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# Symptomatological detection and biochemical changes of the trees infected by apricot chlorotic leafroll phytoplasma

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## Abstract

The ecological conditions in the South-Eastern part of Romania are very favorable for the apricot culture (*Prunus armeniaca*). Unfortunately, the same conditions are favorable also for some pathogens (viruses, phytoplasma, bacteria or fungi), the causal agents for many dangerous diseases. The final syndrome of this diseases complex is known as apoplexy or premature dye back of the apricots. Recognition of this diseases complex is very important for the study and cure of the phenomena. If for the majority of the apricot diseases the symptoms are easy to be evidenced, the ones produced by apricot chlorotic leafroll phytoplasma, a key pathogenic agent in premature dye back of the apricots, are more difficult to be distinguished. This paper refers to the researches carried out in order to realize the symptomatological detection of these phytoplasma presence and the quantification of the biochemical changes in the leaves of some apricot cultivars with different degrees of susceptibility to apricot chlorotic leafroll, compared with the leaf biochemical pattern of the same healthy cultivars. The biochemical changes studied are referred to the leaf cell plasma content in polyphenol-oxidase, total phenols and tannins, free amino-acids, soluble dry mater, water, carbohydrates and assimilation pigments.

**Key words:** biochemical changes, polyphenol-oxidase, phenols, tannins, free amino-acids, photoasimilate pigments.

## Introduction

Every year, in the South-Eastern part of Romania, an important number of apricots die from unknown diseases. Researches carried out in many European countries mention that on this fruit species, 80% of the dead trees or of the suffering ones, are infected by the apricol chlorotic leafroll (ACLR) (Cornaggia *et al*., 1994; Jarausch *et al*., 1998; Labonne *et al*., 2000). ACLR is a quarantine disease (OEPP/EPPO, 1986) belonging to the phytoplasma group (Morvan, 1977). The disease was also evidenced on many fruit species, although some of them (plum) are not showing symptoms (Németh, 1986).

The researches carried out during 2006-2010 were focused mainly on detection of the disease symptoms under the eco-pedo-climatic conditions from Research Station for Fruit Growing Constanța, for early and correct diagnosis of this disease. Moreover, during the period of study, were the biochemical changes that occurred in the cellular juice of the leaves sampled were assessed from the ACLR-diseased trees compared to the biochemical changes occurred in the cellular juice of the leaves sampled from healthy trees.

## Materials and methods

In order to describe the symptoms produced by natural infections with ACLR, periodical observation were done during vegetation and tree dormancy period. Trees with symptoms were marked and details of external and internal macroscopic display of pathogen and their evolution in time were observed for an easier recognition of the disease in the commercial apricot orchards.

For the quantification of the biochemical changes in the leaves of the apricot cultivars the biological material used in this study was represented by some apricot cultivars among those more commonly grown in Romania (Goldrich, Harcot, Dacia, Earliril, Royale and Olimp) integrated in four different classes of resistance (function of the F% and I value faced to the presence of the ACLR) trough natural infection. Biochemical analyses of the cellular juice from leaves were performed during two vegetation periods: in fully activity (June) at the end of the period (October). The determinations were mode using the known methodologies and they refer to the content in:

- Polyphenol-oxidase - titrimetical method.
- Total phenols, spectrophotometrical method.
- Tannins, lowenthol, Neubeur method.
- Free amino-acids, spectrophotometrical method.
- Dry matter, gravimetrical method.
- Assimilate pigments, Hagarsi Bertensath method.
- Carbohydrates-titrimetrie by Bertland, Jijin method.
- Crude protein, spectrophotometrical method.

## Results and discussion

In the spring, the ACLR symptoms are mainly the early leafing, preceding or occurring along with the blooming, the phenomena being spotted first in one or two branches.

When the summer starts, the leaves from the attacked branches are smaller in size with green-yellow color. The typical symptoms occurs at the end of the summer, when the apricot leaves roll along the main stalk and vein, develop a cone (or spoon) shape, and fall prematurely. The fruits, numberless, are smaller, slight asymmetric, and rip later or remain green and prematurely fall. The apricot branches growth slows down, the internodes are shorter and after a variable tie period the internodes are drying. A section trough these branches evidence the phloem oxidation and necrosis, in the structures where the pathogen develops (Morvan, 1977). In the orchard, the disease either occurs in isolated apricots, or affects the trees in groups. As regard the transmission, the researches did not identify *Cacopsylla pruni* species as vector (Carraro *et al*., 1998), but revealed the disease transmission with the shoots used for grafting or budding from diseased apricot trees.

As regard the biochemical changes the content in proteins ferments-polyphenol oxidase was found in higher quantities in cultivars showing resistance to ACLR., compared to smaller quantities in the susceptible ones (table 1). This can be explained by the fact that the polyphenol-oxidase plays a role in the catalase synthesis of the phytoalexins and other components as indicators of the infection process.

**Table 1.** The content of poliphenols, total phenols and tannoid substances of some apricot cultivars.

| Cultivar | Resistance | Polyphenol oxidase | Tannoid substances | Total phenols |
|---|---|---|---|---|
| Goldrich | V.S.A. | 0.35 | 2.98 | 80.0 |
| Harcot | S.A. | 0.30 | 2.78 | 73.0 |
| Dacia | M.A. | 0.25 | 2.67 | 62.6 |
| Royale | H.A. | 0.15 | 2.50 | 50.0 |
| Earliril | V.H.A. | 0.10 | 2.48 | 42.0 |
| Olimp | V.H.A. | 0.05 | 2.40 | 40.0 |

The content in tannoid substances, that plays a role in the inhibition of the infection process is higher in the resistant cultivars. The phenols substances are the first ones opposing to reproduction of the pathogen in the plant. Through their oxidation the chinons obtained, are very toxic substances also for the pathogenic agents.

Regarding the free amino-acids content, our results have evinced that the most susceptible varieties have a higher content. This fact may be explained both by the reduction in the protein synthesis and the increase in the protein decomposition due to the disturbance induced by the pathogenic agent.

As for the dry substance content the higher resistant cultivars have presented an increased content in terms of mean annual value (table 2).

There is a direct correlation between the water content of the leaves and the different behavior of the apricot cultivars under the ACLR infection.

The quantity of a; b; c forms of assimilation pigments, especially of the (a+b)/c ratio are an obvious indicator regarding the appearance of some factors which disturb the normal methabolic processes. Thus, the (a+b)/**c** ratio was much smaller in the susceptible cultivars than in the resistant cultivars.

**Table 2.** The content in dry substance and total water in some apricot cultivars.

| Cultivar | Wet weight | Dry weight | Dry subst. | Total water |
|---|---|---|---|---|
| Goldrich | 53.0 | 18.7 | 35.3 | 64.7 |
| Harcot | 68.0 | 23.2 | 33.6 | 66.4 |
| Dacia | 54.0 | 17.3 | 32.1 | 67.9 |
| Royale | 55.0 | 26.1 | 32.0 | 67.9 |
| Earliril | 41.0 | 12.9 | 31.9 | 68.1 |
| Olimp | 46.0 | 14.2 | 26.3 | 73.7 |

The percentage variation of the glucids in the resistant cultivars presented an elevated quantity of direct reducting and total forms. The mean value of crude protein (%) is decreasing with the increasing of the sensitivity to the ACLR infection. This could be explained by the reduction in the capacity of the proteins synthesis and the intensification of the proteins decomposition in the case of some cultivars whit a higher susceptibility to the disease.

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