New Disease Reports

First detection of *Phytophthora chrysanthemi* on *Chrysanthemum indicum* in Germany

M. Götz [1], R. Ulrich [2] and S. Werres [1]*

[1] *Julius Kuehn Institute – Federal Research Centre for Cultivated Plants (JKI), Institute for Plant Protection in Horticulture and Forests, Messeweg 11/12, 38104 Braunschweig, Germany*; [2] *Regierungspräsidium Gießen, Dezernat 51.4, Schanzenfeldstrasse 8, 35578 Wetzlar, Germany*

*E-mail: sabine.werres@julius-kuehn.de

Received: *09 Jan 2017.* ***Published:*** *07 Feb 2017.*

Chrysanthemum is a commercially important plant in Germany cultivated in both field and greenhouse production. In 2015, approximately 200 potted *Chrysanthemum indicum* hybrids, mostly cultivar 'Palisade', in a nursery in Hessen, Germany showed wilting symptoms (Fig. 1). Oospores were observed microscopically in infected roots (Fig. 2). From the roots of diseased plants a *Phytophthora* sp. (isolate JKI-050-15-8-01-2-0) was recovered on carrot piece agar (CPA), malt extract and SNA agar.

Chlamydospores and very low numbers of sporangia and oospores were observed on CPA or V8 agar amended with calcium carbonate (V8A). Higher numbers of healthy oospores developed on chrysanthemum agar (CA). Low numbers of sporangia were produced on CPA and V8A or after flooding with pure water, pea extract, or Petri solution. Sporangia released zoospores immediately after rinsing and showed nested and internal proliferation (Fig. 3). Cardinal temperatures for vegetative growth on CPA and size and shape of oogonia (mean 35.4 µm; n=50) and oospores (mean 28.4 µm; n=50) grown on CA were similar to those described for *Phytophthora chrysanthemi* (Naher *et al*., 2011). Chlamydospores produced on CPA were slightly larger (mean 44.0 µm; n=50) than reported.

To confirm morphological identification, ITS, 28S rDNA, β-tubulin, TEF1 alpha and COXI loci of JKI-050-15-8-01-2-0 were sequenced with the primers listed in Table 1. The ITS sequence showed 100% identity to a *P. chrysanthemi* isolate from Croatia (GenBank Accession No. KJ508824) and 99-100% to those from Japan and USA (AB437135, AB437136, AB511826, AB511827, AB688343, EU596361). The 28S rDNA sequence showed 99-100% identity to isolates from Japan (AB465508, AB465349, AB511313, AB511314, AB688485) and USA (FJ868725, EU596366) and for the β-tubulin sequence (Japan: AB511995 - AB511998; USA: EU596363, FJ868721). The COXI sequence showed 9% identity to a sequence of a Japanese isolate (AB688212) of *P. chrysanthemi*. However, the TEF1 alpha sequence had 98-99% identity to *P. chrysanthemi* isolates from the USA (EU596364, FJ868722) but only 96% identity to the Japanese reference isolates (AB511925, AB511927 - AB511929).

To fulfill Koch's postulates, ten rooted cuttings of *C. indicum* 'Palisade White' and 'Palisade Yellow' were inoculated by drenching and draining the soil with 40 ml of a mycelium suspension in sterile tap water of isolate JKI-050-15-8-01-2-0 grown on V8A. A homogenate of sterile V8A was applied to ten rooted cuttings of each cultivar as a negative control. The plants were incubated in a growth chamber with a 14 h photoperiod in a 25/20°C day/night regime. Two days after inoculation some inoculated plants started to wilt. After two weeks all inoculated plants showed severe wilting. The roots on these plants were brown and necrosis had spread to the stem base. Oospores were observed in the infected roots and *P. chrysanthemi* was re-isolated from infected plants after surface disinfection. Negative controls remained asymptomatic and the pathogen was not isolated.

Phytophthora chrysanthemi was first described as a new species on *Chrysanthemum* in Japan (Naher *et al*., 2011). In 2015, it was reported from Croatia (Tomić & Ivić, 2015) and in 2016 from the USA (Randall-Schadel, 2016). According to our knowledge, this is the first report of *P. chrysanthemi* in Germany.

Acknowledgements

The authors would like to thank Irmtraut Woggon, Henrike Gottfried, Johanna Wesche and Kirsten Löhr for excellent technical assistance. We are also grateful to Roger Cook for critical reading of the manuscript.

References

Naher M, Motohash K, Watanabe H, Chikuo Y, Senda M, Suga H, Brasier C, Kageyama K, 2011. *Phytophthora chrysanthemi* sp. nov., a new species causing root rot of chrysanthemum in Japan. *Mycological Progress* **10**, 21-31. http://dx.doi.org/10.1007/s11557-010-0670-9

Randall-Schadel B, 2016. NPAG Report - *Phytophthora chrysanthemi* Naher, Hi. Watan., Chikuo Kageyama: Crown and root rot of chrysanthemum. New Pest Advisory Group, United States Department of Agriculture. http://www.canr.org/newsletter/PhytophthorachrysanthemiNPAGReport20160401R.pdf (Accessed 9 January 2017).

Tomić Z, Ivić D, 2015. *Phytophthora chrysanthemi* Naher, Motohash, Watanabe, Chikuo, Senda, Suga, Brasier & Kageyama - new cause of chrysanthemum disease in Croatia. *Glasilo Biljne Zaštite* **15**, 291-300.

Figure 1

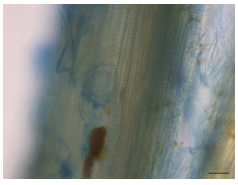

Figure 2

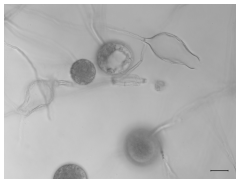

Figure 3

Table 1. List of primers used in this study and GenBank accession numbers of resulting sequences.

Locus	Primer name	Primer sequence (5' -3')	Annealing temperature	Reference[1]	Accession No.
ITS	ITS1 ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	53°C	White *et al*., 1990	KY363520
28S rDNA	NL1 NL4	GCATATCAATAAGCGGAGGAAAAG GGTCCGTGTTTCAAGACGG	52°C	O'Donnell, 1993	KY363521
β-tubulin	TUBUT2_for TUBUR1_rev	CGGTAACAACTCCGCCCAAGG CCTGGTACTGCTGGTCACTCAG	60°C	Kroon *et al*., 2004	KY363523
Elongation factor 1-α (TEF1-α)	ELONGF1_for ELONGR1_rev	TCACGATCGACATTGCCCTG ACGGCTCGAAGAATGACCATG	60°C	Kroon *et al*., 2004	KY363524
mt Cytochrome c oxidase I (COXI)	OomCoxI-Levup OomCoxI-Levlo	TCAWCWGAYTGGCTTTTTCAAC CYTCRKGRTGWCCRAAAAACCAAA	48°C (5 cycles); 51°C (35 cycles)	Robideau *et al*., 2011	KY363522

[1]Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG, 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* 41, 766-782.

O'Donnell K, 1994. *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW, eds. The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics, Wallingford, UK: CAB International, 225-233.

Robideau GP, De Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, Eggertson QA, Gachon CMM, Hu CH, Küpper FC, Rintoul TL, Sarhan E, Verstappen ECP, Zhang Y, Bonants PJM, Ristaino JB, Lévesque CA, 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources* 11, 1002-1011.

White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: InnisMA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*, New York, USA: Academic Press, 315-322.

Figure 4

To cite this report: Götz M, Ulrich R, Werres S, 2017. First detection of *Phytophthora chrysanthemi* on *Chrysanthemum indicum* in Germany. *New Disease Reports* **35**, 6. http://dx.doi.org/10.5197/j.2044-0588.2017.035.006

This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.