

Drought Response of Three Ornamental Herbaceous Perennials

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ABSTRACT. *Boltonia asteroides* L. ‘Snowbank’ (Snowbank boltonia), *Eupatorium rugosum* L. (eastern white snakeroot), and *Rudbeckia triloba* L. (three-lobed coneflower) were subjected to drought for 2, 4, and 6 days during the fall and spring. Leaf gas exchange, leaf water potential, growth, and carbohydrate partitioning were measured during drought and throughout the following growing season. Leaf gas exchange of *B. asteroides* was not affected by drought treatment in the fall, not until day 6 of spring drought, and there were no long-term effects on growth. Transpiration and stomatal conductance of *R. triloba* decreased when substrate moisture decreased to 21% after drought treatment during both seasons. Assimilation of drought-treated *R. triloba* decreased when substrate moisture content decreased to 12% during spring but was not affected by drought in the fall. There was a decrease in the root-to-shoot ratio of *R. triloba* that had been treated for 4 days, which was attributed to an increase in the shoot dry weight (DW) of treated plants. Reductions in spring growth of *E. rugosum* were observed only after fall drought of 6 days, and there were no differences in final DWs of plants subjected to any of the drought durations. Spring drought had no effect on growth index or DW of any of the perennials. *Boltonia asteroides* and *R. triloba* had increases in low-molecular-weight sugars on day 4 of drought, but *E. rugosum* did not have an increase in sugars of low molecular weight until day 6 of drought. Differences in drought response of *B. asteroides*, *E. rugosum*, and *R. triloba* were attributed to differences in water use rates.

During drought stress plants typically minimize water loss. Some methods of water conservation include decreases in stomatal conductance (Farquhar and Sharkey, 1982; Raschke, 1979), differential growth of roots, shoots, and leaves (Finn and Bunn, 1980; Khalil and Grace, 1992; Palta and Gregory, 1997; Sharp and Davies, 1989), and changes in carbohydrate partitioning (Morgan, 1984; Santamaria et al., 1991). Reduced stomatal conductance may lead to reduced photosynthesis (Fischer et al., 1986; Freeden et al., 1991; Kanechi et al., 1996).

Conversion of sucrose to glucose and fructose may facilitate osmotic adjustment in the leaves of plants under water stress (Morgan, 1984; Santamaria et al., 1991). Osmotic adjustment results in cell turgor maintenance necessary for cell expansion (Boyer, 1988), maintenance of stomatal conductance for gas exchange (Davies and Lakso, 1978; Fernandez and McCree, 1991; McCree and Richardson, 1987), and protection of the photosynthetic apparatus from photoinhibition (Downton, 1983).

We sought to investigate drought responses of *Boltonia asteroides* ‘Snowbank’ (Snowbank boltonia), *Eupatorium rugosum* (eastern white snakeroot), and *Rudbeckia triloba* (three-lobed coneflower) which are popular species of ornamental perennials grown commercially in nurseries. *Boltonia decurrens* is moderately drought tolerant (Smith et al., 1993), but drought tolerance of *B. asteroides* ‘Snowbank’ is unknown. *Eupatorium rugosum* is native to the eastern United States and performs best in well-drained soils (Armitage, 1997). *Rudbeckia triloba* is native to North America. There is no definitive report of drought tolerance of *R. triloba*, although *R. hirta* (L.) is reportedly drought tolerant (Moore, 1996).

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All three taxa studied are members of the Asteraceae, which include species that produce fructans (Edelman and Jefford, 1968). The first objective of this study was to determine leaf gas exchange and growth of *B. asteroides* ‘Snowbank’, *E. rugosum*, and *R. triloba* during drought imposed for 2, 4, or 6 d, and through recovery. The second objective was to determine the role of carbohydrate partitioning in maintenance of leaf gas exchange and growth.

Materials and Methods

PLANT MATERIAL. Plugs (2.54 cm²) of *E. rugosum* and *R. triloba*, from a commercial nursery were potted into 1.9 L nursery containers on 30 May 1997 and *B. asteroides* ‘Snowbank’ on 11 June 1997, filled with a substrate of 4 composted pine bark: 1 river sand (v/v) amended with 6 kg·m⁻³ pelletized dolomitic limestone. Plants were liquid fertilized (20N–8.6P–16.6K, Southern Agr. Chemicals, Hendersonville, N.C.) with N at 150 mg·L⁻¹ N at transplant and 1 week before start of treatment. On 16 June 1997, 9 g/container of a slow-release fertilizer (Osmocote 14N–4.2P–11.6K, Scotts-Sierra, Marysville, Ohio) was top dressed to each container. All plants were grown on a gravel nursery area outdoors under natural photoperiod and irradiance at the South Carolina Botanical Garden, Clemson, S.C. Plants were irrigated twice daily to container capacity with overhead sprinkler irrigation of tap water until drought was imposed. Plants were subjected to drought at two times during the growing season: fall to determine if this would affect growth the following spring, and spring to measure effects through the summer.

Plants were placed in a completely randomized design 12 Sept. 1997 for the fall and 1 May 1998 for the spring drought treatments. There were eight replications for all four treatments: 2, 4, or 6-d drought periods and controls for each of the durations for all taxa for a total of 144 plants. Fall drought was imposed 18 Sept. 1997 (day 0) and spring treatment 8 May 1998 (day 0) by withholding water from the containers. Portable tents were constructed by using 0.03-mm-thick white polyethylene over a 13-mm-diameter aluminum conduit frame with a height of 1 m, width of 2 m, and length of 3 m. Tents were placed over the plants at dusk and were left on until

0745 HR to exclude precipitation. Tents also were placed over the plants when there was threat of precipitation during other times. Control plants were irrigated with tap water by hand until runoff twice daily at 0800 and 1500 HR. All treated and control plants for each duration were planted in the field in a completely randomized design the day following the end of each drought duration. Field soil was a Cecil sandy loam. Control plants were planted at each field planting to allow assessment of differences in leaf gas exchange as a result of transplant stress. An additional eight replicates of each treatment (144 plants) were grown in the fall for use in destructive analysis of carbohydrates. Plants grown for the spring study were overwintered in their containers on the nursery area and covered for winter protection. Carbohydrate analysis was not repeated in the spring drought study.

WATER MEASUREMENTS. Moisture content of the upper 6 cm of substrate was randomly measured daily at 1000 HR during drought periods by using a Theta Probe type HH1 measurement device and a Theta Probe type ML1 (Delta-T Devices, Cambridge, U.K.) set on the organic soil setting. Predawn leaf water potential (Ψ_1) of every plant, sampled in random order, was measured on an upper fully expanded leaf by using a pressure bomb (Soilmoisture Equipment Corp, Santa Barbara, Calif.) on days 2, 4, and 6 at 0200 HR. Measurements could not be made on day 2 of spring drought because of rain.

LEAF GAS EXCHANGE. Abaxial carbon dioxide assimilation (A), stomatal conductance (g_s), and transpiration (E) were randomly measured daily between 1000 and 1350 HR during drought periods and through recovery with an infrared gas analysis system (CIRAS-1, PP Systems, Haverhill, Mass.) equipped with a Parkinson broad leaf chamber (model PLC-B; PP Systems). Measurements were taken on the youngest fully expanded leaf of every plant, and plants were assessed in a completely random order. When most leaves of *E. rugosum* had abscised, measurements were taken on the healthiest remaining leaf. Stomatal conductance could not be determined for *B. asteroides* because the leaves did not fill the leaf chamber.

GROWTH. Growth index [(height (cm) + width (cm))/2] was measured every other day during fall and spring droughts, and then weekly after transplanting during the fall and monthly during the spring and summer. Growth index of fall-treated plants was measured until a killing frost on 3 Nov. 1997. Measurements resumed when new growth started 30 Apr. 1998. Plants were harvested for biomass determination after they had reached full bloom. Fall-treated *E. rugosum* were harvested 7 Sept. 1998, and *R. triloba* were harvested 4 Aug. 1998. Fall-treated *B. asteroides* were not harvested because there were <50% of the plants still living after they flowered. Roots were harvested by removing a 20-cm³ volume of soil from beneath the center of the plant with a shovel. Roots and shoots were placed in a drying oven at 60 °C for 7 d.

CARBOHYDRATE ANALYSIS. Three mature leaves were harvested from each plant of the eight replicates per treatment on days 0 and 4 of the fall drought. Leaves were immersed in liquid nitrogen and then stored at -80 °C until lyophilization. Crown samples were taken at 1130 HR on day 0 of drought, and days of planting in the field (days 3, 5, and 7). About 5 g of tissue were cut from the crown, rinsed with water, immersed in liquid nitrogen, and stored at -80 °C until lyophilization. Additional crown tissue samples were taken in midwinter on 30 Jan. 1998 and soon after plants started regrowth, on 5 May 1998.

Carbohydrates were extracted following the method described in Ranwala and Miller (1998) with modifications: samples were boiled at 100 °C for 15 min to denature enzymes and then placed in a hot water bath at 70 °C for 30 min.

To determine the amount of low molecular weight sugars partitioned into fructans, the fructose, glucose, and sucrose values before acid hydrolysis were subtracted from the fructose and glucose values after acid hydrolysis. The result was the glucose and fructose amounts that were in all fructans. A preliminary experiment showed that the leaf tissue contained only minimal amounts of fructan, so acid hydrolysis was not conducted on leaf tissue. The crown tissue extracts were adjusted to a final volume of 10 mL by using high-performance liquid chromatography (HPLC) water at pH 8.0, 2 mL was removed and pipetted into a 15-mL glass test tube, to which 1 mL 0.2 mol·L⁻¹ H₂SO₄ was added. The test tubes were then placed in a water bath at 100 °C for 30 min. The test tubes were removed, and 375 μL of 2 mol·L⁻¹ NaOH was added to each sample to neutralize pH. The natural log of the peak area of each of the fructans was used to reduce variability and to facilitate separation of means of fructan amounts as described in Legnani (1998).

Substrate moisture, predawn Ψ_1 and dry weight (DW) data were analyzed using Tukey's Studentized range test as appropriate after analysis of variance (ANOVA). Growth index, g_s, E, A, and carbohydrate data were analyzed using repeated measures in time, with eight samples per species per treatment per day. Comparisons were made within taxa between the drought and control treatments.

Results and Discussion

Substrate moisture content of treated plants was less than that of controls 2 d after fall drought (DAFD) for all three taxa during the fall treatment (Fig. 1A). Treated *E. rugosum* had a lower substrate moisture content than treated *B. asteroides* or *R. triloba* 2 DAFD through 4 DAFD, indicating that *E. rugosum* developed more intensive stress than the other two taxa. During the spring, substrate moisture of treated plants was less than that of controls for all taxa after 3 d (Fig. 1B).

During the fall drought, there was a difference in predawn Ψ_1 between controls and treated *E. rugosum* 2 DAFD, but not until 4 DAFD for *B. asteroides* and *R. triloba* (Table 1). There was no decrease in predawn Ψ_1 of *B. aster-*

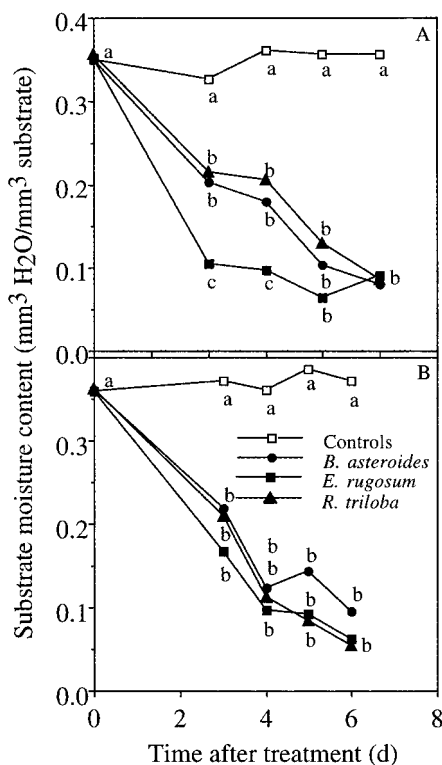


Fig. 1. Substrate moisture content (mm³ H₂O/mm³ substrate) of control (average of controls for all three taxa) (□); *Boltonia asteroides* 'Snowbank' treated (●); *Eupatorium rugosum* treated (■); *Rudbeckia triloba* treated (▲) for (A) fall drought, beginning 19 Sept. 1997 and (B) spring drought, beginning 8 May 1998. Legend in (B) applies to both figures. Comparisons are between the two treatments of all taxa. Means followed by different letters are significantly different at $P \leq 0.05$ by Tukey's Studentized range test. Each point is a mean of eight replicates for controls and treated plants.

Table 1. Predawn leaf water potential (MPa) for *Boltonia asteroides* 'Snowbank', *Eupatorium rugosum*, and *Rudbeckia triloba*, measured at 0200 HR during fall (19 Sept. 1997, day 0) and spring (8 May 1998, day 0) drought periods.

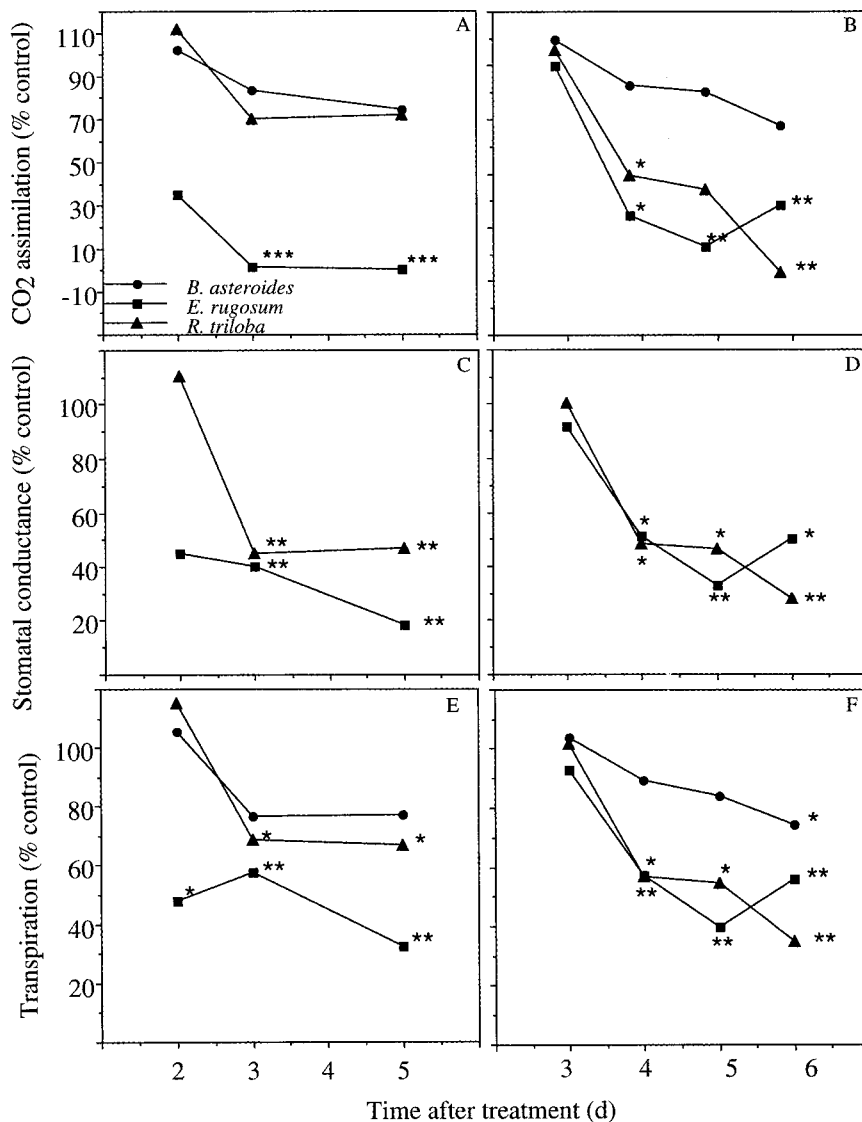
Treatment	Time after fall drought (d)			Time after spring drought (d)	
	2	4	6	4	6
<i>B. asteroides</i>					
Control	-0.1 a ²	-0.4 a	-0.4 a	-0.4 a	-0.5 a
Drought	-0.2 a	-1.1 b	-1.3 b	-0.5 a	-0.5 a
<i>E. rugosum</i>					
Control	-0.1 a	-0.4 a	-0.3 a	-0.3 a	-0.3 a
Drought	-1.2 b	-2.5 b	-2.1 b	-0.7 b	-1.0 b
<i>R. triloba</i>					
Control	-0.2 a	-0.5 a	-0.3 a	-0.3 a	-0.3 a
Drought	-0.2 a	-1.3 b	-2.0 b	-1.0 b	-1.1 b

²Comparisons are between plants in the drought and control treatments within species for each day. Values are means of eight replications. Means separation by Tukey's studentized range test, $P \leq 0.05$.

oides during the spring drought compared to control (Table 1). There were differences in predawn Ψ_1 of *E. rugosum* and *R. triloba* by 4 d after spring drought (DASD) (Table 1). During water stress, many physiological functions are affected before the leaves show signs of wilting, with cell growth being the most sensitive (Boyer, 1988; Boyer et al., 1985; Hsiao, 1973). A decrease in turgor of enlarging cells is thought to be the cause of reduced growth (Boyer et al., 1985; Green et al., 1971; Mayssek et al., 1988). Predawn Ψ_1 has been shown effective as a measure of the maximum soil water potential available to roots (Tardieu and Simonneau, 1998). Predawn Ψ_1 of *E. rugosum* and *R. triloba* were reduced by a greater amount than in *B. asteroides* indicating that *B. asteroides* had a lower water use rate.

None of the drought durations during the fall (Fig. 2A) or spring (Fig. 2B) affected A of *B. asteroides*. Effects of drought were more pronounced on g_s than on A for *R. triloba* during the fall as found for other species (Freeden et al., 1991; Herde et al., 1997). Stomatal conductance of *R. triloba* was reduced 51% 6 DAFD compared to control (Fig. 2C), but A was reduced by only 25% 6 DAFD (Fig. 2A). During the spring drought, g_s and A of *R. triloba* were affected similarly, each reduced by 75% 6 DASD compared to control (Fig. 2). During the fall drought, g_s of *E. rugosum* was reduced to 23% of that of the control 6 DAFD, but A was reduced by 100% 3 DAFD (Fig. 2A and C). Assimilation of fall-treated *E. rugosum* decreased to less than zero 3 DAFD (Fig. 2A), showing that the plants were respiring CO_2 . Because g_s was still occurring, CO_2 was still available for fixation, suggesting that the limitation to A was nonstomatal. As a result of the spring drought, however, g_s and A of *E. rugosum* decreased the same degree suggesting that limitations of A in the spring were due to decreased g_s . Although the exact mechanisms of the control of stomata over E and A are still unknown (Jarvis and Davies, 1998; Jones, 1998), there was a close relationship between E and g_s of *E. rugosum* and *R. triloba* during drought treatment (Fig. 2C–F). Transpiration of treated *E. rugosum* decreased 1 d sooner than g_s during the fall and on the same

Fig. 2. CO_2 assimilation rate, stomatal conductance, and transpiration, during fall drought, beginning 19 Sept. 1997 (A, C, and E respectively) and during spring drought, beginning 8 May 1998 (B, D, and F, respectively), for *Boltonia asteroides* 'Snowbank' (●), *Eupatorium rugosum* (■), and *Rudbeckia triloba* (▲). Legend in (A) applies to all figures. Means ($n=8$) for a particular taxon are for the drought treatment expressed as a percent of control for that taxon. ****Significantly different means from controls within days at $P \leq 0.05, 0.01, \text{ or } 0.001$, respectively. Values are means of eight replicates. Mean separation by repeated measures in time by ANOVA.



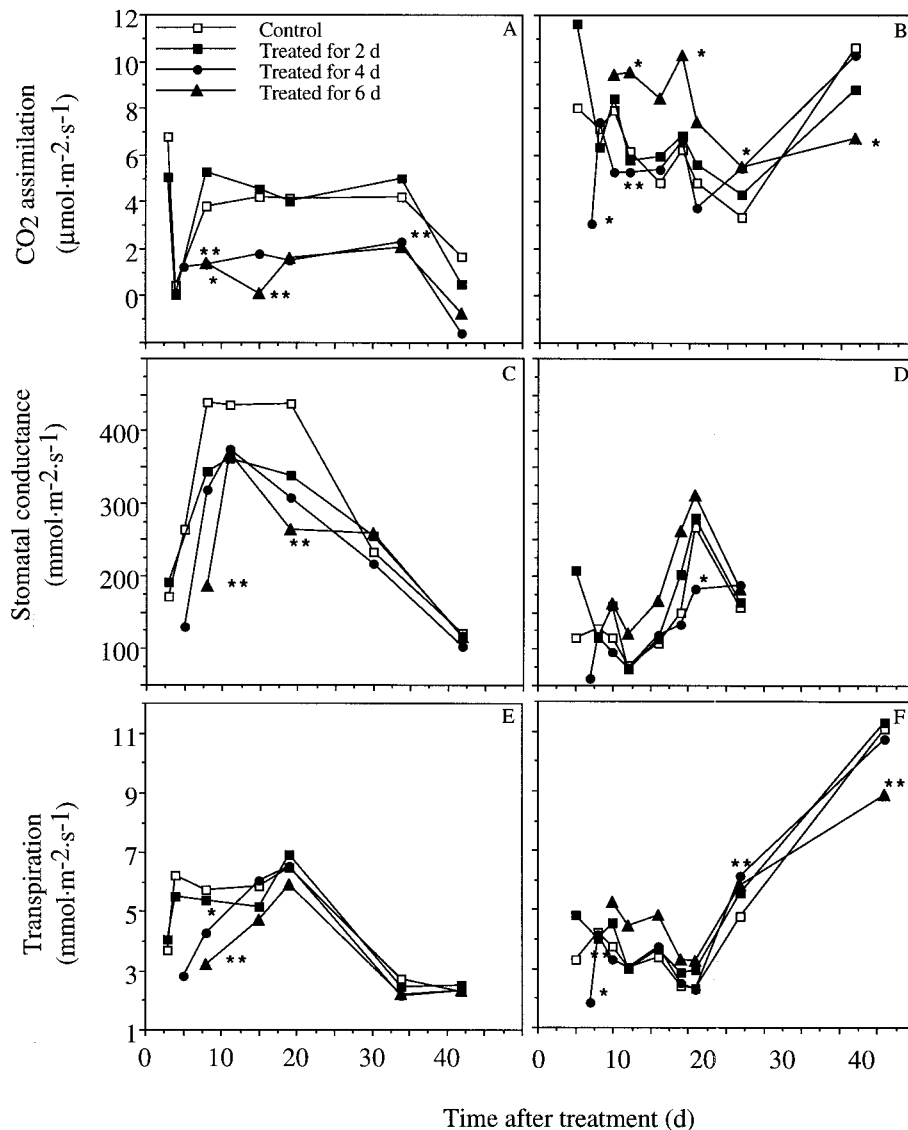


Fig. 3. CO₂ assimilation rate, stomatal conductance, and transpiration of *Eupatorium rugosum* during recovery from fall drought (19 Sept. 1997, day 0) (A, C, and E, respectively), and during recovery from spring drought (8 May 1998, day 0) (B, D, and F, respectively). Legend in (A) applies to all figures. **Significantly different means from controls within days at $P \leq 0.05$ or 0.001, respectively. Values are means of eight replications. Mean separation by repeated measures in time by ANOVA.

day for *E. rugosum* and *R. triloba* during the spring and *R. triloba* during the fall (Fig. 2C–F). There was a reduction in g_s and E of *E. rugosum* when substrate moisture content was 10%, and there may have been a reduction sooner, but leaf gas exchange was not measured until 2 DAFD. *Rudbeckia triloba* had a decrease in g_s when substrate moisture content was 21%. Average values of A for control plants of *B. asteroides*, *E. rugosum*, and *R. triloba* were 14, 6, and 9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during the fall and 20, 8, and 15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the spring, respectively. Average values of g_s for control plants of *E. rugosum* and *R. triloba* were 300 and 375 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during the fall, and 115 and 250 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the spring, respectively. Average values of E for control plants of *B. asteroides*, *E. rugosum*, and *R. triloba* were 8, 5, and 7 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during the fall and 10, 4, and 6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the spring, respectively.

Assimilation, g_s , and E of fall- and spring-treated *B. asteroides*

and *R. triloba* returned to levels of controls the day following rewatering (data not presented). Assimilation of 2-d fall and spring treated *E. rugosum* returned to levels of controls the day following rewatering (Fig. 3A and B). *Eupatorium rugosum* treated for 4 d in fall and spring had lower A until 8 DAFD, at which time it returned to levels of controls (Fig. 3A and B). Unlike A of *E. rugosum* treated for 6 d in the fall, which was less than A of controls for several days during the fall, *E. rugosum* treated for 6 d in the spring was greater than A of the controls on several days (Fig. 3A and B). Stomatal conductance of *E. rugosum* treated for 2 and 4 d in the fall and spring returned to levels of controls the day following rewatering (Fig. 3C and D), however g_s of *E. rugosum* treated for 6 d in the fall was less than g_s of controls for only 2 d after rewatering. After rewatering, E of fall-treated *E. rugosum* returned to levels of controls by 15 DAFD for all treatment periods (Fig. 3E). During recovery from spring drought, E of all drought periods of *E. rugosum* returned to levels of controls by 10 DAFD (Fig. 3F), however, E values of all drought periods were greater than E of controls 25 DAFD.

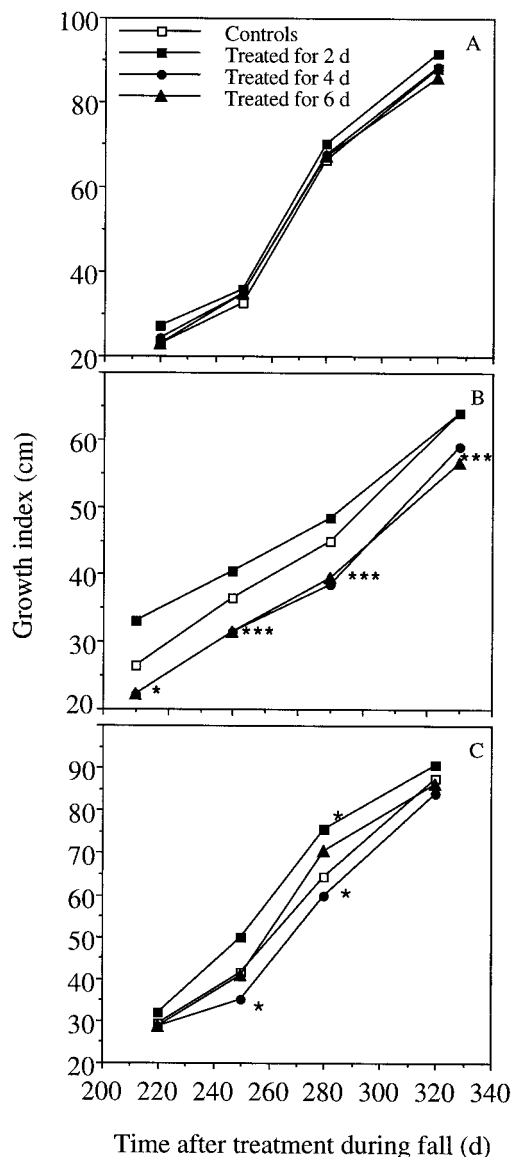
Neither fall nor spring drought affected growth index of *B. asteroides* (Fig. 4A). Growth index of fall-treated *E. rugosum* was not different from that of the controls. Growth index of *E. rugosum* treated for 4 and 6 d in the fall was less than the growth index of controls in measurements taken up to 320 DAFD (Fig. 4B). Growth index of *E. rugosum* had returned to levels of controls by the date of harvest 355 DAFD. Spring drought had no effect on the growth index of *E. rugosum*. Growth index of *R. triloba* treated for 2 d in the fall was greater than growth index of

controls 280 DAFD, but returned to nontreated levels by 320 DAFD (Fig. 4C). Growth index of *R. triloba* treated for 4 d in the fall was less than growth index of the controls from 250 DAFD through 280 DAFD, but had returned to levels of controls by 320 DAFD (Fig. 4C). The growth index of *R. triloba* treated for 6 d in the fall was not different from growth index of the controls (Fig. 4C). The root-to-shoot ratio of *R. triloba* treated for 4 d in the fall was 0.98 compared to 1.8 for the control at harvest. The decrease in the root-to-shoot ratio of treated *R. triloba* was attributed to an increase in shoot DW from 200 g for the control to 290 g for the treated. There was no difference in the root-to-shoot ratio of *R. triloba* treated for 6 d in the fall compared to the control, which may have been due to higher root turnover during the longer stress (Gregory and Atwell, 1991; Smucker, 1984, 1993). Water stress decreases shoot growth more than root growth in many plant species, resulting in a lower shoot-to-root ratio (Finn and Bunn, 1980; Khalil and Grace, 1992; Palta and Gregory, 1997; Sharp and Davies, 1989).

Differences in the ability of species to adjust osmotically during drought stress influence the ability of plants to tolerate drought (Chapman and Augé, 1994; Nguyen et al., 1997; Premachandra et al., 1995). *Helianthus angustifolius* L.) (swamp sunflower) was found to be less drought tolerant than *Rudbeckia fulgida* var.

Fig. 4. Spring growth index ((height + width)/2) (cm) of fall-treated (A) *Boltonia asteroides* 'Snowbank', (B) *Eupatorium rugosum*, and (C) *Rudbeckia triloba*. Legend in (A) applies to all figures. Measurements began on 15 Apr. 1998. ***,**Significantly different means from control within days at $P \leq 0.05$ or 0.001, respectively. Values are means of eight replications. Mean separation by pairwise t test.

Sullivanti (Beadle & Boynt.) Cronq (black-eyed susan), and these differences were attributed to osmotic adjustment (Chapman and Augé, 1994). A study with *H. annuus* L. (sunflower) showed that, during drought stress, starch was hydrolyzed and sucrose concentration increased (Freeden et al., 1991). In *Phaseolis vulgaris* L. 'Tacariqua' (Tacariqua bean) plants under drought stress, the starch/sucrose ratio decreased and the glucose/fructose ratio increased (Castrillo, 1992). There were increased activities of sucrose invertase, sucrose synthase, and starch-hydrolyzing enzymes (amylases) in *Cajanus cajan* L. (pigeonpea) plants under drought stress (Keller and Ludlow, 1993). *Boltonia asteroides* leaves had a higher concentration of glucose, sucrose, and fructose after treatment for 4 d (Table 2). This change in carbohydrate partitioning apparently was not a result of the breakdown of sucrose, however,



because the sucrose level increased as well. Because there were no fructans present in the leaves of *B. asteroides*, the build up of sucrose in the leaves may have been a result of decreased transport of sucrose out of the leaves. Sucrose becomes concentrated in leaves during drought due to reduced sucrose export from the leaves, which is thought to be due to reduced export of protons that are the cotransporters of sucrose in the phloem (Malek and Baker, 1978). In plants of other species, accumulation of photosynthates in the leaves during drought (Ackerson, 1980; Azcon-Bieto, 1983) has acted as a feedback-inhibition mechanism (Janoudi et al., 1993) to *A. Eupatorium rugosum* had the most stable carbohydrate partitioning in the leaves compared to the other taxa, having only an increase in fructose content (Table 2). Glucose and sucrose contents in the leaves of treated *R. triloba* were greater than the content of the controls 4 DAFD (Table 2).

Because there are no standards for fructans, concentration of each individual fructan could not be determined. Comparisons were made between the natural log of the peak areas per gram DW of the control and treated for each fructan. Although no attempts were made to isolate the longer-chain sugars, other workers (Chatterton et al., 1989; Legnani, 1998) have indicated that the peak with a retention time of ≈ 8 min is 1-kestose, a trisaccharide intermediate. Each subsequent peak increases the chain of the fructan by one fructose unit; for example, degree of polymerization 5 (DP 5) having one more fructose than DP 4.

Six days after fall drought, the peak area of sucrose in the crown tissue of treated *B. asteroides* was greater than that of the control

Table 2. Soluble sugars ($\text{mg}\cdot\text{g}^{-1}$ dry weight) in leaf tissue of *Boltonia asteroides* 'Snowbank', *Eupatorium rugosum*, and *Rudbeckia triloba*, on day 0 (19 Sept. 1997) and day 4 of drought during fall.

Treatment	Sucrose		Glucose		Fructose	
	Time after fall drought (d)					
	0	4	0	4	0	4
<i>B. asteroides</i>						
Control	12	12	2	4	1	4
Drought	12	28	2	14	1	11
Significance	---	***	---	***	---	***
<i>E. rugosum</i>						
Control	16	16	8	9	12	12
Drought	16	18	8	10	12	22
Significance	---	NS	---	NS	---	*
<i>R. triloba</i>						
Control	8	10	1	2	1	4
Drought	8	24	1	4	1	6
Significance	---	***	---	*	---	NS

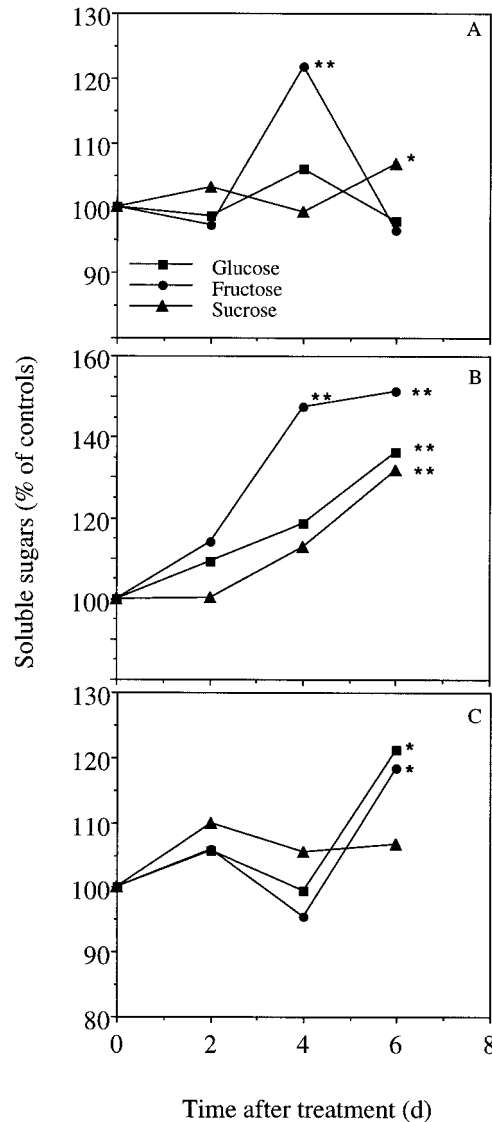
^zThe same tissue was used for the two treatments on day 0.

NS,*,*** Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively. Comparisons are between the treatments within species for each day. Values are means of eight replications. Mean separation by pairwise t test.

Fig. 5. Soluble sugars in the crown tissue of (A) *Boltonia asteroides* 'Snowbank', (B) *Eupatorium rugosum* and (C) *Rudbeckia triloba* during a fall drought as a percentage of control. Treatment began 19 Sept. 1997. Comparisons are between the treated and controls within the same taxon. ***Significantly different means from controls within days at $P \leq 0.05$ or 0.01, respectively. Values are means of eight replications. Mean separation by repeated measures in time by ANOVA.

(Fig. 5A). Fructose of treated *E. rugosum* was greater than that of the control 4 DAFD, and sucrose and glucose were greater than the respective control values 6 DAFD in treated *E. rugosum* (Fig. 5B). On day 6 of drought, fructose and glucose in treated *R. triloba* were greater than those of the control (Fig. 5C). There were no differences in glucose, fructose, or sucrose in crown tissue between treated and control plants for all three species in samples taken 30 Jan. 1997 and 5 May 1998 (data not presented).

There was no difference in the total fructan content or average fructan length between treated and control *B. asteroides* at any measurement date (Table 3). The amount of glucose and fructose attributed to fructans in the crown tissue of *E. rugosum* was less than that of the control starting 4 DAFD. The average fructan length of *E. rugosum* treated for 6 d was DP 3 compared to DP 7 for control plants.



There were no differences in the average fructan length in the crown tissue between control and treated *R. triloba* during drought. The total amount of sugars attributed to fructans in the crown tissue of treated *R. triloba* was greater than that of the control 2 DAFD (Table 3). Total fructan content in crown tissue of *R. triloba* treated for 2 d was 250% of control 120 DAFD and 300% of control 210 DAFD (data not presented). Possibly, as a result of the greater availability of carbohydrates for growth, the growth index of these plants was greater than that of the control throughout the spring but returned to control levels by the beginning of the summer.

On day 6 of drought, 1-kestose and DP 5 content of *B. asteroides* subjected to drought were greater than those of the control (Table 4). Correspondingly, there was a decrease in DP 22, 25, and 26 content in treated *B. asteroides* compared to the control 6 DAFD (Table 4). A similar trend was observed in *E. rugosum* (Table 4). Although treated *E. rugosum* had a more pronounced change in carbohydrate partitioning in crown tissue compared to leaf tissue, the other taxa had changes in carbohydrate partitioning 2 d sooner. Treated *R. triloba* showed little change in carbohydrate partitioning in the leaves, however, there was a more pronounced change in carbohydrate partitioning in the crown tissue. Treated *R. triloba* had an increase in the lower-molecular-weight sugars in crown tissue sooner than the other two taxa. On day 2 of drought, 1-kestose and DP 4 and 5 content had increased in treated *R. triloba*, with DP 5 returning to control levels by day 6 (Table 4), possibly as a result of increased transport into the root tissue. Control values for 1-kestose, DP 4-6, and DP 22-26 for days 0, 2, 4, and 6 of treatment for all taxa are presented in Table 5.

Changes in the amount of a particular fructan can be a result of synthesis of fructans or depoly-

Table 3. Amount of glucose and fructose in fructans ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in crown tissue of *Boltonia asteroides* 'Snowbank', *Eupatorium rugosum*, and *Rudbeckia triloba*.

Treatment	Glucose			Fructose		
	Time after fall drought (d)					
	2	4	6	2	4	6
<i>B. asteroides</i>						
Control	48	80	44	143	221	190
Drought	41	54	49	98	171	192
Significance	NS	NS	NS	NS	NS	NS
<i>E. rugosum</i>						
Control	14	47	20	91	140	156
Drought	33	7	25	95	39	43
Significance	NS	**	NS	NS	**	**
<i>R. triloba</i>						
Control	26	9	22	76	87	102
Drought	60	13	26	244	84	97
Significance	**	NS	NS	**	NS	NS

NS, ** Nonsignificant or significant at $P \leq 0.01$, respectively. Comparisons are between the two treatments within species for each day. Values are means of eight replications. Mean separation by repeated measures in time by ANOVA.

Table 4. Low and high degree of polymerization (DP) of fructans in the crown tissue of *Boltonia asteroides* 'Snowbank', *Eupatorium rugosum*, and *Rudbeckia triloba* during fall drought shown as a percentage of control.

Fructan	<i>B. asteroides</i>				<i>E. rugosum</i>				<i>R. triloba</i>			
	Time after fall drought (d)											
	0	2	4	6	0	2	4	6	0	2	4	6
1-kestose	100 ^z	95	104	111	100	100	109	137*	100	117**	114	119**
DP 4	100	95	102	113*	100	115	114	142*	100	117**	110**	113*
DP 5	100	112	93	115*	100	115	116	154*	100	114*	111	107
DP 6	100	95	99	106	100	115	108	156*	100	112	116*	108
DP 22	100	123	80*	84	100	96	93	76***	100	64	86	111
DP 23	100	114	60*	82	100	96	92	85*	100	68	88	102
DP 24	100	128	75	81	100	95	92	84	100	68	86	103
DP 25	100	119	64*	81	100	96	90	78**	100	65	72	96
DP 26	100	164*	67	76	100	97	90	77**	100	76	90	111

^zComparisons are between the treated and controls within the same taxon.

*,**,***Significantly different means from controls within days at $P \leq 0.05$, 0.01, or 0.001, respectively. Values are means of eight replications. Mean separation by repeated measures in time by ANOVA.

merization of longer-chain fructans (Bielecki, 1993; van den Ende and van Laere, 1996). All taxa in this study had an increase in 1-kestose and the lower-order DP fructans as a result of drought and a corresponding decrease in the highest DP fructans (Table 4). Analysis of the intermediate DP fructans showed no difference in quantities (data not presented). It is apparent that the longest-chain fructans are the ones that depolymerize in response to drought.

Depolymerization of fructans results in shorter-chain fructans and free fructose units, which can contribute to osmotic adjustment (Bielecki, 1993; Hendry, 1993; Pilon-Smits et al., 1995). Following drought stress, growth rate and fresh and DWs were higher in transgenic, fructan-accumulating *Nicotiana tabacum* L. (tobacco) plants than in wild-type plants (Pilon-Smits et al., 1995). The presence of fructans in many species from arid regions and regions of intense cold suggests they may play a role in helping plants survive environmental stresses (Hendry, 1993). The presence of fructans does not guarantee greater drought tolerance. All taxa in this study contained fructans, but only *B. asteroides* and *R. triloba* had rapid changes in carbohydrate partitioning during drought and were able to maintain photosynthesis and growth.

Boltonia asteroides maintained photosynthesis during treatment, and there were no differences in growth index as a result of fall or spring drought. Maintenance of photosynthesis was attributed to

an increase in low-molecular-weight sugars in the leaves, perhaps enabling *B. asteroides* to maintain turgor to greater soil water deficits than the other two taxa. Maintenance of photosynthesis provides essential carbohydrates for growth, and there was no decrease in growth index of treated *B. asteroides* compared to the controls.

Rudbeckia triloba had an increase in sugars of lower molecular weight in leaf and crown tissue in response to drought, which may have provided more available carbohydrates for growth the following spring, when we found a high growth index. *Eupatorium rugosum* had slower changes in carbohydrate partitioning than *B. asteroides* and *R. triloba*. The severe water loss from *E. rugosum* during drought could be attributed to rapid reductions in soil moisture and lack of adjustments of carbohydrates in the leaves. The amount of water lost from *E. rugosum* caused long-term decreases in photosynthesis, resulting in reduced amounts of carbohydrates available for growth. Growth index of *E. rugosum* was decreased for several months as a result of drought. Many factors contribute to drought tolerance or lack of drought tolerance of plants. *Boltonia asteroides* was able to maintain photosynthesis and growth during drought periods that decreased growth of *R. triloba* and photosynthesis and growth of *E. rugosum*. Maintenance of photosynthesis and growth of *B. asteroides* may be the result of a relatively low rate

Table 5. Low and high degree of polymerization (DP) fructans in crown tissue of control plants of *Boltonia asteroides* 'Snowbank', *Eupatorium rugosum*, and *Rudbeckia triloba* on days 0 (19 Sept. 1997), 2, 4, and 6 of fall treatment.^z

Fructan	<i>B. asteroides</i>				<i>E. rugosum</i>				<i>R. triloba</i>			
	Fructans (ln peak area/g dry wt)											
	Time after fall drought (d)											
	0	2	4	6	0	2	4	6	0	2	4	6
1-kestose	125	110	122	128	106	106	106	93	104	96	105	110
DP 4	128	112	118	115	97	81	83	75	103	92	100	106
DP 5	124	113	120	112	99	73	83	64	103	95	97	107
DP 6	125	114	122	115	101	75	86	63	107	97	89	104
DP 22	84	75	90	83	72	83	84	83	74	81	77	74
DP 23	80	71	86	79	68	81	82	78	70	77	74	73
DP 24	69	61	77	69	64	78	78	78	65	74	71	67
DP 25	75	67	82	74	60	74	74	76	60	71	69	65
DP 26	64	57	71	64	56	70	70	72	55	64	61	57

^zValues represent means of eight controls for each taxon.

of water use by *B. asteroides*, which may be attributed to many things including smaller leaves and a less fibrous root system. Further study is needed to determine if, under the same drought intensity that *R. triloba* and *E. rugosum* experienced in this experiment, photosynthesis and growth of *B. asteroides* would also be decreased.

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