Predictive Environmental and Phenological Components of Flower Bud Hardiness in Highbush Blueberry[1]

H. C. Bittenbender and G. S. Howell, Jr.[2]
Michigan State University, East Lansing

Abstract. **Environmental and phenological factors considered potential components of flower bud hardiness of highbush blueberry (*Vaccinium australe* Small) were regressed against hardiness (T_{50}). Three multiple regression equations were derived from 1 year's hardiness and component data on 7 commercial highbush cultivars. Factors considered in the models were air temperature, photoperiod, bud dry weight, bud moisture content, bark color, date of leaf drop and pollen tetrad formation in the field and time to 50% flowering. The standard deviations of the estimated T_{50} values from the actual T_{50}'s were 1°C or less.**

Though the exact freezing resistance mechanism(s) is not known, certain components of hardiness that are responsible for induction, maintenance, and loss of hardiness are known. The effects of environmental stimuli such as photoperiod and air temperature are at least partially understood. The role of short days (SD) for inducing hardiness has been documented with apple (10), *Acer negundo* (11), and *Cornus stolonifera* (28). One theory of hardiness suggests that acclimation occurs in two stages (10, 27, 28); the first stage is induced by SD, the second by frost. Fluctuating air temperatures have been correlated with peach flower bud (6, 16, 18, 20, 22) and apple wood (13) hardiness changes in the winter, both during and after rest.

Pollen maturity and appearance of pink color in the bud are phenological characteristics which indicate rapid transition from a hardy to tender status in peach buds (20). Other factors that may play a role in or correlate with hardiness are moisture content (7, 12, 15), anthocyanin content (16), date of leaf drop, and date of flowering (14).

All of these factors were considered in 3 models dealing with acquisition, maintenance and loss of hardiness in the spring in the blueberry flower bud. The data were analyzed by multiple regression analysis to determine if the models could be used in a predictive manner, as was done for wheat and barley (17).

[1]Received for publication October 19, 1974. Michigan Agricultural Experiment Station Journal Article No. 7017.

[2]Department of Horticulture. The authors wish to acknowledge the help and cooperation of the growers and staff of the Michigan Blueberry Growers Association, Grand Junction, Michigan.

The cultivars used were 'Jersey' (at 2 locations), 'Rubel', 'Northland', 'Berkeley', 'Earliblue', 'Elliot', and 'Bluehaven', located at either South Haven or Grand Junction, Michigan.

The bushes were 10 yrs old and were grown with 4 bushes/replication (rep) and 4 replications for each cultivar in a completely randomized design. Samples were collected at approx monthly intervals from Sept. to April, the flower buds were evaluated for tissue browning of the ovaries following controlled freezing (4), and for hardiness expressed as T_{50} using the Spearman-Kärber method (2).

Components of hardiness

Bud dry wt. Flower bud dry wt (bud dwt) is a measure of size and an indicator of growth. Four 2-node terminal stem pieces (8 buds) were taken per plot, and the buds dried for 24 hr at 65°C. The bud dwt, measured to the nearest tenth mg, is a mean of 4 reps.

Moisture content. The bud moisture content was determined from the bud dwt data, and was expressed in % of the dry wt (g H_2O/g dwt) as the mean of 4 reps.

Bark color. The same stem pieces used for bud dwt and moisture content were also used to determine bark anthocyanin content. The bark was removed with a potato peeler and the anthocyanin extracted with cold acidified methanol for 18 hr at 5°C using 100 ml methanol per g fresh wt of bark. The extract was filtered through Whatman No. 4 filter paper and the absorbance read at 520 nm (23). The mean absorbance of 4 replications was used.

Leaf drop. Date at which a cultivar had dropped 50% of its leaves was determined by observation, and was expressed as the number of days after Nov. 3, 1973.

Tetrad formation. The date of pollen grain formation was used based on the assumption that the interval from microsporogenesis to pollen grain formation was the same for all cultivars (26). Terminal 2-node stem pieces were collected in April and May, 1974, killed and fixed in FAA (5:90:5), and stained with acetocarmine (45% acetic acid) using the squash method (23).

Interval to flowering. Interval to flowering was expressed as the mean no. of days for 50% of the buds on a branch to open at least one flower. Branches with 7-8 laterals were collected on 3 dates in the spring of 1974 for all cultivars. Buds on the laterals were removed so that there were only 2 distal buds per lateral on each branch. The branches were placed with their bases in water at room temp and data recorded as the time from the start of forcing to 50% flowering. The flowering date for a cultivar is the mean of 3 different test periods.

Air temp. Air temp was recorded with a 7 day thermograph 1.6m from the ground, at the Grand Junction plots, and expressed as °C + 100, to make all values positive. In South Haven, the thermograph was 8 km from the plots; thus the weekly temp charts were adjusted using the max and min readings recorded at the experimental plots.

The mean air temp, i.e. max plus min divided by 2, of the 7th day prior to sampling for a hardiness determination, correlated significantly with hardiness in 1973-74. Correlation coefficients were calculated for air temp vs. hardiness, using max, min, and mean temp for day of sampling and for each of the 14 days prior to sampling, and running averages for the same time period. Use of the 7th day mean resulted in the highest r values, regardless of season. In 1972-73, mean temp of the 8th day prior to sampling showed the best correlation with hardiness.

Photoperiod. Correlation coefficients were calculated for hardiness vs. daylength using each of the 14 days prior to sampling. Daylength was defined as hours from sunrise to sunset. These values were for Lansing, Michigan, approx 130 km E and 35 km N of Grand Junction (1).

The daylength of the 8th day prior to sampling in 1973-74, had the highest correlation with hardiness; the 9th day in 1972-73.

Photoperiod x Air Temp. This product was found simply by multiplying the 2 values given above for a given date and location.

Hardiness Equations

The model for a multiple regression equation in which the independent variables correlate linearly with the dependent variable takes this form:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 \ldots . B_NX_N + E$$

where in our model Y is the estimated T_{50}, B_0, B_1 . . . . B_N are partial regression coeficients, X_1 . . . . X_N are independent variables such as bud dwt and moisture content, and E is the residual error. The data for each season were entered with the measured T_{50} and hardiness components for one cultivar at one date as the unit for

regression. The various components were sequentially deleted from the model if their contribution was greater than the .10 level of significance (5). A stepwise deletion multiple regression program in CDC 6500 computer at Michigan State University, was used to calculate the best fit equation. The fall model tested bud dwt, moisture content, bark color, mean air temp, photoperiod, temp x photoperiod, and leaf drop date as possible components of the hardiness equation. The winter model tested the components bud dwt, moisture content, bark color, mean air temp, photoperiod, and temp x photoperiod, and the spring model considered these possible components: bud dwt, moisture content, bark color, mean air temp, photoperiod, temp x photoperiod, date of pollen formation, and mean flowering interval.

Table 1 shows the correlation matrices for the components of hardiness. Using these matrices the partial regression coefficients for the hardiness equations were derived. The resulting prediction equation explain a significant portion of the variation 99, 90, and 98%, respectively. The SD of the observed T_{50} from the values of the estimated T_{50}'s were 1°C or less (Table 2).

The equations contained the following significant components: 1) fall – photoperiod, moisture content, air temp, and temp x photoperiod; 2) winter–bud dwt, moisture content, and bark color; 3) spring–bud dwt, bark color, and photoperiod. The components of each equation give the best fit of the factors measured during each season for estimating T_{50}. The highly significant R values cannot prove cause and effect; these are merely predictive equations. An example of the close agreement between actual T_{50} and estimated T_{50} values can be seen for 'Jersey' (Fig. 1), no estimate differs more than 1°C from the measured T_{50}. Comparison of actual and estimated T_{50} values for other cultivars were made by substituting the specific phenological and environmental values for each cultivar into the hardiness equations (values not shown).

Note that no environmental parameters enters into the winter equation. This is probably due to the limited fluctuation in mean air temp and photoperiod, and the high positive correlations of moisture content with the environmental components. High r values for moisture with these components will reduce their effects on hardiness variation. In the spring equation, photoperiod is significant but not temp. This should not be taken to imply that temp is not important in blueberry dehardening. The dehardening

Table 2. Hardiness (T_{50}) prediction equations, multiple correlation coefficients (R), and the standard deviation of the estimated T_{50} from the observed T_{50} (SD) of multiple regression on selected highbush blueberry cultivars, 1973-74.

FALL

T_{50}^{z} = -45.233 + 12.358 (photoperiod + 0.0323 (H_2O/dwt %) + 0.4695 ($\bar{x}$air temp) - 0.0528 (air temp x photoperiod)

R^{y} = 0.9970

SD = 1.01°C

n^{x} = 24

WINTER

T_{50} = 60.551 + 19.900 (bud dwt) + 0.0892 (H_2O/dwt %) + 8.961 (color)

R = 0.9502

SD = 0.46°C

n = 16

SPRING

T_{50} = -2.530 + 9.556 (bud dwt) + 0.0097 (H_2O/dwt %) + 10.232 (color) + 6.850 (photoperiod)

R = 0.9920

SD = 0.59°C

n = 24

[z] T_{50} is expressed as T_{50} + 100, a coding used to make all temperautures positive.

[y] Significant at the P = 0.1% level.

[x] Number of units in regression analysis.

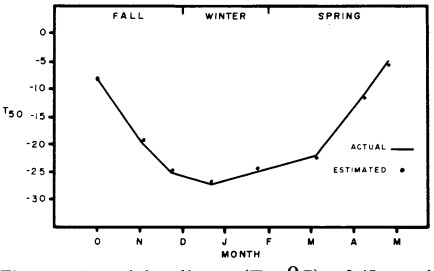

Fig. 1. Actual hardiness (T_{50}°C) of 'Jersey' flower buds as determined by controlled freezing tests and estimated T_{50}'s from hardiness equations, 1973-1974.

Table 1. Correlation matrices for components of highbush blueberry flower bud hardiness (T_{50}) in fall, winter, spring 1973-1974.

Variable	T_{50}^{z}	Bud dwt (%)	H_2O/dwt (%)	Color	Air temp	Photopd	Leaf drop	Pollen	Flowering
FALL									
Bud dwt	-.66								
H_2O/dwt (%)	-.11	.42							
Color	-.35	.62	.57						
Air temp	.85	-.47	.15	.07					
Photopd	.99	-.65	-.12	-.30	.89				
Leaf drop	.04	-.04	-.01	-.32	-.09	.00			
Air temp x photopd	.95	-.58	.00	–.13	.96	.98	-.04		
WINTER									
Bud dwt	.47								
H_2O/dwt (%)	.83	.25							
Color	.39	.15	-.02						
Air temp	.69	.06	.78	.14					
Photopd	.84	.14	.92	.20	.86				
Air temp x photopd	.77	.09	.86	.17	.98	.94			
SPRING									
Bud dwt	.78								
H_2O/dwt (%)	.98	.88							
Color	-.87	-.58	-.80						
Air temp	.72	.31	..57	-.85					
Photopd	.98	.71	.90	-.94	-.81				
Pollen	-.02	-.05	-.18	-.08	-.02	.00			
Flowering	-.08	-.27	-.20	-.14	-.02	.00		.45	
Air temp x photopd	.89	.53	.76	-.94	.95	.94		-.01	-.01

[z] T_{50} is a transformed value, observed T_{50} + 100, therefore positive correlations with T_{50} indicate an inverse relationship with hardiness.

effects of temp have been noted for many species (8, 9, 22) including blueberry (3). Rather, increasing daylength correlated better with decreasing hardiness, also the effect of temp did not remove any additional variation from the equation, and subsequently was deleted. Another explanation is that temp is acting as the trigger for a chain reaction resulting ultimately in dehardening, once the temp rises to a certain level, dehardening starts. Then the dehardening rate remains independent of temp fluctuation within a certain range, in such a case temp would not correlate with hardiness on a per date basis as well as photoperiod (8).

The potential value of hardiness equations is great. Of basic importance are the models, hardiness prediction being a function of several factors not just one. Multiple regression provides a method to study these relationships better. There are several practical applications such as site selection or screening for hardy seedlings. After several years' observations it should be possible to get constant values for partial regression coefficients of significant environmental and phenological components. Then known values of phenological components of commercial cultivars can be put into the equation for determination of survival potential at a site. The breeder can make these measurements on seedlings which will predict their hardiness. From our experience moisture content and bud size measurements are easier to make than any freezing methods currently in use.

Work is being continued in our program to find the constant values for the partial regression coefficients. In the equations, in this paper, the coefficients may not remain constant from year to year. This must be considered as a concern in evaluating the prediction equation's utility at this time.

Literature Cited

1. Anon. 1965. Sunrise and sunset at Lansing, Michigan. No. 1148, Nautical Almanac Office, USN Observatory, Washington, D.C.
2. Bittenbender, H. C., and G. S. Howell, Jr. 1974. Adaptation of the Spearman-Kärber method for estimating T_{50} of cold stressed flower buds. *J. Amer. Soc. Hort. Sci.* 99:187-190.
3. _____ and _____. Interactions of temperature and moisture content on deacclimation of post rest flower buds of *Vaccinium australe* Small, highbush blueberry. *Can. J. Plant Sci.* (In Press).
4. _____ and _____. Cold hardiness of flower buds from selected highbush blueberry cultivars (*Vaccinium australe* Small). *J. Amer. Soc. Hort. Sci.* (In Press).
5. Draper, N. R. and H. Smith. 1966. Applied regression analysis. Wiley & Sons, New York, New York.
6. Edgerton, L. J. 1954. Fluctuations in the cold hardiness of peach flower buds during rest period and dormancy. *Proc. Amer. Soc. Hort. Sci.* 64:175-180.
7. Graham, R. P. 1971. Cold injury and its determination on selected Rhododendron species. MS Thesis, Univ. of Minnesota.
8. Hamilton, D. F. 1973. Factors influencing dehardening and rehardening of *Forsythia* × *intermedia* stems. *J. Amer. Soc. Hort. Sci.* 98:221-223.
9. Howell, G. S. and C. J. Weiser. 1970. Fluctuations in the cold resistance of apple twigs during spring dehardening. *J. Amer. Soc. Hort. Sci.* 95:190-192.
10. _____ and _____. 1970. The environmental control of cold acclimation in apple. *Plant Physiol.* 45:390-394.
11. Irving, R. M. and F. O. Lanphear. 1968. Regulation of hardiness in *Acer negundo*. *Plant Physiol.* 43:9-13.
12. Johnston, E. S. 1923. Moisture relations of peach buds during winter and spring. *Univ. Maryland Agr. Expt. Sta. Bul.* 255.
13. Ketchie, D. O., C. H. Beeman, and A. L. Bullard. 1972. Relationship of electrolytic conductance to cold injury and acclimation in fruit trees. *J. Amer. Soc. Hort. Sci.* 97:403-406.
14. Layne, R. E. C. 1967. Relation of bloom date and blossom temperature to frost injury and fruit set in apricot. *Fruit Var. & Hort. Digest* 21:2.
15. Lumis, G. P. 1970. Winter hardiness of evergreen azalea (*Rhododendron* cv.) flower buds. PhD Thesis, Michigan State Univ., East Lansing.
16. Meader, E. M. and M. A. Blake. 1943. Seasonal trend of fruit bud hardiness in peaches. *Proc. Amer. Soc. Hort. Sci.* 48:91-98.
17. Metcalf, E. L., C. E. Cress, C. R. Olien, and E. H. Everson. 1970. Relationship between crown moisture content and killing temperature of three wheat and barley cultivars. *Crop Sci.* 10:362-365.
18. Parker, J. 1962. Relation among cold hardiness, water, soluble protein, anthocyanins and free sugars in *Hedera helix* L. *Plant Physiol.* 37:8-9-813.
19. Proebsting, E. L. 1963. The role of air temperatures and bud development in determining hardiness of dormant 'Elberta' peach fruit buds. *Proc. Amer. Soc. Hort. Sci.* 83:259-269.
20. _____. 1970. Relation of fall and winter temperatures to flower bud behavior and wood hardiness of deciduous fruit trees. *HortScience* 5:422-424.
21. _____ and H. H. Mills. 1961. Loss of hardiness by peach fruit buds as related to their morphological development during the pre-bloom and bloom period. *Proc. Amer. Soc. Hort. Sci.* 78:104-110.
22. _____ and _____. 1972. A comparison of hardiness responses in fruit buds of 'Bing' cherry and 'Elberta' peach. *J. Amer. Soc. Hort. Sci.* 97:802-806.
23. Sass, John E. 1958. Botanical microtechnique. 3rd Ed. Iowa State Univ. Press, Ames, Iowa.
24. Steponkus, P. L. and F. O. Lanphear. 1967. Factors influencing artificial cold acclimation and freezing tests of *Hedera helix* 'Thorndale'. *Proc. Amer. Soc. Hort. Sci.* 91:735-741.
25. _____ and _____. 1969. The relationship of anthocyanin content to cold hardiness of *Hedera helix* L. *HortScience* 4:55-56.
26. Stushnoff, C. and B. F. Palser. 1969. Embryology of five *Vaccinium* taxa including diploid, tetraploid, and hexaploid species or cultivars. *Phytomorphology* 19:312-331.
27. Van Huystee, R. B., C. J. Weiser, and P. H. Li. 1967. Cold acclimation in *Cornus stolonifera* under natural and controlled photoperiod and temperature. *Bot. Gaz.* 128:200-205.
28. Weiser, C. J. 1970. Cold resistance and injury in woody plants. *Science* 169:1269-1278.

In Vitro Reproductiveness of Asparagus Stem Segments with Branch-shoots at a Node[1]

Hsu-Jen Yang and W. J. Clore[2]

Irrigated Agriculture Research and Extension Center

Prosser, Washington

Abstract. **More rooted *Asparagus officinalis* L. plantlets were obtained *in vitro* from stem segments with 3 or more branch-shoots than from those with 1 or 2 branch-shoots; those without branch-shoots produced the fewest plantlets.**

We reported a technique and procedure for vegetatively propagating asparagus plants *in vitro* (1, 2). Explants for culture were obtained from nonbranching stems of aseptically-cultured stock plants. When stock plants were cultured for more than 1 month, branching usually occurred on the apical portion of stems killed back by heat from overhead lights; occasionally they developed on uninjured stems (Fig. 1A, B). After 3 months 54% of the stems had branched, averaging 3.2 branches per stem. When a multiple branch-shoot formed an enlargement at a node, essentially an aerial crown resulted at the base of the shoots. In a few cases aerial roots were produced (Fig. 1B). This suggested that these structures may have a generative capacity that could be used as another source for developing plants in asparagus tissue culture. The objective was to determine if there were differences in developing plantlets *in vitro* between stem segments from stock plants with and without branch-shoots.

[1]Received for publication January 11, 1975. Scientific Paper No. 4356. Washington State University, College of Agriculture, Project No. 0173.

[2]Research Associate and Horticulturist, respectively, Department of Horticulture. Acknowledgement is given the Washington Asparagus Growers Association for grant funds supporting these studies.