American Journal of Botany

Duration of Cell Cycles in Cultured Roots of Convolvulus
Author(s): Harry L. Phillips, Jr. and John G. Torrey
Source: *American Journal of Botany*, Vol. 59, No. 2 (Feb., 1972), pp. 183-188
Published by: Botanical Society of America
Stable URL: http://www.jstor.org/stable/2441399
Accessed: 23/08/2011 15:40

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at
http://www.jstor.org/page/info/about/policies/terms.jsp

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Botanical Society of America is collaborating with JSTOR to digitize, preserve and extend access to *American Journal of Botany*.

http://www.jstor.org

Amer. J. Bot. 59(2): 183–188. 1972.

DURATION OF CELL CYCLES IN CULTURED ROOTS OF CONVOLVULUS[1]

HARRY L. PHILLIPS, JR. AND JOHN G. TORREY
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT

The durations of the cell cycle in physiologically different regions of the meristem of cultured roots of *Convolvulus arvensis* were determined by the metaphase-accumulation technique involving colchicine. The cell cycle in the root cap increases from 13 hr in the actively dividing initials of the first tier to 155 hr in the slowly dividing initials of tiers 2–4 to an indeterminate value for derivatives of the initials in the root cap columella. The cycle times for the cells of the central cylinder and cortex are 21 and 27 hr, respectively. The cells of the quiescent center have a cycle of an estimated 420 hr. The duration of the cell cycle in these different regions is discussed in relation to the increased duration of G_1 in slowly or non-dividing cells. The possible regulation of cell division by the synthesis of a cell-division factor in the quiescent center is also discussed.

EARLY STUDIES on the duration of the cell cycle in root meristems of intact plants yielded average values for the total elapsed time from one mitosis to the next for cells of the whole meristem. These studies did not consider possible differences in cycle times of populations of cells within the apex which might be physiologically distinct from one another. Then Clowes (1956) showed that a region of infrequently dividing cells, which he called the quiescent center, existed in close proximity to rapidly dividing initials which gave rise to different populations of cells in the apical meristem of roots of *Triticum* and *Zea*. Thereafter, attention shifted to the determination of cell-cycle times in different regions of the apical meristem in an attempt to understand how differential rates of cell division influenced the development and maintenance of an organized meristem.

Clowes (1961) determined the total length of the cycle for cells of the quiescent center, the root cap initials, and the initials of the stele and cortex in seedling roots of *Zea mays* by two different experimental techniques; namely, continuous labelling with tritiated thymidine, and metaphase accumulation with colchicine. He reported that the root cap initials had the shortest cycle (12 hr), while cells of the quiescent center had the longest (239 hr). The initials of the stele and cortex had cycle times of 25–28 hr and 48–55 hr, respectively. Using the pulse-labelling technique with tritiated thymidine, Clowes (1965) found that the duration of the cycle for the root cap initials and the stelar initials in roots of *Zea mays* was 14 and 22 hr, respectively. Also, using the pulse-labelling technique, Barlow (1969) reported that the total cycle time for the cap initials, the stelar initials, and cells of the quiescent center in roots of *Zea mays* was 14, 15–24, and 370 hr, respectively. These data on corn roots, derived from quite different techniques, were consistent with each other and demonstrated a remarkable substructure within the root apex, based on populations of cells with different cell cycles. Further studies on cell cycles in roots of *Sinapis alba, Vicia faba*, and *Allium sativum* by Clowes (1962), Clowes and Hall (1962), and Thompson and Clowes (1968), respectively, showed similar values for the different regions of the root proper. One difference was that in *Vicia faba* the cycle time for the root cap initials was greater than that for the stelar initials.

Cultured roots of *Convolvulus arvensis* were shown to have a quiescent center located subterminally in the root apex (Phillips and Torrey, 1971a). In such roots the cells of the root cap columella terminate DNA synthesis after their formation by the root cap initials, indicating that physiologically different populations of cells exist within the root cap itself (Phillips and Torrey, 1971b). The purpose of the experiments to be described was to determine the cell-cycle times for the different populations of cells within the apical meristem of *Convolvulus* by the metaphase-accumulation technique.

MATERIALS AND METHODS—Cultured roots of *Convolvulus arvensis* L., obtained from a clone of roots maintained in modified Bonner-Devirian medium (Torrey, 1954) over a period of 18 years by sub-culturing root tips and root segments, were cultured in 125 ml Erlenmeyer flasks containing 50 ml of liquid medium in the dark

[1] Received for publication 21 June 1971.
This research was supported in part by research grant GM-08145 and a fellowship GM-41021 to H. L. P. from the National Institutes of Health, USPHS.

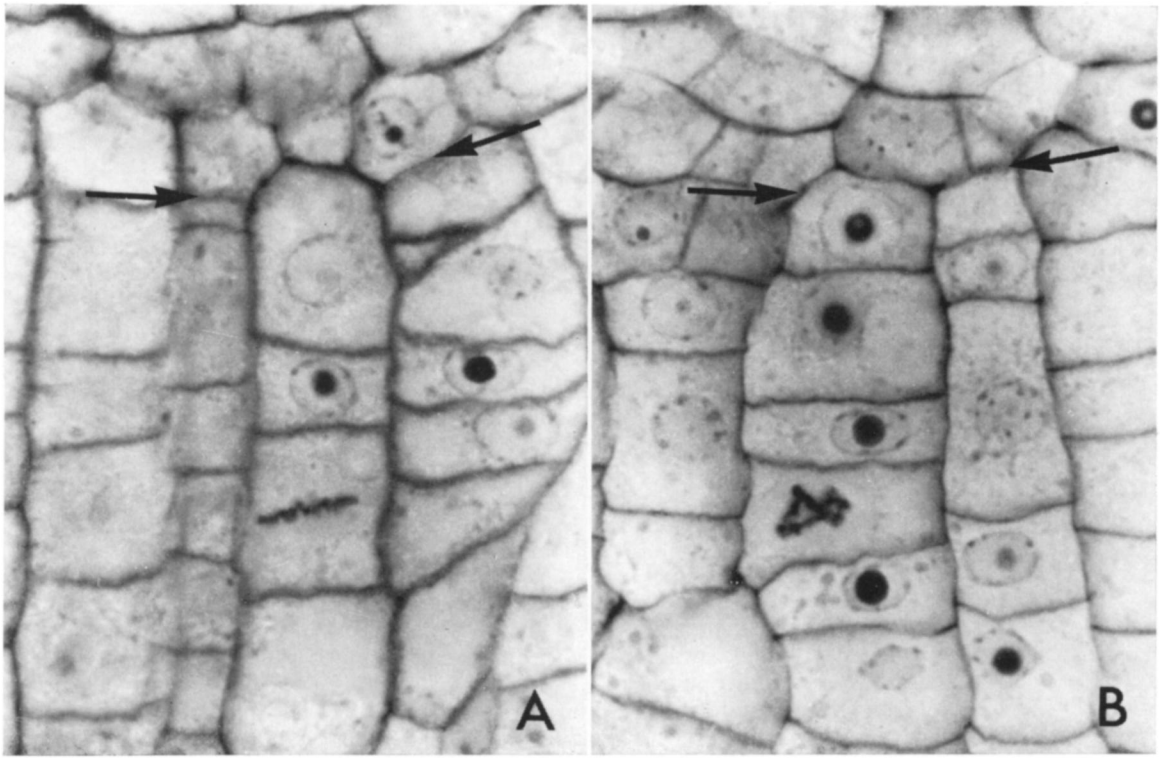

Fig. 1A. Enlarged view of a longitudinal section of *Convolvulus* root grown in control medium fixed at 0 hr, showing a metaphase figure in the third tier of the root cap initials. Arrows mark root cap junction. × 1470.—Fig. 1B. Longitudinal section of *Convolvulus* root treated with 0.05 % colchicine for 5 hr, showing a blocked metaphase figure in the fourth tier of the root cap initials. Arrows mark root cap junction. × 1380.

at 23 C. Cultures were agitated continuously on a horizontal rotary shaker at 80 rpm. Prior to use, root tips were transferred to fresh medium for 3–4 days to determine the approximate rate of elongation. Root tips elongating at a rate of 15–30 mm per day were used in the experiments to be described.

Colchicine treatment—Colchicine was added to sterilized medium to give a concentration of 25 mg/ml. This solution was then Millipore filtered, and 1 ml added per 49 ml of sterilized, modified Bonner-Devirian medium to give a final concentration of 0.05 % colchicine. Evans, Neary, and Tonkinson (1957) showed that metaphase accumulation in roots of *Vicia faba* between 1–6 hr was the same for concentrations of 0.1, 0.05, and 0.025 % colchicine.

The following procedures were used for the experimental treatments:

1) Four untreated cultured roots were fixed to provide the 0 hr sample.
2) Sixteen cultured roots were transferred to four flasks (four roots/flask) to which 50 ml of nutrient medium with 0.05 % colchicine had been added. Four root tips were fixed at 2, 4, 8, and 12 hr.
3) Sixteen cultured roots were transferred to two flasks (eight roots/flask) to which 60 ml of nutrient medium with 0.05 % colchicine had been added. Four root tips were fixed at 0.5, 1, 3, and 5 hr.

Fixation, dehydration, and embedding procedures—Roots were fixed in 3 % glutaraldehyde in 0.025 M phosphate buffer (*p*H 6.8) for either 2–2½ hr at room temperature or overnight in the cold (4 C). They were subsequently stored in 0.05 M phosphate buffer (*p*H 6.8) at 4 C until dehydration. Procedures for dehydration and embedding in Epon-Araldite were the same as those previously reported (Phillips and Torrey, 1971a). Serial longitudinal sections 5 μ thick were obtained with a dry knife on a Huxley Ultramicrotome and mounted on gelatin-coated slides. After heating on a hot plate and allowing to cool, the sections were stained with 0.05 % toluidine blue in phosphate buffer (*p*H 6.8) with 1 % sodium borate for 3 min on the hot plate. The sections were mounted in Permount.

Populations of cells in the root apex—The cells of the root cap can be separated into three regions: the root cap initials, the root cap periphery, and

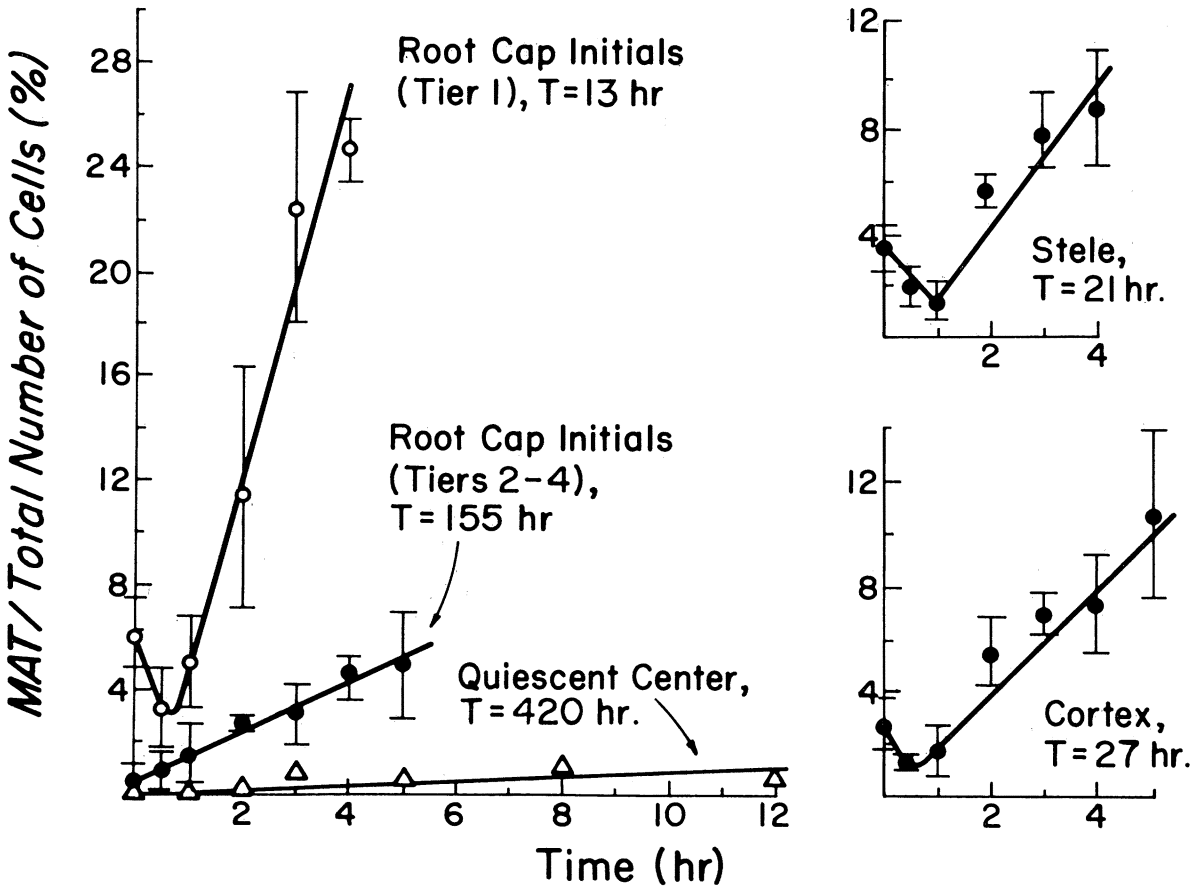

Fig. 2. The percentage of cells at metaphase, anaphase, and telophase (MAT) per total cells counted are plotted as a function of time in colchicine. The duration of the cell cycle (T) is shown for each region of the root apex.

the root cap columella. The root cap initials, in turn, can be subdivided into the rapidly dividing cells (mitotic index, 7.5%) found in the first tier immediately distal to the root cap junction and the more slowly dividing cells (mitotic index, 0.46%) found in the second, third, and fourth tiers from the root cap junction (Fig. 1). In *Convolvulus* the cell wall layer which composes the root cap junction stains more intensely than walls between cells in the root proper and in the root cap, so the root cap initials can easily be recognized. In addition, the initials found in the first tier are morphologically distinct from the cells of the root proper. These initials give rise to the root cap columella and are distinct from the initial cells which give rise to the root cap periphery. About 77% of all the cell division figures observed in the root cap initials in stained serial sections were found in the first of the four tiers immediately distal to the root cap junction.

The cells of the root proper immediately proximal to the root cap junction can be subdivided into the cells of the quiescent center (mitotic index, 0.13%), the initials of the stele and their derivatives (mitotic index, 3.5%), and the initials of the cortex and their derivatives (mitotic index, 3.5%).

Selection of regions of initials for cell cycle calculations—The regions which were selected for calculations of cell cycle times were as follows: the root cap initials in the first tier, root cap initials in tiers 2–4, the cells of the quiescent center, the stelar initials and their immediate derivatives within 250 μ of the root cap junction, and the cortical initials and their derivatives within 250 μ of the root cap junction. Only cells within the 850 μ root tip (including the root cap) were included in these calculations. The data for the cells of the quiescent center, the stele, and the cortex were obtained by counts taken from three alternate 5 μ sections, including the most median one. The data for the root cap initials were obtained from 5 to 11 alternate serial sections (including the most median section) taken from each root.

Values for the cell cycle were calculated according to the method devised by Evans et al. (1957) and modified by Thompson and Clowes (1968). No correction for any cells that may have escaped

TABLE 1. *The percentage of cells at different stages of the cell cycle, the mitotic index, and the number of cells and roots in the sample listed for increasing intervals of time in colchicine*

				Root Cap Initials, Tier 1					
t[a]	P	M	A	T	I	MAT	MI	Cells	Roots
0	1.2	3.0	1.4	1.9	92.5	6.3 ± 1.5	7.5	215	4
0.5	0.0	3.3	0.0	0.0	96.7	3.3 ± 1.6	3.3	162	3
1.0	1.3	4.6	0.0	0.5	93.6	5.1 ± 1.7	6.4	218	4
2.0	0.0	11.4	0.0	0.0	88.6	11.4 ± 4.9	11.4	149	4
3.0	1.8	22.4	0.0	0.0	75.8	22.4 ± 4.4	24.1	202	4
4.0	0.9	24.6	0.0	0.0	74.5	24.6 ± 1.2	25.5	129	3
5.0	1.9	21.0	0.0	0.0	77.1	21.0 ± 9.7	22.9	169	4
8.0	1.6	27.5	0.0	0.0	70.9	27.5 ± 7.9	29.2	119	3
				Root Cap Initials, Tiers 2–4					
t	P	M	A	T	I	MAT	MI	Cells	Roots
0	0.0	0.1	0.0	0.4	99.5	0.5 ± 0.5	0.5	636	4
0.5	0.0	0.7	0.1	0.0	99.2	0.8 ± 0.8	0.8	480	3
1.0	0.0	1.3	0.1	0.0	98.6	1.4 ± 1.1	1.4	662	4
2.0	0.0	2.6	0.0	0.0	97.4	2.6 ± 0.3	2.6	232	2
3.0	0.0	3.0	0.0	0.0	97.0	3.0 ± 1.1	3.0	540	4
4.0	0.0	4.3	0.0	0.0	95.7	4.3 ± 0.8	4.3	261	2
5.0	0.0	4.9	0.0	0.0	95.1	4.9 ± 0.1	4.9	419	4
				Stele					
t	P	M	A	T	I	MAT	MI	Cells	Roots
0	0.0	2.1	0.6	0.8	96.5	3.5 ± 0.9	3.5	1003	4
0.5	0.3	1.0	0.2	0.8	97.7	2.0 ± 0.7	2.3	1002	3
1.0	0.4	1.3	0.0	0.1	98.2	1.4 ± 0.6	1.8	1275	4
2.0	0.1	5.6	0.0	0.0	94.3	5.6 ± 0.6	5.7	655	2
3.0	0.1	7.8	0.0	0.0	92.1	7.8 ± 1.4	7.9	1633	4
4.0	0.0	8.7	0.0	0.0	91.3	8.7 ± 2.1	8.7	978	3
5.0	1.0	7.5	0.0	0.0	91.5	7.5 ± 0.5	8.5	1259	3
8.0	0.0	10.5	0.2	0.1	89.2	10.8 ± 2.2	10.8	796	2
12.0	0.1	10.6	0.0	0.0	89.3	10.6 ± 2.6	10.7	1401	4
				Cortex					
t	P	M	A	T	I	MAT	MI	Cells	Roots
0	0.3	1.3	0.7	0.8	96.9	2.8 ± 0.9	3.5	1253	4
0.5	0.3	0.7	0.0	0.6	98.4	1.3 ± 0.2	1.6	1149	3
1.0	0.2	1.6	0.0	0.0	98.2	1.6 ± 0.9	1.8	1438	4
2.0	0.5	5.4	0.0	0.0	94.1	5.4 ± 1.3	5.9	687	2
3.0	0.2	6.9	0.0	0.0	92.9	6.9 ± 0.7	7.1	1864	4
4.0	0.3	7.3	0.0	0.0	92.4	7.3 ± 1.9	7.7	1191	3
5.0	0.6	10.7	0.0	0.0	88.7	10.7 ± 3.2	11.3	1318	3
8.0	0.1	9.9	0.0	0.1	89.9	10.0 ± 0.6	10.1	906	2
12.0	0.1	8.5	0.0	0.0	91.4	8.5 ± 0.7	8.6	1684	4
				Quiescent Center					
t	P	M	A	T	I	MAT	MI	Cells	Roots
0	0.0	0.1	0.0	0.0	99.9	0.1	0.1	806	2
0.5	0.0	0.0	0.0	0.0	100.0	0.0	0.0	465	3
1.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	516	4
2.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	207	2
3.0	0.0	0.6	0.0	0.0	99.4	0.6	0.6	748	4
4.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	431	3
5.0	0.2	0.4	0.0	0.0	99.4	0.4	0.6	564	3
8.0	0.0	1.1	0.0	0.0	98.9	1.1	1.1	237	2
12.0	0.0	0.7	0.0	0.0	99.3	0.7	0.7	706	4

[a] Column headings: t = time in colchicine (hr), P = prophase, M = metaphase, A = anaphase, T = telophase, I = interphase, MAT = total percentage of metaphases, anaphases, and telophases, MI = mitotic index.

from metaphase to anaphase or interphase was made. In addition, the assumption was made that the cells in the various regions were meristematic and dividing asynchronously.

RESULTS—*The effects of colchicine on the progression of mitosis in different initials regions*—In Table 1 are listed the percentages of nuclei in the various phases of mitosis following the onset of colchicine treatment in different regions of the root. Standard deviations are given for the total number of metaphases, anaphases, and telophases expressed as a percentage of the total number of cells, except for the cells of the quiescent center. No metaphase figures were observed in cells of the root cap columella even after 12 hr.

In the rapidly dividing cells of the stele, cortex, and the first tier of the root cap initials, a decrease in the total number of metaphases, anaphases, and telophases occurred within the first hour of treatment with colchicine, although colchicine-blocked metaphases were apparent by 0.5 hr. The drop in the total number of division figures may have been due to an inhibiting effect of colchicine on mitosis, or more likely to an adjustment of the roots to fresh medium. Between 2–5 hr no cells were found in anaphase or telophase. During 1–5 hr the accumulation of metaphases was linear with time in all the regions investigated, as is shown in Fig. 2. Between 5–12 hr cells started escaping from metaphase, as was evidenced by a greater variability in the percentage of metaphases scored. Evans et al. (1957) reported that during the first 6 hr of treatment of roots of *Vicia faba* with colchicine the accumulation of metaphases was linear, but that after 6 hr it became irregular. They believed that the decrease in the metaphase counts after 6 hr was caused by a suppression of prophase and by cells escaping from metaphase.

From the slopes of the straight lines plotted between 1–5 hr in Fig. 2, the length of the cell cycle for the different regions can be determined following the method of Thompson and Clowes (1968). The value for the quiescent center was obtained from the slope of the straight line plotted for 12 hr and represents merely an order of magnitude. The calculated values of these cycle times (T) are shown in Fig. 2.

DISCUSSION—The cycle time of 13 hr for the sub-population of rapidly dividing initials in the first tier of the root cap is the same as values reported for the root cap initials in *Zea mays* (Barlow, 1969; Clowes, 1961, 1965). Yet, this value is considerably less than that reported for these initials in other species (Clowes, 1962; Clowes and Hall, 1962; Thompson and Clowes, 1968). The cycle time of 155 hr for the cells in the second through fourth tiers of the root cap is the first reported value for a sub-population of root cap initials. One might note that previous investigators did not state precisely which cells were included in their designation of the root cap initials nor did they distinguish differences in cells within this region. The cycle time of 21 hr for initial cells of the central cylinder is the same as, or less than, all previously reported values (Barlow, 1969; Clowes, 1961, 1962, 1965; Clowes and Hall, 1962; Thompson and Clowes, 1968), while the value of 27 hr for initial cells of the cortex is considerably shorter than that reported for the one species in which it was determined (Clowes, 1961). The cycle time of 420 hr for the cells of the quiescent center is longer than any value reported for other species except *Sinapis alba* (Barlow, 1969; Clowes, 1961, 1962, 1965; Clowes and Hall, 1962; Thompson and Clowes, 1968). The experimental data presented above substantiate the evidence from autoradiographic studies, demonstrating differences in labelling with tritiated thymidine in cells of the meristem of *Convolvulus* (Phillips and Torrey, 1971a).

Clowes (1965) and Barlow (1969) found that the differences in the cell cycles in various regions of the meristem of roots of *Zea mays* were caused by a greater duration of the pre-DNA synthetic phase (G_1) in cells of the quiescent center. An increased duration of G_1 also accounted for the low mitotic rate. The other phases of the mitotic cycle were similar in duration in the different regions. Thompson and Clowes (1968) showed that the duration of G_1 was extended in the quiescent center in roots of *Allium sativum*. From absorption microdensitometric measurements of Feulgen-stained sections Clowes (1968) reported that the percentages of cells at 2C, 2–4C, and 4C in the quiescent center of roots of *Zea mays* were 53%, 43%, and 4% respectively. Since pulse-labelling data had shown that only 5% of the cells possessed the 2–4C level of DNA in the quiescent center, Clowes concluded that cells could exist with amounts of DNA between 2C and 4C and yet not be in the DNA-synthetic phase (S) of the mitotic cycle. Thus, in roots of *Zea mays* and *Allium sativum*, cells of the quiescent center are delayed in their passage through the mitotic cycle at the 2C and 2–4C levels of DNA in G_1 and an inactive S (which appears as a component of the G_1 phase in pulse-labelling experiments).

The experimental results in this paper indicate that rapid changes occurred in the cell cycle of root cap cells after their formation by initial cells. The duration of the cell cycle changed drastically from 13 hr in the initials of the first tier to 155 hr in cells found in tiers 2–4. For cells further displaced into the root cap columella the cycle time is extended to infinity. In these cells further DNA synthesis is terminated, and such cells are sloughed off from the root cap between 6–9 days, depending on the length of the root cap (Phillips and Torrey, 1971b). Studies by Barlow (1970) and

Clowes (1968) on the levels of DNA within the root cap of *Zea* showed that the root cap initials were at the 2–4C level, as would be expected for actively dividing cells, while central cap cells were held at the 2C level of DNA.

The published evidence from DNA measurements and from determinations of the relative duration of the various phases of the cell cycle indicate that in two different regions of the apical meristem—the quiescent center of the root proper and the columella of the root cap—slowly dividing or non-dividing cells are primarily delayed in the G_1 phase of the mitotic cycle. Since the quiescent center has been shown to fluctuate in size during development (Byrne and Heimsch, 1970; Clowes, 1958), clearly a mechanism exists within the root for inducing quiescence in cells in the G_1 phase or the early portion of S by preventing further synthesis of DNA. The mechanism of this control remains to be explained.

Thus, it is clear that within the root apex there exists a complex organization based on several populations of cells, each in a different physiological state. The striking patterned differences in cycle times among cells in such close proximity can be most readily explained in terms of gradients within the apex of factors active in inhibiting and/or stimulating cell division. If the quiescent center is the site for the production of such a cell-division factor, one can visualize that the factor would be supra-optimal in the center so as to inhibit cell division, optimal for stimulating cell divisions in the regions of the various initials, then limiting at some distance from the center in a diffusion pattern constrained by internal barriers imposed by cellular differentiation. Such an hypothesis would go far in explaining the complexity of apical organization in the root. To demonstrate its reality at present is a very difficult task.

LITERATURE CITED

BARLOW, P. W. 1969. Phases of the cell cycle in the root meristem of *Zea mays*. *In* W. J. Whittington [ed.], Root growth. Plenum Press, New York.

———. 1970. RNA synthesis in the root apex of *Zea mays*. J. Exp. Bot. 21: 292–299.

BYRNE, J. M., AND C. M. HEIMSCH. 1970. The root apex of *Malva sylvestris*. II. The quiescent center. Amer. J. Bot. 57: 1179–1184.

CLOWES, F. A. L. 1956. Nucleic acids in root apical meristems of *Zea*. New Phytol. 55: 29–34.

———. 1958. Development of quiescent centres in root meristems. New Phytol. 57: 85–88.

———. 1961. Duration of the mitotic cycle in a meristem. J. Exp. Bot. 12: 283–293.

———. 1962. Rates of mitosis in a partially synchronous meristem. New Phytol. 61: 111–118.

———. 1965. The duration of the G_1 phase of the mitotic cycle and its relation to radiosensitivity. New Phytol. 64: 355–359.

———. 1968. The DNA content of the cells of the quiescent centre and root cap of *Zea mays*. New Phytol. 67: 631–639.

———, AND E. J. HALL. 1962. The quiescent center in root meristems of *Vicia faba* and its behavior after acute X-irradiation and chronic gamma irradiation. Radiat. Bot. 3: 45–53.

EVANS, H. J., G. J. NEARY, AND S. M. TONKINSON. 1957. The use of colchicine as an indicator of mitotic rate in broad bean root meristems. J. Genet. 55: 487–502.

PHILLIPS, H. L., JR., AND J. G. TORREY. 1971a. The quiescent center in cultured roots of *Convolvulus arvensis* L. Amer. J. Bot. 58: 665–671.

———, AND ———. 1971b. DNA synthesis in root cap cells of cultured roots of *Convolvulus*. Plant Physiol. 48: 213–218.

THOMPSON, J., AND F. A. L. CLOWES. 1968. The quiescent center and rates of mitosis in the root meristem of *Allium sativum*. Ann. Bot. 32: 1–14.

TORREY, J. G. 1954. The role of vitamins and micronutrient elements in the nutrition of the apical meristem of pea roots. Plant Physiol. 29: 279–287.