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THE PRESENCE OF *ALTERNARIA* SPP. ON THE SEED OF APIACEAE PLANTS AND THEIR INFLUENCE ON SEED EMERGENCE

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Abstract — Considerable damping-off of the seedlings of several commercial Apiaceae plant species was observed in Serbia. The infection of a total of 48 seed samples of nine vegetable and spice plants with phytopathogenic *Alternaria* spp. was established using the deep-freeze-blotter method. Identification of *Alternaria* species was performed using both conventional methods and PCR. Four different plant-pathogenic *Alternaria* species were detected in Serbia: *A. dauci, A. radicina, A. petroselini,* and *A. alternata*, all of which caused reduction of carrot, parsley, parsnip, and celery seed emergence. *Alternaria dauci, A. radicina,* and *A. petroselini* were relatively more aggressive compared to *A. alternata*. Substantial seed infection levels and strong influence of *Alternaria* spp. on seed emergence indicated that production of Apiaceae seed needs to be improved in order to obtain pathogen-free seed.

Key words: Morphological characteristics, PCR detection, seed infection, pathogenicity test

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INTRODUCTION

The Apiaceae are a large plant family consisting of approximately 200 genera and over 2,900 species grown worldwide. Its representative vegetables are carrot, parsley, parsnip and celery, as well as some well-known spice plants such as fennel, anise, caraway, dill, and coriander. Their production is imperiled by numerous fungal pathogens, among which *Alternaria* species are of great importance.

The genus *Alternaria* comprises a great number of species with various pathogenicity that are the most important pathogens for certain host plants, such as carrot (Farrar et al., 2004). Among the *Alternaria* spp., *A. dauci* (David, 1988; Pryor and Strandberg, 2002), *A. radicina* (Ellis and Holliday, 1972; Konstantinova et al., 2002; Pryor, 2002b), and the polyphagous *A. alternata* (Grabarkiewicz-Szczesna and Chelkowski, 1992; Kwasna, 1992) also cause damping-off of the seedlings of numerous other host plants (Otani and Kohmoto, 1992) and are described as carrot pathogens. *Alternaria petroselini* (Pryor, 2002a), *A. alternata* (Strandberg, 1992), and *A. radicina* (Coles and Wicks, 2003; Farrar et al., 2004) are reported as parsley pathogens, although there are some doubts concerning the exact identity of particular isolates (Pryor and Gilbertson, 2002). *Alternaria radicina* (Ellis and Holliday, 1972) is reported as a pathogen on celery, parsnip, and dill, while *A. alternata* is a pathogen with a broad host range. *Alternaria petroselini* (Pryor and Asma, 2007) and *A. alternata* (Strandberg, 2002) are proven fennel seed pathogens, while there is no published literature on *Alternaria* spp. on caraway and anise.

In the period from 2000 to 2005, significant decay of various Apiaceae seedlings was noticed in Serbia. The objective of this research was to establish the presence and level of infection with *Alternaria* species on Apiaceae seed produced or used in Serbia. Morphological and PCR-based methods of detection were employed with the underlying goal of examining the efficacy of several *Alternaria* specific primers against *Alternaria* spp. found in Serbia. To establish their influence on seed emergence, the pathogenicity of *Alternaria* spp. recovered in Serbia was tested on four Apiaceae hosts.

MATERIALS AND METHODS

Commercial seed samples

A total of 48 seed samples of nine Apiaceae plants donated by seed companies or purchased from retailers were tested for fungal infection. Commercially available seed lots were produced in the period from 2001 to 2004 in Serbia. There were 18 seed samples of carrot, 13 of parsley, six of celery, five of parsnip, two of dill, and one each of coriander, caraway, fennel, and anise.

Seed health condition

The standard deep-freeze-blotter method (Sheppard et al., 2003a, 2003b) for assaying Apiaceae seed for *Alternaria* spp. infection was used. Tests were performed in Petri dishes lined with filter paper disks and moistened with sterile water. Of each sample, 4 x 100 seeds were evenly spaced in dishes (20 per dish), incubated in the dark at 23°C for 3 days, frozen at -20°C for 24 h to prevent seed germination, and then incubated at 23°C (under conditions of a 12-h light-dark cycle) to allow fungal growth and sporulation. After 6 days, each seed was examined microscopically for the presence of *Alternaria* spp. conidia. The infection level with the recovered *Alternaria* species was calculated.

Fungal identification

The identification of *Alternaria* species recovered from commercial Apiacae seed samples was based on pure single-spore colony morphology; on the shape, dimensions, and catenulation of conidia; and on beak presence and length (Pryor and Gilbertson, 2002).

The polymerase chain reaction (PCR) assay was performed with eight selected Serbian isolates and three standard isolates to confirm the identity of isolated *Alternaria* species on the one hand and check if the primers have utility with Serbian isolates on the other. Fungal total genomic DNA was extracted from mycelium grown on PDB (Konstantinova et al., 2002) with CTAB extraction buffer (Day and Shattock, 1997). Primer pairs AAF2/AAR3, specific for *A. alternata*, ADF2/ADR1, specific for *A. dauci*, and ARF2/ARR3, specific for *A. radicina* (Konstantinova et al., 2002) were used, in addition to Pa2071/Pa072, developed for the detection of *A. radicina* and *A. petroselini* (Pryor and Gilbertson, 2002) (Table 1). Fragments of DNA amplified by PCR were separated in 1.0% agarose gel.

Fungal influence on carrot, parsley, celery, and *parsnip emergence*

A total of 18 treatments, control and 17 isolates, were chosen, 12 of which were recovered during this investigation in Serbia, as well as five reference ones obtained from the USA and from Europe. The influence of *Alternaria* spp. isolates on carrot, parsley, celery, and parsnip emergence was investigated using a modification of previously described methods (Grogan and Snyder, 1952; Coles and Wicks, 2003). Carrot (cv. Chanteney), parsley (cv. Domaći lišćar), celery (cv. Alabaster), and parsnip (cv. Dugi beli glatki) seeds, previously proved to be non-infected, were selected for the investigation. Seeds of all four host plants were planted in small pots, one seed per pot. The trial was set up in six replications of 50 seeds each, and the experiment was repeated twice. Seeds were soaked in individual spore suspension of

Table 1. Specific primer pairs used in PCR identification of *Alternaria* spp.

Target sequences	Primer name	Sequence 5'- 3'	Fragment size	Source
A. alternata	AAF2	TGCAATCAGCGTCAGTAACAAAT	~340 bp	Konstantinova et al. (2002)
	AAR3	ATGGATGCTAGACCTTTGCTGAT		
A. dauci	ADF2	GCAATCAGCGTCAGTAACAACA	~345 bp	Konstantinova et al. (2002)
	ADR1	CGCAAGGGGAGACAAAAA		
A. radicina	ARF2	AATCAGCGTCAGTAAACAAACG	~251 bp	Konstantinova et al. (2002)
	ARR3	AGAGGCTTTGTGGATGCTG		
A. radicina	Pa2071	GGGCGTTATGCGAGATCAGG	~900 bp	Pryor and Gilbertson (2001)
	Pa2072	GTATTTGTAGGAATTTCCAG		

all 17 isolates. Seeds soaked in sterile water served as a control. Seeds were sown in sterilized sand and watered with the remaining individual spore suspension (10^3 conidia/ml), except for the control, which was watered with sterile water. Conidial suspensions were prepared from 10-day-old cultures and cultivated on PDA at 23°C in darkness. Sown seeds were maintained under glasshouse conditions (temperature 25±3°C) and watered daily. The number of emerged seedlings was recorded 3 weeks after inoculations. Basic statistical parameters were calculated and the obtained information was presented through a box-plot. Homogeneity of variances was analyzed by Levene's test. Since the data variability called for unparameter testing, differences in the effect of fungal isolates on the emergence of each host plant were analyzed using the Kruskal-Wallis model of analysis of variance. Individual differences in seed emergence under the influence of the isolates were compared by the t-test and Mann-Whitney's U-test. On the basis of influence on emergence of the seeds of four host plants, investigated isolates were combined by the cluster analysis model based on Euclidean distances and complete linkage. All statistical analysis was performed using STATISTICA v.6 (StatSoft, Inc.) software.

RESULTS

Identification of Alternaria spp. present on the seed

Morphological characteristics observed *in situ* on the seed, which were the first criteria for differentiation *Alternaria* spp., indicated the presence of four species, and the following obtained single-spore isolates were chosen for futher investigation: *A. radicina* (isolates Cr-68-1, Cr-89, and Cr-104), *A. petroselini* (Pr-69, Pr-98, and Pr-81), *A. dauci* (Cr-68-5, Cr-21-1, and Cr-120) and *A. alternata* (Cr-21-2, Cr-68-4, and Ce-82). Isolates identified as *A. radicina* (Fig. 1) formed noncatenulate, multicellular, beakless conidia (17.90-45.00 x 11.25-23.75 µm) with average conidia dimensions (30.55 x 15.96 µm) corresponding to those reported in the literature (Ellis and Holliday, 1972; Konstantinova et al., 2002; Pryor and Gilbertson, 2002; Farrar et al., 2004). The conidia of *A. petroselini* isolates (Fig. 2) were morphologically very similar, i.e., solitary, multicellular,

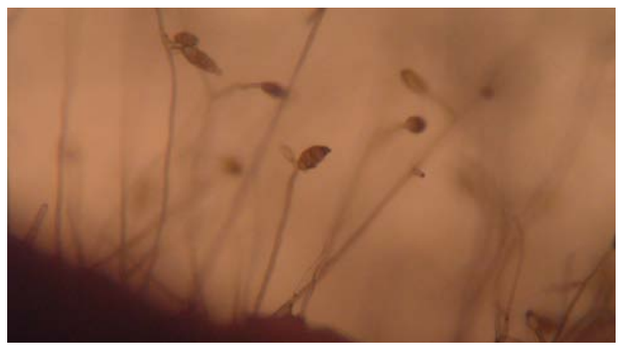

Fig. 1. *A. radicina:* solitary, beakless conidia on the carrot seed.

Fig. 2. *A. petroselini:* solitary, beakless conidia on the parsley seed.

Fig. 3. *A.* *dauci:* solitary conidia with long beak on the carrot seed.

Fig. 4. *A. alternata:* conidia in long chains on the carrot seed.

and beakless (17.90-43.75 x 11.25-23.75 μm) with average dimensions (30.10 x 17.93 μm) corresponding to those reported in the literature (Pryor and Gilbertson, 2002; Pryor and Asma, 2007). Isolates identified as *A. dauci* (Fig. 3) formed solitary, multicellular conidia having a long beak (47.50-90.00 x 11.25-26.25 μm) with average dimensions (66.31 x 18.49 μm) corresponding to those reported in the literature (David, 1988; Yu, 1992; Farrar et al., 2004). *Alternaria alternata* isolates (Fig. 4) formed long chains of small, multicellular conidia that either have a short beak or are beakless (15.00-40.00 x 7.50-15 μm) and whose average dimensions (24.56 x 11.05 μm) correspond to those reported in the literature (Yu, 1992; Mirkova and Konstantinova, 2003).

Molecular detection using PCR confirmed identity of the chosen isolates differentiated on the basis of their morphological features, but showed different specifity and ability to detect all fungal isolates from Serbia. Thus, the expected DNA fragment, of about 340 bp, amplified in two isolates (Cr-21-2 and Ce-82) using the AAF2/AAR3 primer pair, confirmed their identity as *A. alternata* (Konstantinova et al., 2002; Mirkova and Konstantinova, 2003). Similarly, by applying the ADF2/ADR1 primer pair, designed for *A. dauci* detection, amplicons of expected 345 bp occurred in three isolates (Cr-68-5, Cr-120, and BMP155), which confirmed their identity (Konstantinova et al., 2002). Primer pair ARF2/ARR3, specific for *A. radicina*, amplified a DNA fragment to the expected size of 250 bp in two *A. radicina* isolates (Cr-89 and BMP79) and in all three isolates belonging to *A. petroselini* (Pr-69, Pr-98, and BMP139). By using primer pair Pa2071/Pa072, designed for detection of *A. radicina*, the expected DNA fragment, amplified to approximately 900 bp, occurred both in all three isolates identified as *A. radicina* (Cr-68-1, Cr-89, and BMP79) and in those identified as *A. petroselini* (Pr-69, Pr-98, and BMP139) (Pryor and Gilbertson, 2001).

Primer pair AAF2/AAR3, specific for *A. alternata*, repeatedly amplified one of the Serbian *A. dauci* isolates (Cr-68-5), exhibiting cross reactivity. Similarly, primers ADF2/ADR1, specific for *A. dauci*, repeatedly amplified two *A. petroselini* isolates (Pr-69 and BMP139), while primer pair ARF2/ARR3, specific for *A. radicina*, failed to amplify one of the Serbian isolates (Cr-68-1).

Seed infection

A total of 48 commercial samples of the seeds of different Apiaceae were examined microscopically, and the results of seed health testing indicated different levels of infection with *Alternaria* spp. (Table 2), depending on the host plant species.

On carrot seed, three different *Alternaria* spp. were detected, in single and as mixed infections: *A. alternata* (0-89.25%), *A. dauci* (0-21%), and *A. radicina* (0-10%). On parsley seed, two pathogenic species – *A. alternata* (0-100%) and *A. petroselini* (0-100%) – were present in mixed infections. *Alternaria alternata* was predominant on celery seed with infection levels of 0-16.75%, while the presence of *A. petroselini* was detected in only one celery sample (1%). Parsnip seed samples were infected by *A. alternata* (0-30.25%) and *A. petroselini* (0-3.25%). Two dill seed samples were infected with *A. alternata* (56.25%-62.25%) and only one with *A. petroselini* (1.5%). In the other Apiaceae seed samples, the presence of only *A. alternata* was established in coriander (92.75%), fennel (61.75%), anise (25.25%), and caraway (0.5%) seed.

Influence of Alternaria spp. on carrot, parsley, celery, and *parsnip emergence*

All selected isolates, both those obtained during this investigation and the referent ones, caused substantial reduction in the number of emerged carrot, parsley, celery, and parsnip seedlings (Table 3) compared to the control. The lowest rate of carrot seed emergence occurred after inoculation with *A. radicina* (NL1R) and *A. dauci* (Cr-68-5 and Cr-21-1) isolates, while *A. petroselini* (Pr-81) isolate caused the least negative effect. The highest reduction in emergence of parsley was induced by *A. petroselini* (Pr-69 and Pr-98) isolates, while *A. radicina* (Cr-89) and *A. alternata* (Cr-21-2) isolates had the smallest influence on its emergence. The highest reduction in parsnip emergence was caused by one isolate of *A. radicina* (Cr-89), while the isolates of *A. alternata* (Cr-21-2 and Cr-68-4) caused the lowest reduction in the number of emerged parsnip seedlings. Celery

Table 2. Presence and frequency of *Alternaria* spp. (%) on the seed of vegetable and spice plants from Apiaceae family.

Host plant	Cultivar	Sample	Year of produc.	Healthy seed	Single infection				Mixed infection
					A. alternata	*A. dauci*	*A. radicina*	*A. petroselini*	
Carrot	Nantes	Cr-21	2002	9.50	86.00	1.25	0	0	3.25 (*A.dauci* + *A. alternata*)
Carrot	Chanteney	Cr-45	2002	98.25	1.75	0	0	0	0
Carrot	Nantes	Cr-46	2002	56.25	43.75	0	0	0	0
Carrot	Nantes	Cr-68	2003	7.75	78.00	4.25	1.25	0	8.75 (*A.dauci*+ *A. radicina*+ *A. alternata*)
Carrot	Chanteney	Cr-72	2003	100.00	0	0	0	0	0
Carrot	Nantes	Cr-75	2001	100.00	0	0	0	0	0
Carrot	Amsterdamska	Cr-76	2002	94.25	5.75	0	0	0	0
Carrot	Nantes	Cr-77	2003	88.50	11.50	0	0	0	0
Carrot	Chanteney	Cr-78	2003	10.50	86.50	0	0	0	0
Carrot	Nantes	Cr-84	2002	54.25	42.25	1.50	2.00	0	0
Carrot	Danvers 126	Cr-85	2002	23.50	66.25	6.00	4.25	0	0
Carrot	Nantes	Cr-89	2003	56,25	40,50	0	2.25	0	1.00 (*A.radicina*+*A. Alternata*)
Carrot	Nantes SP 80	Cr-104	2004	78.25	20.50	0	1.25	0	0
Carrot	Unknown	Cr-105	2003	100.00	0	0	0	0	0
Carrot	Nantes Improved	Cr-114	2003	100.00	0	0	0	0	0
Carrot	Nantes Improved	Cr-115	2003	100.00	0	0	0	0	0
Carrot	Nantes Improved	Cr-116	2003	100.00	0	0	0	0	0
Carrot	Nantes	Cr-120	2004	1.25	76.50	1.25	1.25	0	19.75 (*A.dauci* + *A. alternata*)
Parsley	Berlinski	Pr-3	2002	92.50	4.25	0	0	3.25	0
Parsley	Domaći lišćar	Pr-44	2002	97.75	0	0	0	2.25	0
Parsley	Berlinski sred-nje dugi	Pr-69	2003	51,00	39.25	0	0	1.50	8.25 (*A.petroselini*+*A.alternata*)
Parsley	Domaći lišćar	Pr-73	2003	75.50	2,25	0	0	2.75	19.50 (*A.petroselini*+*A. alternata*)
Parsley	Muskaraus	Pr-79	2002	100	0	0	0	0	0
Parsley	Berlinski sred-nje dugi	Pr-80	2003	100	0	0	0	0	0
Parsley	Berlinski	Pr-81	2003	60.75	37.75	0	0	0	1.50 (*A.petroselini* +*A. alternata*)
Parsley	Berlinski sred-nje dugi	Pr-86	2003	58.25	40.50	0	0	1.25	0
Parsley	Domaći lišćar	Pr-87	2002	96.50	1.25	0	0	1.25	1.00 (*A.petroselini* +*A. alternata*)
Parsley	Unknown	Pr-93	2003	100	0	0	0	0	0
Parsley	Unknown	Pr-98	2003	55.75	40.25	0	0	2.75	1.25 (*A.petroselini* +*A. alternata*)
Parsley	Berlinski	Pr-121	2004	0	36.25	0	0	0	63.75 (*A.petroselini*+*A. alternata*)
Parsley	Lišćar	Pr-122	2004	0	62.25	0	0	0	37.75 (*A.petroselini*+*A. alternata*)
Celery	Alabaster	Ce-52	2004	83.25	16.75	0	0	0	0
Celery	Praški	Ce70	2003	97.25	1.75	0	0	1.00	0
Celery	Alabaster	Ce-74	2003	100	0	0	0	0	0
Celery	Praški	Ce-82	2003	97.50	2.50	0	0	0	0
Celery	Alabaster	Ce-90	2004	98.25	1.75	0	0	0	0
Celery	Praški	Ce-91	2004	90.25	9.75	0	0	0	0
Parsnip	Panonski glatki	Pn-71	2003	66.50	30.25	0	0	3.25	0
Parsnip	Dugi beli glatki	Pn-83	2003	91.25	8.75	0	0	0	0
Parsnip	Dugi beli glatki	Pn-88	2002	100	0	0	0	0	0
Parsnip	Panonski glatki	Pn-92	2003	98.25	1.75	0	0	0	0
Parsnip	Panonski	Pn-123	2004	91.50	6.25	0	0	2.25	0
Dill	Unknown	An-96	2003	42.25	56.25	0	0	1.50	0
Dill	Unknown	An-99	2003	37.75	62.25	0	0	0	0
Coriander	Unknown	Co-100	2003	7.25	92.75	0	0	0	0
Caraway	Unknown	Ca-101	2003	99.50	0.50	0	0	0	0
Fennel	Unknown	Fo-102	2003	38.25	61.75	0	0	0	0
Anise	Unknown	Pi-103	2003	74.75	25.25	0	0	0	0

Table 3. Number of emerged seedlings of Apiaceae vegetables and spice plants after seed inoculation with Alternaria spp. isolates. *Average value of all repetitions.

Species	Isolate	Carrot	Parsley	Parsnip	Celery
A. radicina	Cr-68-1	15,25	30,92	10,25	12,50
	Cr-89	14,17	34,83	5,83	14,50
	Cr-104	16,25	29,17	13,83	11,17
	NL1R	12,17	33,42	10,25	13,08
	BMP79	13,83	30,33	8,83	12,17
A. petroselini	Pr-69	29,67	11,08	11,17	9,67
	Pr-98	30,92	12,42	11,42	11,42
	Pr-81	34,42	15,83	10,50	10,42
	BMP139	33,33	14,50	8,58	13,67
A. dauci	Cr-68-5	12,50	31,75	10,08	10,08
	Cr-21-1	13,75	34,00	15,25	11,08
	Cr-120	13,25	31,42	11,25	11,42
	NL2D	14,42	32,50	12,75	14,25
	BMP155	13,67	30,83	10,00	13,17
A. alternata	Cr-21-2	33,00	35,92	15,83	31,33
	Cr-68-4	31,33	32,42	20,75	29,33
	Ce-82	28,00	28,33	18,00	35,17
Sterile H_2O	Control	48,42	49,08	33,00	39,08

emergence was the most reduced by several isolates of *A. radicina* (Cr-104), *A. petroselini* (Pr-69 and Pr-98), and *A. dauci* (Cr-68-5 and Cr-120), while one isolate of *A. alternata* (Cr-82) exhibited the lowest pathogenicity on celery seed.

Since the experiment was repeated two times, the model of two-factorial analysis of variance was applied, and there were no statistically significant differences between repetitions or any statistically significant interaction. For that reason, all the obtained data were combined into one analysis, with 12 replicates. The established emergence of carrot and parsley seed was homogeneous (CV<30%), while parsnip and celery exhibited maximum nonhomogeneity of CV=36.6% and CV=56.2%, respectively. In all four host plants, Levene's test showed nonhomogeneity of variances. Comparison among 17 isolates and the control using the Kruskal-Wallis model of analysis of variance revealed that carrot (H=98.43%, $p<0.01$), parsley (H=97.30%, $p<0.01$), and parsnip (H=46.79%, $p<0.01$) seed emergence was statistically very significantly different under influence of the tested isolates, while celery (H=21.59%, $p<0.05$) seed emergence was only statistically significantly different.

Seed emergence in the control was compared with emergence of inoculated seed by the t-test and Mann-Whitney's U-test, and the obtained data (as well as Fig. 5) confirmed that seed emergence in the control was statistically very significantly higher than emergence of seed inoculated with all tested isolates, in all four host plants.

The selected statistical model of cluster analysis grouped the investigated fungal isolates on the basis of their pathogenicity and all four host plant seed emergence. The formed clusters were not illustrative enough to reveal relationships among *Alternaria* species. As shown in Fig 6, the control was clearly separated from all the isolates, although the isolates of *A. alternata* were more similar to the control than isolates of the other species. The isolates belonging to *A. alternata* and *A. petroselini* formed separated clusters, while clear distinction among the isolates of *A. radicina* and *A. dauci* could not be seen. On the basis of parsley and celery seed emergence, the

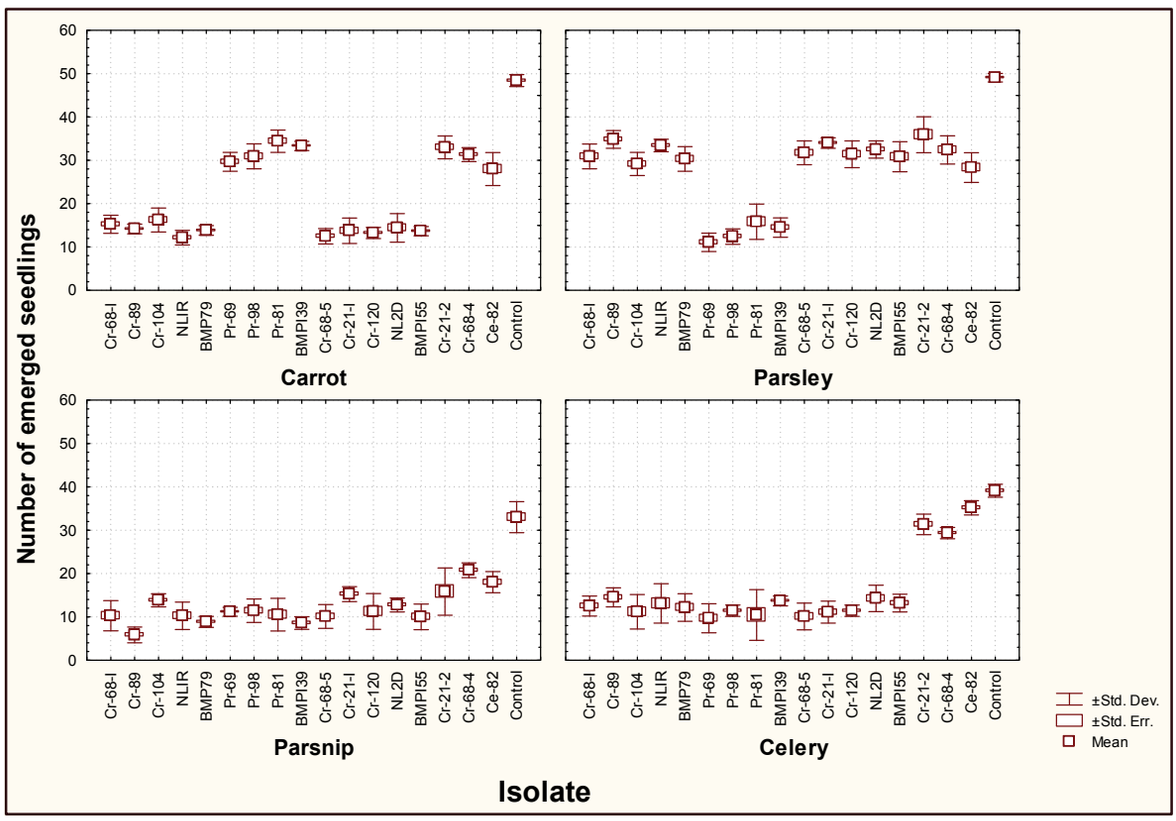

Fig. 5. Influence of *Alternaria* spp. isolates on carrot, parsley, parsnip and celery seed emergence.

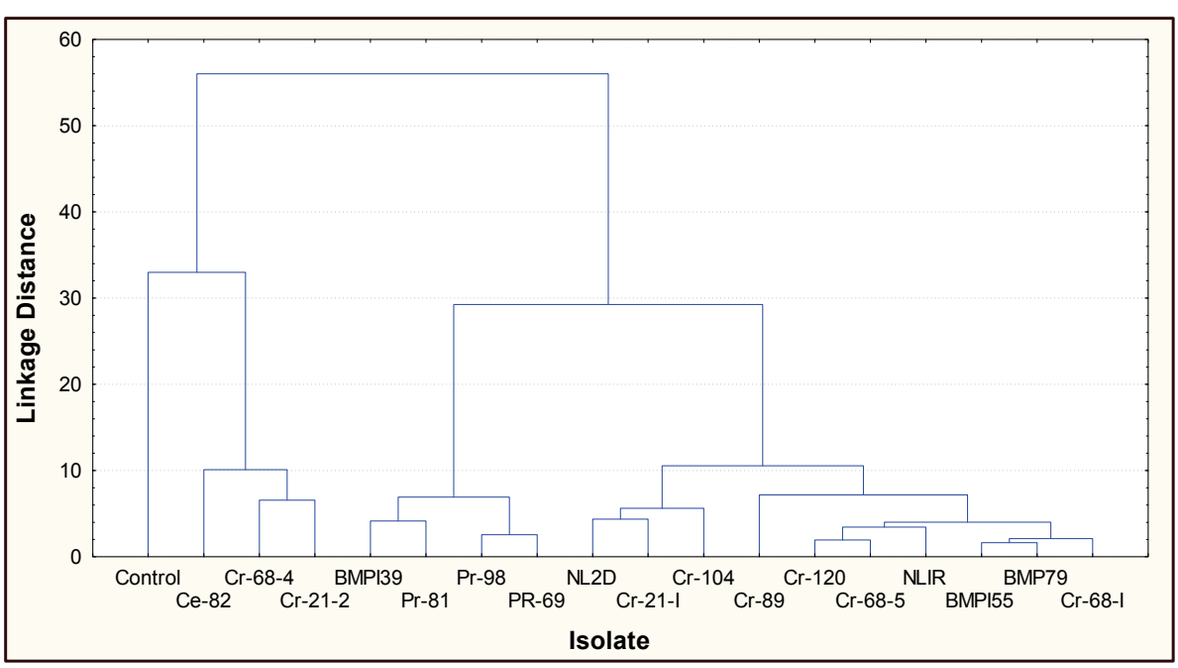

Fig. 6. Grouping of *Alternaria* spp. isolates based on carrot, parsley, parsnip and celery seed emergence.

investigated isolates could not be grouped in accordance with their representative species, while all isolates from all four species were distinct from the control.

DISCUSSION

Infected seeds play a very important role in disease epidemiology. Therefore, determination of seed health status and use of *Alternaria*-free or treated seed are the first and most important steps in disease management. There are numerous reports on seed infection levels with different *Alternaria* spp. in a number of host plants indicating that seed infection is a constant problem. Until this investigation, there was no information on the health status of commercial Apiaceae seed used in production in Serbia.

The results of this investigation showed that commercial Apiaceae vegetable and spice plant seeds produced from 2001 to 2004 in Serbia were infected with four species from the genus *Alternaria*: *A. radicina*, *A. petroselini*, *A. dauci*, and *A. alternata.* Identification of the detected species was performed on the basis of morphological features and catenulation of conidia (Strandberg, 1983; Sheppard et al., 2003a, 2003b) as well as by the PCR molecular test using previously described primer pairs (Pryor and Gilbertson, 2001; Konstantinova et al., 2002; Mirkova and Konstantinova, 2003).

Our investigation to determine suitability of primers for molecular detection of Serbian isolates of *Alternaria* spp. was not extensive and included only 11 isolates belonging to the four species. Nevertheless, the obtained results showed that the four primer pairs used in this investigation exhibited different suitability for detection of Serbian isolates of *A. radicina*, *A. petroselini*, *A. dauci*, and *A. alternata.* One of the primer pairs (AAF2/AAR3), specific for *A. alternata*, repeatedly cross-reacted with one Serbian *A. dauci* isolate. Similarly, the primer pair specific for *A. dauci* (ADF2/ADR1) cross-reacted with two *A. petroselini* isolates, while primer pair ARF2/ARR3, specific for *A. radicina*, failed to amplify one of the Serbian isolates. Both primer pairs specific for *A. radicina* (ARF2/ARR3 and Pa2071/Pa072) repeatedly amplified all *A. petroselini* isolates, both Serbian and referent ones. Only one primer pair (Pa2071/Pa072) amplified all Serbian as well as all referent isolates, but it was not possible to make any distinction between *A. radicina* and *A. petroselini* isolates. This primer pair was reported to amplify both *A. radicina* and *A. petroselini* isolates, but is still recommended for routine usage (Pryor and Gilbertson, 2001).

Molecular detection of *Alternaria* species on Apiaceae seeds could be a powerful tool giving results in a much shorter period compared to the freezing-blotter or similar methods. Furthermore, routine use of molecular methods for *Alternaria* spp. detection would make detection faster with lower costs. It is important to develop a protocol for molecular detection of *A. radicina*, *A. petroselini*, *A. dauci*, and *A. alternata* in Serbia by using newly designed primers against a great number of Serbian isolates.

The levels of seed infection with detected and identified species were variable and in some samples very high. For example, the levels of parsley seed infection with *A. petroselini* detected during this investigation were extremely high, reaching 100% in several samples. Previous findings also indicated seed infection with this species at very high levels of more than 50% (Pryor, 2002a; Bulajić et al., 2005b). Similarly, infection with other *Alternaria* spp. was established on the seed of other investigated Apiaceae host plants within infection levels previously reported worldwide. There are several reports of high infection with *A. radicina* in carrot seed with levels of 0.5-82% (Tylkowska, 1992), an average of 35% (Coles and Wicks, 2003), 1-20% (Grogan and Snyder, 1952), and 0.25-30% (Maude, 1966). Infection by *A. radicina* was detected even in carrot seed treated with iprodione, at levels of 0.2-14% (Coles and Wicks, 2003). The reported carrot seed infection levels caused by *A. dauci* are 0.3-8% (Tylkowska, 1992), an average of 28% (Strandberg, 1983), and 13.2% (Netzer and Kenneth, 1969). There are reports of mixed infections by *A. radicina* and *A. dauci* (Strandberg, 2002). In some samples, even triple infection was recorded, with *A. radicina*, *A. dauci*, and *A. alternata*. Apiaceae seed infection with *A. alternata* is often recorded in different hosts,

especially carrot, with seed infection levels higher than 80% (Strandberg, 1992). There are reports of *A. alternata* seed infection in various Apiaceae hosts, generally at very high levels (Bulajić et al., 2005a).

Apiaceae plant seed infection with *Alternaria* spp. is one of the most important ways of survival and spreading of these fungi in nature (Kwasna, 1992; Agrios, 2005). Seed infection can result in reduced germination (Tylkowska, 1992) and yield, and it can contribute to the introduction of *Alternaria* spp. into new production areas (Pryor et al., 1997). Investigation of infection of commercial Apiaceae seed in Serbia revealed high levels of infection with the four *Alternaria* species. Pathogenicity tests were performed on carrot, parsley, parsnip, and celery in order to investigate influence of *Alternaria* spp. infection on emergence in a controlled environment. Under the experimental conditions, all included isolates exhibited different pathogenicity on all four host plants. Isolates belonging to one fungal species, both those originating from Serbia and the referent ones, showed slightly different pathogenicity. However, such differences were to be expected, considering the known host range and *Alternaria* spp. specialization in different parts of host plants in natural infections. Some of the investigated host plants reacted similarly to infection with two or three detected *Alternaria* spp., while others exhibited a completely different reaction to each of them. Generally, the influence of all four fungal species was in accordance with their respective natural host range. In the case of emergence of four host plants, there were differences both among isolates of the same species and among different species.

On the basis of carrot seed emergence, it can be concluded that the greatest pathogenicity was of *A. radicina* and *A. dauci* isolates, *A. alternata* isolates were less pathogenic, and *A. petroselini* isolates were the least pathogenic. Although pathogenicity somewhat varied among isolates of the same species, all isolates formed clearly separated clusters and were completely separated from the control. Differences in exhibited pathogenicity among the four detected species were expected, since *A. alternata* is described in the literature as a weak pathogen with a broad host range, and natural infection of carrot with *A. petroselini* is not known. Furthermore, even in artificial infection of carrot leaf, *A. petroselini* was not pathogenic (Pryor and Gilbertson, 2002). Also, it was observed that *A. petroselini* exhibited the highest pathogenicity in parsley, and *A. radicina* the lowest. There are controversial data in the literature concerning *A. radicina* as a parsley pathogen. In some early reports (Coles and Wicks, 2003; Farrar et al., 2004), *A. radicina* is recorded as a parsley pathogen, but there is one report indicating its non-pathogenicity even after artificial inoculations (Pryor and Gilbertson, 2002). Considering that these two species are morphologically very similar, if the distinction is based only on conidia morphology, misidentifications would be possible, which should be taken into consideration.

Pathogenicity of the investigated species on parsnip was not uniform, and isolates could not have been clearly distinguished, something that was not in accordance with their taxonomic status. None of the species included in the pathogenicity test showed differences of pathogenicity from the others, but all four reduced seed emergence compared to the control. The situation was similar with celery emergence, with the exception of *A. alternata*, which exhibited lower pathogenicity compared to the other three species. Pathogenicity of all four *Alternaria* species on parsnip and on celery should be confirmed by inoculations of other plant organs and tissues and in different growth stages, as well as by investigations of natural infections.

The influence exerted by the investigated *Alternaria* species on emergence of the seeds of four host plants revealed that their pathogenicity is not related to phylogenetic relationship and mainly cannot be used to illustrate taxonomic status. However, this study clearly confirmed their pathogenicity and implied possible significance in nature.

This investigation of seed infection in several important Apiaceae plants in Serbia revealed different levels of infection with four different species, viz., *A. dauci*, *A. radicina*, *A. petroselini*, and *A. alternata*, all of them pathogenic and able to cause reduction in the number of emerged seedlings. *Alternaria dauci*, *A. radicina*, and *A. petroselini* were

more aggressive than *A. alternata.* The results also revealed an unsatisfactory situation in the Apiaceae vegetable and spice plant seed industry in Serbia and the need to improve seed health status by reduction of phytopathogenic *Alternaria* spp. infection levels. Apart from determination of the health status of commercial seed, the intention of this investigation was to contribute to the development of improved phytosanitary practice in Apiaceae seed production and management of diseases caused by *Alternaria* spp.

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ПРИСУСТВО *ALTERNARIA* SPP. НА СЕМЕНУ БИЉАКА ИЗ ФАМИЛИЈЕ APIACEAE И ЊИХОВ УТИЦАЈ НА НИЦАЊЕ

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У производњи неколико усева биљака из фамилије Apiaceae, уочено је интензивно пропадање сејанаца у Србији. Применом методе замрзавања на филтер папиру, установљена је зараза укупно 48 узорака семена 9 гајених биљака поврћа и зачинских биљака фитопатогеним врстама из рода *Alternaria*. Идентификација *Alternaria* spp. обављена је применом конвенционалних метода и PCR. У Србији је детектовано присуство укупно четири фитопатогене врсте из рода *Alternaria*: *A. **dauci***, *A. radicina*, *A. **petroselini*** и *A. **alternata*** које су изазвале смањено ницање мркве, першуна, паштрнака и целера. *A. **dauci***, *A. **radicina*** и *A. **petroselini*** биле су релативно агресивније у поређењу са *A. **alternata***. Значајан ниво заразе семена, као и испољени утицај *Alternaria* spp. на ницање, указали су да би производњу семена врста фамилије Apiaceae требало побољшати у циљу добијања здравог семена.