Effect of Sublethal and Lethal Temperatures on Plant Cells

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Abstract. Soybean, Glycine max L., and elodea, Elodea canadensis Michx., leaves were exposed to sublethal and lethal temperatures and examined by light microscopy. Loss of chlorophyll and swollen chloroplasts were observed in cells of elodea leaves exposed to sublethal temperatures. At the thermal death point of leaf cells of elodea and soybean, there was a disorganization of the tonoplast membrane, plasmalemma, and chloroplast membranes. Approximately 40% of the cells in elodea and 50% of the cells in soybean leaves exhibited criteria of cell death when exposed to a temperature which induced necrotic leaf tissue. Plasmolysis of leaf cells of elodea and soybean occurred at lethal temperatures, but did not appear to be the primary cause of cellular death. The primary effect of lethal temperatures on the leaf cells used in these experiments is disintegration of the cellular membranes.

Following exposure of attached elodea leaves to lethal temperatures, changes in leaf cells were periodically observed with a light microscope. In low temperature treatments, (43 through 52°), the percentages of cells exhibiting criteria of death 12 days after treatment did not change from the percentages determined immediately after treatment. All treatments above 52° resulted in 40% or more of the cells exhibiting criteria of cell death immediately after treatment. In these treatments, this resulted in all cells exhibiting criteria of death on the fourth day after treatment.

Sun-scald of fruits (6), whitespot lesions on stems (5), and other thermal incited injuries have been reported when plants or fruits have been subjected to superopitimal temperatures. Accumulative heat injury eventually results in total inhibition of the vital functions of plants or individual cells. In initial stages of heat injury, the effect is reversible but finally leads to "thermal death". Nielsen and Todd (13) found that the permeability of cell membranes of potato tubers was increased by heating to sublethal temperatures of 43 to 45°. To determine the injury to cells from exposure to high temperatures, Alexandrov (1) used such criteria as suppression of plasmolysis and deplasmolysis, exit of pigments from the vacuole, loss of vital staining, luminescence of the chloroplasts, and the cessation of protoplasmic streaming. Vital stains have been used by several workers (11,15) to determine the death of cells.

The cause of death by high temperature usually has been explained as the result of denaturation of proteins (9). Other theories have been reviewed by Levitt (10). Heat denatured proteins may aggregate or coagulate, with the coagulation being a secondary phenomenon (14). In 1938, Maximov (12) stated that the action of superoptimal temperature, as explained by the coagulation of protein theory, often is not sufficient since death may begin at 40°, a level which is far below the coagulation point of proteins. There appear to be proteins or enzymes which are relatively stable at high temperatures. Koffler (8) suggests that the relative heat stability of proteins or enzymes in thermophilic organisms depends on more effective hydrogen bonding. This effectiveness of the bonding may depend on the number, strength, or location of the hydrogen bonds as well as other types of bonds.

The purpose of this investigation was to determine some cellular responses to sublethal and lethal temperatures and to obtain information on the effects of superoptimal temperatures at the thermal death point.

Materials and Methods

Soybean plants [Glycine max (L.) Merr. var. Ogdan] were grown from seed in pots in a greenhouse until they were 42 days old. Elodea plants (Elodea canadensis Michx) were obtained from a fresh water stream when needed.

Soybean. Temperature treatments were applied to soybean leaves by exposing the plant tissue to a 1042° flame for 130 msec. In preliminary tests, it
had been determined that leaf tissue exposed to this flame for 130 msec was killed quickly and very pronounced changes occurred within the cells. An assembly consisting of 2 Afro gas burners (one mounted on each of the 2 long sides of the assembly) 18 inches apart was employed. The burners, supplied with liquefied petroleum at a constant pressure of 22 psi, developed a 1042° flame which was 7 inches across and 4 inches wide at a distance of 9 inches from the tip. The belt on the assembly was adjusted to travel at a speed of 4.4 feet per sec. Thus, an object placed on the belt would move past the burners and be exposed to the flame for 130 msec. The soybean plants were placed on the assembly and an attached leaf was held with a utility clamp on the petiole so that the upper epidermis of the leaf was perpendicular to the burner.

Sublethal and lethal temperature treatments were applied to soybean leaves by placing detached mature leaves in a water bath which was maintained at a constant temperature within ±0.1°. The temperature treatments were from 47 to 57° in 1° increments. Control leaves were immersed in water at 24°. Detached leaves were placed in cheesecloth and immersed in the hot water and stirred constantly. At the end of 1 min, the leaves were removed and cooled immediately by placing them in water at approximately 25°. Immediately after treating, 2-cm-square sections of each leaf were cut and fixed in formalin-acetic acid-alcohol solution. Prepared slides were made of these sections as outlined by Jensen (7) using the safranin-fast green staining solution. Cross and longitudinal sections were cut at 9 microns thickness.

*Elodea.* Elodea was selected for this study because the cytoplasm and cellular organelles in living cell cells could be observed under a light microscope without damage to the cells. Temperature treatments were applied to elodea leaves by using hot water treatments similar to those used for soybean leaves. Some leaves were mounted in water on slides for immediate study, and others were stained for 30 min before mounting. The dyes used were neutral red, methyl green, toluidine blue 0, methylene blue, basic fuchsin, and rhodamine B. All of the dyes were used at a concentration of 0.0005 % (w/v) except neutral red, which was used at 0.002 % (w/v). Two of the dyes, neutral red and methylene blue, have been classified as "vital stains." In a living cell these stains are accumulated in vacuoles, but after death the cytoplasm and the nucleus stain intensely and diffusely. The response of cells to these 2 dyes was used as the criterion of cell death. In this study, the other dyes stained the cells apparently only after the differential permeability of the cell membranes had been altered by the high temperature and were, therefore, used as indicators of changes in cell permeability. The cells in the elodea leaf were observed microscopically to determine the effect of high temperature on suppression of protoplasmic streaming; changes in the cytoplasm, nuclei and chloplasts; exudation of pigments from the chloplasts; plasmolysis and deplasmolysis; and suppression of Brownian movement. Photomicrographs were taken with a Leica camera and a Zeiss microscope. The percentage of damaged cells was calculated from counts of 10 replications of the number of damaged and undamaged cells in treated tissue within the field of a microscope with magnification of 150X.

An additional test was conducted on attached elodea leaves using the materials and methods as in the previous test, except that mounts were made of the intact treated leaves at specific days after treatments. After treatment, the attached leaves were placed in beakers containing 500 ml of water and kept in a growth chamber with a 12 hr dark period temperature of 12° and a light period temperature of 17°. Whole mounts were made using 6 leaves from each of the treatments on the first, second, fourth, seventh, and twelfth day after the hot water treatments, and changes in the cells were noted.

**Results**

*Soybean.* Leaf studies in this experiment were directed toward determining the visible effects of excessive heat on cells within leaves. The visible effects of the flame on the palisade parenchyma cells of a soybean leaf are shown in Fig. 1b. The chloplasts, nucleus, and tonoplast appeared to rupture, and leakage of the contents of the cytoplasm into the vacuole occurred.

A second test was designed to determine the minimum temperature at which the above responses could be observed when the leaves were treated in hot water. At 56° and 57°, 90 % or more of the cells exhibited disintegration of the chloplasts and disruption of the vacuole. However, all cells did not exhibit the same degree of injury at a particular temperature. At 55°, approximately 50 % of the palisade cells exhibited the damage described above, and at 54° approximately 40 % of the cells were damaged. Little or no damage was observed with treatments at lower temperatures. Separate tests in which one-half of attached soybean leaves received the hot-water-bath treatment, showed that treatment at 55° or above for 1 min resulted in necrosis of all treated leaf tissue. A sharp line of demarcation was apparent between the necrotic tissue and undamaged, non-treated tissue. Also, treatment at 54° resulted in chlorosis of treated tissue while treatment at 53° and below, resulted in no visible injury.

*Elodea.* In preliminary tests with attached elodea leaves, a 54° treatment for 1 min produced necrosis of all treated tissue, a 53° treatment produced chlorosis and necrosis of the summit imbricated leaves, and treatments below 52° produced no visible injury. In the present study, it was found that all cells
within an elodea leaf did not exhibit the same degree of injury at a particular temperature as was true with soybean leaves. Even in the 54° treatment, cytoplasmic streaming was observed in cells adjacent to cells showing a high degree of plasmolysis.

In the treatments from 48 to 51°, there appeared to be a reversible cessation of cytoplasmic streaming in all of the cells. This may have been a shock effect of the treatments, since the cytoplasm resumed streaming within 6 to 8 hr in the majority of cells observed. Vital stains accumulated in the vacuole of the cells and not the cytoplasm, indicating that the cells were still living. In the check treatment, approximately 3% of the cells exhibited injury. This injury was classed as mechanical injury and this percentage was subtracted from the total cell injury for each treatment.

In the 52° treatment, approximately 60% of the cells showed no apparent evidence of injury. However, 15% of these undamaged cells stained with each of the non-vital stains, indicating that a change in permeability of membranes had occurred. The cytoplasm of approximately 15% of the cells stained when the vital stains were used, indicating they were non-living. Toluidine blue, which has been used to determine disorganized chloroplasts, stained the chloroplasts in these cells a deep blue or brownish blue color. Approximately one-half of the cells that stained with the non-vital stains also exhibited plasmolysis and chloroplast leakage. The remaining 25% of the cells exhibited enlarged chloroplasts and nuclei. Cells with enlarged chloroplasts are shown in Fig. 1c.

In the 53° treatment, approximately 35% of the cells showed no evidence of injury. Approximately 40% of the cells stained with the non-vital stains and approximately the same number of cells were classed as "dead" when the vital stains were used. The majority of cells which stained with the non-vital stains also exhibited plasmolysis and bleached chloroplasts. The remaining 25% of the cells contained seemingly enlarged chloroplasts and nuclei. At 54° and higher temperature treatments, approximately 90% of the cells showed irreversible plasmolysis, bleached chloroplasts, and other criteria of cell death.

Microscopic observations were made with elodea leaves which were mounted on the first, second, fourth, seventh, and twelfth day after the hot water treatments. In the lower temperature treatments (43 through 52°), the percentages of cells exhibiting criteria of death 12 days after treatment did not change from the percentages determined immediately after treatment. Also, in the lower temperature treatments which produced enlargement of chloroplasts and cessation of cyclosis, there appeared to be no enlargement of any chloroplasts after the second day. Approximately 90% of the cells observed in the lower temperature treatments exhibited cyclosis at each observation. All of the leaf cells treated at 53° and above exhibited characteristics of cell death on the fourth day after treatment.

Some of the characteristics of cell death and changes in the cells at or after thermal death which were observed in elodea leaves are shown in Fig. 1d, 1e, 1f, and 1g. In plasmolized cells of elodea leaves observed immediately after treatment (Fig. 1d), there appeared to be chlorophyll in the cytoplasm (indicated by the green color in the cytoplasm) and the chloroplasts appeared shrunken. The chloroplasts stained dark blue with toluidine blue indicating a disorganization of the chloroplasts (Fig. 1e). Cells which had been treated in a water bath at 53° 2 days previously are shown in Fig. 1f and 1g. The chloroplasts appeared intact in some of the cells. The cytoplasm appeared to be coagulated or gelled (also indicated by lack of Brownian movement) and pulled away from the cell wall. Rod-like fibrils were apparent in the cytoplasm. The plasma membrane appeared to be breaking down in some of the plasmolized cells. Leaf cells which had been treated 4 days previously at 54° are shown in Fig. 1h. The chloroplasts were bleached and only remnants of the chloroplasts were apparent in some cells. The protoplasm of the cells showed characteristics of coagulation and the plasma membrane appeared to be disrupted. The nucleus appeared to be breaking down in some of the cells at this time.

Fig. 1a. Cross section of untreated control soybean leaf. Chloroplasts appear normal, abutted to each other, and closely appressed to the cell wall. The vacuoles and nuclei are distinct, X 900.

Fig. 1b. Cross section of soybean leaf which had been exposed to a flame at 1042° for 30 mce. Note the disruption of the chloroplasts and nuclei and the leakage of the contents of the cytoplasm into the vacuoles, X 900.

Fig. 1c. Cells of Elodea leaf treated with sublethal temperature of 52°. The chloroplasts are enlarged (compare 1c with 1d) and show lack of orientation. Cytoplasmic streaming occurred while the microphotograph was taken, X 900.

Figs. 1d and 1e. Leaf cells of Elodea showing plasmolysis and damaged chloroplasts immediately after a lethal treatment. The leaves had been immersed in a hot water bath at 54°. The cells in Fig. 1e were stained with toluidine blue. There appears to be leakage of chlorophyll into the cytoplasm, X 900.

Figs. 1f, 1g, and 1h. Leaf cells of Elodea which had been treated with lethal temperatures by placing in hot water baths for 1 min, X 900. Cells in Fig. 1f had been treated 2 days previously at 54°. Note the plasmolysis and damaged chloroplasts. Cells in Fig. 1g had been treated 2 days previously at 53°. Note that 2 of the cells have intact nuclei while 1 cell exhibits extreme plasmolysis. Cells in Fig. 1h had been treated 4 days previously at 54°. Only remnants of the chloroplasts are apparent. One peripheral cell shows a nucleus in the process of disintegration.
Discussion

In this study, the behavior of “vital stains” was used to identify dead cells. Most of the cells which were classed as “dead” by this method also exhibited other criteria of cell death or injury, such as suppression of protoplasmic streaming, bleached chloroplasts, plasmolysis, and changes in membrane permeability. The other stains were used to show changes in membrane permeability. Some cells showed injury, such as enlargement of chloroplasts and cessation of cytoplasmic streaming, but were not classified as “dead” when the vital stains were used. This injury was considered reversible in this study. The interpretation that some injury was reversible is supported by the test where mounts of elodea leaves were observed at specific dates after treatment. In sublethal temperature treatments resulting in cessation of cytoplasmic streaming and enlargement of chloroplasts, the cytoplasm resumed streaming 6 to 8 hr after the heat treatments. There appeared to be no enlargement of chloroplasts beyond the second day after treatment.

The apparent deleterious swelling and loss of chlorophyll by the chloroplasts observed in this work may not be a criterion of cell death. Hartley (5) observed that excessive heat causes whitspot lesions on stems of plants but not necrosis. Observations made in a thermal death point experiment (Daniell, unpublished data) suggested that chlorosis in vivo of the leaves of soybeans apparently occurs at a temperature of 1° less than that inducing necrosis of the tissue.

An excellent discussion on the effects of high temperature on membranes and membrane constituents has been presented by Chapman (4). Some research supporting the “lipoid liberation” theory is presented. With this theory, high temperature injury, whether reversible or irreversible, is attributed to the melting of lipid constituents in the cell or cell membrane. This theory is linked with the observation that lipid formed by living organisms at high temperatures is more solid than lipid formed at lower temperatures. Changes in lipids within the cell membranes could account for the membrane changes at high temperatures which were observed in the present study.

The plasmolysis observed in most of the cells at lethal temperature treatments did not appear to be a primary event at the thermal death of a cell. Plasmolysis was not apparent in all cells which exhibited other criteria of cell death. It has been suggested that plasmolysis is reversible in most cells (2) and may be a function of living cells (16).

Approximately 40 % of the cells in elodea leaves show cellular disorganization at a temperature treatment which results in death of the whole leaf. This agrees with Berkley and Berkley (3) finding that plasmolysis and deformation of the nuclei were not uniformly distributed in cells of cotton plants which had been subjected to high temperatures.

All enzymes are not inactivated at temperatures which produces necrosis of all treated tissue. Previous tests (Daniell, unpublished data) showed that the respiration rate of tissue treated at lethal temperatures was reduced about 50% but continued at a steady rate for over 2 hr after the lethal treatment. Isolated chloroplasts subjected to lethal temperatures still reduced potassium ferricyanide several hr after treatment, but the Hill reaction was significantly reduced. This could suggest that the action of lethal temperature is not complete denaturation of proteins but is breakage of weak bonds such as those in the membranes of cells.

The results obtained in these cytological tests show that the primary event at the thermal death point of a cell is the structural change of the cellular membranes. It also shows that structural changes within the cells were similar in the 2 types of treatments but were much more pronounced when the temperature of the cells changes rapidly (as in the flame treatments) than when the temperature changes are more gradual (as in the hot-water-bath treatments). Evidence that there are structural changes in the membrane is apparent in the prepared slides of soybean leaves which had been subjected to sublethal or lethal temperatures. In some cells, the tonoplast, the chloroplast membranes, and the nuclear membranes were broken or extensively altered at lethal temperatures.

Literature Cited