JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 46, No. 1 (2006)

PHYTOPHTHORA TROPICALIS, A NEW PATHOGEN OF ORNAMENTAL PLANTS IN POLAND

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Accepted: March 3, 2006

Abstract: From *Hedera helix* and *Epipremnum aureum* showing necrosis of shoot base spread upwards and on leaves *Phytophthora tropicalis* was isolated. The species was obtained from 7/8 of *Hedera* and 3/4 of *Epipremnum* diseased shoot and root parts. Additionally, *Botrytis* cinerea, *Fusarium avenaceum* and *Rhizoctonia solani* was recovered from some of affected plants. The chosen 2 isolates colonised petioles and leaf blades of both host plants.
P. tropicalis caused necrosis of leaves of 11 tested cultivars of *H. helix* and 13 other pot plant species and seedlings of tomato. The fastest spread of necrosis was observed on leaves of *Peperomia magnoliaefolia, Pelargonium zonale* and *Phalaenopsis* x hybridum. The development of disease was observed at temperatures ranged from 10 to 32.5°C with optimum 30°C.

Key words: *Phytophthora* spp., symptoms, occurrence, identification, colonisation, host plants, temperature

INTRODUCTION

In 1997 a new disease was observed by Gerlach and Schubert (2001) on *Cyclamen persicum* Mill. grown in the Netherlands and Germany. Leaf blades changed colour from dark green to olive green and unspecified flagging of leaves followed by a yellowing of margins of older leaves and then yellowing of entire leaves. On lengthwise sectioned corms firm, creamed colored tissues were observed. At the base roots were darkbrown and died. In 2001 Aragaki and Uchida isolated from diseased pepper, tomato, eggplant and cucurbit a new species described as *Phytophthora tropicalis* Aragaki et Uchida. Both, *P. tropicalis* and *P. capsici* Leonian have similar morphological and physiological atributes but molecular distinctions between them indicated on *P. tropicalis* as a seperate species (Zhang et al. 2004). Cac-

ciola et al. (2001) isolated this species from diseased silver-bush in ornamental nurseries in Southern Italy whereas Cerqueira et al. (2005) found it on breadfruit (*Artocarpus altilis*) in Brazil.

In summer of 2003 disease symptoms were observed on *Hedera helix* L. and *Epipremnum aureum* Linden ex Andre in 3 greenhouse farms in Poland. On *Hedera* plants the first obvious symptoms of the disease were loss of luster and colour change in foliage on single shoots from dark to light green. The base of branches were brown or dark brown and necrosis spread upwards on leaf petioles and blades and also on roots. Usually part of plants died. On *Epipremnum* plants base of branches dark-brown spots, in the beginning small, irregular spread upwards, on the periphery and leaves. Infected shoots were light green or yellow-green. Affected plants died during the next few weeks. The objectives of this investigation was: (1) to isolate and identify fungi and *Algae*- like *Oomycetes* inhabiting diseased plants, (2) to determine pathogenicity of *P. tropicalis* isolates toward hosts and other pot plants and tomato seedlings.

MATERIALS AND METHODS

Isolation and identification of fungi and *Algae*- like *Oomycetes*

Partly diseased shoots and roots of *Hedera helix* and *Epipremnum aureum* were individually collected in plastic bags and transferred into laboratory, washed in running tap water, rinsed 3 times in distilled, sterile water and blotted dry with paper towels. Chosen parts of shoots and roots were sterilized over a burner flame, cut into 5 mm pieces and placed on Difco potato-dextrose agar (PDA) in 90 mm Petri dishes (6 pieces/dish and 3 plates/plant). After 24–48 hr incubation at 24°C in the dark, small parts of growing colonies were transferred into PDA slants. Isolation and identification of chosen isolates were performed according to the method described by Orlikowski et al. (2001). Morphology of *P. tropicalis* was studied using the procedure described by Orlikowski and Szkuta (2002). Confirmation of classification to species was performed by DNA analyses. DNA was extracted from pure culture using the method of Aljanabi and Martinez (1997), modified by Wiejacha et al. (2002) and amplified with non-specific primers. RAPD (Operon) and ISSR (UBS – 800 series), were used to generate DNA profiles characteristic of 13 *Phytophthora* species, including *P. alni*, *P. cactorum*, *P. cambivora*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. nicotianae* var. *nicotianae*, *P. palmivora*, *P. quercina*, *P. ramorum* and to compare with *P. tropicalis* profiles. The alternative way of confirmation was the use of the PCR-RFLP method described by Cooke and Duncan (1997). It involves digesting of amplified DNA fragment obtained with primers ITS4 and ITS6 with restriction enzymes, which produce bands of marker value. We used four endonucleases: *Alu*I, *Msp*I, *Rsa*I and *Taq*I (Fermentas) but our sample didn't have DNA profiles such as 13 reference *Phytophthora* isolates. The isolate sample which failed identification was amplified with primers ITS4 and ITS6 and subsequently the amplified product was cleaned using Clean-up (A&A Biotechnology) and sequenced using ABI PRISM Dye Terminator Cycle Sequencing Core Kit in DNA Sequencing and Oligonucleotide Synthesis Laboratory of the Institute of Biochemistry and Biophysics, Warsaw. The sequences obtained by amplification

with the primers ITS 4 and ITS 6 were compared with those available in the Gene Bank using nucleotide-nucleotide BLAST (blastn) software (Figs. 1, 2).

5'ACTTCCCAAAATGGATCGACCCTCGACAGCCGAAGCCGCCACTCTACTTCGCAACAGCA
AAGCCGATTCAAAAGCCAAGCCACACACAGCTACGGTTCACCAGCCCATCACGCCACAGC
AGGAAAAGCATACAATAAGCGCCTGTTCAGCCGAAGCCAACCATACCGCGAATCGAACAC
TCCTCCATTAAACGCCGCAGCAGACAAACCGGTCGCCGACTGGCCACGCAGGCAGCCTC
CACAACCAGCAACACCACGCTTTTCGAGCAAAGAGAAGTACAGTTCAGTACATTTAAAAG
GACTCGCAGACGACCCGAAGGACAAACCGCAAGACACTTCACATCTGGCACATCCTCCA
CCGACTACACGGAAGGAAGAAAGCCAAGTTTGATGTACGGACACTGATACAGGCATACT
CCCAGGACTAACCCGGAAGTGCAATATGCGTTCAAAATTTCGATGACTCACTGAATCCTG
CAATTCGCATTACGTATCGCAGTTTCGCAGCGTTCTTCATCGATGTGCGAGCCTAGACATC
CACTGCTGAAAGTTGCTATCTAGTTAAAAGCAGAGACTTTCGTCCCCACAGTATAATCAGT
ATTGAGAAA3'

Fig.1. Sequence ITS4 of *P. tropicalis*

5'TCTTTCCTTTCCGTGTAGTCGGTGGAGGATGTGCCAGATGTGAAGTGTCTTGCGGTTTGTC
CTTCGGGTCGTCTGCGAGTCCTTTTAAATGTACTGAACTGTACTTCTCTTTGCTCGAAAAGC
GTGGTGTTGCTGGTTGTGGAGGCTGCCTGCGTGGCCAGTCGGCGACCGGTTTGTCTGCTGC
GGCGTTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTTCGGCTGAACAGGCGCTTA
TTGTATGCTTTTCCTGCTGTGGCGTGATGGGCTGGTGAACCGTAGCTGTGTGTGGCTTGGCT
TTTGAATCGGCTTTGCTGTTGCGAAGTAGAGTGGCGGCTTCGGCTGTCGAGGGTCGATCCA
TTTTGGGAACT3'

Fig. 2. Sequence ITS6 of *P. tropicalis*

Colonisation of ornamental plant leaves by *P. tropicalis* and other species

Isolates of *P. tropicalis* from *Hedera* and *Epipremnum*, *P. palmivora* (Butler) Butler from *Hedera* shoots (Orlikowski et al. 2001) and *P. capsici* from *E. aureum* (Orlikowski, unpubl.) stem base were used for inoculation of leaf petioles and blades and tomato (*Lycopersicon esculentum* Mill.) seedlings. Eleven *H. helix* cultivars and 15 pot plant species (Tables 2, 3) were used for study of *P. tropicalis* pathogenicity whereas *P. palmivora* and *P. capsici* were tested additionally towards tomato seedlings (Table 4). The last trial with tomato seedlings has been done because *P. tropicalis* was originally described as species closely related to *P. palmivora* complex (Gerlach and Schubert 2001) and similar to *P. capsici* (Aragaki and Uchida 2001; Zhang et al. 2004)). Stock cultures were grown on PDA at 24°C in the dark.

Three mm diameter mycelial plugs, taken from the edge of colonies grown at 24°C in the dark, were transferred on the base of leaf petioles and central part of leaf blades, placed in polystyrene boxes on sterile moist blotting paper covered with plastic net. Boxes were covered with foil and incubated at 22–25°C in the dark. Length and diameter of necrosis was measured after 3–11-day-incubation.

Additionally, colonisation of *Hedera* leaves in relation to temperature from 7.5° to 35°C was studied using the same procedure as in previous trials. The experimental design was completely randomised with 4 replications and 10 leaves in each rep. Trials were repeated 2–3 times.

RESULTS AND DISCUSSION

Isolation of fungi and *Algae*- like *Oomycetes*

Nine genera and species were isolated from diseased shoots and roots of *H. helix*. On the base of morphological characteristic and sequences of ITS4 and ITS 6 isolates of *Phytophthora* obtained from both plant species were identified as *P. tropicalis* (Figs. 1, 2). This species was detected from shoots of 2/3 of analysed plants and from roots of about 1/4 of plants (Table 1). *Pythium* sp. was isolated from about 1/3 of diseased plants, both, from shoots and roots. *Botrytis cinerea*, known as causal agent of young shoot death, was found on 5 plants (Table 1). *P. tropicalis* was recovered from 3/4 of *E. aureum* whereas *B. cinerea* and *Rhizoctonia solani* from about 1/4 of diseased plants (Table 1).

Table 1. Fungi and *Algae*- like *Oomycetes* isolated from diseased *Hedera helix* and *Epipremnum aureum*; number of settled plants

Isolated species	*Hedera helix* (n=32)		*Epipremnum aureum* (n=20)
	shoots	roots	shoots
Botrytis cinerea Pers.	5	–	4
Cladosporium herbarum (Pers.) Link.	2	3	–
Fusarium avenaceum (Fr.) Sacc.	7	4	–
Mucor spp.	11	14	8
Penicillium spp.	5	9	–
Phytophthora tropicalis Aragaki et Uchida	19	8	15
Pythium sp.	8	11	–
Rhizoctonia solani Kuhn	–	–	5
Trichoderma spp.	6	12	3

Isolation: June – October, 2003

Colonisation of ornamental plants by *Phytophthora tropicalis* and other species

Isolates from *Hedera* and *Epipremnum* colonised leaves and stem parts of both plants and there were no differences in the diameter and length of necrosis. In further study isolate from *H. helix* was used. All 11 cultivars of *H. helix* were colonised by *P. tropicalis* but the fastest necrosis spread was observed on leaf blades of Colibrii, Gitte, Golden Child, Golden Mathilde and Mathilde cvs (about 5,4 mm/24 hr). On leaf petioles necrosis spread at least twice slower than on blades (Table 2).

Development of necrosis was observed on all 15 tested pot plant species, but the fastest spread of disease occurred on *Peperomia magnoliaefolia*, *Pelargonium zonale* and *Phalaenopsis* x hybridum. On *H. helix* cv. Wonder necrosis spread about 1/3 slower than on 3 mentioned plant species. The disease developed slower on leaf petioles than on blades (Table 3).

Table 2. Colonisation of leaves of *Hedera helix* inoculated on 2005.12.13 by *Phytophthora tropicalis* after 5-day-incubation

Cultivars	Diameter/length of necrosis in mm	
	leaf blades	leaf petioles
Colibrii	26.0 d	8.5 a-c
Esa	19.1 b	9.0 a-d
Ester	25.3 cd	7.9 ab
Gitte	28.0 d	8.8 a-c
Golden Child	27.0 d	9.0 a-d
Golden Mathilde	27.0 d	10.3 b-d
Golden Wonder	23.5 b-d	8.3 a-c
Iverlace	25.0 cd	10.5 cd
Mathilde	27.1 d	7.5 a
Schamrock	20.9 bc	7.3 a
Wonder	25.0 cd	8.5 a-c

Means in columns, followed by the same letter, do not differ with 5% of significance using Duncans multiple range test

Table 3. Colonisation of leaves of some ornamental plants by *Phytophthora tropicalis* 3-days after inoculation

Plant species	Diameter/length of necrosis in mm	
	leaf blades	leaf petioles
Cyclamen persicum Mill.	17.3 d	5.3 ab
Diffenbachia maculata (Lodd.) G. Don	4.5 a	3.8 a
Epipremnum aureum (Linden and Andre) Bunt	12.8 c	9.0 b
Euphorbia pulcherrima Willd. ex Klotzsch	18.3 d	20.8 d
Hedera helix Wonder	21.0 e	13.3 c
Impatiens x hybridum	12.8 c	20.8 d
Kalanchoe blossfeldiana Poelln.	5.5 a	3.5 a
Peperomia magnoliaefolia (L.) A. Dietr.	31.6 f	29.8 e
Pelargonium zonale Ait.	30.0 f	15.0 cd
Phalaenopsis x hybridum	28.0 f	12.2 c
Rhododendron sp. Nova Zembla	12.8 c	18.0 d
Rhododendron Simsii	7.8 ab	8.8 b
Rosa sp.	13.0 c	5 ab
Sainpaulia ionantha H. Wendl.	10.0 b	5.8 ab
Syngonium vellozianum Schott.	4.5 a	5.8 ab

Means in columns, followed by the same letter, do not differ with 5% of significance using Duncans multiple range test

Three *Phytophthora* species colonised tomato seedlings (Table 4). After 8 and 11-day-incubation the fastest spread of necrosis was observed when seedlings were inoculated with *P. capsici*. There were no significant differences in length of necrosis when *P. palmivora* and 2 isolates of *P. tropicalis* were used for seedlings inoculation after 8 days. After the next 3 days the slowest spread of necrosis was observed on tomato inoculated with *P. palmivora* (Table 4).

Table 4. Colonisation of tomato seedlings by *Phytophthora* spp.

Phytophthora species	Source of isolate	Length of necrosis (in mm) after days of incubation	
		8	11
P. capsici	*Epipremnum aureum*	26.0 b	48.0 c
P. palmivora	*Hedera helix*	10.8 a	15.8 a
P. tropicalis	*Hedera helix*	12.8 a	21.0 b
P. tropicalis	*E. aureum*	13.7 a	20.0 b

Means in columns, followed by the same letter, do not differ with 5% of significance using Duncans multiple range test

Development of necrosis was observed at temperature ranged from 10–32.5°C with optimum at 20–32.5°C. Leaves were not colonised at 35°C. On leaf petioles necrosis spread slowler than on blades (Table 5).

Table 5. Colonisation of *Hedera helix* leaves by *Phytophthora tropicalis* in relation to temperature of incubation

Temperature °C	Diam/length (in mm) of necrosis after 6-day-incubation	
	Leaf blades	Leaf petioles
7.5	0 a	0 a
10	8.2 b	5.5 b
15	17.4 c	7.5 b
20	24.6 d	14.8 c
25	28.0 de	17.0 cd
27.5	30.7 e	20.5 de
30	37.6 g	26.8 f
32.5	25.0 d	15.2 c
35	0 a	0 a

Means in columns, followed by the same letter, do not differ with 5% of significance using Duncans multiple range test

Results obtained indicated on continuous menace of ornamental plants by a newly isolated *Phytophthora* species. It is possible that *P. tropicalis* was brought to Poland on imported *Hedera* shoot parts used for cuttings production. In case of *E. aureum* the pathogen was probably transferred on that species from diseased *H. helix*, grown in the same greenhouse. In Gerlach and Schubert (2001) pathogenicity trial, besides cyclamen, European isolates of *P. tropicalis* were pathogenic toward *E. aureum*, *Dianthus caryophyllus* L., partially to *Diascia vigilis* Hilliard et B.C. Burtt and *H. helix* but not for *L. esculentum* and some other vegetables. Our trials with some pot plants showed that there are more potential hosts of *P. tropicalis*, including *Peperomia, Pelargonium* and *Phalaenopsis* species. The study with influence of temperature on development of necrosis on *Hedera* leaves, inoculated with *P. tropicalis*, confirmed results of Gerlach and Schubert (2001) indicated on cardinal temperature 10° and 35°C and optimum about 30°C.

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POLISH SUMMARY

PHYTOPHTHORA TROPICALIS – NOWY PATOGEN ROŚLIN OZDOBNYCH W POLSCE

Z *Hedera helix* i *Epipremnum aureum*, z objawami zgnilizny podstawy pędów, rozszerzającej się ku górze i na blaszki liściowe oraz zgnilizną korzeni, izolowano *Phytophthora tropicalis*. Gatunek ten stwierdzono na 7/8 analizowanych roślin *H. helix* i 3/4 *E. aureum*. Ponadto z porażonych tkanek niektórych roślin wyizolowano *Botrytis cinerea, Fusarium avenaceum* i *Rhizoctonia solani*. Wybrane dwa izolaty *P. tropicalis* kolonizowały liście obu roślin – gospodarzy. Omawiany gatunek powodował nekrozę liści 11 odmian *H. helix*, 15 gatunków roślin doniczkowych oraz siewek pomidorów. Najszybszy rozwój nekrozy obserwowano na liściach *Peperomia magnoliaefolia, Pelargonium zonale* i *Phalaenopsis* x hybridum. Rozwój choroby obserwowano w temperaturze od 10–32,5°C przy optimum 30°C.