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

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## INTRODUCTION

Running buffalo clover (RBC) is native to Ohio, however, loss of prairie habitat has resulted in it becoming a federal endangered species [2]. Presumed extinct around 1940, researchers rediscovered small populations in the mid-1980s along the Ohio River corridor. 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 [2].

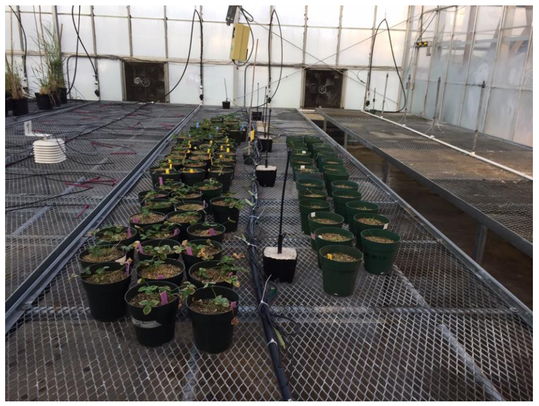
## AIM

The objective of this study is to determine the tolerance of RBC to acidic and sulfuric/ferrous soil [1]. The hypothesis is that a commercial or private utility may encourage larger reintroductions and invigorate new interest in a deserving, endangered endemic species [2, 3, 4]. Previous research has investigated the forage potential of RBC, however, it has failed to outperform related species, white clover (*Trifolium repens*) [3].

## METHODS

In this study, three strains 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/L) in the Kottman greenhouse [1]. The experimental design was a 4 x 2 x 2 factorial treatment structure, with a randomized complete block arrangement for the four replications (64 pots in total). Plants were established from stolons in December 2016, and grow in vermiculite to facilitate measurements on roots. Intermediate shoot measurements were taken on 8 February 2017 and 22 February 2017. Final shoot measurements were taken 24 March and destructive root measurements took place 26 and 28 March. Stolon measurements, as well as plant dimensions, entered the second round of non-destructive measurement. All data went through the SAS program.

### Specimens establishing before treatments



*Study specimens January 13, 2017. Specimens on the left all represent the three strains of running buffalo clover (Trifolium stoloniferum). White clover (Trifolium repens) seen left.*

### Initial root ball observations


*Initial root observations from January 20, 2017. RBC accessions are to the right and two white clover specimens are on the far left.*

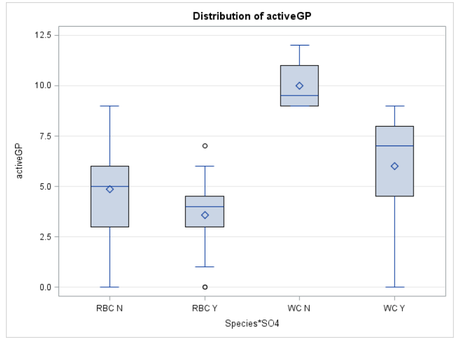
## RESULTS

When the nutrient solution treatments began on 20 January 2017, 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.
The persistence of RBC in vermiculite medium—a first in its research history—allowed for data collection. After a few months pH and sulfur treatments, root mass and aboveground growth was measured. After four weeks eight significant interactions came from statistical analysis. The most significant results from varietal responses to main effects and interactions are displayed here. After nine weeks of treatment, the iron sulfate treatment effect was statistically significant on all dependent variables, though the acid treatment effect was not. Interactive effects between species(variety), acid, and sulfate were documented as significant. Root and shoot masses suggested that varietal differences existed within RBC, though white clover produced more root mass compared to RBC. Root-to-shoot ratios were insignificant.

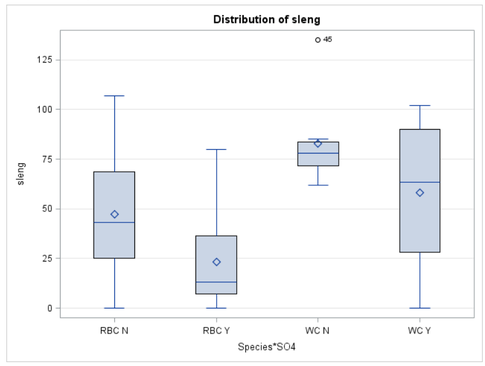
### Replicates four weeks into treatment

*Single replicate of the study. Replicates consisted of all varieties and both species with a specimen from each undergoing one of the four treatments. Replicates were laid out in a randomized complete block design.*

### $FeSO_4$ Interactions: Active grow points and stolon length

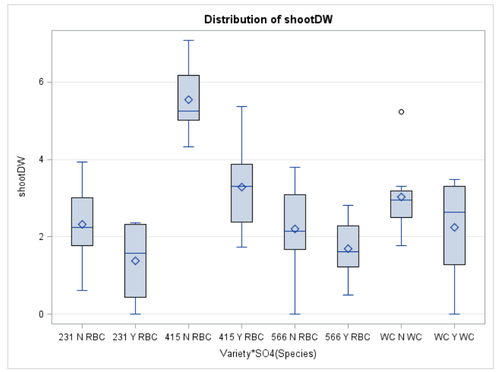



*Active growing point distribution from 24 March 2017. N and Y represent sulfur presence in a yes/no fashion, RBC and WC represent running buffalo clover and white clover respectively. (Pr=0.0244).*

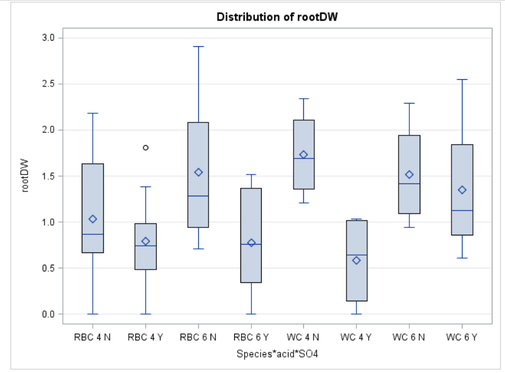



*Stolon length distribution from 24 March 2017. N and Y represent sulfur presence in a yes/no fashion, RBC and WC represent running buffalo clover and white clover respectively. (Pr =0.0009).*

### Treatment Interactions: Shoot and Root dry mass




*Shoot dry mass distribution from 28 March 2017. N and Y represent sulfur presence in a yes/no fashion. (Pr =0.0440).*




*Root dry mass distribution from 28 March 2017. N and Y represent sulfur presence in a yes/no fashion, RBC and WC represent running buffalo clover and white clover) respectively. (Pr =0.0031).*

## CONCLUSIONS

Should running buffalo clover have outperformed white clover, it might have recover introduced, reclamation populations serving to revegetate their endemic range on protected habitat. Specific accessions might be better suited for future commercial applications though further comparative studies would better evaluate the diversity of potential wild varietal candidates [6]. The genetic diversity of RBC offers a suite of potential sources for reclamation, and additional data suggests that accession 415 represents the most agronomically useful accession of this study. At present, white clover appears to best RBC.

### Root ball destructive sampling

*Root balls of Second Replicate organized by accession and treatment March 27, 2017*

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## 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.*

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