

ON THE INFLUENCE OF TRANSPIRATION ON THE DISTRIBUTION OF GROWTH IN THE AVENA MESOCOTYL

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SUMMARY

A study is made of the influence of transpiration on the distribution of growth in dark-grown *Avena* seedlings.

It appears that an increase in transpiration will cause a change in the distribution of growth in the mesocotyl in the same sense as the change that is caused by a small amount of red light *i.e.* the part of the mesocotyl that grows is restricted to the more apical part.

1. INTRODUCTION

To obtain straight seedlings of *Avena sativa* with relatively long coleoptiles and short mesocotyls, useful for research on tropisms, the seedlings usually are grown during the first 20 hours in red light. If cultivated in total darkness the mesocotyl will develop to a far greater length and the coleoptile will remain much shorter in the same period (3–4 days).

MER (1953) showed that cultivation in darkness but under conditions favoring transpiration also resulted in a reduction in the final length of the mesocotyl, although this reduction was far less than that obtained by an irradiation of about 20 hours with red light in the first stages of germination. It is remarkable that always a (substantial) reduction of mesocotyl growth is correlated with an increase in the growth of the coleoptile.

To explain this phenomenon we advanced a hypothesis in 1964, with as a central feature, the transport of auxin from the tip of the *Avena* coleoptile towards the growing zones. The influence of light and transpiration-enhancing environments were postulated to reduce the transport of auxin in basal direction thereby reducing growth in the mesocotyl and increasing that in the coleoptile. If a reduced transport of auxin causes the decrease in growth of the mesocotyl, growth should not only be less but should also be more restricted to apical parts of the seedling. In 1967 we demonstrated that irradiation with red and blue light indeed reduced the growing part of the mesocotyl.

The results of Mer, obtained with seedlings grown from the start of germination under different conditions are not comparable with the results of our experiments on the distribution of growth when influenced by light, because in our experiments the irradiation was given after the seedlings had been grown in darkness for $3\frac{1}{2}$ days and the experiments lasted but 20 hours.

We therefore carried out experiments on the influence of transpiration upon

the distribution of growth in *Avena* seedlings, using the methods of 1967 in our experiments on the influence of light.

We found that under our experimental conditions the part of the mesocotyl that grows is indeed reduced by an enhanced transpiration. To compare this result with the influence of light we also made some experiments with small amounts of red light.

2. METHODS

Avena seedlings (*Avena sativa* "Victory oats") were cultivated (method of BLAAUW & BLAAUW-JANSEN 1964) in total darkness and a relative air humidity approaching 100% at 23°C until they were 3½ days old.

All subsequent manipulations of the seedlings were done in total darkness in a room of high relative humidity and 23°C. The seedlings were cut from roots and seeds and placed in plates with receptacles (*fig. 1*) made of two strips of perspex glued to one another. The upper strip had been bored so as to form water reservoirs with a small hole to hold the excised seedlings (see also HUISINGA 1964). The seedlings fitted in the holes in such a way that air could replace the water taken up by the plants.

The amount of water that could evaporate directly from the reservoir was negligible. Marks, evenly spaced at 0.35 mm, were placed on the coleoptiles as described in HUISINGA 1967. One batch of plants was then placed in a light-tight wooden box in which the relative humidity was high. The other batch was

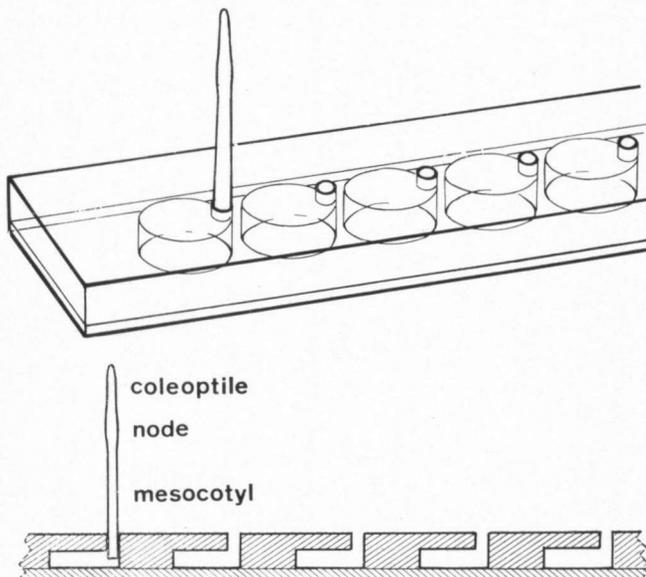


Fig. 1. Plates to hold the excised seedlings.

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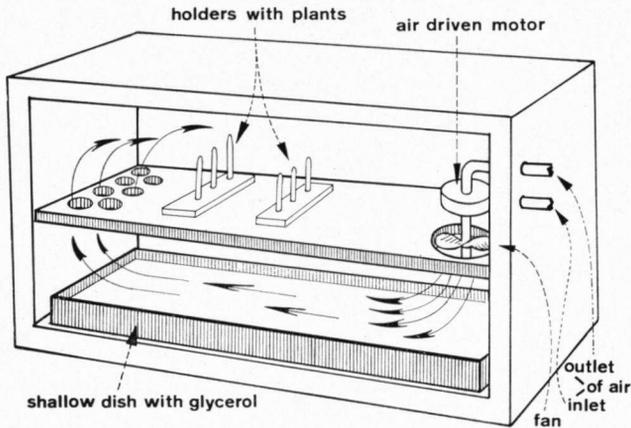


Fig. 2. Schematic drawing of the box used to realise relatively strong transpiration of seedlings. The light-tight door is omitted from the drawing. The air is blown by means of a fan over a large surface of glycerol. The arrows indicate the air flow (the air driven motor does not change the temperature in the box).

placed in a similar box that had been provided with means to maintain a lower relative humidity of the air and a gentle flow of the air (fig. 2).

The relative humidity in the dry box was estimated by means of a hair hygrometer at approx. 80%. The humidity in the moist box was approaching 100%. In order to estimate the combined effects of relative humidity and air flow on the transpiration of the seedlings some experiments were performed in which light could not be excluded. In these experiments the plates with the seedlings were weighed before and after a 24-hour stay in the two boxes. The differences in weight showed that a plant in the dry box evaporated more water than a plant in the moist box.

In another set of experiments excised seedlings were kept either in darkness or were irradiated with different amounts of red light at the start of the experiment. Subsequently they were all kept in the moist box.

The source of red light, with interference filters, is described in BLAAUW & BLAAUW-JANSEN (1964).

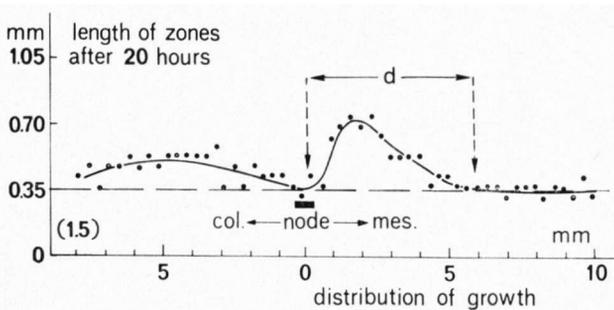


Fig. 3. Graph showing the distribution of growth in an *Avena* seedling and the distance d.

The methods of marking the plants and measuring the results were the same as in our experiments on different relative humidities.

After 20 hours the plants were photographed. The distances between the marks were determined and plotted in a graph (see HUISINGA 1967). Such a graph is shown in *fig. 3*.

The distance between the arrows and designated with the letter *d*, was measured. This distance is the length of the mesocotyl, at the start of the experiment, in which growth could be detected after 20 hours. From each group of at least 10 plants the mean of *d* and the standard error of the mean was calculated.

3. RESULTS

In a number of preliminary experiments in which desiccators and glycerol were used to maintain different relative humidities (the air was not circulated) we got results that, though variable, indicated a reduction in the lengths of the distances *d*. Summing up these experiments demonstrated a significant shortening of the distance *d* in dry conditions. This encouraged us to proceed to the more carefully designed experiments described above. With this improved method less variable results were obtained. They are presented in *table 1*.

Table 1. The length of the growing part (*d*) of the mesocotyl in moist non-moving air ("moist") and in moving air of lower relative humidity ("dry").

	d. (means of at least 10 plants and standard errors)	
	"dry"	"moist"
Exp. 1	35.3 ± 2.3	40.3 ± 3.4
Exp. 2	31.6 ± 1.8	35.4 ± 1.3
Exp. 3	30.2 ± 1.1	40.5 ± 1.3
Exp. 4	33.9 ± 1.1	43.2 ± 1.6

Table 1 shows that the "d" lengths obtained in the dry box are consistently smaller than those obtained in the moist box. In the coleoptiles the different treatments did not cause differences discernable with the methods used. The variability in the results of different experiments can be attributed to differences in the climate in the boxes in different experiments. This can be seen in the experiments made to get some data about the amount of water evaporated by the plants in the two boxes. The results of these experiments are shown in *table 2*. Just as in the experiments on growth-distribution the different experiments show rather great variations in the amount of water lost from the plates.

The results of the experiments with red light are shown in *fig. 4*. It appears that with increasing amounts of red light the distance decreases.

In amounts of about 1 to 10 erg. cm⁻² red light caused a reduction in the distance *d* of the same magnitude as the reduction found in the experiments on increased transpiration.

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Table 2. Amount of water evaporated in dry and moist conditions.

	Amount of water evaporated per plant. Mean of 12 plants.	
	“dry”	“moist”
Exp. 1	29	10
Exp. 2	38	4
	35	0
Exp. 3	36	8
	42	3

The treatment with red light of approx. 1 to 10 erg. cm⁻² did not cause measurable differences in the growth of the coleoptiles.

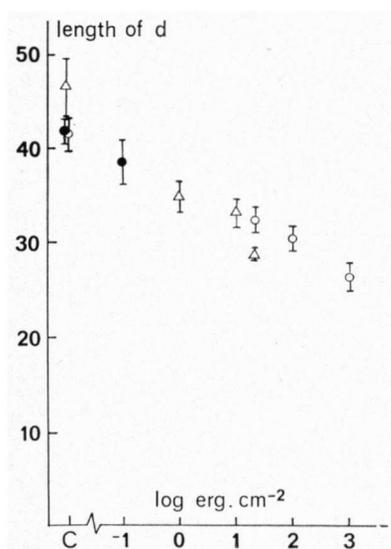


Fig. 4. The influence of red (660 nm) light on d. Means of at least 10 plants.

4. DISCUSSION

The results of the experiments presented show that transpiration affects the growth of the mesocotyl, i.e. the growing part of the mesocotyl is reduced by an increase in transpiration. Irradiation with red light of plants kept in very humid conditions caused the same effect in the mesocotyls as did the enhancement of transpiration. The effect of 1–10 erg. cm⁻² of red light was approximated quantitatively by the effect of the increased transpiration in the “dry” box. Neither treatment caused discernable effects in the coleoptiles.

In a working-hypothesis put forward in 1964 HUISINGA suggested that both light and increased transpiration might cause an increase in water transport in apical direction. The downward transport of auxin might thereby be counteracted.

The results presented in this communication do not contradict this. Since 1964, however, experiments have been published that are at variance with this hypothesis.

In 1968 BLAAUW-JANSEN & BLAAUW published an investigation on the influence the osmotic value of the water supply offered to excised *Avena* coleoptiles had on their geotropic curvature. They found that with increasing osmotic values the curvatures made by the coleoptiles were more restricted to the apical part of the coleoptiles. Growth distribution and distribution of the geotropic curvature have been shown to be correlated (HUISINGA 1967). KRELLE & LIBBERT (1968) reported that the reduction of auxin transport by morphactin will result in the fact that the geotropic curvature of *Avena* coleoptiles is restricted to the apical part of the organ.

In 1968 HUISINGA reported the results of experiments on the influence of red light on transport in apical direction of fluorescein in mesocotyls of excised *Avena* seedlings. He found that red light decreased the rate of transport through the living cells.

Both observations contradict the hypothesis that the supposed reduction of the auxin transport from the tip downward might be caused by the counteraction of an increased water transport in apical direction.

Since a number of observations, to wit:

1. reduction of the transport of auxin by red light in *Avena* coleoptile sections (NAQVI *c.s.* 1966) and in seedlings of *Helianthus* (LAM *c.s.* 1964, 1966),
2. an influence of red light on the distribution of the geotropic curvature and on the distribution of growth (BLAAUW 1963, HUISINGA 1964, 1967) in *Avena* seedlings,
3. influence of the osmotic value of the water given to excised coleoptiles of *Avena sativa* on the distribution of the geotropic curvature (BLAAUW-JANSEN & BLAAUW 1968),
4. influence of red light on the transport of fluorescein in apical direction in *Avena* mesocotyls (HUISINGA 1968),
5. decrease of transport of food reserves from the cotyledons to the hypocotyl and roots in seedlings of *Cucumis* (HALEVY *c.s.* 1964) by light,
6. influence of transpiration on growth-distribution,

seem to be interrelated, it seems probable that all these different factors act through the same, or a similar mechanism.

This mechanism might be the ability to transport substances, and the influence of the factors mentioned may be realised by way of changes in protoplasmic structures.

As to how red light causes changes in protoplasmic structures, resulting in reduced transport rates, no hypothesis can be offered. VIRGIN (1951) by means of centrifugal forces, showed an influence of (among other wavelengths) red light on protoplasmic properties in *Elodea* leaves.

Influences of other colours of light than red on protoplasmic properties have been reported by BOTTELIER (1934).

The action of increased transpiration and osmotic action of the water supply

is more accessible to speculations. Both factors reduce the amount of available water for the living cells. The osmotic values of the cells may therefore increase and these changes may alter the properties of the transport mechanism. Interesting in this connection is the observation of BLAAUW-JANSEN & BLAAUW (1966) that the suction force of *Avena* coleoptile cells will change by irradiation with red light 16 hours prior to analysis.

The experiments on the transport of fluorescein and of food reserves show that the influence of red light on transport is not restricted to the polar transport of auxin. This makes one wonder if also increase in transpiration will act in a nonspecific way. No experiments designed especially to investigate this point have, to the knowledge of the author, been made. However, in the work of CLOR *c.s.* (1962) on translocation of labelled sucrose and 2,4-D we find reproductions of autoradiograms that suggest that increase in transpiration will decrease the translocation of substances even in situations where translocation might be expected to be favoured by a stronger transpiration.

In conclusion, it is very likely that, apart from other actions, red light, and to a lesser extent also other wavelengths, will cause changes in the protoplasmic properties that are correlated with translocation mechanisms and that increased transpiration and high osmotic values of the water will probably act in the same way.

REFERENCES

- BLAAUW, O. H. (1963): Effects of red light on geotropism of *Avena* and their possible relations to phototropic phenomena. *Acta Bot. Neerl.* **12**: 424–432.
- & G. BLAAUW-JANSEN (1964): The influence of red light on the phototropism of *Avena* coleoptiles. *Acta Bot. Neerl.* **13**: 541–552.
- BLAAUW-JANSEN, G. & O. H. BLAAUW (1966): Effect of red light on irreversible and reversible expansion of *Avena* coleoptile sections. *Planta (Berl.)* **71**: 291–304.
- & — (1968): Geotropic curvature of *Avena* coleoptiles in solutions of various osmotic values. *Acta Bot. Neerl.* **17**: 273–280.
- BOTTÉLIER, H. P. (1934): Über den Einfluss äusserer Faktoren auf die Protoplasmaströmung in der *Avena*-Koleoptile. *Rec. trav. bot. néerl.* **31**: 474–582.
- CLOR, M. A., A. S. CRAFTS & A. YAMAGUCHI (1962): Effects of high humidity on translocation of foliar applied labelled compounds on plants. *Plant Physiol.* **37**: 609–617.
- HALEVY, A. H., S. P. MONSELISE & Z. PLAUT (1964): Effects of gibberellin on translocation and on dry matter and water content in several plant species. *Physiol. Plant.* **17**: 49–62.
- HUISINGA, B. (1964): Influence of light on growth, geotropism and guttation of *Avena* seedlings grown in total darkness. *Acta Bot. Neerl.* **13**: 445–487.
- (1967): Influence of irradiation on the distribution of growth in darkgrown *Avena* seedlings. *Acta Bot. Neerl.* **16**: 197–201.
- (1968): Influence of red light on transport of fluorescein in the mesocotyls of darkgrown *Avena* seedlings. *Acta Bot. Neerl.* **17**: 390–392.
- KRELLE, E. & E. LIBBERT (1968): Inhibition of the polar auxin transport by a morphactin. *Planta (Berl.)* **80**: 317–320.
- LAM, S. L. & A. C. LEOPOLD (1964): Effect of light on auxintransport. *Plant Physiol.* **39**: XXXVIII.
- & — (1966): Role of leaves in phototropism. *Plant Physiol.* **41**: 847–851.
- MER, C. L. (1953): An examination of the factors affecting variability in the growth of the mesocotyl and coleoptile of etiolated *Avena* seedlings. *Ann. Bot. N.S.* **17**: 569–578.

- NAQVI, S. M. & S. A. GORDON (1966): Auxintransport in *Zea mays* L. coleoptiles I: Influence of gravity on the transport of indoleacetic acid-2-¹⁴C. *Plant Physiol.* **41**: 1113-1118.
- VIRGIN, H. I. (1951): The effect of light on the protoplasmic viscosity. *Physiol. Plant.* **4**: 255-357.