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Original article

Ophiostomatoid fungi associated with the spruce bark beetle, *Ips typographus*, in three areas in France

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Abstract – The species composition of ophiostomatoid fungi associated with *Ips typographus* was studied in the Vosges, Alps and Massif Central regions of France. In each region, damage caused by bark beetles has increased during recent years. For this study, beetles were collected individually by hand from freshly attacked trees and crushed in healthy *Picea abies* logs. Fungi were isolated from log phloem and sapwood, and identified. The most frequently found species were *Ophiostoma bicolor*, *O. penicillatum*, *Ceratocystiopsis minuta* and *Ceratocystis polonica*. Results are discussed in terms of differences between locations and in relation to previous investigations in which populations of spruce bark beetle have been sparse. The potential role of associated fungi in the population dynamics of the spruce bark beetle is discussed.

associated fungi / *Ceratocystis polonica* / *Ips typographus* / *Ophiostoma* / *Picea abies*

Résumé – Champignons Ophiostomatoides associés au scolyte de l'épicéa, *Ips typographus*, dans trois régions françaises. La composition spécifique des champignons Ophiostomatoides associés à *Ips typographus* a été étudiée dans trois régions françaises, Vosges, Alpes et Massif Central, dans lesquelles les dommages dus aux Scolytes s'étaient récemment accrus. Les insectes ont été récoltés individuellement sur des arbres fraîchement attaqués, puis écrasés dans des rondins frais d'épicéa. Les champignons ont ensuite été isolés du liber et de l'aubier des rondins, et identifiés. Les espèces les plus fréquemment rencontrées ont été *Ophiostoma bicolor*, *O. penicillatum*, *Ceratocystiopsis minuta* et *Ceratocystis polonica*. Les résultats sont discutés en termes de différences entre localités, et en liaison avec des investigations plus anciennes réalisées sur des populations éparses. Le rôle potentiel des champignons associés dans la dynamique des populations d'*I. typographus* est discuté.

champignons associés / *Ceratocystis polonica* / *Ips typographus* / *Ophiostoma* / *Picea abies*

1. INTRODUCTION

European spruce (*Picea* spp.) forests suffer regularly from extensive outbreaks of the Eurasian spruce bark beetle *Ips typographus* L. (Coleoptera: Scolytidae). During recent years, Eurasian spruce bark beetles together with associated pathogenic fungi have killed millions of cubic metres of spruce in western and central Europe. In north-eastern France alone, the damage has been as high as 100 000 m^3 in 1991, 212 500 m^3 in 1992 and 113 000 m^3 in 1993 [1, 27, 28]. Severe beetle damage often follows heavy storm damage and windfall, e.g. as a result of the severe windstorm in December 1999.

Adults of the spruce bark beetle transport spores of blue-staining fungi in the pronota, elytra and digestive tract [7]. When building breeding chambers and galleries, spruce bark beetles introduce the spores of *Ophiostoma* and *Ceratocystis* species into the phloem and cambium of Norway spruce, *Picea abies* (L.) Karsten. Together with the associated fungi, spruce bark beetles can overcome the resistance of vigorous spruce trees. In the most harmful species, *Ceratocystis polonica* (Siemaszko) Moreau, pathogenicity is based on its ability to grow rapidly through the tracheids of moist wood and to disrupt water transport in the tree, finally leading to high levels of mortality [3, 11, 17, 20, 32, 33].

The aim of this investigation was to describe the ophiostomatoid fungi associated with *I. typographus* in France where there have been no extensive surveys. A further aim was to compare the fungal flora associated with spruce bark beetles

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Table I. Study areas used for collection of *I. typographus*.

Location	Forest	Elevation (m.s.l.)	Stand age (yrs)
Col de Praye, Vosges	Val de Senones	910	130
Tête de Nayemont, Vosges	Vologne	840	130
St Pierre de Belleville, Savoie	St Pierre de Belleville	1350	150
St Michel de Maurienne, Savoie	piles	450	150
Boussoulet, Haute-Loire	Meygal	1300	120
Mont d'Alambre, Haute-Loire	Mézenc	1480	120

collected from different regions. This information will provide us with useful details that will help us understand the role of associated fungi as possible regulators of bark beetle epidemics.

2. MATERIALS AND METHODS

2.1. Study areas

Beetles were collected at the beginning of the main swarming period of the first generation, in late May and early June 1996, from three regions in France: Vosges, Alps and Massif Central (Fig. 1 and Tab. I). Two locations in each region were selected on the basis of previously large populations of beetles, and 50 beetles were collected at each location. At all locations, extensive damage due to spruce bark beetles occurred in 1990–1995 [1, 2]. In 1991–1995 in Vosges, where two generations occur each year, the volume of dead Norway spruce varied between 1 200 and 5 900 m^3. In 1995, beetles were collected in pheromone traps and the total catch for three pheromone traps was 2 219 spruce bark beetles, thus indicating a declining trend (Office National des Forêts, Raon l'Étape). In Massif Central, at the Mézenc collecting site, the high altitude reduces reproduction and only one generation of spruce bark beetles occurs annually. In Meygal, depending on weather conditions, 1–2 generations occur per year.

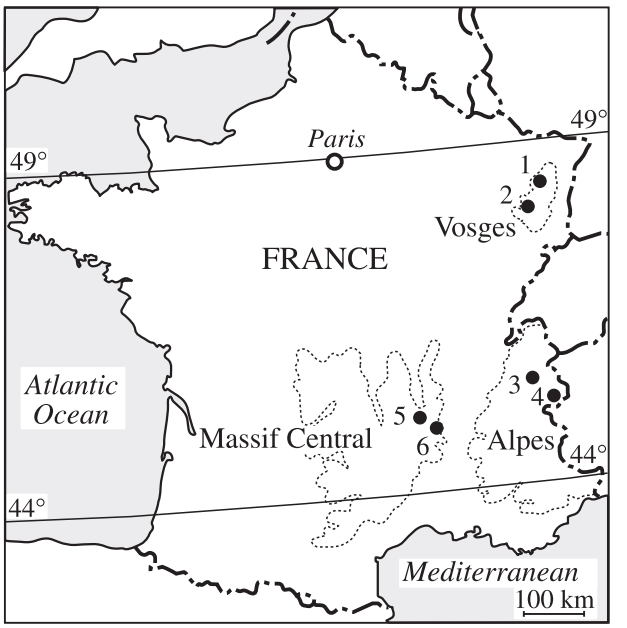

Figure 1. Location of the *I. typographus* collecting areas. 1 = Val de Senones, 2 = Vologne, 3 = St Pierre de Belleville, 4 = St Michel de Maurienne, 5 = Meygal, 6 = Mézenc.

2.2. Collecting beetles

At all locations, except St Michel de Maurienne, beetles were collected individually by digging out adult females and males with a knife and forceps from windblown Norway spruce trunks lying in the forest. In St Michel de Maurienne, beetles were collected in Norway spruce trunks lying in a timber yard. The beetles were placed individually into sterile Eppendorf-test tubes. The equipment used for collection was sterilized after extraction of each individual. The logs had fallen during the previous winter and the beetles had just started to build galleries in them. The construction of nuptial chambers was mostly completed, and the mother galleries, which were less than 4 cm long, had been initiated. The collected beetles were stored individually at +4 °C in Eppendorf-test tubes for a maximum of three days before they were introduced into logs.

2.3. Inoculation, isolation and identification of fungi

Fungi were pre-cultivated in fresh uninfected Norway spruce bolts (one metre long, diameter 15 cm) according to the method described previously by Furniss et al. [7]. The bolts were brushed and the surfaces wiped with 70% alcohol. To prevent drying, the ends of the bolts were dipped in melted paraffin. Then 25 beetles were introduced individually into each log to the level of the cambium through holes (5 mm diameter) bored previously with a cork-borer. After the beetle was introduced, the bark plugs were replaced and the beetles were crushed gently. In each log two control holes without beetles were made and treated similarly.

After 21 days of incubation at room temperature (+20 °C), reaction zones formed with phloem around each inoculation point. These reaction zones were then cut from the logs, wrapped in foil and stored at +4 °C for two weeks until used for isolations. Two phloem samples (50–60 mm^3) were taken from inside each necrotic zone, one at the border of the visible reaction and one 15 mm from the border. Two samples were also taken from a depth of 1 mm in the sapwood. When the reaction zones were less than 20 mm long, all four samples were taken from the edge of the visible reaction zone. When reaction zones were more than 150 mm long, six samples were taken, four from the phloem and two from the sapwood. A total of 1 221 primary samples were taken around the inoculation points.

Samples were cultured in Petri dishes (2% malt and 1.4% agar medium) at room temperature. Occasionally, pieces of fresh autoclaved phloem or sapwood of Norway spruce were added to the dishes to promote formation of sexual stages. The cycloheximide tolerance of one isolate, later identified as *Ophiostoma* sp., was determined on malt extract agar supplemented with 0.1 g L^{-1} cycloheximide [9, 13]. For identification, reproductive structures of the fungi were mounted on a glass slide in lacto-fuchsin, lactic acid or cotton blue. Fungal structures were compared with the species descriptions given in the literature [4, 5, 10, 15, 16, 25, 30, 31, 36, 40, 41].

2.4. Statistics

Frequencies of ophiostomatoid species were analysed with the Kruskal-Wallis test. Since the observed frequencies of some fungi were skewed or sparse, the data were analysed with StatXact^{TM} Version 2.11 software, a statistical package for exact nonparametric inference [26]. As the data sets were too large for exact calculation of *p*-values, the Monte-Carlo estimates of the *p*-value were computed by generating 100 000 tables. The level of significance in the tests was $p < 0.01$.

Table II. Frequencies of occurrence of ophiostomatoid fungi associated with *I. typographus* collected at six locations in France. Locations presented in Table I. $n = 50$ beetles per location.

	Vosges		Alps		Massif Central	
	Senonne	Vologne	Belleville	Maurienne	Meygal	Mézenc
C. minuta	62	36	36	30	28	24
C. polonica	40	32	22	50	42	30
O. ainoae	2	10	28	12	24	10
O. bicolor	54	74	26	44	66	42
O. cucullatum	0	0	0	0	2	0
O. piceaperdum	20	34	30	10	16	28
O. penicillatum	40	40	24	26	60	40
O. piceae	8	12	10	12	8	2
Ophiostoma sp.	2	0	6	4	8	0
Pesotum spp.	36	28	46	46	50	58
Leptographium sp.	2	2	0	0	0	0
Primary isolations	204	204	202	202	205	204

3. RESULTS

The most common and consistently occurring species were *Ophiostoma bicolor* Davidson and Wells, *O. penicillatum* (Grosm.) Siemaszko, *Ceratocystiopsis minuta* (Siemazko) Upadhyay & Kendrick and *C. polonica*. Other frequently isolated species were *O. piceaperdum* (Rumbold) Arx and *O. ainoae* Solheim (Tab. II). Species that were isolated only occasionally were *O. piceae* (Münch) H. & P. Sydow, *O. cucullatum* Solheim and an unidentified *Ophiostoma* species. There was no visible staining on any of the control inoculations, and no ophiostomatoid fungi were detected in the control inoculations.

When the frequencies of nine ophiostomatoid species were compared simultaneously at six beetle-collection locations, the Kruskal-Wallis analysis of variance indicated a highly significant difference between locations ($\chi^2 = 29.04$, $df = 8$, asymptotic p-value = 0.0003). When the five most frequent species (*C. minuta*, *C. polonica*, *O. bicolor*, *O. piceaperdum* and *O. penicillatum*) were compared, the difference between locations was also significant (locations ($\chi^2 = 16.86$, $df = 4$, asymptotic p-value = 0.0021).

4. DISCUSSION

This was the first time the fungal flora associated with *I. typographus* was studied extensively in France. All isolated ophiostomatoid fungi were found the first time as associates of the spruce bark beetle. Previously *C. polonica*, *O. bicolor*, *O. piceaperdum* and *O. penicillatum* have been reported to be associated with *I. typographus*, occurring with various frequencies in different environmental conditions and investigations [8, 19, 31, 34, 35, 38]. As they cannot be distinguished on the basis of morphology, *O. europhioides* (Wright & Cain) Solheim was recently synonomised with *O. piceaperdum* [14].

The most common and consistently occurring fungus in this study was *O. bicolor*, which in Vologne was recovered from 74% of the bark beetles examined. At nearly all locations, *C. minuta*, *C. polonica*, *O. ainoae*, *O. bicolor*, *O. penicillatum* and *O. piceaperdum* occurred at higher frequencies than recorded from the low population density areas of *I. typographus* [35, 38]. The following ophiostomatoid species have previously been reported to be associated with other *Ips* bark beetles in France: *Ceratocystiopsis minima* (Olchow. and Reid) Upadhyay, *C. minuta*, *C. polonica*, *O. bicolor*, *O. brunneo-ciliatum* Mathiesen-Käärik, *O. europhioides*, *O. ips* (Rumbold) Nannf., *O. piceae* and *O. minus* (Hedgcock) H. & P. Sydow [22–24, 29].

Both *C. polonica* and *O. piceaperdum* have been suggested to play a special role in the population dynamics of the spruce bark beetle [8, 35]. It has been proposed that during endemic periods when beetles utilise dead trees and timber for breeding, pathogenic species can be replaced by less harmful ones. In Norway, the frequency of *C. polonica* has been low during periods of low population level, when beetles use dead trees and timber, whereas the frequency has been higher during the epidemic phase, when living trees are attacked [19, 34, 35]. Our results are in agreement with those suggesting that pathogenic species can be replaced by other species during endemic periods. Furthermore, they support the idea that the role of the associated fungi may differ under different environmental conditions. The previous finding that the frequency of the pathogenic species, *C. polonica* [38], in the endemic population of spruce bark beetle is low does not conflict with the fact that associated pathogenic fungi can regulate the damage by spruce bark beetles.

According to surveys made in previous years in all sampling areas, especially in Vosges and Massif Central, population levels of the spruce bark beetle had been high. This had resulted in numerous spontaneous attacks on spruce trees in these areas. Pheromone trapping, although done only in Vosges, showed declining population size already during the year of beetle sampling. Thus in the present study the isolated fungal flora constantly corresponded to a beetle population in the post-epidemic phase. Quantification of the abundance and distribution of forest

pests is a complex problem in forest health management. An attempt has been made to solve this problem by introducing damage surveys, pheromone trapping and evaluation of data from sales of infested trees [6, 37, 39]. According to Weslien et al. [39], fewer than 15 000 spruce bark beetles in a group of three traps correspond to a low population level. In Denmark, Hübertz et al. [12] caught 3 400–12 000 individuals and in Finland, Valkama et al. [37] caught at most 14 000 individuals per season with a group of three traps during a period when the beetle population was low.

In this study the fungal flora differed significantly between locations. However, according to Yamaoka et al. [41], the technique used to isolate ophiostomatoid fungi from various niches can also greatly affect the frequencies of occurrence. Thus when results are compared to those of other authors, discrepancies in fungal frequencies may be partly due to differences in methods of sampling and isolation.

Owing to conflicting results concerning frequency and pathogenicity [8, 18, 21], genetic variation within the species *O. piceaperdum* and *C. polonica* needs to be clarified. The pathogenicity of geographically different strains of *O. piceaperdum* and *C. polonica* should be tested. The success or failure of bark beetle attacks on living trees is ultimately determined by the beetle-fungus-host tree interaction.

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