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INFLUENCE OF TEMPERATURE AND LIGHT CONDITIONS TO COLONY GROWTH OF BROWN ROT FUNGI OF STONE FRUITS IN MONTENEGRO

SUMMARY

This research was performed to determine the influence of temperature and light conditions to colony growth of *Monilinia* isolates collected on stone fruits in Montenegro. The fungi cause brown rot on stone fruits and have a great economic impact in Montenegrin stone fruit production.

Effects of temperature (at 15°, 22°, 27° and 30˚C) and light (light16h /darkness 8h and constant darkness) were studied on *Monilinia laxa (*Aderh. & Ruhland) Honey and *Monilinia fructicola* (G. Wint.) Honey isolates.

Optimal temperature for both *M. laxa* and *M. fructicola* was 22 °C and at this temperature *M. fructicola* isolates grew faster than *M. laxa*. Temperatures higher then optimal favoured *M. fructicola*, while lower temperature favoured *M. laxa*. Isolates of both species developed better in darkness while sporulation was instigated by light/dark alteration.

Keywords: *Monilinia fructicola*, *Monilinia laxa*, brown rot, micelial/colony growth, temperature, light regime, Montenegro

INTRODUCTION

Brown rot is a fungal disease caused by one or more of three closely related fungi – *Monilinia fructigena* Honey, *Monilinia laxa* (Aderh. & Ruhland) Honey and *Monilinia fructicola* (G. Winter) Honey (Lane, 2002). These species are economically very important pathogens of stone and pome fruits. *M. laxa* and *M. fructicola* are mainly spread on stone fruits while *M. fructigena* is widely present on pome fruits (Duduk et al., 2017).

Monilinia species cause similar symptoms on stone fruits – flower and twig blight and brown rot of fruits. *M. fructicola* is common in North and South America, Australia, New Caledonia and New Zealand, while the other two species occur in Europe. Hence, *M. fructicola* is called „American brown rot fungus", while *M. laxa* and *M. fructigena* are designated as „European brown rot fungi" (Ivić and Novak, 2012). Since *M. fructicola* is considered the most destructive, in Europe it was regulated firstly as quarantine pest from the A1 pest

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list of the EPPO region and after its appearance in several European countries it was moved to the A2 pest list (Carstens et al, 2010).

Detailed study of *Monilinia* species distribution on stone fruits in Montenegro was done by Vučinić (1994). In the study performed from 1980 to 1983 and later in 1988 it was concluded that *M. laxa* and *M. fructigena* were present on stone fruits in Montenegro while *M. fructicola* has not been recorded. However, in 2016 Latinović et al. (2017) reported the presence of *M. fructicola* in Montenegro for the first time. It was detected in a commercial orchard of nectarine in the region of Podgorica.

Since the disease is widespread in Montenegro causing blossom, branch and twig blight and fruit brown rot on stone fruits, and especially because the new fungal species (*M. fructicola*) was recently discovered, the aim of our study was investigation of ecological features (temperature and light conditions) of selected *Monilinia* isolates obtained from different regions and hosts in Montenegro. Isolates were compared, identified and examined in different conditions of the abiotic factors for better understanding of their influences on fungal development and reproduction. The results can be useful in prediction of the two species during the season and control of brown rot fungi on stone fruits.

MATERIAL AND METHODS

In order to explore the presence of *Monilinia* species on stone fruits in Montenegro, field survey was conducted from March to October 2018. Orchards of plum, cherry, peach and nectarine at localities: Pljevlja, Žabljak, Bijelo Polje, Nikšić, Podgorica surroundings Zeta, Mataguži, Gornja Gorica, Lješkopolje,Vranj, Ćemovsko polje and Tuzi were checked. In case of apparent disease symptoms, samples were collected and transferred to the laboratory.

The research was performed at the Plant Pathology Laboratory of the Biotechnical Faculty in Podgorica, Montenegro. A total of 18 *Monilinia* spp. isolates were isolated from diseased fruits or flowers. Isolation was performed by placing small pieces of decayed tissue on potato dextrose agar (PDA, Biolife) in Petri dishes (90 mm in diameter) and incubated at 22°C for a week. Four representative isolates have been chosen in order to study influence of different temperatures and light conditions to fungal colony growth.

Determination of the isolates was made according to the synoptic key established by **Lane** (2002).

Influence of temperature to isolates colony growth was examined at 15˚, 22˚, 27˚ and 30˚C on PDA nutrient medium in dark. Effect of light was studied on isolates' colonies grown on PDA at 22˚C in two treatments: in darkness and in natural light/dark rotation (16 h light / 8 h dark long-day photoperiod).

Colony growth of different isolates was evaluated by measurement of diameter of the colonies on the third and the seventh day. Average daily growth of the colonies was calculated according the formula:

$$\frac{D_2\text{-}D_1}{T_2\text{-}T_1}$$

where D_1 and D_2 represent colony diameters in mm after the first and the last measurement while T_1 and T_2 corresponds to the days at which the measurements were made (Brasier and Webber, 1987), in this case at 3 and 7 days after medium inoculation, respectively.

The experiment was set up in four repetitions.

RESULTS AND DISCUSSION

Among 18 obtained isolates, four of them were selected as representative in relation to the fungal species and the host, in order to study influence of different temperatures and light conditions to fungal colony growth. The isolates were determined as *Monilinia laxa* (isolates MT1 and Mšpv-r from cherry and plum, respectively) and *Monilinia fructicola* (isolates MN_1 and MBP from nectarine and peach, respectively). These isolates are presented in Table 1.

Table 1. List of *Monilinia* isolates used in the study

Isolate	Species	Host	Variety	Locality
MT1	*Monilinia laxa*	Cherry	Burlat	Tuzi
Mšpv-r	*Monilinia laxa*	Plum	Zerdelija	Pljevlja
MN1	*Monilinia fructicola*	Nectarine	Unknown	Vranj
MBP	*Monilinia fructicola*	Peach	Early May Crest	Ćemovsko polje

Effect of different temperatures on mycelial growth. Influence of different temperatures on colony growth of the studied isolates is presented in Fig. 1. Development of examined isolates of *M. laxa* and *M. fructicola* colonies at different temperatures is shown in Fig. 2.

Among different temperatures studied (15˚, 22˚, 27˚ and 30˚C), colony growth of the tested isolates was the best at 22°C. *M. laxa* developed better at 15° then at 27° and 30°C while it was the opposite for *M. fructicola*. In general, growth rate of *M. fructicola* isolates were faster than those of *M. laxa*. Those results could be important for the disease epidemiology especially related to aggressiveness of *M. fructicola* which can be considered more certain in warmer climate of the country. On the other hand, *M. laxa* can be expected more at lower temperatures which are common for the northern parts of Montenegro.

Those results are in accordance with findings of the other authors. Vučinić (1994) studied the effects of temperatures on growth of *M. laxa* and *M. fructigena* on stone fruits in Montenegro and revealed that both fungi developed in a broad range of different temperatures with the optimum at 24 °C, well growth at 21° and 18 °C while the temperature at 33 °C completely stopped the fungi development.

As cited in Bernat et al. (2017), *M. laxa* cannot develop at 33 ºC, but it is even able to grow below 0 ºC *in vitro*. *M. fructicola* mycelia can develop at 33 ºC however, no mycelia were observed at 0 ºC. The authors stated that *M. fructicola*

is better adapted to high temperatures, whereas *M. laxa* is better adapted to low temperatures.

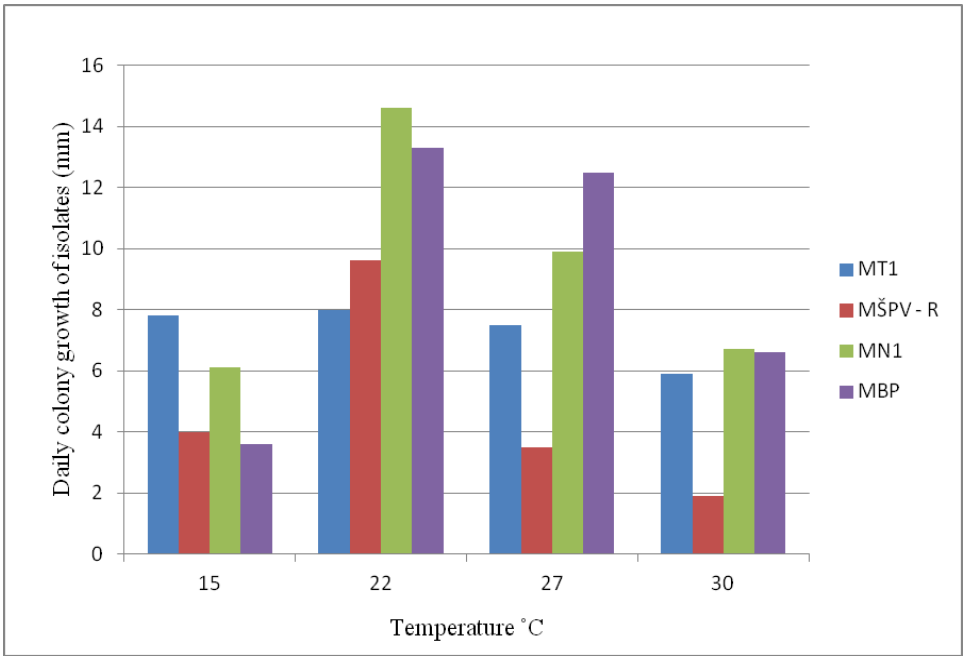

Figure 1. Average daily growth (mm) of the *Monilinia laxa* and *Monilinia fructicola* isolates at different temperatures.

Batra (1979) stated that *M. laxa* can be discriminated from *M. fructicola* by slower growth rate. De Cal and Melgarejo (1999) have also reported that growth rates of *M. fructicola* isolates were faster than those of *M. laxa*. Similar, Lane (2002) founded that in general, colony diameter for *M. fructicola* was greater than for *M. laxa*.

All these findings are important for understanding the disease epidemiology. Based on ecological parameters it can be considered when and in what extent the disease can be expected. According to Hrustić *et al.* (2012), heavy rains in the period of flowering, with temperatures ranging from 20 to 25ºC during the day are ideal conditions for the disease development.

Angeli *et al.* (2017) cited that there are differences in ecological requirements in areas where these two species co-exist. In these areas, *M. fructicola* is mostly reported on fruit, whereas *M. laxa* is mostly prevalent on flowers and twigs (EFSA, 2011). The reason for these differences could be in weather conditions during flowering and fruit ripening, since flowering occurs during spring at lower temperatures, while ripening of the fruit occurs in summer when temperatures are significantly higher. The results obtained in our study are in accordance with this opinion since isolates of *M. fructicola* developed better at higher temperatures then *M. laxa* isolates. Detailed studies done by Angeli *et al.*

(2017) indicate that *M. fructicola* is favoured by warmer weather than *M. laxa*. These authors reported that in Brazil the optimum temperature for development of brown rot caused by *M. fructicola* was 24.5ºC and for *M. laxa* was 19.8ºC. In their experiments *M. laxa* lesions produced more conidia than *M. fructicola* at 10ºC, while at 30ºC *M. fructicola* lesions produced more conidia than *M. laxa*. The temperature which influenced on lesion development was also higher for *M. fructicola* than for *M. laxa*.

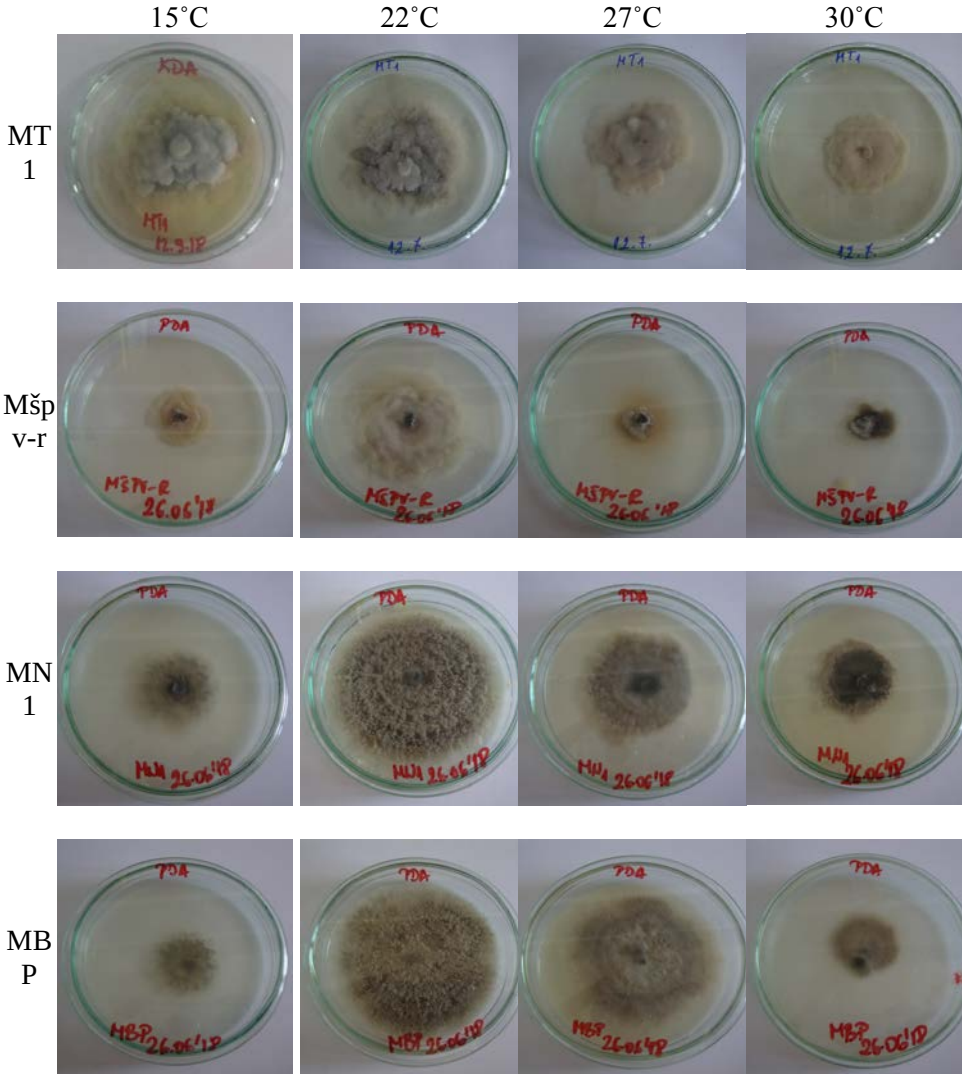

Figure 2. Colony growth of *Monilinia laxa* (MT1, Mšpv-r) and *Monilinia fructicola* (MN_1, MBP) isolates on PDA at different temperatures

Effect of different light conditions on mycelial growth. Influence of different light conditions on colony growth of the studied isolates is presented in Fig. 3. Development of examined isolates of *M. laxa* and *M. fructicola* colonies at different light conditions is shown in Fig. 4.

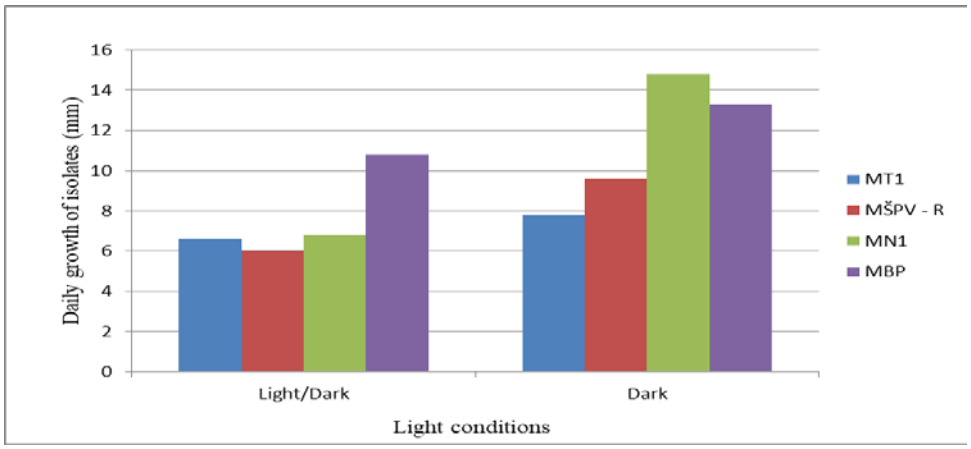

Figure 3. Average daily growth (mm) of the *Monilinia laxa* and *Monilinia fructicola* isolates at different light conditions (16 h light / 8 h dark and darkness)

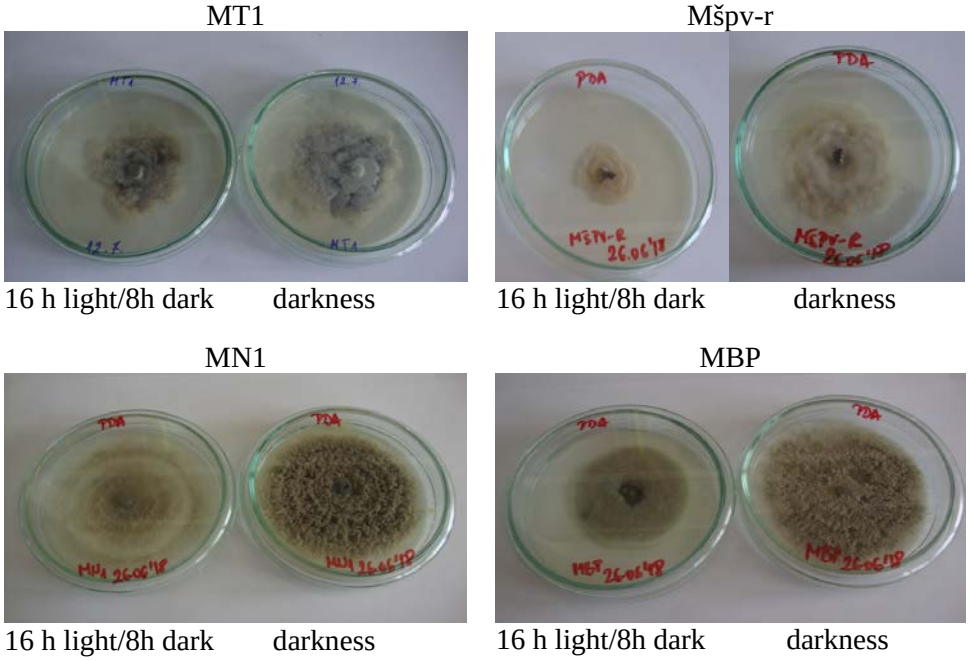

Figure 4. Colony growth of *Monilinia laxa* (MT1, Mšpv-r – upper row) and *Monilinia fructicola* (MN1, MBP – bottom row) isolates on PDA at different light conditions

The colonies of the all studied isolates grew faster in conditions of total darkness. Also, isolates of *M. fructicola* exhibited faster colony growth then isolates of *M. laxa* both in conditions of total darkness, as well as in the conditions of light/dark rotation. Differences in sporulation were noted between the isolates of *M. fructicola* and *M. laxa*. *M. fructicola* isolates sporulated in abundance in both light regimes but more abundant in condition of light and darkness alternation. However, isolates of *M. laxa* sparsely sporulated in darkness but the sporulation was induced with the presence of light.

Effect of light on *Monilinia* species (*M. laxa* and *M. fructigena*) was studied by Vučinić (1994). There were no significant differences between the *Monilinia* isolates grown in total dark or in alternation of light and darkness. However, in total darkness zones in the colony were less pronounced then in a presence of light and in *M. laxa* sporulation was sparse especially in darkness. The influence of light to *Monilinia* species is indubitable, but it is different concerning growth rate, sporulation and zonal colony distribution. Willetts (1969) concluded that total darkness stops conidia formation so when sporulation is inhibited the energy is directed to vegetative growth; Harada (1975) similarly reported that mycelial growth is reduced at light in comparison to darkness while sporulation is more pronounced in the presence of light (as cited in Vučinić, 1994).

Van Leeuwen and van Kesteren (1998) determined growth rate and sporulation intensity under two light regimes (darkness, 12 h light : 12 h dark) among a wide collection of isolates of *M. fructicola* and *M. laxa* and their results showed that increase in colony diameter and sporulation intensity was remarkable higher in *M. fructicola*. According to these authors, sporulation was more abundant in the light : dark regime. In darkness sporulation was the highest in *M. fructicola*, but in the light : dark regime sporulation intensity of some of the isolates of *M. fructicola* overlapped with some of the profusely sporulating isolates of *M. laxa*.

As stated by De Cal and Melgarejo (1999), differences in mycelial growth under long-wave UV may be a useful tool to identify *Monilinia* spp. They investigated the effect of long-wave UV/dark period on mycelial growth of *Monilinia* isolates. Growth in the dark was faster than growth under long-wave UV/darkness. Growth rates of *M. fructicola* were faster than those of *M. laxa* under both test conditions.

CONCLUSIONS

The results showed that ecological factors had significant effects on *Monilinia fructicola* and *Monilinia laxa* isolates from stone fruit in Montenegro.

Growth rates of examined isolates were the highest at 22˚C, however, at this temperature *M. fructicola* isolates grow faster than isolates of *M. laxa*. The temperature below optimum (15°C) is more favourable for the growth of *M. laxa* isolates, while the temperatures above optimum (27° and 30°C) suit more for *M. fructicola* isolates.

The studied isolates developed more intensively under condition of total darkness compared to the condition of natural light and dark alteration. On the contrary, sporulation in both species was more abundant in the light:dark regime then in darkness but this was much more expressed in *M. laxa* isolates. Sporulation of *M. fructicola* isolates was abundant also in darkness while in isolates of *M. laxa* it was sparse.

The effects of climate change, especially changes in temperature can affect the distribution of *Monilinia* species on different stone fruit hosts which should be considered for the future in the relevant risk analysis and strategies for disease management.

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