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Powdery mildew (*Erysiphaceae*) on *Calibrachoa* hybrids in Germany, Nicaragua and the USA

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Abstract

Cultivated *Calibrachoa* hybrids were previously thought to be resistant to powdery mildew, but infections have been recently encountered in Germany, USA and Nicaragua. The exclusive development of asexual morphs (anamorphs) led to the question as to which powdery mildew species might be involved as causal agents. Based on inoculation experiments and molecular sequence analyses, it was determined that powdery mildew infections on *Calibrachoa* in Europe (Germany), North America (USA), and Central America (Nicaragua) were found to be caused by the plurivorous *Podosphaera xanthii*. The anamorph is a typical *Fibroidium* characterized by conidia formed in chains (catenescent), containing distinct fibrosin bodies. *Calibrachoa* powdery mildew caused by *P. xanthii* could be easily transferred to cucumber, squash and *Verbena* ×*hybrida* and vice versa in the latter case. Attempts to inoculate petunias failed. In addition to *P. xanthii*, two additional powdery mildew species were found infecting *Calibrachoa* ×*hybrida* in Germany. The first, characterized by having lobed hyphal appressoria and conidia formed singly, can be assigned to *Pseudoidium neolycopersici*, and the second species, readily distinguishable by its very long conidiophores, conidia in chains with sinuate outline and nipple-shaped hyphal appressoria, belongs to *Euoidium longipes*. In the course of the current examinations, *E. longipes* was also found on *Verbena* ×*hybrida*, which represents the first record of this species on a non-solanaceous host.

Key words – *Erysiphales* – *Euoidium longipes* – *Podosphaera xanthii* – *Pseudoidium neolycopersici* – *Solanaceae* – *Petunia*

Introduction

Since its introduction in the mid-1990s as an ornamental plant, *Calibrachoa* has become the second largest vegetative bedding plant in North America and Europe based on the number of cuttings produced and sold (M. Miller, personal communication). *Calibrachoa* 'Double Ruby' was

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selected as “Balcony Plant of the Year 2012” in Rheinland-Pfalz, Germany (www.gartenbau-rlp.de/index.php/component/item/103-rheinland-pfälzische-balkonpflanze-des-jahres-2012).

Powdery mildew infections on *Calibrachoa* were only recently observed in Germany (on non-commercial genotypes) where it was previously thought that *Calibrachoa* was resistant to powdery mildew. However, observations and examinations of naturally infected *Calibrachoa* hybrids had been made in the USA (Warfield 2011). European as well as North American *Calibrachoa* powdery mildew only occurs as an asexual morph (anamorph), fruiting bodies (chasmothecia) are not formed. Three different powdery mildew species have been encountered in the course of the present examinations. Based on indistinct to nipple-shaped hyphal appressoria, conidia formed in chains and conspicuous fibrosin bodies, one of the mildews can easily be classified as *Fibroidium* (R.T.A. Cook et al.) R.T.A. Cook & U. Braun, the anamorph of *Podosphaera* Kunze (Braun & Cook 2012). However, plants of various genera belonging to the *Solanaceae* may be hosts of two different species of the latter genus, namely the recently described *Podosphaera solanacearum* U. Braun and *P. xanthii* (Castagne) U. Braun & Shishkoff (Braun & Cook 2012). The two species are discriminated by traits of the ascomata, but their asexual morphs are indistinguishable. This led to the question which species might be involved as the causal agent of this *Calibrachoa* powdery mildew disease. Inoculation experiments and molecular sequence analysis were carried out to answer this question. Two additional anamorphic powdery mildews observed on *Calibrachoa* hybrids proved to be morphologically quite distinct from the *Podosphaera* anamorph and pertain to *Euoidium* Y.S. Paul & J.N. Kapoor (*Golovinomyces* (U. Braun) Heluta anamorph) and *Pseudoidium* Y.S. Paul (*Erysiphe* DC. anamorph).

Materials & Methods

The fungal material was examined by standard light microscopy using oil immersion (bright field and phase contrast), but without any staining. Thirty measurements (× 1000 magnification) of conidia and other structures were made, with the extremes given in parentheses.

To confirm the identification of the North American isolate, total genomic DNA was extracted from *Calibrachoa* leaves visibly infected with powdery mildew. Leaves were collected in April 2011 from infected plants growing in a trial greenhouse in coastal California. DNA isolation was based on a modified protocol described by Dellaporta et al. (1983). The complete internal transcribed spacer (ITS) region of rDNA, including 5.8S rDNA, the 3' end of 18S rDNA and the 5' end of 28S rDNA, was amplified and sequenced with primer pair ITS1-ITS4 using procedures outlined in White et al. (1990). PCR reactions were carried out using GoTaq Flexi DNA polymerase (Promega, Madison, Wisconsin) in a thermal cycler (2720 Geneamp, Life Technologies Corporation). The PCR DNA products were cleaned using a commercial kit following the manufacturer’s protocol (QIAquick PCR Purification kit, Qiagen Valencia, CA). Visualization of the PCR products was obtained by electrophoresis in 1.8% agarose gels in TBE buffer. Direct sequencing of the PCR products was performed by the DNA Facility of the Iowa State University Office of Biotechnology (Ames, Iowa). Sequencher software (Gene Codes Corporation, Ann Arbor, MI) was utilized to obtain a consensus sequences for the amplified region. The resulting 536 bp sequence was deposited in GenBank, as *Podosphaera xanthii* (Accession no. KP256814). The obtained sequence was used in the BLASTN 2.2.25+ program (NCBI http://www.ncbi.nlm.nih.gov/) to determine taxon identity based on similarity between sequences expressed as percent sequence identity.

Inoculation experiments were conducted on *Cucumis sativus*, *Verbena ×hybrida* and *Calibrachoa ×hybrida*. Two young cucumber plants ‘Delikatess’, as well as four detached leaves in double petri plates, were inoculated by brush application with the *Podosphaera* anamorph (*Fibroidium*) originating from *Calibrachoa* hybrids. Four young plants of *Verbena ×hybrida* ‘Obsession White’ were treated in the same way. Conversely, 12 *in vitro* plantlets of a *Calibrachoa* genotype with known susceptibility to powdery mildew were inoculated with conidia originating from *Verbena ×hybrida*. Inoculated plant material was incubated in a growth chamber at 20°C and

12 hours light. Non inoculated plants kept separately under the same conditions were used as controls.

Transfer experiments with powdery mildew from *Calibrachoa* to *Petunia* were performed under greenhouse conditions. Two *Calibrachoa* hybrid plants predominantly infected with a *Podosphaera* anamorph were placed between four plants of *Petunia* hybrids known to be susceptible to powdery mildew.

The identity of the developing powdery mildew was microscopically checked. Voucher herbarium specimens are deposited in the Herbarium of the Martin Luther University, Halle (Saale), Germany.

Results

Three different powdery mildews have been observed on *Calibrachoa* ×*hybrida*. One of them, detected in Germany, Nicaragua, and the USA, is morphologically characterized by the formation of thin white patches on leaves, composed of branched superficial hyphae, 3–8 µm wide, septate, thin-walled, smooth, with indistinct to somewhat nipple-shaped hyphal appressoria. The conidiophores are straight, erect, composed of cylindrical foot-cells, 30–70 × 9–12 µm, followed by 1–2 shorter cells, giving rise to catenescent conidia, ellipsoid-doliiform, 25–35 × 15–21 µm, with conspicuous fibrosin bodies (herbarium material deposited as HAL 2611 F: on *Calibrachoa* hybrid, Germany, Niedersachsen, Braunschweig, 9 Oct. 2013, U. Brielmaier-Liebetanz). Based on these morphological characteristics, this powdery mildew can readily be classified as *Fibroidium*, the anamorph of *Podosphaera*. However, the identification of a particular species based only on the anamorph was not possible.

Mixed infections of two additional, morphologically distinct powdery mildews have also been found on *Calibrachoa* in Germany. The most abundant of these differs from *P. xanthii* by the formation of lobed hyphal appressoria and conidia formed singly (hyphae 3–8 µm wide; hyphal appressoria solitary or mostly in opposite pairs, slightly lobed to multi-lobed, 2–9 µm diam.; conidiophores arising from upper surface or slightly lateral from the mother cell of superficial hyphae, more or less centrally or mostly towards one end of the cell, erect, straight, up to about 100 µm long, foot-cells cylindrical 20–45 × 8–10 µm, followed by 1–2 cells, shorter than the foot-cell, about as long or even longer; conidia formed singly, ellipsoid-doliiform, 28–50 × 13–20 µm, position of germ tubes perihilar, short to moderately long, apex with lobed to multi-lobed appressorium). These characteristics closely match those of *Pseudoidium neolycopersici* (L. Kiss) L. Kiss (in Braun & Cook 2012), except for more variability in the length of the cells following the conidiophore foot-cells. Material has been deposited as *P. neolycopersici* at HAL (on *Calibrachoa* ×*hybrida*, Germany, Niedersachsen, Braunschweig, 9 Oct. 2013, U. Brielmaier-Liebetanz, HAL 2612 F). On leaves infected with *P. neolycopersici*, visible colonies of *Euoidium longipes* (Noordel. & Loer.) U. Braun & R.T.A. Cook were also present. *E. longipes*, was easily discernible by its very long conidiophores mostly composed of a relatively short foot-cell followed by a very long cell, about 60–300 µm long, and 0–4 short cells, conidia in chains with sinuate outline, without fibrosin bodies and nipple-shaped hyphal appressoria. The occurrence of *E. longipes* on *Verbena* ×*hybrida* under greenhouse conditions was an additional, interesting observation during the course of the present examinations (material deposited as HAL 2665 F and HAL 2666 F).

Comprehensive descriptions and illustrations of the powdery mildew species concerned have recently been published by Braun & Cook (2012), which can be used as reference and source for comparison.

Inoculation experiments

Inoculation and host range experiments with powdery mildew inoculum from naturally infected *Calibrachoa* hybrids in Germany and the USA showed interesting results. It was possible to transfer this powdery mildew to cucumber (Germany), squash (USA) and *Verbena* ×*hybrida*, all principal hosts of *Podosphaera xanthii*, and vice versa. A *Fibroidium* anamorph from *Verbena*

×*hybrida* was successfully transferred to *Calibrachoa* hybrids in Germany. On the other hand, attempts to transfer the *Calibrachoa Fibroidium* onto petunias failed in all German and North American experiments.

Phylogenetic analysis

A GenBank BLAST search of the complete ITS sequence obtained from the North American *Calibrachoa* isolate showed 100% similarity with the ITS sequence of numerous *Podosphaera* isolates including *Podosphaera xanthii* (AB046985.1, on *Verbena* ×*hybrida*, USA), *P. xanthii* (AB040339.1, on *Saintpaulia* sp., Australia), *P. phaseoli* (Z.Y. Zhao) U. Braun & S. Takam. (GQ927253.1, on *Vigna unguiculata*, China), *P. balsaminae* (Wallr.) U. Braun & S. Takam. (FJ625796.1, on *Impatiens balsamina*, China), and *P. fusca* (Fr.) U. Braun & Shishkoff (KJ698669.1, on *Cucurbita pepo*, Italy). All of these powdery mildews belong to *P. xanthii*. *P. phaseoli* has been reduced to synonyms with *P. xanthii*, and *Podosphaera* on *Impatiens balsamina* refers to *P. xanthii* as well (Braun & Cook 2012). True *P. balsaminae* is confined to *Impatiens nolitangere* in Asia and Europe and is morphologically and genetically distinct. The use of the name *P. balsaminae* for powdery mildew collections on *Impatiens balsamina* is a case of misapplication. *P. fusca* (s. str.) is now confined to powdery mildew on *Doronicum* spp. (Braun & Cook 2012). The nucleotide sequence of the *P. xanthii* collected in December 2014 from calibrachoa plants under cultivation in Nicaragua had 100% identity with the rDNA ITS sequence of the North American (California) isolate.

Key to powdery mildews occurring on *Calibrachoa* ×*hybrida*

The following key is intended to facilitate the identification of asexual morphs of powdery mildews on *Calibrachoa* ×*hybrida*, and includes confirmed and potential species (marked by an asterisk).

1. Hyphal appressoria lobed, mostly in opposite pairs; foot-cells of the conidiophores relatively short, about 20–60 × 6–10 µm; conidia formed singly ..... *Pseudoidium neolycopersici*
1. Hyphal appressoria indistinct to nipple-shaped, mostly solitary; foot-cells of the conidiophores often longer (>60 µm) and wider above, about 10–14 µm; conidia catenescent (formed in true chains) 2
2. Edge line of chained immature conidia crenate; fresh conidia with distinct fibrosin bodies ..... *Podosphaera xanthii*
2. Edge line of chained immature conidia sinuate; conidia without fibrosin bodies 3
3. Width of the conidiophores distinctly increasing from base to top, foot-cells 40–90 µm long, followed by 1–2 often longer cells, about 50–300 µm long ..... *Euoidium longipes*
3. Width of the conidiophores not distinctly increasing, foot-cells followed by 1–2 much shorter cells 4
4. Foot-cells 95–180 µm long; on *Solanaceae*, Australia..... **Euoidium lycopersici*
4. Foot-cells shorter, 30–100 µm; on hosts of various genera of the *Solanaceae* (found worldwide in greenhouses) ..... **Golovinomyces orontii*

Discussion

In conclusion, the powdery mildew detected on *Calibrachoa* ×*hybrida* in Europe as well as North and Central America, characterized by catenescent conidia with fibrosin bodies, was confirmed as the plurivorous *Podosphaera xanthii* based on morphology, pathogenicity assays and molecular analysis of the complete internal transcribed spacer sequence. *P. xanthii* on cucurbits, *Verbena* ×*hybrida* and other hosts of this species should be considered as potential sources of infection on *Calibrachoa* ×*hybrida*. These results suggest a potential threat to calibrachoa by *P. xanthii* wherever this ornamental plant is cultivated.

In the USA as well as in Germany, transfers of the calibrachoa powdery mildew onto several varieties of petunias, using both brush transfers and wet inoculations, failed to infect any of the petunia varieties inoculated, which conflicts with Catlin (2012) who listed *Podosphaera xanthii* as a causal agent of petunia powdery mildew, but without any further information or references.

It appears that *Pseudoidium neolycopersici*, the common tomato powdery mildew, is able to infect calibrachoa, at least under greenhouse conditions. This is the first record of this powdery mildew on *Calibrachoa* ×*hybrida*. *Euoidium longipes* on calibrachoa is also new and hitherto not yet reported.The observation of *E. longipes* on *Verbena* ×*hybrida* represents the first record of this species on a non-solanaceous host.

Euoidium lycopersici (Cooke & Massee) U. Braun & R.T.A. Cook and *Golovinomyces orontii* (Castagne) Heluta, two additional species on hosts of the *Solanaceae*, are potential powdery mildew threats for calibrachoa, although not yet observed on calibrachoa.

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