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Mesocotyl Growth of Etiolated Seedlings of *Avena sativa* and *Zea mays* in Relation to Light and Diffusible Auxin

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Abstract. Diffusible auxin levels were measured in coleoptiles and mesocotyls of dark-grown seedlings of *Avena sativa* (cv. Spear) and *Zea mays* (cv. Golden Cross Bantam) using the *Avena* curvature bioassay. The coleoptile tip was confirmed as the major auxin source in etiolated seedlings. Auxin levels were found to decrease basipetally in sequent sections of the *Avena* coleoptile but not to decrease in apical sections of increasing length. An inhibitor capable of inducing positive curvatures of *Avena* test coleoptiles was discovered in diffusates from the mesocotyls of oat and corn seedlings. The amount of this inhibitor was correlated with the cessation of mesocotyl growth of oat seedlings grown in darkness, and with the inhibition of mesocotyl growth of corn seedlings exposed to red light.

The control of mesocotyl growth in seedlings of *Avena sativa* and *Zea mays* has been attributed to the auxin levels present in the coleoptile (Went, 1928). Many investigators (Went and Thimann 1937, Van Overbeek 1938, Briggs 1963a, Iino and Carr 1982ab) have demonstrated that the coleoptile tip is the greatest source of diffusible auxin in the etiolated seedling. When Van Overbeek (1936) observed a decrease in the amount of diffusible auxin from corn coleoptile tips after exposure to light, he suggested that the light-induced inhibition of mesocotyl growth was through the reduction in auxin production in the coleoptile tip. Recently, Iino (1982) found that red light decreased the level of both diffusible and free auxin in the coleoptile, but had no effect on the amount of IAA conjugates. Iino (1982) also observed that red light caused

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a reduction in the synthesis of IAA (indole-3-acetic acid) from tryptophan. These findings support the view that the regulation of mesocotyl growth is by auxin production in the coleoptile tip.

Other investigators, however, have produced experimental evidence that contradicts this view. Schneider (1941) observed that the growth of excised *Avena* mesocotyl segments was inhibited by red light and concluded that the inhibition was a direct effect on the mesocotyl rather than an effect on auxin levels in the coleoptile. Mer (1951) demonstrated that successive decapitations of the coleoptile had no appreciable effect on subsequent growth in the mesocotyl of dark-grown *Avena* seedlings and provided further evidence that mesocotyl growth was not regulated entirely by the levels of auxin production in the tip of the coleoptile. Because of these reports and the fact that the growth of the mesocotyl has been used as a bioassay for auxin activity (Nitsch and Nitsch 1956), the growth of the mesocotyl in relation to light and diffusible auxin was investigated.

Materials and Methods

Plant Materials

Oat seedlings (*Avena sativa* L. cv. Spear) were used for the measurement of growth and the collection of diffusates. After the removal of the lemma and palea, the grains were placed with the embryo facing upward on platforms made of aluminum wire mesh. The platforms were then placed in Petri dishes (9 cm diameter), and distilled water was added to the level of the platform. This procedure facilitated uniform germination and growth of the seedlings in complete darkness. The seedlings were grown for various periods of time at 24°C and 85% relative humidity. For the collection of diffusates two segments of the coleoptile or the mesocotyl were placed vertically on each agar block (2 × 2 × 2 mm, 1.25%) which had been equilibrated previously in 0.05 M phosphate buffer at pH 3.5 or pH 8.5. Diffusates were collected for 1 or 3 h.

Corn seedlings (*Zea mays* L. cv. Golden Cross Bantam) were grown on paper toweling in clear plastic boxes for 4 days in complete darkness at 24°C and 85% relative humidity. Seedlings exposed to light were given a 30-min exposure to red light (600–750 nm, 4 W m^{-2}) 12 h before the measurement of growth and the collection of diffusates. For the collection of diffusates a single segment of the coleoptile or the mesocotyl was placed vertically on an agar block (2 × 2 × 2 mm, 1.25%) which had been equilibrated previously in 0.05 M phosphate buffer, pH 3.5. Diffusates were collected for 1 h. The diffusible auxin contained in diffusates from seedlings of *Avena sativa* and *Zea mays* was determined by the *Avena* curvature bioassay.

Procedure for Auxin Bioassay

The standard procedure for the measurement of auxin using the *Avena* curvature bioassay was used. Oat grains (*Avena sativa* L. cv. Spear) were soaked

Table 1. Diffusible auxin from sequent segments of the *Avena* coleoptile.

Segment length	Auxin curvature	pg IAA/plant · h
Apical 3 mm	−16.6 ± 1.3°	44
Sequent 3 mm	−8.4 ± 0.4°	24
Sequent 3 mm	−6.4 ± 0.4°	21

Diffusates were collected from dark-grown *Avena* seedlings 4 days old. Two coleoptile segments were placed vertically on each agar block. Diffusion occurred for 3 h. Agar blocks were equilibrated in 0.05 M phosphate buffer at pH 3.5, prior to diffusion. Results are given as the mean of 10 determinations ± SE. Picograms of IAA in diffusates were calculated from the curvature response to standard solutions of IAA.

in tap water for 1 h. The grains were then germinated on moist paper toweling in darkness at 24°C and 85% relative humidity for approximately 38 h. Seedlings with roots 4 mm in length were irradiated with red light (600–750 nm, 4 W m^{-2}) for a period of 8 h, at which time uniform seedlings were planted in wooden trays containing a commercial mix of sphagnum and vermiculite known as Jiffy-mix. When the coleoptiles were 18–20 mm in length, approximately 42 h after planting, the apical 2 mm of the coleoptile including the primary leaf was removed. After 3 h in darkness, a nick 2 mm below the cut surface was made on one side with a razor blade, the coleoptile segment was removed, and the primary leaf pulled up 1 cm. Agar blocks containing diffusates or IAA were applied unilaterally to the coleoptiles. After 90 min in darkness the coleoptiles were removed. The degrees of curvature were determined from shadowgraphs of the coleoptiles. Picograms of IAA in the diffusates were calculated by relating the curvatures induced by the diffusates with curvatures induced by standard solutions of IAA.

Manipulations were conducted under dim green light provided by a 15-W GE cool white fluorescent tube covered with two layers of amber plastic, six layers of green plastic, and a single layer of green masking tape. The light intensity was 1.8 μW cm^{-2} (500–560 nm) at plant level in a controlled environment room kept at 24°C and 85% relative humidity. The manipulations required approximately 6 min each.

Results and Discussion

Since auxin production at the tip of the coleoptile has been implicated as the controlling factor regulating the growth of the mesocotyl in etiolated seedlings of *Avena sativa* and *Zea mays* (Went and Thimann 1937, Van Overbeek 1936, Iino and Carr 1982ab), the diffusible auxin in the coleoptile was examined in relation to mesocotyl growth in these plants.

When the levels of diffusible auxin from sequent segments of *Avena* coleoptiles were determined (Table 1), a definite decrease in auxin content was observed in subapical segments. Although isolated coleoptile segments of *Zea* have been found to increase their content of free (Iino and Carr 1982b) and diffusible (Van Overbeek 1941) IAA with time, this regeneration of IAA synthesis was not observed to be substantial until 4 h after decapitation. Thus the

Table 2. Diffusible auxin from apical segments of the *Avena* coleoptile.[a]

Apical segment length	Auxin curvature	pg IAA/ plant · h
2 mm	−13.7 ± 0.9°	35
3 mm	−15.1 ± 1.2°	40
5 mm	−15.2 ± 0.5°	40
10 mm	−13.7 ± 1.4°	35

[a] Otherwise as for Table 1.

basipetal decline in auxin levels shown in Table 1 can be attributed to the removal of the site of auxin synthesis found in the tip of the coleoptile. Measurements of diffusible auxin from subapical coleoptile segments of *Avena* during a period of 3 h probably represent the exportable auxin in the tissues at the time of excision. Auxin measurements from the apical segment, however, not only represent the initial auxin present in the tissue, but also include the amount of IAA synthesized by the physiologic tip and exported during the 3-h diffusion period. Continuation of auxin synthesis and export by isolated coleoptile tips has been shown to occur for several hours after decapitation (Iino and Carr 1982a, Iino 1982, Van Overbeek 1941).

The measurement of diffusible auxin from progressively longer apical coleoptile segments of *Avena* (Table 2) shows virtually no differences in the amount of diffusible auxin obtained. These findings support the conclusion that the tip is the principal source of auxin production in the *Avena* coleoptile. Shen-Miller (1973) has shown that the velocity of auxin transport is essentially the same throughout the length of the *Avena* coleoptile. Thus, under steady-state conditions of auxin production, the rate of auxin transport is constant in this tissue. The data of Table 2, therefore, dispute the existence of a basipetal gradient of diffusible auxin within the *Avena* coleoptile.

Briggs (1963a), using the *Avena* curvature bioassay, reported a decreasing gradient of diffusible auxin in apical corn coleoptile segments. Similarly, Iino and Carr (1982a) observed a reduction in the amount of auxin that diffused from an entire corn coleoptile as compared to that which diffused from the coleoptile tip alone. They attributed this reduction to decomposition of IAA during transport, as suggested by Goldsmith and Thimann (1961), although most of the experiments indicated no decomposition. Later, Pickard and Thimann (1964) concluded that there was no auxin destruction in the coleoptile. The destruction of IAA observed by Goldsmith and Thimann (1961) in some of their experiments may have been due to the exposure of their samples to light before counting the radioactivity. Such an exposure has been shown to result in a substantial loss of radioactivity (Iino et al. 1980). In this investigation, when the diffusible auxin from the entire *Zea* coleoptile and 3 mm of the mesocotyl was determined (Table 3), the amount found was the same as that found for the coleoptile tip. Iino and Carr (1982a) found diffusible auxin levels from primary leaves of *Zea* seedlings to be approximately one-sixth of that measured from the coleoptile tip. They concluded that mesocotyl elongation is regulated by auxin produced at the coleoptile tip, but that other parts of the seedling, particularly the primary leaves, are also sources of auxin. However,

Table 3. Measurements of diffusible auxin from *Zea mays* seedlings.

Corn segment (length)	Auxin curvature	pg IAA/ plant · h
Apical coleoptile (3 mm)	−22.3 ± 1.4°	391
Apical mesocotyl (3 mm)	−8.9 ± 0.9°	150
Entire coleoptile plus (3 mm) mesocotyl	−24.8 ± 1.4°	512

Diffusates were collected from dark-grown *Zea mays* seedlings 4 days old. Individual apical segments were placed vertically on each agar block and allowed to diffuse for 1 h. Otherwise as for Table 1.

their measurements of diffusible auxin from the primary leaves also included the coleoptile node as well as 1 mm of both the coleoptile and mesocotyl. Measurement of diffusible auxin from the primary leaf alone by the *Avena* curvature bioassay in this laboratory (data not shown) has failed to show the presence of diffusible auxin.

In other respects, the data reported in this investigation are similar to those reported by Iino and Carr (1982a). The values they obtained for diffusible auxin collected in water and measured by the fluorescence method were 1,030 pg IAA/plant · h for the coleoptile tip compared with 391 pg IAA/plant · h found in this investigation (Table 3) by diffusion into agar and measurement with *Avena* curvature. However, their measurement for the entire coleoptile and a portion of the mesocotyl was 615 pg IAA/plant · h which compares closely with the measurement of 512 pg IAA/plant · h found in this investigation (Table 3). Although Iino and Carr (1982a) obtained less diffusible auxin from the entire coleoptile than was obtained from the coleoptile tip. the measurement of diffusible auxin in this investigation (Table 3) gave as much auxin for the entire coleoptile plus 3 mm of the mesocotyl as for the coleoptile tip.

In early investigations of the growth of etiolated *Avena* seedlings (Avery and Burkholder 1936), the coleoptile was found to grow by a combination of cell division and cell elongation to a length of 1 cm, but additional growth of the coleoptile occurred almost entirely by cell elongation. The mesocotyl, however, increased in length by both cell division and cell elongation throughout its grand period of growth (Avery et al. 1937). The relationship of auxin to the growth of these two tissues was examined with separate lots of seedlings which were of different ages at the time of measurement of their length and the amount of diffusible auxin. During the first 4 days of growth of *Avena* seedlings in complete darkness (Table 4), the mesocotyl elongates more rapidly than the coleoptile. By day 5, the mesocotyl ceases growth while the coleoptile is in the middle of its grand period of elongation. The amount of diffusible auxin measured from the coleoptile increased from the second to the third day and then diminished after the fourth day of growth. These changes in diffusible auxin levels from coleoptiles of different ages as well as the measurements of the growth of the coleoptile and mesocotyl correspond well with those of Schneider (1941). The measurement of diffusible auxin from the mesocotyl of seedlings at 2 days was not possible because of the small size of the mesocotyl. A small amount of diffusible auxin was found in apical mesocotyl segments at 3 and 4 days (Table 4). The diffusates from mesocotyl tissues of seedlings at 5, 6, and 7 days induced curvatures toward the side of application in the *Avena*

Table 4. Measurement of growth and diffusible auxin from dark-grown *Avena* seedlings.

Seedling age (days)	Coleoptile		Mesocotyl	
	Length (mm)	Auxin curvatures (pg IAA/ plant · h)	Length (mm)	Auxin curvatures (pg IAA/ plant · h)
2	2.9 ± 0.2	−5.0 ± 0.4° (19)	1.5 ± 0.1	—
3	11.0 ± 0.3	−15.0 ± 0.4° (39)	21.6 ± 1.0	−1.5 ± 0.8° (7)
4	16.7 ± 0.9	−14.7 ± 1.1° (38)	55.1 ± 1.0	−5.0 ± 0.3° (16)
5	31.9 ± 1.7	−8.6 ± 1.0° (25)	75.0 ± 2.2	+3.6 ± 0.2°
6	44.8 ± 2.4	−6.2 ± 0.7° (17)	79.7 ± 1.6	+1.7 ± 0.6°
7	53.8 ± 4.2	−4.2 ± 1.9° (13)	75.6 ± 3.1	+2.8 ± 0.6°

Diffusates were collected by placing two apical 3-mm segments (coleoptile or mesocotyl) vertically on individual agar blocks. Otherwise as for Table 1.

curvature bioassay (positive curvature). Positive curvatures result from less growth on the side of the test coleoptiles to which agar blocks are applied and represent a unilateral inhibition of growth of the test coleoptiles. The development of positive curvatures induced by diffusates from the mesocotyl indicates the existence of an inhibitor of auxin-induced growth in the mesocotyl. The curvatures induced by all diffusates measured at pH 3.5 thus represent the net effect of auxin and the inhibitor. Smaller curvatures of the coleoptiles in the *Avena* curvature bioassay may represent either an actual decline in auxin levels or an increase in inhibitor levels. Auxin measurements are most effective at an acidic pH since the IAA molecules are undissociated (Ferguson 1971) and enter the tissues more readily (Smith and Jacobs 1969). It was observed in preliminary experiments (data not shown) that the diffusates from mesocotyl tissues collected in agar blocks buffered at pH 8.5 prior to diffusion induced a greater positive curvature response than similar diffusates buffered at pH 3.5. This finding indicates that the inhibitor is not acidic in nature and also facilitates the measurement of inhibitor levels in the presence of auxin since at this alkaline pH the IAA molecules are dissociated and do not enter the tissues of the test coleoptiles.

When agar blocks were equilibrated at pH 8.5 prior to diffusion, it was observed that all diffusates from the *Avena* mesocotyl induced positive curvatures in test coleoptiles (Fig. 1). Measurements of mesocotyl growth in complete darkness show a steady increase in length from the second to the fifth day followed by a cessation of growth. Levels of diffusible inhibitor increased from the third day to the fourth day of growth in darkness, 1 day prior to the cessation of mesocotyl growth, and then steadily decreased. These results indicate the existence of an inhibitor of auxin-induced growth in the *Avena* mesocotyl which is correlated with the cessation of mesocotyl growth.

The detection of an inhibitor in the mesocotyl of the *Avena* seedling indicated

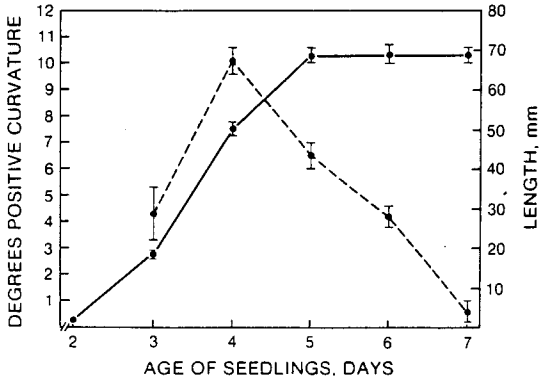

Fig. 1. Measurement of growth and diffusible inhibitor in the *Avena* mesocotyl. Diffusates were collected from dark-grown *Avena* seedlings of various ages. Two apical 3-mm mesocotyl segments were placed vertically on each agar block. Diffusion occurred for 3 h. Agar blocks were equilibrated in 0.05 M phosphate buffer (pH 8.5) prior to diffusion. Solid line: Length of the mesocotyl. Broken line: Positive *Avena* curvatures induced by diffusates from the mesocotyl segments. Results are plotted as the mean of ten determinations ± SE.

Table 5. Effect of red light on the growth of *Zea mays* seedlings in relation to diffusible auxin levels.

	Coleoptile		Mesocotyl	
Treatment	Length (mm)	Auxin curvature (pg IAA/ plant · h)	Length (mm)	Auxin curvature (pg IAA/ plant · h)
Dark	15.6 ± 0.7	−25.2 ± 2.3° (295)	56.2 ± 1.4	−7.3 ± 0.5° (133)
Red light	20.2 ± 1.1	−16.0 ± 1.0° (248)	37.1 ± 1.6	+7.2 ± 0.7°

Seedlings were exposed to 30 min of red light 12 h before measurement of growth and diffusible auxin. Single apical coleoptile or mesocotyl segments (3 mm) were placed vertically on each agar block. Diffusates were collected for 1 h. Otherwise as for Table 1.

that the inhibitory effect of red light on the growth of the corn mesocotyl (Weintraub and Price 1947, Dattaray and Mer 1964, Mandoli and Briggs 1981) may be due to an increased amount of inhibitor caused by the light. Therefore, the growth and diffusible auxin of the corn coleoptile and mesocotyl were determined under conditions of darkness or after exposure to red light. The data of Table 5 show that the red light caused an increase in growth of the coleoptile but decreased the amount of diffusible auxin from the tip, as has been reported by Briggs (1963b) and Iino (1982). The red-light treatment reduced the growth of the mesocotyl by 34% and increased the amount of the inhibitor in the diffusate from the mesocotyl segment such that a pronounced positive curvature was developed. Through the use of a modification of the *Avena* curvature bioassay designed to measure the neutral inhibitor of auxin effects, Muir and Zhu (1983) found that red light causes an increase in the inhibitor content of diffusates from light-grown sunflower plants and a decrease in the auxin content. Thus evidence exists for the control of mesocotyl growth by an inhibitor, in addition to the control by auxin as described by Iino and Carr (1982b).

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