DAILY XANTHOPHYLL CYCLE PHOTOPROTECTION IN DEVELOPING LEAVES PRIOR TO PHOTOSYNTHESIS

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There is widespread agreement that the xanthophyll cycle provides a major photoprotection system for photosynthesis in green leaves (1-8). Indeed this type of photoprotection seems to be ubiquitous for photosynthetic organisms. Photoprotection is provided via a rapid, near 10^{-13} sec, ability of zeaxanthin (Z) to dissipate excess light energy from photosynthesis because the energy of the excited singlet state of Z is lower than that of chlorophyll a (9).

Details of historical and current understandings on the operation of the xanthophyll cycle in green tissues are readily available (3,6,9, A.M. Gilmore - these Proceedings). During initial research efforts to establish the xanthophyll cycle some developing photosynthetic tissues were appropriately used, e.g., to identify the pigments involved or to detect the enzymes required for epoxidation and de-epoxidation. However these developing tissues were not subjected to natural plant diurnal growth conditions and this may have obscured cycle functions during the development of photosynthesis. For example, red kidney bean plants were dark grown for 4 days followed by giving 1-msec flashes of Xenon light every 12 minutes for 6 days (2). Then these primary bean leaves were used to identify the carotenoids involved and to show that the 505-nm absorbance change could be used to monitor them. The 505-nm change correlated closely with the induction of O_2 evolution; but even more closely with the thylakoid fusion process (2). Or 15 to 18 days old completely dark grown primary bean leaves were used to study the de-expoxidase; but the 505-nm change could not be detected in those leaves until infiltration with a pH 5 buffer for at least 4 hours of illumination (7). These and other studies helped establish the pigments and enzymes required for the xanthophyll cycle (1-8).

The ability to rapidly dissipate light energy raised the question in our research of, "When is the xanthophyll cycle functional relative to functional photosynthesis in a normally developing plant tissue?" Here we will show that the daily xanthophyll cycle functions in developing leaves prior to photosynthesis. We propose that this rapid energy dissipation process must be in place, prior to photosynthesis, to protect chlorophyll and other components of the photosynthetic apparatus even during their biosynthesis and proper insertion for the complete biogenesis of chloroplasts. In our early xanthophyll research we obtained results indicating that the diurnal xanthophyll cycle was operating in developing leaves of *Quercus nigra* and *Q. palustris* and in mature leaves of *Q. rubra* and *Q. alba*. The ratio of Z plus antheraxanthin (A) over the total xanthophyll pool (Z + A + Violaxnthin (V) ranged between 0.5 and 0.83 between 10 am and 4 pm and the ratio decreased to near 0.1 at night. To study photoprotection via the xanthophyll cycle in more detail the xanthophyll cycle and photosynthesis were investigated with both developing and mature leaves. We will conclude that xanthophyll cycle photoprotection exists and is required in young leaves

even prior to net photosynthesis and that this requirement is to photoprotect chlorophyll and thylakoid membrane components from photodamages, especially before CO_2 photoassimilation and other photosynthetic processes that can utilize absorbed light energy are functional.

Materials and Methods. All plants were field grown in Athens, GA. Oaks have the useful biological trait of recurrent bud breakage during a growing season with a rapid branch elongation and new leaf formation. Buds may break, rapidly elongate, and develop leaves several times each growing season. Thus mature leaves and a developing leaf sequence (usually containing 12 to 16 leaves per flush) can be studied on the same branch. Leaves were collected and frozen in liquid N_2 over a day to establish the xanthophyll cycle and then in a more detailed study they were collected at 6 am and 12 noon for xanthophyll determinations using a Dionex HPLC with a Zorbax non-end capped ODS column (10). At 6 am, the predominant component of the xanthophyll pool was V. At 12 noon, levels of A and Z increased concurrently with stoichiometric decreases in the V level. Maximum leaf photosynthesis was measured between 11 am and noon using a portable LiCor infrared CO_2 gas analyzer.

Results. A set of results on the daily xanthophyll cycle in different sized developing oak leaves is shown in Fig. 1. Several points should be noted: first the smallest and youngest leaves convert a high fraction of V to A + Z near mid-day; second as leaves age a smaller fraction is converted at mid-day; and third, at night a complete conversion to V does not occur. We have obtained similar data with other oaks and other plant species.

We isolated the pigments, i.e., chlorophylls and carotenoids, from leaves on an elongating flushing oak branch and compared these with mature leaves on the same branch. As shown in Fig. 2 the total carotenoids and chlorophylls a + b increased, as expected, during leaf ontogeny. The ratio between total chlorophylls and total carotenoids ranged between 6:1 and 8:1 in oak leaves. But the xanthophyll pigments increased only slightly from the youngest leaves to mature leaves (Fig. 2).

Knowing that in the daily xanthophyll cycle a maximum amount of Z was converted near mid-day (6, Fig. 1), we examined the noon and dawn conversion values in a flushing oak branch. Fig. 3 clearly shows that the cycle was present in all of the leaves we measured and that the A + Z was highest in the youngest leaves.

We then measured both the noon xanthophyll cycle and photosynthetic CO_2 uptake via leaf gas-exchange techniques. The results of such studies are shown in Fig. 4 where the xanthophyll cycle was functional in all leaves from the youngest to the oldest. Net photosynthesis, in contrast, was not detectable in very young leaves and then progressively developed, as expected, with increasing leaf size (leaf position). Clearly a functioning xanthophyll cycle was present prior to net CO_2 uptake in these developing leaves (Fig. 4). Photosynthesis reached maximum values only in mature leaves. These leaves were irradiated with PPFD values near those in Fig. 1. There was a clear tendency in all studies for the ratio of xanthophyll cycle pigments to be lower in more mature leaves near mid-day. That is expected as other mechanisms for the dissipation of excess light energy become functional in photosynthesis (1-9).

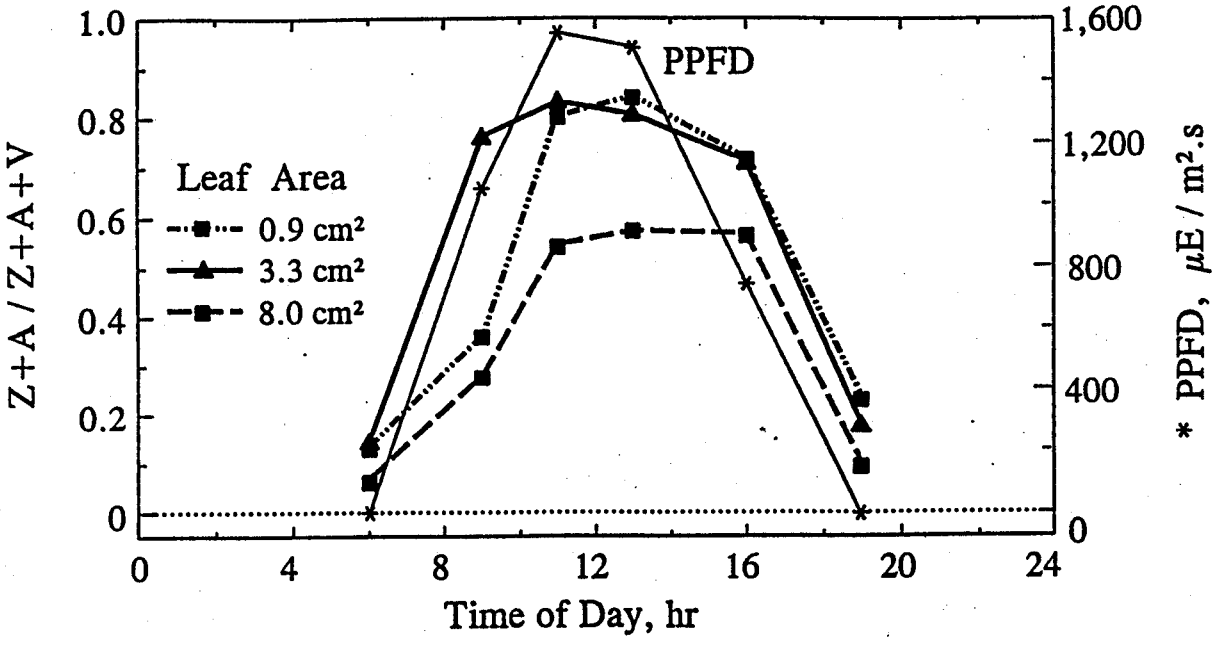

Fig. 1. The daily xanthophyll cycle, as the ratio of Z+A / Z+A+V, in developing leaves of a flushing water oak tree branch.

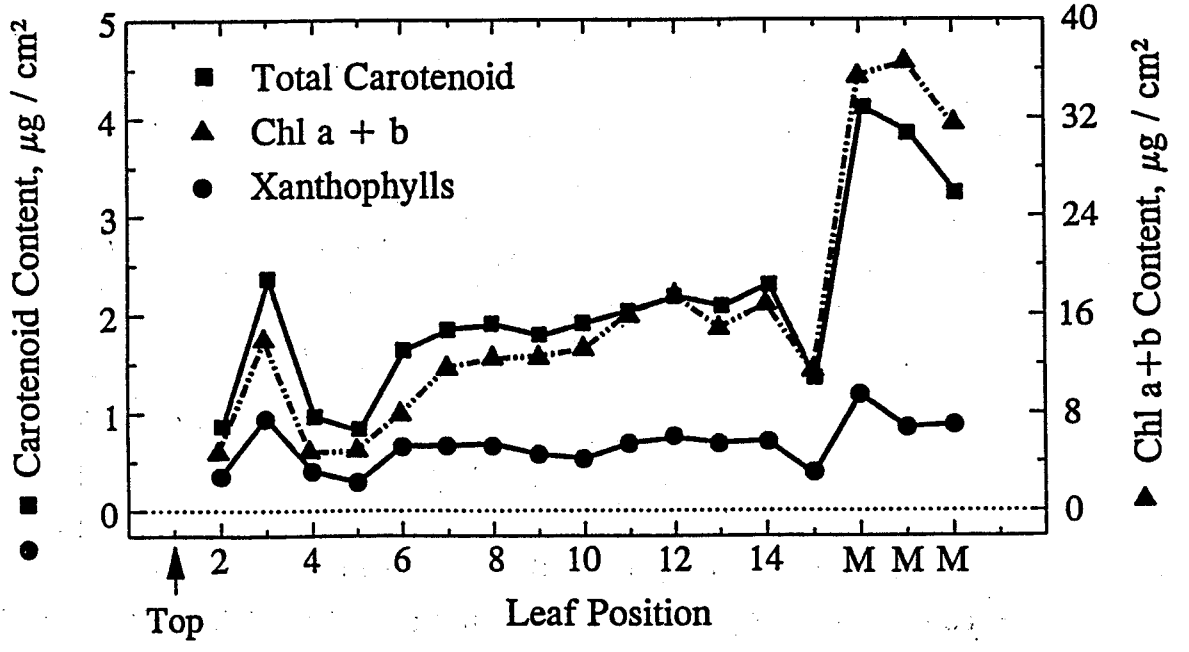

Fig. 2. Changes in carotenoids and chlorophyll contents of developing Northern red oak leaves and the pigment contents of three mature (M) leaves, all on the same tree branch.

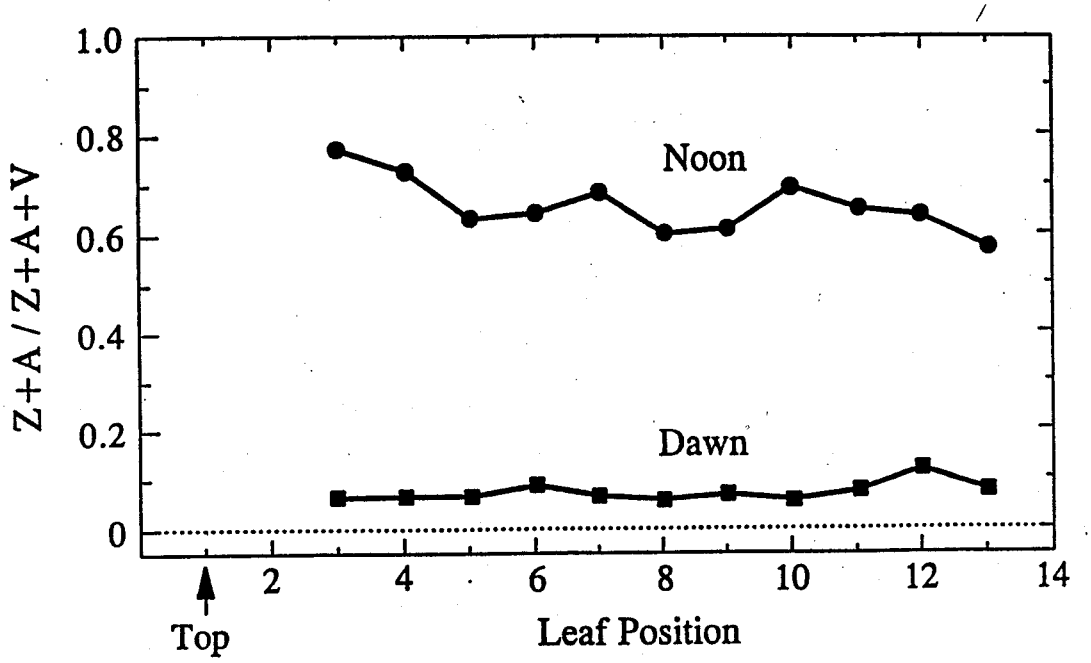

Fig. 3. Noon and dawn values for the ratio of xanthophyll cycle carotenoids in developing leaves of a flushing Northern red oak branch.

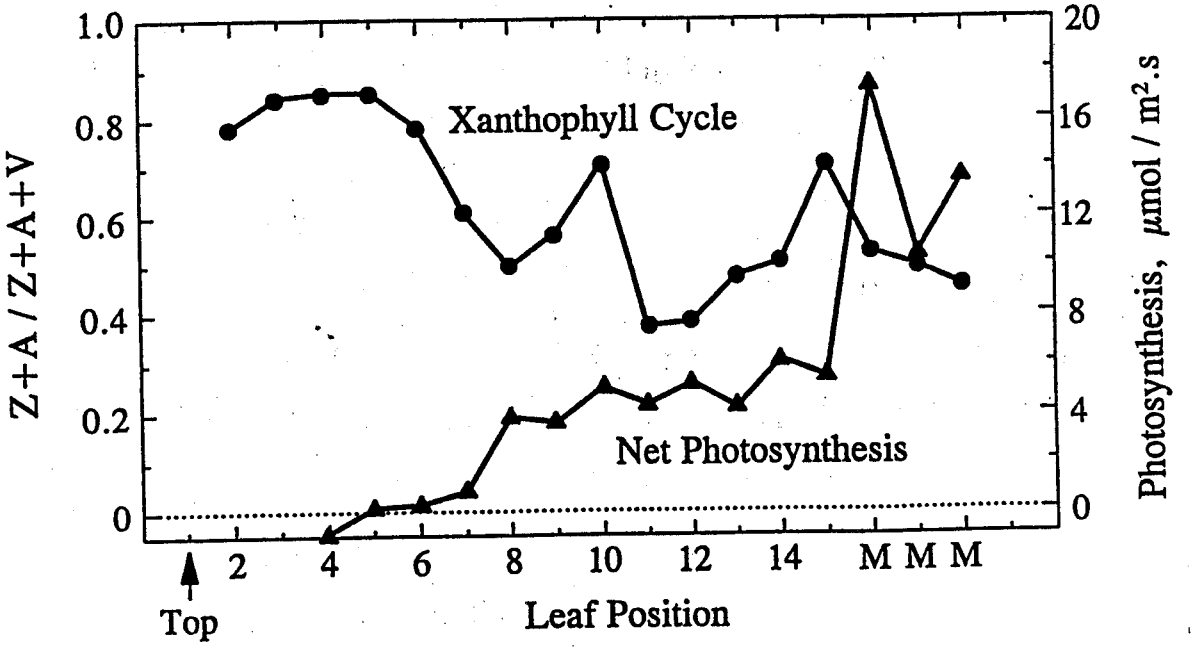

Fig. 4. Noon values for the xanthophyll cycle and for net photosynthesis in developing Northern red oak leaves, and with three mature (M) leaves, all on the same tree branch.

Discussion and Conclusions. Our hypothesis was that the absorption of light energy is so rapid, about 10^{-15} sec (11), that photoprotection mechanisms must be in place as or before chlorophylls are synthesized and thylakoid membranes are completely formed. We interpret those studies to show that the complete xanthophyll cycle is present in the youngest leaves we could obtain. In all of the leaves studied some chlorophyll was present (Fig. 2) along with carotenoids. Of course carotenoids also are well known to be present in many etiolated plant tissues though the xanthophylls have no known function in these tissues. Precisely when the xanthophyll cycle becomes operative in young leaves remains to be determined. However photosynthesis certainly is not functional by gas exchange measurements in the youngest leaves (Fig. 4), all of which have a functional daily xanthophyll cycle (Fig. 1,3,4). These results raise many unanswered questions about how and where the xanthophyll cycle functions prior to photosynthesis, e.g., substrate source, energy source, or the formation of a Δ pH?

We conclude that the xanthophyll cycle is functioning prior to complete photosynthesis and that a major role of the xanthophyll cycle is to protect the components of photosynthesis from light during plastid development. We theorize that the xanthophyll cycle photoprotection, primarily to dissipate light energy, is required for proper plastid development; though this does not exclude other roles for carotenoids such as direct light energy utilization.

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