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Predicted potential distribution of *Sydowia japonica* in Japan

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ABSTRACT

Sydowia japonica, a dothidealean fungus, is a parasite that is specific to the male strobili of Japanese cedar. The fungus is a candidate for the control of Japanese cedar pollen dispersal, which is a cause of pollinosis. To evaluate *S. japonica* for bioherbicidal applications, it is necessary to characterise its potential distribution and environmental niche. Here, we predicted the distribution of *S. japonica* in Japan using field surveys and a maximum entropy model, and identified the environmental variables that influence its distribution. We identified *S. japonica* from a total of 87 localities in Japan through field surveys. Based on presence data and associated environmental variables, our model predicted that *S. japonica* is widely distributed in Japan, but concentrated in the Hokuriku and Kinki areas along the Sea of Japan. The model also predicted that the most important environmental variables influencing fungal distribution were sunshine duration in the winter and precipitation in the summer. This new information will contribute to the development of bioherbicidal applications for *S. japonica*.

1. Introduction

Sydowia japonica (Kasai) Hirooka & Masuya is a fungal species that belongs to Dothideaceae, Dothideals, Ascomycota, although it has previously been treated as *Leptosphaerulina japonica* Kasai (Hirooka, Masuya, Akiba, & Kubono, 2013b). This fungus has a unique ecology as it specifically infects the male strobili of Japanese cedar (*Cryptomeria japonica*) and prevents pollen dispersal. This trait makes the fungus suitable for preventing the spread of *C. japonica* pollen, which causes Japanese cedar pollinosis, a serious allergy. Much of the Japanese population suffers from pollinosis, and the allergy is responsible for significant economic losses (Kawaguchi, Hoshiyama, & Watanabe, 2001). While there is great interest in applying *S. japonica* for pollen control, its ecology is not yet well characterised.

The life cycle of *S. japonica* has been documented in previous reports. In autumn, ascospores and conidia are dispersed from ascocarps and acervuli produced by the male strobili of *C. japonica*. The spores germinate on the living male strobili, invade the pollen sac and pollen, and eventually colonise the interior of the strobili in spring. From spring to summer, the fungus spreads throughout the strobili and decomposes the inner structures, including pollen. The following autumn, new ascocarps and acervuli are produced on the ramenta covering the male strobili (Hirooka et al., 2013a, b). The growth of this fungus is suppressed at temperatures exceeding 28 °C, but there is currently no information about the production of ascospores, ascocarps, conidia and acervuli in relation to temperature (Hirooka et al., 2013a, b). Furthermore, the distribution of *S. japonica* in Japan has not been fully reported, although the fungus has been isolated from several Japanese localities (Hirooka et al., 2013a). The lack of knowledge about the ecology of *S. japonica* poses an obstacle to its use as a bioherbicide for *C. japonica*. Indeed, the establishment of biocontrol agents in applied areas often fails due to the lack of knowledge about their ecology such as suitable environmental condition for their establishment (Harding & Raizada, 2015). The establishment of bioherbicidal applications using the fungus is likely to be affected by many environmental factors, including temperature and precipitation. For this reason, practical application of the fungus as a bioherbicide requires characterisation of its environmental niche.

Species distribution models (SDMs) are mathematical approaches used to predict the potential distribution of a particular species using species occurrence data and measurements of environmental variables (Phillips, Anderson, & Schapire, 2006). There are many types of SDMs, but the models that use presence/absence data are generally more accurate (Phillips & Dudík, 2008; Phillips

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et al., 2006). However, in many cases, absence records are unavailable because of technical limitations. In particular, absence data for many fungi are unreliable because the fungi are microscopic, hard to detect, and/or difficult to identify, sometimes even through molecular analysis. In such cases, species distributions can be predicted by SDMs using presence-only data. Among such models, the maximum entropy (MaxEnt) approach is considered to offer superior performance (Elith et al., 2006), and seems to be well suited for modelling the distributions of fungal species. Galdino et al. (2016) maped the potential risk of the mango sudden decline caused by *Ceratocystis fimbriata* by using MaxEnt approach. Cogliati et al. (2017) used MaxEnt approach to produce the detailed prediction maps of human-pathogenic fungi, *Cryptococcus neoformans* and *C. gattii* species complex in Europe and Mediterranean area. Shrestha and Bawa (2014) showed the impact of climate change on the potential distribution of *Ophiocordyceps sinensis* with MaxEnt approach. These approaches can be contributed in preparation of efficient risk assesmments and disease monitoring, but the prediction of the fungal distribution has never been attempted for the development of the biocontrol agents.

In the present study, we used a MaxEnt approach to predict the potential distribution of *S. japonica* in Japan in relation to relevant environmental variables. To this end, we surveyed 87 localities in Japan for the presence of *S. japonica* and integrated this data into the MaxEnt model along with environmental variables from climatic mesh data (2010 normal annual values) taken from the Japan Meteorological Agency.

2. Materials and methods

2.1. Collection of samples

We observed and collected the tips of twigs with male strobili infected by *S. japonica* from various localities in Japan from 2009 to 2016. The symptoms of infection were unique and easily identifiable (Fig. 1). However, to ensure accurate identification, we used molecular analyses in addition to microscopic observation for some samples. Spatial distribution data (latitude and longitude) for the samples of *S. japonica* was recorded by global positioning system (Supplementary table S1).

2.2. Identification

Two methods for identification were conducted: isolation from samples and direct detection through molecular techniques. To establish pure *S. japonica* cultures, infected male strobili were surface-sterilised by washing with 70% ethanol, sodium hypochloride (1% Cl), and sterilised water. Washed samples were dissected with flame-sterilised tweezers and 2 mm^3 pieces of the inner tissues were placed on 1% malt extract agar emended with 200 μg/L streptomycin. After a week, growing mycelia from the inner tissues were picked and placed on 2% malt extract agar. Obtained pure cultures were identified based on their observed morphology and through molecular analysis as described by Hirooka et al. (2013b).

For direct detection, we developed a species-specific primer pair for *S. japonica* that targeted its internal transcribed spacer 2 region. We used *S. japonica* sequence data from the DNA Data Bank of Japan Genbank (accession no. JQ814698) and designed primers using the Primer 3 plus website (http://www.primer3plus.com). Developed primers (SJF: 5′-GCG TGC CTC GAA GAC CTC-3′ and SJR: 5′-AAA ATT GGT TTA ACG GCT ATG G-3′) were first tested for their specificity using National Centre for Biotechnology Information/Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Total DNA was extracted from infected male strobili using the FastDNA Spin Kit (MP Biomedicals). Polymerase chain reaction (PCR) was performed using 40 μL reaction mixtures containing 1 μL genomic DNA, 2 μM of each primer, 20 μL GoTaq green master mix (Promega), and distilled water. Thermocycling conditions were as follows: initial denaturation at 95 °C for 4 min, 35 cycles of 94 °C for 30 s, 63 °C for 50 s, 72 °C for 50 s, and a final elongation at 72 °C for 7 min, using the Biorad T100 thermocycler. Amplified products were verified by 1% agarose gel electrophoresis.

2.3. Environmental variables

We predicted the potential distribution of *S. japonica* using MaxEnt version 3.4.0 (Phillips, Dudík, & Schapire, 2017) based on presence data of *S. japonica* and climatic data (1 km resolution) for 2010 from the Japan Meteorological Agency (http://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-G02.html). Shape files for each environmental variable were transformed to ascii files using QGIS 2.14 (QGIS Development Team, 2009). A total of 78 factors (monthly average temperatures, maximum temperatures, minimum temperatures, precipitation, amount of global solar radiation, and sunshine duration) were used as environmental variables. One hundred bootstrap replicates were used. Replicated data sets were selected by sampling replaced with random seeds. The accuracy of the resulting model was assessed by calculating the area under the curve (AUC) for the receiver operating characteristic curve. AUC values close to 1 indicate that the model is accurate, whereas values under 0.5 suggest that the model had poor predictive value performance.

3. Result and discussion

We used the MaxEnt model to predict the potential distribution of *S. japonica*. According to the model, *S. japonica* tended to be distributed along the side of the Sea of Japan. The fungus was also predicted to be distributed on the Pacific Ocean side, albeit with a low probability of distribution (Fig. 2). The model predicted that the fungus has a high probability of distribution in the Kinki region (Fig. 2). The average AUC for the replicate runs was 0.768, and the standard deviation was 0.048 (Fig. 3). This suggests that the model's predictions were accurate. Indeed, we were able to easily find the fungus in areas where it had a high probability of occurrence, including the Kinki region (Ichihara & Masuya, 2015, Supplementary Fig.1). We were also able to find *S. japonica* in the Hakodate area, on the southern part of Hokkaido Island, in which the MaxEnt model predicted a high probability of its occurrence. Conversely, while we have repeatedly conducted surveys in areas where the fungus has a low probability of occurrence (e.g., the

Fig. 1. Male strobili of Japanese cedar infected by *Sydowia japonica*.

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