Seasonal Cold-acclimation Patterns of *Sedum spectabile* x *telephium* L. 'Autumn Joy' and *Sedum spectabile* Boreau. 'Brilliant'

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Abstract. Electrolyte leakage and regrowth were measured from September through January to determine cold hardiness of *Sedum spectabile* x *telephium* L. 'Autumn Joy' and *Sedum spectabile* Boreau. 'Brilliant' plants grown outdoors in central Iowa. Crowns were subjected to 0, -3, -6, -9, -12, -15, -18, -21, -24, or -27°C. Regrowth tests were performed on whole crowns and electrolyte leakage was determined on excised tuberous root and crown tissue. Both cultivars were killed at -3°C in September, but they acclimated gradually through January. Maximum hardiness was achieved in January, with killing temperatures of -27°C for 'Autumn Joy' and -21°C for 'Brilliant'. Regrowth quality ratings were significantly correlated with crown and tuberous root electrolyte leakage measurements, although the relationship was stronger for 'Autumn Joy'.

Most herbaceous perennial landscape plants depend on regenerative organs, such as crowns, stolons, or rhizomes, to survive and overwinter successfully in northern climates. Because these regenerative growing points are located at or below the soil surface or are protected by insulating snow cover, little attention has been given to investigating their ability to cold-acclimate or to identifying their specific low-temperature tolerance. As more perennials are produced and held through winter in containers, thereby exposed to potentially lethal low temperatures, cold hardiness information becomes more important.

Cold acclimation is essential to the survival of woody plants growing in temperate regions (Teets et al., 1989), and seasonal changes in freezing tolerance have been reported for various woody species (Flint, 1972; Howell and Weiser, 1970; Irving and Lanphear, 1967; van Huystee et al., 1967). Herbaceous plants have a limited capacity to withstand subzero temperatures during the growing season (Fennell and Li, 1987); however, researchers have identified several species that acclimate extensively when exposed to cold-acclimating environments (Gusta and Fowler, 1976; Kohn and Levitt, 1965; Li, 1984; Peavy and Greig, 1973). Under natural conditions in

the field, the acclimation pattern of some overwintering herbaceous plants seems to approximate that of woody perennials but is more closely associated with the advent of low temperature than with shortening daylength (Burrows et al., 1989; Dexter, 1933; Fennell and Li, 1987; Steponkus, 1978). To our knowledge, seasonal cold-acclimation patterns have not been established for herbaceous perennial landscape plants. Therefore, our principal objective was to determine the seasonal cold-acclimation pattern of *Sedum* 'Autumn Joy' and 'Brilliant' in response to conditions typical of container culture in central Iowa by using electrolyte leakage (EL) viability and regrowth tests as methods for determining cold hardiness and seasonal cold-acclimation patterns. These taxa were chosen because of their reputation for tolerating low temperature (Armitage, 1989; Clausen and Ekstrom, 1989; Praeger, 1967) and observed differences in cold hardiness (Iles et al., 1993).

Materials and Methods

In Apr. 1991, plugs (3.2 cm wide x 5.1 cm deep) of *Sedum* 'Autumn Joy' and 'Brilliant' were obtained from Green Leaf Enterprises, Leola, Pa., and established in 3.5-liter, black plastic containers using a medium of 2 Canadian sphagnum peat : 2 perlite : 1 sandy-loam soil (by volume) amended with ground calcitic limestone (pH 6.5). Plants were grown outside at the Iowa State Univ. Horticulture Research Station, Gilbert (U.S. Dept. of Agriculture hardiness zone 5a) in a shadehouse (20% radiation exclusion) until time of freezing tests. Each plant was irrigated as needed with tap water, received 5 g 17N-2.6P-10K (17-6-12) Sierra fertilizer plus micronutrients (The Scotts Co., Marietta, Ga.), and was fertilized bi-weekly until 15 Aug. with an additional 150 ppm N from a 20N-4.4P-16.6K (20-10-20) water-soluble fertilizer. To prevent low-tem-

perature injury before the freeze tests, plants were covered with a white polyethylene bonded to microfoam thermoblanck (6 mm thick) when ambient air temperatures were predicted to fall below -10°C.

Controlled freezing tests were replicated three times during each test period; 1 to 6 Sept., Oct., Nov., and Dec. 1991 and 1 to 6 Jan. 1992. Twenty-two container-grown plants were selected randomly for each replication and cultivar, 11 each for hardiness determinations using a regrowth procedure and an EL test.

Plants were removed from their containers, stem tissue was trimmed to 3 cm above the crown, and crowns with roots attached were rinsed with cold tap water to remove soil and plant debris. Crowns were placed in moist cheesecloth, wrapped in aluminum foil (McKenzie and Weiser, 1975), transferred to a programmable freezer (Scientemp Corp., Adrian, Mich.), and cooled from 3°C to 0, -3, -6, -9, -12, -15, -18, -21, -24, or -27 ± 0.5°C at 2 ± 0.5°C/h. Two crowns each of 'Autumn Joy' and 'Brilliant' were removed from the freezer at each treatment temperature, transferred to a cooler set at 3 ± 1°C, and allowed to thaw slowly for 24 h. Control plants were held at 3 ± 1°C during the freezing test. Test-tube racks were used to suspend crowns above the freezer floor to facilitate uniform cooling. Copper-constantan thermocouples [0.51 mm in diameter (24 gauge)] (type T, Omega Engineering, Stamford, Conn.) were attached to the surface of three extra crowns to monitor tissue temperature. Thermocouple output was recorded on a 15-multipoint recorder (Honeywell Electronik; Honeywell, Fort Washington, Pa.). Preparation for each replication was completed within 4 to 6 h of collection.

Two procedures were used for determining cold hardiness levels of 'Autumn Joy' and 'Brilliant'. For the regrowth procedure, thawed crowns (one per treatment temperature per replication per cultivar) were planted in 3.5-liter, green plastic containers using a medium of 2 Canadian sphagnum peat : 2 perlite : 1 sandy-loam soil (by volume) and transferred to a greenhouse at 21 ± 3°C. After a regrowth period of 60 days, plants were rated using a 1 to 5 qualitative scale, where 1 = a dead plant, 2 = a live plant with little top growth, and 3 to 5 = a plant exhibiting vigorous regrowth (5 being the highest rating). Killing temperature was defined as the temperature resulting in plant survival percentages ≤ 33%. Shoot regrowth was harvested, dried at 67°C for 96 h, and weighed. Because similar trends were found between dry mass measurements and quality ratings, dry mass data are not presented.

Degree of injury at each temperature also was estimated by EL measurements on excised crown and tuberous root tissue. Three 1-cm sections of tuberous root tissue were excised from thawed crowns. Sections were triple-rinsed with deionized water to remove exogenous electrolytes (Nanaiah and Anderson, 1992), placed into individual 50-ml polypropylene test tubes containing 25-ml of deion-

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ized water, and incubated on an orbital shaker for 24 h at $24 \pm 2^\circ\text{C}$ in darkness. Remaining crown tissue was trimmed to a 3- to 5-cm length with stem, root, and tuberous root tissue removed and treated as previously described for tuberous root tissue. After incubation, initial electrical conductivity (EC) measurements were taken on crown and tuberous root tissues in each test tube using a conductance meter (model 35; Yellow Springs Instrument Co., Yellow Springs, Ohio). Final EC measurements were taken after tissues were killed by immersing test tubes in an 80°C water bath for

1 h and incubating for 24 h on an orbital shaker in darkness. Percent EL at each temperature was expressed as $\text{EL}(\%) = (\text{initial EC}/\text{final EC}) \times 100$. EC measurements were made on one crown per treatment temperature per replication per cultivar. Statistical differences were determined by analysis of variance.

Three extra crowns of each cultivar were evaluated for percentage moisture during each freezing test period. Plants were removed from their containers, stem tissue was trimmed to 3 cm above the crown, and soil was gently removed from crown and root tissue. After

obtaining fresh mass, crowns were dried at 67°C for 96 h and reweighed. Percent crown moisture (CM) for each test period was expressed as $\text{CM}(\%) = (\text{fresh mass}/\text{dry mass}) \times 100$.

Results and Discussion

Sedum 'Autumn Joy' and 'Brilliant' cold-acclimated, as survival percentages increased at successively lower treatment temperatures over the fall and winter acclimation period. By October, the killing temperatures for 'Autumn

Table 1. Effect of low temperature on percent survival, shoot regrowth quality, and percent crown electrolyte leakage (EL) of *Sedum* 'Autumn Joy'.

Treatment month and criterion	Treatment temp ($^\circ\text{C}$)										
	Control	0	-3	-6	-9	-12	-15	-18	-21	-24	-27
September											
Survival (%)	100 ^z	100	0 ^y	0	0	0	0	0	0	0	0
Regrowth quality	3.0 ^x	2.0	---	---	---	---	---	---	---	---	---
Crown EL (%)	21 ^w	29	77	53	79	70	57	75	55	73	82
October											
Survival (%)	100	100	67	67	100	0 ^y	0	0	0	0	0
Regrowth quality	3.0	2.3	2.5	3.0	2.3	---	---	---	---	---	---
Crown EL (%)	23	23	23	18	48	53	69	83	71	76	72
November											
Survival (%)	100	100	100	100	100	100	33 ^y	0	0	0	0
Regrowth quality	3.0	3.0	3.0	3.0	2.7	2.7	3.0	---	---	---	---
Crown EL (%)	34	23 ^x	25	25	41	43	64	82	64	69	81
December											
Survival (%)	100	100	100	100	100	100	100	67	67	0 ^y	0
Regrowth quality	4.7	4.0	4.7	4.0	4.3	4.0	3.7	3.5	3.5	---	---
Crown EL (%)	18	30	27	21	20	28	35	44	42	46	58
January											
Survival (%)	100	100	100	100	100	100	100	100	100	67	0 ^y
Regrowth quality	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.7	3.3	3.0	---
Crown EL (%)	35	26	26	25	24	27	28	28	36	43	72

^zPercentage of test plants surviving ($n = 3$).

^yKilling temperature = plant survival percentages $\leq 33\%$.

^xRatings were on a scale from 1 to 5, where 1 = dead plant; 2 = alive, but little top growth; 3 to 5 = vigorous regrowth, 5 = highest quality. Mean quality ratings do not include dead plants. Least significant difference for regrowth quality ($P \leq 0.05$) = 0.38.

^wLeast significant difference for percent crown EL ($P \leq 0.05$) = 7.1 ($n = 3$).

Table 2. Effect of low temperature on percent survival, shoot regrowth quality, and percent crown electrolyte leakage (EL) of *Sedum* 'Brilliant'.

Treatment month and criterion	Treatment temp ($^\circ\text{C}$)										
	Control	0	-3	-6	-9	-12	-15	-18	-21	-24	-27
September											
Survival (%)	100 ^z	100	0 ^y	0	0	0	0	0	0	0	0
Regrowth quality	3.0 ^x	2.0	---	---	---	---	---	---	---	---	---
Crown EL (%)	19 ^w	25	50	50	68	88	54	64	66	78	54
October											
Survival (%)	100	100	0 ^y	0	0	0	0	0	0	0	0
Regrowth quality	3.0	2.7	---	---	---	---	---	---	---	---	---
Crown EL (%)	13	18	50	51	67	70	65	76	62	73	70
November											
Survival (%)	100	100	100	100	100	100	100	33 ^y	0	0	0
Regrowth quality	3.3	3.0	3.0	2.7	2.7	2.7	2.3	2.0	---	---	---
Crown EL (%)	22	23	24	24	38	37	40	47	61	72	73
December											
Survival (%)	100	100	100	100	100	100	100	67	33 ^y	0	0
Regrowth quality	5.0	5.0	4.7	4.7	4.7	4.7	3.3	4.0	4.0	---	---
Crown EL (%)	16	26	25	28	21	25	28	28	32	49	42
January											
Survival (%)	100	100	100	100	100	100	100	100	33 ^y	0	0
Regrowth quality	5.0	5.0	5.0	5.0	5.0	4.7	5.0	4.7	2.0	---	---
Crown EL (%)	25	28	21	21	27	33	27	25	31	27	56

^zPercentage of test plants surviving ($n = 3$).

^yKilling temperature = plant survival percentages $\leq 33\%$.

^xRatings were rated on a scale from 1 to 5, where 1 = dead plant; 2 = alive, but little top growth; 3 to 5 = vigorous regrowth, 5 = highest quality. Mean quality ratings do not include dead plants. Least significant difference for regrowth quality ($P \leq 0.05$) = 0.41.

^wLeast significant difference for percent crown EL ($P \leq 0.05$) = 7.2 ($n = 3$).

Joy' was -12C (Table 1) and -3C for 'Brilliant' (Table 2). The greatest increase in freeze tolerance for 'Brilliant' occurred in November, when all plants survived exposure to -15C. Only slight changes in cold acclimation were noted thereafter (Table 2). The killing temperature for 'Autumn Joy' in November was -15C (Table 1). The exposure to lower minimum ambient temperatures occurring in November and December presumably was responsible for increased cold hardiness for both cultivars and resulted in maximum cold acclimation by the January test date when killing temperatures were -27C for 'Autumn Joy' and -21C for 'Brilliant'.

In our study 'Brilliant' became cold tolerant later in the season than 'Autumn Joy' (Tables 1 and 2). 'Autumn Joy' began acclimating before significant exposure to freezing temperatures (by the October test), although low temperatures, not short days, are considered by some researchers to be the primary factor for initiating the cold-acclimation process in herbaceous plants (Li, 1984), and unlike 'Brilliant', 'Autumn Joy' continued steady cold acclimation during early winter, achieving greatest hardiness in January.

Shoot regrowth quality ratings, such as survival percentages, suggest a gradual transition from undamaged or viable to killed tissues over several degrees, particularly at the December and January test dates. Quality ratings of 'Autumn Joy' (Table 1) and 'Brilliant' (Table 2) were highest at the January test date. Plants exposed to 3 to -18C regrew vigorously and had similar quality ratings, but ratings were considerably lower for 'Brilliant' exposed to -21C. Quality ratings for 'Autumn Joy' declined after exposure to -21C and -24C, but plants regrew with sufficient vigor to earn quality ratings ≥ 3 .

Regrowth measurements for both cultivars at the September, October, and November test dates were difficult to interpret. Although quality ratings increased at decreasing treatment temperatures from September to November, regrowth was considerably less vigorous than in December and January (Tables 1 and 2). These results suggest 'Autumn Joy' and 'Brilliant' will not regrow vigorously until a chilling requirement has been satisfied. A similar occurrence was noted in cold hardiness studies with *Panicum virgatum* L. (switchgrass) (Hope and McElroy, 1990). Plants freeze-tested during the cold-hardening period in October and November failed to initiate new vegetative

growth when placed in a greenhouse, unless dormancy had been broken by growing them at 30C. Harris (1973) blamed poor regrowth in *Fragaria virginiana* Duch. (strawberry) after a late October freeze test on dormancy and an unsatisfied cold requirement.

Regrowth quality ratings were significantly correlated with crown and tuberous root EL measurements, especially for 'Autumn Joy' (Table 3). The correlation between crown and tuberous root EL measurements for 'Autumn Joy' and 'Brilliant' indicates either tissue would be suitable for estimating viability and survival potential of plants exposed to freezing temperatures. Because of these similar trends, tuberous root EL data are not presented.

Except on the December test date, treatment temperatures resulting in 'Autumn Joy' crown EL $\geq 50\%$ closely matched killing temperatures determined by survival percentages (Table 1). This relationship held for 'Brilliant' at the September and October test dates, but was not as well-defined in November, December, and January (Table 2). Tissue viability determinations using EL data are relatively easy to interpret during the early stages of acclimation, but identifying a single lethal temperature or range of lethal temperatures becomes increasingly difficult as plants approach maximum winter hardiness.

Decreases in plant water content have been correlated with increasing freeze tolerance in small grains (Brule-Babel and Fowler, 1988; Metcalf et al., 1970; Nass, 1983; Siminovitch and Cloutier, 1982; Tyler et al., 1981; Willemot and Pelletier, 1979), *Medicago sativa* L. (alfalfa) (Stout, 1980), turfgrass (Gusta et al., 1980), and woody plants (Levitt, 1980). Percent crown moisture declined by 7% for 'Autumn Joy' and 4% for 'Brilliant' between September and October, but sampling in subsequent months revealed slight increases in moisture content. 'Autumn Joy' crowns were relatively drier than 'Brilliant' crowns from October through January, offering a possible explanation for its slightly greater cold hardiness.

Regrowth measurements and EL tests are useful assays for determining cold hardiness levels after freezing tests, but regrowth data are difficult to interpret when plants are in early stages of acclimation. In our study, after plants had a chilling requirement satisfied (December test date), regrowth measurements were effective in identifying injurious low temperatures. The EL test was fairly accurate in predicting killing temperatures for 'Autumn Joy' but was less reliable for 'Brilliant' and, therefore, will require further refinement before it can be used as the sole indicator of killing temperatures for herbaceous perennial plant tissues. Results from EL tests are quantitative, can be obtained in ≈ 48 h from time of freezing event, and preclude the need for potting materials and greenhouse space. A major disadvantage is the intensive labor required for preparing crown samples for EL determinations. These difficulties could be circumvented if tuberous root tissue were used to estimate whole-plant injury.

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Table 3. Coefficients of correlation among quality rating, crown electrolyte leakage (EL) measurements, and tuberous root EL measurements for *Sedum* 'Autumn Joy' and 'Brilliant'.

Criterion and Cultivar	Crown EL	Tuber EL
Quality rating		
Autumn Joy	-0.71***	-0.73***
Brilliant	-0.67***	-0.63***
Crown EL		
Autumn Joy	---	0.85***
Brilliant	---	0.82***

***Significant at $P \leq 0.001$.

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