OCCURENCE OF PHOMOPSIS SP. ON ACHILLEA MILLEFOLIUM

Vrandečić, Karolina; Jurković, Draženka; Riccioni, Luca; Ćosić, Jasenka; Poštić, Jelena

Source / Izvornik: **Poljoprivreda, 2010, 16, 3 - 7**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:151:710290

ISSN 1330-7142
UDK = 632.4:582.9

OCCURENCE OF PHOMOPSIS SP. ON ACHILLEA MILLEFOLIUM

Karolina Vrandečić [1], Draženka Jurković [1], L. Riccioni [2], Jasenka Ćosić [1], Jelena Poštić [1]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

During a three year period (2004 to 2006) on locations of Eastern Croatia (Kneževi Vinogradi, Osijek, Brođanci and Krndija) we collected plants of Achillea millefolium L. from which we isolated Phomopsis sp. Many of the collected plants exhibited no visible symptoms of infection but in spring pycnidia were noticed on overwintered plants. Isolation was done on PDA from naturally infected plants and morphological and molecular characteristics were studied. Biometrical values of reproductive structures developed on naturally infected plants and grown on PDA were measured. Internal transcribed spacers ITS rDNA of the examined isolates were sequenced. Although molecular analysis showed that isolates were grouped with Diaporthe arctii, we decided to classify isolates from A. millefolium as Phomopsis sp., the details of which were discussed in the paper.

Key-words*: *Achillea millefolium*, *Diaporthe/Phomopsis*, *identification

INTRODUCTION

Throughout many years of studying mycopopulation of weeds, we have found fungi of the *Diaporthe/Phomopsis* complex interesting to research. After years of examining plants of *Achillea millefolium* L., some of them exhibited symptoms of infection typical for *Diaporthe/Phomopsis* species. *A. millefolium* was usually found in uncultivated, pasture and forest flora, but also in or nearby sunflower fields.

Previous studies confirmed that many fungi known to cause diseases of cultivated plants had weeds as alternative hosts (Boland and Hall, 1994, Jurković et al., 2001, Vajna, 2002, Vrandečić et al., 2005, Ćosić et al., 2006).

As sunflower and *A. millefolium* belong to the same botanical family (*Asteraceae*) we intended to determine which *Diaporthe/Phomopsis* species occurred on *A. millefolium* and whether there was a connection between *Phomopsis* spp. that infected these two hosts. The experiments were carried out within the project that aimed to determine the role of weeds in the epidemiology of cultivated plant diseases.

MATERIAL AND METHODS

Plants of *A. millefolium* with and without symptoms of infection with *Phomopsis* sp. were collected during fall and spring from 2004 to 2006 on the locations of Eastern Croatia (Knezevi Vinogradi, Osijek, Brodjanci and Krndija). Stems were washed with tap water, cut into pieces 15 mm long and disinfected with 96% ethanol for 60 sec. Plant material was rinsed three times with distilled water, dried on paper towel and placed in Petri dishes on moist filter paper. Petri dishes were kept at 25°C with 12 h light/dark regime (NUV). Isolation of *Phomopsis* sp. was performed by transferring pycnidia and conidial exudate on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI). Morphological characteristics and biometrical values of reproductive structures as well as cultural characteristics of isolates were examined. Where possible, 3 x 100 conidia and 20 pycnidia per each sample were measured (Olympus microscope, Olympus DP Soft). Water agar with various plant parts was used for perithecia production.

DNA isolation was determined according to Cenis (1992). PCR amplifications were carried out in a total volume of 50 μl, with the following cycling conditions: denaturation for 5 min at 94 °C, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min and a final 7 min extension step at 72 °C to complete the reaction. Purified PCR products were sequenced in both direc-

(1) DSc Karolina Vrandečić (karolina.vrandecic@pfos.hr), Prof. DSc Draženka Jurković, Prof. DSc Jasenka Ćosić, Jelena Poštić, BSc - J. J. Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Trg Sv. Trojstva 3, 31000 Osijek, (2) DSc Luca Riccioni - Istituto Sperimentale per la Patologia Vegetale, I-00156 Rome, Italy

tions by ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GCAAGTAAAAGTCGTAACAAGG-3') primers (in the Gene Lab – ENEA, Rome). The obtained sequences were aligned with CLUSTAL W (Thompson et al. 1994), and manually adjusted using Chromas (version 1.45). In addition, sequences obtained from the GenBank (http://www.ncbi.nlm.nih.gov) were added to the alligned ITS sequences. Phylogenetic analysis was performed by the Neighbour-Joining-Kimura 2-parameter model in MEGA version 3.1 (Kumar et al. 2004). Alignment gaps were treated as missing data. Bootstrap analysis for 1000 replicates was performed in order to estimate confidence for phylogenetic tree topology. Two of our isolates as representatives (Ah1 and Ah2), were taken for the phylogenetic analysis. Isolates originating from the GenBank were listed in the Table 1. *Colletotrichum coccodes* (Wallr.) Hughes and *Colletotrichum dematium* (Pers.) Grove were included as an outgroup. Molecular analyses were completed in Istituto Sperimentale per la Patologia Vegetale in Rome.

Table 1. Isolates from GenBank used for phylogenetic analysis

Tablica 1. Izolati iz Gen Banke korišteni za filogenetsku analizu

Isolate *Izolat*	Species *Vrsta*	Host *Domaćin*	Origin *Porijeklo*	References *Reference*	GenBank number *Gen Banka br.*
978	*C. coccodes*	Paprika	Italy/ Italija		AM422215
AR3563	*C. dematium*	*Lirope muscarii*	Mexico/ Meksiko	Farr et al. (2006)	DQ286154
CBS109490	D. arctii	Ambrosia trifida	USA	Castlebury et al. (2002)	AY196777
MAFF 237054	*D. arctii*	*Dillenia indica*	Japan		AB245064
olrim961	*Diaporthe* sp. olrim961	*Fraxinus excelsior*	Lithuania/ Litva	Lygis et al. (2005)	AY787696
Dh95016	*D. helianthi*	Sunflower/Suncokret	France /Francuska	Say-Lesage et al. (2001)	AF358435
IMI318865	*D. helianthi*	Sunflower/Suncokret	Ex Jugoslavia	Rekab et al. (2004)	AJ312363
95-vs-43	*D.phaseolorum var. caulivora*	Soybean/Soja	USA	Zhang et al. (1997)	AF000212
713	*D.phaseolorum var. caulivora*	Soybean/Soja	USA	Zhang et al. (1997)	AF000567
Su9	*D.phaseolorum*	Sunflower/Suncokret	Croatia	Vrandečić et al. (2009)	GQ149763
Su10	*D.phaseolorum*	Sunflower/Suncokret	Croatia	Vrandečić et al. (2009)	GQ149764

RESULTS AND DISCUSSION

Fungus, lately recognized as *Phomopsis* sp. was isolated from *A. millefolium* plants which did not exhibit any symptoms of infection, as well as from plants that had symptoms of infection typical for *Phomopsis* species.

Plants collected during fall had no symptoms of infection. Stems of overwintered plants collected in April had small, black, randomly scattered pycnidia immersed in host tissue. On those spots plant tissue was pale or lighter in color, while parts without the symptom were dark brown. After 8 days in moist chamber, drops of yellow color occurred on pycnidia of disinfected tissue (Photo 1). Microscopic examination showed that the drops contained only conidia of alpha type characteristic for genus *Phomopsis*. Pycnidia with few beta conidia were seen only sporadically. Table 2 presents biometrical values of reproduction structures of *Phomopsis* sp. isolated from *A. millefolium*.

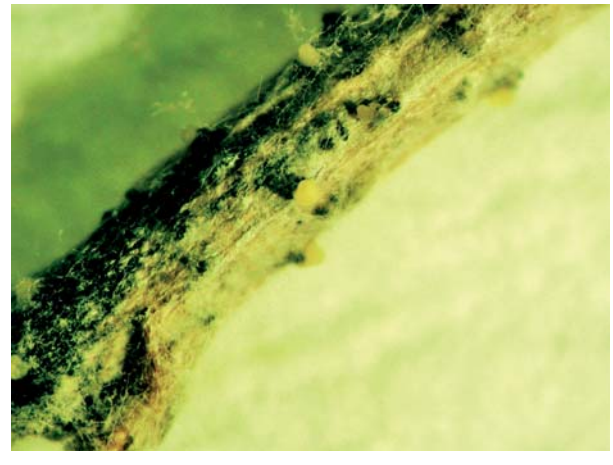

Photo 1. Pycnidia on *A. millefolium* stem

Slika 1. Piknidi na stabljici A. millefolium

Table 2. Biometrical size of reproduction structure of *Phomopsis* sp. from *A. millefolium*

Tablica 2. Veličina reproduktivnih organa Phomopsis sp. izoliranog s A. millefolium

	Pycnidia *Piknidi (µm)*	Alpha conidia - range *A konidije – raspon (µm)*	Alpha conidia - average *A konidije – prosjek (µm)*
Natural infection/*Prirodna infekcija*	400-600 x 450-550	7.58-11.76 x 2.76-4.37	9.56 x 3.46
PDA/KDA	300 x 400	7.94-11.42 x 2.59-4.37	9.27 x 3.22

The fungus was growing fast on PDA and mycelium filled out the Petri dish after 6 days. Aerial mycelium was cottony and especially abundant at the edges of cultures. At the beginning, it spread in circle and was more compact on the edges and in the middle of culture. On the 20th day after inoculation, mycelium was equally compact in the whole culture. The color of mycelium changed as the culture grew older, being white at the beginning, and later grayish, light brown, olive brown or olive green. The color of substrate also changed from beige to brown. Dark spots, i.e. stromatic formations were clearly visible on the backside of the cultures. Black pycnidia of short necks were developed on stromatic formations after 10-15 days. Beige or dirty-white thick drops came out through ostiolum, and later on turned yellow (Photo 2). Pycnidia were usually grouped, scarcely appeared single. They were partially or completely immersed in medium. Number of pycnidia was small to medium. Only alpha conidia was determined in pycnidia. Alpha conidia unicellular, hyaline, biguttulate, fusiform, rounded at both ends (Photo 3). Especially good sporulation was present in the central part of the colony. Perithecia were never found on naturally infected plants of *A. millefolium* or on culture media.

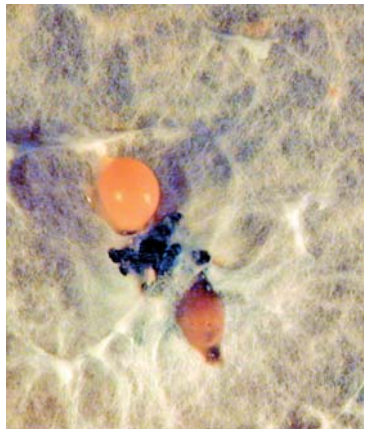

Photo 2. Pycnidia exudate of *Phomopsis* sp./
Slika 2. Piknidijski eksudat Phomopsis sp.

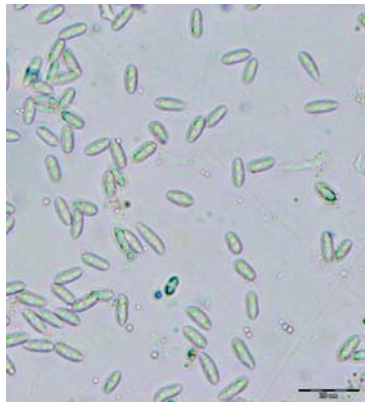

Photo 3 Alpha conidia of *Phomopsis* sp./
Slika 3. Alfa konidije Phomopsis sp.

The recent literature does not provide information on *Phomopsis* species on *A. millefolium*. Wehmeyer (1933) stated that *A. millefolium* is host for *D. arctii* var. *achilleae* (Auers.) Wehm. (sin. *Sphaeria achilleae* Auersw., *Diaporthe orthoceras* (Fr.) Nits.), pointed out that this variety on *A. milefollium* represents an extreme form of *D. arctii*, quote the *P. achilleae* (Sacc.) Höhn as a conidial stage.

Although the teleomorph of the fungus was not confirmed either in nature or culture media (PDA and water agar with supplemented different plant tissues), on the basis of morphological characteristics we supposed that the fungus could be *P. achilleae*, i.e. variety of *D. arctii, D. arctii* var. *achilleae*.

Results of molecular analysis (Photo 4) showed that the examined group (89% bootstrap values) contained our isolates of *Phomopsis* sp. (Ah1 and Ah2) originating from *A. millefolium,* as well as two sequences obtained from the GenBank that grouped together with our sequences. One isolate from Lithuania (99% bootstrap values) determined as *Diaporthe* sp. (AY787696) was isolated from European ash (*Fraxinus excelsior* L.), and the other isolate was *D. arctii* (AF362562) from USA isolated from great ragweed (*Ambrosia trifida* L.).

While studying the taxonomy of *Diaporthales*, Castlebury et al. (2002) stated the reference isolate *D. arctii* (AF362562) as one of the species that characterize the *Diaporthaceae* familiy. However, in the GenBank there is one more isolate *D. arctii* (AB245064) isolated in Japan from elephant apple (*Dillenia indica* L.) which did not belong to this group. Except for the DNA sequence (ITS-1, 5.8S, ITS-2), there are no available information in the literature for that isolate from Japan, so we assumed that the isolate was misidentified. In the GenBank there are no sequences for any other *Diaporthe/Phomopsis* species originating from *A. millefolium*.

According to morphological, cultural and molecular research we found out that there was not a connection between *Diaporthe/Phomopsis* spp. that infected sunflower and *A. millefolium*.

Although after the molecular analysis it was determined that our isolates grouped with *D. arctii*, the name of *Phomopsis* sp. was still kept for isolates from *A. millefolium* because the teleomorph was not found. Apart from molecular characteristics, it is important to study morphological characteristics of parasites if intending to confirm their taxonomic position. Identification of *Diaporthe/Phomopsis* species is very complex because characteristics of some species are not clearly defined and many overlappings are found with respect to morphological and cultural characteristics. ITS region analysis was already successfully used in studying of relations and taxonomy between different fungal species (Bernard 2002, Allain-Boulé et al., 2004, van Niekerk et al., 2005). However, molecular analysis for many *Diaporthe/Phomopsis* species from different hosts is to be carried out because number of available sequences is still relatively small.

Muntanola-Cvetković et al. (1996) concluded that it was important to determine cultural characteristics of a fungus, mycelium pattern and pigmentation, stroma complexity and conidioma structure, variability of conidial type in order to determine particular *Phomopsis* species. Comparative studies of *Diaporthe/Phomopsis* isolated from soybean, sunflower and some weeds underline the obvious heterogeneity within the *Phomopsis* genus (Muntanola-Cvetković et al., 1996). Rodeva et al. (2006) studied *Phomopsis* sp. from *Apiaceae* species, and could not correctly identify particular isolates on the basis of pathogen taxonomic characteristics. They highlight the necessity to revise *Phomopsis* genus by using molecular and morphological methods. Since the morphological characteristics of *Diaporthe/Phomopsis* species are too variable the application of molecular technologies in their taxonomy are necessary in their identification (Brayford, 1990, Vergara et al., 2005, Murali et al., 2006).

Following the fact that no enough data on morphological and molecular characteristics of *Phomopsis* species are available (in literature or in GenBank), we consider that future research of *Diaporthe/Phomopsis* species from *A. millefolium* and other host are necessary.

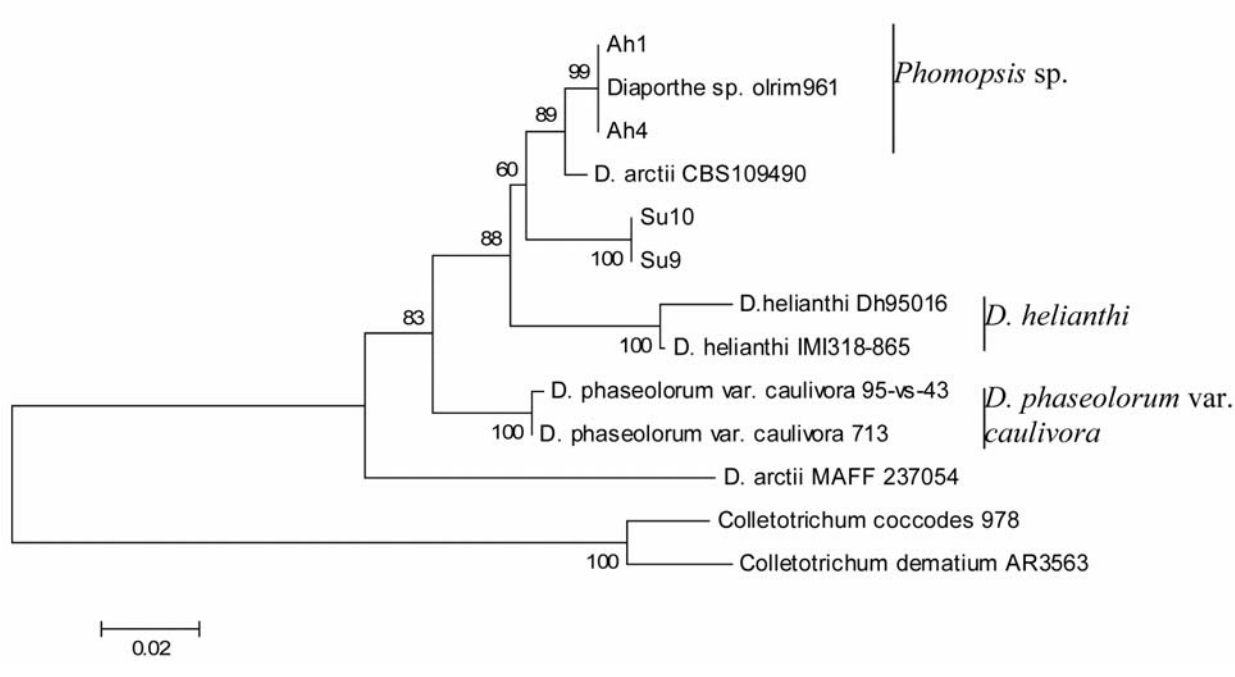

Photo 4. Molecular phylogenetic tree based on ITS1-5.8S gene-ITS2 sequences using Neighbour-Joining -Kimura 2-parameter model. Numbers above each branch represent percentages of 1000 bootstrap repetitions. *C. coccodes* (AM422215) and *C. dematium* (DQ286154) were used as outgroups. The scale bar shows the number of substitutions per site

Slika 4. Molekularno filogenetsko drvo dobiveno na osnovu ITS1-5.8S gen-ITS2 sekvenci koristeći Neighbour-Joining Kimura 2-parameter model. Brojevi iznad svake grane predstavljaju postotak od 1000 bootstrap ponavljanja. C. coccodes (AM422215) i C. dematium (DQ286154) uzeti su kao vanjska grupa. Mjerilo pokazuje broj supstitucija po mjestu

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Croatian Ministry of Science, Education and Sports for financial support awarded to the scientific project No. 079-0790570-2995.

REFERENCES

1. Allain-Boulé, N., Tweddell, R., Mazzola, M., Bélanger, R., Lévesque, P.B. (2004): *Pythium attrantheridium* sp. nov.: taxonomy and comparison with related species. Mycological Research 108: 795-805.
2. Bernard, P. (2002): *Pythium terrestris*, a new species isolated from France, its ITS region, taxonomy and its comparison with related species. Microbiology Letters 212: 255–260.
3. Boland, G.J., Hall, R. (1994): Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16: 93-108.
4. Brayford, D. (1990): Variation in *Phomopsis* isolates from *Ulmus* species in the British Isles and Italy. Mycological Research 94:691-697.
5. Castlebury, L. A., Rossman, A.Y., Jaklitsch, W. J., Vasilyeva, L. N. (2002): A preliminary overview of the

Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia 94: 1017-1031.

6. Cenis, J. L. (1992): Rapid extraction of fungal DNA for PCR ampification. Nucleic Acids Research 20: 2380.
7. Ćosić, J., Jurković, D., Vrandečić, K., Steindl, D. (2006): Pathogenicity of *Fusarium graminearum* isolated from cultivated plants and weeds to wheat seedlings. 9th European Fusarium Seminar, Wageningen, Netherland. Book of Abstracts, 134.
8. Farr, D.F., Aime, M.C., Rossman, A.Y. And Palm, M.E. (2006): Species of *Colletotrichum* on *Agavaceae*. Mycol. Res. 110:1395-1408.
9. Jurković, D., Ćosić, J., Vrandečić, K. (2001): Parazitna mikopopulacija važnijih korovnih vrsta. 37. Znanstveni skup Hrvatskih agronoma, Opatija, Hrvatska, Abstr. 341.
10. Kumar, S., Tamura, K., Nei, M. (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief. Bioinform. 5: 150-163.
11. Lygis, V. Vasiliauskas, R., Larsson, K. H., Stenlid, J. (2005): Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. Scandinavian Journal of Forest Research 20: 337-346.
12. Muntanola-Cvetković, M., Vukojević, J., Mihaljčević, M. (1996): Cultural growth patterns and incompatibility reaction in *Diaporthe* and *Phomopis* population. J.of Phytopathology 144: 285-295.
13. Murali, T.S., Suryanarayanan, T.S., Geeta, R. (2006): Endophytic *Phomopsis* species: host range and implications for diversity estimates. Can. J. Microbiol. 52: 673-680.
14. Rekab, D., Del Sorbo, G., Reggio, C., Zoina, A., Firrao, G. (2004): Polymorphisms in nuclear rDNA and mtDNA reveal the polyphyletic nature of isolates of *Phomopsis* pathogenic to sunflower and a tight monophyletic clade of defined geographic origin. Mycological Research 108: 393-402.
15. Rodeva, R., Stojkov, D., Vitkova, A. (2006): Occurence of *Phomopsis* spp. on wild growing *Apiaceae* host. XII MPU Congress, Rhodes Island, Greece. Book of Proceedings, 578-580.
16. Says-Lesage, V., Roeckel-Drevet, P., Viguié, A., Tourvielle, J., Nicolas, P., Tourvieille de Labrouhe, D. (2002): Molecular variability within Dia*porthe/Phomopsis helianthi* from France. Phytopathology 92: 308-13.
17. Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.
18. Vajna, L. (2002): Downy mildew epidemic on common ragweed in Hungary caused by *Plasmopara halstedii*. Plant Pathology 51: 809-809.
19. Van Niekerk, J. M., Groenewald, J. Z., Farr, D. F., Fourie, P.H., Halleen, F., Crous, P. W. (2005): Reassessment of *Phomopsis* species on grapevines. Australian Plant Pathology 34: 1-13.
20. Vergara, M., Capasso, T., Gobbi, E., Vannacci, G. (2005): Plasmid distribution in European *Diaporthe helianthi* isolates. Mycopathologia 159: 591-599.
21. Vrandečić, K, Ćosić, J., Riccioni, L., Duvnjak, T., Jurković, D. (2005): Isolation of *Diaporthe phaseolorum* var. *caulivora* from *Abutilon theophrasti* in Croatia. Plant Pathology 54 (4): 576.
22. Vrandečić, K. Ćosić, J., Jurković, D., Duvnjak, T., Riccioni, L. (2009): First Report of *Diaporthe phaseolorum* on Sunflower (*Helianthus annuus*) in Croatia. Plant disease 93(10):1074.
23. Wehmeyer, L. E. (1933): The genus *Diaporthe* Nitschke and its segregates. Univ. Mich. Stud., Sci. Ser. 9: 1-394.
24. Zhang, A.W., Hartman, G.L., Riccioni, L., Chen, W.D., Ma, R.Z., Pedersen, W.L. (1997): Using PCR to distinguish *Diaporthe phaseolorum* and *Phomopsis longicolla* from other soybean fungal pathogens and to detect them in soybean tissues. Plant Disease 81:1143-1149.

POJAVA PHOMOPSIS SP. NA ACHILLEA MILLEFOLIUM

SAŽETAK

Phomopsis sp. izolirali smo s biljaka A. millefolium na kojima nije bilo vidljivih simptoma zaraze, kao i s biljaka sa simptomima tipičnima za Phomopsis vrste. Biljke su prikupljene tijekom tri godine (2004.-2006. godine) u jesen i proljeće na lokacijama (Kneževi Vinogradi, Osijek, Brođanci i Krndija) istočne Hrvatske. S prirodno zaraženih biljaka izolacija gljive obavljena je na KDA te su proučene morfološke i molekularne karakteristike. Biometrijske vrijednosti reproduktivnih struktura izmjerene su u kulturi (KDA) i na prirodno inficiranome materijalu. Za proučavane izolate sekvencirane su unutarnje prijepisne razmaknice jezgrine DNA (ITS rDNA) te je utvrđena povezanost analiziranih izolata s Diaporthe arctii. Zbog nedovoljno pouzdanih informacija zadržan je naziv Phomopsis sp. detalji su raspravljeni u radu.

Ključne riječi: Achillea millefolium, Diaporthe/Phomopsis, identifikacija

(*Received* on 1 February 2010; accepted on 17 March 2010 - *Primljeno 01. veljače 2010.; prihvaćeno 17. ožujka 2010.*)