

New hosts for *Lecanosticta acicola* and *Dothistroma septosporum* in Spain

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Abstract

The largest impact of needle diseases in the recorded history of *Pinus radiata* and *Pinus nigra* plantations in the North of Spain, was from 2018 to 2019. The severity of the disease has led to a serious reconsideration of forest management in the area. The main pathogens involved in this historical outbreak were *Lecanosticta acicola* and *Dothistroma septosporum*. Three arboreta were established under the European project REINFFORCE in the Basque Country in recently harvested *P. radiata* plantations. The plants for the arboreta were produced in nurseries located in the Alps of Upper Provence (France). These arboreta were surrounded by plantations seriously damaged by *L. acicola* and *D. septosporum*. The *Pinus* species which were sampled in the arboreta included *P. brutia* (4 provenances), *P. elliottii* (2 provenances), *P. nigra* (8 or 9 provenances), *P. pinaster* (7 provenances), *P. pinea* (6 provenances), *P. ponderosa* (3 provenances), *P. sylvestris* (8 provenances) and *P. taeda* (3 provenances). *Lecanosticta acicola* is reported for the first time infecting *Pinus brutia* (Provenance: Alexandropolis, Greece and var. *eldarica*, Crimea), a new host species for this pathogen. *Pinus elliottii* (Provenance: Georgia, USA) and *P. ponderosa* (Provenance: Central California, USA) are new host reports of *L. acicola* for Spain. *Dothistroma septosporum* was found for the first time on *P. brutia* (Provenance: Marmaris, Turkey) and *P. ponderosa* (Provenance: Oregon, USA) in Spain and was also detected infecting *P. nigra* (Provenance: Slogne Vayrières, France).

Keywords: BSNB, defoliation, *Dothistroma* needle blight, *Mycosphaerella dearnessii*

1. Introduction

The incidence of *Lecanosticta acicola* (Thüm.) Syd., causal agent of brown spot needle blight (BSNB), and *Dothistroma septosporum* (Dorog.) Morelet and *D. pini* Hulbary, the causal agents of red band needle blight (DNB), have increased during the last decades in Europe [1,2]. The first reports of *Dothistroma* spp. and *L. acicola* in Spain were in 1933 and 1942 respectively [3,4]. However, the highest impact of disease severity ever recorded on *Pinus radiata* D. Don and *Pinus nigra* Arnold

plantations in the North of Spain was from 2018 to 2019. High defoliation levels, and the unusual mortality rate, has caused an economic, environmental and social alarmism that has questioned the efficacy of current management strategies carried out in forest plantations.

Lecanosticta acicola and *Dothistroma* spp. have a broad host range and are found in several countries around the world. The presence of *L. acicola* and *Dothistroma* spp. has been detected on 53 and 96 different *Pinus* species, including hybrids, respectively [2,5,6]. At least two putative introductions of *L. acicola* have occurred in Europe from North America [7]. Its intercontinental introduction can most likely be explained as a consequence of the movement of infected plant material [7,8]. The spread of *L. acicola* within Europe appears to be associated with conidial dispersion and ascospore dissemination and also to accidental, human mediated introductions [7,8]. This species is included in the EPPO A2 list. In Spain, *L. acicola* has been reported on *P. radiata*, *P. sylvestris* L. and *P. nigra*; *D. septosporum* on *P. radiata*, *P. sylvestris*, *P. nigra*, *P. pinaster* Ait. and *Pseudotsuga menziesii* (Mirb.) Franco, and *D. pini* on *P. nigra* [6,9].

An important group of organisms causing similar symptoms to the brown spot and red bands on pine, are those belonging to *Phytophthora*. *Phytophthora pluvialis* Reeser, Sutton, and E. Hansen was reported as a new defoliating disease in *Pinus radiata* and *Pseudotsuga menziesii* in New Zealand [10]. *Phytophthora pinifolia* Alv. Durán, Gryzenh. and M.J. Wingf. was detected in 2004 in *P. radiata* plantations in central Chile [11] and in 2006, a total of 60,000 trees were reported to be affected. These days however, the disease is not causing significant damage and symptoms are only observed on sporadic trees [12]. Both *Phytophthora* species may produce common symptoms such as olive-coloured bands on needles, dark resinous spots and reddening and cast of infected needles. In addition, infections by *P. pinifolia* cankers may appear at the base of the needle fascicle [11,13]. Although these *Phytophthora* spp. species are considered absent in the EPPO region, and were removed from the EPPO Alert List in 2013, it cannot be ruled out that the severe damage observed in *Pinus* in the Basque country might also be due to a newly introduced *Phytophthora* spp., and this would need to be investigated.

As part of a wider monitoring plan carried out to determine the possible causes of the most explosive emergence of a forest disease in the North of Spain, arboreta located next to pine plantations with similar severe symptoms of defoliation were examined. These arboreta were established under the European project REINFFORCE in the Basque Country in recently harvested *P. radiata* plantations. Two of them, with an average size of 3 Ha each, are located in Albinabehea (Aramaio, Araba, AR24) and Irisasi (Usurbil, Gipuzkoa, AR22). The third arboretum, 6 Ha in size, is located in Laukiz (Bizkaia, AR20) (Figure 1). The plants in the arboreta were produced in two nurseries placed in the Alps of Upper Provence (France) during 2011 and 2012, and in a nursery located in Guémené (France) in 2013. The material was subjected to phytosanitary controls and it was planted in the winters of 2011, 2012 and 2013. Common European tree species and alternative ones, including several provenances with between five to 12 trees per provenance, were planted in order to determine their adaptability to climate change. After being established for six years, severe needle defoliation was observed. The arboreta were located in an area of oceanic climate and at distances of less than 100 meters from plantations of *Pinus radiata* and *Pinus nigra*, in which the presence of *Lecanosticta acicola* and *Dothistroma septosporum* was previously confirmed [9].

The aim of the present study was to characterise the main defoliating disease agents in these new established arboreta which are of great interest due to the diversity of the *Pinus* species and provenances.

2. Materials and Methods

2.1. Sample collection and fungal identification.

Needle samples from a total of 74 pine trees located in the arboreta of Albinabehea (Araba), Irisasi (Gipuzkoa) and Laukiz (Bizkaia) were collected (Figure 1). A minimum of 100 g of needles per tree were collected from both symptomatic and asymptomatic trees. Defoliation was estimated using a severity degree scale for each tree using the 5% step method (1: <25% 2: 25–<50%; 3: 50–<75%; 4: ≥75%) [14] (Figure 2). The tree species sampled included *P. brutia* Ten. (4 provenances), *P. elliottii* Engelm. (2 provenances), *P. nigra* (8 or 9 provenances), *P. pinaster* Ait. (7 provenances), *P. pinea* L. (6 provenances), *P. ponderosa* Douglas ex C. Lawson (3 provenances), *P. sylvestris* L. (8 provenances) and *P. taeda* (3 provenances). Trees were sampled regardless of whether they exhibited symptoms of defoliation or not (Table 1). All samples were visually inspected for any symptoms resembling spots or bands with fruiting bodies.

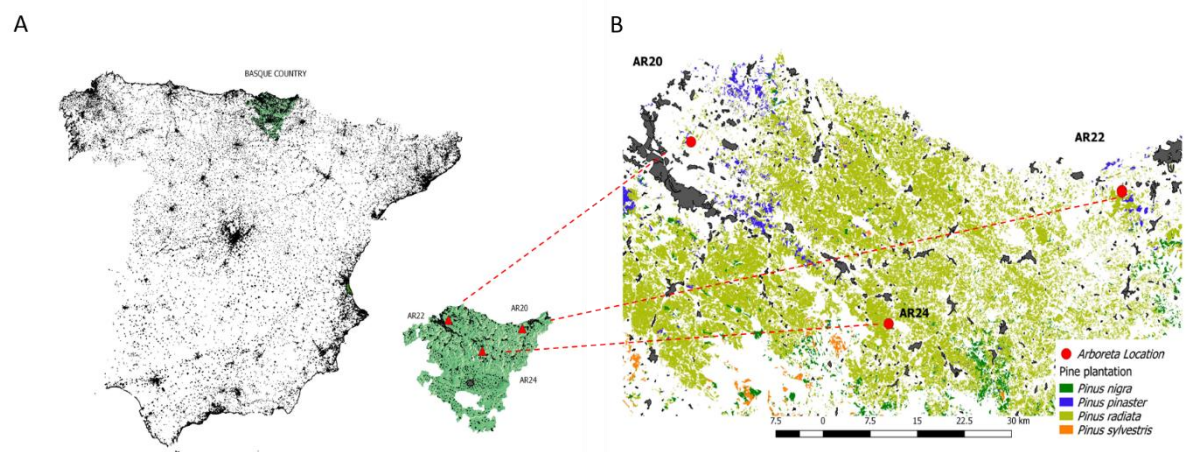


Figure 1. Locations of the three arboreta (AR20, AR22 and AR24) in the Basque Country from which samples were collected for this study (A). Species of pine plantations surrounding the arboretum environment (B).

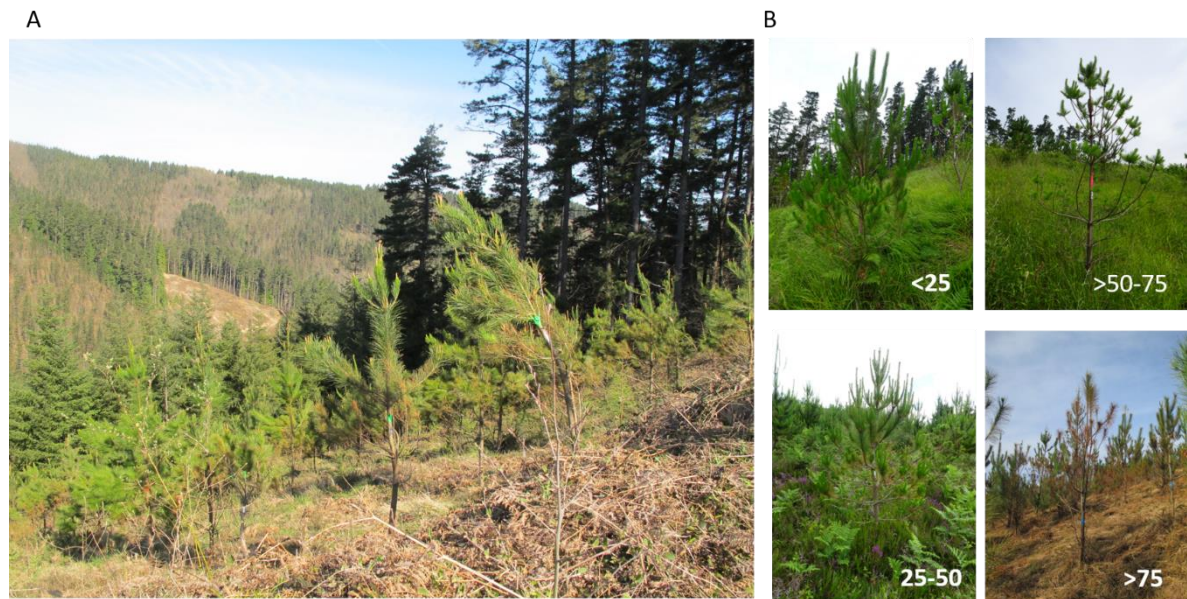


Figure 2. Severe outbreak in adult and young plantations located next to the arboretum (A). Score system applied in the defoliation evaluation (B).

DNA was extracted directly from a 100 mg sample per tree of symptomatic and asymptomatic trees with the innuPREP Plant DNA Mini Kit (Analytic Jena AG, Jena, Germany). The presence of *L. acicola*, *D. septosporum* and *D. pini* was determined by species-specific conventional PCR using 0.4 μ M of each primer (LATef.F/LATef.R , DStub2-F/DStub2-R or DPtef-F/DPtef-R) [15], 10x buffer (Complete II KCl Buffer, IBIAN technologies, Zaragoza, Spain), 200 μ M dNTP, 0.5 U Taq DNA Polymerase (IBIAN technologies), and 1.5 μ L DNA template in a total volume of 20 μ L. The PCR conditions were as follows: 10 min at 94 $^{\circ}$ C, 35 cycles of 30 s at 94 $^{\circ}$ C, 30 s at 60 $^{\circ}$ C, and 45 s at 72 $^{\circ}$ C, and a final 10 min at 72 $^{\circ}$ C. PCR amplicons were visualised on a 2% agarose gel (Conda, Madrid, Spain) stained with GelRed[®] (Biotium Inc., California, USA). The reactions were considered positive for *D. septosporum*, *D. pini* and *L. acicola* if amplicon sizes measured 231 bp, 193 bp and 237 bp respectively.

The detection of *Phytophthora* species was carried out by commercial enzyme immunoassays using the *Phytophthora* ImmunoStrip[®] Test (AGDIA Inc., Indiana, USA), which is recommended as a preliminary screening tool in survey programs for *Phytophthora* species. ImmunoStrips[®] and extraction buffer were warmed to room temperature (18 - 30 $^{\circ}$ C) before use. 150 mg of each needle sample was placed in the extraction bag containing 3 mL of extraction buffer and macerated and crushed with a tissue homogenizer. The ImmunoStrip[®] was inserted into the bag and submerged in the extract for 30 minutes, after which results were recorded.

3. Results

Symptoms of defoliation were mainly located in the lower half of the tree. Despite the high level of defoliation, in the majority of the cases, no visible fungal fruiting bodies of needle blight species were observed.

From 74 samples analyzed, four resulted in positive amplifications for *Lecanosticta acicola* and three for *Dothistroma septosporum*. Positive results for *D. septosporum* were only found in the samples

collected from Irisasi arboretum, on *P. brutia* (Marmaris, Turkey), *P. nigra* (Slogne Vayrières, France) and *P. ponderosa* (Oregon, USA) with levels of defoliation scored 1, 3 and 4 respectively. *Lecanosticta acicola* was detected in all three arboreta, in needle samples from *P. elliotii* (Georgia, USA), *P. ponderosa* (Central California, USA), *P. brutia* (Alexandropolis, Greece) and *P. brutia* (var. *eldarica*, Crimea) with estimated defoliation of 4, 3, 1, and 3 (Table 1) . None of the tested samples yielded a positive result for *D. pini* or *Phytophthora* sp.

Table 1. Detection of *Dothistroma septosporum* and *Lecanosticta acicola* in relation to location (arboretum) and *Pinus* species, provenance, age (in years) and level of defoliation.

Sample ID	Location	Species	Provenance	Age	Defoliation level	<i>L. acicola</i>	<i>D. septosporum</i>
23	Albina	<i>P. brutia</i>	Taurus, Turkey	7	1	0	0
18	Albina	<i>P. brutia</i>	var. <i>eldarica</i> , Crimea	7	2	0	0
42	Albina	<i>P. elliotii</i>	Georgia, USA	5	3	0	0
37	Albina	<i>P. elliotii</i>	Georgia, USA	6	4	1	0
32	Albina	<i>P. elliotii</i>	Louisiana, USA	7	3	0	0
36	Albina	<i>P. nigra</i>	Cazorla Alcaraz, Spain	6	1	0	0
26	Albina	<i>P. nigra</i>	Les Barnes Sivens, France	7	2	0	0
35	Albina	<i>P. nigra</i>	Sistema Iberico Meridional, Spain	6	1	0	0
34	Albina	<i>P. nigra</i>	Slogne Vayrières, France	7	1	0	0
27	Albina	<i>P. nigra</i>	subsp. <i>laricio</i> var. <i>corsican</i> , Haute Serre seed tree orchard, France	7	1	0	0
39	Albina	<i>P. nigra</i>	subsp. <i>salzmannii</i> , Soria, Spain	5	1	0	0
21	Albina	<i>P. pinaster</i>	Mimizan, Landes, France	7	1	0	0
17	Albina	<i>P. pinaster</i>	Picard, Lande Corse, France	7	1	0	0
22	Albina	<i>P. pinaster</i>	Tamjout, Morocco	7	2	0	0
25	Albina	<i>P. pinea</i>	Cordillera Central, Spain	7	1	0	0
29	Albina	<i>P. pinea</i>	Idaho, USA	7	2	0	0
28	Albina	<i>P. pinea</i>	Italy	7	1	0	0
40	Albina	<i>P. ponderosa</i>	Central California, USA	5	3	1	0
31	Albina	<i>P. ponderosa</i>	Southern Rockies, USA	7	3	0	0
38	Albina	<i>P. sylvestris</i>	Haguenau Vayriere, France	6	1	0	0
24	Albina	<i>P. sylvestris</i>	Severozapadna, Slovakia	7	2	0	0
30	Albina	<i>P. sylvestris</i>	Sierra de Guadarrama, Spain	7	3	0	0
33	Albina	<i>P. sylvestris</i>	Turkey	7	3	0	0
41	Albina	<i>P. taeda</i>	Georgia, USA	5	3	0	0
20	Albina	<i>P. taeda</i>	Sur de California, USA	7	3	0	0
19	Albina	<i>P. taeda</i>	Virginia, USA	7	1	0	0
11	Irisasi	<i>P. brutia</i>	Alexandropolis, Greece	7	1	1	0
15	Irisasi	<i>P. brutia</i>	Marmaris, Turkey	7	1	0	1
14	Irisasi	<i>P. brutia</i>	Taurus, Turkey	7	1	0	0
12	Irisasi	<i>P. brutia</i>	var. <i>eldarica</i> , Crimea	7	1	0	0
9	Irisasi	<i>P. elliotii</i>	Georgia, USA	7	1	0	0

4	Irisasi	<i>P. elliottii</i>	Louisiana, USA	7	1	0	0
16	Irisasi	<i>P. nigra</i>	Slogne Vayrières, France	7	3	0	1
2	Irisasi	<i>P. nigra</i>	subsp. <i>laricio</i> var. <i>corsican</i> , Haute Serre seed tree orchard, France	7	3	0	0
10	Irisasi	<i>P. pinaster</i>	Mimizan, Landes, France	7	1	0	0
1	Irisasi	<i>P. pinaster</i>	Picard, Lande Corse, France	7	1	0	0
13	Irisasi	<i>P. pinaster</i>	Tamjout, Morocco	7	1	0	0
8	Irisasi	<i>P. pinea</i>	Italy	7	2	0	0
5	Irisasi	<i>P. ponderosa</i>	Oregon, USA	7	4	0	1
7	Irisasi	<i>P. ponderosa</i>	Southern Rockies, USA	7	3	0	0
3	Irisasi	<i>P. sylvestris</i>	Severozapadna, Slovakia	7	4	0	0
6	Irisasi	<i>P. sylvestris</i>	Turkey	7	3	0	0
31	Umbemendi	<i>P. brutia</i>	Marmaris, Turkey	7	2	0	0
30	Umbemendi	<i>P. brutia</i>	Taurus, Turkey	7	3	0	0
32	Umbemendi	<i>P. brutia</i>	var. <i>eldarica</i> , Crimea	7	3	1	0
19	Umbemendi	<i>P. elliottii</i>	Georgia, USA	7	2	0	0
29	Umbemendi	<i>P. elliottii</i>	Louisiana, USA	7	2	0	0
21	Umbemendi	<i>P. nigra</i>	Cazorla Alcaraz, Spain	7	2	0	0
20	Umbemendi	<i>P. nigra</i>	subsp. <i>laricio</i> var. <i>calabrica</i> , Les Barnes-Sivens, France	7	2	0	0
14	Umbemendi	<i>P. nigra</i>	subsp. <i>laricio</i> var. <i>corsican</i> , Slogne Vayrières, France	7	3	0	0
13	Umbemendi	<i>P. nigra</i>	subsp. <i>salzmannii</i> – ES07b – Sistema Iberico Meridional, Sur de Cuenca, Spain	7	1	0	0
2	Umbemendi	<i>P. pinaster</i>	Cordal de Loba, Spain	7	3	0	0
5	Umbemendi	<i>P. pinaster</i>	Leiria, Portugal	7	1	0	0
3	Umbemendi	<i>P. pinaster</i>	Mimizan, Landes, France	7	4	0	0
7	Umbemendi	<i>P. pinaster</i>	Picard, Lande Corse, France	7	4	0	0
8	Umbemendi	<i>P. pinaster</i>	Serrania de Cuenca, Spain	7	4	0	0
4	Umbemendi	<i>P. pinaster</i>	Sierra de Gredos, Spain	7	2	0	0
6	Umbemendi	<i>P. pinaster</i>	Tamjout, Morocco	7	1	0	0
16	Umbemendi	<i>P. pinea</i>	Italy	7	2	0	0
15	Umbemendi	<i>P. pinea</i>	Région méditerranéenne, France	7	1	0	0
18	Umbemendi	<i>P. pinea</i>	Valles del Tietar and Alberche, Spain	7	2	0	0
17	Umbemendi	<i>P. pinea</i>	Vendas Novas, Portugal	7	2	0	0
27	Umbemendi	<i>P. ponderosa</i>	Oregon, USA	7	2	0	0
28	Umbemendi	<i>P. ponderosa</i>	Southern Rockies, USA	7	2	0	0
23	Umbemendi	<i>P. sylvestris</i>	Montes universales, Spain	7	2	0	0
24	Umbemendi	<i>P. sylvestris</i>	Pinhal da Pedra Bela, Portugal	7	2	0	0
12	Umbemendi	<i>P. sylvestris</i>	Scotland	7	1	0	0
26	Umbemendi	<i>P. sylvestris</i>	Severozapadna, Slovakia	7	1	0	0
11	Umbemendi	<i>P. sylvestris</i>	Sierra de Guadarrama, Spain	7	2	0	0
22	Umbemendi	<i>P. sylvestris</i>	Taborz-Haute Serre, Poland	7	2	0	0
25	Umbemendi	<i>P. sylvestris</i>	Turkey	7	2	0	0

10	Umbemendi	<i>P. taeda</i>	Georgia, USA	7	2	0	0
9	Umbemendi	<i>P. taeda</i>	South of California, USA	7	1	0	0
1	Umbemendi	<i>P. taeda</i>	Virginia, USA	7	2	0	0

4. Discussion

In this preliminary study, we report *Pinus brutia* (Provenance: Alexandropolis, Greece and var. *eldarica*, Crimea) as a new host species for *Lecanosticta acicola*, and *P. elliotii* (Provenance: Georgia, USA) and *P. ponderosa* (Provenance: Central California, USA) are reported as new host reports for Spain. *Dothistroma septosporum* was found for the first time in Spain on *P. brutia* (Provenance: Marmaris, Turkey) and *P. ponderosa* (Provenance: Oregon, USA), and was also detected infecting *P. nigra* (Provenance: Slogne Vayrières, France).

Pinus nigra, *P. ponderosa* and *P. elliotii* are known to be susceptible to brown spot needle blight and *Dothistroma* needle blight [2,5,6]. *Pinus brutia* is a pine species closely related to *P. halepensis* Mill. and adapted to Mediterranean regions where it has been extensively planted [16]. *D. septosporum* was detected on *P. brutia* in Greece and Turkey [17,18], but based on EPPO and the recent review by van der Nest et al. [5], this is the first report of *L. acicola* on this *Pinus* species. Three of the four *P. brutia* provenances screened positive for *L. acicola* and/or *D. septosporum*. The Mediterranean nature of this host could be affecting its adaptability to the Atlantic area and predisposing it to fungal infections. It would be interesting to monitor these species and provenances during the next few years. The absence of *D. pini* in the analyzed samples may be explained by the low detection rates previously observed in the region [9].

It was observed that the level of defoliation was not always related to the positive detection of the pathogenic organisms. There were cases with low levels of defoliation and positive detections of *D. septosporum* or *L. acicola*, and vice versa (Table 1). Other biotic and/or abiotic factors (stress caused by insects flooding, soil nutrient deficiencies, etc.) that can lead to defoliation may be involved. Even though *Phytophthora* sp. were not detected in the current survey, monitoring for these pathogens will continue in the future.

The purpose of this survey was to determine the presence of *Lecanosticta acicola*, *Dothistroma* spp. and *Phytophthora* as causal agents of the needle defoliation symptoms observed on *Pinus* species in three arboreta located in the North of Spain. The arboreta were established as part of the project REINFFORCE to study the impact of the climatic change on forests of the Atlantic region where different species and provenances were planted to determine their adaptability. Arboreta were mainly surrounded by *P. radiata* and *P. nigra* plantations that were severely infected with *Dothistroma* spp. and *L. acicola*. Since the seedlings were sent from the nurseries with their phytosanitary certificate, it is plausible to think that natural infections were the cause of the disease outbreak. In addition, the lack of a positive identification of the pathogens could be due to the low detection level of conventional PCR, which might not be sensitive enough to pick up recent infections, as opposed to predicting the host species exhibit some level of resistance. To better define the possible tolerance of the examined pine trees against these diseases, a controlled inoculation assay is needed.

5. Conclusion

In this preliminary study, we report the detection of *Lecanosticta acicola* in *Pinus brutia* (Alexandropolis, Greece and var. *eldarica*, Crimea), a new host species for this pathogen at a global scale. For the first time in Spain, *L. acicola* was found on *P. elliottii* (Georgia, USA) and *P. ponderosa* (Central California, USA) and *Dothistroma septosporum* was found on *P. brutia* (Marmaris, Turkey), *P. ponderosa* (Oregon, USA) and *P. nigra* (Slogne Vayrières, France). Knowledge regarding differences in host-tolerance is fundamental to manage a disease as these pathogens can lead to serious economic, social and environmental losses. Forestry companies and European organizations need to re-enforce or establish breeding programmes to identify disease-tolerant planting stock.

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