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A COMPARISON OF AUXIN DESTRUCTION BY TISSUE EXTRACTS AND INTACT TISSUES OF THE FERN OSMUNDA CINNAMOMEA L.[1,2]

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Although much work has been done on the nature of enzyme systems which inactivate native auxins and synthetic indole acetic acid (IAA) (5, 6, 10, 11, 15), most of the experiments have been carried out with plant extracts, and many of them were done using non-physiological concentrations of IAA. There are few in vivo studies on the physiological function of such systems. Tang and Bonner (11) have suggested that in normally grown peas, the system found does not destroy auxin within the plant, while it does appear to be functional in etiolated seedlings. Von Abrams (14) found that growth differences between tall and dwarf etiolated pea seedlings could not be correlated with IAA-oxidase activity in tissue extracts of the two varieties. Other experiments involving tissue extracts (4) or auxin diffusion across a cut surface into agar blocks (2, 13) do not convincingly separate the phenomenon of enzymatic auxin destruction by damaged tissue from destruction by living and intact cells. The last three papers (2, 4, 13) show an inverse correlation between growth of tissues and activity of the destruction system, either in tissue extracts or at cut surfaces. Only Van Overbeek (13) attempted to demonstrate a direct causal relationship between the two phenomena. An earlier paper from this laboratory (9) has reported auxin destruction at cut surfaces in *Osmunda cinnamomea* L. In presenting a method for overcoming this inactivation during auxin diffusion studies, the authors considered it to be a cut surface phenomenon only. A second paper (1) has studied the distribution of this system as found in crushed tissue extracts of various parts of the plant, and has dealt with some of its characteristics. The present paper will consider further the physiological significance of this auxin destruction system, and will present evidence which suggests that auxin is not destroyed in the non-growing tissue of this plant, but disappears only in parts which are growing. The somewhat anomalous behavior of the rhizome tissue will be discussed.

MATERIALS AND METHODS

Plants of the fern *Osmunda cinnamomea* were either collected in the spring and used directly, or collected in the fall, kept in a cold room at 4° C for an appropriate period of dormancy, and then grown in a greenhouse the following spring. All of the experiments described below were done on fronds in their fourth and final year of development (1). In

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production and transport experiments, auxin was permitted to diffuse from tissues into 1.5 % agar blocks (8 × 11 × 1.5 mm). These experiments were done at approximately 23° C in a closed chamber lined with wet paper towels to keep humidity high and minimize wilting. Except where otherwise noted, the cut surfaces of tissues were treated with 0.005 M KCN to prevent cut surface auxin inactivation, following the technique described by Steeves et al (9). In experiments involving transport of externally applied IAA, an emulsion of 0.5 % IAA in 50 % each of lanolin and water was used. Ether extractions were made from tissue slices roughly 1 mm thick, at 0° C, for 4 hours, with freshly distilled, peroxide-free diethyl ether. The extracts were then evaporated to dryness over a steam bath, and made up to appropriate volume with agar to a final concentration of 1.5 %. For all auxin bioassays, agar was finally cut into blocks 2.67 × 2.75 × 1.5 mm and assayed by means of the standard Avena test (16). Crude preparations of the IAA-inactivating enzyme system were made as described in a previous paper (1).

NORMAL DISTRIBUTION OF AUXIN IN THE GROWING FROND: In a series of experiments to be published elsewhere, it was shown that all of the auxin measurable by the technique used was produced by the pinnae of developing fronds of Osmunda, and not by the rachis (8). Thus, by removing all of the pinnae from a developing frond, or removing the portion of the rachis bearing the pinnae, it was possible to obtain parts of the rachis in which there was no detectable auxin present at the start of a given experiment. It was also shown that transport either of naturally occurring auxin or of IAA applied in a lanolin-water emulsion to the epidermis was strictly polar, through sections of elongating or mature rachis tissue.

Experiments were then undertaken to determine the course of disappearance of endogenous auxin from the frond rachises during development. Developing fronds were selected on which no pinnae were as yet set off below the crozier, and on which there were

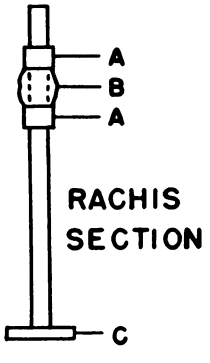

FIG. 1. Method for applying IAA-lanolin-water emulsion to a given area of epidermis of rachis section of Osmunda frond. A. 5 mm sections of rubber tubing. B. Emulsion filling gap between tubes. C. Agar block at basal end of rachis section. Agar block was 2, 4 or 6 cm from center of lanolin.

TABLE I

DISAPPEARANCE OF ENDOGENOUS AUXIN PRODUCED BY PINNAE FROM RACHIS TO RHIZOME

POINT OF RACHIS AT WHICH AUXIN WAS COLLECTED *	EXPERIMENT 1	2	3	4	5
180° apical from base of crozier	...	...	...	...	15.3
Base of crozier	19.3	21.9	19.9	15.6	16.5
Base of zone of elongation	8.9	8.7	10.0	3.9	...
Base of zone of maturation (ground level)	11.5	6.7	13.4	3.1	7.7
⅔ distance from ground level to attachment to rhizome	10.3	9.0	...	...	...

Auxin collected by diffusion. Data expressed as degrees of Avena curvature. Cyanide technique used throughout. Each diffusion from two fronds tied together. Pairs of fronds in each experiment roughly comparable.

* See (1), fig. 1: A, B, C, D and E.

no pinnae on the outer 180° of the crozier (see (1), fig 1). The fronds were cut off just above the first 180° of crozier; or just below the crozier, but above the zone of elongation; or just at the base of the zone of elongation; or at ground level, just above the white leaf base; or at a level 2/3 of the distance between ground level and the attachment of the leaf to the rhizome, a point normally below the ground. The positions of these cuts are shown by the letters A, B, C, D and E, in (1), figure 1. The level dividing the zone of elongation from the zone of maturation was determined fairly accurately by marking the developing rachis at regular intervals with India ink, and recording the progress of rachis elongation through the relative separation of the marks. It was found that the rachis tissue within the crozier elongated only slightly, though enough to bring about uncoiling, while the major elongation was confined to a region just below the uncoiling pinnae-bearing crozier. Auxin was collected from the tissue distal to the cut in each case, and separate fronds, tied in pairs, were used for each individual diffusion. The results of five such experiments are shown in table I. Comparable results were obtained in numerous other experiments. The only region in which a significant disappearance of auxin takes place is the zone of elongation. The amount of auxin travelling through the rachis below the auxin source, the pinnae, but still within the crozier and above the zone of elongation, remained constant within the limits of variability of the material. There is a significant drop in the amount of diffusible auxin from the top to the bottom of the zone of elongation, but there appears to be no drop at all through the entire remainder of the rachis and leaf base. Thus, the amount of endogenous auxin obtainable by diffusion, using the cyanide technique, does not drop in any tissue of the rachis that is not actually growing.

THE DISTANCE WHICH CYANIDE TRAVELS ABOVE

THE POINT OF APPLICATION IN CONCENTRATIONS EFFECTIVE IN PREVENTING CUT SURFACE AUXIN INACTIVATION: In view of the fact that cyanide is such a powerful physiological agent, even at the low concentrations used in these experiments, it was considered desirable to determine just how far above the original cut surface, to which it was applied, cyanide was still effective in preventing cut surface auxin inactivation. Eight fronds, almost fully uncoiled, but still producing considerable auxin, were cut off roughly 10 cm below the zone of elongation, and diffusion experiments were done in the following manner: the fronds were matched into comparable pairs, tied together, a fresh cut made at the basal end, and 0.01 ml of 0.005 M KCN applied to the cut surfaces of each pair. Agar blocks were then placed on the cuts and left for 2 hours. At the end of this time the blocks were removed. On the first pair of fronds, 0.01 ml of water was applied to the original cut surface, and a second block applied for 2 hours. On the second pair, the procedure was repeated, after a new cut had been made 1.5 mm above the original, before applying the water. On the third and fourth pairs, the procedure was repeated, but the fresh cut was made 5 and 10 mm respectively above the original. All eight blocks were then assayed for auxin, and the results are shown in table II. At 1.5 mm above the original cut surface, to which KCN was applied initially, inhibition of cut surface auxin inactivation was still complete, and the second block contained as much auxin as the first; 5 mm above the original cut, the cyanide was less effective, as shown by the lower yield of auxin obtained from the second block; 10 mm above the original cut, there was not enough cyanide to affect the destruction system at all, so that almost no auxin was obtained from the second block. In the second experiment shown in table II, the second cuts were made 1 and 2 cm respectively above the original, previous to the second diffusion, and it was evident that cyanide was ineffective at those distances since the blocks from the second diffusions contained no auxin at all. Thus,

TABLE II

EFFECTIVENESS OF KCN IN PREVENTING CUT SURFACE INACTIVATION OF ENDOGENOUS AUXIN AT DIFFERENT DISTANCES ABOVE ORIGINAL CUT SURFACE TO WHICH 0.01 ML OF 0.005 M KCN WAS APPLIED. DATA FROM TWO EXPERIMENTS. 0.01 ML H_2O ADDED TO CUT SURFACE FOR SECOND DIFFUSION

FROND PAIR	DISTANCE SECOND CUT ABOVE FIRST	IAA COLLECTED 1ST 2-HR DIFFUSION	IAA COLLECTED 2ND 2-HR DIFFUSION
	mm	*Degrees Avena curvature*	*Degrees Avena curvature*
1	0	11.9	13.2
2	1.5	13.0	13.4
3	5	22.6	16.3
4	10	10.4	2.4
1	0	11.8	11.2
2	10	11.7	0
3	20	12.2	0

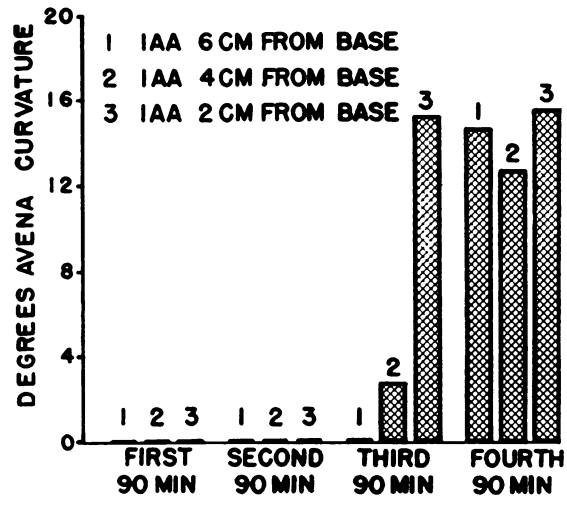

FIG. 2. IAA collected during four consecutive 90-min periods from the bases of sections of the zones of maturation of 3 leaves of *Osmunda cinnamomea*, to which IAA was applied in a lanolin-water emulsion 2, 4 and 6 cm respectively above the bases. Details in text.

under these conditions, cyanide does not move up into the rachis more than 1 cm in 2 hours, at least in concentrations strong enough to inhibit cut surface auxin inactivation.

TRANSPORT OF EXTERNALLY APPLIED AUXIN BY SECTIONS OF MATURE RACHIS: The polarity of auxin transport by the rachis made it possible to determine whether the inactivation is in intact tissue or only at cut surfaces, in the following way: the croziers and laminar tissue were removed from three comparable young fronds, removing the source of endogenous auxin. After 23 hours, or enough time to allow the endogenous auxin to disappear from the remaining rachises, the latter were cut off at ground level, two sections of rubber tubing 5 mm long were slipped over the rachis and placed 1 cm apart, with the center of the 1 cm gap respectively 2, 4 and 6 cm above the basal cut. The gap was then filled with a 0.5 % auxin lanolin-water emulsion, described above. After the application of KCN to the basal end, agar blocks were placed on the cut surface for auxin diffusion, and were replaced every 90 minutes over a period of 6 hours. A diagram of the experiment is shown in figure 1. The results of the assays of the agar blocks for auxin are shown in figure 2.

It was reasoned that since no auxin was produced in the rachis, and none appeared from the basal end during the first three hours, all auxin collected must have been IAA which had diffused into the transport system from the lanolin-water emulsion. Therefore, in the longest section, to which IAA had been applied 6 cm from the basal end, it should have taken it longer to reach the basal end than that applied 4 or 2 cm from the cut, and this is seen to be the case. Furthermore, since the surface area covered by the emulsion was constant in each case, the rate of auxin

diffusing into the tissue at the point of application should also be constant. Therefore, as soon as IAA was diffusing from the basal cut at a constant rate, the amount obtainable in any given length of time, here 90 minutes, should provide a measure of any endogenous destruction. If auxin is being destroyed by the living tissue, the further away from the point of application the auxin is collected, the lower should be the final steady rate of auxin diffusion from the cut surface. If, on the other hand, the tissue is not destroying auxin endogenously, the final steady rate of diffusion should be the same, regardless of the distance from the point of application to the point of collection. As shown in figure 2, the amount of auxin obtained during the fourth 90 minute period is approximately the same, regardless of the length of the transport path, and there is no indication of any endogenous destruction. These results do not preclude the existence of a small region at the base of each section, under the influence of cyanide in these experiments, which normally inactivates auxin within the tissue, but experiments shown below indicate that the destruction system is actually most active in extracts of the upper tissues, the crozier and the zone of elongation. Data from ether extractions, also shown below, rule out such a possibility as well.

A Comparison of Auxin Disappearance in Living Tissue and Auxin Inactivation by Extracts of the Identical Tissue: Since the auxin-destroying ability of leaf extracts varied widely (1), a comparison of in vivo auxin disappearance in rachis tissues and in vitro auxin inactivation by extracts of the identical tissues was made. Six young fronds 29 to 36 cm long, and with no pinnae set off below the croziers (see (1), fig 1) were matched into comparable pairs, and diffusion experiments were done as follows: auxin was collected just below the croziers in one case, just below the zone of elongation in the second, and at ground level, below the zone of maturation in the third (see (1), fig 1: B, C, D). The portions of the fronds below the cuts were left on the plants until later to prevent their wilting. After the diffusions were completed, all 6 croziers were combined and extracted in 0.067 M phosphate buffer, pH 6.1, as were the 6 pieces representing the zones of elongation and the 6 of the zones of maturation. The extracts were centrifuged at 2500 × g for 10 minutes and the supernatants were decanted, made up to volume with buffer, and stored overnight in the cold room at 4° C. The following morning, the extracts were incubated at 23° C with IAA at a final concentration of 125 μgm/ml with tubes for each extract in which IAA was added 5 and 0 hours before the tubes were immersed in boiling water for 5 minutes. The reaction mixtures were then made up into agar blocks and assayed for auxin, together with the agar blocks from the diffusions of the previous day. The data are shown in table III. The results of the diffusions agree with those shown earlier (table I); only in those tissues which are actively growing is there a significant disappearance of diffusible auxin. However, all three extracts destroyed added IAA, although the zone of maturation showed less activity, in vitro, than either the zone of elongation or the crozier. All cut surfaces which had been treated with cyanide were removed by severing and discarding the terminal centimeter (see table II). The data in table III indicate that the same tissue that transports endogenous auxin in vivo with no loss measurable by the Avena test, in this case the zone of maturation, has the ability to destroy added IAA in vitro, upon extraction.

Table III

Comparison of in Vivo Disappearance of Endogenous Auxin in Parts of Developing Fronds of Osmunda cinnamomea with in Vitro Destruction of Added IAA by Water Extracts of the Identical Tissues. Results of Diffusions Expressed as Degrees Avena Curvature (upper table). Residual Auxin in Reaction Mixtures Calculated from Avena Curvatures (lower table)

Level of Diffusion	Auxin yield, 3-hr diffusion *
Below crozier, above zone of elongation	19.9
Just below zone of elongation	10.0
Below zone of maturation above leaf base	13.4

Region of leaf extracted	Auxin added 0 hr	Residual auxin after 5 hr
	μgm/l	μgm/l
Crozier and pinnae	125	77
Zone of elongation	125	94
Zone of maturation	125	100

* Maximum curvature for the day 31.0°.

Auxin Content of Different Parts of Leaves and Rhizome, as Measured by Ether Extraction: Since measurement of auxin diffusing into or out of a given piece of tissue does not necessarily give an indication of the amount of auxin contained within the tissue, and therefore is not an infallible method for demonstrating the presence or absence of auxin destruction in vivo, it was necessary to make ether extractions of various parts of the leaves and rhizome, to determine the content of extractable growth substance in each. Furthermore, all diffusion experiments were done with cyanide, and it was important to know that the auxin obtained in this manner was really the amount that could be accounted for by diffusion techniques under normal circumstances, that is, that the cyanide was not poisoning a very mild endogenous destruction system, well above the cut surface to which it was applied. Plants with fronds 58 to 61 cm high were selected. At this time 3 or 4 pairs of pinnae were set off below the croziers of the fronds extracted. The croziers with pinnae attached, 10 cm sections of the zones of elongation, taken below the lowest pair of pinnae, 10 cm sections from the upper and lower portions of the zones of maturation, the white leaf bases, 5.5 to 7.5 cm long, and 1.5-cm sections of the rhizome, one directly below the point

of attachment of the current year's leaves, and a second 1.5 cm below the first, were all separately extracted with ether, as described above. All extracts were made up to a final volume of 5 gm fresh weight of tissue per ml 1.5 % agar, and assayed by the Avena test. In order to be sure that the auxin extracted was indeed just diffusible auxin, extracts were also made, in another experiment, from the lower zone of maturation and a 3-cm section of the rhizome, taken from just below the attachment of the current year's leaves, from plants from which the croziers and all laminar tissue had been removed 48 hours previously. The assays of these extracts were compared with assays of similar extracts from a comparable plant which was left intact until extraction. In both of these experiments, plants were selected on which the leaves were in a stage of maximum growth and high auxin production. The data from these bioassays are shown in table IV.

Since it was known that no auxin could be obtained by diffusion from rachises from which all laminar tissue had been removed 24 hours previously, it was determined that the auxin extracted was indeed diffusible auxin, since it also disappeared not only from the rachis, but from the rhizome as well, during the 48 hours following decapitation. The content of auxin in normal fronds was lowest in the crozier, which included the pinnae in which the auxin is produced. This auxin is funnelled into the rachis, in which the content of auxin per gram of tissue became higher. Down the entire length of the rachis, through the leaf base, there was no loss of extractable auxin, and in the rhizome, in which auxin from several leaves —in these cases, six—was accumulating, the content of auxin per gram of tissue was even higher.

From a study of diffusion data, one might expect to find the level of auxin per gram of tissue to be considerably higher in the zone of elongation than in the zone of maturation, since the amount of auxin entering the former in a given length of time is greater than that entering the latter. However, as shown in table IV, this is not the case. This apparent inconsistency may be partially explained as follows: the average density of the zone of elongation, determined by measuring both fresh weight and volume of given sections, is roughly two times that of any portion of the zone of maturation. Thus, in order to obtain equal weights of tissue from these two regions, one must have roughly twice the volume of tissue from the zone of maturation as from the zone of elongation. Therefore, if one were to extract equal volumes of these two tissues instead of equal weights, one would find approximately twice as much auxin per unit volume in the zone of elongation as in the zone of maturation. Since the density of the zone of maturation does not change down to the leaf base, the figures given in table IV for regions of rachis below the zone of elongation are valid on both a weight and volume basis. As the reasons for density changes in growing tissue are highly complex, and data on rates of auxin transport are not yet available for this material, the seeming inconsistency mentioned above will not be considered further in this paper.

The data obtained from these extractions show no decrease in the amount of extractable auxin below the zone of elongation, and furthermore, an accumulation of auxin in the rhizome. This evidence, then, supports the conclusions obtained from diffusion data, that at least in the leaves, there is no disappearance of auxin, except in growing tissue.

TABLE IV

AUXIN EXTRACTED WITH ETHER FROM VARIOUS PORTIONS OF DEVELOPING FRONDS AND RHIZOMES OF OSMUNDA CINNAMOMEA. RESULTS EXPRESSED AS PLANT UNITS * PER GM FRESH WT OF TISSUE

TISSUE EXTRACTED	PLANT UNITS PER GM **
Crozier plus pinnae	37.6
Zone of elongation	54.0
Upper zone of maturation	53.5
Lower zone of maturation	54.0
Leaf base	56.3
Rhizome directly below attachment of leaves	68.3
Rhizome 1.5 to 3 cm below attachment of leaves	82.0
Lower zone of maturation, leaves with all laminar tissue removed 48 hours before extraction	4.6
Rhizome from plant above, with all laminar tissue removed 48 hours before extraction	3.2
Lower zone of maturation, normal plant	53.8
Rhizome, normal plant	118.0

* Degrees curvature per Avena plant per gm tissue extracted.

** All values given were below maximum curvature for day.

DISCUSSION

The enzymatic auxin-inactivating system obtained in water extracts of Osmunda leaves was described in a previous paper (1). In view of the possible morphogenetic implications in the activity and distribution of such a system, it becomes important to determine whether or not the system is active in vivo. Furthermore, it must be possible to construct a reasonable hypothesis about the system as controlling growth. Also, in vivo experiments on the activity of the system must correlate with in vitro experiments, if one is to use conclusions from the latter to suggest interpretations for the former. If the system is a growth-limiting factor, one would anticipate finding its activity to be highest in such tissues, in Osmunda, as the zone of maturation, leaf base, and rhizomes below the current year's leaves. These regions have completed their growth, and are no longer responsive to added or endogenous auxin. Tissues such as those of the rachis above the zone of elongation, which have not yet become responsive to added or endogenous auxin, might also show high activity. Rapidly grow-

ing tissues such as the zone of elongation might be expected to show lower activity.

However, none of the data obtained either from in vitro or in vivo experiments support the hypotheses listed above. Auxin destruction activity in leaf extracts is indeed high in the crozier, above the zone of elongation, but there is no disappearance of auxin within the rachis in vivo, in this region (see table I). This activity remains high in extracts of the zone of elongation, but rather than becoming higher in the zone of maturation, it is either considerably reduced, or completely absent. These results appear to be just contrary to those of Galston and Dalberg (4), which showed that extracts of the youngest tissues of etiolated pea seedling epicotyls contained the lowest IAA-oxidase activity, while extracts of progressively older tissues showed increasing activity. In Osmunda, however, one cannot establish a causal relationship between either initiation or cessation of elongation of the rachis and in vitro auxin inactivation from the data presented above.

Studies on the relative activity of the system in vitro, through the growing season (1), present an equally uncertain picture. If auxin inactivation is a growth-limiting factor of importance in rachis tissue either before or after elongation (or both), one might expect the activity of the system to be high in extracts of dormant fronds, or fronds just breaking dormancy, falling to a lower level as the fronds enter their period of most active growth, and rising again as growth tapers off and the fronds become mature. Again, in vitro experiments do not support this premise; activity of the system is low while fronds are dormant, rises early to a high peak as the fronds begin to elongate, and appears to fall off again as the fronds enter their most active growth stage (although this drop could be accounted for by a localization of enzyme activity in the more apical portions in extracts of whole leaves), remaining low as the fronds mature (1). A morphogenetic interpretation of these data is equally difficult to make. The situation must be far more complex than that hypothesized above.

Diffusion data shown also make it difficult to accept the hypothesis of enzymatic auxin inactivation as a morphogenetic factor in this material. As Steeves et al (9) have pointed out, although there are dangers involved in basing any broad interpretations upon auxin yields obtained by diffusion using as powerful a physiological agent as cyanide, it is impossible to obtain consistent yields of auxin by diffusion from any part of the plant when cyanide is not used. Auxin in fairly large quantities clearly passes from the crozier into the zone of elongation, and from there, through the lower tissues into the rhizome. Ether extractions show a high level of auxin in the rhizomes of normal plants with developing leaves, and that this auxin comes from the fronds is demonstrated by the fact that it disappears both from the lower rachis and from the rhizome when laminar tissues are removed 48 hours prior to extraction. Furthermore, it has also been possible to show that auxin can be obtained from the rhizome by diffusion, if this is done within approximately a millimeter below the attachment of the current year's leaves, on certain occasions. The pattern of auxin distribution in fronds of this fern, elucidated by the use of the cyanide technique, provides a reasonable picture which is unobtainable without the use of cyanide. A similar situation was found in shoots of Cercidophyllum, which also gave negligible and inconsistent yields of auxin without cyanide (12).

If one considers that KCN, applied to cut surfaces, poisons endogenous auxin destruction, one must then consider that it poisons it selectively, since neither auxin production by the pinnae, auxin utilization by the zone of elongation, auxin transport by any part of the leaf, nor polarity of auxin transport by the rachis appear to be affected by this treatment. Moreover, diffusion studies on two other ferns, *Adiantum pedatum* L. (maidenhair fern) (9) and *Pteretis pensylvanica* (Willd.) Fern. (ostrich fern), the latter having a growth habit closely comparable to that of *Osmunda cinnamomea*, have indicated that auxin yields from these species are unaffected by the cyanide treatment. It is concluded, therefore, that these ferns lack the inactivation system described for Osmunda, and that, in these plants, no other systems influencing auxin production, transport, and utilization are affected by cyanide, in the concentrations used.

Several authors (2, 4, 13) have suggested morphogenetic interpretations based on the distribution and activity of an auxin destruction system in their material. Other authors (5, 14, 15) have not attempted such interpretations. Their data give a picture of the distribution of the system which, like that described in the present paper, is difficult to reconcile with the concept of auxin destruction as a morphogenetic factor. (We must limit auxin destruction to auxin disappearance without accompanying growth.) Recently Platt (7) has concluded that an auxin destruction system found in extracts of normal and crown gall tissues of *Vitis vinifera* L., grown in sterile culture, is in all probability a cut surface and crushed tissue phenomenon only. Furthermore, although Tang and Bonner (11) have shown IAA-oxidase activity both in vivo and in vitro by epicotyls of etiolated pea seedlings, they were able to show only slight activity by an acetone precipitate of an extract of normal green epicotyls, and none at all either in vitro, by crude extracts, or in vivo, by green epicotyls. Galston and Baker (3) were able to show IAA-oxidase activity by extracts of green epicotyls, but only after acetone precipitation and double washing with acetone to remove a naturally occurring inhibitor (10). Thus, evidence from the literature does not give a convincing argument for a physiological IAA-oxidase system acting in vivo.

Evidence presented above shows a lack of correlation between auxin destruction in vitro and auxin disappearance in vivo, in Osmunda leaves. Tissues which transport auxin without destroying it, particu-

larly the crozier and zone of maturation, will inactivate added IAA in crude water extracts. Nevertheless, neither the diffusion data, particularly that obtained by using the cyanide technique, nor the extraction data, by themselves, are unequivocal. But ether extractions of rhizome tissue show that when the only source of endogenous auxin, the pinnae, are removed, extractable auxin disappears completely from the rhizome. Yet, a water extract of rhizome tissue consistently fails to inactivate added IAA. In this case, the situation is reversed from that in the zone of maturation, and auxin disappears in the living tissue, a water extract of which will not destroy it. Transport of auxin to lower parts of the rhizome cannot explain its disappearance in this tissue, for other experiments indicate that little or no auxin is actively transported by the rhizome more than a millimeter below the current year's leaves. The eventual fate of this auxin is unknown.

Although no one type of experiment by itself is absolutely conclusive, the total evidence presented and discussed above strongly suggests that enzymatic auxin destruction in vivo can play at most a negligible role in control of growth in the leaves of *Osmunda cinnamomea*, and is in all probability an artifact resulting from cutting or otherwise damaging tissues. Therefore, investigations of a different nature must be carried out to determine just what the controlling factors are.

Summary

In the fern *Osmunda cinnamomea* L., endogenous auxin is produced by the pinnae only, and both endogenous auxin and IAA applied in a lanolin-water emulsion move in a strictly polar fashion. Auxin diffusion data show that the only disappearance of endogenous auxin in the rachis is in the zone of active elongation. KCN, applied to cut surfaces throughout, to prevent cut surface auxin inactivation during diffusions, is effective less than 1 cm above the point of application in preventing inactivation, at least by damaged tissues.

The final rate of diffusion of IAA, applied in a lanolin-water emulsion to the epidermis, from sections of the zone of maturation is constant for sections of comparable age and diameter, and independent of the length of the transport path. Since as much auxin comes through long sections as short, it is suggested that there is no endogenous destruction within this tissue.

A comparison of in vivo auxin disappearance and in vitro auxin inactivation by the rachis shows that the crozier and zone of maturation will transport auxin without destroying it, while water extracts from the identical tissue, particularly the crozier, will inactivate IAA. The only auxin disappearing in vivo disappears in growing tissues. The concentration of ether extractable auxin, determined on a weight basis, increases from the crozier, bearing the pinnae, to the zone of elongation and tissues below. It does not diminish down the entire length of the rachis, but accumulates in the rhizome. There is no evidence for inactivation within the rachis, from these data.

Water extracts of rhizome tissue fail to destroy added IAA, but a disappearance of ether-extractable auxin is noted in this tissue following the removal of the auxin-producing fronds. The eventual fate of this auxin is unknown, but it is suggested that neither active transport nor the enzyme system under consideration are responsible for its disappearance.

The enzyme system is considered to be active only at cut surfaces and in tissue extracts, and to be of little importance in the normal growth physiology of this fern.

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EFFECTS OF OXYGEN AND CARBON DIOXIDE LEVELS UPON ABSORPTION OF POTASSIUM BY PLANTS[1,2]

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Soil aeration has been recognized for years as an important factor in plant competition and crop production. Earlier work on the problem has been reviewed by Cannon and Free (6), Clements (8), Leather (18), and Parker (21), and the more recent literature by several writers (9, 10, 15, 20, 23, 27, 30). Most of the research on aeration suggests that plant roots are surprisingly tolerant of reduced O_2 and high CO_2 pressures. Short time experiments, however, might fail to show small differences in root growth or absorption rates which would be accumulative with time.

Interest in the problems of soil aeration has been increased in Iowa by the poor crop yields obtained in some areas with cultural programs which omit plowing during seedbed preparation (5, 17). Corn has shown serious potassium, and possibly other, deficiencies with listing and subsurface tillage on heavier glacial till soils (fig 1). Hoagland and Broyer (13) and others (3, 25, 26) have shown the importance of respiration and oxygen supply in certain types of mineral accumulation by plants. Chang and Loomis (7) and Vlamis and Davis (28, 29) have shown that high concentrations of CO_2 are injurious also, although the question of the relative importance of O_2 deficiency and CO_2 excess in field soils is not yet fully established (10, 12, 14, 19, 24, 30).

FIG. 1. Corn planted on plowed land, *left*, and hard listed, *right*. Potassium fertilizer largely offset the unfavorable effects of listing or other seed bed preparation methods which reduced gas exchange in soils.

The research reported here was undertaken to determine the relative effects of O_2 and CO_2 on potassium absorption by roots of maize plants growing in soil or in water culture. Soybeans were used in one experiment, and incidental data were collected on the effect of soil gases on transpiration rates of both corn and soybeans. However, the emphasis has been on the absorption of potassium by corn because of the striking potassium deficiencies obtained with corn in the field cultivation plots and the sensitivity of potassium absorption to poor aeration as shown in the work of Chang and Loomis (7).

MATERIALS AND METHODS

APPARATUS: Special equipment was designed to discharge various gas mixtures through glazed, one-gallon pots of soil or quart jars of nutrient solution. The mixing of the gases and the control of the flow rate were accomplished by the use of calibrated glass capillaries as described by Bartholomew and Broadbent (4). Gas from tanks of O_2, N_2 and CO_2 was used to obtain the desired mixtures. Figure 2 illustrates the system of mixing and delivery of gases to the bottom of a one-gallon pot. Gas was delivered to the pot at a pressure of 100 cm of water. A differential pressure of 100 cm of water forced the gases through calibrated capillaries to the mixing chamber. In practice it was only necessary to adjust the needle valves at the gas source so that a slight excess of gas escaped through the water pressure towers. By careful adjustment of the water pressure, gases of the desired composition were readily obtained. Figure 3 shows diagrammatically an arrangement of the above type of apparatus for delivery of 6 different mixtures

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