*CHEMICAL CONTROL OF VASCULAR CAMBIUM INITIATION IN ISOLATED RADISH ROOTS**

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Roots of many higher plants increase in diameter through the enlargement of cells formed in a vascular cambium. After the seedling plant is established, thickening begins near the root base by the initiation of cell divisions in the procambial tissue of the central cylinder. How the vascular cambium is initiated, what maintains cambial activity and causes it to extend, with time, along the root, and how the new cells differentiate into secondary xylem and phloem are questions which have received considerable attention from botanists. It is clear from several lines of evidence that the aerial shoot has an influence in some or all of these processes. Dependence upon the shoot involves more than supplying adequate carbohydrate to the heterotrophic root since cambial activity has been observed only rarely in excised roots provided simply with an abundant supply of carbohydrate. Of particular interest are the observations that auxin will induce moderate cambial activity when it is supplied to the stumps of decapitated plants[1] and of excised roots grown in culture,[2, 3] or to the medium in which excised roots are grown.[4, 5]

Interest also centers on several species in which root thickening has been reported to be dependent upon the relative lengths of day and night in the shoot environment. Such dependence can be interpreted as indicating that other substances besides carbohydrate and auxin produced under appropriate photoperiodic stimulus are probably involved in cambial initiation.

Root thickening of intact radish plants, *Raphanus sativus* L., has been reported to be promoted by short days (8–12 hr light)[6, 7] and optimum temperatures. Excised seedling roots of radish fail to thicken noticeably in culture[2, 8, 9] even though a cambium sometimes may be initiated. Jones[2] found that cambial initiation and activity in such roots was promoted by auxin supplied to the root base or by small amounts of stem (hypocotyl) tissue left attached to the root. Thus, radish roots which thicken rapidly from a single vascular cambium appear to be particularly well suited for investigations on cambial activity. We have undertaken a study into their behavior. Excised seedling roots were cultured sterilely for a period of time to deplete them of possible endogenous cambial regulators. Transfer tips were then cultured with their cut bases placed in media containing substances to be tested for their influence on cambial initiation.[3] These experiments have shown that, in addition to auxin, kinins and inositol have striking effects on cambial initiation and activity.

Materials and Methods.—The radish variety "White Icicle"[10] was chosen because of Jones' success with it and since in the intact plant a long well-thickened tap root is produced in contrast to many other commercial radish varieties in which the thickening is confined largely to the hypocotyl. The seeds were surface-sterilized in calcium hypochlorite solution (5% w/v Pittchlor, Columbia-Southern Chemicals Corp., Pittsburgh, Pa.), rinsed repeatedly in sterile distilled water, and germinated sterilely on moistened filter paper in Petri dishes in the dark at 23°C. After 3 days, when the roots were 30–100 mm long, 10-mm tips were excised and transferred to Petri dishes containing a modified Bonner nutrient medium.[11] These initial tips were allowed to grow for 4 days during

this first passage to an average length near 50 mm after which 15-mm first-transfer tips were excised and placed on fresh Bonner medium but with 5 mm of their bases inserted into separate media contained in 12 × 35-mm glass vials. (Fig. 1 shows this arrangement with two vials in an 11-cm Petri dish.) Growth and development of the roots were assayed during this second passage. The roots were cultured in the dark at 23°C except during transfers and observations. White light was found to be slightly inhibitory to growth.

The Bonner medium in the dish contained $Ca(NO_3)_2 \cdot 4\ H_2O$ (242 mg/l), $MgSO_4 \cdot 7H_2O$ (42 mg/l), KNO_3 (85 mg/l), KCl (61 mg/l), and KH_2PO_4(20 mg/l). $FeCl_3 \cdot 6\ H_2O$ (2.5 mg/l) and H_3BO_3 (1.5 mg/l), $ZnSO_4 \cdot 7\ H_2O$ (1.5 mg/l), $MnSO_4 \cdot H_2O$ (4.5 mg/l), $Na_2MoO_4 \cdot 2\ H_2O$ (0.25 mg/l), and $CuSO_4 \cdot 5\ H_2O$ (0.04 mg/l) were included. The organic constituents included sucrose (20 gm/l), thiamin-HCl (0.1 mg/l); pyridoxine-HCl (0.5 mg/l) and nicotinic acid (0.5 mg/l); the medium was solidified with 8 gm of Difco Bacto-agar per liter and was adjusted to a pH after autoclaving of 4.8–5.3. The vials were filled with the same medium but usually with a higher sucrose concentration and with additions of various test substances. All media were sterilized by autoclaving.

In the experiments reported below, each treatment was replicated with 8 or 10 roots. Even after selection during the initial culture period, the first-transfer roots were rather variable in their development (increases in length and the number of lateral roots); this variability is indicated in the tables by standard errors reported with means of 5 or more observations. Measurements from roots which grew poorly or which were unusually vigorous were excluded.

Material for anatomical study was handled by standard procedures; the roots were fixed in formalin-acetic acid-alcohol, dehydrated through an ethyl-butyl alcohol series, embedded in Tissuemat, and sectioned at 10 μ. The sections were stained with iron-haematoxylin and safranin.

Results.—"White Icicle" radish roots cultured on the basic medium declined in vigor with successive passages or transfers to fresh medium. In one case, 10-mm initial tips grew 6.9 ± 0.6 mm per day during a 7-day first passage after a 10-mm tip had been transferred to fresh medium. Only 25 per cent of the roots continued to grow through the third passage at rates of 2–6 mm per day. An explanation for the better growth of these more "vigorous" roots constitutes a problem which will be referred to again.

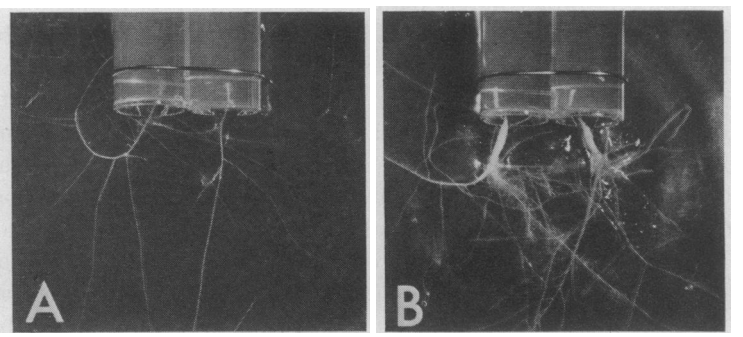

FIG. 1.—Petri dish assemblies for basal feeding of cultured roots; the two vials are fastened together with stainless steel wire. The ends of the wire support the vials underneath and prevent contact between plate and vial media. (*A*) A control culture at one month in which the vial medium contains mineral salts, vitamins, 12% sucrose, 10^{-5} *M* indoleacetic acid, and 100 mg/l *myo*-inositol. (*B*) Same, but with the addition of 1 mg/l of 6-benzylaminopurine in the vial.

Bonner and Devirian[9] reported rates of 30 mm per 2 weeks, while White[8] reported an average growth rate of 13.5 mm per day; these rates were maintained during several passages. The differences between these results and our own may be due to varietal differences or to differences in media and selection for "vigorous" roots. The decline in growth rate with succeeding passages may indicate depletion of some factor(s) originally supplied from the cotyledons (seed leaves). Such a depletion is

the desired situation with regard to those factors specifically required for cambium initiation if one is to achieve a sensitive test system.

In our first experiments using vials, sucrose and auxin were supplied to the cut bases of second-passage roots. Results from such an experiment are shown in Table 1. Sucrose from the vial medium supported a moderate rate of growth when

TABLE 1

GROWTH RESPONSES OF RADISH ROOTS TO SUCROSE AND INDOLEACETIC ACID (IAA)*

Vial medium contained mineral salts and vitamins plus:		Increase in main axis length (mm/day) with plate sucrose at:		Total no. laterals per root with plate sucrose at:	
Sucrose	IAA	0%	2%	0%	2%
0%	0	0.2 ± 0.1	2.3 ± 0.8	0 ± 0	2.7 ± 0.9
8	0	1.5 ± 0.2	2.2 ± 0.3	1.4 ± 0.5	0.6 ± 0.4
8	10^{-5} *M*	0.9 ± 0.1	1.8 ± 0.4	3.9 ± 0.4	4.8 ± 0.8

*Measured 10 days after transfer. Means of 6–8 roots with standard errors (expt. 13). No roots in this experiment showed visible thickening.

it was omitted from the plate medium but did not affect growth when the plate medium contained the optimal level (2%) of sucrose. In other experiments, it was found that when sucrose was supplied only to the vial medium, a concentration of 12 per cent produced maximum elongation. Indoleacetic acid (IAA) provided in the vial at 10^{-5} *M* reduced elongation but increased the number of lateral roots formed. Indoleacetic acid at 10^{-4} or 10^{-3} *M* inhibited root growth more strongly while 10^{-6} and 10^{-7} *M* promoted elongation but were not as effective in inducing the formation of lateral roots. In contrast to the results of Jones[2] and Torrey,[3] none of these roots showed any visible thickening when provided just auxin and sucrose. Thus, these second-passage roots appeared to be a suitable system with which to seek further information on cambial regulation. In subsequent tests, 2 per cent sucrose was added to the plate medium, and the standard vial medium contained 10^{-5} *M* IAA and 8 or 12 per cent sucrose.

A variety of chemical substances including organic nitrogen compounds (casein hydrolysate, L-asparagine, L-glutamine), gibberellin (potassium salt of gibberellic acid, GA_3), kinin (6-benzylaminopurine), and various phenolic acids were tested as vial additives with and without IAA and found to be without influence on visible thickening.[12]

Myo-inositol, although it did not induce thickening, yielded more robust roots and increased growth (Table 2). The proportion of so-called "vigorous" roots increased in treatments with inositol and auxin. Data from such roots, where distinguishable, are not included here, but it is worth noting that when supplied with inositol and IAA via their bases, some "vigorous" roots attained growth rates of 15 mm per day and maintained such rates for over 30 days. This is comparable to the rate reported by White.[8] Such roots, being robust with many laterals, were quite similar in their appearance to roots from intact seedlings. It was found later that these roots thicken in response to the same stimuli as do the "normal" roots, so this problem of variability has not been pursued further.

When various kinins were added to the vial medium at 0.1–1.0 mg/l in the presence of inositol, sucrose, and auxin, marked visible thickening of the transfer root was obtained (Fig. 1*B*). Root thickening was apparent within 7 days after transfer. The largest roots obtained were nearly 3 mm in diameter or 15 times the diam-

TABLE 2

THE INFLUENCE OF *Myo*-INOSITOL AND INDOLEACETIC ACID (IAA) ON RADISH ROOT GROWTH*

	IAA	Vial Medium Contained Mineral Salts, Vitamins, and 8% Sucrose plus: *Myo*-inositol (mg/l) 0	10	50	100	500	1000
Increase in main axis length (mm/day)	0	1.6 ± 0.4	2.4 ± 0.2	3.0 ± 0.6	2.3	2.9 ± 0.5	2.1 ± 0.3
	10^{-5} *M*	1.1	1.6 ± 0.3	1.4	1.8 ± 0.2	3.8	2.1 ± 0.3
Number of laterals per root on the new growth	0	0.1 ± 0.1	0	0.2 ± 0.2	0	0.8 ± 0.3	0
	10^{-5} *M*	0	0.3 ± 0.2	0	1.4 ± 0.5	5.5	1.4 ± 0.7

* Measured 11 days after transfer. Plate medium contained 2% sucrose. Means of 2–8 roots with standard errors (expt. 26). No roots in this experiment showed visible thickening.

TABLE 3

GROWTH AND THICKENING OF RADISH ROOTS AS AFFECTED BY 6-BENZYLAMINOPURINE BA*

Vial medium†	Increase in main axis length (mm/day) with BA at: 0	1 mg/l	Total no. laterals per root on the new growth with BA at: 0	1 mg/l	% roots having visible thickening with BA at: 0	1 mg/l	Maximum no. secondary xylem elements observed per radial row with BA at: 0	1 mg/l
Complete	2.3 ± 0.5	1.2 ± 0.2	3.3 ± 0.1	2.8 ± 0.1	0	80	1	19
Sucrose at 2%	2.0 ± 0.3	0.6 ± 0.3	4.8 ± 0.1	1.6 ± 0.0	0	70	1	17
Minus inositol	2.8 ± 0.2	0.2 ± 0.2	3.8 ± 0.6	0.2 ± 0.2	0	0‡	—	9
Minus auxin	2.3 ± 0.3	1.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.0	0	0‡	0	0

* Length, no. of laterals, and % of roots with visible thickening were measured 10 days after transfer; no. of xylem elements were counted on transections of roots fixed 29 days after transfer. Plate medium contained 2% sucrose. Means of 5–9 roots with standard errors (expt. 23).

† The complete vial medium consisted of the standard Bonner-Devirian salts and vitamins with sucrose at 12% (w/v) to which 10^{-5} *M* indoleacetic acid and 100 mg/l of *myo*-inositol were added.

‡ In other experiments a small number of roots from these treatments were thickened to a limited extent.

eter (0.2 mm) of the roots at the time of transfer. Thickening was usually confined to the length of the transfer piece, i.e., it extended only 5–15 mm along the root from the base. Thickening of the new growth, when present, was less. Auxin, kinin, and inositol all appear necessary for the response (Table 3). In some tests, 5–10 per cent of the roots thickened in the absence of added auxin, possibly by utilizing endogenous auxin; in the absence of added inositol, the thickening was much less or absent, and the roots usually were brown and unhealthy in appearance due to the high levels of kinin and auxin.

Serial transverse sections cut from the basal regions of second-passage roots after vial treatments for 20–30 days showed that roots provided with the basal medium had only primary tissues (Fig. 2*A*). When IAA was added to the vial medium, cambial activity, limited to one or two radial rows of cell divisions, was sometimes observed (Fig. 2*B*). This cambium arose, as in normal plants,[13] in the procambial tissue between primary xylem and phloem tissues and later extended around the tips of the protoxylem in the pericycle. With both auxin and kinin (Fig. 2*C*) the amount of thickening was much greater; as many as 19 cells aligned in radial rows could be recognized in the secondary xylem. As the roots thickened, the epidermis and primary cortex split and were sloughed off (Fig. 2*B*), and an exoderm developed apparently from the pericycle and the secondary phloem.[13] Differentiated vessel elements were very abundant in the secondary xylem, more so than in roots from intact vigorous radish plants grown in a growth chamber in which xylem parenchyma predominated (Fig. 2*D*). The xylem vessels showed normal scalariform or reticulate secondary wall formation. As in intact plants, a broad ray of parenchyma usually developed in both xylem and phloem outside the tips of the protoxylem.

The increase in root diameter due to combined auxin and kinin treatment involved a much greater stimulation of cambial activity than that initiated with IAA alone. Presumably both compounds stimulated divisions in the same cell layers, although we have not proved this. Judging from the timing of the root thickening, cambial activity probably did not continue beyond 10–15 days after the vial treatment began, and no mitotic figures were observed in material fixed 20 days after the beginning of the treatment. A time-course study of cambial activity will be necessary to establish more information on these points.

Other pertinent findings may be summarized briefly. Root thickening of the type described above was obtained with various kinins, including 6-phenylaminopurine, 6-benzylaminopurine (BA), and 6-furfurylaminopurine (kinetin) at 0.1–1.0 mg/l, or when coconut milk which contains natural kinins constituted 10 per cent of the vial medium.

With increasing auxin concentrations at a constant kinin concentration, an increasing proportion of cultured roots showed thickening: with 1 mg/l of kinetin, 12 per cent of the roots thickened at 10^{-7} *M* IAA, 25 per cent at 10^{-6} *M* IAA, and 75 per cent at 10^{-5} *M* IAA. With benzylaminopurine, no thickening was observed with 10^{-7} *M* IAA. High concentrations of kinin (10 mg/l) together with auxin caused browning of the roots and inhibited elongation. Naphthaleneacetic acid at 10^{-6} *M* was quite effective as an auxin in stimulating cambial divisions in the presence of inositol and kinin. Gibberellin at concentrations of 1–100 ppm had no effect on this system.

While several factors are required for marked thickening, the response to one

factor appears to be independent of the level of the others as long as each is in the effective range. In view of the frequent reports of kinin-auxin balances (e.g., Skoog and Miller[14]), the matter of interacting concentrations of these substances should be investigated further. The thickening response seemed to be independent

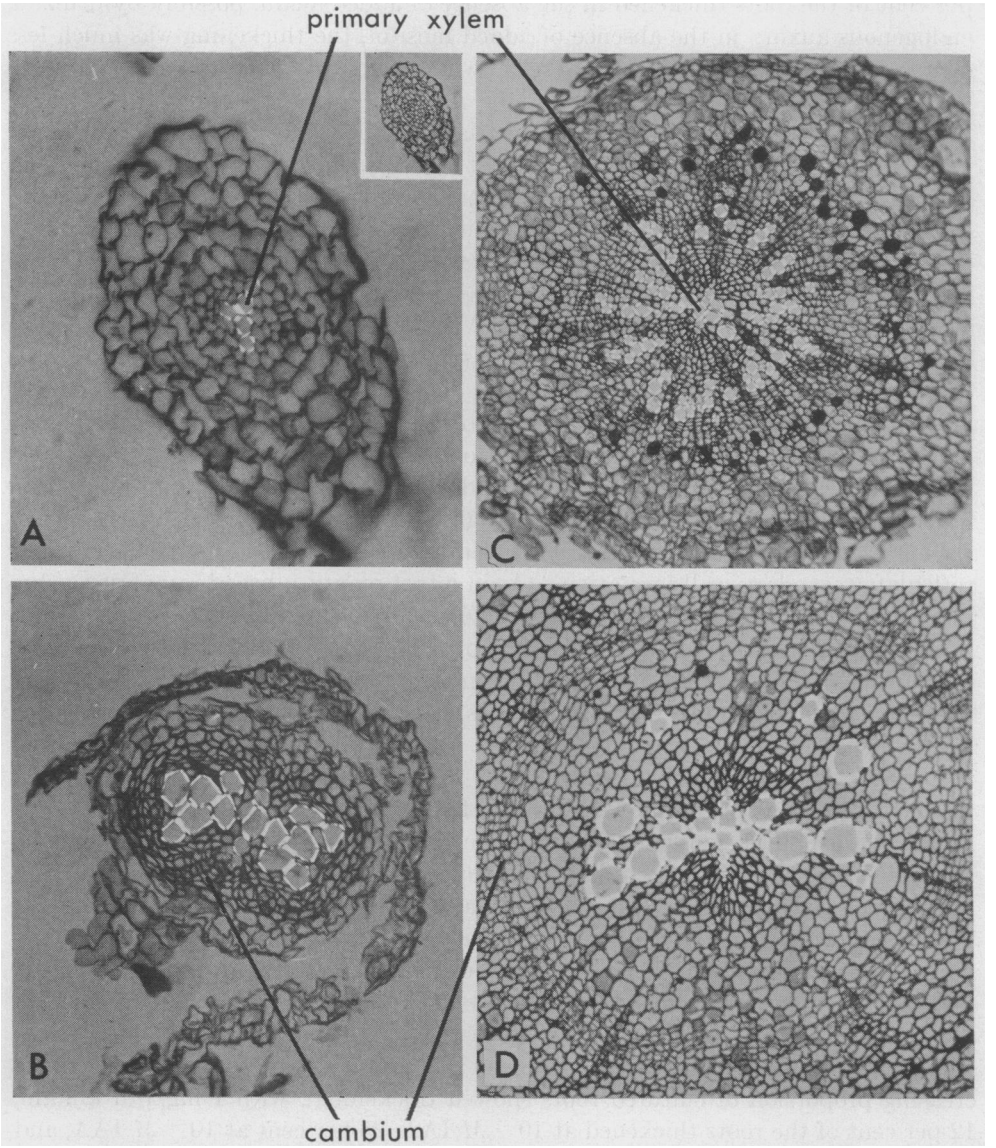

FIG. 2.—Photomicrographs of transections about 10 mm from the base of second-passage radish roots after 20–30 days of culture as shown in Fig. 1. Photographs were taken with polarized light to show the birefringent secondary wall thickening of the xylem elements. (*A*) A root grown on the control medium showing only primary tissues (332 ×; inset at 83 ×). (*B*) With IAA at 10^{-5} *M* and *myo*-inositol at 100 mg/l provided via the base, some cambial activity has occurred (165 ×). (*C*) With IAA at 10^{-5} *M*, *myo*-inositol at 100 mg/l and 6-benzylaminopurine at 1 mg/l provided via the base, pronounced secondary thickening has occurred (83 ×). Compare with the inset in Fig. 2*A* at the same magnification. (*D*) A transection of a normal radish root from an intact plant grown in a controlled environment chamber to a diameter of 1 mm (83 ×). The diarch protoxylem poles are oriented approximately vertical in each figure.

of the position and number of lateral roots except that well-thickened regions of the roots usually did not have an abundance of laterals. Thickening of laterals, particularly those which arose from the new growth of the primary root, sometimes occurred.

Discussion.—The finding that a kinin is required, together with auxin, for significant cambial activity in excised radish roots is an important addition to our knowledge of cambial regulation. The experiments described raise several challenging questions. The first of these concerns the reasons why, in the experimental system described here, cambial activity was limited in duration so that the roots produced were considerably smaller than those obtained in intact plants. Perhaps related to this question was the observation that little thickening occurred on the new growth made by the tip after transfer. Apparently some experimental feature limited the maintenance of activity; it is possible that this was due to the accumulation of some antagonist, to depletion of an unidentified essential factor, or to depletion of one of the known required factors. Thus, for example, a decrease in auxin level could have occurred during the relatively long-term culture. Experiments in which the vial medium is renewed periodically should shed considerable light on this point.

Assuming that kinin is involved in the endogenous regulation of cambial activity in the intact plant, a second question is concerned with the source of the kinin and the kinin transport system. In these experiments, the experimental arrangement between the isolated root and vial medium was such as to allow no other interpretation than that auxin, kinin, sugar, and inositol were absorbed by the root base and moved along at least a 15-mm length of the excised root. That auxin, sugar, and inositol would be transported and utilized is not surprising. The movement of the kinin stimulus even this short distance seems to present some problems since the known kinins are notorious for their low mobility in plant systems.[15] Natural kinins, whose existence is still not fully established, might be more mobile or might be synthesized *in situ* from some mobile precursor translocated from the shoot. Our simplest hypothesis, that in the intact plant a kinin is supplied from the shoot, requires transport of the kinin over only modest distances in a rosette plant such as radish.

Equally intriguing is the question of what organs or tissues might serve as source of kinin, or kinin precursor. The radish axis normally thickens acropetally toward the root tip beginning with hypocotyl tissues which are transitionary between stem and root. Like Brown,[16] one may think of a wave of cambial activity proceeding down the root. The leaves, the stem apex, or the previously initiated cambial tissues might serve to supply both auxin and kinin for extending cambial activity. The idea of autocatalytic activation, i.e., that active cambium stimulates the adjacent tissues, is common in literature on stem cambia.[17] In the present case, the observations and measurements have not been sensitive enough to detect a progressive wave of cambial initiation moving from the root base. There are too many uncertainties extant to justify further speculation on how auxin, kinin, inositol, and sucrose (or their endogenous equivalents) might be involved in the organization of the root cambium and in the normal integration of root and shoot development. Certainly there are ample possibilities for environment, including day length, to exert controlling influences.

Our observations add another example to the rapidly lengthening list of morphogenetic processes which both auxin and kinin can regulate. In fact, the ability of auxin alone to induce cell division in the vascular cambia of stems led some[18] to suggest that endogenous kinins also must be present. It is interesting that Skoog and Miller[14] observed root thickening in bean seedlings supplied with kinetin at 0.5 mg/l in the root environment. Although there is no anatomical evidence, it seems possible that this thickening resulted from the stimulation of a cambium under the influence of kinin and endogenous auxin. Comparison of the present results with earlier experiments with radish,[2] beet,[1] and pea[3] in which auxin alone sufficed to induce cambial activity leads to the hypothesis that subculture of radish (but not of pea) depletes endogenous kinins below some critical level. It seems worth while to seek in similar fashion circumstances under which the gibberellins, which may activate cambium in tree shoots,[19, 20] might be limiting for root enlargement. Whatever the outcome of such questions on the regulation of cell division, the subcultured radish root with basal feeding offers an appropriate experimental system with which to study a variety of other morphogenetic problems.

Summary.—Vascular cambia were induced chemically in excised "White Icicle" radish roots grown in sterile culture on a defined medium. Seedling roots which had been depleted of endogenous substances by one subculture period after excision underwent marked thickening when both kinin (at 1 mg/l) and auxin (at 10^{-5} *M*) were supplied together with sucrose via the base of the root. *Myo*-inositol stimulated root growth and greatly promoted the thickening. The duration and longitudinal extent of the cambial activity was limited, in these experiments, probably because some factors, as yet unidentified, became deficient.

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[1] Winter, H., *Planta*, **44**, 636–668 (1954).

[2] Jones, R. W., "Secondary thickening in isolated roots of White Icicle radish," Ph.D. thesis, Univ. of Calif. (Los Angeles), 1954.

[3] Torrey, J. G., *Symp. Soc. Exptl. Biol.*, **17**, 285–314 (1963).

[4] Torrey, J. G., and Y. Shigemura, *Am. J. Botany*, **44**, 334–344 (1957).

[5] Torrey, J. G., in *Cell, Organism and Milieu*, ed. D. Rudnick (New York: Ronald Press Co., 1959), chap. 7.

[6] Garner, W. W., and H. A. Allard, *J. Agr. Res.*, **23**, 871–920 (1923).

[7] Angell, F. F., and I. G. Hillyer, *Proc. Am. Soc. Hort. Sci.*, **81**, 402–407 (1962).

[8] White, P. R., *Am. J. Botany*, **25**, 348–356 (1938).

[9] Bonner, J., and P. S. Devirian, *Am. J. Botany*, **26**, 661–665 (1939).

[10] Seed was obtained from the Asgrow Co., New Haven, Conn.

[11] Torrey, J. G., *Physiol. Plant.*, **12**, 873–887 (1959).

[12] The gibberellin was obtained from Merck, Sharp and Dohme, Rahway, N. J. The 6-benzylaminopurine was the generous gift of the Shell Development Co. (Dr. J. van Overbeek), Modesto, Calif.

[13] Hayward, H. E., *The Structure of Economic Plants* (New York: Macmillan Co., 1938), chap. 10.

[14] Skoog, F., and C. O. Miller, *Symp. Soc. Exptl. Biol.*, **11**, 118–131 (1957).

[15] Miller, C. O., *Ann. Rev. Plant Physiol.*, **12**, 395–408 (1961).

[16] Brown, A. B., *New Phytol.*, **34**, 163–179 (1935).

[17] Wilcox, H., in *Tree Growth*, ed. T. T. Kozlowski (New York: Ronald Press Co., 1962), chap. 3.
[18] Thimann, K. V., *Ann. Rev. Plant Physiol.*, **14**, 1–18 (1963).
[19] Bradley, M. V., and J. C. Crane, *Science*, **126**, 972–973 (1957).
[20] Wareing, P. F., *Nature*, **181**, 1744–1745 (1958).

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*BEHAVIOR OF PLANTS UNDER EXTRATERRESTRIAL CONDITIONS: SEED GERMINATION IN ATMOSPHERES CONTAINING NITROGEN OXIDES**

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The presence of nitrogen oxides in the atmosphere of Mars has been cited as evidence for the nonexistence of plant life,[1] and this argument has been put forth recently in a critical commentary on the federal space program with respect to exploration of the Martian surface.[2]

The present communication is not intended as a discussion of life on Mars, nor of governmental plans and policies pertaining to its exploration. It is entirely addressed to the validity of the claim that the presence of nitrogen oxides in planetary atmospheres precludes the existence of vegetable life. The conclusion of Kiess *et al.*[1] must have been based upon terrestrial plant life; hence a test of the validity of their assertion requires experimentation with common earthly organisms and has no necessary bearing upon indigenous vegetation elsewhere.

In early 1962, it was observed in this laboratory that winter rye, although capable of remarkably good germination in the absence of oxygen (under argon, for example), was, even more remarkably, enhanced in rate by certain gases usually regarded to be toxic, among them N_2O.[3] This finding alone constitutes evidence contrary to the assertion that oxides of nitrogen exclude plant life, but it is by no means isolated or unique, as our additional experimentation has shown.

Experimental.—In contrast with other studies,[4] no attempt has been made to simulate a Martian environment. The experimental atmosphere selected consisted of N_2 or mixtures of N_2 with nitrogen oxides at a total pressure of 75 mm Hg. In preliminary experiments, CO_2 (up to 3 vol %) was included, but was found to have no effect on germination under these circumstances.

Seeds were germinated in hemispherical Plexiglas chambers on a moist filter paper substratum or on filter paper placed over a 1-cm layer of moist, crushed chalk ($CaCO_3$). Seeds were incubated at 25°C in laboratory light (*ca.* 100 ft-c, daylight fluorescent for 8 hr, darkness for 16 hr).

Germination data were based upon duplicates of 30–50 seeds.

Seeds tested included: bean (*Phaseolus vulgaris*, "Black Valentine"); sweet pea (*Lathyrus odoratus*, "Blue Marvel"); onion (*Allium cepa*, "Southport"); tomato (*Solanum lycopersicum*, "Manalucie"); sorghum (*Sorghum vulgare*, "Combine Kafir 40"); rice (*Oryza sativa*, "Calora"); and rye (*Secale cereale*, "winter"). Other biological materials used in exploratory tests will be noted below.

Results and Discussion.—Among the seven species surveyed (Table 1), all but