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Shot-hole disease on *Prunus persica* – the morphology and biology of *Stigmina carpophila*

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Abstract

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Shot-hole disease caused by *Stigmina carpophila* (Deuteromycetes) is a major limiting factor in peach production, causing foliage shot hole in spring and early summer; fruit-spotting and cankers on limbs and twigs during autumn rains. The fungus overwinters, for at least two seasons, in cankers and killed buds. During spring and summer of 2009–2010, there occurred optimal conditions for manifestation of these symptoms on peach leaves and for the fungus activation. In such humid conditions is activated germination of brown, smooth walled, fusiform conidia with truncate base and rounded apex 16 to 20 µm by 8–10 µm in size, which accounts for the winter infection of buds. The fungus *Stigmina carpophila* isolated from damaged leaves of *Prunus persica* formed in culture sub-hyaline, septate and smooth walled mycelium, and dark brown stromata, partly superficial and partly immersed. The evaluation of mycelium growth suggested a significant effect of cultivation media on the assessed mycelium size on each of the eight days of the experiment. Since the third cultivation day, the size of mycelium on CzD was significantly smaller than the mycelium size on PDA and V-8. The variability of mycelium size on all media decreased with the time of cultivation. There was observed formation of terminal, intercalary, often chained chlamydospores on PDA in the dark. The most serious aspect of shot hole disease on peach is leaf infection leading to defoliation, as severe defoliation during the early fruit development can cause falling young fruits, and repeated defoliation weakens the trees and reduces their yield.

Key words

shot hole blight, *Stigmina carpophila*, stone fruit trees

Introduction

Shot hole blight or shot hole disease is a fungal disease of stone fruit trees including peach, nectarine, apricot, plum, cherry and almond. The most commonly affected are apricot, peach and nectarine, and to lesser degree cherries.

Stone fruit trees are economically important landscape plants. The most limiting factor to their production is shot-hole disease. On leaves, the symptoms of shot-hole disease range from small reddish or purplish, with yellow halo bordered spots, the centre of which drops out as the spot ages, to larger, irregular, reddish-brown spots occurring usually along the leaf margin – where the affected area also drops out (Woodward, 1999). On twigs, the symptoms are small black spots which later enlarge and become sunken. The disease is the most harmful in intensified cool and wet conditions of spring, although it can occur and cause damage at any time during prolonged wet weather (Evans et al., 2008). Overhead sprinkler irrigation and closely spaced plants favour the disease development.

The primary causal pathogen of shot-hole disease was described as *Xanthomonas pruni*. This bacterium, in association with another bacterium *Xanthomonas campestris*, has been blamed for all the damage. Preliminary evidence suggests that shot-hole disease is not caused solely by bacteria but also by one or several fungal pathogens (Woodward, 1999). Identical symptoms can be caused by a minor fungal pathogen known as *Stigmina carpophila* (Lév.) M.B. Ellis, [syn. *Thyrostroma carpophilum* B. Sutton, *Coryneum beyerinckii* Qud., *Wilsonomyces carpophilus* (Lév.) Adask., *Ascospora beijerinckii* Qud., *Clasterosporium carpophylum* (Lév.) Aderh.], (*Dothideomycetes*, *Capnodiales*, *Mycosphaerellaceae*). The fungus was first observed in France in 1846, later in Africa, Asia, Europe, North, Central and South America, Australia and Oceania (Văcăroiu et al., 2008).

On some *Prunus* species (*P. amygdalus, P. armeniaca, P. avium, P. cerasus, P. communis, P. domestica, P. dulcis, P. italica, P. laurocerasus, P. persica*) particularly laurel, shot-holing may occur following any factor damaging leaf tissue. In addition to *Pseudomonas* and *Stigmina,* such factors include powdery mildew, other leaf-spotting fungi, pests, nutritional problems and damage from adverse soil or weather conditions. Morphological and physiological characters of affected leaves may be necessary for identifying the precise cause.

Material and methods

During spring and summer 2009–2010, leaves of *Prunus persica* (L.) Batsch, syn. *Persica vulgaris* Mill. showing symptoms of discoloration, piercing, brown spots or necroses were sampled from affected plants in private gardens of the town Nitra. Visual characteristics of necrotic and chlorotic leaves were examined with a stereomicroscope SZ51 (Olympus). Investigation of fungal structures immersed in water was performed with a clinical microscope BX41 (Olympus), under 400× and 1000× magnification.

The leaf pieces cut from the diseased plants were surface-sterilized with a 3% sodium hypochlorite solution for 20 min., rinsed in sterile distilled water (2–3 times) and dried carefully with filter paper. After the surface sterilization, the tissue samples were cut to small pieces, placed on potato-dextrose agar (3% PDA) and subsequently incubated in Petri dishes. There followed cultivation in a versatile environmental test chamber MLR-351H (Sanyo) at 24 ± 1 °C temperature, 45% humidity and photoperiod 12/12 hours, and finally isolation on potato-dextrose agar (3% PDA). Pure fungal cultures were obtained after multiple purifications. The growth rate of mycelium was balanced on three growth media: PDA, V-8 and CzD, each in 30 Petri dishes, for eight days. The mycelium size was assessed daily, based on two diameter values measured perpendicular each to other. The obtained data were evaluated for each day separately, by analysis of variance – to assess the influence of the three media on growth of *S. carpophila*. Subsequently, multiple range test of growth means for media was performed. The package Statgrafic was used for statistical analysis.

The colonies of fungi were identified using various keys for identification**:** Adaskaveg et al. (1990), Ellis and Ellis (1997) and Kirk (1999), working with micro- and macroscopic symptoms. Based on morphological and physiological characters and optimal temperatures for growth in the culture, all the isolates were identified as *S. carpophila*. Samples of material have been deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology in Nitra.

Results and discussion

In our experiments, during the spring and summer, red dots were scattered all over the leaves; then they expanded into larger circular lesions. These lesions had a necrotic brownish centre and purple margins. The central necrotic area gradually gave way and droppped out, resulting in a hole (Fig. 1a, b). At the beginning, the lesions were small, round purplish-black spots on the surface of the affected leaf parts (Fig. 1c). On young leaves, the diseased areas sometimes expanded rapidly (Fig. 1d) and killed large areas of the blade (Fig. 1e). We have confirmed the results of Smith et al. (1988) that shot hole disease of stone fruit trees, caused by the fungus *S. carpophila,* produces lesions on leaves, fruits, flowers and succulent shoots. Across all the growing areas of stone fruits, this serious pathogen causes large circular purple-brown spots with chlorotic haloes on leaves. Buds and twigs are affected, too.

Leaf infection leading to defoliation in the most serious aspect of shot hole diseases, because severe defoliation during early fruit development can cause the young fruits to fall, and repeated defoliation weakens the trees and reduces their yield (Teviotdale et al., 1999). With lesions on the petiole, the leaf is killed outright (Kotte, 1941; Kirk, 2005). Frequently, large numbers of young leaf clusters are killed by lesions that develop on the base of the petioles. In our observations, the affected areas of the blades of mature leaves separated quickly from the non-affected tissue by abscission zones and immediately fell away. Newly formed leaves with only a few lesions dropped (Fig. 1f), but older leaves commonly remained on the tree, despite a number of lesions. The association between defoliation and shot-hole infections was reported earlier by Wilson (1953). According to his study, if a leaf infection causes an early defoliation and if the early defoliation adversely affects the tree growth or vigour, then defo-

liation over several years may cause stress to the trees or reduce the amount of their fruiting wood.

During spring and summer 2009 and 2010, there were very suitable conditions for the fungus growth. According to the climatic data of SHMI (2011), the average sums of rainfall in Slovakia in the of spring-summer periods 2009 and 2010 were above normal. The year 2009 in Slovakia had an 871 mm atmospheric precipitation total, the year 2010 an about 164% precipitation total compared to the normal. These conditions resulted in an extensive damage to *Prunus* trees by the fungus *S. carpophila*, as the spores of this fungus infect susceptible plant tissues during periods of persistent moisture. According to EVANS et al. (2008), the disease is most harmful in extended cool and moist periods in spring, while it can occur and cause damage at anytime during long lasting wet weather.

The infection is spread by conidia, in dry conditions viable for several months but not possible to detach and spread by the wind. Rain is necessary for their dispersal. In humid conditions, they can germinate at highly varying temperatures above 2 °C, which accounts for the winter infection of buds. Temperature and duration of wet periods during the inoculation influenced the development of shot-hole disease on leaves of the *Prunus* species caused by *S. carpophila*. (VĂCĂROIU et al., 2009).

GROVE (2002) detected the influence of temperature and high humidity on the infection of cherry and peach foliage by this fungus. The effects of temperature and wetness duration on infection of *P. avium* and *P. persica* were examined under controlled conditions. In cherry, the disease severity increased with wetness duration. After 24 h of wetness presence, the maximum disease severity of 10.5 lesions cm^{-2} was obtained at 20 °C. Although severity values were different, the general responses to temperature and wetness period were similar on peach. SHAW et al. (1990) observed in controlled environment studies that a 14-hr wetness period resulted in 0.1 and 45.0 lesions per leaf after 10 days at 8 °C and 22 °C, respectively. Extended wetness periods during the infection period increased the number of lesions per a leaf regardless of the temperature. According to LARSEN (1999), temperature from 70 °F to 80 °F (21–27 °C) is optimum for *Coryneum* infection. Lesions can develop at 45 °F (7 °C), however, at a much slower rate. It takes from two to five days for a spore to initiate infection and cause a visible lesion. According to HICKMAN (2001) and EVANS et al. (2008), in spring under sustained moisture, the pathogen can actively colonize host tissues at temperature as low as 36 °F (2 °C) in as few as 24 hours, while at 77 °F (24.5 °C) the fungus can infect a suitable host's tissues in as few as 6 hours. Infection periods are determined by duration of moisture conditions and the temperature. At cooler temperatures, longer periods of moisture are required (SHAW et al., 1990).

In our experiments, the fungus *S. carpophila* isolated from the damaged leaves of *Prunus persica* formed sub-hyaline, septate and smooth walled mycelium (Fig. 1g, h, i) with septate, thin-walled, branched hyphae growing in a culture on 3% PDA (at 24 ± 1 °C temperature, 45% humidity and 12/12 hours of photoperiod), and dark brown stromata which were partly superficial and partly immersed. No growth was observed in the culture above 30 °C.

The evaluation of mycelium growth by ANOVA has suggested a significant effect of cultivation media on assessed mycelium size on each of the eight days of the experiment. Beginning with the third day of cultivation, the size of mycelium on CzD was significantly smaller than the mycelium size on PDA and V-8 (Table1). In addition, the mycelium on CzD showed a lower growth rate: its size on the seventh day was 6 to 7-times smaller than the mycelium size on the other two media. The differences in mycelium size between PDA and V-8 substrates were smaller, significant, however, in most of the cultivation days. On the first and the seventh day, the mycelium on PDA was smaller than on V-8; contrarily, on the second, fifth and sixth day it was larger than on V-8 (Fig. 1j-k). Statistically insignificant differences in mycelium size between PDA and V-8 were observed on the second, third and eighth day. Different growth rates of mycelia on individual media are demonstrated in Fig. 2. Fluctuation in mycelium size on a single medium was the highest on the CzD substrate where the mycelium was of the smallest size on average. This medium stopped the fungus vegetative development and fructifications were reduced. The second biggest size fluctuation in mycelia cultivated on 30 Petri dishes was obtained on medium PDA. The lowest mycelium size fluctuation was on medium V-8. Generally, on all media, the fluctuation of mycelium growth decreased with cultivation time (see the coefficient of variation in Table 1).

The conidia were brown, fusiform, with a truncate base and rounded apex, smooth walled with 3, occasionally 4 dark transverse and 1–2 oblique septa with dimensions from 16 to 20 µm by 8–10 µm (Fig. 1l-n). These pigmented spores are extremely durable and can survive dormant on leaf or bud surface for months, waiting just for the right temperature and moisture conditions to germinate and infect its host (EVANS et al., 2008). Formation of chlamydospores on PDA was obtained in the dark. Chlamydospores were single, terminal and intercalary, often chained (Fig. 1o).

The mycelium of this fungus obtained by KAFI and RIZVI (1971) in culture from apricot fruits was septate, sub-hyaline and smooth walled. The conidiophores, outgrowing from the upper cells of the dark brown stromata, were straight, sub-hyaline to pale brown. The conidia produced acrogenously were clavate elipsoid or fusiform, with truncate base and rounded or acute apex, sub-hyaline to brown. The basal cells of conidia were

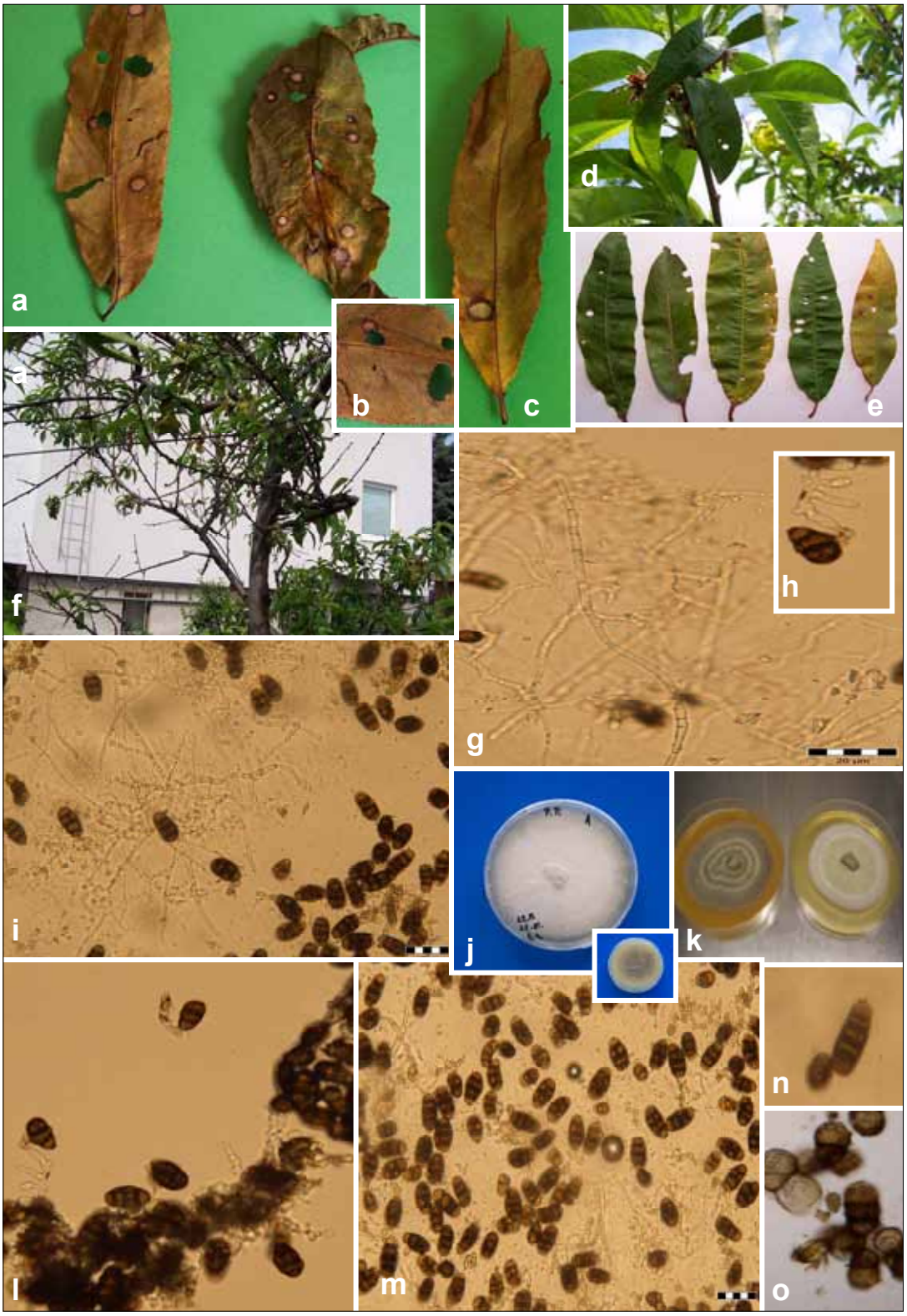

Fig. 1. Fungus *Stigmina carpophila* isolated from affected leaves of *Prunus persica*: a, b, Central necrotic area gradually gives way and drop out resembling in a hole; c, The lesions at first were small, round purplish-black spots on the surface of the affected part; d, e, On the young leaves, the diseased areas may expand rapidly and kill large areas of the blade; f, Newly formed leaves with only a few lesions will drop; g, h, i, Sub-hyaline, septate and smooth walled mycelium in culture; j-k, Culture of *Stigmina carpophila* after 36 days cultivation on PDA (reverse side-detail) and V-8 media; 1-n, Brown, fusiform, smooth-walled conidia with 3–4 dark transverse and 1–2 oblique septa; o, Formation of chlamydospores on PDA in the dark conditions.

hyaline, sometimes forming a beak. The conidia were smooth walled with 2 to 7 dark transverse and occasionally 1 to 2 oblique or longitudinal septa, measuring from 21.6 µm to 65.6 µm by 9.6 µm to 14.4 µm. ELLIS and ELLIS (1997) obtained similar results. The conidia produced in culture were brownish, fusiform with a truncate base, 3–7-septate, with dimensions of 30–60 × 9–18 µm.

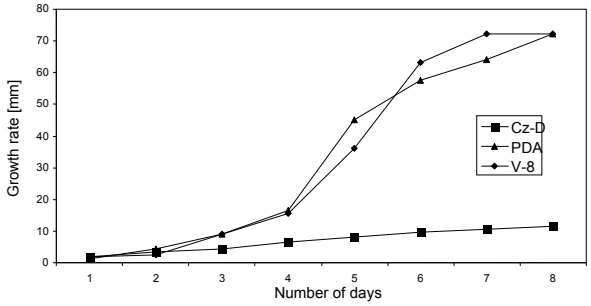

Fig. 2. Comparison of growth rate of the mycelium of the fungus *Stigmina carpophila* isolated from *Prunus persica* on three different media.

The fungus *S. carpophila* develops very well in laboratory conditions, on different crop substrates. The fungus acted best on PGA (potato-glucose-agar), followed by PDA (potato-dectrose-agar), where colonies (from morphological aspect, specific to the fungus and with a very good fructification) were formed. The MA environment produced bigger conidia, the progress, however, was rather slow. The Czapek-Dox environment stopped the fungus vegetative development, and fructifications were cut (VĂCĂROIU et al., 2009). AHMADPOUR et al. (2009) isolated from infected leaves, fruits and twigs of different *Prunus* species (apricot, almond, peach, nectarine, plum, sweet cherry and sour cherry) hyphae of the fungus *Wilsonomyces carpophilus* (syn. *S. carpophila*). The hyphae were septate, thin-walled, branched, 2.5–7.5 µm in diameter, sub-hyaline to light brown. Conidiophores in the sporodochia were sympodial, scars on conidiophores inconspicuous to conspicuous, conidia fusoid with apical cells ovate and basal cells truncate, 2.5–5 µm at the base, with 2–4 transverse septa (occasionally 0–8 septa), holoblastic, rhexolytic, 20–67.5 × 7.5–15 µm in size, sub-hyaline

Table 1. Descriptive statistics for the mycelium growth of *Stigmina carpophila* on three different media and multiple range test of means

Day	*Medium	No. of dishes	Mean size	Standard error	Minimum	Maximum	Coeff. of variation
1	PDA	30	1.235a	0.068	0.700	2.210	30.428
	V-8	30	1.994b	0.062	1.440	2.720	17.022
	CzD	30	1.794b	0.164	0.800	5.060	50.083
2	PDA	30	4.539b	0.189	2.080	6.720	22.816
	V-8	30	2.612a	0.065	1.960	3.420	13.745
	CzD	30	3.376a	0.389	0.990	9.000	63.194
3	PDA	30	9.352b	0.300	5.750	13.200	17.579
	V-8	30	8.622b	0.219	6.500	11.700	13.939
	CzD	28 (*2)	4.304a	0.488	1.200	9.920	60.037
4	PDA	30	16.878b	0.548	9.000	25.650	17.793
	V-8	30	15.523b	0.162	13.200	17.480	5.700
	CzD	27(*3)	6.450a	0.455	1.800	10.540	36.668
5	PDA	30	45.234c	0.658	36.000	53.290	7.973
	V-8	30	36.014b	0.355	32.940	40.800	5.413
	CzD	27(*3)	8.108a	0.543	2.560	13.200	34.793
6	PDA	30	57.697c	0.773	49.000	64.000	7.332
	V-8	30	48.648b	0.443	42.600	56.000	4.989
	CzD	27(*3)	9.649a	0.608	4.560	15.910	32.760
7	PDA	30	64.000b	0.000	64.000	64.000	0.000
	V-8	30	72.250c	0.000	72.250	72.250	0.000
	CzD	27(*3)	10.683a	0.602	5.630	16.900	29.276
8	PDA	30	72.250b	0.000	72.250	72.250	0.000
	V-8	30	72.250b	0.000	72.250	72.250	0.000
	CzD	27(*3)	11.577a	0.603	6.200	18.000	27.051

PDA – Potato-dextrose-agar, V-8 agar (V-8 juice, $CaCO_3$, agar); CzD – Czapek-Dox agar; (*2) or (*3), the number of contamined PM.

to golden brown, dark olivaceous to black in mass. No growth was observed in culture below 5 °C or above 30 °C. Văcăroiu et al. (2008) observed conidium germination starting at a temperature of 2 °C (1–3%), the optimal temperature was recorded between 16 and 24 °C (25–80%) and it decreased to 5% at 30 °C. Progressive spore growth was recorded starting from 2 °C, the highest colony growth rate was reached at 20 °C, decreasing until 30 °C.

Only one type of propagative structure is regularly produced. It is three- to six-celled, ovoid, yellowish conidium, borne on a short stalk (conidiophore) emerging from a simple cushion of fungal cells. The conidia generated by inoculum source are transported by rain and infect flowers and young leaves. On the leaves, the infection hypha penetrates directly through the cuticle, and it is seldom if ever found entering stomata. After the entry of the infection hypha, the fungus produces mycelium between the walls of the host tissue. From this mycelium, loosely packed cushions of hyphal cells emerge to the surface, and give rise to conidia (Văcăroiu et al., 2008).

Adaskaveg (1995) studied the morphology and ultrastructure of shot hole disease of almond infected by conidia *Wilsonomyces carpophilus* using light, scanning and transmission electron microscopy. The multicelled conidia of this fungus were thick-walled and darkly pigmented. The conidial wall was multilayered and mainly consisted of an electron-dense cuter-wall layer, and an electron-translucent inner-wall layer. Septa of conidia were also multilayered. The conidia lacked true septa and germinated by rupturing their outer-wall layers. The germination hyphae penetrated, indirectly through stomata or directly through the cuticle, into leaf tissue from appressoria that were produced terminally or on lateral branches of germ tubes. In cankers, the fungus may persist for several years. The pathogen overwinters on infected dormant leaves and blossom buds on twigs. According to Highberg (1986), *S. carpophila* conidia can survive the dormant season in association with healthy dormant buds, thereby contributing to the overwintering population of the fungus on the almond tree.

More serious and widespread diseases of *Prunus* leaves caused by other fungi may appear similar. Three species cause a shot-hole symptom, in which the necrotic tissue in limited spots dries and falls out of the leaf. All these fungi cause repeated defoliation which makes the tree more susceptible to winter injury and may eventually kill it.

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Dierkovitosť listov *Prunus persica* – morfológia a biológia huby *Stigmina carpophila*

Súhrn

Dierkovitosť alebo suchá škvrnitosť listov je všeobecne známe ochorenie kôstkovín, ktoré postihuje zástupcov rodu *Prunus* rastúcich v sadoch a záhradách, v ovocných a okrasných škôlkach. Ochorenie vyvoláva huba *Stigmina carpophila* (Lév.) M. B. Ellis. Jej výskyt podporuje daždivé počasie na jar a skoro v lete, ako aj vlhké počasie na jeseň a v zime. Pôvodca ochorenia prezimuje tzv. pučiacim mycéliom v škvrnách, v nádoroch na konárikoch, v púčikoch, v mumifikovaných plodoch, ale aj konídiami v opadaných listoch a na kôre stromov. Na jar 2009–2010 boli na sledovanom území mesta Nitra zaznamenané priaznivé teplotné podmienky na aktiváciu huby. Príznaky na listoch sa prejavili skoro na jar po vypučaní listov vo forme okrúhlych, niekoľko milimetrov veľkých, oranžových, neskôr ostro ohraničených hnedých škvŕn rozsiatych po celej listovej čepeli. Huba tvorila sub-hyalinné, delené, hrubostenné mycélium a valcovité, 3–4 priehradkové konídie veľké 16 – 20 × 8–10 µm, ktoré vďaka vlhkému počasiu infekciu ďalej rozširovali na pučiace listy. Vyhodnotenie rastu hýf mycélia huby *in vitro* analýzou variancie poukázalo na štatisticky významný vplyv kultivačného média na veľkosť mycélia počas 8-dňovej kultivácie. Od tretieho dňa kultivácie bola veľkosť mycélia na médiu CzD preukazne nižšia v porovnaní s dosiahnutou veľkosťou mycélia na médiách PDA a V-8. Predlžovaním kultivácie sa variabilita veľkosti mycélia huby na všetkých testovaných médiách znižovala. Na PDA agare pri kultivácii v tme sa pozorovala tvorba jednotlivých, terminálnych a interkalárnych chlamydospór často tvoriacich retiazky. Ochorením vznikajú na ovocných drevinách hospodársky významné škody. Silne poškodené listy predčasne opadávajú, čím sa znižuje asimilačná plocha, klesá intenzita fotosyntézy ako aj tvorba zásobných látok. Viacročné napadnutie predovšetkým mladých stromov spôsobuje ich oslabenie, výnimočne aj ich odumretie.

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