THE INDUCTION OF LATERAL ROOTS BY INDOLEACETIC ACID AND ROOT DECAPITATION [1]

John G. Torrey

THE ANATOMY of lateral root formation has been known for a considerable time from the classic histological work of van Tieghem and Duliot (1888) which showed the endogenous origin of laterals within the primary root axis. Yet relatively little is known even now concerning the physiological processes involved.

That artificially applied auxin stimulates lateral root formation in many plants was early recognized. Bouillenne and Went (1933) attributed increased root branching in the roots of *Acalypha* cuttings to the applied hormone not utilized in the initiation of the adventitious roots induced on the stem itself. Zimmerman and Hitchcock (1935) reported that lateral root formation on aerial roots of *Cissus* was induced by applied artificial auxin or by root decapitation. They postulated the formation of a factor by the primary root tip which prevented lateral branching but which became inoperative when the tip was interfered with or no longer actively growing.

The fact that auxin is definitely one factor controlling root branching was shown by Thimann (1936) in experiments treating the roots of *Avena* and *Pisum* seedlings with indoleacetic acid. He also found that removal of 1 mm. root tips from these seedlings resulted in increased lateral root formation in both species. In *Avena,* which has large amounts of natural auxin, Thimann reported that the principal influence on root branching is exerted by the tip. Thus it was indicated in these experiments that auxin is not the only factor controlling lateral root formation. In his study of auxin production in isolated pea roots grown in nutrient solution, van Overbeek (1939) found that 10 mm. root decapitation after 2 weeks' subculture resulted in a marked increase in lateral root formation on the basal portion of such roots.

Delarge (1941) reported that, although indoleacetic acid caused branching in initially excised root tips of *Zea* and *Triticum* grown in sterile culture, subsequent retreatment with the auxin caused no further lateral root formation. Delarge suggested that there was a depletion of some factor necessary for lateral root formation. Recently, Nutman (1948) has proposed that root meristems, both those of the root and those of effective root nodules, produce a substance which is inhibitory to the initiation of lateral root primordia.

With a view to further elucidating the physiological process of lateral root formation and the part played by auxin in it, experiments using sterile cultures of pea root tips were carried out. It is the purpose of this paper to report on the effect of indoleacetic acid treatment and decapitation on lateral root formation in isolated pea roots grown in nutrient culture.

METHODS.—Methods essentially as described by Bonner and Devirian (1939) for the sterile culture of pea roots were used. Seeds of the garden pea, *Pisum sativum,* variety Alaska, were sterilized in mercuric chloride solution, 0.1 per cent, washed several times with sterile distilled water, and allowed to germinate in the dark at 20°C. At the end of 48 hr., 3–4 mm. root tips were excised aseptically and transferred to culture dishes.

The medium of Bonner and Devirian modified from that of Bonner and Addicott (1937) contains per liter of solution: 242 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 42 mg. $MgSO_4 \cdot 7H_2O$; 85 mg. KNO_3; 61 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. ferric tartrate; 0.1 mg. thiamin; 0.5 mg. nicotinic acid; and 40 g. sucrose. The salts were added to the final solution from stock solutions made up at ten times the final concentration. Indoleacetic acid (IAA), when used, was added from a sterile stock solution of 100 mg. per liter after the nutrient solution had been autoclaved. Water distilled in a block tin still and redistilled in pyrex glass was utilized throughout in preparing the culture medium. When using the agar medium, powdered Bacto-agar was added to the medium mixture to give a final agar concentration of 0.5 per cent. The final medium was steam autoclaved at 15 lb. pressure for 25 min.

All glassware was cleaned in dichromate cleaning solution, passed through successive thorough rinses in tap water, detergent solution (Calgolac), tap water, distilled water, and was finally air dried. Petri dishes were sterilized using dry oven heat at 175°C. for 2 hr. Two root tips were grown per Petri dish, using 20 cc. of solution per dish. Cultures were maintained in the dark at an average temperature of 25°C. Weekly measurements of root length were made with a millimeter rule to an accuracy of 1 mm.

EXPERIMENTS.—*The growth of initial and first transfer root tips.*—Tips of Alaska pea roots, 3–4 mm. in length, were excised after 48 hr. germination and transferred to culture dishes, one half to agar control medium, the other half to medium containing 1 mg. IAA per liter. Following 3 days of IAA treatment, the latter group of root tips was transferred to control medium. At the end of 1 week in the dark at 25°C., this series of "initial" root tips was measured and lateral roots counted. In a

[1] Received for publication May 31, 1949.

The writer is indebted to Dr. Kenneth V. Thimann for his interest and helpful criticism during the course of this study.

[The Journal for March (36: 185–255) was issued May 3, 1950]
AMERICAN JOURNAL OF BOTANY, VOL. 37, NO. 4, APRIL, 1950

second series, initial root tips were grown on control medium for 1 week. At this time, 10 mm. "first transfer" tips were excised and transferred, one half of the tips to control medium, the other half to IAA medium for 3 days, and then to control medium. At the end of another week, measurements were made. The results of a typical experiment are shown in fig. 1.

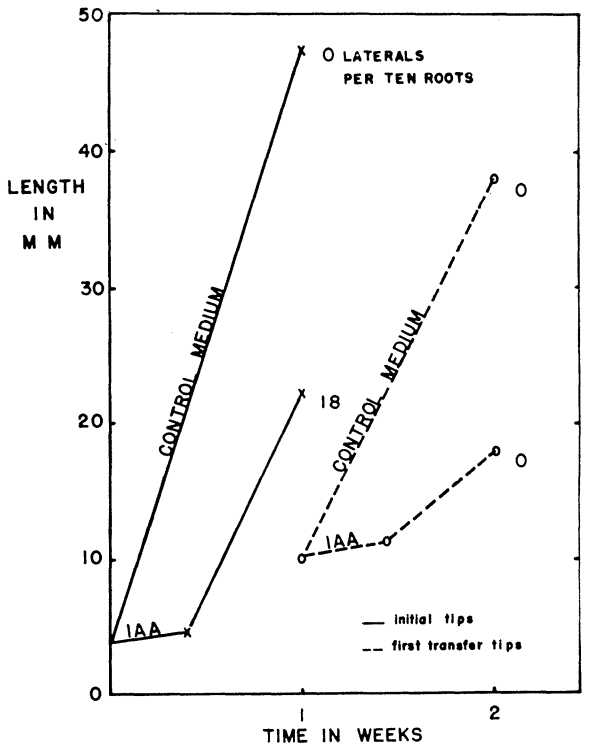

Fig. 1. Growth and lateral root formation in isolated initial and first transfer pea root tips in nutrient medium. Series of initial and first transfer tips cultured for 1 week each on control medium compared with a series of initial and first transfer tips grown for 3 days on medium containing 1 mg. IAA per liter and then transferred to control medium. The number of laterals per ten roots is indicated by the number after each growth curve.

On the initial tips, the effect of the applied IAA is twofold. It causes (1) marked inhibition of growth by elongation, and (2) the initiation of lateral roots. In contrast, first transfer root tips treated with IAA, while inhibited in elongation, show no lateral root formation. It appears that some factor necessary for lateral root formation, which is present in the initial root tip, is no longer present in the first weekly transfer root tip, or at least is no longer effective with IAA in causing lateral root initiation.

Optimal concentration and duration of IAA treatment for lateral root formation.—Several series of initial and first transfer root tips were grown on nutrient agar medium containing added concentrations of IAA from 10^{-4} mg.–20 mg. per liter. In no case were lateral roots initiated in the first transfer tips, whereas initial tips similarly treated formed laterals as before, showing a decrease in the number of laterals formed at the lower IAA concentrations. The fundamental difference between initial tips and first transfer tips in regard to lateral root formation therefore cannot be explained in terms of auxin concentration. Further, the concentration of approximately 1 mg. IAA per liter of solution was optimum for lateral root formation in the initial pea root tips under the conditions of these experiments, inducing an average of eighteen to twenty lateral roots per ten roots treated.

TABLE 1. *Growth and lateral root formation of isolated pea roots grown for varying periods on nutrient medium containing 1 mg. IAA per liter and then transferred to control medium. Initial tips of 3–4 mm. length excised from 48-hr. germinated seeds. Each series is an average of ten roots.*

		Average total length in mm.		Average number of laterals per ten roots		
Days of treatment	After IAA	At 1 week	At 2 weeks	1 week	2 weeks	3 weeks
0		43	84	0	0	..
1	4	32	62	0	1	..
2	4	25	46	12	12	12
3	5	22	43	18	21	21
7	7	7	27	11	20	22

In order to study the effect of the duration of IAA treatment on lateral root initiation, initial root tips were placed on nutrient agar medium containing 1 mg. IAA per liter for varying periods, and then were transferred intact to control medium. The results summarized in table 1 indicate that the treatment with IAA for 3 days gives maximum effect, and the number of laterals is not increased by longer IAA treatment.

Prolonged culture of excised root tips.—When cultured under optimum conditions for prolonged periods of time in nutrient solutions, isolated pea roots will form lateral roots without the addition of IAA to the medium. It seemed of interest to consider the conditions under which this occurred. Initial and first transfer root tips were grown on control medium, transferring the entire root intact at the end of each week to fresh nutrient medium. The results of a typical series are shown in table 2. It is apparent that, on continued culture, initial and first transfer tips ultimately do produce lateral roots, a phenomenon which is associated in some way with the elongation of the primary root. The lateral roots thus formed without artificial stimulation appear in the apical third of the growing root and are readily distinguished from the basally-located laterals which form behind the apex of the short initial tip when it is treated with IAA.

In an attempt to establish the relationship between elongation of the primary root and the formation of lateral roots, several series of roots were

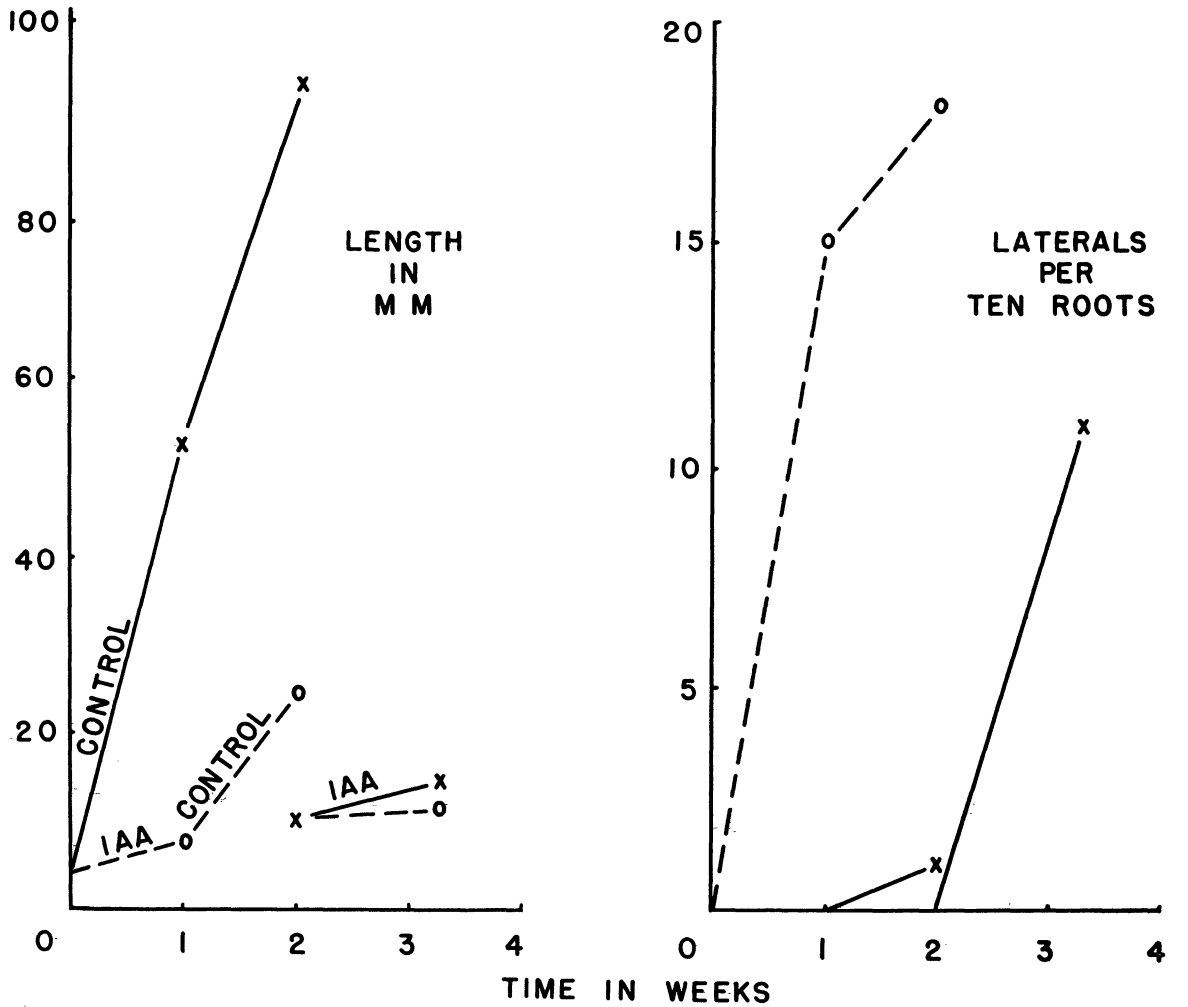

Fig. 2. Growth and lateral root formation in isolated initial and first transfer pea root tips grown in nutrient culture. Two experiments: solid line—initial tips grown for 2 weeks on control medium, then 10 mm. tips transferred to IAA medium; broken line—initial tips grown for 1 week on IAA medium, transferred intact to control medium for 1 week, then 10 mm. tips transferred to IAA medium. IAA treatment at 1 mg. per liter.

grown on IAA medium and then transferred to control medium where they were continued for a prolonged period. Another group of root tips were grown on control medium for several weeks without excision, then treated with IAA, and returned to control medium. The results of such an experiment are shown in table 3.

In the first case, the laterals apparent at the end of 2 weeks at the extreme bases of the roots are due to the stimulation of the applied IAA. After continued elongation of the primary root on control medium, additional laterals appear after 5 weeks, when they are found uniformly distributed in the normal manner along the apical third of the root. These apically-located lateral roots appear to be initiated independently of the IAA stimulation, and are under the control of factors within the elongating root itself. In the second case, the delayed IAA treatment induced lateral root formation in the apical region only. These laterals were formed in a very restricted region behind the apex, and did not show the normal acropetal distribution. Noirfalise (1940) reported what may be a similar phenomenon in intact roots of *Vicia Faba* which showed localized lateral root formation when treated with high IAA concentration (1,500 p. p. m.), but a scattered distribution of the lateral roots when treated with more dilute IAA solution (1 p. p. m.).

In further experiments on the root-forming capabilities of excised root tips, it was found that 10 mm. first transfer tips excised from initial tips grown on control medium for 2 weeks, formed lateral roots when placed on IAA medium. The first transfer tips excised from intact initial tips which had first been treated with IAA 1 week and then continued a second week on control medium, showed no lateral root formation on IAA medium. These results are represented in fig. 2. It was found that only in those first transfer tips, which were

TABLE 2. *Prolonged culture of initial and first transfer pea root tips grown in agar control medium without excision. Initial length: 3–4 mm.; first transfer length: 10 mm. Each series represents an average of twenty roots.*

Days elapsed	Average total length in mm.	Average number of laterals per ten roots
	Initial tips	
7	42	0
15	74	0
20	80	10
	First transfer tips	
7	32	0
30	63	0
44	88	7

excised from elongating roots about to form or already forming lateral roots in the apical region, was there lateral root formation when the excised tip was placed on IAA medium. It is evident that such first transfer tips exhibit a recovery of lateral root forming capacity which is correlated with the elongation of the root itself. This evidence, together with the facts described concerning lateral root formation in unstimulated roots grown for long periods, suggests that the factor necessary for root branching ultimately may be produced within the growing root itself.

The effect of root decapitation.—Preliminary experiments indicated that 10 mm. decapitation of initial pea roots which had been growing 1 week on control medium resulted in the formation of lateral roots in the apical portion of the remaining root bases. Intact control roots formed no lateral roots during the same period. In table 4 are recorded the results of such a decapitation experiment. In this experiment, one half of the roots were washed following decapitation to remove substances released at the cut surface.

In the light of the evidence of Thimann (1936) and of the experiments reported above concerning the action of IAA when applied to isolated pea roots, it is a noteworthy fact that removal of the tips from these roots produces the same apparent results as does treatment with auxin, namely, inhibition of root growth, and a marked increase of lateral root formation. The former effect is to be expected since in pea roots grown in culture, excision of the apical 10 mm. removes the apical meristem and the region of the elongating cells, leaving only mature differentiated tissue. In reference to the latter effect, it would seem not unlikely that these two different stimuli produce the same result, lateral root formation, through some common effect on the growing root.

Two possible explanations of the decapitation effect suggest themselves: (1) that the apical meristem of the root produces a substance which inhibits lateral root formation, or (2) that there is an accumulation of a specific substance in the cut root stump which is necessary to lateral root formation. The release of a wound substance at the excision which stimulates lateral root formation is probably not the basis of the decapitation effect. This is evident from the results of the washing experiments (table 4) and from the fact that laterals were never formed at the basal end of the excised root. It has been shown that first transfer tips, *i.e.*, 10 mm. tips excised from initial roots at the end of 1 week's growth in culture, have lost temporarily the capacity to form lateral roots under the stimulation of applied IAA. It was of interest, therefore, to test the first possible explanation by studying the effect of decapitation on lateral root formation in these first transfer root tips.

After initial root tips had been grown for 1 week in culture, the apical 10 mm. tips were transferred as first transfer tips to control medium. In one case, 10 mm. decapitation was made at the end of 1 week, and then observations made on the "1st transfer bases" for an additional week. In the other case, the first transfer tips were allowed to grow 3 weeks on control medium, and then the 10 mm. tips were excised and the first transfer bases continued for an additional week. The results of

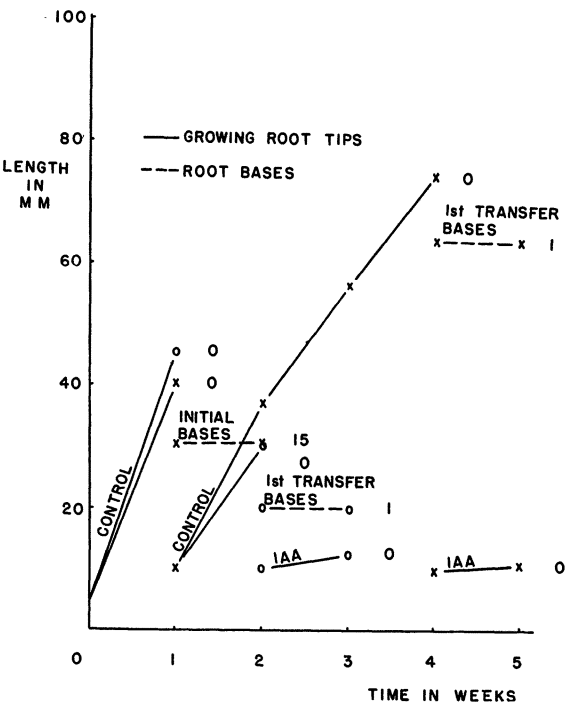

Fig. 3. Growth and lateral root formation in isolated initial and first transfer pea root tips and decapitated root bases grown in nutrient medium. Decapitation of 10 mm. apical portion from initial tips made after 1 week. First transfer tips decapitated after 1 week (circles) and 3 weeks (crosses). Second transfer tips treated with 1 mg. IAA per liter. The number of laterals formed per ten roots is indicated by the number after each growth curve.

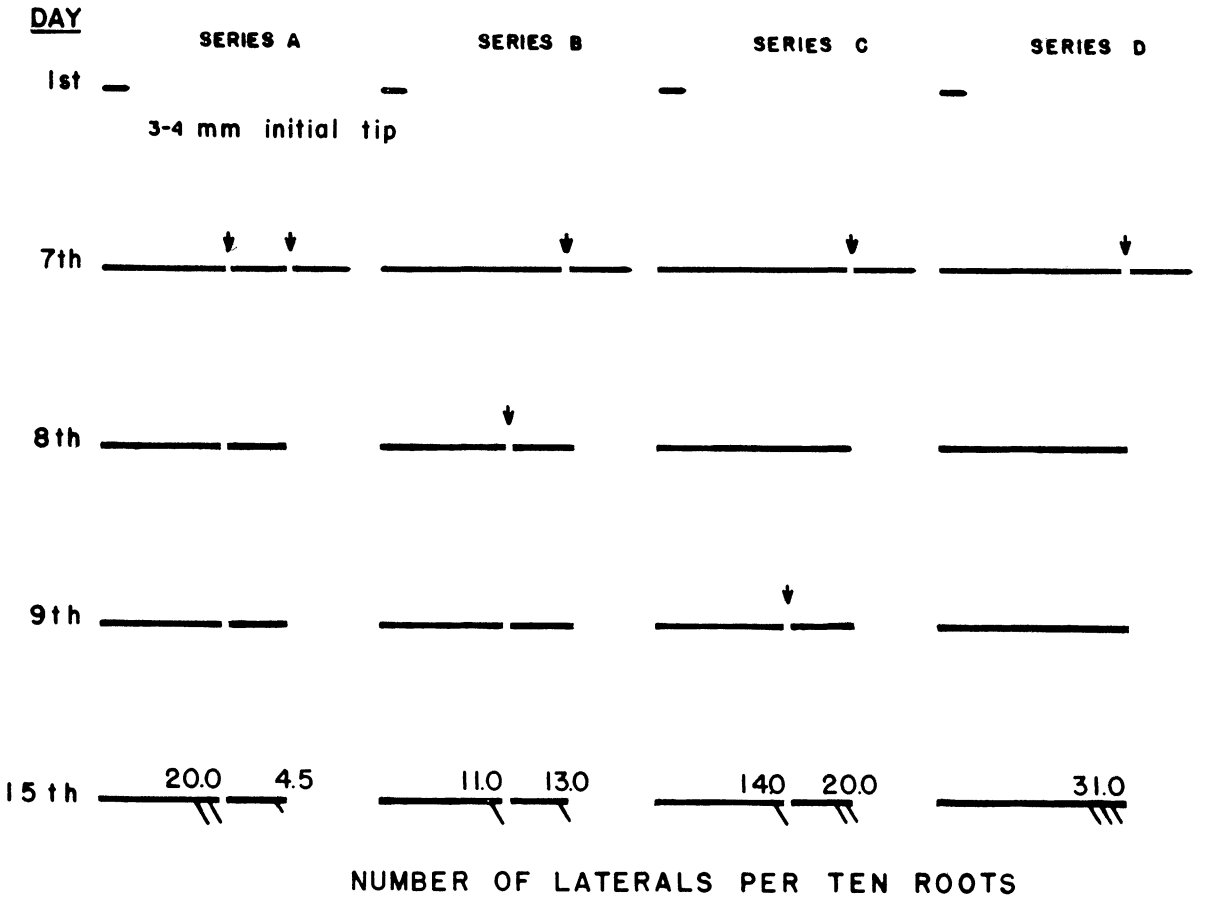

Fig. 4. Lateral root formation in segmented initial pea root tips grown in control nutrient medium. Arrows indicate the points of excision on the days noted. Ten millimeter decapitation of all roots on the seventh day.

TABLE 3. *Root elongation and lateral root formation by isolated initial pea roots in culture after initial or delayed IAA treatment at 1 mg. per liter for 1 week. Each series represents an average of twenty roots.*

Treatment	Time in days	Average root length in mm.	Average number of laterals per ten roots
Immediate IAA treatment			
IAA	7	8	6
Control medium	14	34	10
Control medium	28	99	10
Control medium	40	152	44
Delayed IAA treatment			
Control medium	7	33	0
Control medium	14	80	0
IAA	21	85	15
Control medium	29	115	18

these experiments are recorded diagrammatically in fig. 3. It is apparent that, although decapitation of initial tips after growth for 1 week results in the formation of the usual number of lateral roots at the cut end of the initial bases, decapitation of first transfer tips, after 1 or 3 weeks of growth, does not cause the initiation of lateral roots. Hence, one must conclude that absence of the root apical meristem does not result necessarily in lateral root formation. Apparently the factors necessary for the formation of lateral roots are lacking in the first transfer tips. Stimuli which will cause the initiation of lateral roots in an initial root tip have no such effect on the first transfer tip.

From the evidence presented above, it seems safe to conclude that some substance other than auxin is necessary for lateral root formation in pea roots. The substance present in the initial tip apparently comes from the germinating seed, but is exhausted or lacking in the first transfer tip. Root decapitation may cause an accumulation of this substance at the cut end, and lateral root formation in this area results. In the first transfer tip there is no such accumulation and decapitation only acts to stop root elongation.

Further experiments were made using the initial tip, which is known to be capable of forming lateral roots under the stimulus of decapitation. Initial tips were grown on control medium for 1 week. At the end of this time, 10 mm. tips were excised

TABLE 4. *Lateral root formation in isolated initial pea root tips grown in nutrient medium. Decapitation at the seventh day by excision of the 10 mm. tip. Bases continued in situ. Roots washed when indicated with sterile distilled water for 1 hr., then continued on nutrient medium. Each series represents an average of twenty roots.*

			Decapitation series			
	Intact series		Unwashed roots		Washed roots	
Day	7th	14th	7th	14th	7th	14th
Average total length in mm.....	43	84	41	31	40	30
Average number of laterals per ten roots...	0	0	0	18	0	15

and discarded in all roots. An additional 10 mm. section was cut from each root base in each of three series on 3 successive days. The sections were continued in place on the agar medium. A fourth series was continued with only the initial decapitation. Eight days after decapitation, observations were made and the distribution of lateral roots noted. The results are shown diagrammatically in fig. 4.

In the first place, it should be noted that the total number of laterals in each series is of the same order. It should be noted also that all laterals were formed at the most apical end of the segment in every case. No laterals appeared at the basal ends of the sections. In the first series (A), the apical sections produced considerably fewer laterals than the basal sections. In the cases where excision was made 1 and 2 days after the initial decapitation, the basal sections produced fewer laterals, while the short apical sections showed a successive increase in number of laterals formed. This experiment and others like it strongly suggest the movement from the root base of a substance which accumulates at the most apical region of the section. Similar studies show that the movement of the substance toward the apical cut is complete in 3 days after decapitation. Experiments in which a wedge-shaped cut was made half-way across the diameter of the root indicate that a partial blockage of the moving substance can be accomplished. A given isolated root thus appears to be capable of producing a limited number of laterals in culture, dependent upon the amount of substance present in the root.

Experiments designed to study the effect on lateral root formation of successive IAA treatment and decapitation were made. A series of initial tips was cultivated on control medium for a period of 2 weeks, then the apical 10 mm. tips were removed and observations were made for 10 days. A parallel series of initial tips was grown on IAA medium for 1 week, and then the tips were transferred intact to control medium for a week. At the end of this period, the apical 10 mm. tips were excised, and the bases continued *in situ* for a further week. As a test for the presence of lateral root forming capacity, the excised tips in each series were placed on IAA medium for 1 week, and then transferred intact to control medium to observe lateral root formation. The results of these experiments are recorded in fig. 5.

In the initial tips treated with IAA, there occurs a characteristic root growth inhibition and the initiation of a number of lateral roots at the extreme basal ends of the intact root (nineteen laterals per ten roots—series C). Removal of the 10 mm. tip after a further week on control medium results in the formation of very few additional lateral roots at the cut surface, *i.e.*, one per ten roots. Decapitation of an untreated root after 1 week results in the formation of approximately the same number of laterals as in the IAA treated roots but these, in contrast, appear at the apical end in the region of the cut (bases A). Decapitation after 2 weeks of prolonged growth results in the formation of a large number of laterals by the root bases (bases B), a number well above that of the IAA treated

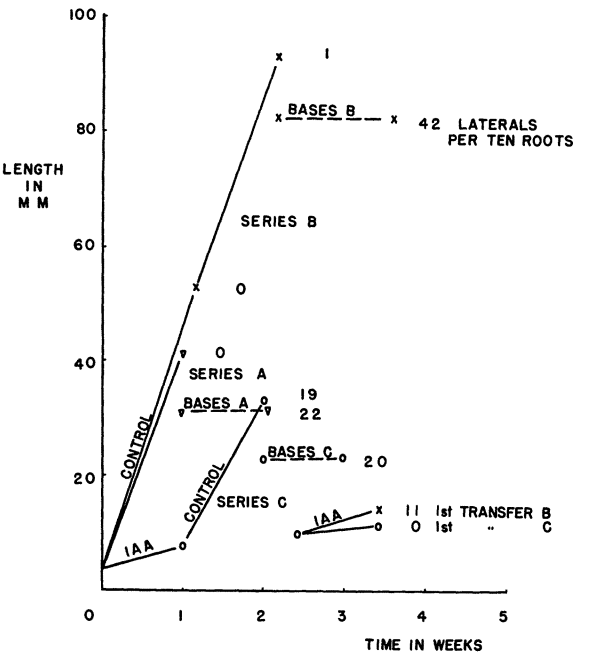

Fig. 5. Growth and lateral root formation in isolated initial and first transfer pea root tips and decapitated root bases grown in nutrient medium. Ten millimeter decapitation of initial tips after growth on control medium for 1 week (series A), 2 weeks (series B), and 1 week IAA treatment followed by 1 week on control medium (series C). First transfer tips treated with IAA for 1 week. IAA treatment at 1 mg. liter. The number of laterals formed per ten roots is indicated by the number after each growth curve.

roots or the initial tips decapitated after only 1 week. It is evident that these roots are no longer limited by the root forming substance present in the initial tips on excision, but have begun to provide the substance by their own synthetic activity. That this is the case is indicated by the beginning of lateral root formation by these tips on the control medium after 2 weeks. Further evidence comes from the fact that first transfer tips from these roots, when treated with IAA, form lateral roots (first transfer B), unlike the week-old first transfer tips when similarly treated (fig. 1, 3) or first transfer tips from roots whose growth has been inhibited (first transfer C). Thus in this experiment the initial IAA treatment causes lateral root development, and subsequent decapitation produces almost no additional lateral roots, as if all the root forming substance had already been utilized in response to the first stimulus. Experiments in which decapitated root bases were placed on IAA medium were also made. In no case was there an increase in the number of laterals formed by these combined stimuli. It appears, therefore, that the two stimuli are active via the same substance.

Discussion.—A consideration of these experiments has led to the formulation of an hypothesis concerning the factors controlling the formation of lateral roots in *Pisum sativum*. Support is given to the original hypothesis of Delarge (1941) that a substance other than auxin in the root is necessary for lateral root formation. This unidentified substance is present in the initial 3–4 mm. root tip excised from the germinating pea seed, presumably having been carried over in the root tip from the seed. Under the stimulatory influence of applied IAA, such tips form lateral roots. The substance is lacking in the first weekly transfer tip at the time of its transfer to new medium and has either been exhausted from the tip during growth or has never reached the apical region. The growing root is initially dependent upon the seed for the lateral root forming substance but, as growth becomes well established, the root itself assumes the function of producing the substance. Thus lateral roots become apparent in acropetal succession when isolated roots are grown in culture for a prolonged period without transfer. First transfer tips as well as initial tips, when cultivated for sufficient periods to assure optimum growth, will form lateral roots without external stimulus.

The experiments of Noll (1900), deHaan and Petrick (1935), and Rippel (1937) suggest the movement within the root of a substance upon which lateral root formation is dependent. It appears that the effect of decapitation is to cause an accumulation of this substance. It seems possible that IAA similarly may cause accumulation of the substance which causes lateral root formation at the site of its mobilization. In the intact plant, lateral root formation may depend upon the relative concentrations of auxin, probably produced by the root meristem, and of the root forming substance, initially coming from the cotyledons, and subsequently produced in the growing root itself. Any upset in the balance between these two factors within the root, which tends to cause accumulation of the root forming substance, results in the formation of lateral roots in the region of concentration.

SUMMARY

An optimum concentration of 1 mg. of indoleacetic acid per liter of solution, applied for 3–7 days to initially excised 3–4 mm. tips of the roots of *Pisum sativum* grown in nutrient culture, produces a maximum and uniformly reproducible response in number of lateral roots formed. When IAA is applied at the same concentration to first transfer tips excised from roots grown in culture 1 week, no laterals are formed. After a period of prolonged growth in control medium, initial root tips or transfer tips eventually will form lateral roots without IAA treatment. In such prolonged culture, it is shown that lateral root formation, either with or without IAA stimulation, is correlated with the elongation of the primary root. Removal of the 10 mm. tip from initially excised pea roots grown in nutrient culture 1 week, results in the initiation of lateral roots near the cut surface of the basal section within 3 days. Decapitation of first transfer tips grown for 1 week causes no lateral root formation, indicating the absence from these roots of a factor necessary for lateral root formation. Evidence is given for the progressive movement in initial root tips of this factor toward the apical region. Initial root tips show a limited lateral root forming response after a given period of growth, whether stimulated by applied IAA, decapitation, or a combination of the two stimuli. It is postulated that an unidentified substance other than auxin is necessary for lateral root formation in pea roots, but that the substance becomes active within the root under the influence of auxin. A balance between naturally produced auxin within the root and the lateral root forming substance is suggested as the mechanism controlling normal lateral root formation in the intact plant.

Department of Botany,
University of California,
Berkeley, California

LITERATURE CITED

Bonner, J., and F. Addicott. 1937. Cultivation *in vitro* of excised pea roots. Bot. Gaz. 99: 144–170.

———, and P. S. Devirian. 1939. Growth factor requirements of four species of isolated roots. Amer. Jour. Bot. 26: 661–665.

Bouillenne, R., and F. W. Went. 1933. Recherches expérimentales sur la néoformation des racines dans les plantules et les boutures des plantes supérieures. Ann. Jard. Bot. Buitenzorg. 43: 25–202.

Delarge, L. 1941. Étude de la croissance et de la ramification des racines in vitro. Mém. Soc. Roy. Sci. Liège 2e Sér. 5: 1–221.

de Haan, I., and L. Petrick. 1935. Polaire wortelvorming. Natuurw. Tijdschr. 17: 117–127.

NOIRFALISE, A. 1940. Recherches sur le développement des racines de *Vicia Faba* traitées à l'heteroauxine. Cellule 48: 307–333.

NOLL, F. 1900. Über den bestimmenden Einfluss von Würzelkrümmungen auf Entstehung und Anordnung von Seitenwurzeln. Landw. Jahrb. 29: 361–426.

NUTMAN, P. S. 1948. Physiological studies on nodule formation. I. The relation between nodulation and lateral root formation in red clover. Ann. Bot. 12: 81–96.

RIPPEL, K. 1937. Umkehr der Seitenwurzelgenese bei Leguminosen als korrelative Störung. Ber. Deutsch. Bot. Ges. 55: 288–292.

THIMANN, K. V. 1936. Auxins and the growth of roots. Amer. Jour. Bot. 23: 561–569.

VAN OVERBEEK, J. 1939. Evidence for auxin production in isolated roots growing *in vitro*. Bot. Gaz. 101: 450–456.

VAN TIEGHEM, P., AND H. DULIOT. 1888. Recherches comparatives sur l'origine des membres endogènes dans les plantes vasculaires. Ann. Sci. Nat.—Bot. 7 Sér. 8: 1–660.

ZIMMERMANN, P. W., AND A. E. HITCHCOCK. 1935. Responses of roots to "root-forming" substances. Contrib. Boyce Thompson Inst. 7: 439–445.

TOBACCO CALLUS RESPIRATION AND ITS RESPONSE TO 2,4-DINITROPHENOL [1]

Eldon H. Newcomb

THIS PAPER describes some of the general respiratory characteristics of tobacco callus tissue and its response to different concentrations of 2, 4-dinitrophenol. Evidence concerning respiratory pathways in this tissue, including data on the effects of other poisons and on the identity of the terminal oxidase, will be presented in a later paper.

The tobacco tissue used in the investigation was derived from cultures of White (1939a). White grew fragments of procambial tissue excised from the stem tip of a hybrid tobacco, and sub-cultured the resultant callus indefinitely in an undifferentiated state on a simple agar medium of known composition (White, 1939b). The cultures used by the writer have not formed organs either on solid or in liquid media, although White (1939b) induced transplants to form leafy shoots by immersion in a liquid nutrient, and Skoog (1944) controlled undifferentiated growth and organ formation in cultures of White's tobacco tissue by manipulation of external factors.

In seeking similarities between animal neoplasia and sunflower and tobacco "tumors" of various origins, White (1945) determined the respiratory activity and quotient of the hybrid tobacco callus tissue. He found a somewhat lower level of oxygen consumption by the callus tissue in comparison on a dry weight basis with certain normal parts of tobacco, such as internodal tissue and stem tips. The respiratory quotient of the callus tissue proved to be 1.1.

An extensive literature now exists on the effects of 2,4-dinitrophenol (referred to hereafter as DNP) on the respiratory processes of animals and microorganisms, though its influence on higher plant respiration has been studied very little. Experiments with this poison have therefore been conducted on tobacco callus tissue to determine whether its effects are qualitatively similar to those observed for other living material. While the results do not furnish evidence for specific respiratory pathways, they concern the relationship between respiration and fermentation, indicate parallels between the respiratory mechanisms of higher plants and of other organisms, and provide data for the interpretation of the effects of catechol, p-nitrophenol, and related substances on plant respiration.

TABLE 1. *Oxygen consumption of tobacco callus slices at 25°C. on fresh and dry weight bases. Slices suspended in M/20 phosphate buffer of pH 5.3.*

Age of callus in days	Date of experiment	μl. O_2 uptake per g. fr. wt. per hr.	Q_{O_2} (μl. O_2 uptake per mg. dry wt. per hr.)		
		1st hr.	1st hr.	2nd hr.	3rd hr.
39	2/28/49	79	1.7	1.7	1.7
45	2/19/49	73	1.8	1.9	1.8
48	12/2/48	88	1.6	1.7	1.7
48	12/10/48	70	1.8	1.8	1.8
49	12/11/48	75	1.7	1.7	1.6
59	1/24/49	91	2.0	2.0	2.1
60	1/25/49	86	2.2	2.1	2.1
71	1/16/49	55	1.5	1.4	..
72	1/17/49	58	1.4	1.5	..
75	1/20/49	56	1.5	1.4	1.5
92	1/15/49	69	1.7	1.6	..

The literature on the effects of DNP on microbial respiration and assimilation has been reviewed by Clifton (1946). Clifton (1937) discovered that DNP blocks oxidative assimilation in microorganisms. The partial conversion of metabolites into cell material by resting microorganisms was completely interrupted by DNP, so that breakdown continued until all material was oxidized.

[1] Received for publication July 15, 1949.

This work was done during the tenure of a National Research Council Predoctoral Fellowship in Biology, and represents a portion of a thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author takes pleasure in thanking his adviser, Professor J. F. Stauffer, for his interest and advice. Appreciation is also expressed to Dr. Folke Skoog for providing the original callus transplants and for aid in other ways, and to Dr. P. J. Allen for reading the manuscript.