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Comparing Gompertz and Richards Functions to Estimate Freezing Injury in *Rhododendron* Using Electrolyte Leakage

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ABSTRACT. **Seasonal patterns in freezing tolerance of five *Rhododendron* cultivars that vary in freezing tolerance were estimated. Electrolyte leakage was used, and raw leakage data were transformed to percent leakage, percent injury, and percent adjusted injury. These data were compared with visual estimates of injury. Percent adjusted injury was highly correlated (0.753) to visual estimates. Two asymmetric sigmoid functions—Richards and Gompertz—were fitted to the seasonal percent adjusted injury data for all cultivars. Two quantitative measures of leaf freezing tolerance—LT_{50} and T_{max} (temperature at maximum rate of injury)—were estimated from the fitted sigmoidal curves. When compared to the General Linear Model, the Gompertz function had a better fit (lower mean error sum of squares) than Richards function. Correlation analysis of all freezing tolerance estimates made by Gompertz and Richards functions with visual LT_{50} revealed similar closeness (0.77 to 0.79). However, the Gompertz function and T_{max} were selected as the criteria for comparing relative freezing tolerance among cultivars due to the better data fitting of Gompertz function (than Richards) and more descriptive physiological representation of T_{max} (than LT_{50}). Based on the T_{max} (°C) values at maximum cold acclimation of respective cultivars, we ranked 'Autumn Gold' and 'Grumpy Yellow' in the relatively tender group, 'Vulcan's Flame' in intermediate group, and 'Chionoides' and 'Roseum Elegans' in the hardy group. These relative rankings are consistent with midwinter bud hardiness values reported by nurseries.**

Measuring electrolyte leakage following a freeze–thaw stress has been widely used to estimate freezing injury and tolerance of various plant tissues. Some discrepancies however, have been found in laboratory estimates of freezing tolerance when compared to field data (Pellet et al., 1986). Electrolyte leakage is usually calculated as the ratio of ion leakage from freeze injured to the total ions in the tissue and expressed as a percent leakage (Flint et al., 1967).

Researchers often rely on empirical mathematical models to predict plant response to environmental stress over time. When using a computerized simulation for estimating any plant response, the accuracy of predictions depends on the appropriateness of the mathematical function (Hopper et al., 1996). Plant response to temperature stress is characterized by an asymmetric sigmoid function (von Fircks and Verwijst, 1993). Simple straight line or interpolation techniques (Holt and Pellet, 1981), however, have also been used. Each plant tissue has its own unique sigmoidal response curve that may or may not differ during the initial and final rate of injury. Therefore, one limitation with using the logistic function (Andrews and Morrison, 1992; Oldum and Blake, 1996; Zhu and Liu, 1987) is that it considers the rate of initial and final injury to be identical (symmetric sigmoid function). A more appropriate choice for a function to be used in data fitting of plant response to temperature stress would be an asymmetric sigmoid one that would take into account differing rates of injury.

Conventionally, the temperature giving an index of 50% ion leakage (or injury) is termed LT_{50} (Burr et al., 1990). Most reports use LT_{50} as a measure of relative freezing tolerance (Arora et al., 1992; Palta et al., 1978; Sakai et al., 1986; Sutinen et al., 1992). When working with woody plants, however, even the lowest treatment temperature used in the experimental protocol often fails to reach the LT_{50} level (Zatylny et al., 1996). Alternatively, others have used the rate of freezing injury and the point of inflection (T_{max}) as a measure of maximum rate of injury due to freeze–thaw stress (Anisko and Lindstrom, 1995; von Fircks and Verwijst, 1993). Frequently, the quantitative values for freezing tolerance are based on single-point estimates (Hummer et al., 1995; Sakai et al., 1986), and no variances or standard errors are provided due to complex experiment protocols and limitations on the availability of ample plant sample. Therefore, there is a need to reevaluate methods of data analysis for estimating the relative freezing tolerance using the electrolyte leakage method that would provide more consistent and reliable results.

The amount of winter injury to *Rhododendron* cultivars may vary from year to year depending on the severity of the winter season and the level of bud and leaf cold hardiness (Holt and Pellet, 1981). Information is available on midwinter cold hardiness of various *Rhododendron* species and cultivars (Holt and Pellet, 1981; Sakai et al., 1986; Wagner, 1994); however, it is mostly based on sampling from a single source or period at a particular location (Pellet et al., 1986). Apart from visual estimations of freezing tolerance, limited data are available on seasonal changes in freezing tolerance of *Rhododendron* (Anisko and Lindstrom, 1996a; Holt and Pellet, 1981; Sakai et al., 1986). *Rhododendron* breeders are particularly interested in cold hardiness, but the

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Table 1. Example of ion leakage data calculations and transformation for 'Vulcan's Flame' *Rhododendron* leaf tissues in December 1995.[z]

Treatment temp (°C)	Initial leakage[y]	Final leakage[y]	Leakage[x] (%)	Injury[w] (%)	Adjusted injury[v] (%)	Visual estimate[u]
0	1.9	26.9	7.1	0.0	0.0	0.0
–13	3.9	22.6	17.2	10.9	14.1	0.0
–15	4.2	19.5	22.3	16.4	21.3	16.7
–17	5.9	24.3	24.3	18.5	24.0	16.7
–19	7.1	21.3	33.5	28.3	36.9	25.0
–21	7.7	21.4	36.0	31.1	40.5	41.7
–23	11.3	26.3	43.7	39.4	51.2	75.0
–25	12.0	25.1	47.9	43.8	57.1	83.3
–27	15.9	23.1	68.6	66.2	86.1	91.7
–80	20.4	26.0	78.5	76.8	100.0	100.0

[z] Values are mean of three separate measurements at each treatment temperature.
[y] Raw data (µmhos).
[x] (Initial leakage/final leakage) × 100.
[w] $[(\%\ \text{Leakage}_{(T)} - \%\ \text{leakage}_{(C)})/(100 - \%\ \text{leakage}_{(C)})] \times 100$.
[v] $(\%\ \text{Injury}_{(T)}/\%\ \text{injury}_{(-80)}) \times 100$.
[u] Percentage browning and water soaked.

irregular occurrence and unpredictability of severe or test winters has limited the breeding selection. Hence, there is a need to develop laboratory protocols under controlled conditions that produce reliable relative injury estimates and enable comparison among cultivars (Wagner, 1994). Therefore, a study was undertaken to evaluate two asymmetric sigmoid functions—Richards (von Fircks and Verwijst, 1993) and Gompertz (Messori, 1997)—to estimate the seasonal patterns for two quantitative measures—LT_{50} and T_{max}—of the leaf freezing tolerance of *Rhododendron* cultivars (that vary in freezing tolerance) using the electrolyte leakage method. Additionally, data on LT_{50} and T_{max} (obtained by fitted curve) were compared with visual estimates of LT_{50}.

Materials and Methods

Plant material. *Rhododendron* cultivars—'Autumn Gold', 'Chionoides', 'Grumpy Yellow', 'Roseum Elegans' and 'Vulcan's Flame'—were obtained as 2-year-old rooted cuttings and potted in 18-cm pots with artificial mix (70% pine bark, 15% sand, and 15% sphagnum peat). The plants were fertilized with Azalea Special 21–7–7 (W.R. Grace, Fogelsville, Pa.) at 1.5 g·L^{-1} plus Fe chelate (Sequestrene 330 Fe, 10% Fe; Ciba-Geigy, Greensboro, N.C.) at 0.25 g·L^{-1} to maintain pH at 4.5 to 5.5 and electrical conductivity (EC) at 0.5 to 0.9 dS·m^{-2} of the potting medium. The plants were irrigated as needed and maintained under natural photoperiod and temperature conditions. These cultivars are adapted to hardiness zones 4 to 7 and are expected to have a varying degree of nonacclimated freezing tolerance and cold acclimation ability [Van Veen Nursery, Portland, Ore., and Appalachian Nurseries, Waynesboro, Pa. (from where cuttings were obtained) provided a ranking of midwinter bud hardiness]. Current-year leaves from five *Rhododendron* cultivars were collected monthly from September 1995 until June 1996. Fifteen to twenty-two leaves were randomly collected from ten to fifteen plants of each cultivar, kept on ice, and brought to the laboratory for freezing tolerance estimation.

Relative freezing tolerance estimation and data transformation. Leaves were cut into 1-cm^2 discs and placed into test tubes (25 × 200 mm) containing 50 to 75 µL water. Leaf margins and midribs were excluded from the discs. Six replications (one disc per tube) per temperature per cultivar were placed in a glycol freezing bath (model 2325 CH/P; Forma Scientific, Marietta, Ohio) and frozen to various treatment temperatures as described by Arora et al. (1996). Tubes were removed at various treatment temperatures, first placed in ice (2 h minimum), and then transferred to 4 °C (overnight) to allow slow thawing. A set of three discs per cultivar was removed from the freezing bath (after reaching at least –10 °C) and placed in a –80 °C freezer for 4 to 5 h and thawed similarly to other samples. Following thaw, discs from three replications were placed on a wet filter paper in a petri dish at 100% relative humidity and 20 °C. After 5 to 6 d, visual injury (browning and water soaking) was estimated in these discs and given a ranking of 0%, 25%, 50%, 75%, or 100% injured. Discs from the other three replications were placed in 20 mL of distilled deionized water immediately after thaw, vacuum infiltrated for 3 to 5 min, and shaken at 250 rpm on gyratory shaker (model G10; New Brunswick Scientific, Edison, N.J.) for 3 h for ion leakage measurements as described by Arora et al. (1996). A preliminary

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OPTIONS LS=72;

DATA; SET IN.RHODO; IF MONTH=12;

PROC NLIN METHOD=MARQUARDT BEST=1 ITER=1000;

BY MONTH CUL;

PARMS B=1 TO 100 BY 1 K=-0.30 TO -0.01 BY .01;

    EF=EXP (-K*TEMP);

    EEF=EXP (-B*EF);

    MODEL AD_INJ=100*EEF;

    DER.B=-100*EF*EEF;

    DER.K=100*B*TEMP*EF*EEF;

    OUTPUT OUT=EST P=GOMPERTZ;

PROC PLOT;

   BY MONTH CUL;

   PLOT AD_INJ*TEMP='X' GOMPERTZ*TEMP='*' / OVERLAY;
```

Fig. 1. A SAS program for finding the best starting parameters of the Gompertz function.

Table 2. Empirical calculations used to determine freezing tolerance.[z]

Function	Equation	Slope	LT_{50}	T_{max}
Richards	$100(1-e^{(b-kT)})^{-1/d}$	$[(100ke^{(b-kT)})(1-e^{(b\ kT)})^{(\ 1/d+1)}]/d$	$(b-\log(1-(1/2)^{d}))/k$	$(b-\log(-d))/k$
Gompertz	$100e^{-be-kT}$	$100bke^{-kT}e^{-be-kT}$	$[-\log((\log(100)-\log(50))/b)/k$	$-\log(1/b)]/k$

[z]Where b, d, k are function parameters, a =100, T = temperature, and e = exponential.

time-course study revealed no significant change in the percent ion leakage from leaf discs when shaken for 3 or up to 6 h (data not shown). Percent injury (as described by Arora et al. 1992) was then calculated using the percent ion leakage data.

The percent injury data transformations take into account the ion leakage from unfrozen control samples but do not adjust for ion leakage from 100% freeze-injured leaf discs. Therefore, percent injury data were transformed to percent adjusted injury using the following method. Ion leakage and percent injury measurements were also made on discs frozen to –80 °C (representing 100% freeze-injured samples by extreme freezing). The percent adjusted injury was then calculated by the following equation:

Percent adjusted injury = [percent injury (t)/percent injury (–80 °C)] × 100

where, percent injury (t) is the measurement of injury at respective freeze treatment temperature. An example of data calculations and transformations is provided in Table 1. A full statistical correlation analysis between different data transformations cannot be conducted because the plants' response to freeze–thaw stress is not linear. Therefore, a partial correlation analysis (holding treatment temperatures and months constant) was conducted to find the closest correlation between the different data transformations and the visual estimates.

COMPARISON OF RICHARDS TO GOMPERTZ FUNCTIONS. The Gompertz and Richards functions were fitted to the percent adjusted injury data by the Marquardt method using the NLIN procedure of SAS (SAS Institute, Cary, N.C.). The obstacle in using the Richards or Gompertz function for fitting data lies with estimating the initial starting parameters. These parameters are crucial for the function to converge and for a good fit to the data. A SAS program was written for finding the best starting parameters of the Gompertz function, an example of which is provided in Fig. 1.

That leaf tissues are completely killed (100% injured) at –80 °C was assumed, which eliminated the need to estimate parameter A. Parameter A represents the maximum injury level, where it is always 100% when percent adjusted injury data are used. The modified Richards and Gompertz equations (without parameter A) are listed in Table 2. Using the monthly data for all cultivars, Gompertz and Richards functions were compared to the General Linear Model for lack of fit using an F test.

COMPARISONS OF FREEZING TOLERANCE QUANTITATIVE EXPRESSIONS. The LT_{50} and T_{max} (the point of inflection of a fitted curve of freezing tolerance) were estimated based on the computer simulated sigmoid curves of Richards and Gompertz functions fitted to adjusted injury data. The LT_{50} in adjusted injury data will always be at the 50% injury level and T_{max} is located at the highest point of the first derivative of the equation (equate the second derivative to zero). The LT_{50} and T_{max} equations are listed in Table 2. A partial correlation analysis (holding month constant) was conducted to evaluate the closeness of all freezing tolerance quantitative expressions in comparison with the visual LT_{50}.

LEAF FREEZING TOLERANCE. A single estimate of LT_{50} and T_{max} was assessed for each month. These estimates cannot be distinguished from each other without some information provided by its

Fig. 2. A SAS program using the Jackknife method and Gompertz function to find LT_{50} and T_{max} means and standard errors.

```
OPTIONS LS=72; DATA LIM; SET IN.RHODO; BY MONTH CUL TEMP; IF MONTH=12;
IF FIRST.CUL THEN NUM=0; NUM=NUM+1; RETAIN NUM;
PROC MEANS NOPRINT; BY MONTH CUL; VAR TEMP; OUTPUT OUT=MN N=N;
DATA ; MERGE LIM MN; BY MONTH CUL;
   DATA BIG; SET ; DO JACK=1 TO N; IF NUM NE JACK THEN OUTPUT; END;
PROC SORT; BY MONTH CUL JACK; PROC PRINTTO UNIT=20 NEW;
PROC NLIN METHOD=MARQUARDT BEST=1 ITER=1000 OUTEST=EST;
   BY MONTH CUL JACK;
   PARMS B=15.18,6.53,9.04,17.88,13.48
         K=-.158, -.062, -.131,-.101,-0.136;
   EF=EXP (-K*TEMP); EEF=EXP (-B*EF);
   MODEL AD_INJ=100*EEF;
   DER.B=-100*EF*EEF; DER.K=100*B*TEMP*EF*EEF;
   DATA; SET EST; IF _TYPE_='FINAL'; IF _TYPE_='FINAL' THEN CONVERGD=1;
      GOMPLT50=-LOG ((LOG (100)-LOG (50))/B)/K;
      GOMPTMAX=-LOG(1/B)/K;
   PROC PRINTTO; /*PROC PRINT; VAR MONTH CUL JACK _SSE_--GOMPLT50; */
   PROC MEANS NOPRINT; BY MONTH CUL; VAR GOMPLT50 GOMPTMAX CONVERGD;
   OUTPUT OUT=FORJACK N=N MEAN=GOMPLT50 GOMPTMAX CSS=LT50CSS TMAXCSS
      SUM=S1 S2 CONVERGD;
      DATA ; SET ;
         GOMP50ER=SQRT((N-1)*LT50CSS/N);
         GOMPTMER=SQRT((N-1)*TMAXCSS/N);
         PROC PRINT; ID MONTH CUL;
         VAR N CONVERGD GOMPLT50 GOMP50ER GOMPTMAX GOMPTMER;
```

Table 3. Partial correlation coefficients derived from the error sum of squares for the comparison of three different freeze–thaw injury estimates with visual estimates[z][y]

	Leakage (%)	Injury (%)	Adjusted injury (%)	Visual estimate
Visual estimates	0.693	0.699	0.753	1.000

[z]df = 1467, correlation probability = 0.0001.

[y]General Linear Model multivariate ANOVA, holding treatment temperatures and sampling dates constant.

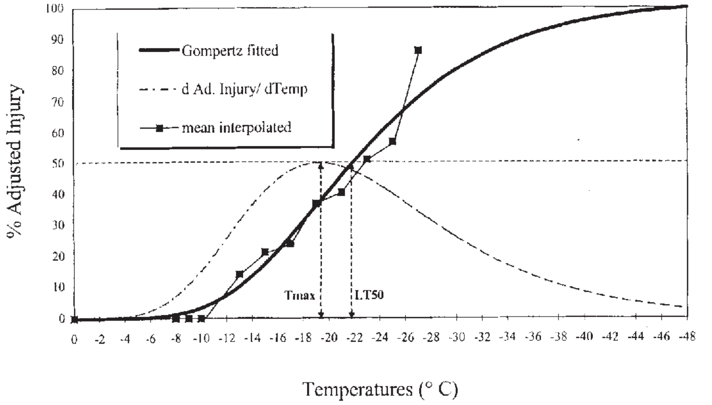

Fig. 3. Determinations of LT_{50} and T_{max} (quantitative expressions of freezing tolerance) using the Gompertz function fitted to percent adjusted injury data and by interpolating the mean of three data points per treatment temperature (LT_{50} only). LT_{50} is always at 50% injury for percent adjusted injury, whereas the inflection point of the slope of the Gompertz curve corresponds to T_{max}. The slope of the curve (d adjusted injury/d temperature) bears no units and does not correspond to percent adjusted injury units on the y-axis.

variance. Repeating the entire experiment to obtain the LT_{50} and T_{max} variances is impractical and inefficient. Therefore, the Jackknife method (Efron, 1982) was used to estimate the standard error of LT_{50} and T_{max}. The Jackknife method involved taking out one data point at a time and estimating the LT_{50} and T_{max}. The removed data point was replaced by another data point and LT_{50} and T_{max} were reestimated. This process was repeated until all data points were removed and reincorporated. By the end of this process, there were at least 30 estimates of LT_{50} and T_{max} per cultivar per month, thus giving a mean and standard error. Multiple *t* tests at $P = 0.05$ were used to compare all the estimates. An example of a SAS program using the Jackknife method and Gompertz function to find LT_{50} and T_{max} means and standard errors is provided in Fig. 2.

Results

Data transformation analysis. Partial correlation analysis (for a linear model comparing each treatment temperature for all cultivars and every month in the entire sampling season) revealed the highest correlation (0.753) between the percent adjusted injury data and the visual estimate data (Table 3). The percent leakage (0.693) and percent injury data (0.699) were not much different from each other when compared with the visual estimate data. Therefore, the asymmetric functions were fitted using percent adjusted injury data.

Comparison of Richards to Gompertz functions. When compared with the General Linear Model, the adjusted injury data fit better using the Gompertz function (Fig. 3) than the Richards function; 82.2% vs. 75.5%, respectively (Table 4). Of the 45 functions (5 cultivars × 9 months) fitted to percent adjusted injury data, 37 of Gompertz functions (compared to 34 of Richards functions) fitted as adequately as the General Linear Model. In addition, using the Richards function produced a higher overall mean error sum of squares (117.21) than the Gompertz function. Although both functions produced similar but not identical estimates of LT_{50} and T_{max} (data not shown), the Gompertz function was less complicated (one less parameter to be estimated) and it was easier to produce freezing tolerance estimates than with the Richards function.

Comparisons of quantitative expressions for freezing tolerance. Partial correlation analysis of all freezing tolerance quantitative estimates made by Gompertz and Richards functions in comparison with visual LT_{50} (per month) revealed similar closeness (Table 5). The LT_{50} estimations made by the Richards function and T_{max} estimations made by the Gompertz function were the two best correlated to visual LT_{50} estimate (0.793). Although statistical analysis could not distinguish a significant difference between freezing tolerance estimators, the Gompertz function's estimations of T_{max} were selected as the criteria for comparison of freezing tolerance among cultivars. We believe that T_{max} may be more descriptive than LT_{50} in representing plant stress-response (injury) from a physiological standpoint because it is an estimated point where the rate of injury is maximum.

Leaf freezing tolerance. The seasonal fluctuations of T_{max} for all cultivars revealed maximum acclimation by December 1995 followed by slow deacclimation (Fig. 4). The cultivars were divided into two groups based on freezing tolerance of nonacclimated leaf tissues and into three groups at the maximum cold acclimation (Table 6). In September (when the leaves are nonacclimated), 'Autumn Gold', 'Grumpy Yellow', and 'Vulcan's Flame' were assessed as the tender group and 'Chionoides' and 'Roseum Elegans' as the hardy group. At fully cold-acclimated state by December, 'Autumn Gold', 'Grumpy Yellow', 'Chionoides', and 'Roseum Elegans' were still grouped in their respective groups but 'Vulcan's Flame' was significantly different from the other cultivars and was placed in the intermediate group by itself. When the leaves were deacclimated in June, 'Autumn Gold', 'Chionoides', and 'Roseum Elegans' remained in their grouping as of when nonacclimated; however, 'Grumpy Yellow' and 'Vulcan's Flame' were in a transitional group overlapping the other groups. Data indicated that 'Grumpy Yellow' and 'Vulcan's Flame' had yet to fully deacclimate (to the levels in September 1995) to the nonacclimated state by June.

Table 4. Comparison of two asymmetric functions to General Linear Model (GLM) using F test for lack of fit.

Function	N	Adequate fit as GLM (no.)	Percentage	Overall mean ESS[z]
Gompertz	45	37	82.2	112.97
Richards	45	34	75.5	117.21

[z]Overall mean error sum of squares for GLM = 105.47.

Table 5. Partial correlation coefficients derived from the error sum of squares for comparison of four freezing tolerance quantitative expression with visual LT_{50}.[y][z]

	Richards		Gompertz		Visual
	LT_{50}	T_{max}	LT_{50}	T_{max}	LT_{50}
Visual LT_{50}	0.793	0.769	0.790	0.793	1.000

[y] General Linear Model multivariate analysis of variance, holding month constant.

[z] df = 36, correlation probability = 0.0001.

Discussion

Data transformation analysis. Estimation of stress response using ion leakage is favored because of its nondestructiveness (i.e., requires only a portion of and not the entire plant) and rapidity. Stuart (1939) first described the transformation of percent ion leakage data to percent injury and it was further refined by Flint et al. (1967). Percent injury calculations have since been widely used in many electrolyte leakage studies to assess plant injury. Although the method of Flint et al. (1967) takes into account and adjusts for ion leakage from unfrozen (control) tissues, it assumes that tissues exhibit 100% ion leakage (similar to heat killed) when maximally freeze injured. Whereas this may be a safe assumption for herbaceous plants, all too often cold-acclimated tissues of woody plants exhibit a relatively low percent ion leakage even when frozen to temperatures that cause lethal injury (assessed visually). This is indicated in a study by Sutinen et al. (1992), where the ion leakage from cold-acclimated (7 Dec.) *Pinus rensinosa* needles was only 20% when slowly frozen to –196 °C (LN_2). In comparison, visual browning caused by identical freeze–thaw stress was assessed at 64.2%. Therefore, we believe that using percent injury data in Flint's equation may result in biased estimation of LT_{50} for freeze-injured tissues of woody plants.

In the present study, not all ions were leaked during freeze–thaw treatments, as evidenced by percent ion leakage and percent injury of leaf tissues exposed to –80 °C, which caused 100% visual browning (Table 1). Thus, we implemented the percent adjusted injury data transformations. The percent adjusted injury data also allow for the ease of sigmoidal fitting with a fixed initial point of zero and final point of 100. Thus, the LT_{50} will always be at 50% adjusted injury. Furthermore, the percent adjusted injury showed a higher correlation to the injury data based on visual observations (Table 3). This makes percent adjusted injury data transformation more appropriate and not just arbitrary.

Comparison of Richards to Gompertz functions. Many reports that use ion leakage to assess relative injury to plant tissues do not include the analysis of data fitting to an asymmetric sigmoidal curve partly because it involves many complex calculations. Some have chosen to fit the data in a straight line, while others have interpolated the mean of three or more replications. Hence, the typical quantitative measure of freezing tolerance is somewhat biased with a single estimate obtained from one sampling period and provides no ranges of the estimate. In conducting freeze–thaw experiments, the supply of tissue samples and the coldest temperature to which tissue can be cooled (limited by the freezing bath) are often a limitation. Therefore, in some reported experiments, the coldest treatment temperatures have failed to reach LT_{50} and prompted researchers to use LT_{40} estimates (Zatylny et al., 1996). Fitting the data with an asymmetrical curve would eliminate this problem, as the curve would begin with 0% and end with 100% injury. There have been a few reports that used the Richards function (Anisko and Lindstrom, 1996b; von Fircks and Verwijst, 1993) in fitting the freezing tolerance data; however, no reports were found that used the Gompertz function to determine a plant's response to freezing stress.

The most difficult part in getting the Richards or Gompertz function to fit data is estimating the initial starting parameters. These parameters are crucial for the function to converge and for a good fit to the data. A SAS program was written in this study for the grid search of the best starting parameters. Alternatively, the availability of a commercial program, (PCNONLIN) may be used for the complex matrix search of the parameters (Messori, 1997). Based on the assumption we made that leaf tissue was 100% injured at –80 °C, we eliminated the need to estimate parameter A. However, this assumption may not hold up when plant material other than leaves is used as experimental units. The elimination of parameter A simplified both asymmetric functions in that the modified functions have one less parameter each to be estimated, which reduced the size of the matrix grid search of parameters. Our report estimated 45 different sets of parameters for each function. No significant difference was found between the two functions used, however, the Gompertz function was chosen to estimate the quantitative expressions (LT_{50} and T_{max}) of freezing tolerance because it has only two parameters to be estimated (compared to three in the Richards function) and fitted better than the Richards function when compared with the GLM (Table 4). It is noteworthy that the Richards function is expected to be more flexible than the Gompertz function with an additional parameter to fit the data; however, the extra parameter may also introduce more error and use an extra degree of freedom (Table 4).

Comparisons of quantitative expressions for freezing tolerance. T_{max} was selected (instead of LT_{50}) as the quantitative measure of freezing tolerance because we believe that, physiologically, T_{max} is more descriptive. Conventionally in the literature, LT_{50} is regarded as the temperature that causes 50% injury and the critical temperature of cold hardiness of the particular tissue evaluated (Arora et al., 1992; Sakai et al., 1986; Zhang and Willison, 1987). However, it may be argued whether LT_{50} repre-

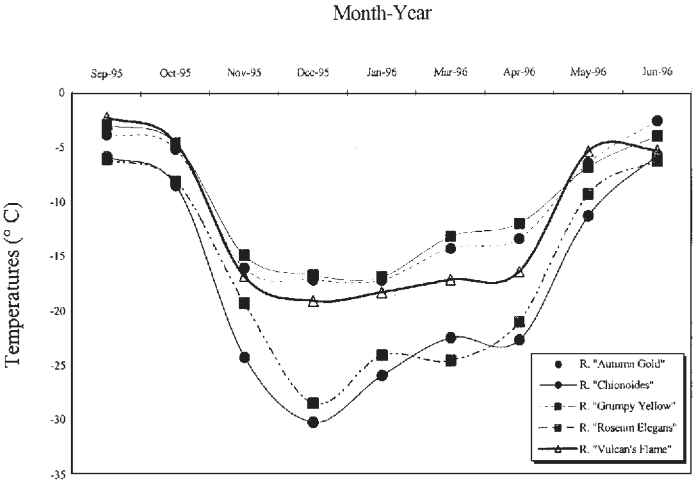

Fig. 4. Seasonal fluctuations of leaf freezing tolerance (T_{max}; °C) of five *Rhododendron* cultivars.

Table 6. Leaf freezing tolerance (T_{max}; °C) of five *Rhododendron* cultivars.[z]

	Nonacclimated (September 1995)	Cold acclimated (December 1995)	Deacclimated (June 1996)
Cultivars	Mean ± SE[y]	Mean ± SE	Mean ± SE
Grumpy Yellow (GY)	–2.9 ± 0.43 a	–16.8 ± 0.42 e	–4.1 ± 0.28 bc
Autumn Gold (AG)	–3.8 ± 0.51 ac	–17.2 ± 0.40 e	–2.7 ± 0.12 a
Vulcan's Flame (VF)	–2.2 ± 0.86 ab	–19.1 ± 0.38 f	–5.3 ± 0.58 cd
Chionoides (CH)	–5.8 ± 0.41 d	–30.3 ± 1.1 g	–5.8 ± 0.37 d
Roseum Elegans (RE)	–6.0 ± 0.34 d	–28.5 ± 1.3 g	–6.3 ± 0.41 d

[z] Using the Gompertz function fitted to adjusted injury data, standard error estimated by the Jackknife method.

[y] Mean separation in matrix by multiple *t* test, $P = 0.05$. Midwinter bud hardiness of these cultivars (provided by nurseries) is as follows: –17.8 °C (GY), –20.6 °C (AG), –26.1 °C (VF), –28.9 °C (CH), and –31.7 °C (RE).

sents a temperature that causes injury to only 50% of the total tissue area or causes all cells to be half injured. On the other hand, T_{max} is the temperature that causes maximum rate of injury where any lowering of temperature beyond T_{max} results in diminishing rates (Fig. 3). Evidence is accumulating in the literature supporting the use of T_{max} as a quantitative cold hardiness index (Anisko and Lindstrom, 1995; Repo et al., 1996; von Fircks and Verwijst, 1993; Zhu and Liu, 1987).

Leaf freezing tolerance. A rather unique portion of this study relies on the Jackknife method (Efron, 1982) for predictions of T_{max}'s standard errors. von Fircks and Verwijst (1993) used 100 simple random samplings with replacement for each data set to predict the standard errors. Instead of using random samplings, the Jackknife method involves manipulation of the actual data set. For example, a nine-point data set could potentially produce nine different data sets, each having one less data point if the Jackknife method is used. This would allow for the estimation of mean T_{max} with standard errors that were derived from the nine different data sets. Therefore, the standard errors of T_{max} should be proportional to the fluctuations of each experiment data point. Also, the standard errors are generated from the number of sigmoidal curves fitted by the computer (which are equal to experimental data points). Hence, each T_{max} value in this report represents at least 30 estimates per cultivar per month, providing a statistically sound estimate of freezing tolerance and saving experimental cost and time.

Data indicated that the *Rhododendron* cultivars exhibited an increase in freezing tolerance during the fall, reached a maximum in December, and gradually decreased thereafter (Fig. 4). All cultivars exhibited a 4- to 5-fold increase (except for about an 8-fold increase for 'Vulcan's Flame') in freezing tolerance during cold acclimation. Based on T_{max} values, we ranked 'Autumn Gold' and 'Grumpy Yellow' in the tender group, 'Vulcan's Flame' in the intermediate group, and 'Chionoides' and 'Roseum Elegans' in the hardy group at maximum cold acclimation. Sakai et al. (1986) consistently reported *Rhododendron* leaf tissues to be significantly more freeze tolerant than bud tissues. In this study, however, the relative leaf freezing tolerance (cold acclimated) estimates were somewhat similar to midwinter bud hardiness of the respective cultivars (Table 6). We were unable to evaluate bud hardiness due to the lack of flower buds in relatively younger plants (2 to 3 years old). The closeness between the leaf freezing tolerance (assessed in this study) and the presumed bud hardiness (reported by nurseries for mature plants) of these cultivars may be due to the use of relatively younger plants that have yet to express their maximum cold acclimation ability. Many reports have attempted to associate laboratory freezing tolerance estimates to the field data and found that the electrolyte leakage method tends to overestimate cold hardiness of plant tissues (Anisko and Lindstrom, 1995; Oldum and Blake, 1996; Repo et al., 1996; Sutinen et al., 1992; Zhang and Willison, 1987). Others have abandoned the electrolyte leakage method entirely and used more complex techniques, such as ionic conductance as a measure of membrane competence, to determine plant cold hardiness (Whitlow et al., 1992). Manley and Hummel (1996), however, concluded that electrolyte leakage method was simpler and did not require additional extensive measurements such as tissue ionic conductance as suggested by Whitlow et al. (1992).

This study provides baseline information on the relative freezing tolerance of five *Rhododendron* cultivars at nonacclimated and cold-acclimated state as well as cold-acclimation ability. We have shown that the percent adjusted injury data fitted to the Gompertz function coupled with the Jackknife method (in estimating standard errors) allows for reliable and statistically sound estimations of freezing tolerance (T_{max}) of *Rhododendron* leaf tissues.

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