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Patterns of Chlorophyll Fluorescence Kinetics in Relation to Growth and Expansion in Cucumber Leaves[1]

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ABSTRACT

Photochemical development was studied in developing cucumber (*Cucumis sativus* L. cv Hokushin) leaves to determine if the spatial pattern coincided with relative growth rates of expanding leaves, intercalary cell division, or position relative to the vascular tissue. Both leaf surfaces undergo a series of similar changes in chlorophyll *a* fluorescence kinetics, but the upper surface more quickly achieved the characteristic response. Imaging of fluorescence showed an individual developing leaf has four regions differing in kinetics, but these regions do not coincide with areas of increasing relative growth rate. Two of these regions lie at the leaf edge and their divergent kinetics may be related to structural and physiological features present at this position. A third area with different kinetics, in the basal region of the leaf, is spatially consistent with primordial regions that are clonal during development. The correspondence between areas of clonal growth and specific fluorescence kinetics indicates that cells of common ancestry show functional uniformity. No evidence was found that the proximity of the vascular tissue influenced development of photochemical function.

The attainment of photosynthetic competency in angiosperm leaves is regulated by light and by developmental factors. Unlike monocots such as wheat (2), cucumber leaves are not photochemically competent when first exposed to light, but acquire competency over an extended time period, 6 d or more in the light (5). Within these leaves photochemical activity is not homogeneous, but develops basipetally as shown by Chl *a* fluorescence transients. These data indicate that the primary regulation of the development of photochemical activity lies with developmental factors, not light (5).

Although the attainment of characteristic fluorescence transients progresses in a basipetal direction, mirroring the general direction of expansion and growth of the leaf (5), the influence of developmental factors from cells and tissues within the leaves has not been studied. The objective of the present research was to determine if the spatial pattern of photochemical development coincided with leaf expansion and relative growth rates, intercalary cell division, or position relative to the vascular tissue. With the availability of dynamic imaging of Chl *a* fluorescence (10), a precise, nondestructive means of studying these influences exists.

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MATERIALS AND METHODS

Plant Materials and Leaf Area Measurements

Cucumber (*Cucumis sativus* L. cv Hokushin) plants were grown as previously described (10) for up to 5 weeks. For fluorescence and gas exchange measurements, plants were brought from the controlled-environment greenhouse to the laboratory and allowed to acclimate for about 2 h. Plants were dark-incubated for at least 30 min before fluorescence induction. No appreciable differences in induction kinetics were observed with longer dark periods. Leaves monitored for fluorescence and assimilation were intact and remained attached until all measurements on the plant were completed. Leaf area was measured with a LI 3100 area meter (LI-COR, Lincoln, NE).

Chl *a* Fluorescence Measurements

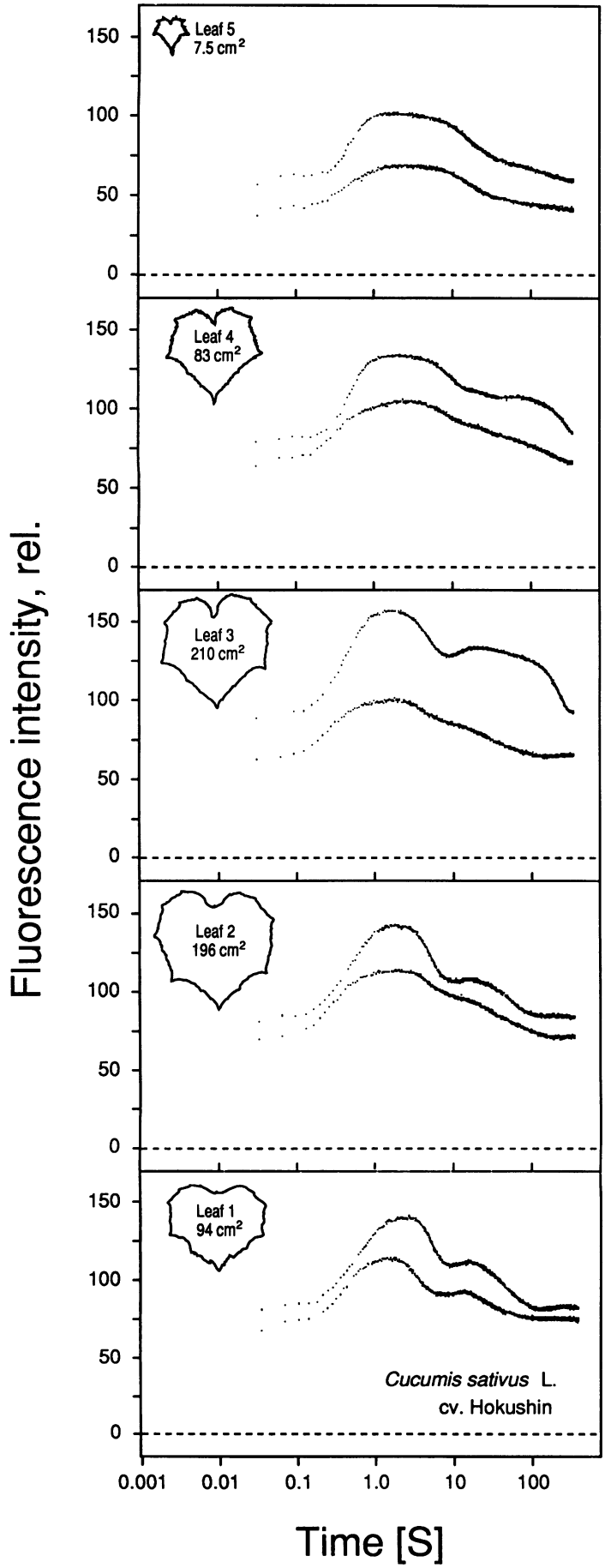
A portable Kautsky apparatus (Richard Branker Research Ltd., Plant Productivity Meter, SF-20) was used to monitor fluorescence on both leaf surfaces at a position midway between the margin and midvein. Light of 670 nm at 40 μmol photons m^{-2} s^{-1} was used for excitation and fluorescence was measured at >710 nm. Data were collected at 30 ms intervals over a period of 400 s, stored, and analyzed by computer.

Imaging System for Measuring Chl Fluorescence Induction

As previously described in detail (10), this system consists of a video camera with a charge coupled device imager and appropriate filters interfaced to a time code generator and video tape recorder. Images of the entire leaf were collected at intervals of 17 ms, digitized by an analog to digital convertor, stored on an optical disc, and then analyzed by computer. Two xenon lamps (380–620 nm) were used to illuminate evenly (±10%) the entire upper leaf surface at a flux density of 150 μmol photons m^{-2} s^{-1}; fluorescence was measured at 683 nm. Five data points from each measured area were analyzed and the data averaged. The kinetics of each data point were examined prior to averaging in order that spatial deviations in kinetics were not overlooked.

Fluorescence Data

The kinetic data are displayed as single curves on a log time scale. While this time basis changes the overall appearance of



fluorescence curves, the presentation gives good resolution of the fast phases of fluorescence and compresses the slow phases of the signal, but without loss of important characteristics of fluorescence induction. The terminology of Lavorel and Etienne (10) is used to identify the consecutive phases of the fluorescence induction signals (I, D, P, S, M, T[2]) and their interpretation is based on established criteria (4, 7, 8, 11, 19, 27).

Assimilation Measurements

Measurements were made by an apparatus previously described (17). Briefly, air was mixed from N_2, O_2, and 3% v/v CO_2 in air using mass flow controllers. Some of this synthetic air passed through an aluminum leaf chamber. The air flow through the chamber was controlled by a mass flow controller. Some of the synthetic air and air from the leaf chamber was compared for water content and CO_2 content with an IR gas analyzer. Cross-sensitivity of the CO_2 measuring section to water was eliminated by condensing the water out of the air as the air passed from the water measuring tube to the CO_2 measuring tube. Leaf temperature was measured with a copper-constantan thermocouple probe. Calculations of assimilation were made according to von Caemmerer and Farquhar (26). Light was provided by a quartz halogen lamp and flux density was the same as that used for fluorescence measurements. Water content was measured with a Dew 10 (General Eastern) dew point hygrometer.

RESULTS

The development of photochemical activity in the Hokushin cultivar of cucumber (*Cucumis sativus* L.) was established by measuring fluorescence transients on the upper and lower leaf surfaces of 3-week-old plants (Fig. 1, upper traces are from the upper leaf surfaces). Three-week-old plants have 5 leaves expanded away from the main bud. Only the first leaf produced, leaf 1, has completed its expansion; the remaining leaves are still growing. Only on the oldest leaf (Fig. 1, leaf 1) did both leaf surfaces show characteristic fluorescence transients. The slow and fast phases of fluorescence kinetics appeared more quickly on the lower surface of this leaf than on the upper surface; however, the initial level of fluorescence and its peak value were greater on the upper side of the leaf.

In developing leaves (Fig. 1, leaves 5 to 2), characteristic

[2] Abbreviations: I, D, P, S, M, T, inflection points of Chl *a* fluorescence kinetics where I is intermediary level, D is dip, P is peak, S is quasi-stationary level, M is second maximum, and T is terminal level.

Figure 1. Fluorescence induction transients on the upper and lower surfaces in the five leaves of a 21-d-old cucumber plant. Plants were dark-incubated for at least 30 min before measurement at 40 μmol photons m^{-2} s^{-1}; upper traces are from the upper leaf surfaces. An outline of each measured leaf appears with its leaf number and area to the left of its fluorescence induction traces; the outlined images are reductions, but not to scale.

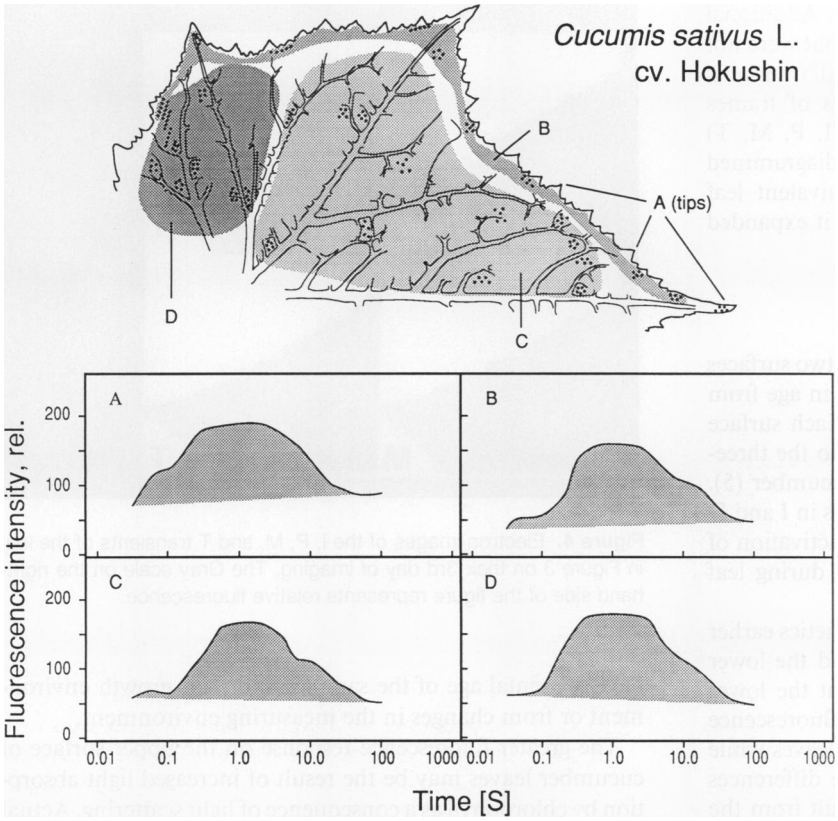


Figure 2. Diagram of a developing leaf (the eighth leaf from the bottom of a 31-d-old cucumber plant) in which Chl *a* fluorescence was dynamically imaged; the sample points within areas are marked with solid circles on the diagram. The regions with areas of common kinetics (A, B, C, and D) are differentially shaded on the diagram. A curve representative of each region appears below the leaf diagram; the area under each curve has been shaded for ease in distinguishing the kinetics among regions. The cucumber plant was dark-incubated for 30 min prior to measurement at 150 μmol photons m^{-2} s^{-1}.

fluorescence transients developed slowly on each surface with the upper side achieving typical fluorescence kinetics sooner than the lower surface. On the upper surface the kinetics first consisted of I, D, and a broad P on the smallest leaf and were then followed by the presence of an extended S region and an expanded M peak on leaf 4. In the next older leaf, P was quenched more rapidly, but M remained broad. The two oldest foliage leaves showed an increase in the time to P and a slight decrease in the rate of rise; the S to M to T transients were typical of mature leaves. On the lower surface, P was broad in the beginning and showed slow quenching throughout development. Typical transients including M were present only on the oldest leaf. Variable fluorescence was greater on the upper surface throughout leaf development (Fig. 1) with leaf 3 showing the largest difference between the two leaf surfaces.

The regional differences in fluorescence response of developing leaves known in the Natsusairaku cultivar of cucumber (5) were studied in this variety by dynamic imaging. A single leaf (similar to leaf 4 in Fig. 1) was used to determine the exact locations of variation in fluorescence kinetics. Analysis of approximately 150 points within representative areas of the upper side of the leaf showed four areas with different kinetics (Fig. 2). The different transients occurred at the tips (Fig. 2A), in a submarginal position (Fig. 2B), in the distal two-thirds of the blade (Fig. 2C), and in the basal one-third of the blade (Fig. 2D).

In the tip areas, at the terminus and along the leaf margin (Fig. 2A), the fluorescence response consisted of a single, broad P peak. These extreme tip regions may never become photochemically competent because equivalent areas of older, mature leaves showed the same kinetics (data not shown). In the submarginal position (Fig. 2B), the I, D, P, and M transients were present, but the initial level of fluorescence was reduced relative to the tip areas, although peak fluorescence was approximately the same. The interveinal areas in the distal portion of the leaf (Fig. 2C) showed typical fluorescence kinetics, with all transients present. However, the proximal region of the leaf (Fig. 2D) was characterized by kinetics without a well-defined M peak; I and D were also absent.

To examine the development of the spatial and temporal patterns of fluorescence in greater detail, a single area of a young leaf from a plant 33 d old was imaged on 3 consecutive d (Fig. 3). The leaf was the tenth from the base and appeared at the edge of the main bud when the plant was between 28 and 31 d old. The leaf area measured was 20% of full expansion on the first day it was imaged and 35% expanded on the third day of imaging. Thirteen sampling regions were selected for analysis and the sample points were relocated each day on the digitized images (Fig. 3). Within the area imaged, data are presented from three of the thirteen regions based on their position with respect to the leaf tip, the midvein, and the margin. Data from the other ten regions showed the same pattern of changing fluorescence kinetics. There were no discernible changes in kinetics that were position dependent.

The three regions showed similar kinetics on each day and similar changes from day to day. On the 1st d, the transients

consisted of a single, broad peak, P (Fig. 3, d 1). Additional transients began to appear on the following day, but were not clearly distinguishable (Fig. 3, d 2). By the 3rd d, all transients were evident (Fig. 3, d 3). Figure 4 is a series of frames showing representative fluorescence transients (I, P, M, T) from the videotape images of the fluorescing leaf diagrammed in Figure 3. Assimilation measured in an equivalent leaf increased linearly from 2 to 10 μmol m^{-2} s^{-1} as it expanded over a 5 d period (Fig. 5).

DISCUSSION

The fluorescence kinetics were different on the two surfaces of developing cucumber leaves of plants ranging in age from 5 to 30 days old (Fig. 1, 30 plants measured). Each surface showed changes in fluorescence kinetics similar to the three-step sequence reported in another cultivar of cucumber (5). Similar changes in variable fluorescence, increases in I and D levels, and an increase in the DP rise caused by activation of the water splitting enzyme system are apparent during leaf development (5).

The upper leaf surface showed characteristic kinetics earlier and developed greater peak fluorescence than did the lower surface. Previous measurements have shown that the lower side has greater F_O and F_M (3, 16) and that fluorescence response on the lower surface is like that of sun leaves while the upper surface is similar to shade leaves. The differences among these species may be taxa specific, or result from the developmental age of the samples and their growth environment or from changes in the measuring environment.

The greater fluorescence response on the upper surface of cucumber leaves may be the result of increased light absorption by chlorophyll as a consequence of light scattering. Actual

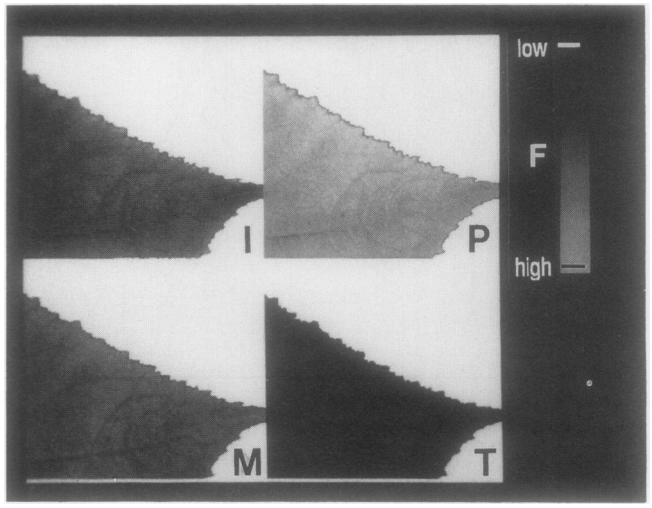


Figure 4. Electron images of the I, P, M, and T transients of the leaf in Figure 3 on their 3rd day of imaging. The Gray scale on the right-hand side of the figure represents relative fluorescence.

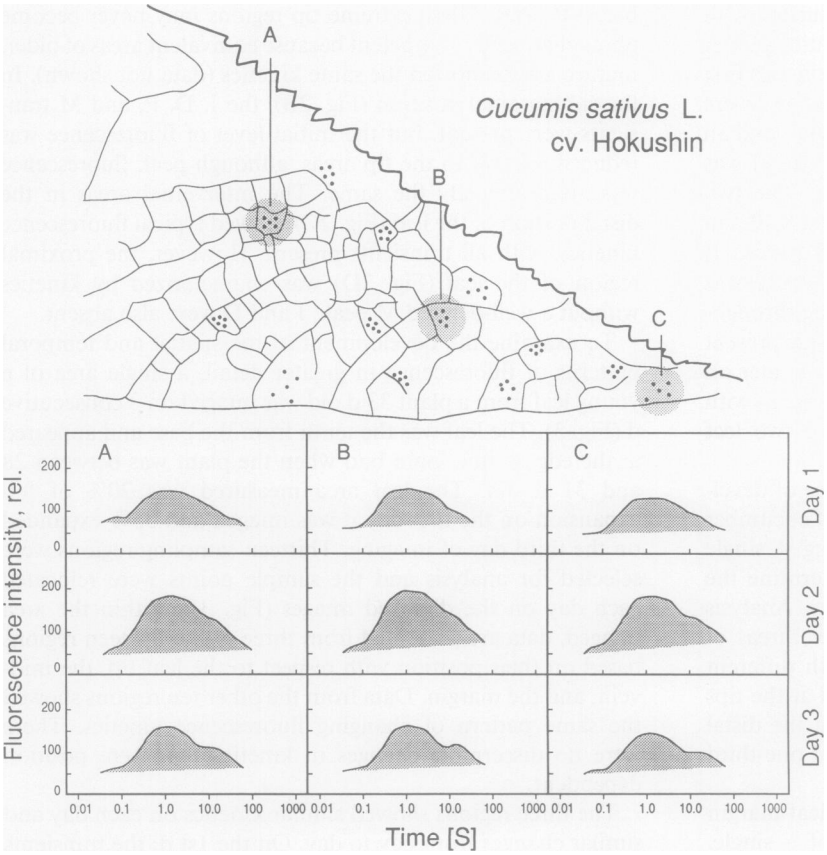


Figure 3. Diagram of a young leaf tip (the 10th leaf from the bottom of a plant 33 d old) that underwent dynamic imaging of Chl *a* fluorescence transients on 3 consecutive d; the sample points within regions are marked with solid circles. Three regions have been shaded and their fluorescence kinetics appear below; the regions were selected based on their position relative to the leaf tip, the midvein and the margin. The plant was dark-incubated for 30 min prior to measurement at 150 μmol photons m^{-2} s^{-1}.

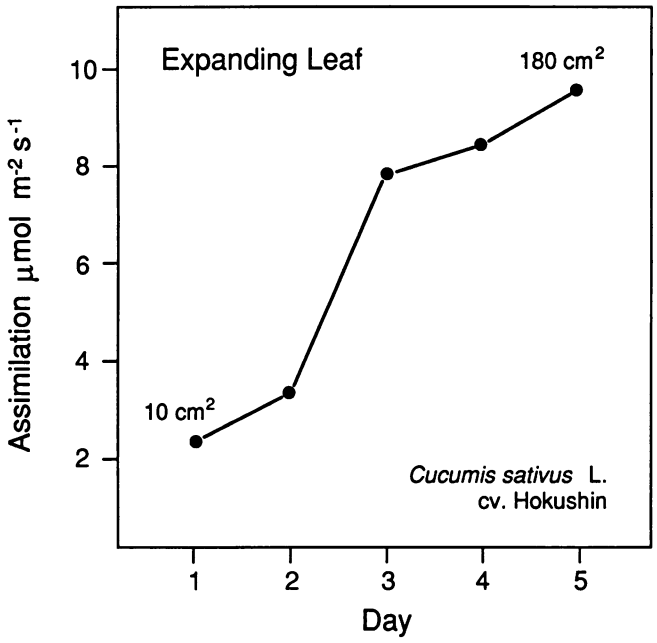


Figure 5. Carbon assimilation measured at the same intracellular CO_2 partial pressure over a 5 d period in a single developing leaf which changed in area from 10 to 180 cm^2.

measurements in the interior of cucumber cotyledons (24) and bifacial *Medicago sativa* leaves (25) support this view. The initial level of fluorescence was always greater on the upper side of developing cucumber leaves. This could occur if the number of antenna molecules were greater or if energy distribution between the reaction centers and photosystems favored an increased density of excited PSII pigments (17).

As cucumber leaves expanded, the time to P increased as the rate of the rise to P declined, indicating changes in PSII acceptors (7, 15). The maximum changes in the time and rise to P coincided with the narrowing of the M peak in leaves 1 and 2 (Fig. 1). In contrast, in developing pea leaves the time to P declines linearly with age (20). Quenching mechanisms in cucumber developed over an extended time period on both sides of expanding leaves (Fig. 1), but were more protracted on the lower leaf surface. The delay may be a response to the spectral environment seen by these chloroplasts (25) and would be consistent with photosynthetic properties of chloroplasts isolated from successive, vertical regions of the mesophyll (21–23).

The fixation of CO_2 was minimal or lacking in younger cucumber leaves, even though they had been exposed to light for one or more days. Evidence of this was shown by the absence of M (Figs. 1, 2, and 4) (27) and the direct measurement of carbon assimilation (Fig. 5). The M transient appeared first on the upper side of leaf 4 and later on the lower surface in the older leaves, 2 and 3. Young pea leaves also lack M (20). However, once present the time to M declines with leaf age in both peas and cucumber (Fig. 1). While M appears and/or oscillates under certain physiological conditions in mature leaves (19, 27), its presence is also a normal feature of leaf development and a useful indicator of carbon metabolism.

The pattern of fluorescence kinetics within a developing cucumber leaf (Fig. 2) does not accurately coincide with the pattern of dicot leaf expansion and growth. Four regions with different fluorescence responses occur in this leaf, but these regions do not correspond spatially to areas of growth. Although a comparison of the two large interveinal areas (Fig. 2, C and D) showed that the distal region achieved characteristic kinetics prior to the proximal area signifying basipetal development, two other areas of fluorescence (Fig. 2, A and B) were present at the leaf margin and each extended longitudinally from the tip to the base. Studies of dicot leaf expansion (6, 18), including cucumber (1), show that sectors differing in growth rate extend horizontally across the entire blade and frequently curve basally at the leaf margin. Thus, there are qualitative differences between the location of maxima in fluorescence kinetics and in relative growth in the leaf.

The marginal areas of the leaf which developed photochemical activity according to a program independent of the central blade region have spatial correspondence with known patterns of division within leaves (12, 14). In a clonal analysis of tobacco leaf development, selected clones are restricted to the leaf edge and contributed to elongation growth at the margin and not growth in blade width. These clonal regions occupy the same location as the marginal areas with different fluorescence induction kinetics (Fig. 2B). The tips that are found along the entire length of the leaf margin never developed the fluorescence response characteristic of mature leaves (Fig. 2A). This is probably related to the structural differences of tissue at the leaf edge. The two-tiered mesophyll region at the interior of the leaf gradually changes to a more or less homogenous group of cells without intercellular spaces at the leaf margin. The venation and stomatal distribution changes at the leaf edge may also serve to isolate the tips from CO_2 and nutrients carried by the conducting tissue. The lack of a characteristic fluorescence response in the leaf tips may be a reflection of the changes in structural and physiological aspects in this area.

The proximal area of the developing cucumber leaf (Fig. 4) is reminiscent of primordial areas occurring between the lateral veins of young tobacco leaves (12). The homogeneity of this region in cucumber may reflect a clone of cells specified to function early in leaf development. The specification of function may be carried through cell division to yield a cell group of photochemical uniformity. Although all groups eventually achieve characteristic fluorescence responses, during development several regions of different photochemical activity appear on a single leaf. According to this scheme, occasionally adjacent cells should be functionally asynchronous because each belongs to a different clone and each was originally specified at a different time. This could be explicitly demonstrated using a cucumber mutant with variegated foliage leaves or by inducing clones on developing leaves (13).

Differences in the micro growth environment within these expanding leaves may also be involved in the spatial development of photochemical activity. Mesophyll near vascular bundles or the leaf margin are likely locations for such differences. Light intensity, pigment composition and concentra-

tion, water potential and stomatal function undoubtedly differ in cells from different regions of the leaf, thus contributing to the spatial differences in photochemical activity. Water potential must be different throughout developing leaves in order that immature areas can continue to extend. Some of these physiological parameters may be coupled to cell ancestry and development of the photochemical apparatus, while others are regulated differently and not in synchrony with photosynthetic development.

There was no evidence that the proximity of the vascular tissue influenced development of photochemical function as in corn (9). Imaging of the tip of a young cucumber leaf on consecutive days showed homogeneity in kinetics in the interveinal areas with similar changes occurring at all points on a day to day basis (Fig. 4). The fluorescence responses did not vary with distance from leaf veins but were uniform throughout the intercostal areas. Thus, position influences or positional sensing appears not to modify the attainment of function.

Dicotyledonous leaves have complex patterns of photochemical activity during their expansion and maturation. These patterns do not coincide spatially with areas of relative growth rate in leaves, although there is a basipetal progression in the acquisition of the characteristic phases of fluorescence induction. The configuration of areas with comparable fluorescence responses shows the greatest resemblence to cell clones established early in leaf growth. The resulting mosaic of photochemical activity indicates that cells of common ancestry show functional uniformity.

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