Vegetative compatibility groups of *Amylostereum areolatum* and *A. chailletii* from Sweden and Lithuania

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Mycelial interactions were studied between 28 isolates of *Amylostereum areolatum* and 15 of *A. chailletii* from wounded spruce in 10 sample plots in Sweden and Lithuania. Based on somatic incompatibility, nine vegetative compatibility groups (VCGs) of *A. areolatum* were detected. Five of these VCGs were isolated from two or more trees and included 24 (80%) of all isolates.

In *A. chailletii*, 12 VCGs were found and three contained two isolates each. Grouping of isolates into VCGs was more pronounced in *A. areolatum*. Two of the *A. areolatum* VCGs had wide geographical distribution. VCG A2 was isolated from 14 different trees in two Swedish and four Lithuanian sample plots. VCG A1 was found in one plot in Sweden and in two in Lithuania. VCGs of *A. chailletii* had a local distribution and were confined to a single sample plot or to a forest stand. In *A. areolatum* occurrence of compatible pairings between isolates was uniform in all spatial scales of investigation: within a plot, within each country and among both countries. The proportion of somatically compatible *A. chailletii* isolates was significantly lower and decreased with geographic distance.

*Amylostereum areolatum* (Fr.) Boidin and *A. chailletii* (Pers.: Fr.) Boidin are saprotrophic decay fungi occurring on fallen trunks and stumps of *Picea* and *Abies* (Eriksson & Ryvarden, 1973; Eriksson, Hjortstam & Ryvarden, 1978; Breitenbach & Kränzlin, 1986). In central and northern Europe *A. areolatum* is also regarded as important cause of wound decay of *Picea abies* (L.) H. Karst., usually infecting 5–20% of open bark wounds on living trees (Pechmann & Aufsess, 1971; Schönhar, 1975; Vasiljauskas, Stenlid & Johansson, 1996). *A. chailletii* seems to be less frequent in spruce injuries than *A. areolatum* (Bonnemann, 1979; Vasiljauskas et al., 1996). With time, both fungi are able to develop an active rot within the damaged stems (Siepmann, 1971; Bonnemann, 1979; Vasiljauskas, 1999).

Both *A. areolatum* and *A. chailletii* are associated with woodwasps (*Sirex* and *Urocerus*) that are capable of introducing the fungi into living trees (Stillwell, 1966; Coutts & Dolezal, 1969). During 1940s and 1950s, combined attacks by *Sirex noctilio* Fabre and *A. areolatum* devastated *Pinus radiata* D. Don. plantations on thousands of hectares in New Zealand, Tasmania and Australia (Talbot, 1977), showing the high efficiency of insects to disseminate this fungus over large areas. Since the females of *Sirex* carry and introduce the fungus in form of vegetative mycelium fragmented into oidia or arthrospores (Francke-Grossmann, 1939), the single genotypes of *A. areolatum* may become widely spread, thus forming dispersive clones (Anderson & Kohn, 1995). With this effective means of dispersal the production of fruit bodies becomes almost superfluous (Francke-Grossmann, 1939). In fact, basidiocarps of *A. areolatum* have never been found in nature in Australia and New Zealand (Hood, 1992), although isolates have not lost the ability to fruit in culture (Talbot, 1977).

A self–non-self rejection mechanism known as somatic or vegetative incompatibility operates to delimit individual genotypes from one another in many fungal species (Rayner et al., 1984). On this basis isolates can be assigned to vegetative compatibility groups (VCGs) that are likely to represent groups of closely related mycelia or even single clones or genets (Anderson & Kohn, 1995). Studies on the distribution of genets in nature by means of somatic incompatibility have revealed the formation of territorial clones of root rotting fungi due to vegetative spread among trees (Korhonen, 1978; Kile, 1983, 1986; Stenlid, 1985, 1987; Dickman & Cook, 1989; Lewis & Hansen, 1991; Swedjemark & Stenlid, 1993) and the presence of several genets of stem decay species within trunks of individual trees due to abundant infections by airborne basidiospores (Adams & Roth, 1969; Rayner & Todd, 1979; Holmer, Nitar & Stenlid, 1994).

Dispersive fungal VCGs have been detected mainly in plant pathogenic ascomycetes that can be distributed over considerable geographical range due to conidia, insects, homothallic ascospores, sclerotia or plant material trade (Webber, Brasier & Mitchell, 1987; Milgroom, MacDonald & Double, 1991; Leslie, 1993; Mitchell & Brasier, 1994; Meijer, Megnegneau & Linders, 1994; Anderson & Kohn, 1995;...
In Lithuania inoculum are the more important for infection of wounded wood decaying snow mould *Typhula* (Matsumoto & Tajimi, 1993) and for the wood decaying *Stereum sanguinolentum* that is likely to possess homokaryotic fruiting bodies (Rayner & Turton, 1982; Ainsworth, 1987; Vasiliauskas & Stenlid, 1998a).

As a result of symbiosis between insect and fungus, fungal VCGs or genets may become widespread over a considerable geographical range (Mitchell & Brasier, 1994). If the species is ‘unit-restricted’, incapable of mycelial migration (Rayner & Boddy, 1988), the physiological boundaries of each spatially separated representative of a genet will be confined within the physical boundaries of individual resource units which in our study equals a tree stem. Detection of VCGs in insect-dispersed, unit-restricted basidiomycetes as *A. areolatum* and *A. chailletii* can, therefore, provide information concerning scale and spatial distribution of genetic variation in natural populations. The aim of the present investigation was to study the distribution of VCGs among Swedish and Lithuanian populations of *A. areolatum* and *A. chailletii*. The second aim was to determine if basidiospores or woodwasp-transmitted inoculum are the more important for infection of wounded *P. abies* by these fungi.

**MATERIALS AND METHODS**

Isolates of *A. areolatum* and *A. chailletii* were collected from 43 *P. abies* trees in three sample plots located in Sweden and seven sample plots in Lithuania (Fig. 1). The plots were situated in pure *P. abies* stands approx. 50 y old that were previously damaged during logging and extraction or by bark stripping by moose (*Alces alces L.*). A total of about 750 living stems 8–40 cm diam. at breast height, bearing wounds 2–4815 cm² in size made 1–23 y ago were randomly selected, and samples were taken by inserting an increment borer 6–8 cm into stems at the vicinity (1–3 cm) of a wound (Vasiliauskas *et al*., 1996). Bore cores were brought to the laboratory in sterilized glass tubes. Sampled trees in plots C and V were numbered, mapped and distances between every isolation within these plots were estimated.

In the laboratory, all samples were surface sterilized by flaming and placed on Petri dishes containing Hagem agar (HA) medium (Stenlid, 1985). Colonies of *A. areolatum* and *A. chailletii* were subcultured after 10–15 d growth. The total number of isolates obtained from each sample plot is shown in Table 1. All isolates exhibited numerous clamp connections and were, therefore, assumed to be heterokaryotic.

Somatic incompatibility tests were carried out as described by Vasiliauskas & Stenlid (1999a). All isolates were confronted pairwise in all possible combinations. For compatibility controls, each isolate was self-paired using two pieces from the same mycelium. A total of 406 pairings was made for *A. areolatum* and 120 for *A. chailletii*. In all tests 4 mm mycelium discs cut together with HA medium were cut from the margin of actively growing colonies and placed pairwise 1.5–2 cm apart in the centre of 9 cm Petri dishes containing approx. 20 ml HA. These were incubated for up to 60 d at room temperature (18–23 °C) and examined periodically. Interactions between two mycelia were regarded as compatible when a continuous mycelial mat was formed between isolates, corresponding to that of self-pairing controls. Antagonistic types of mycelial interactions following contact were classed as incompatible.

Distribution of VCGs per resource unit was analysed by the Poisson distribution (Mead & Curnow, 1983) and $\chi^2$-tests

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**Table 1.** Origin of *Amylostereum areolatum* and *A. chailletii* isolates and their distribution accordingly to VCG

<table>
<thead>
<tr>
<th>VCG</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>V</th>
<th>All</th>
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</thead>
<tbody>
<tr>
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<td>Total number</td>
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<td>10</td>
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**Fig. 1.** Map of Baltic region showing the sample plots for *Amylostereum areolatum* and *A. chailletii*. Arrow from square indicates area of a local forest stand. Sample plots are identified according to Table 1.
Vegetative compatibility in *Amylostereum* spp. were calculated to compare proportions of compatible isolates from spatially different sampling scales (Clarke, 1989).

**RESULTS**

Somatically compatible isolates were of similar morphology and exhibited similar growth rates. Tests among three and more isolates were always transitive; when one isolate intermingled with two or more others, the latter were compatible with each other.

A total of nine VCGs was detected among the 28 *A. areolatum* isolates. Five of these VCGs were isolated from 2–14 trees and included 24 (86%) of all isolates. In *A. chailletii*, 12 VCGs were found among 15 isolates and six (40%) of these were assigned to three VCGs with two isolates each. Thus the clustering of isolates into VCGs was much more pronounced in *A. areolatum*. Distribution of *A. areolatum* and *A. chailletii* isolates on the sample plots is shown in Table 1. The data indicate wide geographical distributions of *A. areolatum* VCGs A1 and A2. VCG A2 was the largest, isolated from 14 trees on two Swedish and four Lithuanian sample plots. VCG A1 was found on one sample plot in Sweden and on two in Lithuania. In contrast, all three VCGs of *A. chailletii* were confined to a single sample plot or to a forest stand (Table 1).

In *A. areolatum*, occurrence of compatible isolates was similar at all spatial scales of investigation – within a plot, within each country and among both countries, with the exception of plots I, K, L, M and V in a forest stand (Table 2).

### Table 2. Proportions of compatible *Amylostereum areolatum* and *A. chailletii* pairings within spatially different sampling areas

<table>
<thead>
<tr>
<th></th>
<th><em>Amylostereum areolatum</em></th>
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<th><em>Amylostereum chailletii</em></th>
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<tbody>
<tr>
<td></td>
<td>Total number</td>
<td>Compatible number (%)</td>
<td>Total number</td>
<td>Compatible number (%)</td>
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<tr>
<td><strong>Within all plots</strong></td>
<td>69</td>
<td>23</td>
<td>46</td>
<td>2</td>
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<td><strong>Among plots</strong></td>
<td>68</td>
<td>10</td>
<td>45</td>
<td>2</td>
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<tr>
<td>I, K, L, M, V</td>
<td>70</td>
<td>19</td>
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<td><strong>Among plots in countries</strong></td>
<td>171</td>
<td>50</td>
<td>14</td>
<td>0</td>
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</table>

### Table 3. $\chi^2$-values for comparison of proportions of somatically compatible pairings between *Amylostereum areolatum* (*A.a.*) and *A. chailletii* (*A.c.*) isolates within different spatial scales, calculated according data in Table 2; w.p., within plots; w.f., within forest stand (among plots I, K, L, M, V); w.c., among plots within countries; a.c., among countries; blank spaces, not compared

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<td>A.a. w.f.</td>
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<tr>
<td>A.a. w.c.</td>
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<tr>
<td>A.a. a.c.</td>
<td>0–39</td>
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<td>A.a. w.f.</td>
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<tr>
<td>A.a. w.c.</td>
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<td>A.c. w.p.</td>
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<td>A.c. w.f.</td>
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<td>A.c. w.c.</td>
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*Significance levels: $P < 0.001$; **$P < 0.01$; *$P < 0.05$.  

Fig. 2. Spatial distribution of *Amylostereum areolatum* (○) and *A. chailletii* (△) isolates within sample plots C and V. Numbers identify the VCG of the isolate (Table 1).

In *A. areolatum*, occurrence of compatible isolates was similar at all spatial scales of investigation – within a plot, within each country and among both countries, with the exception of plots I, K, L, M and V in a forest stand (Table 2). These data were supported by statistical analysis using $\chi^2$ test (Table 3). When compared to *A. areolatum*, the proportions of somatically compatible *A. chailletii* isolates were significantly lower at all spatial scales (Tables 2 and 3). There was a decreasing occurrence of somatically compatible *A. chailletii* among isolates over geographical distance, but the
DISCUSSION

Our earlier study in the Baltic Sea region revealed several VCGs of *S. sanguinolentum*, some of them having wide geographical distribution across Sweden, Finland and Lithuania, and it has been argued the long range dispersal mechanisms by airborne homothallic or nearly homothallic (inbred) basidiospores may operate in that case, although generally short range dispersal prevailed (Vasiliauskas & Stenlid, 1998a). The mating system in both *A. areolatum* and *A. chailletii* is outcrossing tetrapolar (Boidin & Lanquetin, 1984). Since in our work 86% of *A. areolatum* isolates were assigned to VCGs containing two or more spatially separated individuals, the role of basidiospores seems less important in spread of the fungus. Other observations also suggest dispersal of *A. areolatum* by means of airborne basidiospores unlikely, at least in the area of our investigations. Studies on distribution of the fungus based on the occurrence of fruiting bodies showed that *A. areolatum* is moderately common in Switzerland and southern Germany (Jahn, 1979; Breitenbach & Kränzlin, 1986), but its occurrence decreased abruptly towards northern parts of Germany (Jahn, 1979). In Sweden and Lithuania *A. areolatum* was reported to be very rare (Mazelaits, 1976; Eriksson et al., 1978). Despite that, we isolated the fungus from 5-2% and 3-5% of wounded spruce trees in Sweden and Lithuania respectively (Vasiliauskas et al., 1990; Vasiliauskas & Stenlid, 1998b). Results of the present study show rather even spatial distribution of *A. areolatum* VCGs both over small and large geographical areas (Tables 2 and 3). This indicates that the occurrence of *A. areolatum* in forest stands does not depend greatly on the presence of basidiocarps and that most probably woodwasps are responsible for spread and introducing mycelium into wounded stems, resulting in dispersive clones of the fungus. Indeed, wounded stems are reported to be highly attractive habitat for various Sirex species (Schwerdtfeger, 1957).

One possible explanation for wide geographical distribution of *A. areolatum* VCGs is that they were gradually spread by insects across Europe from one forest stand to another, and therefore, represent very old clonal lineages in a well adapted Sirex – *A. areolatum* – *P. abies* association. Additional facilitation of spread could be by exports of wood from one country to another. Since the life cycle of Sirex woodwasps usually lasts 3–4 y (Schwerdtfeger, 1957), it is possible that their larvae, along with mycelium of *A. areolatum*, would be transported between countries within wood. Emerging insect females could, therefore, have entered new areas and established local clonal populations of the fungus, as happened in New Zealand and Australia (Talbot, 1977). Export of wood from Lithuania to Sweden only started in 1992 (V. Vaiciunas, Ministry of Agriculture and Forestry of Lithuania, personal communication), however, and it is unlikely that wood transport had strongly influenced the fungal population structure as it is presently reported.

The living trees in our study were infected via wounds caused by bark peeling by moose or inflicted during forest operations. Since the age of the wounds from which *A. areolatum* was isolated was uniform both within stand C (3 y) and stand V (9 y), isolates belonging to the same VCGs might have entered the trees during a single season, when wounds were particularly attractive and/or susceptible to insect attack. A single Sirex female could be responsible for the infections in one separate stand, attacking stepwise one tree after another. During one attack 1–8 eggs are laid in the wood and one female can produce up to 250–350 eggs in total (Schwerdtfeger, 1957). Alternatively, the infection source might have been several insects carrying the same fungal genotype.

The present work showed that in *A. chailletii* clustering of isolates into VCGs was significantly lower as compared with *A. areolatum* and no case of somatic compatibility was noted among isolates separated by the Baltic Sea (Tables 2 and 3). Pairings within local populations of *A. chailletii* showed prevalingly incompatible reactions, closely resembling results obtained in populations of non-outcrossing unit-restricted basidiomycete as *S. sanguinolentum* (Vasiliauskas & Stenlid, 1998a). Despite the small number of *A. chailletii* isolates studied, this indicates that greater diversity exist in populations.
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of *A. chailletii* as compared with *A. areolatum* and that short-range dispersal mechanisms of genets prevail. On the other hand, the present study provided evidence also for somatic compatibility between *A. chailletii* isolates from spatially separated resource units. The role of woodwasps in spread of *A. chailletii* clones in nature still might be important, therefore, since fruitbodies had been found only occasionally in Sweden (Eriksson & Ryvarden, 1973; Ryman & Holmäsen, 1984) and have not been reported from Lithuania (Mazelaitis, 1976). We have, however, isolated *A. chailletii* from 0.5% and 2.6% of wounded spruces in both countries respectively (Vasiliauskas et al., 1996; Vasiliauskas & Stenlid, 1998b).

Among 42 stems with *Amylostereum* spp., only one yielded an isolate of both *A. areolatum* and *A. chailletii*, suggesting that mainly one *Amylostereum* genet is present within each tree. This situation reflects restricted establishment of *A. areolatum* genets within living trees from individual spore sources, indicating rather limited access of woodwasps to wounded *P. abies*.

The possibility cannot be excluded that VCGs detected in the stands do not represent clones in both *Amylostereum* spp. Situation, where isolates from the same VCG are not genetically identical, has been reported in several basidiomycetes (Jacobson, Miller & Turner, 1993; Matsumoto, Uchiyama & Tsuchima, 1996; Stenlid & Vasiliauskas, 1998). Future studies will assess genetic variation between and within *A. areolatum* and *A. chailletii* VCGs, and structures of geographical populations of the fungi (Vasiliauskas, Stenlid & Thomsen, 1998).

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RECENTS


