Gremmeniella abietina in North-western Spain: Distribution and Associated Mycoflora

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Abstract – *Gremmeniella abietina*, in its conidial state (*Brunchorstia pinea*), was recently reported in Palencia (north-west Spain) on *Pinus halepensis* in 1999. For that reason, the main aim of the present study was to determine the distribution of *G. abietina* in areas next to that where it was first recorded in order to evaluate there the current spread of the pathogen. Fungal mycoflora occurring in trees showing symptoms of *G. abietina* was also recorded with the goal of discussing the possible role it plays in the disease expression observed in the field. The isolation method consisted of finding fruitbodies on plant tissues after incubating them in wet and warm conditions. *G. abietina* was found in five out of a total of 40 stands that were examined. Furthermore, in another 25 stands, trees showing symptoms similar to those caused by *G. abietina* were also recorded. In addition to that, another 22 fungal species were isolated from plant fragments. *Thyriopsis halepensis*, *Sclerophoma pythiophila* and *Cenangium ferruginosum* were frequently isolated from injured plant fragments and were recovered from many stands (up to 70% of the total stands). These fungal species could play a role in the disease symptoms expression observed in the field, which were initially attributed exclusively to *G. abietina*. *Lophodermium pinastri*, *Naemacyclus niveus* and *Pestalotia stevensonii*, previously reported to be secondary pathogens on pine, were also occasionally recovered.

Brunchorstia pinea* / *Cenangium ferruginosum* / *Pinus halepensis* / *Sclerophoma pythiophila* / *Thyriopsis halepensis

Kivonat – A *Gremmeniella abietina* Északnyugat-Spanyolországban: elterjedése és a kapcsolódó mikoflóra. A *Gremmeniella abietina* konídiumos alakját (*Brunchorstia pinea*) Palencia-ban (Északnyugat-Spanyolország) *Pinus halepensis*-en jelezték először 1999-ben. Jelen tanulmány fő célkitűzése a *G. abietina* előfordulásának meghatározása az első megtaláláshoz közeli területeken, a kórokozó jelenlegi elterjedésének felmérése érdekében. Felvettük a *G. abietina* tüneteit mutató fák mikoflóráját is, a betegség terepi megnyilvánulásában játszott esetleges szerepének megismerése érdekében. A kitenyésztést nedves és meleg körülmények közötti inkubáció során a szövetekben kifejlődő termőtestekből végeztük. A *G. abietina* a vizsgált 40 állomány közül ötben fordult elő. További 25 állományban a *G. abietina* tüneteihez hasonlókat találtunk. A növényi részekből 22 gombafajt tenyésztettünk ki. A *Thyriopsis halepensis*, *Sclerophoma pythiophila* és *Cenangium ferruginosum* fajokat gyakran izoláltuk károsodott növényi részekből (az összes állomány 70%-ában). Ezek a gombafajok részt vehetnek az eredetileg csak a *G. abietina*-nak tulajdonított terepi tünetek kialakulásában. A korábban a *Pinus*-ok másodlagos kórokozóiként ismert *Lophodermium pinastri*, *Naemacyclus niveus* és *Pestalotia stevensonii* fajokat is alkalmanként megtaláltuk.

Brunchorstia pinea* / *Cenangium ferruginosum* / *Pinus halepensis* / *Sclerophoma pythiophila* / *Thyriopsis halepensis

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1 INTRODUCTION

Gremmeniella abietina (anamorph *Brunchorstia pinea*) is an ascomycete fungus that causes stem canker and shoot blight on many conifer species (Donaubauer 1972). The pathogen has been responsible for the destruction of many plantations in North and Central Europe, North America and East Asia in recent decades (Yokota et al. 1974, Dorworth 1979, Kaitera – Jalkanen 1992, 1994). In Spain *G. abietina* was first reported on *Pinus pinaster* causing seedling mortality in 1929 (Martinez 1933). It was not recorded again until 1999, when dieback caused by *G. abietina* was seen on *Pinus halepensis* (Santamaría et al. 2003), tree species that is currently undergoing a severe decline in Spain.

Since then, several studies about this fungus have been conducted in Spain: in both physiological and morphological experiments (Santamaría et al. 2004), it was determined that Spanish isolates of *G. abietina* developed well on malt agar added with pine needle extract at 15 ºC. The length and the width of those isolates ranged between 10.7-44.8 μm and 1.5-4.4 μm, and most of them had 3 septa. The results also suggested that the isolates from Spain do not belong to the Alpine biotype, and the disease symptoms caused by these isolates resembled those of the European biotype. The isolates from Spain were also genetically characterized (Santamaría et al. 2005) using RAPD markers and comparison of RAPD patterns for Spanish isolates and those originating from different regions of Europe and North America. The results showed that the Spanish isolates represent the European race of *Gremmeniella abietina* var. *abietina* and not the Alpine or Northern biotype. Spanish isolates appeared to be clearly separated from all other biotypes within the EU race and preliminary data suggested that Spanish isolates have low genetic variability.

In inoculation tests (Santamaría et al. 2006) performed on the more representative conifer species from Spain, Spanish isolates of *G. abietina* were shown to be pathogen on seedlings of all the pine species tested. *P. halepensis* were consistently the most susceptible one, although it is important to take into account that all the isolates used in the experiments were isolated from *P. halepensis*, suggesting a certain host specificity of *G. abietina*. The susceptibility of the other pine species was regarded with the age of the seedlings. In a preliminary study, it was observed that fungi *Sclerophoma pythiophila* and *Cenangium ferruginosum* were frequently recovered from diseased twigs by *G. abietina*; then, it was decided to study the interactions between *Gremmeniella abietina* and both fungi (Santamaría et al. 2007) in order to evaluate the role that each fungus plays in the symptom expression observed in the field. The results of that study suggested that, even though it can be considered as a weak pathogen, *S. pythiophila* might be involved in disease symptoms caused by *G. abietina* on pine trees in Spain, since it was able to cause damage on *P. halepensis* seedlings and, more importantly, it was able to increase the damage severity caused by *G. abietina* on plants. On the contrary, *C. ferruginosum*, which was not able to damage healthy seedlings, showed antagonism against *G. abietina*.

Finally, several control strategies, including fungicides, fungal endophytes and fungal filtrates, were evaluated *in vitro* (Santamaría et al. in press) as a first step to develop a management control programme against the pathogen before a potential spread of the disease. In that study, chlorothalonil and chlorothalonil-carbendazim would be the most suitable fungicides at low doses to reduce growth of *G. abietina* isolates from Spain. Results also indicated that four of the endophytes tested *in vitro* showed a strong antagonistic activity against *G. abietina* which deserve further testing *in vivo*.

Since the pathogen was observed in 1999 in Palencia (North-west Spain), new records of *G. abietina* have not been made in another areas of Spain yet, therefore the actual distribution of the pathogen in Spain is unknown. In that way, to evaluate the actual spread level of the pathogen, the main aim of the present study was to determine the distribution of *G. abietina* in areas next to that where it was first recorded. Fungi occurring in plant fragments collected

from trees showing symptoms of *G. abietina* was also recorded with the goal of discussing the possible influence they could play in the disease expression observed in the field.

2 MATERIAL AND METHODS

2.1 Sampling

Samples were collected at forty *P. halepensis* stands in the north-west of Spain at altitudes between 800-900 m in transitional areas, where both evergreen sclerophyll broad-leaf and coniferous forest occur within the temperate zone, and the soil is less than 50 cm thick containing limestone with basic pH (> 7). Both hot and dry summers, and a lot of frost days in winter (about 60 per year) are common, but snow is rare in these areas. The localization, altitude and some topographic characteristics of the forty sampling stands are given in *Table 1*. Stands were sampled twice, in spring and summer 2001, and samples were collected from a total of 160 trees (4 trees per stand). Within each stand, trees were chosen among those showing typical symptoms of *G. abietina*: drying up of needles and branches with some distortion of terminal twigs, and dieback. From each tree, 2- to 3-yr-old recently diseased twigs, located at 3-4 m above the ground, were collected from the periphery of the canopy. The samples were brought to the laboratory, stored at 4ºC and processed within 24 h.

2.2 Fungal isolation and identification

From each tree, six twig segments (0.5 cm diam., 0.5-1 cm thick, including bark) and six needles were randomly selected and processed according to the moist chamber method, as it has been suggested in previous mycoflora studies (Santamaría - Diez 2005). The method consisted of finding fruitbodies on plant tissues (twigs and needles) after incubating them, within Petri dishes with wet paper, at room temperature (22ºC ± 2ºC) in diffused daylight until fruitbody production. The samples used in this method were not surface sterilised in order to find endophytes as well as fungal epiphytes. Cultures were identified according to morphological characteristics.

2.3 Statistical data analyses

The comparison between the species distribution of each sampling stand was made using a cluster analysis, with the statistical package STATISTICA´ 99, STATSOFT®, Ink. (Tulsa, OK. USA). To construct the dendogram, levels of similarity between the stands were calculated by using Dice coefficient (Dice 1945) and the cluster analysis of similarity matrices was calculated with the unweighted pairgroup method with arithmetic averages (UPGMA). For the comparison among the fungal species recovered in each stand a correspondence analysis was applied by means of 'corresp procedure' of SAS statistical package (Anonymous 1989).

3 RESULTS

The fungal species isolated from *Pinus halepensis*, as well as the stands where they were recovered, are shown in Table 2. *G. abietina*, in its anamorphic state *Brunchorstia pinea*, was recorded in five out of a total of 40 stands that were examined, although in another 25 stands, similar symptoms to those caused by *G. abietina* were observed (*Table 1*). In addition to that, another 22 fungal species were isolated from plant fragments. From the total, four taxa, *Thyriopsis halepensis*, *Sclerophoma pythiophila*, *Alternaria* sp. and *Cenangium ferruginosum* were very frequently isolated (in more than 70% of the stands) whilst seven fungal species,

Dichomera sp., *Fusarium* sp., *Hendersonia acicola*, *Hysterographium elongatum*, *Pithomyces chartarum*, *Rhizopus stolonifer*, and *Trichoderma viride* (*Table 2*) were isolated in a quite low frequency (lower than 10% of the stands). Another species, like *Cladosporium* sp., *Lophodermium pinastri* (it was recorded in both teleomorphic and anamorphic state *Leptostroma pinastri*) and *Trichothecium roseum*, were also frequently isolated.

Table 1. Sampling stands location and description

Stand	Location	***UTM coordinates***		***Altitude (m)***	Slope	Cardinal direction	*G. abietina* symptoms
		X	Y				
00-VAL	Valle del Cerrato	4640475	386450	880	high	South	20
01-MEL	Melgar de Yuso	4676700	393775	850	medium	West	5
02-SAN	Santoyo	4674300	390450	810	medium	North	15
03-AST	Astudillo	4673075	398625	790	high	West	5
04-AST	Astudillo	4670250	391025	810	medium	South-East	0
05-AST	Astudillo	4668950	392100	860	high	East	5
06-AST	Astudillo	4671775	393550	818	high	North	15
07-PAL	Palencia	4655800	373500	810	medium	South- West	15
08-PAL	Palencia	4649575	368725	825	high	South-East	5
09-AST	Astudillo	4647465	390225	845	medium	North-East	10
10-AMU	Amusco	4665625	381900	860	medium	South-East	0
13-VIL	Villamediana	4655075	385075	840	Low	South-West	0
14-VAL	Valdeolmillos	4655925	382975	825	medium	West	10
15-VLB	Villalobón	4653775	377825	835	high	West	15
16-TOR	Torquemada	4653525	397425	820	high	South-West	0
18-VLH	Villahán	4657800	406650	825	medium	West	10
19-TAB	Tabanera de Cerrato	4652625	409675	855	high	South	10
20-VLM	Villamuriel de Cerrato	4647375	372175	856	high	East	0
21-TOR	Torremormojón	4644175	352700	820	high	West	10
22-AMP	Ampudia	4643425	356100	845	high	West	15
23-VLM	Villamuriel de Cerrato	4642700	371075	865	flat	North-East	0
24-AMP	Ampudia	4642475	354650	830	high	North-East	15
27-REI	Reinoso de Cerrato	4647725	385750	824	high	North-West	5
28-BAL	Baltanás	4646850	395275	874	high	North-East	15
29-BAL	Baltanás	4643075	398075	820	high	South-West	10
30-TAR	Tariego de Cerrato	4638675	379600	861	high	South-West	0
31-CEV	Cevico de la Torre	4637375	386475	835	high	South	0
32-VAL	Valle del Cerrato	4638450	388075	845	medium	South	5
33-CAS	Castrillo de Onielo	4635825	393100	885	high	South-West	0
34-VER	Vertavillo	4633650	389175	800	low	North-East	15
35-TAB	Tabanera de Cerrato	4649450	406325	887	high	South-West	20
36-CUB	Cubillas de Cerrato	4630850	378225	840	high	South-West	15
37-POB	Población de Cerrato	4630475	382125	857	high	South	15
38-ALB	Alba de Cerrato	4628600	387675	838	high	South-West	5
40-VER	Vertavillo	4630750	397100	830	high	South	15
41-HER	Hérmedes de Cerrato	4631200	399325	860	high	South	5
42-POB	Población de Cerrato	4626675	381825	835	high	South-West	10
44-HER	Hérmedes de Cerrato	4630775	403225	880	high	South	0
45-CAS	Castrillo de Don Juan	4627575	410800	905	high	South-West	20
out1	Hontoria de Cerrato	4639825	382900	790	high	South-West	15

Stand.- Code to designate each stand.
Location.- Village the stand is located in.
UTM coordinates.- Universal Transverse Mercator, UTM, coordinates (in meters).
A.- Altitude, in meters above sea level, of each sampling site.
G. abietina symptoms.- Approx. percentage of the total stand area showing symptoms similar to those caused by *G. abietina* (drying up of needles and branches with some distortion of terminal twigs, and dieback).

In all the stands where *G. abietina* was recovered, three species, *T. halepensis*, *S. pythiophila* and *C. ferruginosum*, were consistently isolated too (*Table 2*). This fact was confirmed by the correspondence analysis (*Figure 1*). The plot representing the first two dimensions of the model (which explain 17.03% and 12.36% respectively of the total

variance), showed the scores corresponding to those four species (*G. abietina*, *T. halepensis*, *S. pythiophila* and *C. ferruginosum*) to be very related in some way, since all of them were found in the negative quadrant of both dimensions.

Table 2. Fungal species recovered from twigs and needles of Pinus halepensis *and stands where they occurred.*

Fungi	N[1]	Stands where fungus was recovered
Alternaria complex.	34	01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 08-PAL, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 22-AMP, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 45-CAS, OUT1
Brunchorstia pinea (Karst.) Höhn.	5	00-VAL, 09-AST, 15-VLB, 24-AMP, 41-HER,
Camarosporium propinquum (Sacc.) Sacc.	10	01-MEL, 07-PAL, 14-VAL, 20-VLM, 21-TOR, 24-AMP, 28-BAL, 34-VER, 36-CUB, 37-POB.
Cenangium ferruginosum Fr.: Fr.	28	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 07-PAL, 08-PAL, 09-AST, 10-AMU, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 24-AMP, 28-BAL, 29-BAL, 31-CEV, 32-VAL, 34-VER, 35-TAB, 36-CUB, 37-POB, 40-VER, 41-HER, 45-CAS, OUT1
Cladosporium sp.	19	01-MEL, 02-SAN, 03-AST, 06-AST, 07-PAL, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 22-AMP, 24-AMP, 27-REI, 32-VAL, 34-VER, 36-CUB, 37-POB, 38-ALB, 42-POB
Cytospora sp.	14	00-VAL, 03-AST, 06-AST, 15-VLB, 18-VLH, 20-VLM, 28-BAL, 31-CEV, 32-VAL, 33-CAS, 36-CUB, 37-POB, 38-ALB, 42-POB
Dichomera sp.	2	13-VIL, 15-VLB
Epicoccum nigrum Link	5	01-MEL, 14-VAL, 24-AMP, 36-CUB, 37-POB
Fusarium sp.	1	14-VAL
Gonatobotrys sp.	9	04-AST, 06-AST, 13-VIL, 14-VAL, 27-REI, 32-VAL, 36-CUB, 37-POB, 42-POB
Hendersonia acicola Münch et Tub.	4	00-VAL, 01-MEL, 02-SAN, 04-AST
Hysterographium elongatum (Wahl.) Corda	2	13-VIL, 38-ALB,
Leptostroma pinastri (Desm.)	19	00-VAL, 02-SAN, 03-AST, 05-AST, 06-AST, 13-VIL, 14-VAL, 18-VLH, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 42-POB, OUT1
Lophodermium pinastri (Schard. ex Hook.) Chev.	19	00-VAL, 02-SAN, 03-AST, 05-AST, 06-AST, 13-VIL, 14-VAL, 18-VLH, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 42-POB, OUT1
Naemacyclus niveus (Pers. ex Fr.) Fuck. ex Sacc.	5	03-AST, 14-VAL, 15-VLB, 35-TAB, 36-CUB
Penicillium sp.	14	00-VAL, 01-MEL, 02-SAN, 03-AST, 05-AST, 06-AST, 10-AMU, 15-VLB, 30-TAR, 31-CEV, 32-VAL, 34-VER, 38-ALB, 45-CAS
Pestalotia stevensonii Peck	5	00-VAL, 03-AST, 06-AST, 13-VIL, 32-VAL
Pithomyces chartarum (Berk. & Curt) M. B. Ellis	1	06-AST
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	1	22-AMP
Sclerophoma pythiophila (Corda)	35	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 09-AST, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 18-VLH, 19-TAB, 21-TOR, 22-AMP, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 45-CAS, OUT1
Thyriopsis halepensis (Ck.) Theiss y Syd.	40	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 08-PAL, 09-AST, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 16-TOR, 18-VLH, 19-TAB, 20-VLM, 21-TOR, 22-AMP, 23-VLM, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 44-HER, 45-CAS, OUT1
Trichoderma viride Pers.: Fr.	3	36-CUB, 37-POB, 38-ALB
Trichothecium roseum (Pers.: Fr.) Link	20	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 08-PAL, 10-AMU, 13-VIL, 15-VLB, 18-VLH, 22-AMP, 27-REI, 32-VAL, 34-VER, 36-CUB, 37-POB, 38-ALB, 42-POB

[1] - Number of stands where the fungus was recovered

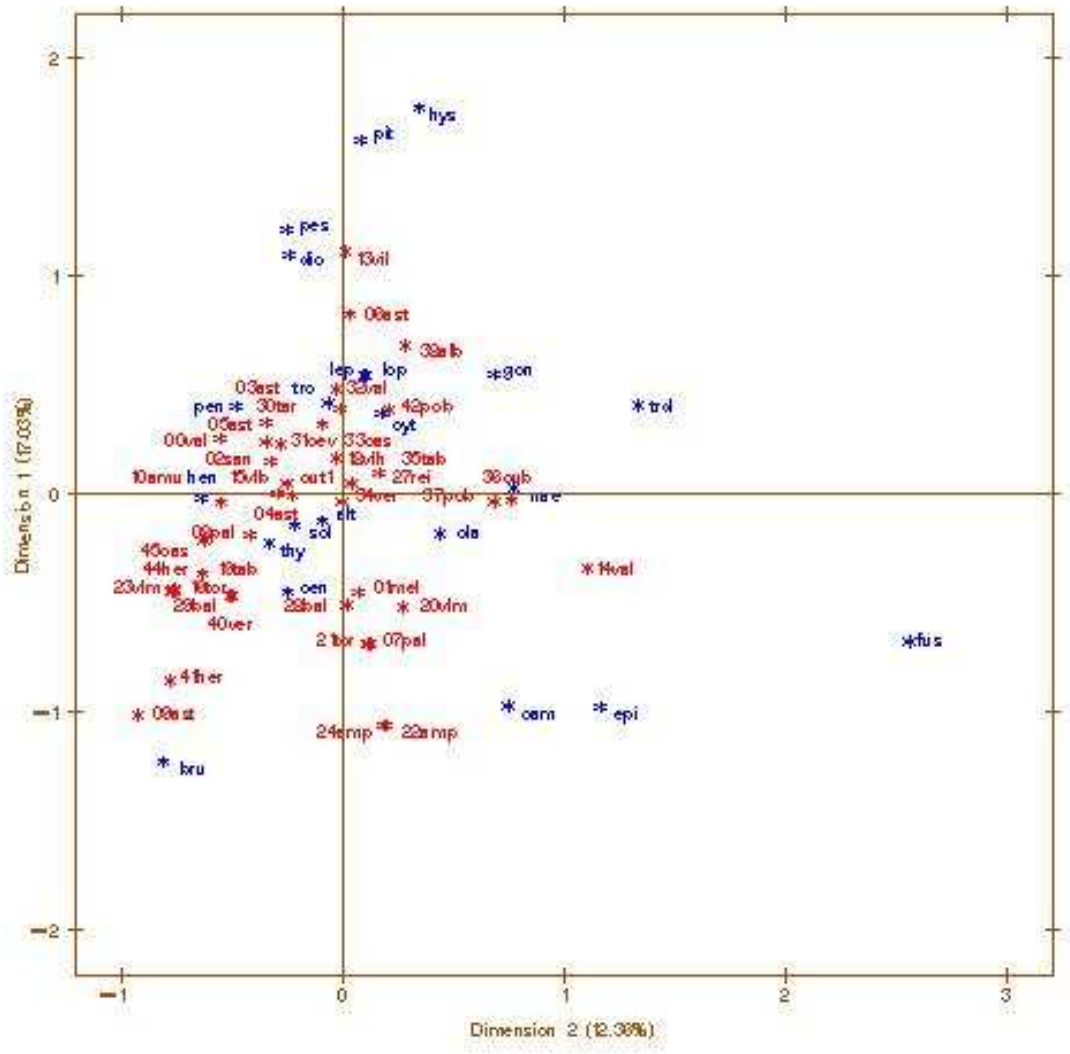

Figure 1. Plot of the first and second dimension scores from a correspondence analysis based on species distribution from each stand.
Fungi are designed in the plot as the three first letters of the species latin name

The number of fungal species recovered per stand ranged between 1 (in 16-TOR, 23-VLM and 44-HER) and 14 (in 36-CUB stand). In 32.5% of the total stands were recovered more than nine fungal species, whilst in 20% only were found less than five taxa. The stands, where a lower number of species was recovered, corresponded to those showing good forest health and grouped together in the cluster analysis (*Figure 2*). Only *T. halepensis* and *S. pythiophila* were found in such stands. However, in general terms, no correlation was found between species richness and cluster grouping, as well as no correlation was observed between topographic conditions of the stands and the fungal species distribution recovered. The stands where *G. abietina* was recovered are not very related among them with regard species distribution, as it is shown in dendrogram (*Figure 2*). Nevertheless, in the correspondence analysis, scores corresponding those stands were located in the negative part of the dimension 2.

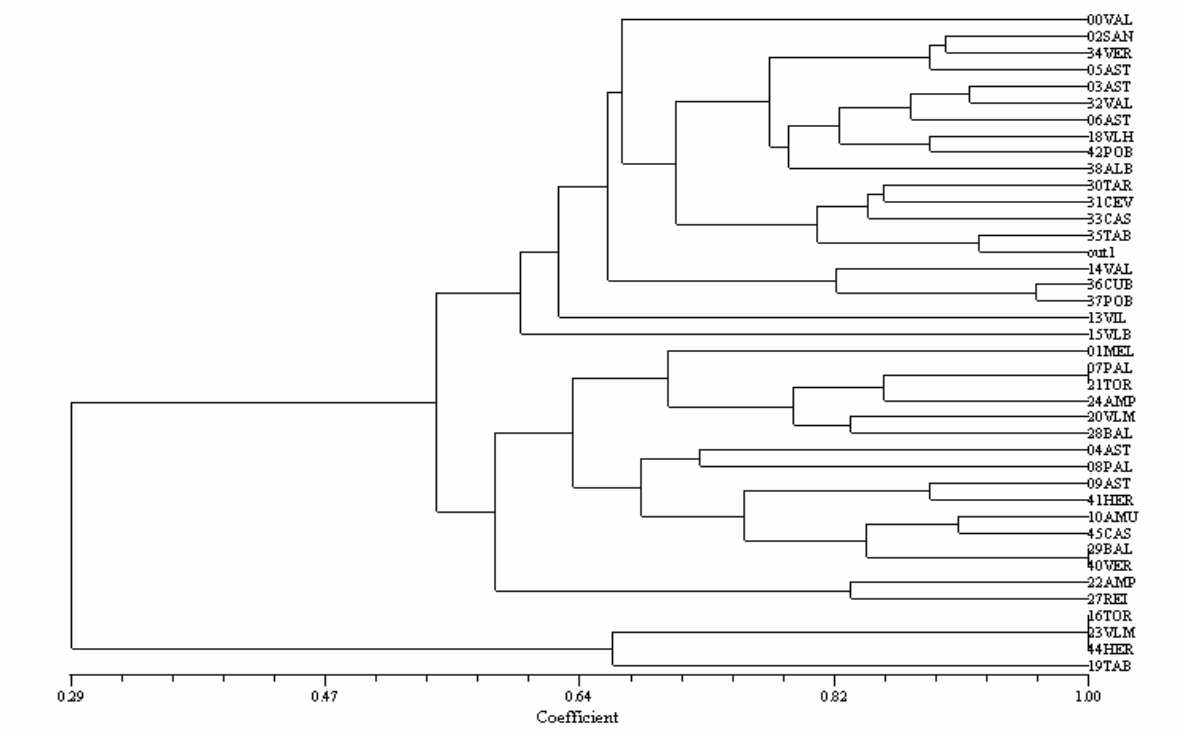

Figure 2. Dendrogram showing the similarity among the stands regarding to the species distribution

4 DISCUSSION

Thirty stands showed symptoms similar to those described in previous works for the European race of *G. abietina* (Uotila 1983, 1993, Virtanen et al. 1997, Santamaría et al. 2006); however, *G. abietina* only was recovered from five out of these 30 stands. To explain this fact, two possibilities are suggested: the first one might consist of that *G. abietina* was indeed the primary pathogen that caused the disease observed in the field, but later, either secondary pathogens or opportunist fungi colonized the necrotic tissues making it difficult to isolate the primary agent. The other possibility might be that symptoms observed in the field were not as specific to *G. abietina* as initially thought and they were caused by another fungal pathogen alone or by a combination of several fungi (including *G. abietina*).

In this sense, the only fungi recovered in almost all of the stands showing those symptoms were *T. halepensis*, *S. pythiophila*, *Alternaria* and *C. ferruginosum*. Among those, *Alternaria* complex is known as saprobe and ubiquitous; therefore, it is not very probably its implication in the symptoms expression. *T. halepensis* has previously shown a pathogenic behaviour on pine needles (Muñoz et al. 2003), so it could play a certain role on the defoliation observed in diseased trees. The other two fungi, *S. pythiophila* and *C. ferruginosum*, have been shown to be generally weak pathogens on several conifers (Brener et al. 1974, Phillips – Burdekin 1992), although *C. ferruginosum* has also been reported to cause severe damage on pine species (Koiwa et al. 1997). Furthermore, they have been found associated to *G. abietina* infections so frequently (Dorworth 1971, Barklund 1989, Duda – Sierota 1997) that *C. ferruginosum* was even thought to be the teleomorphic state of *Brunchorstia pinea* early last century (Dorworth 1971).

In addition to that, in a previous work (Santamaría et al. 2007), it was observed that *S. pythiophila* was able to cause damage on healthy seedlings but inoculated by wounding and, what is more, it was able to increase the damage severity caused by *G. abietina* on plants

of *P. halepensis*. This is in agreement with results obtained in the present study, as it is observed in the correspondence analysis plot where scores, representing these fungi, are grouped together. Therefore, these four fungi, *T. halepensis*, *S. pythiophila*, *G. abietina* and *C. ferruginosum*, could be involved in some way in disease symptoms observed in the field and initially attributed exclusively to *G. abietina.*

From the rest of fungal species listed in *Table 2*, several of them are well-known pine pathogens and therefore they could cause damage on *P. halepensis* if climatic conditions turn adverse to the plant and increase their populations. *Lophodermium pinastri*, which has been previously reported as a pine needle pathogen (Lanier et al. 1978, Phillips – Burdekin 1992), was frequently isolated in many stands. *Naemacyclus niveus* and *Pestalotia stevensonii* have been shown to be secondary pathogen on pine needles (Lanier et al. 1978, Phillips – Burdekin 1992), although, they were isolated at a low level.

Differences in the species richness among stands could be explained by the different forest health conditions among them, as it has been widely stated by other authors (Petrini et al. 1989, Bettucci et al. 1999, Frohlich et al. 2000) that dead or dying tissues can be usually colonised by a highest number of fungal species than the healthy or slightly damaged ones. In addition to that, the effect that the local environmental conditions have on the species richness, has been already stated on diverse tree species (Elamo et al. 1999, Ragazzi et al. 2003).

5 CONCLUSION

In conclusion, *Thyriopsis halepensis*, *Sclerophoma pythiophila* and *Cenangium ferruginosum*, which were very frequently recovered from almost all the stands, could be involved, in adittion to *G. abietina*, in the disease symptoms expression observed in the field, which were initially attributed exclusively to *G. abietina*.

REFERENCES

ANONYMOUS (1989): SAS/STAT® User´s guide. Version 6, 4th edn., Vol. 1-2. Cary, NC: SAS Institute Inc.

BARKLUND, P. (1989): Occurrence of and interaction between *Gremmeniella abietina* and endophytic fungi in two conifers. Ph. D. Thesis. Swedish University of Agricultural Sciences. Dept. of Forest Mycology and Pathology. Uppsala, Sweden.

BETTUCCI, L. – ALONSO, R. – TISCORNIA, S. (1999): Endophytic mycobiota of healthy twigs and the assemblage of species associated with twig lesions of *Eucalyptus globolus* and *E. grandis* in Uruguay. Mycol. Res. 103: 468-472.

BRENER, W. D. – SETLIFF, E. C. – NORGREN, R. L. (1974): *Sclerophoma pythiophila* associated with a tip dieback of Juniper in Wisconsin. Plant Dis. Rep. 58: 653-657.

DICE, L. R. (1945): Measures of the amount of ecologic association between species. Ecology 26: 297–302.

DONAUBAUER, E. (1972): Distribution and hosts of *Scleroderris lagerbergii* in Europe and North America. Eur. J. For. Path. 2: 6-11.

DORWORTH, C. E. (1971): Diseases of conifers incited by *Scleroderris lagerbergii* Gremmen., a review and analysis. Can. For. Serv. Publ. 1289.

DORWORTH, C. E. (1979): Influence of inoculum concentration on infection of red pine seedlings by *Gremmeniella abietina*. Phytopathology 69: 298-300.

DUDA, B. – SIEROTA, Z. (1997): Diseases caused by *Gremmeniella abietina* (Lagerb.) Schlapfer – Bernhard and *Cenangium ferruginosum* Fr. ex Fr. in Scots pine (*Pinus sylvestris* L.) stands in Poland. In: Foliage, Shoot and Stem Diseases of Trees. Proc Meet. IUFRO Working Party

7.02.02, Quebec City, Canada. May 25-31, 1997. Ed. by LAFLAMME, G. - BÉRUBÉ, J. A. - HAMELIN, R. C.: Can. For. Serv. 90-94.

ELAMO, P. – HELANDER, M. L. – SALONIEMI, I. – NEUVONEN, S. (1999): Birch family and environmental conditions affect endophytic fungi in leaves. Oecologia 118: 151-156.

FROHLICH, J. – HYDE, K. D. – PETRINI, O. (2000): Endophytic fungi associated with palms. Mycol. Res. 104: 1202-1212.

KAITERA, J. – JALKANEN, R. (1992): Disease history of *Gremmeniella abietina* in a *Pinus sylvestris* stand. Eur. J. For. Path. 22: 371-378.

KAITERA, J. – JALKANEN, R. (1994): The old and fresh damage of Scots pine by *Gremmeniella abietina* in eastern Lapland in 1992. Silva Fenn. 28: 107-113.

KOIWA, T. – SAKUYAMA, T. – TAKAHASHI, K. (1997): Damage to Japanese pines caused by *Cenangium ferruginosum* in northern Honshu, Japan. In: Foliage, Shoot and Stem Diseases of Trees. Proc Meet. IUFRO Working Party 7.02.02, Quebec City, Canada. May 25-31, 1997. Ed. by LAFLAMME, G. - BÉRUBÉ, J. A. - HAMELIN, R. C.: Can. For. Serv. 95-102.

LANIER, L. – JOLY, P. – BONDOUX, P. – BELLEMERE, A. (1978): Mycologie et pathologie forestieres. Vol. I. Paris: Masson.

MARTÍNEZ, J. (1933): Una grave micosis del pino observada por primera vez en España. [A serious pine disease observed for the first time in Spain]. *Boletín de la Sociedad Española de Historia Natural* 33: 25-9. (In Spanish)

MUÑOZ, C. – PÉREZ, V. – HERNÁNDEZ, R. – SÁNCHEZ, G. (2003): Sanidad Forestal. Guía en imágenes de plagas, enfermedades y otros agentes presentes en los bosques. [Forest Health. Guide to pest, diseases and abiotic damages of Spanish forests]. Madrid: Ediciones Mundiprensa (In Spanish)

PETRINI, L.E. – PETRINI, O. – LAFLAMME, G. (1989): Recovery of endophytes of *Abies balsamea* from needles and galls of *Paradiplosis tumifex*. Phytoprotection 70: 97-103.

PHILLIPS, D.H. – BURDEKIN, D.A. (1992): Diseases of Forest and Ornamental Trees. London: The MacMillan Press LTD.

RAGAZZI, A. – MORICCA, S. – CAPRETTI, P. – DELLAVALLE, I. – TURCO, E. (2003): Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. For. Path. 33: 31-38.

SANTAMARÍA, O. – DIEZ, J. J. (2005): Fungi in leaves, twigs and stem bark of *Populus tremula* from northern Spain. For. Path. 35: 95-104.

SANTAMARÍA, O. – ALVES-SANTOS, F.M. – DIEZ, J.J. (2005): Genetic characterization of *Gremmeniella abietina* var. *abietina* isolates from Spain. Plant Pathol. 54: 331-338.

SANTAMARÍA, O. – GONZÁLEZ, M. A. – PAJARES, J. A. – DIEZ, J. J. (in press): Effect of fungicides, endophytes and fungal filtrates on in vitro growth of *Gremmeniella abietina* from Spain. For. Path.

SANTAMARÍA, O. – PAJARES, J. A. – DIEZ, J. J. (2003): First report of *Gremmeniella abietina* on *Pinus halepensis* in Spain. Plant Path. 52: 425.

SANTAMARÍA, O. – PAJARES, J. A. – DIEZ, J. J. (2004): Physiological and morphological variation of *Gremmeniella abietina* from Spain. For. Path. 34: 395-495.

SANTAMARÍA, O. – PANDO, V. – DIEZ, J. J. (2006): Susceptibility of six pine species to *Gremmeniella abietina* isolates from Spain.. For. Path. 36: 349-359.

SANTAMARÍA, O. – TEJERINA, L. – PAJARES, J. A. – DIEZ, J. J. (2007): Effects of associated fungi *Sclerophoma pythiophila* and *Cenangium ferruginosum* on *Gremmeniella abietina* dieback in Spain. For. Path. 37: 121-128.

UOTILA, A. (1983): Physiological and morphological variation among Finnish *Gremmeniella abietina* isolates. Commun. Inst. For. Fenn 119: 1-12.

UOTILA, A. (1993): Genetic variation of *Gremmeniella abietina* in Finland. Metsäntutk. Tied. 451: 119-122.

VIRTANEN, T. – RANTA, H. – NEUVONEN, S. (1997): Shoot-feeding aphids promote development of *Gremmeniella abietina*, the fungal pathogen causing Scleroderris canker disease in conifers. J. Phytopathology. 145: 245-251.

YOKOTA, S. – UOZUMI, T. – MATSUZAKI, S. (1974): Scleroderris canker of Todo-fir in Hokkaido, Nothern Japan. II. Physiological and pathological characteristics of the causal fungus. *European Journal of Forest Pathology* 4: 155-166.