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ASCOCHYTA BLIGHT (*Ascochyta syringae*) OF LILAC (*Syringa vulgaris* L.)

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Abstract. Lilac (*Syringa vulgaris* L.) is a popular ornamental woody plant grown for its very decorative flowers and large, dark-green leaves. The leaves remain on the shrubs for a long time. The fungus, *Ascochyta syringae*, is a pathogen which deteriorates the decorative value of the leaves. It causes brown irregular spots on leaves. In this study, 20 fungal isolates were tested in terms of their pathogenicity towards the leaves of *S. vulgaris*, and mycelium growth rate, while genetic variability was determined by RAPD-PCR. It was found that some isolates do not cause the formation of brown spots on leaves. Isolates differed considerably in terms of mycelium growth rate, ranging from 0.5 mm day^{-1} (B96 at 30°C) to 8.8 mm day^{-1} (B92a at 25°C). A positive dependence between mycelium growth and the capacity to cause leaf spots was observed. No close dependence was found between the genetic variability of isolates and the other examined traits of the isolates.

Key words: disease, RAPD, genetic variability

INTRODUCTION

Syringa vulgaris L. is a shrub commonly grown for its decorative flowers. The flowering period is very short. The dark-green leaves form the compact canopy of the shrub, and are also a decorative element. The shrub grows up to a height of 5 m and is not demanding in terms of locality or cultivation. Many cultivars and varieties differ in the colour of the inflorescences, habit, and size of the shrubs. This species is cultivated in gardens but also frequently found in the wild. It is of particular importance to maintain the good health of the ornamental plants over a considerable part of the vegetation season. The decorative value of the shrubs may be reduced as a result of Ascochyta blight. This blight may be caused by a deficiency of nutrients or the action of pathogens. For this reason brown spots appearing on leaves need to be considered as highly unde-

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sirable. Such spots reduce the decorative value, and they decrease the size of the assimilating organ, thus weakening the affected shrubs. Ascochyta blight caused by pathogens, is observed mainly in the second half of the vegetation period. The formation of brown spots on the leaves of *S. vulgaris* may by caused by the bacterium *Pseudomonas syringae* pv. *syringae* [Canfield et al. 1986, Canfield et al. 1997].

Ascochyta blight of *Syringa vulgaris* may also be caused by the fungus *Ascochyta syringae* Jaap and *A. orientalis* Bondartsev, or the closely related *Boeremia exigua* var. *lilacis* (Sacc.) Aveskamp, Gruyter & Verkley and *Boeremia exigua* var. *pseudolilacis* Aveskamp, Gruyter & Verkley [Aveskamp et al. 2010]. These fungi cause brown spots on leaves, which can occasionally be extensive. In the course of their vegetative development, these fungi produce pycnidia. Conidial spores form inside the pycnidia. Although hardly visible to the casual observer, pycnidia are produced within the brown spots.

MATERIAL AND METHODS

Experiment on pathogenicity. Analyses were conducted on 20 isolates of *A. syringa*. Isolates came from three locations: Kościelec (52.79282°N, 18.17198°E) (isolates B19–B38), Wielowieś (52.82602°N, 18.13433°E) (B71) and Poznań (52.41795°N, 16.88477°E) (B90a–B120). Single-spore cultures were obtained which were later used in further analyses. Species affiliation was determined on the basis of available keys [Melnik 2000]. Fungal cultures were run for 4 weeks on PDA medium. After this, a spore suspension was prepared (10^6 spores ml^{-1}) by removal of pycnidia and spores from the medium. Leaves coming from one shrub of *S. vulgaris* were washed in tap water and rinsed in distilled water. A single leaf was placed on a Petri dish containing 18 ml agar and kinetin (5 ppm). A needle was used to make a small wound on one half of the leaf. A spore suspension (50 µL) was prepared for each of the wounded leaves. One drop of the spore suspension was put on the wound and a second drop was placed on the other side of the leaf where no wound had been inflicted. Each combination was repeated 3 times. After 10 days, the size of the formed spots was recorded, while the length and width of the formed spots were measured. Next, the data were calculated using the formula: radical ($lenght^2$ + $width^2$). Such obtained data were subjected to a two-way ANOVA, factor 1 – isolate, factor 2 – presence or lack of wound on leaf.

Genetic analyses. In addition, DNA analyses of fungal isolates were also performed. Fungi were grown for 10 days on liquid glucose medium with a yeast extract. DNA was isolated using CTAB [Doyle 1991, Ii et al. 2012]. Polymorphisms of the examined isolates were determined by applying RAPD-PCR [Weber et al. 2005, Baturo-Ciesniewska 2011, Bayazit et al. 2011, Baturo-Ciesniewska et al. 2012, Ii et al. 2012] using primers A01, A02, A06 and A08 [Gutman et al. 1999, Frazzon et al. 2002]. The reaction mixture of 20 µL contained 10 µL – 2x PCR Master Mix (A&A Biotechnology), 30 ng of primers and 30 ng of DNA. Amplification was carried out in a thermocycler (S1000™ Thermal Cycler – Bio-Rad) as follows: one cycle lasting 1 min at 94°C, followed by 45 cycles: 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C [Frazzon

et al. 2002]. Amplification products were separated by electrophoresis in 2% (w/v) agarose gel containing 1 µg of ethidium bromide per ml. The DRAMIX molecular marker was used (A&A Biotechnology). The dendrogram result was obtained from 80 different DNA fingerprinting profiles out of 20 isolates. Statistical calculations were performed with the use of the Statistica ver. 10 programme.

RESULTS AND DISCUSSION

Ascochyta syringae causes spots on leaves as a result of pathogen infestation. The spots may be of a regular shape resembling an oval, but may also be irregularly shaped. The irregular spots formed in this experiment were compared by applying a transformation using the formula given in the Material and Methods section. The transformation allows for the comparison of the various lengths and widths of the irregular spots. The product of the transformation is an approximation of spot size. Isolates of *A. syringae* differed considerably in their pathogenicity towards leaves of *S. vulgaris* (tab. 1). The difference was assessed on the basis of spot size (IS). Only isolate B90a was capable of causing brown spots on leaves with no prior wounding. Despite leaf wounding, some isolates did not cause disease symptoms. This phenomenon may be considered characteristic of a considerable proportion of fungal species from the genus *Ascochyta*, which are considered to be "weakness pathogen". This means that these fungi most frequently cause disease when a plant is weakened e.g. by abiotic stress or wounding [Punithalingam 1979, Sałata 2002]. From all the wounded leaves, the largest spot was found to be caused isolate B92a (IS = 212.9). Analysis of variance (ANOVA) showed an interaction between the analysed factors, between isolates, and damage or a lack of leaf damage (ANOVA for isolate (S): df = 19, F = 130.20, p = 0.000, for damage (D): df = 1, F = 170.46, p = 0.000, for interaction S*D: df = 19, F = 26.85, p = 0.000).

Tested isolates differed in the mycelium growth rate on PDA medium (tab. 2). An interaction was recorded between temperature and the tested isolate of *A. syringae* (ANOVA for isolate (S): df = 19, F = 153.34, p = 0.000, for temperature (T): df = 4, F = 949.50, p = 0.000, for interaction (S*T): df = 76, F = 29.29, p = 0.000). The fastest growth was recorded for isolate B92a at 25°C. This isolate also exhibited the fastest growth irrespective of temperature. The slowest growth was observed at a temperature of 30°C for isolate B96, and this isolate also grew slowest irrespective of temperature. The fastest growth (6.4 mm day^{-1}) for all isolates (the mean) was recorded at 25°C, while growth was slowest at 30°C (2.8 mm day^{-1}). Studies on growth rate at different temperatures, are used to determine optimum fungal growth in the environment in terms of a major factor occurring in that environment, i.e. temperature [Kosiada 2012, Machowicz-Stefaniak et al. 2012]. Despite the growth differences observed between isolates, it may be stated that the optimal temperature for mycelium growth (most probably also for the entire development period) ranges from 25°C and 20°C, i.e. temperatures found in the summer. A slightly slower growth was recorded at 15°C, which is the temperature recorded in early autumn.

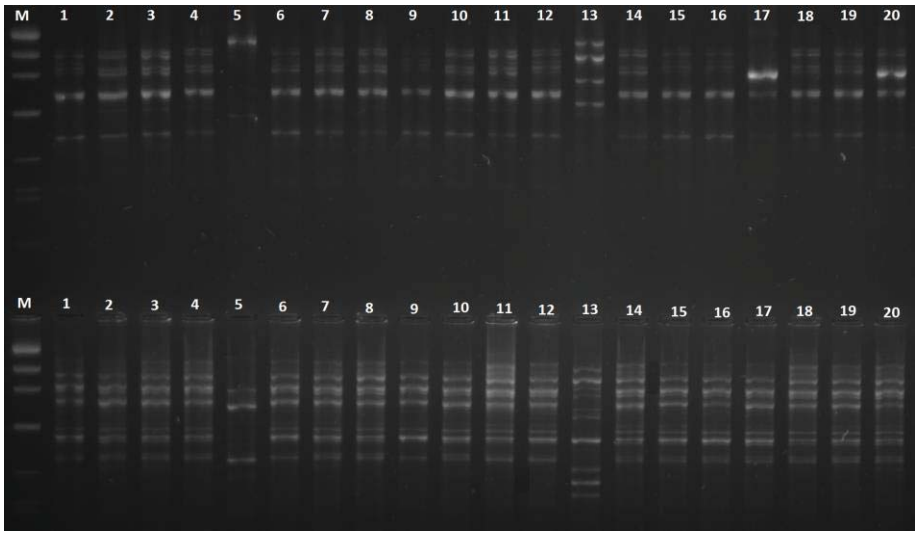
Symptoms on *S. vulgaris* leaves were observed in late summer and early autumn, which indicates the consistence of optimal mycelium growth temperature which was found in summer and autumn. Isolate B92a exhibited the fastest mycelium growth (8.8 mm day^{-1} at 20°C) and isolate B92a caused the greatest spots (IS = 212.9) on lilac leaves. The isolate characterised by the slowest growth (B96) did not infect leaves. Similar dependencies could be observed in the case of the other isolates, except for isolate B91b, which had a relatively slow (1.9 mm day^{-1}) mean growth rate yet still caused the formation of a relatively large spot (IS = 179.9). This could be explained by the fact that this isolate showed much greater differences in growth rates at different temperatures. At 25°C, the growth of isolate B91b was 3.9 mm day^{-1}. Such a dependence may indicate that mycelium growth rate is a major characteristic of *A. syringae* in pathogenesis.

Table 1. Leaf damage in *S. vulgaris* caused as a result of inoculation with *A. syringae*

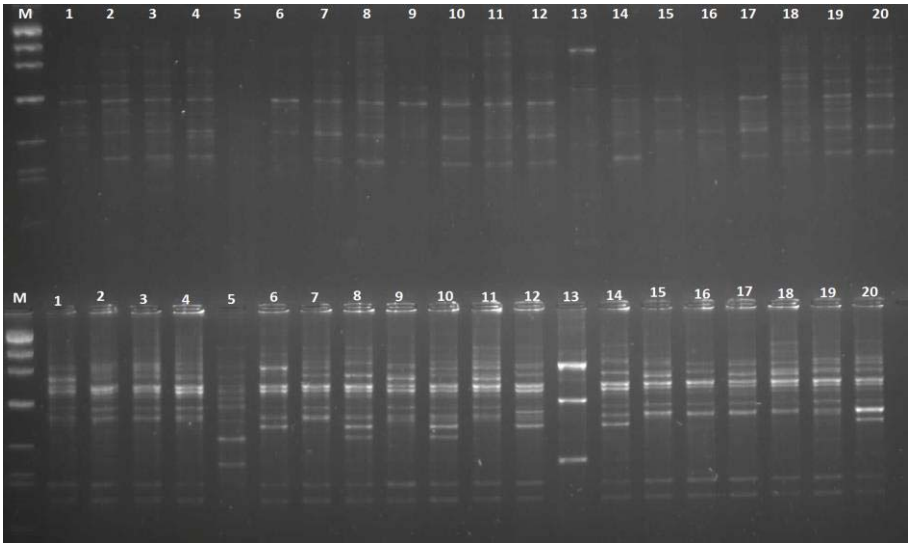
Isolate	Index Spot (IS) (mm)	
	damage	
	–	+
B19	0	9
B24	0	49.4
B27a	0	0
B27b	0	0
B29	0	0
B32	0	0
B33	0	0
B36b	0	0
B38	0	0
B71	0	0
B90a	38.2	113.1
B91a	0	171.9
B91b	0	179.9
B92a	0	212.9
B96	0	0
B102a	0	7.1
B103b	0	57.2
B118a	0	0
B119	0	0
B120	0	9
$LSD_{N\text{-}K,\ 0.05}$	3.28	
	1.91	40.48
$LSD_{N\text{-}K,\ 0.05}$	2.45	

Table 2. Growth rate of *A. syringae* at different temperatures

Isolate	Mycelium *in vitro* growth (mm day^{-1})					
	10°C	15°C	20°C	25°C	30°C	mean
B19	4.2	3.3	4.0	5.2	2.3	3.8
B24	3.2	4.4	5.1	5.5	2.4	4.1
B27	3.9	4.9	5.4	6.2	2.8	4.7
B27b	5.3	6.0	5.4	7.9	4.3	5.8
B29	5.2	5.6	6.5	1.0	0.6	3.8
B32	3.6	4.3	6.0	6.4	1.9	4.4
B33	3.7	5.6	5.8	7.5	5.2	5.6
B36b	5.2	5.4	6.4	7.3	3.4	5.5
B38	2.6	3.3	3.8	5.6	2.3	3.5
B71	4.3	4.7	6.3	6.1	2.5	4.8
B90a	3.6	4.2	5.8	6.5	1.4	4.3
B91a	2.9	4.6	4.6	6.8	4.9	4.7
B91b	1.9	1.2	1.9	3.9	0.6	1.9
B92a	6.7	6.9	8.4	8.8	3.4	6.8
B96	1.2	2.2	2.6	8.5	0.5	3.0
B102a	4.5	5.1	4.7	7.6	3.4	5.1
B103b	3.9	4.0	5.0	6.5	4.2	4.7
B118a	3.4	4.3	4.9	5.9	3.7	4.4
B119	5.8	5.4	6.6	8.4	2.8	5.8
B120	3.3	5.0	6.1	6.4	3.8	4.9
$LSD_{N–K, 0.05}$			0.86			0.27
	3.9	4.5	5.3	6.4	2.8	
$LSD_{N–K, 0.05}$			0.21			



Phot. 1. Electrophoregram of RAPD-PCR products using primers A01 (top), A02 (bottom) isolate sequence 1–20 as in Table 1



Phot. 2. Electrophoregram of RAPD-PCR products using primers A06 (top), A08 (bottom), isolate sequence 1–20 as in Table 1

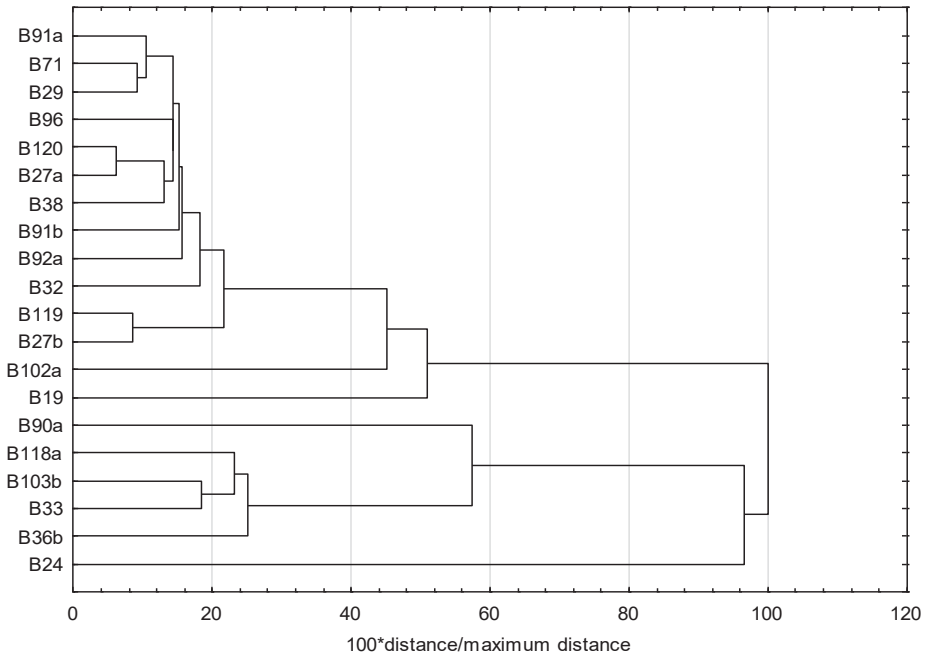


Fig. 1. Dendrogram presenting genetic similarity of *A. syringae* isolates

A dendrogram was prepared (fig. 1) for the obtained RAPD-PCR products (phots 1, 2). The tested isolates mainly belong to one large group (B91a, B71, B29, B96, B120, B27a, B38, B91b, B92a, B32, B119, B27b), and also to two smaller less numerous groups (group 2: B119, B27b, and group 3: B118a, B103b, B33, B36b). Isolates B24 and B90a showed the least similarity to other isolates. Isolate B24 was the only one which came from the Kościelec infested lilac leaves. In turn, isolate B90a from Poznań, was the only one which infected the unwounded leaves. Thus, a high genetic diversity of these isolates was confirmed by certain phenotypic traits.

These analyses explain the dependencies between some of the factors responsible for the development of Ascochyta blight in lilac caused by *A. syringae*. The mycelium growth rate in the tested isolates is definitely such a factor.

CONCLUSIONS

1. Not all of investigated *Ascochyta syringae* isolates were pathogenic to *Syringa vulgaris*, although they were isolated from the leaves with symptoms.
2. Lilac leaf damage contribute to pathogenesis by *A. syringae.*
3. Optimum temperature for growth of *A. syringae* is about 25°C.

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ASKOCHYTOZA (*Ascochyta syringae*) LILAKA (*Syringa vulgaris* L.)

Streszczenie. Lilak (*Syringa vulgaris* L.) należy do powszechnie uprawianych krzewów ozdobnych. Uprawiany jest ze względu na bardzo dekoracyjne kwiaty oraz duże ciemnozielone liście długo utrzymujące się na krzewach. Jednym z patogenów obniżających wartość dekoracyjną liści jest grzyb *A. syringae*. Powoduje on powstawanie na liściach brązowych nieregularnych plam. Przebadano 20 izolatów grzyba pod względem patogeniczności wobec liści *S. vulgaris*, szybkości wzrostu grzybni oraz określono zróżnicowanie genetyczne przy pomocy RAPD-PCR. Stwierdzono, że cześć izolatów w ogóle nie powoduje powstawanie brunatnych plam na liściach. Izolaty różniły się znacznie szybkością wzrostu grzybni: od 0,5 mm dzień$^{-1}$ (B96 w temp. 30°C) do 8,8 mm dzień$^{-1}$ (B92a w temp. 25°C). Zaobserwowano dodatnią zależność wzrostu grzybni ze zdolnością do wywoływania plam na liściach. Nie stwierdzono ścisłej zależności pomiędzy zmiennością genetyczną izolatów a pozostałymi badanymi cechami izolatów.

Słowa kluczowe: choroba, RAPD, zmienność genetyczna

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