

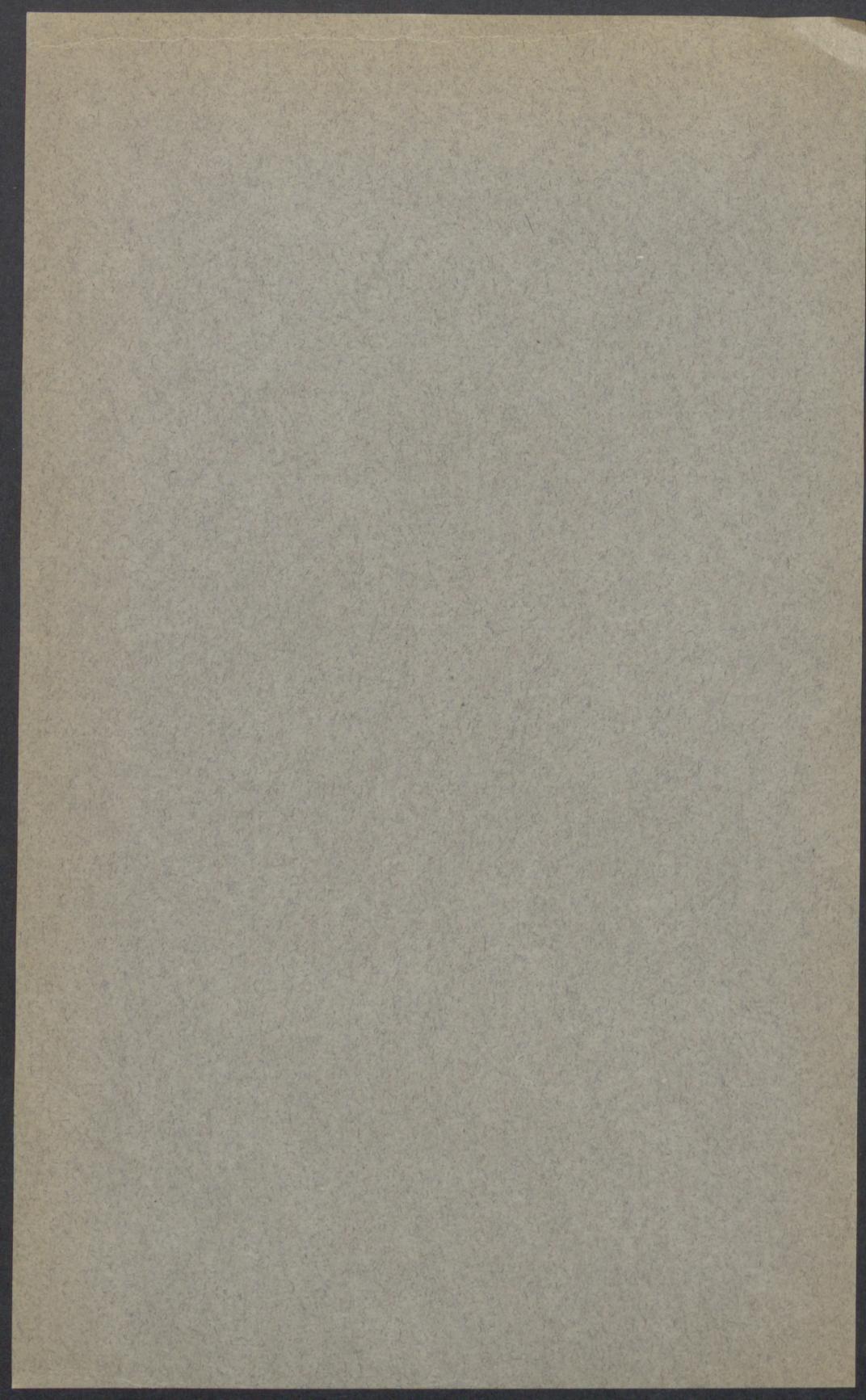
High Temperature Tolerance of Forest Trees

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High Temperature Tolerance of Forest Trees¹

RALPH W. LORENZ²

NATURAL heat injury to forest nursery stock and young plantations during recent years in the Lake States and other parts of the country has emphasized the importance of properly understanding the relation between the growth of plants and high temperature. True indices of heat injury, which make it possible to determine accurately the extent to which the life functions of plants have been impaired, are noticeably wanting. The object of this study was to determine the time and temperature necessary to kill the cells of the cortical parenchyma of various trees. The intake of neutral red dye by these cells was used as an index of vitality after microscopic sections had been subjected to various durations of high temperature in a controlled-temperature water bath.

LITERATURE REVIEW

According to Heilbrunn (38) and Miller (52), the thermal death point for most plant cells has been found to vary between 45° and 55° C. Considerable variation from these two temperature limits can be found in the literature because of differences in the species of plants used, the method of application of heat to the test plants, the duration of the experiment itself, the age of the test plants or tissues, and also the variations in the interpretation of the heat injury produced in the test material. Many records pertaining to lethal high temperatures have lost their significance because the time factor was neglected.

Some of the first systematic investigations of high temperature relationships of plants were conducted by Sachs (63) in

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1860 and in the decade following. It was known to Nägeli as early as 1855 that plant tissues killed by hot water lose their turgor, and that natural pigments diffuse out from them.

Methods for Estimating the Vitality of Tissues

Use of Vital Stains

The classical work of Růžička (62) describes methods for the use of vital stains in estimating the vitality of tissues. By using equimolecular mixtures of neutral red and methyl blue, the living cells stain red and the dead cells stain blue.

Levitt and Scarth (43) used neutral red in their frost-hardening studies with living cells. They state: "Since only living cells are stained by this dye, it removes the danger of mistaking dead cells with coagulated protoplasm for plasmolyzed living cells, or empty cell walls for unplasmolyzed living cells. Furthermore, the slightest incipience shows up sharply." One interested in staining technique and cell structure *in vivo* should consult Zirkle (71), Bailey (2), and Strugger (66).

Electric Resistance of Tissues

The study of the electric resistance of living cells in relation to variation of frequency of alternating current has been used chiefly in determining the physical characteristics of various tissues such as muscle, liver, skin, red corpuscles, and plant cells. The method has not been generally applied to problems concerning the growth and death of plant cells (46, 47).

It is apparent that the use of the electric resistance of plant tissue has a limited application in high-temperature studies as an indicator of vitality where one is concerned with individual cells in a microscopic section.

Ultraviolet Absorption by Living and Dead Cells

Luyet and Gehenio (48) were able to distinguish between the dead and the live cells of the outer skin of the onion by ultraviolet photograph. The living cells appear black in an ultraviolet photograph, while the dead cells are white. This is due to differences in ultraviolet transmission. In a later article they attribute the difference in ultraviolet absorption between living and dead cells to the presence of a pigment (49).

Direct or Microscopic Observations

No special criterion of vitality for the injured tissues has been used for the majority of high-temperature studies concerning tree seedlings. They have merely been placed under direct ob-

servation for study of the lethal effects that become evident with time. In some cases this may require from ten days to two weeks. Previous investigations have been conducted under such varying conditions that the results are not strictly comparable. Careful scrutiny of the past investigations of heat injury on the higher plants will show that most plant tissue is killed at between 45° C. and 55° C., irrespective of the method employed.

That the direct heat of the sun was able to injure young seedlings was probably first suggested by Mayr (50). Hartig (33) in 1883 noted a "stem girdle" in coniferous stock which he attributed to the freezing of shallow pools of water in which the trees stood. A rather interesting concurrence of incidents leading up to the determination of the true nature of heat injury occurred in German and American studies. The German ideas from 1883, until the work of Mayr (50) in 1909, and that of Münch (54, 55) in 1913 and 1914, closely parallel the American ideas from the turn of the century up to the time of Hartley's (35) work in 1918. In Germany an effort was made to establish the fungus *Pestalozzia* as the causal organism producing the injury resulting from heat. Repeated inoculation experiments with this organism as well as with other fungi failed, and the theory had to be abandoned, Tubeuf (69), Münch (54). The cultures made from "white spot" lesions by Hartley (34) also failed to show regularly the presence of any definite organism as the causal agent. A clear picture of heat injury is presented by Hartley (35), who gave the name "white spot" to it in a previous publication (34, p. 5). He states, "The white spot lesion is very light in color, and this characteristic color continues to the very edge, making a sharp line of demarcation from the healthy tissue. Lesions may continue definitely limited for some days, and the upper stem and cotyledons remain turgid. In this early stage most cases of white spot injury are easily distinguished from damping off. Typical damping off in porous soils is primarily a root rot, which may attack above the ground line, but which more commonly attacks below."

This same type of injury to tree seedlings was described by Münch (54, 55) in Germany, who attributed it to the heat at the soil surface.

That excessive temperatures from direct insolation may be a deciding factor in the survival between one species and another was claimed by Bates (4). This idea was further enlarged upon by him in a later paper (5) concerning the physiological requirements of Rocky Mountain trees, and in a still later paper on forest types in the central Rocky Mountains as affected by climate and soil (6). He estimated that exposure for one minute at approximately 141° F. would cause critical injury to all the seedlings.

The scale of heat tolerance suggested by Bates for the four species studied stands in the following order: lodgepole pine, yellow pine, spruce, and Douglas fir. Baker (3, p. 957) criticizes Bates' work because of the unnatural conditions of injury brought about by the use of wet soil and the uncertainty as to what his recorded temperatures mean in terms of plant-tissue temperature.

Comprehensive tests of certain western conifers, ranging from one to three months in age, were carried out by Baker (3) with respect to their ability to withstand heat. He found that the living tissues of one- to three-month-old seedlings of representative conifers of western America were quickly killed when subjected to a temperature of about 54° C. (130° F.), but could withstand a temperature only a few degrees lower for some time.

Roeser (61) digressed somewhat from the usual approach to the study of the heat resistance of tree seedlings by sprinkling fine heated sand over them to a depth of one-fourth inch while their roots were immersed in water. Seedlings approximately 58, 71, and 110 days old were used. When using 71-day-old material, most western yellow pine seedlings could tolerate 133° F. but would usually be killed at 158° F. The same treatment showed corresponding temperatures of 128° and 152° F. for lodgepole pine, 124° and 154° F. for Douglas fir, and 120° and 152° F. for Engelmann's spruce.

A recent and intensive investigation of the lethal high-temperature relations existing in conifers is that of Shirley (65). Experiments were designed to determine the lethal high temperatures for both roots and tops, and the influence of variations in relative humidity upon these temperatures. Exposures were made in a water bath, in moist air, and in dry air. The four conifers most commonly employed in forest planting in the Lake States region were used in the test: red pine, *Pinus resinosa* Ait.; white pine, *Pinus strobus* L.; jack pine, *Pinus banksiana* Lamb.; and white spruce, *Picea glauca* (Moench) Voss. Conclusions drawn from these tests are quoted directly from Shirley's work:

"From tests of Norway pine, white pine, jack pine, and white spruce one to four years old for killing temperatures in a water bath, it may be concluded that: (1) Resistance to excessive heat increases with increasing age and increasing size or mass of plant and tissue; (2) tops are more resistant than roots; (3) with 2-hour exposures the maximum temperature that needles can withstand is 49° C.; (4) temperatures as low as 44.3° C. do not cause severe damage to tops in exposures up to 5 hours' duration, but may result in death to roots; (5) there is little difference among the species tested in their ability to withstand heat.

"For comparable plant material the external killing temperature was higher in air than in water and higher in dry air than in moist air. The

maximum temperature which needles withstood for 5 hours' exposure in moist air (relative humidity, 85 per cent) was 50° C., while in dry air (relative humidity, 15 per cent) they withstood 54° C. Exposures of 5 hours in dry air caused little injury at temperatures of 50° C."

Shirley believed that the cooling effect of transpiration was probably the most important factor involved in the greater re-

Table 1. Influence of High Temperatures on Conifer Seedlings

Test material	Temperature		Exposure	Injury	Authority	Notes
	° C.	° F.				
Seedlings	52	125.6		No injury	Münch (54, 55)	
Seedlings	54-55	129-131		Death	Münch (54, 55)	
Seedlings	51-55	124-131		Death	Baker (3)	Artificial insulation
Seedlings	60.5	141	1 minute*	Death	Bates (7)	Moist air
Seedlings	57.2	135	2 minutes*	Death	Bates (7)	Moist air
Seedlings (needles)	49	120.2	2 hours	Withstood temp.	Shirley (65)	Water bath
Seedlings (tops)	44.3	111.7	5 hours	Withstood temp.	Shirley (65)	Water bath
Seedlings (needles)	50	122	5 hours	Withstood temp.	Shirley (65)	85% relative humidity
Seedlings (needles)	54	129.2	5 hours	Withstood temp.	Shirley (65)	15% relative humidity
Seedlings (roots)	44.3	111.7	5 hours	Severe injury or death	Shirley (65)	Water bath

* Approximately.

sistance of plants to heat in dry air. He found that recovery from excessive heat injury was associated with the ability to send out epicormic shoots from the uninjured stem.

A condensed summary of the influence of high temperatures on coniferous seedlings is given in table 1.

Soil Temperatures

Many high-temperature studies in tree seedlings have been made because of the excessively high soil temperatures often found in nurseries and in exposed planting sites. A close parallel exists between soil temperature and heat injury to tree seedlings.

Temperatures ranging as high as 58° C. (136° F.) in seedbeds were found by Mayr (50) to cause injury to the seedlings. Toumey and Neethling (68) demonstrated that a sustained temperature of 121° to 123° F. in the surface soil is sufficiently high to cause typical heat lesions on white and red pine seedlings. Münch (56) has shown that the temperature of the surface soil varies with its dryness, looseness, and color. Other factors being equal, the darker, the drier, and the looser the soil, the higher its temperature. In publications of Münch (54) and Hartley

(35) diagrammatic sketches are presented to show the point of injury on the seedlings in relation to their position in the soil. The point of injury is at the surface, or immediately above or below it. Toumey and Neethling (67) determined the effect of cover over coniferous seedbeds in southern New England. Although stem lesions were apparent in open beds because of high temperatures, no lesions were in evidence on any of the seedlings in beds protected by half shades. Bates and Roeser (7) and Roeser (61) report soil temperatures of 67° to 70° C. on the south slopes of the Rocky Mountains in Colorado. Shirley (65) has measured a temperature of 53° C. in the Forest Service nursery at Cass Lake, Minnesota.

EXPERIMENTAL

Source of Material

The high-temperature relationship of the cortical parenchyma tissue in northern white pine, *Pinus strobus* L.; red pine, *Pinus resinosa* Ait.; white spruce, *Picea glauca* (Moench) Voss.; American elm, *Ulmus americana* L.; and hardy catalpa, *Catalpa speciosa* Ward. has been determined. The coniferous seedlings were 2-1 stock (two years in seedbed, one year in transplant) obtained from the nursery at the Cloquet Forest Experiment Station, Cloquet, Minnesota. They were planted in six-inch pots and remained in the greenhouse one year before they were used. The elms and catalpas were raised from seed in the greenhouse and were approximately one year old when the experiments were conducted.

Equipment

Temperature Stage for Microscope

The first experimental approach made in this study was with a high-temperature stage patterned after that of Levitt and Scarth (44, p. 293), with the addition of a thermocouple for greater precision in temperature measurements. Although this type of stage has many valuable applications in both low- and high-temperature studies, it was discarded because it was too difficult to maintain precise temperatures over long periods.

The Water Bath

A five-gallon water bath, equipped with an electric stirrer and an electric immersion heater controlled by a sensitive mercury thermoregulator, was used to maintain constant temperatures within $\pm 0.1^{\circ}$ C.

Immersion Baskets

Immersion baskets were made of very fine copper gauze, 1.5 cm. in diameter and 1 cm. in depth, with a fine wire handle soldered to them. Detachable covers of the same material were made for each basket. Absolutely no change in temperature of the water bath could be noted on a precision thermometer calibrated to 0.1° C., even though as many as six immersion baskets were placed in it at one time. The deKhotinsky type of thermo-regulator was used because temperature changes could be made with ease and rapidity.

Procedure

Sectioning

Tests were made by immersing microscopic sections of the cortical parenchyma in the water bath for definite periods of time. The sections were cut by hand and put directly into distilled water previous to placing them in the water bath. A short time in the distilled water had no apparent effect on their ability to withstand high temperatures. Six microscopic sections were placed in a basket for the test and then immersed in the constant-temperature bath. At the end of the specific time period, the sections were removed and immediately placed in the dye solution.

Staining

Sections were allowed to stain for one-half hour in a solution of 0.2 molar calcium chloride containing 5 ppm of neutral red. The concentration of neutral red is not important as regards its staining ability. Good results were obtained with varying concentrations. A more intense red color is obtained by prolonged staining. The dye used was Grubler's Neutralrot. Bailey (2), who has made a comprehensive study of the staining of vacuoles in various angiosperms and gymnosperms with neutral red, was able to keep the fusiform initials alive and actively streaming for 500 hours after an intense staining of the vacuome.

Plasmolysis

Neutral red is a distinct aid in determining incipient plasmolysis. Well-defined plasmolysis can easily be induced by adding concentrated calcium chloride in small quantities until the proper hypertonic concentration is attained. This results in a smooth, well-rounded protoplast in contrast to the irregular type produced by placing the plant tissue directly in a highly concentrated hypertonic solution. The calcium chloride is a further aid to

clear observation, since it prevents the cell walls from being stained. Fitting (30) and others have shown that calcium salts do not penetrate the cell. Scarth (64) found that calcium prevents the penetration of other ions. It should, therefore, reduce exosmosis to a minimum. Levitt and Scarth (44) tested sections before and after a six-hour period in calcium chloride. No change in osmotic pressure could be detected. At the higher temperatures, close to the lethal point of the tissue in question, a well-defined plasmolysis is often lacking, although a thorough staining of the vacuome manifests itself. In all probability this is due to an increase in protoplasmic viscosity at higher temperatures.

Estimation of Degree of Injury

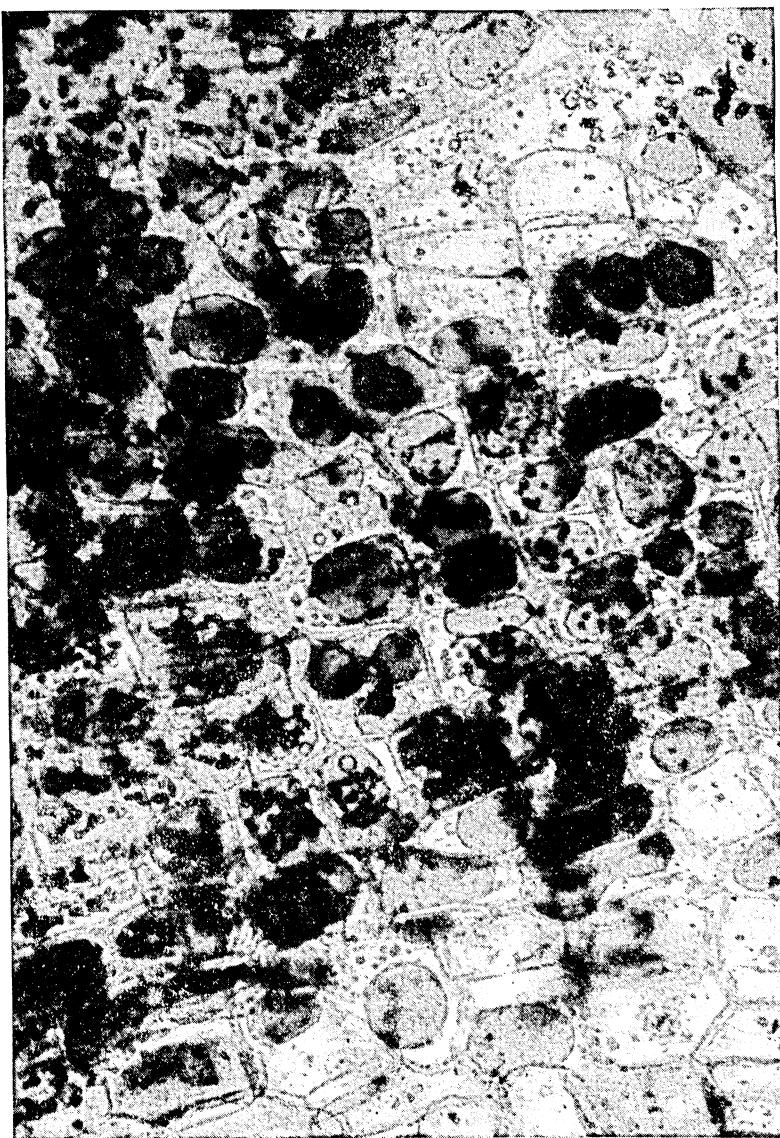
The degree of injury resulting from high-temperature exposures in the water bath was determined by the per cent of cells in the section that would stain with neutral red. This was easily determined under the low power of the microscope. The total number of stained cells in unheated tissue was taken as the base figure from which the percentage of injury was calculated. It is apparent that a number of the cells will not stain because of mechanical injury resulting from sectioning. The degree of injury was classified as follows according to per cent of the stainable cells staining: 100, 75-100, 50-75, 25-50, 0-25, 0.

The microscopic sections of the cortical parenchyma were cut by hand and placed in distilled water until they could be immersed in the water bath for the heat treatment. After the sections were subjected to the heat treatments, they were placed directly into the neutral red solution. The cells staining red in the tissue served as an index of vitality. Every effort was made to handle and conduct each experiment in a comparable manner so as to reduce the variables to a minimum.

The Application of van't Hoff's Rule (Q_{10}) and the Arrhenius Formula

The effect of varying temperatures on the rate of biological processes has been much studied with the hope that a clue to the real nature of the process might be furnished by its temperature coefficient. This has been realized only to a limited extent.

The literature pertaining to the influences of moderate temperatures on biological processes was adequately reviewed by Kanitz (41) in 1915. It is not within the scope of this paper to review the literature on temperature coefficients in biological processes. For a recent survey of this subject, one may consult the monograph of Bělehrádek (10) and also the work of Heilbrunn (39, 40).



STAINING AND PLASMOLYSIS OF THE CORTICAL CELLS OF *Catalpa speciosa*
WARD., USING NEUTRAL RED AND CALCIUM CHLORIDE

It has been found that the velocity of chemical reactions is doubled or trebled by each rise of 10° C. This temperature effect of van't Hoff is usually designated as Q_{10} . The importance of the value of Q_{10} lies in its ability to indicate the nature of the factors which determine the rate of reactions. The rate of biological processes are often expressed in terms of Q_{10} . This is the ratio of the speed of a process or reaction at a given temperature to the speed at 10° C. lower. Thus,

$$Q_{10} = \frac{K_t + 10}{K_t}$$

where K_t is the speed of the process at the temperature t , and $K_t + 10$ the speed at a temperature 10° C. higher. If the rate of reaction is known for any two temperatures, then Q_{10} may be calculated from the following formula:

$$Q_{10} = \frac{10}{\frac{(K_1)}{(K_2)} \frac{t_1 - t_2}{t_1 - t_2}}$$

in which K_1 and K_2 are the speeds at temperatures t_1 and t_2 , respectively. The above formula is conveniently used in logarithmic transcription as follows:

$$\log Q_{10} = \frac{10 (\log K_1 - \log K_2)}{t_1 - t_2}$$

It is now generally recognized that systematic variations of Q_{10} with temperature are a constant and general feature of temperature action. Considerable difference of opinion exists among investigators in regard to the application of the Q_{10} rule in biological reactions. The consensus of opinion seems to be that the rule of van't Hoff does not apply to the majority of biological reactions. When it does apply, it is only within a narrow temperature range. For a complete discussion of the significance of Q_{10} in biological processes see Bělehrádek (10).

Q_{10} determinations were made on the rate of killing of the cortical cells for each tree species studied. When distinct breaks in the curves resulted from the application of the Arrhenius formula in the determination of the temperature characteristic, Q_{10} values were determined for both slopes of the line.

Physical chemists usually employ the so-called Arrhenius formula (1) in the study of the temperature relations of chemical reactions. The formula takes the following form:

$$K_2 = K_1 \cdot e^{\frac{\mu}{2} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

in which K_1 and K_2 are the velocity constants at the absolute temperatures T_1 and T_2 , e is the base of natural logarithms, and μ is a constant which is the temperature coefficient. It is often called the "temperature characteristic" or "critical thermal increment." For further information on its history see Glaser (32). The constant μ designates the energy of activation of the process or the heat required to activate inactive molecules. The greater μ is, the more rapidly the velocity of reaction increases with temperature. This law was introduced into biology in order to obtain more accurate and comparable results than could be obtained with the Q_{10} rule. Probably no less than 100 applications of the Arrhenius formula to the rate of biological phenomena have made their appearance since the turn of the century. To list the processes whose temperature coefficients have been studied would cover the whole field of biological phenomena. Following the Q_{10} rule, the logarithm of velocity gives a straight line when plotted against temperature in degrees centigrade, while following the law of Arrhenius the logarithm of velocity gives a straight line when plotted against the reciprocal value of the absolute temperature. Bělehrádek (8) states that as far as the biokinetic zone is concerned, the reciprocal of absolute temperature is practically a linear function of temperature in degrees centigrade, which means that there is no practical difference between μ and Q_{10} in biology. Heilbrunn (40, p. 343) introduces a conversion factor of 6286, which if multiplied by Q_{10} will result in μ , or conversely, dividing μ by 6286 will give Q_{10} . This conversion factor seems to be accurate only within certain prescribed limits.

Two schools of thought now prevail concerning the true significance of the Arrhenius formula as applied to biological phenomena. The proponents of the first school maintain that Q_{10} values, giving the ratio of velocities for an interval of $10^{\circ}\text{ C}.$, are imperfect means of identifying a process, but that the Arrhenius formula might lead to a method of characterizing a definite biochemical mechanism, since a similar value of μ may denote similar protoplastic reactions controlling its activity. A second point in this hypothesis explains an abrupt change in the value of μ at a definite temperature which is "critical," on the assumption that in the "catenary series" of reactions underlying the process in question different members may become slowest at different temperatures, and these therefore limit the velocity of the whole chain. The most enthusiastic supporters of this viewpoint are Crozier and his co-workers. See Crozier (16, 17, 18, 19); Crozier and Stier (24, 25, 26, 27, 28); Crozier and Federighi (20, 21, 22, 23); Navez (57); Orr (58), and many others.

That the use of the Arrhenius formula is applicable to biological processes is seriously questioned by Heilbrunn (39, 40); Emerson (29); Burton (14); Ponder and Yeager (59); Bělehrádek (8, 9, 10), and Fulmer and Buchanan (31).

The Arrhenius formula was applied to the data for each tree species studied. The time and the temperature necessary to kill 50 per cent of the cells of any microscopic section from the cortex was used in the calculations.

RESULTS AND DISCUSSION

Time and Temperature Relationships

The data resulting from the experimental investigations are presented for each tree species investigated, including a temperature range from one which will show no injury to one which will result in complete killing in periods of 1, 3, 5, 10, 20, and 30 minutes. Approximately 90 specific time-temperature relationships were established for each species. A well-defined relation-

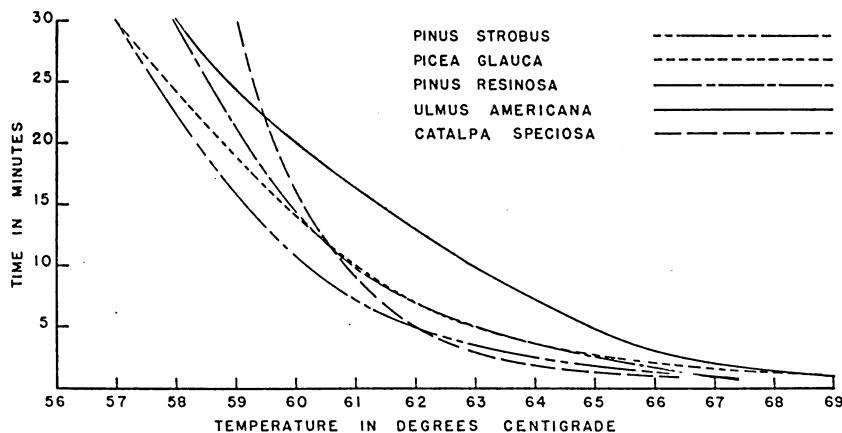


FIG. 1. THE TIME AND TEMPERATURE RELATIONSHIP OF 5 TREE SPECIES

Neutral red was used as an index of vitality after the microscopic sections of the cortical parenchyma were subjected to heat treatments in a controlled water bath. The curves represent complete killing.

ship was found to exist between high-temperature injury in the cells of the cortical parenchyma tissue of the tree species investigated and their ability to take up neutral red. The ability of the cells to take up neutral red was used as an index of vitality of the tissues.

An analysis of the composite results for northern white pine, *Pinus strobus* L.; white spruce, *Picea glauca* (Moench) Voss.; red pine, *Pinus resinosa* Ait.; American elm, *Ulmus americana* L.; and catalpa, *Catalpa speciosa* Ward., as presented in figure 1,

Table 2. The Per Cent of Cells of the Cortical Parenchyma in Catalpa, *Catalpa speciosa* Ward., Staining with Neutral Red After Microscopic Sections Were Placed in a Constant-Temperature Water Bath for the Time Periods and Temperatures Given

Temperature °C.	Time in minutes					
	1	3	5	10	20	30
Per cent of cells living						
54.....	100	100	100	100	100	100
55.....	100	100	100	100	100	100
56.....	100	100	100	100	100	100
57.....	100	100	100	100	100	75-100
58.....	100	100	100	100	75-100	25-50
59.....	100	100	100	50-75	0-25	0
60.....	100	100	50-75	25-50	0	0
61.....	100	50-75	0-25	0	0	0
62.....	75-100	0-25	0	0	0	0
63.....	50-75	0	0	0	0	0
64.....	25-50	0	0	0	0	0
65.....	0-25	0	0	0	0	0
66.....	0	0	0	0	0	0

Table 3. The Per Cent of Cells of the Cortical Parenchyma in American Elm, *Ulmus americana* L., Staining with Neutral Red After Microscopic Sections Were Placed in a Constant-Temperature Water Bath for the Time Periods and Temperatures Given

Temperature °C.	Time in minutes					
	1	3	5	10	20	30
Per cent of cells living						
55.....	100	100	100	100	100	100
56.....	100	100	100	100	100	75-100
57.....	100	100	100	75-100	50-75	0-25
58.....	100	100	100	75-100	50-75	0
59.....	100	100	100	75-100	0-25	0
60.....	100	75-100	75-100	50-75	0-25	0
61.....	100	75-100	75-100	25-50	0	0
62.....	100	75-100	50-75	0-25	0	0
63.....	75-100	75-100	25-50	0	0	0
64.....	75-100	25-50	0-25	0	0	0
65.....	50-75	0-25	0	0	0	0
66.....	25-50	0	0	0	0	0
67.....	0-25	0	0	0	0	0
68.....	0-25	0	0	0	0	0
69.....	0	0	0	0	0	0

Table 4. The Per Cent of Cells of the Cortical Parenchyma in White Pine, *Pinus strobus* L., Staining with Neutral Red After Microscopic Sections Were Placed in a Constant-Temperature Bath for the Time Periods and Temperatures Given

Temperature ° C.	Time in minutes					
	1	3	5	10	20	30
Per cent of cells living						
54.....	100	100	100	100	100	100
55.....	100	100	100	100	100	75-100
56.....	100	100	100	100	75-100	25-50
57.....	100	100	100	75-100	0-25	0
58.....	100	100	75-100	50-75	0-25	0
59.....	100	75-100	50-75	0-25	0	0
60.....	100	75-100	25-50	0	0	0
61.....	75-100	25-50	0-25	0	0	0
62.....	75-100	0-25	0	0	0	0
63.....	50-75	0-25	0	0	0	0
64.....	50-75	0	0	0	0	0
65.....	25-50	0	0	0	0	0
66.....	0-25	0	0	0	0	0
67.....	0	0	0	0	0	0

Table 5. The Per Cent of Cells of the Cortical Parenchyma in White Spruce, *Picea glauca* (Moench) Voss., Staining with Neutral Red After Microscopic Sections Were Placed in a Constant-Temperature Water Bath for the Time Periods and Temperatures Given

Temperature ° C.	Time in minutes					
	1	3	5	10	20	30
Per cent of cells living						
53.....	100	100	100	100	100	100
54.....	100	100	100	100	75-100	75-100
55.....	100	100	100	100	50-75	25-50
56.....	100	100	100	75-100	25-50	0-25
57.....	100	100	100	75-100	0-25	0
58.....	100	100	75-100	75-100	0-25	0
59.....	100	75-100	75-100	25-50	0	0
60.....	75-100	50-75	25-50	0-25	0	0
61.....	75-100	25-50	0-25	0	0	0
62.....	75-100	25-50	0-25	0	0	0
63.....	75-100	0-25	0	0	0	0
64.....	75-100	0-25	0	0	0	0
65.....	50-75	0-25	0	0	0	0
66.....	25-50	0-25	0	0	0	0
67.....	25-50	0	0	0	0	0
68.....	25-50	0	0	0	0	0
69.....	0-25	0	0	0	0	0
70.....	0	0	0	0	0	0

Table 6. The Per Cent of Cells of the Cortical Parenchyma in Red Pine, *Pinus resinosa* Ait., Staining with Neutral Red After Microscopic Sections Were Placed in a Constant-Temperature Water Bath for the Time Periods and Temperatures Given

Temperature °C.	Time in minutes					
	1	3	5	10	20	30
	Per cent of cells living					
55.....	100	100	100	100	100	100
56.....	100	100	100	100	100	75-100
57.....	100	100	100	100	75-100	50-75
58.....	100	100	75-100	50-75	0-25	0
59.....	100	100	50-75	25-50	0	0
60.....	100	75-100	50-75	0-25	0	0
61.....	100	50-75	0-25	0	0	0
62.....	75-100	50-75	0-25	0	0	0
63.....	50-75	0-25	0	0	0	0
64.....	50-75	0-25	0	0	0	0
65.....	25-50	0	0	0	0	0
66.....	0-25	0	0	0	0	0
67.....	0	0	0	0	0	0

shows that no staining is evident in the cortex cells after they have been exposed for 30 minutes at temperatures of 57° to 59° C. Temperatures of 66° to 69° C. resulted in complete killing in one minute. The curves in these figures represent complete killing, as no cells were stained with neutral red. The relation of the time factor to the thermal death point at the various temperatures is well illustrated by figure 1. This same effect is given in tabular form in tables 2, 3, 4, 5, and 6.

The data from which the graphs were compiled in figures 2 and 3 were obtained by interpolation from the original percentage figures found in tables 2, 3, 4, 5, and 6.

No particular difficulty was encountered in estimating the degree of injury by using the ability of the cells to stain with neutral red. This seemed to be a good index of their vitality, for the cells would be either definitely stained or would show no indication of staining whatsoever. Distinct plasmolysis could be induced in those cells staining with neutral red up to temperatures of about 54° C. The cells that were subjected to temperatures of about 54° C. for durations much over 15 minutes showed only traces of incipient plasmolysis although staining completely with neutral red. This may be explained by the increase of the viscosity of the protoplasm with increasing temperature. Heilbronn (36) observed an increase in the viscosity of the protoplasm of certain plant cells that were subjected to high temperatures and examined by the method of observing the falling starch grains. Similar phenomena were later reported by

other workers using various types of cells. Heilbrunn (37) centrifuged the eggs of *Cumingia* and *Arbacia* while Bělehrádek and Melichar (11) based their conclusions on the shape of plasmolyzed cells in *Elodea canadensis* Rich. The feeble, irregular type of plasmolysis resulting at higher temperatures can be mentioned as additional evidence of the increased viscosity of the protoplasm with temperature. Bělehrádek and Melichar (11), using a modification of Růžička's (62) staining method to differentiate living and dead cells, in studying the effect of high temperatures on the cells of *Elodea canadensis* Rich., always found the plasmolyzed cells to be stained red and that the stained cells always showed some traces of plasmolysis. They found that the cells would survive a temperature of 65.2° C. for three seconds.

The fact that the death point of tree tissues at high temperatures, using the method employed in this work, has never been determined previously makes direct comparison of the results with those of investigators using other methods somewhat difficult. The statement so often seen in the literature that protoplasm is usually killed at about 54° C. is incorrect when neither the nature of the protoplasm nor the time for such killing is mentioned. The data presented in this study show that for every tree species investigated, temperatures between 57° and 59° C. for a duration of 30 minutes were necessary to kill completely all the cells of the cortical parenchyma. These results are no proof that the plant as a whole will respond in the same manner to similar treatments.

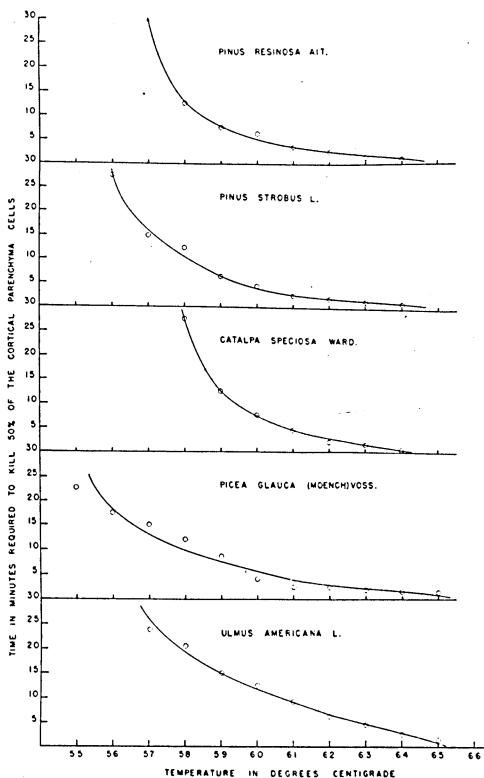


FIG. 2. TIME AND TEMPERATURE RELATIONSHIPS OF FIVE TREE SPECIES

Neutral red was used as an index of vitality after microscopic sections of the cortical parenchyma were subjected to heat treatments in a controlled water bath. The curves are for data representing 50 per cent killing of the cells.

Shirley (65) found that red pine, jack pine, white pine, and white spruce one to four years old withstood exposures of 54° C. for five hours in dry air (relative humidity 15 per cent). His results seem to compare favorably with those of the present

study. Shirley used the whole plant as test material and made exposures of five hours, while the material used in the present study was never exposed over 30 minutes.

Bates and Roeser (7) estimated the exposure of 30-day-old seedlings of Douglas fir, Engelmann's spruce, western yellow pine, and lodgepole pine to a temperature of 141° F. (60.5° C.) in a saturated atmosphere for approximately one minute would cause critical injury.

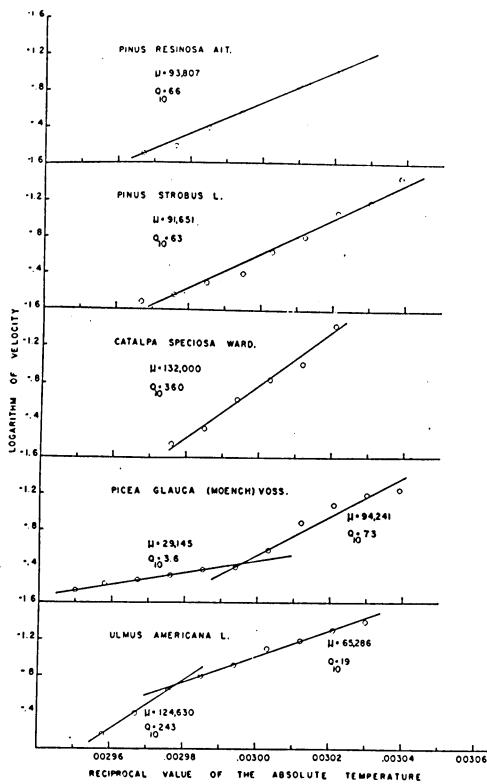


FIG. 3. THE APPLICATION OF THE ARRHENIUS FORMULA TO THE CORRESPONDING DATA AS GIVEN IN FIG. 2

The graphs denote the temperature characteristic (μ) and the Q_{10} values for each resultant slope of the line.

tree species used in this work with the corresponding values of μ and Q_{10} .

Few time-temperature relationships have been determined for plant cells over a sufficient range to enable the investigators to calculate accurately either the Q_{10} or to apply the Arrhenius formula to such data. Although investigators report Q_{10} values of 2 to 3 for biological processes within the biokinetic zone, Q_{10} values over 3,000 have been reported by Moore (53) for the killing temperatures of *Tubularia crocea*. The Q_{10} values for the killing of the cortex cells in the tree species studied compare some-

The Application of the Q_{10} and the Arrhenius Formula

To obtain the proper significance between the Q_{10} and the Arrhenius formula as applied to the time-temperature relationship of the killing of the cortex cells, one must examine figure 3. In table 7 are listed the

what favorably to those obtained by Collander (15) for *Tradescantia discolor*, *Beta vulgaris*, *Brassica oleracea*, *Elodea densa*, *Draparnaldia glomerata*, and *Pisum sativum*. Loeb (45) obtained Q_{10} values of 240 to 1,450 for the killing of the fertilized eggs of the sea urchin, *Strongylocentrotus purpuratus*, between temperatures of 20° to 30° C. The lower Q_{10} values were obtained at the higher temperatures while the Q_{10} of 1,450 was obtained at the lower temperature range, i.e., between 20° and 25° C.

Table 7. Values of μ and Q_{10} Corresponding with Each Tree Species

Species	μ	Q_{10}
American elm		
lower temperature slope	65,286	19
upper temperature slope	124,530	243
Catalpa	132,000	360
Northern white pine	91,651	63
White spruce		
lower temperature slope	94,241	73
upper temperature slope	29,145	3.6
Red pine	93,807	66

The Q_{10} values found for the killing of bacteria at high temperatures lie mostly between 5 and 15, and at times even higher than this, as shown by the work of Blau (13), Meyer (51), and Weiss (70). The fact was emphasized by Meyer (51) and later by Bigelow (12) that the time of killing increases approximately in a geometrical progression as the temperature decreases in an arithmetical progression.

Bělehrádek and Melichar (11) determined the lethal temperatures for *Elodea canadensis* Rich. at different durations of time. A modification of Růžička's (62) staining method, supplemented with plasmolysis was used as an index of vitality. They found that the cells would survive a temperature of 65.2° C. for a period of three seconds. Their Q_{10} and μ values are as follows:

Constant	Value of the constant for the temperatures of—	
	22°-44° C.	44°-65° C.
Q_{10}	7.6	50.0
μ	50,700	90,000

It is noteworthy that when using much the same technique as Belehrádek and Melichar, both as to the application of heat to the test material and the method of determining the degree of injury, the Q_{10} and μ values for the killing of the cortex cells of the trees used in this work compare very favorably with the above-mentioned results.

A previous discussion in this paper has already pointed out how some investigators have used the Arrhenius formula to characterize a definite biochemical mechanism. Crozier (17), the outstanding protagonist of this viewpoint, concluded that processes associated with a μ value of 11,500 are limited by hydroxyl-ion catalysis; those yielding a μ value of 16,700 are limited by iron catalysis, while those in which $\mu = 20,000$ are based upon hydrogen-ion catalysis.

From the tabulated values of μ as listed by Arrhenius (1, pp. 54 and 55) it is apparent that μ is in general greater for spontaneous decomposition, and particularly coagulation by heat, than for processes in which a substance acts on another catalytically. He has listed a μ value of 135,000 for the coagulation of egg white by heat.

The temperature characteristics for the tree species studied, as obtained by the Arrhenius equation, are listed in table 7 as well as occurring in figure 3. The majority of them lie between 91,000 and 132,000. Figure 3 indicates that the data for catalpa, white, and red pine fit the Arrhenius equation quite well with μ values of 132,000, 91,651, and 93,807, respectively. The application of the Arrhenius formula to such data as found for white spruce and American elm can be seriously questioned. It appears that the forces acting to destroy protoplasm at high temperatures tend to act gradually and continuously rather than to produce resultant effects which manifest themselves in distinct or abrupt breaks in the Arrhenius equation. For a complete discussion of the literature pertaining to the Arrhenius formula and its application in biological processes, consult Bělehrádek's monograph, "Temperature and Living Matter," page 50.

Further criticisms are given by Bělehrádek (8, 9), Heilbrunn (39, 40), Emerson (29), Burton (14), Ponder and Yeager (59), and Fulmer and Buchanan (31).

Microscopic Observations of Heat Injury

The physical and chemical changes occurring in protoplasm from the resultant effects of high temperatures were not the objects of special study. During the course of investigation of the high-temperature relationships occurring in the cortical parenchyma of the trees studied, several observations were made which might suggest the nature of heat killing.

Five general theories explaining the mechanism of heat injury to protoplasm may be found in the literature. They are: (a) coagulation theory, (b) heat destruction of enzymes, (c) asphyxiation theory, (d) intoxication theory, and (e) lipid liberation theory.

The coagulation of protoplasmic proteins is probably the oldest and most widely accepted theory of heat injury. It was quoted by Sachs (63) as early as 1864 as a current view.

Lepeschkin (42) distinguishes four stages in what he calls "the heat coagulation of the protoplasm" as may be observed in *Spirogyra* at 43° C.:

(1) Invisible changes in dispersity with a marked increase in permeability, (2) visible coagulation of the superficial protoplasmic layers, (3) coagulation of the chloroplast, and (4) complete coagulation of the whole cell.

At the beginning of this experiment a high-temperature stage was used which permitted constant microscopic observation of the cortex cells as heat was applied to them. Changes took place somewhat similar to those described by Lepeschkin. The first and most pronounced effect of heat was the increase of viscosity of the protoplasm. This manifestation was made apparent by lack of plasmolysis at higher temperatures, although staining was thorough with neutral red. At the higher temperatures, granules in the cytoplasm made their appearance. Above a given time and temperature the cell membrane seems to lose its permeability characteristics. At this point the cortical cells would no longer stain with neutral red. Cells previously stained with neutral red and given the same heat treatment in a water bath would abruptly lose their dye content at this point.

After the function of the cell membrane was impaired by high temperature, the coagulation of the entire cell contents took place.

Two common objections often raised against the coagulation theory are (a) that proteins coagulate by heat at higher temperatures than that which is injurious to protoplasm, and (b) that coagulative changes in the beginning are reversible in protoplasm and irreversible in proteins.

SUMMARY

1. A method is described whereby the living and the dead cells of woody stems may be differentiated.
2. The cortical parenchyma cells of catalpa, American elm, red pine, northern white pine, and white spruce seedlings are killed in 30 minutes when exposed to temperatures between 57° and 59° C.
3. One minute is necessary to kill the cortical parenchyma cells of the above-mentioned species at 65° to 69° C.
4. The five tree species do not vary markedly in their relative heat resistance.

5. The Arrhenius formula gives μ values of 93,807, 91,651, and 132,000 for the heat killing of the cortical parenchyma cells in red pine, northern white pine, and catalpa, respectively. These values lie within the zone characteristic for heat coagulation.

6. Q_{10} values lying between 3.6 and 360 were obtained for the heat killing of the cortical parenchyma cells in catalpa, American elm, red pine, northern white pine, and white spruce.

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