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Wood nematode species spectrum in the Mediterranean pine forests of Croatia

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Abstract

Background and purpose: *This work presents the first research on wood nematodes in Croatia and it was done in order to address the question of the impact of dendropathogenic nematodes in pine stands in the coastal region of Croatia. To reveal this impact, the first step is the isolation and proper identification of the species spectrum of the existing wood nematodes. The aim of this study was to identify wood nematode species isolated from different pine tree species (*Pinus halepensis, P. nigra, P. maritima*) throughout the coastal region of Croatia and to identify their status as either pathogens or saprofits, which should build the basis for further research and understanding of the negative influence of this organisms on the decline of Mediterranian pine stands.*

Materials and methods: *The research was carried out in 2013 and 2014 and it was done along the entire coastal region of Croatia. The experiments were carried out at two levels: by sampling wood nematodes and by monitoring pine longhorn beetles. Wood samples of dry or partly dry trees of* P. nigra, P. halepensis *and* P. pinaster *were collected throughout the year in the form of rings, branches, sawdust or wood chips without bark on different heights on the trees. Two techniques were used for the extraction of nematodes: Baermann funnel technique and Cobb`s method. In samples with an increased number of nematodes, some nematodes were separated and preserved in DESS solution for further morphological and molecular analysis. In the spring of 2014, the monitoring of pine longhorn beetle* Monochamus galloprovincialis *was done at 6 locations along the coastal region of Croatia were traps with pheromones were installed. The traps were placed in the treetops at a height of about 7 m in* P. halepensis *stands. The collected pine longhorn beetles were inspected for nematodes in the laboratory.*

Results: *Saprophytic nematodes from the families Rhabditidae, Diplogasteridae and Cephalobidae dominated in the samples. In 9 wood samples pathogenic nematodes which belong to the genus* Bursaphelenchus*:* B. mucronatus, B. sexdentati *and* B. minutes *were detecte. The pine wood nematode* B. xylophilus *was not found in the collected samples. A total of 109 pine longhorn beetles have been caught in traps with pheromones and their analysis showed that neither* B. xylophilus *nor native wood nematodes were present on them.*

Conclusions: *This is the first record of wood nematodes in the coastal region of Croatia. The results of this research confirm the presence of nematodes and their possible influence on the process of pine decline of the Mediterranean pine species in Croatia. The presence of* M. galloprovincialis*, the vector of highly damaging* B. Xylophilus*, has been confirmed during this research.*

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INTRODUCTION

Pine stands in the Mediterranean have an important ecological and sociological role (1-7) and special attention is given to their hydrological, erosive and water protection functions (8). Along with forest fires which destroy pine stands every year (9-10), some biotic factors are also responsible for the physiological weakening of host trees and sometimes a stronger decline of trees (11). Increased shedding of needles and a decline of some Mediterranean pine species, such as aleppo pine (*Pinus halepensis* Mill.), maritime pine (*Pinus pinaster* Ait.) and black pine (*Pinus nigra* Arn.) was reported in 2009 in the coastal region of Croatia (11). There was not only one specific negative factor responsible for this decline, even though the damages were severe. There is very little data about the causes of such complex decline in the references (12). Usually they are tied to one specific biotic factor, such as pine processionary moth – *Thaumetopoea pityocampa* Denis & Schiffermüller (Lepidoptera, Thaumetopoeidae) (13), fungi *Sphaeropsis sapinea* (Fr.) Dyko et Sutton (14) and *Mycosphaerella dearnesii* Barr. (15-16) and bark beetles, especially Mediterranean pine shoot beetle – *Tomicus destruens* Wollaston (Coleoptera, Curculionidae) (17). Along with biotic, abiotic factors, especially climate extremes can affect the pine stands through physiological weakening, whose consequences are evident in the forest in the next few years (18-19). Alien species can also have a negative influence on native pine species and one such example which causes decline of pine stands on large areas is pine wood nematode – *Bursaphelenchus xylophilus* Nickle (Aphelenchida, Parasitaphelenchidae). This species was first discovered on the North American continent and some studies indicate the problem of particularly difficult

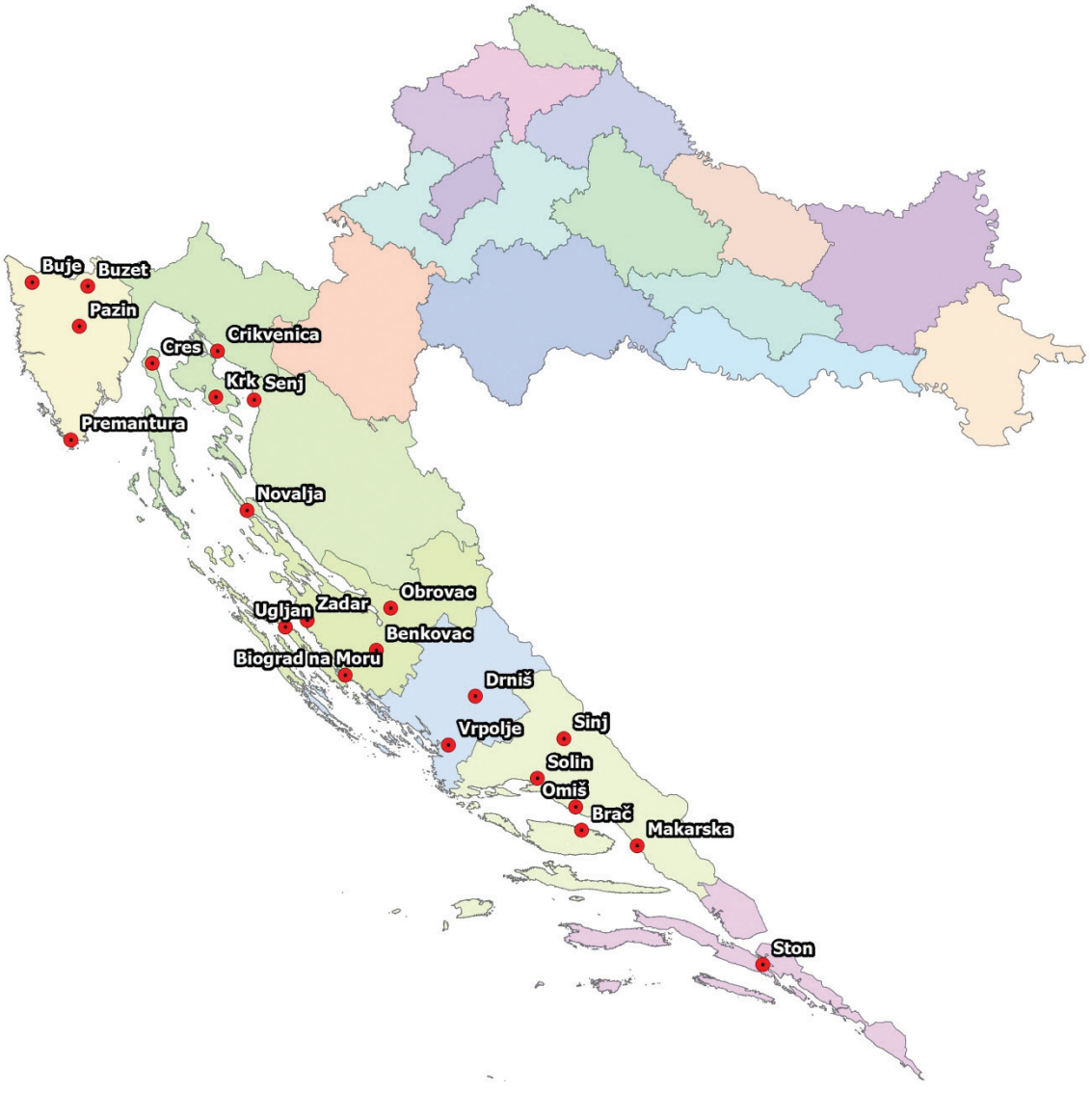

Fig 1 *Geographical distribution of sampling locations in the coastal region of Croatia*

Table 1 *Samples of wood for laboratory analysis of wood nematodes by location, pine species and sample type, collected in 2013*

NUMBER	LOCATION	DATE	SPECIES-sample type	NUMBER	LOCATION	DATE	SPECIES-sample type
1	Senj	May 27	*Pn-S	17	Biograd na moru	May 28	*Pp-R
2				18			
3				19	Vrpolje	May 28	*Ph-S
4	Crikvenica	May 27	*Pn-S	20			
5				21	Benkovac	July 16	*Pp-S
6	Krk	May 27	*Pn-R	22			*Ph-S
7				23			
8				24	Benkovac	July 16	*Pn-S
9	Benkovac	May 28	*Pp-R	25	Obrovac	July 16	*Ph-S
10				26	Zadar	July 17	*Ph-S
11				27			*Pp-S
12				28	Zadar	July 17	*Pp-S
13	Benkovac	May 28	*Pn-R	29	Novalja	July 18	*Ph-S
14				30			
15	Biograd na moru	May 28	*Pp-R	31			
16							

*Pn – *Pinus nigra*; Pp – *Pinus pinaster*; Ph – *Pinus halepensis*; S – sawdust; R – ring

control in the infected area (20) and the importance of early detection of this harmful organism. Since *B. xylophilus* successfully uses longhorn beetles of genus *Monochamus*, especially *Monochamus galloprovincialis* Olivier (Coleoptera, Cerambycidae) for its dispersal, the importance of this insect species has significantly increased (21-24). *M. galloprovincialis* is a native European species which is mainly a secondary pest and attacks trees which are physiologically weakened by drought, fire or some other factor (25), but it sometimes attacks completely healthy trees, and the mass occurences could be significant (26). Furthermore, according to the model of *B. xylophilus* spreading, the invasion is unsuccessful unless the minimum population density of longhorn beetles is met, even if the minimum density of pine trees is satisfied (27).

Since almost nothing is known about the presence of wood nematodes in Croatia and because it can be assumed that pine stands in the Croatian coastal region are most threatened, wood nematodes were studied in order to record pine wood nematode species spectrum on different species of pines along the entire coastal region of Croatia

MATERIALS AND METHODS

Wood nematode study was carried out in 2013 and 2014 at two levels. The first level was the sampling of wood nematodes on 22 separate locations along the coastal region of Croatia (Fig 1). Wood samples were collected throughout the year, primarily on trees with symptoms of decline, i.e. trees with dried needles and visible exit holes from longhorn beetles, trees with stronger resin production as a sign of decline in vitality and trees with fruit bodies of fungi visible on the trunk. The samples were collected in the form of rings, branches, sawdust or wood chips without bark on different heights on the tree from 0.5 m to treetop, but mainly at 1.3 m (Tables 1 and 2). In total, 109 samples were collected from trees of aleppo, maritime and black pine.

In 2013, 31 samples were taken in two sampling periods, the first in May and the second in July. For sampling we used two techniques: the first technique was with a drilling machine, where holes were made after which the sawdust was collected, while the second technique consisted of cutting tree rings from fallen trees on different heights. The samples were then transported to the entomological laboratory of Croatian Forest Research Institute (CFRI) for incubation at room temperature in a period of at least 2 weeks, followed by the extraction. Samples of sawdust were stored in plastic bags in the refrigerator at 4 °C for two weeks. Occasionally, water was sprayed on the samples to prevent them from drying out. Samples collected in May were then delivered in Research Centre for Agrobiology and Pedology (CRA) in Firenze, Italy, where nematodes were extracted and microscoped. Further processing of samples was conducted in entomo-

Table 2 *Samples of wood for laboratory analysis of wood nematodes by location, pine species and sample type, collected in 2014*

NUMBER	LOCATION	DATE	SPECIES-sample type	NUMBER	LOCATION	DATE	SPECIES-sample type
32	Premantura	April 25	*Ph-R	71	Brač	August 22	*Ph-W
33				72			
34				73	Solin	October 29	*Ph-W
35	Premantura	April 25	*Ph-R	74			
36				75			*Ph-B
37				76			
38	Solin	May 28	*Ph-R	77	Omiš	October 29	*Ph-W
39				78			*Pp-W
40				79	Makarska	October 29	*Ph-W
41				80			
42				81			
43				82			*Ph-B
44	Omiš	May 28	*Pn-R	83	Zadar	October 31	*Pp-W
45				84			
46				85			
47			*Cs-R	86			
48				87	Zadar	October 31	*Ph-R
49				88	Cres	May 25	*Pn-B
50	Ston	May 29	*Pp-R	89			*Ph-B
51				90			*Pn-B
52				91			
53				92	Buzet	December 9	*Pn-W
54				93			
55	Makarska	May 30	*Ph-R	94			
56				95			
57				96			
58	Sinj	May 30	*Pn-R	97	Pazin	December 14	*Pn-W
59				98			
60				99			
61	Zadar	June 26	*Pp-W	100			
62				101			
63				102	Buje	December 10	*Pn-W
64				103			
65			*Ph-W	104			*Ps-W
66	Omiš	August 14	*Ph-W	105			
67				106			*Pn-W
68				107	Ugljan	December 21	*Ph-W
69	Drniš	August 14	*Pn-W	108			*Ph-B
70	Brač	August 22	*Ph-W	109			

*Pn – *Pinus nigra*; Pp – *Pinus pinaster;* Ph – *Pinus halepensis;* Ps – *Pinus sylvestris;* S – sawdust; R – ring; W – wood chips; B – branch

logical laboratory of CFRI. In 2014, 78 samples were taken from the Istrian peninsula and central and southern Dalmatia. For the extraction of nematodes two techniques were used: Baermann funnel technique and Cobb`s method (28). The samples were stored and incubated at 22 °C for a minimum of 2 weeks, after which they were crushed to a particle size of 0,5×1×1 cm, wrapped in a three layer paper kitchen towel and immersed in plain water. After 48 hours, the samples were extracted by the modified method with metallic sieves with holes of 38 µm and washed with fresh water. In this way, the nematodes can be kept alive in the tap water at 4 °C for several weeks. The samples which contained nematodes were then microscoped with stereoscopic microscope Olympus® BX53 and photographed with Olympus® XC30 camera connected to a computer with a software for measurements and data processing. On samples with an increased number of nematodes, the nematodes were separated and preserved in DESS solution for further morphological and molecular analysis.

The second level was the monitoring of pine longhorn beetle species, especially *M. galloprovincialis*, which started in the spring of 2014. For monitoring we used 8 WitaPrall Multi Funnel Traps® and 2 WitaPrall IntPT-Wet Traps® at six locations in the coastal region of Croatia (Pula, Zadar, Solin, Makarska, Ston). The location of the last trap was in Omiš and it was installed later that year in August. The traps were placed inside an aleppo pine stand in the tree canopy at a height of about 7 m. Ten traps were equipped with pheromones for pine longhorn beetles (Witasek Gallowit® and Witasek Gallohost®). Pheromones were replaced with the new ones in mid-summer. Multi Funnel traps for pine longhorn beetles were modified with a larger funnel and container. Due to unfavorable weather conditions and heavy rains, catchment with original traps did not perform well, because the containers were quickly filled with water and the drainage holes were blocked. Original containers were replaced with the modified containers in the middle of summer. The collected samples were inspected for nematodes in the CFRI laboratory. Each specimen's head and elytra were removed in a separate Petri dish, their wings were spread and the beetles were flooded in a small amount of plain water and left for 2 hours. If there were nematodes present on the beetle, they would emerge into the water. The water surrounding the longhorn beetle was then examined under the microscope (Olympus® SZX7).

Table 3 *Taxonomy of wood nematodes found in 2013 by location*

NUMBER	TAXONOMIC GROUPS	NUMBER	TAXONOMIC GROUPS
1	Aphelenchoidae *Cephalobus* sp. *Rhabditis* sp.	17	/
2	Neotylenchidae *Cephalous* sp. *Lamiaphelenchus* sp.	18	*Laimaphelenchus* sp. *Rhabditis* sp. *Plectus* sp.
3	Aphelenchoidae	19	*Laimaphelenchus* sp.
4	*Laimaphelenchus* sp.	20	*Laimaphelenchus* sp.
5	Aphelenchoidae *Cephalobus* sp. *Ditylenchus* sp. *Laimaphelenchus* sp.	21	*Rhabditis sp.*
6	*Laimaphelenchus* sp. *Ditylenchus* sp.	22	Rhabditis sp.
7	*Laimaphelenchus* sp. *Ditylenchus* sp.	23	*Laimaphelenchus* sp. *Rhabditis* sp.
8	*Laimaphelenchus* sp. *Ditylenchus* sp.	24	*Laimaphelenchus* sp. *Rhabditis* sp.
9	Aphelenchoidae Diplogasteridae *Rhabditis* sp. *Laimaphelenchus* sp. *Bursaphelenchus minutes*	25	Diplogasteridae
10	Neotylenchidae *Rhabditis* sp. *Laimaphelenchus* sp. *Bursaphelenchus minutes* *Cephalobus* sp.	26	Aphelenchoidae *Plectus* sp. *Rhabditis* sp.
11	*Laimaphelenchus* sp. *Plectus* sp.	27	*Laimaphelenchus* sp. *Plectus* sp. *Bursaphelenchus mucronatus* *Bursaphelenchus sexdentati*
12	*Laimaphelenchus* sp. *Plectus* sp.	28	/
13	*Laimaphelenchus* sp. *Rhabditis* sp. *Cephalobus* sp.	29	Aphelenchoidae
14	*Laimaphelenchus* sp.	30	Diplogasteridae
15	*Laimaphelenchus* sp.	31	Diplogasteridae
16	/		

RESULTS

The collection of samples was done in a period of two years in which 109 samples were collected. In 37 samples no nematodes were found. The presented data (Tables 3 and 4) show that along the entire coastal region of Croatia saprophytic nematodes (Rhabditidae, Diplogasteridae and Cephalobidae) dominate in the collected samples. Along with saprophytic nematodes, in more than 8 % of samples pathogenic species from genus *Bursaphelenchus* were found: *B. mucronatus*, *B. sexdentati* and *B. minutes*. *B. xylophilus* was not detected in any of the collected samples. The pheromone traps caught a total of 101 pine longhorn beetles at seven locations (Table 5). During the extraction of 88 pine longhorn beetles none of the native or alien wood nematode species were found.

Table 4 *Taxonomy of wood nematodes found in 2014 by location*

NUMBER	TAXONOMIC GROUPS	NUMBER	TAXONOMIC GROUPS
32	/	71	*Rhabditis* sp. *Cephalobus* sp.
33	/	72	*Rhabditis* sp. *Cephalobus* sp.
34	Aphelenchoidae	73	*Tylenchida* sp.
35	/	74	Aphelenchoidae *Laimaphelenchus* sp. *Bursaphelenchus* sp.
36	/	75	/
37	Diplogasteridae	76	Aphelenchoidae
38	Aphelenchoidae	77	*Rhabditis* sp.
39	/	78	Aphelenchoidae
40	/	79	*Tylenchida* sp.
41	/	80	Diplogasteridae *Tylenchida* sp. *Rhabditis* sp.
42	*Rhabditis* sp. *Tylenchida* sp.	81	/
43	/	82	Aphelenchoidae Diplogasteridae *Laimaphelenchus* sp.
44	/	83	Diplogasteridae *Rhabditis* sp.
45	*Tylenchida* sp.	84	/
46	/	85	Diplogasteridae *Rhabditis* sp. *Cephalobus* sp.
47	Aphelenchoidae	86	Diplogasteridae *Cephalobus* sp.
48	/	87	/
49	/	88	Aphelenchoidae *Laimaphelenchus* sp.
50	Aphelenchoidae *Laimaphelenchus* sp.	89	Aphelenchoidae *Bursaphelenchus* sp. *Laimaphelenchus* sp.
51	/	90	*Rhabditis* sp. *Plectus* sp.
52	/	91	Diplogasteridae *Plectus* sp. *Bursaphelenchus* sp.
53	/	92	Aphelenchoidae Diplogasteridae *Rhabditis* sp. *Cephalobus* sp.
54	/	93	Diplogasteridae *Rhabditis* sp. *Cephalobus* sp.
55	/	94	/
56	*Laimaphelenchus* sp.	95	Aphelenchoidae Diplogasteridae *Mononchus sp.*
57	/	96	Diplogasteridae *Tylenchida* sp.
58	Aphelenchoidae	97	Diplogasteridae Aphelenchoidae *Bursaphelenchus* sp.
59	/	98	/
60	/	99	Diplogasteridae *Rhabditis* sp.
61	Diplogasteridae *Cephalobus* sp. *Rhabditis* sp.	100	Aphelenchoidae *Bursaphelenchus* sp.
62	/	101	Diplogasteridae *Cephalobus* sp.
63	/	102	*Rhabditis* sp.
64	/	103	*Rhabditis* sp. *Cephalobus* sp.
65	Aphelenchoidae	104	Diplogasteridae Aphelenchoidae *Laimaphelenchus* sp. *Rhabditis* sp. *Cephalobus* sp.
66	Aphelenchoidae *Rhabditis* sp. *Bursaphelenchus* sp.	105	Aphelenchoidae *Rhabditis* sp. *Bursaphelenchus* sp. *Cephalobus* sp.
67	Aphelenchoidae	106	*Rhabditis* sp.
68	/	107	/
69	/	108	Aphelenchoidae
70	/	109	/

DISCUSSION

Pathogenic nematode species of genus *Bursaphelenchus* were found on all sampled pine species along the coastal region of Croatia. Quarantine nematode *B. xylophilus* was not found in any sample, but three other wood nematode species from that genus were found (*B. mucronatus*, *B. sexdentati* and *B. minutes*).

Nematode *B. mucronatus* is very similar to the *B. xylophilus*. It has two known genotypes: East Asian and European type (29), and it is widespread throughout the Euroasia. East Asian genotype of this nematode could pose a threat if it is found in Europe, primarily threatening *P. sylvestris*, *P. nigra* and *P. pinaster*. *B. mucronatus* appeares to be pathogenic to plants when inoculated in the 3-year old seedlings of genera *Picea*, *Abies*, *Larix* and

Table 5 *The description of used traps and pheromones with the number of caught pine longhorn beetles in 2014 and 2015*

LOCATION	TRAP TYPE	PHEROMONES	NUMBER OF PINE LONGHORN BEETLES
Pula	2 IPM 1 multifunnel	2 Gallohost 1 Gallowit	14
Zadar	1 multifunnel	1 Gallowit	27
Solin	3 multifunnel	2 Gallohost 1 Gallowit	1
Makarska	1 multifunnel	1 Gallowit	4
Ston	1 multifunnel	1 Gallowit	34
Omiš	1 multifunnel	1 Gallohost	1
Lučice, Brač	/	/	20* caught by hand on dead tree
Σ			101

Pseudotsuga. Various strains of *B. mucronatus* inoculated in 11 – 29-year old pines in their natural environment in Germany and Austria did not cause a decline of the trees. However, it has been proved that the fungi cultures of nematode population are negligibly increasing at 20 °C, moderately at 25 °C and vigorously at 30 °C (30).

The second species which proved to be highly pathogenic for the trees in the laboratory (at 25 °C with relative humidity of 60 %) was *B. sexdentati (31)*. It is reasonable to think that these two species of nematodes can contribute to the decline of pine trees in the coastal region of Croatia. The third species, *B. Minutes*, is considered to be widespread in the Mediterranean without having a pathogenic impact on the pine trees (30).

The degree of pathogenicity of nematodes depends on temperature, humidity and other conditions which can be seen from the results of this research. The samples that were collected in spring while the temperatures are lower didn`t have nematodes, while those from summer and autumn had an increased number of nematodes. Also, the degree of pathogenicity depends on the habitat, the age of the tree, the presence of the appropriate vector and the provenance and virulence of nematodes (30). This is especially important because of the variations of climate in terms of the increase in temperature and the decrease in precipitation, which will be especially noticable in the coastal region of Croatia according to climate change predictions (32).

The results of this research confirm the presence of nematodes and their possible influence on the process of pine decline of the Mediterranean pine species in Croatia. The presence of *M. galloprovincialis*, the vector of highly damaging *B. xylophilus* has been confirmed during this research and the application of pheromone based traps could be considered as a part of the monitoring system for the detection of this invasive species. This is the first research of wood nematodes in Croatia, which indicates the presence of a rich species spectrum and of potential harmful species that could play a vital role in pine decline in the Mediterranean area. Further and detailed research is suggested with regular monitoring of invasive *B. xylophilus*. Some of the wood nematode species found during this research could not be morphologialy identified and further research should involve molecular analysis

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REFERENCES

1. SABADI R, KRZNAR A, JAKOVAC H 1988 Koliko društvena zajednica pridonosi održanju i proširenju potencijala općih koristi od šuma? Šum list (5-6): 255-265

2. PRPIĆ B 1992 Ekološka i gospodarska vrijednost šuma u Hrvatskoj. In: Rauš Đ (ed) Šume u Hrvatskoj, Šumarski fakultet Sveučilišta u Zagrebu and"„Hrvatske šum"", 237-256, Zagreb
3. PRPIĆ B 1992 O vrijednostima općekorisnih funkcija šume. Šum list (6-8): 301-312
4. SABADI R, PRPIĆ B, JAKOVAC H 2001 Ukupna vrijednost jelovih šuma u Hrvatskoj. In: Prpić B (ed) Obična jela (*Abies alba* Mill.) u Hrvatskoj, Akademija šumarskih znanosti and"„Hrvatske šum"" 792-826, Zagreb
5. PRPIĆ B 2001 Šuma"„tvornic"" vode i zraka. Šum. list, (5-6): 300-302
6. PRPIĆ B 2003 Općekorisna uloga bukovih šuma. In: Matić S (ed) Obična bukva (*Fagus sylvatica* L.) u Hrvatskoj, Akademija šumarskih znanosti, 213-227, Zagreb
7. PRPIĆ B, JURJEVIĆ P, JAKOVAC H 2005 Procjene vrijednosti protuerozijske, hidrološke i vodozaštitne uloge šume. Šum list (13): 186-194
8. MATIĆ S, ANIĆ I, ORŠANIĆ M 2005 Uzgojni zahvati u funkciji poboljšanja protuerozijske i vodozaštitne uloge šume. Šum list (13): 17-30
9. MARGALETIĆ J, MARGALETIĆ M 2003 Požari u šumi i na šumskom zemljištu kao čimbenici degradacije staništa. Šum list (9-10): 475-482
10. MAMUT M 2011 Veza prirodnogeografske i sociogeografske osnove Dalmacije s ugroženošću otvorenog prostora požarom. Šum list (1-2): 37-50
11. PERNEK M, NOVAK-AGBABA S, LACKOVIĆ N, ĐOĐ N, LUKIĆ I, WIRTH S 2012 Uloga biotičkih čimbenika u sušenju borova (Pinus spp.) na području Sjeverne Dalmacije. Šum list (7-8): 343-354
12. GLAVAŠ M, HARAPIN M, HRAŠOVEC B 1992: Zaštita šuma. In: Rauš Đ (ed) Šume u Hrvatskoj. Šumarski fakultet Sveučilišta u Zagrebu i Hrvatske šume, 340 str., Zagreb
13. HARAPIN M 1984 Parazitski kompleks borova četnjaka (*Thaumetopoea pityocampa* Schiff.) i njegov utjecaj na dinamiku populacije. Doktorska disertacija, Šumarski fakultet Sveučilišta u Zagrebu, pp 186
14. DIMINIĆ D 1996 Gljiva *Sphaeropsis sapinea* (Fr.) Dyko et Sutton na borovima sjevernojadranskog područja. Šum list (11-12): 463-468
15. JURC, D, JURC M 2010 Mycosphaerella dearnessii occurs in Slovenia. Plant Pathology 59 (4): 808 http://dx.doi.org/10.1111/j.1365-3059.2010.02262.x
16. GLAVAŠ M, MARGALETIĆ J 2001 Smeđa pjegavost iglica alepskoga bora i mjere zaštite. Znanost u potrajnom gospodarenju hrvatskim šumama. In: Matić S, Krpan APB, Gračan J (ed) Šumarski fakultet i Šumarski institut, Zagreb, 636
17. HRAŠOVEC B, PERNEK M, MATOŠEVIĆ D 2008 Spruce, Fir and Pine bark beetle outbreak development and Gypsy moth situation in Croatia in 2007. Forstschutz Aktuell 44: 12-13
18. LOGAN JA, POWELL A 2001 Ghost forests, global warming, and the mountain pine beetle. Am Entomol 47: 160-173 http://dx.doi.org/10.1093/ae/47.3.160
19. MALANSON GP 2001 Complex response to global change at alpine treeline. Physio Geog 22: 333-342
20. ØKLAND B, SKARPAAS O, SCHROEDER M, MAGNUSSON C, LINDELOW Å, THUNES K 2010 Is eradication of the pinewood nematode (*Bursaphelenchus xylophilus*) likely? An evaluation of current contigency plans. Risk Analysis 30 (9): 1424-1439 http://dx.doi.org/10.1111/j.1539-6924.2010.01431.x
21. MOTA MM, BRAASCH H, BRAVO MA, BRAVO AC, PENAS J, BURGMEISTER W, METGE K, SOUSA E 1999: First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. Nematology 1(7-8): 727-734
22. PERNEK M, MATOŠEVIĆ D 2003 Karantenski štetočinja *Bursaphelenchus xylophilu* – vektorski odnos oprema rodu *Monochamus* i opasnosti za hrvatsko šumarstvo. Glasilo biljne zaštite 6: 378-383
23. JURC M, JURC D, GOGALA N, SIMONČIĆ P 1996 Air pollution and fungal endophytes in needles of Austrian pine. Phyton (Horn) 36(3): 111-114
24. SOUSA E, BRAVO M, PIRES M, NAVES P, PENAS A, BONIFACIO L, MOTA MM 2001 *Bursaphelenchus xylophilus* (Nematoda; Aphelenchoididae) associated with *Monochamus galloprovincialis* (Coleoptera; Cerambycidae) in Portugal. Nematology 3: 89-91 http://dx.doi.org/10.1163/156854101300106937
25. PAJARES JA, IBEAS F, DIEZ JJ, GALLEGO D 2004 Attractive responses by *Monochamus galloprovincialis* (Col., Cerambicidae) to host and bark beetle semiochemicals. J Appl Ent 128 (9-10): 633-638 http://dx.doi.org/10.1111/j.1439-0418.2004.00899.x
26. KOVAČEVIĆ Ž 1956 Primijenjena entomologija: Šumski štetnici. Poljoprivredni nakladni zavod, 535 str., Zagreb
27. YOSHIMURA A, KAWASAKI K, TAKASU F, TOGASHI K, FUTAI K, SHIGESADA N 1999 Modeling the spread of pine wilt disease caused by nematodes with pine sawyers as vector. Ecology 80 (5): 1691-1702 http://dx.doi.org/10.1890/0012-9658(1999)080[1691:MTSOPW]2.0.CO;2
28. VAN BEZOOIJEN J 2006 Methods and techniques for nematology. (https://www.wageningenur.nl/upload_mm/4/e/3/f9618ac5-ac20-41e6-9cf1-c556b15b9fa7_MethodsandTechniquesforNematology.pdf). Accesed on December 19, 2015
29. HAN H, CHUNG YJ, SHIN SC 2008 First Report of the European Type of *Bursaphelenchusmucronatus* on Korean Pine (*Pinus koraiensis*) in Korea. Plant Disease 92 (8): 1248 http://dx.doi.org/10.1094/PDIS-92-8-1248C
30. BRAASCH H 2000 Pest risk analysis of pinewood nematod – related *Bursaphelenchus* species in view of South European pine wilting and wood imports from Asia: final report. BBA
31. MICHALOPOULOS-SKARMOUTSOS H, SKARMOUTSOS G, KALAPANIDA M, KARAGEORGOS A 2003 Surveying and recording of nematodes of the genus *Bursaphelenchus* in conifer forests in Greece and pathogenicity oft he most important species. Nematology Monographs & Perspectives 1: 113-126
32. BRANKOVIĆ Č, GÜTTLER I, PATARČIĆ M, SRNEC L 2009 Scenarij klimatskih promjena. In: DHMZ – Peto nacionalno izvješće Republike Hrvatske prema Okvirnoj konvenciji Ujedinjenih naroda o promjeni klime (UNFCCC) (http://klima.hr/razno/publikacije/klimatske_promjene.pdf) Accesed on January 12, 2016