PHYSIOLOGICAL EFFECTS OF TREATMENT WITH FUNGICIDES IN *Malus domestica* BORKH. ATTACKED BY *Venturia inaequalis* (COOKE) WINT

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Abstract. Research regarding the physiological effects of treatment with fungicides were carried out on apple **Jonagold** variety cultivated in the region of Oltenia (Banu Mărăcine, Dolj). There have been three treatments with contact fungicide **Dithane** M 45-0.2%, after the appearance of the symptoms of the attack produced by the pathogen on leaves. The first treatment has been carried out at the beginning of fruit formation (May 10th 2011) and the following treatments were applied to the period of 10 days. The physiological analyses were conducted on the attacked leaves by pathogen, before the application of treatments with fungicide and then after the last treatment with fungicide. It was found that the intensity of photosynthesis and the intensity of transpiration vary depending on the degree of the attack and the climatic conditions. Intensity of photosynthesis in attacked leaves is lower, correlated with a low chlorophyll content, and intensity of transpiration has higher values compared to the leaves of the analysed plant after the treatment with fungicides. There has been a lower water content and a higher of dry substance content in the leaves of plants attacked by pathogen.

Keywords: apple tree, attacked leaves, fungicide, healthy leaves, pathogen.

Rezumat. Efectele fiziologice ale tratamentului cu fungicide la *Malus domestica* BORKH. atacat de *Venturia inaequalis* (COOKE) WINT. Cercetările privind efectele fiziologice ale tratamentului cu fungicide s-au efectuat la soiul de măr **Jonagold** cultivat în regiunea Olteniei (Banu Mărăcine, Dolj). S-au realizat trei tratamente cu fungicidul de contact **Dithane** M 45-0,2 %, după apariţia simptomelor atacului produs de patogen pe frunze. Primul tratament s-a efectuat la începutul formării fructelor (10 mai 2011) şi următoarele tratamente s-au efectuat la interval de 10 zile. Analizele fiziologice s-au realizat la frunzele atacate de patogen, înainte de aplicarea tratamentelor cu fungicide şi apoi după ultimul tratament cu fungicide. S-a constatat că intensitatea fotosintezei şi intensitatea transpiraţiei variază în funcţie de gradul de atac şi condiţiile climatice. În frunzele atacate intensitatea fotosintezei este mai scăzută, fapt corelat cu conţinutul scăzut în clorofilă, iar intensitatea transpiraţiei are valori mai mari, în comparaţie cu frunzele plantelor analizate după tratamentul cu fungicide. S-a înregistrat un conţinut mai scăzut de apă şi un conţinut mai mare de substanţă uscată, în frunzele plantelor atacate de patogen.

Cuvinte cheie: măr, frunze atacate, fungicid, frunze sănătoase, patogen.

INTRODUCTION

Original from Central Asia, the apple is frequently attacked by disease and pests, and this is why a large number of chemical treatments have been carried out.

Apple scab produced by *Venturia inaequalis* (COOKE) WINT. represents a major problem in apple production and to ensure high yields and fruit quality requires the application of fungicides.

Application of fungicides may affect crop physiology by various disruptions such as growth reduction, perturbation in the development of reproductive organs, alteration of nitrogen, and/or carbon metabolism leading to a lower nutrient availability for plant growth. The sensitivity of some plant species may depend on the developmental stage (more sensitive to the treatments at young stages or during critical events such as reproduction) or the type of pesticides used (PETIT et al., 2012).

The net photosynthetic activity is subjected to seasonal changes and to diurnal changes, which are mainly influenced by the stage of shoot development, leaf ageing, hormones and carbohydrates accumulation in leaves, as well as by light intensity fluctuations, leaf temperature, air temperature and humidity (LAKSO, 1985).

Other research conducted on apple leaves shows there was a slight decrease in the intensity of photosynthesis at midday (LANDSBERG et al., 1975).

The intensity of the photosynthesis process is higher in the case of the apple leaves located at a height of 1.8 m compared with those located at 1.0 m above the ground (CORELLI & SANSAVINI, 1989).

The young leaves have the highest intensity of the transpiration process and as they get older, the transpiration intensity decreases, the lower values being recorded at senescent leaves (BURZO et al., 1999).

The intensity of transpiration process proportionally increases with that of photosynthesis, both processes being dependent on solar radiation intensity (BIGNAMI & NATALI, 1992).

The intensity of photosynthetic active radiations is higher near the edge of the crown and close to the stem axis and decreases from higher to lower levels (MARINI & MARINI, 1983).

Positive correlations were established between the intensity of the physiological processes and the photosynthetic active radiation, the leaf temperature and stomatal conductance of CO_2 (NICOLAE, 2010).

The chlorophyllian pigment content was higher in plant leaves analysed after treatments done with fungicide, compared with the leaves attacked by the pathogen, there being a positive correlation between the chlorophyllian pigment content and the photosynthesis intensity (NICOLAE & BUŞE-DRAGOMIR, 2012).

MATERIAL AND METHODS

The research regarding of the physiological changes produced by the *V. inaequalis* were carried out in the *M. domestica* (**Jonagold** variety) cultivated in the climatic conditions specific to the region of Oltenia (Banu Mărăcine, Dolj).

M. domestica grows in all countries with temperate, warm climates and rain during winter, but also in the vegetation period. This fruit tree has a thick trunk, the leaves have oval or elliptical form, have serrate edge and are petiolated. The flowers are hermaphrodite, arranged in corymb inflorescence. The apple is the fruit of the *M. domestica* and it differs in shape, size, colour, texture of peel and time of maturity.

The apple **Jonagold** variety comes from the crossing of the species *Jonathan x Golden*. It is a variety with vigorous growth, the fruit is spherical, with a sea of red colour, smooth, ripen in the second half of September.

The physiological processes were established with the ultra-compact photosynthesis measurement system - Lci. The results obtained were graphically represented and statistically interpreted. The water contents and dry substance were determined by the gravimetric method. The chlorophyll content was estimates by Minolta SPAD 502.

The estimate of the attack was made using the calculation formulae by SĂVESCU & RAFAILĂ, 1978.

The treatment with the fungicide **Dithane** M 45 (0.2%) was applied in three stages on the leaves, after the appearance of the symptoms on the attacked leaves by the pathogen. The first treatment has been carried out at the beginning of the formation of fruit and other treatments have been carried every 10 days. The physiological analyses were conducted on the attacked leaves by pathogen before the application of treatments and then two weeks ~~later~~ after the last treatment with fungicides.

Dithane M 45 is a contact fungicide with a very broad spectrum in combating pathogens agent in vegetables, flowers, fruit trees, vines, and treating seeds.

RESULTS AND DISCUSSIONS

Apple scab appears in orchards from all regions, being considered one of the most damaging diseases due to high both quantitative and qualitative crop losses.

The symptoms are generally most noticeable and serious on leaves and fruit. On the leaves spots more or less circular in shape, undefined, light gray are being observed at the beginning of the attack. Later, with the formation of conidiophores with conidia, the spots acquire an olive brown colour and velvety appearance (Figs. 1; 2).

The fruit attack manifests itself through the appearance of gray-olive spots and cracks in the tissues corresponding to spots.

V. inaequalis contains mycelium which is developing under cuticle, is brownish-olive, is septal, branched and forms brown stroma that appear on conidiophores and conidia. Conidiophores are short, cylindrical, brown, and on these at each end a brown conidia is formed, initially unicellular, and then bicellular (Fig. 3).

The autumn in fallen leaves form ascogonium and antheridium and then form asci with ascospores, closed in the perithecia (MITREA, 2006).

Figure 1. The *M. domestica* (Jonagold variety) attacked by *V. inaequalis* (original).

Figure 2. Detail of the leaf in *M. domestica* (Jonagold variety) attacked by *V. inaequalis* (original).

The *V. inaequalis* is usually controlled with fungicides applied in the period between leaf fall and bud break, or during the period of vegetation. After treatments with **Dithane** M 45 fungicide of the leaves with specific symptoms (light gray spots or the olive brown spots) the attack was stopped and the symptoms produced on leaves due to the pathogen have gradually disappeared (Fig. 4).

Figure 3. *V. inaequalis* - brown conidia (oc. 10 x ob. 20) (original).

Figure 4. The *M. domestica* (Jonagold variety) after treatment with fungicide (original).

The physiological analyses were performed on the leaves of the plants attacked by *V. inaequalis* before the application of three treatments with fungicide (May 10th 2011) and then two weeks after the last treatments.

The estimation of the attack (frequency, intensity and degree of attack) caused by *V. inaequalis* in apple **Jonagold** variety, before the application of treatments with fungicide, is presented in Fig. 5.

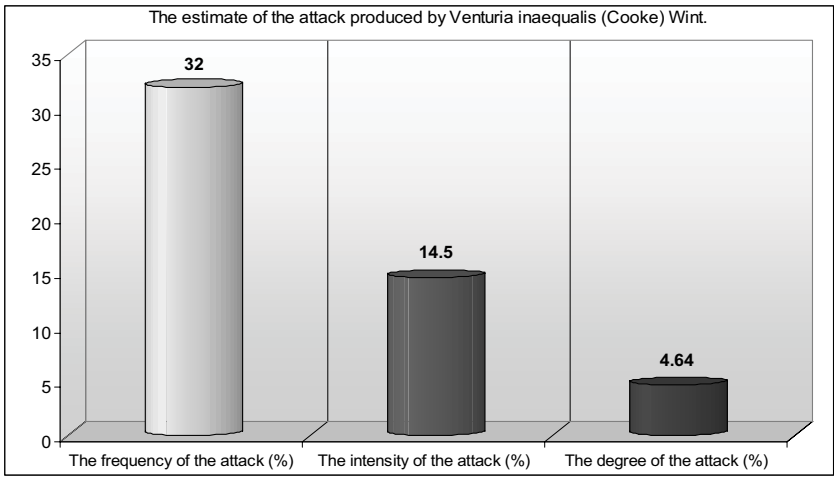

Figure 5. The estimate of the attack produced by *V. inaequalis* (COOKE.) WINT. in *M.domestica* BORKH.

The diurnal dynamics of photosynthesis and transpiration in the attacked leaves plants is similar to that in the plant leaves analysed after treatments, but the recorded values are lower in the attacked leaves (Figs. 6; 7).

Physiological processes intensity is lower in the leaves attacked by the pathogen as a result of the reduction of the assimilation surface due to the reaction of plants to pathogen, reduction of leaf surface due to the formation of light gray spots or the olive brown spots, deterioration of the chlorophyll pigments and stomata coverage of the pathogen.

The intensity of the physiological processes (photosynthesis and transpiration intensity) at the apple leaves depends on the photosynthetic active radiation received by leaves, the leaf temperature, the stomatal conductance for CO_2, etc.

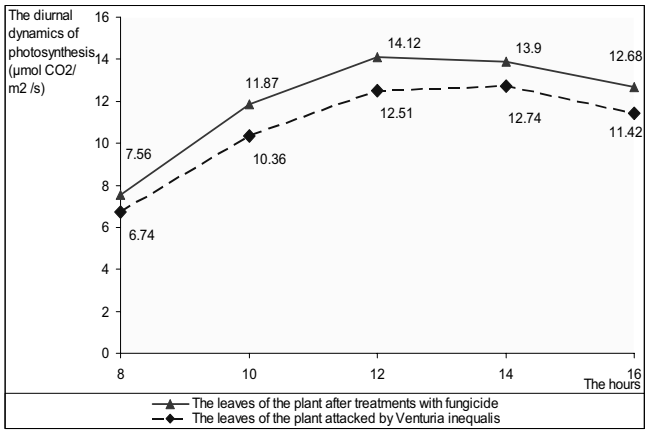

Figure 6. The intensity of photosynthesis in leaves of *M. domestica* - Jonagold variety.

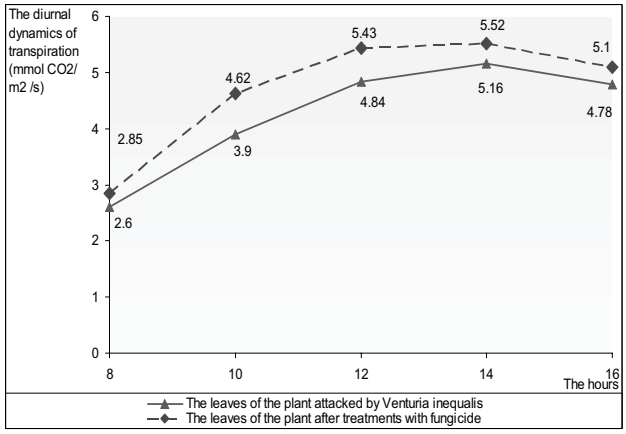

Figure 7. The intensity of transpiration in leaves of *M. domestica* - Jonagold variety.

At the analysed plant, one can observe an increase of the **photosynthetic active radiations** present on the surface of the leaves beginning in the morning (8 a.m.), when it records the values of 1292 µmol / m^2 / s in the leaves attacked by *V. inaequalis* and of 1150 µmol / m^2 / s in the plant leaves after treatments with fungicide, their growth after lunch (12 a.m.), when it records the values of 1460 µmol / m^2 / s in the attacked leaves and of 1415 µmol / m^2 / s in the leaves after treatments with fungicide, and decreases in intensity towards evening (4 p.m.), when it records the values of 1465 µmol / m^2 / s in the attacked leaves and of 1410 µmol / m^2 / s after treatments with fungicide.

Linear regression performed between the photosynthesis intensity and photosynthetic active radiations shows a good positive correlation between these; the coefficient of determination (R^2) is 0.94 for the attacked leaves and 0.96 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and photosynthetic active radiations shows a good positive correlation between these; the coefficient of determination (R^2) is 0.96 for the attacked leaves and 0.98 for the plant leaves after treatments with fungicide (Figs. 8; 9).

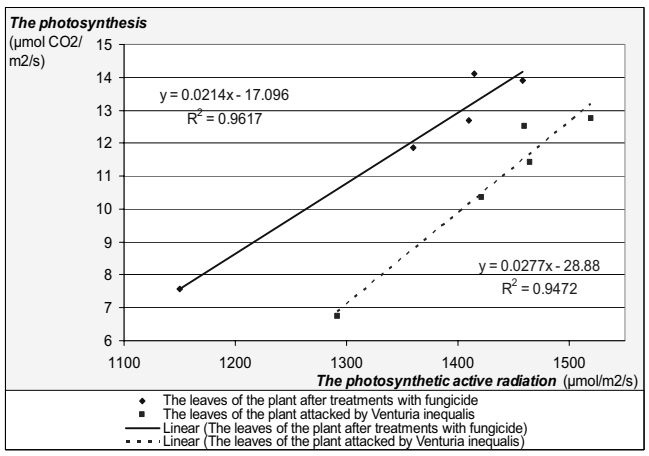

Figure 8. The correlation between the intensity of photosynthesis and the photosynthetic active radiation in *M. domestica* - Jonagold variety.

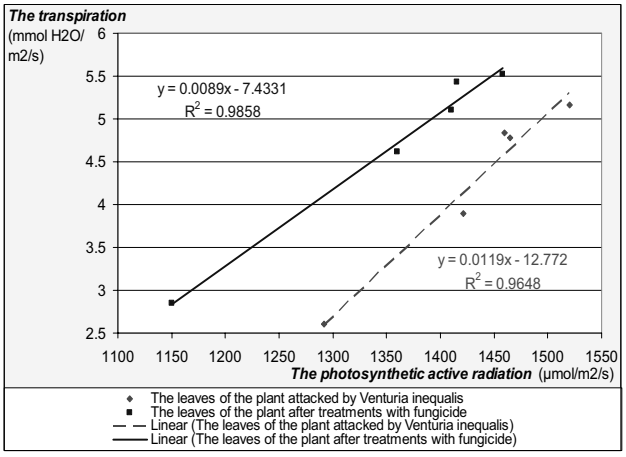

Figure 9. The correlation between the intensity of transpiration and the photosynthetic active radiation in *M. domestica* - Jonagold variety.

The *leaf temperature* increases beginning in the morning (8 a.m.), when it records the values of 27.9 ^oC in the attacked leaves and of 28.4 ^oC in the plant leaves after treatments, their growth after lunch (12 a.m.), when it records the values of 32.5 ^oC in the attacked leaves and 33.4 ^oC in the leaves after treatments and decreases towards the evening (4 p.m.), when it records the values of 31.6 ^oC in the attacked leaves and of 32.5 ^oC in the plant leaves after treatments.

Linear regression performed between the photosynthesis intensity and leaf temperature show a good positive correlation between these; the coefficient of determination (R^2) is 0.92 for the attacked leaves and 0.97 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and leaf temperature show a good positive correlation between these; the coefficient of determination (R^2) is 0.95 for the attacked leaves and 0.97 for the leaves plant after treatments with fungicide (Figs. 10; 11).

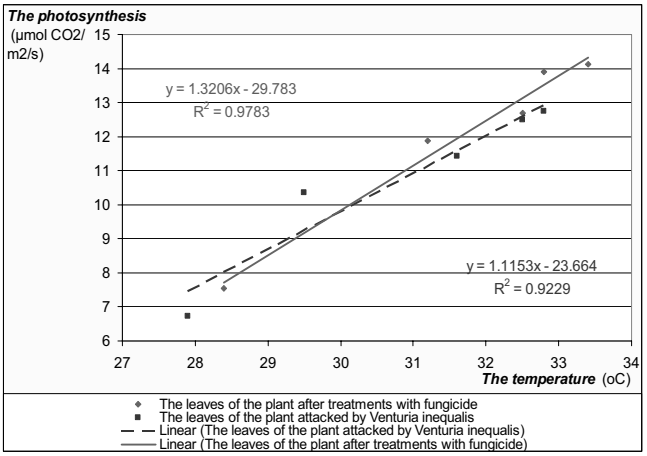

Figure 10. The correlation between the intensity of photosynthesis and the leaf temperature in *M. domestica* - Jonagold variety.

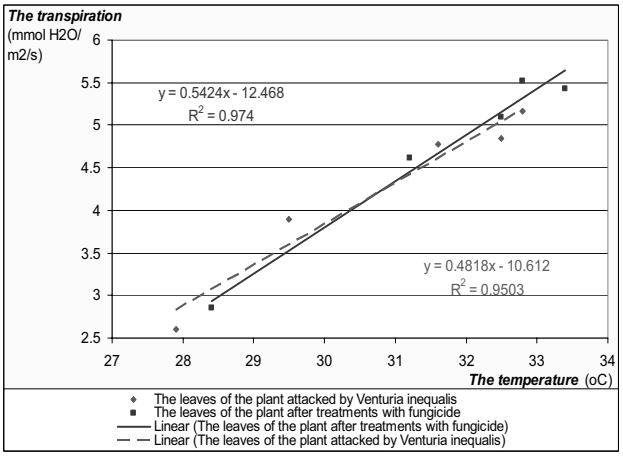

Figure 11. The correlation between the intensity of transpiration and the leaf temperature in *M. domestica* - Jonagold variety.

The **stomatal conductance** of CO_2 increases beginning in the morning (8 a.m.), when it records the values of 0.12 mol / m^2 / s in the attacked leaves and 0.2 mol / m^2 / s in the plant leaves after treatments with fungicide, their growth after lunch (12 a.m.), when it records the values of 0.32 mol / m^2 / s in the attacked leaves and 0.45 mol / m^2 / s in the leaves after treatments and decreases towards the evening (4 p.m.), when it records the values of 0.23 mol / m^2 / s in the attacked leaves and 0.31 mol / m^2 / s in the plant leaves after treatments.

Linear regression performed between the photosynthesis intensity and stomatal conductance of CO_2 shows a good positive correlation between these; the coefficient of determination (R^2) is 0.84 for the attacked leaves and 0.86 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and stomatal conductance of CO_2 shows a good positive correlation between these; the coefficient of determination (R^2) is 0.73 for the attacked leaves and 0.79 for the leaves after treatments with fungicide (Figs. 12; 13).

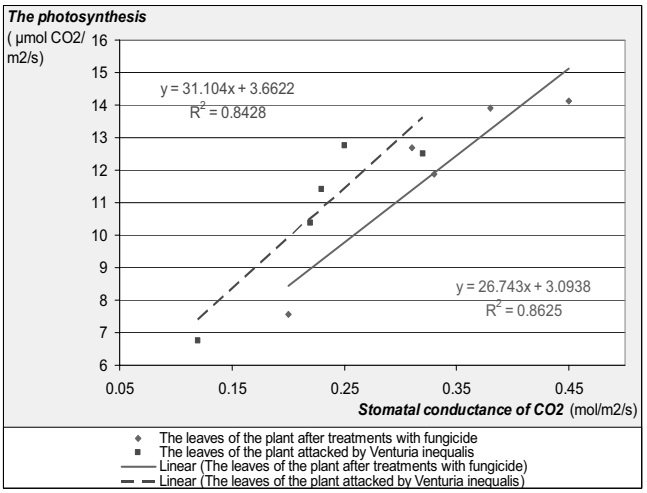

Figure 12. The correlation between the intensity of photosynthesis and the stomatal conductance in *M. domestica* - Jonagold variety.

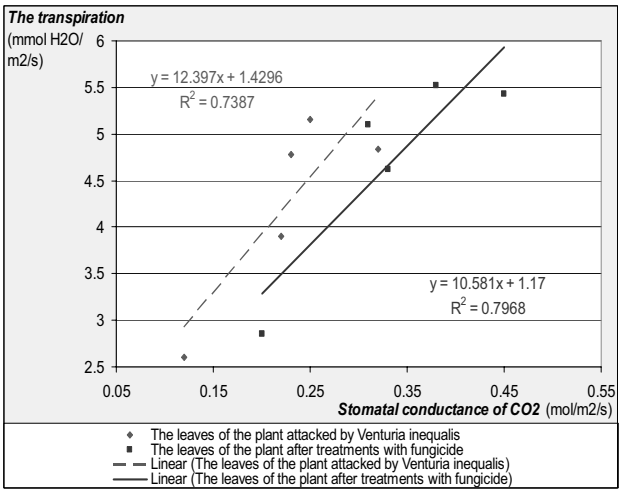

Figure 13. The correlation between the intensity of transpiration and the stomatal conductance in *M. domestica* - Jonagold variety.

In the leaves plant attacked by pathogen, in comparison with the leaves analysed after treatments, it is noticed a lower chlorophyll content by 11.80 % as a result of the reductions of biosynthesis chlorophyll and the deterioration of the chlorophyllian pigments (Fig. 14).

The leaves attacked by *V. inaequalis* present a lower water content by 2.08 % and a higher dry substance content by 4.23 % in comparison with the leaves analysed after the treatments with fungicide (Fig. 15).

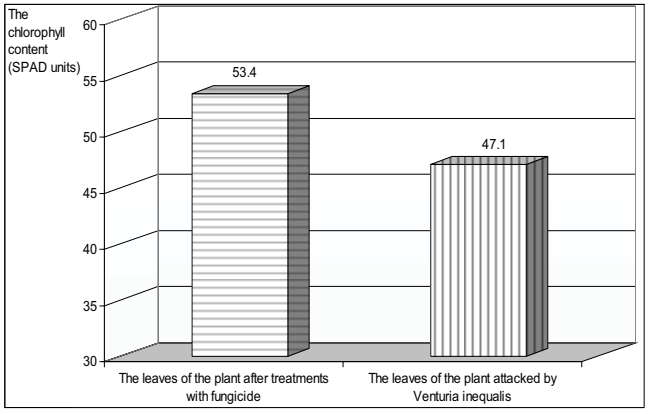

Figure 14. The chlorophyll content in leaves of *M. domestica* - Jonagold variety.

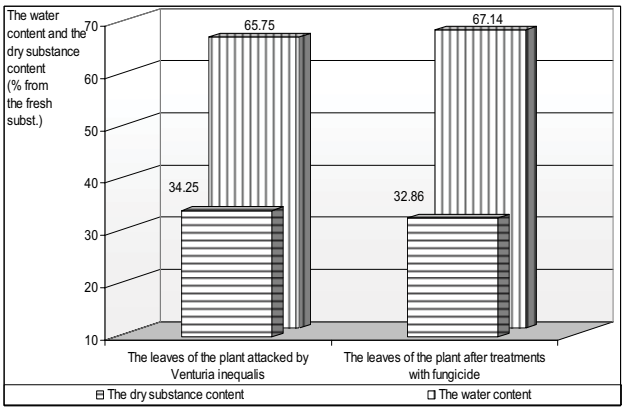

Figure 15. The water content and the dry substance content in leaves of *M. domestica* - Jonagold variety.

CONCLUSIONS

In *M. domestica* - Jonagold variety, it has been noticed that the dynamics of photosynthesis and transpiration, during the day, in the leaves attacked by *V. inaequalis* is similar to that in plant leaves analysed after treatments with fungicide **Dithane** M 45, but the recorded values are lower in the attacked plant leaves.

In the leaves attacked by the pathogen it was noticed that physiological processes intensity is lower as a result of the reduction leaf surface due to the formation of light gray spots or the olive brown spots, deterioration of the chlorophyll pigments and stomata coverage of the pathogen.

The chlorophyll content presents a lower value in attacked leaves, in comparison with the leaves analysed after treatments, as a result of the pathogen action. The attacked leaves present a lower water content and a higher dry substance content and this causes the hydric and metabolic imbalances.

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