A Temporal Phase Mutation of Chlorophyll Fluorescence in Triazine-Resistant *Brassica napus*

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The objectives of this study were to determine if there were variations in triazine-resistant and -susceptible *Brassica napus* leaf disc chlorophyll fluorescence (LCF) intensity in terms of: age of leaf on the plant and time of day. In growth room and field experiments triazine-susceptible *B. napus* cv. "Tower", and triazine-resistant *B. napus* cv. "OAC Triton" were used. Chlorophyll fluorescence intensity measurements were made 30 min after disc removal. In both environments, two periods of reduced photosynthetic efficiency occurred in the diurnal. The times that these periods occurred during the diurnal differed between biotypes. A phase shift in LCF maxima between resistant and susceptible biotypes resulted in two periods, early and late in the light period of the diurnal, of increased LCF in resistant tissue. This differential pattern in LCF is support for the hypothesis that triazine resistance chloroplast alterations could imply an alteration in the temporal organization of chloroplast physiological function.

Introduction

The discovery of s-triazine resistance in plants and its mode of action has provided a system with which to study the effects of an altered chloroplast structure. The development of a triazine-resistant spring oilseed rape *(Brassica napus)* from its triazine-susceptible derivative provides an isonuclear model system to compare this altered protein. A rapid technique to study leaf chlorophyll fluorescence (LCF) was developed by Ali and Souza Machado [1] and Ahrens *et al.* [2].

Past research has demonstrated a diurnal, or circadian, variation in photosynthesis [3, 4]. Whether these endogenous rhythms occur in fluorescence chlorophyll is unknown. The objectives of this paper were to determine if there were diurnal variations in triazine-resistant and susceptible *Brassica napus* LCF in terms of time of day and age of the leaf on the plant.

A preliminary (not reported), one day, experiment was conducted under controlled conditions, similar to those described in the following section. The data from that experiment indicated a different daily LCF oscillation in the resistant tissue relative to that in the susceptible tissue. These observations raised the question whether triazine-resistant *B. napus* may be a phase chronomutant, a biotype of a species with a genetic alteration of the temporal organization of some specific physiological activity [5–7]. Therefore, we hypothesized that alterations in chloroplast structure that confer triazine resistance may also imply an altered temporal organization of chlorophyll fluorescence activities.

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Materials and Methods

Two experiments were performed: one under controlled environmental conditions and one in an agricultural field. Means were calculated from the four leaf-disc samples taken from several plants, and a standard error of the mean (S.E.) calculated. The species tested were triazine-resistant *B. napus* cv. "OAC Triton" and triazine-susceptible *B. napus* cv. "Tower". In both experiments, all the plants were developmentally at the late vegetative to early reproductive stages of growth. Discs were obtained every three hours from young (the youngest fully expanded leaf at the top of the plant) and old leaves (oldest leaf at the bottom of the plant without evidence of scenescence). Two 8 mm diameter discs were punched from either side of the leaf midvein and were then placed in the dark in petri dishes with 0.05 M phosphate buffer at pH 7.5. LCF intensity measurements were taken 30 min later with a Plant Productivity Fluorometer Model SF-110 from

Richard Brancker Research Ltd., Ottawa, Ontario, Canada. Fluorometer illumination time was 20 s.

Controlled environment experiment

The first experiment was conducted in a large phytotron and the plant material was established on April 20, 1983. The experiment commenced at 2000 (EDT) on Sunday, June 19, 1983 and ended at 2300 (EDT) Wednesday, June 22, 1983. The photoperiod was 16 h light, 8 h dark. The temperature was 21 °C in the light period and 16 °C in the dark period. The light fluence during the light period was approximately 450 μM $M^{-1}sec^{-1}$. The relative air humidity was 61%.

Field experiment

The second experiment was conducted at the Elora Research Station, University of Guelph, Ontario, Canada. The plant material was established on July 1, 1983, and suffered some subsequent predation by the crucifer flea beetle. The experiment commenced at 1700 (EDT) on Sunday, August 7, 1983, and ended at 2200 (EDT) on Friday, August 12, 1983. The photoperiod was approximately 14 h light, 10 h dark with full, natural sunlight. Air temperature was quite variable, ranging from 8.5 °C to 29.5 °C. The air humidity was also quite variable, ranging from 43% to 97%.

Results

Controlled environment experiment

Both the susceptible and resistant biotypes had a rhythm characterized by two daily LCF maxima (Fig. 1 and 2). The maxima for the susceptible tissues occurred once in the mid-light period and the second in the dark period. The resistant tissue maxima occurred at different times in the daily light-dark cycle than those associated with the untreated susceptible biotype: one occurred late in the light period, or early in the dark period, the second late in the dark period or early in the light period. Susceptible tissue fluoresced less than resistant tissue at most times of the day. Young leaves of both biotypes fluoresced more than older tissue, and the amplitude of the LCF was greater.

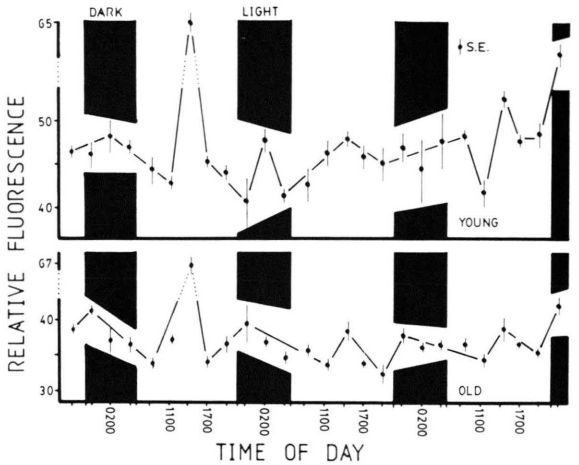

Fig. 1. Diurnal oscillations in relative chlorophyll leaf disc fluorescence in different aged leaves on the same plant (young = most recent fully expanded leaves; old = oldest leaves with no evidence of senescence) with triazine-susceptible *Brassica napus* grown under controlled conditions. Each data point is the mean of four separate leaf discs with the associated standard error of the mean (S.E.).

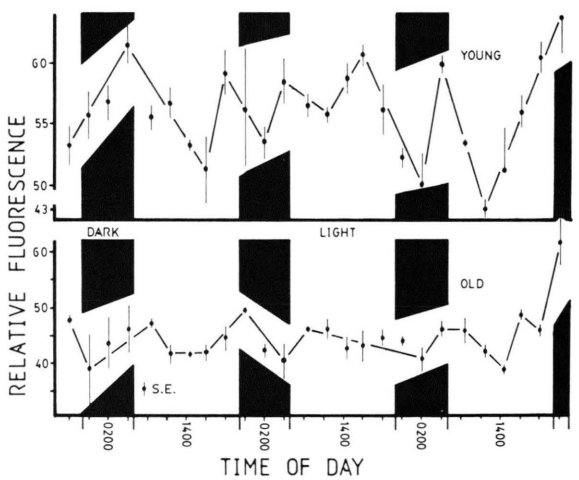

Fig. 2. Diurnal oscillations in relative chlorophyll leaf disc fluorescence in different aged leaves on the same plant (young = more recent fully expanded leaves; old = oldest leaves with no evidence of senescence) with triazine-resistant *Brassica napus* grown under controlled conditions. Each data point is the mean of four separate leaf discs with the associated standard error of the mean (S.E.).

Diurnal differences in LCF between biotypes

In both environments, the differences in LCF between biotypes was often greatest at two times in the

daily cycle: once early in the light period, and once late in the light period (Fig. 3 and 4). At other times in the day, mid-light and mid-dark period, the LCF of the two biotypes approached equality. The differences in LCF, and the amplitude of LCF, were greater with the young leaves relative to older leaves when grown under controlled conditions.

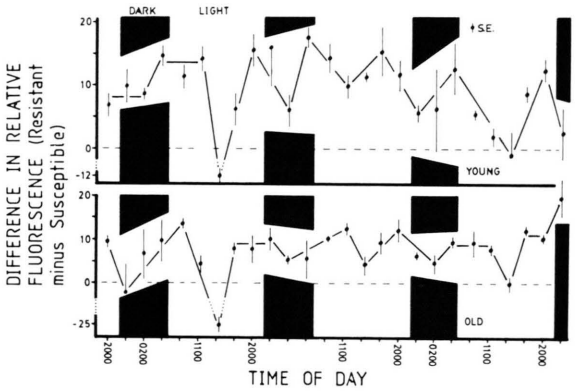

Fig. 3. Difference in relative chlorophyll fluorescence (resistant minus susceptible) found in triazine-resistant and susceptible biotypes of *Brassica napus* grown under controlled conditions. Young = most recent fully expanded leaves; old = oldest leaves with no evidence of senescence. Each data point is the mean of the differences in fluorescence, with the associated standard error of the difference (S.E.).

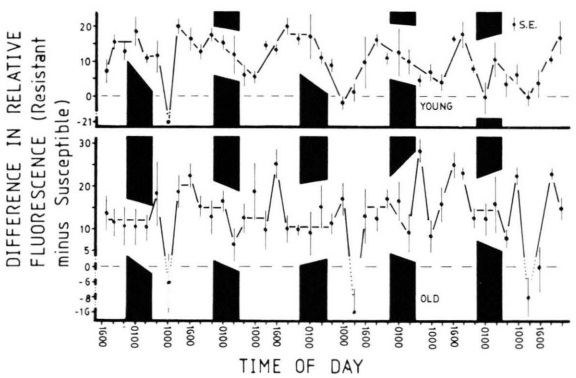

Fig. 4. Difference in relative chlorophyll fluorescence (resistant minus susceptible) found in triazine-resistant and susceptible biotypes of *Brassica napus* grown under field conditions. Young = most recent fully expanded leaves; old = oldest leaves with no evidence of senescence. Each data point is the mean of the differences in fluorescence, with the associated standard error of the difference (S.E.).

Discussion

In both environments studied, two periods of reduced photosynthetic efficiency in leaves of the susceptible and resistant biotype usually occurred in the daily cycle. This relationship can be seen in Fig. 5. The times these maxima occurred during the diurnal differed between biotypes. A comparison of LCF at each sample time indicated a phase shift of LCF maxima between the biotypes. The resistant biotype usually was less photosynthetically efficient than the susceptible analogue early and late in the light period. The susceptible biotype was usually less photosynthetically efficient in the middle of the light and dark periods of the diurnal. These differences in efficiency are evidence in support of the hypothesis that the mutation of the 32 kDa quinone-binding chloroplast protein that confers triazine resistance also confers an alteration of the temporal organization of photosynthetic function. The close, reciprocal, relationship between fluorescence and photosynthetic carbon assimilation [8, 9] may imply that the resistant biotype has the greatest photosynthetic activity early and late in the light period. The susceptible biotype may have maximum photosynthetic activity in the middle of the light period, similar to that reported for other species [3, 4]. There appears to be two different temporal organizations of photosynthetic function in the two biotypes of *Brassica napus*. The resistant mutant is better adapted to the low illuminance regimes of the early and late portions of the light period in the diurnal. The more numerous susceptible biotype in

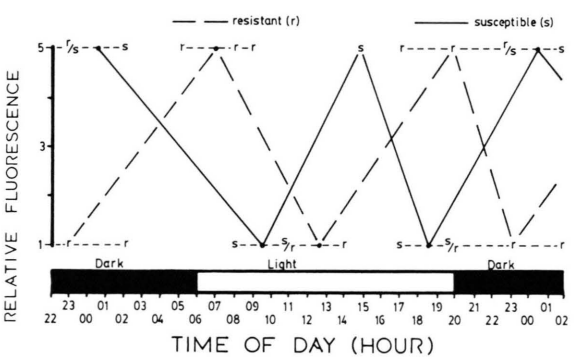

Fig. 5. Diurnal oscillations in triazine-resistant and susceptible *Brassica napus* leaf disc chlorophyll fluorescence; daily maxima and minima combined over three days of a controlled environment experiment; r = resistant means, s = susceptible means; – – – connects associated maxima and minima means; variations in fluorescence amplitude removed.

natural populations has maximum photosynthetic function at peak illuminance periods in the diurnal. Two observations from other work support this hypothesis.

Vaughn has reported that several triazine-resistant species, including *Brassica napus* have a shade-adapted chloroplast ultrastructure [10, 11]. In the chloroplasts of these species, a greater proportion of the resistant chlorophyll was associated with the light harvesting chlorophyll *a*/*b* protein, and it had a lower chlorophyll *a*/*b* ratio. The resistant mutant had a larger volume of the chloroplast as grana lamellae and more thylakoids per granum. This shade-adapted chloroplast ultrastructure is consistent with the hypothesis that the resistant mutant is an adaptation for altered temporal organization of photosynthetic function.

Pillai and St. John [12] concluded that triazine-resistant biotypes have an altered, more cold-tolerant, lipid constitution. Enhanced cold tolerance, and adaptation to the cooler temperatures associated with the early and late portions of the light period, also support the hypothesis that the resistant mutant is adapted to increased physiological function at those times.

Although triazine-resistant mutants have been shown to be less "fit" than their susceptible analogues [13] in a natural environment these alterations of photosynthetic function could enable the resistant mutant to have adaptive advantage, and to ensure its survival in the population.

The alteration of chloroplast membrane structure in the resistant mutant, and the concurrent phase shift forward in LCF, implies a major preturbation in the clock mechanism regulating the activities in this species. This is evidence in support of the clock mechanism being membrane-bound.

[1] A. Ali and V. Souza Machado, Weed Res. **21,** 191–197 (1981).
[2] W. H. Ahrens, C. J. Arntzen, and E. W. Stoller, Weed Sci. **29,** 316–322 (1981).
[3] A. S. Chia-Looi and B. G. Cumming, Can. J. Bot. **50,** 2219–2226 (1972).
[4] J. E. Pallas, Y. B. Samish, and C. M. Willmer, Plant Physiol. **53,** 907–911 (1974).
[5] K. Hansunuma, Proc. Japan Acad. **60,** 260–264 (1984).
[6] C. P. Kyriacou and J. C. Hall, Nature **314,** 171–173 (1985).
[7] H. S. Shin, T. A. Bargiello, B. T. Clark, F. R. Jackson, and M. W. Young, Nature **317,** 445–448 (1985).
[8] T. J. Delieu and D. A. Walker, Plant Physiol. **73,** 534–541 (1983).
[9] D. A. Walker, M. M. Sivak, R. T. Prinsley, and J. K. Cheesbrough, Plant Physiol. **73,** 542–549 (1983).
[10] K. C. Vaughn and S. O. Duke, Physiol. Plant **62,** 510–520 (1984).
[11] K. C. Vaughn, Plant Physiol., in press (1986).
[12] P. Pillai and J. B. St. John, Plant Physiol. **68,** 585–587 (1981).
[13] J. Gressel and G. Ben-Sinai, Plant Sci. **38,** 29–32 (1985).
[14] G. F. Ryank, Weed Sci. **18,** 614 (1970).