little variation in shape or size between the isolates. The β-conidia were filiform, sigmoidal, hyaline, with average size 23.7 x 1,3 μm (Fig. 2 b-c).

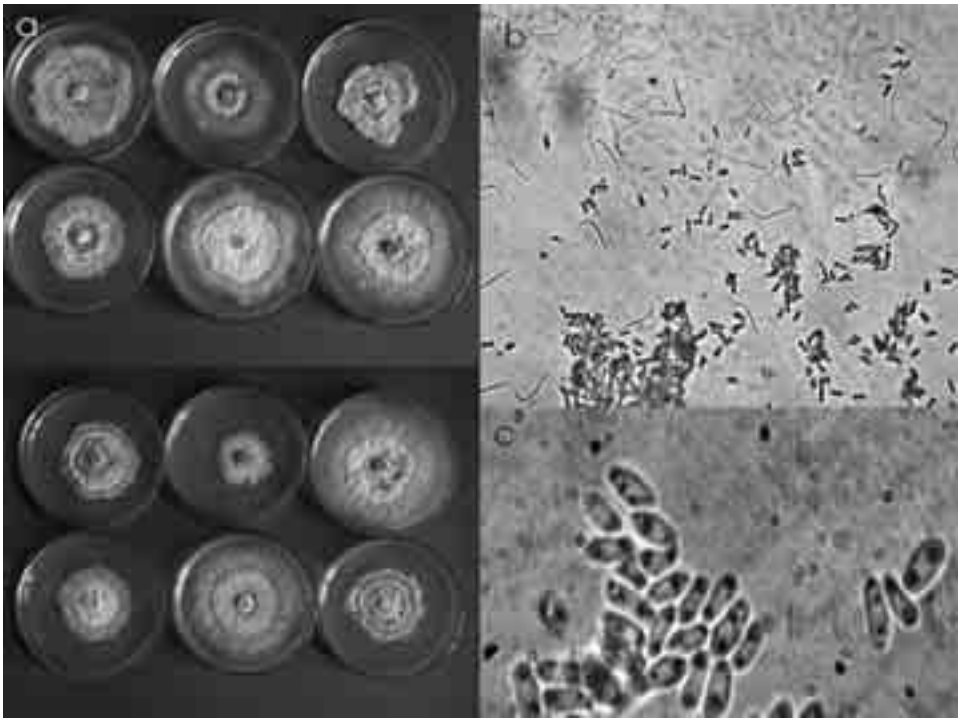

Fig. 2 — Colonies of *Phomopsis* sp. isolates from plum 7 days after incubation on PDA at 25°C (first upper row: SL-1, SL-M and SL-T-1, second row: SL-B-2, SL-Br-2 and SL-K1, third row: SL-K-3, SL-K4 and SL-K-5, fourth row: SL-K-7, SL-K8 and SL-U-4) (a); Alfa and beta conidia of isolate SL-U-4 (x 600) (b); Alfa conidia of isolate SL-K-1 (x 1000) (c).

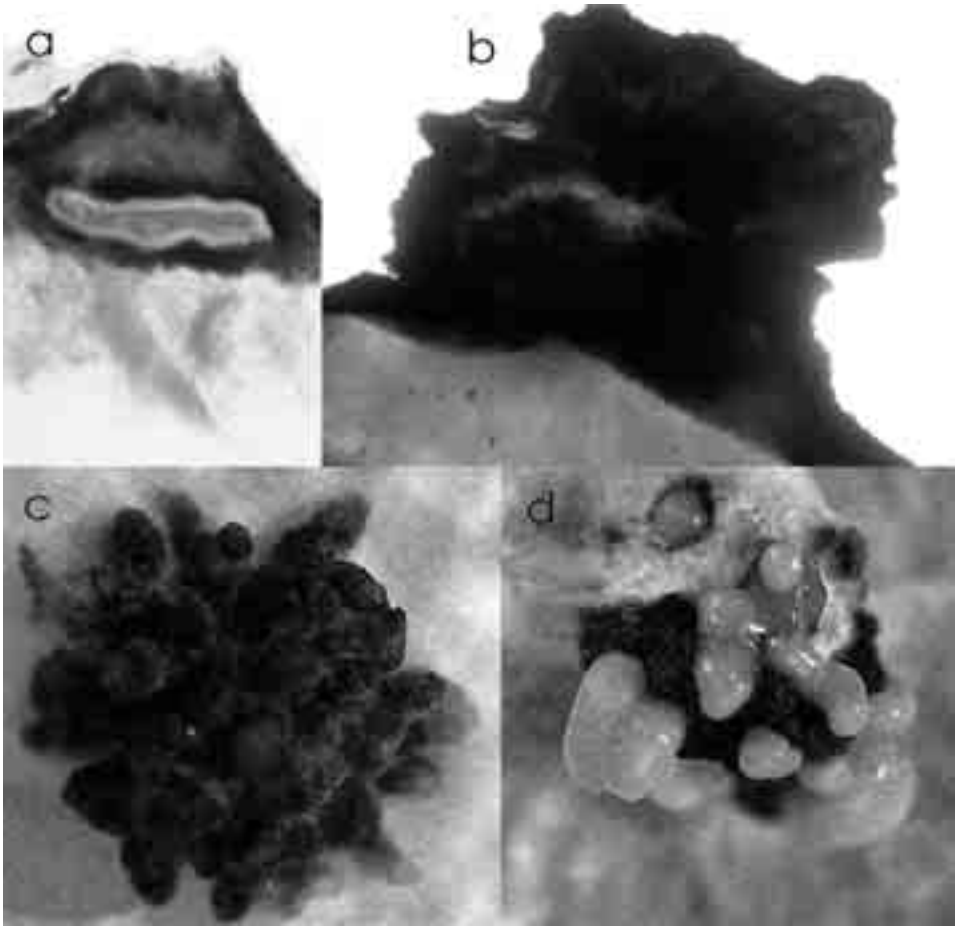

Fig. 3 — Cross section of pycnidium formed on plum branches (a); Cross section of pycnidium formed on stromatic structure on PDA (b); Botriose pycnidium on PDA (c); Cirri produced by pycnidia on PDA (d).

Pathogenicity. All isolates showed extensive virulens and aggressivnes to plum branches and fruits. Inoculated young green branches in the field, and the one-year-old shoots under laboratory conditions, showed necrosis of bark tissue around inoculate seats. Isolates SL-1, SL-T-1, SL-Br-2, SL-K-1, SL-K-3, SL-K-4, and SL-U-4 and SL-B-2, SL-Br-2, SL-M and SL-T-1 showed the greatest pathogenicity in the laboratory and field test respectively, after 6 weeks (Fig. 4 a-b). On the vertical sections of infected shoots the brown necrosis of woody tissue could be seen.

7 days after inoculation, plum fruits showed settled necrotic spot around the inoculation seat. Later on, the fruits became wrinkled, and after 15 days they were completely wrinkled and watery. Control fruits were healthy and fresh. The numerous pycnidia were formed on the inoculated plum fruits. Even the isolate SL-M, which did not form pycnidia in culture, formed abundant co-

nidimata on inoculated plum fruits. The pathogen was reisolated from the inoculated shots, branches and plum fruits (Fig. 4 c-d).

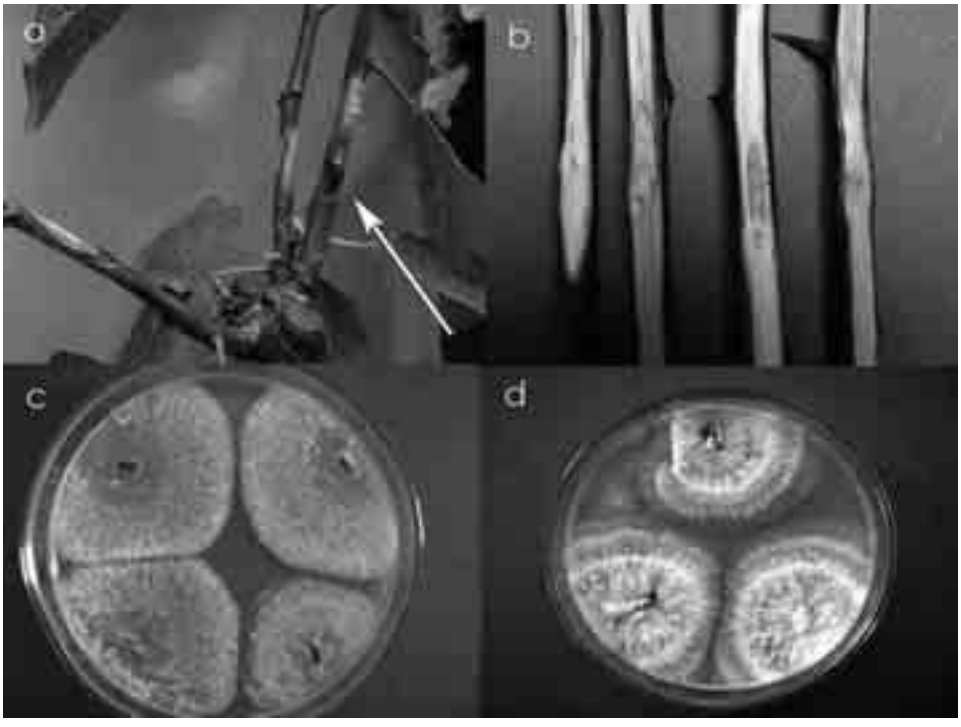

Fig. 4 — Necrosis of bark 6 weeks after artificial inoculation of green plum braches with isolate SL-B-2 in the field (a); Longitudinal section of plum shoots artificial infected in laboratory conditions (from left to right: control, SL-1, SL-M and SL-T-1) (b); Cultures obtained by pathogen reisolation from artificial inoculated green plum branches with isolate SL-B-2 (c) and plum shoots with isolate SL-K-3 (d).

Pathogen development *in vitro*. All media tested, except PA, were favorable for pathogen development (Sl. 5 d). The majority of isolates had the best growth on PDA. For isolates SL-T-1, SL-K-4 and SL-U-4, SL-B-2 and SL-K-7 and Sl-K-3 the most suitable media were CA, MA and OA, respectively. The average radial growth (mm) of tested isolates was 30.0—71.8, 27.5—60.7, 42.2—60.8, 41.7—71.2 i 25.7—63.3 on PDA, PA, OA, CA and MA, respectively. Isolate SL-K-4 had the slowest growth on all media tested. None of the mediums was suitable for pycnidial formation of isolates SL-M and SL-B-2. All isolates formed sparse pycnidia on PA. The abundant pycnidial formation was on CA (isolates SL-1, SL-K-3, SL-K-4, SL-K-7, SL-K-8 and SL-U-4), on OA (isolates SL-T-1, SL-K-1, SL-K-5 and SL-U-4), on MA (isolates SL-T-1, SL-K-1, SL-K-4, SL-K-5 and SL-U-4) and on PDA (isolates SL-K-4 and SL-K-5).

The optimal temperature for pathogen radial growth was 25°C, except for isolates SL-1 (20—25°C) and SL-U-4 (20°C). Minimal and maximal tempera-

tures for the growth of all isolates were 5°C and over 35°C, respectively (Sl. 5 a). The average radial growth (mm) of tested isolates was 5.0—5.5, 6.0—10.2, 14.2—36.7, 26.5—60.2, 35.0—71.8, 12.2—33.7 and 5.2—12.2 at 5, 10, 15, 20, 25, 30 and 35°C, respectively. Temperature in the range of 20—25°C was the most favorable for the formation of pycnidia, but for isolates SL-T-1, SL-Br-2, SL-K-5 and SL-U-4 temperature of 15°C was also suitable. The other tested temperatures were unfavorable for pycnidial production.

The pathogen growth was the largest at medium pH 5,5 (Sl. 5 c). The average radial growth (mm) of tested isolates was 11.5—29.0, 34.0—64.2, 56.7—88.3, 32.8—71.5, 21.5—53.8 and 18.5—42.5 at pH 3.5, 4.0, 5.5, 7.0, 8.5 and 9.5, respectively. The isolates SL-1, SL-K-1, SL-K3 and SL-K-5 did not produce pycnidia at pH 3,5. Medium pH seems to have no effect on the production of pycnidia. Mostly pH values in the range of 4.0—7.0 were equally suitable for pathogen sporulation, but for isolate SL-U-4 the maximal pycnidial formation occurred at pH 8,5.

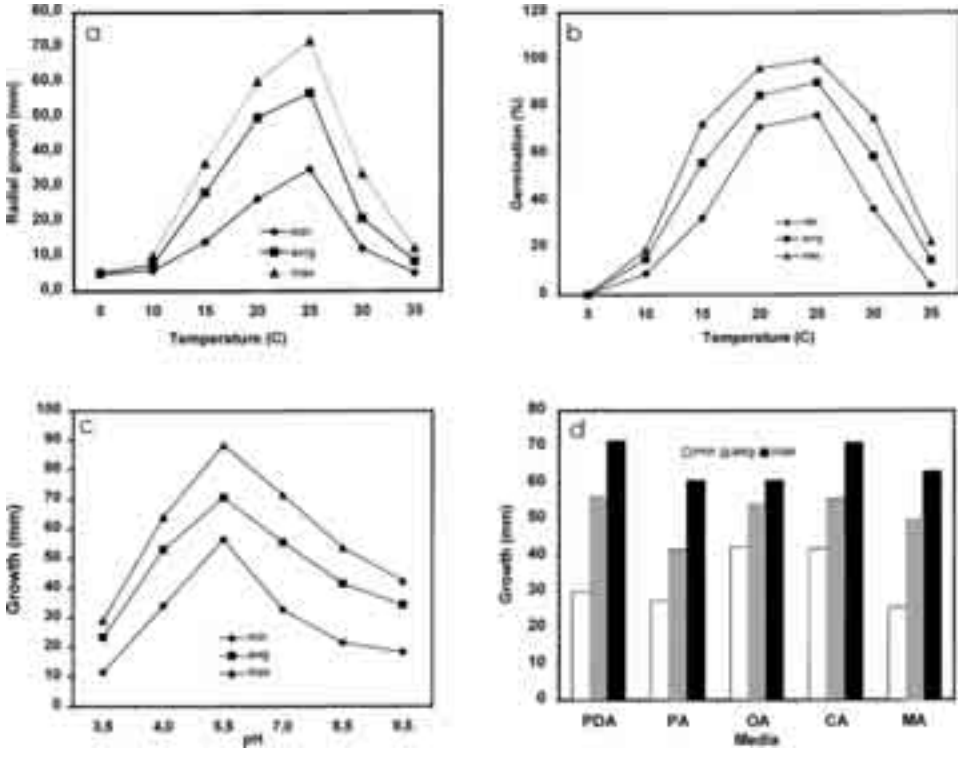

Fig. 5 — Pathogen development *in vitro*. Cultures growth at different temperature (a); Effect of temperature on conidial germination (b); Cultures growth at different pH of medium (c); Pathogen development on different media (d).

The greatest conidial germination occurred at 25°C, except for the isolate SL-1 (20°C). Minimal and maximal temperatures for conidial germination of all isolates were between 5 and 10°C and over 35°C, respectively (Fig. 5 b).

Germinating conidia produced 1—2, rarely 3, terminal, subterminal or basal, rarely lateral germ tubes. The average percentages of conidial germination were 9.0—19.0, 32.8—72.8, 71.4—96.4, 76.4—100.0, 36.4—75.0 and 4.0—22.4 at 10, 15, 20, 25, 30 and 35°C.

DISCUSION

Disease symptoms expressed on plum trees in Serbia were similar to those described by Harris (1988) in the United Kingdom, which were caused by *Diaporthe perniciosa*. Recently, an anamorph of this fungus (*Phomopsis perniciosa*) was described as a rot pathogen of storaged apple fruits in Serbia (Arsenijević and Gavrilović, 2005).

According to morphology, the isolates from plum trees in Serbia belonged to genus *Phomopsis*. The species from this genus were described as the bark pathogens of numerous hosts in our country (Arsenijević, 2005), but plum was not mentioned.

Distinct differences in the colony morphology (color, texture and fruiting), pathogenicity and development at different media, temperature and pH, were observed between the *Phomopsis* isolates from plum. Variability in *Diaporthe perniciosa* from plum was noted before (Caley, 1923 loc. cyt. Harris, 1988).

Diaporthe ambiqua was identified as a cause of cancer disease in apple, pear and plum rootsocks in South Africa (Smit et al., 1996). Uddin et al. (1998) showed that apple, plum and pear were susceptible to the pathogen *Phomopsis* sp., causing shoot blight of peach in Georgia, USA. They concluded that the isolates of *Phomopsis* from peach were not host-specific. *Phomopsis perniciosa* from fruits and *P. mali* from branches of plum, sour cherry and sweet cherry were isolated in Lithuania (Valiuškaite, 2002).

Further investigations should be conducted in order to identify pathogen at species level, as there are three species of genus *Phomopsis* isolated from plum.

REFERENCES

Arsenijević, M. (2005): *O vrstama rodova Diaporthe i Phomopsis parazitima voćaka i vinove loze*, Mycologia Montenegrina VII: 105—117.

Arsenijević, M., Gavrilović, V. (2005): *Phomopsis perniciosa Grove — uzročnik truleži uskladištenih plodova jabuke*, Pesticidi i fitomedicina 20: 189—194.

Arsenijević, M., Špica, G., Filipović, M. (1995): *Nesazreli plodovi voćaka kao test patogenosti Phomopsis spp. poreklom sa kupine i oraha*, VI kongres mikrobiologa Jugoslavije, Herceg Novi 12—16. 06. 1995. god. Zbornik rezimea VI:133.

Harris, D. C. (1988): *Diaporthe perniciosa associated with plum dieback*, Plant Pathology 37: 604—606.

Smit, W. A., Vilioen, C. D., Wingfield, B. D. and Wingfield, M. J. (1996): *A New Cancer Disease of Apple, Pear and Plum Rootsocks caused by Diaporthe ambiqua in South Africa*, Plant Disease 80:1331—1335.

Stojanović, S., Gavrilović, V., Aleksić, G., Pavlović, S., Starović, M. (2004): *Etiologija sušenja šljive*, V Kongres o zaštiti bilja, Zlatibor, 22—25. 11. 2004. Zbornik rezimea, str. 154.

Uddin, W. and Stevenson, K. L. (1998): *Pathogenic and molecular characterization of three Phomopsis isolates from peach, plum and pear*, Plant Disease Vol. 82, No. 7: 732—737.

Valiuškaite, A. (2002): *Micromycetes infecting stone fruit trees*, Biologija, Nr. 1: 18—21.

Weingartner, D. P. and Klos, E. J. (1975): *Etiology and symptomatology of cancer and dieback disease on highbush blueberris caused by Fusiccocum cassandre and Phomopsis vaccinii*, Phytopathology 65: 105—110.

КАРАКТЕРИСТИКЕ ИЗОЛАТА *PHOMOPSIS* SP. ПОРЕКЛОМ СА ШЉИВЕ

Светлана Т. Живковић[1], Саша Д. Стојановић[1],
Јелица Балаж[2], Вељко П. Гавриловић[1]
[1] Институт за заштиту биља и животну средину, Теодора Драјзера 9, 11000 Београд, Србија
[2] Пољопривредни факултет, Департман за заштиту биља и животне средине, Трг Доситеја Обрадовића 8, 21000 Нови Сад, Србија

Резиме

Дванаест изолата *Phomopsis* sp. добијено је са грана и стабла шљиве (*Prunus domestica* L.) са симптомима пропадања у околини Ваљева, Љига, Коцељеве и Уба током 2004—2006. године. Проучене су морфолошке, патогене и одгајивачке одлике ових изолата.

Први симптоми уочавају се у време цветања и листања шљиве уколико се инфекција десила у близини пупољака и основе лисних петељки. У том случају листови су ситнији и хлоротични. Касније и листови и цветови некротирају и суше се. Тек формирани млади изданци обољевају, постају некротични и пропадају. На гранама и стаблу формирају се улегнуте некротичне пеге, које се брзо шире и у оквиру којих кора уздужно пуца, тако да се формирају рак-ране. Некроза се шири на дрвенасти део, где бивају захваћени спроводни судови, што доводи до увенућа и изумирања грана.

Патоген се добро развија на свим подлогама, осим на подлози од сувих шљива. Подлоге чија је pH 5,5 показале су се као најбоље за пораст патогена. Температуре од 25°C су најповољније за развој патогена и клијавост конидија.