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CHLOROPHYLL α FLUORESCENCE AS A DIAGNOSTIC TOOL FOR ASSESSMENT OF APPLE RESISTANCE AGAINST TWO-SPOTTED SPIDER MITE (*Tetranychus urticae* Koch.)

Wojciech Warabieda, Bożena Borkowska
Research Institute of Pomology and Floriculture, Skierniewice, Poland

ABSTRACT

The chlorophyll fluorescence measurements were used as a method of assessing the influence of *Tetranychus urticae* feeding on photosynthetic apparatus of 'Lobo' and 'Jester' apple cultivars. The photochemical efficiency measured with such parameters like: the initial fluorescence (F_0), the maximum fluorescence (F_m), the ratio of variable to maximum fluorescence (F_v/F_m), and the area under the fluorescence curve (S_c) decreases continuously with increasing infestation by mites. The only parameter not influenced by mites feeding was the half-rise time from F_0 to F_m (T_{fm}). Antennas of both 'Lobo' and 'Jester' cvs. were similarly injured by mites (comparable decrease of F_0) but photochemical reactions of the harvested energy of 'Jester' leaves were less influenced by this pest (lower decrease for F_v/F_m). It suggests a higher tolerance of the photosynthetic apparatus of 'Jester' cv. to *T. urticae* feeding, as compared to 'Lobo' cv.

Key words: apple, biotic stress, mite, resistance, chlorophyll fluorescence

INTRODUCTION

Spider mites are common pests on many woody and herbaceous plants. Some species of that group, e.g. Two-spotted spider mite (*Tetranychus urticae* Koch.), Often building a huge population on different crops, including fruit trees. Chemical control of this species is expensive, highly controversial from ecological point of view and often far from full success, caused mainly by high fecundity and its capability to develop acaricide-resistant races. Growing apple resistant cultivars in addition to augmentation and introduction of natural enemies can form a base of pro-ecological approach in controlling *T. Utricae* in the apple commercial orchards. Differences in the level of infestation of the particular apple cultivars by mites were already observed in some previous studies indicating different levels of natural resistance to the mites [1, 3, 4, 11]. However a breading programme aimed to release new cultivars with higher level of resistance to the two-spotted spider mite would be rather different task. One of the main reason is that the resistance of the plants against this mite species is probably of oligo or polygenic nature [5, 6].

Among the mechanisms of plant resistance to mites, the tolerance seems to be particularly important from practical point of view. Planting more tolerant cultivars may leads to avoid of needless pesticide applications and would be profitable for farmers and environment.

Screening techniques and methods have been developed, in order to detect and characterise the degree of plant resistance against pathogens or pests including spider mites [5]. Studies of biotic stresses using the chlorophyll α (Ch) fluorescence method revealed presence of heterogeneous photosynthetic activities in leaves that had been exposed to a pathogen. Using this method, an effect of biotic stress can be detected before any symptoms of it can be observed visually. This method allows also to evaluate the plant's natural defence mechanism against infection [9].

The paper presents interactions between the two-spotted spider mite and the photosynthetic apparatus, using chlorophyll fluorescence analysis and checks the possibility of utilising this technique to assessment of apple tree tolerance to mite feeding.

MATERIALS AND METHODS

Plant material and infestation with mites. The experiment was conducted on one-year apple trees of 'Lobo' and 'Jester' cultivars grafted on M.9. These two apple cultivars characterize different level of resistance to *T. urticae*. 'Lobo' cv. represents the apple being more heavily infested by mites compared to 'Jester' cv. [12]. The trees were planted into 20 l pots filled with a mixture of peat and light soil in ratio 2:1. The experiment was carried out under greenhouse conditions.

Thirty trees of both cultivars were infested with mites at different level, on 25th May, 1998. Every 10-14 days, the pest population density was measured by counting all the mobile mites on three leaves of each apple tree. The level of mites infestation of each tree was calculated as the *cumulative mite days* (CMD), according to the formula:

$$CMD = \sum 0.5\ (P_a + P_b)\ D_{a-b}$$

where P_a and P_b are number of mites per leaf at time a and b, respectively, and D_{a-b} is the number of days between date a and b [13].

The area all of the checked leaves was measured using a Portable Leaf Area Meter CI 202 (ADC, England) device and the level of mite infestation was recalculated to 1 cm^2 of the leaf area.

Chlorophyll *α* fluorescence measurements. The chlorophyll fluorescence measurements were used as a method of assessing the influence of two-spotted spider mite feeding on photosynthetic apparatus of two selected apple cultivars. Ch fluorescence was measured on the abaxial side of the leaves by the fast direct fluorometer PEA (Hansatech, England) on 9th August, 1998. The leaves were placed into clip, darkened for 20 min and then illuminated for 5 s with red light diodes (peak 650 nm, maximum PPFD at leaf surface was 3000 μmol m^{-2}s^{-1}). The samples were characterised by three basic parameters: the initial fluorescence (F_0), the maximum fluorescence (F_m), the half-rise time from F_0 to F_m (T_{fm}). The fast fluorescence rise starts from F_0 and reaches a maximum value F_m. The full kinetics of the fluorescence rise is given by value T_{fm}. Out of these parameters, several expressions were calculated. The basis of the calculated expressions is the ratio F_v/F_m. The value of this

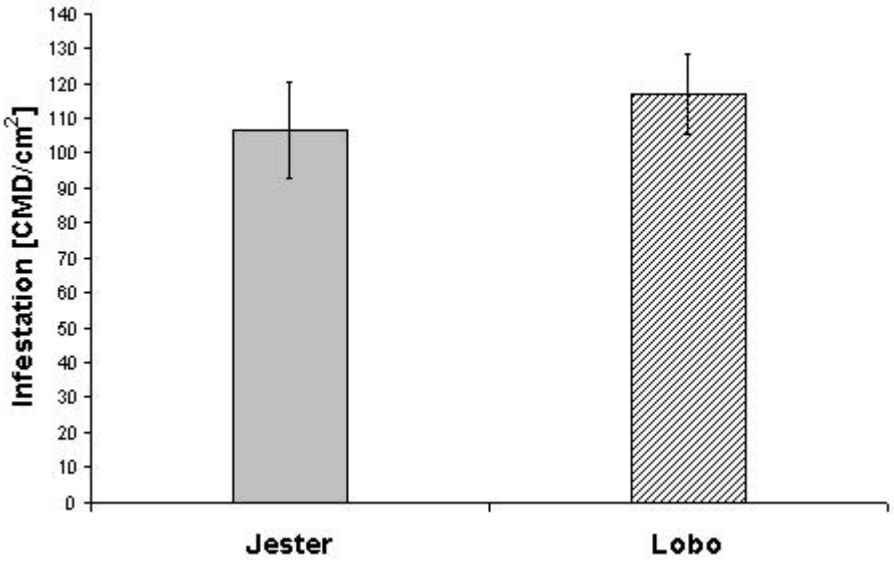
parameter is proportional to the quantum yield of photochemical reactions, where F_v (variable fluorescence) = F_m – F_0. Additionally, the parameter S_c was sampled. This parameter is a sum of non-reduced electron transfers between PSII and PSI [10].

Statistical analyses. The levels of infestation with mites of 'Lobo' and 'Jester' cultivars were compared using the Student's t-test. Linear regression analysis was applied to determine relationship between the level of mite infestation of apple trees and particular parameters of chlorophyll fluorescence. Outliers (atypical observations), were determined using Cook's distance. Significance of differences between regression coefficients across both apple cultivars were evaluated using the method of comparison of few regression lines ("STAT" computer program). Other statistical analyses were conducted using "Statistica for Windows ver. 4.0" software package.

RESULTS

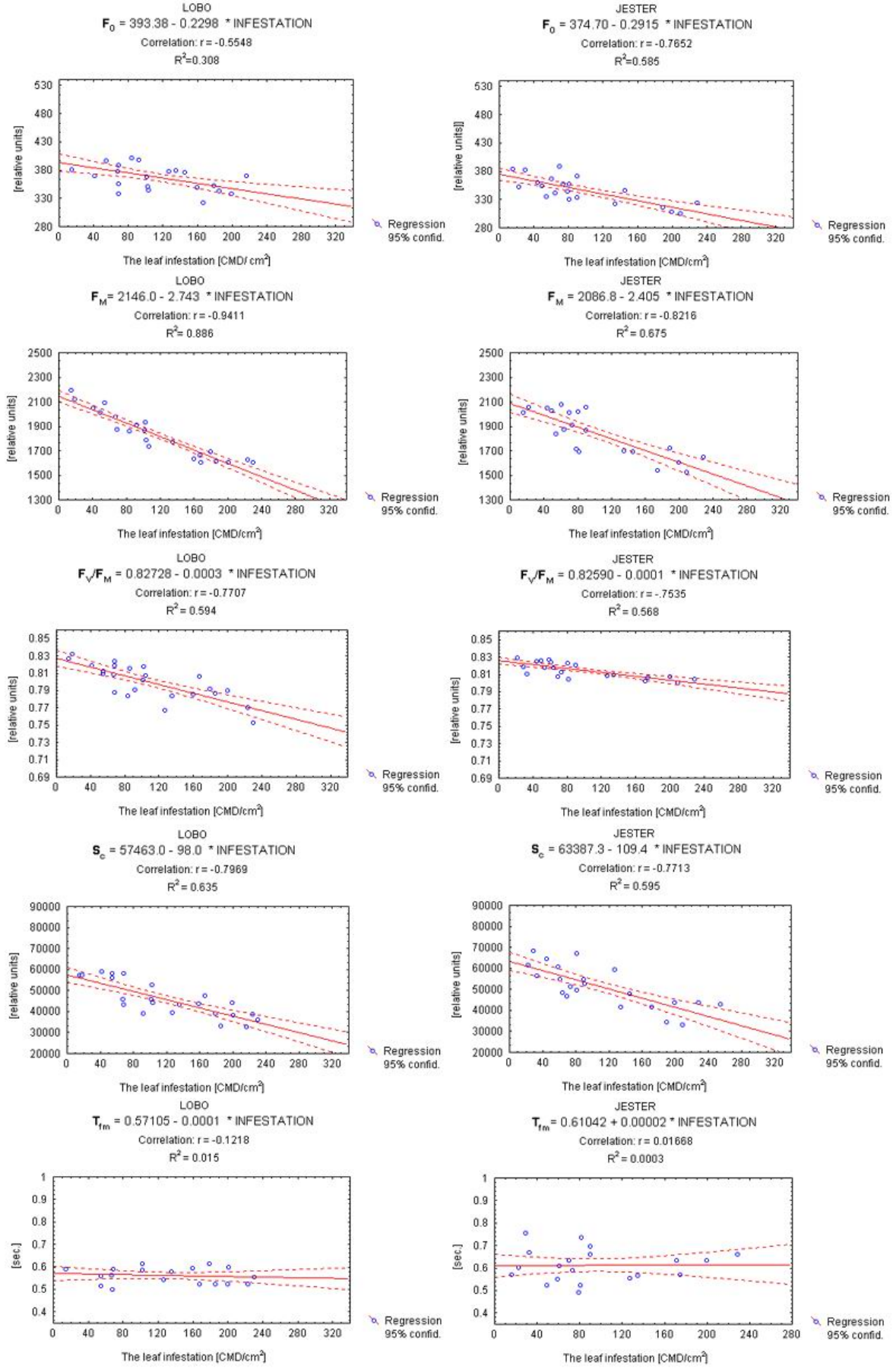
Experimental trees of both apple cultivars infested with various initial mite population have shown from 0.2 to 22 *T. urticae* specimen per cm^2 of leaf area, at the peak of the population growth on the June, 20. The mean level of leaf infestation with mites measured by CMD was similar for 'Lobo' and 'Jester' apple trees (fig. 1) allowing compare both cultivars with respect to the mites influence on the chlorophyll fluorescence parameters.

Fig. 1. Infestation of the two apple cultivars with mites. Data are given as mean ± 1SE



It was shown that the photochemical efficiency has decreased continuously with the increasing infestation by mites on both apple cultivars. It was expressed by the negative correlation between the value of most Ch fluorescence parameters and the level of mite infestation of leaves (fig. 2a, b, c, d). The only parameter that was not correlated with mite infestation was T_{fm} (fig. 2e).

Fig. 2a, b, c, d, e. Regression lines, data points, R^2 values for the response of fluorescence parameters and mite infestation. All models and slope parameters are significant at $p < 0.0001$ except T_{fm}. All the intercept parameters are significant at $p < 0.0001$



In the regression graph, the intercept constitutes the predicted value of the analyzed parameter when the level of mite infestation measured with CMD is equal to zero. In this case the values of predicted basic parameters: F_0, F_m were similar for both compared cultivars (tab. 1). These findings indicate well functioning antennas and lack of non-radiative energy lost (heat dissipation). The value of the parameter F_v/F_m above 0.8, expresses a high potential activity of PSII in both apple cultivars (tab. 1). On the contrary, the lower S_c value for 'Lobo' cv. ($p < 0.1$) may indicate the handicapped electron transport between PSII and PSI (tab. 1). Comparison of T_{fm} for both apple cultivars using Student's t-test revealed the significantly higher value of this parameter in 'Jester' cv. ($p < 0.05$).

Table. 1. Comparison of the regression parameters of two apple cultivars

	'Lobo'	'Jester'	'Lobo'	'Jester'
	intercept		slope	
F_0	393.38 a	374.701 a	-0.2298	-0.29149 a
F_m	2145.989 a	2086.814 a	-2.74256	-2.40522 a
F_v/F_m	0.827 a	0.826 a	-0.00025 a[2]	-0.00011 b[2]
S_c	57463.105 a[1]	63387.330 b[1]	-98.00478 a	-109.36406 a
T_{fm}	0.571 a[2]	0.610 b[2]	-0.00007	0.00002

Means signed with the same letters do not differ statistically
[1] Difference between means at p = 0.1
[2] Difference between means at p = 0.05

Using the slope coefficients obtained in regression analysis seemed to be accurate in evaluation of the tolerance of both apple cultivars to mite infestation. Significant differences between slope coefficients of both apple cultivars were observed for the parameter F_v/F_m (tab. 1). The slope of the regression line for F_v/F_m was greater for 'Lobo' than for 'Jester' cv. (fig. 2c). Similar trend was found for the value of F_m, but unlike F_v/F_m the difference between slopes of the compared regression lines was not statistically significant (tab. 1, fig. 2b). On the contrary, the slopes of the regression lines for F_0 and S_c decreased faster in 'Jester' cv. as compared to 'Lobo', but no statistical differences were observed (tab. 1, fig. 2a, d).

DISCUSSION

Tolerance is the ability of a plant to yield and growth without any reduction, despite pest, pathogen or other stress presence. The plant response to biotic stresses have been poorly understood, mainly because its physiological effects are highly variable. An increasing number of reports suggest that the photosynthetic activity (especially light phase) is reduced when plants are exposed to environmental stresses, including pathogen or pest attack [2, 8]. Study of stress induced by *T. urticae* conducted on bean plants revealed a drop in F_0, F_m, F_v/F_m and T_{fm} parameter values on leaves that had been exposed to the pest. [8]. This confirms the results of our experiments. However, we have not noticed any influence of mites on T_{fm}. This unconformity is probably caused by different conditions of both experiments. In the experiment conducted on bean plants, the levels of mite density were fixed up from 1 to 32 mite specimen per 1.5 cm^2 of leaf area, and fluorescence measure was made after 1-5 days of mite feeding. In our study the infestation of a particular apple trees with mites was differentiated and fluorescence measurements were made about 10 weeks after mite introduction.

The comparison of both apple cultivars with regard to the T_{fm} parameter indicates the better kinetic of electron transport for 'Jester' cv. Furthermore, the higher value of S_c intercept parameter for 'Jester' means that the photosynthetic apparatus of the cultivar is highly effective in electron transfer between PSII and PSI. These findings suggest a better efficiency of light reaction in 'Jester' cv. as compared to 'Lobo'. Antennas of both 'Lobo' and 'Jester' cvs. were injured by mites to a similar degree (comparable decrease of the slope for F_0) but photochemical reactions of harvested energy of 'Jester' leaves were less influenced by mites (the lower slope for F_v/F_m). It suggests a higher tolerance of photosynthetic apparatus of 'Jester' cv. to *T. urticae* feeding, as compared to 'Lobo' cv.

In our former publication we stated that differences in the level of tolerance of leaves to mite damage are accompanied by some anatomical characters of apple leaves [12]. After short period of feeding, intensive injuries of spongy mesophyll and epidermis of the lower side of the leaf were observed in cultivars Close and 'Lobo'. Leaves of these cultivars are characterised by a loose structure of the spongy mesophyll and by a thin layer of cuticle covered epidermis. On the contrary, leaves of the cultivars 'Jester', 'Marvit' and 'Piros', with a tight cell arrangement of the mesophyll and a thick layer of cuticle were less damaged by this pest. We suggest that these

anatomical differences create not only a physical barrier against the pest but also play an important role in the efficiency of the photosyntetic apparatus. The fluorescence measurement seems to be a useful tool for studies on plant – pest interactions, but must be used carefully and in combination with other techniques [7].

CONCLUSIONS

Results of the presented studies concerning the apple physiological reaction to infestation with two-spotted spider mite leading to the following conclusions:

1. The photochemical efficiency measured with Ch fluorescence method decreases continuously with increasing infestation by mites.
2. The negative correlation between the value of particular parameters of Ch fluorescence and the level of mite infestation was higher for 'Lobo' than 'Jester' cv.
3. The slope of the regression line for F_v/F_m decreased faster for 'Lobo' than for 'Jester' cv.
4. Results of the experiment suggest a higher tolerance of the photosynthetic apparatus of 'Jester' cv. to *T. urticae* feeding , as compared to 'Lobo' cv.

REFERENCES

1. Anasiewicz A., Winiarska W., 1993. Występowanie przędziorków i ich drapieżców na intensywnie chronionej kolekcji odmian jabłoni [Occurrence of spider mites and their predators on trees of an intensively protected collection of apple cultivars]. Prace ISiK. 117-118 (1-2), 71-73 [in Polish].
2. Balachandran S., Hurry V. M., Kelley S. E., Osmond C. B., Robinson S. A., Rohozinski J., Seaton G. G. R., Sims D. A., 1997. Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. Physiol. Plant. 100 (2), 203-208.
3. Bielak B., 1979. Wpływ wybranych roślin z rodziny *Rosaceae* na biologię przędziorka owocowca *Panonychus ulmi* (Koch) [Influence of some chosen plant species of the family Rosaceae on biology of red spider mite, *Panonychus ulmi* (Koch)]. PhD Thesis at SGGW-AR, 54-67 [in Polish].
4. Dąbrowski T. Rejman A., 1975. Some aspects of host – plant relationships of the tree red spider mite, *Panonychus ulmi* (Koch). Zesz. Probl. Post. Nauk Rol. 171, 73-79.
5. Dąbrowski Z. T., 1988. Podstawy odporności roślin na szkodniki [Principles of plant resistance against pests]. PWRiL, Warszawa. 2nd Ed. 51-64 [in Polish].
6. Easterbrook M. A., Simpson D. W., 1998. Resistance to two-spotted spider mite *Tetranychus urticae* in strawberry cultivars and wild species of *Fragaria* and *Potentilla*. J. Hort. Sci. Biotech. 73(4), 531-535.
7. Govindjee, 1995. Sixty-three years since Kautsky: chlorophyll *a* fluorescence. Aust. J. Plant Physiol. 22, 131-160.
8. Iatrou G., Cook C. M., Stamou G., Lanaras T., 1995. Chlorophyll fluorescence and leaf chlorophyll content of bean leaves injured by spider mites (Acari: Tetranychidae). Experim App. Acarol. 19, 581-591.
9. Soukupova J., Smatanova S., Jegorova A., Ferimazova H., Kupper H., Setlik S., Maldener C., Whitmarsh C., Tritlek M., Nedbal L., 2001. Early detection of biotic and abiotic stress by kinetic imaging of chlorophyll fluorescence. Int. Congress Photosynthesis. Brisbane, Australia.
10. Strasser R. J., Srivastava A., Tsimilli-Michael M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Probing Photosynthesis: Mechanism, Regulation and Adaptation (M. Yunus, U. Pathre and P. Mohanty, eds.), Taylor and Francis, London and New York, chapter 25, 445-483.
11. Warabieda W., Olszak R. W., 1995. Research on factors responsible for population extent of spider mites (Tetranychidae) on selected apple cultivars. Proceedings of the Symposium on "Advances of Acarology in Poland". 161-166.
12. Warabieda W., Olszak R. W., Dyki B., 1997. Some aspects of tolerance of apple cultivars to two-spotted spider mite (*Tetranychus urticae*). In: Workshop on Arthtopod Pest Problems in Pome Fruit Productions. Einsiedeln, Switzerland. IOBC wprs Bulletin 22 (7), 211-215.
13. Wratten S. D., Lee L., 1979. Duration of cereal aphid populations and the effects on wheat yield and quality. Proc. Brit. Crop Prot. Conf. – Pest and Diseases, 1-9.

Wojciech Warabieda, Bożena Borkowska
Research Institute of Pomology and Floriculture
18 Pomologiczna Street, 96-100 Skierniewice, Poland
tel. (+48 46) 833 20 21, fax (+48 46) 833 32 28
e-mail: wwarab@insad.pl