HCS 4193

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Investigating the potential for use of the endangered species, running buffalo clover (*Trifolium stoloniferum*) on reclaimed mine-land.

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Abstract

Running buffalo clover (RBC; *Trifolium stoloniferum*) is native to Ohio, however, loss of habitat has resulted in it becoming a federal endangered species. Though the U.S. Fish and Wildlife Service implemented management plans and state agencies attempted reintroductions, the species remains in a perilous state in the wild. The objective of this study was to determine the tolerance of RBC to acidic and sulfuric/ferrous soil such as reclaimed mine-land. The hypothesis was that a commercial or private utility in mine reclamation may encourage larger reintroductions and invigorate new interest in a deserving, endangered endemic species. In this study, three accessions of RBC were treated with nutrient solutions in a factorial arrangement of two pH (4.0 and 6.0) and two FeSO4 levels (0 and 1000 mg S/L) in a greenhouse setting. After four months, pH and S treatments, root mass and aboveground growth were measured. Should running buffalo clover outperform white clover in mine conditions, it might recover as a species with introduced, reclamation populations serving to revegetate their endemic range on protected habitat.

Introduction

Running buffalo clover (RBC) is native to Ohio, however, loss of prairie habitat has resulted in it becoming a federal endangered species. Presumed extinct around 1940, researchers rediscovered small populations in the mid-1980s along the Ohio River corridor (USFWS 2007, USFWS 2011). Previous research has investigated the forage potential of RBC, however, it has failed to outperform related species, white clover (*T. repens)*.

Previous investigators have documented natural populations after the species was rediscovered in the mid-1980s (Yatskievych 2006). Before 1985, it was considered to be extinct. Ohio State University students and faculty studied much of this federally endangered species. Twenty years before the present study, graduate students in the former Department of Plant Biology as well as the School of the Environment and Natural Resources studied the soil characteristics of RBC areas, , as well as the fecundity of natural populations (Hattenbach 1996, Franklin 1998).

Failing to find a rhizobia symbiont, but seeing a forage potential in the RBC historical range, focus on the species shifted to potential applications as means to save the species from extinction (Morris et al 2006). Ohio State alumni worked on the U.S. Fish and Wildlife Recovery Plan for the species (Selbo 2007). In the past five years, Dr. David Barker, Department of Horticulture and Crop Science, worked in the RBC system to develop applications that might encourage RBC’s preservation (Sparks and Barker 2014, Barker and Sparks 2013). By advocating for further research, RBC continues to exist in research laboratory populations (Barker and Sparks 2013).

The present study departed from the forage utility paradigm to investigate potential use on reclaimed mine-land. Given the limited use of reclaimed mine areas, as well as the unfavorable growing conditions, a reintroduction scheme could revegetate the sites and take advantage of de facto protected habitat. Left alone, or managed within a revegetation regime, RBC might recolonize former habitat if proven tolerant of such conditions and suitable for revegetation standards.

Materials and Methods

In this study, three accessions of RBC were treated with nutrient solutions in a factorial arrangement of two pH (5.0 and 6.0) and two FeSO4 levels (0 and 1000 mg S/L) in following an experimental design with a 4 x 2 x 2 factorial treatment structure and a randomized complete block arrangement for the four replications (64 pots in total). Plants were established from stolons on 16 December 2016, and grown in vermiculite to facilitate measurements on roots. RBC plant material came from three USDA accessions [PI numbers: 641566, 22231, and 31415]. The responses for RBC were compared with *T. repens*, under the same treatments. Plants were supplied with Peters 200 ppm nutrient solution approximately weekly during the study.

The $FeSO_4$ solution treatments began on 20 January 2017, and continued until 26 March 2017 (See Appendix 1). Shoot measurements at intervals throughout the study provided initial data before destructive sampling for root mass data. Plant diameter was calculated as the average of the longest and smallest dimensions of individual plants. In addition, pH measurements of root media took place 22 February (See Appendix 1). On 26 March 2017, the authors harvested the specimens and dried roots and shoot sections for 40 hours. Mass measurements for root and shoot measurements proceeded 28 March 2017. All data collected throughout the species went into Microsoft Excel spreadsheets, and then into the SAS statistical program for data analysis.

Results

Figure 1. Species/Accession effect for active growing points per plant (p=0.0329)

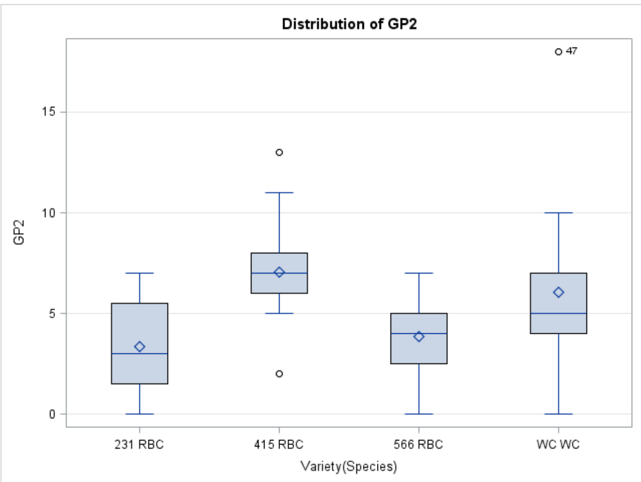


Figure 2. Active growing points species by sulfate interaction (p=0.0244)

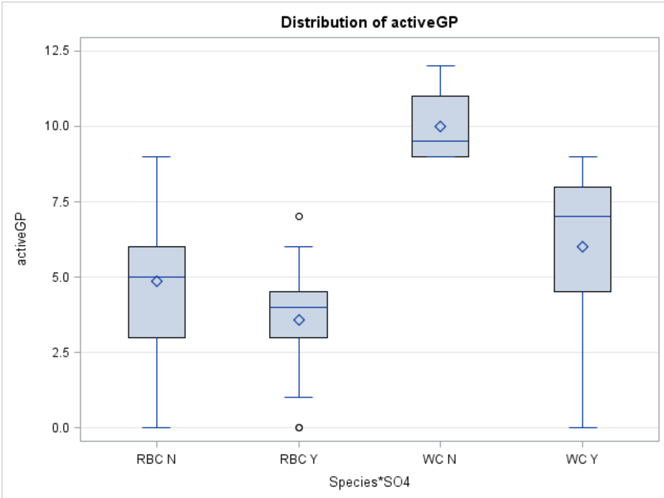


Figure 3. Leaf count species by acid by sulfate interaction (p=0.0006)

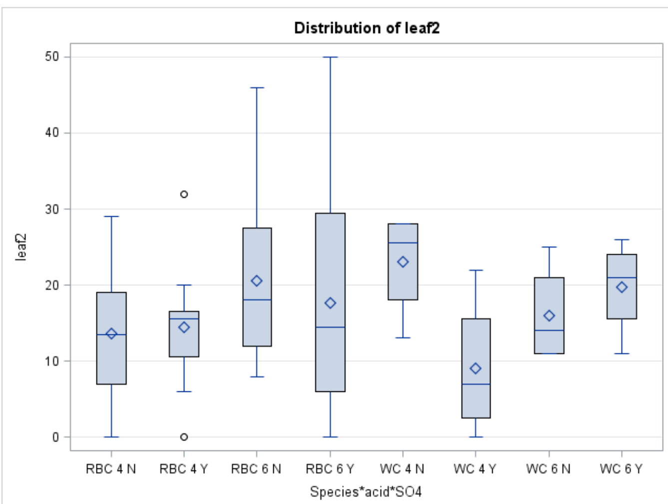


Figure 4. Diameter species by acid by sulfate interaction (p=0.0266)

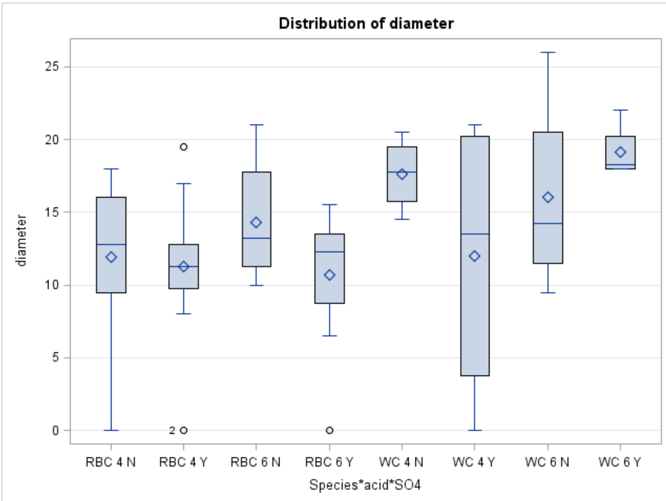


Figure 5. Shoot dry weight variety by sulfate interaction

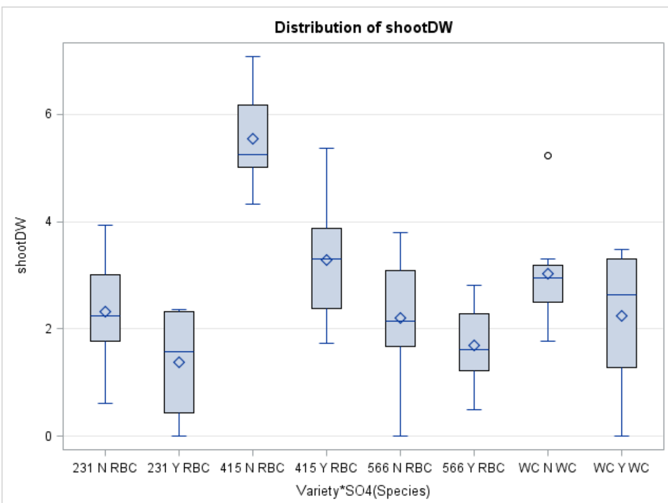
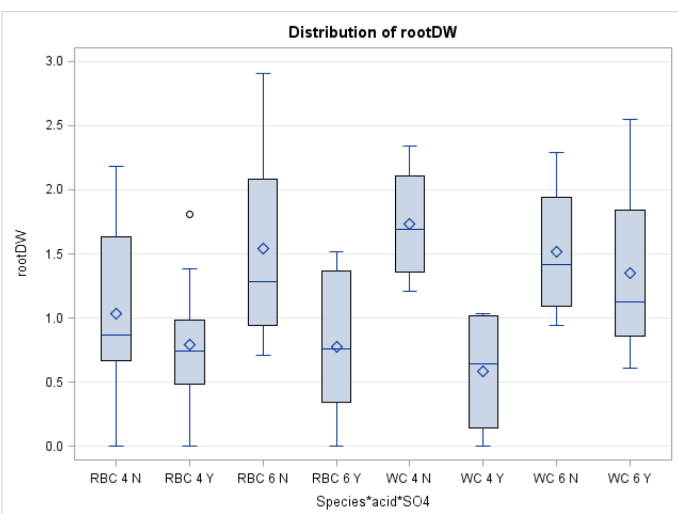


Figure 6. Root dry weight species by acid by sulfate interaction (p=0.0031)



When the nutrient solution treatments began on 20 January 2017, RBC plants averaged 0.549 g of shoot, and 0.481 g of root, whereas white clover averaged 0.0825 g of shoot, and 0.0694 g of root. After nine weeks of treatment, the iron sulfate treatment effect was statistically significant on all shoot metrics, though the acid treatment effect was not (Table 1). Interactive effects between species, acid, and sulfate were documented as significant in leaf counts, plant diameter, and stolon length metrics (Table 1, Figures 1-4). All metrics had significant sulfate treatment effects (Table 1, Figure 1). The species by acid by sulfate interactions recurred for several measurements (Table 1, Figures 3-4, 6).

Variety and iron sulfate direct effects proved significant on shoot dry weight (Table 2, Figure 5). Notably, species effects proved insignificant on the aforementioned metric. Interactions of variety and sulfate, as well as species by acid by sulfate, also proved significant (Table 2). Root dry weight exhibited significant species, varietal, and iron sulfate effects, though a species by acid by sulfate interactions was also documented as significant (Table 2, Figure 6). Root and shoot masses suggested that varietal differences existed within RBC, though white clover produced more root mass compared to RBC (Figure 6). Root-to-shoot ratios were insignificant.

Discussion

White clover outperformed running buffalo clover on most metrics of growth, vigor, and dry weight in all treatment conditions (Figures 1-4, 6). Given the poorer performance of running buffalo clover relative to white clover, it appeared that running buffalo clover presently wouldn't perform well in mine range applications. However, some specific accessions might be better suited for future commercial applications. Further comparative studies would better evaluate the diversity of potential wild varietal candidates (Figures 1 and 5). The genetic diversity of RBC offers a suite of potential sources for reclamation, and additional data suggests that accession 31415 represents the most useful agronomical accession of this study. Given white clover's superior agronomical performance, running buffalo clover appears an ineffective candidate for mine reclamation purposes (Tables 1 and 2).

The persistence of running buffalo clover in vermiculite medium—a first in its research history—as well as the prolific root mass suggest a suitability for reclamation applications. Initial and final evaluation of root-balls suggested a firm establishment in the destitute media. Given the historical challenges of repatriation efforts in favorable sites, the study's experimental protocol might aid future investigations into proper transplantation and repatriation protocols (Smith 1998; Windus and Finfera, personal communication).

Running buffalo clover performance varied among accessions. Whereas USDA 31415 performed comparably to white clover, accessions 641566 and 22231 failed to grow as prolifically (Figures 1 and 5). As a wild plant, this variation would be expected from a plant with geographically and ecologically isolated gene pools (USFWS 2007, USFWS 2011). This variance in performance under common conditions suggests that running buffalo clover populations vary greatly genetically. Importantly, given all accessions used came from Kentucky material, range diversity might in fact surpass the presently observed diversity (Figures 1 and 5).

With additional accessions from other source populations across the species' range, further investigations might determine both agronomic and conservation information to explore the species. The present study compared only three accessions, all hailing from Kentucky. Sampling may not encompass the diversity in other Kentucky populations and may not provide the present results in additional replicates; the species diversity may be much more variable than presently represented in greenhouse examinations (Crawford 1998, USFWS 2011). More vigorous, hardy populations might perform better than the accessions currently available, or investigations may prove 31415 to be an outlier of the species (Figures 1 and 5). Morphological characterization might stand in for genetic analysis to determine inter and intra-population responses to similar adverse conditions. Further investigations might include unique RBC populations from elsewhere in the species' range (Crawford 1998). Ohio or West Virginia material might better persist than the Kentucky accessions used.

Despite the present conclusions, future studies may find running buffalo clover accessions that may encourage mine reclamation applications, and conservation strategies may experiment with mixed repatriation systems, such as using a grass nurse crop to hold soil during the clover's establishment. Field studies of establishment dynamics in similar conditions should follow from greenhouse evaluation of accession; ideally monitoring programs similar to current conservation measures would improve such field studies (Perkins 2015).

Acknowledgements

The author would like to thank the USDA Germplasm Resources Information Network, Department of Horticulture and Crop science, Justin Morse, and the Kottman Hall greenhouse staff.

Additionally the author would like to thank the conservationists protecting running buffalo clover populations, including Rick Gardner, Jennifer Finfera, and Jennifer Windus. Their management of the species directs future research and conservation efforts for the species.

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Appendix 1: Study Timeline, December 2016 to March 2017

16 December	RBC specimens potted up in vermiculite
11 January	White clover specimens potted up in vermiculite
17 January	White clover specimens topped off with 2-3 cm additional vermiculite RBC and White clover pots rotated
20 January	Treatments start Initial destructive root and shoot measurements
8 February	Intermediate shoot measurements Pots rotated
22 February	Intermediate shoot measurements Media pH measurements Pots rotated
10 March	Pots rotated All pots topped off with additional vermiculite
24 March	Intermediate shoot measurements
26 March	Final destructive root and shoot measurements

Appendix 2: Treatment Schedule

1-Pure 6 N Treatment

Batch	Sulfur weight (g)	Volume solution (L)	Applied volume (mL)	pH	Dates
1			100	6.5	20
					23
2			100	7.15	25
					27
			50		30
					1
3			25	6.9	3
					6
					8
			50		10
					13
					15
					17
					20
					22
					24
					27
4			50	7.5	1
					3
					5
					7
					10
					13
					15
5			50	7.13	17
					20
					22

2-Acidic 4 N Treatment

Batch	Sulfur weight (g)	Volume solution (L)	Applied volume (mL)	pH	Dates
1			100	4.6	20
					23
2			100	3.8	25
					27
			50		30
					1
					3
					6
3			25	4	8
			50		10
					13
					15
					17
					20
					22
					24
4			50	4	27
					1
					3
					5
					7
					10
					13
					15
5			50	4.2	17
					20
					22

3-Sulfuric 6 Y Treatment

Batch	Sulfur weight (g)	Volume solution (L)	Applied volume (mL)	pH	Dates
1	8.6866	3	100	6	20
					23
2	17.3737	6	100	5.97	25
					27
			50		30
					1
			25		3
					6
3	17.4664	6	25	6	8
			50		10
					13
					15
					17
					20
					22
					24
					27
4	17.3912	6	50	6.15	1
					3
					5
					7
					10
					13
					15
5	17.3615	6	50	6.04	17
					20
					22

4-Acidic and Sulfuric 4 Y Treatment

Batch	Sulfur weight (g)	Volume solution (L)	Applied volume (mL)	pH	Dates
1	8.8683	3	100	4.9	20
					23
2	17.3763	6	100	4.85	25
					27
			50		30
					1
					3
					6
3	17.3328	6	25	5.3	8
			50		10
					13
					15
					17
					20
					22
					24
4	17.3744	6	50	5.2	27
					1
					3
					5
					7
					10
					13
					15
5	17.3548	6	50	4.85	17
					20
					22

Appendix 3: Significant Effects and Interactions Tables

Table 1. Significant effects and interactions on shoot variables

Shoot Variable	Treatment Effect or Interaction	Pr>F
Vigor	Species	0.0129
	Variety	0.0329
	Sulfate	0.0284
Active Growing Points	Species	<0.0001
	Variety	0.0375
	Sulfate	0.0003
	Species by Sulfate	0.0344
Leaves	Sulfate	<0.0001
	Species by Acid by Sulfate	0.0006
Diameter	Sulfate	0.0002
	Species by Acid by Sulfate	0.0266
Stolon Length	Sulfate	0.0009
	Species by Acid by Sulfate	0.0114
Stolon number	Sulfate	0.0004

Table 2. Significant effects and interactions on dry weight variables

Dry Weight	Treatment Effect or Interaction	Pr>F
Shoot	Variety	<0.0001
	Sulfate	<0.0001
	Variety by Sulfate	0.0440
	Species by Acid by Sulfate	0.0003
Root	Species	0.0378
	Variety	<0.0001
	Sulfate	<0.0001
	Species by Acid by Sulfate	0.0031

Appendix 4: Pedagogical results from the study and its implications for running buffalo clover applications

This experimental study led the author to investigate the basic biology, natural history, and conservation strategies of running buffalo clover. As a consequence of the species' relatively unexplored biology, the author developed a better understanding of the experimental and policy explorations undertaken regarding the clover, as well as the specific needs of future investigations. Experimental design, planning, and execution allowed for a better grasp of agronomic research and approaches.

The current regulatory restrictions on running buffalo clover present challenges to best characterize the species in its present state. Understanding pollinator dynamics, repatriation protocols, and characterizing genetic diversity require specimen access as well as proliferation ex situ (Perkins 2015). The present study highlighted the importance of accessibility to plant material in exploratory research (Perkins 2015).

Due to the relatively unexplored basic biology of the species, running buffalo clover may serve as a suitable species for classroom investigations (Perkins 2015). With such simple biology to presently understand, the species may serve as a pedagogical tool to biological, agricultural, and environmental education in future years. Perhaps the species may end up as a model organism for botanical conservation.