European Journal of Plant Pathology (2007) 117:89–93
DOI 10.1007/s10658-006-9072-x

Short Communication

Host range expansion in a powdery mildew fungus (*Golovinomyces* sp.) infecting *Arabidopsis thaliana*: *Torenia fournieri* as a new host

Pál Vági[1], Gábor M. Kovács[1], and Levente Kiss[2,]*
[1]*Department of Plant Anatomy, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117, Budapest, Hungary;* [2]*Plant Protection Institute of the Hungarian Academy of Sciences, H-1525, Budapest, P.O. Box 102, Hungary *Author for Correspondence (Fax: +36-1-4877555; E-mail: lkiss@nki.hu)*

Accepted 25 September 2006

Key words: Erysiphales, *Golovinomyces orontii*, host-jumping, phylogenetic analysis

Abstract

Since 2003, *Torenia fournieri* plants grown for experimental purposes were repeatedly infected by powdery mildew in a laboratory in Hungary. Based on morphological characteristics, the pathogen belonged to the mitosporic genus *Oidium* subgen. *Reticuloidium*, the anamorph stage of *Golovinomyces*. The rDNA ITS sequence was identical to that of two other powdery mildew fungi, infecting *Arabidopsis* and *Veronica*, respectively, in different parts of the world. According to a previous phylogenetic analysis of ITS and 28S rDNA sequences, those two powdery mildews belong to a recently evolved group of *Golovinomyces* characterized by multiple host range expansions during their evolution. Both the ITS sequence and the morphological data indicate that the powdery mildew anamorph infecting *Torenia* also belongs to this group. It is likely that the powdery mildew infections of the experimental *T. fournieri* plants, native to south-east Asia, were the result of a very recent host range expansion of a polyphagous *Golovinomyces* because (i) *T. fournieri* is absent from our region, except as an experimental plant grown in the laboratory, (ii) the powdery mildew fungus infecting this exotic plant belongs to a group of *Golovinomyces* where host range expansion is a frequent evolutionary scenario, (iii) cross-inoculation tests showed that this pathogen is also able to infect other plant species, notably *A. thaliana* and tobacco, and (iv) no *Golovinomyces* species are known to infect *T. fournieri* anywhere in the world. Although host range expansion has often been proposed as a common evolutionary process in the Erysiphales, and also in other biotrophic plant pathogens, this has not been clearly demonstrated in any case studies so far. To our knowledge, this is the first convincing case of a host range expansion event in the Erysiphales.

Torenia fournieri (Scrophulariaceae) has been used widely in plant reproductive biology research and has long been grown from seeds for this purpose at our department at Eötvös University (e.g., Kristóf and Imre, 1996; Imre and Kristóf, 1999; Kristóf et al., 1999; Vági et al., 2004). It is native to south-east Asia and it is not known to occur in the field in Hungary and neighbouring countries. Since 2003, severe powdery mildew infections were repeatedly observed on our potted *T. fournieri* plants in a laboratory. Intensively sporulating powdery mildew mycelia covered the stems, petioles, leaf lamina, and sepals of the plants (Figures 1 and 2). The infected parts withered and eventually died 3–4 weeks after the first symptoms appeared. As the disease was unwanted, we destroyed all the infected plants whenever the infection occurred, and produced new ones from seeds in different locations. In spite of these measures, severe infections occurred four times during the past 3 years.

Figure 1. Symptoms of the powdery mildew infection on the stems and sepals of *T. fournieri.*

Light microscopic studies of the pathogen revealed that its mature conidia measured 25–38 × 12–18 μm, contained no fibrosin bodies and developed in chains on conidiophores consisting of a 65–150 μm long foot-cell, followed by 2–4 shorter cells, each 12–20 μm long and a number of maturating and mature conidia. When placed on 1.5% water agar, conidia germinated in less than 24 h with germ tubes arising from one end of the conidium. Based on these morphological characteristics, the pathogen was identified as an *Oidium* subgen. *Reticuloidium*, sensu Cook et al. (1997), the anamorph stage of the recently erected powdery mildew genus *Golovinomyces* (Braun et al., 2002). Herbarium specimens of powdery mildew-infected *T. fournieri* plants were deposited in the herbarium of Martin Luther University, Halle, Germany under the accession number HAL 1922 F.

Figure 2. Symptoms of the powdery mildew infection on the sepals, stems, petioles and leaf lamina of *T. fournieri.*

The taxonomic positions and exact host ranges of powdery mildew fungi belonging to the genus *Golovinomyces* are still uncertain (Matsuda and Takamatsu, 2003). In order to know more about the pathogen found on *T. fournieri*, we determined its internal transcribed spacer (ITS) sequence of the nuclear ribosomal DNA (nrDNA), as described in Szentiványi et al. (2005). The ITS sequence, compiled from electropherograms of both strands, and deposited in GenBank under the accession number DQ538345, was 100% identical to two ITS sequences found in GenBank: that of *Golovinomyces orontii* strain UCSC1 (named as '*E. cichoracearum* strain UCSC' in the original report, Adam and Somerville, 1996), found on *Arabidopsis thaliana* in a laboratory in the USA (accession no: AF031282), and that of *G. orontii* collected from *Veronica arvensis* in Japan (accession no: AB077652). In addition, the ITS sequence of the powdery mildew fungus found on *Torenia* differed in only one nucleotide from that of *G. cichoracearum* infecting *Lactuca scariola* (accession no: AB077688), *G. orontii* strain Ecr-3 infecting *Cucurbita pepo* (accession no: AF229017) and *G. orontii* infecting *Mycelis muralis* (accession no: AB077661). The single nucleotide differences were found in different positions of the ITS1 and ITS2 regions. Both *Veronica* and *Torenia* belong to the plant family Scrophulariaceae, while *Arabidopsis* belongs to the Brassicaceae, *Cucurbita* to the Cucurbitaceae, and *Lactuca* and *Mycelis* to the tribe Lactuceae of the Asteraceae.

Recently, Matsuda and Takamatsu (2003) carried out a comprehensive phylogenetic analysis of *Golovinomyces* based on ITS and 28S rDNA sequences. All the fungal specimens mentioned above were included in that analysis, except the one we found on *Torenia*. The analysis showed that the species of the Asteraceae are probably the most ancient hosts of *Golovinomyces*. Matsuda and Takamatsu (2003) have also indicated that all the *Golovinomyces* spp. found on host plants other than the Asteraceae, together with those found on species of the tribe Lactuceae of the Asteraceae, belong to the most recently evolved group of *Golovinomyces*. According to Matsuda and Takamatsu (2003), the evolution of this large and diverse group of *Golovinomyces* powdery mildews, designated by them as the 'LAC group', was characterized by two distinct evolutionary processes: divergence in accordance to the evolution of the asteraceous species belonging to the tribe Lactuceae on the one hand and multiple host range expansions on the other. All the above mentioned five *Golovinomyces* powdery mildew fungi, infecting *Arabidopsis, Veronica, Lactuca, Mycelis* and *Cucurbita*, belong to the LAC group according to Matsuda and Takamatsu (2003).

Since the ITS sequence of the powdery mildew anamorph on *Torenia* is identical to that of *G. orontii* strain USCS1 infecting *Arabidopsis*, and also to that of *G. orontii* infecting *V. arvensis*, the pathogen on *Torenia* should also belong to the recently evolved LAC group. Thus, it belongs to a group characterized by frequent host range expansions as an evolutionary process. This process could also have been valid for the *G. orontii* strain UCSC1, as that strain was also found accidentally on *A. thaliana* grown as an experimental plant in a US laboratory (Adam and Somerville, 1996; Plotnikova et al., 1998). Thus, that strain could have 'jumped' to *Arabidopsis* from another host plant occuring in that environment. It is likely that the powdery mildew fungus infecting our experimental *Torenia* plants also originated from another host plant found in our surroundings because *T. fournieri* is not known to occur in Hungary and neighbouring countries as a wild plant. Of course, the presence of horticultural varieties of *T. fournieri* grown as ornamentals in Hungary cannot be excluded, as their seeds can be purchased from international companies. However, powdery mildew fungi are not seed-borne pathogens, and thus a powdery mildew fungus specialized to *Torenia* cannot be introduced to new places via plants grown from seeds. The import of potted and powdery mildew-infected *T. fournieri* plants is another possibility for the introduction of the pathogen to our region although it is unlikely that the fungus infecting our experimental *Torenia* plants came from such a source. This possibility cannot be totally excluded as diseased plants are sometimes transported accidentally through the international horticultural trade, or they are transported inadvertently by tourists, from one place to another (e.g., Palm, 1999, 2001), but this does not seem to be a real option in our case either, because practically no *Golovinomyces* species are known to infect *Torenia* anywhere in the world. Amano (1986) listed '*Erysiphe* spp.' on *T. fournieri* in Japan, but no further information is available for that record; thus, it is not clear whether that fungus was a *Golovinomyces* species. Powdery mildew fungi belonging to the genus *Golovinomyces* are not listed as pathogens of *Torenia* spp. in a world-wide monograph of the Erysiphales (Braun, 1987). There is only one European record of a powdery mildew fungus on *T. fournieri* from Finland (Amano, 1986; Braun, 1995); however, that fungus, *Podosphaera xanthii* (formerly known as *Sphaerotheca fusca* or *S. fuliginea*), can easily be distinguished from *Golovinomyces* spp. based on both morphological and ITS patterns. In addition, there are two records of *Podosphaera* species on *T. fournieri* from Japan (Amano, 1986). Thus, it seems that there is no clear evidence that *Golovinomyces* species have so far infected *Torenia* anywhere in the world.

To test whether the powdery mildew fungus found on *T. fournieri* can infect *A. thaliana*, similar to the *G. orontii* strain UCSC1, cross-inoculation tests were carried out in two places: in our laboratory where the infected *Torenia* plants were found and in a greenhouse compartment where plants can be grown in isolation. As tobacco is also a known host of *Golovinomyces* spp. with an ITS sequence differing in only two nucleotides from that of *G. orontii* strain UCSC1, *Nicotiana tabacum* cv. Xanthi was also included in the cross-inoculation tests. Mildew-free *T. fournieri*, wild-type *A. thaliana* and *N. tabacum* plants, eight pots for each plant species, were grown in isolation from seeds, until mature leaves developed, and then half of them were inoculated by touching mildew-infected *Torenia* leaves to their leaves. Non-inoculated plants, continuously kept in isolation, served as negative controls while the inoculated healthy *Torenia* plants were the positive controls. Sporulating powdery mildew colonies appeared on all the inoculated plants, in all the tests, but these remained localized and were characterized by sparse sporulation on tobacco cv. Xanthi (Figure 3), in contrast to those developing on *A. thaliana* (Figure 4) and *T. fournieri* (Figures 1 and 2). No symptoms developed on the

Figure 3. A tobacco leaf, cv. Xanthi, artificially infected with *Golovinomyces* sp. found on *T. fournieri*.

non-inoculated plants. These tests suggested that the pathogen found on *T. fournieri* could also naturally infect plants other than *Torenia*. Thus, this powdery mildew fungus could be polyphagous, with a wider host range, and able to parasitize different host plants. The polyphagous nature of some powdery mildews, currently belonging to *Golovinomyces*, was demonstrated earlier by cross-inoculation experiments (e.g., Hammarlund, 1945). This is in accordance with the evolutionary patterns characteristic of the LAC group of *Golovinomyces* (Matsuda and Takamatsu, 2003).

Host range expansion has often been proposed as a common evolutionary process in the Erysiphales (e.g., Braun, 1987; Takamatsu et al., 2000; Takamatsu, 2004; Hirose et al., 2005; Takamatsu et al., 2006), and also in other biotrophic plant pathogens (e.g., Voglmayr, 2003). However, to our knowledge, such an event has not been clearly documented in the Erysiphales to date. Whenever a powdery mildew fungus characterized by distinct morphological and molecular patterns, and associated with a certain host plant, was found on another, yet unreported plant species, it was impossible to exclude the possibility that it had long co-existed with the 'new' host and this relationship had remained unreported. Thus, in all those cases, a host range expansion event cannot be proven. In contrast, the case study presented here is different because (i) *T. fournieri* is absent from our region, except as an experimental plant grown in the laboratory, (ii) the powdery mildew fungus found on this exotic plant belongs to a group of *Golovinomyces* where host range expansion is a known evolutionary scenario, (iii) cross-inoculation tests showed that this pathogen is also able to infect other plant species, and (iv) no *Golovinomyces* species are known to infect *T. fournieri* anywhere in the world. These arguments taken together strongly suggest that the repeated powdery mildew infections on our experimental *T. fournieri* plants were the result of a very recent host range expansion of a polyphagous *Golovinomyces* occurring in our surroundings. This indicates that at least some powdery mildew fungi possess pathogenicity factors that allow them to quickly establish a compatible parasitic relationship even with some allopatric plant species, i.e. those that have evolved in a different geographical region, and introduced to their area of distribution. Such host-jumping events should be different from the much slower host range expansion processes thought to be achieved through a gradual accumulation of pathogenicity genes that finally leads to acquisition of parasitism to a new plant species that is usually closely related to the original host plant. These two processes have repeatedly been indicated to be the major driving forces in powdery mildew evolution: for example, Hirose et al. (2005) suggested that, in general, *Sawadaea* spp., powdery mildew fungi parasitic on maples, expanded their host ranges according to the phylogeny and geographical distribution of *Acer* spp., but at least one of them might have 'jumped' onto *A. negundo* and *A. platanoides* soon after these two species were introduced to Japan as ornamental trees. However, to our knowledge, the present

Figure 4. Leaves of a wild-type *A. thaliana* artificially infected with *Golovinomyces* sp. found on *T. fournieri*.

work is the first case study where a clear host range expansion event was documented convincingly in the Erysiphales.

Acknowledgements

The support of the János Bolyai Research Fellowship, awarded to GMK and LK, is acknowledged. GMK is a postdoctoral research fellow of the Hungarian Research Fund (OTKA, D048333). We are grateful to the anonymous reviewers for their helpful comments on this work.

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