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The Response of Several Plum Cultivars to Natural Infection with *Monilinia laxa, Polystigma rubrum* and *Stigmina carpophila*

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Abstract

The response to the attack of *Monilinia laxa, Polystigma rubrum* and *Stigmina carpophila* in natural conditions of infection of 13 plum cultivars were evaluated during three years, in a commercial orchard located in North-Western of Romania. The studied cultivars were: 'Topfirst', 'Nectarina rosie', 'Tuleu timpuriu', 'Hangata', 'Toptaste', 'Tuleu gras', 'Vinete de Italia', 'Stanley', 'Vinete romanesti', 'Tophit', 'Jojo','Anna Späth', 'Topend'. The highest degree of flower attack by *Monilinia laxa* was recorded for 'Stanley', while the lowest attacks were registered on 'Topend', 'Jojo', 'Tophit' and 'Nectarina rosie'. The best response regarding the attack on fruits proved to be with 'Topend', 'Jojo', 'Tophit' and 'Topfirst'. These cultivars registered small values of attack degree (0.7-3.3%). The cultivars with the lowest attack degree of red staining caused by *Polystigma rubrum* were 'Top End', 'Jojo', 'Tophit', 'Toptaste', 'Nectarina rosie' and 'Topfirst'. A high degree of attack was calculated on 'Anna Späth', 'Vinete romanesti', 'Vinete de Italia' and 'Tuleu timpuriu'. These cultivars prove to be very sensitive to the red staining disease in natural conditions of infections. Cultivars 'Top End', 'Jojo', 'Tophit', 'Toptaste', 'Nectarina rosie' and 'Topfirst' were slightly attacked by *Stigmina carpophila* (5.3-7.3% attack degree). The low infections levels recommend the mentioned cultivars as possible genitors in plum breeding programs, for creating new genotypes with good response to diseases attack.

Keywords: blossom blight, disease resistance, pathogen, plum breeding, plum-leaf blister, shot-hole, tolerance

Introduction

There are over 60 major pests and diseases that attack plums, including 4 bacteria, 19 fungi, 6 viruses, 4 nematodes and 36 insects (Janick and Paull, 2008). Plum diseases that commonly occur year after year in both commercial and backyard plantings of plum in Romania are brown rot, red spots and shot-hole.

Blossom blight and brown rot of stone fruit is caused by *Monilinia fructicola* or *M. laxa*, being a common and destructive disease of stone fruit (Rungjindamai *et al.*, 2014).

Plum-leaf blister is caused by *Polystigma rubrum*, a pyrenomycetous fungus which forms thick, fleshy and reddish patches on leaves. 'Vinete romanesti' a well-known cultivar in Romania, is considered very susceptible to plum leaf blister attack in ecological condition of Transylvania (Sestras *et al.*, 2007).

Shot-hole disease of plum is caused by the fungus *Stigmina carpophila* (Lév.) (Ellis, 1959); it is also known as *Wilsonomyces carpophilus* (Lév.) (Adask *et al.*, 1990) and *Coryneum beijerinkii* Oudem., anamorph of the genus Mycosphaerella (Ascomycota, Mycosphaerellaceae) (Bubici *et al.*, 2010).

These plum diseases are most difficult to control in years with high temperature, high humidity, abundant rainfall and intense cloud-cover. Usually, these diseases can be effectively managed by combining culture technology and growing resistant cultivars. A proper disease management involves selection and planting of varieties with genetic resistance to specific diseases. Genetic forms of control will become more important than pesticide resistance, as declining access to registered chemicals and consumers' demand for pesticide-free fruit will combine in order to remove current chemical solutions (Topp *et al.*, 2012).

Summaries of resistant cultivars (Ramming and Cociu, 1991) and genetic sources of resistance (Okie and Weinberger, 1996) are available for the economically important diseases and pests.

Hartmann and Neumüller (2009) presented the stages in breeding resistant cultivars; primarily, the first step is detecting genetically fixed differences in the behaviour of a single genotype of the respective species against a particular pathogen. The more genotypes can be tested, the higher is the probability of finding resistance and/or tolerance to the disease or pest. National gene banks can be used for obtaining a broad spectrum of different genotypes.

For this kind of large-scale testing, a reliable resistance test has to be developed. Resistant genotypes must be selected in order to use them as crossing partners (Okie and Ramming, 1999). Either in advance or simultaneous with a resistance breeding programme, the life cycle of the pathogen and the kind of reaction of the plant against it must be investigated. Before releasing a new variety, the respective genotype has to be tested under natural inoculation conditions on different sites, for several years.

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The aims of this investigation were to study the response of 13 plum cultivars regarding the most important fungus diseases of plum under conditions of natural infection, to record penetration and diseases expression. A better understanding of these aspects will be useful in selecting suitable genitors in order to obtain new plum cultivars resistant to diseases.

Materials and methods

The research has been carried out at Lunca Farm, Calacea, Sălaj County, North-Western of Romania, in a commercial orchard, during 2012-2014. The orchard had a density of 1,000 trees/ha.

As biological material there have been taken under study 13 old and new cultivars of plum as follow: 'Topfirst', 'Nectarina rosie', 'Tuleu timpuriu', 'Hangata', 'Toptaste', 'Tuleu gras', 'Vinete de Italia', 'Stanley', 'Vinete romanesti', 'Tophit', 'Jojo', 'Anna Späth', 'Topend'.

A complete randomized experimental design with five replicates (trees) was used for sampling trees.

The level of attack was determined by Frequency (F%) and Intensity (I%) of attack, in natural conditions of infections. Thus, the Attack Degree (AD%) was calculated with the formula: AD%=(F% x I%)/100 (Baciu *et al*., 2010; Cociu and Oprea, 1989), representing expressly the extension of the attack seriousness, as a mean for the tree years under study.

The Frequency of attack (F%) was determined by dividing the number of organs (leaves or fruits) affected by disease (n) to the total number of organs analysed (N), the formula being: F% = n / N x 100.

The Intensity of attack (I%) represents percentage assessed for every tree, with the formula: $I\% = \sum(i \times f) / n$, where 'i' represented the percentage of coverage with symptoms per organs, 'f' was the number of cases with symptoms framed in certain percentage and 'n' was the number of disease affected organs.

The method used to identify the infections was based on visual observation, considering the signs and symptoms shown by infected plants.

The symptoms observed on the plum trees were noted on fruits, twigs and blossoms, and were caused by *Monilinia laxa,* red spots (caused by *Polystigma rubrum)* and red spots and shot-hole (produced by *Stigmina carpophila* (Lév) M.B. Ellis, sin. *Ascospora beijerinckii*, newly named as *Wilsonomyces carpophilus*).

The observations on the intensity and frequency of attack were made on leaves, fruits, flowers and fruit. Interpretation of results was done by analysis of variance (ANOVA test). Climatic data from Salaj Water Management System were provided.

Results and discussions

From the climatic point of view one can say that clime was within the normal conditions for the experimental region. The variations of temperature ranged between the typical limits. In June and July the value of temperature became higher. Also, a pattern was noted, that the temperature increased year by year (Fig. 1).

In the period 2012 and 2014 mean temperature values were high and similarly during April, period when the conidia started to germinate and grow. The temperature values created good conditions for evolution of the studied fungus.

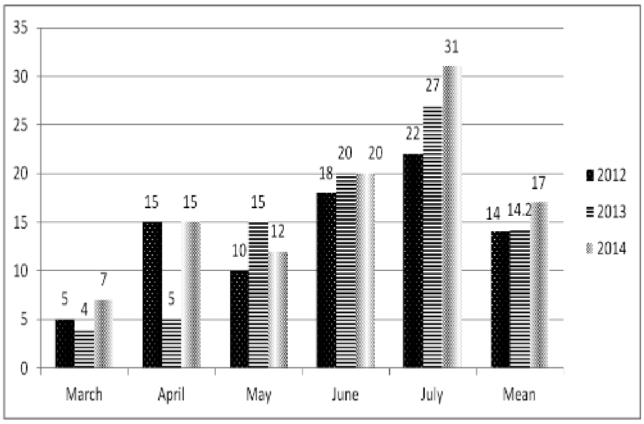

Fig. 1. Mean monthly temperatures in the experimental years (°C), Salaj Water Management System

In terms of fallen precipitation level, one can say that the mean monthly values decreased year by year (Fig. 2). Never the less, the humidity needed for fungus development was assured by natural conditions, therefore the evolution of fungus could be considered to have normal climatic conditions.

Brown rot, caused by the fungus *Monilinia laxa*, is a problem in all regions with rainfall conditions during flowering or fruit development. There are no commercial cultivars with complete resistance, so that do not require fungicide treatments under wet conditions.

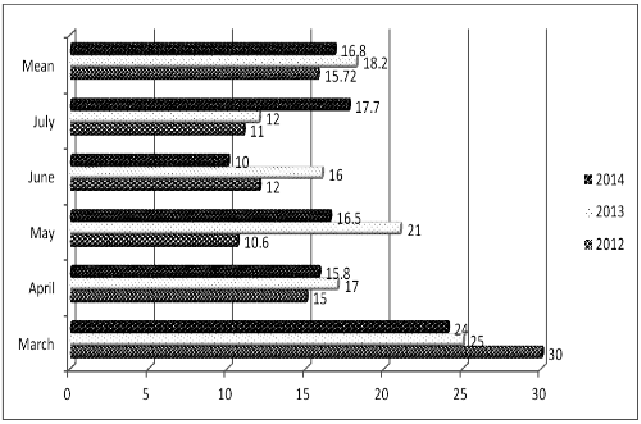

Fig. 2. Mean monthly precipitations in the experimental years (mm), Salaj Water Management System

Brown rot is the most common and destructive disease of plum in north-western part of Romania. The disease is especially severe in wet, humid weather. Brown rot causes blossom blights, twig blights, twig cankers and fruit rots.

The attack of brown rot on the flower is presented in Fig. 3. The highest degree of attack was recorded on 'Stanley', this cultivar registering very significant differences compared to the control (mean of experience). The results are in agreement with the data obtained by Minoiu (1997). On the other hand, Crawford (1997) listed 23 European plum cultivars which had some level of resistance and describeed 'Stanley' as very resistant. In the natural infections conditions of the current experiment

Table 1. The response of plum cultivar to leaf blister (*Monilinia laxa*) attack on fruits, under natural infection conditions

Cultivar	Degree of attack (%)	Degree of attack as to control (%)	Differences as to control (%)	Significance of difference
'Topfirst'	3.3	26.6	-9.2	ooo
'Nectarina rosie'	6.7	53.2	-5.9	oo
'Tuleu timpuriu'	34.3	273.8	21.8	***
'Hangata'	18.0	143.6	5.5	*
'Toptaste'	6.0	47.9	-6.5	oo
'Tuleu gras'	27.3	218.0	14.8	***
'Vinete de Italia'	8.0	63.8	-4.5	o
'Stanley'	31.3	249.9	18.8	***
'Vinete romanesti'	7.0	55.8	-5.5	o
'Tophit'	1.3	10.6	-11.2	ooo
'Jojo'	1.3	10.6	-11.2	ooo
'Anna Späth'	17.7	140.9	5.1	*
'Topend'	0.7	5.3	-11.9	ooo
Mean of experiment	12.5	100.0	-	

LSD 5% = 4.3
LSD 1 % = 5.9
LSD 0.1 % = 7.9

'Stanley' proves to be very sensitive to brown rot on flowers, and therefore this cultivar is not recommended as suitable genitor for inducing plum resistance to *Monilinia laxa*. In Bulgaria, 'Stanley', which used to be widely grown (Vitanova *et al.*, 2004) was reduced as surface coverage percentage in orchards because of sharka (*Plum pox virus*), but along with other cultivars like 'Čačanska Najbolja', 'Californian blue', 'Kishinevskaya rannaya' and 'Vengerka krupnaya sladkaya' could be grown in industrial plum orchards, if good horticultural practices are applied and secure monitoring assure an optimum seasonal fungicide treatment management (Iliev *et al.*, 2010).

The most resistant cultivar was 'Topend', which registered very significant negative differences compared to the control; it was followed by 'Jojo', 'Tophit', 'Nectarina rosie' with distinct significant differences. Statistically assured differences to the control were noted also for cultivars 'Topfirst' and 'Hangata'. All these cultivars could be considered as proper genitors for inducing resistance to *Monilinia laxa* on regard with the flower attack behaviour in this study.

The data presented in Table 1 show the response of the 13 plum tree cultivars to the natural infection with *Monilinia laxa* on fruits.

The cultivars 'Tuleu timpuriu', 'Stanley' and 'Tuleu gras' registered the highest attack degree of *Monilinia laxa* on fruits

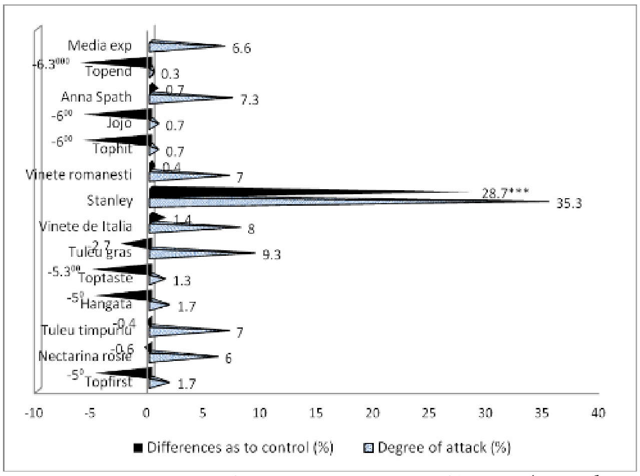

Fig. 3. The response of plum cultivar to leaf blister (*Monilinia laxa*) attack on flowers

(34.3-27.3%) showing very significant differences compared with the control (mean of experience).

The most resistant cultivars compared to the control, with very significant differences, proved to be 'Topend', 'Jojo', 'Tophit' and 'Topfirst'. These cultivars registered small values of attack degree (0.7-3.3%). The varieties 'Vinete de Italia' and 'Vinete romanesti' had a similar response to *Monilinia laxa* attack on fruits.

The cultivars which were very little attacked by brown rot on fruits are recommended in plum breeding programs, in order to obtain new varieties with tolerance or resistance to this fungus. Some cultivars, e.g. 'Jojo', can be a valuable genitor in plum breeding programmes aimed at obtaining varieties resistant also to the *Plum pox virus* disease (Żurawicz *et al.*, 2013).

Regarding red staining of plum (caused by *Polystigma rubrum*) the cultivars having the lowest degree of attack were 'Top End', 'Nectarina rosie', 'Jojo', 'Tophit', 'Topfirst' and 'Toptaste' (Table 2). The differences registered when compared with the control of the experiment were very significant, therefore these cultivars could be recommended for inducing plum tolerance or resistance to red staining attack. The cultivar 'Hangata' also registered statistically assured differences as to the control.

Higher degrees of attack were noted on 'Vinete romanesti', 'Anna Späth', 'Vinete de Italia' and 'Tuleu timpuriu', which had very significant differences as to the control. These cultivars proved to be very sensitive to red staining attack in natural conditions of infections in the experiment.

'Stanley' registered a medium attack degree (22.5%), but the differences registered as to the control are not statistically assured. Similar results were obtained by Berekmeri and Puia (2013) in climatic conditions of Reghin, Romania.

The response regarding the infections with shot-hole (or stone fruit, or stone hole), caused by *Stigmina carpophila* (Lév.) was significantly influenced by the cultivars (Table 2). The cultivars 'Top End', 'Jojo', 'Tophit' 'Toptaste', 'Nectarina rosie' and 'Topfirst' were very little attacked (5.3-7.3% attack degree) by this fungal pathogen. These low infections recommend the cultivars for breeding in plum disease resistance.

On the opposite, the cultivars 'Vinete romanesti', 'Anna Späth', 'Vinete de Italia', 'Tuleu timpuriu' and 'Tuleu gras' proved to be very sensitive to shot-hole caused by the fungus *Stigmina carpophila*.

Table 2. The response of plum cultivars to red staining of plum (*Polystigma rubrum*) and shot-hole (*Stigmina carpophila*) attack on the leaves, under natural infection conditions

Cultivar	Degree of attack (%)	
	Polystigma rubrum	*Stigmina carpophila*
'Topfirst'	7.3 ooo	7.7 ooo
'Nectarina rosie'	6.7 ooo	8.3 ooo
'Tuleu timpuriu'	37.0***	13.7***
'Hangata'	14.7 o	14.7 o
'Toptaste'	9.7 ooo	5.7 ooo
'Tuleu gras'	36.0***	15.3 -
'Vinete de Italia'	37.3***	54.7***
'Stanley'	22.0 -	17.3 -
'Vinete romanesti'	56.0***	56.3***
'Tophit'	7.0 ooo	5.0 ooo
'Jojo'	7.0 ooo	5.0 ooo
'Anna Späth'	51.0***	57.0***
'Topend'	5.3 ooo	3.7 ooo
Mean of experiment	22.8	20.3
LSD 5% =	6.6	6.9
LSD 1 % =	8.9	9.4
LSD 0.1 % =	11.9	12.6

Conclusions

The diseases caused by *Monilinia laxa, Polystigma rubrum* and *Stigmina carpophila* occur in natural infection conditions in the climatic conditions of North-western of Romania and produce significant damages. Because EU regulations imposed the reduction of fungicides in controlling plant diseases, alternative control methods became more emphasised (Rungjindamai *et al*., 2014). Among other methods, using resistant or tolerant cultivars to diseases attack can contribute to this goal. Creating new varieties tolerant or resistant to these diseases is a permanent goal in plum breeding, so that monitoring of the existing germplasm fund in the region, in terms of resistance to major diseases, in order to use them in hybridization, is a permanent objective in breeding activity. The low levels of infections indicated some cultivars as tolerant or with an adequate response to the attack of pathogens (e.g. 'Topend', 'Jojo', 'Top Hit' for *Monilinia laxa*; 'Top End', 'Jojo', 'Top Hit', 'Top Taste', 'Nectarina rosie' for *Polystigma rubrum* and *Stigmina carpophila*). These cultivars could be used in plum breeding for inducing tolerance or resistance to diseases caused by the pathogens.

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