A METHOD TO DETERMINE HEAT-CAUSED MORTALITY IN BUNCHGRASSES[1]

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Abstract. This paper describes a method for separating plant mortality due to heat from mortality caused by herbage removal; squirreltail (*Sitanion hystrix* (Nutt.) J. G. Smith) and needle-and-thread (*Stipa comata* Trin. and Ruper.) are used as examples. After plants are burned in the field under controlled conditions, ratios are calculated between field-measured durations of temperatures in plants and laboratory-measured thermal death times for plant tissue. If the sum of the ratios for the various temperatures is 1.0 (designated 1 necrotherm) or greater, mortality is attributed to heat; if the sum is less than 1.0, to herbage removal. Further testing of the "predictor" on more species, sites, years, etc., is needed, but the method appears very promising. For the two bunchgrasses tested, heat alone accounted for all mortality by fire, except during July. The time required for a specific temperature to kill plants varied with season. To predict thermal death times for bunchgrasses and thus simplify the laboratory determinations, an empirical equation was developed.

INTRODUCTION

Death of perennial grasses following fire is usually attributed to heat. Yet we do not know whether death was caused solely by heat or by some simultaneous shock such as loss of herbage. If the effect of heat could be separated from the effect of herbage removal, perhaps the mortality of grasses in control burns could be minimized, since we would know when to be most concerned about damage from each cause. This paper describes a tentative method for differentiating heat-induced mortality from that resulting from herbage removal. Also, the data show that, for the two species studied, heat, measured by time-temperature relations, accounts for all mortality by fire except during months when herbage removal alone will kill plants.

Combinations of temperature and exposure time have been used by many researchers to measure death by heat in plants (Schmidt 1954, Hare 1961). However, time-temperature relations can be expressed in several ways, and the interpretation of terms may vary. Much of the early work on death by heat was done with respect to a "thermal death point," which is the lowest temperature that results in no survival after a fixed period of exposure, usually 10 min (Schmidt 1954). A later and more sophisticated method by Bigelow and Esty (1920) resulted in a "thermal death time." For each temperature at which microorganisms could be killed, the exact number of minutes of exposure required for death of the organism was found. Then, by a logarithmic plotting of these thermal death times against their respective temperatures, Bigelow (1921) produced a "thermal-death-time curve."

Thermal death points for about 20 plants, compiled from several investigators by Baker (1929), ranged from 117° to 139°F (47.2° to 59.4°C). Only cactus survived temperatures over 144°F (62.2°C). Baker concluded that the thermal death point at the cellular level for herbage of average mesophytic plants lies between 122° and 131°F (50° and 55°C). Roots, however, seemed to be more sensitive to temperature; pea roots died at 113°F (45°C). From his own work Baker stated that tissues of conifer seedlings were quickly killed at 130°F (54.4°C), but withstood temperatures a few degrees lower for some time. This indicates that the relation between time and temperature is an exponential function.

By using a water bath to gradually raise the temperature in dry test tubes until plant tissue was killed, Jameson (1961) found that "lethal temperatures" for culms of four grass species—*Bouteloua curtipendula, B. eriopoda, B. gracilis,* and *Hilaria jamesii*—varied between 140° and 165°F (60° and 74°C). This method is not like that of any other temperature studies. Moreover, Jameson's term, lethal temperature, does not account for the time factor. His lethal temperatures might have been lower if he had maintained the water bath at lower temperatures for a longer time. Hare (1961) states that the term lethal temperature has little meaning unless it is related to time. He reasoned that the exponential relation between temperature and thermal death time indicates that plants can be killed throughout a wide range of

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temperatures, if any given temperature is maintained for the appropriate length of time. Hare suggests that three general factors, each of which includes several variables, determine the amount of heat necessary to kill plants: (1) physiological condition of the protoplasm; (2) initial temperatures of the vegetation; and (3) capacity of dead tissue (such as leaves or bark) to insulate living cells from the heat source.

Assumptions

To assume, as we did in this study, that certain combinations of temperature and time will induce mortality in plants, we must also assume (1) that "a rise in temperature is essentially an increase in molecular activity resulting from the addition of energy" (Brey 1958); (2) that a rise in temperature increases "rate processes" such as ". . . the decomposition of hydrocarbons at elevated temperatures, the oxidation of organic compounds in the living cell, and the reaction of nitrogen and hydrogen to form ammonia" (Brey 1958); (3) that some of these rate processes, if sufficiently accelerated for a suitable length of time, denature proteins, causing death of the plant; (4) that we can distinguish dead tissue from living tissue with tetrazolium; and (5) that the characteristics of a plant that affect the rate of a reaction are the same in situ as in the laboratory when temperatures of the surrounding mediums are the same. Specific heat, vapor pressure, and conductivity were not measured in situ or in the laboratory; possible differences were assumed to be negligible and not of practical importance.

Procedure

The procedure discussed in this paper for determining heat-induced mortality consists of four steps.

1) Thermal-death-time curves for plant tissue are determined over the range of 48.9°–93.3°C in the laboratory.

2) While plants are burning in a self-sustaining manner in the field, the durations of specific temperatures at the growing point 0.6 cm within the perimeter of the plant are recorded.

3) Ratios are calculated between the field-recorded durations of temperatures and the corresponding laboratory-determined thermal death times. Theoretically, if the ratio is greater than 1.0, death of plants is caused by heat.

4) Validity of this theory is evaluated by comparing mortality after fire with mortality predicted from the calculated ratios and with mortality after clipping comparable plants at 1 cm above the soil surface. For this study, plants are considered dead if they had no new basal tillers 1 year after treatment.

Laboratory techniques

Durations of temperatures necessary to kill growing points of squirreltail (*Sitanion hystrix* (Nutt.) J. G. Smith) and needle-and-thread (*Stipa comata* Trin. and Ruper.) were determined on five dates at 5.5°C intervals from 48.9° to 93.3°C. Fresh plants were collected every 4 hr and kept in their sod at room temperature before treatment. The culm bases, 2.5 cm long and stripped of dead leaves, were placed in dry stoppled test tubes and heated for various lengths of time in a constant temperature bath at nine temperatures between 48.9° and 93.3°C. The culm bases were not placed in the test tubes until the air temperature in the test tubes was equal to the bath temperature. After the heat treatment, live tissue was detected with a 1% solution of tetrazolium (U.S. Department of Agriculture 1961). Tissue was considered dead only if absolutely no red or pink coloring showed. The time required to kill the tissue was plotted against temperature (Fig. 1 and 2).

Much of the seasonal variation in heat resistance in this study is related to moisture content of plants, but moisture is confounded with other variables such as content of bound water, pectin, lignin, salt, sugar, and density of plant tissue. As

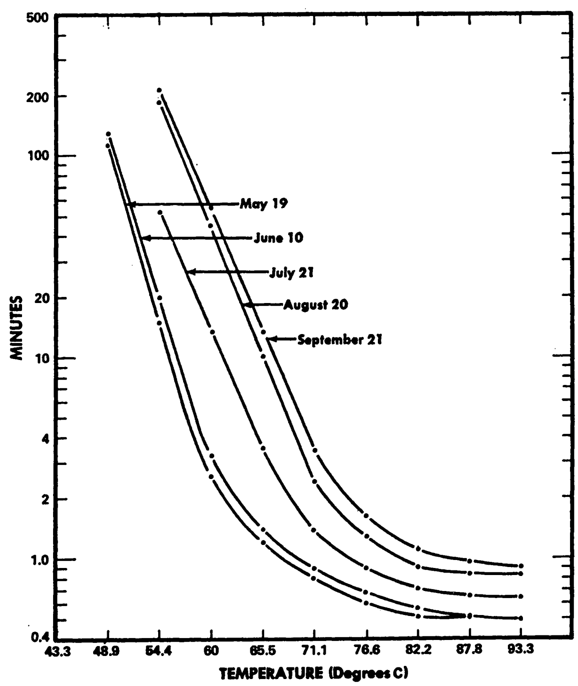

FIG. 1. Time required to kill tissue of needle-and-thread (*Stipa comata*) at specific temperatures on five dates.

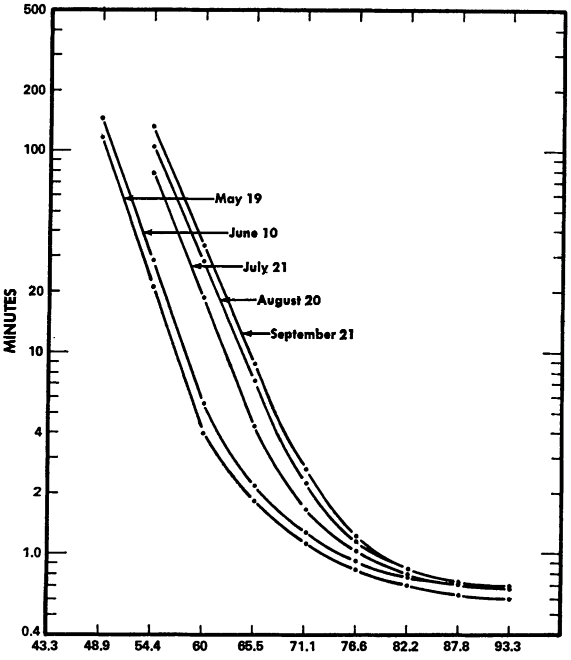

FIG. 2. Time required to kill tissue of squirreltail (*Sitanion hystrix*) at specific temperatures on five dates.

TABLE 1. Moisture content (as percentage of ovendry[a] weight) of living tissue of squirreltail and needle-and-thread at time of thermal-death-time determinations

Treatment date	Squirreltail	Needle-and-thread
May 19	166	158
June 10	127	129
July 21	53	82
August 20	38	66
September 21	28	61

[a]Plant tissue was dried at 70°C for 24 hr.

the season progresses, plant tissue becomes drier (Table 1), and a longer time is required to kill tissue at a given temperature (Fig. 1, 2). This result agrees with work by Richardson (1958).

Field operations

Plants of squirreltail and needle-and-thread were burned individually in the field with a propane burner (Fig. 3). Two sets of hinged plywood (one shown in photo) were placed around the burner. The burner was removed 1 min after heat was applied, but the plywood was not removed until 10 min after heat was applied. Each plant was burned for 30 sec with propane gas held at either 5- or 6.5-lb pressure; half of the plants were burned at one pressure and half burned at the other. These burn treatments provided enough heat to raise the maximum temperature of the soil surface to 260°C and 538°C, respectively, when no plant was being burned. However, these treatments did not produce enough heat to change the initial temperature at the growing-point edge (about 4 cm below the soil surface and 0.6 cm within the edge of a plant) more than 5.5°C. After convective heat was applied, the plants usually burned from 1 to 30 min during May and June, and as long as 2½ hr during July, August, and September. Temperatures above 37.8°C at the growing-point edge were caused by conductive heat from a self-sustaining burn within the bunchgrass. The growing-point edge was chosen as a location to measure temperatures because if temperature-time combinations were lethal at this location, the rest of the plant would have been exposed to much more than lethal combinations.

An iron-constantan thermocouple wired to a panel meter measured burning temperatures at the growing-point edge. At this location (4 cm below soil surface) the thermocouple was in a very dense medium (living material, dead material, and some soil) and was measuring some combination of the surface temperature of the living culms and temperature of the medium surrounding the living culms. Since heat reached this area of the plant very slowly, it seems reasonable to assume that all material in the vicinity of the thermocouple was always very near the same temperature.

Recorded data included preignition temperature at the thermocouple location and postignition time to reach 43.3°, 48.9°, 54.4°C, etc. at 5.5° intervals to maximum temperature during both the heating and cooling periods.

Computations

Since time required to kill a plant is related to temperature, the first step was to calculate the number of minutes that a plant was subjected to lethal temperatures. The exposure time was computed for 5.5°-temperature intervals from 46.1°C to the maximum temperature recorded by the thermocouple. The plant used for illustration was subjected to temperatures above 46.1°C for 159.5 min (Table 2). The total exposure time, including heating and cooling, for each temperature interval is given in Table 2, column a. The number of minutes required to kill plant tissue at the median point of each temperature interval was determined in the laboratory (Table 2, column b). For example, it took 215 min to kill plant tissue at 54.5°C (the median for 51.7°–57.2°C), 55 min at 60°C, etc., to 1.6 min at 76.7°C.

The ratio "minutes of burning time in plant" to "number of minutes required to kill tissue" was calculated for each temperature interval (Table

FIG. 3. Propane gas burner with five jets, used to burn plants in situ for 30 sec. One of the five panel meters served to measure magnitude and duration of temperatures.

2, column c). If the sum of these ratios for all temperature intervals is 1.0 or greater, the plant theoretically died from heat. This ratio of 1.0 has been called a necrotherm (necro = death, therm = heat), which can be defined as any combination of temperature and exposure time that will kill tissue of living organisms. The sum of the necrotherms computed in Table 2 is 36.110; consequently, this plant was subjected to 36 times the lethal temperature-time exposure.

Test of validity

Necrotherms were computed for each of the field-burned plants (60 squirreltail and 60 needle-and-thread), and predicted mortality based on plants subjected to more than 1 necrotherm is given in Table 3. Mortality from fire is caused by heat alone except during those months when herbage removal alone kills plants. In July, mortality of needle-and-thread burned in the field greatly exceeded mortality predicted by necrotherm values derived in the laboratory. But during this month the kill from clipping was also significant. When herbage removal alone can kill plants, we cannot assume that heat necessarily causes the mortality of needle-and-thread.

Frequently, fire removes more herbage than a clipping treatment 1 cm above the soil surface. This more severe herbage removal might account for the higher mortality after burning than was predicted from the additive effect of heat and clipping during July. Logically, the same reasoning should be applied to mortality from burning in August, when actual and predicted mortality were identical, but when some additional mortality could have been expected from herbage removal alone. This discrepancy indicates some inconsistency and shows that the method is not yet adequately tested. The method did, however, indicate the minimum percentage mortality that can be expected for needle-and-thread.

TABLE 2. Computations for one thermocouple placed at growing point 0.6 cm within edge of one needle-and-thread plant

Temperature interval (°C)	Minutes of burning time in plant (field) (a)	Minutes required to kill tissue[a] (lab) (b)	Necrotherms (ratio of a/b) (c)
46.1–51.7	19.4	—	—
51.7–57.2	23.4	215.0	0.109
57.2–62.8	19.9	55.0	0.362
62.8–68.3	26.2	13.3	1.970
68.3–73.9	31.6	3.4	9.294
73.9–79.4	39.0	1.6	24.375
		Sum of ratios	36.110[b]

[a]Taken from thermal-death-time curve for September shown in Fig. 1.
[b]If the sum of the ratios is greater than 1.0, the plant is assumed to have been killed by heat.

TABLE 3. Percentage mortality[a] of needle-and-thread and squirreltail by month of treatment, observed 1 year after burning in the field, predicted from actual temperature records, and observed 1 year after clipping

	Squirreltail			Needle-and-thread		
Month	After burning	Predicted	After clipping	After burning	Predicted	After clipping
May	0	0	0	0	0	0
June	0	0	0	0	0	0
July	8	8	0	83[b]	33	33[c]
Aug	17	33	0	58	58	17[d]
Sep	0	8	0	50	50	0

[a]Based on a total of 12 plants per treatment per month.
[b]Significantly different from predicted mortality at 1% level.
[c]Significantly different from zero mortality at the 5% level.
[d]Not significantly different from zero mortality.

This method of separating the effect of fire from the effect of herbage removal was also tested on squirreltail (Table 3). Here, fewer plants died than would be expected from the predicted mortality by heat, although these differences were not significant. The lower-than-expected mortality by heat may be explained by uneven temperature distribution on the periphery of the plants, a condition observed frequently in squirreltail. This question may be resolved by taking more temperature measurements on the periphery of plants.

When plants can be killed by herbage removal alone, the interaction between herbage removal and heat appears complex. Detailed studies of heat effects on plants during critical stages of growth must be conducted before this method of predicting mortality from heat can be used in the field. This method should also be tested to evaluate the importance of site, year-to-year variation, and soil moisture.

Deriving Equations for Thermal-Death-Time Curves

Constructing thermal-death-time curves for the two plant species studied required about 3 days of laboratory time each month. A desire to shorten this laboratory time prompted development of an equation to approximate the killing time for any given temperature. The equation below approximates points on thermal-death-time curves within 15% of their actual value 75% of the time. This accuracy is satisfactory only for making initial estimates of points on the curve. However, this first approximation can eliminate the step of making exploratory tests to determine the range of time required to kill tissue at a given temperature (°C).

An equation of the general form

$$Y = \frac{a}{(X - c)e^{d(X-c)}} + b$$

expresses the minutes required to kill plant tissue (Y) as a function of temperature (in °C) (X), where b is the time required to kill tissue at an infinite temperature, c is the lowest temperature (in °C) at which tissue can be killed, and a and d are unknown but seem to represent variables such as total moisture, salts, and sugars, or combinations of these variables. Bound moisture, lignin, and pectin are other variables that could be involved. The numerical value of e is 2.7183. . . .

Initial attempts to derive values for these parameters from actual data by using differential corrections and allowing all parameters to vary simultaneously were not successful. The corrections were always divergent. When d was estimated and held fixed, convergence was obtained on the other parameters, but b and c had no biological meaning. When b and c were fixed (approximating their values as will be shown later), a and d varied. Good estimates of a and d were obtained. But it became apparent that all parameters could be approximated empirically with about the same accuracy as the differentially corrected parameters. Since it is easier to approximate parameters empirically than to determine them mathematically, only the approximation procedure will be discussed in this paper.

A good estimate for b in squirreltail and needle-and-thread (and perhaps in many other plant tissues) is the time required to kill tissue at 93°C; at this temperature the time required to kill bunchgrass tissue approaches a horizontal asymptote. Parameter c is the minimum temperature at which squirreltail and needle-and-thread tissue can be killed. It is approximated in relation to percentage moisture of the plant tissue. If the percentage of moisture of squirreltail and needle-and-thread tissue on an ovendry weight basis is more than 100, a value of 46° approximates c; if the moisture percentage is less than 100, 49°–51° approximates c. The value 0.18 is a constant for d during all seasons for both species. The constant a is computed by letting Y equal the time required to kill tissue at 65.5°C; the variable X in this case equals 65.5°. From these constants (determined for each species within season) reasonable estimates could be computed of times required to kill plant tissue for temperatures from 60° to 93°C. Reasonable estimates of threshold values (less than 60°C) can be extrapolated from the straight-line portion of semilogarithmic curves (Fig. 1, 2).

The extent to which this equation will predict thermal-death times for other grasses and plants depends on how much their parameters depart from those of squirreltail and needle-and-thread. Further development of this proposed equation appears worthwhile. We should seek an equation that will predict accurately all points on thermal-death-time curves within 10%. This tolerance would be within our usual sampling variation in biological material. Moreover, the parameters should be easily measured.

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