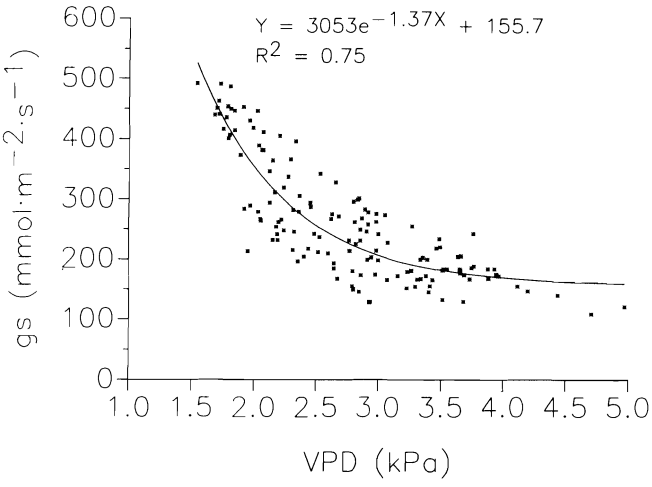

Fig. 3. Relationship of eastern redbud stomatal conductance to water vapor (gs) to leaf-to-air vapor pressure deficit (VPD). Measurements were performed during midday during 1987 and 1988. Treatments were initiated on June 17, 1987.

In conclusion, eastern redbud fully acclimated to 100% to 47% sun, and partially to 29% sun without detrimental effects on plant appearance. Our results are consistent with observations of horticulturists that state that eastern redbud grows equally well in full sun or shade (3, 11). There was no evidence that it prefers heavy shade here in the southern part of its range as has been suggested (12), at least under nonlimiting soil moisture. Also, because eastern redbud is seed propagated, there may be considerable intraspecific variation in its ability to acclimatize to sun and shade; thus ecotype differences must be considered.

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Asexual Propagation of *Shepherdia canadensis* and *S. rotundifolia*[1]

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Abstract

Shepherdia canadensis and *S. rotundifolia* were asexually propagated by hardwood cuttings and aseptic micropropagation. *S. canadensis* showed greatest rooting of 46.5% with 0.3% IBA. *S. rotundifolia* showed greater proliferation in woody plant media (WPM) with 0.89 μM BA and optimum rooting in WPM with 5.4 μM NAA.

Index Words. buffaloberries, Elaeagnaceae, *S. argentea*, russet buffaloberry, desert buffaloberry, IBA, BA, NAA

Significance to the Nursery Industry

Results from these experiments indicate that *S. canadensis* can be rooted from cuttings which might be an alternative to using seeds, especially given the low viability previously experienced by the authors. *S. rotundifolia*, on the other hand, can be successfully propagated in vitro. These asexual propagation methods would be valuable if a plant exhibiting unique characteristics was found. Cloning could reliably be accomplished by the procedures described.

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Introduction

The Shepherdia species, commonly known as buffaloberries, are members of the Oleaster family, Elaeagnaceae. The genus *Shepherdia* comprises 3 species, *S. argentea* (Pursh) Nutt, *S. canadensis* (L.) Nutt, and *S. rotundifolia* Parry. *S. canadensis*, russet buffaloberry, is a thornless small to medium sized deciduous shrub reaching up to 9 m (29.5 ft.) in height (4). An important characteristic of *S. canadensis* is its ability to form root nodules and fix atmospheric nitrogen (10). *S. rotundifolia*, desert buffaloberry, is a low growing, densely branched, evergreen shrub with silvery branches.

The buffaloberries are extremely hardy shrubs capable of surviving drought and harsh winters. Since these are native to Colorado and have adapted to the climatic and soil conditions of the state, they could be useful as water efficient plants in the landscape. They also have considerable potential for the revegetation of disturbed sites, roadsides, and shelterbelt (10), and have value for erosion control (3).

Most of the research on *S. canadensis* has been with seed germination with little on rooting of cuttings. Nothing has been reported on the propagation of *S. rotundifolia* (5). Tissue culture propagation has not been reported for either species of *Shepherdia*. Seeds of *Shepherdia* species have been reported to exhibit dormancies due to the presence of hard seed coat and/or dormant embryos (10). *S. canadensis* has shown erratic behavior in seed germination tests (7). In Canada, McTavish (7) obtained 50–60% germination by treating seeds for 5–7 minutes with sulfuric acid and stratifying for 30 days. No germination occurred when seeds were scarified in acid for 15 minutes with a 30 day cold stratification. However, Cram, as reported by McTavish (8), documented a germination of 89% using the identical treatment. Although these researchers found stratification necessary, Heit (2) reported a maximum germination of 78% by scarifying seeds for 30 minutes in sulfuric acid without stratification.

Propagation of *S. canadensis* by rooting of softwood and hardwood cuttings has had limited success (8). Fung (1) obtained the highest rooting of 4.7% when using semihardwood terminal cuttings treated with 0.8% IBA.

The objectives of this research were to assess asexual propagation methods for *S. canadensis* and *S. rotundifolia*.

Materials and Methods

Propagation of S. canadensis *by cuttings*. Hardwood cuttings were collected in May 1989 from the Lake Dillon rest area off I-70 in Summit county Colorado. These consisted of 2-year-old wood with 2.5–5.0 cm (1–2 in) of new growth. From these, 7–13 cm (2.7–5 in) cuttings were made. Leaves from the lower two-thirds of the stem were removed. The cuttings were dipped in a Benlate solution prepared by dissolving 0.5–1.0 gm of Benlate 50% WP in 1 liter of water. Cuttings were treated with one of 4 concentrations of IBA: 0 ppm (control), 1000 ppm, 3000 ppm, and 8000 ppm (w/v).

Approximately one-third of the basal portion of the cuttings were dipped into the IBA and placed in a 1:1 perlite:vermiculite medium (by volume) under intermittent mist set to spray water for 10 seconds every 3 minutes. There were 43 cuttings per treatment in a completely randomized design with each cutting considered as a replication for each treatment. Significant differences between treatments were determined using Duncan's new multiple range test.

In vitro shoot multiplication and rooting of S. rotundifolia. Shoot-tips, 0.5–1.0 cm (0.25–0.50 in) long, of *S. rotundifolia* were taken from greenhouse-grown seedlings. The mother plants were approximately 16 months from germination. Explants from the greenhouse were washed under running water for 2–4 hours. The outer scales were thoroughly removed. Tissue was then surface sterilized under the laminar flow hood by dipping in 70% ethanol for 30–60 seconds and placing them in a 20% chlorox with 0.1% Tween-80 solution for 10–12 minutes followed by three sterile distilled water rinses. For shoot multiplication, most of the leaves were removed and only the terminal buds were used for culture. The explants were initially placed in either basic Murashige and Skoog medium (MS-9) or woody plant medium (WPM-6) without hormones. The explants produced brown phenolic exudates within 24 hours in culture and had to be transferred to new media. Transfer procedures continued until no further exudates were observed (generally 4 subcultures). After 3–4 weeks in the basic media, the explants were transferred to one of the following four hormone media with 30 ml per baby food jar with 20 cultures per treatment; MS + 0.89 μM benzyl adenine (BA) + 0.04 μM IBA, MS + 0.89 μM BA, WPM + 0.89 μM BA + 0.04 μM IBA, or WPM + 0.89 μM BA. Observations of numbers of axillary shoots, leaf numbers and plant height were made at 7, 9, and 11 weeks.

The plantlets that developed were subdivided and maintained in WPM + 4.5 μM BA for about 3 months with transfers at 4–6 week intervals. These were then transferred to 8 different hormone media with 30 ml per vessel for rooting. Concentrations of NAA and IBA were added to the WPM as follows: 2.7 μM NAA, 5.4 μM NAA, 10.7 μM NAA, 21.5 μM NAA, 2.5 μM IBM, 4.9 μM IBA, 9.8 μM IBA, and 19.7 μM IBA with 20 cultures initially per treatment. Observations on percent rooting and approximate number of roots per rooted plantlet were taken at 8, 9, and 10 weeks in culture.

All data from both experiments were analyzed with a one-way analysis of variance with unequal replication (this resulted when contamination was high) with mean separation by Duncan's new multiple-range test at 5%.

Results and Discussion

Propagation of S. canadensis by cuttings. The 3000 ppm IBA resulted in the highest rooting (46.5%) of *S. canadensis* (Table 1). It was significantly different from the other treatments at $P = 0.05$. The addition of auxin increased rooting at 3000 ppm but inhibited rooting at 8000 ppm. Number of roots per cutting were not significantly different among the treatments. However, the 8000 ppm IBA showed a tendency to have greater numbers of roots. Average of root length was significantly greater in the control, 3.5 cm (1.5 in); however, a level of 3000 IBA showed a length of 1.0 cm (0.5 in).

A ten-fold increase in rooting of cuttings of *S. canadensis* was obtained in this research as compared to previous research. Perhaps even greater rooting may be obtained with further research on type of cuttings, type of rooting hormone, and proper rooting medium.

Table 1. Percent rooting, average number of roots, and average root length of *Shepherdia canadensis* propagated by cutting after 8 weeks under mist.

Treatment	Percent rooting[z]	Average number of roots	Average root length (cm)[z]
Control	14.0 bc[z]	4.3	3.5 a
0.1% IBA	6.8 c	6.3	0.8 b
0.3% IBA	46.5 a	6.8	1.0 b
0.8% IBA	27.9 b	10.8	1.1 b

[z]Means not followed by a common letter differ significantly from one another at the 0.05 level of significance.

In vitro shoot multiplication and rooting of S. rotundifolia. The WPM + 0.89 μM BA treatment tended to have the greatest number of axillary shoots and leaves for *S. rotundifolia* averaging 2.3 and 20.3 respectively (Table 2). The WPM also tended to be better than the MS media with the same growth regulator level. BA alone tended to give better shoot multiplication than using it in combination with IBA. Cultures were highly variable within each treatment and unequal replication (due to contamination) resulted in no significant difference according to the F test despite a ten-fold difference in numbers of axillary shoots between the MS + BA + IBA as compared to the WPM + BA.

The highest percentage of rooting (53.3%) was obtained with the 5.4 μM NAA treatment (Table 3). This treatment also produced the maximum number of roots, averaging about 7.8. Even though all the NAA treatments produced greater numbers of rooted plantlets than the IBA treatments, they also produced callus. Further research is advisable to eliminate callus formation during in vitro rooting with NAA. Again, high variability within treatments and unequal replications (due to contamination) resulted in no significant difference in number of roots despite a three-fold difference between 0.5 mg/l NAA and 4.0 mg/l IBA. These differences may also be related to the use of seedlings, which were genetically different, as an explant source.

Table 2. Average number of axillary shoots, average number of leaves, and average plant height of *Shepherdia rotundifolia* grown in vitro after 11 weeks in culture.

Treatments	No. of axillary shoots[z]	No. of leaves	Plant height (cm)
MS + BA + IBA	0.2	5.9	0.7
MS + BA	1.7	15.9	0.8
WPM + BA + IBA	1.7	15.9	0.6
WPM + BA	2.3	20.3	0.6

[z]Due to contamination problems there were unequal replications. This factor as well as a wide degree of variability were associated with a lack of statistical differences.

Table 3. Percent rooting and average number of roots (approximate) of *Shepherdia rotundifolia* rooted in vitro after 10 weeks.

Treatment	Percent rooting[z]	Number of roots
0.5 mg/l NAA	41.2 ab	6.0
1.0 mg/l NAA	53.3 a	7.8
2.0 mg/l NAA	38.9 abc	5.7
4.0 mg/l NAA	35.3 abc	3.7
0.5 mg/l IBA	15.4 bc	3.5
1.0 mg/l IBA	16.7 abc	2.0
2.0 mg/l IBA	5.9 c	4.0
4.0 mg/l IBA	18.8 abc	2.0

[z]Means not followed by a common letter are significantly different from one another at 0.05 level of significance in the arc sine scale. Unequal replications due to contamination and high variable data influenced the degree of statistical differences.

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