Original article

Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity

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Summary — Mass attacks by *Tomicus piniperda* were induced in young Scots pines of varying vitality by baiting the trees with split, fresh pine bolts. Trees were felled at different times to determine the development of blue-staining of sapwood. Fungi were isolated from samples of inner bark and blue-stained sapwood in connection with galleries of *T piniperda*. Samples were also taken from beetle-attacked pine timber. In addition, 4 stem-pruned trees were inoculated with the 2 most important species isolated from trees attacked by *T piniperda*. Three species of fungi were rather frequently isolated, *Hormonema dematioides*, *Leptographium wingfieldii* and *Ophiostoma minus*. The latter 2 species were most active in invading the sapwood. Blue-staining of sapwood occurred rather late in the season, 1–2 months after attack. One tree in each pair of trees inoculated with *L wingfieldii* and *O minus* were dying when harvested more than 4 months after mass inoculation. Thus, these fungi may play a role in overcoming the resistance of trees under beetle attack.

blue-stain fungi / *Tomicus piniperda* / *Pinus sylvestris* / insect–fungus relationship / pathogenicity

Résumé — Champignons du bleuissement associés à *Tomicus piniperda* en Suède et observations préliminaires sur leur pathogénicité. *Des attaques massives de* Tomicus piniperda *ont été provoquées sur des jeunes pins sylvestres de vitalité variée, en appâtant les insectes avec des fragments de rondins de pin frais. Les arbres ont été abattus à différentes dates pour suivre le développement des champignons du bleuissement dans l'aubier. Les champignons ont été isolés à partir d'échantillons d'écorce interne et d'aubier bleui, situés en correspondance avec des galeries de* Tomicus piniperda. *Des échantillons ont aussi été prélevés sur des grumes attaquées. De plus, 4 arbres complètement élagués ont été inoculés avec les 2 plus importantes espèces précédemment isolées des arbres attaqués par* T piniperda. *Trois espèces de champignons ont été assez fréquemment isolées,* Hormonema dematioides, Leptographium wingfieldii, *et* Ophiostoma minus. *Les 2 dernières nommées se sont avérées les plus actives à envahir l'aubier. Le bleuissement de l'aubier est intervenu plutôt tardivement, 1 à 2 mois après l'attaque.* L wingfieldii *et* O minus *ont tué au moins un arbre chacun après inoculations massives. Il est donc possible que ces champignons jouent un rôle pour vaincre les arbres attaqués par les Scolytes.*

champignon du bleuissement / Tomicus piniperda / Pinus sylvestris / relation insecte–champignon / pathogénicité

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INTRODUCTION

Many bark beetles attacking conifers are associated with blue-stain fungi, which play a key-role in success or failure of beetle establishment. This has been shown for several bark beetle–fungus associations, *eg* the Eurasian spruce bark beetle *Ips typographus* (L) and the blue-stain fungus *Ophiostoma polonicum* Siem (Horntvedt *et al*, 1983; Christiansen and Horntvedt, 1983; Christiansen, 1985; Solheim, 1988).

Some of the bark beetles associated with Scots pine (*Pinus sylvestris* L) have long been known to carry blue-stain fungi (Rennerfelt, 1950; Mathiesen-Käärik, 1953; Francke-Grosmann, 1967). These had not been considered pathogenic until a beetle outbreak in Central France caused considerable mortality in Scots pine, and the interactions between fungi and beetles came into focus (Lieutier *et al*, 1988). A complex of 2 bark beetles, *Tomicus piniperda* (L) and *Ips sexdentatus* (Börn) and associated blue-stain fungi has been held responsible for the pine mortality in France, stress and low tree vitality probably being important predisposing factors (Lieutier *et al*, 1989; Piou and Lieutier, 1989).

In Sweden, Scots pines were found to produce distinct reaction zones in response to induced stem attacks by *T piniperda*, and fungi were apparently present in the sapwood of successfully colonized trees (Långström and Hellqvist, 1988). This finding initiated a series of experiments to clarify the defensive system of Scots pine against bark beetles and their possible fungal associates. In the present paper, we report on the species of fungi found in association with *T piniperda* in Sweden, including some remarks on their ecology and pathogenicity.

MATERIAL AND METHODS

Study areas

Field work was conducted in 2 study areas in the province of Gästrikland in Central Sweden (≈ 61° N lat, 16° E long). One site was situated on a pine-covered moraine at Norrsundet close to the Baltic sea, and the other on a dry pine heath at Jädraås (≈ 185 m above sea level). Both sites were pure pine stands, ≈ 35 and 25 yr old, and stocked with ≈ 2 500 and 1 000 stems per hectare, respectively. Tree diameter range was 5–8 cm (including bark) at Norrsundet, and 4–5 cm at Jädraås. Some of the trees at Norrsundet were heavily damaged by shoot-feeding of *Tomicus* beetles, originating from the timber store of an adjacent pulp mill. The stands at Jädraås were free of any visible beetle damage.

Isolation of fungi

In 1988, attacks by *T piniperda* at Norrsundet were induced in 88 young Scots pine trees, representing 4 different vigour classes, by attaching split pine bolts to the stem. The vigour classes were as follows: unpruned trees in good condition; unpruned trees with reduced crown due to previous shoot-feeding by *Tomicus* beetles; similar beetle-damaged trees pruned (from below) to 50 and 25% crown length, respectively. The trees were pruned on 30 March 1988, ≈ 1 wk prior to beetle flight and attack. Beetle attacks were induced in trees by attaching a split bolt of fresh pine timber to the stem. The attack pattern of the beetles as well as the defence reactions of the trees were similar to those reported by Långström and Hellqvist (1988), and will be reported in detail elsewhere (Långström *et al*, submitted).

From April to September 1988, a total of 60 trees were felled on 5 occasions (table III) (the remaining 28 trees were felled in August 1989). The upper and lower ends of sample bolts taken from the felled trees (cut at 0.3, 0.8, 1.3 and 1.8 m stem height), were visually checked for the occurrence of blue-stain. If present, the stained percentage of the cross-sectional area was estimated.

At the felling carried out on 6 September 1988, stem sections between 1.0–1.3 m stem height were taken for isolation of fungi. Isolations were made in blue-stained wood inside galleries of *T piniperda*, 0.5, 1.5, 2.5 and 3.5 cm inside the cambium. Small pieces of wood, 5–10 mm^3, were taken aseptically, placed on plates with malt agar (2% malt, 1.5% agar) and incubated at room temperature in darkness.

In 1989, beetle attacks were again induced in pine trees of different vigour and pruning history in the low-vigour stand at Norrsundet. Three sets of 20 similar-looking trees had previously been pruned to ≈ 40% crown length on 21 June 1988, 9 September 1988 and 9 March 1989, respectively. On 20 March 1989, half of these trees were baited with split pine bolts in order to attract more beetles to attack these trees than the pruned but unbaited ones. In addition, 72 unpruned trees, representing the full range in tree size in the stand, were selected and baited.

Ten trees (*ie*, 5 baited and 5 unbaited) from each pruning group were felled in June and in August 1989. In addition, unpruned trees were felled in August and October (table III). Blue-stained sapwood was estimated as in the previous year. Stem sections between 80–130 cm in stem height were taken for fungal isolations in June and August.

At Jädraås, stem-pruned pines (intended for a caging experiment) were spontaneously and unintentionally attacked by *T piniperda* in the spring of 1989. Nine of these attacked trees were felled on 2 and 13 June, and stem sections were taken for fungal isolations.

On 2 june, fungal samples were also taken from a pile of logs at Jädraås, ≈ 200 m away from the attacked standing trees.

From all samples taken in June, fungi were isolated in the phloem reaction zone around galleries, and 1 and 3 mm inside the wood beneath galleries. From samples taken in August, isolations were made from blue-stained sapwood as in the previous year. Mostly 4 or 5 galleries were chosen for isolations from each tree or log.

Inoculation experiment

On 2 June 1989, 4 stem-pruned pine trees at Jädraås were inoculated with cultures of *Leptographium wingfieldii* Morelet and *Ophiostoma*

minus (Hedgc) H et P Syd. Two of the trees had been pruned on 2 September 1988, and the others on 24 May 1989, in both cases up to and including whorl 1985. One tree of each pruning class was inoculated with each fungus. All 4 trees had escaped beetle-attack in spring 1989.

The inoculations were made with a 5-mm cork borer in 6 rings encircling the stem 10 cm apart from each other (Solheim, 1988). Each ring consisted of 5–6 inoculations, set 2 cm apart. Each tree thus received 30–36 inoculations over a 50-cm section from 1.2–1.7 m stem height, corresponding to a density of 600 per m^2.

The fungal cultures originated from previous samples from trees attacked at Norrsundet in 1988, and were grown on standard malt agar medium.

The trees were felled on 17 October 1989, taken to the laboratory, and immediately placed in buckets with a water suspension of Fast Green (0.25 g in 1 l water) in order to check the water conducting capacity of the sapwood (see also Parmeter *et al*, 1989).

RESULTS

Fungal flora

Three species of blue-stain fungi were often isolated in association with galleries of *T piniperda* in June. (The mean attack density on these trees was generally high, ranging from 150–400 galleries per m^2.) These fungi were *Hormonema dematioides* Lagerb et Melin, *Leptographium wingfieldii* and *Ophistoma minus*. The frequency of their association was rather variable (table I). *L wingfieldii* and *O minus* were never isolated around the same galleries. *H dematioides* frequently occurred together with the 2 others. All 3 were mostly isolated only from reaction zones in the bark, even though *L wingfieldii* was also isolated from sapwood on about half the occasions. *Ophiostoma piceae* (Münch) H et P Syd and *O pilifera* (Fr) H et P Syd

Table I. Frequency of fungi isolated in association with galleries of *T piniperda* in June 1989 from standing trees or logs.

	No of galleries/trees	H dematioides (%)	L wingfieldii (%)	O minus (%)	Ophiostoma spp (%)
Norrsundet, trees	107/15	1	9	8	3
Jädraås, trees	44/9	44	16	30	5
Jädraås, logs	30/6	5	0	10	0

were isolated a few times. In addition, yeasts, bacteria, different sterile mycelia and some species of Sphaeropsidales were isolated.

Isolations from the wood in autumn, after blue-stain had developed, showed that *L wingfieldii* and *O minus* caused most of the staining (table II). *H dematioides, O europhioides* (Wright et Cain) H Solheim, *O piceae* and *O pilifera* were also isolated, but always together with one of the 2 others. At this time, however, it was rather difficult to determine from which gallery the blue-staining had spread.

Blue-staining

Visible sapwood blue-stain developed slowly and only in a few trees (table III). In both years, only minor patches of blue-stain were seen in a few of the trees felled

in May/June, whereas extensive blue-staining occurred in successfully attacked trees felled in August/September.

In 1988, blue-stain in sapwood was observed only in 5 of the severely pruned trees. In the following year, 4 pruned trees of each pruning date displayed blue-stain at felling, whereas 8 of the 32 unpruned trees were stained.

Pathogenicity

At harvest on 17 October 1989, 3 of the 4 inoculated trees were green and looked healthy, whereas the fourth was yellowish and in poor condition. The Fast Green test, however, revealed that none of the trees had normal water uptake and 2 of them were apparently dying, since 80–90% of the sapwood was non-conducting. Both dying trees had been pruned in May 1989,

Table II. Frequency of fungi isolated in blue-stained sapwood under galleries of *T piniperda* in autumn 1988 and 1989 at Norrsundet.

	No of galleries/trees	H dematioides (%)	L wingfieldii (%)	O minus (%)	Ophiostoma spp (%)
1988	21/3	0	90	62	19
1989	48/12	2	71	46	10

Table III. Blue-stain at 30 cm height of stem-pruned and unpruned trees attacked by *T piniperda* and felled during 1988 and 1989 at Norrstundet.

Date of felling	No of stained trees	Mean % of stained sapwood	Total No of trees
1988			
20 April	0	–	12[a]
4 May	0	–	12[a]
24 May	1	< 5	12[a]
21 June	1	10	12[a]
6 sept	3	45	12[a]
1989			
1 June	1	6	30[b]
24 Aug	11	67	30[b]
24 Aug	2	71	15[c]
17 oct	6	83	17[c]

[a] Includes 3 trees of each of four vigour classes.
[b] Pruned trees, 10 trees of each of 3 pruning dates. [c] Unpruned trees.

and one of the dying trees was inoculated with *L wingfieldii* (the yellowish tree mentioned above), and the other with *O minus*.

DISCUSSION

Although our material was limited, it seems that *H dematioides*, *L wingfieldii* and *O minus* are associated with *T piniperda* in Sweden. The frequency of the fungi in the galleries seems to be low and rather variable. We did not attempt to isolate fungi from the beetles. Previously, the same species have been demonstrated to occur together with *T piniperda* in France, where the fungi have been isolated both from beetles and galleries (Lieutier *et al*, 1989; Piou and Lieutier, 1989). The association

with *H dematioides* is high and uniform; with *L wingfieldii* it is low and uniform and with *O minus* very variable (Lieutier *et al*, 1989).

The first record of blue-stain fungi associated with *T piniperda* was made by Mac-Callum (1922) in Scotland, who found *O minus* and *O piceae* there. In Germany, Grosmann (1931) mentioned *O minus* and *H dematioides*. Siemaszko (1939) found *O minus* as a constant component in Poland, and other species more sporadically, *eg O piceae*, *O pilifera* and *Aureobasidium pullulans* (de Bary) Arnaud. Studies in Sweden have paid special attention to *O minus* and *A pullulans*, but many other species have been found in connection with attack of *T piniperda* (Mathiesen, 1950; Rennerfelt, 1950; Mathiesen-Käärik, 1953).

Most of the species mentioned in association with *T piniperda* are also isolated in connection with other bark beetles, especially species attacking pines. *O minus*, which is always mentioned together with *T piniperda*, is associated with different bark beetles both in Europe and North America (Käärik, 1980; Upadhyay, 1981).

Since *H dematioides* has been synonymized with *A pullulans* (Robak, 1932), and then again considered a distinct species (Roback, 1952; Butin, 1963; Hermanides-Nijhof, 1977), these 2 species have often been confused. Today it is impossible to know which species the different authors may have meant, since no cultures are available. Records on *A pullulans* associated with *T piniperda* in Poland (Siemaszko, 1939) and Sweden (Mathiesen, 1950; Rennerfelt, 1950; Mathiesen-Käärik, 1953) may thus in fact refer to *H dematioides*.

L wingfieldii is a recently described species (Morelet, 1988). Earlier this species may have been included in another *Leptographium* species, *eg L lundbergii* Lager et Melin, found in association with *T piniper-*

da and other bark beetles in Sweden (Mathiesen, 1950).

Our data show that L wingfieldii and O minus were the most important invaders of sapwood, and that the former species occurred more frequently than the latter. In contrast, Lieutier et al (1989) found O minus more frequently than L wingfieldii in galleries of T piniperda as well as in sapwood inside the galleries.

In studies using single inoculations, both L wingfieldii and O minus produced long reaction zones and long fungal extensions in the bark, longest in the case of L wingfieldii (Lieutier et al, 1988, 1989). In contrast, H dematioides yielded short reaction zones and hardly any fungal extension (Lieutier et al, 1988, 1989). Thus, Lieutier et al (1989) concluded that despite its low frequency in beetle galleries, L wingfieldii may play an important role in the tree-killing process due to its high aggressivity to Scots pine and uniform occurrence with T piniperda. As regards O minus, the association with T piniperda was variable and fortuitous, but O minus may still be involved in the tree-killing process (Lieutier et al, 1989). In North America, O minus has repeatedly been shown to be capable of killing seedlings, saplings and older trees (Nelson and Beal, 1929; Nelson, 1934; Caird, 1935; Bramble and Holst, 1940; Mathre, 1964; Basham, 1970; Owen et al, 1987).

In our pilot study, both L wingfieldii and O minus seem to be able to kill trees when mass inoculated. The dose used was rather high, 600 inoculations per m^2 within a 50-cm belt, but comparable to the inoculum dose needed to kill healthy spruce trees with O polonicum (Christiansen, 1985). No control inoculations were carried out, but compared with mass inoculation of O polonicum in Scots pine (Christiansen and Solheim, 1990) its seems that

a control inoculation will not affect the trees much. The pruning itself would not have killed the trees, as indicated by the fact that all trees pruned in 1988 were still alive at the time of inoculation. In a similar study in the same areas, Långström and Hellqvist (1988) demonstrated that trees pruned in a similar way in autumn and spring did not differ in resistance to beetle attacks. Furthermore, they found that even severely pruned trees survived despite heavy beetle attack. Thus, it is reasonable to assume that the 2 dying trees in the present study were killed by the mass inoculation.

In laboratory tests L wingfieldii has been shown to grow faster than O minus at low temperatures (Lieutier and Yart, 1989), and since the beetles attack early in the season (early and late April in 1988 and 1989, respectively in the study area), L wingfieldii may be better adapted to the conditions prevailing during the attack than O minus. In trees, however, Lieutier et al (1990) could not explain all the differences in kinetics of growth between fungi and between seasons by temperature and defence reaction alone; other factors might interfere.

Despite the early date of attack, the first signs of blue-stain development were not seen until 1–2 months later. This may be due to low temperature inhibiting fungal growth and high tree resistance in spring. Horntvedt (1988) found in a seasonal inoculation study with O polonicum on Norway spruce (Picea abies L) that temperature had a great influence on blue-stain development in sapwood, but in spring and early summer tree resistance was high and delayed blue-staining. Thus further studies are needed to clarify the influence of weather conditions and host resistance on the development of blue-stain fungi associated with T piniperda..

ACKNOWLEDGMENTS

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