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MICROORGANISMS COLONIZING THE LEAVES, SHOOTS AND ROOTS OF BOXWOOD (*Buxus sempervirens* L.)

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ABSTRACT

Buxus sempervirens (L.) is an evergreen shrub often used in urban plantings intended for forming the trimmed hedges, as well as for creating geometrical shapes. Its decorative qualities are often diminished by the effects of pathogens colonizing the roots, shoots and leaves. The study was conducted in three consecutive growing seasons. The experimental material originated from a nursery located in southern Poland. The subjects under study were diseased leaves, stems and roots of the boxwood variety 'Suffruticosa'. Samples consisting of 720 diseased fragments of the shrub were taken for mycological examination. Mycological isolations were performed according to the standard methods used in phytopathology. A total of 1059 colonies of fungi and fungus-like organisms were isolated from the diseased boxwood plants. The fungus *Alternaria alternata* dominated among all the isolates. The pathogens frequently isolated from the leaves included *Macrophoma candollei*, *Volutella buxi* and *Fusarium buxicola*. Those frequently isolated from the shoots included: *Pestalotiopsis sydowiana*, *Volutella buxi* and *Fusarium avenaceum*. The root system, in turn, was colonized in large numbers by: *Fusarium oxysporum*, *F. avenaceum*, *Rhizoctonia solani*, *Botrytis cinerea* and *Phytophthora cinnamomi*. Results of the three-year study show a comprehensive participation of phytopathogens in the disease process affecting the boxwood variety 'Suffruticosa'.

Key words: boxwood, isolations, microorganisms

INTRODUCTION

Boxwood (*Buxus sempervirens* L.) decorates every garden, terrace, balcony, and lane. The shrub tolerates pruning well and is mainly used for forming trimmed hedges, flowerbed borders, and planting along alleyways. It is ideal for forming all kinds of geometric shapes, most commonly a cone, a sphere, or a cylinder. Among the many varieties, 'Suffruticosa' is one of the more popular ones. The shrub of this variety is characterized by dense growth. The leaves are small, leathery, ovate in shape, 1–2 cm long.

A serious threat to the decorative organs of shrubs, including boxwood, are fungal pathogens. They are the reason for the degradation of the decorative qualities of boxwood. The literature shows that the pathogens include: *Volutella buxi*, *Macrophoma candollei*, *Mycosphaerella patouillardii*, *Fusarium buxicola*, *Cylindrocladium buxicola* and *Phytophthora* spp. [Saracchi et al. 2008, Stompor-Chrzan and Gargała 2011, Łabanowski at al. 2011, Kurzawinska et al. 2012].

During the growing seasons in 2014–2016, numerous 3-year-old 'Suffruticosa' boxwood shrubs were observed to exhibit clear symptoms of disease on the leaves, shoots and root system. Light-brown round lesions with a dark-brown border could be seen on the diseased leaves. The leaf blades wilted and shrivelled up. Brown, necrotic, rapidly growing lesions could

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also be seen at different heights on the shoots. On some bushes, the necrosis spread towards the base of the shoot.

Due to the worsening health condition of boxwood shrubs in nurseries and high demand for knowledge in this regard, research was undertaken to determine the species composition of the microorganisms colonizing the diseased leaves, shoots and root system of 'Suffruticosa' boxwood shrubs.

MATERIAL AND METHODS

The material for the study came from a nursery located in southern Poland. In the years 2014–2016 (twice in each growing season), diseased leaves, shoots and roots of 'Suffruticosa' boxwood shrubs were sampled by taking 3 mm fragments at the border between the necrotic lesions and healthy tissue. In total, 720 fragments of this plant were collected for mycological studies. Mycological isolation was carried out in accordance with the standard methods used in phytopathology [Kurzawińska et al. 2012]. Selected representative cultures were identified using the available mycological keys and monographs: Booth [1971], Domsch et al. [1980], Ellis and Ellis [1985], Nelson et al. [1983], Rifai [1987] and Kwaśna et al. [1991].

RESULTS AND DISCUSSION

A total of 1059 colonies of fungi and fungus-like organisms were isolated from diseased 'Suffruticosa' boxwood plants. Of all the isolates, the fungus *Alternaria alternata* was decidedly the dominant pathogen. It colonized to the largest extent the diseased root system – 25.3%, and the base of boxwood shoots – 22.5%, with a slightly smaller percentage found on the leaves of the shrub – 14.4% (Tab. 1).

Fungi of the genus *Alternaria* are common, and the pathogenic species cause diseases of ornamental plants, fruit trees and shrubs [Chase 2005, Kakalikova et al. 2009]. As reported by Horst [2008], *Alternaria alternata* causes Alternaria leaf blight or brown spot on the leaves of many ornamental shrubs (including the species under study). According to Wagner and Jamiołkowska [2004], *Alternaria alternata* has long been a serious problem, especially for weakened plants. Earlier results of the co-author's research [Kurzawińska et al. 2012] indicate high pathogenicity of *A. alternata* for boxwood shoots.

In addition, the following pathogens were isolated from the diseased boxwood leaves: *Hyponectria buxi* (syn. *Macrophoma candollei*) (6.6%), *Pseudonectria buxi* (syn. *Volutella buxi*) (5.8%), and *Cyanonectria buxi* (syn. *Fusarium buxicola*) (5.1%). Kopacki [2014], on the basis of his research results, draws attention to the extensive colonization of boxwood leaves by *Macrophoma candollei*. The infected leaves become deformed and eventually wither away. The fungus also causes necrotic lesions to appear at different heights along the shoots. After extending around the entire circumference of the shoot, the lesions cause the part of the shoot above the infection site to die [Hüseyin and Selcuk 2014].

Stompor-Chrzan and Gargała [2011] found a high percentage of fungi of the genus *Fusarium* isolated from boxwood leaves. In particular, the authors recorded very high frequency of *F. buxicola*, regarding it as the main causal agent of the disease symptoms occurring on the leaves of the boxwood variety 'Elegans'. Moreover, according to Werner and Kwaśna [1998], the leaves of decorative shrubs and trees can be infected by *Alternaria alternata*. Saracchi et al. [2008] reported on the emergence in Italy of a new disease of the leaves of the boxwood variety 'Suffruticosa' caused by *Cylindrocladium buxicola*. The presence of this pathogen on boxwood leaves had been detected in England and New Zealand in the 1990s. [Henricot and Culham 2002].

As a result of the mycological analysis, it was shown that the shoots were colonized by a complex of different species of microorganisms. The most frequently isolated (except *A. alternata*) were: *Pestalotiopsis sydowiana* (syn. *Pestalotia sydowiana*) (14.2%), *Pseudonectria buxi* (syn. *Volutella buxi*) (8.5%) and *Fusarium avenaceum* (6.0%) – Table 1. According to Werner and Kwaśna [1998], fungi of the genus *Pestalotiopsis* can infect shoots, especially young or weakened ones, and cause them to wither. The authors also report that *Fusarium avenaceum* can infect not only the leaves but also the shoots of many shrubs. Due to the long-term nature of boxwood cultivation, those species of fungi that cause diseases of shoots and leaves should be considered particularly danger-

Table 1. Microorganisms isolated from leaves, shoots and roots of boxwood

Microorganism	Leaves					Shoots					Roots				
	2014	2015	2016	total	share (%)	2014	2015	2016	total	share (%)	2014	2015	2016	total	share (%)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Alternaria alternata* (Fr.) Keissl.	17	19	21	57	14.4	29	24	26	79	22.5	27	24	28	79	25.3
2. *Ascochyta medicaginicola* Qian Chen & L. Cai	2	2		4	1										
3. *Botrytis cinerea* Pers.	6	6	5	17	4.3	4	3	3	10	2.8	6	8	6	20	6.4
4. *Calonectria* sp. De Not.	3		2	5	1.3	5	2	2	9	2.6					
5. *Cladosporium cladosporioides* (Fresen.) G.A. de Vries	4	5	3	12	3	4	2	2	8	2.3	2	4	3	9	3
6. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.						3	4	2	9	2.6	4	3	4	11	3.5
7. *Cyanonectria buxi* (Fuckel) Schroers, Gräfenhan & Seifert	6	8	6	20	5.1										
8. *Epicoccum nigrum* Link	3	6	8	17	4.3	4	2	4	10	2.8			2	2	0.6
9. *Fusarium avenaceum* (Fr.) Sacc.	2	3		5	1.3	9	4	8	21	6	10	10	9	29	9.3
10. *Fusarium culmorum* (Wm. G. Sm.) Sacc.	2	6	5	13	3.3						2		2	4	1.3
11. *Fusarium lateritium* Nees	1			1	0.3	2			2	0.6	2	2		2	0.6
12. *Fusarium oxysporum* Schltdl.						3	6	8	17	4.8	14	19	16	49	15.7
13. *Humicola fuscoatra* Traaen	7	3		10	2.5										
14. *Hyponectria buxi* (Alb. & Schwein) Sacc.	8	9	9	26	6.6	4	7	6	17	4.8	5	2	2	9	3
15. *Juxtiphoma eupyrena* (Sacc.) Valenz. – Lopez, Crous, Stchigel, Guarro & Cano	3	2	2	7	1.8	4		2	6	1.7					
16. *Mortierella alpina* Peyronel	6	4	5	15	3.8										
17. *Mucor hiemalis* Wehmer	3	2	1	6	1.5										

Table 1 cont.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
18. *Paraconiothyrium fuckelii* (Sacc.) Verkley & Guyter	4	2	2	8	2										
19. *Penicillium aurantiogriseum* Dierckx	7	5	4	16	4.1			4	4	1.1			2	2	0.6
20. *Penicillium brevicompactum* Dierckx	4	4	5	13	3.3		4		4	1.1		4		4	1.3
21. *Penicillium expansum* Link	6	4	2	12	3		8	4	12	3.4		2	2	4	1.3
22. *Pestalotiopsis sydowiana* (Bres.) B. Sutton	8	6	4	18	4.6	12	22	16	50	14.2					
23. *Phialophora cyclaminis* J.F.H. Beyma	4	3	3	10	2.5										
24. *Phoma leveillei* Boerema & G.J. Bollen	3	4	5	12	3	2	4		6	1.7					
25. *Phytophthora cactorum* (Lebert & Cohn) J. Schröt.						6	2	4	12	3.4	4	2	3	9	3
26. *Phytophthora cinnamomi* Rands						4	6	5	15	4.3	7	8	5	20	6.4
27. *Pseudonectria buxi* (DC.) Seifert, Gräfenhan & Schroers	9	8	6	23	5.8	10	11	9	30	8.5					
28. *Rhizoctonia solani* J.G. Kühn	2	1		3	0.8	7	2	5	14	4	8	7	8	23	7.4
29. *Sclerotinia sclerotiorum* (Lib.) de Bary						2			2	0.6	6	2	7	15	4.8
30. *Sordaria fimicola* (Roberge ex Desm.) Ces. & de Not.	6	7	5	18	4.6										
31. *Trichocladium griseum* (Traaen) X. Wei Wang & Houbraken	2	4		6	1.5						3		2	5	1.6
32. *Trichoderma koningii* Oudem.	4	2	2	8	2		4		4	1.1		2	2	4	1.3
33. *Trichoderma piluliferum* J. Webster & Rifai	3	2		5	1.3										
34. *Trichoderma viride* Pers.	4	2		6	1.5	2	2	1	5	1.4	2	3		5	1.6
35. *Truncatella truncata* (Lév.) Steyaert	6	4	4	14	3.5										
Other species	2	3	3	8	2	2	2	2	6	1.7	3	2	2	7	2.3
Total	147	136	112	395	100	118	121	113	352	100	105	102	105	312	100

Table 2. Thermal conditions and rainfall during the investigation period

Experimental year	Month	Average air temperature (°C)				Total rainfall (mm)			
		for 10-day period			for the month	for 10-day period			for the month
		I	II	III		I	II	III	
2014	May	12.9	13.1	13.6	13.2	0.2	58.4	65.3	123.9
	June	13.7	15.3	17.9	15.6	31	62.6	121.6	215.2
	July	19.4	19.2	19.4	19.3	61.5	45.3	40.3	147.1
	August	19.3	18.4	17.3	18.3	1.3	12.3	56.6	59.8
2015	May	12.7	10.9	14.5	12.7	79.4	188	35	302.4
	June	18.6	17.9	16.9	17.8	1.6	28	22	51.6
	July	19.8	23.6	21.3	21.6	8.6	31.6	72.2	112.4
	August	19.9	19.6	18.6	19.4	27.8	38	72.4	138.2
2016	May	9.6	15.4	17.2	14.1	16	12.2	26.4	54.6
	June	19.4	18.4	17.9	18.6	26	29.6	11.4	67
	July	17.3	20.1	16.4	17.9	45	62.2	55.8	163
	August	18.7	19.2	20.3	19.4	23	5.8	8.4	37.2

ous. The frequently listed pathogenic species include *Volutella buxi* and *Macrophoma candollei* [Farr et al. 1989, Kopacki 2014].

Apart from the most frequently isolated colonies of *A. alternata*, the diseased root system was found to be also colonized by: *Fusarium oxysporum* (15.7%), *Fusarium avenaceum* (9.3%), *Rhizoctonia solani* (7.4%), *Botrytis cinerea* and *Phytophthora cinnamomi* (6.4% each) – Table 1. As reported by Lesisz [2005], the well-known pathogens, such as *Botrytis cinerea*, *Fusarium oxysporum* or *Fusarium avenaceum*, can, under favourable conditions (without sufficient protection in the nursery, and later in a permanent place in an urban area, without the use of appropriate fungicides) cause significant losses in plantings. Earlier results of the co-author's research [Kurzawińska et al. 2012] indicate a growing threat to boxwood plantings by both *Phytophthora cinnamomi* and *Rhizoctonia solani*.

The weather conditions during the boxwood growing season were also analyzed. It is worth noting that the year 2015 was characterized by relatively high temperatures and high rainfall, which are conditions conducive to the development of pathogenic fungi (Tab. 2). During the wet weather, prevailing especially in the middle of May of that year, dead parts of shoots were largely colonized by *Alternaria alternata*, *Pestalotiopsis sydowiana* and fungi of the genus *Fusarium*. According to Kopacki [2014], the increase in the occurrence of disease symptoms on the aerial organs of boxwood plants was observed after prolonged periods of rainfall and high temperatures. When training boxwood shrubs, it is very important to thoroughly cut off, remove and burn the frost-damaged and yellowing shoots after winter. The action of low temperatures and winds drying out plant tissues at the end of winter, and then a rapid increase in air temperature can also be the cause of injuries. Later, there is a risk associated with pathogens developing on dying shoots and the risk of secondary colonization of healthy tissues.

CONCLUSIONS

1. The results of the three-year study indicate a comprehensive participation of phytopathogens

in the disease process affecting the boxwood variety 'Suffruticosa'.

2. Diseased leaves, shoots and roots of 'Suffruticosa' boxwood shrubs were most frequently colonized by *Alternaria alternata*.

3. Other pathogens frequently isolated from the diseased organs of boxwood included: *Fusarium oxysporum*, *F. avenaceum*, *Pseudonectria buxi*, *Pestalotiopsis sydowiana*, *Rhizoctonia solani*, *Hyponectria buxi*, *Botrytis cinerea*, *Phytophthora cinnamomi* and *Cyanonectria buxi*.

4. *A. alternata* can, under favourable conditions, become a facultative pathogen of boxwood.

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REFERENCES

Booth, C. (1971). The genus *Fusarium.* Commonwealth Mycological Institute Kew, Surrey.

Chase, A.R. (2005). Advanced treatment of *Alternaria*. Pests Dis., 6, 38–44.

Domsch, K.H., Gams, W., Anderson, T.H. (1980). Compendium of soil fungi. Acad. Press. London, New York, Toronto, Sydney, San Francisco.

Ellis, M.B, Ellis, J.P. (1985). Microfungi on land plants. An identfication handbook. Croom Helm, London–Sydney.

Farr, D.C., Bills, G.F, Chamuris, G.P, Rossman, A.Y. (1989). Fungi on plants and plant products in the United States. APS Press, St. Paul, 117–118.

Henricot, B., Culham, A. (2002). *Cylindrocladium buxicola*, a new species affecting *Buxus* spp., and phylogenetic status. Mycologia, 94(6), 980–997, https://doi.org/10.1080/15572536.2003.11833155

Horst, R.K. (2008). Westcott's plant disease Handbook. Springer, New York.

Hüseyin, E., Selcuk, F. (2014). Coleomycetous fungi in several forest ecosystems of Black Sea provinces of Turkey. Agric. Forest., 60(2), 19–32. DOI: 10.17707/AgricultForest

Kakaliková, L., Janura, E., Šrobárová A. (2009). First report of *Alternaria* bunch rot of grapevines in Slovakia. Australas. Plant Dis. Notes, 4(1), 68–69, https://doi.org/10.1071/DN09029

Kopacki, M. (2014). Grzyby zasiedlające bukszpan w nasadzeniach miejskich. Ann. UMCS sec. EEE Horticultura, 24(4), 25–30.

Kurzawińska, H., Nadziakiewicz, M., Muras, P. (2012). Mikozy – narastające zagrożenia dla bukszpanu oraz przydatność niektórych naturalnych substancji w ich zwalczaniu. Progr. Plant Prot./ Post. Ochr. Rośl., 52(3), 634–637, http://dx.doi.org/10.14199/ppp-2012-110

Kwaśna, H., Chełkowski, J., Zajkowski, P. (1991). Grzyby (Mycota), tom 22. Sierpik (*Fusarium*). PAN, Warszawa–Kraków.

Lesisz, J. (2005). Ważniejsze choroby grzybowe występujące na drzewach i krzewach w Ogrodzie Botanicznym w Łodzi. Biul. Ogr. Bot., 14, 93–100.

Łabanowski, G., Soika, G., Orlikowski, L., Wojdyła, A. (2011). Pielęgnacja roślin ogrodowych, choroby i szkodniki. Multico Oficyna Wydawnicza, Warszawa.

Nelson, P.E., Toussoun, Y.A, Marasas, W.F.O. (1983). Fusarium species. In: An illustrated manunal for identification. Pensylwania State University Press, University Park.

Rifai, M.A. (1969). A revision of the genus *Trichoderma*. Mycol. Pap., 116, 1–56.

Saracchi, M., Roschi, F., Pizzatti, C., Cortesi, P. (2008). Box blight, a new disease of *Buxus* in Italy caused by *Cylindrocladium buxicola*. J. Plant Pathol., 90(3), 581–584. DOI: http://dx.doi.org/10.4454/jpp.v90i3.703

Stompor-Chrzan, E., Gargała M. (2011). Zdrowotność ozdobnych krzewów liściastych w produkcji szkółkarskiej. Zesz. Probl. Post. Nauk Rol., 562, 229–234.

Werner, M., Kwaśna, H. (1998). Choroby pędów i liści różanecznika. Ochr. Rośl., 6, 10–11.

Wagner A., Jamiołkowska A. (2004). First report of *Alternaria alternata* causing stem blight of compass plant (*Silphium laciniatum*) in Poland. Plant Dis., 88, 1045, http://dx.doi.org/10.1094/PDIS.2004.88.9.1045D