STATE-1 STATE-2 TRANSITION INFLUENCED BY HERBICIDES WHICH MODIFY FATTY ACID COMPOSITION IN LEAVES.

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INTRODUCTION

Interactions of herbicides with photosynthetic membranes are still not solved in many respects. Three different modes of action have been reported for pyridazinone herbicides: inhibition of (1) photosynthetic electron transport (2) carotenoid biosynthesis and (3) fatty acid desaturation in the galactolipid fraction of chloroplasts (ST. JOHN, HILTON 1976). The pyridazinone BASF 13-338 (=SAN 9785) used in our investigations has no effect on pigmentsynthesis and photosynthetic activity but affects fatty acid desaturation in leaves (TREBST, HARTH 1974). Cerulenin an antibiotic from the fungus Cephalosporium caerulens inhibits fatty acid synthesis generally. Both herbicides act indirectly on photosynthesis because they alter the mobility of photosynthetic units in the membrane.

MATERIAL AND METHODS

Plants of Petunia hybrida were treated with the herbicides 4-chloro-5(dimethylamino)-2-phenyl-3(2H)pyridazinone (BASF 13-338, SAN 9785) 40 or 160 µM or Cerulenin 4.5 µM for 18 hours or up to 7 days. Kinetin (4.7, 47, 118 µM) was preincubated for 2 days before the herbicide was added at a final concentration of 40 µM. Fluorescence kinetic experiments at low as well as room temperature were carried out using exitation light of 633 nm (Ne He Laser, $5W/m^2$). The fluorescence signals which were measured at 694 nm (PSII) and 735 nm (PSI) were digitized and analyzed with an IMSAI 8080 microcomputer. For state-1 state-2 transition experiments leaves were kept dark for 15 minutes. The electron transports H_2O to MV in red light and ASC to MV in red and far red ($>705^2$nm) light in phosphorylated or nonphosphorylated stage were measured according to SOLIS, STRASSER 1983 in 10 mM $MgCl_2$. For fatty acid analysis lipids were extracted from dried material with chloroform/methanol 2:1 (v/v) esterfied in methanol with H_2SO_4 conc. and analyzed by gas chromatography.

RESULTS

Figure 1 shows the influence of 4.5 µM Cerulenin and 160 µM BASF 13-338 on low temperature fluorescence kinetics. The ratio F2(V)/F2(M) decreases in both cases continuously while the controls remain constant for several days. The term α_N which tells how much energy in PSI originates from own light absorption ($1-\alpha_N$ is the fraction due to spill over) is not changed significantly by the pyridazinone. Cerulenin however causes a decrease after 3 days treatment, that means, the spill over increases. State-1 state-2 transitions measured as room temperature fluores-

Sybesma, C. (ed.), Advances in Photosynthesis Research, Vol. IV. ISBN 90-247-2945-9.

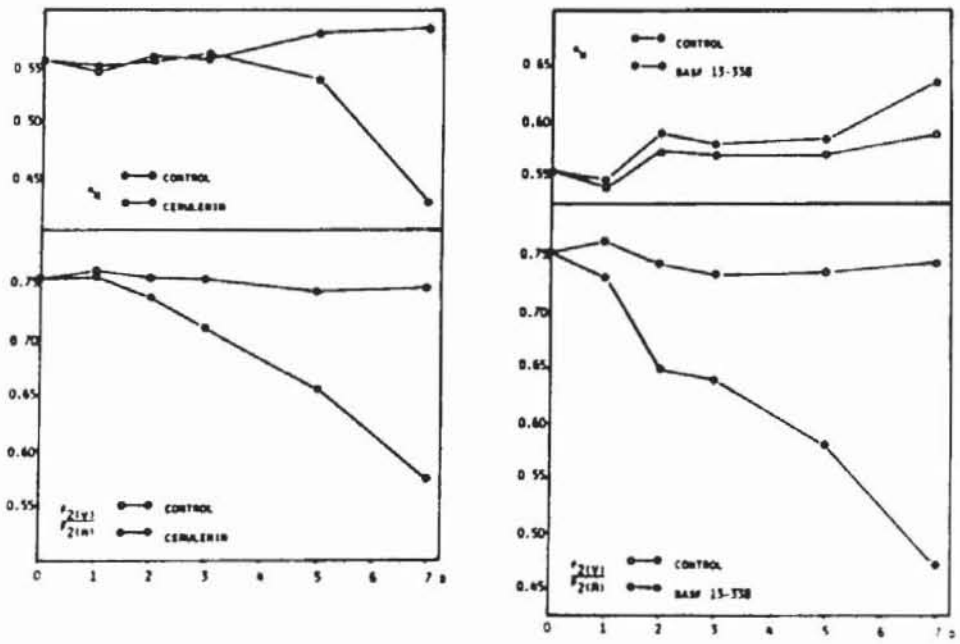


Figure 1 Influence of Cerulenin and BASF 13-338 on low temperature fluorescence kinetics depending on time of treatment.

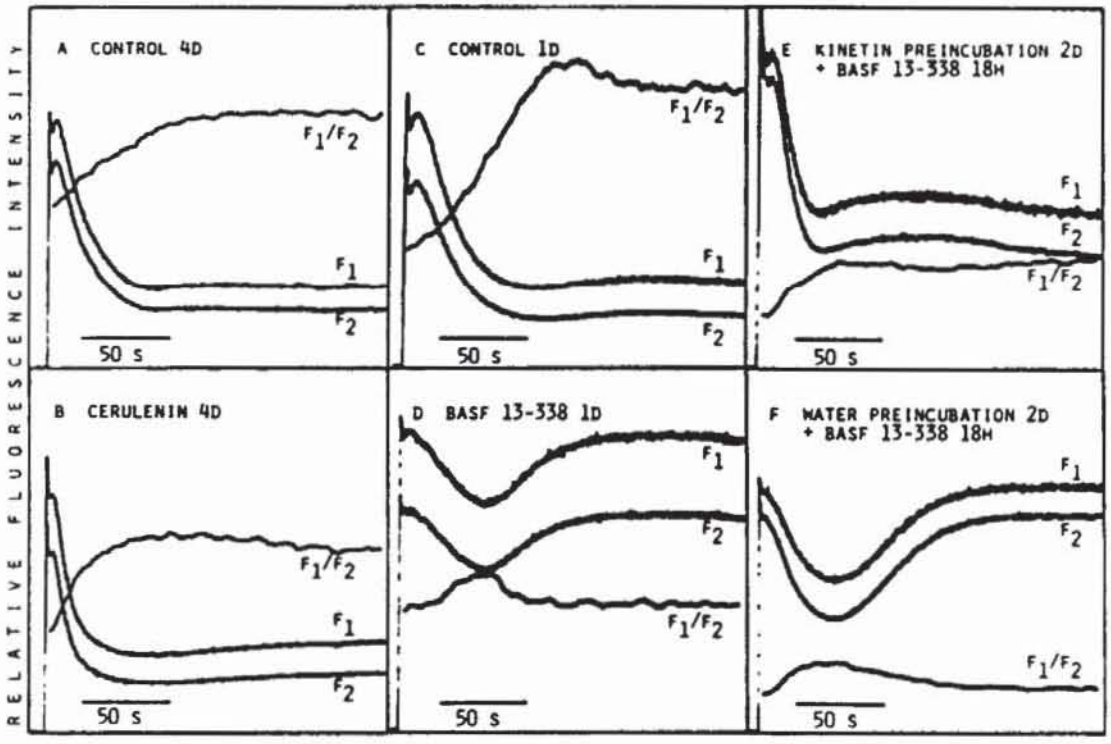
$F_1 = F_{735}$

$F_2 = F_{695}$

cence kinetics are disturbed by both BASF 13-338 and Cerulenin (Figure 2 A - D). The division F1/F2 is besides a constant proportional to the spill over rate constant k21 (equation 1, see also LOMBARD, STRASSER 1983). F1/F2 is not increasing in plants

$$(F1/F2)_{experimental} \sim k21 \quad \text{(equation 1)}$$

Figure 2 State-1 state-2 transitions measured as room temperature fluorescence kinetics.



treated with BASF 13-338 as compared to the controls. Leaves treated with the pyridazinone cannot hold new steady state (state-2) and F1 and F2 run through a minimum and increase again. $\tau/2$ the time necessary to reach half F1/F2 ascent is reduced by both BASF 13-338 and Cerulenin. Figure 3 shows the variation of state-1 state-2 transitions measured as room temperature fluorescence kinetics depending on the time of treatment with the pyridazinone BASF 13-338. The first effects are seen after a lag phase of 1 hour. After that time a minimum in the kinetics become evident and the fluorescence signals increase again. Figure 2 E,F indicates that the phytohormone Kinetin influences the BASF 13-338 effect on state-1 state-2 transition. 2 days preincubation with the cytokinin using concentrations ranging from 4.7 to 118 µM abolishes the herbicide effect clearly. $\tau/2$ shows values comparable to those of untreated plants. When plants are simultaneously treated with Kinetin and the pyridazinone for 18 hours the phytohormone has no influence on the herbicide effect (GRAF, unpublished).

The influence of BASF 13-338 on the phosphorylation of the light harvesting complex is shown in table 1. After light harvesting phosphorylation in the control chloroplasts the electron transport H_2O to MV (PSII + PSI) decreases in red light whereas the electron transport ASC to MV (PSI) in red and far red light increases as compared to the nonphosphorylated state. In experiments with chloroplasts isolated from herbicide treated plants the electron transports remain unchanged in all cases. 40 µM BASF 13-338 has no significant effect on the photosynthetic Hill activities measured as H_2O to MV and H_2O to FeCy in coupled as well as in uncoupled states (data not shown). After 18 hours incubation with BASF 13-338 the fatty acid composition of glyco-

Figure 3 Variation of state-1 state-2 transitions depending on time of treatment.

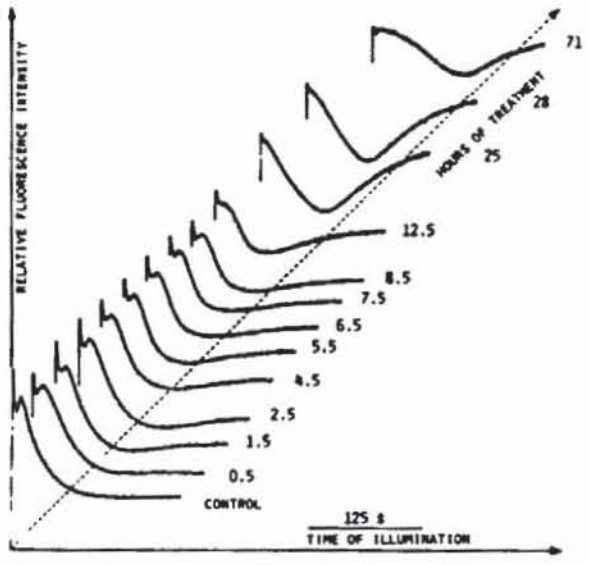
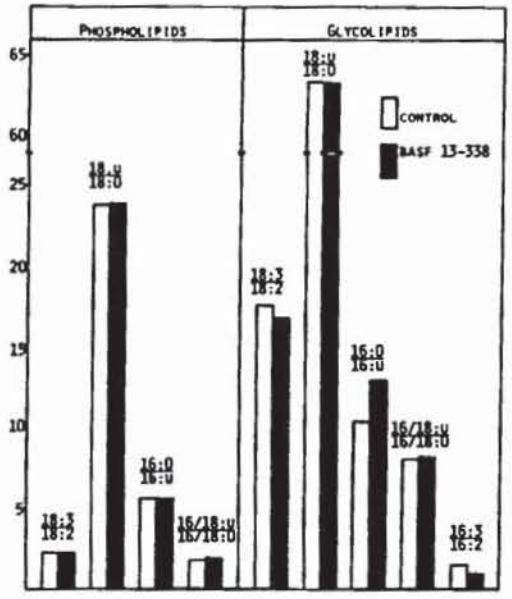


Figure 4 Influence of BASF 13-338 on fatty acid composition.



lipids is changed but not of phospholipids (Figure 4). The ratios linolenic acid/linoleic acid (18:3/18:2) and hexadecatrienoic acid/hexadecadienoic acid (16:3/16:2) decrease whereas the ratio palmitic acid/unsaturated C16 acids (16:0/16:u) increases, that means, desaturation is inhibited.

Table 1 Effect of BASF 13-338 on electron transport in phosphorylated and nonphosphorylated stages of the light harvesting complex (nMol O_2/mg Chl min).

PREPARATION	±ATP	1 $H_2O \rightarrow MV$ RED	2 ASC→MV RED	3 ASC→MV FAR RED	$\frac{2^+:1^+}{2^-:1^-}$	$\frac{2^+:3^+}{2^-:3^-}$	$\frac{3^+:1^+}{3^-:1^-}$
CONTROL CHLOROPL. 6' PREILL.	+ATP	470	705	558	1.46	0.90	1.62
	-ATP	602	617	441			
BASF 13-338 CHLOROPL. 6' PREILL.	+ATP	338	588	294	1.01	1.05	0.96
	-ATP	323	558	294			

CONCLUSIONS

Based on the Energy Flux Theory for Biomembranes (STRASSER 1978, 1980) the low temperature fluorescence kinetics indicate that both BASF 13-338 and Cerulenin force the energy flux constants k2b to decrease or/and k23 to increase. k21 is affected only after 3 days treatment with Cerulenin but not with BASF 13-338 because αN remains constant. From state-1 state-2 transitions measured at room temperature as well as light harvesting phosphorylation experiments we conclude that the pyridazinone BASF 13-338 inhibits dynamic changes within and between photosynthetic units during state-1 state-2 transition (STRASSER 1983) by altering the matrix structure of the membrane. The photosynthetic units are primarly not attacked because spill over remains constant although mobility in the membrane is changed. Variations in the fatty acid composition alter the fluidity of the membranes. Kinetin which influences the fatty acid metabolism (GRAF et al. 1982) acts against the effect due to the pyridazinone herbicide by causing the synthesis of more unsaturated fatty acids thus maintaining mobility of photosynthetic units.

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