

## STUDIES OF VIRAL PROLIFERATION ON APPLES CULTIVARS: JONATHAN, VOINEA AND GOLDEN DELICIOUS

*Ioan Viorel Rați, Dumitra Raducanu, Nicoleta Bădăluță*

**Key words:** *Malus domestica*, resistant cultivars, *Mycoplasma sp*

### INTRODUCTION

Apple's proliferation is known in Europe as one of the most dangerous disease being on 7<sup>th</sup> place in order of it's intensity of distribuion and causing (in extreme cases) death of diseased trees. It is caused by *Mycoplasma sp* germ and it develops itself inside the cell, in those cells that compose phloem canals from: petals of flowers, nervures of leaves, shoots, limbs and roots (EFTIMIA GH., 1981).

This disease is spread in all countries and the percentage of infected trees it has been raised in nursery garden, young plantations, intensive or superintensive plantations (COCIU V., OPREA ȘT., 1989) In intensive plantations, young trees are weak symptomatically: at the age of 6-7 years the frequency of the morphologic symptoms is 0,004 – 3,4%, and at the age of 7 - 12 years it is 7 – 37% (PANTEA S. D., 2012).

Aberrations that constitute the specific symptoms of this disease were noted for the first time in Italy by Rui (1905), then in Holland by Mulder (1951). The primary symptoms of proliferation disease of apples were noted by Pop (1962) in Romania (Transilvania) on wild trees of apple in 1958. The association of Pop, Coman, Gheorghiu, Motoi and Apetri (1967) was the one that obtained the transmtion of apple's proliferation on indicator woody plants, in field and vegetation station and they certified the existence of this disease in different plantations and nursey gardens from this country (EFTIMIA GH., 1981).

The spread of this disease it has accomplish very easy in the same time of breeding apple trees by plantation of root suckers (very used technique in old pomiculture). The strong plantation of root suckers is typical for diseased trees being a breeding technique that encourages the ampleness of proliferation. Another posibility of spreading was the using of branches or shoots for graft, took from diseased trees and using the vegetative stock (RAȚII V., 2001).

The studies and investigations concerning the symptomatology of apple's proliferation (*Mycoplasma sp*) cause to the host complex symptoms: morphological, anatomic, histological, biological, physiological and biochemical.

The aim of this study is to present some aspects of scientific investigation and methodological concerning:

- The identification of apple's proliferation disease in S.C. FRUCTEX S.A fruit garden from Bacau and in mixed fruit gardens from population's farms.
- To estabilish and describe the manner of manifestation of apple's proliferation disease
- To make obvious the behaviour of different types of apple species to the germ that produce apple's proliferation
- The efficiency of techniques for controlling the apple's proliferation disease in fruit gardens
- Another aim was to analyse the quality of the fruits for species' selection for production and for amelioration.

### MATERIALS AND METHODS

The study has been done into a crop competition that is situated in Experimental polygon of S.C. FRUCTEX S.A. from Bacau (Fig. 1).



Fig. 1. Experimental polygon of S.C. FRUCTEX S.A. from Bacau

The material of study was represented by apples plantation landscaped in crop competition with 13 species and hybrids of apple, situated in the

fruit garden of investigation of S.C. FRUCTEX S.A. Bacău.

The observations have been done on an alluvial field situated on the bottom land of Bistrita river, with an average temperature of 9 C and with average rainfalls of 553 mm. The soil has a pH of 8,1 – 8,6 and an organic content of 1,0 – 1,3. The trees have been grafted on M 106 and seeded after the plan with a distance between them of 4/2,5 m.

From those 13 species and hybrids of apple seeded in the crop competition, 3 of them have been selected: *Jonathan*, *Voinea* and *Golden Delicious* cultivars.

The next parameters have been studied: the evolution of apple's phenophases; production (t/ha); the quality of fruits; the amount of affected trees by apple's proliferation.

Also, phenological observations have been done once of two days from the beginning of shoots bagging; once of three days from fruits' maturation, and at the end of vegetation time, once of five days. The determination of frequency, intensity and the amount of affected trees are the most suitable standards for evaluation of species' resistance of apple's proliferation disease. Also, 3 trees of each species have been studied with three observations in different cycles of the year.

#### Methods used for investigation of biological symptoms

*The symptoms of apple's proliferation disease* were established in daily comparative observations on healthy and affected trees' phenology from two species.

*The quantity of pollen* has been extracted from 300 flowers, from 3 affected trees and 3 healthy trees, from 2 species: *Voinea* and *Jonathan*.

*The pollen's capacity of germination* analysed on the extracted material of flowers from the same branches, and the same exposure and time of flowering. For each replicate the pollen of 30 flowers was extracted.

*The establishment of flowers' binding amount* which has been done counting the flowers per branch of control, with the same exposure, of 3 trees per replicate. Bound fruits were marked after the second physiological fall.

*The growing rhythm of affected trees* was determined by decadal measurements of length growing of annual sprouts.

*The quantity of production per tree* was determined by fruit's production balance on affected trees and healthy trees from the same species.

*The number of seeds from one fruit and the atrophy proportion of the seeds* was observed also at two species of apple trees.

To establish the seeds' capacity of germination, seeds with normal development from affected trees and healthy trees were studied.

The dried substance from apple juice was determined by refractometer.

The biological material for studying *Mycoplasma sp* germ of proliferation disease was represented by: sprouts, leaves, flowers, fruits, seeds.

## RESULTS AND DISCUSSIONS

### Determination of frequency, intensity and the amount of affected trees by apple's proliferation (*Mycoplasma sp*)

The determination of frequency, intensity and the amount of affected trees are the most suitable standards for evaluation of species' resistance of apple's proliferation disease.

After the obtained results (table 1) we can conclude that *Jonathan* cultivar has the highest frequency of attack 55,5%, and the lowest frequency is present on *Voinea* cultivar 22,2% - somewhat resistant of proliferation disease.

The highest intensity of proliferation attack is present in *Golden Delicious* cultivar 20% and the lowest intensity is in *Voinea* cultivar 5%. No species was identified as very resistant or resistant to proliferation, the amount of attack being more than 1%. After these results we can observe that *Voinea* species is somewhat resistant of proliferation attack, and *Golden Delicious* and *Jonathan* are sensible cultivars of proliferation attack.

Table 1. The behaviour of studied affected trees by the attack of proliferation (*Mycoplasma sp*). Results obtained on S.C. FRUCTEX S.A. Bacău

Studied species	Frequency of attack (F%)	Intensity of attack (I%)	Damage of attack (GA%)
Golden Delicious	44,4	20	8,88
Jonathan	55,5	10	5,55
Voinea	22,2	5	1,11

### Proliferation affected trees phenology (*Mycoplasma sp.*)

After all the investigations done on a 3 years period (2011-2013) (table nr. 2), we concluded:

- Phenological phases since shoots' expansion until petals' falling were succeeded with 2 – 7 days earlier in affected trees than in healthy trees;
- Since petals' fall until maturation of harvesting the fruits, the phenophases of affected and healthy trees are overlapping;
- The vegetative repose emerges with 9 – 11 days later at affected trees by apple's proliferation disease.

From the observations made in these 3 years at *Jonathan* species attacked by proliferation, we can conclude that in 2013 the acceleration of phenological phases was more obvious and with a bigger number of days (inbudding – 7 days) comparatively with 2011 (inbudding – 2 days), year when phenological phases since shoots' expansion until petals' falling were succeeded with 2 days to 7 days earlier than 2012.

Table 2. Time (in days) of increasing phenological phases at affected trees by apple's proliferation (*Mycoplasma sp*) comparatively with healthy trees. Results obtained at S.C. FRUCTEX S. A Bacau

No	Phenophase	2011		2012		2013	
		Jonatan	Voinea	Jonatan	Voinea	Jonatan	Voinea
1	Shoots expansion	2	2	2	3	3	3
2	Inburgeoning	2	2	2	3	7	7
3	Foliating vegetative shoots	2	4	3	4	6	6
4	Foliating flowers shoots	2	3	2	2	5	5
5	Ascension of blossom	2	3	4	3	5	5
6	Distribution of blossom	3	3	3	2	3	4
7	Increasing the corolla	1	1	2	2	2	2
8	Blooming	2	1	0	0	2	2
9	Petals falling	1	1	0	0	2	2
10	Normal size fruits	0	0	0	0	0	0
11	Dough ripeness	0	0	0	0	0	0
12	Maturation of harvesting	0	0	0	0	0	0
13	Vegetative repose	9	10	11	10	11	11

The phenophases of health and affected trees from Jonathan cultivar are overlapping since petals falling to maturation of harvesting the fruits, but the vegetative repose succeeds with 9 – 11 days later at proliferation affected trees, the average being 11 days.

At Voinea cultivar attacked by proliferation it is observed the same case like in Jonathan cultivar affected by proliferation where the phenological phases since shoots expansion to petals falling are succeeded from 2 to 7 days earlier at affected trees. Next is the periode where phenophases from health and affected trees are overlapping, and then is the periode of vegetative repose that is late with 9 -11 days at proliferation affected trees having an average of 10 days earlier.

#### ***Determination of germination power of pollen at Voinea and Jonathan species affected by proliferation in 2012***

When the pollen is provided from the affected trees (Tabel nr 3) the power of germination was highest than the healthy trees in both investigated cultivars (Voinea - Jonathan).

At affected Voinea cultivar, the germination power of pollen with abnormal flowers was higher than the one with pollen from normal flowers of the same tree (75,1%).

At Jonathan species the difference was lower: 18,1% at normal flowers and 14,9% at abnormal flowers.

At normal flowers of Jonathan species was registred a very advanced aberrance.

We can hypothesize that pollen's granules of flowers from proliferated trees have some biochemical characteristics being induced by the germ or having the natural bent of preserving the natural weak characteristics.

Between these 2 species of apple affected by proliferation the highest power of germination was at Voinea cultivar with a precentage of 75,1 % at abnormal flowers comparatively with 67,2% at normal flowers. Jonathan cultivar has registred a very high range of aberrance at abnormal flowers, the

difference being lower: 18,1% at normal flowers and 14,9% at abnormal flowers. For abnormal flowers of Jonathan cultivar was registred a high grade of aberrance.

Table 3. Determination of germination power of pollen's granules provided from affected trees by apple's proliferation (*Mycoplasma sp*). Results obtained at S.C. FRUCTEX S.A Bacau

No	Species	Total number of investigated pollen's granules	Number of pollen's granules imburgeoned	Germinated pollen's granules (%)
1	Healthy Voinea	14 056	8 266	41,2
3	Normal flowers	14 908	4 890	67.2
4	Abnormal flowers	15 183	3 784	75,1
5	Healthy Jonathan	<b>7542</b>	<b>6938</b>	<b>13,3</b>
7	Normal flowers	9 550	8 777	18,1
8	Abnormal flowers	8 493	7 234	14,9

#### ***Level binding determination of affected flowers by apple's proliferation at Voinea and Jonathan species***

At affected trees the binding of trees was a lot reduced in Voinea and Jonathan cultivars. Affected trees of Jonathan cultivar have bound 14 fruits comparatively with 128 bounded fruits at healthy tree of Jonathan cultivar. Affected trees of Voinea cultivar have bound 44 fruits comparatively with 184 at healthy trees.

In proliferated trees the bound of flowers was done in 0,3% at Jonathan species comparatively with 3,4% at healthy trees and 2,3% at Voinea species comparatively with 5,3% at healthy trees.

Jonathan species affected by proliferation presents a lower precentage of binding the flowers 0,3% comparatively with healthy tree 3,4% and

Voinea species affected 2,3% comparatively with 5,3% at healthy tree. The binding of trees attacked by proliferation was reduced in Voinea and Jonathan species.

**Determination of quality characteristics of fruits at grafted trees and healthy by apple's proliferation (*Mycoplasma sp*)**

The average production on healthy trees at investigated species (table nr 4) was 12,42 t/ha (Jonathan cultivar) and 17,14 t/ha (Voinea cultivar). The weight of fruits registered normal values of each species: 129,70g (Jonathan cultivar); 169,40g (Golden Delicious cultivar) and 182,28g (Voinea cultivar). The dried substance content when the fruits were harvested was determined by refractometer and the values were: 11,5% (Golden Delicious cultivar) and 13,1% (Jonathan cultivar).

Table 4. Physico-chemical characteristics of healthy apple's fruits

Species	Average production (tone/ha)	Fruit's weight (g)	Dried substance (%)
Golden Delicious	14,48	169,40	11,5
Jonathan	12,42	129,70	13,1
Voinea	17,14	182,28	12,8

Similar measures have been done to some fruits provided from affected trees by proliferation's germ. We concluded that the proliferation's germ decreases qualitative and quantitative the production of apples.

The fruits provided from trees affected by proliferation (table nr 5) are small, have abnormal color, and the biochemical content and alimentary values are very low, qualitative gustative being decreases.

Table 5. Physico-chemical characteristics of apple's fruits provided from affected trees by proliferation (*Mycoplasma sp*)

Species	Average production (tone/ha)	Fruit's weight(g)	Dried substance (%)
Golden Delicious	12,47	159,63	12,4
Jonathan	9,07	98,34	14,7
Voinea	15,12	175,82	14,5

**Determination of quantity production of fruits provided from infected trees by apple's proliferation (*Mycoplasma sp*)**

The determination of production from investigated species has been done on infected and healthy trees with an age of 20 years old, with a volume and a treetop with a good development (table nr 6).

For the affected trees comparatively with healthy trees were registered productions of 24,9 – 28,3% lower than healthy trees.

Table 6. Fruits production from healthy and affected trees by apple's proliferation (*Mycoplasma sp*)

No	Species	Quantity kg/tree	% comparatively with healthy tree
1	Affected Jonathan	34,3	28,3
2	Healthy Jonathan	121,0	100
3	Affected Voinea	42,9	24,9
4	Healthy Voinea	173,7	100

From Jonathan affected tree was registered 34,3 kg/tree and for the healthy tree 121kg/tree. For Voinea cultivar affected was registered 42,9% kg/tree and for the healthy one 173,7 kg/tree.

The pronounced decreasing of production is an effect of decay because of proliferation's *Mycoplasma* which is decreasing the biological potential of the affected tree, which determines a bad bound of the fruits, advanced physiological fall and the fruits are small, degenerated, with long stalk, weak colors and the fruits can be chapped sometimes.

Voinea and Jonathan cultivars affected by proliferation have registered lower productions: 24,9% – 28,3% comparatively with healthy trees.

**Determination of germination power of the seeds provided from affected trees**

The seeds provided from affected trees had a higher power of germination comparatively with the ones from the healthy trees. For Jonathan cultivar (affected) the germination was 87,0% and for the healthy tree was 42,4%. For Voinea cultivar (affected) the germination was 79% comparatively with healthy tree 43,3%. These results affirm the ones analyzed for pollen's germination.

**Determination of dried substance content in apple's juice of the affected trees by proliferation**

The determination of dried substance content has been done with hand-refractometer in fruits' juice provided from affected and healthy trees for Jonathan and Voinea species. The affected Jonathan species registered 11,5% d.s., a lowest quantity than in the healthy tree 13% d.s. For affected Voinea species were registered 12% d.s. comparatively with 14,5% d.s. in healthy tree.

We can observe that the attacked trees by proliferation on fruits the content of dried substance was lower than in healthy trees.

**CONCLUSIONS**

The proliferation's germ produces negative effects in all the life's levels of the host. These effects reflect in qualitative and quantitative

decreasing of production, decrease that can provide no fruits for the trees.

After the obtained results we can say that Jonathan species presents the highest frequency of attack 55,5% and the lowest frequency is on Voinea species 22,2%, a species someway resistant for the attack of proliferation. The highest intensity of proliferation's attack is in Golden Delicious cultivar 20% and the lowest one is in Voinea cultivar 5%.

No species have been indentified to be very resistant or resistant on proliferation, the amount of attack having values over 1%.

In affected trees the symptoms are intensifying form year to year and the malady is spreading quickly in big proportions representing a danger for apples production. The spread of proliferation of apples has an important economical importance because the apples production is decreased in different proportions which can increase over 90%.

The fruiterers adjudge frequently the damages provided by proliferation to some normal causes like advanced age of the trees even if the symptoms appeared does not coincide always with the age. Sometimes the symptomatical manifestation appears even when the trees are very young.

Because Mycoplasmas are common germs of plants and animals, the risk of passing to people in a particular phase of their life's cycle it is possible.

#### ABSTRACT

This paper presents the results obtained in monitoring three apple-tree cultivars Voinea, Jonathan and Golden Delicious, from experimental polygon of of S.C. FRUCTEX S.A. from Bacau affected by apple's proliferation (*Mycoplasma sp*).

As a results of numerous observations concerning the evolution of apple's phenophases; production (t/ha); the quality of fruits; the amount of affected trees by apple's proliferation, no species have been indentified to be very resistant or resistant on proliferation, the amount of attack having values over 1%.

#### REFERENCES

1. COCIU V., OPREA ȘT., 1989 - Metode de cercetare în ameliorarea plantelor pomicele, Editura Dacia, Cluj-Napoca;
2. EFTIMIA GH., 1981- Boala proliferării la măr în România, Editura Ceres, București;
3. PANTEA S. D., 2012 - "Efectul diferitelor sisteme de lucrare a solului asupra calității și cantității producției la mărul cultivat în sistem superintensiv" rezumat al tezei de doctorat, Cluj-Napoca;
4. RAȚI I. V., 2001 - Mărul, pasiune și afacere, Editura Moldavia, Bacău.

#### AUTHORS' ADDRESS

RAȚI IOAN VIOREL, NICOLETA  
BADĂLUȚĂ, DUMITRA RĂDUCANU -  
University „Vasile Alecsandri” Bacau, Marasesti  
Street, no. 157, Bacau, Romania, e-mail:  
[ratiioanviorel@yahoo.com](mailto:ratiioanviorel@yahoo.com);  
[dumitra\\_manea@yahoo.com](mailto:dumitra_manea@yahoo.com);  
[nicoleta\\_dogaru@yahoo.com](mailto:nicoleta_dogaru@yahoo.com)