SEASONAL FLUCTUATIONS IN GROWTH RATES OF EXCISED TOMATO ROOT TIPS

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(WITH FOUR FIGURES)

Previous to 1934 ROBBINS (10, 11), ROBBINS and MANEVAL (12, 13), KOTTE (6, 7), CHAMBERS (1, 2), MALYSCHEV (8, 9), GAUTHERET (4, 5), and WHITE (14–17) had reported the cultivation of excised root-tips of various species of plants over periods in some cases as great as 5 months. In such of these studies as extended over considerable periods of time, the growth rates showed a more or less continuous diminution, ending in loss of all cultures. Fluctuations in growth rates were apparently random in nature and of no demonstrable significance. In 1934 WHITE (18) reported experiments in which two clones of tomato root-tips were successfully grown for one year. Aside from fluctuations due to known variations in environmental factors, the cultures showed not a diminution but an increase in growth rate. The improvement in result is probably attributable to a more satisfactory nutrient and technique. The observed increase in growth rate was tentatively attributed to adaptation of the cultured organs to their artificial environment, and it was presumed that the growth rate would ultimately reach a constant level. The present paper proposes to report the behavior of one of these clones (clone C, 18) over an additional two years.

Since a standard procedure had not yet been developed at the time the cultures were started, the first 10 passages were of irregular length and were maintained under a variety of experimental conditions. Passages 11–23 were poor for reasons discussed elsewhere (18). With the 11th passage a standard procedure (18) was adopted which has since remained essentially unchanged. The present paper, then, records the behavior of this clone through 177 passages. Of these, 167 have had a duration of one week each and have been maintained under as uniform conditions as were available in an ordinary laboratory room. In passages 11–52, 100 roots were grown in each passage so that each growth rate is the mean of 100 measurements. In all passages subsequent to 52 this number was reduced to 25.

Figure 1 shows the growth rates of this clone of cultures during 177 passages. The records for the last 125 weeks have not been previously published. The line $AA'$ represents the average growth level for the entire 177 weeks, 6.2 mm. per culture per day. The measured total increment recorded, exclusive of branches, is about 30,000 times the magnitude of the tissue fragment with which the clone was started. The clone is being maintained and, after 3 years' cultivation in vitro, is still in excellent condition.
Increment rates of isolated tomato root-tips over a period of 3½ years. The average weekly increments are represented by the zigzag line. The line AA' represents the average increment level for the entire period. The heavy lines at values of the sine curve \( \theta = +1 \) and \( \theta = -1 \) represent the average increment rates of each 26-week period beginning on September 21 and March 21, each period corresponding to a half-wave of the curve. Measurements were not recorded for passages 1, 2, 99, and 100 (marked "x").

In figure 1, the observed fluctuations in growth rate are rather uniformly grouped around the mean level AA'. The clone has, therefore, after the first period of adaptation, settled down to a uniform growth level, as was anticipated. Nevertheless, analysis of the growth curve shows it to contain two types of deviation from the mean. Random deviation from week to week is the most evident component. It seems probable that inequalities in the amount of trauma, inflicted in the manipulations of excision and transfer, may affect the subsequent growth rates. It has also been noted that the position of the explant in the parent culture—whether taken from the tip, from a branch near the center of the culture, or from the base—may affect its subsequent growth rate. There are also marked differences in the vitality of individual branches at the same level, which may be evident in the color of the meristematic region. Doubtless there are other differences which are not visible. These, and probably other factors contribute to the variability of the cultures. The range of this deviation can be greatly reduced by the use of large numbers of cultures, as is evident in passages 27 to 39 where 100 cultures were maintained. It might conceivably be eliminated by the use of sufficiently large numbers or by more careful selection than has been practiced in preparing this routine stock.

When these week to week deviations are smoothed out by averaging together the indices of several passages, there still remains a second component of the curve which shows a regular cyclic fluctuation with maxima lying just a year apart. The horizontal blocks in figure 1 indicate the positions of such means when groups of 26 passages beginning with passage 23 are so averaged. If a flattened sine curve be drawn with AA' as its axis, the wave...
crests spaced 52 weeks apart, with minima (sine θ = −1) falling on December 21, the curve will be seen to coincide rather closely with the observed growth rates, strongly suggesting that these fluctuations must follow some simple law related to the seasons.

Yearly cycles are, of course, common in the plant kingdom. In some cases these are obviously the result of the annual cyclic fluctuations of light and temperatures resulting from the alternation of seasons, and disappear under controlled environmental conditions. In others, Asparagus medeoloides Thunb., for example, the cycle is inherent and so firmly established in the make-up of the plant that in the northern hemisphere it continues to undergo its dormant period in May to September in spite of a completely inverted environmental cycle, and years of cultivation in the north will not alter this behavior pattern. It has seemed desirable to determine whether the cycle observed in these root-tips can be shown to be correlated with uncontrolled factors in the environment, or if it may be inherent.

The cultures in question were maintained under as uniform conditions as were available for large numbers. But, since they were kept in a laboratory room where other work was being carried on simultaneously, they were subject to two variables known to be only partially controlled and perhaps to other unknown ones. These two variables were light and temperature, both of which undergo seasonal cyclic fluctuations.

The cultures under consideration were at no time exposed to direct sunlight but, since they were placed in a well-lighted room, they received diffuse light of greater intensity and longer daily duration in summer than in winter. Robbins and Maneval (13), White (15), and Felber-Pisk (3) had reported moderate illumination to be beneficial to similar cultures of root-tips of other plants, so that light might conceivably have been an important factor in determining the observed fluctuations. However, the studies of these authors were all of too short duration to be of undoubted significance. Malyschev, moreover, was unable to substantiate this conclusion (9). To test the question more thoroughly, a double-walled box was built of opaque matte-black cardboard, large enough to hold 25 cultures. It was so arranged as to allow free circulation of air, but light was completely excluded. Fifty cultures were prepared from passage 143, clone C. Twenty-five of these were placed in the dark box and set alongside of the other 25 which represented passage 144 of figure 1. Both sets of cultures were then transferred at weekly intervals for 10 weeks, being measured as usual at the beginning and end of each passage. Measurements and transfers of both “dark” and “light” cultures were made by daylight. The “dark” cultures were thus exposed to diffuse light for somewhat less than an hour each week, that is, about 0.5 per cent. of the elapsed time, while the “light” cultures were similarly exposed approximately 100 times this length of time (50 per cent.
of the elapsed time). The growth rates obtained are shown in figure 2. The mean difference in growth rates between the two sets for the entire 10 weeks was only 0.6 per cent., in favor of the "dark" cultures. This is too small a difference to be significant. It is worth noting, however, that had only passage 151 been measured, the result—a 40 per cent. increase of the "light" cultures over the "dark" ones—would have been in agreement with the findings of the earlier authors cited above, while observation of passage 152 alone would have led to an entirely contrary conclusion. The danger in
drawing conclusions from single passages is thus evident. To make the result doubly sure, transfers were made for an additional 5 weeks, the "dark" cultures being transferred by the light of an orange Wratten Safe Light no. 0, without other exposure at any time. Throughout these 5 weeks (fig. 2), the cultures grown in the dark were consistently somewhat better than those provided with diffuse daylight. Since 15 weeks of greatly reduced illumination did not appreciably reduce the growth rates of these cultures nor cause any visible abnormalities in their behavior (fig. 3), it may safely be concluded that light is neither essential nor significantly beneficial to isolated tomato root-tips. Seasonal variations in illumination can, therefore, be ruled out as a factor in determining the cyclic variation in growth rate recorded in figure 1.
The room in which the cultures were maintained was thermostatically controlled as to minimum temperature, so that the temperature never fell below 20° C. Since, however, no provision was made for cooling when the outside air temperature exceeded this figure, the room temperature sometimes rose to 32° C. White had earlier shown (15) that isolated wheat roots are sensitive to variations in temperature, growing best at about 27° C., so that this also might have been an important factor in determining the observed cycle.

Had light proved to be a significant factor in determining growth rates, the exact evaluation of temperature effects would have been difficult, since, in order to obtain comparable results, it would have been necessary to maintain a uniform light intensity at all temperatures studied. Since, however, variations in illumination over a rather wide range of intensity are without significant effect, this factor can be ignored in temperature studies so long as the light intensity is kept low. To test the effects of temperature variations, constant temperature ovens providing no illumination and controllable to ± 2° were used for temperatures 5°, 8°, 10°, 15°, 20°, 25°, and 28° C. Temperatures of 30°, 31°, 32°, 33°, 35°, and 40° C. were provided in a water bath which admitted a low intensity of light to the cultures and was controllable to ± 0.5°. Twenty cultures were grown at each temperature, and measurements made at the end of one week. The mean growth rates for single passages at these temperatures are shown in figure 4. They are to be compared with those shown in figures 2 and 3 in the author's earlier work on wheat root-tips (15). The optimum at 30°—slightly higher than for
wheat—is extraordinarily sharp, an increase in maintained temperature from 28° to 30° causing a 20 per cent. increase in growth rate, while a further temperature increase from 30° to 31° caused a 30 per cent. drop in growth rate. Unlike variations in illumination, variations in temperature are obviously of great importance in determining growth rates.

The range of temperatures to which the cultures, whose growth rates are shown in figure 1, were subjected was, as has been said, from 20° to 32° C. As shown in figure 4, this is sufficient to determine a range of mean growth rates from 5 mm. per culture per day (at 20°) up to 15 mm. per culture per day (at 30°) and then back to 9 mm. per culture per day at 32°. Cultures grown at 32° were of poor color, often misshapen, and obviously in unsatisfactory condition. If the growth rates recorded in figure 4 be compared with the mean growth rates for the various seasons, as shown in figure 1, it will be seen that the growth rate for 20° C.—5 mm. per culture per day—corresponds very closely to the rates obtained during three consecutive winters—5.1, 5.1, 6.0—when, owing to low outdoor temperatures, it was possible to maintain the room temperature at 20–22°. The growth rate for 24°, which may be considered an average room temperature for summer,—8 mm. per culture per day—corresponds closely to the rates observed during the three summers covered by the record—7.2, 7.7, 7.2. Moreover, the maximum growth rate recorded in figure 4—15 mm. per culture per day at 30° C.—differs only slightly from that recorded in figure 1, 13.4 mm. per

![Graph showing the relation between mean growth rate and maintained temperature, recorded over a single passage.](figure)
culture per day in June, 1935, at a time when the mean temperature of the culture room may easily have approached such a value.

From the figures presented above it is evident that variations in temperature of the range to which the cultures under consideration are known to have been subjected, a range characteristic of a moderately well controlled laboratory, are quite sufficient to explain the cyclic fluctuations recorded in figure 1. Whether this is, as the data seem to indicate, the sole explanation or whether, when this variant is eliminated by more careful control of the temperature, there will remain a residual cyclic variation which is inherent, must await further study over an additional period of a year or more. The record presented here, however, serves to emphasize what has been repeatedly noted before, that growth which is sufficiently active for experimental purposes and which is reproducible can be obtained with root-tip materials only when all cultural conditions are rigidly controlled. Sensitivity to small variations in temperature and to slight variations in the concentrations of many of the nutrient ions must be taken into consideration in the interpretation of all results. The importance of these particular variables can hardly be over-emphasized; but as is evident from the results with various intensities of illumination, there also exist variables which are not important.

Summary

The growth rates of a clone of isolated tomato root-tips cultivated in vitro and measured at weekly intervals for a period of 3 years show a random fluctuation around a mean due to variations in the behavior of the individual cultures. In addition, these growth rates show a cyclic fluctuation correlated with the seasons of the year. Investigation of the effects of light vs. darkness showed that seasonal fluctuations in illumination are a negligible factor in producing this cycle. Investigation proved, however, that such cultures are very sensitive to temperature differences and that the observed fluctuations in temperature are sufficient to account for the observed seasonal variations in growth rate. It will probably be necessary to control the room temperature more accurately than has been done in the past if uniform cultures are to be maintained throughout the year.

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