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CALORIMETRIC DETERMINATION OF TISSUE RESPONSES TO THERMAL EXTREMES AS A FUNCTION OF TIME AND TEMPERATURE *

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SUMMARY

Calorimetric procedures have been developed for defining the effects of high and low temperature stresses on the metabolic activities of plant tissues and cell cultures. Inactivation of metabolism was shown to be a complex function of both temperature and time of exposure to thermal extremes. Equations developed to fit the activity loss data can be used to (a) predict residual activity after any regime of thermal exposure, (b) select plant cultivars with differences in thermal stabilities, and (c) provide models for the nature of responses associated with thermal inactivation. Equations describing thermal inactivation at both high and low temperature ranges have a form which suggests a second or higher order dependence on temperature. Results on tomato leaflets and cells are used to illustrate the applicability of the method.

INTRODUCTION

Thermal injury is a common problem for many commercially important plants. The injury may result from exposure to either high or low temperature. The extent of injury caused by thermal extremes is species specific and is a complex function of both the temperature and duration of exposure (ref. 1). Considerable effort has been directed to selecting plants with extended temperature hardiness. Defining precise time-temperature relationships for plant injury has proved difficult, however. Data on the correlation of extreme excursions in temperature with plant damage has traditionally relied on rather gross field observations which do not take into consideration such factors as microclimatic differences, or on laboratory studies which generally have major limitations on the numbers of temperatures and times of exposure which can be evaluated (ref. 2). Even with these difficulties, the time lapse between cellular thermal damage and macroscopic

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physiological expression of the damage, difficulties in quantifying the extent of damage, and the numbers of additional environmental variables which can be controlled and examined for their effects on temperature stability may be more important limitations.

Calorimetric measurement of metabolic rates of plant tissues simultaneously monitors responses of integrated plant metabolism to temperature extremes and precisely manages temperatures and times of thermal exposures of plant tissues (ref. 3). Moreover, measured changes in metabolism can be used to quantitatively assess thermal damage. It is quite obvious, for example, that killing cells will reduce the net metabolic heat rate of a tissue. Non-lethal shifts in the relative contributions of metabolic pathways may be observed as the stressed cells change.

In principle, three different methods for measurements of plant metabolic responses to thermal stress can be considered. The simplest is to measure metabolic rates isothermally at a test temperature. A second is to measure essentially instantaneous changes in metabolic activity as a continuous function of changing temperatures to look for regions where activity is altered. This method was described previously (ref. 4). The third is to measure recovery of activity at a reference temperature following exposure to a given thermal regime (ref. 3). All provide useful, but different information. Examination of recovered activity, after exposure to time-temperature stress treatments, yields data that is most similar to responses in the field.

In this paper, we present methods for measurement of residual tissue activity following thermal stress. Mathematical functions are developed for tomato leaves and cells in tissue culture that (a) describe temperature-time-activity relationships, (b) define parameters that can be used to compare thermal sensitivities of different plant species or cultivars, (c) guide selection of conditions for screening plants for thermal tolerant or sensitive strains, and (d) suggest possible mechanisms of thermal inactivation.

The calorimetric method described here relies on the direct linear relationship between the metabolic rate and the heat rate measured by isothermal calorimetry. Decreases in the heat rate following exposure to extreme temperature conditions are then a measure of thermal inactivation. Increases in metabolic heat rate can also result from thermal damage that causes shifts in metabolic pathways.

MATERIALS AND METHODS

All calorimetric measurements were conducted in a Hart Model 7707 differential scanning calorimeter. Tissue sample heat rates were measured in 1 cm^3 internal volume, screw-top Hastelloy ampules. Three samples were run simultaneously. Sample volumes were generally about 0.2 to 0.4 ml, leaving 0.8 to 0.6 ml of head space. In experiments continued for more than 5 hr, O_2 was used as the head space gas to avoid anaerobic conditions. Otherwise, either air or O_2 was used interchangeably without measured effect on metabolic rates.

Isothermal measurements of metabolic heat rates were examined at a selected reference temperature in the mid-range of normal growth for the plant tissues (e.g. 25°C). When the thermal signal had stabilized (approximately 45 min), the calorimeter temperature was rapidly (99°C/hr) adjusted to a selected stress temperature in a range causing thermal inactivation. This temperature was maintained for a preselected time, heat rates were measured, and the temperature rapidly readjusted to the reference temperature. Heat rate measurements following this cycle of stress treatment recorded the activity loss at the reference temperature due to the time at the selected stress temperature. The calorimeter was programmed to cycle repeatedly between reference and stress temperatures until activity was greatly reduced. A curve of residual activity at the reference temperature could then be plotted vs time at the stress temperature. This sequence of measurements was then repeated at additional stress temperatures to obtain a family of curves illustrating inactivation with time, and ultimately an activity-time-temperature relationship. A surface plot of the data was prepared and equations which describe the plot were developed using the SYSTAT® statistical program (ref. 5).

Tomato cells (Tomato-45), growing in liquid culture (ref. 6) supplemented with Percoll® (registered trademark of Pharmacia) to float the cells (ref. 7), and tomato leaflets with no added nutrients were used in studies to demonstrate the usefulness of the cycling procedures for a typical chilling-sensitive plant and in different types of tissue preparations. Thermal inactivation was studied at both the high and low ends of the range of temperature adaptation of tomato.

RESULTS

A diagram of the protocol for a typical high temperature cycling experiment is presented in Fig. 1. Measurement of metabolic heat rates at the reference temperature required about 30 min. The temperature and duration of the high (or low) temperature treatments and heat rate measurements were varied from run to run.

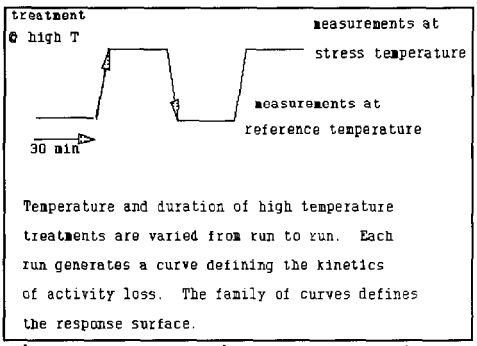

Fig. 1. Schematic of protocol for cycling measurements of stress effects.

Repeated measurements of metabolic heat rates of a 0.4 ml suspension of tomato cells as temperature was cycled repeatedly between 25° and 40° are shown in Fig. 2. Upon returning to 25° after each high temperature cycle, there was only a partial recovery of metabolic activity observed in the previous cycle. The rate of loss of activity at the reference temperature increased as the stress temperature increased. All temperatures above about 35°C had measurable inactivation effects. Loss of activity at each high stress temperature was first order with respect to the time of exposure of the cells to stress temperature, i.e. activity decreased according to e^{-kt} (Fig. 3). The rate of activity loss was independent of the number of cycles of temperature change and of the relative lengths of times for reference temperature or high temperature exposures.

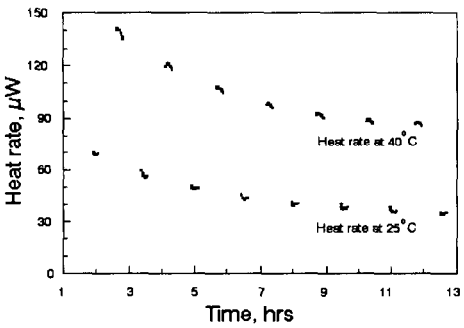

Fig. 2. Metabolic heat rates at 25°C and 40°C.

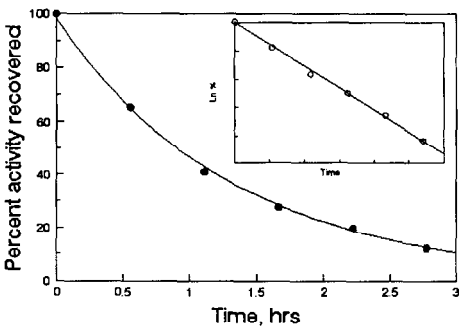

Fig. 3. Loss of activity at 25°C as a function of cumulative time at 40°C.

Activity loss as a function of both time and temperature is shown in Fig. 4. The loss of activity was a complex function of temperature. The surface described by the experimental data points is fit by equation 1,

$$\ln(\text{percent of initial activity}) = 4.6 + t(A(T-T_m)^N+B) \quad (1)$$

where t = time at the stress temperature, T = temperature in degrees C, A and B are constants for a given tissue, and T_m is the temperature at the inflection point of the activity-temperature curve. Our interpretation is that N indicates the degree of molecular cooperativity in the process of thermal inactivation. When N is significantly less than one, some type of higher order of molecular interaction is suggested. The values of these constants which fit the high temperature in activation of the particular strain of tomato cells studied are A = 0.19, B = -0.43, N = 0.30 and T_m = 41.5°C.

The fit of the data points to the surface described by the above equation and set of constants is also indicated in Fig. 4. The intensity of shading of each spot is proportional to the closeness of fit of the mathematical model to the data point. The darker the spots, the poorer the fit of that point. The correlation coefficient, R^2, for the overall fit of the data to the model is 0.996. A plot of experimental values of activity vs predicted values from the equation is given in Fig. 5.

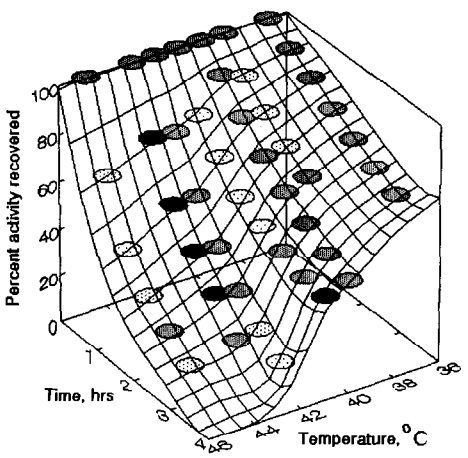

Fig. 4. Activity response surface as a function of cumulative time at the stress temperature.

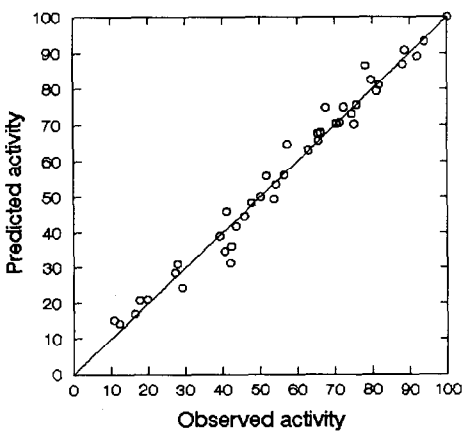

Fig. 5. "Goodness of fit" plot indicating goodness of fit of surface to time-temperature data.

Inactivation rates of tomato leaflets at fixed low temperatures are not described by the simple first order exponential decay of tomato-45 cells shown in Fig. 3. Early events following chilling cause a transient increase in the overall metabolic rates which reaches a maximum after about 30 min cold exposure (Fig. 6). This transient increase is superimposed on a first order exponential loss of activity with time. The transient increase above the first-order decay ends within two hours. This increase in activity is reflected in the response surface.

Data for cold inactivation of tomato cells as a function of time and temperature are summarized in Fig. 7. Equation 1, used to describe high temperature responses, also describes cold inactivation responses, with A, B, N, and T_m values of 0.28, -0.083, -0.83, and 1.12, respectively. Note that N, and thus cooperativity, is different than the value obtained for heat inactivation.

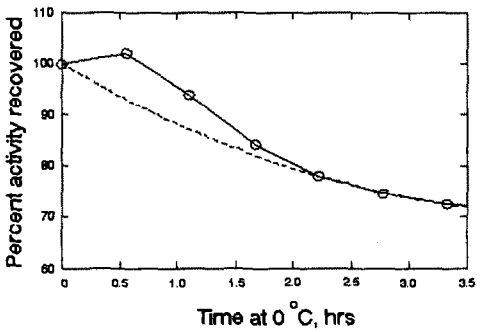

Fig. 6. Activity response surface as a function of cumulative time at the stress temperature.

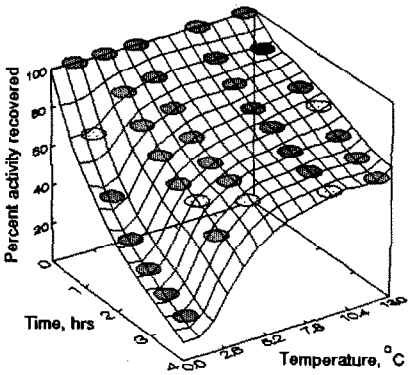

Fig. 7. Experimental data at 25°C showing transient increase in metabolic rate superimposed on inactivation by chilling at 4°C.

DISCUSSION

A temperature cycling method has been developed to determine the complex relationships among metabolic activity, temperature and time of exposure to temperature extremes. Calorimetric data can be obtained rapidly enough to allow investigation of plant metabolic temperature dependencies for moderately large numbers of plants. Each cycling experiment takes about 10 hours, and can include three different plants. Activity measurements at five or six different carefully chosen stress temperatures are sufficient

to produce reliable response surfaces in the time-temperature domain. The mathematical parameters describing the response surfaces can be used to quantitatively compare thermal sensitivities of different ecotypes, cultivars, and species. With the semiautomated procedures described here, detailed data on thermal responses of three different plants can be obtained in 3 to 4 days. Although the data presented here are for cells in tissue culture and leaflets, the method works equally well when applied to leaf cuttings, roots, and other whole tissues.

The data on plant thermal responses obtained by calorimetry may not translate precisely to plants growing in the field. However, the general form of the time-temperature relationship derived here is likely to be directly applicable to field studies and should be of value in further development of models of field crop responses to chilling or high temperature injury. Laboratory measurements of thermal sensitivities will provide accurate information on relative stabilities of test strains of select plants and therefore should be a useful guide to strain selection.

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