Notes

Outbreak of Powdery Mildew on *Zinnia elegans* by *Golovinomyces cichoracearum* in Korea, 2008-2010

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Podosphaera fusca (syn. *Sphaerotheca fusca*, *S. fuliginea*) is a causal agent of powdery mildew infections on *Zinnia elegans*, and has been documented in Korea since 1967. An outbreak involving about 1,000 plants in October 2008, in Yangpyeong, Korea, was caused by *Golovinomyces cichoracearum*. Further surveys of zinnia powdery mildew from 2008-2010 confirmed the widespread occurrence of *Zinnia-Golovinomyces* association in Korea. Chasmothecia of this fungus have not been found on *Z. elegans* in Korea. Analysis of internal transcribed spacer rDNA from five Korean collections confirmed the powdery mildew as *G. cichoracearum*. Comparison with the those from GenBank revealed 100% identity with sequences from zinnia powdery mildew, forming a monophyletic clade with 100% bootstrap support, and with those of *G. cichoracearum* parasitic on the hosts belonging to the tribe Heliantheae of the family Asteraceae.

Keywords : Heliantheae, ITS sequence, *Podosphaera fusca*, *Reticuloidium*

Zinnia (*Zinnia elegans* Jacq.) is a fast-growing annual plant native to Mexico that comprises many cultivars. The plants are widely cultivated in Korea in flower beds for ornamental purposes. In October 2008, about 1,000 zinnia planted around the ponds in Dumulmeori Park, Yangpyeong, Korea, were affected by a heavy infection of powdery mildew. Only a few leaves remained alive in the diseased plants. Microscopic examination of the leaves revealed that the powdery mildew was associated with an *Oidium* subgenus *Reticuloidium*, which is an anamorphic state of the genus

Golovinomyces. The finding of a *Zinnia-Reticuloidium* association was surprising, because zinnia occurring in Korea had previously been thought to be subject to mildew only by *Podosphaera fusca* (Fr.) U. Braun & Shishkoff (Lee and Lee, 1967; Shin, 1994, 2000). Furthermore, such a widespread occurrence of powdery mildew infection of zinnia plants had never been observed (unpublished data).

Surveys of powdery mildew on zinnia. We conducted further surveys of zinnia powdery mildew from 2008-2010, and confirmed *Golovinomyces* infections in many localities of Korea. The voucher specimens collected during these surveys have been deposited in the herbarium of Korea University, Seoul. To our surprise, there was no evidence of powdery mildew infection due to *P. fusca*, which was previously thought to be main cause of powdery mildew infections in Korea. The surveys revealed two further zinnia widespread infections of *Golovinomyces*. Beginning on September 7, 2009, about 500 plants were heavily infected in a flower garden at Bukssang-ri, Nam-myeon, Yeongwol, Korea. A few days later, on September, 17, 2009, about 3,000 nearly-dead plants were discovered in ornamental zinnia flower gardens on the roadside at Dongmak-ri, Hwado-myeon, Ganghwa, Korea (Fig. 1A).

Morphological characteristics of powdery mildew fungus. Twenty four zinnia powdery mildew fungal samples were collected from many Korean localities during the 2008-2010 surveys. Morphological characteristics were ascertained by light microscopy examination using a model BX51 microscope (Olympus, Tokyo, Japan), based on a representative sample (KUS-F23894, Korea, Hongcheon, leg. M.J. Park & H.D. Shin). Images were recorded using a Zeiss Axio imager microscope (Carl Zeiss, Göttingen,

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Fig. 1. Powdery mildew infections of *Zinnia elegans* by *Golovinomyces cichoracearum*. (A) Severe infections and early death of the plants were found in September 17, 2009, at a flower bed in Ganghwa, Korea. (B) Close-up view of infections on leaves and petals. (C) Hypha with nipple-shaped appressoria. (D) Conidiophore producing conidia in chains. (E) A first-formed conidium. (F) Germinating conidium. (G) Conidia. (H) Reticulate network of the conidium surface viewed with DIC microscopy. Bar = 30 μ m for C-G and 10 μ m for H.

Germany). Mycelia on both sides of leaves and also on stems and flower petals were subpersistent to subevanescent, forming circular to irregular white patches (Fig. 1B). Hyphae were sub-straight to wavy, occasionally geniculate, 40–80 μ m long, 4–7 μ m wide, mostly branching at a right angle, with a septum near the branching point. Hyphal appressoria were poorly developed, nipple-shaped, and single (Fig. 1C). Conidiophores were single on a hyphal cell, arising from the upper part of mother cells, 120–220 $\times$ 10–13 μ m, simple, straight in foot-cells, producing 2–6 immature conidia in chains with a sinuate edge, followed by 2–3 straight cells, with a basal septum at the branching point or slightly displaced from the mycelium (Fig. 1D). Conidia were ellipsoid to oval, 30–42 $\times$ 16–22 μ m (length/breadth ratio = 1.4–2.5), lacking distinct fibrosin bodies, producing germ tubes on the perihilar position, with reticulate wrinkling of the

outer walls (Figs. 1F, 1G and 1H). First-formed conidia were apically conical, basally round to more or less subtruncate, and generally shorter (30–36 μ m long) than the secondary conidia (Fig. 1E). No chasmothecia were found. The aforementioned structures were typical of the *Oidium* subgenus *Reticuloidium* anamorph of the genus *Golovinomyces*, and the measurements of the present fungus were compatible with those of *G. cichoracearum* (DC.) V. P. Heluta described previously (Braun, 1987; Nomura, 1997; Shin, 2000).

Additional specimens examined. Korea, Yangpyeong, 27 Oct 2008, M.J. Park & H.D. Shin, KUS-F23914; Gangneung, 25 Oct 2008, B.S. Kim, KUS-F23934; Seoul, 6 Nov 2008, H.D. Shin, KUS-F23968; Suncheon, 2 Nov 2008, H.B. Lee, KUS-F23969; Suncheon, 1 Nov 2008, Y.J. Koh, KUS-F23970; Daejeon, 15 Nov 2008, H.G. Kim, KUS-F23996; Daejeon, 16 Nov 2008, H.G. Kim, KUS-F23997; Yeongwol, 7 Sep 2009, J.H. Park & H.D. Shin, KUS-F24513; Ganghwa, 17 Sep 2009, M.J. Park & H.D. Shin, KUS-F24615; Goesan, 20 Sep 2009, M.J. Park & H.D. Shin, KUS-F24637; Chuncheon, 3 Oct 2009, H.D. Shin, KUS-F24699; Yangpyeong, 15 Oct 2009, J.H. Park & H.D. Shin, KUS-F24753; Hongcheon, 18 Oct 2009, M.J. Park & H.D. Shin, KUS-F24767; Seoul, 5 Aug 2010, H.D. Shin, KUS-F25131; Hoengseong, 12 Sep 2010, J.H. Park & H.D. Shin, KUS-F25273; Seoul, 30 Sep 2010, H.D. Shin, KUS-F25380; Gwangju, 29 Sep 2010, H.B. Lee, KUS-F25381; Cheongju, 5 Oct 2010, B.J. Cha, KUS-F25426; Gangneung, 5 Oct 2010, B.S. Kim, KUS-F25427; Suwon, 11 Sep 2010, S.G. Lee, KUS-F25475; Gongju, 10 Oct 2010, H.G. Kim, KUS-F25587; Geumsan, 11 Oct 2010, H.G. Kim, KUS-F25588; Daejeon, 11 Oct 2010, H.G. Kim, KUS-F25589.

Molecular analysis of powdery mildew fungus. To confirm the tentative identification based on morphological characteristics, molecular analysis of internal transcribed spacer (ITS) rDNA sequences from five representative materials (KUS-F23894, 23934, 23968, 23970, and 23997) was done. The complete ITS regions of rDNA were amplified with primers ITS5 and P3 as described by Takamatsu et al. (2009), and directly sequenced. All five ITS sequences were identical to each other and a resulting sequence of 508 bp from KUS-F23968 was deposited in GenBank (acc. no. HQ214032). A GenBank Blast search with the present data revealed an exact match for several sequences of *G. cichoracearum* including Australian powdery mildew on *Zinnia* sp., with a 100% sequence similarity. A phylogenetic tree was constructed with a representative sequence generated in this study and 24 sequences of *G. cichoracearum* retrieved from GenBank, using the neighbor-joining method

Fig. 2. Neighbor-joining tree showing phylogenetic relationship among *Golovinomyces cichoracearum* isolates inferred from the ITS rDNA regions. Bootstrap values based on 1000 replications are indicated above the branches and the scale bar represents 0.02 nucleotide substitutions per site.

in MEGA4 (Tamura et al., 2007). In a neighbor-joining tree (Fig. 2), the *G. cichoracearum* isolates were divided into five lineages according to the tribe of host plants, as demonstrated by Matsuda and Takamatsu (2003) and Cunnington et al. (2010). Korean powdery mildew on *Z. elegans* (Asteraceae, Heliantheae) was clustered into a clade that mainly contained *G. cichoracearum* isolates infecting hosts belonging to the Heliantheae (bootstrap value, 100%).

Identification of powdery mildew fungus. Based on these morphological and molecular data, the fungus was identified as *G. cichoracearum* DC. During our extensive 3-year surveys of zinnia powdery mildew in Korea, all of 24 materials collected were identified as *G. cichoracearum*. Although we could not conclude that *P. fusca* infections of zinnia had disappeared in Korea, such infections could be very few in number.

Powdery mildew infections of *Z. elegans* associated with *G. cichoracearum* are nearly circumglobal, including Europe, North America, South America, Africa, Oceania, and Western Asian localities like India, Nepal, Jordan, and Israel (Braun, 1987; Bolay, 2005; Farr and Rossman, 2010; Voytyuk et al., 2009). On the other hand, *P. fusca* causes powdery mildew infections on zinnia in East Asia regions such as Japan (Nomura, 1997), China (Zheng and Yu, 1987), Taiwan (Hsieh, 1983), and Korea (Lee and Lee, 1967; Shin 1994, 2000).

Very interestingly, Hoshi et al. (2007) reported the occurrence of zinnia powdery mildew by *Oidium* subgenus *Reticuloidium* in Tokyo, Japan. Since the subgenus *Reticuloidium* is a sole anamorphic group of powdery mildew corresponding to the teleomorphic genus *Golovinomyces* (Cook et al., 1997; Glawe, 2008), the finding of zinnia powdery mildew in Japan in 2007 is, to our knowledge, the first report of *Zinnia-Golovinomyces* association in the Far East.

The present work confirms the occurrence or invasion of *G. cichoracearum* infecting *Z. elegans* in the Far East by an extensive, 3-year-long survey in Korea and by detailed morphological and molecular evidence.

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