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Study on the effect of deuterium depleted water (DDW) on the life cycle of phytopathogen eucariota

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INTRODUCTION

Global and local climate changes and also the intensive use of fruit tree plantations involve biocenosis changes with repercussions on the plant health. Pathogens agents that cause diseases exercise constant pressure of infection correlated with climatic factors determines the emergence of new species.

These species, through their virulence, change the way of response of the main varieties to diseases attack or become resistant to the implementation of fungicides. Taking into account these considerations and the new concepts of agriculture (sustainable, environmental, biological) I have approached this issue.

A first objective pursued in this doctoral thesis was that of conducting fundamental research, orientated towards the knowledge of the effects of deuterium depleted water on the biological cycle of some fungi found in apple and plum.

In fruit tree plantations with apple varieties susceptible to scab and powdery mildew, and at plum susceptible to monilia is inconceivable to obtain a high production of fruit without using pesticides. Chemical treatments applied for years in a row, cause environmental pollution, lead to the disturbance of natural biological balance, which externalizes through the emergence of more virulent forms of pathogens and to some resistant sources to fungicides.

Therefore, this paper presents observations on the effect of deuterium depleted water on the evolutionary cycle of the following fungi: scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucothrica*) in apple and monilia (*Monilinia laxa*), red leaf blotch (*Polistygma rubrum*) in plum.

Experimental research in the years 2004 - 2009 were held in the Gorj county in experimental apple parcel at Gorj Phytosanitary Unit and plum culture at the Research and Development Horticulture Station from Târgu-Jiu, Farm no. 4, Bălăneşti.

To respond to the theme of this thesis were carried out treatments mainly with deuterium depleted water to control fungi: *Venturia inaequalis* and *Podosphaera leucothrica* (apple culture), *Monilinia laxa* and *Polystigma rubrum* (plum culture).

The thesis is divided into 9 chapters and bibliography, 75 tables within 55 original, 78 figures (51 originals) and 7 original drawings, totalling 180 pages.

CHAPTER I
THE ECONOMIC IMPORTANCE OF APPLE AND PLUM CULTURE

In agriculture, fruit growing is one of the most intensive sectors, it fully exploiting the conditions of climate, soil and terrain, sometimes improper to cultivate cereals and other industrial crops.

Over the years, Romania fruit growing has undergone major transformations. The area occupied by fruit trees decreased significantly after 2000 and has had major fluctuations since 2001. Fruit production has declined; the reasons for this decline are multiple.

On the world market apple production ranks the third place and in Europe the second (**FAO**, 2008).

Also in our country the largest areas are the plum plantations (98,000 ha/2000) followed by apples (82 000 ha/2000) (FAO, 2008).

The plum and apple have favourable conditions for growth and diversification. In various areas of the

high hills are important varieties adapted to local conditions.

From 2000 area occupied by fruit tree species have slightly increased, being introduced new species with greater adaptability and even transgenic forms, that bearing the gene for resistance to the *Plum-pox* virus, (plum blotch): *Anna Spath, Centenar, Reclod Ouliu*.

From 2000 to 2009 areas occupied by fruit tree species, plum and apple have not exceeded 100,000 ha, respectively 75,000 and the productions oscillated from 220.6 tonnes in 2002, up to 909.6 tonnes in 2003 to plum, and at apple from 475.4 tonnes in 2007 to 1097.9 tonnes in 2004.

CHAPTER II
GENERAL MEASURES IN COMBATTING THE DISEASE AT FRUIT TREES

In our country every year, the losses caused by plant parasites and the pests in fruit growing exceed 205 of production. Control measures of plant disease must be well known and applied differently, rational, integrated and economically, taking into account the specific of the diseases, the biology of phytopathogenic agent, the plant phenophase in correlation and interdependence with environmental factors.

It is now inconceivable that an advanced agriculture not to give due importance to the phytopathology factor. In combating plant diseases, particular importance has the means of prevention (prophylactic).

The primary objective of prophylactic measures is the prevention of infection, amending plant predisposition to disease, management of natural and artificial factors.

The most important means of prevention are: phytosanitary hygiene measures and culture work.

To prevent the spread of pathogens agents from one region to another or from one country to another plant quarantine measures are taken.

An important role in preventing and combating disease to plants has forecasting and warning measures

Curative means (therapeutic) have limited efficiency. Among preventive and curative treatment cannot be a clear separation, however, some preventive treatments have curative action and vice versa, some curative treatments perform the role of disease prevention.

Among the curative means are mechanical and physical means, chemical measures, biological measures (hyperparasitism, antibiotics) and control measures using unconventional materials (nanomaterials and deuterium depleted water).

CHAPTER III
UNCONVENTIONAL MATERIALS WHICH WERE USED IN EXPERIENCE

Interest in magnetisable fluids first appeared at NASA between 1960 -1970, about the necessity of handling missiles liquid fuel in conditions of weightlessness.

Magnetic fluids called also magnetic liquids or ferrofluid are defined as very stable colloidal suspensions of ultrafine particles (<10 nm), from ferro magnetic materials in liquid mediums such as hydrocarbons, esters etc.

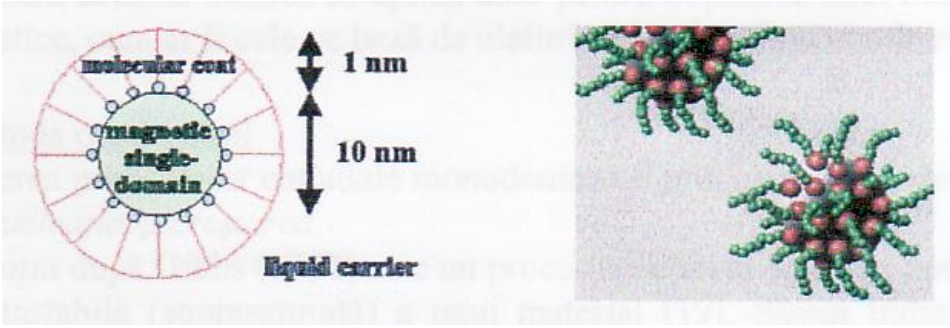

Fig.3.1.Schematic representation of the magnetic fluids

Magnetic fluids are used: in biology, primarily in human medicine and veterinary biotechnology (gene transfer) in vegetal field, in the determination of changes in the cellular organits, chromosomes, and in the growth of plants.

Another unconventional material used was deuterium depleted water (DDW). Deuterium depleted water is used in fighting cancer in humans and animals (**Somalyai,** 1993). Research has been undertaken in order to determine the action of deuterium depleted water on plant organisms (**Butnaru** și colab.,1997).

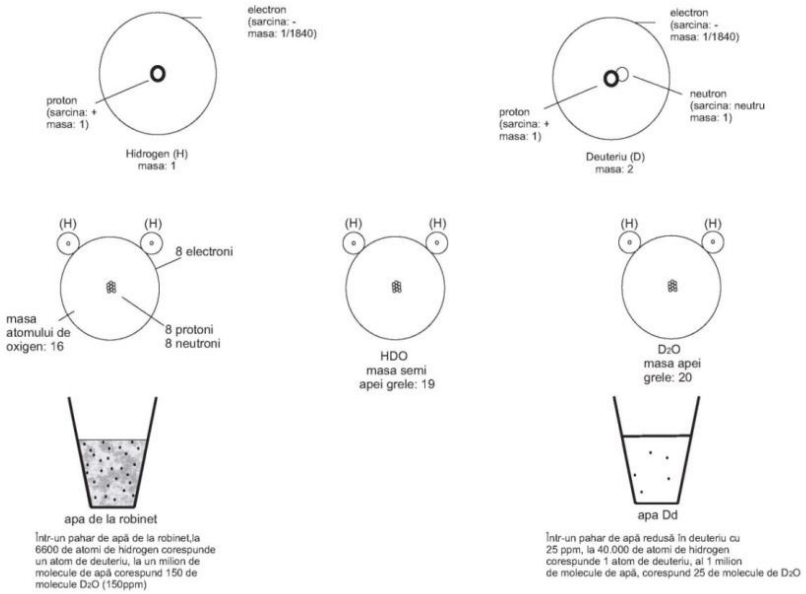

Fig.3.2 Organisation mode of different types of water: tap water and deuterium depleted water
(**Somlyai** și colab., 1993)

Influence of deuterium depletion on the physiology of plant cells was studied on the *Elodea canadensis* leaves. At plants were observed more physiological processes like respiration, photosynthesis, membrane potential, modification of intra-and extracellular pH measured, which led to the conclusion that plant, in the first half hour of the decreased concentrations of deuterium presents biochemical changes as if it came in the dark (**Cachiță** și colab., 2003).

CHAPTER IV
THE ENVIRONMENT AND THE CONDITIONS WHERE WERE CARRIED OUT THE EXPERIENCES (2004 – 2009)

4.1 The land in the Gorj County

The objectives set, to highlight the influence that different new products have, patented in our country (Magnetic Fluids - Fm and deuterium depleted water - DDW) were observed in the Gorj County, at fruit tree farms.

To highlight the effect of different fungicides it had been used Stanley variety at plum and variety Jonathan at apple, which are the most popular in the area.

Thus the land of the Gorj County is 572,576 ha, of which 262,656 ha is agricultural land of which 100,463 hectares are arable land. Fruit trees occupy an area of 4,838 ha.

The plum crop researches were made at the Research and Horticulture Development Station, Târgu-Jiu, geographically located between the meridians of 23^o17'N and 24^o east longitude and just above the 45^o17' parallel north latitude. The experiments were located at 3 km from Tg-Jiu, DN 67.

4.2 Pedological conditions in Gorj County

The experimental field No. 1 (apple culture) was placed in Gorj Phytosanitary Unit, located in the northern city of Targu-Jiu, on Route 66, on a Luvisol (LV). This type of soil is specific to hills area and plateaus, with annual average temperatures ranging from 6 - 9^oC and average rainfall of 600 – 900mm. Aridity index has values of 35-60. These values are characteristic of humid and cooler climate.

The second experimental field (apple culture) was placed in Gorj Phytosanitary Unit, located in the northern part of Targu-Jiu, on Route DN 66.

Experimental field no. 1 (apple culture) was placed at Gorj Phytosanitary Unit on Luvisol soil (LV) and the experimental field no. 2 (plum culture) at the Research and Horticulture Development Station, Târgu Jiu (Farm no. 4) on a land that is the third terrace of the river Jiu, the predominant soil type is eutricambosol soil (EC).

4.3 General conditions of experimental years

The climate conditions are representative for the fruit growing area of the Gorj County, the general climate is temperate continental and is characterized by mild winters and cool summers, except in mountain areas.

The average annual temperature in the Gorj County is 10.2˚C and rain fall depend on microzone, every year range values between 700-1000 mm, the annual average being 909.3.

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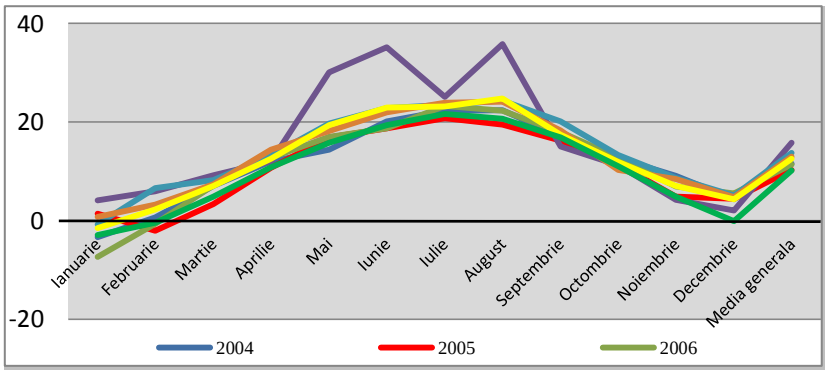


Fig.4.1 Monthly average temperatures in experimental year 2004-2009

Rainfall according to microzone, every year record values between 700 - 1000 mm, the annual average being 909.3

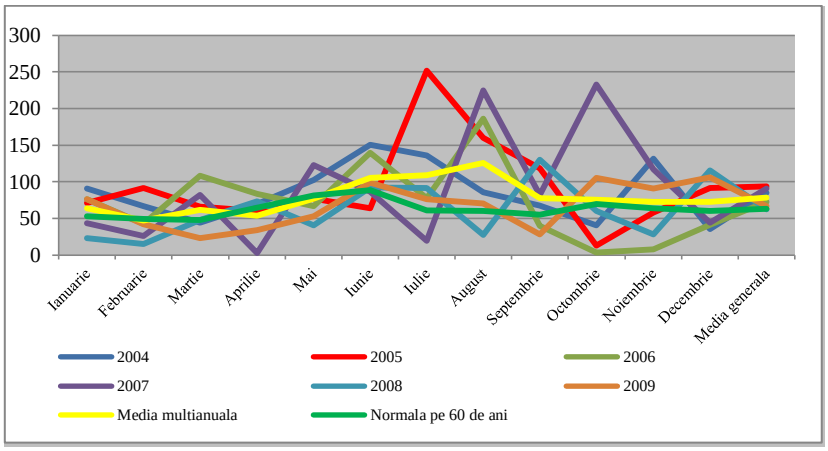


Fig.4.2 The average of rainfall (mm) between 2004 – 2009

In the paper was used the Walter Leight climatogram, where the evaluation is done by parallel curve representing the average monthly temperature and precipitation curve at different scales. Intervals result from their intersection where there is water shortage with different intensities, periods of dryness and drought (**Bogdan** și **Niculescu**, 1999)

Representative for the experimental years are climatogram from 2006 and 2007, where they recorded also periods of droughts (**Fig.4.3-4.4**).

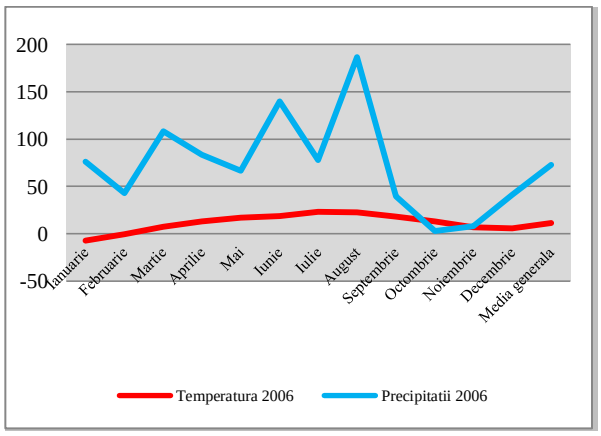


Fig.4.3 Climograma Walter Leight pe anul 2006
Fig.4.3 Walter Leight climatograme – year 2006

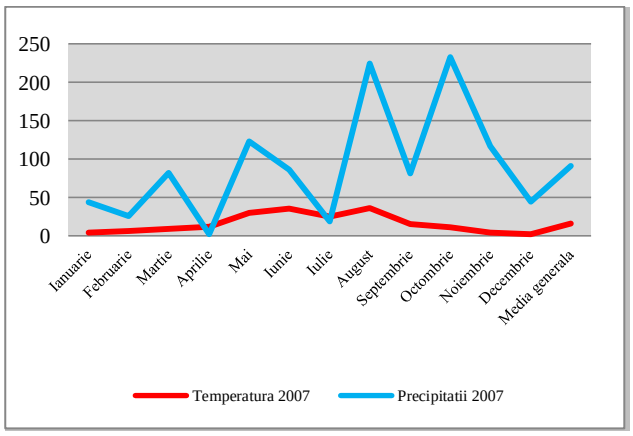


Fig.4.4 Climograma Walter Leight pe anul 2007
Fig.4.4 Walter Leight climatograme – year 2007

In the hill area of the Gorj county, prevailing winds are Crivăţul, Băltăreţul şi Vântul Mare, (Fig.4.5) which affect through speed and frequency the plant growth, changing the main climatic factors (temperature and humidity).

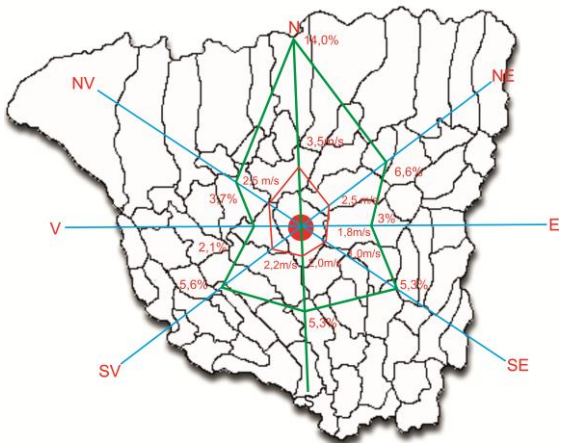


Fig.4.15 Roza vânturilor la Staţia Meteorologică Tg-Jiu
Fig.4.15 Cardinals at Weather Station Tg-Jiu

CHAPTER V
BIOLOGICAL MATERIAL AND WORKING METHODS

5.1 Biological materials used in exeperiences

Experimental research in the years 2004 - 2009 used as biological material Jonathan variety at apple culture, being the variety with the highest sensitivity to powdery mildew and scab and at plum culture was chosen Stanley variety, being the variety with the highest sensitivity to monilia.

5.2 Experimental variants

In 2004 and 2005 at apple culture to control *Venturia inaequalis* (apple scab) and *Podosphaera leucotricha* (apple powdery mildew) and for plum culture to control *Monilinia laxa* (plum monilia disease) and *Polystigma rubrum* (red leaf blotch) was used the following treatment scheme: Control; V1 (DDW 30ppm + H_2O); V2 (DDW 30ppm); V3 (wept grape vine leaf); V4 (wept grape vine leaf θ 3,7 x 10^{-3} g/cm^3); V5 (wept grape vine leaf θ 37 x 10^{-3} g/cm^3); V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3).

In the years 2006-2009 apple experience consisted of 5 variants, each with 3 repetitions and plum culture experience consisted of 5 variants, each with 4 repetitions.

To assess the treatments effectiveness with chemical products and deuterium depleted water (DDW - 30ppm), each variant of treatment remained the same in the experimental years 2006-2009.

Experimental treatments scheme in 2006 – 2009, at apple and plum culture was the following: Control; V1 (water + fungicide); V2 (DDW - 30ppm + fungicide); V3 (DDW+30ppm); V4 (water + DDW 30ppm (1:1)).

Phytosanitary used products are systemic nd contact and were used the concentrations recommende by Plant Protection Codex

5.3 Working methods

The research was carried out using field and laboratory methods.

Fungi biological reserve studied at apple and plum culture was made according to "Methods for forecasting and warning" (**Ministry of Agriculture and Food**, 1969).

During the vegetation at apple culture treatments were performed according to data issued by AgroExpert system stations. The plum culture was performed according to warning bulletins issued by the Gorj Phytosanitary Unit, after the observations made in the field.

5.3.1 Evaluation method of fruit quality in laboratory

5.3.1.1 Quantitative determinations of apple and plum fruits

Characteristics determination was made by weighing the fruit (fruit weight - grams) and measurement (height – H, large diameter - D and small - d) in the years 2007 to 2009.

5.3.1.2 Qualitative determinations of apple and plum fruits

In laboratory were made determinations on water content, dry substance and sugar as well as measurements of their physical traits. For determinations of sugar content of fruit was used RE 40 refractometer, which displays the *refractive index* (nD Index).

Depending on the value of this index it is being determined the sugar content of the sample directly in Brix degrees. The dry substance (ds%) was determined by the difference between 100 - water. Water was determined by thermobalance.

5.4 Methods of statistical calculation

The statistical methods applied in quantitative genetics are based on the study of individuals' distribution in the sample taken into account toward the ideal distribution of the entire population. The frequency distribution study is being used the arithmetic media (x), math error (sx), variance (s2) and coefficient of variability (s%), index calculated by Ciulcă.

Biological efficacy of phytosanitary treatments carried out in apple and plum culture was calculated by gravimetric method

Attack frequency (F) is the relative number of plants or attacked plant organs (n) related to the number of plants or organs observed. Frequency value was determined by direct observations on the number of plants or organs, depending on the circumstances and conditions.

Intensity attack (I) is the relative value by which is given the grade of coverage or extend of the attack on the plant, reporting the control area to total area observed. As criteria for intensity are being used scale with notes of 1-6.

The degree of attack (GA) is an expression of expansion of culture attack or the total number of plants that carry out observations.

CHAPTER VI
FUNGI ANNUAL EVOLUTION CYCLE DURING THE EXPERIMENTAL YEARS 2004 – 2009 AT APPLE AND PLUM CROP

6.1 Evolutionary cycle of the *Venturia inaequalis* fungus (apple scab) and *Podosphaera leucotricha* (apple powdery mildew)

6.1.1 Brown leaf blotch, apple scab fruit and shoots caused by *Venturia inaequalis* (Cke) Wint (sin *Endostigme cinerascens* (Fleisch) Jorst

The fungus is a facultative parasite, showing two phases, a parasitic and a saprophytic phase, each having a role in the evolutionary cycle of disease

Parasitic phase corresponds to the development of imperfect shape (asexual) of the fungus, which develops during the vegetation on the living organs of the tree, known as conidia *Fusicladium dentriticum* (Wallr) Fuck.

Saprophytic phase corresponds to a perfect shape development, (sexual) *Venturia inaequalis* (Cke.) Wint and evolve in the months of repose in dead leaves fallen on the soil, under the tree (perfect form) (**Popescu**, 1989).

Scab infection occurs once the green tissues appear, leaves being the most vulnerable until the end of their development; at 5-8 days after release they become resistant to infection.

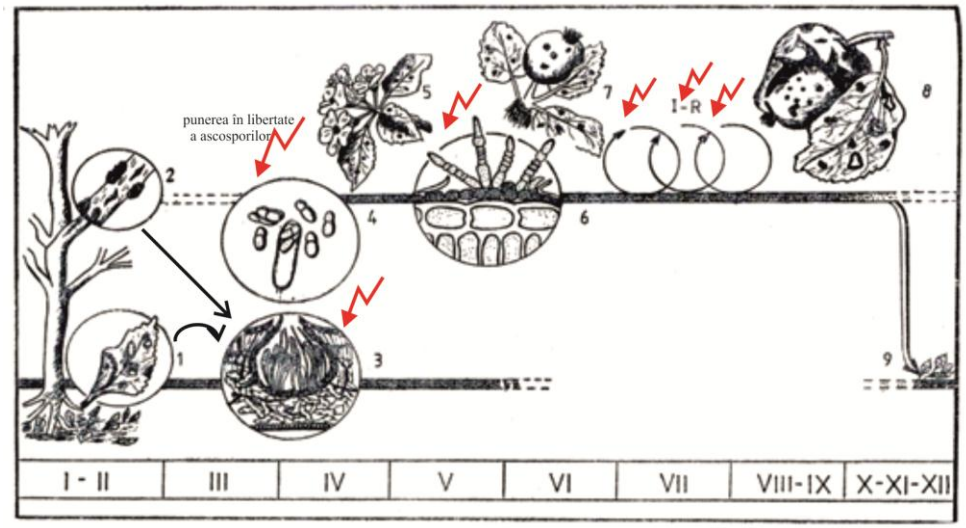


Fig.6.1. Development life cycle phases and the fungus Venturia inaequalis (Cke) Wint *according to* ***Bobeş****,1983*)
(moment of maximum efficiency of treatment)

6.1.2 Apple powdery mildew – *Podosphaera leucotricha* (Ell Et Ev) Salmi

Apple powdery mildew is caused by the pathogen *Podosphaera leucotricha*. Conidian form *Oidium farinosum Cke.*, makes the fungus spread throughout the vegetation period, reaching a maximum in May, June and July in the morning and lunch hours and from one year to another.

Multiply powdery mildew is favoured by frequent rain, fog and strong dew, providing a humidity of 90-100% and average daily temperatures between 16-22°C, when the incubation period lasts 5-6 days, or 3-4 days at average temperatures of 18-25°C.

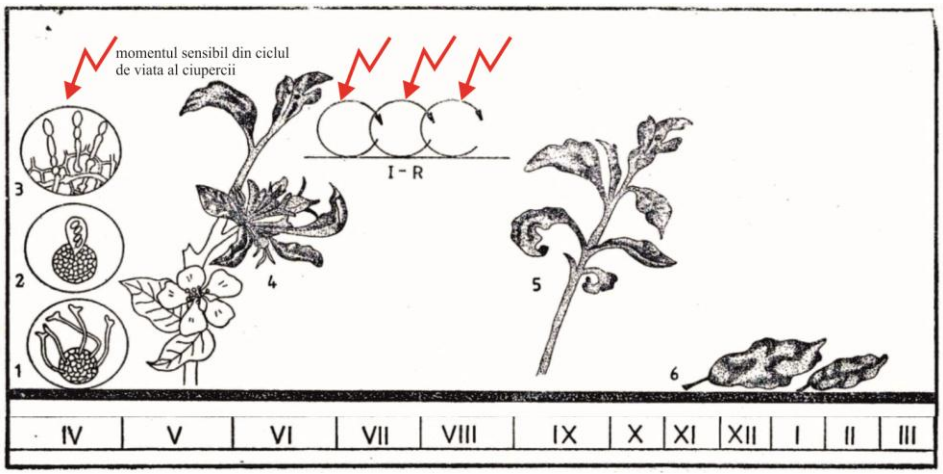


Fig.6.2. Fungus development lifecycle - Podosphaera leucotricha (Ell. Et Ev.) Salm. *(according to* ***Bobeş****, 1983)*
(moment of amximum efficiency of treatment)

6.2 Evolutionary cycle of the *Monilinia laxa* (monilinia disease plum) and *Polystigma rubrum* (red leaf blotch)

Monilia desease is caused by the fungus *Monilinia laxa*. The pathogen is conserved as sclorot in mummified fruits and as mycelium in the ulceration of the branches.

Biological threshold of *Monilinia laxa* fungus is about 4°C. In spring on attacked organs (mummified fruits, dried flowers and sprouts) are formed fungus sporodochium which can be formed also in the winter if the temperature is above 4°C.

6.2.1 Red leaf blotch of plum leaves - *Polystigma rubrum* (Pers.) D.C.

The pathogen that causes red leaf blotch at plum is *Polystigma rubrum*. Fungus mycelium is pigmented and forms in the parasite tissue - stroma, highly coloured, in which differs pycnidia with pycnospores. The dynamics of ascospores is one of the most important elements to make a good warning of treatments against *Polystigma rubrum* fungus. The moment when we must act to mitigate the attack occurred when the leaves appeared at rain at least 0.1 mm and the air temperature was at least 6°C.

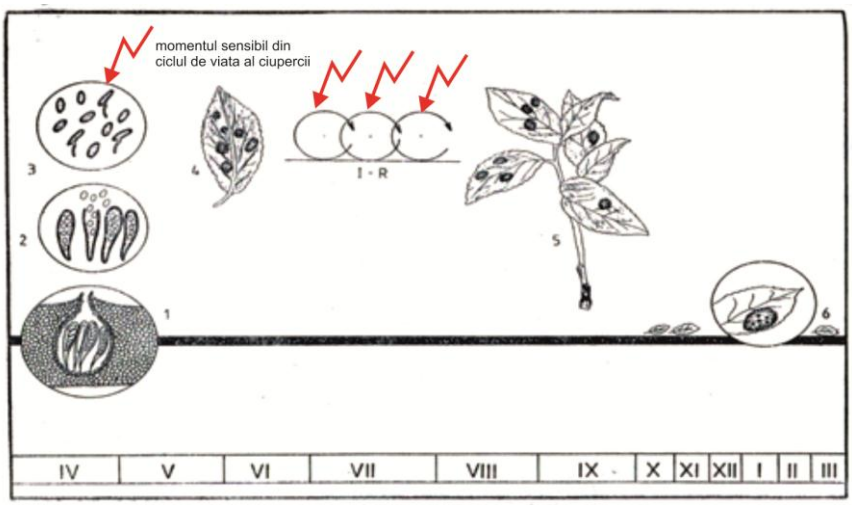


Fig.6.3. Stages of evolution of pathogen Polystigma rubrum (Pers) D.C. (1,2 and 3) and phenotypic expression of disease on leaves (4,5 and *6); (according to* ***Bobeş****,1983)*
(moment of maximum efficiency of treatment)

CHAPTER VII
RESULTS AND DISCUSSIONS

Treatments were made in compliance with specific environmental rules and safety. The choice of fungicides was done taking into account a number of issues such as mode of action of fungicides (systemic and contact action), fungicides efficiency and cost of fungicides.

7.1 Treatment effect with unconventional materials on fungus apple culture - *Venturia inaequalis* (apple scab) and *Podosphaera leucotricha* (apple powdery mildew) in 2004-2005

After analyzing the leaves, before applying the treatments is determined a high attack at variant V2 of 70.68%, and at V6 and V7 of 64.67%, and variants V1, V4, V5, V8 and V9 the attack has values between 17.67% and 41.35% (**Tab.7.1**).

Making the difference between the percentage of attack to control after treatment and the one before treatment is has been observed that the treatment of variant V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3) is different, followed by V7 (DDW FM θ 37 x 10^{-3} g/cm^3), and V4 (wept grape vine θ 3,7) și V5 (wept grape vine θ 37). (**Tab.7.1**).

Tab.7.1 Data on scab at apple before and after treatments in 2004

No.	Apples trees chosen for experiment	Total leaves analyzed	% no. of leaves attacked by scab				
			Before treatment		After treatment		Dif.
			% attack	% to control	% attack	% to control	
1.	Martor	600	2,83	100	3,33	100	0
2.	V1 (apă + DDW) (1:4)	600	1,66	41,35	1,16	65,2	+23,86
3.	V2 DDW	600	0,83	70,68	0,66	79,9	**+9,3**
4.	V3 (plâns viță de vie)	300	2	29,38	1,66	50,1	+20,72
5.	V4 (plâns viță de vie θ 3,7)	300	1,66	41,35	1,66	50,1	**+8,76**
6.	V5 (plâns de viță de vie θ 37)	300	1,66	41,35	1,66	50,1	**+8,76**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	1	64,67	1,33	60,0	**-4,66**
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	1	64,67	1	69,96	**+5,32**
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	1	41,35	1,33	60,0	+18,65
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	2,33	17,67	1,66	50,1	+32,5

The scab attack in 2005 before treatment, had values of 100% at control, V4 (wept grape vine θ 3,7) and V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3), and at the others variants the attack had values between 17% și 33,5% to control (**Tab.7.2**).

After applying the treatments one of the most important was the one made at variant V6 (DDW FM θ3,7 x 10^{-3} g/cm^3), followed by V4 (wept grape vine θ 3,7).

At powdery mildew the treatment from variant V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3) is different in V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3), followed by the treatments from the variants V2 (DDW) andV5 (wept grape vine θ 37).

In variant V2 the treatment with DDW had maximum effect also at scab and powdery mildew and in variant V7 (DDW FM θ 37 x 10^{-3} g/cm^3) had efficiency of 69,96% ats cab and reduced totally the attack at powdery mildew.

Tab.7.2 Data on scab at apple before and after treatments in 2005

No	Apple trees chosen for experiment	Total leaves analyzed	% no of leaves attack by scab				
			Before treatment		After treatment		Dif.
			% attack	% to control	% attack	% to control	
1.	Martor	600	2	100	3,33	100	0
2.	V1 (apă + DDW) (1:4)	600	1,33	33,5	0,66	79,9	+46,4
3.	V2 DDW	600	1,33	33,5	1,16	65,2	**+31,7**
4.	V3 (plâns viță de vie)	300	1,66	17	1,66	50,1	**+33,1**
5.	V4 (plâns viță de vie θ 3,7)	300	2	100	1,66	50,1	**-49,9**
6.	V5 (plâns de viță de vie θ 37)	300	1,66	17	1,66	50,1	**+33,1**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	2	100	1,33	60,0	-40,0
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	1,33	35,5	1	69,96	36,46
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	1,66	17	1,33	60,0	+43
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	1,66	17	1,66	50,1	+33,1

The attack of the *Podosphaera leucotricha* pathogen was after treatment lower in the control being of 1.16% to *Venturia inaequalis* which was 3.33%. In variant V9 (DDW CoFe2O4 θ 37x10-3 g/cm3) the attack was totally reduced. A high level of treatment effectiveness is recorded at variants V2 (DDW) and V5 (wept grape vine θ 37) (**Tab.7.3 – Tab.7.4**).

Tab.7.3 Data on powdery mildew attack at apple before and after treatments in 2004

No.	Apple trees chosen for experiment	Total leaves analyzed	% no of leaves attacked by powdery mildew				
			Before treatment		After treatment		Dif.
			% attack	% to control	% attack	% to control	
1.	Martor	600	0,5	100	1,16	100	0
2.	V1 (apă + DDW) (1:4)	600	0,33	34	0,33	71,2	+37,2
3.	V2 DDW	600	0,5	100	0,33	71,2	**-28,8**
4.	V3 (plâns viță de vie)	300	0,66	32	0,66	42,5	**+10,5**
5.	V4 (plâns viță de vie θ 3,7)	300	0,34	34	0,33	71,2	+37,2
6.	V5 (plâns de viță de vie θ 37)	300	1	100	0,33	71,2	**-28,8**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	0	0	0	0	0
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	0	0	0	0	0
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	0	0	0	0	0
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	0	o	0,34	0	-0,34

Tab.7.4 Data on mildew attack at apple before and after treatments in 2005

No.	Apple trees chosen for experiment	Total leaves analyzed	% no. of leaves attacked by powdery mildew				
			Before treatment		After treatment		Dif.
			% attack	% to control	% attack	% to control	
1.	Martor	600	0,83	100	1,16	100	0
2.	V1 (apă + DDW) (1:4)	600	0,33	60,25	0,33	71,2	**+11,01**
3.	V2 DDW	600	0,33	60,25	0,66	42,6	**-17,65**
4.	V3 (plâns viță de vie)	300	0,33	60,25	0,33	71,2	**+11,01**
5.	V4 (plâns viță de vie θ 3,7)	300	0,33	60,25	0,33	71,2	**+11,01**
6.	V5 (plâns de viță de vie θ 37)	300	0,33	60,25	0,66	42,6	-17,65
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	0	0	0	0	0
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	0,33	60,25	0	0	-60,25
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	0	0	0	0	0
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	0,33	60,25	0	0	-60,25

7.2 The treatment effect with fungicides and DDW 30ppm (deuterium depleted water) on pathogen agents at apple culture in 2006-2009

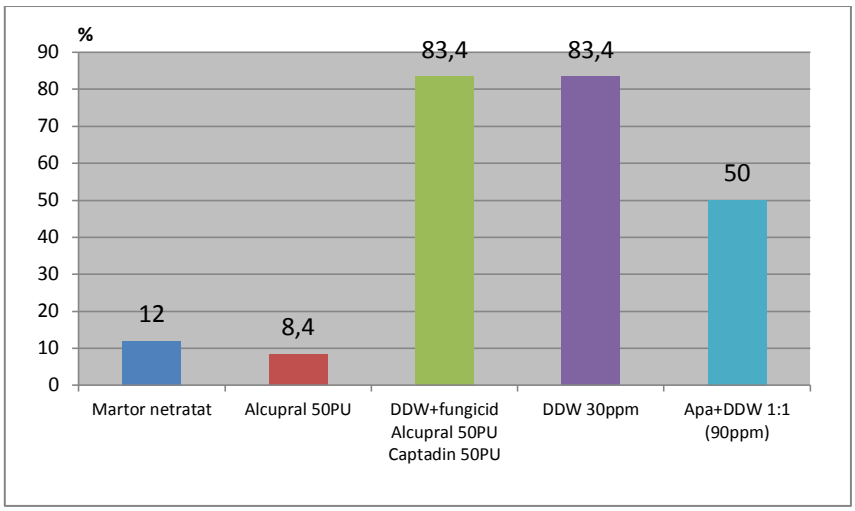


Fig.7.1 Treatment efficiency applied at apple for Venturia inaequalis (%) - 2006

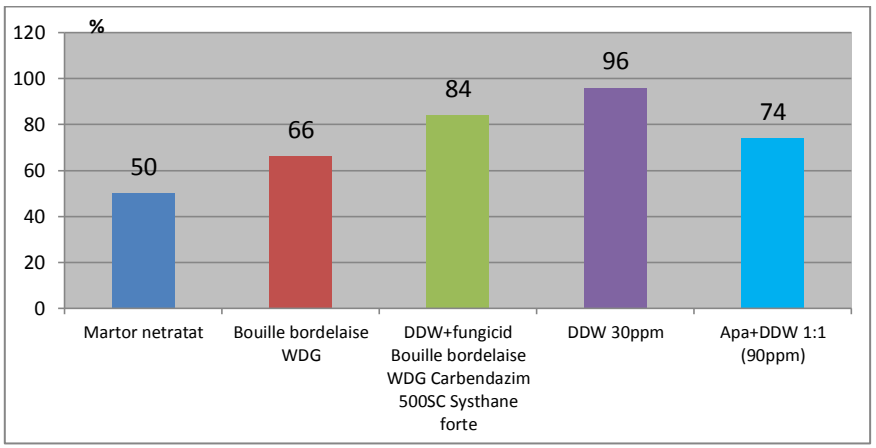


Fig.7.2 Treatment effectiveness applied ar apple for venturia inaequalis (%) - 2007

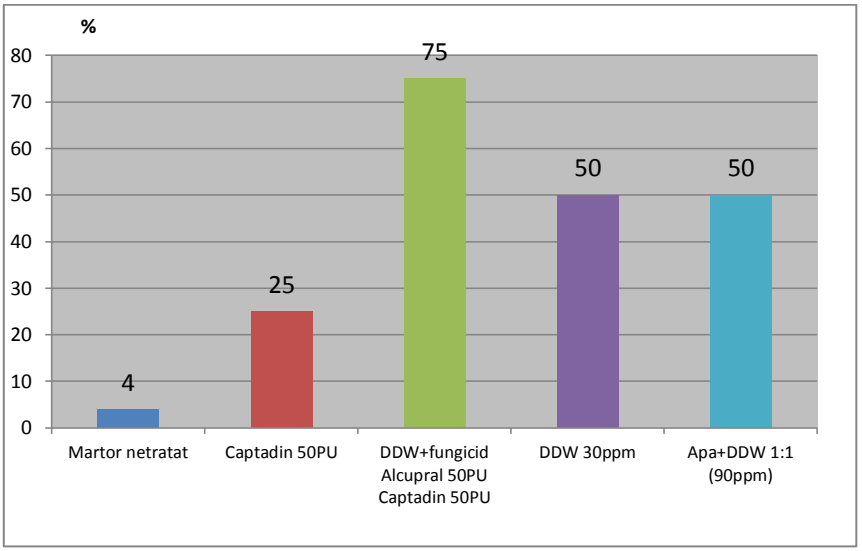


Fig.7.3 Treatment effectiveness applied at apple for Podosphaera leucotricha (%) – 2006

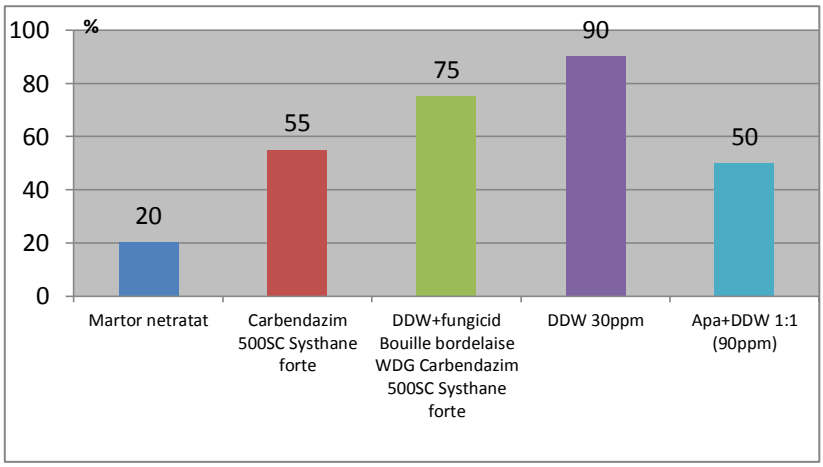


Fig.7.4 Treatment effectiveness applied at apple for Podosphaera leucotricha (%) - 2007

The effectiveness of the products tested for *Venturia inaequalis* (apple scab) and *Podosphaera leucotricha* (apple powdery mildew) in the years 2006-2007 when it was the lowest attack at control and the highest degree of attack (**Fig.7.1 –Fig.7.4**).

The results recorded and presented in **Fig.7.1-7.4** show that there is a significant positive difference in the treatments carried out with DDW + fungicides and DDW simple, so as to *Venturia inaequalis* and *Podosphaera leucotricha*.

7.3 The treatment effect with unconventional materials on plum fungi culture - *Polystigma rubrum* (red leaf blotch) and *Monilinia laxa* (plum monilinia) in 2004-2005

After applying the treatments for combating red leaf blotch, significant effectiveness in treatments had the variants V3 (wept grape vine), V2 (DDW) and V5 (wept grape vine θ 37).

Tab.7.5 *Data on red leaf blotch and attack at plum trees before and after treatments – 2004*

No.	Plum trees chosen for the experiment	Total leaves analyzed	% no. of leaves attacked by red leaf blotch				Dif.
			Before treatment		După tratament		
			% attack	% to control	% attack	% to control	
1.	Martor	300	1,66	100	3,33	100	0
2.	V1 (apă + DDW) (1:4)	300	2,66	-60	1,66	50,16	+110,16
3.	V2 DDW	300	1,66	100	1,66	50,16	**-49,84**
4.	V3 (plâns viță de vie)	300	1,66	100	1,33	60,06	**-39,94**
5.	V4 (plâns viță de vie θ 3,7)	300	2	-20	1,66	50,16	70,16
6.	V5 (plâns de viță de vie θ 37)	300	1,66	100	1,66	50,16	**-49,84**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	1,33	19,87	1,33	60,06	**+40,19**
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	1,33	19,87	1,33	60,06	**+40,19**
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	2	-20	1,66	50,16	+70,16
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	1,33	19,87	1,33	60,06	+40,9

Analysing the data from **Tab.7.5 –Tab.7.6** we can see a significant effectiveness of treatments from variants V2 (DDW), V5 (wept grape vine θ 37), followed by V6 (wept grape vine θ 37).

Tab.7.6 *Data on red leaf blotch attack at plum trees before and after treatments - 2005*

No.	Plum trees chosen for experiment	Total leaves analyzed	% no. of fruits attacked by red leave blotch				Dif.
			Before treatment		After treatment		
			% attack	% to control	% attack	% to control	
1.	Martor	300	3,33	100	3,33	100	0
2.	V1 (apă + DDW) (1:4)	300	2,66	-20,12	1,66	50,16	+70,28
3.	V2 DDW	300	3,33	100	1,66	50,16	**-40,84**
4.	V3 (plâns viță de vie)	300	1,66	50,16	1,33	60,06	**+10,06**
5.	V4 (plâns viță de vie θ 3,7)	300	2	39,93	1,66	50,16	**+10,23**
6.	V5 (plâns de viță de vie Θ 37)	300	3,33	100	1,66	50,16	**-49,84**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	300	2	39,93	1,33	60,06	+20,13
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	300	1,33	60,06	1,33	60,06	0
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	300	2	39,93	1,66	50,16	**+10,23**
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	300	1,33	60,06	1,33	60,06	0

For monilinia the best effectiveness was at treatments from the variants V1 (water + DDW) (1:4), followed by V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3), V7 (DDW FM θ 37 x 10^{-3} g/cm^3) and V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3) (**Tab.7.7.).**

Tab.7.7 *Data on monilinia attack at plum trees before and after treatments – 2004*

No.	Plum trees chosen for experiment	Total leaves analyzed	% no. Of fruits attacked by monilinia				Dif.
			Before treatment		After treatment		
			% attack	% to control	% attack	% to control	
1.	Martor	100	8	100	18	100	0
2.	V1 (apă + DDW) (1:4)	100	8	100	6	66,6	**-33,4**
3.	V2 DDW	100	6	25	6	66,6	+41,6
4.	V3 (plâns viță de vie)	100	3	62,5	3	62,5	0
5.	V4 (plâns viță de vie θ 3,7)	100	2	75	2	88,8	13,8
6.	V5 (plâns de viță de vie θ 37)	100	2	75	2	88,8	13,8
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	100	1	87,5	1	94,4	6,9
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	100	1	87,5	1	94,4	6,9
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	100	1	87,5	1	94,4	6,9
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	100	3	62,5	2	88,8	**26,3**

Tab.7.8 *Data on monilinia attack at plum trees before and after treatments - 2005*

No.	Plum trees chosen for experiments	Total leaves analyzed	% of fruits attacked by monilinia				Dif.
			Before treatment		After treatment		
			% attack	% to control	% attack	% to control	
1.	Martor	100	8	100	18	100	0
2.	V1 (apă + DDW) (1:4)	100	8	100	6	66,6	**-33,4**
3.	V2 DDW	100	6	25	6	66,6	+41,6
4.	V3 (plâns viță de vie)	100	3	62,5	3	83,3	+20,8
5.	V4 (plâns viță de vie θ 3,7)	100	2	75	2	88,8	**+13,8**
6.	V5 (plâns de viță de vie θ 37)	100	2	75	1	94,4	**+19,4**
7.	V6 (DDW FM θ 3,7 x 10^{-3} g/cm^3)	100	1	87,5	1	94,4	**+6,9**
8.	V7 (DDW FM θ 37 x 10^{-3} g/cm^3)	100	1	87,5	1	94,4	**+6,9**
9.	V8 (DW $CoFe_2O_4$ θ 3,7x10^{-3} g/cm^3)	100	1	87,5	1	94,4	**+6,9**
10.	V9 (DDW $CoFe_2O_4$ θ 37x10^{-3} g/cm^3)	100	2	75,0	3	62,5	25

7.4 The treatment effect with fungicides and DDW 30ppm (deuterium depleted water) on pathogen agents at plum culture

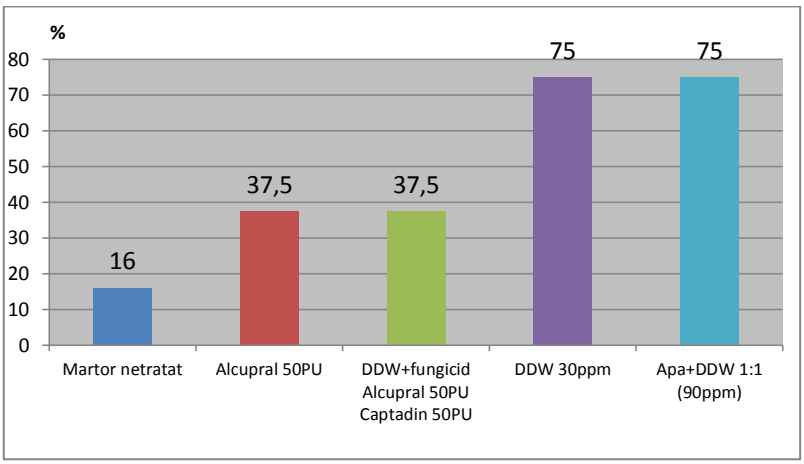


Fig.7.5 Treatment effectiveness applied at plum for Polystigma rubrum - 2006

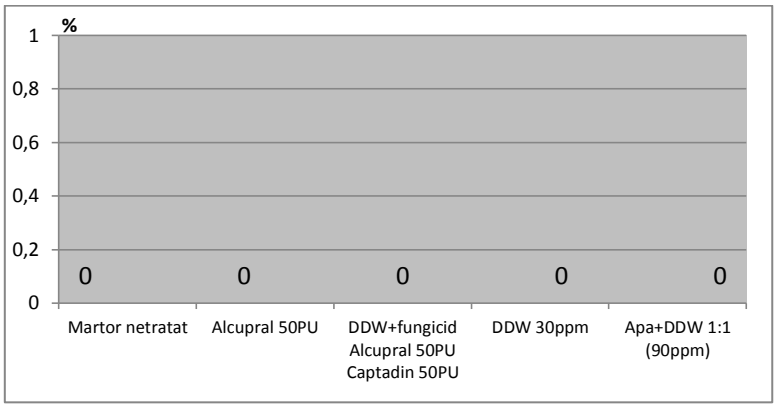


Fig.7.6 Treatment effectiveness applied at plum for Polystigma rubrum 2007 - 2009

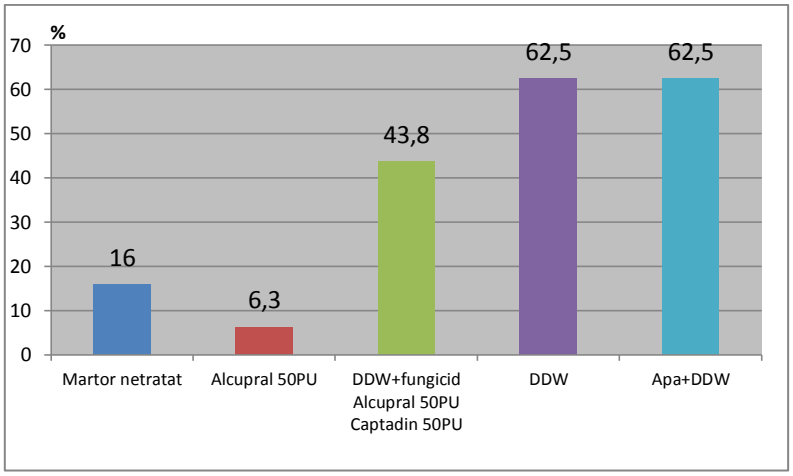


Fig.7.7 Treatment effectiveness applied at plum for Monilinia laxa - 2006

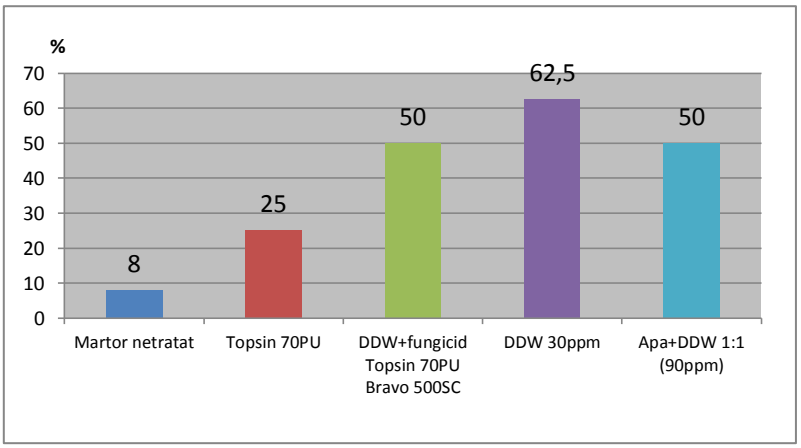


Fig.7.8 Eficacitatea tratamentelor aplicate la prun pentru *Monilinia laxa* 2008 și 2009
Fig.7.8 Treatment effectiveness applied at plum for Monilinia laxa 2008 și 2009

Analysing the **Fig.7.5 - 7.6** we can see that in the case of variants treated with DDW simple and water + DDW (1:1) the effectiveness was significant different in combating the *Polystigma rubrum* pathogen agent. In the case of the *Monilinia laxa* pathogen the lowest degree of attack was registered in 2008 and 2009 and the highest was recorded in 2006. Best efficiency in combating this pathogen agent have had treatments with fungicides and DDW + fungicides and DDW simple

7.5 The answer to phytosanitary treatment – apple culture

7.5.1 The treatment effect on production elements at apple

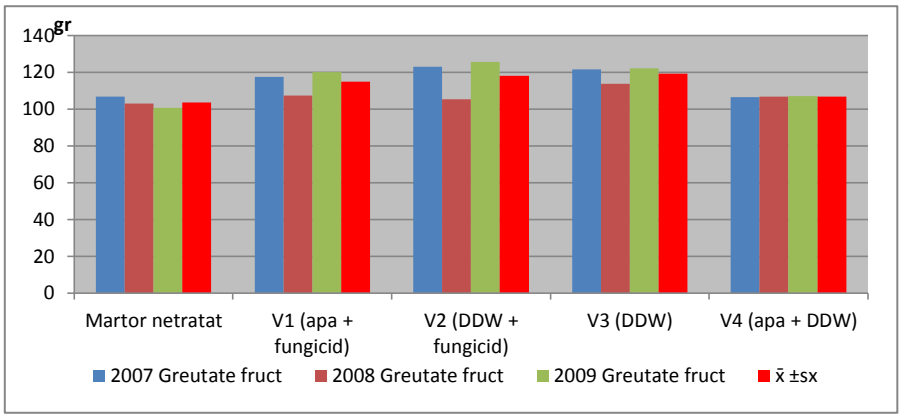
During the experimental years 2007 - 2009 had been observed the treatment effect on some aspects of production, namely, fruit size and production kg / tree or hectare.

7.5.1.1 Treatment effect on quantitative values

The main quantitative characters of apple fruit are weight, diameter and height. Weight an important factor of productivity and quality. The character has determinism polygenic, so variability is high and very high not only between varieties but even within the same variety **(Sestraș**, 2004). The fruit weight has values between certain limits (eg Jonathan variety: 110-140g) and not average values.

The diameter of the apple fruit, is a very important quality factor, apart from the hereditary influence is strongly conditioned by environmental factors. Height and apples diameter were measured with gauge and are the characteristics that define the shape of the fruit (**Sestraș**, 1997).

From the data recorded in Fig.7.9 we can observe the treatment influence in the variants V2 (DDW+fungicide) and V3 (DDW) on fruit dimensions no matter the experimental years. These presents significance differences to control.



Variant	Fruit apple weight (gr)			$x \pm s_x$
	2007	2008	2009	
Martor netratat	106,6	103	100,6	103,4 ±0,78
V1 (apa + fungicid)	117,5	107,23	120,06	114,93 ±1,75
V2 (DDW + fungicid)	123	105,38	125,5	117,99 ±2,84
V3 (DDW)	121,5	113,6	122	119,03 ±1,22
V4 (apa + DDW)	106,45	106,66	107	106,7 ±0,07

Fig.7.9 Fruit weight – Jonathan variety 2007-2009 and the media

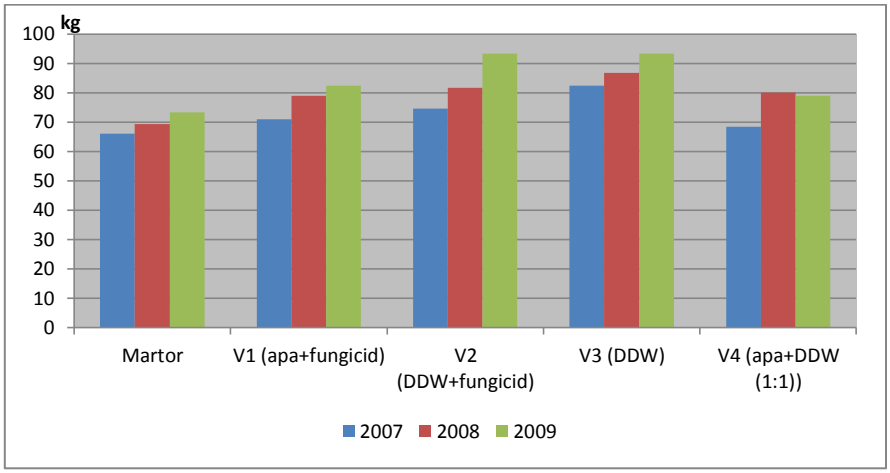


Fig.7.10 Fruit production kg/tree of the variety Jonathan 2007-2009

The treatment made with DDW+fungicide and DDW simple had a positive influence on the fruit production (**Fig.7.10**).

7.5.1.2 The treatment effect on qualitative values

In the laboratory was determined the sugar content (Brix degrees) and dry fruits of apple for each variant using the RE 40refractometer and thermobalance.

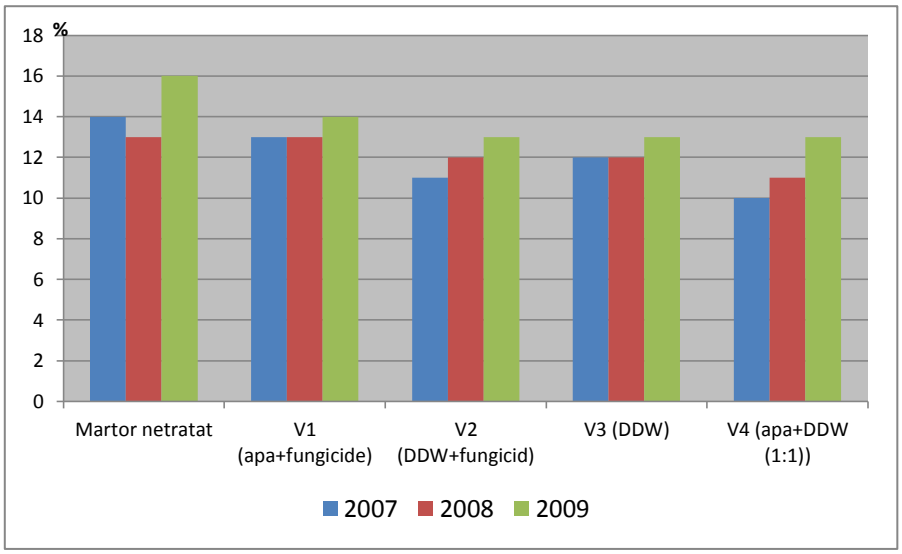


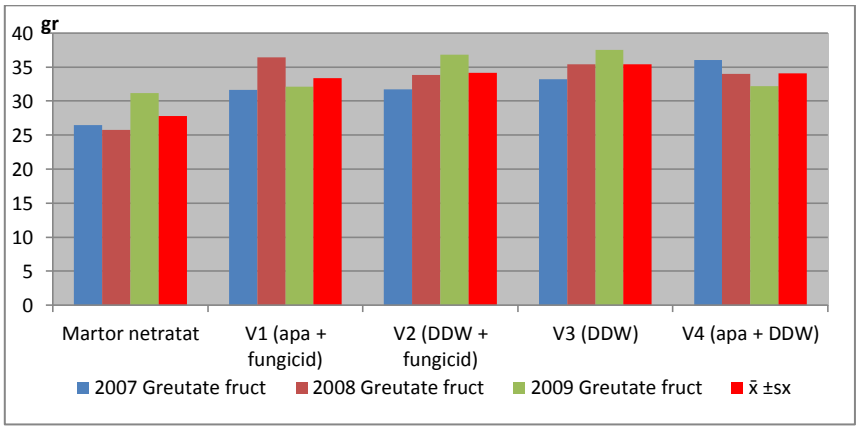
Fig.7.11 Sugar from apple fruits – Jonathan variety 2007 – 2009

7.6 The plant response to phytosanitary treatment – plum culture

7.6.1 The treatment effect on production elements at plum culture

7.6.1.1 The treatment effect on quantitative values

Plum fruit dimensions had suffer obvious changes to control regarding the weight of the fruit, in variants V3 (DDW) and V4 (DDW + water (1:1)). Dimensions were determined through weighting and measuring with gauge. (**Fig.7.12**).



Variant	Plum fruit weight			
	2007	2008	2009	$\bar{x} \pm s_x$
Martor netratat	26,48	25,75	31,15	27,79 ±0,76
V1 (apa + fungicid)	31,6	36,39	32,13	33,37 ±0,68
V2 (DDW + fungicid)	31,72	33,85	36,81	34,13 ±0,66
V3 (DDW)	33,21	35,38	37,54	35,38 ±0,56
V4 (apa + DDW)	36	34	32,19	34,06 ±0,49

Fig.7.12 Fruit weight dimensions – Stanley variety 2007-2009 and the media

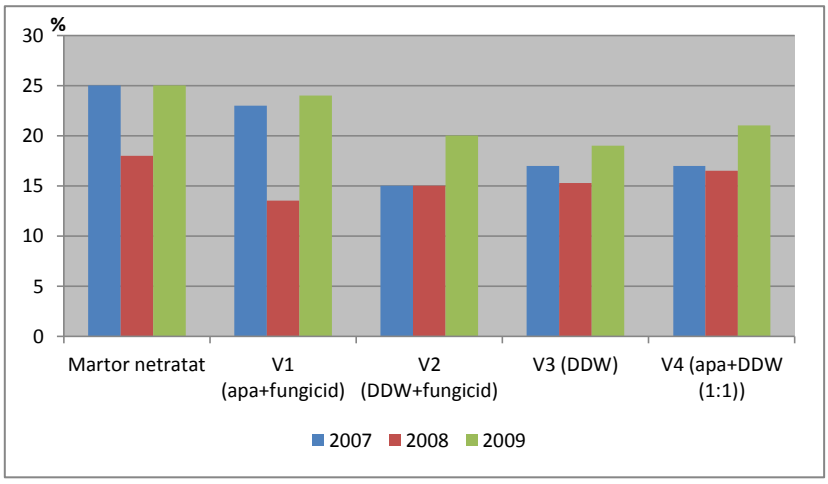
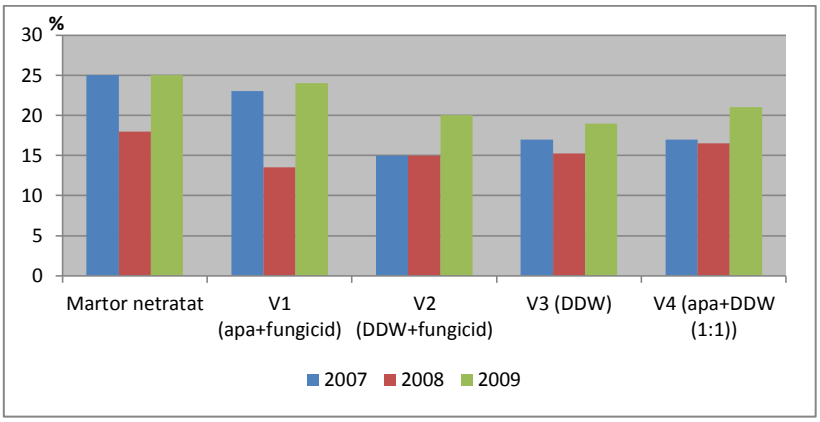


Fig.7.13 Fruit production kg/tree at Stanley variety 2007-2009

In 2007-2009 was determined the tree fruit production for each variant through weighting, and in variants V2 (DDW+fungicide) and V3 (DDW) the fruit production is significant to control in all these years (**Fig.7.13**).

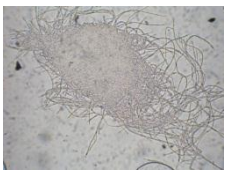


Tab.7.14 Sugar fromm plum fruits – Stanley variety 2007 - 2009

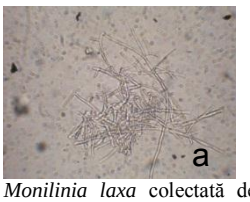
From **Fig.7.14** we can see that the sugar content, in variants where was used DDW is lower than in control variant and in V1 (water+fungicide).

7.7 The effect of different nanomaterials (PNM) and on deuterium depleted water (DDW) on *Monilinia laxa fungus*

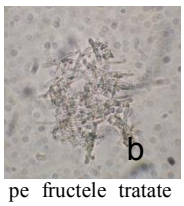
To obtain biological material, *Monilinia laxa*, to test the effectiveness of NPM and DDW on hypha growth and capacity for fructification of the fungus were conducted several experimental cycles. *Monilinia laxa* was chosen because it is easily identifiable and was a previous experience in microscopic and phenotypic identification of the fungus.



Monilinia laxa crescută pe mediul de cultură standard după 10 zile (60x; original)

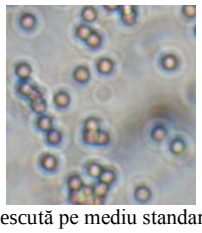


Monilinia laxa colectată de pe fructele tratate cu DDW 30ppm (a) şi crescută pe mediul cu DDW 30 ppm (b) după 10 zile de la inoculare (100x; original



Monilinia laxa colectată de pe fructe stropite cu γ-Fe_3O_4 θ = 3.7x 10^{-3}g/cm^3 /DDW 30ppm şi crescută pe mediul cu DDW şi γ-Fe_3O_4 θ = 3.7x 10^{-3}g/cm^3 /10 zile de la inoculare (100x; original)

Compared to control where it is observed that the hypha are abundant, in the case of application of these treatments they are short, and when on the medium were added namomaterials the increase is very small.



Conidii de la *Monilinia laxa* crescută pe mediu standard de cultură. Dispunere tipică a acestora

Conidii de la *Monilinia laxa* crescută pe mediul de cultură preparat în DDW 30 ppm. Dispunere atipică a conidiilor: sub formă de cerc (a) şi comprimate (b)

If *Monilinia* laxa it is developed on standard medium the conidia have a typical arrangement. When grows on the prepared culture medium in DDW 30ppm, conidia have an unusual arrangement like a circle and film, which shows that their germination is inhibited.

CAPITOLUL VIII
ECONOMIC EFFICIENCY

The economic efficiency is the report from effect and effort. Economic efficiency is calculated in plant protection more difficult than in other sectors, since many factors are involved which, are conditioned by environmental, biotic complex interrelationships, which cannot be integrated into the formula, having the largest qualitative values with very different implications.

Economic effectiveness of the tested products was calculated by gravimetric method, correlated with the degree of attack of fungi and fruit quality. The economic efficiency in the variants where was used DDW is smaller than the control and variant V1 (water+fungicide) (**Tab.8.1**).

Tab.8.1 Eficienţa economică a tratamentelor fitosanitare la cultura mărului în 2007-2009
Tab.8.1. Economic effectiveness of fitosanitary treatments at apples in 2007-2009

Variant	2007	2008	2009	$x \pm s_x$	
	Profit/ Lei	Profit/ Lei	Profit/ Lei		Difference to control
Martor	41.250	43.331,25	45.831,25	43.470,83 ±592,69	0
V1 (apa+fungicid)	58.894,75	48.799,04	51.313,81	53.002,53 ±1357,93***	9531,69
V2 (DDW+fungicid)	32.257,25	36.967,79	44.688,81	37.971,28 ±1621,69	-5499,55
V3 (DDW)	37.956,25	40.668,75	44.831,25	41.152,08 ±894,76 **	**-1799,25**
V4 (apa+fungicid)	36.406,25	43.700	43.075	41.060,42 ±1044,58**	**-2318,75**

At plum culture the best economic efficiency to control was at variant V4 (water+DDW (1:1) and V2 (DDW+fungicide) (**Tab.8.2**).

Tab.8.2 Eficiența economică a tratamentelor fitosanitare la cultura prunului în 2007-2009
Tab.8.2. Economic effectiveness of fitosanitary treatments at plum in 2007-2009

Variant	2007	2008	2009		
	Profit/ Lei	Profit/ Lei	Profit/ Lei	$x \pm s_x$	Difference to control
Martor	38.625	41.625	48.750	43.000 ±1343,77	0
V1 (apa+fungicid)	47.827,5	46.814,98	54.903,4	49.849,63 ±1138,64***	6848,63
V2 (DDW+fungicid)	34.702,5	35.752,48	42.341	37.600,66 ±1069,47**	**-5399,34**
V3 (DDW)	35.062,5	35.437,5	41.437,5	37.312,50 ±924,31	-5687,5
V4 (apa+fungicid)	39.825	40.425	45.825	42.025 ±853,84**	**-975,0**

The difference in profit at both cultures is offset by the fact that organic fruit is being used in ecological food for people.

CHAPTER IX
CONCLUSIONS AND RECOMMENDATIONS

1. The average values of attack degree between 2006-2009 at all the pathogen agents studied decreased

- *Venturia inaequalis (apple* scab*)*, decreased compared to the control by 10% in 2006, by 42% in 2007, by 9% in 2008 and by 16% in 2009 at variants treated with DDW 30ppm + fungicide and DDW simple
- *Podosphaera leucotricha* (powdery mildew) the attack degree was reduced by 10-11% in 2006, by 45-48% in 2007, 16% in 2008 and 16-17% in 2009, at variants treated with cu DDW 30ppm + fungicide and DDW 30ppm simple.
- *Monilinia laxa* (plum monilinia) is a decrease by 7% in 2006, by 10-13% in 2007, 4% in 2008 and 2-4% in 2009, at the variants treated with DDW 30ppm
- *Polystigma rubrum* (red leaf blotch) in 2006 the attack degree decreased by 12% to control at the treatment applied with DDW 30ppm + fungicide.

2. Fruit tree production was influenced by the applied treatments with DDW 30ppm.

- at apple culture fruit tree production registered differences to control at variant V3 (DDW) and at variant V2 (DDW+fungicide).
- at plum culture fruit tree production registered differences to control at variant V2 (DDW+fungicide) and V3 (DDW).
- apple and plum fruit dimensions (fruit weight) were little influenced by the DDW treatment, their values maintenaind in normal limits for the varieties used in experiences.

Phytosanitary treatment had influence on the sugar content and dry substance from fruits.

- in the variants (V2-DDW+fungicide, V3-DDW, V4-water+DDW) where were applied unconventional treatments with values between 1-3% at apple and between 1-6% at plum.
- the fruits with low content of sugar can be used for diabetic people.
- the high content of dry substance allows good preservation of fruit over winter.

3. Economic efficiency

- economic profit lei / ha was obtained in the variants treated with phytosanitary products (conventional), followed by untreated variant.
- variations in which were applied treatments with unconventional products (DDW 30ppm), the profit is less, given the high value of this product, but yields per hectare are higher and fruit can be kept longer.

4. Ecological efficiency

- are recommended treatments with DDW 30ppm taking into account that they maintain the level of attack below economically damaging pathogens.
- the use of these unconventional materials has an unpolluted effect and increasingly gaining ground as more technology and science will provide the cheapest methods of obtaining them.
- the use of these materials in tree orchards is recommended because the most fruits are consumed fresh.