

could be further resolved into several anthocyanins.

- c) The simple monoglycosides obtained by other workers are probably products of acidic degradation of the pigment complex.

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1. Nuclear cycle - a correction.

In our previous contribution (MGCNL 42:175-178, 1968) we presented incorrectly the data of Clowes (1965). The correct data attributed to Clowes in our Table should read:

	"Cap. initials	Quiescent center	stele just above quiescent center	stele 200u from quiescent center
T	14	(174)	22	23
G <sub>1</sub>	-1	(151)	2	4
S	8	9	11	9
G <sub>2</sub>	5	11	7	6
M	2	(3)	2	4

Values in parentheses are derived from a value of T obtained by metaphase accumulation."

Clowes, F. A. L. 1965. The duration of the G<sub>1</sub> phase of mitotic cycle and its relation to radiosensitivity. The New Phytologist 64:355-359.

G. R. Douglas

2. Temperature and nuclear cycle in maize root tips.

Douglas reported (MGCNL 42:175-178, 1968) on the nuclear cycle in root tips of 'Seneca 60' at 28°C. These studies have been extended to undertake an examination of some of the factors which might influence the duration of the components of the cycle. Hereditary and environmental factors are being considered. We report at this time data from three temperatures, 20°, 30°, and 35°C, respectively.

Our materials and methods are as described by Douglas with the following modifications:

- i) Roots were grown in constant light (ca.  $8 \pm 2$  ft. c.)
- ii) exposure following  $^3\text{H}$ -thymidine pulsing was for 14 days.
- iii) five primary radicles (3-4 cm) were employed for each hour of each temperature.
- iv) random samples of nuclei were scored on each slide.

Table 1

Classification and frequency of nuclei scored from root tips (*Zea mays* L., hybrid 'Seneca 60') following pulse labeling ( $^3\text{H}$  thymidine, 30 minutes) at three temperatures

Class	Temperature		
	20°C	30°C	35°C
Interphase Labeled	173607	145893	40255
Prophase Labeled	3579	5417	986
Unlabeled	11903	12644	3196
Metaphase Labeled	1052	1408	336
Unlabeled	4215	4444	1166
Anaphase Labeled	359	484	97
Unlabeled	1620	1820	437
Telophase Labeled	1331	2346	325
Unlabeled	6236	6102	1369
TOTAL	203902	180558	48140

The number and classification of nuclei scored are listed in Table 1. The 30°C treatment experiment was conducted first followed by the 20°C and 35°C experiments. Computation of sampling error variances demonstrated that sample sizes could be reduced to the level recorded for the 35°C treatment without sacrifice of homogeneity. Our "rule of thumb" requires about 100 nuclei in the least frequent category, i.e. labeled anaphase.

Table 2  
Duration of the cell cycle in the root tips of Zea mays L.  
(hybrid 'Seneca 60') at three temperatures

Phase	Calculated Duration					
	20°		30°		35°	
	hrs.	%	hrs.	%	hrs.	%
Interphase:						
G <sub>1</sub>	-2.18	-17.1	-0.93	-14.1	-0.73	-19.7
S	7.00	55.1	4.00	61.1	2.00	54.1
G <sub>2</sub>	6.70	52.7	2.59	39.5	2.05	55.4
sub-total	11.52	90.7	5.66	86.4	3.32	89.8
Mitosis:						
Prophase	0.60	4.7	0.46	7.1	0.20	5.4
Metaphase	0.20	1.6	0.15	2.3	0.07	1.9
Anaphase	0.08	0.6	0.06	0.9	0.03	0.7
Telophase	0.29	2.3	0.22	3.3	0.08	2.2
sub-total	1.17	9.3	0.89	13.6	0.38	10.2
Total	12.69		6.55		3.70	

Wimber's (1960) proportion method for calculating the duration of M has been used to project the estimates presented in Table 2. Until we finish collecting the data at 25°C, we have refrained from applying the more extensive probit regression analysis which will yield weighted mean values and proper standard deviations.

In the absence of statistical analyses we have formed only a few impressions of the influence of temperature on nuclear cycle: Firstly, reference to Table 2 indicates that in general the cycle components retain a proportional relationship at all temperatures tested. Since independent calculations of various components have not yet been made, the percentage values reflect not only any relationship existing in the cycle but also reflect the manner in which the component-duration values were computed. Secondly, the decreasing duration intervals with increasing temperature appears consistent with the general growth habit of corn. These data would predict little or no mitotic activity at temperatures consistently below 15°C and/or above 40°C.

The greater changes in the duration of cycle components occur in the interphase nucleus; however, the percentage changes remain constant in both the interphase and mitotic nucleus. The changes in S and G<sub>2</sub> we report are in general agreement with the less extensive studies of Evans and Savage (1959) with Vicia faba and Wimber (1966) employing Tradescantia paludosa.

We consistently obtained negative estimates of G<sub>1</sub> using Wimber's (1960) proportion method. Whether or not this is an artifact of the method of computation remains to be determined. Clowes (1965) reported that G<sub>1</sub> is absent in maize cap initials at 18°C.

We have begun a genotype/temperature interaction study.

#### Literature cited

- Clowes, F. A. L. 1965. The duration of the G<sub>1</sub> phase of the mitotic cycle, and its relation to radiosensitivity. New Phytol. 64:355-9.
- Evans, H. J. and J. R. K. Savage. 1959. The effect of temperature on mitosis and on the action of colchicine in root meristem cells of Vicia faba. Exp. Cell Res. 18:51-61.
- Wimber, D. E. 1960. Duration of the nuclear cycle in Tradescantia paludosa root tips as measured with H<sup>3</sup>-thymidine. Amer. J. Bot. 47:828-834.

Wimber, D. E. 1966. The duration of the nuclear cycle in Tradescantia paludosa root tips at three temperatures as measured with  $H^3$ -thymidine. Amer. J. Bot. 53:21-24.

R. S. Verma

### 3. Estimates of the replication patterns of individual chromosomes.

In MGCNL 42:175-178 (1968) we presented a preliminary study of the nuclear cycle in maize root tip nuclei. This investigation has been extended to determine  $^3H$ -thymidine incorporation patterns in individual chromosomes of maize root tip nuclei.

The stock used was a chromosome 3 tester in which each cell contained one B chromosome. Autoradiographs were prepared in the manner previously reported (MGCNL 42:175-178, 1968) with the following modifications:

- 1) root tips were fixed 8, 9 and 10 hours after pulse labeling.
- 2) root tips were immersed in 0.002 M 8-hydroxyquinoline for 3 hours prior to fixation.

After photographing labeled cells the silver grains were removed in the following manner:

- 1) Slides were soaked in absolute ethanol until the coverslips were removed, then were passed through an alcohol series to distilled water.
- 2) Slides were transferred to 7.5%  $K_3Fe(CN)_6$  for 3 min. and then to 20%  $Na_2S_2O_3 \cdot 5H_2O$  for 3 mins.
- 3) Following 3 changes of distilled water, slides were passed through an alcohol series to absolute ethanol and mounted in Eupanol.

Cells previously photographed were rephotographed and individual chromosomes were identified on these photographs.

Mean silver grain counts over each chromosome (Table 1) were plotted against time (between fixation and pulse labeling). A linear regression coefficient was calculated for each chromosome (Table 2). We interpret a regression coefficient of a chromosome to represent its mode of replication during that portion of the S period in which the chromosome is replicating. Thus the chromosomes demonstrating initial rapid uptake of label will possess the most positive coefficients.