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SHOOT BLIGHT OF *VINCA MINOR* L. CAUSED BY *PHOMA EXIGUA* DESM. VAR. *INOXYDABILIS* BOEREMA ET VEGH VAR. *NOV.*

LESZEK B. ORLIKOWSKI

RES. INSTITUTE OF POMOLOGY AND FLORICULTURE,
POMOLOGICZNA 18, 96-100 SKIERNIEWICE, POLAND
e-mail: lorlikowski@insad.isk.skierniewice.pl

Abstract. *Phoma exigua* var. *inoxydabilis* var. *nov.* predominated among fungal isolates obtained from diseased stem runners and leaves of periwinkle (*Vinca minor*). The growth of the fungus was observed at temperature ranges from 7.5 to 30°C with optimum at 25°C. Abundant formation of picnidia was noticed mainly on malt extract agar at temp. 15-25°C. On potato-dextrose agar picnidia were observed 3-5 days later. On inoculated leaves of periwinkle, development of necrosis was observed at temperature 10-25°C with optimum 20°C. On field grown periwinkle the first necrosis on the base of stem runners was observed 2 weeks after inoculation and during the next 10 weeks discoloration of tissues occurred on about 1/2 of their length.

Key words: periwinkle, fungi, isolation, *Phoma*, growth, sporulation, pathogenicity

I. INTRODUCTION

Periwinkle known also as ground myrtle (*Vinca minor*) is perennial, evergreen species grown mainly as shade tolerant, ground cover ornamental plant. Periwinkle is grown also as the source of alkaloid – vincamine for medicinal properties (Vegh et al. 1974) and anticancer agent (Wang and Lee 1997). Extracts from *V. minor* and *V. major* have also antifungal and antibacterial properties (Mehrabian et al. 1995).

In recent years a dieback or blight has been observed on periwinkle in Polish ornamental nurseries. In 1998 disease symptoms in 3 nurseries were observed on 5 to 70% of container – grown plants (Orlikowski 1999). On most of plant single shoots were usually affected, rarely all periwinkles. Disease symptoms were observed during the whole vegetation period but especially in spring as brown or brown – black lesions on stems of old overwintered runners near the base or at different distance from the substrate. Development of disease symptoms often resulting in wilting and death of entire shoots. Lesions developed on new runners where they came in contact with other infected shoots or with substrate. Necrosis spread from stem lesions into the leaf petioles and the base of leaf blades. Dark, often spherical spots may develop on the leaves, causeing discoloration and death of blades. Affected plants usually defoliate. Black, often abundant pycnidia were observed on invaded stems and leaves, especially during the spring time.

First report connected with the occurrence of stem and leaf spot on *Vinca minor* was published by Desmazieres (1843). The causal agent of the disease was described by the author as *Phoma lirella*, which Grove (1917) transferred later to the genus *Phomopsis*. Similar organism was described by Bresadola and Krieger (1900) as *Phyllosticta vincae – minoris* Bresadola et Krieger. Boerema and Howeler (1967) used first time the name

P. exigua Desm. var. *exigua* for the fungus isolated from diseased *V. minor*. This name of the causal agent of *Vinca* blight was also used by Paulson and Schoeneweiss (1971). Three years later Vegh et al. (1974) described the pathogen isolated from *V. minor* as *P. exigua* Desm. var. *inoxydabilis* Boerema et Vegh var. *nov*.

This paper reports studies undertaken to isolate the causal organisms of stem and leaf blight of *Vinca minor* and to investigate the development of *P. exigua* on artificial media and on periwinkle leaves and stem runners.

II. MATERIALS AND METHODS

1. Mycological analysis of diseased plants

Three periwinkle nurseries were surveyed for the occurrence of stem and leaf blight in 1997-1999. Diseased plant parts were collected from 157 plants between April and October. Invaded stem and leaf parts were washed in distilled water, dried and sterilised above the burner. About 5 mm long parts of diseased stem runners and 5 mm diam leaf parts were placed on potato – dextrose agar (PDA) in 90 mm Petri dishes (4 plates for each plant). Cultures growing around diseased parts, after separation and cleaning, were identified to species. Monograph of Vegh et al. (1974) was used for identification of *Phoma* to species.

2. Pathogenicity trials

Representative strain of *Phoma exigua* var. *inoxydabilis* var. *nov.* (V1) was maintained on PDA at 25°C. Three mm diam disks of mycelium were taken from the edge of 7-day-old culture for inoculation of leaves and shoots.

For in vitro trials leaves of periwinkle were taken from the middle part of shoots and put into humidity chambers on moist blotting paper covered with plastic net. On each leaf mycelium disk was put in the centre. Additionally, the base, middle and top parts of stem runners were inoculated with the pathogen and incubated in humidity chamber. After 6, 10 and 13 day-incubation at temperature ranging from 5 to 35°C diameter of necrosis was measured.

In nursery trial mycelium disks were put on the base of needle damaged young stem runners and plants were covered with plastic bags for 3 days. After that time sacs were removed and plants were watered and fertilised as required. During 3 months at 2-week-intervals the length of necrosis on invaded shoots was measured.

Experimental design was completely randomised with 4 replications and 5 leaves/stem runners and plants in each rep. Experiments were repeated twice at 2-week-intervals.

3. Relationship between temperature, medium and growth of *Phoma exigua* var. *inoxydabilis* var. *nov*.

Five-mm mycelial disks taken from the edge of 7-day-old culture were transferred into the middle of 90 mm Petri dishes with potato-dextrose agar (PDA) and malt extract agar (MEA). After 6, 10 and 13-day-incubation at temperature ranging from 7.5 to 30°C the

diameters of colonies were measured. Five Petri dishes constituted one experimental unit and the trials were repeated twice. In all trials the data obtained were subjected to an analysis of variance. Duncan's multiple range test was used for separation of means.

III. RESULTS AND DISCUSSION

Disease symptoms observed on naturally infested periwinkle tissues, growth of culture on PDA and MEA, size of pycnidia and pycnidiospores classified isolates of *Phoma*, obtained from diseased stems and leaves, as *P. exigua* var. *inoxydabilis* var. *nov.* (Vegh et al 1974). The fungus dominated among 11 fungal species on diseased stems and leaves of periwinkle (Tab. 1). The species was isolated during 3 years from at least 4/7 of analysed periwinkle stems and 2/5 of leaves (Tab. 1). *Chaetomium globosum*, *Fusarium oxysporum*, *Mucor* and *Trichoderma* species were isolated from at least 1/5 of analysed stems whereas other 7 fungi occurred rarely or only sporadically (Tab. 1). *Mucor* spp., *Trichoderma* spp., *Chaetomium globosum*, *Alternaria alternata* and *Botrytis cinerea* (Tab. 1) mainly colonised invaded leaf tissues, besides *P. exigua*. Vegh et al. (1974), besides *P. exigua*, isolated from invaded periwinkle shoots *Colletotrichum gloeosporioides*, *Phomopsis lirella*, *Septoria vincae*, *Puccinia vincae*, *Fusarium* spp. and *Rhizoctonia solani*. In this study, beside *Fusarium oxysporum* and *F. roseum* the other fungi, mentioned by Vegh et al. (1974), were not found on analysed periwinkle in 3 nurseries. It was found, however, that *F. oxysporum* and *Ch. globosum* were usually isolated from the same stem tissues as *P. exigua*.

Inoculation of periwinkle, however, with mycelium plugs of *F. oxysporum* did not cause any disease symptoms on leaf tissue as well as on the stem base in field grown plants. On leaf tissues inoculated with *P. exigua* var. *inoxydabilis* var. *nov.* development of necrosis

Table 1

Number of diseased stems (a) and (b) settled by fungi

Fungal species	1997 n=42		1998 n=50		1999 n=65	
	a	b	a	b	a	b
Alternaria alternata Keissler	–	6	1	16	2	9
Aspergillus fumigatus Fres.	–	–	–	–	–	1
Botrytis cinerea Pers.	4	6	6	6	8	6
Chaetomium globosum Kunze	12	8	10	6	13	8
Cladosporium herbarum Link.	1	1	2	1	1	4
Fusarium oxysporum Schlecht	15	2	16	1	14	3
Fusarium roseum (Lk.) Sny et Hans.	–	7	2	–	3	1
Gliocladium roseum (Link.) Thom	2	2	5	3	3	2
Mucor circinelloides van Tieghem	3	5	2	4	3	4
Mucor hiemalis Wehmer	3	8	14	12	8	11
Penicillium spp.	4	5	2	2	6	6
Phoma exigua Desm. var. *inoxydabilis* Boerema et Vegh var. *nov.*	24	20	30	20	43	36
Trichoderma spp.	9	10	13	7	8	3

Table 2

Development of necrosis on periwinkle leaves infested with *Phoma exigua* var. *inoxydabilis* var. *nov.*; mean diam of necrosis in mm

Temperature of incubation °C	Days after incubation		
	6	10	13
10	0 a	2 a	2.5 a
15	2.1 bc	3.6 b	12.1 b
20	2.2 c	10.3 c	21.4 c
25	1.8 b	2.1 a	2.6 a

Note: Means in column followed by the same letter do not differ with 5% of significance

Table 3

Development of necrosis on stems of field grown periwinkle with *Phoma exigua* var. *inoxydabilis* var. *nov*; length of necrosis in mm

Inoculation time	Weeks after inoculation			
	2	4	8	12
1999.04.22	6.7 a	15.3 b	33.3 c	50.4 d
1999.05.06	10.4 a	17.5 b	29.9 c	44.2 d

Note: See Table 2

Table 4

Growth of *Phoma exigua* var. *inoxydabilis* var. *nov.* in relation to medium and temperature: mean values from 3 observations

Temperature °C	Potato – dextrose agar	Malt extract agar
7.5	4.2 a	7.5 a
10	7.8 a	9.0 a
15	18.6 ab	17.6 ab
20	29.8 bc	32.9 b
25	46.3 c	63.5 c
30	10.8 ab	13.4 a

Note: See table 2

was observed at temperature range between 10 and 25°C with optimum at 20°C (Tab. 2). On inoculated stem runners development of necrosis was connected with the degree of tissue lignification. On the base of stem, necrosis reached length about 3 mm whereas on top parts about 26 mm after 13-day-incubation. On field grown plants the first necrosis on the base of stem runners was observed 2 weeks after inoculation and during the next 10 weeks and discoloration of tissues occurred on about 1/2 of their length (Tab. 3). On shoots inoculated on April necrosis spread faster than on plant tissues inoculated 2 weeks later. This process was probably connected with higher temperature in the second trial. In Paulson and Schoeneweiss (1972) trial 60% of periwinkle runners were infected at 10°C whereas 80% when temperature reached 18°C. At 27°C only 10% of shoots showed disease symptoms. In Vegh et al. (1974) trial temperatures 15° and 20°C were optimal for the development of disease on leaves whereas necrosis spread very slowly at 12 and 25°C.

Analysis of *P. exigua* var. *inoxydabilis* var. *nov.* growth in relation to temperature and medium showed that the pathogen developed at temperature ranging from 7.5 and 30°C with optimum 25°C (Tab. 4). Abundant pycnidia were observed on MEA already after 10 day incubation at 20 and 25°C. On PDA pycnidia were observed a few days later. At 15°C pycnidia were observed on MEA after 13-day-incubation. The growth of the pathogen was not observed at 5 and 32,5°C even after 13 days of incubation. Vegh et al (1974) observed the growth of the fungus at temperature ranging from 2 to 31°C with optimum 26°C. In Paulson and Schoeneweiss (1972) trial the optimum temperature for growth of the fungus on MEA was 27°C. The above authors study as well as

my own trials indicate that optimal temperature for the pathogen growth in vitro do not correspond with optimal temperature for disease development on periwinkle runners and leaves. The minimum and maximum temperature for disease development is about 5°C higher or lower than for the pathogen growth.

Phoma exigua has been described by Boerema and Howeler (1967) as ubiquitous soil-borne fungus and common inhabitant of dead and dying periwinkle materials. In Polish conditions pycnidia were produced on affected plant organs in nurseries from spring to autumn besides hot, summer months provide a source of inoculum. Pycnidiospores are easily spread during watering of plants, often 2-3 times a day. Paulson and Schoeneweiss (1972) found the growth of the fungus throughout the soil at moisture content above 50%. It indicates that in periwinkle nurseries substrate could be the source for inoculum splash with water and spread during plant cultivation.

The question arises about a source of inoculum of *P. exigua* in Poland. Observation of periwinkle grown in natural conditions and in cemeteries have not shown any disease symptoms on shoots. It suggests that the pathogen could be transferred to Polish nurseries on mother plants imported from other countries. Unusually high substratum moisture resulting from frequent irrigation of periwinkle plantations as well as weather conditions favour the fast spread of the pathogen.

IV. CONCLUSIONS

1. *Phoma exigua* var. *inoxydabilis* var. *nov.* dominated among fungal isolates obtained from diseased stem runners and leaves.
2. The species was pathogenic for periwinkle in laboratory and field trials.
3. Optimal growth and sporulation of the fungus on potato – dextrose agar and malt extract agar was observed at 25°C whereas temperature 20°C was the most advantageous for the disease development.

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Leszek B. Orlikowski

ZARAZA BARWINKA POWODOWANA PRZEZ *PHOMA EXIGUA* DESM. VAR. *INOXYDABILIS* BOEREMA ET VEGH VAR. *NOV.*

STRESZCZENIE

Barwinek (*Vinca minor*) jest zimozieloną rośliną okrywową. Uprawiany jest również dla przemysłu farmaceutycznego jako surowiec do otrzymywania alkaloidu vincaminy oraz związków antyrakowych.

Badania przeprowadzone w 3 polskich szkółkach wykazały występowanie zamierania pędów na 5 do 70% roślin uprawianych w pojemnikach. Analiza mykologiczna 157 chorych roślin wykazała występowanie *Phoma exigua* var. *inoxydabilis* var. *nov.* na co najmniej 4/7 analizowanych pędów i 2/5 porażonych liści. *Chaetomium globosum*, *Fusarium oxysporum* oraz grzyby z rodzajów *Trichoderma* i *Mucor* uzyskiwano z około 1/5 analizowanych pędów.

Badania wykonane w laboratorium oraz w polu wykazały chorobotwórczość *P. exigua* w stosunku do barwinka. Na zainokulowanych liściach i łodygach nekroza rozwijała się w temperaturze od 10 do 30°C przy optimum 20°C. Na pożywkach ziemniaczano-glukozowej i maltozowej grzyb rozwijał się w temperaturze od 7,5 do 30°C przy optimum 25°C. Grzyb tworzył liczne piknidia na pożywce maltozowej w temperaturze 15-25°C. Na pożywce ziemniaczano-glukozowej piknidia tworzyły się kilka dni później.

Częste zraszanie barwinka w okresie wegetacji sprzyja rozprzestrzenianiu zarodników patogena w nasadzeniach.

Z piśmiennictwa wynika, że grzyb może również zasiedlać podłoże, co stanowi dodatkowe źródło inokulum.