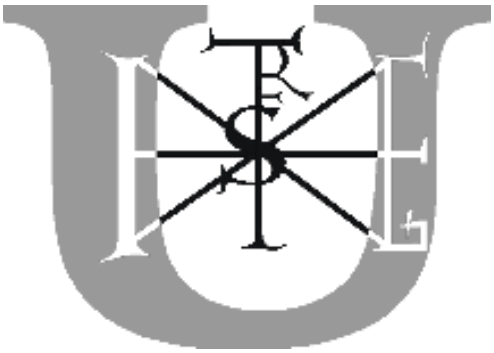

SZENT ISTVÁN UNIVERSITY

ASPECTS OF PHYTOPATHOLOGIC, GENETIC AND RESISTANCE EXAMINATION OF PATHOGENS CAUSING MONILINIA DISEASE IN STONE FRUITS

Thesis of PhD dissertation

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1. ANTECEDENTS AND OBJECTIVES OF THE WORK

Monilinia species are one of the most dangerous enemies of fruit plantations. Fruit rot caused by Monilinia species in stone fruit and pome fruit orchards and blossom- and twig-blight present at greater and greater scale in stone fruits draw our/the attention to these pathogens (Pintér, 1998). These deseases wide-spread in Hungary and Europe can be caused by three fungi-species. Species triggering fruit rot apart from blossom- and twig-blight are *Monilinia laxa* (Aderh. & Ruhl.) Honey / *Monilia laxa* (Ehrenb.) Sacc. & Voglino well-known in Hungary, the quarantine-pathogen *Monilinia fructicola* (Wint.) Honey (=*Sclerotinia fructicola* (Wint.) Rehm. / *Monilia fructicola* Batra and *Monilinia fructigena* (Aderh. & Ruhl.) Honey (=*Sclerotinia fructigena* Aderh. & Ruhl.) / *Monilia fructigena* Pers damaging various fruit-species.

It is easy to notice the presence of the pathogen, however, finding out which it is out of the three species requires further examination. Owing to rapid development of biotechnological research specification of the pathogens with molecular examination on species-level have become fast and precise (Ioos and Frey, 2000; Cote et al. 2004).

The most effective and most economic form of pathogen control is plant-resistance. In the resistance-developing breeding work resistance-sourses – with the use of which full or partial resistance can be developed in new varieties – must be produced or tracked down. According to our knowledge the grown sour-cherry varieties are not resistant to *Monilinia* pathogens, however, there are considerable differences between the susceptibilities to *Monilinia* diseases.

Wide range of fungicides was available for people persuing integrated and conventional farming owing to the chemical industry boom in the 20th century, thus *Monilinia* control appeared to be resolved. However, it was found that the pathogens became more and more aggressive. Even back in the 1970-s it was recognised that the regular use of fungicides developed resistance and in terms of plant-protection it is a serious challenge (Josepovits, 1991). Application of chemical pesticides has been developing several critiqes. For example, during the plantation-treatments the pesticides can be drifted away and can pollute the air, the soil and the subsoil water. In many cases they can do damage to non-target organisms as well, and chemical residues infood can cause problems. Because of the aforementioned reasons the EU withdrew the authorization of number of agents in the past years.

Only few chemicals are allowed for people persuing ecological farming and at present there is no antagonist agent against *Monilinia* species available in Hungary.

Based on the aforementioned my priorities were as follows:

1. I studied the variability of the populations of *Monilinia* isolates collected from different plant-parts, host-plants and fruit-producing areas in Hungary with molecular genetic methods, and this variability might be responsible for the differences in the infections caused by the pathogens. I wanted to know how much the subpopulations within a species are various; how big differences can be observed among the species; if they can be distinguished genetically and if so, how far the species are from one another genetically. I was examining if there is genetically demonstrable host-plant specialisation, population-change traceable in years and if the less fungicide-sensitive sub-populations are distinct from one another.

2. Other object of my work was to examine the susceptibility of seven sour cherry varriations (Érdi bőtermő, Csengődi, Cigánymeggy 59, Érdi jubileum, Kántorjánosi 3, Pándy 279, Újfehértói fürtös) wild-spread in public cultivation and to compare the pathogenity and aggressivity of *Monilinia* isolates collacted from different plants.

3. I studied if *Clonostachys rosea* antagonist - succesfully applied against many pathogens - takes part in preventing blossom blight caused by *Monilinia* species threatening the whole tree. Besides preventing blossom blight it is extremely important to also prevent the development of fruit infections, therefore I studied if *C.rosea* hyperparasite takes part in suppressing the development of fruit mummies, thus reducing the inoculum production.

4. I also studied if the more and more frequently applied fungicide treatments increased the danger of the development of less sensitive or resistant *Monilinia* isolates to fungicides. There are no bibliographic data on fungicide resistance or the decreased sensitivity of the isolates, therefore I aimed to study the fungicide-resistance of pathogens isolated from different stone fruits.

2. MATERIALS AND METHODS

2. 1. Identification of isolates

Collecting isolates

The 69 isolates used in the examinations were collected from different plant parts, nine different hostplants and different orchards in Hungary between 2002 and 2006. Among the production sites there were plantations, public areas and gardens at privately owned houses. The *Monilinia fructicola* isolate was collected, isolated, identified and made available to me by The Central Agriculture Office Central Laboratory for Pest Diagnosis.

Identification of the isolates traditionally with diagnostic methods and molecularly with specific primers

The isolates from different host-plants were sporulated at 5°C then we measured the length and width of the developed conidia and compared them to the bibliographic data.

The growth-speeds of the colonies were measured for six days and were given at millimeter precision.

Besides the traditional mycologic diagnostic methods (Lane, 2002) specific PCR technology was used for the identification on species-level. After the DNA extraction species-specific primers (Ioos and Frey, 2000) were applied in the PCR method.

2.2. Examination of *Monilinia* species on genetic diversity

For the studying of the genetic diversity within and between the species there were iSSR tests carried out with 45 isolates (Fan et al., 2010). Amongst the isolates used in the study 24 were *M. laxa*, 20 were *M. fructigena* and one was *M. fructicola*. There were five microsatellite primers $(GAG)_4RC$, $(CAC)_4RC$, $(GTG)_5$, $(GATA)_4$, $(GTC)_5$ and two minisatellite primers M13 (Heath et al., 1993), T_3B (McClelland et al., 1992) used. The primers were applied independently, not in combinations.

Following the PCR method I evaluated the pattern by determining the size of each band. I represented the existence or absence of the bands with binary code. I grouped the data into a matrix and – using the Treecon program pack (Van de Peer and De Wachter, 1997) - made a dendogram.

I determined the genetic diversity of the whole population (HT) based on the diversity within and between the subpopulations: (Nei, 1987; Takezaki and Nei, 1996): $H_T = H_S + D_{ST}$.

2.3. Examination of the pathogenicity of *Monilinia* isolates and susceptibility of sour cherry varieties

Artificial infections were carried out in *in vivo* and *in vitro* circumstances. Stigmata and two-year old branches were infected on the examined sour cherry varieties. Besides the different sensitivities of the sour cherry variations the possible different pathogenities of the *Monilinia* isolates were studied.

The examinations were carried out with the following sour cherry variations: Érdi bőtermő, Újfehértói fürtös, Kántorjánosi 3, Cigánymeggy 59, Érdi jubileum, Pándy 279 and Csengődi at the experiment-farm of Research Institute for Fruit Growing and Ornamentals in Érd-Elviramajor.

2.3.1. Artificial infection through the stigma, *in vitro*

The artificial stigmainfections were carried out with the mixed suspension of the conidia of *Monilinia laxa* isolates from host-plants of five different stone fruits. A drop of conidium suspension was dropped with Pasteur-pipette on each stigma. Following this the flowers were incubated in climate-chambers (at 22°C, at 87 per cent humidity, at permanent illumination). I carried out the evaluation on the three consecutive days following the treatment in the course of which I assessed the stigma-necrosis visually.

2.3.2. Study of the stigma-tissue

During the tissue-study of the stigma infections the plant-tissue reactions were examinerd at different time-intervals. It was presumed that the more resistant variety produced more substance against the fungi, which showed greater fluorescence stained with aniline-blue. This test was carried out with the Érdi bőtermő, Pándy 279 and Cigánymeggy 59 varieties. At the appearance of the secretum-drop on the stigma suspension made of pollen and conidia was dropped on the top of the stigma with Pasteur pipette. After that the stigmata – with the peduncles - were fixed in 70 % FPA solutions 1, 2, 4, 8, 12, 21, 24, 48 hours after the inoculation. The 70 % FPA solution consisted of formaldehyde, propionic acid and ethanol at the proportion of 1:1:8. The flowers in the fixing solutions were stored at 4°C. There were Quetsch preparation and preparation embedded in resin made. After that I took photos of each preparation then evaluated the degree of fluorescence with Canon Digital Photo Professional (Ver. 2.2) program.

2.3.3. Artificial twig inoculation, *in vitro* and *in vivo*

The aggressivity of *Monilinia* isolates and the susceptibility of the sour cherry varieties – based on the expansion of the necroses in the phloem - were examined. The laboratory examinations were conducted on the aforementioned seven sour cherry variations in intese growing period, at blossoming at the end of April and at recovery period at the end of October. There were 6 mm holes created on the twig, 6-mm and 8-day old mycelial plugs were placed into the holes, then they were covered with moist cotton wool pieces and were fixed with parafilm.

The twigs were placed into big glasses filled with moist sand, and the evaporated water was regularly supplemented. The in vivo artificial twig inoculations were performed in Érd-Elviramajor in 2006 and 2007. I cut off the inoculated twigparts and fixed them in FPA solutions (formaldehyde, propionic acid and ethanol at the proportion of 1:1:8). Then I took photos of each preparation for the assessment.

2.4. Application of *Clonostachys rosea* mycoparasite

2.4.1. Identification of the antagonist and efficience study

Clonostachys rosea isolate was used in the examinations, which was isolated from a *Botrytis cinerea* colony infecting grape and was identified on morphological characteristics.

The antagonistic potential of mitosporic fungus *C. rosea* against *M. laxa* and *M. fructigena* and the antibiotic production of the antagonist was studied. The hyperparasitic activity was examined on malt-agar media. After eight days, when the hyphae of the two fungus approached each other so much that the two ends well-visibly met microscopic specimen was created from the spot of the meeting point. Glycerine and aniline-blue were dropped on the top of the mycelia and covered with cover plates, then the preparations were examined under light-microscope.

For the determination of antibiotic substance production, *C. rosea* was cultured in shaked culture at 90 rpm at 25°C in tomato media (140 g smashed tomato (22 – 24 ref%) in 1000 ml tap water) for 8 days. The fermented liquid was sterile-filtrated (0.2 μm) and the filtrate was added to fresh tomato – agar medium (10% v/v). *M. laxa* and *M. fructigena* isolates were inoculated on Petri dishes and incubated at 25°C. The growth (colony diameter) was measured daily for a week and compared to that on normal tomato – agar medium.

2.4.2. Artificial stigma inoculation treated with the antagonist, *in vitro*

The flowers – before the blossoming period – were placed in 1% water-agar medium (Honty et al., 2004). The artificial stigma infection was performed as it is described at 2.3.2. The concentration of the *C.rosea* antagonist conidium suspension was set at $5*10^6$ cell/ml, and the other conidium suspension concentration was set at $6*10^6$ cell/ml with distilled water.

2.4.3. Artificial fruit inoculation treated with the antagonist

Fruit infection was carried out with inoculation of ripen 'Golden' apples of equal size. 6 mm wide and 4 – 5 mm deep holes were cut out of the apples. The following pathogen – antagonist combinations were set up in the experiment:

1. only a mycelium disc of *C. rosea* was placed in the wound (Cr (only)),
2. only a mycelium disc of *M. laxa* or *M. fructigena* was placed in the wound (M (only)),
3. mycelium discs of *C. rosea* and *Monilia* were placed in the wound at the same time (Cr at same time as M),
4. a mycelium disc of *C. rosea* and 24 h later a mycelium disc of *Monilia* was placed in the wound (Cr 24 h before M),
5. a mycelium disc of *C. rosea* and 48 h later a mycelium disc of *Monilia* was placed in the wound (Cr 48 h before M),
6. in the control the apples were wounded with a sterilised cutter and the wounds were covered with parafilm without any inoculum (C).

2.5. Fungicide sensitivity of the *Monilinia* isolates

42 *M. laxa* and *M. fructigena* isolates were used for the fungicide sensitivity examinations. The fungicide sensitivity of the iosolates was studied at different concentrations against 10 different chemicals by using the poisoned media method (**Table 1**.).

Table 1. Active agent concentrations applied in the fungicide sensitivity examinations

concentration, ppm		
captan	10	20
vinclozolin	3	10
procimidone	3	5
triadimefon	3	10
iprodione	2	5
fenarimol	1	5
benomyl	2	5
pirimetanil	3	50
boscalid	3	50
copper	20	200

I measured the length and width to determine the size of the colony and I calculated the area with the $T_{ellipse} = a*b*\pi/4$ formula. To calculate the lowest concentration (MIC – Minimal Inhibitory Concentration) (Andrews, 2001) necessary for the full inhibition I determined the zero point of the straight line fitted onto the y_1, y_2 area-values belonging to the x_1, x_2 concentration-variables (assuming linear relation).

I used the following formula:

$$x_{sz} = \frac{y_2 x_1 - y_1 x_2}{y_2 - y_1}$$

ahol:

x_1 - the lower agent-concentration (ppm)

x_2 - the higher agent-concentration (ppm)

y_1 - $T_{ellipse}$ value belonging to the x_1 agent-concentration (mm^2)

y_2 - $T_{ellipse}$ value belonging to the x_2 agent concentration (mm^2)

x_{sz} - the lowest concentration necessary for the full inhibition (ppm)

I determined the relative sensitivity of the isolates based on the data. Based on the obtained data I categorized the isolates into three groups according to their sensitivity: High (HS), Medium (MS) and Low (LS) (Leroux et al., 1999).

3. RESULTS AND CONCLUSIONS

3.1. Identification of the isolates

The identification of the isolates was conducted with traditional diagnostic methods and molecularly specific primers. Out of the 69 isolates 48 were *M. laxa*, 20 were *M. fructigena* and 1 was *M. fructicola*.

3.2. Variability study of Monilinia species

Seven microsatellite primers were used to chart the 45 isolates. Out of the 45 isolates 24 were *M. laxa*, 20 were *M. fructigena* and 1 was *M. fructicola*. In the case of two primers /T_3B and $(GATA)_4$/ out of the seven primers, reactions did not give amplification products. In the course of the testing of the 45 isolates altogether 52 bands were obtained in the case of five primers /$(GAG)_4$RC, $(CAC)_4$RC, $(GTG)_5$, M13 and $(GTC)_5$/.

The three species could be distinguished from one another by analysing MP-PCR data. The dendrogram was obtained from the 52 amplicons. *M. fructicola* forms a different branch in the genetic tree whereas *M. laxa* and *M. fructigena* proceeded from a common branch. In addition, both *M. laxa* and *M. fructigena* can be distinguished well on cluster level.

The studied year- and host- specialisations could not be distinguished on the genome level. Analysis of genetic structure applying the Nei's gene diversity (Nei, 1987) based on 47 ISSR loci, the population structure analysis revealed that genetic diversity within *M. laxa* subpopulations was H_S= 0.1599, while within *M. fructigena* subpopulations was H_S= 0.2551. Diversity within *M. fructigena* was greater than in *M. laxa* populations. The total genetic diversity was H_T= 0.3846, while genetic diversity between *M. laxa* and *M. fructigena* subpopulations was D_{ST}= 0.1771. Förster and Adaskaveg (2000) reported low degree of genetic diversity within *M. laxa* populations in Californian orchards. Gell et al. (2007) conducted first European study to determine degree of genetic diversity in *M. laxa* within and among the orchards in Spain. It was concluded that the genetic diversity within subpopulations (orchards) was HS= 0.567, while genetic diversity between subpopulations

was DST= 0.018. Szalóki (2011) was also examining *M. fructigena* isolates from Hungary with 10 iSSR primers and found similarly considerable genetic variability.

In addition to the degree of the developing variability it is also important to deal with host specialization. It is worth observing the possible host specialisation during the genetic mapping and it is also worth studying of the genetic evolution of the populations. In the study, similarly to the one conducted by Gell et al. (2007), no relationship was found in clustering among isolates according to the year of isolation or original host plant. According to this fact and the considerable variability within the populations revealed with MP-PCR, it might be concluded that there was no host specialisation in the investigated *Monilinia* population in Hungary. Gril et al (2008) carried out analysis with AFLP marker system, shows that *Monilinia* isolates were not grouped according to geographic origin. My results – just as the results of Szalóki (2011) working with isolates also from Hungary – have not revealed grouping by geological locations.

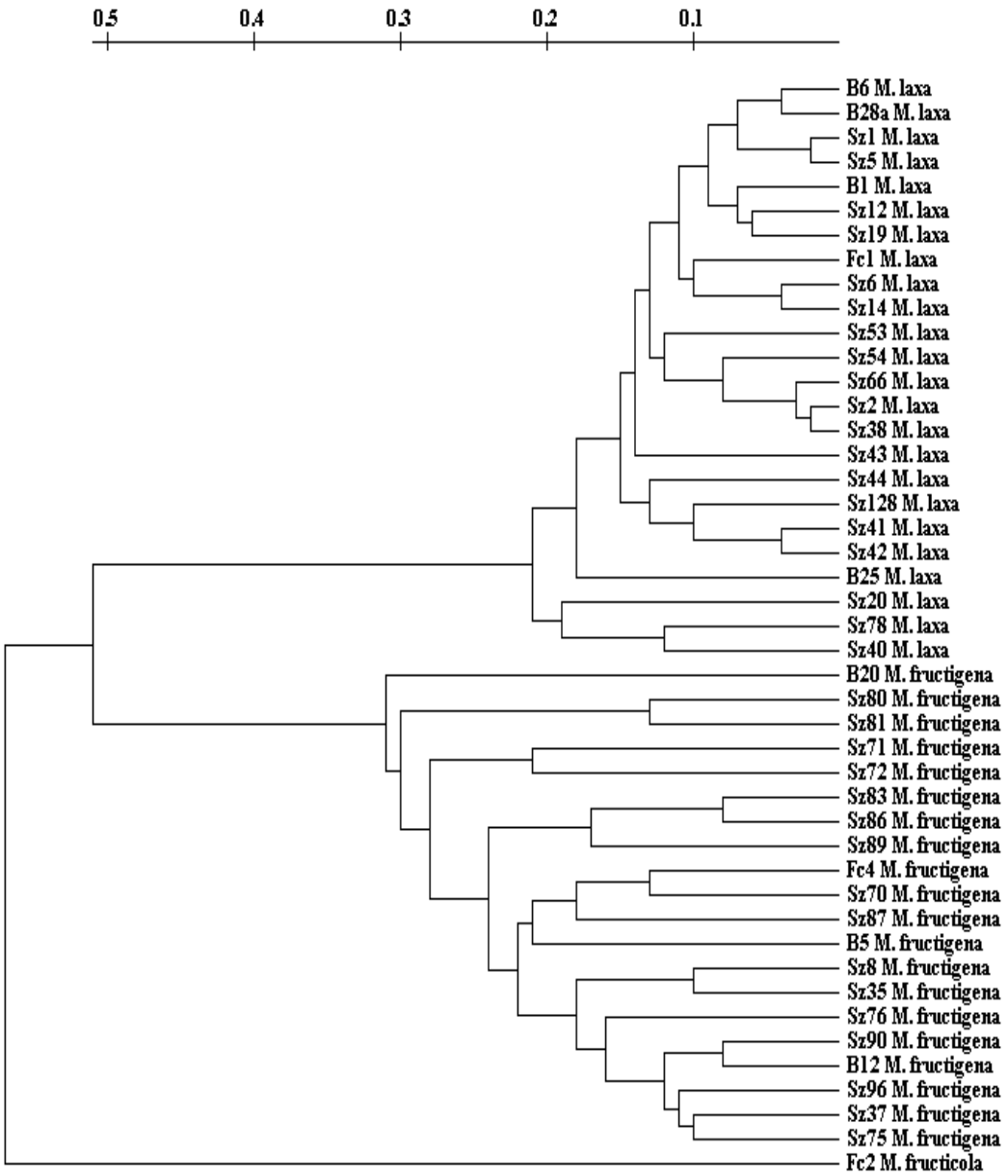

Figure 1: Phenogram generated with the unweighted pair-group method with average cluster analysis of micrasatellite-primer PCR data set from isolates of *M. laxa*, *M. fructigena* and *M. fructicola* from different host plants in Hungary

3.3. Study of the aggressivity of the *Monilinia* isolates and of the susceptibility of the sour cherry variations

3.3.1. Artificial infection through the stigma, *in vitro*

The stigma is protected with an antibiotic-like substance – which is secreted by the plant itself - from the direct infection (Ubrizsy, 1965), still there are differences between the sensitivity of each variation. Considering the results of the stigma infections I supposed that the more resistant stigma withers sooner. However, since the control stigmata also started to

wither by the third day, I assume that the removal of the flowers proved to be such great stress on the stigmata that it has negatively influenced the activity of them. Tóth (2008) also gained similar results concerning his/her apricot in vitro blossom infections. Egea et al. (2002) also established in their pollen tube growth examinations that the removal of plant parts have a negative effect on vital processes. There were significant differences between the pathogenity of each *Monilinia* isolate used in the experiments.

3.3.2. Examination of the stigma-tissue

In the cases of the preparations created with Quetsch technique I found that among the variations there are considerable differences in luminosity concerning the fluorescence. Furthermore, I established that by increasing the time of the incubation the value of the fluorescence reduced. In all the three variations studied the values of the fluorescence were higher in the treatments with pollen. I arrived at the same conclusion regarding the fluorescent illumination of stigma-preparations embedded in resin.

3.3.3. Artificial twig infection, *in vitro* and *in vivo*

It is important to also study the resistance of the ligneous parts in order to determine the sensitivity of the sour cherry variations to *Monilinia* pathogens. Based on the results I pointed out that the intensive growth in the phloem increases the susceptibility of the sour cherry trees and the aggressivity of the isolates, since in the given time period the necrosis was greater than it was in the recovery period. The difference between the aggressivity of each isolate was slight.

In my opinion in the infections performed in spring the tree contributed to the nourishing of the fungus and the “discovery” of organic compounds necessary for the energy-generation with the fast transportation of the assimilates and the active sugar-transport (Vetter, 2003; Pethő, 1993).

I summarized the resistance-features of the varieties in **table 2**. In the cases of the *in vitro* twig infections at the treatments both in blossoming and recovery periods therewere significant differences among each variations. My results indicate that the degree of infection in the phloem depends on the phenological state of the plant rather than the year of origin. However, the year of origin had more pronounced effect on the aggressivity of the isolates in the phloem, than the phenological state.

Table 2. According to the results of each test the variations have been categorized on their sensitivity, 1 – meaning the least infected (the most resistant amongst the variations studied) and 7 – meaning the most infected (the most susceptible amongst the variations studied)

	Significant difference among isolates	**Significant difference among varianties**	**Érdi bőtermő**	**Újfehértói fürtös**	**Kántorjánosi 3**	**Cigánymeggy 59**	**Érdi jubileum**	**Pándy 279**	**Csengődi**
Twig infection in blossoming period *in vitro*	no	yes	5	5	7	1	6	5	1
Twig infection in recovery period *in vitro*	yes	yes	4	4	7	1	2	6	1
Twig infection *in vivo* 2006	yes	no	6	7	7	7	6	6	4
Twig infection *in vivo* 2007	yes	no	6	5	5	6	7	5	7

At the infections performed in spring the value of fluorescence is considerably lower than at the infections performed in June, because by the summer the amount of callose in the phloem is higher.

3.4. Application of *Clonostachys rosea* mycoparasita

3.4.1. Identification of the antagonist and efficience study

We studied the behaviour of *Clonostachys rosea* antagonist, applied successfully against *Botrytis cinerea* on many hosts and plant parts, used against *M. laxa* and *M. fructigena* pathogens. There was not reference found on the microscopic examination of the infection between *Monilinia* species and the antagonist. Our results supported that *C. rosea* was capable of coiling around the hyphae of the host fungus and penetrated it. These observations are contrast to Pachenari's and Dix's (1980) who claim that the contact of *C. rosea* (syn.: *Gliocladium roseum*) hypha with the pathogen takes place without penetration. Li et al. (2002) - referring to the fact that the used cultures were young - did not find any proof concerning enzymatic digestion of the cell walls. In our examination we could not show antibiosis with the method applied.

3.4.2. Artificial stigma inoculation treated with the antagonist, *in vitro*

C. rosea antagonist was applied against different pathogens in plantations in many cases, although there is not much data on *Monilinia* available. Witting et al. (1997) reported in their experiments that they applied the antagonist against *M.fructicola* during the blossoming period of sour cherry. According to their results the antagonist was only able to suppress the blossom-infection to a low extent. In my study I examined the antagonistic effect against stigma infections in *in vitro* circumstances. Similarly to the results obtained from the stigma-infections in the resistance examinations in sour cherry variations, I assume that the removal of the flowers proved to be such great stress on the stigmata that it has negatively influenced the activity of them.

3.4.3. Artificial fruit inoculation treated with the antagonist

In our experiments *C. rosea* did not cause necrotic symptoms on the fruit of 'Golden' apple. It proved to be an ideal model-system in the interaction-examinations of antagonist and pathogen fungi in the experiments. Further tests can be carried out on other host plants of the pathogen i.e. fruit of peach, on which the pathogens used cause considerable economic damage.

In our experiments there were notable results obtained in 8 days. If wounds were treated with mycelium of *C. rosea* 24 h earlier than mycelium of *Monilia*, the extension of the necrosis caused by *Monilia* isolates was reduced in each case. During the treatment with the antagonist and the pathogen the *M. fructigena* isolate deriving from apple fruit produced as extensive necrotic symptoms at the presence of the antagonist as it produced without the presence of it. The fact that the isolate was from apple fruit might account for it. The isolate from almond shoot produced necrosis half the size as the other isolates did. In spite of it in the treatment infecting the isolate with the antagonist at the same time and in the treatment infecting the isolate 24 h earlier the reduction of the necrosis was a lot smaller than in the cases of the other isolates. The fact that the isolate was from stone fruit might supposedly account for it. All the isolate used in our study were from 2004. In that year there were particularly favourable climatic conditions to brown rot and it caused unexperienced infection on almond. However the antagonistic effect clearly visible at the 48-h treatments since there is no noticeable necrosis in any cases. *Botrytis cinerea* was suppressed by the antagonist similarly in Yu's and Sutton's (1997) examinations on raspberry leaf discs, stem segments, and stamens when it was applied at the same time or 32 h before they were infected with the

pathogen. Our results indicate that *C. rosea* could be a promising control agent of both *M. laxa* and *M. fructigena* by preventing the formation of fruit mummies thus reducing the amount of the surviving inocula.

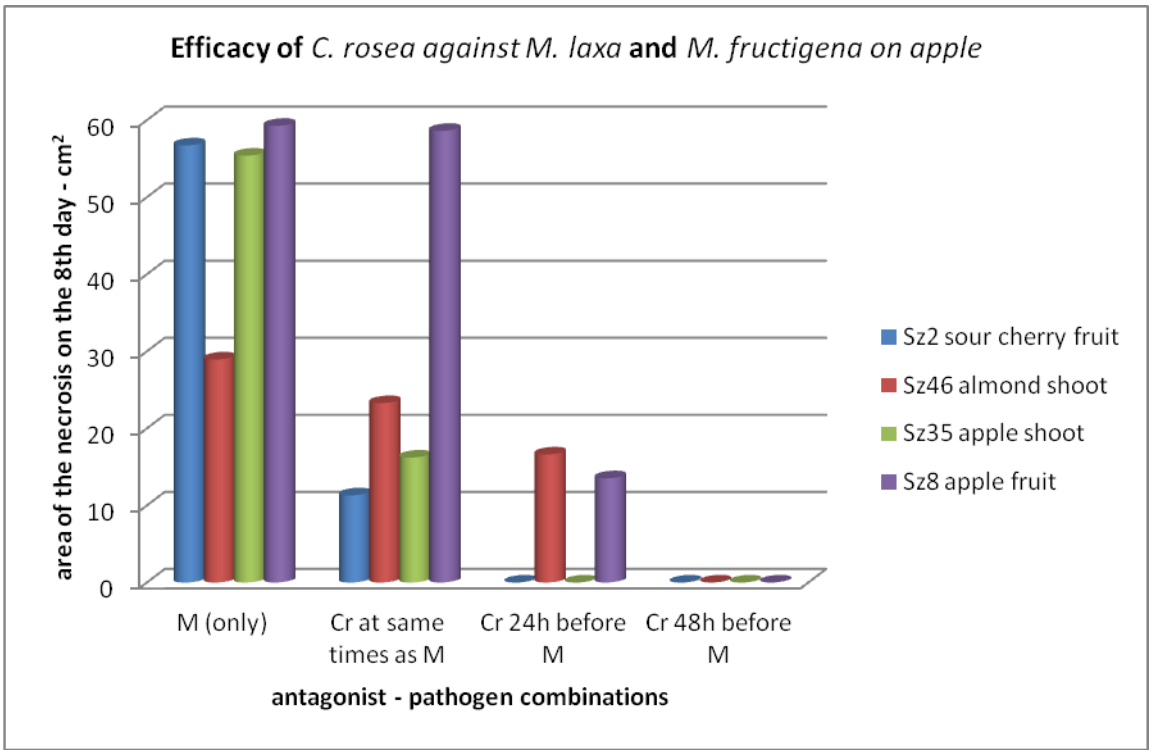

Figure 2. Efficacy of *Clonostachys rosea* against *Monilinia laxa* and *Monilinia fructigena* on apple (M (only): only a mycelium disc of *M. laxa* or *M. fructigena* was placed in the wound; Cr at same time as M: mycelium discs of *C. rosea* and *Monilia* were placed in the wound at the same time; Cr 24 h before M: a mycelium disc of *C. rosea* and 24 h later a mycelium disc of *Monilia* was placed in the wound; Cr 48 h before M: a mycelium disc of *C. rosea* and 48 h later a mycelium disc of *Monilia* was placed in the wound)

3.5. Fungicide sensitivity of the *Monilinia* isolates

Fungicide treatments are key components in the integrated plant-protection against *Monilinia*, however the treatments becoming more and more frequent increase the risk of the reduction of the sensitivity to fungicides (Enisz, 1989). There were 42 *Monilinia* isolates studied from eight host-plants against ten fungicides.

The active agents were grouped into three groups based on the lowest concentracion necessary for the full inhibition (MIC) in the growth of *Monilinia* isolates on poisoned media.

I represented the different sensitivity of the isolates in a diagram by taking the average values of the data for all the fungicides as 0%. So, the degree of the differences of each isolates compared to the average is well-visible in **figure 3**. It is noticeable that Sz1, Sz19 and Sz33 require twice as great concentration as the average does for the full inhibition, while in the cases of Sz7, Sz8 and Sz89 half the concentration of the average is alredy sufficient for the full inhibition.

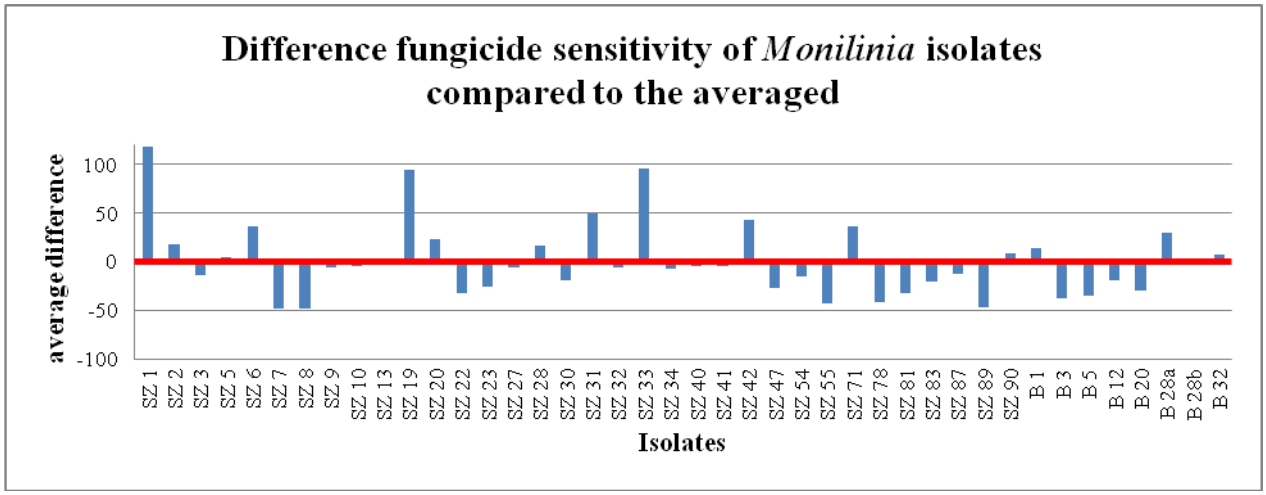

Figure 3. Difference in the fungicide sensitivity of each isolates compared to the average. 0% is the average sensitivity for all the fungicides

My results show that the *Monilinia* populations in Hungary are various, namely sub-populations which are less sensitive to fungicides have already developed. I compared these results with those of the genetic examination, but on the genomic level I was carrying out my research I could not find distinguishable patterns, namely I could not demonstrate selectivity pressure concerning the fungicide sensitivity.

Molecular research could help in understanding the mechanism of fungicide resistance and can develop an effective and fast method in finding the resistant genotypes (Ma and Michailides, 2005). Such an allel-specific PCR method was developed by Ma et al. (2003; 2005) in detecting benzimidazol-resistent *M.fructicola* and *M. laxa*. Hereafter it would be worth treating the isolates from Hungary with these primers, because in this way a single PCR test could reveal the resistance.

4. NEW SCIENTIFIC RESULTS

In this study I formulated the following new scientific results:

1. Based on the genetic examination I established that the genetic diversity in the *Monilinia fructigena* population in Hungary is greater than the diversity in the *Monilinia laxa* population. Within these species I could not distinguish specialisation either on host, year or geographic origin on the genomic level I was studying.
2. I was the first to examine the stigma-tissue with fluorescent tehnique infected with *Monilinia laxa* and I established that the stigma-tissues infected with the pathogen in each case showed lower fluorescence value than the ones treated with pollen. I established that there is no correlation between the extent of necrosis and the degree of fluorescence.
3. I established that the resistance of the phloem of the sour cherry variations and the durability of this is mostly influenced by the ontogenetic state of the plant, while the aggressivity of the isolates is influenced by weather conditions.
4. I was the first to examine the phloem with fluorescent technique infected with *Monilinia* pathogen and I established that the extent of the infection and the degree of the fluorescence is greatly influenced by the differentiation of the phloem.
5. I was the first to reveal the interaction between *Clonostachys rosea* mycoparasite and *M. laxa* and *M. fructigena* with light-microscope. I established that the antagonist was capable of coiling around the hyphae of the pathogen, penetrating it and reaching the ontogenetic phase of sporulation inside.
6. I established that *C. rosea* mycoparasite is an effective antagonist of *Monilinia* species. It is able to inhibit the development of the fruit-infection. During my examinations I revealed hyperparasitism but I did not find antibiosis.
7. I was the first to examine the fungicide sensitivity of *M. laxa* and *M. fructigena* isolates from Hungary and I found considerable dispersion in the concentrations necessary for the full inhibition of each isolate.
8. I established that the *M. laxa* isolates are normally less sensitive to fungicides than the *M. fructigena* isolates, that is higher concentration of the agents is necessary for the full inhibition.

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6. PUBLICATIONS RELATED TO THE DISSERTATION

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