

INTRA-SEASONAL CYCLES OF GROWTH*

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The growth process of organisms is often rhythmic, resulting in the production of well-marked growth cycles. If the growth cycles overlap to any great extent their individual forms are not readily distinguishable, but their presence is generally evident, at least in the more complex organisms. Their study gains interest from the fact that certain physical chemists³ have initiated a noteworthy study of periodic phenomena in heterogeneous systems. The complicated processes of organic growth are admittedly more difficult to study than the simpler inorganic systems of the chemist's beaker, yet there are resemblances which cannot be disregarded. Annual fluctuations in the growth rate of plants are well known, but the intra-seasonal cycles are less prominent. In the cases studied the oscillations of the growth rate diminish from the beginning to the end of the annual growing season.^{5,6,8} One might infer that some agent (in either the environment or the heredity of the organism) damps the oscillations. Evidence has been presented^{7,9} to show that these cycles of growth are actually related to other physiological conditions, e.g., the growth rate of the shoots showed a strong negative correlation with their sap concentration.

I have recently undertaken further studies on the problem of intra-seasonal growth cycles, utilizing measurements of the growth of 94 shoots of lemon trees (*Citrus limonia* Osbeck), growing in a grove at Riverside, California.

Dr. F. F. Halma kindly assisted in taking the measurements during the growing season, which extended from May 1st to October 23rd. The three distinct cycles of growth, each of which ran for about eight weeks, are shown in figure 1. The shoots made approximately the same amount of new growth in the first and second cycles, but fell short of that amount in the third. The curve of seasonal growth of the population is shown by a composite of the three sigmoid curves and shows the characteristic periodic fluctuations.

The rate of growth in each cycle is expressed by the differential equation

$$\frac{dx}{dt} = kx(A - x).$$

The differential equation may also be written

$$\frac{dx}{dt} = k_1(C)$$

where the activity of the catalyst (C) is proportional to $x(A - x)$, increasing from a minimum to a maximum (Fig. 4) and again approaching zero at the end of the growth cycle.

The growth process of each cycle conforms to a curve expressed by the integral equation

$$\log \frac{x}{A - x} = K(t - t_1)$$

in which x = length of shoots at time t ; A = final length; t_1 = time at which $x = \frac{A}{2}$, i.e., when the cycle of growth is half completed; k = a specific constant of the process, and $K = Ak$.

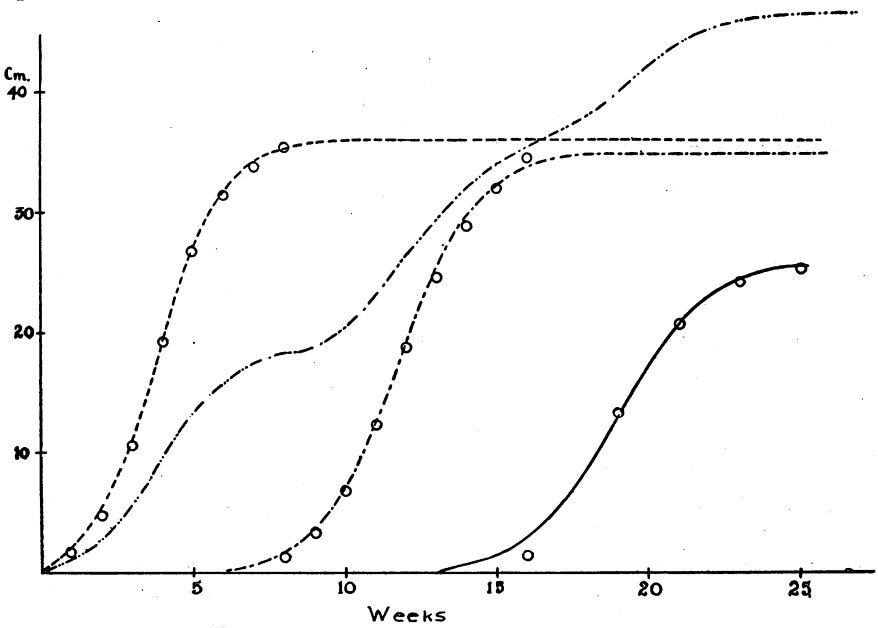


FIGURE 1

Growth curves of lemon shoots, - - - - first cycle, - · - · - second cycle, ——— third cycle, ····· summation of growth of the individual cycles plotted on a scale of ordinates half as great as that employed for the separate cycles. Circles represent observed values.

The equations for the three curves are

$$\text{Cycle I, } \log \frac{x}{36 - x} = 0.411 (t - 3.85)$$

$$\text{Cycle II, } \log \frac{x - 34}{69 - x} = 0.336 (t - 11.75)$$

$$\text{Cycle III, } \log \frac{x - 67}{93 - x} = 0.311 (t - 19.00).$$

The values of A and of t_1 were obtained by approximation, those of K are the averages of the individual constants for the respective cycles. The values of x calculated from these equations are given in table 1. The experimentally obtained values of K were larger at the outset of each cycle and declined, at first rapidly, then more slowly as the cycle progressed. The values of $\log K$ plotted against values of $\frac{x}{A}$, the per cent of growth attained at any given interval, give approximately parallel straight lines (Fig. 2). This results in a slight asymmetry of the growth curve, but it seems advisable to leave this asymmetry uncorrected, although equations giving a closer fitting curve (10) may be used.

TABLE I
LEMON SHOOTS. OBSERVED AND CALCULATED VALUES OF LENGTH

WEEKS	Cycle I			Cycle II			Cycle III				
	x (OBSERVED) CM.	K	x (CALCULATED) CM.	t WEEKS	$x - 34$ (OBSERVED) CM.	K	$x - 34$ (CALCULATED) CM.	t WEEKS	$x - 67$ (OBSERVED) CM.	K	$x - 67$ (CALCULATED) CM.
0	0.09	8	1.19	0.388	1.82	16	1.49	0.406	2.73
1	1.69	0.459	2.27	9	3.26	0.360	3.74	17	5.13	0.305	5.02
2	4.81	0.438	5.32	10	6.89	0.348	7.17	19	13.30	...	13.00
3	10.59	0.447	11.11	11	12.35	0.351	12.56	21	20.64	0.293	20.98
4	19.26	0.406	19.25	12	18.72	0.244	19.18	23	24.28	0.288	24.60
5	26.62	0.395	26.91	13	24.53	0.296	25.34	25	25.32	0.262	25.66
6	31.33	0.385	31.83	14	28.90	0.301	29.78				
7	33.72	0.372	34.25	15	31.96	0.314	32.37				
8	35.19	0.388	35.30	16	34.49	0.427	33.74				

Since the equations indicate that growth proceeded like a series of autocatalytic reactions in which the activity of the catalyst (C) is proportional to $x(A - x)$, it is logical to adopt the idea of an autocatalyst of growth as a working hypothesis. The values of K then represent the activity of some growth-promoting agency in the system, without which none of the external substrates would be converted into organized substance. The general slope of each growth curve represents the activity of the autocatalyst of that particular cycle. The mean values of K were progressively smaller in successive cycles (table 2) and indicate that the activity of the autocatalyst had a downward trend from the beginning to the end of the growing season (Fig. 3). The individual values of K at the outset of the second and third cycles rose, however, to a point nearly level with that at the beginning of the first cycle. The value of K determines the slope of the growth curve (Fig. 1) and may be taken as a constant of the growth process for each cycle. The value of $k \left(= \frac{K}{A} \right)$ is,

however, independent of A and represents the actual constant of growth in any cycle. The values of $\log k$ in the different cycles show a similar parallelism.

The distinct cycles and their sequence is well shown by the derivatives of the growth curves (Fig. 4). The cycles, although distinct, overlap to a slight extent, owing in most instances to the fact that the growth of various individual shoots was not absolutely synchronous. A few shoots continued after the majority of the population had ceased growing, causing the skewness which became pronounced in the third cycle.

TABLE 2
CONSTANTS OF THE CURVES OF GROWTH OF THREE INTRA-SEASONAL CYCLES OF LEMON SHOOTS FOR THE GENERAL EQUATION

$$\text{LOG} \frac{x}{A-x} = K(t-t_1)$$

ALL SHOOTS	CYCLE		
	I	II	III
A	36 cm.	35 cm.	26 cm.
K	0.411 ± 0.0072	0.336 ± 0.012	0.311 ± 0.0222
k	0.0114	0.0096	0.0120
Root-mean-square deviation of calculated values of x	0.43 cm.	0.59 cm.	0.57 cm.
"ONE-CYCLE" SHOOTS			
A	29 cm.		
K	0.473 ± 0.021		
k	0.0163		
Root-mean-square deviation of calculated values of x	0.48 cm.		
SHOOTS AFTER EXCLUDING "ONE-CYCLE" SHOOTS			
A	37 cm.	37 cm.	31.5 cm.
K	0.471 ± 0.011	0.418 ± 0.020	0.361 ± 0.021
k	0.0127	0.0113	0.0115
Root-mean-square deviation of calculated values of x	0.55 cm.	0.74 cm.	0.67 cm.

If the group of meristematic cells in the apical bud of a lemon shoot are killed, growth is subsequently continued from a lateral bud in close proximity to the apex. Apparently the autocatalyst is diffused through many cells of the shoot, since when changed conditions bring lateral meristem into activity, it produces the same type of tissues as those produced by the apical meristem. So far as we can see, the physiological behavior of the shoots gives credence to the idea that the successive cycles of growth are due to the rhythmic activity of one and the same catalyst, having slightly smaller values in succeeding cycles and producing homologous structures in each.

The increase in size of the shoot was produced by the activity of a group of meristematic cells situated at the apex. These cells produced other cells which differentiated into various tissues, e.g., wood, leaves and buds, through which most of the materials necessary for growth were transported to the meristem. It is, therefore, apparent that the growth process of the meristem is regulated to some extent by one of the products of the reaction.

When the shoots begin their first cycle of growth in the spring, they draw largely on the substrates present in the tree, but as the cycle proceeds they supplement their supply of substrates with material drawn from the environment. The shoots may accumulate materials (especially during the latter part of the cycle) which serve as the substrate for the following cycle of growth. The amount of substrate accumulated depends to a large extent upon external factors, but the amount transformed by the plant seems to be more dependent upon internal factors. The processes of growth in succeeding cycles would be a repetition, to some extent, of the steps indicated for the first.

In the present state of our knowledge the analysis of the growth curves and their algebraic forms will supplement the analysis of the physiological data. The growth of these shoots shows a periodicity which is in harmony with the idea that the catalysis involved is a unimolecular reaction consisting of two consecutive reactions, one of which periodically retards the other. An attempt to describe the dynamical aspects of these consecutive reactions in plant growth has been made previously,^{4,6} in which strong evidence of this very process was adduced. I examined the equation for growth proposed by Crozier,² which assumes that there may be two constants of the growth process, and calculated the values

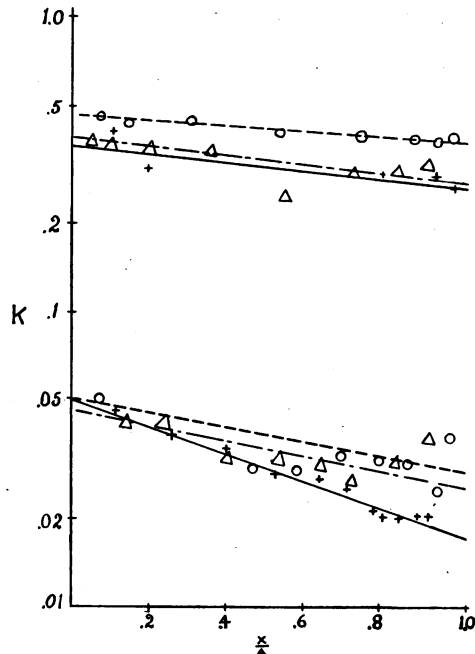


FIGURE 2

Values of K in successive cycles, showing the relation of $\log K$ to values of a/A in successive cycles. Upper graphs, lemon shoots. Lower graphs, apricot shoots; data previously published.⁶ — — — first cycle, - - - second cycle, ——— third cycle. Observed values; ○ ○ ○ first cycle, △ △ △ second cycle, + + + third cycle.

of k_1 and k_2 for it. The values of the constants for the three cycles are given in table 3. The difference between the first and second cycles seems to lie chiefly in the values of k_1 , the second and third differ in the values of A and of k_2 . The interpretation waits for more detailed studies on the effects of environmental conditions. As yet there is no direct or

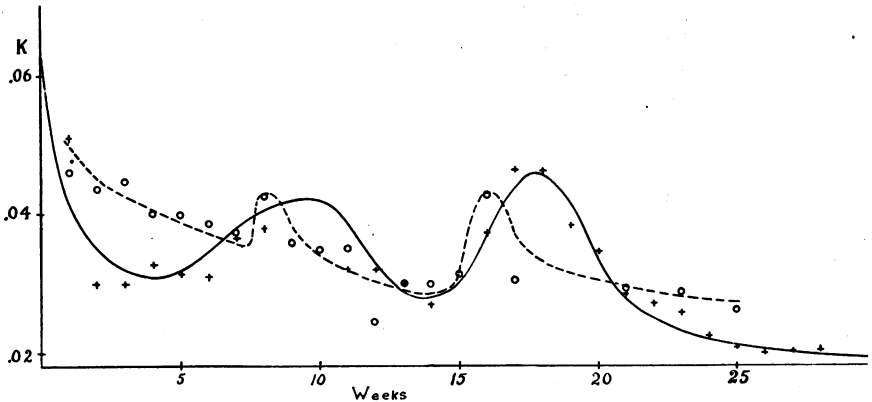


FIGURE 3

Oscillations in the values of K in three intra-seasonal growth cycles. —, values of K for apricot shoots, taken from data in⁶ — — — same $\times 10$ for lemon shoots; + + + actual values of K for apricot shoots, O O O circles same for lemon shoots.

simple means of measuring the intensity of the retarding factor. One of the principal difficulties lies in the fact that many environmental conditions retard growth and their effect may mask that of the internal inhibitory agent. The nutrition of animals, however, may be controlled very accurately, and the occurrence of growth cycles in animals has frequently been reported.^{1,10}

TABLE 3

CONSTANTS OF THE CURVES OF GROWTH OF THE LEMON SHOOTS FOR THE GENERAL

$$\text{EQUATION } t = \frac{1}{k_1 + k_2 A} \text{ LOG } \left(\frac{A(k_2 x + k_1)}{k_1(A - x)} \right)$$

	CYCLE		
	I	II	III
A	36	35	26
k_1	0.014	0.0085	0.009
k_2	0.010	0.010	0.012
Root-mean-square deviation of calculated values of t (weeks)	0.086	0.398	0.698

The growth process of apricot shoots⁶ likewise had three intra-seasonal cycles which could be expressed by the general equation now discussed. The work cited shows that the mean values of K also showed a progressive decrease in successive cycles, though the individual values rose to approximately similar levels at the commencement of the later cycles (Fig. 3).

The logarithmic values of K plotted against values of $\frac{x}{A}$ give approximately parallel straight lines (Fig. 2) and indicate that the factors which retard the growth process are approximately the same at a corresponding place in a cycle. It now seems that the ideas advanced in the paper just cited were only partially adequate to describe the activity of the catalyst, although it seems highly probable that the fluctuations in growth are due primarily to variations in the activity of the catalyst.

One-Cycle Shoots.—There were 17 shoots in the population under discussion which grew rapidly at first but ceased early in the season. They will be designated as "one-cycle" shoots. These one-cycle shoots were so well distributed throughout the population that they may be regarded as a normal phase of development of these trees. Their measurements were included in the averages thus far discussed and diminished somewhat the averages for the second and third cycles of growth of the entire lot.

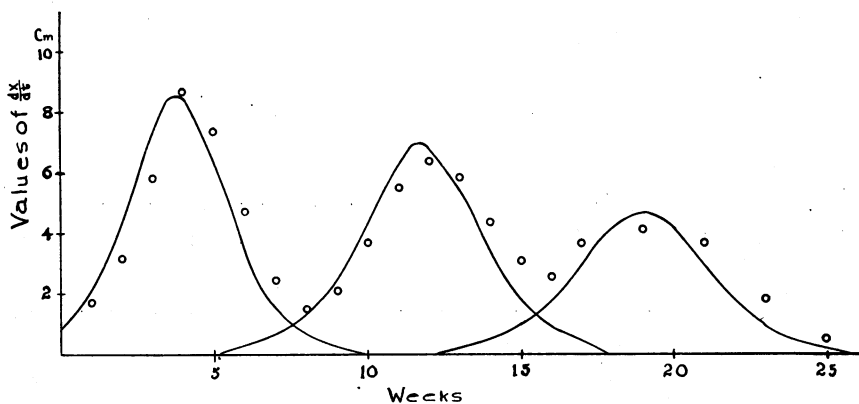


FIGURE 4

Increments of growth of lemon shoots plotted as values of dx/dt .

These one-cycle shoots reached a mean length of 28.11 cm. on the seventh week when the general population had a mean length of 33.72 cm. but the maximum length of the former was 28.93 cm. for the season, whereas the latter attained a length of 92.32 cm. Their growth is of interest because, although they were so closely parallel to the general population in the first four weeks of the season, they were completely arrested at the end of that time. When the values of K were plotted against $\frac{x}{A}$ the values of K for the one-cycle shoots were larger at corre-

sponding stages of development than corresponding values of K for the general population (Fig. 5). This agrees with the fact that these one-cycle shoots completed their growth earlier than the remainder of the

population. The autocatalyst of growth in the three-cycle shoots resumed its activity in a short time and started a new cycle of growth. The constants of the equations for growth led one to the conclusion that the growth of the one-cycle shoots differed from that of their neighbors during the first cycle chiefly in the possession of a greater amount of autocatalyst. Some agency, however, terminated the activity of the autocatalyst at the end of the first cycle. Whether this agency is one of the products of the growth process, or whether it migrates into the shoots from other parts

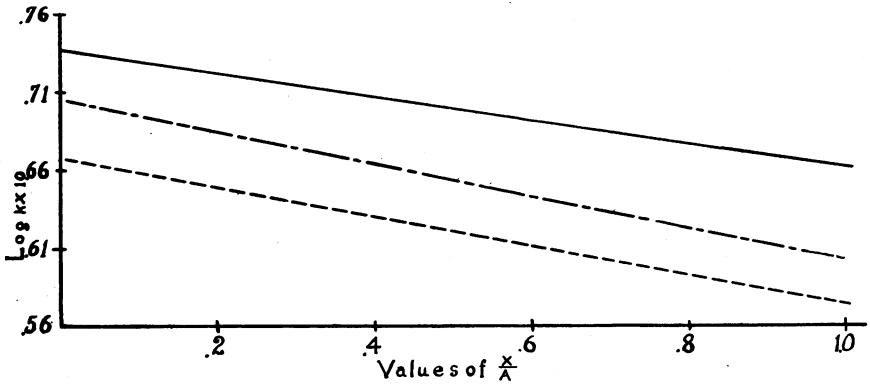


FIGURE 5

Comparison of values of K for one-cycle lemon shoots and those making three cycles. Upper, one-cycle shoots; middle, after excluding one-cycle shoots; lower, average of entire population.

of the tree remains to be determined. We know that starch is often associated with dormancy and its hydrolysis occurs when buds begin to grow. If conditions which favor the activity of carbohydrate-hydrolyzing enzymes also favor the activity of the growth catalyzers there might be renewed activity, resulting in the growth of a new cycle.

Summary.—There were three distinct cycles of growth of lemon shoots during a single season. During the first and second cycles the shoots made nearly equivalent amounts of growth, but they produced less in the third. The successive cycles of growth are assumed to be due to the periodic activity of a specific growth-promoting substance which catalyzed the growth process.

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² Crozier, W. J., *Ibid.*, 10, 53-73, 1926-27.

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¹⁰ Robertson, T. B., *J. Gen. Physiol.*, **8**, 463-507, 1926.

STUDIES ON THE BIOCHEMICAL DIFFERENCES BETWEEN SEXES IN MUCORS

4. *Enzymes Which Act upon Carbohydrates and Their Derivatives*

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Our studies on the biochemical differences between sexes in *Mucors* have been carried along various lines during the past few years.^{1,2,3} Most of the comparative tests which were previously made in an endeavor to discover the fundamental biochemical differences between the (+) and (-) races were quantitative in nature. In another series of tests, attempts have been also made to find out whether or not the (+) and (-) *Mucor* races could be distinguished qualitatively, whether, in other words, some substances which are present in races of one sex are absent in the races of the other sex. Some of the results obtained from such qualitative tests are presented in this paper.

A number of investigators have stated that the *Mucor* species which they tested are dimorphic in respect to certain qualitative biochemical differences. Thus, according to Breslauer⁴ (*Mucor hiemalis*⁵), as well as Kostytschew and Eliasberg⁶ (*M. racemosus*), only races of one sex contain sucrase while this enzyme is absent in the races of the other sex. This conclusion was based in each case on tests with one pair of races only. No evidence, however, is given to show whether the tested (+) and (-) races of *M. hiemalis* correspond to the same sexes of *M. racemosus*. In consequence, the results obtained by these authors are not directly comparable. It seemed desirable, therefore, to repeat the tests with a larger number of races not only to confirm the results just mentioned regarding the presence of sucrase in one sex and not in the other but also to discover how far such a qualitative biochemical difference could be demonstrated throughout this group of fungi. Since such a sexual difference might also be expected in respect to various carbohydrases, we made, in addition to tests for sucrase, a comparative study with a number of other carbohydrases, such as trehalase, amylase, emulsin and others which act upon