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Susceptibility of sour cherry cultivars to isolates of *Monilia laxa* (Ehrenbergh) Saccardo et Voglino

Sződi, Sz.[1], Rozsnyay, Zs.[2], Rózsa, E.[1] & Turóczi, Gy.[1]

[1]*Institute for Plant Protection, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103 Gödöllő, Páter K.u. 1, Hungary*
[2]*Research Institute for Fruit Growing and Ornamentals, H-1223 Budapest, Park u. 2, Hungary*

Summary: In this study, the susceptibility of 7 commercially important sour cehrry cultivars to Monilinia laxa was studied. Artificial inoculation was made with M. laxa isolates,which were isolated from different woody plants. Artificial inoculation was prepared int he laboratory and int he field. In laboratory, flowers of sour cherries while int he field, the two-year old twigs were inoculated in 2006 and 2007. According to results of stigmata inoculation, there were infection ability differences among the isolates originated from five different stone fruit host. Cultivars could be sorted into two susceptibility groups. In the field, twig inoculation in 2007 was made at blossom period and in 2007 at harvest. Seven sour cherry cultivars were inoculated with 8-day-old mycelial culture of M. laxa originated from sour cherry and almond. The agressivity and pathogenicity of the two isolates were measured by the degree of floem death: Results showed that year and phenological stage considerably influenced the degree of symptoms caused by the fungus. After artificial inoculation, tissue death progression was studied by fluorescent microscope. According to results, sour cherry cultivars were sorted into disease susceptibility groups. Susceptibility orders were identical to results on stigmata inoculation.

Key words: *Monilia laxa, blossom and twig blight, sour cherry*

Introduction

The most damaging plant protection problem is ultimately caused by *Monilinia laxa* (Aderhold et Ruhland) Honey / *Monilia laxa* (Ehrenbergh) Saccardo et Voglino for sour cherry growers. There are wide ranges of contact and systemic fungicides for growers, which able to prevent infection and epidemic development (*Ogawa* et al., 1995). Chemical control does not give suitable control efficacy in every case, therefore, the yearly occuring infection causes death of sour cherry trees. Several fungicide applications during blossom increase production cost and fungicide load to environment is also negligible. Therefore, the breeding of minilinia resistant sour cherry cultivars are essential task for achieving environmentally-friendly plant protection (*Rozsnyay*, 2004).

In the last two decades, epidemics occured at several occasion. The wider host group during epidemics also occured to the pathogen. Severity of epidemics is dependent upon susceptibility of sour cherry cultivars. From this point of view, pathogenicity process can be divided into two groups. First is susceptibility of sour cherry flowers to *M. laxa* conidia. Landing on stigmata, conidium produces hyphae and it gets into ovary through the style then into floem thorugh the stalk. Cold, mositer and reainy weather during flowering influences considerably the processes and mass blossom infection can reach 60–90%. Domestic sour cherry cultivars can be sorted into three flowering groups: early, early-middle, and late (*Nyéki* et al., 2003) but the longevity of flowering is largely influenced by the weather (*Stösser & Anvari*, 1983).

The second part of the pathogenicity process is caharcterized by the spreading ability of the pathogen in the floem. On these places, floem starts to collapse in most cases under fruit bearing shoot (*Virányi*, 2003). Penetration and spreading degree int he floem can show large differences among isolates and sour cherry cultivars (*Sződi* et al., 2006). Death of shoots and two-three year sold twigs can reach 40–80% (*Rozsnyay & Vajna*, 2001).

Cultivar feature plays essential role int he resistance against the disease. Role of defense reaction is that it denays the spread of the pathogen from infection site. Plant defese materials built a protection wall in the injured tissues and their neighbourhood. This defense mechanism starts to work after the attack of the pathogen. Succes of pathogen attack dependent upon pathogen virulence (pathogenicity), environmental consitions, and inherited and gotten resistance of the host. Both cultivar resistance ability and virulence of pathogen biotype continuously changes depending upon outer factors (*Király* et al., *1972*).

According to results on artificial blossom infection, 'Csengődi' and 'Kántorsjánosi' is resistant, 'Cigánymeggy 59' is poorly susceptible, Erdi bőtermő, Pándy 259 és Újfehértói fürtös is susceptible to brown rot (*Apostol &*

Rozsnyay, 2003; *Rozsnyay*, 2004). 'Érdi jubileum' is tolrenat against brown rot infection in twigs (*Brózik & Kállay*, 2000).

Spontaneous resistance is normally not connected to good production features, and hence as long as breeders payed attention only to production quality, susceptibile cultivars had been introduced into growing practice. Resistance sources have to be produced or searched and full or partial resistance can be introgressed into new cultivars by using these sources (*Apostol* et al., 1998).

In this study, susceptibility of improtant sour cherry cultivars to *M. laxa* as well as aggressivity of M. laxa isolates from our isolate collection were comapred in 2006 and 2007 using artificial inoculation in laboratory and blossom and twig inoculation in the field.

Materials and methods

Origin of isolates: Comparison of agressivity of *Monilinia* isolates were done on 7 isolates (*Table 1*). Species identification of isolates were done by morphological characters and by using specific primers (*Fulton* et al., 1999; *Ioos & Frey*, 2000; *Leeuwen* et al., 2002). *Monilinia* isolates were grown on tomato-agar media (140 g tomato, 1 l water, 20 g agarose, 10 g glucose).

Table 1. Origins of the isolates

Fungusj	Host	Plant part	Location	Name of the isolate
M. laxa	Plum	fruit	Kisvárda	B22
M. laxa	Cherry	fruit	Tiszabura	Sz10
M. laxa	Apricot	fruit	Kunmadaras	Sz13
M. laxa	Almond	shoot	Érd	Sz46
M. laxa	Sour cherry	fruit	Érd	Sz100
M. laxa	Sour cherry	fruit	Érd	Sz101
M. laxa	Sour cherry	fruit	Tiszabura	Sz14

Artificial inoculation in laboratory: According to previous knowledge, *M. laxa* insects its host during flowering when it penetrate to ovary thrughout the style, then to the stalk and finally to floem tissue. During the artificial inoculation experiment, we wanted to know that whether the susceptibility of cultivar style and agressivity of some *Monilinia* isolates differ from each other. We collected shhots with popcorn phenological stages from the above mentioned 7 cultivars. Flowers from their stalk were placed into 1% agarose media (*Honty* et al., 2004) by put the stalk into the agarose gels. First cultivar 'Érdi bőtermő' started bloom and then the order of flowering was as followed: 'Pándy 279', 'Kántorjánosi', 'Újfehértói fürtös', 'Cigánymeggy 59', 'Csengődi' és az 'Érdi jubileum'.

Artificial inoculation was peoposed with *M. laxa* isolates originated from 5 differnt stone fruit hosts by using conditional susception. These were the following Sz10, Sz13, Sz14, Sz46, B22. Conidia were produced on tomato agar media for 3 monthes at 5 C and they were suspended in 1.5 ml distilled water. Conidial concentration was measured with Bürker-boksz and was adjusted to 5×10^6 conidia / ml for each isolate (*Ubrizsy & Vörös*, 1968).

During artificial inoculation, when oose appeared on stigmata a droplet of conidial suspension were placed on the stigmata by Pauster pipette. After this flower were incubated at 22 C and 87 RH providing continuous light. The experient was replicated 12 times by cultivars and fungal isolates as well as 4 times for the control.

During the assessment,death of stigmata was assessed through 3 days. Degree 0 represented fully green and healthy stigmata. At degree 1, top of the stigmata was brown, at degree 2, 25% of the style was brown, at degree 3. 50% of the stíle was brown and at degree 4, the whole stigmata died *(Figure 1)*.

Artificial inoculation in the field: Artificial twig inoculation was prepared in Érd-Elviramajor in spring of 2006 and 2007. The experiment was made ont he above shown 7 sour cherry cultivars. Artificial inoculation was made on two different dates referring to intensive growth periods. Inoculation was made at the end of flowering in 2006 and 7 weeks later at fruit ripening stage in 2007 in oder to know that wheather infection ability is influenced by phenological stage of sour cherry cultivars.

The weather moist and rainy in 2006 which was favourable for spontanous brown rot infection (temperature 13.75 °C; relative humidity 76,8%, mean rainfall 44.85 mm). Artificial inoculation was made at 18 April after flowering or at full bloom for some cultivars. The weather was warm at 2007, which resulted in short flowering (temperature 15.6 °C; relative humidity 66%, mean rainfall: 38.6 mm). Therefore spontanous brown rot infection did not occur int he experimetal area. Artificial twig inoculation was made at 6 June when fruit was mature for some cultivars.

Four trees were infected for each cultivar. Inoculation was made with SZ46 (almond) and Sz100 (sour cherry) *M. laxa* isolates in spring of 2007. Artificial inoculation was perepared as follows: weak surface of two-year old twig was

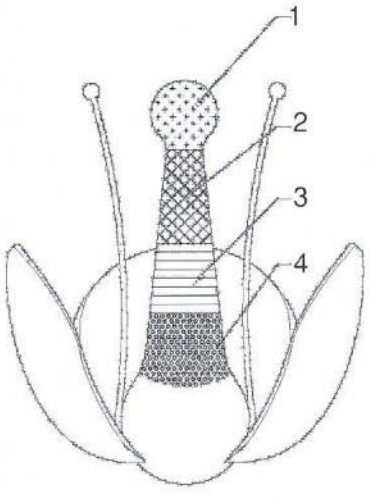

Figure 1 Evaluation of pistil necrosis: (1-top of the pistil is brown, 2-one fourth of the style from the top is brown, 3-half of the pistil is brown from the top, 4-the whole pistil is brown)

removed at 1–1.5 cm distance and a 6-mm and 8-day old mycelial plug was placed into the cut surface, then was covered with moist paper and finally it was fixed with parafilm. In case of control treatments, most paper was placed on the wounds and was fixed with parafilm. Assessment was made 43 days after inoculation by removing surface and measuring the lenght of necroses int he floem caused by the fungus.

Tissue study: Processes took part in the floem was the aim int he tissue study. We believed that defense material produced in resistance cultivars where the fungus caused death and a defense mechanism start sin the host. Produced defense material was followed by flourescens microscope and according to this, resistance of cultivars was tried to differentiate as well as further relationship was searched between infection conditions and phenological stage. Infested twigs were cut and fixed in FPA solution (8 ethyl alcohol: 1 propionic acidand 1 formaldehid). A thin tissue at the margin between infested and healthy tissue of collected twigs was cut and placed on objective. During tissue study, infected and healthy tissue parts of collected twigs were examined with Olympus BX50 microscope under fluorescence light. Photocopies were made from all preparates then degree of flourescency was evaluated by Cannon digital Photo Professional (Version 2.2) software.

Results

Artificial inoculation in laboratory: Susceptibility of stigmata of 7 sour cherry cultivars as well as agressivity of *M. laxa* isolates from 5 different hosts were evaluated. Under laboratory conditions, stigmata of control flowers were healthy and green, at the first two days but various degrees of death were detected at the third day. Largest death was observed on stigmata of cultivar 'Érdi jubileum' (mean 1.25) while the lowest one on stigmata of cultivar 'Újfehértói fürtös' (mean 0.00).

Stigmata death of 7 sour cherry cultivars at day 1,2, and 3 caused by articial inoculation of *Monilinia* isolates can be seen on *(Figure 2)*. Degree of stigmata death correspond to two susceptibility groups *(Figure 2)*. Three early-flowering cultivars can be sorted into the first 'Cigánymeggy 59', a 'Csengődi' és az 'Érdi jubileum' and the late flowering 'Pándy 279', 'Kántorjánosi', 'Újfehértói fürtös' and ''Érdi bőtermő'' can be sorted into the other group. Degree of infection was identical for all cultivars.

The strongest symptoms were on stigmina of 'Cigánymeggy 59' when was inoculated with (Sz10) *M. laxa* isolated originated from sweet cherry (mean 2.55). This cultivar was the most susceptible to brown rot int he stigmata inoculation experiment. Isolate sz10 caused the lowest symptoms on cultivar 'Pándy' (mean 1.33). Stigmata of cultivar 'Csengődi' was nfested uniformly by Sz14 (sour cherry) and Sz13 (apricot) isolates (mean 2.02). sz13 isolates originated from apricot caused the largest infection on cultivar 'Csengődi' out of the 7 sour cherry cultivars. Agressivity order of isolates during artificial stigmata inoculation was the following: Sz10(sweet cehrry) (mean: 1.79), Sz46 (almond) (mean: 1.56), Sz13 (apricot) (mean: 1.48), Sz14 (sour cherry) (mean: 1.40) and final B22 (plum) (mean: 1.28). Results was analysed with two way ANOVA. Cultivars (F=52.96; Fcrit=1.57), isolate (F=20.67; Fcrit=2.37) and interacions (F=3.03; Fcrit=1.28).

Artificial inoculation in the field: The degree of artificial inoculation was influenced greatly by the phenological stage of the host and the weather conditions of the two years. In 2006, the largest and smallest necrosis was observed on cvs. 'Cigánymeggy 59' (value: 15 cm), and 'Csengődi' (value: 6.37 cm), respectively, in the case of the isolate Sz100 from sour cherry, while in 2007, the largest and smallest necrosis was observed on cvs. 'Érdi jubileum' (value: 4.2 cm) and 'Pándy 279' (value: 12.8 cm), respectively, in the case of isolate Sz101 from sour cherry. In, 2006, the greatest necrosis in cv. 'Kántorjánosi' was caused by the isolate Sz46 from almond (value: 28.37 cm). In our experiment, this was the greatest necrosis.

The *M. laxa* isolates infested the host in all cases. *M. laxa* isolated from almond (Sz46) resulted in total shoot necrosis. The leaves and flowers on the infected shoot also died. Int he case of infection from sour cherry (Sz100 és Sz101) dead flowers were observed only on a few shoots. However, the set flowers fell from the tree later after touching.

In the artificial field inoculation experiment the susceptibility order of the cultivars from the most resistant to the most susceptible one was: 'Csengődi' (average: 4.87 cm), 'Pándy 279' (average: 6.13 cm), 'Érdi bőtermő' (average: 6.45 cm), 'Érdi jubileum' (average: 6.48 cm), 'Újfehértói fürtös' (average: 7.13 cm), 'Kántorjánosi'

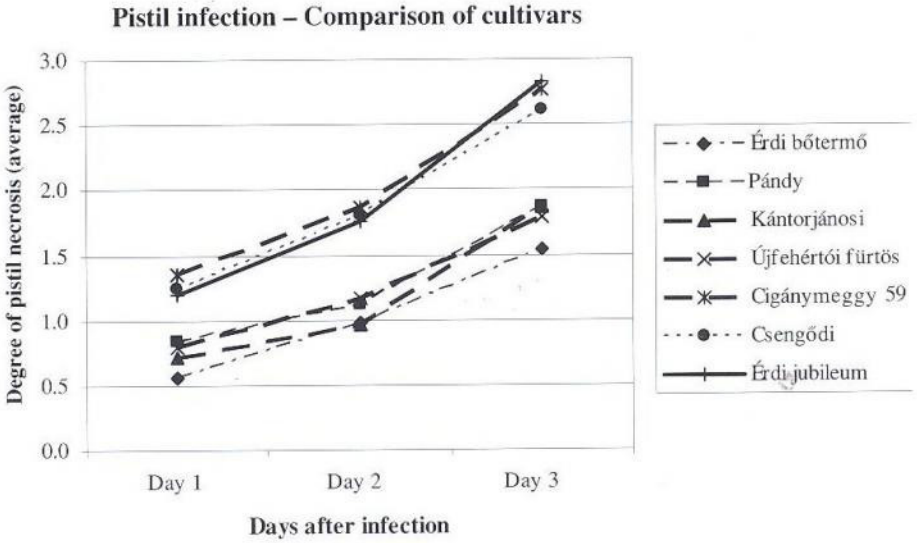

Figure 2 The degree of pistil necrosis formed in three days on seven sour cherry cultivars

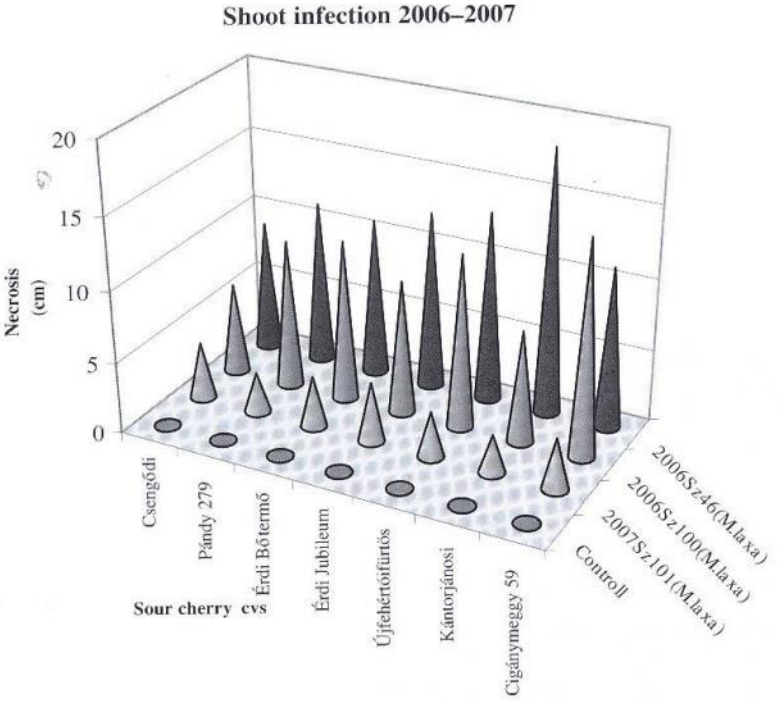

Figure 3 The results of artificial fiel inoculation with three Monilia laxa isolates on seven sour cherry cultivars

(average: 7.21 cm), Cigánymeggy 59 (average: 7.45 cm) *(Figure 3)*.

The obtained results were analysed by analysis of variance. In 2006, there were no significant differences in the aggressiveness of the two *Monilia laxa* isolates (F=1.55; Fcrit=5.98) and int he susceptibility of cultivars (F=0.74; Fcrit=4.28) In 2006 (Sz100) and 2007 (Sz101), there was a significant difference in the agressiveness of M. laxa (F=38.31; Fcrit=4.28), but there was no significant difference between the cultivars.

Histological examination: The phloem necrosis resulting from field infection was examined with a fluorescence microscope *(Figure 4)*. The obtained values are given at 192 – 256 corrected fluorescence value.

In 2007, the highest and lowest fluorescence values were observed in the control treatment for cvs. 'Cigánymeggy 59' (value: 2560) and 'Kántorjánosi' (value:11), respectively. In 2006, the highest and the lowest fluorescence values were measured for cv. 'Érdi bőtermő' as a reaction to the isolate from sour cherry (Sz100) (values: 510 and 55).

Evaluating the isolates based on the fluorescence values, the strongest *M. laxa* isolate was Sz101 (sour cherry) from 2007, followed by Sz46 (almond) used for infection in 2006 and the last one was Sz100 (sour cherry). There are significant differences in the fluorescence values of field shoot infection performed at two different phenological phases in 2006 and 2007.

Table 2 shows the mean of the fluorescence microscope illumination values for the artificial inoculation in 2007. Similarly to the pistil infestation, the values can be classified into two groups. One group contains the cultivars 'Pándy 279', 'Kántorjánosi' and 'Érdi bőtermő' with values under 200. The other group contains the cultivars 'Csengődi', 'Érdi jubileum', 'Újfehértói fürtös' and 'Cigánymeggy 59' with values above 200.

The explanation of the lower fluorescence values of 2006 can be that due to the higher flower infection the trees could produce less antibodies for the protection of the shoots than

Table 2. Fluorescence illumination results of the artificial shoot inoculation in 2007

Fluorescence average	2007
Pándy 279	129.1808
Kántorjánosi	134.4025
Érdi bőtermő	177.3883
Csengődi	233.894
Érdi jubileum	252.9348
Újfehértói fürtös	260.384
Cigánymeggy 59	285.2115

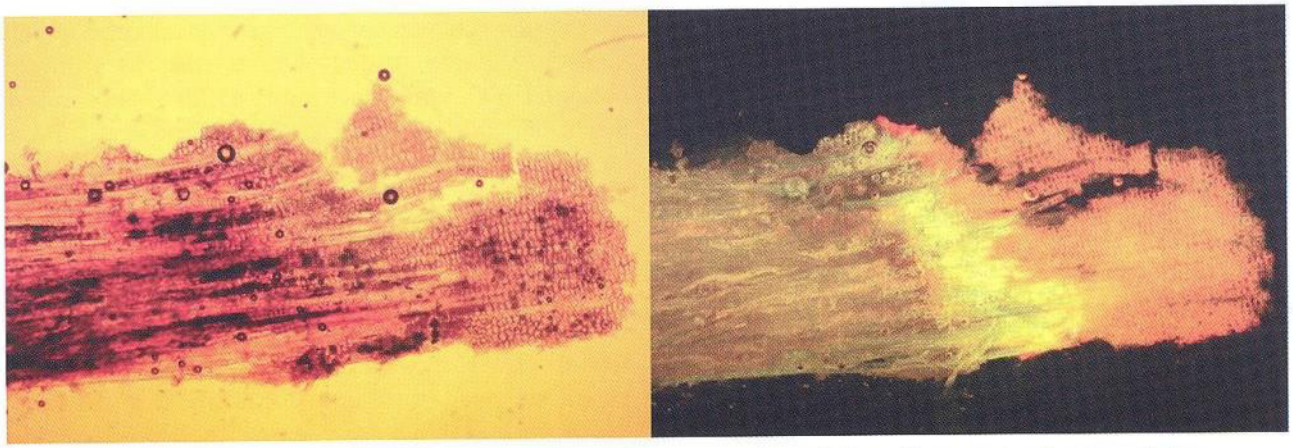

Figure 4 Autofluorescence in the phloem of sour cherry: (left: normal microscope photo, right: autofluorescence microscope photo) (Photo: Sződi, Sz.)

in 2007. A further explanation of the higher fluorescence values in 2007 can be that the trees were two months ahead regarding the phenological phase and the shoots were in a different physiological status.

Discussion

The susceptibility of seven sour cherry cultivars (most frequently grown in Hungary) to *Monilinia laxa/Monilia laxa* was tested. In the course of our work, we studied cultivar susceptibility and the aggressiveness of the fungi in the different infection processes and under different environmental conditions. Based on the pistil infection, the sour cherry cultivars could be classified into two groups. One group consisted of the cultivars with later blooming but with larger necrosis: 'Cigánymeggy 59', 'Csengődi' and 'Érdi jubileum'. The other group consisted of the cultivars 'Pándy 279', 'Kántorjánosi', 'Újfehértói fürtös' and 'Érdi bőtermő'. The artificial pistil inoculation method seems to be suitable for detecting significant differences among the isolates, however, this experiment alone is not suitable for judging the susceptibility of the cultivars.

The lifespan of sour cherry trees is greatly dependent on the fact to what degree they can prevent the spread of *Monilia*. In our research, we studied the susceptibility of sour cherry cultivars in two years with different meteorological characteristics and in different phenological stages. Based on the results it can be stated that temperature, relative humidity and precipitation greatly influence the tolerance of the sour cherry cultivar. When the weather conditions were favourable for natural flower infection (18. 04. 2006), the defense of the tree against the artificial shoot infection was weaker. The defense mechanism of the tree was stronger when the artificial shoot inoculation was performed at a later phenological stage (06.06.2007) under such weather conditions which were less favourable for natural flower infection. The defense process in the phloem of the sour cherry trees (host-parasite relationship) was monitored with histological examination with a fluorescence microscope. The examinations verified the differences between years and phenological stages.

Shoot infection was not always correlated with flower infection. Regarding the cultivars, cvs. 'Cigánymeggy 59' and 'Csengődi' had a strong flower infection, while shoot infection was the weakest and strongest in cvs. 'Csengődi' and 'Cigánymeggy 59', respectively. This example indicates that among the sour cherry cultivars there are some with a relatively strong tolerance, but the temperature, relative humidity and precipitation greatly influence this ability. Based on our experiments, we recommend the utilization of this characteristic of cv. 'Csengődi' in the future breeding programmes in the hope of reducing the number of chemical applications.

Our experiments partly verify and partly contradict the previous opinions ont he susceptibility of the studied sour cherry cultivars to *Monilia*.

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