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Geographical distributions of rhytismataceous fungi on *Camellia japonica* leaf litter in Japan

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ABSTRACT

Rhytismataceous fungi (Ascomycota) exhibit ligninolytic activities during the initial stages of litter decomposition. We quantitatively investigated the geographical distributions of rhytismataceous fungi on *Camellia japonica* leaf litter across Japan. We found three rhytismataceous species (*Coccomyces* sp., *Lophodermium jiangnanense*, and a Rhytismataceae sp.) on bleached leaves of *C. japonica*. The *Coccomyces* sp. was distributed at all 40 sites investigated. On the other hand, *L. jiangnanense* was restricted to the southwestern region, and the Rhytismataceae sp. was localized to part of the warm-temperate zone. *L. jiangnanense* and the Rhytismataceae sp. were more common at sites with higher annual temperatures and greater precipitation. The relative abundance of rhytismataceous fungi revealed that either *Coccomyces* sp. or *L. jiangnanense* predominated at all sites, with a distribution related to annual precipitation. These results suggest that the geographical distributions and abundances of rhytismataceous fungi are influenced by climatic conditions.

1. Introduction

It is of fundamental importance to understand the geographical distributions and abundances of fungal species in leaf litter, as well as their diversity and ecological function (Osono, 2011), when it is sought to understand the organization of fungal communities associated with decomposition. Such data would yield substantial information on how climate influences the growth characteristics and the abundance of fungi. However, the identification and quantification of fungi are methodologically difficult because the featureless hyphae extend into the substrate (Arnolds, 1997). Previous studies have semi-quantitatively investigated the distributions of saprotrophic fungi using the frequencies of occurrence (the proportions of colonized substrates), an index of fungal abundance. van Maanen et al. (2000) found that the frequencies of occurrence of three saprotrophic fungi on *Pinus sylvestris* litter correlated with the altitudinal gradient, suggesting that temperature and/or precipitation affected fungal distribution and frequency. In addition, in Japan, the distributions of saprotrophic fungi on coniferous litter are positively correlated with the mean annual temperature (Iwamoto and Tokumasu, 2001; Tokumasu, 2001). However, the frequency of occurrence is not an appropriate metric for comparing the abundances of various fungal species on a single substrate. Adequate study has not been conducted on either geographical variation in the relative abundance of fungi sharing a common substrate or on the factors influencing such variation. Quantitative studies on fungal species, distributions and factors possibly affecting such distributions are very few in number compared to the wealth of data available for macroorganisms (Pirozynski, 1968; Arnolds, 1997; Tokumasu, 2010).

Rhytismataceae is a family of Ascomycota that includes 44 genera (Lumbsch and Huhndorf, 2007). Rhytismataceous fungi are

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pathogens, endophytes, and saprotrophs of plants (Cannon and Minter, 1986). Species in the genera *Lophodermium* and *Coccomyces* are thought to decompose litter (Sherwood, 1980; Minter, 1981). Some *Lophodermium* species are endophytic fungi that dominate coniferous needles (Hata et al., 1998; Deckert and Peterson, 2000; Ganley and Newcombe, 2006). A few endophytic rhytismataceous fungi have ligninolytic capacities and (as saprotrophs) decompose leaves after leaf fall (Koide et al., 2005a, 2005b; Osono, 2007; Osono and Hirose, 2011; Yuan and Chen, 2014).

Rhytismataceous fungi often bleach portions of fallen leaves, and zone lines separate different neighboring species or strains. The zone lines are assumed to mark the edges of individual colonies on substrates (Rayner and Todd, 1979; Minter, 1981). Hirose and Osono (2006) used dual culture tests to confirm that different isolates of *Lophodermium pinastri* formed zone lines on pine needles, and applied this knowledge to quantitatively evaluate seasonal variation in *Lophodermium* populations.

Camellia japonica is a common tree that dominates evergreen broad-leaved forests in Japan. The tree is widely distributed through the cool- and warm-temperate zones and the subtropical zone, attaining its northern limit in the region of Aomori prefecture (Horikawa, 1972). Thus, *C. japonica* is a suitable substrate for assessment of the fungal distribution on the same host species at various climatic sites. Previous studies have shown that rhytismataceous fungi on *C. japonica* leaves play important roles in the forest ecosystem; the fungi are the primary selective decomposers of lignin (Koide and Osono, 2003; Koide et al., 2005a, 2005b). Two rhytismataceous species belonging to the genera *Coccomyces* and *Lophodermium* have been recognized on *C. japonica* leaf litter, accompanied by pronounced bleaching (Koide and Osono, 2003; Koide et al., 2005a, 2005b; Hirose et al., 2013). Strains of Rhytismataceae have been reported to have bleached portions of sterilized leaves of *C. japonica* under pure culture conditions (Koide et al., 2005b). In addition, Koide et al. (2005a) showed that these rhytismataceous fungi infected only living leaves of the tree (as endophytic fungi) and bleached the leaves after leaf fall. However, to date, there have not been any studies on the species composition, distribution, and abundance of rhytismataceous fungi on *C. japonica* leaf litter.

The objective of this study was to reveal the species compositions and geographical distributions of rhytismataceous fungi on *C. japonica* leaf litter in Japan, based on a quantitative investigation. We focused on the areas of fungal colonies, defined as bleached portions of leaves surrounded by zone lines. These areas were used as quantitative measures of mycelial abundance. We employed both this method and the frequency of occurrence (the proportions of colonized leaves) to investigate the distributions of rhytismataceous fungi. Measurements of colony areas allowed us to compare the relative abundances of fungal species coexisting within a single leaf. Then we calculated relative abundances to investigate the predominant species on *C. japonica* litter at each site. We analyzed the effects of climatic factors (annual mean temperature and annual precipitation) on the geographical distributions and abundances of rhytismataceous species.

2. Materials and methods

2.1. Study sites and collecting samples

We collected bleached, fallen leaves of *C. japonica* that had been detached from the tree in the current year and had been colonized by rhytismataceous fungi (Fig. 1) at 40 sites across Japan in 2009–2013 (Table 1). In each forest, we selected five trees approximately 4 m away from each other and picked 10 leaves per tree. A total of 50 leaves were collected at each site, for a total of 2000 leaves (40 sites × 50 leaves). All samples were placed in paper bags, transported to the laboratory, and held at 4 °C until treated as described below.

2.2. Measurement of areas colonized by rhytismataceous fungi

All leaves were put between newspapers, dried for 2 weeks at 40 °C in a drying machine, and then stored at room temperature under moisture-free conditions. Images of the pressed leaves were captured using a scanner (CanoScan 8400F; Canon, Japan). The bleached portions of *C. japonica* leaves were separated into small zones by black zone lines (Fig. 1) formed between colonies of different strains or species. We measured the areas of all bleached zones.

To identify the fungi colonizing each bleached zone, we observed the morphological characteristics of ascomata under a light microscope and the fungi were assigned to morphological groups. In addition, we sequenced the rRNA internal transcribed spacer (ITS) regions of each morphological group. We thus recognized three taxa of the Rhytismataceae (Fig. 2; Appendices 1 and 2). We measured the areas of bleached zones surrounded by zone lines using image analysis software (Microanalyzer ver. 1.1d; Nihon-Poladigital, Japan). All bleaching was considered to be caused by one of the three rhytismataceous fungi, although we could not identify the fungi causing bleaching of a minority of zones because the zones did not contain ascomata. Bleached areas for each taxon were summed to calculate the colony area of each rhytismataceous fungus on each leaf (the 'colony areas').

2.3. Indices used to evaluate fungal distributions

We calculated three indices to assess the level of each rhytismataceous fungus at each site: the frequency of occurrence, the colony area ratio, and the relative abundance. Frequency of occurrence was calculated using the following formula: frequency of occurrence (%) = the number of bleached leaves that had ascomata of the rhytismataceous species/the number of bleached leaves collected at each site (n = 50) × 100. Each colony area ratio was calculated as the colony area per unit area (to allow for variation in whole leaf areas among sites) using the following formula: colony area ratio = integrated colony area of a given rhytismataceous species at each site (mm^2)/integrated whole leaf area at each site (mm^2). In addition, the relative abundance of the colony area of each rhytismataceous fungus at each site was calculated using the following formula: relative abundance (%) = integrated colony area of a given rhytismataceous species at each site (mm^2)/integrated colony areas of all rhytismataceous species at each site (mm^2) × 100.

2.4. Statistical analysis

Generalized linear models (GLMs) were used to perform single and multiple regression analyses exploring relationships between the distributions of rhytismataceous fungi and climatic factors. The response variables were frequency of occurrence, the colony area ratio, and the relative abundance of each rhytismataceous fungus. The 'frequency of occurrence' data at all sites included absence of a fungus, whereas colony area ratios and relative abundances were calculated only for sites at which fungi were present. We analyzed frequencies of occurrence using the binomial error distribution and the logit link function. We analyzed colony area ratios and relative abundances using the Gaussian error distribution and the log link function, because these data were not normally distributed (Shapiro-Wilk test, $P < 0.05$). The explanatory variables were the average (over the past 30 y) of the annual mean temperature (AMT)