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THE INFLUENCE OF SHADING ON THE DEVELOPMENT OF *PODOSPHAERA LEUCOTRICHA* UNDER FIELD CONDITIONS

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Abstract

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The influence of shading on the incidence and development of *P.leucotricha* was examined at the locality of Belgrade, during 2008 and 2009. Investigation was carried out on two-year old apple nursery trees, cultivar Idared, sensitive to causal agent of apple powdery mildew. The experiment included three variants, two with different conditions of reduced light (24h and 48h shading, with 24h in the natural photoperiod) and the third variant, which included the cultivation of plants in natural photoperiod, without shading. Reduced light conditions were achieved by growing the plants under wooden experimental boxes. The boxes provided minimum differences in air temperature and RH values, inside and outside them. Inoculum was provided from two trees that were close to the experimental boxes, and the infection was carried out by direct application of conidia from infected young shoots and naturally, during growing period.

Four assessments were made, three during shading period and one after removal of the experimental boxes. In both years of investigation, the highest intensity of infection was manifested in the first variant (48h shading, 24h natural photoperiod), (14.0 - 100%), slightly lower in the second (24h shading, 24h natural photoperiod), (12.7 – 93.5%) and the lowest in variant of normal photoperiod, (0.1 - 16.6%). In shading variants, intensity of infection was rapidly increasing from the first to the third assessment, while in the fourth assessment, after the removal of boxes, it significantly decreased. The results of these studies indicate that light intensity significantly affects the appearance and development of *P. leucotricha*, and that apple powdery mildew develops intensively in the shade environmental conditions.

Key words: Podosphaera leucotricha, apple, shading, photoperiod

Introduction

Podosphaera leucotricha (Ell. and Ev.) is the causal agent of apple powdery mildew, one of commercially the most important fungal diseases in apple. Its occurrence is rather regular and, in susceptible cultivars (Jonatan, Idared), it causes leaf necrosis and drying, decelerates growth and

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reduces the formation of buds. Apple powdery mildew is spread in all apple-growing regions throughout the world (Butt et al., 1983; Yoder and Hickey, 1983). The disease also occurs regularly in Serbia, especially because of the great presence of susceptible Idared cultivar and favorable agro-ecological conditions. In years favorable for its growth, *P. leucotricha* can cause significant economic losses (Vojvodić, 1964; Miletić, 1999).

The occurence and development of apple powdery mildew is influenced by several factors, and the most important are: cultivar's susceptibility, plant defensive mechanism, as well as various abiotic factors (temperature, relative air humidity and light). Although each of them can significantly influence the occurence intensity of apple powdery mildew, it is still not clear which of them is the most important at a particular moment. Besides that, abiotic factors can influence the disease development in two ways – through the pathogen (fungus) and through a host plant. Because of that, in natural conditions it is very difficult to determine if abiotic factors have more important influence to the fungus or to the host plant (Miletić, 1999).

Temperature can greatly influence the occurrence and growth of *P. leucotricha*. The pathogen grows within temperature range of 10-25ºC (opt. 19-22ºC), (Yoder and Hickey, 1983). Minimal temperature for sporulation and dispersion of spores is 12ºC (Stephan, 1988), while the germination of its conidia is very poor above 32ºC (Molnar, 1971). Temperature conditions for the growth of other powdery mildew causal agents are similar (Xu, 1996).

Prevailing opinion is that the most favourable relative air humidity (RH) for the growth of powdery mildew causal agents is between 50 and 99%, depending on the species. Optimal air RH for the growth of *P. leucotricha* is 90% (Butt, 1995).

The intensity of light could be the possible dominant factor in the occurrence and growth of powdery mildew causal agent. Like other abiotic factors, light can affect both the pathogen and the host plant. In natural conditions, it is difficult to distinguish between these two effects, especially if we take into account that apple powdery mildew causal agent, like all other Erysiphaceae family members, is an obligate parasite which cannot be grown on artificial media. Two groups of authors have opposite opinions on the influence of light to the growth of powdery mildew causal agents. Results of many investigations show that increasing of light intensity causes greater formation and better germination of the conidia (Stephan, 1988; Xu et al., 1995; Jacob et al., 2008; Meislerova and Lebeda, 2010), although, in sunny days, a large number of conidia formed in sunny days may be destroyed by UV rays and reducing the infective potential of the pathogen (Willocqet et al., 1996). Contrary to that, in the conditions of reduced light the formation of conidia is also reduced, and even inhibited in total dark (Meislerova and Lebeda, 2010). From these facts, one group of authors draw the conclusion that powdery mildew causal agents grow more intensively in the conditions of increased light intensity. However, taking both the plant and the pathogen into consideration, and especially their interaction, the results of many other authors show that powdery mildew is, primarily, the disease "of reduced light" (Cimanowski et al., 1975; Aust and Hans-Jürgen, 1986; Edwards, 1993; Zahavi, 2003). In their investigations, Schnathorst (1965), Leibovich et al. (1996), Austin (2010) and Austin et al. (2011) concluded that shading was very favorable for the growth of powdery mildew causal agents. The question, which remains open, is if powdery mildew causal agents grow better in shading conditions because of lower temperatures and higher relative humidity, or because of the increased plant susceptibility.

The objective of this investigation was to examine the influence of shading on the occurrence and growth of *P. leucotricha* in nursery apple trees of susceptible Idared cultivar, in natural conditions. The results could contribute to better understanding of *P. leucotricha* occurrence and growth, which could make the control of this commercially important pathogen more efficient.

Material and Methods

Experimental design: In order to determine the influence of light (shading) on the occurrence and growth of *P. leucotricha* in natural conditions, a trial was set at the locality of Belgrade, in 2008 and 2009. The trial was carried out on two-year old apple nursery trees of Idared cultivar (rootstock M9), which is naturally highly susceptible to *P. leucotricha*. The trial consisted of three variants of investigation: two in different shading conditions, while in the third variant nursery trees were grown in natural photoperiod (full sunlight), without shading. Nursery trees for all three variants were planted on March 13, 2008 and on March 16, 2009 (the first and the second year of investigation). Those examined in the first two investigation variants were grown in plots under wooden experimental boxes of 2x2m in base and 2m high. The boxes were placed on legs 30cm above the ground, in order to provide free air flow and to reduce temperature and RH differences between their inside and outer environment (where nursery trees were grown in the variant of natural photoperiod). Within plots, nursery trees were planted in six rows at the distance of 30cm (30x30cm), so there were 36 nursery trees under each box, i.e. in each shaded experimental plot (6 rows x 6 nursery trees = 36 nursery trees). Trees grown in the variant of natural photoperiod were planted in plots at the same distance, so each investigation variant of the trial included a total of 144 nursery trees (36 nursery trees x 4 replications = 144 nursery trees). Within the trial field, experimental plots were arranged according to the model of full randomized block system in four replications, in accordance to the instructions of the method PP 1/152 (2) (EPPO, 1997). Distance between each plot was one meter, which provided enough space for necessary operations.

All nursery trees within the experimental field were nursed equally, using usual agro-technical measures, which included optimal soil nutrition and irrigation. Chemical control included pesticides, which do not affect powdery mildew causal agents, so that they could not influence the results of the investigation. Insecticide acetamiprid was used for the control of aphides, as well as fungicide captan, for the control of the pathogen *Venturia inaequalis*. Weeds were regularly removed using mechanical measures.

Investigation variants: The trial included three variants of investigation. In experimental plots of the first variant, trees were covered for 48 hours, followed by 24 hours of normal photoperiod. In experimental plots of the second variant, nursery trees were covered with experimental boxes for 24 hours, every other day (24h of shading followed by 24h of normal photoperiod). In experimental plots of the third variant, trees were grown in the conditions of normal photoperiod (without shading), and they were used as a control. In both years of investigation, periodical shading was carried out during the period of about six weeks (15/05/2008 – 01/07/2008 and 18/05/2009 – 01/07/2009). After that, experimental boxes were removed and trees were grown without shading.

Source of inoculum and inoculation: About 10m far from the trial field, there were two 16-year old apple trees of Idared cultivar, which served as the source of *P. leucotricha* inoculum. They had a high level of natural primary infection with *P. leucotricha*, with more than 100 young infected shoots at the beginning of vegetation. On each of the three consecutive days before the first covering of nursering trees with experimental boxes, artificial inoculations were carried out by shaking conidia from the fresh infected shoots (three times for three days). Application of conidia was performed evenly onto all nursery trees within the trial field, including all control trees. Because the infected trees were in close vicinity to the experimental trees, they were also exposed to spontaneous natural infection.

Trial assessment: The occurrence and growth of *P. leucotricha* were monitored daily during vegetation, through secondary infections. The intensity of secondary infections was assessed four times in each year of the trial, on three fully developed top leaves (on 01/06, 14/06, 30/06 and 08/08 in 2008, and on 03/06, 13/06, 28/06 and 09/08 in 2009). The infection intensity was assessed according to methods of Butt (1978) and OEPP (1984), and it was calculated using Towsend and Heuberger formula (Juhasova, 2004). Statistical analysis was performed using Duncan and LSD tests (Duncan, 1975). Each year, three assessments were performed in the period when the trees from the first and second investigation variants were shaded, and one was performed about a month after shading was over.

Results and Discussion

The influence of light intensity on the occurrence and growth of *P. leucotricha* in apple nursery trees was investigated in 2008 and 2009. The trial was carried out under favourable weather conditions for the occurrence and growth of this pathogen. In both years of investigation, limiting temperatures for the growth of this pathogen were not recorded (below 10ºC and above 32ºC) and the average relative air humidity (RH) was over 50%. In addition, in both years of investigation, there were no significant fluctuations of temperature and RH values between the inside and outside of the experimental wooden boxes, which provided the objectivity of the results. The first symptoms appeared on infected experimental nursery trees 7-10 days after the period of artificial inoculations.

In the first year of the trial (2008), the highest average intensity of the infection was recorded in the plots of the first investigation variant (48h of shading / 24h of normal photoperiod), ranging from 14.0 to 97.2%. It was slightly lower in the plots of the second variant (24h of shading / 24h of normal photoperiod), ranging from 12.7 to 91.0%, and by far the lowest in the control plots of the third variant (normal photoperiod), ranging from 0.1 to 10.7%. Within the first investigation variant (48h of shading / 24h of normal photoperiod), the highest infection intensity was recorded at the third and the second assessments (97.2% and 96.0%, respectively), while it was significantly lower at the first (68.6%), and the lowest at the fourth (last) assessment (14.0%), when shading was over. Similarly, in experimental plots of the second investigation variant (24h of shading / 24h of normal photoperiod), the highest infection intensity was also recorded at the third and the second assessments (91.0% and 81.6%, respectively), significantly lower at the first (68.6%), and by far the lowest infection intensity (14.0%) was recorded at the fourth (last) assessment. In plots of the third (control) investigation variant (normal photoperiod), the highest infection level was recorded at the second assessment (10.7%), it was lower at the first (5.0%), while the lowest infection levels were recorded at the third and the fourth (last) assessments (0.6% and 0.1%, respectively) (Table 1). The results of Duncan and lsd tests indicate statistically very significant differences between the variants of investigation (Table 1).

The results obtained in the second year of the trial (2009) were similar. The highest average infection intensity was recorded in the first investigation variant (36.1-100%), slightly lower in the second variant (27.2-93.5%), and the lowest in the control plots of the third investigation variant (1.5-16.6%). As it was in the first year of the trial, the highest levels of infection with *P. leucotricha* were recorded at the third assessment of the first and the second investigation variants (100% and 93.5%, respectively), lower at the second (97.1%

and 87.3%, respectively) and the first assessments (75.7% and 45.8%, respectively), and the lowest at the fourth assessment, after shading was over (36.1% and 27.2%, respectively). In the plots of the third (control) investigation variant, infection intensity was the lowest (1.5-16.6%) (Table 2).

In both years of the trial, the obtained results on the influence of light on *P. leucotricha* occurrence and growth in apple nursery trees were very similar. Taking into account that there were not any significant temperature and RH differences between the inside and the outside of the experimental boxes, increased infection intensity recorded in the two investigation variants of shading, in comparison with the control one, could be attributed solely to reduced light. In the plots of these two variants of reduced light (shading), the highest levels of the infection with *P. leucotricha* were recorded at the first three assessments, which were made during the period of shading. In addi-

Table 1
Investigation variants and intensity of infection with pathogen *P. leucotricha* on apple leaves in four different assesments in 2008

Assesment dates in 2008	Average infection intensity levels on apple leaves in different investigation variants, %							
	48h shading + 24h normal photoperiod	Sd	24h shading + 24h normal photoperiod	Sd	control (normal photoperiod)	Sd	$lsd_{0,05}$	$lsd_{0,01}$
June 01st	68.6 a*	1.07	4.3 b	1.43	5.0 c	0.42	2.13	3.23
June 14th	96.0 a	1.09	81.6 b	1.44	10.7 c	1.12	1.98	3.0
June 30th	97.2 a	0.87	91.0 b	1.15	0.6 c	0.11	1.34	2.03
August 08th	14.0 a	0.56	12.7 b	0.43	0.1 c	0.08	0.8	1.21

Legend: Sd – standard deviation; a* - Duncan test marks; $lsd_{0,05}$ and $lsd_{0,01}$ – significance levels of lsd test; gray rows refer to the assessments made during the shading period; the lowest row refers to the assessment made after shading was over

Table 2
Investigation variants and intensity of infection with pathogen *P. leucotricha* on apple leaves in four different assesments in 2009

Assesment dates in 2009	Average infection intensity levels on apple leaves in different investigation variants, %							
	48h shading + 24h normal photoperiod	Sd	24h shading + 24h normal photoperiod	Sd	control (normal photoperiod)	Sd	$lsd_{0,05}$	$lsd_{0,01}$
June 03rd	75.7 a*	2.10	45.8 b	0.73	7.2 c	0.46	2.03	3.08
June 13th	97.1 a	1.24	87.3 b	0.81	16.6 c	0.38	1.27	1.92
June 28th	100.0 a	0	93.5 b	0.69	2.1 c	0.26	0.66	1.00
August 09th	36.1 a	0.47	27.2 b	1.35	1.5 c	0.39	1.11	1.68

Legend: Sd – standard deviation; a* - Duncan test marks; $lsd_{0,05}$ and $lsd_{0,01}$ – significance levels of lsd test; gray rows refer to the assessments made during the shading period; the last row refers to the assessment made shading was over tion, in the plots of the first variant, with longer shading intervals, the intensity of the infection was much higher. The results also indicate that the infection intensity increases correspondingly with the length of shading period, because it increased rapidly from the first until the third assessment. The results of the fourth assessment (after shading was over) showed significant decrease in the infection intensity after the boxes had been removed from the plots of both shading variants of investigation. It could be explained by the fact that natural sunlight in July, with high level of UV rays and high temperatures, is unfavorable for the development of the disease. The findings of some other authors were also the same (Cimanowski et al., 1975; Aust and Hans-Jűrgen, 1986; Edwards, 1993; Willocqet et al., 1996; Zahavi, 2003; Austin, 2010; Austin et al., 2011). Not long ago, similar investigations were carried out in America, on the influence of shading on the occurrence and growth of the grape-vine powdery mildew causal agent *Erysiphe necator*, and the obtained results were also very similar to the results of this investigation (Austin, 2010; Austin et al., 2011). This confirms the opinion of most researchers who study powdery mildew causal agents, that *Erysiphaceae* family is a homogeneous entity whose members have very similar common characteristics.

Most plants have defensive mechanisms against powdery mildew causal agents, which can reduce or completely inhibit the infection process. One of defensive mechanisms includes the formation of papillae (callus) at the spot of infection, which was described in detail in articles on the causal agents of powdery mildew in barley (*E. graminis sp. hordey*) and grape-vine (*E. necator*) (McKeen and Rimer, 1973; Prat et al., 1984). Papillae are formed during the formation of infection pore, by aggregation of cytoplasmic content (McKeen and Rimer, 1973; Aist, 1976). The formation of papillae directly depends on the intensity of photosynthesis in the cells of a host plant (Weinhold and English, 1964; Aust and Hans-Jűrgen, 1986), i.e. reduced intensity of photosynthesis causes reduced papilla formation, and plant defensive mechanism becomes weaker (Weinchold i English, 1964). It is also confirmed that defensive mechanism of apple against *P. Leucotricha* increases with ageing of its leaves, because of greater presence of papillae (Aust and Hans-Jűrgen, 1986; Schlösser, 1990), which have been detected in epidermal cells of older apple leaves. All the mentioned researches could be related to the results of this investigation, because reduced light and photosynthesis under the experimental wooden boxes could also weaken the defensive mechanism of apple nursery trees. To confirm that, further detailed researches into the influence of light on the presence and formation of papillae are needed in laboratory conditions.

Conclusion

It could be concluded from the results of this investigation that reduced light significantly influences the occurrence and growth of *P. leucotricha* in a way that this apple powdery mildew causal agent grows more intensively in the conditions of shading. Although further researches are needed, this conclusion could probably refer to the other powdery mildew causal agents. On the basis of this, more intensive occurrence of powdery mildew causal agents cuold be expected in years with a larger number of cloudy days, which could be helpful in the forecast of the disease occurrence. These results also indicate that the occurence and growth of powdery mildew causal agents could be reduced by undertaking some measures for better exposure of orchards to sunlight, such as the choosing of optimal crown shape and proper direction of rows.

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References

Aist, J., 1976. Papillae and related wound plugs of plant cells. *Ann. Rev. Phytopathol*., **14**: 145-163.

Aust, H., D., 1986. Hans-Jűrgen, Microclimate in relation to epidemics of powdery mildew. *Ann. Rev. Phytopathol*., **24**: 491-510.

Austin, C. N., 2010. Sunlight's Influence on Grapevine Powdery mildew: Direct effects on pathogen development and attendant consequences of canopy management and vineyard variability. *Ph.D. Thesis, Cornell Univ., Ithaca, NY*.

Austin, C. N., J. Meyers, G. G., Grove and W. F. Wilcox, 2011. Quantification of powdery mildew severity as a function of canopy variability and associated impacts on sunlight penetration and spray coverage. *Amer. J. Enol. Vitic.*, **62**: 23–31.

Butt, D., 1978. An apple mildew assessment method for use in supervised control. *Rep. East Malling Res*., **14**: 1-157.

Butt, D., 1995. Apple powdery mildew - an overview of the biology. In: Apple and Pear Mildew and Canker, *East Malling Research Association*.

Butt, D., M. Jeger, A. Swait, 1983. Biometerological forecasting scheme for apple mildew. *Proceedings of the 10th International Congress of Plant Protection,* 184.

Cimanowski, J., J. Novacki, D. Millian, 1975. Effect of light on the development of powdery mildew and some physiological and morphological changes in apple leaf tissue. *Phytoprotection,* **56**: 96-103.

Duncan, D. B. T., 1975. Tests and intervals for comparisons suggested by the data. *Biometrics,* **31**: 339-59.

Edwards, H. H., 1993. Light affects the formation and development of primary haustoria of Erysiphe graminis hordei in leaf epidermal cells of *Hordeum vulgare*. *Physiological and Molecular Plant Pathology,* **42**: 299 – 308.

EPPO, 1997. Guidelines for the efficacy evaluation of plant protection products: Design and analysis of efficacy evaluation trials – PP 1/152(2), in EPPO Standards: *Guidelines for the efficacy evaluation of plant protection products,* **1**: 37-51.

Jacob, D., D. Rav David, A. Sztjenberg and Y. Elad, 2008. Conditions for Development of Powdwery Mildew of Tomato Caused by Oidium neolycopersici. *Phytopathology,* **98**: 270-281.

Juhasova, G., H. Ivanova, K. Adamcikova, M. Kobza, and A. Cerevkova, 2004. Scab disease of firethorn at selected localities in Slovakia. *Plant Protection Science,* **40**: 42-48.

Leibovich, G., R. Cohen and H. S. Paris, 1996. Shading of plants facilitates selection for powdery mildew resistance in Squash. *Euphytica,* **90**: 289 – 292.

Mc Keen, W. and S. Rimmer, 1973. Initial penetration process in powdery mildew infection of susceptible barley leaves. *Phytopathology,* **63**: 1049-1053

Mieslerova, B. and A. Lebeda, 2010. Influence of temperature and light conditions on germination, growth and conidiation of Oidium neolycopersici. *Journal of Phytopatology,* **158**: 616 – 627.

Miletić, N., 1999. System of apple control against *Podosphaera leucotricha* (Ell. And Ev.) Salm. in some major phases of apple growth. *Ph.D Thesis, University of Belgrade, Faculty of Agriculture*.

Molnar, L., 1971. The effect of environmental factors on the germination of conidia of Podosphaera leucotricha (Ell. et Ev.) Salm. *Ochrana Rostlin*., **7**: 203 -210.

OEPP, 1984. Guideline for the biological evaluation of fungicides – *Podosphaera leucotricha. OEPP Bull*., **14**: 235-242.

Pratt, C., M. Goffinet, M. Welser and R. Pearson, 1984. Powdery mildew of vitis: papillae (wall appositions) as a host response to infection. *Vitis,* **23**: 225-229.

Schlöser, E., 1990. Horizontal resistance of some species to powdery mildew. *Medelingewn van de Fac Landbouwetenschappen Rijksuniversitet Gent,* **2**: 203-206.

Schnathorst, W. C., 1965. Environmental relationships in the powdery mildews. *Annual Rev. of Phytopathology,* **3:** 343 – 366.

Stephan, S., 1988. Untersuchungen zu Sporulation und Sporenflug des Apfelmehltaus (*Podosphaera leucotricha* (Ell. ev Ev.) Salm.). *Arch. Phytopathol. Pflanzenschutz, Berlin*. **24**: 491-501.

Vojvodić, Đ., 1964. Study of some biological and ecological factors (*Podosphaera leucotricha* (Ell. et Ev.) Salm.), for the purpose of control. *Ph.D Thesis, University of Novi Sad, Faculty of Agriculture*.

Weinhold, A. and H. English, 1964. Significance of morphological barriers and osmotic pressure in resistance of mature peach leaves to powdery mildew. *Phytopathology,* **54**: 1409-1414.

Willocqet, L., D. Colombet, M. Rougier, J. Fargues, and M. Clerjeau, 1996. Effects of radiation, especialy ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. *European Journal of Plant Pathology,* **102**: 441-449.

Xu, X-M., 1996. The effects of constant and fluctuating temperatures on the length of the incubation period of apple powdery mildew (*Podosphaera leucotricha*). *Plant Pathology,* **45**: 924-932.

Xu, X-M., D. Butt, and M. Ridout, 1995. Temporal patterns of airborne conidia of *Podosphaera leucotricha*, causal agent of apple powdery mildew. *Plant Pathology,* **44**: 944-955.

Yoder, S. and D. Hickey, 1983. Control of apple powdery mildew in the mid-atlantic region. *Plant Disease,* **67**: 245-248.

Zahavi, T., M. Reuveni, D. Scheglov and S. Lavee, 2001. Effect of grapevine training systems on development of powdery mildew. *European Journal of Plant Pathology,* **107**: 495 – 501.

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