Research Paper

Myxomycete occurrence on living bark of Cryptomeria japonica in relation to tree Vitality

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ABSTRACT

Corticolous myxomycetes are protists that inhabit the bark surface of living trees. However, there is no information available regarding the influence of tree decline on the occurrence of myxomycete species. Japanese cedar trees (Cryptomeria japonica) have recently undergone a decline and reduction in vitality, which has been attributed to environmental factors such as air pollution and lower rainfall. The objective of the present study was to investigate the influence of C. japonica vitality on myxomycete diversity, relative abundance, and richness in urban areas of Okayama Prefecture, Western Japan. Bark samples collected from 57 healthy to severely declining C. japonica trees were examined in 650 moist chamber cultures. A total of 19 myxomycete species were identified, averaging 4.1 ± 1.7 species per tree. The most abundant species on the bark were Paradiacheopsis rigida, Cribraria confusa, and Enerthenema berkeleyanum. Several myxomycete species, such as P. rigida, Enerthenema papillatum, and Comatricha laxa, were relatively abundant on healthy trees. In contrast, Physarum nutans, Clastoderma debaryanum, and Diderma chondrioderma occurred significantly on the bark of trees with decreased vitality, a distribution that is affiliated with a decrease in acidity caused by the deterioration of healthy bark. These findings provide new insights into the relationship between tree vitality and myxomycete distribution and reveal that in terrestrial ecosystems, the provision of bark habitats for myxomycetes is dependent on maintaining tree vitality.

Key words: Bark pH, Corticulous myxomycete, Japanese cedar, tree decline.

INTRODUCTION

Forest and tree declines have become a recent feature in Japan, the causal factors of are thought to be pathogens and/or long-range transport of atmospheric aerosols from East Asia (Nagafuchi, 2000; Kume et al., 2011; Miyake et al., 2013; Shimadera et al., 2009). For example, the Japanese cedar tree Cryptomeria japonica (Thunb. ex L.f.) D. Don. has been damaged due to air pollution (Kato 1991; Kaneko et al. 1994; Matsumoto et al. 2002). The decline of C. japonica has been conspicuous in lowland areas around the Setouchi Inland Sea and the Kanto district of Japan (Nashimoto and Takahashi, 1991; Matumoto et al., 2002). Estimation of tree vitality has been used as an indicator for the assessment of air pollution since the 1970s (Yambe, 1978; Nashimoto and Takahashi, 1991); however, there is little information available regarding the association between tree vitality and the microbial inhabitants of tree bark.

Many myxomycetes, members of the Protista, have been found on the bark of living trees (Gilbert and Martin, 1933), and the bark of C. japonica has been shown to provide a microhabitat for 21 myxomycete species (Takahashi, 2014). To the best of my knowledge,
however, the influence of tree vitality on the myxomycete inhabitants of *C. japonica* has never been studied. Myxomycete species are known to act as phagotrophic bacteriovores and fungivores during the amoeba and plasmodium stages (Keller and Brooks, 1973), and are presumed to play a role in maintaining bark vitality. Although it is known that the acid stem flow from *C. japonica* creates a specific habitat for microbes in the soil surrounding trees (Yambe, 1978), little information is available regarding the effects of tree vitality on the occurrence of bark living myxomycetes.

A survey of corticolous myxomycetes in the urban green spaces of south-western Japan identified 40 myxomycete species on the bark of seven tree species (Takahashi, 2014). Several of these myxomycete species were strongly associated with bark pH among the different tree species, which is consistent with the findings of other studies (Everhart et al., 2008, 2009). Some myxomycetes prefer particular microhabitats (Takahashi and Hada, 2010; Rollins and Stephenson, 2011), and there has been recent progress in determining the restricted distribution of protists (Foissner, 2007). Tree vitality of *C. japonica*, which is an endemic tree species in Japan, is assumed to influence the occurrence of corticolous myxomycetes due to microhabitat modification brought about by a change in bark conditions. The present study aimed to investigate the influence of differences in *C. japonica* bark pH on the diversity, relative abundance, and species richness of corticolous myxomycetes.

**MATERIALS AND METHODS**

**Study area**

The decline of *C. japonica* is considered to be extensive along the coastal area of the Setouchi Inland Sea in Western Japan (Takahashi et al., 1991). The study site used for the present investigation is located in the western part of Okayama Prefecture, and mainly comprises an area extending from the Mizushima industrial complex in the city of Kurashiki to the upper mountain area of Niimi City. The area, which extends along the Takahashi River (Figure 1), is approximately 10 km wide and 70 km long, and is characterized by diverse environments, that is, Industrial, midtown, suburban regions, farmland, and an intown to intermontane area.
Differences in tree vitality were investigated in 28 plots within 8 sites, located in different environments covered by an altitudinal range of 2–645 m (Table 1).

Within these sites, solitary C. japonica trees (≥20-cm diameter at breast height) found in open areas such as green spaces, Buddhist temples, and Shinto shrines and parks were surveyed. In each plot, bark samples were collected from 2–3 trees. A total of 57 samples were collected from trees with diameters at breast height ranging from 20 to 135 cm.

**Tree vitality categories and bark sampling**

Tree vitality was categorized in terms of mild to severe decline based on the observed differences in tree shape, which is a recognizable and simple trait for determining tree vitality (Urushibara et al., 2004). The sampled trees were classified into four categories according to tree anterior shape and damage to canopy branches (Figure 2): V1, steeple-crowned tree canopy with healthy branches; V2, round and declined crown of tree canopy with fairly healthy branches; V3, significantly degenerated crown with blasted upper branches; and V4, severely damaged crown with degenerated upper branches.

Of the 57 bark samples, 15 were collected from V1 trees, 15 from V2 trees, 18 from V3 trees, and 9 from V4 trees. V1 trees were distributed in a mountain area with an elevation of approximately 20–645 m, whereas V4 trees were distributed in an area with an elevation of 2–67 m, indicating that there was a greater extent of tree decline in the lowland region. Average tree diameters at breast height were not significantly different among the four tree vitality categories (Table 2).

Old bark of the sampled trees without epiphytes was manually peeled off from the stem surface from areas 1–2 m above ground. Sampling was carried out during May–October 2015, a period when germination occurs under favorable conditions and is not affected by seasonal cues (Harkonen and Ukkola, 2000). Bark samples collected from tree trunks were placed in paper bags (23 cm W × 35 cm L) and individually pooled by tree trunk. Samples were transported to the laboratory and maintained under dry conditions at room temperature for approximately 1 month before culture.

**Myxomycete culture**

Moist chamber cultures (Gilbert and Martin, 1933) were prepared for the 57 bark samples to assess the occurrence of corticolous myxomycetes. At least 10 moist chamber cultures were prepared for the samples from each tree, as described by Takahashi (2014). Barks were cut into pieces, placed in plastic Petri dishes (diameter, 9 cm), and arranged on a single layer of sterile filter paper (diameter, 7 cm). Petri dishes were filled with 25 ml of distilled water (pH 6.9) and incubated at room temperature in ambient light (Härkön, 1977). After approximately 3 d, the Petri dish covers were opened almost halfway to allow the slow drying of chambers. Within 7 d, fruiting bodies started to appear on the barks and these were examined under a dissecting stereomicroscope. If no sporangia were observed, barks were re-wetted and allowed to dry in order to perform a second set of observations. The number of myxomycete species per tree was recorded, and the relative abundance of each species was quantified as the number of culture dishes in which each species occurred compared with the total number of culture dishes.

Myxomycete species classification was performed according to Yamamoto (1998; 2006), and binomial nomenclature is according to the most recent literature (http://eumycetozoa.com; Hernandez and Lado, 2005). Voucher specimens of the myxomycetes were prepared using separate collection boxes for each species. Bark containing the myxomycete fruiting bodies was glued into the bottom of each box, and these were then maintained in the laboratory.

A total of 650 moist chamber cultures were prepared from the 57 C. japonica bark samples from V1–V4 trees. The pH of each culture was measured using a compact pH meter (Horiba, Kyoto, Japan), and the median bark pH was computed for each tree.

**Data analysis**

The occurrence, mean number, and richness of myxomycete species were estimated for each tree vitality.
category (Table 2), and significant differences were determined using Tukey’s test and Fisher’s exact test in contingency tables of V1–V4 in ESUMI Excel Statistics 5.0 (ESUMI Co., Ltd, Tokyo, Japan). The frequency of species occurrence on trees in each of the four vitality categories is shown in Table 3. Species richness was quantitatively determined from species numbers based on field observations using Chao1 (Chao, 1984), which provides species richness estimates that include undetected species. Species diversity \( (H') \) was estimated using the Shannon-Wiener index (Shannon and Weaver, 1963), and equitability \( (J'; \text{Pielou 1966}) \) was calculated using PAST (Hammer et al. 2001; http://folk.uio.no/ohammer/past/), as previously described by Takahashi (2014). The myxomycete communities of V2, V3, and V4 trees were compared with that of V1 to calculate the percentage similarity \( (PS) \) as follows: \( PS = \Sigma \min (a, b, c,...x) \), where \( \min \) is the lowest percentage composition of species \( a, b,...x \) between the myxomycete communities in two different tree decline categories. \( PS \) is considered an effective index for comparing the structure of myxomycete communities (Stephenson, 1989).

RESULTS

Species occurrence and diversity

Myxomycetes grew in 86% of the moist chamber cultures, ranging from 83 to 92% among the tree vitality categories. Observed species richness on the barks of 57 C. japonica trees was 19 (Table 3), which represents 95% of the species richness estimated using Chao1 (Chao, 1984). The overall mean number of species per tree was 4.1 ± 1.7. The occurrence (%) of myxomycetes in culture
Table 2: Information of Cryptomeria japonica bark samples and myxomycete occurrence, mean number of species, species richness, diversity and equitability in relation to tree vitality categories (V1 to V4).

<table>
<thead>
<tr>
<th>Bark samples</th>
<th>Tree vitality</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>Number of trees</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Bark pH range</td>
<td>3.0 - 4.0</td>
<td>3.3 - 4.2</td>
</tr>
<tr>
<td>Mean</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Myxomycetes

<table>
<thead>
<tr>
<th>Occurrence (%)</th>
<th>Number of species/Cultures</th>
<th>Total number of species</th>
<th>Chao-1</th>
<th>Shannon &lt;i&gt;H′&lt;/i&gt;</th>
<th>Equitability &lt;i&gt;J′&lt;/i&gt;</th>
<th>Percentage similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>83</td>
<td>87</td>
<td>92</td>
<td>87</td>
<td>4.1 ± 1.7</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Tree vitality categories (V1 to V4). V1, good health tree; V2, fair health tree; V3, significant decline tree; V4, severe decline tree. Different superscript letters indicate significant differences at <i>p</i> < 0.05.

Table 3: Myxomycete species and occurrences associated with the tree vitality categories (V1 to V4), significantly different by independent test, **<i>p</i> < 0.01.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tree vitality</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;i&gt;Paradiacheopsis rigida&lt;/i&gt; (Brandza) Nann.-Bremek.</td>
<td>102&lt;sup&gt;**&lt;/sup&gt;</td>
<td>322</td>
</tr>
<tr>
<td>&lt;i&gt;Cribraria confusa&lt;/i&gt; Nann.-Bremek. and Y. Yamam.</td>
<td>36</td>
<td>208</td>
</tr>
<tr>
<td>&lt;i&gt;Enarthne bekeleyanum&lt;/i&gt; Rostaf.</td>
<td>32</td>
<td>199</td>
</tr>
<tr>
<td>&lt;i&gt;Enarthne papillatum&lt;/i&gt; (Pers.) Rostaf.</td>
<td>54&lt;sup&gt;**&lt;/sup&gt;</td>
<td>98</td>
</tr>
<tr>
<td>&lt;i&gt;Arzycacinerea&lt;/i&gt; (Bull.) Pers.</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>&lt;i&gt;Physarum mutans&lt;/i&gt; Pers.</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>&lt;i&gt;Macbrideola cornea&lt;/i&gt; (G. Listar and Cran) Alexop.</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>&lt;i&gt;Collaria elegans&lt;/i&gt; (Racib.) Dhillon and Nann.-Bremek.</td>
<td>21&lt;sup&gt;**&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td>&lt;i&gt;Clastdermadebaryanum&lt;/i&gt; Blytt</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>&lt;i&gt;Cribraria minitissima&lt;/i&gt; Schw.</td>
<td>15&lt;sup&gt;**&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>&lt;i&gt;Echinostela minuscula&lt;/i&gt; Bary</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>&lt;i&gt;Comatricha pulchella&lt;/i&gt; (C. Bab.) Rostaf.</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>&lt;i&gt;Macbrideola confusa&lt;/i&gt; Nann.-Bremek. and Y. Yamam.</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>&lt;i&gt;Comatrichalaxa&lt;/i&gt; Rostaf.</td>
<td>9&lt;sup&gt;**&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>&lt;i&gt;Arctriapomiformis&lt;/i&gt; (Leers) Rostaf.</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>&lt;i&gt;Didermachondrioderma&lt;/i&gt; (de Bary and Rostaf.) G. Lister</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>&lt;i&gt;Macbrideoladubia&lt;/i&gt; Nann.-Bremek. and Y. Yamam.</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>&lt;i&gt;Paradiacheopsis cribibra&lt;/i&gt; Nann.-Bremek.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&lt;i&gt;Perichaenapedata&lt;/i&gt; (A. and G. Lister) G. Lister</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total occurrences</td>
<td>308</td>
<td>1223</td>
</tr>
</tbody>
</table>

showed no significant differences among the four tree vitality categories. However, mean species number per tree was markedly different among the vitality categories; that is those for V4 (5.1 ± 1.8) and V3 (4.3 ± 1.5) were higher than those for V2 (3.6 ± 1.5) and V1 (3.9 ± 1.6). Species richness was highest on V3 tree bark (17 species), followed by V1 (15 species), V4 (14 species), and V2 (13 species).

The overall species diversity (<i>H′</i>) and equitability (<i>J′</i>) was 2.27 and 0.77, respectively. Species diversity and equitability by tree vitality category were as follows: V1 (2.05 and 0.76, respectively), V2 (1.89 and 0.74), V3 (2.38 and 0.80), and V4 (2.32 and 0.88). This indicates a greater diversity of myxomycete species on the barks of trees with lower vitality (V3 and V4).

The different species of myxomycete identified from C.
Table 4: Upper part: Total occurrences and species richness in relation to bark pH class of Cryptomeria japonica tree. Lower part: Relative abundance (%) of the six species in relation to bark pH increasing and correlation ratio between the increasing bark pH and relative abundance. *, significance at $p < 0.05$; **, significance at $p < 0.01$.

<table>
<thead>
<tr>
<th>Bark pH</th>
<th>&lt;=3.2</th>
<th>&lt;= 3.5</th>
<th>&lt;=3.8</th>
<th>&lt;=4.1</th>
<th>&lt;=4.2</th>
<th>Correlation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trees</td>
<td>4</td>
<td>12</td>
<td>18</td>
<td>19</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Number of Occurrences</td>
<td>70</td>
<td>229</td>
<td>400</td>
<td>303</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Species richness</td>
<td>10</td>
<td>15</td>
<td>14</td>
<td>16</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Species
- Physarum nutans
- Clastodermadebaryanum
- Cribraria confusa
- Arcyria cinerea
- Enerthenema papillatum
- Paradiacheo psisrigida

Myxomycete communities were compared using differences in percentage similarity ($PS$) among different tree vitalities. The relative abundance of individual species was found to either increase or decrease from V1 to V4 (Table 3). For example, compared with V1, the $PS$ score decreased in V2 (0.72) and V4 (0.57). Most species showed little difference between different vitality classes, whereas several species occurred significantly on trees of a particular vitality. An independence test statistically demonstrated the significant difference in species inhabitancy. Five species, that is, *P. rigida*, *E. papillatum*, *Collaria elegans* (Racib.) Dhillon and Nann.-Bremek., *Cribraria minutissima* Schw., and *Comatricha laxa* Rostaf., had a typical preference for V1, whereas *Enerthenema japonica* bark are listed in order of abundance in Table 4. The most widespread and abundant myxomycete species were *Paradiacheopsis rigida* (Brandza) Nann.-Bremek. (Figure 3A), *Cribraria confusa* Nann.-Bremek. and Y. Yamam. (Figure 3B), and *Enerthenema berkeleyanum* Rostaf., followed by *Enerthenema papillatum* (Pers.) Rostaf. (Figure 3C), *Arcyria cinerea* (Bull.) Pers., and *Physarum nutans* Pers (Figure 3D).

### Tree vitality and myxomycete community

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berkeleyanum Rostaf. and Comatricha pulchella (C. Bab.) Rostaf. had preference for V2 tree bark. Similarly, P. nutans and Echinostelium minutum de Bary had a preference for V3 tree bark, and P. nutans, Clastoderma debaryanum Blytt, and Diderma chondrioderma (de Bary and Rostaf.) G. Lister showed a preference for V4 tree bark. Thus, tree vitality appears to influence the subsistence of corticolous myxomycete species and community composition on the bark.

Bark pH and myxomycete species

Bark pH ranged from 3.0 to 4.4, and the average pH was found to differ among vitality categories: 3.5 ± 0.3 for V1, 3.8 ± 0.3 for V2 and V3, and 3.9 ± 0.4 for V4. There was an increasing in bark pH between vitality categories V1 to V3 and V4, and there was a significant difference between the bark pH of V4 (pH 3.9 ± 0.4) and V1 (pH 3.5 ± 0.3), according to Tukey's test (Table 2). The deterioration of healthy bark caused a decrease in bark acidity. Myxomycete communities of 57 trees were categorized into five pH groups based on 0.3 increments in the bark pH ranged from 3.1 to 4.4. The species richness was 10 in the community on bark with a pH of less than 3.2, and then increased with increasing bark pH, but subsequently decreased at 4.2 and above (Table 4). The highest number of species (16) was found on bark with a pH between 3.8 and 4.1.

Relative abundance (%) of species was examined in relation to bark pH increasing. It was found that six dominant species varied in relation to bark pH classes (Table 4). The relative abundance of P. nutans, C. debaryanum, C. confusa, and A. cinerea was positively associated with bark pH classes, whereas that of E. papillatum and P. rigida was negatively associated. Thus, a decline in tree vitality was associated with an increase in bark pH, which in turn influenced species occurrence of myxomycetes on the bark.

DISCUSSION

Specialists on C. japonica

Throughout the world, many myxomycetes have been identified on the bark surfaces of living trees, which can be attributed to the passive dispersal of spores by wind (Tesmer and Schnitteler, 2007; Kamono et al., 2009). Corticolous myxomycete species complete their life cycle from spore to fruiting body on the bark surface of living trees (Keller and Brooks, 1973; Snell and Keller 2003). However, there has been limited study on the biodiversity of corticolous myxomycetes in North-East Asia (Liu et al., 2013) and Japan (Takahashi, 2014), where their distribution and ecology is poorly known. The present study of the myxomycete communities associated C. japonica tree bark provides further knowledge of myxomycete occurrence on this tree, including six species found for the first time on C. japonica; namely, E. berkeleyanum, C. elegans, E. minutum, C. pulchella, Macbrideola confusa Nann.-Bremek. & Y. Yamam., and Perichaena pedata (A. & G. Lister). Together with the species identified in a previous study (Takahashi, 2014), the total number of myxomycete species found on the bark of C. japonica was 29.

The bark of C. japonica, a tree that is endemic to Japan, has characteristic habitats for myxomycetes. The four dominant corticolous species identified in the present study, P. rigida, C. confusa, E. berkeleyanum, and E. papillatum, are specialist on C. japonica. They feed on microbes or organic matter on the acidic C. japonica bark during the amoebo and plasmodium stages, and subsequently the plasmodium gave rise to fruiting bodies at maturity.

Influence of bark pH

Trees of the same or different species with similar bark pH have been found to harbour almost identical myxomycete assemblages (Everhart et al., 2008). Takahashi (2014) arranged corticolous myxomycetes identified on seven Japanese tree species into three groups based on increasing bark pH, ranging from 2.8 (strongly acidic) to 7.9 (moderately alkaline). The distribution of corticolous myxomycetes depends to a large extent on bark pH, which in C. japonica has narrow range from 2.8 to 4.4. In the present study, the pH of C. japonica bark ranged from 3.0 to 4.6, and six species of myxomycetes that occurred relatively abundantly responded sensitively to bark pH, with their relative abundance either increasing or decreasing in relation to pH (Table 4). Overall, P. rigida was the most abundant and acid tolerant, inhabiting bark with pH values ranging from 3.1 to 4.0, whereas it was scarce on bark with a pH of 4.4. E. papillatum was more abundant under strongly acidic conditions, whereas P. nutans, C. debaryanum, C. confusa, and A. cinerea became more abundant with increasing bark pH (Table 4). Accordingly, it was clearly demonstrated that pH plays an important role in determining colonization by myxomycete species on the bark of tree.

These results are mostly consistent with those reported in previous studies (Härkönen, 1977; Stephenson, 1989; Snell and Keller, 2003). For example, among corticolous species in southern Finland, the relative abundance of E. papillatum and C. pulchella peaked at pH 3, whereas that of Arcryia pomiform peaked at pH 4, and that of A. cinerea and E. minutum peaked at pH 4.5 or higher (Härkönen, 1977), In North America, the relative abundance of Cribraria minutissima peaked at pH 3.7–3.8, whereas that of C. confusa and E. papillatum peaked at pH 4.1–4.5, that of Macbrideola cornea peaked at pH 5.4–5.8, and that of D. chondrioderma peaked at approximately pH 7 (Stephenson, 1989).
Tree deterioration leads to a loss of vitality and causes the bark to change pH. This in turn influences the colonization of myxomycete species, despite the pH being in the narrow strong to weak acid range.

**Tree vitality and bioindicator**

Corticolous myxomycetes colonize on tree barks using as a substrate affecting varied environments. To the best of my knowledge, this study is the first to present evidence of an association between tree vitality and their colonization. Air pollution and low precipitation have been cited as a causal factor of *C. japonica* tree decline in the present study area (Takahashi et al., 1991). A few studies have already examined the influence of air pollution on the occurrence of corticolous myxomycetes (Härkönen, 1977; Wrigley, 2000; 2004), and the findings have been compared with observation made after recent decades of recovery from air pollution (Härkönen and Vänskä, 2004). These studies suggest that corticolous myxomycetes can be used as bioindicator species for air pollution. However, the variation in species distribution is not necessarily directly affected by air pollution, which is a cause of tree decline and might also obliquely alter the quality of the bark surface.

The living tree bark of *C. japonica* forms a flat fibrous surface and has a characteristic acidic nature (Tazaki et al., 2004). The bark also contains antimicrobial compounds and a low cellulose content (Kofujita et al., 2001), which creates a relatively infertile and sterile substrate that is particularly resistant to degradation. Tree bark plays a critical role in defending trees against pathogens and herbivores, thereby preserving tree vitality (Shibutani et al., 1998; Kofujita et al., 2001). In this study, tree vitality categories were found to occasionally differ along the Takahashi River, influenced either by differences in climate or human activity. When the vitality of healthy trees begins to decline, there is a concomitant weakening of bark acidity, which in turn causes changes in the occurrence of myxomycete species, and presumably also changes in the microbial fauna and flora on bark.

Myxomycetes typically have global distribution (Martin and Alexopoulos, 1969) because their spores are extensively dispersed worldwide by wind. However, several myxomycete species are specialist inhabitants of a particular pH niche on *C. japonica* bark. Their preference for certain bark conditions presumably limits their distribution to this specific microhabitat, and this probably explains the moderately endemic distribution of myxomycetes, as well as protists in general (Foissner, 2007). The present study provides new insights into the relationship between tree vitality and the distribution of corticolous myxomycetes. Thus, monitoring of corticolous myxomycetes may provide a specific and quantitative understanding of tree vitality, and also yield information on bioindicator species that can be utilized to detect changes in the environment. These observations will also contribute to enhancing tree conservation in green areas of city parks and forests in local terrestrial ecosystems.

**Conclusion**

The Japanese cedar tree (*C. japonica*), which is an endemic tree species in Japan, has been used as an indicator for the assessment of air pollution. Moist chamber cultures revealed that a reduction in tree vitality influences the occurrence of corticolous myxomycetes due to microhabitat modification brought about by a change in bark pH. The species density and diversity of corticolous myxomycetes tended to increase in relation to a decline in tree vitality. The dominant species found on healthy tree bark were different to those found on declined tree bark. The distribution of several myxomycete species changed in relation to a change in bark pH.

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**REFERENCES**


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