High Temperature Acclimation in Pepper Leaves

Jeffrey Anderson, Greg McCollum, and Warren Roberts

Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078

Additional index words. *Capsicum annuum*, deacclimation, electrolyte leakage, heat stress, thermotolerance

Abstract. Electrolyte leakage was used to quantify heat stress injury in 'Early Calwonder' pepper (*Capsicum annuum* L.) leaf disks. Lethal temperatures were estimated from the midpoint of the sigmoidal response curve. An interaction between exposure temperature and duration was observed, with lethal temperatures decreasing linearly from 53 to 46°C as exposure duration increased exponentially from 5 to 240 min. Exposure to two 7.5-min periods at 51.5°C, interrupted by 4 hours at 21°C, resulted in the same injury as a continuous 15-min exposure to 51.5°C. Plants grown at 22/20°C day/night cycles and held 24 hours at 38/30°C had increased their heat tolerance by 3°C, 51 to 54°C; these plants reacclimated to 52°C 48 hours after having been transferred back to 22/20°C. Leaf disks acclimated significantly in vitro in 1 hour and were fully acclimated by 4 hours at 38°C.

High temperature tolerance of plants varies with genotype (Ingram and Buchanan, 1984; Lester, 1985; Wanner et al., 1982), previous temperature exposure (Chen et al., 1982; Lester, 1986), and tissue age (Lester, 1986; Martineau et al., 1979). Studies of membrane integrity following high temperature stress have revealed exposure duration-temperature interactions (Inaba and Crandall, 1988; Ingram, 1985; Wanner et al., 1982). Tissue responses to heat stress can be measured following exposure to a series of temperatures for a fixed duration or by varying the duration at a fixed temperature. Generally, heat-killing time is the more sensitive indicator of high temperature tolerance (Chen et al., 1982).

Heat tolerance levels can be influenced by growth temperature and exposure to nonlethal doses of heat stress before assay. The heat-killing time of turfgrasses was increased by exposing plants to 38/24°C day/night cycles for 10 days before assay (Wanner et al., 1982), while damage to melon leaves was reduced by prior exposure to 35°C for 2 to 4 hr (Lester, 1986).

Our objective was to determine the capacity of pepper leaves to acclimate to high temperature. We also examined the relationship between exposure temperature and duration. Acclimation was studied by placing entire plants into a growth chamber at an elevated temperature and by exposing excised disks to acclimating treatments in vitro. In addition to experiments involving series of ex-

posure temperatures and intervals, the time factor was further explored using discontinuous periods of high temperature stress to determine whether damage was cumulative over time.

'Early Calwonder' pepper seeds were planted in 1.5-liter pots using commercial potting mix (Fafard GP Mix, Springfield, Mass.) and grown in a growth chamber at 22/20°C day/night cycles. Photosynthetic photon flux of $\approx 500 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ at canopy height was supplied by fluorescent and incandescent bulbs. Relative humidity ranged

from 45% to 65%. Plants were fertilized at each watering with 0.75 g 20N-8.7P-16.6K soluble fertilizer/liter (Peters Professional, W.R. Grace & Co., Fogelsville, Pa.) and repotted to 7-liter pots ≈ 8 weeks after planting. Watering was based on soil color and pot weight.

Heat stress. Preliminary experiments indicated that plants younger than ≈ 8 weeks tended to be less hardy than older plants. In addition, immature leaves were found to be $\approx 1^\circ\text{C}$ less tolerant of high temperature than fully expanded leaves on the same plant (data not presented). Therefore, sampling was restricted to fully expanded leaves from plants 60 to 80 days old. Leaf disks were excised with a 9-mm-diameter cork borer and rinsed with at least three changes of distilled water. Individual disks were placed in 25×150 -mm test tubes containing ≈ 0.5 ml distilled water to prevent secondary water stress. Three tubes per treatment level were placed in a heated, circulating water bath for 15 min at each temperature unless stated otherwise. Tissue temperature was measured with 36-gauge (0.13-mm diameter) thermocouples threaded through an extra disk at each exposure temperature.

Tissue response. Distilled water (20 ml) was added to each tube following high temperature exposure. Samples were placed on an orbital shaker for 24 hr at 21°C, and then electrolyte leakage was measured with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs, Ohio). All samples then were killed in an autoclave, cooled to 21°C, and incubated an additional 24 hr before final conductivity measure-

Table 1. Electrolyte leakage from pepper leaf disks following exposure to 51.5°C for 0, 7.5, or 15 min at time 0, after 4 hr, and following two 7.5-min exposures interrupted by 4 hr at 21°C.

Treatments administered (hr into experiment)	Exposure duration (min at 51.5°C)	Electrolyte leakage (initial/final) ^a
0	0	0.16 a
4	0	0.17 a
0	7.5	0.35 b
4	7.5	0.25 ab
0	15	0.81 c
4	15	0.83 c
0 + 4	7.5 + 7.5	0.88 c

^aMeans followed by the same letter are not significantly different at $P = 0.05$.

Fig. 1. Predicted killing temperatures (T_{mid}) for pepper leaf disks exposed to high temperature stress for durations ranging from 5 to 240 min. Killing temperatures for each exposure duration were determined from the midpoint of the sigmoidal response curve of electrolyte leakage plotted vs. exposure temperatures ranging from 42 to 58°C. Values represent means $\pm$ SD of three replicates.

Received for publication 16 Oct. 1989. Oklahoma Agricultural Experiment Station publication no. 5673. Research supported in part by USDA Special Grant no. 88-34146-3401. Pepper seeds were kindly supplied by Sunseeds Genetics, Hollister, Calif. We gratefully acknowledge the technical assistance of Susan Kenna and Anthony Dillard. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

Fig. 2. Electrolyte leakage from pepper leaf disks exposed to 52C for 0 to 60 min. Samples were acclimated in vitro for 0 (-----), 1 (————), or 4 (— · —) hr at 38C before heat stress.

Table 2. Acclimation-deacclimation sequence of pepper leaf disks from plants grown at 22/20C, transferred to 38/30C, then moved back to 22/20C. Heat-killing temperatures (T_{mid}) were determined at 24-hr intervals from immediately before transfer from 22/20C to 38/30C, during 48 hr at 38/30C, then during 72 hr back at 22/20C.

Duration at temp given (hr)		T_{mid} (°C)*
38/30C	22/20C	
0	0	51.1 a
24	0	54.1 c
48	0	54.7 c
48	24	53.6 c
48	48	52.0 ab
48	72	52.3 b

*Means with the same letter are not significantly different at $P = 0.05$.

ments were taken. Electrolyte leakage data were expressed as initial readings (following treatment and incubation)/final readings (following autoclaving and incubation). Ingram's model (1985) was used to estimate heat-killing temperatures by fitting data to a sigmoidal response curve and calculating the inflection point (T_{mid}) using the Gauss-Newton method (SAS Institute, 1985).

Time-temperature interaction. In one experiment, the interaction between exposure temperature and duration was examined by subjecting leaf disks to 42 to 58C in 2C increments for 5, 15, 30, 60, 120, 180, or 240 min. Killing temperatures were estimated for each of three replicates. The relationship between killing temperature and natural log of exposure duration was determined using linear regression analysis.

In a second experiment, samples were exposed for 0, 7.5, or 15 min to 51.5C. One set of disks was subjected to heat stress at the beginning of the experiment (time 0), while a second set was treated 4 hr later. An additional treatment consisted of an interrupted exposure with 7.5 min at time 0 followed by 4 hr at 21C, then an additional 7.5 min at 51.5C. This experiment was repeated five times with arcsin-transformed data analyzed by analysis of variance (ANOVA) and means separated by least significant difference.

Acclimation-deacclimation. Acclimation experiments were carried out with entire plants

and excised leaf disks in vitro. In whole plant studies, pepper plants were transferred from a growth chamber at 22/20C to another chamber at 38/30C and held there 48 hr. Heat-killing temperatures were determined at time of transfer, then after 24 and 48 hr at 38/30C. After 48 hr, plants were moved back to the 22/20C chamber to monitor deacclimation for three 24-hr intervals. This experiment was repeated four times with data analyzed by ANOVA and means separated by least significant difference.

In another series of experiments, leaf disks from plants grown at 22/20C were held in test tubes placed in a water bath at 38C for 1, 4, 7, or 24 hr before their exposure to 52C for 0, 5, 10, 15, 30, 45, or 60 min. Heat-killing times were estimated using the previously described model with time, rather than temperature, as the independent variable. This experiment was repeated four times with the effects of exposure duration at 52C, duration at the acclimating temperature of 38C, and replicate on electrolyte leakage examined in an analysis of covariance using the General Linear Models procedure (SAS Institute, 1985).

Time-temperature interaction. Electrolyte leakage from pepper leaf disks following high temperature stress yielded sigmoidal response curves in agreement with results for *Pittosporum tobira* roots (Ingram, 1985), tomato fruit (Inaba and Crandall, 1988), and leaves of sorghum (Sullivan, 1972), soybean (Martineau et al., 1979), melon (Lester, 1985), and citrus (Ahrens and Ingram, 1988). In addition, an interaction between treatment duration and temperature was observed similar to that reported by Ingram (1985, 1986) and Wanner et al. (1982). Lethal temperatures decreased linearly from 53 to 46C as exposure duration increased exponentially from 5 to 240 min (Fig. 1). The natural log of exposure duration plotted vs. killing temperature had an r^2 of 0.95.

We also found that interrupted exposures yielded the same degree of injury as continuous exposure, suggesting that injury was cumulative over time. Little or no damage resulted from 7.5 min at 51.5C, while 15 min at this temperature killed the tissue (Table 1). Electrolyte leakage values were similar for a continuous 15-min exposure and

two 7.5-rein periods separated by 4 hr at 21C. We did not observe evidence of acclimation or repair of damage in the interrupted exposure treatment during 4 hr at 21C.

Acclimation-deacclimation. Plants grown at 20/22C were heat-killed at 51.1C (Table 2). Twenty-four hr at 38C increased heat tolerance to 54.1C. An additional 24 hr produced no further increase in hardiness. Leaf tissue reacquainted after being transferred back to 22/20C with mean heat-killing temperatures declining to 52.0C after 48 hr. An additional 24 hr did not result in further deacclimation. Similarly, Chen et al. (1982) reported that reacquainted tomato, bean, and potato plants were harder than nonacclimated plants, but soybeans reacquainted fully. Although our sampling interval of 24 hr was too long to calculate deacclimation rates, our data suggest that deacclimation was a slower process than acclimation. Lester (1986) also found acclimation to proceed more rapidly than deacclimation.

Acclimation of excised tissue in vitro, as expressed by electrolyte leakage, was also observed (Fig. 2). Time to achieve heat-killing at 52C increased from 8.4 min for controls to 19.3 min for tissues exposed to 38C for 1 hr. Four hours at 38C increased the heat-killing time to 34.7 min. No additional increase in hardiness was observed after 7 or 24 hr at 38C (data not presented). The rate and/or extent of acclimation may be different at other temperatures. Chen et al. (1982) found 35 to 40C to be the optimum temperature range for acclimation of tomato, soybean, and potato. Lester (1986) reported that 2 to 4 hr at 35C was sufficient to acclimate melon leaves.

The present study is the first we are aware of demonstrating the capacity for in vitro acclimation of leaf disks. An earlier report described heat acclimation in tissue culture derived from the pericarp of avocado fruit (Schroeder, 1963). High temperature tolerance has also been examined in cell suspensions derived from pear (Wu et al., 1984) and wheat (Wang and Nguyen, 1989). These systems may prove useful in studying the acclimation process.

Literature Cited

- Ahrens, M.J. and D.L. Ingram. 1988. Heat tolerance of citrus leaves. *HortScience* 23:747-748.
- Chen, H. H., Z.Y. Shen, and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. *Crop Sci.* 22:719-725.
- Inaba, M. and P.G. Crandall. 1988. Electrolyte leakage as an indicator of high temperature injury to harvested mature green tomatoes. *J. Amer. Soc. Hort. Sci.* 113:96-99.
- Ingram, D.L. 1985. Modeling high temperature and exposure time interactions on *Pittosporum tobira* root cell membrane thermostability. *J. Amer. Soc. Hort. Sci.* 110:470-473.
- Ingram, D.L. 1986. Root cell membrane heat tolerance of two dwarf hollies. *J. Amer. Soc. Hort. Sci.* 111:270-272.
- Ingram, D.L. and D. W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstock. *J. Amer. Soc. Hort. Sci.* 109:189-193.
- Lester, G.E. 1985. Leaf cell membrane thermo-

- stabilities of *Cucumis melo*. J. Amer. Soc. Hort. Sci. 110:506-509.
- Lester, G.E. 1986. Physiology of melon leaf membrane thermostability during heat conditioning. J. Amer. Soc. Hort. Sci. 111:561-564.
- Martineau, J. R., J.E. Specht, J.H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19:75-78.
- SAS Institute. 1985. SAS/STAT guide for personal computers. version 6 (cd.). SAS Institute, Inc., Cary, N.C.
- Schroeder, C.A. 1963. Induced temperature tolerance of plant tissue *in vitro*. Nature (London) 200:1301-1302.
- Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement, p. 247-264. In: N.G.P. Rao and L.R. House (eds.). Sorghum in the seventies. Oxford & IBH Publishing Co., New Delhi.
- Wanner, S. J., M.R. Becwar, and J.D. Butler. 1982. Measurement of turfgrass heat tolerance *in vitro*. J. Amer. Soc. Hort. Sci. 107:608-613.
- Wang, W.C. and H.T. Nguyen. 1989. Thermal stress evaluation of suspension cell cultures in winter wheat. Plant Cell Rpt. 8:108-111.
- Wu, M., S.J. Wanner, and J.W. Waddell. 1984. Heat stress responses in cultured plant cells. Plant Physiol. 74:944-946.