Effects of Chilling on Episcia and Dieffenbachia

Margaret J. McMahon, A.J. Pertuit, Jr., and James E. Arnold
Department of Horticulture, Clemson University, Clemson, SC 29634

Additional index words. carbon dioxide, chlorophyll, fluorescence, photosynthesis, transpiration

Abstract. Leaves of chilled ‘Moss-Agate’ Episcia (Mart.) plants exhibited direct chilling injury (i.e., watersoaked browning of leaf blade interveinal areas within 24 h of exposure to low temperature) immediately following exposure in darkness to 10C for 0.5 or 1.0 h. Chlorophyll fluorescence peak: initial ratios and terminal: peak ratios of chilled Episcia were reduced 20% and 25%, respectively, 3 h after chilling, a result suggesting possible photosystem II damage. Total leaf chlorophyll content was reduced by 17% within 3 h of chilling and CO₂ uptake also was reduced at this time. Leaves of chilled ‘Rudolph Roehrs’ Dieffenbachia maculata (Lodd.) (D. Roehrsii Hort.) plants expressed no visible injury within 24 h of 1.2C chilling in darkness for 36, 48, or 60 h, but CO₂ uptake was reduced by 70% compared to the control 3 h after chilling. Visible injury began to appear 27 h after chilling, and the older leaf blades of all chilled plants exhibited a watersoaked appearance 75 h after chilling. Chlorophyll fluorescence peak: initial ratios of chilled Dieffenbachia did not vary, and terminal: peak ratios were not reduced until 147 h after chilling, when the injured tissue was extremely flaccid and translucent. Chilling reduced the chlorophyll content of Dieffenbachia by 10% in some plants 27 h after chilling and by 35%, in all plants 75 h after chilling. Transpiration rate was reduced and stomatal diffusive resistance increased 27 h after chilling.

Marketed tropical and subtropical plants often are inadvertently exposed to chilling temperatures during production, transport, storage, and display. Exposure to these temperatures can induce chilling injury (CI), resulting in reduced yields or an unsightly, unsalable product.

All plant parts can be affected by chilling temperatures (Abbott and Massie, 1985, Bauer et al., 1985, Caldwell, 1987). Visible symptoms may include wilting, necrotic lesions, pitting, chlorosis, or a watersoaked appearance on portions of the damaged plant (Saltveit and Morris, 1990). Physiological symptoms, often the result of membrane destruction or dysfunction (Murphy and Wilson, 1981; Yoshida, 1991), include altered photosynthetic and transpiration rates (Bauer et al., 1985). Symptoms appear immediately in some species, but develop over several days in others (Abbott and Massie, 1985; Bauer et al. 1985; Caldwell, 1987; Semeniuk et al., 1986).

Understanding some of the physiological effects of chilling on plants has helped develop methods of reducing CI. The process of hardening plants by gradually exposing them to lower temperatures is based on the premise that this procedure subtly alters the membrane to a form less affected by low temperatures (Murata, 1990; Wilson, 1987; Yakir et al., 1986). Applying an antitranspirant to the plants to prevent or reduce transpiration also protects plants from cold. These techniques, however, have not been successful with all plants. For example, Episcia reptans does not harden (Wilson, 1976). Also, antitranspirants did not reduce CI of Dieffenbachia maculata (Semeniuk et al., 1986). Qualifying and quantitating physiological changes resulting from CI could help detect plant injury before it becomes visually apparent and help develop techniques to ameliorate CI.

This study was undertaken to determine the effects of chilling on transpiration, CO₂ uptake, chlorophyll content, and fluorescence of ‘Moss-Agate’ Episcia and ‘Rudolph Roehrs’ D. maculata (D. Roehrsii Hort.) and to determine if these measurements could be used as a rapid method for detecting CI in these species.

Received for publication 28 Dec. 1992. Accepted for publication 25 May 1993.

Technical contribution no. 3009 of the South Carolina Agricultural Experiment Station. The use of trade names does not imply endorsement by the South Carolina Agricultural Experiment Station of products named, nor criticism of similar ones not mentioned. “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.”
Chlorophyll fluorescence, chlorophyll content, and CO\(_2\) uptake were recorded 21 h before and 3 h after chilling. All measurements were taken between 2200 and 0100 h in the glasshouse under light (300 to 310 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\)) provided by a high-intensity discharge (HID) lamp (Lithonia Lighting Co., Crawfordsville, Ind.) using a 400-W multivapor bulb (General Electric Co., Cleveland). The light intensity chosen was the previously determined light saturation point for Episcia. The initial fluorescence level, the peak, and the terminal value received by the fluorescence diode were recorded.

Chlorophyll fluorescence was evaluated according to Shaw et al. (1985) using a plant productivity fluorometer (model SF-20; Richard Brancker Research, Ottawa, Canada). The sensing probe was placed on the adaxial surface of a leaf resting on a flat black surface. The leaf and probe were covered to exclude light. After 3 min of dark acclimation, the monochromatic (670 nm \(\lambda\) max) light-emitting diode of the probe illuminated the leaf for 50 sec (15.6 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\)). The initial fluorescence level, the peak, and the terminal value received by the fluorescence diode were recorded.

Five 3-mm-diameter disks from each leaf being evaluated were placed in 5 ml of \(N,N\)-dimethylformamide and kept in darkness at 10C for 48 to 72 h to extract chlorophyll. Absorbance was measured spectrophotometrically (Spectronic 1001; Bausch and Lomb, Rochester, N.Y.) at 664 and 647 nm. Total chlorophyll, chlorophyll \(a\), and chlorophyll \(b\) were determined by the method developed by Moran and Porath (1980) and Moran (1982).

Net photosynthesis (CO\(_2\) uptake) was measured with a portable infrared gas analyzer (IRGA) system (Analytical Development Co.) consisting of a Parkinson leaf chamber, leaf chamber analyzer, and air supply unit. The leaf chamber was clamped to a leaf until a steady state was achieved, then the CO\(_2\)-differential (compared to a reference) in the chamber was recorded as ppm and converted to mg·m\(^{-2}\)·s\(^{-1}\).

Episcia CO\(_2\) uptake, chlorophyll content, and fluorescence were measured on the proximal end of fully expanded leaves in the top third of the plant, where preliminary tests indicated injury first occurred.

Two measurements of each criterion were taken on separate leaves of each Episcia. To sample chlorophyll content, tissues were removed, thus preventing repetitive sampling of exactly the same area; therefore, all measurements were done randomly. Once visible injury developed, measurements and sampling were confined to visibly injured areas.

Analysis of variance was performed on each measured variable to estimate experimental error variances. Analysis of variance revealed a chilling duration \(\times\) day of evaluation interaction; hence, chilling durations were compared for each day of evaluation. Mean separation of chilling duration for each evaluation day was by Dunnett’s test (\(\alpha < 0.05\)).

Dieffenbachia. Rooted ‘Rudolph Roehrs’ Diffenbachia cuttings were planted the week of 16 Sept. in 22.8-cm-diameter, round, opaque, green, plastic pots containing 1400 cm\(^3\) of washed river sand. Cultural practices were the same as for Episcia, except internal shade was not installed. The oldest cane of each plant was removed 4 months before the experiment began to reduce plant height and promote lateral cane development.

Dieffenbachia experiments were conducted on 13 and 27 Oct. Twenty-four uniform Dieffenbachia were selected for each date and divided into four treatment groups of six plants each. Dieffenbachia were exposed to 22 ± 2C (control) or 1.2 ± 0.2C for 24.36, or 60 h. These chilling treatments had produced moderate injury in preliminary experiments.

All plants to be chilled were placed in the cooler at staggered times, as for Episcia. The control remained at 22 ± 2C in the room housing the cooler until chilling treatments were completed. The room and the cooler were kept dark during the treatment period and thermocouples were placed as previously described for Episcia.

Dieffenbachia transpiration rate, diffusive resistance, chlorophyll fluorescence, chlorophyll content, and CO\(_2\) uptake were recorded 21 h before and 3, 27, 75, and 147 h after chilling. Attempts to collect data during the night failed when the Dieffenbachia did not evolve measurable oxygen after 1 h of exposure to artificial light. Cool fall days allowed us to collect data between 1000 and 1400 h, when vehicular exhaust was not a problem. Natural light (in the glasshouse) was augmented with the previously described HID to provide 390 to 400 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\); this was the previously determined light saturation point for Dieffenbachia.

Chlorophyll fluorescence, chlorophyll content, and CO\(_2\) uptake were measured with the same equipment as for Episcia. Transpiration and diffusive resistance were measured with with a steady-state porometer (model 1600; LI-COR, Lincoln, Neb.) clamped onto a leaf until a steady state was obtained.

Preliminary tests indicated that the initial injury site was on fully expanded leaves in the lower two-thirds of the plant. Measurements were taken in the middle third of the leaf blade along, or adjacent to, its margin. Chlorophyll content and fluorescence were measured along the margin, where preliminary tests indicated a higher chlorophyll content and less chlorophyll variation than in the interior of the blade. Because the designs of the porometer and IRGA chambers precluded their attachment at the exact margin, measurements were made slightly inside the dark green margin. Stomate counts were similar for the leaf margin and interior.

Two measurements of each criterion were taken on separate leaves of each Dieffenbachia. Chlorophyll content was sampled as for Episcia. Once visible injury developed, measurements and sampling were confined to the visibly injured area.

The design, analysis of variation, and mean separation were the same as for Episcia.

Results

Episcia. Temperatures in chilled plant petioles reached 10C within 15 min after chilling began and remained there (within 0.5C) during treatment. Medium temperatures fell to 14C during 0.5 h chilling and to 13C during 1.0 h of chilling.

CI in both Episcia treatments was apparent when the plants were removed from the cooler. Damage was more severe as exposure time increased. The common visible injury was a water-soaked browning of leaf blade interveinal areas, with younger, almost fully expanded leaves most severely injured. The amount of injured area did not increase with time. The injured portion of the leaf eventually desiccated but, unless severely damaged, the injured leaves survived and the plant continued growing.

Table 1. Effects of three chilling durations (0.0, 0.5, and 1.0 h) at 10C on fluorescence ratios (peak: initial), CO\(_2\) uptake, and leaf chlorophyll content of ‘Moss-Agate’ Episcia. Measurements were taken 3 h after chilling ended.

| Chilling (h) | Fluorescence ratio | CO\(_2\) uptake (µg·cm\(^{-2}\)·s\(^{-1}\)) | Chlorophyll content (µg·cm\(^{-2}\)) |
|-------------|--------------------|--------------------------------------|-----------------------------------|
| 0.0         | 1.4 a              | 0.7 a                                | 40 a                               |
| 0.5         | 1.1 b              | -0.2 b                               | 33 b                               |
| 1.0         | 1.1 b              | -0.2 b                               | 33 b                               |

Mean separation within columns by Dunnett’s test, \(P < 0.05\).
The chlorophyll fluorescence peak: initial ratio decreased ≈ 20% from the control within 3 h after chilling ended (Table 1). The terminal: peak ratio also was reduced by this time (≈ 25% from the control; data not shown). After 3 h, there was CO₂ evolution rather than a net uptake for treated plants (Table 1). Total chlorophyll content of both chilled treatments had decreased ≈ 20% from the control within 3 h (Table 1).

Dieffenbachia. Temperatures of chilled Dieffenbachia petioles reached 1.3 ± 0.5°C within 25 min of chilling and remained there during treatment. Growing-medium temperatures reached 1.2 ± 0.5°C within 4 h and remained constant.

Dieffenbachia had no visible injury when chilling ended; however, visible injury had developed in the leaf blades at the basal two-thirds of some chilled plants 27 h after chilling ended. The damage first appeared on the underside of the leaf blade as a watersoaked area at or near the leaf margin. Injury was visible in all chilled plants after 75 h and continued to develop. Most damaged leaves became brown, wilted, then died. The upper third of the Dieffenbachia was not visibly affected and growth continued. Lateral shoots developed normally from lateral nodes where the damaged petioles were attached.

Chilled Dieffenbachia fluorescence peak: initial ratios did not vary from the control during the evaluation period (data not shown). Net CO₂ uptake was reduced to near zero 3 h after treatment in all chilled Dieffenbachia, and it remained low during the evaluation period (Fig. 1).

Dieffenbachia total chlorophyll concentration was reduced by ≈ 35% in all chilled plants 75 h and ≈ 65% 147 h after chilling ended (Fig. 2).

Chilled Dieffenbachia had a much reduced transpiration rate (≈ 75% of the control) 27 h after chilling ended, and transpiration was barely detectable for the rest of the experiment (Fig. 3). Diffusive resistance of all chilled Dieffenbachia had increased about threefold 27 h after exposure compared to the control and remained much higher than that of the control (Fig. 4) until the experiment ended.

Discussion

Episcia. Visible injury provided an easy and rapid method of detecting CI. Reduced CO₂ uptake and the loss of leaf chlorophyll within hours after chilling indicates that the photosynthetic or carbon fixation system or both had been damaged. The normal fluorescence pattern induced by illumination following a dark period is an initial fluorescence level, followed in less than a
nanosecond by a fluorescence peak, then a decline to near initial levels (Kautsky, 1931). In chilled Episcia, the reduced fluorescence peak suggests that photonsystem (PS) II was damaged and unable to hydrolyze water. Additionally, no decline from the peak indicates that the oxidation–reduction chain linking PS II to PS I is inhibited or that PSI is unable to accept electrons (Conroy et al., 1982).}

Increased diffusive resistance and decreased transpiration in chilled plants indicates that stomata were not locked open, although it is possible that water loss though transpiration was greater than could be replaced by uptake from chilled roots. However, noninjured leaves never wilted and injured leaves did not wilt until 75 h after chilling, a result indicating that transpiration was not excessive within the first 2 or 3 days after chilling.

The reduced CO₂ uptake after chilling is a means of detecting CI before it is visible. Variability in transpiration and diffusive resistance decreases their effectiveness as CI indicators.

At this time, the specific mechanism of CI remains unknown in Dieffenbachia. However, further investigation of the physiology of immature Dieffenbachia leaves may provide a means of ameliorating CI, as these leaves apparently were resistant to our chilling treatments.

Literature Cited

Abbott, J.A. and D.R. Massie. 1985. Delayed light emission for early detection of chilling injury in cucumber and bell pepper fruit. J. Amer. Soc. Hort. Sci. 110:42-47.

Anderson, J.M. 1982. The significance of chlorophyll stacking in b-containing chloroplasts. Photobiochem. Photobiophys. 3:225-241.

Bauer, H., R. Wierer, W.H. Hathaway, and W. Larcher. 1985. Photosynthesis of Coffea arabica after chilling. Physiol. Plant. 64:449-454.

Caldwell, C.R. 1987. Temperature-induced protein conformations changes in barley root plasma membrane-enriched microsomes. Plant Physiol. 84:926-929.

Conroy, J.P., R.M. Smillie, M. Kuppers, D.I. Bevege, and E.W. Barlow. 1986. Chlorophyll a fluorescence and photosynthetic growth responses of Pinus radiata to phosphorus deficiency, drought stress and high CO₂. Plant Physiol. 81:423-429.

Kautsky, H. and A. Hirsch. 1931. Neue Versuche zur Kohlenstaurausscheidung. Naturwissenschaften 19:964.

Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol. 69:1376-1381.

Moran, R. and D. Porath. 1980. Chlorophyll determinations in intact tissues using N,N-dimethylformamide. Plant. Physiol. 65:478-479.

Murata, T. 1990. Lipids in relation to chilling sensitivity of plants, p. 201-210. In C.Y. Wang (cd.). Chilling injury of horticultural crops. CRC Press, Boca Raton, Fla.

Murphy, C. and J.W. Wilson. 1981. Ultrastructural features of chilling injury in Episcia reptans. Plant Cell Environ. 4:261-265.

Ogren, E. and G. Oquist. 1985. Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition in intact willow branches. Planta 166:380-388.

Saltveit, Jr., M.E. and L.L. Morris. 1990. Overview of chilling injury of horticultural crops, p. 3-16. In: C.Y. Wang (cd.). Chilling injury of horticultural crops. CRC Press, Boca Raton, Fla.

Semeniuk, P., H.E. Moline, and J.A. Abbott. 1986. A comparison of ABA and an antitranspirant on chilling injury of coleus, cucumbers and dieffenbachia. J. Amer. Soc. Hort. Sci. 111:866-868.

Shaw D.R., T.F. Peeper, and D.L. Nofziger. 1985. Comparison of chlorophyll fluorescence and fresh weight as herbicide bioassay techniques. Weed Sci. 33:29-33.

Wilson, J.M. 1976. The mechanism of chill- and drought-hardening of Phaseolus vulgaris leaves. New Phytologist 76:257-270.

Wilson, J.M. 1987. Chilling injury in plants, p. 271-292. In: B.W.W. Grout and G.J. Morns (eds.). The effects of low temperatures on biological systems. Edward Arnold Publishers, London.

Yakir, D., J. Rudich, and B.A. Bravo. 1986. Adaptation to chilling: Photosynthetic characteristics of the cultivated tomato and a high altitude wild species. Plant Cell Environ. 9:477-484.

Yoshida, S. and C. Matsuura-Endo. 1991. Comparison of temperature dependency of tonoplast translocation between plants sensitive and insensitive to chilling. Plant Physiol. 95:504-508.