Chlorophyll Fluorescence Responses to Pesticides with Copper Active Ingredient in Pannon frankos and Narancsízű grape varieties

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Abstract
Pulse Amplitude Modulated (PAM) fluorometry is a sensitive and rapid method used to assess toxic effect of chemical components in plants. 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 (copper hydroxide), Rézoxiklorid (copper-oxychloride) and Ridomil Gold Plus (mefenoxam+copper-oxychlorid) and their physiology were studied 4 times, on the 2nd, 4th, 8th and 12th days after treatments. These pesticides caused proportional decrease in the photosynthetic efficiency.

Keywords: chlorophyll fluorescence, copper toxicity, variety sensitivity

1 Introduction

Copper is considered as a toxic heavy metal ion to plants and is a potent inhibitor of photosynthesis1. Gledhill et al. realized the significance of regulatory and biological perspectives of bioavailable copper in seawater and copper speciation, and discussed its deleterious effects. 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.

2 Material and methods

Methods

The pulsed amplitude modulation (PAM) fluorometer 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). 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.

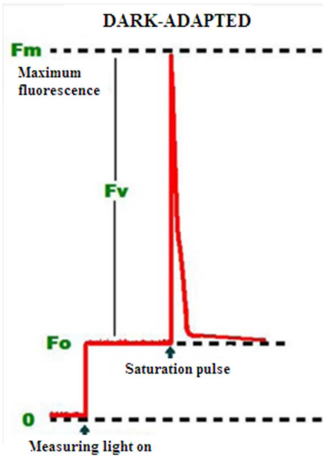

Figure 1: The theory of chlorophyll fluorescence measurement

$F_v/F_m = (F_m-F_o)/F_m$
F_m = maximum fluorescence (Reaction centers-RC's are closed)
F_o = minimum fluorescence (RC's open)

3 Results

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

Table 1.

ANOVA table of Rézoxiklorid treatment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,107(a)	7	,015	,898	,531
Intercept	1,324	1	1,324	77,829	,000
VARIETY	,016	1	,016	,957	,342
DAY OF MEAS.	,085	3	,028	1,667	,214
VARIETY * DAY OF MEAS.	,006	3	,002	,109	,953
Error	,272	16	,017		
Total	1,703	24			
Corrected Total	,379	23			

As significance coeffient, p>0.05 there is no significant differences varieties, day of measurement and their combination. It means that Réxoxiklorid, 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.

Pannon frankos and Narancsízű older leaf copper toxicity by Champion (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 2.

ANOVA table of Champion treatment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,379(a)	7	,054	3,821	,013
Intercept	2,549	1	2,549	180,102	,000
VARIETY	,066	1	,066	4,649	,047
DAY OF MEAS.	,312	3	,104	7,339	,003
VARIETY * DAY OF MEAS.	,001	3	***,000***	,027	,994
Error	,226	16	,014		
Total	3,154	24			
Corrected Total	,605	23			

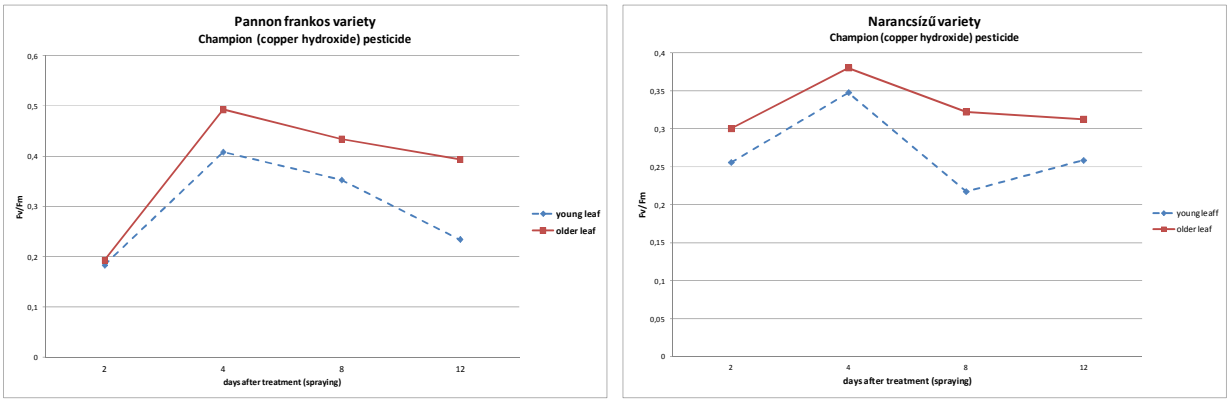

Figure 2: The effect of Champion pesticide on the Y(II) of older leaves of Pannon frankos and Narancsízű varieties

Table 4. indicates that there is a significant difference between each measuring days.

When we look at the FV/Fm 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. In both varieties Champion 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 results are only explained by the effect of the pesticide.

Table 4.

Multiple Comparisons of measurement day

Dependent Variable: FVPERFM
Tukey HSD

(I) DAY	(J) DAY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1,00	2,00	-,3009(*)	,06868	***,002***	-,4974	-,1044
	3,00	-,2427(*)	,06868	***,013***	-,4392	-,0462
	4,00	-,2176(*)	,06868	***,028***	-,4141	-,0210
2,00	1,00	,3009(*)	,06868	,002	,1044	,4974
	3,00	,0582	,06868	,831	-,1383	,2547
	4,00	,0833	,06868	,628	-,1132	,2798
3,00	1,00	,2427(*)	,06868	,013	,0462	,4392
	2,00	-,0582	,06868	,831	-,2547	,1383
	4,00	,0251	,06868	,983	-,1714	,2216
4,00	1,00	,2176(*)	,06868	,028	,0210	,4141
	2,00	-,0833	,06868	,628	-,2798	,1132
	3,00	-,0251	,06868	,983	-,2216	,1714

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

Table 3.

ANOVA table of control

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,238(a)	7	,034	1,720	,174
Intercept	2,599	1	2,599	131,655	,000
VARIETY	,005	1	,005	,255	,621
DAY OF MEAS.	,071	3	,024	1,201	,341
VARIETY * DAY OF MEAS.	,162	3	,054	2,729	,078
Error	,316	16	,020		
Total	3,152	24			
Corrected Total	,554	23			

4 Conclusions

- 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

References

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