Theses of PhD dissertation

FIRE BLIGHT SUSCEPTIBILITY OF PEAR CULATIVARS AND CHARACTERIZATION OF THE DISEASE PROCESS BY SOME BIOCHEMICAL PARAMETERS

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1. INTRODUCTION

Pear production in Hungary exceeded 100,000 tons yearly, however, this amount decreased to 30-40,000 tons by this time. In 1996 the total growing area was also significantly reduced because of serious fire blight infection. Although the area of pear has been growing somewhat in the last few years it is still not enough to supply the Hungarian market. Pear import has been gratuitously increased in the recent years, however, Hungarian market supply has not been raised.

The pathogen *Erwinia amylovora* (Burrill) Winslow causes a huge problem all over the world. The Effective Control System against the fire blight disease so far has not been implemented. The most effective chemical is the streptomycin, which is not allowed to use because of human health reasons. Therefore, utilization of environment protective, alternative and biological control methods play an important role. The most effective way to control the fire blight could be the use of resistant cultivars. It is very important to renew the cultivar sortiment, because only few varieties are grown worldwide.

There are many results about fire blight resistance of pear cultivars from other countries, however it must be considered that data sometimes can differ depending on different ecological circumstances and different *Erwinia amylovora* strains might occur in each country.

Therefore our most important aim is to sort the pear cultivars by their degree of resistance to blight. Field tests are not permitted, because of actual quarantine regulations in Hungary. Experiments for evaluation of susceptibility of pear cultivars to fire blight were conducted under controlled greenhouse conditions, at the laboratory of *Erwinia* at the Department of Fruit Science of Corvinus University of Budapest from 2001 to 2006.

In the plants free radicals arise after infection, which can be eliminated by antioxidative enzymes, phenols and other non enzymatic defense components. In the literature there are several but different data about the changes of these components in connection with disease development. For this reason another aim of my work was to monitor the host response after an infection by different biochemical parameters. These experiments were conducted at the Department of Plant Breeding and Genetics and at the Department of Applied Chemistry.

2. AIM OF THE STUDY

The susceptibility/resistance of cultivars can be partly characterized by development of visual symptoms, with its intensity and rapidity. In addition it can be also characterized by the changes in plant tissue after an infection.

Our objectives were:

- to determine the degree of susceptibility of flowers, shoots and fruits of pear cultivars by inoculation in the laboratory. We examined the most important commercial cultivars, some newly bred cultivars, not grown yet in Hungary and Japanese pear cultivars comparing with resistant cultivars known by literature;
- to elaborate inoculation and evaluation methods for each plant organs (flower, shoot and fruit) for the above mentioned objective;
- to determine plant organs those susceptibility characterize best the susceptibility/resistance of pear cultivars;
- to monitor the susceptible/resistant host response in infected plant tissue by evaluating the changes of biochemical parameters (antioxidant enzymes, total polyphenol content and carbohydrate fractions);
- to determine which biochemical parameter examined is the most suitable for detecting resistance.

3. MATERIALS AND METHODS

Materials for inoculation

Bacteria

In a preliminary test we have scored the virulence of different isolates and two bacterial isolates have been selected that display a high virulence, originating from Sarkad (Ea 21) and Nagykanizsa (Ea 23). Test-inoculations were performed with a mixture of these isolates at a concentration of $5x10^8$ cells/ml.

Pear cultivars

Among the pear varieties tested important commercial-, old traditional-, newly bred-, allegedly resistant- and Japanese pear cultivars were chosen, including almost 40 cultivars. Plant material (flowers, shoots and fruits) was collected from the gene bank of the Experimental Station of the Corvinus University of Budapest, in Szigetcsép and from the Experimental Station of Pölöske of the Central Agricultural Office. Micropropagated plants were performed at Research Institute for Fruitgrowing and Ornamentals of Budapest (Érd).

Methods of inoculation

The applied methods were developed according to papers published previously by other investigators (van der Zwet and Keil, 1979), or partly modified (Hevesi et al, 2000), or developed by us. Shoots of container-grown grafts, flowers and unripe fruits collected from trees and micropropagated plants were inoculated from 2001 to 2006.

For **flower** inoculation two different methods were compared: short and medium long branches at the beginning of flowering were sprayed with bacterial suspension ($5x10^8$ cells/ml, 50 flowers/cultivars). After this with the capillary technique we could inoculate the flowers separately, when infection was carried out by placing the bacterial suspension ($5x10^8$ cells/ml) on the pistil through a capillary vessel (20 flowers/cultivar).

Shoot inoculations were performed on 20-30 cm long shoots (8 shoots/cultivar) that developed from grafts. Shoots were punctured by a needle connected to a syringe containing the bacterial suspension at the axil of the third leaf below the shoot tip.

Unripe fruits (2-2.5 cm diameter) were pricked with a needle dipped into the bacterial suspension ($5x10^8$ cells/ml, 10 fruits/cultivars).

Micropropagated plants were inoculated with scissors dipped into the bacterial suspension ($5x10^8$ cells/ml), cutting the lowest leaves.

All the inoculated plant parts were kept at 25°C with high humidity in order to maintain the favoring bacterial multiplication.

Materials for biochemical analysis

Unripe pear fruits, leaves and shoots of grafted trees, and micropropagated plant were analyzed.

For measuring carbohydrate and total polyphenol content **in fruit**, a susceptible ('Max Red Bartlett') and a resistant ('Vicar of Winkfield') cultivar was chosen for analysis. For determination of enzyme activity, a moderate resistant Japanese pear cultivar ('Hosui') was also included.

Samples of flesh cutouts (1 cm ø) were taken around the inoculation point (a) and from 1 cm far (b), and 2 cm far from the neighboring tissue (c), in the intervals of 0-72 hours (2003-2004) and 0-168 hours (2005).

We also measured the peroxidase enzyme activity, carbohydrate content and total polyphenol content in the **leaf and shoots** of grafted trees (resistant: 'Kieffer' and susceptible: 'Packham's Triumph').

The biochemical changes were measured (in 2003) on the 1st and 5th days after inoculation (for susceptible cultivar) and also on the 14th day (for resistant cultivar). In the second experiment (2007) the changes were analyzed in the intervals of 0-48 hours in the top leaves (L_1) the leaves above the inoculation point (L_2), the leaves below the inoculation point (L_3) and in shoots (3 cm long) above and below the inoculation point (H).

Samples were taken from **micropropagated plants** on the 2nd, 4th, and 6th days - (2005) and on the 1st, 4th, 6th, 8th and 12th days (2006) after infection.

Methods of biochemical analysis

Enzyme activities were measured in the Department of Applied Chemistry of the Corvinus University of Budapest. Peroxidase activity was determined by spectrophotometry with H_2O_2 as a substrate and ortho-dianidizine as a choromegenic reagent (ε = 11.3), at λ = 460 nm (Shannon et al., 1966). Changes in polyphenol-oxidase (PPO) enzyme activity were also followed by spectrophotometry using catechol at λ=420 nm (Jen and Kahler, 1974).

The total poliphenol content was determined by spectrophotometry using Folin-Ciocalteu reagent (Singleton and Rossi, 1965) at the Department of Applied Cemistry.

Determination of carbohydrates was measured with overpressured layer chromatographic techniques (OPLC, in 2003, 2004) at the Department of Genetics and Plant

Breeding of the Corvinus University of Budapest (Sárdi et al., 1996) Determination of carbohydrates by HPLC (High Pressure Liquid Chromatography) was made at the Department of Fruit Science (2005 and 2007).

Evaluation of experiments

Evaluation of fire blight infection of **flowers** was performed four days after inoculation. For the method of spraying of branches, the progression of bacterial infection was monitored visually based on the extent of browning in the receptacle, calyx, petal, pistil, and stamen. The disease rating on flowers was expressed by the formula: $DR_f = (n_b/n) \times 100$ (DR_f : the severity of disease on flowers, n_b: numbers of infected flower part, n: number of flower parts examined).

For the capillary technique the disease rating on flowers was expressed by the formula: $DR_f = \sum f_i * n_i/n$ (DR_f : the severity of disease on flowers, f_i: scale value (index of infection), n_i: frequency referring to the infection index; n: number of flower parts examined). Susceptibility of flower parts was expressed on a scale (index of infection) of 0 – 3: (0: symptomless, 3: the whole flower organ died - total browning).

For evaluation of the susceptibility of the flowers, cultivars were grouped into four categories, 0 – 0,75 (0 - 25%): moderately resistant, 0,76 – 1,5 (26 - 50%): moderately susceptible, 1,51 – 2,25 (51 - 75%): susceptible and 2,26 – 3 (76 - 100%): very susceptible.

Fire blight symptoms on **shoots** were evaluated one week after inoculation and repeated every fourth day over a period of three weeks. We measured the length of the infected part (cm), this way the spreading of the disease could be recorded. We determined:

- the % of the necrosis N (%) = (length of the necrosis/length of infected shoot) x 100
- The severity of fire blight symptoms on leaves, leaf veins and shoots was rated according to a scale of 0-5 (infection index) (0: the inoculation (puncture) site dries, the infection does not spread further, 5: the whole shoot and leaves are brown). The disease rating on shoots is expressed by the formula: $DR_s = \sum f_i * n_i/n$ (DR_s: the severity of disease on shoots, f_i: scale value - index of infection, n_i: frequency referring to the infection index, n: number of plant parts/shoot examined, Horsfall and Barratt, 1945; Bertrand and Gottwald, 1978). Cultivars were grouped into four categories, 0 - 1,25: moderately resistant, 1,25 - 2,5: moderately susceptible, 2,51 - 3,75: susceptible and 3,76 - 5: very susceptible.
- For evaluation of bacterium cell multiplication in infected plant tissue, an 1 cm long shoot part was used, cultivars were grouped into categories as follows: $0–10^5$ bacteria cells/1cm: modatetely resistant, $10^5 + 1 – 10^6$ bacteria cells /1cm: moderate

susceptibility, 10^6 +1-10^7 bacteria cells /1cm: susceptible, more than 10^7: very susceptible

Evaluation of infection of **unripe fruits** was rated 4 days after inoculation. The character and the diameter of water soaked/necrotic spots and the size of mucilage drops appearing on the surface served as parameters of the degree of susceptibility. Disease severity on fruit (DR_{fr}) $DR_{fr} = \Sigma f_i \times n_i / n$, as well as categorization of cultivars has been calculated by the same formula as the one used in shoot tests.

Statistical analysis was carried out with the aid of the SPSS 14.0 software with Hierarchical Cluster analysis (based on the infection of calyx, receptacle, shoots and flowers). Results were represented on dendrograms.

4. RESULTS AND DISCUSSION

Susceptibility of pear cultivars

We concluded that susceptibility/resistance of the **flower** is the most important measure of the cultivar, because the infections occur through the flower at the time of blooming in spring. At the beginning of the experiments (2001) the flowers of branches were inoculated spraying with *E. amylovora* suspension. This method was not enough sophisticated, too much bacteria got into flowers, petals turned into brown untimely and fall down, symptoms of flower organs did not differentiate hence their evaluation was difficult.

With introduction of capillary method a determinate quantity (20 µl/flower) of bacterial suspension got into flowers, which method resembles more to natural infection. We could evaluate precisely the flower infection with this inoculation method. According to our opinion, when evaluating the cultivars, susceptibility of the receptacle and the calyx of flowers are most indicative because the flower is the gate to the infection of the shoot. Therefore, cultivars were grouped according to these organs.

After flower infection completely resistant flowers are very rare to find. Considering our experiments, the flowers of 'Hosui', 'Bonne Luise d'Avranches', 'Flemish Beauty' and 'Kieffer' were moderately resistant. Moderately susceptible flowers proved to be 'Beurre Bohusné', 'Bronzovaja', 'Clapp's Favorite', 'Harrow Delight', 'Ilonka', 'Nijisseiki', 'Max Red Bartlett', 'Star' and 'Williams'.

The second most important indicator of cultivar-resistance is the susceptibility of **shoots**. By the fire blight symptoms on shoot, we found the cultivars: 'Harrow Delight',

'Harrow Sweet', 'Kieffer', 'Moonglow' and 'US 65062-13' as moderately resistant. 'Bonne Luise d'Avranches', 'Beurre Bohusné', 'Cascade', 'Clapp's Favorite', 'Beurre Giffard', 'Magness' and 'Star' belong to the moderately susceptible group. We observed that most of the examined cultivars (71%) were susceptible or very susceptible, included the most important cultivars in Hungary. We got similar conclusion after inoculation of micropropagated plants, for example the very susceptible 'Packham's Triumh' and 'Beurre Durondeau'.

For characterization of the fire blight susceptibility of the cultivars it is necessary to evaluate together the susceptibility of the flowers and shoots (Table 1). Considering the symptoms observed on shoots and flowers, only 'Kieffer' showed moderate resistance. This cultivar is not primarily for fresh consumption, but still it can be used as gene source for resistance breeding. 'Bonne Luise d'Avranches' had moderately resistant flowers and moderately susceptible shoot, and 'Harrow Delight' had moderately resistant shoots and moderately susceptible flowers. We could mark other three cultivars, 'Star', 'Clapp's Favourite' and 'Beurre Bohusné' – with Hungarian origin – , which displayed only moderate susceptibility based on shoot and flower's infection.

Shoot and flower susceptibility of several cultivars showed significant differences, and in case of some cultivars the infection of the two organs was completely different. 'Moonglow', 'Harrow Sweet' and 'US 65062-13' had moderately resistant shoots but very susceptible flowers, whereas 'Hosui' and 'Flemish Beauty' had moderately resistant flowers and susceptible or very susceptible shoots.

Table 1. Susceptibility of pear cultivars based on shoot and flower infection (2001-2005)

Shoot ↓				
VS	Flemish Beauty	Bronzovaja	Beurre Bosc Eldorado Beurre Durondeau	Baki Bosc, Conference Fertilia Delbard, Beurre Hardenpont, Packham's Triumph, Vicar of Winkfield, Porporata
S	Hosui HP25	Ilonka Nijissejiki Williams	Harvest Queen NP41	Dr. Jules Guyot Mosoly
MS	**Bonne Louise d'Avranches**	**Beurre Bohusné Clapp's Favourite Star**	Beurre Giffard Magness Magyar kobak	Cascade
MR	**Kieffer**	**Harrow Delight**		Harrow Sweet Moonglow US 65062-13
***Flowers*→**	MR	MS	S	VS

MR: moderately resistant, **MS**: moderately susceptible, **S**: susceptible, **VS**: very susceptible

Few data are available on susceptibility of pear fruits, however, the transported fruits can be a source of infection, Fire blight susceptibility of fruits therefore can be an important feature of the cultivars. Based on the data of **fruit** tests, 'Bronzovaja', 'Cascade', 'Eldorado', 'Beurre Hardenpont', 'Hosui', and its hibrid (HP 25), 'Packham's Triumph', 'Vicar of Winkfield', and 'Beurre Durondeau' showed moderately resistant response. Fruits of 22 cultivars were tested out of 35 cultivars and they were moderately or very susceptible to the disease.

We found that susceptibility of fruits differs significantly from the susceptibility of the shoots and flowers in the orchard. According to our laboratory test results, the flowers and shoots were very susceptible but unripe fruits showed moderately resistant of some cultivars e.g. 'Packham's Triumph', 'Vicar of Winkfield' and 'Beurre Hardenpont'.

Evaluation of fire blight susceptibility and grouping cultivars on the basis of susceptibility of the three organs examined was not easy. In some years the plant organ examined infected at different level. The organ showed moderately resistance in one year, and in another year it was very susceptible, this was also cited by other authors (Thibault et al., 1989; Le Lezec et al., 1998). In the other hand the plant organs showed different susceptibility after inoculation by some cultivars.

Similar degree of fire blight susceptibility of the different plant organs was observed at 'Bonne Louise d'Avranches', 'Beurre Bohusné', 'Clapp' Favourite' and 'Harrow Delight' cultivars. and the plant organs examined showed moderate resistance, or moderate susceptibility. The organs of the cultivars 'Harvest Queen', 'Magness', 'Magyar kobak', 'Nijisseiki', 'Star' and 'Williams' showed the same level of susceptibility – but they were moderate susceptible or susceptible. 'Beurre Bosc', 'Conference', 'Dr. Jules Guyot', 'Fertilia Delbard' and 'Republica'. were highly susceptible. However, in case of some cultivars - e.g. 'Cascade', 'Flemish Beauty', 'Moonglow', Harrow Sweet' and 'US 65062-13' - susceptibility varied among different organs. The results of the relative fire blight susceptibility of the cultivars examined are presented in Table 2.

Table 2. Relative fire blight susceptibility of the shoots, flowers and fruits of pear cultivars (2001-2005)

Cultivar	shoot	flower	fruit
Árpával érő		VS	
Bonne Louise d'Avranches	MS	MR	MS
Baki Bosc	VS	VS	
Beurre Bohusné	MS	MS	MS
Beurre Bosc	VS	S	S
Bronzovaja	VS	MS	MR
Cascade	MS	VS	MR
Clapp's Favourite	MS	MS	MS
Conference	VS	VS	S
Dr. Jules Guyot	S	VS	S
Eldorado	VS	S	MR
Flemish Beauthy	VS	MR	S
Ferenc vérbélű	VS		
Fertilia Delbard	VS	VS	VS
Abate Fetel	VS		
Beurre Giffard	MS	S	MS
Beurre Hardenpont	VS	VS	MR
Harrow Delight	MR	MS	MS
Harrow Sweet	MR	VS	MS
Harvest Queen	S	S	MS
HW 620		VS	MS
Hosui	S	MR	MR
HP12	S		
HP25	S	MR	MR
Ilonka	S / MS	MS	VS
Kieffer	MR	MR	S
Magness	MS	S	MS
Magyar kobak	S / MS	S	
Marik kedveltje	VS		MS
Moonglow	MR	VS	MS
Mosoly körte	S	VS	MS
Nijisseiki	S	MS	MS
NP1	VS		
NP41	S	S	MS
Orsolya	S		
Packham's Triumph	VS	VS	MR
Vicar of Winkfield	VS	VS	MR
Porporata	VS	VS	
Max Red Bartlett		MS	VS
Red Sensation	VS		
Republika	VS		S
Star	MS	MS	S
Doyenne de Comice	VS		
Beurre Durondeau	VS	S	MS
US 65062-13	MR	VS	S
Williams	S	MS	S

MR: moderately resistant,
MS: moderately susceptible,
S: susceptible,
VS: very susceptible

Monitoring the biotic stress by biochemical markers

Biochemical changes in unripe pear fruits

Every cultivar responded with an increase of enzyme activity (Peroxidase: POD, polipehenol oxidase: PPO) after bacterial infection, but the susceptible and resistant host response was different. The susceptible cultivar ('Max Red Bartlett') reacted faster with an increased POD activity, than the resistant one ('Vicar of Winkfiled'), which result was mentioned also by other authors (Torres et al., 2003). In susceptible cultivar the POD enzyme activity increased – as a sign of stress – 24 (2005) and at 48 hours (2003, 2004) after inoculation. As time passed (72 and 168 hours after inoculation), in accordance with symptom development a decrease of enzyme activity was detected, which can be attributed to tissue death. At that moment the amount of free radicals were so high that the antioxidant defense system was not able to eliminate them and tissue death occurred. In *tissues close to the infection* the increase of enzyme activity was observable with some time lag (72 hours after inoculation), which could indicate delayed mobilization of plant defense processes in healthy neighboring tissues.

In the inoculation points the resistant cultivar reacted later – 48 hours (2005) or 72 hours (2003, 2004) after inoculation – with an increase of POD activity, which indicates that infection caused less stress. Another explanation is that at that time there were less reactive oxygen species to start the antioxidant defense system. After 72 hours the POD activity started to increase in the inoculation point, showing that its antioxidant defense system reacted against the later developed reactive oxygen species. We measured the highest POD activity in the control tissues of the moderate resistant cultivar ('Hosui', Japanese pear). The host response of this cultivar to biotic stress corresponded to the susceptible cultivar, which showed that the inoculation was higher stress than for the resistant 'Vicar of Winkfield' cultivar.

Several authors reported that the higher level of **polyphenol-oxidase** (PPO) enzyme activity associated with higher level of resistance to various pests and pathogens. In our experiments the control PPO activity level in the fruits of the resistant cultivar ('Vicar of Winkfield') was higher than in the susceptible cultivar ('Max Red Bartlett'). In the susceptible cultivar 'Kieffer' we measured similarly high level of enzyme activity than in the resistant cultivar. Similar to POD activity, the highest PPO enzymatic activity was measured in the moderate resistant 'Hosui' Japanese pear cultivar. According to our opinion, the PPO levels in control tissues could rather be determined genetically than a marker of resistance, which was also described in earlier works by Melo et al. (2006).

Similar to POD activity, the susceptible cultivar responded earlier to stress, the PPO enzymatic activity significantly increased *in the infection point* 48. hours after inoculation, that further increased later. In the resistant cultivar the PPO activity increased later – only at 72. hours –, and it reacted with less increase in PPO enzyme activity than in the susceptible cultivars was measured.

First of all the changes (decrease) of glucose but also of the sucrose content characterized well the disease process in the tissue of susceptible/resistant fruit. Our experiment showed that in the fruit tissues of the susceptible cultivar the glucose and sucrose content dropped faster – what the bacterium used for the metabolic process – than in the resistant one.

Changes of **polyphenol content** can give us information about defense mechanism, while plants produce these components for their own defense system in synthesis of secondary metabolites. In earlier works there are many publications about accumulation of phenolic components and its quantity increases faster after infection in resistant plants than in susceptible cultivars.

In the unripe fruits the polyphenol content also showed significant correspondence with the stress response. In the resistant cultivar the amount of polyphenols raised around the inoculation point, which seemed to be enough protection during the disease process. However, the susceptible cultivar did not synthesize enough polyphenols for self protection.

Biochemical changes in leaves and shoots

In our first experiment higher control **peroxidase** enzyme activity (POD) was measured in the shoots of the resistant (’Kieffer’) cultivar than in the susceptible ('Packham’s Triumph') cultivar. After inoculation an increase in POD activity could be detected after 1 and 5 days after infection in the susceptible cultivar. In contrast, in the resistant cultivar the POD activity decreased by the first day, and later it did not either change. So, in these dates the inoculation could not cause any stress response regarding enzyme activity.

Thus, in order to detect the early stress response, we included more sample taking dates in the intervals of 0-48 hours to monitor the changes of the biochemical parameters in the leaves and shoots of grafted trees.

After *E. amylovora* infection both genotypes responded with increased peroxidase enzyme activity, but the level and start of increase was different in the susceptible (’Packham’s Triumph’) and the resistant cultivar (’Kieffer’).

First increase of POD activity was detected in the resistant cultivar in the leaves above the inoculation point; in control tissues (treated only with distilled water) it was occurred 1

hour after inoculation, and in the infected leaves it was measured 30 minutes after inoculation. This correlates with visual symptoms that appear also above inoculation. We could conclude that the plant reacts both to abiotic and biotic stresses, but higher increase in POD enzyme activity could be measured after biotic stress.

In the susceptible cultivar less and the same level of increase in POD activity was measured in control and in infected tissues 48 hours after inoculation. Similar host response to mechanical stress and inoculation was described also in earlier works (Torres et al., 2003). In the resistant cultivar in every leaves the POD activity reached the maximum level 24 hours after infection. In the susceptible cultivar we measured less increase 12 hours (leaves below inoculation), and 48 hours (in other leaves) after infection.

In the shoots and the leaves the change of the total **polyphenol content** did not prove to be such a good marker for biotic stress as the peroxidase enzyme.

In the **micropropagated plants** the changing peroxidase activity also proved to be a good marker for detection of susceptible/resistant host response.

There are numerous literature about the change of the **carbohydrate** metabolism due to a mechanical stress or a pathogen infection. After biotic stress, changes of sugar fractions were observed in leaves in various plant - pathogen interactions.

In the leaves of the grafted plants the changing of glucose and sucrose content after the infection characterized well the development of the disesase. In the leaves of the susceptible cultivar the glucose content decreased, which occurred half an hour after inoculation. After the initial decrease – probably due to a transport process – the starting glucose content set back to original content, then decreased again till 24 hours after infection. Decrease and exhaustion of resources can be explained by the fast metabolism due to infection. Another cause of the decrease was that the bacterium multiplied in the tissues and used glucose for its own metabolic process

In the contrary, in the resistant cultivar the glucose content raised even 1 hour after inoculation, which continuously increased by 12 hours in leaves below inoculation. In the leaves above the infection point the glucose concentration was higher than the initial by 24 hours.

Different trends were observed in **sucrose** content: in the susceptible cultivars the sucrose content diminished while in the resistant cultivars it rose. In the **shoots** the stress response did not show significant difference between the resistant and the susceptible cultivars, since in both genotypes the sucrose content increased after inoculation. Therefore we do not consider shoots to be suitable for testing susceptibility/resistance of the cultivars.

New scientific results

1. In Hungary, fire blight susceptibility of various pear cultivars was evaluated the first time. In the experiments more than 40 cultivars were tested.
2. Flower organ inoculation and evaluation methods of pear cultivars to *Erwinia amylovora* were elaborated. Degree of resistance/susceptibility of cultivars can be accurately characterized by symptoms on receptacle and on the calyx.
3. Inoculation and evaluation methods in literature were modified and completed with bacteria cell multiplication and reisolation in shoots.
4. Little information is available on susceptibility of pear fruits; my work fills this gap by reporting new data.
5. Pear cultivars were grouped according to their summarized data of susceptibility (flowers, shoots and fruits) into categories (moderately resistant, moderately susceptible, susceptible, very susceptible).
6. The disease process could be monitored by means of biochemical markers:

   a, Among the biochemical parameters examined the changes of peroxidase enzyme activity characterized best the susceptible/resistant host response to fire blight infection in fruits, shoots, leaves and in the micropropagated plants.

   b, Among carbohydrates, the changes of glucose and sucrose monitored well the disease process of susceptible and moderate resistant cultivars, in the fruits and leaves.

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Publications in conference reviews

International conferences, full paper

1. Hevesi, M., Göndör, M., G. Tóth, M., Kása, K., **Honty, K.** 2002. Susceptibility of pear cultivars commercially grown in Hungary to the bacterial pathogen *Erwinia amylovora*. Proceedings 8th Symposium „New Aspects of Resistance Research on Cultivated Plants" Bacterial Diseases, November 15-16, 2001. Achersleben, Germany Beitrage zur Züchtungsforschung Bundensanstalt für Züchtungsforschung an Kulturpflanzen, pp. 74–77.
2. Hevesi, M., Göndör, M., Kása, K., **Honty, K.,** G. Tóth, M., 2004. Traditional and commercial apple and pear cultivars as sources of resistance to fire blight. EPPO Bulletin 34: 377-380.
3. Hevesi, M., G. Tóth, M., Göndör, M., Papp, J., **Honty, K.,** Kása, K. 2006. Development of eco-friendly strategies for the control of fire blight in Hungary.
4. **Honty, K.,** Göndör, M., Hevesi, M., G. Tóth, M., Kása, K. 2006. Susceptibility of pear cultivars to fire blight in Hungary. Acta Horticulturae, 704: 583-587.
5. **Honty K.,** Hevesi, M., Sárdi, É., Stefanovits-Bányai, É., Tóth, M. 2008. Effect of *Erwinia amylovora* infection in biochemical changes if different pear fruits. Acta Horticulturae, 800: 879-884.

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1. Göndör, M., **Honty, K**., Hevesi, M., Szabó, N., Sárdi É. 2004. Study of carbohydrates on infected pear fruits with *Erwinia amylovora*. International Conference on Horticulture Post-graduate (PhD.) Study System and Conditions in Europe, Lednice, 2004. november 17-19. Abstracts, p. 19.
2. **Honty, K.,** Nagyné-Sárdi, É., Stefanovitsné Bányai É., Tóth, M. 2007. Effect of *Erwinia amylovora* infection on biochemical changes of different pear fruit, 10th International Pear Symposium 22-26. May. 2007. Peniche, Portugalia, Abstracts, p. 78.

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4. **Honty, K**., Göndör, M., Hevesi, M., Tóth, M., Kása, K. 2004. Susceptibility of pear cultivars to fire blight in Hungary, 10th International Workshop on fire blight, Bologna, Italy 5-9. July 2004. Abstracts, p. 110.

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1. Hevesi M., Papp J., Göndör Jné, G. Tóth M., Kása K., **Honty K**. 2002. Termesztett körtefajták fogékonysága az *Erwinia amylovora* baktériummal szemben. Növényvédelmi Tudományos Napok. Növénykórtan Szekció. Budapest. Összefoglaló, p. 77.
2. Hevesi M., Jámbor-Benczúr E., Göndör M., **Honty K.**, Balla I., G. Tóth M., Kása K., Deli Zs. 2003. Mikroszaporított alma- és körtefajták fogékonysága tűzelhalás (*Erwinia amylovora*) betegséggel szemben. XIII. Keszthelyi Növényvédelmi Fórum. Keszthely. Összefoglaló, p. 62.
3. **Honty K**., Göndör M., Hevesi M. 2003. Újabb eredmények a körtefajták *Erwinia amylovora* baktériummal szembeni fogékonyságáról. Növényvédelmi Tudományos Napok. Növénykórtan Szekció. Budapest. Összefoglaló, p. 98.
4. **Honty K.,** Göndör M., Hevesi M. 2003. Körtefajták fogékonyságának vizsgálata az *Erwinia amylovora* fertőzés hatására. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. 2003. november 6-7. Összefoglaló, p. 320.