SZENT ISTVÁN UNIVERSITY

EVALUATION OF SOUR CHERRY GENOTYPES SELECTED IN THE COURSE OF RESISTANCE BREEDING, BASED ON THE TESTING OF POMOLOGICAL TRAITS AND ENDOGENOUS COMPOUNDS

SÁNDOR ISTVÁN SZÜGYI

GÖDÖLLŐ

2017

Postgraduate School

Name:	Postgraduate School for Horticultural Sciences
Branch of Science:	Crop Production and Horticultural Sciences
Head:	Professor Éva Zámbori-Németh, DSc Head of the Department of Medicinal and Aromatic Plants Faculty of Horticultural Sciences Szent István University
Supervisor:	Éva Sárdi, DSc, Hon. Professor Department of Genetics and Plant Breeding Faculty of Horticultural Sciences Szent István University

Authorisation of the head
of the postgraduate school

Authorisation of
the supervisor

1. BACKGROUND AND AIMS

Fruit production (including sour cherry production) is a strategic branch of Hungarian agriculture, and the importance of sour cherries can be compared with that of apples. The growing requirements of sour cherry trees are not demanding, so they can be grown in almost all regions of the country. The cultivars selected or bred via hybridisation in Hungary are familiar worldwide, as they have been successfully introduced into many countries (e.g. USA, Poland, Germany) in recent decades and some have been exploited in breeding programmes. Sour cherry cultivars bred in Hungary represent important export items. The development of dual-purpose cultivars (suitable for both fresh consumption and industrial uses) would further improve the competitiveness of this species. This can be achieved by broadening the genetic basis through crossing and the selection of landraces.

The pathogens most frequently attacking stone fruits such as sour cherry are *Monolinia* species. The damage they cause to flowers and shoots is a widespread problem, which has often reached epidemic proportions in Hungary over the last 15–20 years. However, sour cherry cultivars have proved to exhibit great variability in their resistance to *Monolinia* infection.

Sour cherry production is a costly activity, making it essential to improve crop safety and reduce financial risks. One way of doing so is to plant disease-resistant trees, leading to a direct reduction in production costs (lower pesticide requirements). The other is to grow cultivars with good yield potential and crop safety, thus reducing the production costs per unit fruit. In order to achieve these aims, a joint breeding programme was commenced in 1991 with the cooperation of Prof. Amy Iezzoni and funding from the US Department of Agriculture with the title "Breeding of disease-resistant sour cherry cultivars" (APOSTOL et al. 1995). In this programme 'Érdi bőtermő' has been consistently used as the female cultivar and 'Csengődi' as the resistant donor. In consequence of this continuous crossing programme, an 'Érdi bőtermő' × 'Csengődi' hybrid generation currently consisting of 120 trees is now available, which has been selected for resistance to the *Monilinia laxa* pathogen under the leadership of János Apostol and Zsuzsanna Rozsnyay since 2008.

Cultivar breeding, including the development of disease-resistant cultivars, is a long-term process requiring 15–20 years, which needs to be speeded up due to the great mutability of pathogens. For this purpose targeted research is underway to analyse endogenous compounds or groups of compounds (carbohydrates, endogenous formaldehyde, methyl donors), shown in the literature to play an important role in the resistance and defence responses of plants to various abiotic and biotic factors. Although fewer papers have been published on carbohydrate

analysis aimed at studying host–pathogen relationships than on the investigation of abiotic stress effects, it has nevertheless been demonstrated that the quantity of carbohydrates, especially monosaccharides, in various organs of the host plant acts as an indicator of defence responses to pathogens.

Investigations on the role of endogenous methylation and demethylation processes, and of the formaldehyde arising as an intermediate product of these processes, in plant defence against stress factors, is still a priority research area. Monitoring quantitative changes in endogenous formaldehyde (HCHO) and its potential natural generators (e.g. betaine, choline, trigonelline, L-carnitine, N^{ε}-trimethyl-L-lysine) in response to infection is a relatively new approach for comparing the stress responses of cultivars with different levels of resistance.

In the present experiments a comparison was made of the detectable carbohydrates, endogenous HCHO and methyl-donor compounds in the phloem tissues and leaves of the resistant 'Csengődi' and susceptible 'Érdi bőtermő' cultivars to *M. laxa* infection, and of four resistant and four susceptible hybrids produced from them. The overpressured layer chromatographic separation (OPLC) technique was used for the quantitative and qualitative determination of the endogenous compounds, followed by densitometric evaluation

Aims

The experiments were designed with the following aims:

- selection of new, promising hybrids suitable for inclusion in the Hungarian cultivar list from the hybrid sapling population developed in the breeding programme begun over 20 years ago, which should be self-fertile, with the excellent fruit quality of the 'Érdi bőtermő' cultivar but earlier maturity, and with the resistance of the 'Csengődi' cultivar;
- selection based on the tolerance of the parental cultivars and their hybrids to spontaneous infection with the pathogenic fungus *M. laxa* in the field and to artificial inoculation under field and laboratory conditions;
- analysis of the carbohydrate composition of the phloem and leaf tissues of selected genotypes and its correlation with resistance to *M. laxa* infection;
- monitoring of quantitative changes in the carbohydrate content of phloem tissues over time in response to infection in hybrids with different levels of resistance and in the parental cultivars;
- comparison of the phloem and leaf tissues of sour cherry genotypes selected on the basis of disease resistance by measuring methyl-donor compounds and endogenous formaldehyde;

- analysis of the defence response induced by *M. laxa* infection by monitoring time-dependent quantitative changes in methyl donors and endogenous formaldehyde in hybrids with different levels of resistance and in the parental cultivars;
- analysis of possible correlations between the resistance/susceptibility of sour cherry trees to the *M. laxa* fungus and changes in the quantities of the endogenous compounds tested in response to infection.

2. MATERIALS AND METHODS

2.1. Experimental location

The crosses required for resistance breeding were performed at the Elvira Major nursery of the Fruitculture Research Institute of the National Agricultural Research and Innovation Centre in Érd. The work was begun in 1991 in the framework of cooperation between the institute and Michigan State University.

2.2. Experimental material

The experimental orchard was established in 1996 in order to examine hybrid saplings developed from crosses in the breeding programme. Most of the trees in the nursery are sweet cherry hybrids, only a smaller proportion being made up of the sour cherry hybrids discussed here. The thesis evaluates the data of the most promising fruit-bearing hybrid saplings selected from the 'Érdi bőtermő' × 'Csengődi' population (9/5-6, 9/21, 9/24, 9/79-80, 9-91, 7/47, 7/67-68, 7/141).

2.3. Experimental methods

2.3.1. Analysis of self-fertility

The analysis of self-fertility involves isolating branch sections of selected hybrids in the full-blown bud stage of flowering using water-resistant wax parchment bags. The buds are counted before bagging and the bags are removed after the petals fall by tearing them with a quick pull 5–10 cm above the attachment site, leaving a paper collar on the shoots that makes them easier to find. By the time the bags are removed fruit setting has already taken place. During the ripening period the number of fruit on the treated branch sections are counted and compared with the number of flowers to give the percentage of self-fertilisation.

2.3.2. Fruit mass measurements

The fruit were picked when fully ripe and the number of fruit required to make up 1 kg was recorded for each genotype. The weight of each individual fruit was also recorded using a digital balance. These measurements were repeated each year between 2010 and 2014.

2.3.3. Resistance analysis

M. laxa fungal strains originating from various host plants were available for the artificial inoculation of the cultivars and of the hybrids selected on the basis of spontaneous field infection. The isolates were tested on the susceptible parental cultivar 'Érdi bőtermő'.

In order to evaluate resistance to the *M. laxa* pathogen, parallel artificial inoculations were performed in the laboratory and field (2010–2012), and an evaluation was also made of spontaneous field infection (2011–2014).

2.3.4. Analytical studies

Cultivar comparison

The analytical studies were performed in the Department of Genetics and Plant Breeding of Szent István University, where responses to abiotic and biotic stress have been investigated for more than 20 years. Samples were collected for the comparative analysis in various seasons (spring, summer, winter) in several replications. In addition to the parental cultivars, 4 resistant and 4 susceptible hybrids were tested in spring (May) and summer (July), while in winter phloem samples were collected from the cultivars 'Csengődi' and 'Érdi bőtermő', and from the most resistant (7/67-68) and most susceptible (9/79-80) hybrids.

Monitoring the effect of M. laxa infection

Time-dependent changes in the quantities of carbohydrate, endogenous formaldehyde and methyl donors in response to infection were analysed after artificial shoot inoculation in the field and laboratory. The most susceptible ('Érdi bőtermő', 9/79-80) and most resistant ('Csengődi', 7/67-68) cultivars/hybrids represented the extreme values of disease resistance level, so the effect of inoculation was studied on these four genotypes. Samples were collected 1, 2, 3, 6, 12, 24, 48 and 72 hours after inoculation, and after 11, 15 and 19 days. The quantities of endogenous compounds in samples collected after 1, 2, 3, 6, 12 and 24 hours were interpreted as being early responses, while those recorded after 1, 2, 3, 11, 15 and 19 days were considered to represent the normalisation phase. After removing the phloem tissues from the whole circumference of the inoculated shoots, tissue samples not yet exhibiting symptoms were

collected from the 5 mm zone on the border of infected and healthy tissues, assuming that the most intensive interaction between the pathogen and the plant would occur in this zone.

2.3.4.1. Analysis of endogenous compounds

Endogenous compounds (carbohydrates, methyl donors, endogenous formaldehyde) were analysed using the over-pressured layer chromatography or optimum performance layer chromatography (OPLC) technique (TYIHÁK 1987). The quantitative and qualitative determination of carbohydrates was performed using the method described by SÁRDI et al. (1996), while quaternary ammonium compounds (choline, betaine, carnitine, trigonelline, N^{ε}-trimethyl-lysine) and endogenous formaldehyde were analysed with the methods of GERSBECK et al. (1989), SÁRDI (1994) and SÁRDI & TYIHÁK (1998).

3. RESULTS

3.1. Self-fertility analysis

The cultivar ‘Érdi bőtermő’ was found to be 19.8% self-fertile, while for ‘Csengődi’ this value was 15.5%. Among the selected hybrids, self-fertility was exhibited by the combinations 7/67-68, 7/47, 9/5-6 and 9/79-80. The 10% minimum self-fertility required by the production technology was achieved by 7/67-68 (11.6%) and 9/79-80 (17.5%). The combinations 9/91, 9/24, 7/141 and 9/21 were not self-fertile.

3.2. Fruit mass analysis

The sour cherry cultivar ‘Érdi bőtermő’ was found to have a mean fruit mass of 6 g, which is in agreement with the findings of other authors. The cultivar ‘Csengődi’ had smaller fruit, as also reported by APOSTOL (1998), with a mean weight of 5 g in the present work. Among the hybrids, the fruit weight of 9/91 and 7/67-68 significantly exceeded that of the heavier parental cultivar (reaching 8 g in the case of 7/67-68).

3.3. Determination of ripening time

All the hybrids ripened earlier than ‘Érdi bőtermő’. The ripening time of 9/21 fell between those of the two parental cultivars. Depending on the genotype and the year, hybrids 9/79-80, 7/67-68, 9/91, 9/5-6, 9/24 and 7/141 ripened 7–16 days earlier than the female parent ‘Érdi bőtermő’, which is an important cultivar from the production point of view.

3.4. Analysis of the pathogenicity of the *Monilinia laxa* isolates

Test inoculations were performed on the cultivar ‘Érdi bőtermő’ in the laboratory using the five isolates available (M22, M16, M10, M4, M14) in order to determine their pathogenicity. Of the five strains, M4, isolated from sour cherry, and M14, isolated from apricot, had the greatest pathogenicity, with no significant difference between them. The M4 isolate was used for the further tests.

3.5. Spontaneous infection in the field

The results of spontaneous field infection indicated that the parental cultivars responded to the pathogen as expected from production experience, with 54.8% shoot infection for ‘Érdi bőtermő’ and 1.6% for ‘Csengődi’. Six of the selected hybrids gave susceptibility values between those of the parents, while 7/67-68 exhibited no shoot symptoms arising from spontaneous infection, thus proving to be more resistant than the resistant parent ‘Csengődi’.

3.6. Artificial inoculation in the field

The greatest shoot decay in response to artificial inoculation in the field was observed for hybrid 9/79-80, where phloem lesions measuring over 50 mm on average were observed on infected shoots, compared with 44 mm for ‘Érdi bőtermő’. The extent of phloem decay averaged 30.9 mm for hybrid 9/24, 20 mm for 9/5-6 and 9/91 and above 10 mm for 9/21, while hybrids 7/47, 7/141 and 7/67-68 and cultivar ‘Csengődi’ exhibited lesions measuring less than 10 mm. Hybrids 7/67-68 and 7/141 had outstanding tolerance, with values very similar to that of the male parent (1.5 mm).

3.7. Laboratory inoculations

Intense shoot decay was recorded on both hybrids and parental cultivars after artificial inoculation in the laboratory. Nevertheless, differences in susceptibility between the individual genotypes could be clearly seen. Cultivar ‘Érdi bőtermő’ was again in the most susceptible group, with phloem decay of 27.3 mm, which was only exceeded by that of hybrid 9/79-80 (30.3 mm). The susceptibility of hybrids 7/141, 7/67-68, 9/5-6, 9/91 and 9/24 was intermediate to that of the parental cultivars, and of these 9/24 and 9/91 were significantly less susceptible than ‘Érdi bőtermő’, with phloem decay of less than 20 mm, a value very similar to that of ‘Csengődi’. Two hybrid combinations, 9/21 and 7/47, were less infected than ‘Csengődi’. Of these, hybrid 7/47 was significantly the most resistant.

3.8. Analysis of endogenous compounds

As regards the purpose of analysis, the analysis of endogenous compounds can be divided into two groups:

- Comparative analysis of cultivars and hybrids in homeostasis to study correlations between resistance to *M. laxa* infection and the quantity of the endogenous compounds tested.
- Monitoring the effect of *M laxa* infection to study time-dependent responses by measurement changes in the quantities of endogenous compounds.

Comparison of cultivars and hybrids in homeostasis

The analysis was made on the two cultivars and eight hybrids included in the inoculation experiments. With the exception of one hybrid (9/79-80) all the hybrids proved to be more resistant to the disease than the cultivar 'Érdi bőtermő', but there were considerable differences in their resistance levels. For the purposes of this work, hybrids exhibiting over 40% infection compared with 'Érdi bőtermő' (9/5-6, 9/91, 9/24, 9/79-80) were considered to be susceptible and those with less than 40% infection (7/47, 7/141, 7/67-68, 9/21) were regarded as resistant.

3.8.1. Analysis of carbohydrates in homeostasis

Leaf and phloem samples were compared in spring (May), leaf samples in summer (July) and phloem samples in winter (December).

Glucose, fructose and sucrose could be reproducibly detected in the plant samples, while xylose was also present in the spring samples and raffinose in those collected in winter.

In spring there were larger quantities of glucose in the leaves of all the cultivars and hybrids than in the phloem tissues. This was also true of fructose for all but two of the hybrids (9/21 and 7/47, where the quantities were almost the same). In the leaves disease resistance was primarily correlated with the glucose concentration, with a significantly higher quantity in susceptible genotypes than in resistant ones. In phloem tissues the glucose quantity also declined as the level of resistance increased, but significant quantitative differences and correlations were only observed in the case of the fructose concentration, which was almost twice as high in susceptible genotypes as in resistant ones.

The combined quantity of glucose and fructose in the leaves exhibited the same correlation, with larger quantities of these carbohydrates in susceptible genotypes than in resistant ones. This correlation was also observed for the phloem tissues. The ratio of the two

monosaccharides to sucrose was significantly higher in both the leaves and phloem tissue of the susceptible cultivar and hybrids than in those with better resistance.

The analysis of samples taken in the summer revealed a significant drop in the quantities of glucose and fructose in the leaves of susceptible genotypes compared with those recorded in spring. The glucose and fructose contents in the leaves of susceptible genotypes also declined, but to a lesser extent. It was also observed that the comparative quantities of glucose and fructose in susceptible and resistant cultivars were opposite to those recorded in spring.

With the exception of the 'Érdi bőtermő' cultivar, the concentration of sucrose decreased similarly to those of glucose and fructose in July, compared with the spring values.

In winter homeostasis there were significantly higher glucose and fructose contents in susceptible than in resistant genotypes, in agreement with the results obtained in spring.

3.8.2. Changes in carbohydrate quantities over time in response to *Monolinia laxa* infection

Early responses

As suggested by various authors (SÁRDI 1994, SÁRDI et al. 2006, BAKER and ORLANDI 1995, SZARKA 2008) changes occurring in the first 24 hours after infection were regarded as early responses.

It could be seen from the results that responses related to disease resistance could be monitored by tracing changes in the glucose level. In susceptible genotypes the glucose concentration dropped in the first hour after infection, after which it exhibited cyclic changes. After 24 hours the glucose concentration was lower than the control value in 'Érdi bőtermő', but was similar to the control in the susceptible hybrid. Among the resistant genotypes, the glucose level of 'Csengődi' did not change significantly in the first hour after infection, but unlike the susceptible hybrid, the resistant hybrid exhibited an increase, after which a reduction was detected in both cases. The glucose quantity was considerably smaller than the control value (less than half as much) in samples taken after 24 hours.

The changes in fructose and sucrose concentrations over time were not suitable for characterising the responses of the susceptible and resistant groups.

Investigation of the normalisation phase

Studies carried out up to now aimed to measure changes in the carbohydrate composition in the alarm phase in order to determine the early responses of plants to biotic stress. The later phases of disease progress are greatly influenced by how quickly the infected plant is able to

respond to the penetration of the pathogen. In order to investigate these later phases, the time-dependent studies were extended to the 2nd, 3rd, 11th and 15th days, and, in the case of hybrids, to the 19th day.

Visible signs of phloem necrosis were observed on the 3rd day in the most susceptible genotype, while no necrosis was observed even on the 19th day in the case of resistant genotypes. In the susceptible cultivar the decrease in glucose concentration was greatest in the third hour following the infection, while in the susceptible hybrid the glucose minimum was measured at the next sampling time. The time interval between the two sampling times was so big that it was impossible to draw conclusions. In samples of susceptible genotypes on the 15th day the glucose concentration was significantly lower than the control values. The glucose quantity in the resistant cultivar and hybrid also reached a minimum on the first day after infection, after which a continual increase was detected. On the 15th day, the glucose level approached the control value.

There was again no correlation between the time-dependent changes in fructose and sucrose concentrations and the level of disease resistance.

3.8.3. Effect of artificial inoculation in winter

In addition to glucose, fructose and sucrose, raffinose was also detected in the samples collected in winter. The laboratory determination of carbohydrates in homeostasis revealed significant differences in the glucose and fructose contents, with higher quantities in susceptible than in resistant genotypes. When time-dependent changes in the quantity of glucose and fructose were monitored, significant differences were observed between the two tolerance groups in the first hour. In the first hour no significant change was detected in the case of resistant genotypes, while the susceptible genotypes showed a significant decrease in the quantity of monosaccharides. At later sampling dates a decreasing tendency was observed for the resistant genotypes. In the 6th hour after infection, the 'Érdi bőtermő' cultivar showed an increase in glucose content, but later this value decreased or stagnated in both the resistant and susceptible cultivars. On the 11th day a significant decrease was detected in all the genotypes.

3.8.4. Comparative analysis in homeostasis by measuring methyl-donor compounds

Among the methyl-donor compounds used as standards for analysing phloem tissue (N^{ε}-trimethyllysin, choline, carnitine, trigonelline, betaine) choline could be clearly detected under the OPLC-conditions.

In spring (May), the concentrations of choline in the phloem tissues of resistant genotypes were significantly higher than in the susceptible ones (except in 'Érdi bőtermő', probably due to a sampling or analytical error).
In summer (July), the concentrations of choline in the phloem tissues of the resistant hybrid and cultivar were significantly higher than in the susceptible ones.
The choline concentration in the 'Csengődi' cultivar was 47% higher than in 'Érdi bőtermő', and 57% higher than in the susceptible hybrid, where the choline concentration in the resistant hybrid was 53% higher than in 'Érdi bőtermő' and 64% higher than in the susceptible hybrid.

3.8.5. Monitoring the effect of artificial inoculation

In the first hour after *M. laxa* inoculation, a reduction in the quantity of choline in the phloem tissue could be observed in both the resistant and susceptible cultivars, but the extent of the change differed, being 63,2% in the susceptible and 57,8% in the resistant genotype. A further decrease was detected two hours after inoculation (71,8% in the resistant and 83,2% in the susceptible cultivar, compared to the control values). In the resistant cultivar, the choline level reached a maximum in the 6^{th} hour following inoculation, while in the susceptible cultivar no significant change could be detected. After that the concentration of choline decreased significantly in the resistant cultivar, while the susceptible cultivar still exhibited no significant change. In the 48^{th} hour, the choline concentration was 10,4% of the control values in 'Csengődi' and 13,3% in 'Érdi bőtermő'. Changes in the hybrids were similar to those in the cultivars, between the same tolerance groups, quantitative changes could only be detected.

Correlations between endogenous formaldehyde content and disease resistance

Methylation and demethylation processes and the HCHO originating as a transient product from easily mobilizable methyl groups play an important role in compensating for stress effects. In response to biotic or abiotic stress methylated compounds may be demethylated, while HCHO participates in the methylated reactions that protect the stress-sensitive parts of biological systems (enzyme proteins, nucleic acids).

3.8.6. Comparative analysis in homeostasis by measuring endogenous HCHO

The comparative analysis of HCHO was performed on the same samples and at the same time as that of methylated compounds. Due to a sample storage error, the adduct compound of HCHO could not be observed in the cultivar 'Érdi bőtermő' or in hybrids 9/5-6 and 9/91. However, it could be demonstrated, that the quantity of HCHO, as a dimedone adduct, was

significantly lower in the resistant cultivar and hybrid that in hybrid 9/79-80 (which is the most susceptible hybrid among the examined genotypes).

Monitoring the effect of artificial inoculation

In the first hour following the inoculation, an increase in the quantity of HCHO was observed in both the susceptible and resistant cultivars, but the rate of change differed significantly the increase being 81,8% in 'Érdi bőtermő', and 25,9%. in 'Csengődi'. In the 2nd hour after inoculation a further increase (88,2%, compared to the control) was detected in the cultivar 'Csengődi', where the HCHO level exhibited a negative peak in the 3rd hour. However, in the 6th hour its quantity returned to the control value, after that it was decreased continuously. Finally, in the 48th-hour samples a significant decrease in the HCHO level was measured (88%, compared to the control). In the cultivar 'Érdi bőtermő', the HCHO level reached a 2nd peak in the 24th hour, after which a significant decrease could be detected. In the 48th-hour samples the concentration of HCHO was 0,44 µg/g in 'Csengődi', and 11,9 µg/g in 'Érdi bőtermő'.

In relation to the time at which the maximum values were recorded, the time-dependent changes in the concentration of HCHO in the phloem tissue of the susceptible hybrid, showed the same tendency as in the susceptible cultivar 'Érdi bőtermő'.

In the first-hour samples the increase in HCHO in the resistant hybrid was greater than in the resistant cultivar. In the resistant genotypes, a negative peak in the HCHO level was observed in the 3rd hour. However, 48 hours after inoculation there was a significant reduction in the quantity of this compound, in the cultivar 'Csengődi', while in the resistant hybrid the HCHO concentration approached the control value.

3.9. New scientific results

- Among the carbohydrates analysed in the phloem and leaf tissues of sour cherry cultivars and hybrids in homeostasis *M. laxa* resistance was most closely associated with the quantity of glucose. In the leaf and phloem tissues of susceptible genotypes the glucose concentration was significantly higher than in the resistant genotypes.
- The monitoring of time-dependent change in monosaccharides, especially glucose, was found to be suitable to detect differences between *M. laxa*-induced stress responses of susceptible and resistant sour cherry genotypes

- Comparative analysis of methyl-donor compounds in sour cherry phloem tissues in homeostasis revealed significant differences, in the choline concentration, which was lower in susceptible and higher in resistant genotypes.
- The endogenous formaldehyde level in phloem tissues showed the reverse tendency, its quantity being higher in susceptible and lower in resistant genotypes so this compound could also be suitable to detect differences between the *M. laxa*-induced stress responses of susceptible and resistant sour cherry genotypes
- The analysis and monitoring of quantitative changes in formaldehyde (a transient product of methylation-demethylation processes induced by infection) and choline (a methyl-donor compound detected in the plant tissues of sour cherry) could thus be used to depict the diverse stress-reactions of resistant and susceptible genotypes.

4. CONCLUSIONS AND DISCUSSION

4.1. Analysis of *Monilina laxa* resistance and pomological characteristics

Spontaneous field infection and artificial *in vivo* inoculation showed a similar order of resistance. On this basis, it was found that all hybrids except one were significantly more resistant than the susceptible cultivar 'Érdi bőtermő', furthermore, clearly detectable differences could be observed between the resistance levels of the genotypes. On the basis of a comparison of infection methods, artificial *in vivo* inoculation was deemed more appropriate for the study of *M. laxa* resistance than *in vitro* (laboratory) infection, because laboratory conditions were too favourable for the pathogen, making objective assessment more difficult.

The analysis of self-fertility showed that both cultivars reached the 10% level (APOSTOL 1994) desirable from the technological point of view ('Csengődi': 15%, 'Érdi bőtermő': 20%). The self-fertility of hybrids 7/47 and 9/5-6 was below 10%, but that of hybrids 7/67-68 and 9/79-80 exceeded 10%.

The fruit weight of three hybrids (7/141, 9/5-6, 9/21) averaged 6 g, similar to that of 'Érdi bőtermő', while hybrids 9/91 and 7/67-68 had and average fruit weight of over 7 g, even reaching 8 g in the case of hybrid 7/67-68. Although this analysis provided information on the fruit size of genotypes, it is not suitable to establish their productivity, for which hybrid seedling need to be tested under commercial orchard conditions. The most promising hybrids were therefore grafted on *Mahaleb* rootstocks and are currently being evaluated under both commercial and experimental orchard conditions.

With regard to the ripening time, all the selected hybrids ripened earlier than the cultivar 'Érdi bőtermő' and seven combinations (7/141, 9/5-6, 9/24, 7/47, 9/91, 7/67-68, 9/79-80) ripened earlier than the medium early cultivar 'Csengődi'. The ripening time of these hybrids was 7-12 days earlier than that of 'Érdi bőtermő'.

4.2. Analysis of carbohydrates in homeostasis

Comparisons in homeostasis were made in spring (May), summer (July) and winter (December). The results obtained in spring and winter led to the same conclusions: among the carbohydrates detected, the quantity of glucose was most closely associated with the *M. laxa* resistance of the sour cherry cultivars and hybrids. In addition a clear correlation was found based on the relative ratio of the individual carbohydrates. These results confirm the observations of SÁRDI et al. (1996, 1999) who compared bean cultivars susceptible and resistant to *Pseudomonas*, and the findings of studies on the pepper-*Xanthomonas* (SZARKA et al. 2002, SÁRDI et al. 2006), grape-*Botrytis* (KOVÁCS-NAGY et al. 2008, NÉMETH et al. 2009) and apple-*Erwinia* (MILCEVICOVA et al. 2010) host-pathogen interactions. The analysis of carbohydrate fractions also revealed that the quantity of glucose, fructose and sucrose in the leaves was higher than in phloem tissues. These observations are in agreement with those of HONTY (2010), who compared the glucose and fructose quantities in susceptible and resistant pear cultivars.

In summer (long-lasting drought, strong UV radiation) the differences in the relative contents of glucose and fructose in the susceptible and resistant genotypes did not exhibit the correlations observed in spring, probably due to environmental stress.

4.3. Time-dependent changes in carbohydrates in response to *Monolinia laxa* infection

The disease resistance of sour cherry cultivars and hybrids was found to be correlated with the quantity of monosaccharides, the quantitative changes occurring in response to infection and the tendencies exhibited by these changes. The time-dependent changes in the glucose content revealed clear distinctions between the susceptibility groups. Compared with the control values, the glucose concentration was higher in susceptible and lower in resistant genotypes as an early response to infection. After an initial significant decline, the glucose level in susceptible genotypes exhibited cyclical changes after the third hour. One reason for this was that the interaction with the pathogen in the alarm phase led to a drop in vitality due to the disturbance of the homeostatic balance, with a consequent increase in catabolic processes. In the case of resistant genotypes, on the other hand, a rise in the glucose level was observed in

the first hour after infection, so an increased carbohydrate concentration after infection may signal the launch of defence reactions (BERGER et al. 2007).

During the normalisation phase, the glucose concentration in the resistant genotypes fell to a minimum by the end of the first day, followed by a slow rise. After a stabilisation phase, the glucose level then returned to the initial level by the end of the test period in both the resistant cultivar and the hybrid. In the susceptible genotypes the minimum glucose level was not reached until the third day or later, and at the end of the test period the glucose concentration was still significantly lower than the initial level. SÁRDI et al. (2006) and SZARKA (2008) reported similar results when investigating the pepper–*Xanthomonas vesicatoria* host–pathogen relationship in susceptible and resistant genotypes and in *gds* (general defence system) lines. The results were also in agreement with the findings of HONTY (2010), who analysed the connection between glucose and fructose content and the bacterial resistance of pear cultivars and suggested that the depletion of carbohydrate resources could be the result of the accelerated metabolism caused by infection and of the glucose consumption of the pathogen.

The glucose content recorded in control samples in winter, under *in vitro* conditions, and the changes occurring in response to inoculation also exhibited clear differences between the responses of the susceptible and resistant genotypes, which confirmed the results obtained after *in vivo* infection.

The various approaches used to test plant responses all suggest that monitoring the quantitative changes in carbohydrates after infection could make it possible to demonstrate differences in the defence responses of susceptible and resistant cultivars and hybrids to *M. laxa*. This is in agreement not only with the findings of SÁRDI et al. (2006) and SZARKA (2008) for the pepper–*Xanthomonas vesicatoria* host–pathogen relationship, but also with those of DANIELE et al. (2003), who examined the relationship between the resistance of potato plants to *Phytophthora infestans* and the time-dependent changes in carbohydrates after artificial inoculation. A similar opinion was reported by MANDAL et al. (2012), who found that the effect of infection with *Tobacco mosaic virus* on the metabolism of tobacco plants could be detected in the first hour after infection as a change in the quantity of carbohydrates. The rapidity of detectable defence responses was also proved by SÁRDI (1994), BAKER and ORLANDI (1995), SZARKA et al. (2002) and SÁRDI et al. (2006).

Further research will be required for a precise explanation of the biochemical background of these changes, but the present results confirm the fundamental role of glucose in the mutual responses characteristic of the pathogen–host relationship.

4.4. Correlations between methyl donors and disease resistance

4.4.1. Comparative analysis in homeostasis

The theoretical background of the present research was formed by previous results on host–pathogen relationships (SÁRDI 1994, SÁRDI and TYIHÁK 1998, SÁRDI 2006, SZARKA 2008). As a consequence of the stress-induced demethylation of various methyl donors, readily mobilisable methyl groups are liberated, from which HCHO is formed as a transient product. This leads to a reduction in the measurable quantity of methylated compounds, with a parallel increase in the concentration of endogenous formaldehyde. However, the experiments cited were performed on watermelon and pepper plants. No data have been found in the literature on this approach to defence responses in woody plants.

In the present work the concentration of choline was considerably greater in the phloem tissues of resistant sour cherry genotypes than in susceptible ones. Similar results were reported by ROZSNYAY et al. (1988) in comparative tests on peach and apricot trees, where most of the cultivars found by experience to be resistant contained higher quantities of choline, trigonelline and trimethyl-lysine (TML) than susceptible cultivars. TYIHÁK et al. (1989) reached a similar conclusion: the level of fully methylated compounds in the leaves of resistant 'wild' genotypes and of cultivars found in practice to be resistant was higher than in those of susceptible cultivars. The relationship between the quantity of methyl donors and disease resistance was observed when analysing peach and apricot leaves, and was confirmed after the artificial inoculation of herbaceous plants grown under controlled conditions (SÁRDI 1994, SÁRDI and TYIHÁK 1998, SZARKA et al. 2006).

4.4.2. Comparative analysis based on the quantity of endogenous HCHO

The samples taken for the analysis of methylated compounds were also used for the determination of endogenous HCHO in homeostasis. A comparison of the results of choline analysis and HCHO concentrations indicated an inverse trend for the correlation between easily mobilisable CH_3 groups and disease resistance than was found for the methyl donor choline. In the case of European Turkey oak, formaldehyde and its natural precursors were also found to be suitable signal molecules for the characterisation of environmental effects (NÉMETH 2002).

4.4.3. Monitoring the effect of artificial inoculation

Transmethylation, the removal or incorporation of methyl groups, has low energy requirements, thus facilitating rapid, reversible regulation. Resistant and susceptible genotypes

could be clearly distinguished on the basis of the dynamics of quantitative changes in choline and endogenous formaldehyde (SÁRDI 1994, 2006, SZARKA et al. 2006, NÉMETH 2002).

In the first hour after inoculation the quantity of choline decreased significantly in all four genotypes, accompanied by a rapid rise in the formaldehyde content. At later sampling times, however, the choline level differed considerably in resistant and susceptible genotypes. The choline level reached a maximum after 6 hours in the cultivar 'Csengődi' and hybrid 7/67-68, while in the susceptible genotypes there was no significant change during the rest of the test period. In parallel to the changes in choline content, the changes in the quantity of endogenous formaldehyde also showed clear differences between the two susceptibility groups. Similar correlations were published by SÁRDI (1994, 2006) on the defence responses taking place in the roots of watermelon cultivars sensitive or resistant to infection with *Fusarium oxysporum*.

In response to infection, changes with a similar tendency but differing as regards time and quantity were observed. The choline and TML levels were similar, while the HCHO content exhibited an opposing trend. Correlations detected when studying the watermelon–*Fusarium* host-pathogen relationship were later confirmed in tests on the bean–*Pseudomonas* and pepper–*Xanthomonas* relationships (SÁRDI 2006, SZARKA 2008). However, no similar reports have been found on woody plants.

In summary, all the endogenous compounds examined were suitable for characterising the *M. laxa* resistance of sour cherry genotypes with various levels of resistance. Among the carbohydrates, glucose gave the best demonstration of differences, while in the case of methyl donors the best results were achieved with choline. It is important to note that changes in the concentration of endogenous formaldehyde in response to infection indicated differences in the defence responses of susceptible and resistant genotypes within a short time of infection, substantially earlier than the appearance of visible symptoms.

The eventual aim of this research is the improvement of the analytical methods and the use of plant pathological techniques to elaborate a testing method that could be employed to abbreviate the time-consuming process of resistance breeding.

5. PUBLICATIONS CONNECTED TO THE DISSERTATION

Article in impact factored journals:

1. **SZÜGYI, S**., SÁRDI, É. (2017): Health-affecting compounds in sour cherry (*Prunus cerasus* L.) at the beginning of fruit burgeoning stage. *Acta Alimentaria*. (in press). IF:0,357

Journals without IF:

2. **SZÜGYI, S**., ROZSNYAY, ZS., SÁRDI, É. (2017): A meggy gazdanövény és a *Monilinia laxa* kórokozó kapcsolatának tanulmányozása szénhidrátok mérésével. *Kertgazdaság* 49 (1) 35-43. p.
3. **SZÜGYI S**., ROZSNYAY ZS., SÁRDI É. (2017): Meggyfák betegségellenállósága és a metilezési körfolyamat egyes komponensei közötti kapcsolat. *Kertgazdaság* 49 (3) 23-31. p.

Other journal articles:

4. **SZÜGYI, S.,** ROZSNYAY, ZS., APOSTOL, J. (2012): Betegségellenálló meggy hibridek jellemzése. *Agrofórum Extra* 43, 28-31. p.

Conference papers (full papers):

5. **SZÜGYI, S.,** ROZSNYAY, ZS., APOSTOL, J. (2014): Monília ellenálló meggyfajták nemesítése. XX. Növénynemesítési Tudományos Napok,. Budapest, Március 18. 439-443. p.

6. APOSTOL, J., **SZÜGYI, S**., BÉKEFI, ZS. (2014): Nemesítési alapanyagként használható meggy genotípusok vizsgálata. XX. Növénynemesítési Tudományos Napok. Budapest, Március 18. 45-49. p.

7. **SZÜGYI, S.**, APOSTOL, J., ROZSNYAY, ZS., BUJDOSÓ, G., SÁRDI, É. (2015): Examination of disease resistant sour cherry genotypes bred in Hungary. Proceedings of the III Balkan Symposium on Fruit Growing Volume 1. *Acta Horticulturae*, 1139. 13-18. p.

8. **SZÜGYI, S.**, ROZSNYAY, ZS., APOSTOL, J., BÉKEFI, ZS. (2015): Breeding of *Monilinia laxa* resistant sour cherry varieties in Hungary. *Acta Horticulturae*. (in press)

Conference papers (abstracts):

9. **SZÜGYI, S.,** APOSTOL, J., ROZSNYAY, ZS. (2009): Az 'Érdi bőtermő' és a 'Csengődi' meggyfajták utódnemzedékeinek jellemzése. Lippay János – Ormos Imre – Vas Károly Tudományos ülésszak. Budapest, Október 28-30, 242-243. p.

10. **SZÜGYI, S.,** APOSTOL, J., ROZSNYAY, ZS. (2010): 'Érdi bőtermő' és 'Csengődi' meggyfajták utódnemzedékeinek vizsgálata betegségellenállóság és termesztési érték szempontjából. XVI. Növénynemesítési Tudományos Napok. Budapest, Március 11, 133. p.

11. APOSTOL, J., **SZÜGYI, S.** (2014): Sour cherry breeding in Hungary. COST meeting on sour cherry breeding, Novi Sad, Serbia September 15-17. 2-4. p.

12. **SZÜGYI, S**., APOSTOL, J., ROZSNYAY, ZS., LANTOS, E., SÁRDI, É. (2015): *Monilinia laxa* gombával szemben ellenálló meggyfajták nemesítése. XXI. Növénynemesítési Tudományos Napok, Martonvásár Március 11-12, 38. p.

13. **SZÜGYI, S**., APOSTOL, J., ROZSNYAY, ZS., LANTOS, E., SÁRDI, É. (2015): Sour cherry breeding at Research Station Érd of NARIC Fruitculture Research Institute. COST meeting on sour cherry breeding, Dresden-Pillnitz, Germany, July 13-16, 1. p.

14. **SZÜGYI, S**., ROZSNYAY, ZS., SÁRDI, É. (2017): Meggyfák betegségellenállósága és a körfolyamat egyes komponensei közötti kapcsolat. 63. Növényvédelmi Tudományos Napok. Budapest 2017.02.21-22, 98. p.

15. KOVÁCS, K., **SZÜGYI, S**., TURÓCZI, GY. (2017): Meggyfajták és fajtajelöltek moníliával szembeni ellenállóképessége. 63. Növényvédelmi Tudományos Napok. Budapest 2017.02.21-22, 65. p.

16. **SZÜGYI, S.,** ROZSNYAY, ZS., SÁRDI, É. (2017): Transzmetilezési folyamatok nyomon követése meggyfák háncsszöveteiben *Monilinia laxa* fertőzés hatására. XXIII. Növénynemesítési Tudományos Napok. Budapest 2017.03.07, 34. p.

REFERENCES

1. APOSTOL J. (1994): A meggynemesítés eredményei Budatétényben. Kandidátusi értekezés. MTA. Budapest.
2. APOSTOL, J., VÉGHELYI, K., IEZZONI, A., JONES, A. L: (1995): Magyar- amerikai együttműködés a betegségellenálló meggyfajták nemesítése érdekében. *Új Kertgazdaság*, 1 (1-2) 1-3. p.
3. APOSTOL, J. (1998): Meggy. In: Soltész M. (Szerk): *Gyümölcsfajta-ismeret és -használat.* Budapest. Mezőgazda kiadó, 288-307. p.
4. BAKER, C. J., ORLANDI, E. W. (1995): Active oxygen in plant pathogenesis. *Annual review of phytopathology*, 33 (1) 299-321.p.
5. BERGER, S., SINHA, A. K., ROITSCH, T. (2007): Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. *Journal of Experimental Botany*, 58 (15-16) 4019-4026. p.
6. DANIELE, E., DOMMES J., HAUSMANN, J. F. (2003): Carbohydrates and resistance to *Phytophtora infestans* in potato plants. *Acta Physiologiae Plantarum*, 25 (2) 171-178. p.
7. GERSBECK, N., SCHÖNBECK, F., TYIHÁK, E. (1989): Measurement of formaldehyde and its main generators in *Erysiphe graminis* infected barley plants by planar chromatographic techniques. *Journal of Planar Chromatography* (2) 86-89. p.
8. HONTY, K. (2010): A körtefajták tűzelhalással szembeni ellenállósága és a betegség folyamatának jellemzése néhány biokémiai paraméter vizsgálatával. BCE Gyümölcstermő Növények Tanszék, *Doktori értekezés*, 106-107 p.

9. KOVÁCS-NAGY, E., BILEK, A., LACZ, E., BODOR, P., SÁRDI, É. (2008): Grape variety comparison of different stress tolerance based on the quantitative measurement of carbohydrates. *International Journal of Horticultural Sci*ence, 14 (4) 7–10. p.
10. MANDAL, R., KATHIRIA, P., PSYCHOGIOS. N., BOUATRA S., KRISHNAMURTHY, R., WISHART, D., KOVALCHUK, I. (2012): Progeny of *tobacco mosaic virus*-infected *Nicotiana tabacum* plants exhibit trans-generational changes in metabolic profiles. *Biocatalysis and Agricultural Biotechnology*, 1 (2) 115–123. p.
11. MILCEVICOVA, R., GOSCH, C., HALBWIRTH, H., STICH, K., HANKE, M-V., PEIL, A., FLACHOWSKY, H., ROZHON, W., JONAK, C., OUFIR, M., HAUSMAN, J. F., MATUSIKOVA, I., FLUCH, S. (2010): *Erwinia amylovora*-induced defense mechanisms of two apple species that differ in susceptibility to fire blight. *Plant Science*, 179 (1-2) 60-67. p.
12. NÉMETH ZS, I., (2002): A formaldehid és természetes generátorai, mint a környezeti hatások jelző molekulái a csertölgy ontogenezisének korai szakaszaiban. PhD értekezés, Sopron. Nyugat-magyarországi Egyetem
13. NÉMETH, ZS. I, SÁRDI, É., STEFANOVITS-BÁNYAI, É. (2009): State dependent correlations of biochemical variables in plants. *Journal of Chemometrics*, 23 (3-4) 197-210. p.
14. ROZSNYAY, ZS., TYIHÁK, E., CMOREK, É., KEREK, M., NYUJTÓ, F., PEDRYC, A. (1988): A tejes N-metilezett vegyületek, mint potenciális rezisztencia faktorok vizsgálata kajszi és őszibarack fajták növényi részeiben. III. Magyar Növényélettani Kongresszus 1988. július 5-7. Szeged.
15. SÁRDI, É. (1994): A formaldehid és egyes generátorainak tanulmányozása *Fusarium oxiosporum f. sp. niveum*-mal fertőzött görögdinnye növényeken. MTA Kandidátusi értekezés. 127. p.
16. SÁRDI, É., VELICH, I., HEVESI, M., KLEMENT, Z. (1996): The role of endogenous carbohydrates in the Phaseolus-Pseudomonas host-pathogene interaction. 1, Bean ontogenesis and endogenous carbohydrate components. *Horticultural Science,* (28) 65-69. p.
17. SÁRDI, É., TYIHÁK, E. (1998): Change of biotransformation steps of formaldehyde cycle in water-melon plants after infection with *Fusarium oxysporum*. *Acta Biologica*, 49 (2-4) 353-362. p.

18. SÁRDI, É., VELICH I., HEVESI M., KLEMENT, Z. (1999): Ontogenesis- and biotic stress dependent variability of carbohydrate content in snap bean (*Phaseolus vulgaris L.*). *Zeitschrift für Naturforschung*, (54) 782-787. p.
19. SÁRDI, É. (2006): A növények betegségellenállósága és az endogén transzmetilezési folyamatok kapcsolata. MTA doktori értekezés
20. SÁRDI, É., SZARKA, E., CSILLÉRY, G., SZARKA, J. (2006): Biochemical examination of the General Defense System of plants by OPLC. *Journal of Planar Chromatography*, 19 (109) 233-237. p.
21. SZARKA, E. (2008): Növények általános védekezési rendszerének biokémiai és genetikai vizsgálata. BCE Genetika és növénynemesítés Tanszék, *Doktori értekezés* 116-123. p.
22. SZARKA, J., SÁRDI, É., SZARKA, E., CSILLÉRY, G. (2002): General defense system in the plant kingdom III. *International Journal of Horticultural Science,* 8 (3-4) 45-54. p.
23. SZARKA, J., TOLDI, O., SZARKA, E., REMENYIK, J., CSILLÉRY, G. (2006): General defense reaction in the plant kingdom. *Acta Agronomica Hungarica,* 54 221-232. p.
24. TYIHÁK, E. (1987): Overpressured layer chromatographyc methods in the study of the formaldehyde cycle in biological systems. *Trends in Analytical Chemistry,* (6) 90-94. p.
25. TYIHÁK, E., ROZSNYAY, ZS., TIMON, B. (1989): A formaldehid természetes főgenerátorainak vizsgálata kajszi- és őszibarack leveleiben. Növényvédelmi Tudományos Napok, 1989. febr. 21-22, Budapest.