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Note

Development of a model system comprising *Populus* as a model tree and *Colletotrichum gloeosporioides* as a model pathogen for studying host–pathogen interactions

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Abstract To develop a model system for studying host tree–fungal pathogen interactions, we focused on *Populus* as a model tree and the fungus *Colletotrichum gloeosporioides* as a model pathogen. Although hybrid *Populus* is currently planted worldwide for biomass production and other purposes, the hybrid often lacks the tolerance of native species to cold, drought, and disease. *Populus* diseases have increased with the rapid development of *Populus* plantations worldwide. To investigate the susceptibility of hybrid aspen plants to the fungal pathogen *Colletotrichum*, which has a wide range of hosts, hybrid aspen plants were inoculated with *Colletotrichum* species such as *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. higginsianum*, and *C. orbiculare*. *C. gloeosporioides* strains isolated from strawberry plants could infect hybrid aspen. These results indicate that monoculture of hybrid *Populus* may be potentially susceptible to local pathogens. The experimental system using hybrid aspen and *C. gloeosporioides* presented here will bring a lot of fruitful information, as first model system of host tree–fungal pathogen interactions.

Key words: Aspen, *Colletotrichum gloeosporioides*, hybrid *Populus*, strawberry anthracnose

A model system for studying host-pathogen interactions is one of strong tools to develop our knowledge on the field of plant pathology. To establish such a system, there are several requirements for both pathogen and its host plant; ease of handling in the laboratory, and availability of genomic information and genetic engineering technology. To establish a host–pathogen model system is very difficult because it deals with an interaction between two organisms. However, recent advances in biological analysis technologies are changing the situation. The present study aimed to search for a candidate for developing a model system to study host tree–fungal pathogen interactions. We focused on *Populus* as a model tree and *Colletotrichum* species as model fungal pathogens.

Populus as a model tree is an important tool for research in the genomics and molecular biology of woody plants. Recently, such research has been advanced by complete sequencing of the *Populus trichocarpa* Nisqually-1 genome (Brunner et al. 2004; Tuskan et al. 2006).

In tree breeding, the production of hybrids is considered a promising strategy for developing novel industrially useful tree varieties. Hybrid species of *Populus*, a genus containing 25–35 species of deciduous flowering plants in the family Salicaceae, are among the most successful examples. Hybrid *Populus* plants are currently planted in many regions worldwide for purposes such as production of wood, energy, and fiber resources because of their typically rapid growth of 5–8 feet per year (Stettler et al. 1996).

However, hybrid *Populus* often lacks cold, drought, and disease hardiness of native species. *Populus* diseases have increased with the rapid development of *Populus* plantations (Xiang and Zhu 2000; Yu et al. 2004). Diseases such as leaf spot, canker disease (Ostry 1987, 1994), leaf rust, and black spot (Xiang and Zhu 2000) cause high biomass loss. These facts are increasing the demand for effective control of *Populus* diseases, based on the understanding of the molecular mechanisms of *Populus*–pathogen interactions.

Colletotrichum species serve as excellent models for studying the molecular basis of infection structure differentiation and fungus–plant interactions because

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of tractability of the fungal culture and development of techniques for genetic manipulation (Perfect et al. 1999). Pathosystems between *Colletotrichum* species and model plants have been reported in previous studies. For example, *C. higginsianum* infects the model plant *Arabidopsis thaliana* (Narusaka et al. 2004; O'Connell et al. 2004) and *C. orbiculare* infects the model plant *Nicotiana benthamiana* (Shen et al. 2001). In addition, genome and transcriptome analyses of *C. higginsianum* that infects *A. thaliana* and *C. graminicola* infecting maize (*Zea mays*) have been reported (O'Connell et al. 2012). Genomes of *C. orbiculare*, the causative agent of cucurbit anthracnose, and *C. gloeosporioides*, isolated from strawberry, have also been sequenced and analyzed (unpublished data).

Anthracnose is a highly destructive plant disease worldwide caused by the fungi *Colletotrichum* spp.. *Colletotrichum* species have a wide range of hosts, including cereals, legumes, vegetables, perennial crops, shade trees, and tree fruits (Bailey and Jeger 1992). In particular, strawberry anthracnose, caused by *C. gloeosporioides* (teleomorph; *Glomerella cingulata*), is an economically serious disease worldwide (Xie et al. 2010). *C. gloeosporioides* infects various hosts, including many fruit and vegetable species, regardless of the host origin (Kim et al. 1999). Poplar anthracnose is also caused by *C. gloeosporioides* (Xiang and Zhu 2000). However, genetic manipulation for *C. gloeosporioides* isolated from *Populus* has not been established till date and its genome has not been sequenced. Here we report that strains of *C. gloeosporioides* isolated from strawberry plants can infect hybrid aspen, and we describe the development of a model system for studying host tree–fungal pathogen interactions.

First, for evaluating the susceptibility of hybrid aspen plants to *C. gloeosporioides*, hybrid aspen plants were inoculated with *C. gloeosporioides*. Seven *C. gloeosporioides* strains isolated from anthracnose lesions on strawberry plants (Nara-gc5, 2007 han-6-10, RSGC09-1-B, RSGC09-1-C, RSGC09-1-D, and RSGC09-2-B) and from *Glycine max* Merrill (MAFF 238875) were used in this study (Table 1). Cultures of the isolate were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 24°C in the dark. Conidia were obtained by gently scraping cultures incubated for 10–14 days under a 16-h light/8-h dark cycle with a blacklight blue fluorescent lamp (FL10BLB; Toshiba Corp., Tokyo, Japan) and filtered through two layers of sterile cheesecloth. The strawberry plants (*Fragaria×ananassa* Duchesne, cvs. Sachinoka and Hokowase) were grown in Soil Mix (Sakata Seed Corp., Yokohama, Japan) and expanded vermiculite (1.5–2-mm granules) at a ratio of 1 : 1 in a growth chamber at 25°C during daylight. Hybrid aspen plants (*Populus tremula×tremuloides*, T89; Nilsson et al. 1992) were grown in Soil Mix and expanded vermiculite (1.5–2-mm granules) at a ratio of 1 : 1 in a growth chamber at 22°C under a 12-h light/12-h dark cycle. Approximately 4–105-μl drops of a spore suspension containing 5×10^5 spores ml^{-1} in distilled water were placed on detached leaves of strawberry and hybrid aspen. Alternatively, for a whole plant assay, hybrid aspen was sprayed with a spore suspension containing 5×10^5 spores ml^{-1} in distilled water. The inoculated leaves or whole plant were then placed in a growth chamber at 22°C under a 12-h light/12-h dark cycle and maintained at 100% relative humidity. Control leaves were treated with only distilled water.

All the six *C. gloeosporioides* strains isolated from strawberry caused anthracnose disease symptoms on strawberry cv. Sachinoka (Figure 1A); however, strawberry cv. Hokowase was resistant to infection by these strains. On hybrid aspen leaves, all these *C. gloeosporioides* strains isolated from strawberry incited anthracnose disease symptoms similar to those incited on strawberry plants (Figure 1). When challenged with the fungus, hybrid aspen leaves developed brown necrotic lesions. These lesions expanded from the inoculation site within 3 days post inoculation (dpi). *C.*

Table 1. Origins of the *Colletotrichum* isolates used in this study and their virulence on hybrid aspen

Culture collection numbers	*Colletotrichum* sp.	Host plant of origin	Virulence on hybrid aspen*
Nara-gc5	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
2007 han-6-10	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
RSGC09-1-B	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
RSGC09-1-C	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
RSGC09-1-D	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
RSGC09-2-B	*C. gloeosporioides*	*Fragaria×ananassa* Duchesne ex Rozier	+
MAFF 238711	*C. dematium*	*Raphanus sativus* L. var. *longipinnatus* L. H. Bailey	−
MAFF 238712	*C. dematium*	*Raphanus sativus* L. var. *longipinnatus* L. H. Bailey	−
MAFF 240106	*C. destructivum*	*Perilla ocymoides* L.	−
MAFF 238875	*C. gloeosporioides*	*Glycine max* Merrill	−
MAFF 305635	*C. higginsianum*	*Brassica rapa* L. Perviridis Group	−
MAFF 240422 (104-T)	*C. orbiculare*	*Cucumis sativus* L.	−

*The detached leaves were inoculated with approximately 4–10 5-μl drops of a spore suspension of *Colletotrichum* isolates (5×10^5 spores ml^{-1}). + Indicates lesion formed, − indicates no lesion formed.

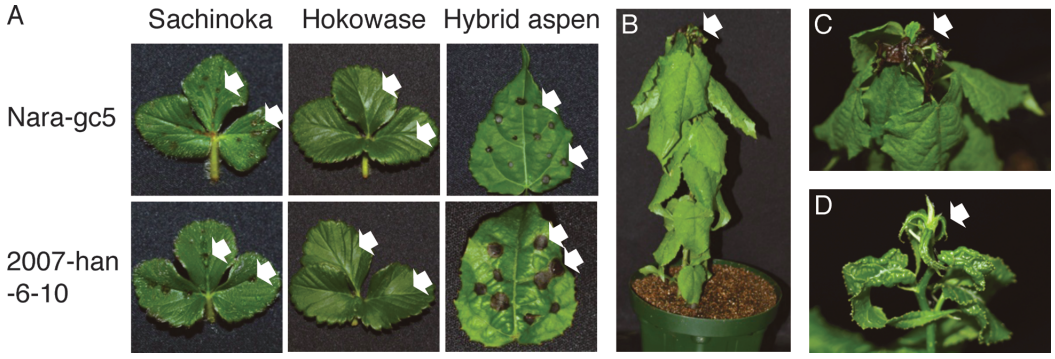

Figure 1. Strawberry and hybrid aspen leaves infected with the fungus *C. gloeosporioides* strains Nara-gc5 and 2007 han-6-10 isolated from anthracnose lesions on strawberry plants. (A) The detached leaves were inoculated with approximately 4–10 5-μl drops of a spore suspension of *C. gloeosporioides* (5×10^5 spores ml^{-1}). For a whole plant assay, hybrid aspen was sprayed with a spore suspension of *C. gloeosporioides* strain Nara-gc5 (5×10^5 spores ml^{-1}) (B, C) or distilled water as a control (D). Lesion phenotypes at 4 dpi are shown. Arrows indicate the inoculation sites and lesions. Each picture is representative of three independent experiments.

gloeosporioides spores germinated and formed melanized appressoria on the strawberry and hybrid aspen leaf surfaces (Figure 2A, B). Orange–pink to brownish spore masses were observed on the lesion surface on hybrid aspen leaves 5 dpi (Figure 2C), and the fungus recovered from infected plant material was used to complete Koch's postulates. There was no obvious difference among the fungal strains in their ability to cause disease symptoms, and accordingly, the strain Nara-gc5 was chosen for further experiments. In contrast, *C. gloeosporioides* isolated from *G. max* did not cause anthracnose disease symptoms on hybrid aspen leaves (Table 1).

For investigating the host range, the spore suspension of the *C. gloeosporioides* strain Nara-gc5 was put onto *Brassica rapa* L. Perviridis Group (Japanese mustard spinach, Komatsuna), *N. benthamiana*, tomato (*Solanum lycopersicum* L. cv. Moneymaker), and *A. thaliana* ecotype Columbia. None of these plants showed any disease symptoms at 7 dpi. Thus, among the crops tested, only strawberry and hybrid aspen plants were susceptible to *C. gloeosporioides* isolated from anthracnose lesions on strawberry. Although *C. gloeosporioides* generally has a wide host range, the *C. gloeosporioides* Nara-gc5 used here were host specific.

To further investigate the susceptibility of hybrid aspen plants to *Colletotrichum* species, hybrid aspen plants were inoculated with different *Colletotrichum* species. Hybrid aspen was resistant to *C. dematium* (Persoon : Fries) Grove (MAFF 238711 and 238712) isolated from *Raphanus sativus* L. var. *longipinnatus* L. H. Bailey, *C. destructivum* O'Gara (MAFF 240106) isolated from *Perilla ocymoides* L., *C. higginsianum* Saccardo (MAFF 305635) isolated from *Brassica rapa* L. Perviridis Group, and *C. orbiculare* (Berkeley et Montagne) Arx (MAFF 240422, 104-T) isolated from *Cucumis sativus* L. (Table 1). In contrast, these fungal pathogens were virulent on their respective host plants.

Among the plant species tested for susceptibility to *C.*

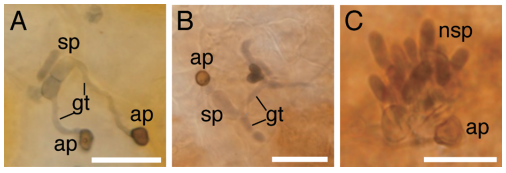

Figure 2. Infection phenotypes of leaves of strawberry (cv. Sachinoka) and hybrid aspen with the *C. gloeosporioides* strain Nara-gc5. The detached leaves were inoculated by placing approximately 4–10 5-μl drops of a *C. gloeosporioides* spore suspension (5×10^5 spores ml^{-1}). Leaves at 5 dpi were cleared and stained with trypan blue. *C. gloeosporioides* spores germinated and formed melanized appressoria on (A) strawberry and (B) hybrid aspen leaf surfaces. (C) The fungus sporulated on hybrid aspen leaves. ap: appressorium, sp: spore, gt: germ tube, nsp: newly formed spores. Bars = 25 μm. Each image is representative of three independent experiments.

gloeosporioides, aspen (Malpighiales) is phylogenetically the closest to strawberry (Rosales) (Stevens 2001, http://www.mobot.org/MOBOT/research/APweb/). In contrast, hybrid aspen was resistant to *Colletotrichum* species isolated from cucumber (Cucurbitales) and *G. max* (Fabales), making it difficult to predict the host range of *Colletotrichum* species from phylogenetic relationships of host plant species. Whether natural infections of hybrid *Populus* by *C. gloeosporioides* occur in the field remains unclear; however, we showed that *C. gloeosporioides* isolates from strawberry can infect hybrid aspen in laboratory inoculation experiments.

Populus plants are exposed to attacks by many pathogens in the field. However, stands in *Populus* plantations are monoclonal and the diversity of cultivars planted in a given region is limited (Gérard et al. 2006). Furthermore, most hybrid *Populus* plants are not native to the growing area; thus, the plants may be potentially susceptible to endemic pathogens. In addition, because the pathogen produces conidia, even disease-free *Populus* fields can become contaminated with inoculum from neighboring strawberry fields. *Populus* may become a secondary host or an intermediate host that transfers

a pathogen to strawberry. We recommend a search for pathogenic variation in microorganisms affecting *Populus* and analysis of mechanisms of disease resistance in *Populus*. These measures will also be important for the development of potential fungicides and breeding of disease-resistant *Populus* cultivars. The experimental system using hybrid aspen and *C. gloeosporioides* presented here will bring a lot of fruitful information for these aspects, as first model system of host tree–fungal pathogen interaction.

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