A STUDY OF FORMATION, ELONGATION, LONGEVITY AND DEATH OF BARLEY ROOT HAIRS

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This is to certify that the

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ABSTRACT

A STUDY OF FORMATION, ELONGATION, LONGEVITY AND DEATH OF BARLEY ROOT HAIRS

by James Douglas McElgunn

The root hairs of 1 month old barley (Hordeum vulgare L.) seedlings, grown in a growth chamber (14-hour day at 20 C and 10-hour night at 10 C), were observed after the roots of intact seedlings were placed in a modified Hoagland Number 2 nutrient solution at three different constant temperatures (15, 18, 26 C).

Formation and elongation were observed for 24 hour periods. Longevity was observed until death occurred as indicated by a neutral red staining technique.

The number of root hairs formed was greater at 18 and 26 C than at 15 C. Formation was periodic, with three peaks and two minimums. The changes in formation rate did not coincide precisely at each temperature.

Elongation was greater at 26 C than at the 15 C temperature. The elongation rate was a constant for each of the temperatures over a 24 hour period.

Longevity of root hairs was 40, 45 and 55 hours at 26, 18 and 15 C respectively. A distinct peak period of death occurred at 7 to 9 p.m.

It was concluded that the phasic formation and death of root hairs may affect diurnal water absorption due to changes in the living and the dead root hair areas. Elongation rate may also be a factor in water uptake as it is affected by soil conditions, although the elongation was constant in this research.

A STUDY OF FORMATION, ELONGATION, LONGEVITY AND DEATH OF BARLEY ROOT HAIRS

By

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INTRODUCTION

Root hairs are ephemeral tubular outgrowths of the exterior wall of root epidermal cells. They function as water absorption surfaces and aid in anchorage.

Previous work has shown root hair formation and elongation to be affected by a host of physical and chemical factors. Research is lacking as to the time of formation and elongation of root hairs and their longevity. If their formation, elongation, or death is phasic it could shed some evidence as to the nature of the phasic rate of water absorption evident in plants.

The purpose of this research was to study the hourly rate of formation and elongation of barley (Hordeum vulgare L.) root hairs growing under three different temperatures.

Longevity was studied to determine the length of life of root hairs and also to ascertain if time of death is phasic.

LITERATURE REVIEW

Occurrence

The occurrence of root hairs on the roots of plants is not universal. Some aquatic plants possess root hairs whereas some arid and desert plants lack root hairs. Plants may produce root hairs under one set of conditions yet be devoid of root hairs when grown under another set.

Root hairs do not occur over the entire surface of the root and are lacking on the terminal portion of the root, which consists of the root cap and regions of cell division and elongation (Farr 1928 c). Also the older portions of the root, those nearest the stem, are usually devoid of root hairs in that the root hairs have collapsed and sloughed off (Farr 1928 c). Limited to a definite zone on the root, and arising only by acropetal succession (Cormack 1949), root hairs are an effective means of increasing the absorptive surface area of roots. Dittmer (1937 a) in his study of the root system of a 4 month old rye plant (Secale cereale L.) found 14 billion living root hairs with a surface area of 37 m^2. Rosene (1943) has determined that individual root hairs of Allium can absorb water at a rate of 1.86 X 10^{-3} mm^3 / mm^2 / hour. If the rye root hairs were all functional

and in contact with available water the capacity for water uptake would be phenomenal.

Formation

In many plants it cannot be predicted which epidermal cells can or will develop root hairs. Leavitt (1902) noted that certain higher plants have short cells, trichoblasts, which give rise to root hairs, interspersed among longer non root hair producing epidermal cells. Numerous other studies (Bardell 1915, Roberts 1916, Cormack 1935, 1945, 1947, 1949, 1962, Wilson 1936, Sinnot and Block 1939 a, 1932 b, Stebbins 1956, Avers 1957), have verified this and in addition have shown that trichoblasts have a denser protoplasm (Addoms 1923, Sinnot 1939), a larger nucleus (Wilson 1936), and a greater intensity of enzymatic activity (Avers 1958, 1961, Avers and Grimm 1959 a, 1959 b) even prior to hair formation. In plants not having these characteristic trichomatous cells all epidermal cells may be capable of producing hairs with proper internal and external conditions (Snow 1905, Arber 1934, Esau 1960). These conditions are not all known.

Cormack (1949, 1962) has proposed a theory that encompasses the results of many studies (Snow 1905, Jeffs 1925, Farr 1925, 1927 a, 1927 b, 1928 a, 1928 b, Cormack 1935, 1937, 1944, 1945, 1947, Ekdahl 1953) as to the cause of root hair formation. He believes that formation results from retardation of vertical elongation

of the root epidermal cells and that root hairs are produced by internal pressure on weaker portions of an unequally hardened cell wall as affected by calcium. Burstrom (1952) is not in agreement with the latter part of Cormack's theory and explains root hair formation as occurring in two phases:

> During the first phase auxin dissolves the cell wall and elongation occurs without new formation of wall material. Calcium then acts, as assumed by Cormack, by antagonizing auxin during the second phase of elongation and elongation depends on deposition of new material which is prevented by auxin and the action of calcium is reversed accordingly.

Whatever their differences, root hair formation seems to occur after retardation of epidermal cell elongation.

Elongation

The phenomenon of cell elongation has often been found to be a periodic event with a maximum rate taking place when cell division is at a minimum (Lewis 1901, Kellicott 1904, Karsten 1919, Friesner 1920, Beatty 1941, 1944, Bunning 1956, Jensen and Leroy 1958). Diurnal rhythms of cell division and elongation are well documented in Allium, Vicia and other plants. In Allium, the most studied plant, two maximum rates of root cell elongation occur, one at 4 to 5 p.m. and the other at 6 to 8 a.m. (Lewis 1901, Kellicott 1904, Karsten 1919). Elongation is at its maximum in Vicia at 7 to 9 a.m. and 5 to 8 p.m. (Karsten 1919, Friesner 1920, Beatty 1941,

Erickson 1961). In grasses the diurnal rhythm of elongation and cell division of roots, if it exists, has not been studied extensively. Brumfield (1942) found no evidence of rhythmic cell elongation in Phleum pratense L. when grown under continuous light and at a constant temperature. Karsten (1919) found two maximum periods of cell elongation (5 to 7 p.m. and 7 to 11 a.m.) in Zea mays Sturt. grown under an 18 hour day with a day temperature of 25 C and a night temperature of 15 C. Hill and Carnahan (1954) have found that the best time to collect root tips for maximum mitotic figures in orchard grass (Dactylis glomerata L.) and meadow fescue (Festuca elatior L.) is 1 p.m. on a cloudy day at 20 C. Hageman (1956) found no rhythm of cell division or elongation in root tips of barley grown for 1 week in constant darkness and at a constant temperature. His conditions were somewhat unrealistic and could have caused no rhythm to develop or caused it to deteriorate. (Bunning 1956, Leopold 1964).

Root hair formation and elongation probably occur in a phasic pattern if retardation of elongation of epidermal cells is the "trigger." Root hairs were observed for continuous 24 hour periods by Reinhardt (1892), yet he failed to report if growth was periodic and only concluded that they grew at a rate of 0.3 to 0.7 microns per minute. Devaux (1888) has observed, in the presence of light, that root hairs of Lolium and other grasses form cone shaped masses of

hairs and he interpreted this as periodicity produced by enhanced growth in light periods. Goosens and Stapleberg (1933) observed similar cones on Eragrostis plana L. but were of the opinion that these were the result of daily fluctuations of temperature as plants grown in the dark also produced cones. Farr (1925) suggested that root hairs may grow by pulsations and noted a slight retardation of elongation at 9 to 12 a.m. and 4 to 7 p.m. in studies lasting about 15 hours.

Longevity

The longevity of root hairs has not been ascertained. I would like to distinguish between "persistence" and "longevity." The criterion commonly used to indicate persistence has been the lack of decay of root hairs, but whether the root hairs are alive or even functional is not considered. Longevity, although it does not imply functionality, means that the root hairs are still alive as evidenced by certain metabolic activities.

In studies of the persistence of root hairs Whitaker (1923) found that in some Compositae they are not always ephemeral and may persist 1 to 3 years if secondary thickening of the cell wall has occurred. McDougall (1923) reported persistent root hairs in some members of the Leguminosae but observed that the hairs were thick walled and brown colored within a few days after being formed.

Weaver (1925) found that the root hairs of some Gramineae persisted over the whole length of a grass root but failed to mention secondary thickening of walls or brown coloration. Dittmer (1937 a, 1937 b) in his study also found persistence of hairs and used all the hairs present in his calculation of the root surface area. The ability of root hairs to absorb water once having undergone secondary thickening is questioned by Cormack (1949).

The longevity of root hairs is commonly stated to be days in many textbook discussions. Whether any of these reports are based on adequate experimentation is not known. Schaede (1923) has determined that the pH of living Hydrochans root hairs is basic and acid when the root hairs are dead. Cormack (1935) has found that the root hairs of Georgia collards (Brassica oleracea L.) are slightly acid (pH 5.8 to 6.8) at initiation and approach neutrality (pH 6.6 to 7.2) with age. No literature was found where the longevity of root hairs had been carefully studied even though good vital stains which are operative in this pH range are available.

MATERIALS AND METHODS

One month old barley seedlings (Hordeum vulgare L., var. Betzes), were grown in plastic envelopes (DiS PO Growth Pouch, Northrup, King and Co.). The seedlings were grown in 20 ml of No. 2 Hoagland solution to which was added 1500 units of streptomycin sulfate and adjusted to a molar concentration of 0.10 M (about 6.5 atm) with calcium chloride. Calcium chloride was used to aid in attaining a workable number of root hairs and in the neutral red staining procedure used in the longevity study. The pH was adjusted to 6.8 with sodium hydroxide. A 14-hour day with a temperature of 20 C and a 10-hour night with a temperature of 10 C was used for growing the seedlings in a growth chamber. The light source was both incandescent and flourescent lamps.

Individual seedlings were placed in an observation chamber (see Figure 1) which contained 25 ml of the aerated solution described above. The solution was changed every 4 hours. Roots were allowed to equilibrate 4 hours prior to the initial observation.

The desired root temperatures (15, 18, 26 C) were maintained using a Bronwill No. 20 constant temperature circulator. The two lower temperatures were obtained by placing the water reservoir

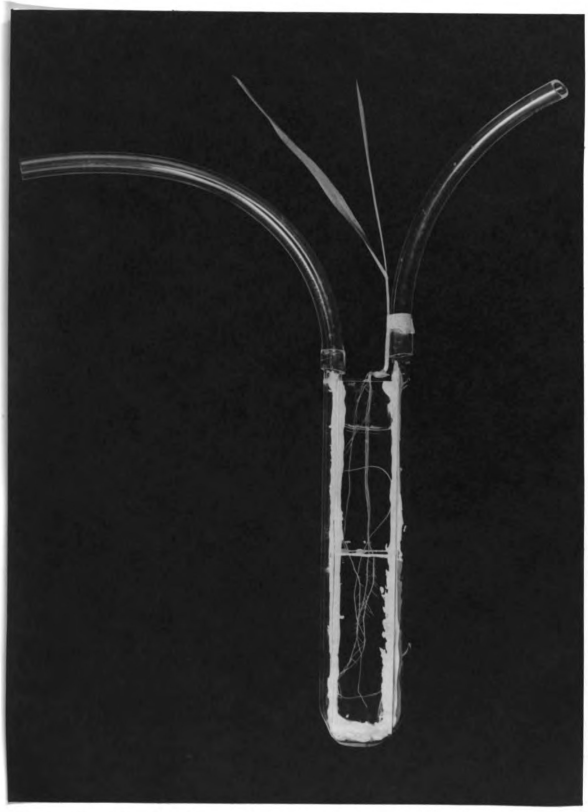

Figure 1. Chamber for continuous observation of root hairs at a constant root temperature.

in an ice bath because the constant temperature circulator had no cooling system to permit attaining temperatures less than ambient temperature. The tops of the plants were maintained at 20 $\pm$ 1 C both day and night in an air conditioned room. Illumination intensity was about one-half that of the growth chamber with the same day and night period.

Observation of Root Hairs

Using a Baush and Lomb projecting microscope (magnification 145X), hourly observations were made by tracing a given portion of a root and later measuring the length of the root hairs with a ruler or ascertaining if new root hairs had appeared. In the formation study a distance of 0.8 mm below the last formed root hair was observed and the data were transformed into the number of new root hairs per 1 mm of root length per row of epidermal cells per hour. In the elongation study, the elongating root hairs 0.8 mm above the most recent formed root hair were observed and the data were recorded as the change in length of a root hair per hour. The root hairs observed in the elongation study ranged from 0.02 to 0.17 mm in length initially. Five to ten root portions (0.8 mm X 1 row of epidermal cells) per temperature were studied for the rate of root hair formation and rate of elongation.

Care was taken to initially select two to four root hairs for use in refocusing at each of the 24 hourly observations so as to positively identify previous root hairs. When studying root hair formation, it was necessary to establish if they arose from the row of epidermal cells under study or if they were merely root hairs from adjacent cells coming into view.

Determination of Longevity

The longevity study used the same procedures as above except that 15 minutes prior to each hourly observation 1 ml of 1% neutral red (pH 6.9) was added to the observation chamber reservoir and staining was allowed to proceed for 5 minutes. This colored solution was then withdrawn and the reservoir flushed twice with medium at the proper temperature and then the medium was replaced a final time. Living cells take up neutral red and retain it within their vacuoles whereas the stain is lost from the vacuoles of dead cells. The Ca^{++} present in the media prevents the cell walls from staining and thus does not obscure observation of the vacuoles. At least 12 newly formed root hairs per temperature were observed until their death. A newly formed root hair was less than 7.0×10^{-3} mm at the initial observation times of 8 a.m., 4 p.m. or 8 p.m. Four root hairs were observed per temperature at each of the three starting times.

RESULTS

In the course of observing formation, elongation and longevity of root hairs certain other observations were noted:

1) As the root hair begins to form, the nucleus is located adjacent to the section of the cell wall from which the protrusion occurs.

2) Numerous small vacuoles appear in the very young root hairs and the vacuoles tend to be located close to the base of the hairs.

3) Vertical elongation of epidermal cells in the root hair zone had all but ceased at beginning of root hair production and no further vertical growth of these cells occurred after the hairs were about 0.25 mm long.

4) Root hairs grown under the conditions used in this study did not show any abnormalities as far as branching, swelling of the tips, discoloration or lack of turgor or plasmolysis when alive.

5) The cone shaped masses of root hairs reported by Deveaux (1888) and Goosen and Stapleberg (1933) were observed

when grown in fluctuating conditions in a non temperature controlled laboratory during preliminary studies.

Formation

The formation rate of root hairs was highly significantly affected by root temperature. An average of 34.8 root hairs per 1 mm per one row of epidermal cells (root segment) per 24 hours formed at a root temperature of 26 C as compared to 16.4 and 17.4 root hairs per root segment per 24 hours formed at 18 and 15 C respectively (Figure 2).

Figure 2 shows the number of root hairs formed per 5 root segments at each hourly observation. The 26 C root temperature showed three distinct peaks and two distinct minimum times of formation. At 18 and 15 C the peaks and minimum did not coincide precisely in time or magnitude with the 26 C readings. The 18 C temperature was somewhat lacking in distinct changes in the rate of root hair formation.

Elongation

The average rate of elongation at 15, 18 and 26 C was 7.25, 7.9 and 8.3×10^{-2} mm per hour respectively. The 18 and 26 C rates were significantly greater than the 15 C rate.

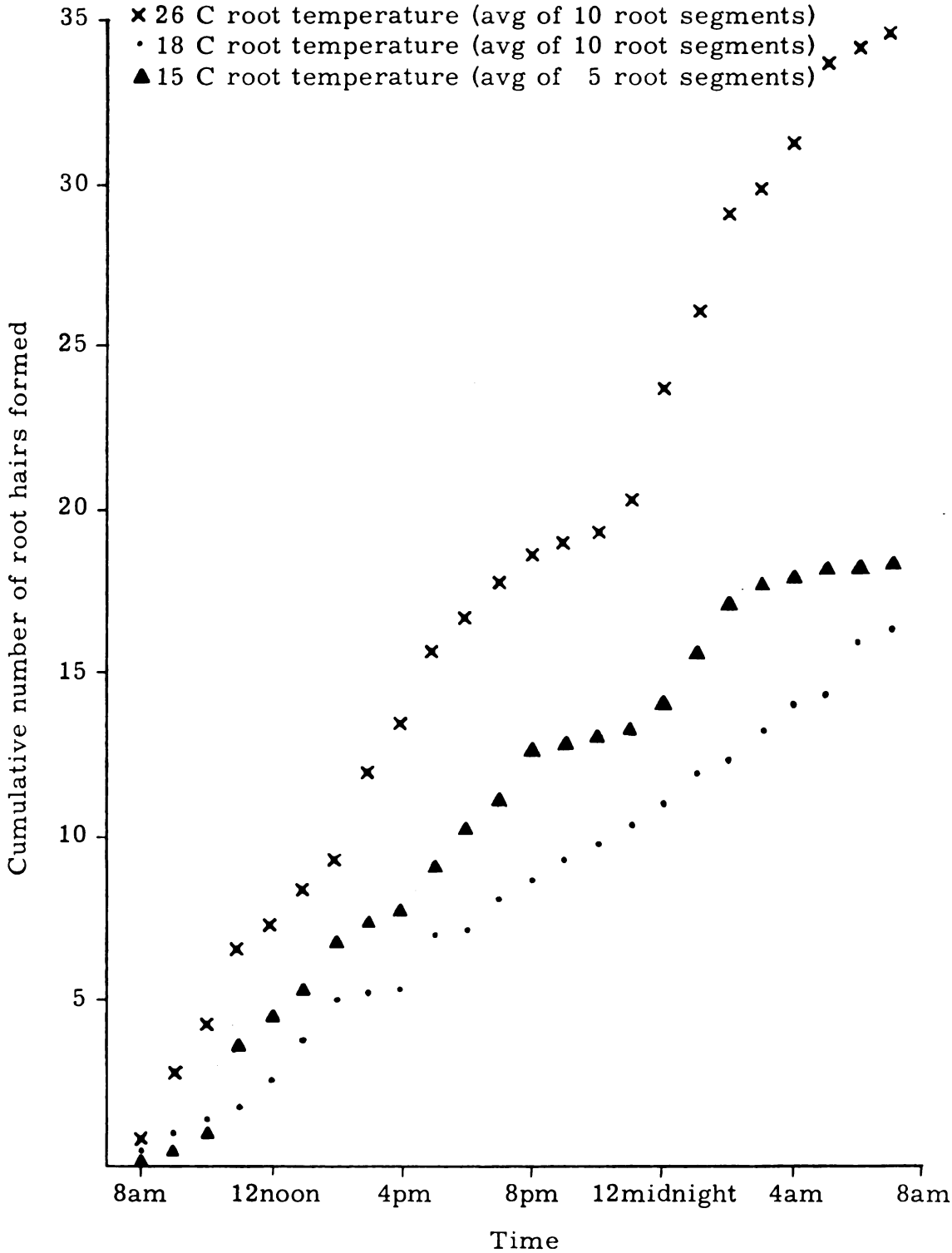

Figure 2. The average number of root hairs formed on a root segment in 24 hours at 3 temperatures.

Figure 3 shows the average change in length on an hourly basis. No diurnal change in elongation rate was found in that no distinct changes were visible. This was also verified by the F statistic in the analysis of variance.

It was found that root hair elongation was rather variable. The initial length of the root hair did not affect the elongation rate significantly. Variation of elongation rate of adjacent root hairs was also noted to be large in spite of their close proximity.

Fluctuations of root temperature, caused by an accident involving the hose connecting the circulator to the observation chamber, caused the elongation rate to increase as the temperature rose from 15 to 19 C in the course of about one-half hour. The elongation rate was also elevated during the period in which the temperature was returned to 15 C. Readjustment to a steady rate of elongation occurred within 1 hour after returning to the constant root temperature.

Longevity

The average length of life of a root hair, as shown by neutral red staining, was 40, 45[1] and 55 hours when grown at root temperatures of 26, 18 and 15 respectively. A Duncan's Multiple

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[1]Three of 15 root hairs died extremely early at 18 C and were excluded from the calculation.

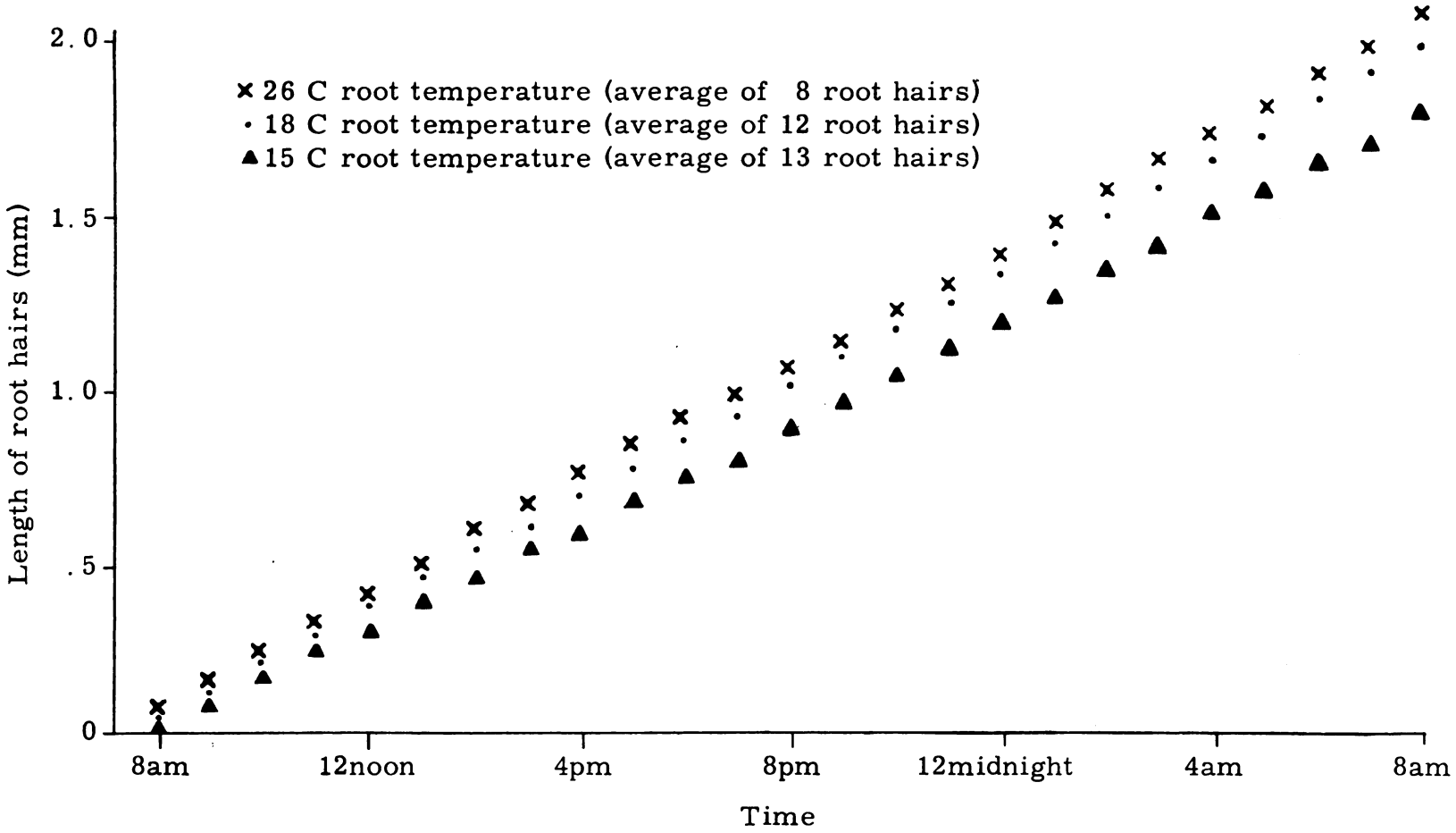

Figure 3. The average length of root hairs at hourly intervals during 24 hours grown at 3 constant root temperatures.

Range test (Duncan 1955) showed that root hairs lived significantly longer at a root temperature of 15 C than at the other two temperatures.

By calculating the time of day a root hair died (irrespective of the day of death) and plotting the results (Figure 4), at least one distinct peak period of death occurred.

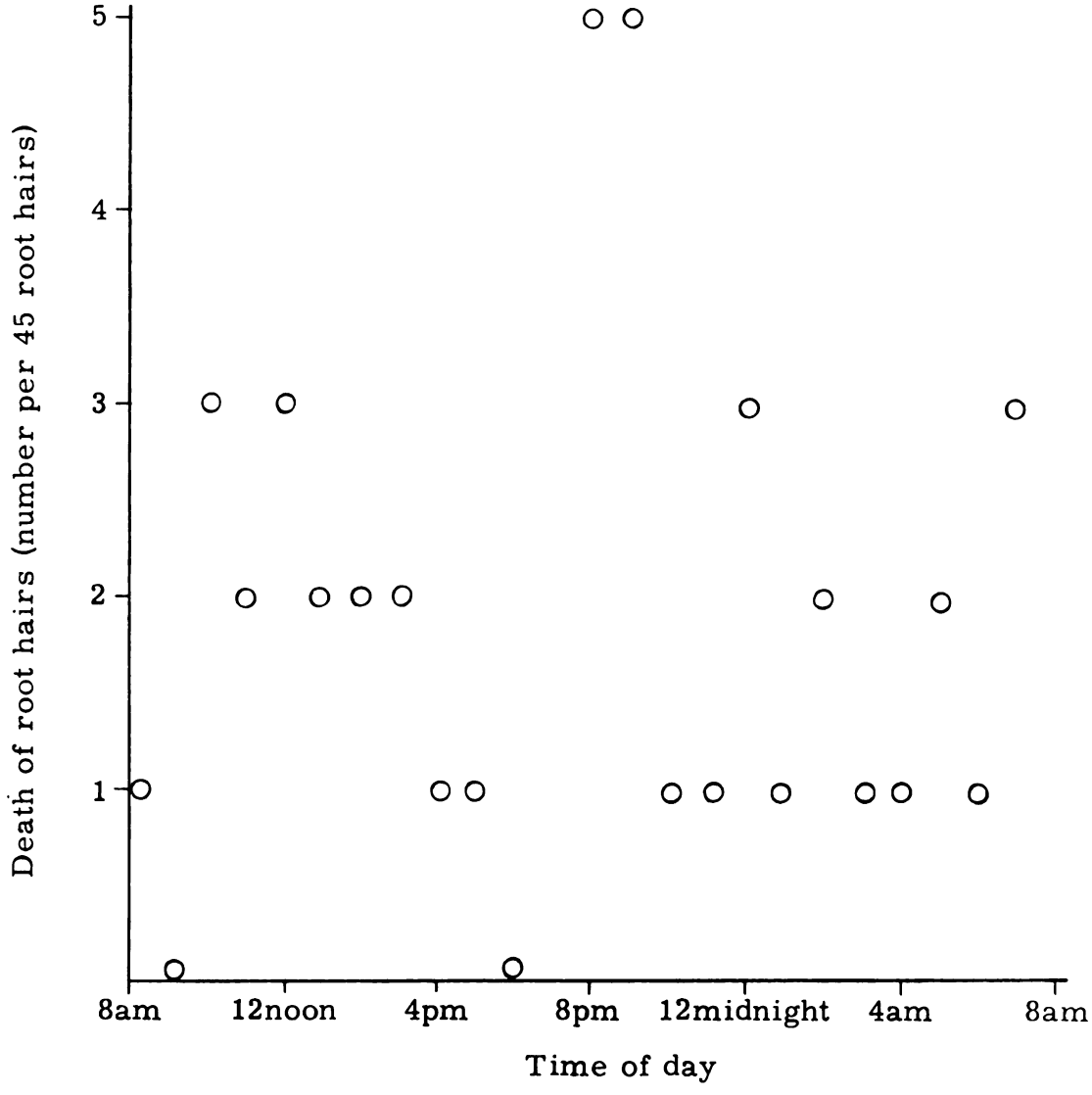

Figure 4. The frequency of death of root hairs at various times of the day (sum of 3 temperatures with 15 root hairs from each).

DISCUSSION

Similar observations of the location of the nucleus as noted here during root hair formation has been reported by Haberlandt (1914) and Esau (1960). Haberlandt (1914) and Addoms (1923) observed numerous small vacuoles in young root hairs as was found in this study. The observation that vertical elongation of epidermal cells ceased prior to root hair production has been reported by Haberlandt (1914) and Cormack (1949, 1962) and has been ascribed essentially to the fact that the root hairs would be damaged if the epidermal cells continued to elongate.

The abnormal root hairs observed when roots were grown or placed in acid solutions (Addoms 1923, Farr 1928 a, Cormack 1935, 1949), hypo- and hypertonic solutions (Snow 1905, Bardell 1915, Farr 1925, 1927 a, 1927 b, 1928 c, and Ekdahl 1953), calcium deficient solutions (Farr 1927 a, 1928 a, 1928 c, Cormack 1935, 1937, 1944, 1945, and Burstrom 1952) and extremes of temperature (Jeffs 1925, Farr 1928 c) were not observed in root hairs of barley grown under the conditions used in this research. Preliminary studies, prior to actual 24-hour observations, had been conducted to ascertain the pH, osmotic concentration and stain concentration

that would not produce abnormal root hairs in barley and would give near optimum rates of formation and elongation.

The cone shaped mass of root hairs observed in the laboratory was probably due to temperature fluctuations as reported by Gossen and Stapleberg (1933) but did not occur when the temperature was changed rapidly in the growth chamber. In the laboratory the temperature varied more frequently and at a slower rate, giving the root hairs a greater time period under a changing temperature and allowing for a cone shaped mass of root hairs to be produced.

Formation

The greater number of root hairs produced at 26 C than at 18 or 15 C does not agree with Snow (1905) who found maximum production at 4.5 to 11.5 C, few at 16 to 29.5 C and no root hairs formed above 29.5 C in wheat and corn grown in soil. She reported that the moisture content of the soil was difficult to control and available moisture does affect root hair formation greatly. Also in using soil, many more variables which are difficult to control or account for were introduced. The greater rate of formation of root hairs at higher root temperatures suggests some adaptive value in that the need for water is greater at higher temperatures and more root hairs would be one method of increasing the water absorptive surface.

If the elongation of root epidermal cells of barley is phasic, as it is in other plants, then the data presented here regarding the phasic nature of formation may support the theory of Cormack (1949, 1962) if the retardation of epidermal cell elongation and maximum root hair formation occurs in the same time periods. More conclusive proof that the elongation of epidermal cells in barley is phasic would be desirable. The phasic formation of root hairs also suggests that water absorption lag and root hair formation may be related in that a minimum rate of formation occurred between 12 noon and 4 p.m. when absorption lag generally is greatest (Kramer 1938, 1947) and maximum formation occurred when water absorption rates are high.

Elongation

The greater root hair growth per hour found at 26 C (see Figure 3) shows that root hair activity increased with temperature and produced a larger water absorptive surface.

The steady rate of elongation seemingly is not in agreement with Jeffs (1925), Farr (1925, 1928 c) and Snow (1905), who found that small changes in temperature (about 2 to 6 C) caused decisive changes in growth rate. Jeffs (1925) found that a decrease in temperature of 1 to 6 C decreased the growth rate and sometimes even contraction of the root hair occurred. In some cases recovery

of the growth rate is partial or entire and in other elongation ceases and is not resumed (Farr 1925, Jeffs 1925). Jeffs (1925) found that a 2.5 to 3 C increase in temperature temporarily increases elongation but it soon returns to that before the temperature increase. Although the root temperature remained constant in the observation chamber, a daily temperature change of 10 C between day and night periods occurred for 1 month in the growth chamber. No latent periodic root hair growth rate was evoked by these conditions. The results suggest that elongation rate in barley is not strongly rhythmic or if it is rhythmic it cannot persist without external stimulus. Bunning (1956) has stated that some plant rhythms can be followed under constant environmental conditions only for a relatively short time; "sometimes only two or three periods can be registered if no further impulses from some environment stimulus follow." An examination of Figure 3, during the early part of the observation period (8 a.m.) shows no indication of residual rhythm if it ever existed.

There should be no doubt that root hair elongation rate is not a constant in the field and this rate is probably mediated by changes in temperature and other root environmental factors such as changes in oxygen supply and osmotic tensions. Also physiological conditions may influence the elongation rate.

Longevity

The short life span of root hairs does not necessarily mean that the function of root hairs is completely lost in a short time, as it is known that dead roots (and probably root hairs) can absorb water (Kramer 1933). The short life may in fact enhance water uptake. Kramer (1938) has reported that living cells between the epidermis and xylem offer considerable resistance to the passage of water and death reduces the resistance. Also by early death, secondary thickening of root hairs is prevented or reduced and they remain permeable to water. The dead root hairs must be in contact with available soil moisture to remain functional.

A shorter life may be in part counteracted by a greater rate of formation and elongation. This would be beneficial in drier soils as the hairs would be exploiting new areas of the soil for moisture.

The peak period of death does not coincide with the peak periods of formation or vice versa (see Figure 5) but formation was accelerated shortly after the maximum time of death. The large number of root hair deaths at 7 to 8 p.m. may account for the decrease in absorption lag found by Kramer (1938) and Evans (1963) during this time period.

It may be postulated that the phasic formation and death of root hairs may affect diurnal water absorption. Elongation rate,

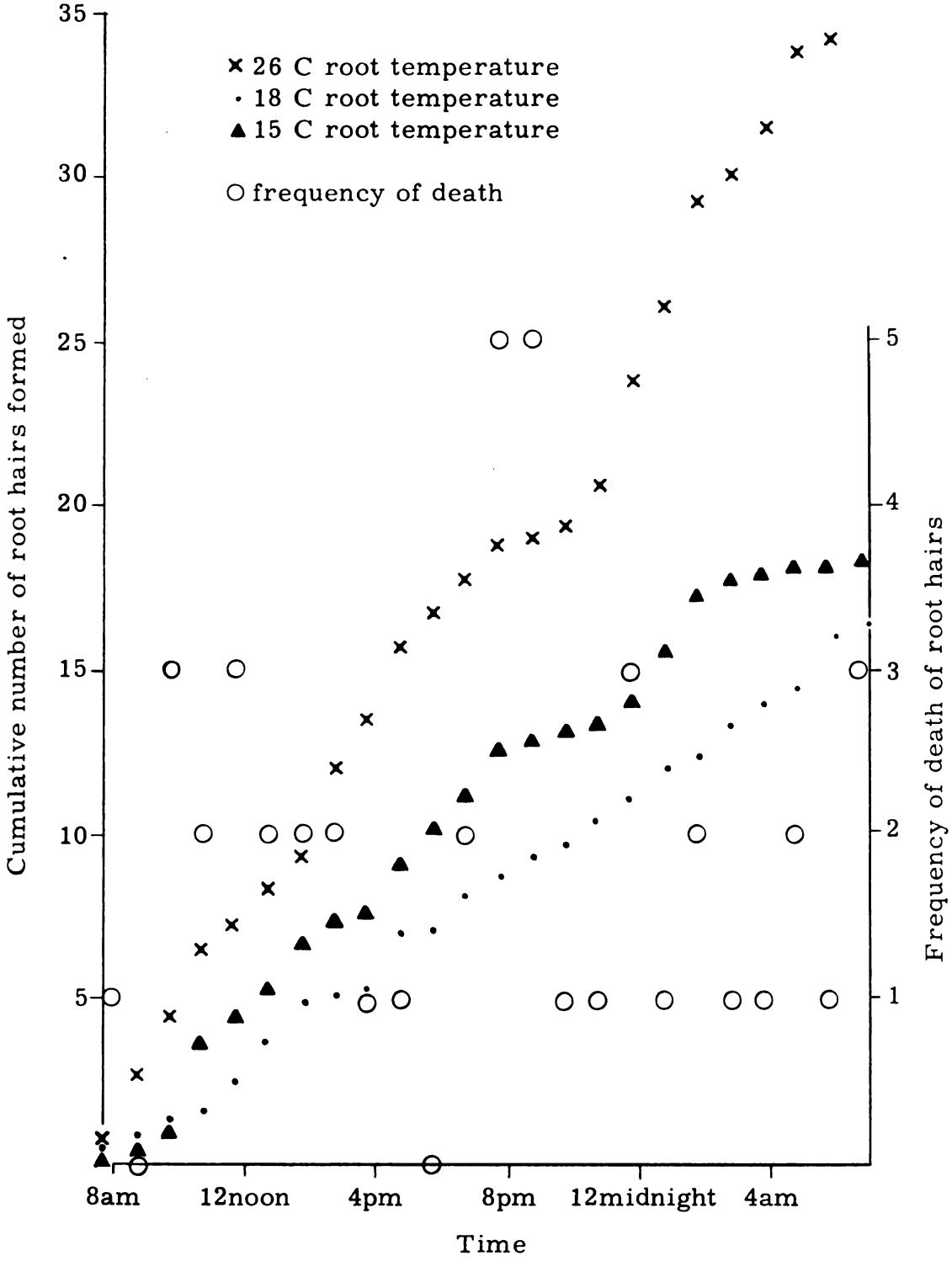

Figure 5. The number of root hairs formed and the frequency of death of root hairs in the course of 24 hours.

as affected by temperature and other changes, may also be a factor in the periodic uptake of water by plants in that soil temperature (and no doubt other soil and plant factors) follow diurnal cycles.

SUMMARY

Root hairs of 1 month old barley seedlings were observed to determine the rate of formation, elongation and death at various hourly intervals. Longevity was also ascertained using a neutral red staining procedure.

The seedlings in all cases were grown for 1 month in a 14-hour day at 20 C and a 10-hour night at 10 C in a modified nutrient solution. Individual seedlings were then placed in an observation chamber in which the root temperature remained constant (15, 18, or 26 C).

More root hairs formed during 24 hours at a root temperature of 26 C than at the other two temperatures. Three peaks and two minimum formation periods occurred but the 15 and 18 C peaks or minimums did not occur at precisely the same time or with the same magnitude as at 26 C.

The elongation rate was greater at 26 C than at the 15 C temperature. No changes in the elongation rate between different hour intervals were found.

Longevities of 40, 45 and 55 hours occurred at 26, 18 and 15 C respectively. A distinct peak death period occurred at 7 to 9 p.m.

The phasic rate of formation and death of root hairs may affect the diurnal water absorption rate in that the absorption area changes with time. Elongation rate probably varies with changes in soil conditions and as such may also affect the rate of water uptake by plant root systems.

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APPENDIX

Table 1. Analysis of variance of root hair formation at 3 root temperatures.

Source of Variation	df	SS	MS	F
		15 C		
Time	23	48.4	2.10	4.38**
Root	4	3.5	0.88	1.83
Error	92	54.1	0.48	
Total	119	106.0		
		18C		
Time	23	21.8	2.42	6.4 **
Root	9	28.0	1.22	3.2 *
Error	207	78.8	0.38	
Total	239	128.6		
		26C		
Time	23	15.7	1.74	3.35**
Root	9	7.0	2.18	4.20*
Error	207	108.2	0.52	
Total	239	140.9		
		Combined		
Time	23	47.8	1.74	3.35**
Temp.	2	31.2	2.18	4.20**
Error	46	82.5	0.52	
Total	71	161.5		

Table 2. Combined analysis of variance of root hair elongation at 3 root temperatures

Source of Variation	df	SS	MS	F
Time	23	62.8	2.72	0.84
Root	2	26.4	13.22	4.08**
Error	46	149.5	3.25	
Total	71	238.7		

Table 3. The effect of root temperature on root hair longevity.

Source of Variation	df	SS	MS	F
Temp.	2	2750.1	1375.1	6.02**
Error	42	9830.6	228.6	
Total	44	12580.7		

Table 4. The time of day root hairs die (irrespective of day of death).

Time	Frequency
8 a.m.	1
9 a.m.	0
10 a.m.	3
11 a.m.	2
12 noon	3
1 p.m.	2
2 p.m.	2
3 p.m.	2
4 p.m.	1
5 p.m.	1
6 p.m.	0
7 p.m.	2
8 p.m.	5
9 p.m.	5
10 p.m.	1
11 p.m.	1
12 midnight	3
1 a.m.	1
2 a.m.	2
3 a.m.	1
4 a.m.	1
5 a.m.	2
6 a.m.	1
7 a.m.	3