Review on Agriculture and Rural Development 2014. vol. 3 (1) ISSN 2063-4803

COPPER FLUORESCENCE BASED COPPER TOXICITY ASSESSMENT OF TWO GRAPE VARIETIES

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ABSTRACT
Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists and ecophysiologists. This review aims to provide an introduction for the novice into the methodology and applications of chlorophyll fluorescence. After a brief introduction into the theoretical background of the technique, the methodology and some of the technical pitfalls that can be encountered are explained. A selection of examples is then used to illustrate the types of information that fluorescence can provide. In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. This study evaluates the difference in leaf sensitivity of two grapevine varieties, *Pannon frankos* and *Narancsízű*, to copper. The photosynthetic efficiency of the varieties was measured as the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm). Young and older leaves of these varieties were exposed to four different pesticides with copper active ingredients in the recommended dosage: Bordói por (copper sulphate), Champion 50 WP (copper hydroxide), Rézoxiklorid 50 WP (copper-oxychloride) and Ridomil Gold Plus 42,5 WP (mefenoxam+copper-oxychlorid) and their physiology were studied 4 times, on the 2^{nd}, 4^{th}, 8^{th} and 12^{th} days after treatments. These pesticides caused proportional decrease in the photosynthetic efficiency.

Keywords: chlorophyll fluorescence, copper toxicity, variety sensitivity

INTRODUCTION

Copper is considered as a toxic heavy metal ion to plants and is a potent inhibitor of photosynthesis. In grapevine, copper is essential for metabolic processes like electron transport in photosynthesis and in various enzyme systems (e.g. amine oxidase, cytochrome c oxidase). However, excess copper results in toxic responses, including subtle changes in enzymatic activity to gross changes in cell structure and function and inhibits photosynthesis. The type and extent of the responses of grapevine to copper vary according to the varieties under consideration.

MATERIAL AND METHOD

Material

Four different kinds of pesticides were used *(Table 1)* and two grape varieties were sprayed in the recommended dosage *(Figure 1)*.

Table 1. The pesticides used in the experiment

Name of product	Active ingredient	Dosage
Bordói por WP	20 % copper equivalent réz /II/-calcium-double-salt	4-6 kg/ha
Champion 50 WP	77% copper-hydroxide	2-3 kg/ha
Rézoxiklorid 50 WP	50% Rézoxiklorid	2-3 kg/ha
Ridomil Gold Plus 42,5 WP	2,5% mefenoxam + 40% Rézoxiklorid	3.5–4.0 kg/ha

the vineyard Narancsízű Pannon frankos

Figure 1. Kecskemét-Máriahegy vineyard and the varieties

Methods

The pulsed amplitude modulation (PAM) fluorometer *(Figure 2)* is one of the instruments available for use in measuring chlorophyll fluorescence as an indicator of primary productivity. The PAM fluorometer uses the saturation pulse method, in which dark adapted leaf is subjected to a short beam of light that saturates the PS II reaction centers of the active chlorophyll molecules (see Schreiber, 1986 for a detailed discussion). This process suppresses photochemical quenching, which might otherwise reduce the maximum fluorescence yield (SCHREIBER ET AL, 1994) (Figure 3). A computer subsequently records fluorescence yield measurements. A ratio of variable to maximal fluorescence (Fv/Fm) can then be calculated which approximates the potential quantum yield of PS II (BILGER ET AL, 1995).

Statistical analysis was carried out with the SPSS statistical computer package (SPSS for Windows, Version Release 11,5). Statistically differences in F_o/F_m were analyzed by GLM procedure and factor level was established according to factor significance and interactions. Studies of instantaneous comparisons were carried out by analysis of variance (ANOVA). Significant effect of means was identified with Tukey-test at 0.05 probabilities.

Figure 2. MINI-PAM fluorometer

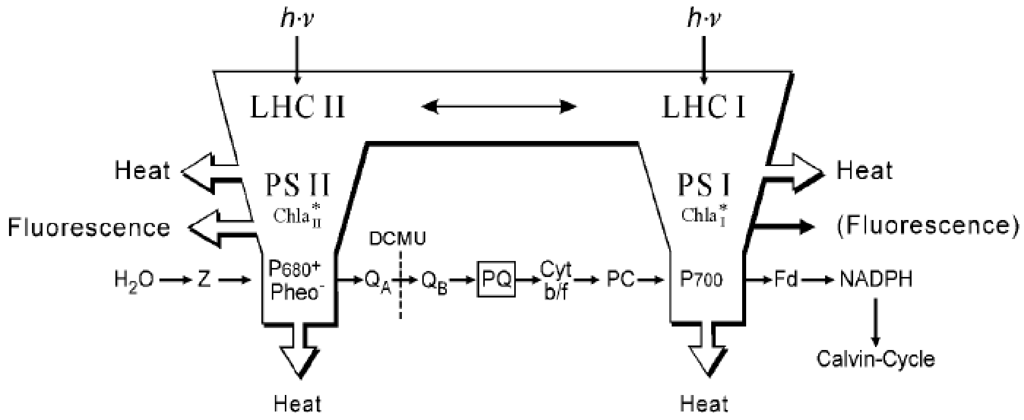

Figure 3. Schematic view of primary energy conversion and primary electron transport in photosynthesis

LHC, light harvesting pigment-protein complex; P680 and P700, energy converting special chlorophyll molecules in the reaction centers of photosystem II (PSII) and photosystem I (PSI), respectively; Pheo, pheophytin; DCMU, PSII inhibitor (diuron); PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin (http://www.walz.com/downloads/manuals/mini-pam/MINIP_1EB.pdf)

RESULTS

Pannon frankos and Narancsízű young leaf copper toxicity by Rézoxiklorid 50 WP (copper-oxychloride) measured in four days after spraying

As significance coefficient, $p>0.05$ there is no significant differences varieties, day of measurement and their combination. It means that Copper-oxychorid, which is known to be the most toxic of all cupriferous pesticides are not toxic on young leaves if it is sprayed in the recommended dosage.

Table 2. ANOVA table of Rézoxiklorid 50 WP treatment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.107(a)	7	0.015	0.898	0.531
Intercept	1.324	1	1.324	77.829	0.000
VARIETY	0.016	1	0.016	0.957	0.342
DAY OF MEAS.	0.085	3	0.028	1.667	0.214
VARIETY * DAY OF MEAS.	0.006	3	0.002	0.109	0.953
Error	0.272	16	0.017		
Total	1.703	24			
Corrected Total	0.379	23			

Pannon frankos and Narancsízű older leaf copper toxicity by Champion 50 WP (copper-hydroxide)

As *Table 2* indicates $p<0.05$ so we can say that there is a statistical difference between the days of measurement.

Table 3. ANOVA table of Champion 50 WP treatment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.379(a)	7	0.054	3.821	0.013
Intercept	2.549	1	2.549	180.102	0.000
VARIETY	0.066	1	0.066	4.649	0.047
DAY OF MEAS.	0.312	3	0.104	7.339	0.003
VARIETY * DAY OF MEAS.	0.001	3	*0.000*	0.027	0.994
Error	0.226	16	0.014		
Total	3.154	24			
Corrected Total	0.605	23			

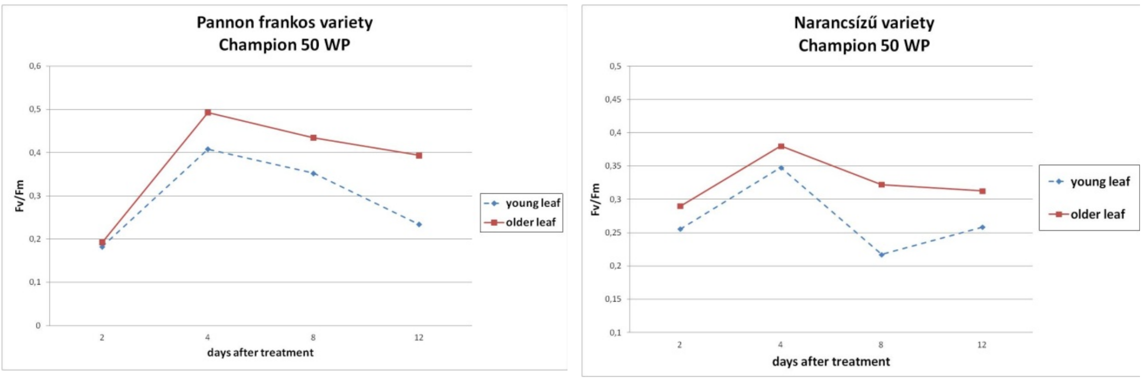

Figure 4. The effect of Champion 50 WP pesticide on the Y(II) of older leaves of Pannon frankos and Narancsízű varieties

Table 4. Multiple Comparisons of measurement day

(I) DAY	(J) DAY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-0.3009(*)	0.06868	*0.002*	-0.4974	-0.1044
	3.00	-0.2427(*)	0.06868	*0.013*	-0.4392	-0.0462
	4.00	-0.2176(*)	0.06868	*0.028*	-0.4141	-0.0210
2.00	1.00	0.3009(*)	0.06868	0.002	0.1044	0.4974
	3.00	0.0582	0.06868	0.831	-0.1383	0.2547
	4.00	0.0833	0.06868	0.628	-0.1132	0.2798
3.00	1.00	0.2427(*)	0.06868	0.013	0.0462	0.4392
	2.00	-0.0582	0.06868	0.831	-0.2547	0.1383
	4.00	0,0251	0.06868	0.983	-0.1714	0.2216
4.00	1.00	0.2176(*)	0.06868	0.028	0.0210	0.4141
	2.00	-0.0833	0.06868	0.628	-0.2798	0.1132
	3.00	-0.0251	0.06868	0.983	-0.2216	0.1714

Based on observed means.
* The mean difference is significant at the ,05 level.

Table 4 and *Table 5* indicate that there is a significant difference between each measuring days.

When we look at the F_V/F_m lines it is seen that in Pannon frankos Y(II) values were always higher than the control while in case of Narancsízű there was an inhibiting effect 2 days after the treatment *(Figure 4).* In both varieties Champion 50 WP had a positive effect on photosynthesis.
A question arises whether the results are influenced by the change of Y(II) of the control leaves. We investigated it by running a two-way ANOVA between variety and control's older leaves. *Table 3* of ANOVA shows that there is no statistical difference in the measuring days so the Champion 50 WP results are only explained by the effect of the pesticide.

Table 5. ANOVA table of control

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.238(a)	7	0.034	1.720	0.174
Intercept	2.599	1	2.599	131.655	0.000
VARIETY	0.005	1	0.005	0.255	0.621
DAY OF MEAS.	0.071	3	0.024	1.201	0.341
VARIETY * DAY OF MEAS.	0.162	3	0.054	2.729	0.078
Error	0.316	16	0.020		
Total	3.152	24			
Corrected Total	0.554	23			

CONCLUSIONS

Based on our research we can conclude the followings:

- in therapy dosage copper is not toxic to varieties, however the date of recovery is significantly different
- control Y(II) did not change in the measurement period
- according to the ANOVA calculations, it is possible that copper-hydroxide has a positive effect on enzymatic activities
- the hypothesis that young leaves can be burnt by copper did not prove to be true
- difference between variety's copper sensitivity was not observed
- temperature and sunshine largely influenced the data obtained

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