Nodulation and Plant Growth of *Shepherdia* × *utahensis* ‘Torrey’ Topdressed with Controlled-release Fertilizer

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Abstract. *Shepherdia* × *utahensis* ‘Torrey’ (‘Torrey’ hybrid buffaloberry) is an actinorhizal plant that can fix atmospheric nitrogen (N₂) in symbiotic root nodules with *Frankia*. Actinorhizal plants with N₂-fixing capacity are valuable in sustainable nursery production and urban landscape use. However, whether nodule formation occurs in *S. ×utahensis* ‘Torrey’ and its interaction with nitrogen (N) fertilization remain largely unknown. Increased mineral N in fertilizer or nutrient solution might inhibit nodulation and lead to excessive N leaching. In this study, *S. ×utahensis* ‘Torrey’ plants inoculated with soils containing *Frankia* were irrigated with an N-free nutrient solution with or without added 2 mM ammonium nitrate (NH₄NO₃) or with 0.0 to 8.4 g·L⁻¹ controlled-release fertilizer (CRF; 15N–3.9P–10K) to study nodulation and plant morphological and physiological responses. The performance of inoculated plants treated with various amounts of CRF was compared with uninoculated plants treated with the manufacturer’s prescribed rate. Plant growth, gas exchange parameters, and shoot N content increased quadratically or linearly along with increasing CRF application rates (all *P* < 0.01). No parameters increased significantly at CRF doses greater than 2.1 g·L⁻¹. Furthermore, the number of nodules per plant decreased quadratically (*P* = 0.0001) with increasing CRF application rates and nodule formation were completely inhibited at 2.9 g·L⁻¹ CRF or by NH₄NO₃ at 2 mM. According to our results, nodulation of *S. ×utahensis* ‘Torrey’ was sensitive to N in the nutrient solution or in increasing CRF levels. Furthermore, plant growth, number of shoots, leaf area, leaf dry weight, stem dry weight, root dry weight, and N content of shoots of inoculated *S. ×utahensis* ‘Torrey’ plants treated with 2.1 g·L⁻¹ CRF were similar to those of uninoculated plants treated with the manufacturer’s prescribed rate. Our results show that *S. ×utahensis* ‘Torrey’ plants inoculated with soil containing *Frankia* need less CRF than the prescribed rate to maintain plant quality, promote nodulation for N₂ fixation, and reduce N leaching.

Nursery production of native plants has increased tremendously because of increasing interest in using native plants in urban gardens and landscapes (Thomas and Schrock, 2004). The growing interest in native plants is attributable to their ornamental potential, including aesthetic appearance, bio-diversity, and, most importantly, water conservation (Hooper et al., 2008). In the Intermountain West, native plants are used in low-water-use landscapes with sustainable and low-water-use features (Mee et al., 2003). However, it is difficult to establish native plants in disturbed and poorly drained soils (Edmondson et al., 2011; Mee et al., 2003). Native plants in the Intermountain West, such as *Arctostaphylos patula* (greenleaf manzanita), *Artemisia nova* (black sagebrush), *Ceratoides*

lanata (syn. *Krascheninnikovia lanata*) (winterfat), and *Cercocarpus montanus* (alder-leaf mountain mahogany), are susceptible to overwatering and wet rooting substrates (Mee et al., 2003). Parkinson et al. (2003) also reported that adequate drainage is essential for growing native plants in the Intermountain West, citing *Agave parryi* (Parry’s agave), *Aquilegia caerulea* (Colorado blue columbine), *Eriogonum niveum* (snow buckwheat), and *Eriophyllum lanatum* (woolly sunflower) as examples.

A similar scenario was discovered for *Shepherdia* species (buffaloberry). *Shepherdia argentea* (silver buffaloberry) and *Shepherdia rotundifolia* (roundleaf buffaloberry) are both native plants in the Intermountain West (Mee et al., 2003). *Shepherdia rotundifolia* is an evergreen shrub with a tidy,

rounded form and outstanding drought tolerance, but it is highly sensitive to excessive landscape irrigation (Sriladda, 2011; Sriladda et al., 2014). However, *S. argentea* is a fast-growing, deciduous shrub that adapts to a wide range of soil conditions, but it is less aesthetically acceptable due to its thorny and unkempt appearance (Sriladda et al., 2016). To enhance the adaptability of *Shepherdia* to wet and poorly drained soils while maintaining drought tolerance and aesthetic appearance, *S. ×utahensis* Torrey, an interspecific hybrid cultivar of *S. argentea* and *S. rotundifolia*, was created by Sriladda et al. (2016). This hybrid cultivar has the potential for use in low-water landscapes because of its tolerance to drought conditions as well as the occasionally wet soils found in residential landscape environments (Sriladda et al., 2016). Furthermore, *S. argentea* and *S. rotundifolia* are actinorhizal plants (Benson and Silvester, 1993), and *S. ×utahensis* ‘Torrey’ can form a symbiotic association with *Frankia* to fix N₂ in its root nodules (J. Chen, unpublished data). This biological N₂-fixing capacity may reduce the need for nitrogenous fertilization of nodulated actinorhizal plants, thus solving two primary concerns in the nursery industry: mineral N runoff and leaching to groundwater (Urbano, 1989). For example, nodulated *Alnus maritima* (seaside alder) had better fertilizer use efficiency when inoculated with soils containing *Frankia* than uninoculated plants (Beddes and Kratsch, 2010). In another study, *Frankia*-induced nodulation improved plant performance and N use efficiency of *Alnus incana* (gray alder) (Sellstedt and Huss-Danell, 1986). Laws and Graves (2005) reported that nodulated *A. maritima* sustained plant vigor and quality with a lower NH₄NO₃ concentration than uninoculated plants. Therefore, actinorhizal plants with symbiotic nodules have commercial potential in nursery production and urban landscapes (Kratsch and Graves, 2004).

Slow-release fertilizer and CRF gradually deliver mineral nutrients (mainly N) to plants and have been widely used in nursery production (Adams et al., 2013; Beddes and Kratsch, 2010), but excessive N fertilization reduces nodule formation of actinorhizal plants inoculated with *Frankia* (Huss-Danell, 1997). For example, *A. maritima* exhibited a decreased nodule number when NH₄NO₃ increased from 0 to 8.0 mM (Laws and Graves, 2005). In addition, Beddes and Kratsch (2010) reported that *A. maritima* plants had a decreased nodule number when CRF (15N–3.9P–10K) levels increased from 0 to 1.8 g·L⁻¹, and 3.6 g·L⁻¹ completely inhibited nodule formation. Unfortunately, although N fertilization significantly influences nodule formation, the impact of fertilizers on plant growth and nodule development of *S. ×utahensis* ‘Torrey’ is largely unknown. Research investigating the effects of CRF and its application rate on nodulation is needed to inform best practices for nurseries.

The objectives of this research were to 1) investigate the impacts of NH₄NO₃ and CRF on nodule number and plant growth of

S. xutahensis ‘Torrey’, and 2) to determine CRF application rates that maintain acceptable plant quality with minimal nitrate–nitrogen (NO₃-N) leaching. In addition, the effects of inoculation on growth and gas exchange parameters were studied by comparing inoculated *S. xutahensis* ‘Torrey’ plants treated with 0 to 8.4 g·L⁻¹ CRF with uninoculated plants treated with the manufacturer’s prescribed rate of 3.2 g·L⁻¹.

Materials and Methods

Shepherdia xutahensis ‘Torrey’ plants were clonally propagated using cuttings collected from the Utah State University Greenville Research Farm (North Logan, UT) on 16 July 2019. Except for the two to three pairs of leaves at the top, leaves were removed from cuttings. Cuttings were dipped in 1000 mg·L⁻¹ indole-3-butyric acid (Hormodin 1; OHP, Mainland, PA) and placed in a tray filled with perlite (Hess Perlite, Malad City, ID). Trays were kept on a mist bench in a greenhouse with temperatures set at 25/20 °C (day/night). On 1 Oct. 2019, nodule-free rooted cuttings were transplanted into 3.8-L injection-molded, polypropylene containers (PC1D-4; Nursery Supplies, Orange, CA) filled with calcined clay (Turface MVP; Profile Products, Buffalo Grove, IL), which is an inorganic growing substrate that is used for native plants (Beddes and Kratsch, 2009). Plants were irrigated with deionized water before the experiment.

On 15 Oct. 2019, the experiment was initiated and plants of uniform size were randomly assigned to 12 groups. In previous studies, soil collected from the rhizosphere of an actinorhizal plant was used as a source of *Frankia* inocula (Tortosa and Cusato, 1991). In this study, field soils (≈8 L) were collected from the rhizosphere of a *S. xutahensis* ‘Torrey’ plant (lat. 41°45′ N, long. 111°48′ W) at Utah State University Greenville

Research Farm. Plants in groups 1 to 10 were inoculated with 50 mL of field soil layered on the surface of the substrate. For groups 1 to 8, each plant was topdressed with 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 g of Osmocote 15N–3.9P–10K (Osmocote Plus 15–9–12; Israel Chemicals, Tel Aviv-Yafo, Israel, hereafter referred to as CRF), resulting in CRF levels of 0, 0.1, 0.3, 0.5, 1.1, 2.1, 4.2, and 8.4 g·L⁻¹, respectively. Plants in groups 9 and 10 were irrigated with 500 mL modified N-free nutrient solution (Bugbee, 2004) supplemented with (N-supplement) or without (N-lacking), respectively, added 2 mM NH₄NO₃ at pH 7.5 every other day, resulting in NO₃-N concentrations of 114.9 ± 6.8 and 13.0 ± 1.1 mg·L⁻¹ (mean ± SE), respectively. Plants in group 11 had no inoculation but received 3.2 g·L⁻¹ of CRF following the manufacturer’s recommended application rate for plants in a 3.8-L container. Plants in group 12 were uninoculated and not fertilized to confirm whether uninoculated, unfertilized plants form nodules. Except for the plants in groups 9 and 10, plants were irrigated with 500 mL tap water (pH = 7.8) every other day. A saucer was placed under a container before irrigation to collect leachate for measurements. A NO₃-N meter (LAQUA Twin; Horiba, Kyoto, Japan) and a pH meter (LAQUA Twin) were used to record the NO₃-N concentration and pH of the leachate weekly. The NO₃-N concentration in the leachate of plants treated with CRF was calculated using the difference between NO₃-N concentrations in irrigation water and in leachate to correct the variations from background N.

Gas exchange parameters, including the leaf net photosynthesis rate (P_n), stomatal conductance rate (g_s), and transpiration rate (E), were recorded for plants treated with 0, 2.0, 8.0, and 32.0 g of CRF 1 week before the experiment was ended. Parameters were recorded using a Portable Photosynthesis System with a PLC3 Universal Leaf Cuvette (CIRAS-3; PP Systems, Amesbury, MA) on a sunny day between 1000 and 1400 hr. Within the cuvette, the intensity of photosynthetic photon flux density was set at 1000 μmol·m⁻²·s⁻¹ with 38% red, 37% green, and 25% blue light provided from light-emitting diodes, whereas the carbon dioxide level and leaf temperature were controlled at 400 μmol·mol⁻¹ and 25 °C, respectively.

The experiment was ended on 8 Dec. 2019. Plant height was recorded at the initiation and termination of the experiment to determine plant growth. Before all plants were destructively harvested, the number of shoots (longer than 5 cm) was recorded for each plant. All *S. xutahensis* ‘Torrey’ plants were destructively harvested, and the leaf area was recorded using a leaf area meter (LI-3000; LI-COR Biosciences, Lincoln, NE). After roots were harvested and washed using deionized water, plants were checked for nodule number. Leaves, stems, and roots were dried in an oven at 80 °C for 3 d, and the dry weight was recorded. Dry leaves and stem samples were ground and analyzed at

the Utah State University Analytical Laboratories for N content with an elemental analyzer (vario MAX cube; Elementar Analysensysteme GmbH, Langensfeld, Germany).

The experiment used a randomized complete block design with 10 blocks for all groups. An analysis of variance was performed to test the effects of treatments on plant morphological and physiological responses. The means separation among treatments was adjusted using the Tukey–Kramer method for multiplicity at α = 0.05. All statistical analyses were performed using the PROC Mixed procedure in SAS Studio (SAS Institute, Cary, NC).

Results

Leachate. The N-supplement and N-lacking treatment led to leachate NO₃-N concentrations of 188.3 ± 13.5 and 19.8 ± 1.4 mg·L⁻¹ (mean ± SE), respectively. The pH values of the leachate solutions were 7.3 ± 1.3 and 7.6 ± 1.3 (mean ± SE) for N-supplement and N-lacking treatment, respectively (data not shown). However, for plants irrigated with tap water, the pH of the leachate solution was 7.9 ± 0.01 (mean ± SE). For plants treated with CRF, the NO₃-N concentration of leachate solution increased as the applied CRF levels increased (Fig. 1). The NO₃-N concentrations of leachate solutions from plants treated with 0 to 2.1 g·L⁻¹ CRF were 28% to 80% less than that from uninoculated plants treated with the manufacturer’s prescribed rate (Fig. 1). However, inoculated plants treated with 4.2 and 8.4 g·L⁻¹ CRF had 42% and 179% greater NO₃-N leachate concentrations, respectively, than uninoculated plants treated with the manufacturer’s prescribed rate.

Plant growth, number of shoots, and leaf area. Plant growth of *S. xutahensis* ‘Torrey’ plants receiving the N-supplement treatment was three times greater than that of plants receiving the N-lacking treatment (Table 1). A regression analysis indicated a quadratic relationship between CRF and plant growth (P = 0.002; R² = 0.95) (Fig. 2). Plant growth and visual quality are shown in Fig. 3. Plant growth of inoculated *S. xutahensis* ‘Torrey’ plants treated with 1.1 to 8.4 g·L⁻¹ CRF increased one to six times compared with plants treated with 0 g·L⁻¹ CRF. A quadratic trend in CRF and the number of shoots of *S. xutahensis* ‘Torrey’ (P = 0.003; R² = 0.95) was observed (Fig. 2). Except for the plants receiving 0.3 g·L⁻¹ CRF, *S. xutahensis* ‘Torrey’ plants treated with 0.1 to 8.4 g·L⁻¹ CRF had one to four more shoots than plants treated with 0 g·L⁻¹ CRF. The leaf area of *S. xutahensis* ‘Torrey’ plants receiving N-supplement treatment was four times more than that of N-lacking treatment (Table 1). When plants were fertilized with increasing amounts of CRF, a quadratic relationship was found between the leaf area and CRF (P = 0.007; R² = 0.98) (Fig. 2). When *S. xutahensis* ‘Torrey’ plants were treated with 1.1 to 8.4 g·L⁻¹ CRF, the leaf area was one to three times greater than those treated with

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Fig. 1. Nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations of leachate recorded after *Shepherdia xutahensis* 'Torrey' plants topdressed with 0 to $8.4 \text{ g}\cdot\text{L}^{-1}$ controlled-release fertilizer (CRF; 15N-3.9P-10K) were irrigated with tap water. The error bars represent the SE of five samples. The background nitrogen in tap water was corrected by calculating the difference between $\text{NO}_3\text{-N}$ concentrations in leachate and tap water.

Table 1. Plant growth, number of shoots, leaf area, dry weights (DW) of leaf, stem, and root, and number of nodules of inoculated *Shepherdia xutahensis* 'Torrey' treated with nutrient solution supplemented with (N-supplement) or not supplemented with (N-lacking) 2 mM ammonium nitrate (NH_4NO_3) for 8 weeks.

Treatment	Plant growth ² (cm)	Shoots (no.)	Leaf area (cm^2)	Leaf DW (g)	Stem DW (g)	Root DW (g)	Nodules (no.)
N-supplement	8.4	2.7	154.2	1.6	0.6	1.0	0.1
N-lacking	2.2	0.1	34.6	0.5	0.2	0.2	10.8
P value	****	***	****	****	**	****	****

²Plant growth was calculated as the difference between plant height at the initiation (15 Oct. 2019) and termination (8 Dec. 2019) of the experiment.

, *, ****Significant at $P \leq 0.01$, 0.001, or 0.0001, respectively.

0 $\text{g}\cdot\text{L}^{-1}$ CRF. No significant difference was found for these parameters of uninoculated *S. xutahensis* 'Torrey' plants treated with the manufacturer's prescribed rate and inoculated plants treated with 1.1 and $2.1 \text{ g}\cdot\text{L}^{-1}$ CRF.

Dry weight of leaf, stem, and root. Leaf dry weight of *S. xutahensis* 'Torrey' plants receiving N-supplement treatment was two times greater than plants with the N-lacking treatment (Table 1). A quadratic relationship was found between leaf dry weight and CRF amounts in inoculated plants ($P = 0.0002$; $R^2 = 0.97$) (Fig. 2). Leaf dry weight of *S. xutahensis* 'Torrey' plants treated with 2.1, 4.2, and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF was three, four, and five times greater, respectively, than those treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF. The N-supplement treatment increased the stem dry weight of *S. xutahensis* 'Torrey' plants by 130% compared with plants given the N-lacking treatment (Table 1). When inoculated *S. xutahensis* 'Torrey' plants were treated with increasing amounts of CRF, a quadratic relationship was found between CRF and stem dry weight ($P = 0.03$; $R^2 = 0.98$) (Fig. 2). Compared with plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF, the stem dry weight of *S. xutahensis* 'Torrey' treated with 4.2 and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF increased two and three times, respectively. *Shepherdia xutahensis* 'Torrey' had four times greater root dry weight when N-supplement

treatment was applied compared with N-lacking treatment (Table 1). A quadratic relationship was observed between CRF and root dry weight of *S. xutahensis* 'Torrey' ($P = 0.01$; $R^2 = 0.91$) (Fig. 2), and the root dry weight increased approximately five times for plants treated with 4.2 and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF compared with plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF (Fig. 3). These parameters of uninoculated *S. xutahensis* 'Torrey' treated with the manufacturer's prescribed rate were not different from those of inoculated *S. xutahensis* 'Torrey' plants treated with 1.1 and $2.1 \text{ g}\cdot\text{L}^{-1}$ CRF (Fig. 2). However, no significant difference in the root:shoot ratio was observed between inoculated and uninoculated plants receiving no CRF ($P = 0.3$) (data not shown). In addition, no correlation was observed between the root:shoot ratio and nodule and growth parameters in this study (data not shown).

Gas exchange and shoot N content. A positive linear relationship was exhibited between CRF and the P_n of *S. xutahensis* 'Torrey' ($P < 0.0001$; $r^2 = 0.74$) (Fig. 4). The P_n of *S. xutahensis* 'Torrey' plants treated with 2.1 and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF increased 12 and 16 times, respectively, compared with plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF. The g_s of *S. xutahensis* 'Torrey' increased linearly with increasing CRF ($P = 0.004$; $r^2 = 0.66$) (Fig. 4), and the g_s of plants treated with

$8.4 \text{ g}\cdot\text{L}^{-1}$ CRF was 72% greater than that of plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF. The E of *S. xutahensis* 'Torrey' had a positive linear trend with increasing CRF ($P = 0.005$; $r^2 = 0.69$). *Shepherdia xutahensis* 'Torrey' plants treated with $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF had a 54% increase in E compared with plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF. No statistical difference was found in the P_n , g_s , and E of *S. xutahensis* 'Torrey' plants receiving 2.1 and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF.

The shoot N content of inoculated *S. xutahensis* 'Torrey' plants increased linearly with CRF ($P < 0.0001$; $r^2 = 0.84$). When *S. xutahensis* 'Torrey' was fertilized with 2.1 and $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF, the shoot N content increased by 44% and 71%, respectively, compared with plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF. There was no statistical difference in the shoot N content of inoculated *S. xutahensis* 'Torrey' treated with 2.1 or $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF and uninoculated plants that received the manufacturer's prescribed rate (Fig. 4).

Nodulation. Nodules formed on *S. xutahensis* 'Torrey' plants inoculated with *Frankia*-infected soils in our experiment ($P < 0.0001$), whereas plants without inoculation and treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF did not form any nodules (data not shown). Furthermore, uninoculated plants treated with the manufacturer's recommended rate did not form nodules. The NH_4NO_3 in the nutrient solution and CRF affected the nodule number of *S. xutahensis* 'Torrey' (both $P < 0.0001$) (data not shown). When the N-supplement treatment was applied, one nodule appeared on 1 out of 10 inoculated *S. xutahensis* 'Torrey' plants (Table 1). In contrast, for plants receiving N-lacking treatment, all plants formed nodules, with 10 nodules per plant on average. For the inoculated plants treated with CRF, the number of nodules per plant decreased quadratically as CRF increased ($P < 0.0001$; $R^2 = 0.74$) (Figs. 3 and 5). When inoculated with soils containing infective *Frankia* strains, *S. xutahensis* 'Torrey' topdressed with $8.4 \text{ g}\cdot\text{L}^{-1}$ CRF did not form any nodules, whereas 1 out of 10 plants treated with $4.2 \text{ g}\cdot\text{L}^{-1}$ CRF had one nodule. According to the regression analysis, $2.9 \text{ g}\cdot\text{L}^{-1}$ CRF completely inhibited nodule formation. The dry weight of nodules increased quadratically as CRF increased ($P = 0.006$; $R^2 = 0.94$). The dry weight of nodules on plants treated with $2.1 \text{ g}\cdot\text{L}^{-1}$ CRF was 13 times greater than that of plants treated with 0 $\text{g}\cdot\text{L}^{-1}$ CRF (Fig. 5).

Discussion

Leachate. Factors such as environmental temperature and physical properties of CRF coating affect the release rate of CRF; however, generally, Osmocote has a relatively rapid release rate initially, followed by a steadily decreasing rate of release over time (Adams et al., 2013). A similar pattern was found in our study; leachate $\text{NO}_3\text{-N}$ concentrations increased at an early stage of the experiment and then decreased over time in

Fig. 2. Plant growth (A), number of shoots (B), leaf area (C), leaf dry weight (D), stem dry weight (E), and root dry weight (F) of *Shepherdia xutahensis* 'Torrey' inoculated with soil containing infective *Frankia* and topdressed with 0 to 8.4 g·L⁻¹ controlled-release fertilizer (CRF; 15N-3.9P-10K) for 8 weeks. The round markers represent means of the plants inoculated with *Frankia*, whereas the square markers represent the uninoculated plants treated with the manufacturer's prescribed rate of 3.2 g·L⁻¹. A regression analysis was conducted using the plants inoculated with *Frankia*. The error bars represent the SE of 10 samples. No significant difference was found between the inoculated plants treated with 1.1 and 2.1 g·L⁻¹ CRF and uninoculated plants treated with the manufacturer's prescribed rate according to the Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

plants receiving 2.1 to 8.4 g·L⁻¹ CRF (Fig. 1). The trend of increasing NO₃-N concentrations in the leachate at the early stage of the experiment followed by declining concentrations was also reported by Glenn et al. (2000) and Niemiera and Leda (1993). This pattern was also reported for *A. maritima* plants grown in a substrate containing peat and vermiculite and treated with CRF; the NO₃-N concentration in the leachate increased from days 1 to 14 for plants receiving 3.6 and 7.3 g·L⁻¹ Osmocote 15N-3.9P-10K and then decreased (Beddes and Kratsch, 2010). The increasing NO₃-N concentration in growing substrates during the early period of the experiment could delay nodule formation.

Morphological and physiological responses. In our study, although morphological parameters of *S. xutahensis* 'Torrey' increased quadratically with increasing CRF levels, these parameters did not change significantly when fertilizer levels exceeded 2.1 g·L⁻¹ CRF (≈ 0.3 g·L⁻¹ N) (Fig. 2). This may be because plant growth responses do not correlate well with nutrient availability when the substrate nutrient concentration exceeds a certain range (Taiz et al., 2015). For example, for morphological parameters, the shoot dry weight of *Salvia farinacea* (mealycup sage) increased quadratically with Osmocote 39N-0P-0K, increasing from 0.5 to 3.0 g·L⁻¹ N, but increasing the rate of Osmocote 39N-0P-0K did not contribute to the shoot dry weight when it exceeded 2.0 g·L⁻¹ N (Knowles et al., 1993). Beddes and Kratsch (2010) also reported that leaf area, shoot dry weight, and root dry weight of *A. maritima* did

Fig. 3. The shoot (A) and root (B) of *Shepherdia xutahensis* 'Torrey' plants inoculated with soil containing infective *Frankia* and topdressed with 0 to 8.4 g·L⁻¹ controlled-release fertilizer (CRF; 15N-3.9P-10K) and the uninoculated plants that received the manufacturer's prescribed rate of 3.2 g·L⁻¹. Inoculated *S. xutahensis* 'Torrey' plants that received 1.1 and 2.1 g·L⁻¹ CRF had plant vigor similar to that of uninoculated plants that received the manufacturer's prescribed application. Photos were obtained at week 8 of the experiment.

Fig. 4. The photosynthesis rate (P_n) (A), stomatal conductance rate (g_s) (B), transpiration rate (E) (C), and nitrogen content of shoot (D) of *Shepherdia xutahensis* 'Torrey' topdressed with 0 g·L⁻¹, 0.5 g·L⁻¹, 2.1 g·L⁻¹, and 8.4 g·L⁻¹ of controlled-release fertilizer (CRF; 15N–3.9P–10K) for 8 weeks. The error bars represent the SE of four measurements or samples. The round markers represent means of the plants inoculated with *Frankia*, whereas the square marker in the shoot nitrogen content represents the uninoculated plants that received the manufacturer's prescribed rate of 3.2 g·L⁻¹ CRF. A regression analysis was conducted using the plants inoculated with *Frankia*. No significant differences were found in the nitrogen contents of shoots of the inoculated plants treated with 2.1 g·L⁻¹ CRF and uninoculated plants treated with the manufacturer's prescribed rate according to Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

Fig. 5. The number of nodules per plant (A) and nodule dry weight (B) of *Shepherdia xutahensis* 'Torrey' inoculated with soil containing infective *Frankia* and topdressed with 0 to 8.4 g·L⁻¹ controlled-release fertilizer (CRF; 15N–3.9P–10K) for 8 weeks. A regression analysis was conducted using the plants inoculated with *Frankia*. The error bars represent the SE of 10 samples.

not increase when Osmocote 15N–3.9P–10K was more than 3.6 g·L⁻¹ (≈ 0.5 g·L⁻¹ N).

Physiological parameters of *S. xutahensis* 'Torrey' increased linearly with increasing CRF levels (Fig. 4), which is inconsistent with a previous report indicating that a quadratic relationship generally occurs between plant growth and nutrient availability (Taiz et al., 2015). Actually, our data points strongly resemble quadratic trends (Fig. 4) because the physiological parameters did not change significantly when CRF levels exceeded 2.1 g·L⁻¹ CRF (≈ 0.3 g·L⁻¹ N). This discrepancy might have resulted from numerous variations in the physiological data that were recorded and the limited number of treatments. The P_n of *Abies fraseri* (fraser fir), *Picea glauca* (white spruce), *Picea pungens* (blue spruce), and *Pinus strobus* (eastern white pine) increased when Osmocote 15N–

3.9P–10K ranged from 0.25 to 0.5 g·L⁻¹ N, but it did not change at levels greater than 0.5 g·L⁻¹ N (Klooster et al., 2010). In a study by Zhang et al. (2011), the P_n of *Hosta clausa* (hosta) did not increase when controlled-release N fertilizer 46N–0P–0K exceeded 0.3 g·L⁻¹ (≈ 0.1 g·L⁻¹ N) at 90 and 120 d after treatment. Therefore, it is important to define the minimum application rate of CRF to maintain plant vigor and photosynthesis while reducing fertilizer costs and the potential for groundwater contamination (Poole and Conover, 1989). Based on the results of our study, an effective CRF application rate for inoculated *S. xutahensis* 'Torrey' is 2.1 g·L⁻¹ (≈ 0.3 g·L⁻¹ N) for maintaining plant vigor and reducing NO₃-N leaching.

Nitrogen content and nodulation. The N concentrations in plant tissues in our study increased linearly with increasing fertilizer

levels. Klooster et al. (2010) reported that the N contents of *A. fraseri*, *P. glauca*, *P. pungens*, and *P. strobus* needles increased when the Osmocote 15N–3.9P–10K application increased from 0.25 and 0.5 g·L⁻¹ N. The N contents of *Pilea* 'Silver Tree', *Aphelandra squarrosa* (zebra plant), *Chamaedorea elegans* (parlor palm), and *Dieffenbachia maculata* 'Camille' ('Camille' dumb cane) increased linearly with the 14N–6.2P–11.6K slow-release fertilizer level increasing from 0.5 to 1.5 g·L⁻¹ (≈ 0.07 to 0.2 g·L⁻¹ N) (Poole and Conover, 1989). In the study by Knowles et al. (1993), the shoot N content of *Salvia farinacea* showed a quadratic trend with increasing Osmocote 39N–0P–0K. The N content of *A. maritima* increased quadratically with increasing application rates of Osmocote 15N–3.9P–10K, but the shoot N contents of nodulated *A. maritima* plants receiving 0.9 to 1.8 g·L⁻¹ CRF (≈ 0.1 to 0.3 g·L⁻¹ N) were not different from those of uninoculated plants receiving the manufacturer's prescribed rate of 2.7 g·L⁻¹ CRF (≈ 0.4 g·L⁻¹ N) because of the N₂-fixing ability of *Frankia* (Beddes and Kratsch, 2010). The leaf N content of *A. maritima* plants also increased along with NH₄NO₃ levels in the nutrient solution (Laws and Graves, 2005). In our study, the shoot N content of nodulated *S. xutahensis* 'Torrey' plants fertilized with 2.1 g·L⁻¹ CRF was similar to that of uninoculated plants receiving the manufacturer's prescribed rate of 3.2 g·L⁻¹. These results indicate that nodulation induced by *Frankia* may help accumulate N. Because the N₂-fixation of *Frankia* enhances the N content, inducing nodules in novel actinorhizal plants using field soil is a preferred practice to reduce fertilizer applications in sustainable nurseries concerned with the economic and ecological impacts of chemical fertilizers (Kratsch and Graves, 2004).

Soils from actinorhizal plant habitats have dense populations of infective *Frankia*, and host plants are the primary factor amplifying *Frankia* populations in the soil (Benson and Silvester, 1993; Schwencke and Caru, 2001). In our study, soil used for inoculation was collected from the rhizosphere of a *S. xutahensis* 'Torrey' in North Logan, UT. Our results suggest that this soil has infective *Frankia* to induce symbiotic nodules of *S. xutahensis* 'Torrey', which is similar to previous reports indicating that soils collected from wild populations have significant effects on inducing nodules on actinorhizal plants (Beddes and Kratsch, 2010; Jeong and Myrold, 2001; Laws and Graves, 2005).

Although soil can induce nodules on *S. xutahensis* 'Torrey', such nodule formation was inhibited by 2.9 g·L⁻¹ CRF (≈ 0.4 g·L⁻¹ N) or 2 mM NH₄NO₃. This might be associated with the significant impact of N fertilizer on the infection process of actinorhizal plants. Depending on the host plant species, *Frankia* strains infect host plants by either root hair infection or intercellular penetration (Schwencke and Caru, 2001). Nitrate has a negative effect on root hair development;

therefore, the nodule number is more sensitive to nitrate in actinorhizal plants with root hair infection. For instance, *Alnus glutinosa* (black alder), *Casuarina cunninghamiana* (river oak), and *Myrica cerifera* (southern wax myrtle) are infected by *Frankia* via root hair infection, and their nodule formation is more sensitive to nitrate when compared with *Elaeagnus angustifolia* (Russian olive), which is infected by *Frankia* via intercellular penetration (Kohls and Baker, 1989). Ammonium ions in substrate may impact nodule formation, but the substrate used for growing *S. xutahensis* 'Torrey' is well-drained, a condition under which N fertilizer tends to transform to nitrate (Norton and Ouyang, 2019). This nitrate, together with those released from fertilizer, would significantly inhibit the *Frankia* infection process of *S. xutahensis* 'Torrey'. However, further investigations should be conducted to confirm the mechanism of *Frankia* strains infecting *S. xutahensis* 'Torrey' because they infect most *Shepherdia* species via intercellular penetration (Racette and Torrey, 1989) and, therefore, would not be sensitive to nitrate.

Additionally, the reliance of host plants on the symbiont decreases when the plant N status increases; therefore, autoregulation is triggered to suppress nodule formation and prevent excessive export of photosynthate (Kratsch and Graves, 2004). The sensitivity of nodule formation to N is different among actinorhizal plants. Thomas and Berry (1989) reported that the nodule number on *Ceanothus griseus* (carmel ceanothus) was significantly reduced at 0.714 mM NH_4NO_3 and completely inhibited at 2.68 mM NH_4NO_3 . However, *Purshia mexicana* (Mexican cliffrose) and *Purshia tridentata* (antelope bitterbrush) did not form nodules when exposed to 6 mM NH_4NO_3 (Righetti et al., 1986). For *A. maritima*, the nodule number decreased linearly when the NH_4NO_3 concentration increased from 0.25 to 4 mM, and it was completely inhibited at 8 mM NH_4NO_3 (Laws and Graves, 2005). In another study, the nodule number of *A. maritima* declined when Osmocote 15N-3.9P-10K exceeded 1.8 g·L⁻¹ (≈ 0.3 g·L⁻¹ N), and it was completely inhibited at 3.6 g·L⁻¹ CRF (≈ 0.5 g·L⁻¹ N) (Beddes and Kratsch, 2010). Compared with *A. maritima*, the nodule formation of *S. xutahensis* 'Torrey' is completely inhibited by a relatively lower concentration of NH_4NO_3 or CRF level. Therefore, *S. xutahensis* 'Torrey' may be sensitive to environmental N content.

Apart from N, mineral nutrients in the CRF and substrate pH might also affect the nodule number of *S. xutahensis* 'Torrey'. Although $\text{NO}_3\text{-N}$ and ammonium-nitrogen ($\text{NH}_4\text{NO}_3\text{-N}$) are the primary nutrients in Osmocote 15N-3.9P-10K, it also contains mineral nutrients such as phosphate and iron (Adams et al., 2013). A previous study has documented that phosphate, cobalt, iron, calcium, and sodium affect the nodule number of actinorhizal plants (Huss-Danell, 1997). For instance, low phosphorus availability significantly impaired the nodule formation

of *Discaria trinervis* (Valverde et al., 2002). Although substrate pH was not controlled in this experiment, it might affect the availability of macronutrients and micronutrients to inhibit nodulation (Huss-Danell, 1997; Taiz et al., 2015).

However, the N fixing ability of actinorhizal plants is related to nodule biomass instead of nodule number (Dawson and Gordon, 1979; Gordon and Wheeler, 1987). For *S. xutahensis* 'Torrey', nodule dry weight increased with increasing CRF levels (Fig. 5), and a similar result was reported by Beddes and Kratsch (2010). These results indicate that the N fixation ability of *S. xutahensis* 'Torrey' may increase with increasing CRF levels up to 2.1 g·L⁻¹. Although the N_2 fixation capacity of nodules of *S. xutahensis* 'Torrey' was undefined in our study, our results indicate that *Frankia* inoculation could improve plant performance and reduce fertilizer use. Further investigations are needed to test the N_2 fixation of *S. xutahensis* 'Torrey' using acetylene reduction assays or ¹⁵N-labeling techniques (Huss-Danell, 1997; Laws and Graves, 2005).

For acceptable plant quality and minimal $\text{NO}_3\text{-N}$ concentrations in leachate, Beddes and Kratsch (2010) concluded that 0.9 g·L⁻¹ Osmocote 15N-3.9P-10K was the proper application rate for producing nodulated *A. maritima*. In addition, NH_4NO_3 at 0.5 to 2.0 mM in nutrient solution was recommended by Laws and Graves (2005) for *A. maritima* with symbiotic nodules to enhance leaf N as well as maintain plant vigor. In our research, plant growth was similar between the inoculated plants fertilized with 1.1 g and 2.1 g·L⁻¹ CRF and the uninoculated plants receiving the manufacturer's prescribed rate. Furthermore, inoculated plants treated with 2.1 g·L⁻¹ CRF had tissue N content similar to that of the uninoculated plants receiving the manufacturer's prescribed rate. Therefore, 1.1 or 2.1 g·L⁻¹ CRF may be the proper application rate for the production of nodulated *S. xutahensis* 'Torrey'.

Conclusions

Although growth and physiological responses of *S. xutahensis* 'Torrey' were improved by increasing CRF, increased CRF led to higher $\text{NO}_3\text{-N}$ concentrations in the leachate. No significant differences in morphological and physiological parameters and shoot N contents were observed in the inoculated plants when CRF levels exceeded 2.1 g·L⁻¹. Furthermore, compared with the uninoculated plants treated with the manufacturer's prescribed rate, nodulated plants treated with 1.1 g·L⁻¹ CRF had similar morphological and growth responses, and nodulated plants at 2.1 g·L⁻¹ had similar shoot N contents. The number of nodules on *S. xutahensis* 'Torrey' was inhibited with increased CRF application rates, and it was completely inhibited by 2.9 g·L⁻¹ of CRF or 2 mM NH_4NO_3 . Therefore, when nodulated *S. xutahensis* 'Torrey' plants are produced in the nursery, CRF lower than 2.9 g·L⁻¹ or

NH_4NO_3 lower than 2 mM should be applied. Rates between 1.1 and 2.1 g·L⁻¹ CRF may be sufficient for nodulated *S. xutahensis* 'Torrey' to sustain acceptable visual quality and promote nodulation for N_2 fixation with minimal nitrate leachate.

Literature Cited

- Adams, C., J. Frantz, and B. Bugbee. 2013. Macro- and micronutrient-release characteristics of three polymer-coated fertilizers: Theory and measurement. *J. Plant Nutr. Soil Sci.* 176:76–88.
- Beddes, T. and H.A. Kratsch. 2009. Seed germination of roundleaf buffaloberry (*Shepherdia rotundifolia*) and silver buffaloberry (*Shepherdia argentea*) in three substrates. *J. Environ. Hort.* 27:129–133.
- Beddes, T. and H.A. Kratsch. 2010. Nodulation of seaside alder topdressed with controlled-release fertilizer. *HortTechnology* 20:740–745.
- Benson, D.R. and W.B. Silvester. 1993. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol. Rev.* 57:293–319.
- Bugbee, B. 2004. Nutrient management in recirculating hydroponic culture. *Acta Hort.* 648:99–112.
- Dawson, J.O. and J.C. Gordon. 1979. Nitrogen fixation in relation to photosynthesis in *Alnus glutinosa*. *Bot. Gaz.* 140:70–75.
- Edmondson, J.L., Z.G. Davies, S.A. McCormack, K.J. Gaston, and J.R. Leake. 2011. Are soils in urban ecosystems compacted? A citywide analysis. *Biol. Lett.* 7:771–774.
- Gordon, J.C. and C.T. Wheeler. 1987. Whole plant studies on photosynthesis and acetylene reduction in *Alnus glutinosa*. *New Phytol.* 80:179–186.
- Glenn, J.S., C.H. Gilliam, J.H. Edwards, G.J. Keever, and P.R. Knight. 2000. Recycled waste paper mulch reduces available container N. *J. Environ. Hort.* 18:188–191.
- Hooper, V.H., J. Ender-Wada, and C.W. Johnson. 2008. Theory and practice related to native plants: A case study of Utah landscape professionals. *Landsc. J.* 27:1–8.
- Huss-Danell, K. 1997. Actinorhizal symbioses and their N_2 fixation. *New Phytol.* 136:375–405.
- Jeong, S.C. and D.D. Myrold. 2001. Population size and diversity of *Frankia* in soils of *Ceanothus velutinus* and Douglas-fir stands. *Soil Biol. Biochem.* 33:931–941.
- Klooster, W.S., B.M. Cregg, R.T. Fernandez, and P. Nzokou. 2010. Growth and photosynthetic response of pot-in-pot-grown conifers to substrate and controlled-release fertilizer. *HortScience* 45:36–42.
- Knowles, T.C., B.W. Hipp, and M.A. Hegemann. 1993. Container medium and slow-release nitrogen fertilizer influence growth and quality of *Salvia farinacea*. *HortScience* 28:623–625.
- Kohls, S.J. and D.D. Baker. 1989. Effects of substrate nitrate concentration on symbiotic nodule formation in actinorhizal plants. *Plant Soil* 118:171–179.
- Kratsch, H.A. and W.R. Graves. 2004. Nitrogen fixation as a stress-avoidance strategy among actinorhizal (non-legume) trees and shrubs. *J. Crop Improv.* 10:281–304.
- Laws, M.T. and W.R. Graves. 2005. Nitrogen inhibits nodulation and reversibly suppresses nitrogen fixation in nodule of *Alnus maritima*. *J. Amer. Soc. Hort. Sci.* 130:496–499.
- Mee, W., J. Barnes, R. Kjellgren, R. Sutton, T. Cerny, and C. Johnson. 2003. Water wise: Native plants for intermountain landscapes. Utah State University Press, Logan, UT.

- Niemiera, A.X. and C.E. Leda. 1993. Nitrogen leaching from Osmocote-fertilized pine bark at leaching fractions of 0 to 0.4. *J. Environ. Hort.* 11:75–77.
- Norton, J. and Y. Ouyang. 2019. Controls and adaptive management of nitrification in agricultural soils. *Front. Microbiol.* 10:1931.
- Parkinson, H., A. DeBolt, R. Rosentreter, and V. Geertson. 2003. Landscaping with native plants of the Intermountain Region. U.S. Bureau of Land Management, Boise, ID.
- Poole, R.T. and C.A. Conover. 1989. Fertilization of four indoor foliage plants with Osmocote or Nutricote. *J. Environ. Hort.* 7:102–108.
- Racette, S. and J.G. Torrey. 1989. Root nodule initiation in *Gymnostoma* (Casuarinaceae) and *Shepherdia* (Elaeagnaceae) induced by *Frankia* strain HFPGp11. *Can. J. Bot.* 67:2873–2879.
- Righetti, T.L., C.H. Chard, and R.A. Backhaus. 1986. Soil and environmental factors related to nodulation in *Cowania* and *Purshia*. *Plant Soil* 91:147–160.
- Schwencke, J. and M. Caru. 2001. Advances in actinorhizal symbiosis: Host plant-*Frankia* interactions, biology, and applications in arid land reclamation. A review. *Arid Land Res. Manage.* 154:283–327.
- Sellstedt, A. and K. Huss-Danell. 1986. Biomass production and nitrogen utilization by *Alnus incana* when grown on N₂ or NH₄⁺ made available at the same rate. *Planta* 167:387–394.
- Sriladda, C. 2011. Ecophysiology and genetic variation in domestication of *shepherdia* and *shepherdia* species for the Intermountain West. Utah State University, Logan, UT. 2 June 2020. <<https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=2027&context=etd>>.
- Sriladda, C., H.A. Kratsch, S.R. Larson, T.A. Monaco, F. Shen, and R.K. Kjellgren. 2016. Interspecific hybrid of xeric *Shepherdia rotundifolia* and riparian *Shepherdia argentea*: Description, and traits suitable for low-water urban landscapes. *HortScience* 51:822–828.
- Sriladda, C., R. Kjellgren, H. Kratsch, T. Monaco, S. Larson, and F. Shen. 2014. Ecological adaptation of the endemic *Shepherdia rotundifolia* to conditions in its Colorado Plateau range. *West. N. Amer. Nat.* 74:79–91.
- Taiz, L., E. Zeiger, I.M. Møller, and A. Murphy. 2015. *Plant physiology and development*. 6th ed. Sinauer Associates, Sunderland, MA.
- Thomas, A.L. and D. Schrock. 2004. Performance of 67 native midwestern U.S. perennials in a low-maintenance landscape. *HortTechnology* 14:381–388.
- Thomas, K.A. and A.M. Berry. 1989. Effects of continuous nitrogen application and nitrogen preconditioning on nodulation and growth of *Ceanothus griseus* var *horizontalis*. *Plant Soil* 118:181–187.
- Tortosa, R.D. and M. Cusato. 1991. Effective nodulation of rhizomatous actinorhizal plants induced by air dry soils. *Plant Soil* 131:229–233.
- Urbano, C.C. 1989. The environmental debate: An industry issue. *Amer. Nurseryman* 169:69–73, 76–79, 81, 83, 85.
- Valverde, C., A. Ferrai, and L.G. Wall. 2002. Phosphorus and the regulation of nodulation in the actinorhizal symbiosis between *Discaria trinervis* (Rhamnaceae) and *Frankia* BCU110501. *New Phytol.* 153:43–51.
- Zhang, J., W. Wang, G. Sun, H. Liu, and X. Li. 2011. Photosynthesis of *Hosta* under light and controlled-release nitrogen fertilizer. *Russ. J. Plant Physiol.* 58:261–270.