

## SUSCEPTIBILITY OF APPLE CULTIVARS TO *BOTRYOSPHAERIA OBTUSA*

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**Abstract.** The study was carried out in an apple orchard and a laboratory of the Institute of Agriculture, Kyustendil, Bulgaria in seven consecutive years from 1998 to 2004. The apple cultivars Liberty, Cortland, Freedom, Gloster, Florina, Gala, Granny Smith, Smoothy, COOP – 10 and Golden Delicious were evaluated for susceptibility after artificial inoculation of annual growths (shoots) and fruits. Susceptibility of the cultivars Granny Smith, Smoothy and COOP 10 on rootstock M9 and MM106 were evaluated at natural infection in the orchards. The annual growths (shoots) and fruits of all evaluated cultivars were susceptible to *Botryosphaeria obtusa*. The shoots of the cultivars Liberty and COOP 10 were most susceptible to the black rot. It was established that the rootstock had a strong influence over the cultivars susceptibility. The cultivars COOP 10, Granny Smith and Smoothy were more susceptible when was grafted on rootstock M9 than on MM106.

**Key words:** apple, cultivars, diseases, black rot, susceptibility

### INTRODUCTION

Fundamentally three *Botryosphaeria specias* were established as agents of cancer and fruit rot on pome and stone fruits (Arauz and Sutton, 1989; Sutton, 1981; Britton and Hendrix, 1981, 1882; Brown and Britton, 1986; Rumbos, 1987). In Kyustendil region, Bulgaria, the most important cancer disease in apple orchards is *Botryosphaeria* cancer caused by *B. obtuse* (Borovinova, 2000). This pathogen causes cancers on trunks, branches, twigs and annual growths and black rot of fruits. The articles with results about susceptibility to *B. obtusa* of apple cultivars are not enough. Foster (1937) tested twenty-two apple varieties for comparative susceptibility of leaves to *Physalospora obtusa* and was founded that no variety of apple observed was immune, but some were more susceptible than others. The results of Latorre and Toledo (1984) showed that trunk susceptibility of apple to *B. dothidea* was dependent on cultivar, isolate and inoculation position. Red King Oregon was the most susceptible cultivar, followed by Red Spur and Starkrimson. Granny Smith, Granny Smith Spur and Golden Spur were the most resistant cultivars.

### MATERIAL AND METHODS

#### **Susceptibility of the cultivars to artificial infection**

The study was conducted in an apple orchard and a laboratory of the Institute of Agriculture Kyustendil, Bulgaria, in seven consecutive years from 1998 to 2004.

#### **Isolation and apple annual growths (shoots) inoculation**

Isolations were performed on potato-dextrose agar and inoculations were made with one week old mycelial disk with 6 mm diameter. Apples cultivars Liberty, Cortland, Freedom, Gloster, Florina, Gala, Granny Smith, Smoothy, COOP – 10 and Golden Delicious were

evaluated for susceptibility after artificial inoculation of annual growths. At the end of the June ten annual growths from each cultivar were wounded with a sterile scalpel at 8-10 cm distance from the base. The inoculum consisted of a disk of mycelium with agar and it was deposited in artificial wound. Before wounding the annual growths were surface – disinfected with 90° ethanol. Inoculation sites were covered with wet cotton and were wrapped with a polyethylene ribbon. In the wounds of the control annual growths were put disks of sterile agar. The lengths and widths of cankers were measured two months after the inoculation. The susceptibility of the apple fruits was tasted in the laboratory. Six fruits (picked at the end of July) from each cultivar were wounded and inoculated as annual growths. The fruits for control were wounded but not inoculated. Fruits were incubated in plastic bags at chamber temperature 18- 25°C.

The data were analysed using the method of variance (Maneva, 2006), statistical significance of observed differences was estimated using F-coefficient for the test significance and LSD values for significance of the mean differences at level  $P=0.05$ , 0.01 or 0.001; Duncan's multiple range test to be estimated the differences among the variants (Duncan, 1955) and for clustering the variants – Cluster analysis.

#### **Susceptibility of the cultivars at natural infection**

The investigations were done in an experimental apple orchard (1.5 ha) with cultivars Granny Smith, Smoothy and COOP 10 on rootstock M9 and MM106, planted at 4/2.5 m in 1986. Fungicides were used for controlling scab and powdery mildew.

In autumn, 132 – 136 trees of each cultivar were examined for cankers caused by *Botryosphaeria obtusa*.

## RESULTS AND DISCUSSION

#### **Symptoms on annual growths (shoots) after artificial infection**

The symptoms of all cultivars were identical. The area around the place of infection formed cancer was brown – reddish coloured. The infected tissue was sunken and sharply separated from healthy tissue. Picnidia were produced on the cankered area. Some of cankers girdled the shoots after two months and a part of the shoots above the cankers died. Seven of 10 infected shoots from the cultivar COOP 10 died; two from Smoothy and Gala; one from Golden Delicious, Freedom, Liberty, Florina. Only for Granny Smith no dead shoots were found. The control shoots were without cankers and the artificial wounds were surrounded by callus tissue.

#### **Susceptibility of annual growths (shoots) after artificial infection**

The investigated cultivars were separated to three groups according to their canker area using k-means cluster analysis (Table 1).

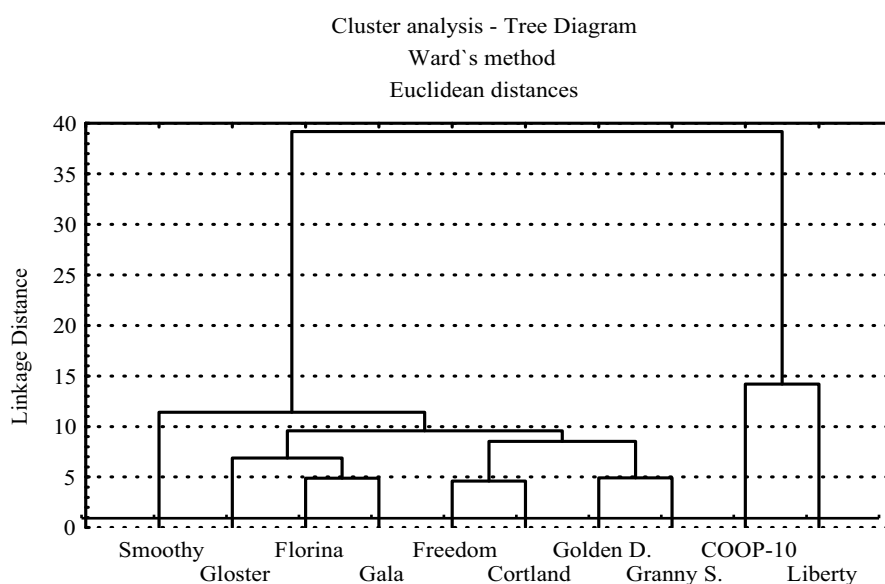
Poor susceptibility of shoots to *Botryosphaeria obtusa* showed cultivars Gala, Golden Delicious, Cortland, Freedom, Gloster 69 and Florina (I group), medium susceptibility - Granny Smith and Smoothy (II group), and with strong susceptibility - Liberty and COOP 10 (III group). According to the Duncan's analysis the cultivars belonging to the I and III group were with well manifested level of susceptibility (a or c). The cultivar Granny Smith of the second group had the mixed characteristics belonging to I and II group (a,b). Only three of the cultivars were with statistically significant differences from the most poorly susceptible cultivar Gala – Smoothy ( $P<0.05$ ) and the belonging to the group of strong susceptibility - Liberty and COOP 10 ( $P<0.001$ ). The detailed relationships between the investigated cultivars in the clusters groups are presented by the tree clustering analysis (Figure 1).

Table 1

Mean canker area (cm<sup>2</sup>) after artificial wound infection of shoots with *Botryosphaeria obtusa*

Cultivars	Mean canker area (cm <sup>2</sup> )	Analysis of variance	Duncan's methods*	K – means Clustering
Gala	2.57	K	a	I
Golden Delicious	2.71	N	a	
Cortland	2.99	N	a	
Freedom	3.07	N	a	
Gloster 69	3.13	N	a	
Florina	3.34	N	a	
Granny Smith	3.65	N	ab	
Smoothy	4.44	+	b	II
COOP 10	7.72	+++	c	III
Liberty	8.51	+++	c	
LSD5%=0.93      F= 1.04				

\*The differences between two variants with a common letter are not significant

Figure 1. Clustering of the apple cultivars in dependence of susceptibility of shoots to *Botryosphaeria obtusa*

### Susceptibility of the cultivars at natural infection

The symptoms of all infected trees at the three cultivars were similar. Fundamentally the trunks and branches were damaged. All damaged trunks and branches were with wounds caused by cold injury, mechanical injury by cutting or machines. Our data confirmed that *Botryosphaeria spp.* is wound pathogens.

The results of natural infection showed that trunk susceptibility of apple to *B. obtusa* was dependent on cultivar and rootstock (Table 2). Cultivar COOP 10 was more susceptible to *B. obtusa* than Granny Smith and Smoothy. The results from natural infection were similar to those of artificial infection. It was established that rootstock had a strong influence over the susceptibility of cultivars. The rootstock M9 provoked a stronger susceptibility than MM106. All trees on M9 after 18 years of planting were with reduced vitality and symptoms for

incompatibility especially at COOP 10. The stronger susceptibility of cultivars on M9 can be explained by the reduced vitality and the wounds caused by cold injury which is very favourable for infection and development of *B. obtuse*.

Table 2

Damages on trunks and branches of apple cultivars by *Botryosphaeria obtuse* by natural infection in the orchard

Cultivars	Rootstocks	% of infected trees
COOP 10	MM 106	7.35
	M9	34.85
Smoothy	MM 106	3.68
	M9	28.57
Granny Smith	MM 106	2.21
	M9	18.80

### Symptoms on fruits after artificial infection

The lesion around the place of infection was dark brown. The rot was firm and was not sunken. Later the lesions turned to black. Picnidia were produced on the rotted area. The control fruits were without rotted areas.

### Susceptibility of the fruits after artificial infection

The k-mean clustering shows that the cultivars could be separated in three groups: I - with poor susceptibility: Granny Smith and Gala; II - with medium susceptibility: Smoothy, Gloster 69, Golden Delicious and Liberty; III - with strong susceptibility: Cortland, Freedom, Florina and COOP 10 (Table 3).

Table 3

Mean rotten area (cm<sup>2</sup>) after artificial wound infection of fruits with *Botryosphaeria obtusa*

Cultivars	Mean rotten area (cm <sup>2</sup> )	Analysis of variance	Duncan's methods*	K-means clustering
Granny Smith	10.30	K	a	I
Gala	12.01	N	b	
Smoothy	13.28	N	bc	II
Gloster 69	13.75	N	cd	
Golden Delicious	14.50	N	d	
Liberty	15.04	++	de	
Cortland	15.76	++	e	III
Freedom	15.92	++	e	
Florina	15.95	++	e	
COOP 10	16.04	+++	e	
LSD5% = 1.59      F = 3.96				

\*The differences between two variants with a common letter are not significant

The cultivars that belonged to II group are without clearly manifested difference of susceptibility compared to the cultivars in the I and III group. They were separated in several subgroups, with mixed characteristics (bc, cd, de). In the I group the cultivars had different characters according Duncan's. Only the cultivars of the III group were significantly different compared to the least susceptible cultivar Granny Smith (P<0.01 and P<0.001). The detailed relationships between the investigated cultivars in the clusters groups are presented by the tree clustering analysis (Figure 2).

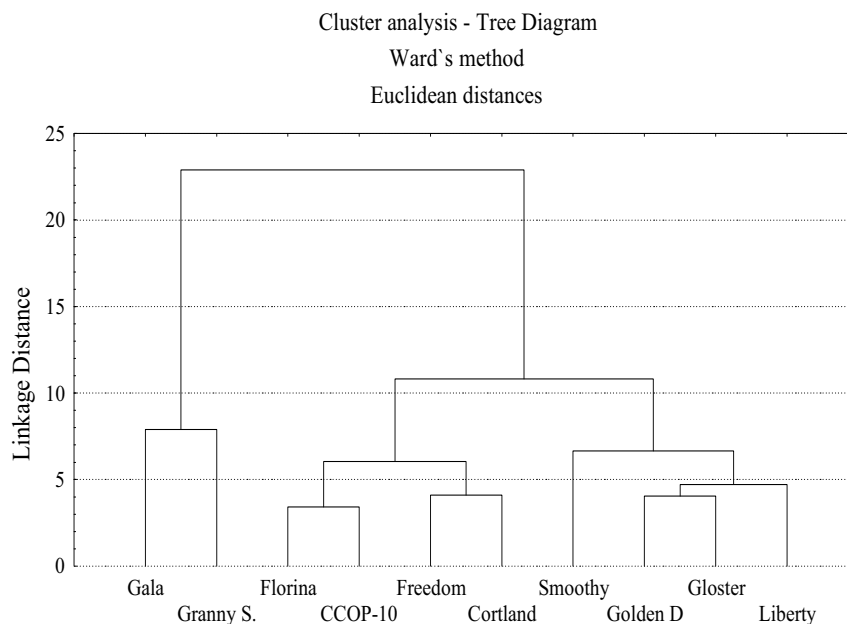


Figure 2. Clustering of the apple cultivars in dependence of susceptibility of fruits to *Botryosphaeria obtusa*

## CONCLUSION

The annual growths (shoots) and fruits of all evaluated cultivars were susceptible to *Botryosphaeria obtusa*. The shoots of cultivars Liberty and COOP 10 were the most susceptible to the black rot. The rootstock had a strongly manifested influence over the cultivars susceptibility. The rootstock M9 provoked a stronger susceptibility of the cultivars COOP 10, Granny Smith and Smoothy, than MM106.

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## REZUMAT

### SENSIBILITATEA UNOR SOIURI DE MĂR LA ATACUL DE *BOTRYOSPHAERIA OBTUSA*

Studiul privind comportarea unor soiuri de măr la atacul de cancer provocat de *Botryosphaeria obtuse* s-a desfășurat în livada experimentală și laboratorul de specialitate de la Institutul de Agricultură Kyustendil, din Bulgaria, în șapte ani consecutivi, între 1998 și 2004. Soiurile Liberty, Cortland, Freedom, Gloster, Florina, Gala, Granny Smith, Smoothy, COOP-10 și Golden Delicious au fost evaluate pentru susceptibilitatea lor la inocularea artificială a lăstarilor anuali și a fructelor. Cultivarurile sensibile Granny Smith, Smoothy și COOP-10 altoite pe portaltoi M9 și MM106 au fost evaluate și în livadă, în condiții de infecție naturală. Atât lăstarii anuali, cât și fructele tuturor cultivarurilor au prezentat susceptibilitate (sensibilitate) la atacul de *Botryosphaeria obtusa*. Lăstarii soiurilor Liberty și COOP-10 au prezentat cea mai mare sensibilitate. S-a constatat că portaltoiul a influențat puternic comportarea pomilor la atac. Soiurile COOP-10, Granny Smith și Smoothy au fost mult mai sensibile când au fost altoite pe portaltoiul M9 decât pe MM106.