# Susceptibility of Austrian Apricot and Peach Cultivars to ESFY

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**Abstract**

1548 stone fruit trees (1435 trees of *P. armeniaca*, 113 trees of *P. persica)* were examined by PCR for ESFY to get information on spread and susceptibility of cultivars and rootstocks used in Austrian stone fruit production. Cultivar susceptibility seems to be less important for tolerance to ESFY than rootstock resistance. Apricot cultivars on rootstocks of myrobalan, commonly used in Austria, are more infected than cultivars on plum rootstocks. Data on peach and apricot rootstocks are not representative as both are not commonly used in Austrian apricot production. In addition, the presence of peroxidase activity in shoot sieve tubes of infected apricot trees (Hungarian Best) reveals that peroxidase is involved in defense mechanisms in plant-pathogen interaction.

**Keywords**: ESFY; stone fruit trees; cultivar susceptibility; rootstock susceptibility; Austria

## INTRODUCTION

Apricot chlorotic leaf roll (ACLR) first described by MORVAN (1977) occurs in most countries of Central and Southern Europe. This economically important disease, which belongs to European stone fruit yellowing (ESFY – LORENZ *et al*. 1994), affects the vigour of stone fruit trees (*P. armeniaca*, *P. persica*) whose fruits cannot be commercialized because of their small size and their poor taste. Symptoms of decline, yellowing, longitudinally upward rolling of leaves, and sparse foliage are typical for infected apricot and peach trees. However, as many infected apricot trees are symptomless (RICHTER 1999), spread of disease in Austrian orchards is enormous. In Austria, 35.5% of apricot and 41.7% of peach orchards are infected with ACLR; the amount of contaminated apricot trees per orchard ranges from 7 to 30%, that of peach trees from 6 to 15% (SCHÜTZ *et al*. 2002, unpubl.). As direct measures against ESFY pathogens are not available or registered, the most promising means is to grow tolerant or even resistant plants. Thus, cultivars and rootstocks used in Austria for apricot and peach production were tested for their apricot susceptibility. Furthermore, the localization of peroxidase activity was investigated by histochemical technique to check the causal effect of susceptibility and defense mechanisms in plant-pathogen interaction.

## MATERIALS AND METHODS

***Plant material***. 1435 apricot and 113 peach trees collected from all major apricot growing regions in Austria (from 109 apricot and 14 peach orchards or sites) were sampled in late summer 1999–2001. Samples were collected randomly without taking notice of ESFY symptoms.

***Diagnosis***. Total DNA was isolated from shoots and leave midribs according to the protocol published by AHRENS and SEEMÜLLER (1992). A fragment of the 16S rDNA gene was used as template DNA for PCR amplification with the universal primer pair fU5/rU3 (positive signal: 874-bp) and the ESFY specific primer pair fAT/rPRUS (positive signal: 505-bp, LORENZ *et al*. 1995).

***Histochemical investigations (peroxidase activity)***. Stem tissue of one-year old apricot shoots of infected and healthy Hungarian Bests was subsequently dissected into little, 1 $mm^2$ large pieces and put after vacuum infiltration (p = 55 mbar) for 2h in a freezing compound (Tissue Freezing Medium, Jung). The tissue, chosen for the experiment, was optically free

of necrotic cells. The samples, frozen at 16°C, were then cut into 12$\mu$m thick sections with a freezing-stage microtome (Leica CM 1800). The sections were subsequently stained with 3,3',5,5'-tetramethylbenzidine (TMB, Sigma) according to the protocol of DAI *et al*. (1995). Sections were mounted in glycerine: water (15:85, v/v) and examined by light microscopy (Nikon Labophot-2) with two filter sets, a UV filter set with 330–380nm excitation and a 420nm barrier filter and a blue filter set with 450–490nm excitation and a 520nm barrier filter.

## RESULTS

***PCR amplification***. All apricot and peach cultivars examined are listed in Table 1, rootstocks are summarized in Table 2. As peach is not important in Austrian stone fruit production data on peach cultivars are spare. The target sequence of all positives could be amplified with primer pair fU5/rU3 and/or fAT/rPRUS. In apricot, amplification products could be obtained in ca. 50% samples derived from trees showing non-specific symptoms whereas in peach the presence of ESFY phytoplasms was highly correlated with symptoms. For percentage of infection in peach and apricot cultivars and apricot rootstocks see Figures 1 and 2; for data in rootstock/cultivar combination of diseased apricot trees (Figure 3).

Table 1. Number of tested, infected and healthy cultivars

| Tested cultivars | *n* | Neg. | Pos. |
|---|---|---|---|
| Hungarian Best | 609 | 470 | 139 |
| Klosterneuburger/Hungarian Best | 125 | 97 | 28 |
| Bergeron | 251 | 181 | 70 |
| Goldrich | 152 | 138 | 14 |
| Hargrand | 87 | 71 | 16 |
| Rouge de Fournes | 56 | 52 | 4 |
| Wahre Große Frühe | 28 | 20 | 8 |
| Orangered | 19 | 13 | 6 |
| Dürkheimer (from two orchards, not used for statistic) | 16 | 4 | 12 |
| Aurora | 14 | 12 | 2 |
| Polonais | 12 | 7 | 5 |
| Kremser Rosenmarille | 12 | 11 | 1 |
| Unimported Cultivars (Pisana, Ananas-marille, Harval, Kittseer Frühe etc.) | 54 | 38 | 16 |
| *n* | 1435 | 1114 | 321 |
| Red Haven | 94 | 84 | 10 |
| Unimported Cultivars (Sun Crest, Dixi Red, Spring Lady etc.) | 19 | 18 | 1 |
| *n* | 113 | 102 | 11 |

Table 2. Number of tested, infected and healthy rootstocks

| Tested apricot rootstocks | *n* | Neg. | Pos. |
|---|---|---|---|
| Torinel Avifel | 198 | 155 | 43 |
| St. Julien (655-2, INRA 2) | 201 | 174 | 27 |
| *Prunus domestica* | 32 | 29 | 3 |
| Brompton | 6 | 6 | 0 |
| Myrobalane | 302 | 202 | 100 |
| Mariana GF8-1, Plumina | 2 | 2 | 0 |
| BVA | 63 | 61 | 2 |
| *Prunus armeniaca* | 15 | 13 | 2 |
| *Prunus pumila* | 15 | 15 | 0 |
| *n* | 834 | 657 | 177 |

***Histochemistry***. Sieve tube cells of shoot tissue of Hungarian Best reacted with TMB, indicating peroxidase activity. Cell walls and protoplast emitted a yellowish-blue color under UV and a blue fluorescence under blue light. No reaction was observed in shoot sieve tubes of healthy apricot trees.

## DISCUSSION

Symptom expression of ACLR is known to be considerably influenced by rootstock and cultivars. Previous studies by artificial pathogen inoculation (AUDERGON *et al*. 1991; KISHON & SEEMÜLLER 2001) have shown that apricot, Japanese plum, and peach are more susceptible to ACLR than rootstock Mariana GF8-1 (*P. cerasifera* × *P. munsoniana*), and that myrobalan (*P. cerasifera*) and genotypes of *P. domestica* (Ackermann, Brompton) are little affected by this phytoplasmic disease. The data obtained in the present study largely confirm these results. Cultivars on rootstocks of myrobalan, commonly used in Austria, are more infected than cultivars on plum rootstocks (Figures 2 and 3). For plum, St. Julien (especially St. Julien 655-2 in comparison to St. Julien INRA 2) and some *P. domestica* varieties like Brompton are more tolerant than Torinel. Cultivars on BVA seem

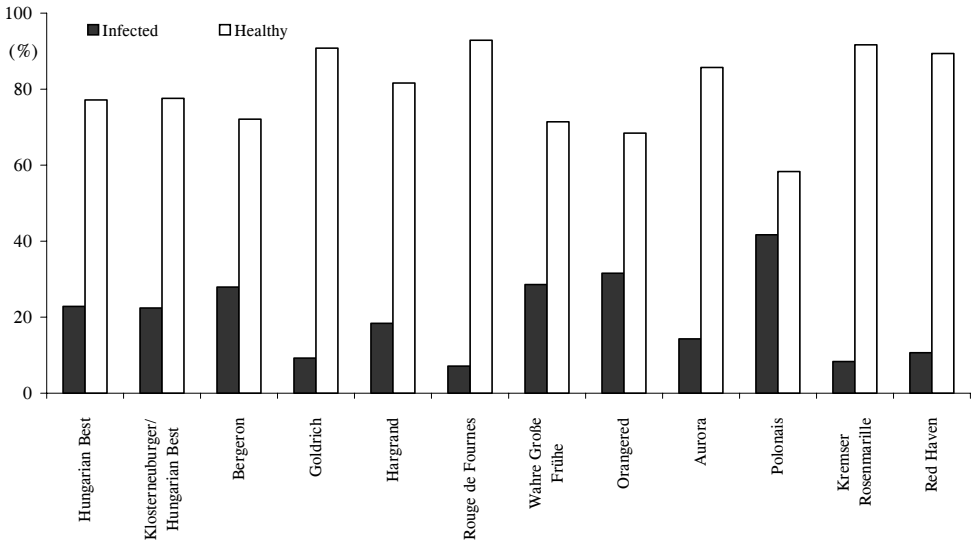


Figure 1. Percentage of infected apricot and peach cultivars in Austrian stone fruit orchards

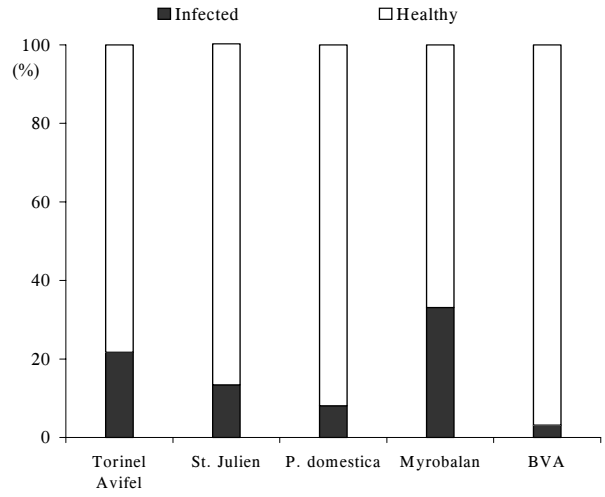


Figure 2. Percentage of infected apricot rootstocks

to be less infected; but be aware, in contrary to the other rootstocks, BVA plants originate from few commercial growers. A comparison between peach and plum/myrobalan rootstocks is therefore not efficient. The same situation is found within cultivars with less than 30 samples. In general, cultivar susceptibility (Figure 1) seems to be less important for tolerance to ESFY than rootstock resistance; infection of Bergeron results in high mortality of trees whereas Rouge de Fournes seems to be rather insensitive to phytoplasm infection. Mortality and expression of symptoms was moderate in Hungarian Best, Klosterneuburger and Hargrand. Goldrich is in Austria mainly grafted on Torinel and St. Julien; therefore the low mortality of Goldrich may be rather due to high tolerance of plum rootstocks than to cultivar susceptibility.

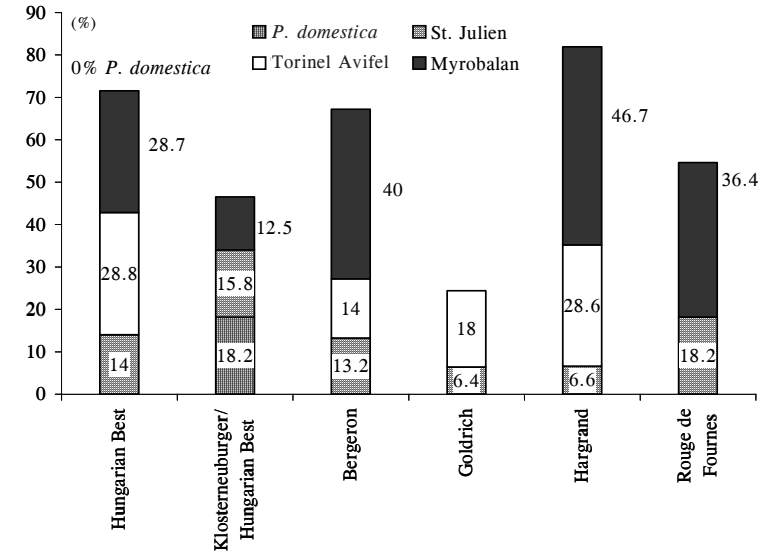


Figure 3. Percentage of infected apricot trees: rootstocks – cultivars

Histochemical tests, made to gain information on cultivar susceptibility, showed that shoot sieve tubes of infected apricot trees of Hungarian Best contained abundant isoenzymes of peroxidase. Peroxidase activity involved in defense mechanisms is thought to take part in an intensified process of production of toxic compounds postulated to cause early killing of infected cells and formation of a barrier to microbial pathogen spread during plant pathogen interaction (MÄDER & AMBERG-FISHER 1982; MÄDER & FÜSSL 1982; STICH & EBERMANN 1988). The necrosis of sieve tube cells in heavily phytoplasm infected plant tissue may support this hypothesis.

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