Don Minore
and
Alan W. Smart
USDA Forest Service
Pacific Northwest Forest and Range Experiment Station
Portland, Oregon 97208

Frost Tolerance in Seedlings of Vaccinium membranaceum, Vaccinium globulare, **and** Vaccinium deliciosum

Abstract

Frost tolerances of *Vaccinium globulare, V. deliciosum, and V. membranaceum* seedlings were compared at controlled temperatures of 0°, –3°, –6°, and –9°C. Subsequent shoot growth decreased with decreasing temperature in all species, but growth trends differed. Seedings of *V. deliciosum* were more frost tolerant than the others. *V. deliciosum* seedlings also began spring growth earlier when all three species were grown in the same environment.

Introduction

Wild huckleberry fields occupy extensive areas in the mountains of Oregon and Washington. Many of these areas produce large quantities of high quality fruit, and some are heavily utilized by berry pickers (Minore, 1972). Unfortunately, huckleberry production is often reduced by frost damage. Similar crop reductions occur in the blueberry fields of eastern North America (Johnson, 1951; Hall, Aalders, and Barker, 1964). Sometimes most of the autumn berry crop is destroyed by spring frosts that occur when the huckleberry bushes are actively growing, but a few huckleberry shrubs remain uninjured and productive after severe frosts. Many of these shrubs escape damage because they grow in protected locations or under local heavy snow accumulations where active growth is delayed. Others seem to be frost resistant—field observations after the severe frosts of 1973 indicated that some northwestern *Vaccinium* species are more frost tolerant than others. As it is difficult to separate species differences from environmental differences in the field, we compared the frost tolerances of three common northwestern *Vaccinium* species under controlled conditions. Our working hypothesis was that frost damage would increase with decreasing temperatures. Further, we expected damage to differ among species.

Vaccinium membranaceum grows at moderate to high elevations (1000-1800 m) on both eastern and western slopes of the Olympic Mountains and the Cascade Range. *V. globulare* also grows at moderate elevations, but usually occurs east of the Cascade crest. Both *V. membranaceum* and *V. globulare* are large shrubs, 0.5-1.0 m tall when mature. In contrast, *V. deliciosum* is a low, matted shrub less than 0.5 m tall. It is common at elevations above 1300 m in the Olympics and Cascades. All three species produce palatable berries, and all three occur in the heavily used huckleberry fields near Mt. Adams, Washington.

Methods

V. membranaceum, V. globulare, and *V. deliciosum* berries were collected from the

Sawtooth huckleberry field near Mt. Adams, Washington, in September, 1974. A single shrub (the only one found in this locality) provided the *V. globulare* berries. *V. membranaceum* and *V. deliciosum* berries were collected from many (>50) shrubs. Viable seeds were obtained from these fresh berries by pulping them in a laboratory blender, then decanting the lighter pulp from the heavier seeds by running water through a gently agitated dish. After drying, the unstratified seeds were sown on moist peat surfaces in a growth chamber set for 18°C, 12-hour days and 13°C, 12-hour nights. The seeds of all three species germinated in 16 to 21 days. Seven weeks after germination, growth chamber conditions were changed to 20°C, 14-hour days and 14°C, 10-hour nights. After seedlings were 10 weeks old, the peat substrate was saturated periodically with a nutrient solution based upon the macronutrient proportions published by Ingestad (1973) and the micronutrients listed by Minton, Hagler, and Brightwell (1951).

Twelve-week-old seedlings of all three species were transplanted to individual pots filled with a peat-sand soil (50 percent peat, 50 percent soil by volume). These pots were arranged in three randomized blocks within the growth chamber. Each block contained 72 pots (24 of each species) randomly arranged by species and future freezing treatment. Temperatures, photoperiods, and nutrient additions were maintained at the levels used before transplanting. All seedlings were grown under these conditions for an additional 20 weeks. All three species responded well and appeared to be equally healthy and vigorous when 32 weeks old.

Dormancy was induced in the 32-week-old seedlings by gradually lowering growth chamber temperatures and shortening photoperiods over four weeks. (Temperatures were lowered 2.8°C, and photoperiods shortened 1.5 hours per week.) At the end of this adjustment period, temperature was maintained at a constant 3°C, with 8 hours of illumination in every 24-hour period. These chilling conditions were maintained for 16 weeks, with one exception—4 weeks into the dormancy period, a growth chamber malfunction lowered the temperature to –1°C for an unknown period during a weekend. Although both soils and plants appeared to be frozen solidly in this accidental occurrence, seedlings were not adversely affected. When growth chamber temperatures and photoperiods were readjusted to pre-chilling levls (20°C, 14-hour days; 14°C, 10-hour nights) 12 weeks later, the dormant seedlings responded by breaking buds and commencing growth.

After chilling conditions had been terminated for 10 days, the new leaves on each seedling were counted. Five weeks after chill termination, the vigorously growing seedlings were transferred to a 1°C conditioning room for 24 hours, then subjected to 1-hour freezing treatments at 0°, –3°, –6°, and –9°C. Pots were embedded in sawdust to prevent soil freezing during these treatments. Beginning at 1°C, the freezing-treatment temperatures were attained gradually by lowering air temperatures at a uniform rate of 4°C per hour in a freezing chamber. After being held at the treatment temperature for 1 hour, temperatures were raised again to 1° at the same rate of 4°C per hour (1° every 15 minutes). Six seedlings of each species were treated at each temperature —in each of the three randomized blocks. The blocks were treated sequentially in the same freezing chamber. Treatments in each block were randomly ordered and applied in successive freezing-chamber cycles. In this way, 216 *Vaccinium* plants were frozen— 3 blocks x 3 species x 4 treatments x 6 seedlings per treatment.

Immediately before being subjected to the freezing treatments, the length of the longest live shoot on each of the 216 *Vaccinium* seedlings was measured and recorded. Immediately after treatment, all seedlings were transferred back to normal growing conditions in their original, randomized growth chamber positions. They were maintained in this growth-chamber environment for an additional eight weeks following treatment, after which the length of the longest live shoot on each seedling was again measured. Shoots of all seedings then were cut at the soil surface, oven-dried at 65°C for 48 hours, and weighed. Shoot response was calculated by subtracting shoot lengths eight weeks after treatment from shoot lengths immediately before treatment.

To test our hypothesis, main effects of temperature treatments and their interactions with species were partitioned into linear, quadratic, and cubic components and subjected to analyses of variance (Snedecor and Cochran, 1961, pp. 349-359). Response curve equations were then constructed for each species using as coefficients only those values that were significant at $P<.05$. Finally, the calculated curves were used to illustrate graphically any statistically significant frost response trends.

Results

Phenological differences between species became evident soon after the dormant *Vaccinium* seedlings resumed growth at the end of the chilling period (Fig. 1). *V. deliciosum* buds expanded more rapidly than buds of the other two species. Average numbers of new leaves per plant 10 days after chill termination were:

V. membranaceum—10.0
V. globulare—10.3
V. deliciosum—36.2

These differences between *V. deliciosum* and the other species are statistically significant at the .05 level of probability.

Figure 1. *Vaccinium globulare* (left), *V. deliciosum* (center), and *V. membranaceum* seedlings 10 days after termination of chilling conditions. *V. deliciosum* breaks dormancy faster than the other two species.

Seedlings subjected to 0° and −3°C treatments were not visibly affected, and shoot response did not differ appreciably among species. In contrast, frost damage was evident immediately when seedlings were removed from the −6° and −9°C treatments. Leaves were dull in color or wilted, with an odor reminiscent of fresh peas. Although all plants were injured at these lower temperatures, *V. deliciosum* seedlings seemed less affected than seedlings of the other two species. This difference between *V. delicio-*

Figure 2. *Vaccinium globulare* (left), *V. deliciosum* (center), and *V. membranaceum* seedlings four weeks after freezing at −6°C. *V. deliciosum* is less damaged than the other two species.

TABLE 1. Average **Vaccinium** shoot response (length difference) measured 8 weeks after freezing.[1]

Species	Freezing Temperature (C°) 0°	-3°	-6°	-9°
	cm			
V. globulare	+2.08	+2.28	-6.58	-11.75
V. membranaceum	+2.25	+1.28	-6.50	-9.22
V. deliciosum	+2.61	+3.08	-1.19	-6.36

[1]Response=length of longest live shoot 8 weeks after freezing minus length of longest live shoot before freezing. Negative values indicate shoot length decreases. Each tabular figure is the average of 18 plant measurements.

TABLE 2. Average oven-dry **Vaccinium** shoot weights measured 8 weeks after freezing.[1]

Species	Freezing Temperature (C°) 0°	-3°	-6°	-9°
	g			
V. globular	3.37	3.75	1.41	1.51
V. membranaceum	3.29	3.61	1.61	1.43
V. deliciosum	3.18	3.34	2.24	1.54

[1]Each tabular figure is the average shoot weight of 18 plants.

sum and the other species was quite apparent 4 weeks after treatment (Fig. 2). None of the seedlings died, and the 8-week measurements summarized in Tables 1 and 2 confirmed our earlier observations. Shoot response trends and species-treatment interactions were significant at the .001 level of probability. Temperatures of 0° and –3°C did not seem to damage any of the species. All shoot lengths were reduced by the –6° and –9°C treatments, but the species trends differed (Fig. 3). *V. deliciosum* shoot lengths were less affected by –6°C than shoots of other species. The superior frost tolerance of *V. deliciosum* at –6°C also is demonstrated by the shoot-weight trends shown in Figure 4. Both shoot length and dry weight response curves indicate converging species trends at –9°C.

Discussion

Phenological differences among species may be partially responsible for the observed differences in frost tolerance. Because *V. deliciosum* began spring growth earlier than the other species, its new leaves and shoots had a somewhat longer time to mature and harden before being subjected to the freezing treatments. All three species experienced

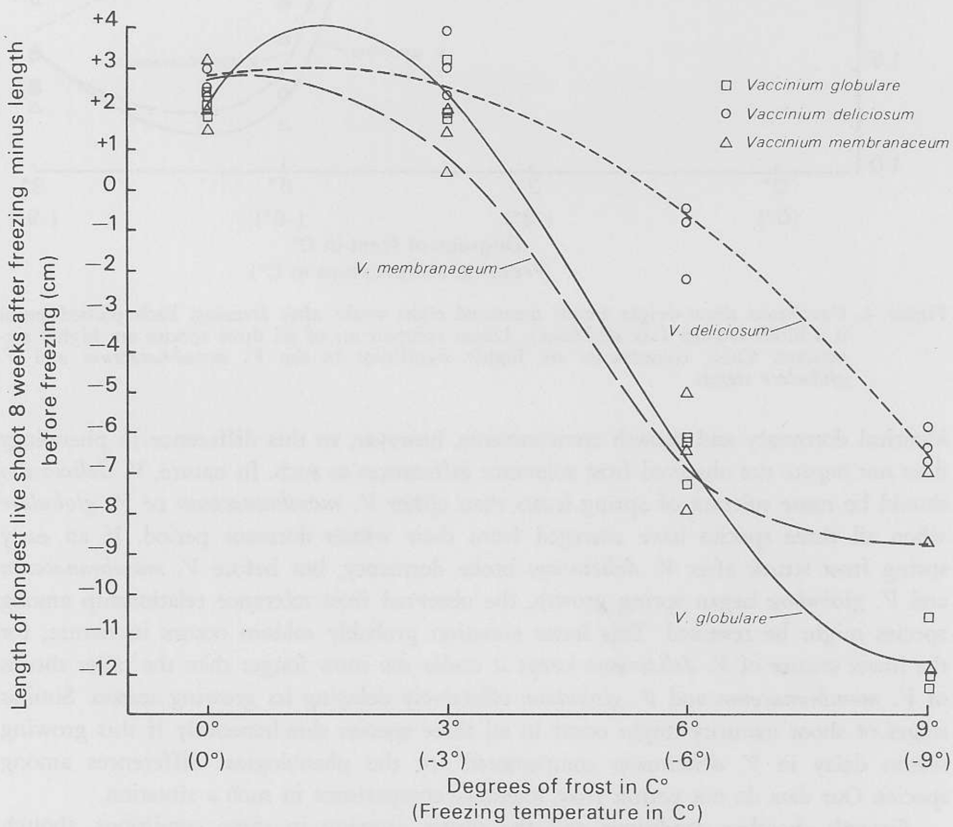

Figure 3. Response curves showing decreased growth (length difference) measured eight weeks after freezing. Each plotted point is a block average (six seedlings). Linear components of all three species trends are highly significant. Quadratic components of the *V. deliciosum* and *V. globulare* and cubic components of the *V. globulare* and *V. membranaceum* trends are also highly significant.

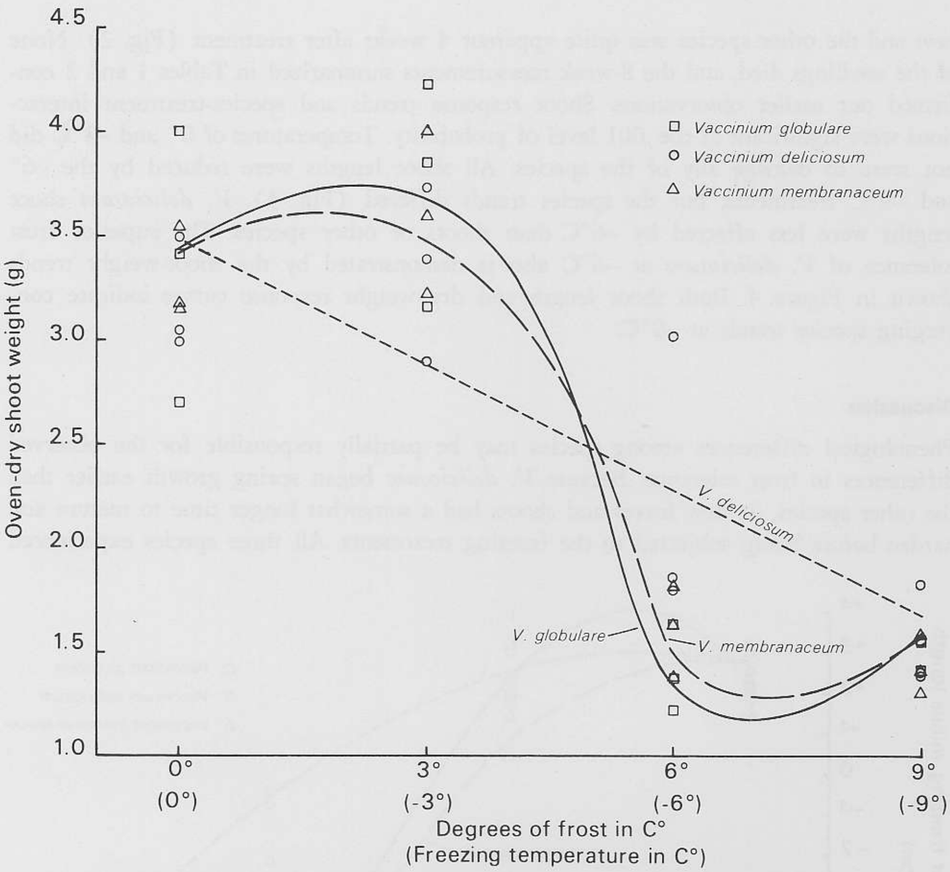

Figure 4. *Vaccinium* shoot-weight trends measured eight weeks after freezing. Each plotted point is a block average (six seedlings). Linear components of all three species are highly significant. Cubic components are highly significant in the *V. membranaceum* and *V. globulare* trends.

identical dormancy and growth environments, however, so this difference in phenology does not negate the observed frost tolerance differences as such. In nature, *V. deliciosum* should be more tolerant of spring frosts than either *V. membranaceum* or *V. globulare* when all three species have emerged from their winter dormant period. If an early spring frost struck after *V. deliciosum* broke dormancy, but before *V. membranaceum* and *V. globulare* began spring growth, the observed frost tolerance relationship among species might be reversed. This latter situation probably seldom occurs in nature, for the lower stature of *V. deliciosum* keeps it under the snow longer than the taller shrubs of *V. membranaceum* and *V. globulare,* effectively delaying its growing season. Similar stages of shoot maturity might occur in all three species simultaneously if this growing season delay in *V. deliciosum* compensated for the phenological differences among species. Our data do not permit frost tolerance comparisons in such a situation.

Growth chamber conditions and the plants growing in these conditions, though uniform and comparable, are not exactly the same as field conditions and plant ecotypes existing in nature. The absolute levels of frost response measured in our growth chamber may or may not occur at similar temperatures in nature, even when similar preconditioning regimes and identical rates of cooling and warming occur. The relative species differences observed here, however, should be similar to those in nature. When all three species are growing vigorously in the spring, *V. deliciosum* is more frost tolerant than either *V. membranaceum* or *V. globulare*. *V. deliciosum* probably should be favored where frost-prone areas are managed for wild huckleberry production.

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