ON SOME QUANTITATIVE RELATIONSHIPS BETWEEN ANATOMY AND LIGHT INDUCED FORMATIVE DIFFERENCES IN GLADIOLUS STEMS

by

E. C. WASSINK

Laboratory of Plant Physiological Research, Agricultural University, Wageningen, Netherlands, 232nd Communication 1)

(Received 27.11.63)

1. INTRODUCTION

Some years ago the author described effects of light intensity (obtained by different degrees of screening) on dry weight production of various organs and on some morphogenetic characteristics (leaf shape, leaf area, stem length, etc.) in a field experiment with Gladiolus (G. [hybr.] gandavensis v. HOUTTE, the ordinary large garden gladiolus) 1). The experiment was repeated in subsequent years; these results are not yet sufficiently worked out for publication. However, the picture is mainly the same as obtained in 1959 while some additional observations, e.g., on day-length effects, were added. In 1961, material for anatomical studies was again preserved, now including stem material.

A preliminary anatomical study of this stem material is the object of this paper.

1) The contents of this paper have been presented at a Joint Meeting of the “Society for Experimental Biology”, at Oxford (England), July 16–20, 1963.
2) Some preliminary results have been communicated at a meeting of the Roy. Netherl. Botanical Society, at Nijmegen, November 1960.
Perception of an external factor inducing morphogenetic differences takes place in some part or organ of a plant, and causes initial biophysical and biochemical effects. These initial effects may be supposed to start an amplifier chain (2), in which biochemical connections come into being between the initial effect and the ultimately observed change in the external appearance of the plant (e.g. a change in leaf shape, stem length, stem size, etc.).

The basic unit of structure of a plant is the cell; every change in size or shape of a plant organ ultimately belongs to a change in number or size of individual cells, i.e., to cell division, and cell extension, the latter including and presupposing plasma growth, i.e. increase in dry weight. The various cell types and tissues may react similarly or differently, this can hardly be foreseen. The behaviour of these cell types translates the initial biochemical amplifier chain into observable effects of size and shape.

The quotation in our motto is from Bonnier's preface to a volume (3) in which he has united three classical papers on formative and structural differences in plants, grown in the plains and in the mountains, and also in continuous and discontinuous electric light.

In the latter, by the way, he reports on what may be deemed to be the first successful attempt at growing plants for a long time in electric light only — previous experiments having answered such elementary questions as to whether chlorophyll is formed in electric light, and if so, whether it would be apt for photosynthesis — at fairly constant temperature and humidity, thus establishing the prototype of the contemporary 'phytotron'. The light was of 8 amp. — probably carbon — arcs, filtered by glass to eliminate harmful ultraviolet; the plants were at 1.5 to 4 m distance.

BONNIER extensively studied the formative changes occurring in his plants, and supplemented his study with detailed anatomical observations.

Certainly, BONNIER neither was an absolute pioneer in this respect. Already BONNET, in his experiments on etiolation (4, see also 5) states that 'il est bien manifeste que ce prolongement excessif [des tiges dans l'étiolement] provient de l'excès de ductilité des fibres de la tige. Ces fibres conservent trop long-temps le degré de souplesse qui leur permet de s'étendre; elles s'endurcisissent trop tard . . .' (4, p. 212).

An extensive survey of the earlier literature is given by McDOUGAL (5) who contributed many anatomical observations pertaining to normal and etiolated structures himself.

Recent studies of anatomy in relation to deliberately induced variations in the environment of plants are rather scattered and often restricted to fairly general items (e.g. average cell size and cell number) (see, e.g. 6–14 for some examples).

More elaborate studies are concerned with leaf anatomy adaptation to variations in environment (see, e.g. 15–20).

A detailed survey of data is not attempted in this preliminary note which only serves to outline the type of observations we are envisaging.

The study of quantitative anatomical differences in connection with morphogenetic effects induced by quantitative differences in environmental factors offers an extensive field which may lead to a more detailed understanding of the reaction of a plant to its environment, and, moreover, to a reincreased interest in anatomical structures among plant physiologists. Also submicroscopical studies, e.g. of cell walls, are of interest in this connection. One may

2 Meded. Landbouwhogeschool, Wageningen 63 (16), 1–8 (1963)
well predict that studies along these lines will receive a strongly increased attention in the near future. The rapid extension of controlled environment facilities will no doubt strongly contribute to the availability of material, suitable for experimental anatomy on a quantitative basis.

2. OBSERVATIONS; PRESENTATION AND DISCUSSION

Cross sections were made at about the middle of the 3rd internode of a representative stem, collected shortly after flowering, in 1961, from the material grown at each light intensity applied, viz., 12, 37, 75, and 100% of natural daylight. The light intensities were obtained by screens as described earlier (1), and pictured, e.g., in (21). Some data on weight and size of such stems, as obtained in 1959, are in (1).

The following presentation is mainly concerned with properties of the vascular bundles.

Large, if possible complete cross sections, made by hand, were used for low magnification, and photographed (plates I and II); these photographs served for counting the number of vascular bundles.

Not all bundles have been counted, notably not the very small ones towards the circumference. Those counted have been connected by a full-drawn line, for separate quarters of the sections, on plates I and II. If the quality of the sections allowed, all four quarters have been counted, if not, at least two. (In the case of 37% light two inner bundles have been schematically added. The 100% 'cross section' shown consists of two parts not exactly belonging together; counting has been restricted to one of these, containing slightly over 1/2 of the whole area).

Plates III, IV, V, and VI present photographs of still rather large parts of cross sections, suited for somewhat higher magnification. These pictures were used for measuring length and width of separate bundles with the aid of the image of an object micrometer photographed at the same magnification. For an estimation of the area of each bundle, \( \pi \times \frac{1}{2} l \times \frac{1}{2} b \) was taken. The shape of the bundle cross section is not exactly that of an ellipse, but it is a sufficient approximation for mutual comparison of average bundle surfaces developing under different conditions.

The same pictures were used for determining bundle distance. The bundles were redrawn on a piece of transparent paper laid upon the photographs. Certainly, the drawing of the bundle circumference is somewhat arbitrary; the small cells surrounding the bundle have mainly been taken to belong to it; it is only hoped that it has been done correspondingly in the various cases. The bundles thus represented were connected by lines to their neighbouring ones; the pictures thus obtained are shown in plates III–VI along with the photographs. The bundle distances as drawn were measured on the pictures with an ordinary ruler, divided in mm.

Plate VII and VIII present some sufficiently clear parts of sections at still higher magnification. They were used for measuring some more details, e.g. phloem surface and xylem surface per bundle, diameter of cortex, diameter of sclerenchyma layer. Phloem cross section is estimated by measuring 'length' and 'width' of its roundish surface. Xylem surface is more difficult to determine; to achieve this, the circumference of the main xylem vessels of 4 bundles...
were copied together on transparent paper, transferred on millimeter paper, and the number of square millimeters covered was counted. The picture obtained and used is shown on plate IX.

The principal data collected along the lines indicated above are presented together in figure 1, using different units in relation to light intensity. Curves

![Graph showing characteristics of vascular bundles in relation to stem cross section in Gladiolus, 3rd internode, field experiment 1961, at different light intensities (12, 37, 75, and 100% daylight).]

Fig. 1. Characteristics of vascular bundles in relation to stem cross section in Gladiolus, 3rd internode, field experiment 1961, at different light intensities (12, 37, 75, and 100% daylight).

show, e.g., the stem diameter, or rather its radius, $r$, the number of internodes, the number of the vascular bundles on the cross section, average length and width of the bundles, average stem surface, average bundle surface, total bundle surface, and average bundle distance, all obtained as explained in detail above. Vascular bundle data, as may be seen from plates III–VI, are derived from about 25 bundles for each light intensity.

In figure 2 the same items have been redrawn, recalculating the values after putting the 12% light value at '20' (in one case the 37% light value was adjusted).

The following conclusions may be drawn from figures 1 and 2. Obviously, the number of internodes, the number of vascular bundles, visible at the cross
section, the average length and width of the cross section of the bundles all show the same light intensity dependence as the stem diameter; they all 'run with $r$'. That indeed the slope of the various curves is very similar is especially evident from figure 2.

![Graph showing characteristics of vascular bundles in relation to stem cross section in Gladiolus.](image)

**Fig. 2.** Characteristics of vascular bundles in relation to stem cross section in *Gladiolus*, as fig. 1; relative values, most of them fixed at '20' for 12% light intensity.

Correspondingly, bundle surface, composed of length $\times$ width, may be expected to run with $r^2$ or stem cross section; this indeed clearly shows up in figures 1 and 2, and confirms the conclusion that the linear bundle dimensions indeed closely run with $r$.

Since also the number of bundles per cross section was found to run with $r$, it is obvious that the total bundle surface per cross section of the stem runs with $r^3$.

Since average bundle dimensions run with $r$ or stem diameter, without anything else bundle distance would also run with $r$ (see scheme, fig. 3). Because, however, also bundle number runs with $r$, one may expect that bundle distance actually will approximately run with $r/r$ or $r^0 = 1$, which is really shown in figs. 1 and 2.

Figure 4 presents, with broken lines, data on some additional items, mainly collected from the photographs plates VII, VIII. These items so far are only related to a small number of data per light intensity, and thus should be re-

*Meded. Landbouwhogeschool, Wageningen 63 (16), 1-8 (1963)*
garded as provisional. Full drawn lines repeat some data of figure 1, for
reference.

Remarkably, the diameter of the sklerenchyma layer shows little de­pen­
dence on light intensity; the diameter of the cortex layer, on the con­trary,

fairly runs with $r^2$. The latter relation is shown also in dry weight of stem, with
a somewhat steeper dependence in the low light intensity region. This is in
accordance with the findings on dry weight and length of stem, published
earlier (1). (Since stem length is little affected by light intensity, only some­what reduced at the lowest intensity, one should expect dry weight to run
roughly with cross section area, viz. $r^2$.)

![Diagram](image-url)

**Fig. 3.**
Scheme of stem cross section with some (4) vascular
bundles. Stem and bundle diameter in right figure
twice those in left figure.

![Graph](image-url)

**Fig. 4.** Characteristics of dimensions of some tissues in relation to other data in _Gladilus_;
material as in fig. 1.
Curiously, phloem surface, and even more so, xylem surface per bundle, appeared less affected by light intensity than total bundle surface. The data scatter rather much, but they suggest a relation to $r$ at most (see, e.g. the dotted line, averaging each 2 neighbouring points), and not to $r^2$ as one would be inclined to expect. However, these data need further extension and confirmation, the more so since they are at variance with the data on the average surface of the whole bundles, obtained from a larger number of measurements, which runs with $r^2$, as discussed above. The possibility exists that at low light intensities, lumens show a tendency to extend, as an etiolation effect. Moreover, the bundles chosen for these additional measurements (4 per light intensity) belong to the largest ones available in the section and may show relationships different from the average.

Rather concomittant with the constancy of average bundle distance, discussed above, the average diameter of parenchyma cells seems fairly independent of light intensity.

SUMMARY

Representative transverse sections of representative stems of *Gladiolus* plants, grown at different intensities of natural daylight (12, 37, 75, 100%, realized by metal gauze screens, see ref. 1) have been studied with regard to a quantitative comparison of some anatomical features.

The diameter of the stems, represented by the radius ($r$) is distinctly related to light intensity, with a certain slope; it increases about 2-fold in the mentioned range of light intensities.

The number of internodes, the number of vascular bundles seen on the cross section, the average length and width of the bundles all show the same relationship with light intensity as $r$; consequently, the average bundle surface runs with stem cross section, or $r^2$, total bundle surface with $r^3$, and average bundle distance with $r^0$, i.e., it remains more or less constant over the range of light intensity studied.

According to some preliminary additional data, the diameter of the peripheral sclerenchyma layer shows only little dependence on light intensity; cortex diameter appears related to $r^2$, whereas phloem and xylem surface per bundle seem to increase less than average total bundle size. These data require extension.

Thanks are due to Miss J. Bos and Mr. D. Stedelaar for experimental assistance.

REFERENCES

7. ———, ibid. 19, 538-546 (1932).

Meded. Landbouwhogeschool, Wageningen 63 (16), 1-8 (1963)
Sections of *Gliadiolus* stems (3rd internode), grown at different light intensities (12, 37, 75, and 100% daylight; field experiment, 1961), showing counting of vascular bundles.
Sections of *Gladiolus* stems, cf. Plates I and II, as used for measuring average surface and distance of vascular bundles. Bundle dimensions estimated in photographs with object micrometer scale, distances measured in drawings with ordinary ruler, and corrected with magnification factor.
PLATE V

PLATES V AND VI
Legend: see Plate III.
PLATES VII AND VIII

Sections of *Gladiolus* stems, as before; used for measuring some details, e.g. in 4 numbered bundles.
**PLATE IX**

Xylem surfaces in 4 selected vascular bundles at each light intensity, 3rd internode stem *Gladiolus*, field experiment 1961, at different light intensities (100, 75, 37, 12% daylight).