

# *Alternaria alternata* – the main causal agent of disease symptoms in juniper, rose, yew and highbush blueberry in nurseries in southern Poland

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## ABSTRACT

Symptoms of discoloration and necrosis of the leaves/needles and shoots of plants are an increasingly common phenomenon in nurseries. They necessitate the withdrawal of the affected plants from sale, which has significant economic consequences. In 2010-2011, observations were conducted of the health of shrubs in nurseries of the Małopolska province. Disease symptoms were mostly found in juniper (*Juniperus horizontalis* 'Wiltonii'), rose (ground-cover rose 'Star Profusion'), yew (*Taxus × media* 'Hillii') and highbush blueberry (*Vaccinium corymbosum* 'Patriot'). These species were selected for further study. The affected shrubs represented more than 46% of the population of a given species. Fragments of the diseased organs: the leaves or needles, the base of the shoots, and the roots, were collected from the borderline between healthy and diseased tissue, and used to isolate and identify the microorganisms colonizing the diseased parts. The affected organs were found to be inhabited to the largest extent by the fungus *Alternaria alternata* (Fr.) Keissl., which accounted for 19.7 to 47.5% of the isolates from the tested species of shrubs. There were also large populations of fungi of the genera: *Botrytis* (up to 9.82%), *Cladosporium* (up to 5.66%), *Colletotrichum* (up to 5.13%), *Fusarium* (up to 18.38%), *Mortierella* (up to 7.26%), *Pestalotia* (up to 5.36%), *Rhizoctonia* (up to 5.36%), *Sclerotinia* (up to 6.99%), and *Trichoderma* (up to 17.09%). The fungus *A. alternata*, being by far the dominant pathogen, was tested for its pathogenicity for the shoots of the chosen species of shrubs. The test was conducted for 14 days in a chamber with parameters so programmed that they reflected the natural conditions at the height of the growing season. The fungus *A. alternata* exhibited pathogenicity for all of the tested species of shrubs. Necrosis developed on all the inoculated fragments of shoots. The surface area of necrotic lesions was larger on the shoots of juniper and blueberry.

Key words: *Alternaria alternata*, DNA, pathogenicity

## INTRODUCTION

The nature of plant production is such that it is not possible to separate it from the influence of external factors. Among biotic factors, phytopathogenic

fungi are a major infectious agent in plants, as more than 70% of major diseases of crops are caused by fungi (Agrios, 2005; Larrañagal et al., 2012)

Fungi of the genus *Alternaria* are ubiquitous, occurring in large numbers in the organic matter

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in the soil (Lou et al., 2013). Many species are saprotrophs, plant pathogens or crop pathogens. Pathogenic *Alternaria* fungi cause diseases of various crop plants and are present on all continents (Chase, 2005; Perello and Sisterna, 2006). They also infect ornamental plants and fruit trees and shrubs (Windham, 2008; Kakalikova et al., 2009; Pegg et al., 2014; Andersen et al., 2015).

*Alternaria* fungi, due to their prevalence and genetic variation, constitute a major threat in the cultivation of plants (Kakvan et al., 2012). Already in 1960, Pomerlau and Nadeau named the fungi of *Alternaria* spp. as the cause of withering of 20-65% of seedlings of coniferous plants in nurseries. Likewise, James and Woo (1987) found that seedlings of conifers infected by *Alternaria alternata* withered away completely.

Sharma et al. (2013) found that analyses performed on genomic DNA from *A. alternata* isolates derived from various host plants showed a high level of genetic variation. When planting ornamental plants, our primary goal is the decorative effect, and in the case of fruiting plants it is their ability to produce a valuable crop, so the choice of appropriate species and varieties of plants introduced into our garden is as important as their health. The quality of plant material produced by nurseries is therefore a factor that largely influences subsequent vegetative growth of all the plantings. For several years, nursery producers have been reporting disease symptoms appearing on the aboveground parts of some species of shrubs. Initially, it is a change in colour of the shoot tips and development of lesions on the leaves of deciduous plants, followed by withering of the affected organs. This phenomenon is more and more common and necessitates the withdrawal of large numbers of plants from sale, which makes it increasingly important economically. In this situation, it has become necessary to determine the causes of the observed symptoms. The objects of the study were plants of the species in which disease symptoms in nurseries had been observed most frequently: juniper, rose, yew, and highbush blueberry.

The aim of the study was to investigate the effect of mycorrhizal inocula on the growth, development and health of these plant species.

## MATERIAL AND METHODS

In 2010-2011, disease symptoms on the leaves/needles and the shoot base of plants in nurseries of the Małopolska province were most often found in creeping juniper, rose, yew and highbush

blueberry. This was the basis for the selection of shrubs of these species for thorough examination and analysis. Five nurseries, where all of these shrubs were grown, were selected. In 2010 and 2011, from April to October, monthly observations of the health condition of three-year-old plants of the chosen species were conducted. In 2011, plants with disease symptoms: discoloration, necrotic lesions, withering of the leaf blade/needles and shoots, were collected from the nurseries. Five plants of each species were selected for the study. From each plant, six leaves or needles, depending on the plant, were taken. In the laboratory, all of the samples were initially rinsed with water. Then, using a sterile scalpel, four small fragments were taken from the borderline between necrotic lesions and healthy tissue; these were cut into 0.5-1.0 cm pieces and subjected to surface disinfection. The disinfected plant material was thoroughly dried with sterile absorbent paper and laid out, four pieces at a time, on a glucose-potato medium and a pea medium in Petri dishes. After a period of incubation in a thermostat at a temperature of 22-23°C for four to seven days, successively appearing colonies of fungi were split off and inoculated onto PDA slants. After homosporous cultures had been obtained, they were transferred onto suitably selected media and identified to the level of species. In addition to the PDA medium, rice and potato media were also used to identify fungi of the genus *Fusarium*. The isolated fungi were identified using mycological keys (Riffai, 1969; Ellis and Ellis, 1988; Kwaśna et al., 1991; Dugan, 2006; Domsh et al., 2007; Klaus et al., 2008). The frequency of occurrence of the different species of fungi was determined on the basis of the number of the obtained isolates of a given fungus, which was expressed as a percentage.

Pathogenicity of the isolates for the shoots of plants was tested with five isolates of *A. alternata* originating from the shrubs under study. The experiment was set up in five replicates, each with 5 fragments of shoots (8 cm long) for each plant species. Five-millimeter diameter discs were cut out with a cork borer from 14-day-old mycelium of *A. alternata*. Transparent plastic boxes were bottom-lined with a double layer of filter paper, and 20 ml of sterile distilled water was poured onto it. Next, five plant fragments were placed inside each of these boxes; the fragments were pricked with a sterile needle and the prick spots were covered with the discs overgrown with the mycelium of *A. alternata*; the inoculation was with the isolate derived from

the same plant species. The control consisted of the shoots of the same species of plants, inoculated with discs of the glucose-potato medium only.

The boxes with the fragments of plants were snap-closed and transferred to a Sanyo Versatile Environmental Test Chamber MLR-351H. The parameters programmed in the chamber were chosen to reflect the natural conditions at the height of the growing season (Tab. 1). The test in the chamber was conducted for 14 days. While in progress, the conditions of the test were checked regularly. The discolorations that appeared were measured: the length and width of the necrotic lesions on the shoots. After the completion of the experiment, re-isolation of the fungi from the shoots was performed to confirm the presence of the infectious agent in plant tissue. The obtained results were subjected to statistical calculations (computer program Statistica 12) by ANOVA variance analysis. The validity of the differences between the combinations was

checked by the Tukey test, with a significance level of  $p = 0.05$ .

The *A. alternata* isolates obtained from the diseased parts of the tested species of shrubs were subjected to molecular analysis. For this purpose, DNA was isolated from 14-day-old cultures of *A. alternata* grown on the potato-glucose medium. The mycelium of each isolate separately was collected from the Petri dish and homogenized by grinding in a ceramic mortar in liquid nitrogen. DNA was then isolated using the Invisorb Spin Plant Mini Kit (Symbios). RAPD-PCR amplification was performed using DNA of four *A. alternata* isolates with OPAD12 primer (AAGAGGGCGT). RAPD-PCR mixture was prepared according to Table 2. The dye used was ethidium bromide. The products of RAPD-PCR amplification were separated by 1.5% agarose gel electrophoresis in  $1\times$  TBE buffer at a voltage of 80 V for 1.5 hours. Visualization of the results was carried out by irradiating the agarose gel with UV light in a Gel Doc EZ Imager (Bio-Rad).

**Table 1.** Parameters in the pathogenicity test of *Alternaria alternata*

Hours	Temperature (°C)	Degree of exposure	Relative humidity RH (%)
05.00 - 06.00	15	1	85
06.00 - 07.00	15	2	85
07.00 - 08.00	18	3	80
08.00 - 09.00	20	4	70
09.00 - 10.00	22	5	70
10.00 - 12.00	24	5	60
12.00 - 14.00	26	5	50
14.00 - 16.00	28	5	50
16.00 - 18.00	24	4	60
18.00 - 20.00	22	2	60
20.00 - 21.00	18	1	70
21.00 - 05.00	15	0	80

**Table 2.** Composition of RAPD-PCR mixture with the DNA of *Alternaria alternata* isolates and OPAD12 primer

Reagents	Concentration in the reaction mixture	Content in the reaction mixture for 1 sample (μl)
Taq buffer [750 mM Tris-HCl; pH 8.8; 200 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ; 0.1% Tween20]*	1×	2.0
MgCl <sub>2</sub> 25mM*	1.5 mM	1.2
dNTP 2.5 mM*	0.1 mM	0.8
Taq polymerase*	0.5 U	0.4
Primer	0.2 mM	0.4
Distilled water	-	13.2
DNA	20 ng μl <sup>-1</sup>	2.0
Total	-	20.0

\*Thermo Scientific

## RESULTS AND DISCUSSION

Symptoms of discoloration and withering of the leaves/needles and shoots, and sometimes of entire plants, occurred on all of the examined shrubs in all of the nurseries selected for the study. In both years of observations of the health of shrubs of the selected species, the percentage of infected plants was high, reaching values of more than 46.0% in all of the nurseries. Information on the pathogenic effect of *Alternaria alternata* on ornamental plants can also be found in the literature. According to Atilano (1983), the lesions caused by *Alternaria* spp. on the leaves are endemic to many species of plants grown in nurseries.

In the creeping juniper variety Wiltonii there appeared initially light green sections of withering shoots. Later, their colour changed to light brown. The number of shoots infected in this way increased with time. The lesions on the leaves of the rose variety Star Profusion were unevenly distributed on the surface of the leaf blade; they were light brown in colour, and their shape was irregular. The needles of yew shrubs of the variety Hillii changed their colour initially to light celadon, then to yellow, and in the final stage to light brown. With time, the plants withered away. The tips of the shoots of the northern blueberry variety Patriot initially became claret-coloured, but remained alive. Towards the end of the growing season they withered only as far as the boundary of discoloration, and then crumbled away. The remaining part of the plant resumed vegetative growth in the spring, but the new growth was shorter, and the branching not as extensive.

As a result of the mycological analysis of the leaves and needles of the test plants collected in 2011 and 2012, a total of 592 fungal isolates and 18 bacterial isolates were obtained (Tab. 3). On the basis of the frequency of occurrence, *A. alternata* was among the dominant fungi isolated from the leaves/needles of the diseased shrubs. This fungus was isolated in the largest numbers from the needles of juniper – 72.22%, in considerably smaller numbers from yew needles – 42.11%, and in the lowest percentages from the leaves of blueberry – 25.32% and rose – 23.08% (Tab. 3). Fungi of the genus *Fusarium* were also isolated in considerable amounts: 24.59% from yew needles, 17.72% from blueberry leaves, 17.31% from rose leaves, 9.26% from juniper needles (Tab. 3). Colonies of *Botrytis cinerea* were isolated in smaller numbers: 17.31% from rose leaves; 8.77% from yew needles; 5.56% from juniper needles; 3.80% from blueberry leaves (Tab. 3).

In the creeping juniper shrubs of the variety Wiltonii, the bark of the shoot base was very dark. The base of the stems in the rose variety Star Profusion was heavily discoloured to a dark, brown and purple colour. These changes extended high above the ground surface. The shoot base in the yew variety Hillii was dark brown, almost black. The lowest part of the base of shoots of the 'Patriot' northern highbush blueberry was discoloured to an intense brown.

As a result of the mycological analysis of the diseased base of the shoots of the test plants collected in 2011-2012, a total of 604 fungal isolates and 20 bacterial isolates were obtained. Among all the isolates, except for blueberry, the most dominant was the fungus *Alternaria alternata*. The largest numbers of it inhabited the diseased base of rose stems – 56.92% (Tab. 4). Slightly fewer isolates of *A. alternata* were isolated from the diseased base of yew shoots – 42.86%, and the fewest from the diseased base of the shoots of juniper – 35.14% (Tab. 4).

Fungi of the genus *Fusarium* also had a large share in the total number of the isolates obtained: 26.39% from the diseased base of blueberry, 18.46% from the diseased base of rose, 17.57% from the diseased base of juniper, and the smallest number – 9.89% from the diseased base of yew (Tab. 4). *Botrytis cinerea* was also isolated in considerable amounts: 13.51% from the diseased base of juniper and slightly less – 9.89% from the diseased base of yew (Tab. 4). The most numerous fungus isolated from the diseased base of blueberry was *Sclerotinia sclerotiorum* – 19.44% (Tab. 4). Among the dominant fungi colonizing the diseased base of the shoots of the test shrubs were fungi of the genus *Trichoderma* – from 23.61% in blueberry to 6.15% in rose (Tab. 4). The dominant fungi colonizing the diseased base of yew shoots were the fungi *Pestalotia sydowiana* – 13.19%, and *Chaetomium globosum* – 9.89% (Tab. 4). The dominant, potentially pathogenic, fungus colonizing the diseased base of rose shoots was *Verticillium albo-atrum* – 6.15% (Tab. 4).

The root system of the tested shrubs exhibited symptoms of disease. After removing the creeping juniper variety Wiltonii from the pot, it was found that the roots were well-developed, with many lateral branches, but some of them had turned brown. The roots of the rose variety Star Profusion were poorly branched, with clearly visible dark brown sections. The root system of the yew cultivar Hillii was brown, poorly developed, with



**Table 3.** Microorganisms colonizing diseased needles/leaves of the shrubs studied in 2011-2012

Microorganism	<i>Juniperus horizontalis</i> Wiltonii			Ground cover rose Star Profusion			<i>Taxus x media</i> Hillii			<i>Vaccinium corymbosum</i> Patriot		
	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years
	2010	2011		2010	2011		2010	2011		2010	2011	
<i>Alternaria alternata</i> (Fr.) Keissl.	84	72	72.25	13	11	23.08	25	23	42.11	19	21	25.32
<i>Alternaria botrytis</i> (Preuss) Woudenb. & Crous (syn. <i>Ulocladium botrytis</i> Preuss)	-	-	-	-	-	-	6	4	8.77	-	-	-
<i>Boeremia exigua</i> (Desm.) Aveskamp. Gruyter & Verkley (syn. <i>Phoma exigua</i> Desm. var. <i>exigua</i> )	3	1	1.85	-	-	-	-	-	-	-	6	3.80
<i>Botrytis cinerea</i> Pers.	8	4	5.56	12	6	17.31	8	2	8.77	4	2	3.80
<i>Chaetomium globosum</i> Kunze	2	2	1.85	-	-	-	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	2	4	2.78	9	7	15.38	2	2	3.51	15	11	16.46
<i>Cladosporium herbarum</i> (Pers.) Link	-	-	-	1	3	3.85	-	-	-	-	-	-
<i>Colletotrichum gloeosporioides</i> (Penz.) Penz. & Sacc.	-	-	-	-	-	-	-	-	-	14	10	15.19
<i>Epicoccum nigrum</i> Link (syn. <i>Epicoccum purpurascens</i> Ehrenb.)	-	-	-	-	-	-	2	2	3.51	-	-	-
<i>Fusarium culmorum</i> (Wm. G. Sm.) Sacc.	7	5	5.56	-	-	-	7	9	14.04	5	7	7.59
<i>Fusarium oxysporum</i> Schltdl.	-	-	-	2	4	5.77	5	7	10.53	-	-	-
<i>Fusarium poae</i> (Peck) Wollenw.	-	-	-	5	7	11.54	-	-	-	-	-	-
<i>Gibberella pulicaris</i> (Kunze) Sacc. (syn. <i>Fusarium sambucinum</i> Fuckel)	-	-	-	-	-	-	-	-	-	9	7	10.13
<i>Gibberella zeae</i> (Schwein.) Petch (syn. <i>Fusarium graminearum</i> Schwabe)	5	3	3.70	-	-	-	-	-	-	-	-	-
<i>Mortierella horticola</i> Linnem.	-	-	-	-	-	-	-	-	-	8	10	11.39
<i>Mortierella isabellina</i> (Oudem.) W. Gams.	2	2	1.85	-	-	-	-	-	-	-	-	-
<i>Paraphaeosphaeria minitans</i> (W.A. Campb.) Verkley. Göker & Stielow (syn. <i>Coniothyrium minitans</i> W.A. Campb.)	-	-	-	-	4	3.85	-	-	-	-	-	-
<i>Penicillium</i> spp.	4	6	4.63	4	2	5.77	3	1	3.51	1	3	2.53
<i>Septoria albopunctata</i> Cooke	-	-	-	-	-	-	-	-	-	2	4	3.80
<i>Torula herbarum</i> (Pers.) Link.	-	-	-	4	6	9.62	-	2	1.75	-	-	-
<i>Trichoderma viride</i> Pers.	-	-	-	2	2	3.85	-	-	-	-	-	-
<i>Umbelopsis isabellina</i> (Oudem.) W. Gams (syn. <i>Mortierella isabellina</i> Oudem.)	-	-	-	-	-	-	1	3	3.51	-	-	-
Total	117	99	100.00	52	52	100.00	59	55	100.00	77	81	100.00
Bacteria	2	-	-	2	-	-	-	4	-	6	4	-

**Table 4.** Microorganisms colonizing the diseased base of shoots in 2011-2012

Microorganism	<i>Juniperus horizontalis</i> Wiltonii			Ground cover rose Star Profusion			<i>Taxus x media</i> Hillii			<i>Vaccinium corymbosum</i> Patriot		
	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years	Number of colonies		Percentage in both years
	2010	2011		2010	2011		2010	2011		2010	2011	
<i>Alternaria alternata</i> (Fr.) Keissl.	27	25	35.14	41	33	56.92	41	37	42.86	-	-	-
<i>Alternaria botrytis</i> (Preuss) Woudenb. & Crous (syn. <i>Ulocladium botrytis</i> Preuss)	5	3	5.41	-	-	-	-	-	-	-	-	-
<i>Botrytis cinerea</i> Pers.	14	6	13.51	-	-	-	14	4	9.89	-	-	-
<i>Chaetomium globosum</i> Kunze	-	-	-	-	-	-	7	11	9.89	-	-	-
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	2	2	2.70	-	-	-	-	-	-	-	-	-
<i>Cylindrocarpum magnusianum</i> Wollenw.	2	4	4.05	-	-	-	-	-	-	9	7	11.11
<i>Fusarium culmorum</i> (Wm. G. Sm.) Sacc.	-	-	-	-	-	-	3	5	4.40	6	8	9.72
<i>Fusarium oxysporum</i> Schltdl.	6	4	6.76	-	-	-	-	-	-	8	10	12.50
<i>Fusarium solani</i> (Mart.) Sacc.	-	-	-	-	4	3.08	-	-	-	-	-	-
<i>Gibberella avenacea</i> R.J. Cook (syn. <i>Fusarium avenaceum</i> (Fr.) Sacc.)	7	9	10.81	12	8	15.38	5	5	5.49	-	-	-
<i>Mortierella alpina</i> Peyronel	-	-	-	-	-	-	-	4	2.20	11	5	11.11
<i>Penicillium</i> spp.	2	4	4.05	2	4	4.62	2	4	3.30	3	3	4.17
<i>Pestalotiopsis sydowniana</i> (Bres.) B. Sutton (syn. <i>Pestalotia sydowniana</i> Bres.)	-	-	-	-	-	-	11	13	13.19	-	-	-
<i>Rhizopus arrhizus</i> A. Fisch. (syn. <i>Rhizopus oryzae</i> Went & Prins. Geerl.)	-	-	-	3	3	4.62	-	-	-	-	-	-
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	4	2	4.05	1	3	3.08	-	-	-	12	16	19.44
<i>Septoria albopunctata</i> Cooke	-	-	-	-	-	-	-	-	-	6	-	4.17
<i>Trichoderma harzianum</i> Rifai	5	5	6.76	-	-	-	-	-	-	4	8	8.33
<i>Trichoderma koningii</i> Oudem.	4	6	6.76	-	-	-	-	-	-	7	9	11.11
<i>Trichoderma polysporum</i> (Link) Rifai	-	-	-	3	5	6.15	-	-	-	-	-	-
<i>Trichoderma virens</i> (J.H. Mill. Giddens & A.A. Foster) Arx (syn. <i>Glilocladium virens</i> J.H. Mill. Giddens & A.A. Foster)	-	-	-	-	-	-	2	2	2.20	-	-	-
<i>Trichoderma viride</i> Pers.	-	-	-	-	-	-	5	7	6.59	-	-	-
<i>Trichothecium roseum</i> (Pers.) Link	-	-	-	-	-	-	-	-	-	2	4	4.17
<i>Verticillium albo-atrum</i> Reinke & Berthold	-	-	-	5	3	6.15	-	-	-	-	-	-
Total	78	70	100.00	67	63	100.00	90	92	100.00	68	76	100.00
Bacteria	4	2	-	2	-	-	3	1	-	6	2	-

Table 5. Microorganisms colonizing diseased roots in 2011-2012

Microorganism	<i>Juniperus horizontalis</i>			Ground cover rose			<i>Taxus x media</i>			<i>Vaccinium corymbosum</i>		
	Wiltonii			Star Profusion			Hillii			Patriot		
	Number of colonies 2010	2011	Percentage in both years	Number of colonies 2010	2011	Percentage in both years	Number of colonies 2010	2011	Percentage in both years	Number of colonies 2010	2011	Percentage in both years
<i>Alternaria alternata</i> (Fr.) Keissl.	29	25	28.72	42	34	55.07	32	30	40.79	29	23	31.33
<i>Aspergillus brasiliensis</i> Varga. Frisvad & Samson (syn. <i>Aspergillus niger</i> Tiegh.)	-	4	2.13	-	-	-	-	-	-	-	-	-
<i>Botrytis cinerea</i> Pers.	14	6	10.64	-	-	-	12	4	10.53	-	-	-
<i>Chaetomium globosum</i> Kunze	-	-	-	2	4	4.35	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	4	6	5.32	-	-	-	-	-	-	3	5	4.82
<i>Epicoccum nigrum</i> Link (syn. <i>Epicoccum purpurascens</i> Ehrenb.)	-	-	-	-	-	-	-	-	-	5	7	7.23
<i>Fusarium equiseti</i> (Corda) Sacc.	8	6	7.45	-	-	-	-	-	-	-	-	-
<i>Fusarium oxysporum</i> Schldt.	12	6	9.57	-	-	-	2	8	6.58	9	7	9.64
<i>Gibberella avenacea</i> R.J. Cook (syn. <i>Fusarium avenaceum</i> (Fr.) Sacc.)	5	7	6.38	4	-	2.90	3	3	3.95	-	-	-
<i>Gibberella intricans</i> Wollenw. (syn. <i>Fusarium equiseti</i> (Corda) Sacc.)	-	-	-	-	-	-	-	2	1.32	-	4	2.41
<i>Ilyonectria destructans</i> (Zinssm.) Rossmann. L. Lombard & Crous (syn. <i>Cylindrocarpum destructans</i> (Zinssm.) Scholten)	-	-	-	-	-	-	-	-	-	9	7	9.64
<i>Paraphaeosphaeria minitans</i> (W.A. Campb.) Verkley. Göker & Stielow (syn. <i>Coniothyrium minitans</i> W.A. Campb.)	-	-	-	-	-	-	-	-	-	5	1	3.61
<i>Penicillium</i> spp.	5	3	4.26	4	2	4.35	3	1	2.63	2	-	1.20
<i>Purpureocillium lilacinum</i> (Thom) Luangsa-ard. Houbraken.	-	-	-	-	-	-	-	-	-	-	-	-
Hywel-Jones & Samson (syn. <i>Paecilomyces lilacinus</i> (Thom) Samson)	4	-	2.13	-	-	-	-	-	-	-	-	-
<i>Rhizoctonia solani</i> J.G. Kühn	9	7	8.51	-	-	-	14	10	15.79	-	-	-
<i>Rhizopus arrhizus</i> A. Fisch. (syn. <i>Rhizopus oryzae</i> Went & Prins. Geertl.)	-	-	-	3	5	5.80	-	-	-	-	-	-
<i>Sarocladium strictum</i> (W. Gams) Summerb. (syn. <i>Acremonium strictum</i> W. Gams)	-	-	-	-	-	-	-	-	-	-	4	2.41
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	-	-	-	12	10	15.94	4	-	2.63	-	-	-
<i>Torula herbarum</i> (Pers.) Link.	3	3	3.19	-	-	-	-	-	-	-	-	-
<i>Trichoderma harzianum</i> Rifai	-	-	-	-	-	-	5	7	7.89	2	6	4.82
<i>Trichoderma koningii</i> Oudem.	6	10	8.51	-	-	-	-	-	-	8	12	12.05
<i>Trichoderma polysporum</i> (Link) Rifai	2	4	3.19	3	7	7.25	4	8	7.89	7	11	10.84
Total	101	87	100.00	73	65	100.00	79	73	100.00	79	87	100.00
Bacteria	3	1	-	4	2	-	2	2	-	2	2	-

short intertwined roots without new growth. After removing the northern highbush blueberry 'Patriot' from the container, the roots were found to be well-developed, with numerous branches, and small dark brown sections.

As a result of the mycological analysis of the root system of the test plants collected in 2011-2012, a total of 644 fungal isolates and 18 bacterial isolates were obtained. Among all the isolates, *Alternaria alternata* was by far the most dominant fungus. It inhabited in the largest numbers the roots of rose – 55.07%, in a smaller percentage the roots of yew – 40.79, the roots of blueberry – 31.33%, and juniper – 28.72% (Tab. 5). A large share of the total number of isolates was taken by fungi of the genus *Fusarium*. 23.40% of these fungi were isolated from the diseased roots of juniper, a lower percentage from the roots of blueberry – 12.05% and yew – 11.85% , and the lowest from the roots of rose – 2.90% (Tab. 5). Next in the order of fungi colonizing the diseased root system of the test shrubs was the genus *Trichoderma*: 27.71% in blueberry, 15.78% in yew, 11.70% in juniper, up to 11.60% in rose (Tab. 5). Among the dominant fungi colonizing the diseased root system of both juniper and yew shrubs was the fungus *Botrytis cinerea* – 10.64% and 10.53%, respectively (Tab. 5). Next in the order of dominant fungi isolated from the root system of the above-mentioned shrubs was *Rhizoctonia solani* – 15.79% in yew, 8.51% in juniper (Tab. 5).

On the basis of the results obtained from the isolation of fungi colonizing the aboveground parts, the base of the shoots, and the roots of the test shrubs, it was found that the most numerous of all the isolates obtained both from the aerial parts and the roots, as well as the base of the shoots of the test shrubs, was *A. alternata*. Such a high number of isolates of *A. alternata* among the fungi colonizing the tested parts of plants is a major threat to their health. This is consistent with the information in the literature (Abeer et al., 2014), indicating the prevalence of fungi of the genus *Alternaria*. Goetz and Dugan (2006), pointing out the presence of *Alternaria* spp. in the rhizosphere of coniferous plants, draw attention to their pathogenicity for such plants. Many authors (Kurzawińska and Duda-Surman, 2008; Ghosh et al., 2009; Nasim et al., 2012; Pegg et al., 2014; Kurzawińska et al., 2015) have indicated that *Alternaria* spp. can infect the leaves, stems, flowers, fruits, and the roots of ornamental plants.

**Table 6.** Mean necrotic lesion dimensions in the pathogenicity test

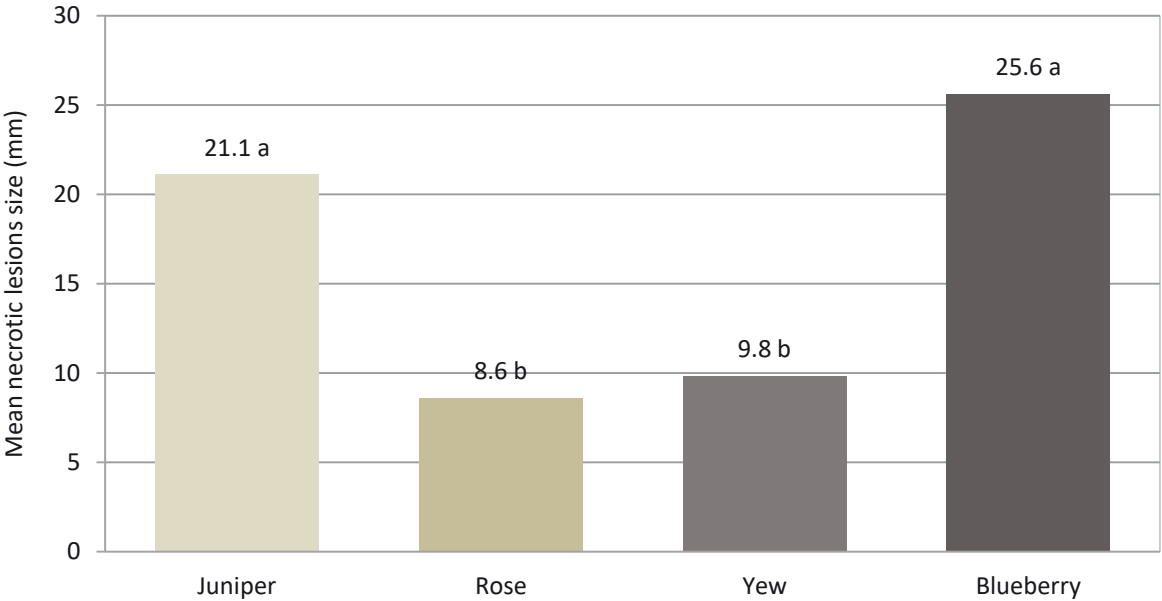
Plant species	Combination	Mean lesion dimensions (mm)	
		length	width
Juniper	<i>Alternaria alternata</i>	6.1	3.3
	Control	0	0
Rose	<i>Alternaria alternata</i>	2.7	2.7
	Control	0	0
Yew	<i>Alternaria alternata</i>	3.8	2.3
	Control	0	0
Blueberry	<i>Alternaria alternata</i>	6.9	3.2
	Control	0	0

Analysis of the results of the pathogenicity test showed that the isolates of *Alternaria alternata* had caused disease symptoms on the shoots of the test plants of the same species from which they had been isolated. The necrotic lesions varied in terms of size (Tab. 6). The smallest were those on the stems of rose, and the largest on the shoots of juniper and blueberry. The tissues around the site of penetration of the pathogen became dark-coloured and died. The isolates of *A. alternata* were characterized by statistically significant pathogenicity for the shoots of the shrubs under study (Fig. 1). Significantly the highest pathogenicity of *A. alternata* was found on the shoots of highbush blueberry and juniper, and the lowest, but also statistically significant, on rose and yew shoots (Fig. 1).

The necrotic lesions on the shoots of juniper were brown, with a distinct small depression formed at the prick site, filled with the mycelium. They had an elongated shape. The necrotic spots on rose stems were grey-brown, and had a clearly round regular shape. Within the spot, there were visible signs of the dead skin tissue peeling off and a pronounced depression of the prick site. The mycelium of *A. alternata* was visible on the surface of the necrotic lesions, but not very abundant. On yew shoots, the colour of the necrotic spots was dark brown to black. Their edges were broad and indistinct, changing at the rim to a light brown colour. The shape of the spots was elongated. The necrotic lesions of the shoots of highbush blueberry were brown, almost black, elongated, with a clear edge, without a rim. The tissues of the skin within the lesions were cracked. In the control combination of all the species of shrubs there were no visible changes around the prick site on the shoots.

The re-isolation of fungi from the inoculated shoots of the test shrubs carried out after the



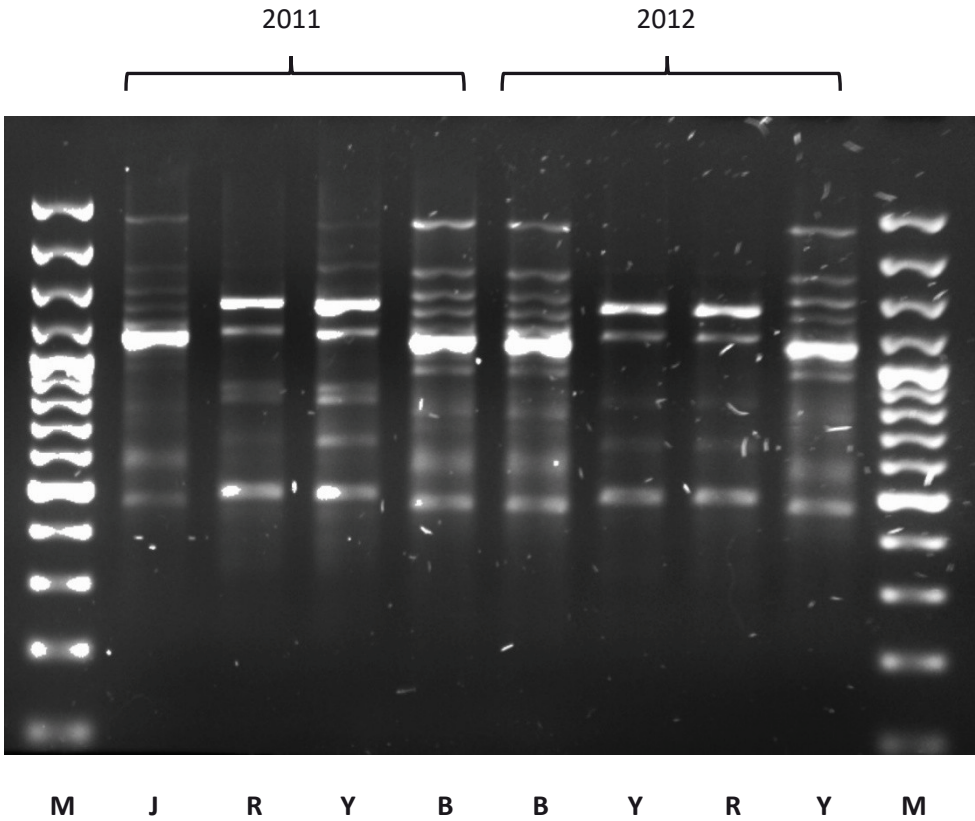


Means followed by the same letter do not differ significantly at  $p = 0.05$  according to Tukey's multiple range test

**Figure 1.** Size of necrotic lesions on the tested plant shoots

completion of the experiment confirmed the presence of the tested isolates of *A. alternata*. The analysis performed on genomic DNA of the *A. alternata* isolates obtained from the tested species of shrubs showed a similarity between the isolates obtained from juniper and blueberry, and

a similarity between the isolates obtained from rose and yew. Comparable results were obtained in both 2011 and 2012. Comparison of the electrophoretic images of RAPD-PCR amplification products of the isolates obtained from juniper and blueberry with the images of the isolates obtained from rose



**Figure 2.** RAPD-PCR amplification products with OPAD12 primer using DNA of *Alternaria alternata* isolates obtained from the tested shrubs in the years 2011 and 2012 for: M – marker, J – juniper, R – rose Y – yew, B – blueberry

and yew provided evidence of genetic variation among *A. alternata* fungi colonizing these shrubs (Fig. 2). Many authors (Tran-Dinh and Hocking, 2006; Sharma et al., 2013; Aneja et al., 2014) have found genetic variation among isolates of *A. alternata* based on analysis of RAPD bands. Sharma et al. (2013), by analyzing genomic DNA, have also demonstrated genetic variation among isolates of *A. alternata* obtained from different host plants. PUSZ (2009), on the basis of his results, has indicated that similar pathogenicity is associated with genetic similarity within *A. alternata* populations originating from different host plants and geographic locations. He believes that this finding suggests the ability of *A. alternata* to easily adapt to closely related host plants.

## CONCLUSIONS

The results of the study are the basis for formulating the following conclusions:

1. The most common disease symptoms in juniper, rose, yew and highbush blueberry were discoloration and withering of the leaves/needles and the shoots of the plants.
2. The diseased leaves/needles, the base of the shoots, and the roots of the tested shrubs were dominated by *Alternaria alternata*.
3. The *A. alternata* isolates were pathogenic for the shoots of all the tested species of shrubs.
4. *A. alternata* can, under favourable conditions, become a facultative pathogen of juniper, rose, yew and highbush blueberry.

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## AUTHOR CONTRIBUTIONS

M.N. and H.K. – designed and performed experiments, analysed data and wrote the paper; M.S. and D.T. – designed and performed experiments, gave suggestions and corrected the manuscript.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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