PLOIDY AND FITNESS LEVELS OF BIG BLUESTEM (*Andropogon gerardii*) POPULATIONS IN SOUTH-CENTRAL ONTARIO: IMPLICATIONS FOR SEED QUALITY AND RESTORATION

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Abstract

Ploidy and fitness levels of Big bluestem *(Andropogon gerardii*) populations in South-Central Ontario: Implications for seed quality and restoration

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Ecological restoration of grassland ecosystems is increasing in scope in Ontario, as a result the demand for genetically appropriate, high-quality seeds of native plants is also increasing. This mixed methods study characterized fitness and seed quality traits using genetic, demographic and growth trial data for a keystone tallgrass prairie species Big bluestem (*Andropogon gerardii)*. To estimate the ploidy levels in Big bluestem, our flow cytometric results indicated an average of 6.32 picograms of nuclear DNA within sixteen populations surveyed showing that hexaploid (6x) cytotypes are dominant in Southern and Central Ontario populations, aside from one 9x occurrence in Norfolk county, ON. Seed quality, measured through germination and viability testing did not change based on whether a population was remnant or restored. Concerningly, our study shows that remnant populations of Big bluestem are at risk of being lost as high quality seed sources likely because of the absence of stewardship and the resulting loss in population fitness. Workshops with prairie restoration practitioners suggest that there is significant vision behind the future of this work in the province, and that an ecosystem wide seed strategy for keystone tallgrass prairie species is a necessary next step to increase the sustainability of seed-based restoration strategies and preserve remnant site genetics.

Keywords: Ecological Restoration, Big bluestem (*Andropogon gerardii*), fitness, seed quality, polyploidy, flow cytometry, remnant populations, practitioners.

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General Introduction

Tallgrass Ecosystems in North America

Pre- European settlement, Central North America was once blanketed in grasslands that produced biodiverse communities of plants and animals. These grassland systems were part of a landscape mosaic of open prairies, wet meadows, semi-treed savannas and open woodlands. Water features intertwined throughout these forb and graminoid dominated communities to create ideal breeding zones for birds and amphibians. Potholes, rivers and streams supported large populations of fish (Jacobson et al., 2017). These interconnected communities were maintained for millennia by Indigenous peoples, who sustained this patchwork ecosystem through active stewardship. Cultural burning invigorated grassland plants, which attracted game to the area and prevented tree encroachment (Andersen 2009; Spiesman et al., 2018; Bach & Kleiman, 2021).

Tallgrass prairies species composition is unique compared to the short grass and mixed grass prairies of the more arid mid west regions of North America (Watkinson, 2023, verbal comm). Tallgrass ecosystems feature lush tall warm season grasses and a high diversity of forbs. Their vegetative diversity is immensely important for supporting the hundreds of species that rely on the dense graminoid cover and consistent blooms of forbs for habitat, life cycle cues and food sources (Gayton, 1992). Both plant and animal species that thrive within these complex ecosystems are highly adapted for the extremes of the landscape; high winds and light, drought conditions, fire, as well as grazing pressure and killing frosts are all typical of prairie environments (Waramit, 2010). Warm season grasses, also known as C4 species are the dominant vegetation type in tallgrass ecosystems and they have evolved a photosynthetic system that allows them to thrive in hot, drought prone environments (Gayton, 1992). During photosynthesis they assimilate carbon dioxide (CO^2) within their bundle sheath cells, instead of the mesophyll cells where C3 or cool season photosynthesis takes place. The result is the plants' ability to accumulate CO^2 molecules in large amounts, limiting the production of oxygenase which leads to less stomatal opening pressure and a two fold reduction in transpiration rates and water loss, compared to C3 plants (Waramit, 2010). This efficient use of resources allows C4 plants to allocate resources to below ground growth when water availability is scarce and explosive above ground growth when water availability is high. Leaf tissue in C4 plants can also be produced at a low carbon cost due to lower leaf tissue density compared to C3 plants (Edwards et al., 2010; Spiesman et al., 2018). This essential function is responsible for one of the most productive ecosystems in North America (Packard & Mutel, 2005).

Grasslands are globally important to carbon storage and act as carbon sinks. Their Net Primary Productivity (NPP) often exceeds carbon losses from soil respiration which means they sequester more carbon than they release (McKee et al., 2019). According to a recent global review on the state of grassland soil carbon sequestration, sixty percent of grassland systems' NPP is allocated belowground, producing extensive root systems that make up to ninety percent of the systems total biomass. (Bai & Cotrufo, 2022). Not unlike old growth forest ecosystems, plant species in remnant prairies have significant relationships with arbuscular mycorrhizal fungi (AMF). AMF mine hard to access nutrients from the soil and deliver them in accessible forms to plant root systems, receiving sugars, amino acids and organic acids in return (Ohsowski, 2015; Koziol et al, 2022). These extensive root systems, and the AMF community they support catalyze the formation of mineral-associated-organic matter (MAOM). MAOM, is a very stable form of soil organic carbon (SOC) that is strongly bonded to minerals in soil and therefore has a long (decades to centuries) residence time before it re enters the atmosphere as CO^2, resulting in long term carbon sequestration (Bai & Cotrufo, 2022). Mature grasslands can accumulate larger and more stable pools of carbon below ground than many forest ecosystems above and below ground growth combined (Tölgyesi et al., 2022). Grasslands provide clear benefits to humans through ecosystem services like soil erosion control and flood mitigation, they obviously support massive biodiversity and carbon storage but receive almost no protection compared to other ecosystems like forests and wetlands (Zhao et al.,2020). This "Grassland Awareness Disparity" coined by Csaba Tolgyesi (2022) suggests that this under valuing of non forest vegetation , both presently and in the past is based in a European ideology. During colonization, the European sentiment of forests being the epitome of ecosystem succession was spread around the globe and resulted in the mis categorization of grasslands and savannahs as degraded forests. Europeans' arrival in North America rapidly converted grasslands to agricultural fields and urban developments and this phenomenon persists today. In Canada, crop land continues to replace pasture and grasslands at a rate of 250,000 ha per year. Between 2011- 2016, 4 million hectares of grassland was converted to annual agricultural practices. Sadly, less than 5% of tallgrass prairie exists in its historic range in North America, making it one of the most endangered ecosystems (Bai & Cotrufo, 2022).

Grassland Restoration

The primary goal of grassland restoration is to establish native herbaceous vegetation to catalyze the return of the essential ecosystem functions, be it in a historical prairie location or a new constructed location (Whillans, 2022, verbal comm). As an ecological application prairie restoration is a new and developing field. One of the first recorded attempts at a tallgrass prairie restoration took place at the University of Wisconsin -Madison in 1934. Curtis Prairie was a 25-hectare parcel of land that had been under field crop and pasture cultivation since the early 1920's.. For the first planting installment Conservation Corps workers harvested sod from remnant prairies nearby and planted straight into a dense cover of Kentucky blue grass. Much to the chagrin of local farm boys, the ecologist working on the Curtis project requested that the adjacent stone farm fence be scattered amongst the prairie site to replicate the scattering of stones left behind by glacial retreat. (Anderson, 2009; Jordan & Lubick, 2011). Since that time grassland restoration has evolved and practitioner insights have given rise to several effective restoration method, including prescribed burning, land preparation, invasive species management, seeding and planting strategies are used in combination to produce high quality prairie habitats (Rowe, 2010; Bach & Kleiman, 2021; Higgs et al, 2018).

Prairie protection and restoration has a long history in Ontario involving Indigenous and non-Indigenous groups. Indeed, in some areas like Walpole island the Ojibway have never stopped managing their prairies through burning (Bachowsky, verbal comm, 2022). Prescribed burning, in a western land management context was brought back onto the Ontario landscape in the 1970's. Since that time regional practitioner insights and research coming out of the American prairie states have given rise to several effective restoration methods including prescribed burning, land preparation, invasive species management and seeding and planting strategies which are used in combination to produce high quality prairie habitats (Rowe, 2010; Bach & Kleiman, 2021; Higgs et al, 2018). Collaborations across Ontario municipalities have resulted in a network of prairie researchers and practitioners and is well exemplified by the charity Tallgrass Ontario (TgO). TgO is a federally recognized charity whose mission is to identify and facilitate the conservation of tallgrass ecosystems in Ontario. It was officially established in 2000 with the aim to connect groups and individuals engaged in recovery efforts, promote scientific research into tallgrass prairies and accrue funding for undertaking restoration projects. In 1998, TgO produced a landmark document that detailed the state of remnant prairies as well as conservation priorities of Ontario's grasslands. The recovery report was updated in 2019 with pertinent research and restoration initiatives highlighted. Specific knowledge gaps identified in the report include two broad categories: The in-situ genetic diversity and quality of remnant prairies in the province and seed sourcing best practices for restoration. This thesis document endeavours to provide data to reduce the knowledge gaps on these key research priorities.

Quality Seed Sourcing for Ecological Restoration

Seed and plant material for ecological restoration must be well adapted and genetically fit enough to thrive in challenging landscapes. Certainly, the quality of the seeds used underlies the success of the restoration planting. Best practices for where and how to source native seed for restoration is considered a growing field of research and is scientifically unresolved due to the differing schools of thought and lack of empirical data surrounding its guidelines. (Bower et al., 2014; Pedrini & Dixon, 2020; Woolridge et al., 2022).

Previously, championing locally collected seed directly adjacent to the site in question was considered the only acceptable method based on the principles of local adaptation. This well known theory suggests that plants adapt to their native environments, giving local genotypes a "home site advantage" (Magnoli, 2020, Weber, 2021). Local adaptation is well documented in some species especially where dramatic environmental clines exist. In the perennial alpine forb, Firecracker penstemon (*Penstemon eatoni*) seeds from populations at high elevations where harsh winters are observed produced seeds with longer cold stratification requirements, while those from lower elevations required much shorter stratification time before germination (Meyer et al. 1995). This trait could inhibit high elevation ecotypes to establish a second generation at lower elevation sites with dissimilar winter conditions (Lesica & Allendorf, 1999). Therefore, as a starting point for vegetation restoration, championing local material makes good sense.

Increasingly, local seed transfer agreements with considerations for how patterns of genetic variation differ between species, scale and landscape type are being adopted by practitioners. Incorporating this level of complexity is valuable as some plants are strong habitat specialists and differentiate rapidly over macro scales and others are widely outcrossing generalists with much larger climate tolerances and show no fitness declines when moved away from the home site (Bower et al., 2014).

A nonnegotiable for sourcing seeds is that the material collected for use has good genetic diversity and is genetically adapted ; Seed should be of high fitness that leads to good seed quality (i.e. seed is viable and has an understood dormancy) (Bischoff et al., 2010; Gibson et al., 2016; Espeland et al., 2017; Galliart et al., 2020).

Intraspecific Ploidy Variation (IPV)

One of the primary concerns regarding moving native plant materials for restoration is the potential for outbreeding depression. Although infrequently recorded, outbreeding depression occurs when crossing occurs between far genetically differentiated sources of the same species and yields next generation hybrids with lower fitness traits, essentially obliterating the allele frequencies that were shaped by the local environment. A particularly damaging form of outbreeding depression can occur when species with Intraspecific ploidy variation (IPV) are mixed at a restoration site. Species with differentiated cytotypes or ploidies (different chromosome numbers in the DNA) are often breeding incompatible, this condition is referred to as Intraspecific ploidy variation (IPV) (Gibson et al., 2017). These cytotype crosses can result in extreme losses of fitness and fecundity in progeny and can destabilize resulting restored populations (Gibson et al. 2017). IPV is common in the Poaceae and Asteraceae families which are both are used widely in restoration. In a 2012 study in Iowa assessed 5 restoration plantings and found mismatched ploidy levels for three co-dominant grasses; *Panicum virgatum, Sorghastrum nutans and Andropogon gerardii* and one species of forb; *Amorpha canescens* (Delaney & Baack, 2012) . Considering the prevalence of IPV in common restoration plant families and the potential for it to cause significant outbreeding depression in resulting populations it is pertinent to explore the occurrence and distribution of polyploidy across tallgrass species.

Research Objectives and Research Questions

Inspired by the research priorities identified in Tallgrass Ontario's 2019 Recovery Plan for Tallgrass Ecosystems, this study aimed to gather genetic, demographic, laboratory, and social data towards understanding the quality of seed sources and fitness trait variation in the keystone tallgrass prairie species Big bluestem (*Andropogon gerardii).* The Three objectives for Chapter 1 and Chapter 2 of this Mixed Methods study are stated below with the accompanying research question:

1- The objective for Chapter 1 was to identify the cytotypes present within Big bluestem populations in South -Central Ontario. We hypothesize that prairie restoration could have resulted in cytotype mixing at restoration sites. A better understanding of ploidy distribution in Ontario could help avoid outbreeding depression in this species. Specifically, we asked:

i) What polyploid cytotypes are present within remnant and restored Big bluestem populations in South-Central Ontario; and what are the implications for grassland restoration?

2- The objectives for Chapter 2 were first to characterize the fitness trait qualities of remnant and restored Big bluestem populations to understand if both population types are good sources of seed for restoration. Specifically, we wanted to know:

i) Are there fitness differences between Big bluestem plants in remnant and restored populations?

The second objective for Chapter 2 was to identify if there are ideal seed sources and adaptive traits within remnant Big bluestem populations in South -Central Ontario. To address this, we asked:

i) Are there early growth fitness trait differences between Big bluestem seedlings from remnant Ontario populations when grown in a common climate chamber?

The objectives of Chapter 3 were to integrate the results of the quantitative chapters through a participatory action workshop. The results of Chapters 1 and 2 were presented to a group of tallgrass prairie practitioners and researchers in a participatory workshop. Chapter 3 summarizes and reports on the findings of this workshop. This data could ultimately be used to assist in developing a seed sourcing strategy for Big bluestem and other tallgrass prairie species in South-Central Ontario.

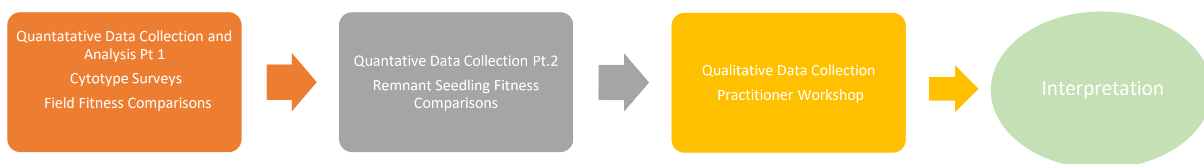
Methodology

Methodological Framework

To provide a concrete methodological foundation for this research study I used a Mixed Methods Research design. Mixed Methods designs are useful as a research approach because they can provide a richer understanding of complex research questions. Through meaningfully integrating quantitative and qualitative data, mixed methods provide a more complete picture of the research question and study than either method alone. This approach to research allows for contextual understandings of data that are culturally and situationally influenced (Creswell, Klassen, Plano Clark & Smith 2011).

Explanatory Sequential Mixed Methods

An Explanatory Sequential approach was used to structure the collection of different types of data for a more thorough understanding of the research topic and to inform any recommendations after the research process. The framework I employed had two interconnected phases. The first research sequence (Chapter 1 & 2) was a two-part biological study that sought to characterize genetic, demographic and laboratory features of Big bluestem populations, seeds, and seedlings. The quantitative data produced was analyzed to provide the foundation for the nature of the inquiry in the qualitative data collection. In the second and qualitative research phase a practitioner workshop session was hosted with a small set of knowledge holders to provide an opportunity to get to a deeper explanation of the concepts and add richness to the quantitative results. I chose an explanatory sequential mixed methods approach for my study because there is significant expertise around Ontario's tallgrass prairie ecosystems and the input of local experts is integral to include in any study on this rare ecosystem to ensure integrated context and accurate fact finding.



Above is a flow diagram depicting the research methods used in their respective order in each phase of the study. The first quantitative phase involved cytographical and fitness surveys of Big bluestem (*Andropogon gerardii*) in the field. The second quantitative step was a controlled observational study of remnant seedling fitness traits grown in a climate chamber. Qualitative data collection was used to follow up and ground truth quantitative findings in the form of a thematic practitioner workshop. The last phase of research is the "Interpretation" step where the qualitative findings are used to explain in more detail the quantitative results.

Chapter 1: Ploidy levels in remnant and restored populations of Big bluestem (*Andropogon gerardii*) in South-Central Ontario

1.1 Introduction

Plant biologists have been aware of the phenomenon of polyploidy since 1907 and it is still of research interest today especially in evolutionary and restoration biology today (Leitch et al., 2013). The occurrence of complex ploidies in common plant families has been described as the 'missing link' within the dialogue of seed sourcing for restoration. Cytotype variation is usually not determined prior to plant material use in revegetation projects but considering its major impacts on the reproductive capacity and ecological tolerances of species, more research and screening is required to avoid potentially lowering the long-term stability of restored populations (Gibson et al., 2017).

Polyploidy is the multiplication of whole chromosome pairs within the nucleus, resulting in a duplication of the whole genome. It is widespread among plants especially in the Asteraceae and Poaceae families and is generally characterized as either an allopolyploid or autopolyploid. Autopolyploids present as individuals with genomic multiplications within the same species while allopolyploids are plants with genome duplications because of hybridization between species or genome doubling of those hybrids. The biological mechanism responsible for autopolyploidization events occurs through meiotic error. The error occurs during meiosis through the production of unreduced gametes; unreduced simply meaning that pollen or ovule (gametes) produced will have double the number of normal chromosomes. When unreduced gametes combine during sexual reproduction the resulting progeny have double or in some cases nine times the base number of chromosomes. Research into meiotic processes suggests that this misfiring is not a random error, but inextricably linked to speciation and polyploidization. Increases in meiotic errors in plant families are positively correlated with environmental stressors, suggesting that polyploidization is triggered in response to changing environments and much less random than previously thought (Norrmann et al, 1997; Mason & Pires, 2015) .While it is unknown the exact mechanisms that cause species to develop ploidy systems, it has been shown that polyploid generation is strongly linked to harsh climate zones at the edge of natural ranges, indicating that it is a climate driven adaptation event. (Mason & Pires, 2015; Stuessy & Weiss-Schneeweiss, 2019).

Ploidy polymorphism, which is the maintenance of two or more types of ploidies or-cytotypes within a species, provides a platform for evolution through which species can extend their ranges and establish in new climatic niches. Possibly because each set of genomes can undergo selection and developing new gene combinations with differential expressions producing novel phenotypic traits. (Mason & Pires, 2015; Stuessy & Weiss-Schneeweiss, 2019; Husband et al, 2013; Karunarathne et al., 2018). Evidence for this phenomenon can be seen by the distribution of polyploids on the landscape; where more dense frequencies of higher order cytotypes can be found at the edge of species natural ranges.(Tompkins et al., 2015; McAllister et al., 2019) . In the species *Themeda triandra*, a foundational C4 graminoid species of the Australian grasslands, tetraploids are found only in the semi-arid reaches within its range and the levels of heterozygosity (genetic variability within the genome) between populations is positively correlated to higher temperature values and lower precipitation variables, therefore it's persistence in harsh environments seems reliant on auto-polyploidization events and maintenance of tetraploid individuals (Eichorn & Evert, 2013; Karunarathne et al., 2018).

Polyploids can show fitness advantages when compared to their diploid progenitors, these advantages could be attributed to high levels of heterozygosity within their populations. Extra sets of chromosomes create genetic redundancy in the genome which provides a buffer against the effects of inbreeding depression. This is because the fixation and inactivation of one set of genes by deleterious alleles is buffered by its multiple sets, so in order to experience the negative effects of a recessive mutation, there needs to be more than two or four copies of the deleterious allele before the effects are not masked by the dominant allele (Freeland, 2019). A test of this theory by experimental crossings of *Centaurea stoebe* tetraploids versus their non invasive diploid counterpart found that tetraploid individuals were much less likely to suffer from genetic inbreeding than their conspecific diploids (Rosche, 2017).

Polyploidy relevance in restoration

Restoration of grasslands in North America is increasing with wider recognition of their ecosystem services such as improving biodiversity and carbon sequestration, in turn generating higher demand for genetically appropriate seed to complete these projects. Restoration seed strategies include collecting and mixing seed and seedling population sources as well as combining provenances in seed production areas for maximizing heterozygosity at a site (Nevill et al., 2016; Delaney & Baack, 2012). Inter-ploidy variation (IPV) is common in plant families used for grassland restoration but identifying materials with IPV is extremely challenging in the field, therefore there is a risk of unintentionally mixing cytotypes at project sites (Gibson et al., 2017). Interbreeding between cytotypes has resulted in reduced fitness in resulting offspring and it is often the case that previously differentiated cytotypes are no longer able to successfully cross at all. Progenies of crosses can exhibit reduced seed set and poor seed viability. (Marsden et al., 2013; Gibson et al., 2017; Kramer et al., 2018).Crosses of cytotypes of *Andropogon gerardii* result in sterile or low fitness hybrids that are virtually nonexistent on the natural landscape (Keeler, 1990) .In other cytotype crossing experiments, progeny of specimens presented with seed endosperm abnormalities and low vigor Gibson et al., 2017). Interbreeding between different cytotypes can reduce reproductive potential within a restored population and result in reduced seed set, poor seed viability, and offspring that display low fitness. These factors could ultimately contribute to instability in grassland restoration plantings. (Tompkins et al., 2015). It is pertinent then for restorationists to understand the occurrence and distribution of IPV in all species being restored but especially keystone species that determine so many functions of the ecosystem.

Study Species: Big bluestem (*Andropogon gerardii*)

Big bluestem is a C4 perennial grass that is a keystone species in tallgrass prairie habitats. In some prairies it forms dense populations growing with other prairie grasses like *Sorghastrum nutans* and creates 80% of the ecosystem biomass (Moser & Vogel, 1995). Its natural range is extensive, spanning from Central Mexico to central Canada where it is typically found on deep well aerated soils (Owsley et al.,2020). Big bluestem florets are usually hermaphroditic or male and are largely self-incompatible, so it relies on out crossing through wind pollination to sexually reproduce and set seeds. This outcrossing nature results in high genetic diversity within the genome. Big bluestem will also reproduce vegetatively through tillers that produce clonal stands that often contain more than one genotype, in fact this is the dominant form of population persistence in Big bluestem (Gustafson et al., 2004) As well as being visually interesting with a unique three spikelet inflorescence that resembles a turkey foot, Big bluestem is genetically interesting. It is an autopolyploid complex with two predominant cytotypes. Hexaploids (2n=6x=60) are the dominant cytotype and are prevalent in the northern and eastern extents of tallgrass prairie's range. Enneaploids, (2n=9x=90), are theorized to have generated from hexaploids through several separate autopolyploidization events (McAllister & Miller, 2016). They occur intermixed with hexaploidy populations but increase in frequency in the southern and western portions of its range to form pure enneaploid stands (McAllister et al., 2015. Morphologically, it is difficult to distinguish any differences between the cytotypes, but they do have significantly distinct reproductive traits. Crosses between two cytotypes produces aneuploid progeny that are almost never found in natural populations, likely because of extremely low fitness(Keeler, 1990; Norrmann & Keeler, 2003; Tompkins et al., 2015).

Research Context:

In South-Central Ontario, Big bluestem is a keystone species in the pockets of tallgrass prairie remaining and is a major component of restoration seed mixes and planting projects, but little is known about its polyploid distribution in the province. To avoid outbreeding depression consequences between the hexaploidy (6x) cytotype and the enneaploid (9x) within seed production areas and at restoration sites, more information should be available to guide practitioners' seed sourcing decisions. Currently there has been no cytographic research in Ontario that investigates the distribution of Big bluestem polyploids and the resulting restoration implications. For these reasons this chapter will investigate**:**

a) What cytotypes are present within remnant and restored Big bluestem populations in South-Central Ontario?

b) What are the implications for grassland restoration efforts in Ontario?

1.2 Methods

Study Site Selection

With the help of the Nature Conservancy Canada (NCC), Tallgrass Ontario (TgO), Alderville Black Oak Savanna (ABOS) and the Ontario Ministry of Natural Resources and Forestry (OMNRF). I established sixteen study sites: eight remnant natural prairie and eight restored sites within OMNRF seed zones 34-38 on a general east to west gradient from Roseneath to Essex, Ontario. These sites were chosen because of their population size, enough individuals of reproductive age had to be present to ensure adequate pollination and seed set (Minimum population size = .2 ha) . Appropriate vegetation community also had to be present. In this case, one other tallgrass prairie indicator species including but not limited to : *Sorghastrum nutans, Panicum virgatum, Lupinus perennis.* The sites were grouped and sampled in a paired approach; each restored site corresponds to a geographically close by remnant site that is within the same eco-district to control for macro environmental variables like precipitation, soil type and topography. Initial site visits in the summer of 2020 were made to each site to determine that these features were present. Locations and coordinates of sampling locations can be found in **Table 1.**

Cytotype Surveys

For the ploidy surveys, 10-15 plants were subsampled randomly along a population transect. Sampling transects were established using the “Ignorant Man” sampling method cited in Hamelin (2015). Two metre wide transects were established using a randomly selected compass bearing, across the widest section of a population. This method attempted to ensure that researcher bias was mitigated and that the extent of each population was sampled. Two metre wide transects were used so that individual assessments were not performed on the same clone. Big bluestem plants encountered within the transect were arbitrarily assigned a number as the sampler moved along the compass bearing. Fifteen plants that corresponded to a list of random numbers were tagged and included in this study. The length of the transect corresponded to how quickly the random numbers came up within the plant counts. Individual plants (genets) were distinguished from other individuals within the populations through observing if stems were clustered or spatially discrete from there neighbours. Other visual characteristics to identify individuals included finding the growing crown of the genet in the middle of a cluster of tillers as there is often a ring of senescence around a central crown in mature individual plants. (Gartshore, verbal comms, 2020). If it was impossible to separate the edge of an individual from a neighbour, the sampler randomly selected the next apparent genet for measurement.

Ten plants were selected for ploidy tissue sampling and were collected according to Kron et al (2007). A minimum of two - five cm of green viable leaf tissue was sampled in the field from each plant and initially placed in coin envelopes. Samples were then transported to Trent University and immediately placed on silica gel and stored in a freezer until analyses were completed (Kron et al., 2007). All leaf samples were analyzed for ploidy DNA content using the standard methods according to the Husband Lab at Guelph University, which are briefly described below by McAllister (2015):

"DNA content estimates for all samples were made using flow cytometry (FCM), following the basic method of Galbraith et al. (1983). Ploidy levels were assigned to individual plants by comparing their 2C nuclear DNA contents with published 2C values for plants of known ploidy; we used rye (*Lolium multiflorum)* as the DNA content standard (16.19 pg/2C; Doležel et al., 1998). We also compared our DNA content estimates with previously reported DNA content measurements from chromosome-counted 6 *x* and 9 *x* plants of *A. gerardii* (Norrmann et al., 1997). Preliminary testing demonstrated that high-quality dried leaf tissue from *A. gerardii* produced estimates of DNA content very close to those of fresh tissue. Consequently, for this study, approximately 2 cm^2 of dried leaf tissue with midveins removed and 1 cm^2 fresh leaf tissue from the DNA content standard (S.

cereale) were chopped with a new razor blade in a petri dish with 1 mL ice-cold LB01 buffer (Doležel et al., 1989) containing 100 μg/mL propidium iodide and 50 μg/mL RNAse). After chopping for approximately 20 s to release nuclei into the buffer, the sample was filtered through a 30 μm Partec Celltrics filter. Samples were then centrifuged at 7600 × *g* for 10 s. After centrifugation, 300 μL of the supernatant was removed, and the pelleted nuclei were resuspended. The nuclei were stained for 30–60 min before testing. Samples were analyzed using a BD FACS Calibur flow cytometer. Samples were run for up to 5 min to acquire at least 1000 nuclei per G1 phase for both the standard and the test plant. We used the FL2 detector (585/42 nm) to measure propidium iodide fluorescence and analyzed fluorescence area (integrated fluorescence) histograms using ModFit LT for Mac software (Vers. 3.3.11, 2011; Verity Software House, Topsham, Maine, USA). We measured peak means, coefficients of variation (CV), and number of nuclei per peak."

1.3 Data Analysis

Ploidy and DNA Content Calculations

Flow Cytometry estimates the DNA content in relative units as an indicator of ploidy level. Cell nuclei are stained with DNA-binding fluorochromes, and the amount of DNA present is determined by measuring the fluorescence emitted when the nuclei are excited with light of a particular wavelength (Suda and Kron et al., 2007). DNA content is interpreted from the CV peaks in the FCM output and calculated from the equation:

Sample DNA content= Ratio (between Andropogon mean and standard mean) x Standard DNA content (pg/2c) =0.39 x 16.19 pg/2c **=** *6.3 pg/2c*

1.4 Results

Ploidy Estimates

Ploidy was estimated for 106 plants from 16 populations. N = 8 - 10 usable samples per population. Four samples were removed from the data set from the Ojibway Prairie Provincial Park populations because their DNA content far exceeded known and feasible ranges for *Andropogon* (> 15.0pg/2C). These values almost certainly represent biological material that is not *Andropogon gerardii* with sampling error likely being the cause (P. Kron, personal comm, March 2021). The remaining 102 plants in the data set showed distinguishable ploidy variation typical of the two common cytotypes (6x and 9 x) .

The first data range with histogram peaks between 5- 7.8 g / 2C represents 101 individuals out of the 102 sampled **(Figure 1**). This cluster is made up of individuals with an estimated DNA content between 5.3 and 7.7 pg of DNA per nucleus. The average DNA content for the individuals in this data set is 6.32 pg/2C +/- 0.41 SD. These values are consistent with results from other flow cytometry studies of *Andropogon gerardii*. DNA content and estimated ploidies from several eastern *Andropogon gerardii* populations in North Carolina was reported by Tompkins et al in 2015. Their sampled populations showed a mean DNA content of 6.36 pg/2C with a range from 5.96–6.70. Other results from midwestern hexaploid populations have reported value ranges between 5.39 (McAllister, 2015) and 7.47 (Keeler et al 1990, 1992, 1997, 2002, 2004). The second outlying peak in our FCM showed one individual with a DNA content of 9.64 which is aligned with reported values for enneaploid DNA ranges and cytotypes (2n = 9x= 90 chromosomes). Midwestern enneaploid populations, both intermixed and enneaploid have been shown to consistently produce nuclear DNA content ranging from 8.44-11.0 pg/2C (Norrmann & Keeler, 2003; McAllister & Miller, 2016; Tompkins et al., 2015).

Ploidy Trends in South-Central Ontario

Cytotype surveys taken from remnant and restored prairies on a general East to West gradient in South-Central Ontario revealed that the overall pattern within the 16 sites and the Big bluestem plants sampled was hexaploid (n=106).

This result was expected for remnant sites as the general distribution of higher order polyploids (9x's) tend to be correlated with hotter more arid environments within its continental range. South-Central Ontario is typified by a warm and moist climate, however, we hypothesized that with the last 50 years of prairie restoration activity in the region enneaploid populations could have been established from non-regional seed. This was overwhelmingly not the case, with restored prairies in the survey displaying similar nuclear

DNA content to similar remnant sites. All sites displayed 100% hexaploid plants except for one sample found at a restored site in Norfolk County which interestingly showed the occurrence of a single enneaploid plant. Our results fit into the general understanding of Big bluestem's cytotype distribution, indeed surveys from North Carolina, Michigan, and further Midwestern States show a general East to West hexaploidy to enneaploid trend, likely driven by precipitation. (Gray et al., 2014; McAllister et al., 2015).Our findings complete a missing piece in the understanding Big bluestem's ploidy distribution across North America **(Figure 2).**

1.4 Discussion

Ploidy levels of Big bluestem Populations in Ontario

The FCM data indicates that there is a dominant cytotype of Big bluestem (*Andropogon gerardii*) within our study area. Our surveys show that 102/103 samples had a mean of 6 pg of nuclear DNA, which is indicative of a hexaploidy plant.

Our cytotype surveys took place on the eastern edge of Big bluestem's range associated with tallgrass prairie ecosystems in Ontario. At the time of this research no other data set exists for polyploid characterization of Big bluestem plants in Ontario, however there are several research studies from the Midwestern States and the Carolina's that have characterized ploidy distribution within restored and remnant tallgrass prairie populations. Specifically, Keeler provided foundational research into understanding polyploid variation in Big bluestem, they surveyed 600 plants from 15 prairies in Midwestern states and found hexaploid plants to make up the majority of plants sampled (Keeler,1990) . McAllister built upon Keeler's work in 2015 and mapped the distribution of polyploid polymorphism within remnant prairies from Minnesota to Texas and Kansas to Ohio. They reported that populations of the higher order enneaploid plants could be found at the far south and west of the species range in Texas but populations in the Great Lakes – Northern Plains region in Michigan, Nebraska and the Dakota's were predominantly hexaploidy in cytotype (McAllister et al., 2015) Tompkins' study adds another piece to the polyploid geography puzzle, in his research characterizing the chromosomal DNA content (ploidy) levels of eastern Big bluestem individuals in small pockets of remnant prairie in the Carolinas, he noted that out of the six remnant sites he sampled from, the three northern accessions were hexaploid but the three more southern sites were enneaploid dominant. Finding enneaploids east of the Missouri River somewhat contradicts the theory that ploidies of Big bluestem are spatially organizing on an east to west gradient but instead suggests that a warmer climate and lower precipitation volumes could be a better predictor of the occurrence of enneaploid plants. It is evident from these studies that there is a clear trend toward hexaploid plants monopolizing populations in the northern and eastern portions of Big bluestem's range in the United States and higher order polyploids (9x's) occurring in much higher frequencies on the western and southern edges of the species range. Our results are consistent with this general cytogeography pattern of Big bluestem.

We found one enneaploid plant at a restored site in the Norfolk sand plain watershed in Norfolk County, ON. This occurrence gives rise to two theories; the first is that there is natural interploidy variation being maintained within some remnant populations of Big bluestem in Southwestern Ontario or this could also indicate that seed from elsewhere was translocated in the past and became established.

The first theory is supported by existing knowledge on the distribution and maintenance of multiple ploidy populations. Researchers Keeler (1990), Norman (1997), McAllister (2015) and Tompkins (2016), noted that as well as existing in homogenous cytotype populations, variation in ploidy is found within Big bluestem populations as well and that mixed populations occur more frequently than previously thought. Mixed cytotype populations seem to follow the same general spatial patterns in so far as the proportion of hexaploids to enneaploids decreases as you travel west and south in the species range. DNA content data produced in McAllister's surveys in the Northern and Great Plains, USA, indicated that enneaploid plant frequency ranged from 1 or 2 plants per site on the Michigan border with Windsor, ON to making up 80-100% of some populations at the western extreme of their range in Texas. Further, in 2015, McAllister provided novel and significant research towards understanding environmental and climate correlates of different ploidies of Big bluestem. They found that the occurrence of higher order polyploids was in fact not random and that they were ecologically sorting. Decreased summer precipitation and increased variation in diurnal and seasonal temperatures at locations were significantly correlated with the occurrence of 9x plants (McAllister et al., 2015). Norfolk County, and Ontario's Southwest corner falls within Ontario ecoregion 7E, the mildest Ontario climate, typified by long, hot and humid summers and ample precipitation.(Crins et al., 2009). It also has a higher diurnal and seasonal temperature variation, when compared to the eastern adjacent ecoregion 6E so it could provide the conditions for enneaploid generation and maintenance in low frequencies.

Enneaploid plants develop from hexaploids through autopolyploidization events. During meiosis a normal gamete fuses with an unreduced one, resulting in mixed populations that are diploid: triploid complexes. (Bretagnolle & Thompson, 1995; Normann,1997). Interestingly, stress in the growing environment, such as drought, temperature extremes and pathogens has been correlated with an increase in meiotic errors in many plant taxa, which results in the production of unreduced gametes. Polyploid development theories recognize this unreduced gamete production and subsequent autopolyploidization events as an adaptive response to environmental edges and a step towards speciation (Husband et al, 2013; Mason &Pires, 2015; Karunaratne et al, 2018).

The second theory to explain the occurrence of an enneaploid plant is that seed from another region was transplanted into the restored site. Although based on the restoration history available for this site, establishment seed can be traced to remnant populations in Norfolk County, therefore it seems more likely that the enneaploid plant was a product of autopolyploidization rather than seeds transfer. Based on the solitary occurrence of an enneaploid plant in this study and the fact that enneaploid plants have an inferior fecundity and low seedling establishment rates (Normann & Keeler, 2003); it seems fair to say that enneaploid plants do not contribute a significant genetic load to Ontario populations of Big bluestem. It does suggest that South-western Ontario is a region where enneaploids are generated and maintained in low frequencies.

1.5 Restoration Implications

Reproductive fitness of cytotypes

Restored plant communities reflect the values of the people involved with establishing them and they often serve multiple functions. Some of these include returning ecological processes to the ecosystem, connecting fragmented habitats, and providing a refugia for high quality seed sources. For populations to persist over time and meet the goals of restoration, plant material needs to be appropriately adapted and contain adequate levels of reproductive fitness (Oldfield, 2015; Nevill, 2016; Espeland,2017; Weber, 2021).

In the case of Big bluestem, ploidy type should be considered when matching the plant material to the goals of the restoration project. From a reproductive fitness perspective, Big bluestem cytotypes are dramatically different. Hexaploids, the eastern dominant type, are described as the more robust cytotype based on their reproductive traits. They typically have higher seed viability and seedling establishment rates while enneaploids tend to be bigger plants with more vegetative growth which might contribute more seed per plant, but the viability of seed is quite reduced. (Keeler, 1990; McAllister et al., 2015). Enneaploids make better pollen parents than seed parents as fertility or seed producing capacity is related to the quality of the gametes, which in the case of 9 x is abnormal and prone to abortion, meaning there are less available for seed production. (Norrman, 1997).

It is common that post autopolyploidization events, differing cytotypes are no longer capable of interbreeding successfully at all. Cytotype crosses can result in extreme losses of fitness and fecundity in progeny and can destabilize resulting restored populations. In a literature review compiled by researchers at the University of Montana, they noted that crosses between diploid (2x) and tetraploid (4x) plants were shown to reduce seed set compared to the same-cytotype crosses by up to 66%. As well, they reported that seeds from interploidy crosses have reduced fitness and can suffer from abnormalities in seed endosperm (Gibson et al., 2017). Crosses of cytotypes of *Andropogon* have been reported to have similar fitness reductions; In a breeding experiment, hexaploid plants crossed with hexaploids were more fertile than hexaploids crossed with higher order polyploid plants, which were more fertile than enneaploids crossed with enneaploids so, relative reproductive rates in hexaploid populations are reduced with the introgression of enneaploids (Tompkins, 2015;Gibson & Nelson, 2017).

Hexaploids provide pollen with n=30 and enneaploids would provide n=35-50 so resulting seeds, and therefore plants could contain anywhere from 2n=65 to over 100 and are not predictably one cytotype or another. Typically, these crosses produce offspring of abnormal chromosome numbers (7x or 8x) and are referred to as aneuploids. *Andropogon* aneuploids are rare to find in mixed populations. Keeler's (1990) extensive study on mixed ploidy populations of *Andropogon gerardii* suggest that less than 0-5% of mixed ploidy populations are made up of these aneuploid individuals. Aneuploid plants' inability to persist in populations suggests that the crosses have low fitness levels, at least in comparison to their hexaploidy and enneaploid progenitors, whether that is because of sterility, low seed viability or poor seedling establishment is unclear and probably a combination of all three (Tompkins et al, 2016).

If the goal is to establish populations of Big bluestem for the purpose of seed collection, then matching ploidy types should be prioritized. Mixing ploidies where they are not naturally occurring could lead to outbreeding depression that would result in reduced fertility within the stand. Characterizing the cytotypes of Big bluestem plant and seed material used for restoration is recommended as a best practice especially if the seed is coming from a new supplier, is coming from outside of Ontario or from commercial cultivars with unknown ploidy levels. From the literature, the 6x plant has shown to be more fecund than the 9x plant and in the scope of this study and others, to be the dominant-native cytotype to the eastern tallgrass prairies.

1.6 Tables and Figures

Table 1: Sample populations location and site type.

Site	n	Coordinates	Ecoregion- District	Type
Alderville Black Oak Savanna (ABOS)	9	44°10'22.39" 78° 5'6.58"	6E-13	Remnant
Red Cloud	8	44° 9'6.81" 77°56'17.16"	6E-13	Restored
Pinery	10	43°14'53.65" 81°49'20.77"	7E-1	Remnant
Hazel Bird	10	44° 5'55.73" 78° 9'40.84"	6 E-13	Restored
Black Oak Heritage Park (BOH)	10	42°16'07.7" 83°05'14.9"	7E-5	Restored
Peters' woods	10	44°07'32.2" 78°02'25.7"	6E-13	Restored
Ganaraska East	10	44°05'10.9" 78°21'44.3"	6E-8	Remnant
Holland Landing	10	44° 7'15.76" 79°29'29.33"	6E-6	Remnant
Delhi Railway	10	42°50'48.1" 80°31'12.7"	7E-2	Remnant
Dutton	10	42°38'35.0" 81°32'08.0"	6E-6	Remnant
Van-Hove	10	44° 8'52.10" 77°56'32.62"	6E-6	Restored
Kenesserie prairie	10	42°29'12.20" 81°51'36.75"	7E-1	Restored
Ducks Unlimited (DULP)	10	42°35'21.8" 80°28'01.9"	7E-2	Restored
Nature Conservancy (NCC)	10	42°37'41.5" 80°28'46.8"	7E-2	Restored
Mary's Farm	10	42°38'33.84 80°34'29.15"	7E-2	Restored
Ojibway Prairie (OJP)	13	42°15'26.59" 83° 4'11.80"	7E-5	Remnant
Rondeau	8	42°19'2.50" 81°50'49.55"	7E-1	Remnant

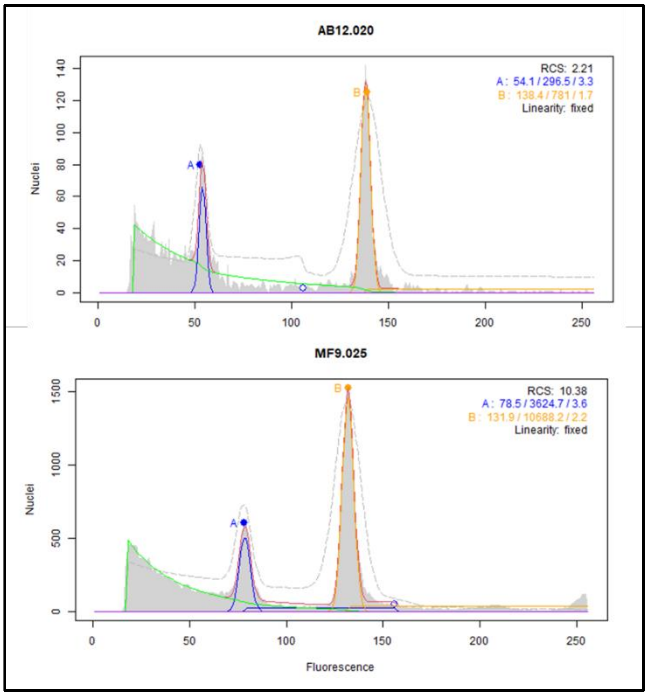


Figure 1: Cytometric profile of nuclear DNA content for Big bluestem *(Andropogon gerardii)* plants relative to the standard Ryegrass (*Lolium perenne)*. Greater fluorescence values are indicative of more DNA content in the nucleus. Top Profile: Peak 'A' has 6.3 pg/ 2C. Bottom Profile: Peak B has 9.6 pg/2C.

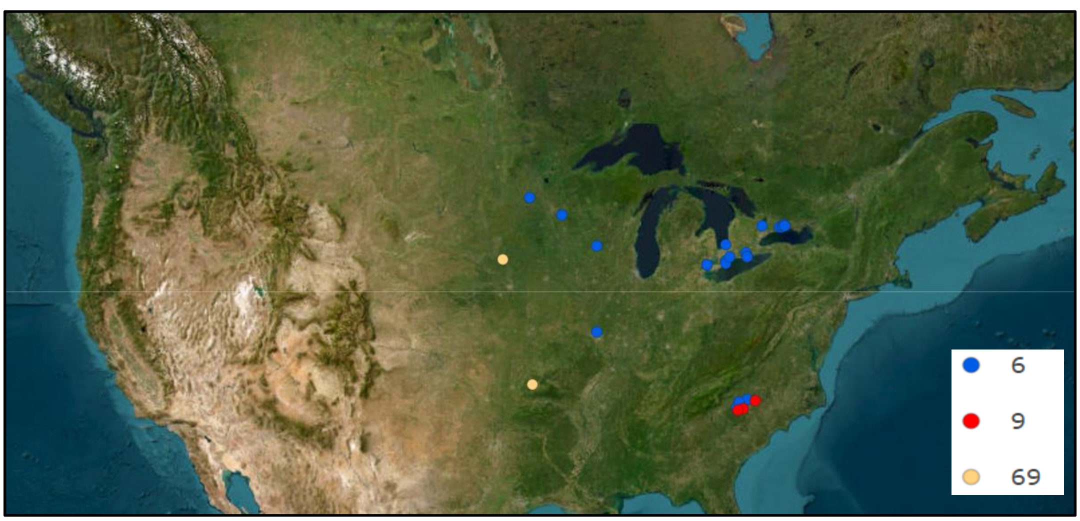


Figure 2: Distribution of cytotypes of Big bluestem across North America. Blue points indicate hexaploid (6x) populations, red points indicate enneaploids (9x) and yellow points indicate a mixed population of 6:9's. Data pooled from this study's data set as well as McAllister et al (2015) and Tompkins et al (2015).

Chapter 2: Fitness Traits of Big bluestem (*Andropogon gerardii*) populations in South - Central Ontario

2.1 Introduction

The quality of seed sources used directly affects restored population outcomes (Pedrini & Dixon 2020; Schmidt et al., 2019). Therefore, understanding the fitness traits of plants and seeds within population sources and how they vary on the landscape is beneficial for selecting high quality native plant materials and informing seed sourcing strategies (Woolridge et al., 2022).

Fitness is an expression that has different meanings depending on the school of science. In population genetics, relative fitness is a calculation that quantifies the contribution of a genotype to a population by dividing absolute fitness (reproductive contribution) by average fitness of the population. Ecologists primarily use "fitness" as a term to describe reproductive success and how well adapted an individual is to its environment (Primack and Kang, 1989; Reed et al, 2003).

For the purpose of this study, fitness is characterized as the reproductive success of a particular genotype and is a function of a plants' successful establishment and survival. Ecologists commonly use proxies to estimate plant fitness in the field and in greenhouse settings. Frequently used metrics include size and reproductive measurements as the capacity to acquire resources and put them towards growth and the next generation is strongly positively correlated with long term survival and greater resistance to competition. A large body of work on Big bluestem has shown that biomass is positively associated with fecundity measurements, which increase the chance of plants contributing successful offspring (Primack & Kang, 1989; Reed et al., 2003; Tompkins.,2011; Hamelin.,2012;

Gibson et al., 2013; Anderson, 2016, Younginger et al., 2017). Traits that are borne to individuals with high average fitness will increase in frequency in successive generations. Alternatively, when fitness in individuals is reduced, the frequency at which beneficial traits can be passed on is constrained and can result in contracting populations with low adaptive potential, making these poor sources for restoration material (Anderson, 2016; Higgs et al., 2018).

Numerous factors contribute to, and impact traits related to fitness in plant populations. Habitat fragmentation can increase inbreeding instances and reduce progeny seedling vigour, competition from non native plants can reduce the biomass and survival rate of native plants and maladaptation from seed to site mismatches can constrain seed production.(Hamelin, 2012; Jauni & Ramula, 2015 ; Aguilar et al., 2019). Traits can also vary naturally between plant populations and is a result of breaks in gene flow and differential selection that fixes beneficial alleles responsible for traits onto the genome, stabilizing the phenotype in place resulting in ecotypic variation between populations. (Anderson, 2016; Woolridge et al., 2022).

From a vegetation restoration standpoint, useful fitness traits to quantify are those of seeds and seedlings considering they contribute significantly to overall population survival (Stevens et al., 2020). Seed dormancy developed to ensure seeds delay their germination until the appropriate environmental conditions are present for seedling survival. The degree of dormancy and conditions required for germination is a relevant fitness trait to consider in restoration; seed with a more ready germination (lower dormancies) may germinate in untimely conditions in the field, so require careful planning on environmental windows for sowing and seeds with greater dormancies are better candidates for seeding in marginal windows (Stevens et al., 2020). A common misconception is that seed dormancy is a resting state of seeds but rather it reflects the seed being prevented by an exogenous or endogenous mechanism to germinate under favorable conditions (Copeland, 1976).

Viability is a measure of the degree to which a seed is capable of germinating in the first place and producing a seedling under regular conditions. To be viable seeds must contain essential structures and enzymes to catalyze the necessary reactions. Many factors impact the production of viable seed from year to year in plant populations, it is dependent on the previous and current growing season and can be impacted by environmental and genetic stressors. Seed viability is a true reflection of the seed collection's capacity to produce a next generation (Spearing, personal comms, 2022; Chambers et al.,1982).

An understanding of seed dormancy and viability is critical for understanding overall seed quality and has many practical applications for restoration practitioners (Pedrini and Dixon, 2020). Other fitness traits that impact restoration outcome are the vigor of seedlings. Seedling establishment is crucial stage of plant growth and is often the bottleneck for population establishment in restoration environments (Turner et al., 2022; Larson et al., 2022). Early growth traits impact a seedling's ability to acquire critical resources and survive into reproductive age. Rapid growth rates are beneficial at restoration sites with competitive and unpredictable environments as are early biomass acquisition strategies (Hamelin, 2012).

Understanding fitness trait variation between populations on a landscape can help identify advantageous seed sources (provenances) as well as unique gene pools for genetic conservation and seed increase strategies. Identifying seed collection provenances with ideal fitness traits can help narrow the focus of collection programs and reduce wasted efforts. Certainly, for Big bluestem, a plant that is established primarily by seed in large scale tallgrass prairie restorations, understanding fitness traits of the seed sources available on the landscape could be instrumental in sustainable tallgrass prairie restorations.

This chapter of the study endeavoured to compare fitness traits between restored and remnant populations of Big bluestem in tallgrass prairies, to understand if population status impacts their utility as a seed source for restoration. We also characterized fitness traits in seeds and seedlings grown out from remnant provenances to identify differentiation between populations and any ecologically relevant fitness traits.

Research Questions:

a) Are there fitness trait differences between Big bluestem seed and plants in remnant and restored populations and what are the restoration implications?
b) Are there population level differences in fitness traits of seeds and seedlings from remnant populations of Big bluestem.

2.2 Methods

Soil Samples

Within the same sites 16 sites described in Chapter1, to characterise the physio-chemical parameters I collected 3 soil samples from each site in a randomly selected location for a total of 54 soil sub samples. Each sample was taken with a 5 cm diameter soil corer to a depth of 30 cm. Soil samples were stored in a cooler on ice and transported back to Trent University. Prior to C-N analysis, sub-samples were dried at 105 ℃ for 24 hours sieved (2 mm) and pulverized. Sub samples allocated for Organic Matter (OM) analysis were placed in a muffle furnace at 375 ℃ for 16 hours, and % OM was calculated with the following formula:

$$\% \text{ OM} = \frac{\text{Pre-Ignition weight- Post ignition weight}}{\text{Pre ignition weight}} * 100$$

Total Carbon and Nitrogen were calculated with a CNS analyzer at Trent University in the Geoscience lab.

Field Fitness Measurements

For this study we chose the common performance measures of plant height, the number of vegetative and flowering tillers as well as seed head weight (inflorescence) to gather an estimate of fitness characteristics in restored and remnant populations of Big bluestem.

In October of 2020, 15 plants were subsampled randomly along a transect, described in Chapter 1 Methods. For each Big bluestem individual, plant height, tiller counts (reproductive and vegetative) and total inflorescence were recorded. We measured plant height as the distance from the soil surface to the top of the tallest panicum, if a grass culm was on an angle or drooping, I righted it to get its fully expressed height. For the seed collections, all inflorescences on the surveyed plants were collected into paper bags by snipping off the entire seed head. Collection bags were labelled by site and individual number, samples were dried in a low humidity environment at Trent University and weighed prior to seed extraction.

Seed Testing: Germination and Viability

To characterize the fitness levels in seeds; germination and viability trials were performed for extracted seeds from each population. First, seeds were threshed from inflorescence for 3-5 minutes, and collected with sieve sets. Post extraction, seeds were chilled at 4 ℃ in a fridge at Trent university for 90 days.

To initiate the germination trials, seeds were cleaned with a 30% Hydrogen Peroxide solution then rinsed well with deionized water to eliminate any fungal pathogens, 100 seeds from each population were divided into 10 replicates, 10 seeds were placed in Petri dishes on moistened bleach free filter paper, para-filmed shut and observed for germination events for 21 days in a climate chamber at Trent University. Germination was measured as the emergence of the coleoptile from the seed. (Methods adapted from Gibson et al, 2013). Environmental parameters of the climate chamber were set according to ISTA seed testing protocols, a diurnal temperature cycle of 18-24 ℃ in alignment with an 18:6 photoperiod was established under low intensity metal halide lights. Relative Humidity in the chamber was kept at 60% to ensure dishes didn't dry out and water was applied to dishes as needed when filter paper edges appeared dry. (Guidelines for ISTA Rules Proposals, 2020).

Viability assessments were performed via Tetrazolium (TZ) testing according to Gibson et al and Peters et al. Sub samples of 50 seeds per population were tested in two separate viability trials. Seeds were placed between two sheets of filter paper in a Petri dish and soaked with 1% 2,3,5-triphenyl tetrazolium chloride solution. Seeds were then placed in the dark at a slightly warm temperature (22 °C) for 24 hours then removed for dissection to observe the seed endosperm and embryo, for Big bluestem a red stained endosperm indicated a viable seed (Gibson et al., 2013; Miller & Ars, 2004).

Seedling Fitness Trait Measurements: Biomass, Height, Relative Growth Rate

For the seedling fitness trait observations, we planted 30 seeds from each of the eight remnant provenances into seedling pots in a climate chamber for 28 days to identify and compare early seedling fitness traits and ascertain if they differentiated between provenances. Seedlings grew up in the climate chamber for 28 days under an 18:6 photoperiod and were watered as needed with equivalent volumes. 10 seedlings from each population were selected randomly out of the 30 planted for trait measurement, we over sowed seed initially to make up for low germination rates. Seedling height was monitored every 4 days to calculate growth rates and final seedling height was recorded on the day of harvest. On day 28, all seedlings were harvested, rinsed and dried in a Thermo-Fisher drying oven at 70 °C for 48 hours. Post drying, shoot, and root biomass were recorded from dry mass (Rees et al, 2010; NECI,2012).

2.3 Data Analysis

For the statistical analyses, I used the SPS program, a significance level of $\alpha = 0.05$ was used for all tests. A nested ANOVA was used for the fitness comparisons between Population types (Remnant and Restored) This model contained populations nested within restoration type and treated them as random effect term. Student T tests were used for comparing mean soil parameters between remnant and restored sites, a linear regression correlation was then used to check for associations between soil parameters and fitness measurements. To detect and compare differences in seedling mean fitness traits in the climate chamber trial, a One Way ANOVA and Tukey Post Hoc Tests were used.

Fitness Trait Nested ANOVAS

I compared mean fitness trait values among remnant and restored populations for five fitness proxies; Plant Height, Inflorescence Weight, Tiller Counts of plants and Percent Germination and Percent Viability of seeds collected from remnant and restored populations. To determine the effect of the independent variable (Site Status and Population) on the dependent variables (Fitness Proxies), several Nested ANOVAs were used. Nested ANOVA's are an extension of a one-way ANOVA and it was chosen as the statistical model because one dependent measurement variable was used (ex: Mean Plant Height) and more than one nominal independent variable (Status) and (Population). Populations were nested within Status to form subgroup (random effects). The models test for significant variation among subgroups and within groups. For example, REMNANT (PINERY). Using a regular 1- or 2-way ANOVA would have treated every data point as an independent observation and would have violated the terms of pseudo replication. In order to run this model, the data sets had to meet assumptions of: Normality, Homogeneity of Variances, Balanced Sample Design.

Seedling Trait One Way ANOVAS and Post Hoc Test

I tested for differences in the mean fitness traits of seedlings from different populations in a growth chamber. To determine the effect of the independent variable (in this case population) on the dependent variables (seedling biomass, height, and relative growth rate) I used a One-Way ANOVA. To follow up on the significant differences in means identified in the ANOVA, a Tukey Post Hoc test was run to make pairwise comparisons of population mean fitness traits.

2.4 Results

Site Soil Characteristics

In terms of site characteristics, soil parameters did not differ significantly between the remnant and restored sites (Student T-test; C: N Ratio $p= 0.793$; % Organic Matter $p= 0.84$). Mean C:N ratios for all sites was 25.5 (SD= 20.5; Min= 7; Max: 90). Mean site % OM was 5.0 (SD= 2.8; Min=1.6; Max= 10.8). We performed a linear correlation analysis to investigate if there were associations between soil characteristics at remnant and restored sites and field fitness parameters (Plant Height, Seed Production and Reproductive Tiller No.). The analysis showed that there were no significant associations. Interestingly, field height had a significant ($p= 0.041$) moderately positive ($r= 0.516$) correlation with seed production.

Field Fitness Traits between Remnant and Restored Sites : Mean Height, Inflorescence Weight, Reproductive Tillers

Mean plant height across all populations was 174.5 cm. (n= 240, SD=36.8, Min=89.0 Max=321.0) The population with the tallest plants on average was Black Oak Heritage Park (Restored) and the population with the smallest plants were found at Peters Woods (Remnant). Mean height of plants in remnant areas was 153.8 cm and in restored populations mean height was 192.3 cm. Individual plants at restored sites were **s**ignificantly taller than plants at remnant sites (p= 0. 0276) and there were also significantly more reproductive tillers on plants at restored sites (p=0.043). Mean inflorescence weight differed across sites and between remnant (3.6 g) and restored (5.64 g) populations. The heaviest seed heads were produced at Mary's Farm (restored) and the lightest were found at the Peters Woods (remnant) site, but the nested ANOVA did not detect that site type, Remnant or Restored was a significant predictor of variation in the production of seed between sites (p =0.289).

Climate Chamber Seed Testing

Germination Trials

The results of the germination tests of collected seed between each population revealed that seed produced in both population statuses had moderately low germination (Figure 5). On average 44% of seeds germinated. In the Pinery seed lot, only 16% of seed collected germinated under climate chamber conditions. On the high end, seed collected from Alderville Black Oak Savanna had a 68% germination rate. The Nested ANOVA results showed a negligible difference between average germination rates of seed collected from remnant populations (44.5%) and restored ones (43.5%) and that population status (remnant or restored) was not a good explanation for differences in seed germination rates (p=0.387). (**Figure 5).**

Viability Assessments

Tetrazolium assessments of seed viability revealed that all seed collections had high viability (Average, 83%). The seed with the most viable embryos came from the Ojibway Prairie, followed by the Black Oak Heritage Park (95%) and Hazel Bird (95%). The population with the lowest amount of viable seed in the collection came from the restored Ducks Unlimited property in Norfolk County (58%) followed by seed from Pinery (75%) and Delhi remnant prairie (70%) .The Nested ANOVA results indicate there is no statistical difference in seed viability between populations (p=0.119). **(Figure 6).**

Fitness Traits of Seed and Seedlings of Remnant Provenances: Biomass, Height, Relative Growth Rate, Seed Germination.

There were significant differences in Relative Growth Rates (p = 0.006) , Total Biomass (p=< 0.001), and Height (p=< 0.001) of seedlings from different remnant provenances in our climate chamber study. The Tukey post hoc test revealed that the seedlings from the Ojibway Prairie (9.19 cm), were significantly taller than seedlings from: Alderville Black Oak Savanna (6.07 cm, p=0.003), Ganaraska East (5.3 cm, p= 0.001), Holland Landing (5.73 cm, p= 0.001), Pinery PP (3.59 cm, p= 0.001), Rondeau PP (4.57 cm, p= 0.001) and Dutton (6.3 cm, p = 0.036). Interestingly seedlings from Pinery PP, were significantly smaller than seedlings from Delhi (6.54 cm) and from ABOS (6.07 cm).

In terms of Biomass, all seedlings but Holland Landing (0.016 g) and Delhi (0.015 g) had significantly (p=<0.05) less biomass than Ojibway Prairie seedlings (0.025 g). Relative Growth Rates (RGR) calculated for all seedings did not differ along the same trends as Height and Biomass. Out of the eight remnant provenances sampled, seedlings from Ganaraska East (3.0%) had the lowest RGR. They were significantly lower than 4 populations: OJP (p=0.015), Rondeau (p = 0.007), ABOS (p = 0.024) and Delhi (p =0.015) lower but not significantly so from Pinery (10.6%) Dutton (13.6 %) and Holland Landing (14.6%) **(Figure 6).** See table of Tukey -Post Hoc tests in Appendix A2-1.

For seed germination, the ANOVA did not detect significant variation between remnant seed provenances and did not detect that variation partitioned onto any individual site (p =0.101). In summary, Ojibway prairie seedlings had increased size characteristics compared to all other seedlings and Ganaraska East seedlings exhibited low growth vigor (RGR) respectively in this climate chamber study.

2.5 Discussion

Fitness Comparisons Between Remnant and Restored Big bluestem Populations.

The objective of this research was to characterise and compare the field fitness traits of Big bluestem plants in remnant and restored populations to determine if population status was indicative of seed source quality. In general, fitness traits were significantly different in demographic fitness traits but not significant for seed quality measures. Plants in restored sites were taller with more flowering tillers and on average produced more seed than remnant sites, differences in seed production were not significant.

Restored populations of Big bluestem are taller with more reproductive tillers.

Restored plants were on average, larger than plants surveyed in remnant prairies. A likely theory to support this observation, is that the actions of tallgrass prairie restorations are creating conditions for plants to achieve higher fitness levels relative to their unmanaged remnant counterparts. Specifically, the presence and absence of fire may be influencing plant physiological processes responsible for height and tiller production. In this study, remnant sites visited had a wide range of fire intervals and we were not able to confirm records of management history for all sites, however from what we could tell, remnant sites had far less regular fire management, and some had no record or evidence of contemporary burning at all. On the other hand, management history for restored sites in this study showed that every site had been burned at least once in the last 10 years.

Fire has a significant regenerative effect on grasslands; therefore it would have been beneficial to rigorously quantify in this study (Towne & Owensby, 1984). Without fire disturbance, thatch produced in tallgrass systems builds up and reduces available light levels and subsequent plant growth. (Wagle & Gowda, 2018). This litter accumulation can change the microclimate of the entire prairie, lowering soil temperatures thus giving a growing edge to cool season agronomic grasses which are prolific competitors to Ontario's remaining tallgrass prairie. In a local study within Northumberland County, Ontario, restoration actions were quantified over a 20-year period over 14 tallgrass prairie and savanna research sites and a correlation analysis revealed that prescribed burning had a significant positive effect on the abundance of prairie indicator species on any given site. (Lefort., 2023).

Litter incineration results in enhanced below and above ground productivity in C4 grasses as well as stem tiller density. Fire adapted grasses like Big bluestem rapidly resprout from basal area tissue after fire, fueled by their significant root systems and rhizomes (Towne & Owensby, 1984). This result is seen consistently in grassland ecosystems. One study showed that not only did vegetative tiller density increase after fire intervals in savanna grass species, but flowering (reproductive) tillers were significantly related to fire intervals and that grass flowering declined when it was excluded from the system. Indeed, this same trend has been observed many times for Big bluestem. It is postulated that flowering may be triggered by a hormonal reaction in buds that is catalyzed by heat, or indirectly through increased soil temperatures. C4 grasses in the Andropogoneae lineage have co evolved with fire which has resulted in a specific suite of traits. Big bluestem leaf tissues have developed a high tannin content which decreases palatability for herbivores and slows decomposition, resulting in a high volume of senesced tissue which results in a large dry fuel load in the spring, so when fire passes through hardly any living tissue is damaged.

As well leaf sheaths at the plant base protect meristematic tissue during fire passage and a tall caespitose growth form encourages flames away from growth nodes (Pilon et al., 2021). Fire is an intrinsic part of Big bluestem productivity thus, the exclusion of burning on remnant sites could be responsible for the significantly lower number of vegetative and flowering tillers observed on Big bluestem plants within remnant populations in our study.

Exclusion of fire also heavily contributes to woody species encroachment into grasslands; indeed, it is a global phenomenon in grassy biomes. As woody species increase into grasslands, canopy cover (shade) increases leading to a decrease in herbaceous species richness(García Criado et al., 2020; Wagle & Gowda, 2018b) In Pilon et al's study, tree encroachment into Brazilian savanna ecosystems are changing the fire regime (flammability) of the region. They assessed grassland vegetation composition along a gradient of increasing canopy cover and found a strong species compositional pattern. C4 grass species would decrease in frequency regardless of species type as shade increased, while C3 species increased with increasing cover(García Criado et al., 2020; Pilon et al., 2021). This is unsurprising as C4 species like Big bluestem have evolved pathways to efficiently store water and slow photorespiration, but these same mechanisms create a slower photosynthetic system under low light conditions in comparison to C3 plants. Specifically, Carbon 'leakiness' within the cells of C4 plants has been shown to increase at low photon levels, it is this leakiness, defined as the rate of CO^2 diffusion into bundle sheath cells relative to the rate of carboxylation, that has been identified as the mechanism that leads to reductions in photosynthetic efficiencies, plainly this means that C4 plants cannot photosynthesize at a high enough rate to sustain positive growth in low light conditions. Some C4 plants have evolved underground storage structures that allow them to live off reserved starches, but these are not long-term solutions as reserves will eventually be used up (Kromdijk et al., 2014; Li et al., 2021; Pilon et al., 2021). Although we did not quantify the percent shade at our sites it is well understood that frequent fire intervals reduce shade from woody species encroachment and keeps tallgrass habitats open of cool season grass competition allowing C4 species to maximize their productivity and achieve positive growth. The absence of contemporary burning on remnant tallgrass habitats could be contributing to the reduced stature and fitness of Big bluestem plants in remnant sites compared to restored (managed) sites in this study.

Seed Production and Quality in Restored and Remnant Big bluestem Populations.

In this study, seed production measured by inflorescence mass at restored sites (5.64 g) generally exceeded seed produced at remnant sites (3.6 g), but the variation wasn't significant. It is possible that Big bluestem; a widely adapted, plastic plant with a polyploid complex is inherently resistant to the deleterious effects of fragmentation which-could explain the equivalent rates of seed production and high seed viability across most of the sites.

Broad ecological tolerances allow plants to grow and persist in wide climatic envelopes, indeed Big bluestem spans over a significant precipitation gradient across the U.S Midwest and east into Canada (Owsley et al., 2021). Trait plasticity can also contribute to an individual's ability to morphologically adjust based on local environmental conditions. Plastic traits are not genetically fixed on the genome and can change based on conditions; one individual's genome can support a range of morphologies (phenotypes) making plants with this type of adaptive variation hardy to environmental change and extremes (Galliart et al., 2018). Many graminoid species adjust to dry climates and precipitation gradients through plasticity or ecotype development (Droste et al., 2011). Some trait plasticity has been observed for Big bluestem, specifically changes in leaf and stem width were associated with drier site conditions, adaptations to site moisture conditions were also reflected in the results of Galliart et al, that observed co-gradient variation in Big bluestem vegetative traits over a 1000 km precipitation gradient (Galliart et al., 2018).

It is also possible that equivalent levels of seed production between remnant and restored sites indicate that the reproductive nature of Big bluestem provides resistance to the effects of maladaptation common at restored sites and buffers the effects of fragmentation at remnant sites. Fragmented populations characteristic of many remnant tallgrass prairie communities are at risk for genetic drift due to small population size and gene flow disruption (Williams et al., 2014; Mijangos et al., 2015; Aguilar et al., 2019). Similarly restored populations can experience low genetic diversity because of population bottlenecks in the seed collection and seedling production supply chain, resulting in reduced fecundity (Hamelin, 2012; Bucharova et al., 2017, 2019; Nevill et al., 2016; Espeland et al., 2017).

However, studies of Big bluestem have shown that compared to other outcrossing species, they harbour high levels of genetic diversity within small populations. Likely, this is a result of Big bluestems' polyploid status; genome rearrangements and extra sets of alleles increase heterozygosity, genomic diversity and lower rates of inbreeding depression.(Soltis & Soltis, 2000).The clonal and long lived nature of Big bluestem also contributes to high within population genetic variation, as genets have more chances over a long life span to pass down alleles and aren't totally reliant on seed recruitment for population persistence. (Kramer et al., 2018; Gaynor et al., 2019).

Our results show that fitness, observed as seed production is intact at restored and remnant sites in this study and is not starkly different between population status'. We interpret that this is because Big bluestem's inherent reproductive and genetic characteristics decrease the risks of maladaptation at restored sites and loss of genetic diversity at remnant sites which can affect seed production.

Seed Quality

Seed Quality, measured in our study through seed viability and germination rates did not differ significantly between remnant and restored populations, Seed quality is at the core of successful native plant restorations so understanding variations in its quality is crucial (Pedrini, 2020).

Big bluestem seeds are borne in a fertile, long awned spikelet, the caryopsis is 3-5 mm long and smooth and brown after being debearded. Like other C4 grasses its seed has a moderate to high reported physiological or embryo dormancy (15-50%) (Adkins et al, 2002).
A cold-dry chilling for 90 days is used to improve germination for this species. The exact mechanism of breaking physiological dormancy is not well studied in Big bluestem, but it is hypothesized that like other warm season grasses embryos must undergo a period of "after ripening" off the plant before it can germinate (Adkins et al, 2002; Houseal, 2007). Post chilling, germination rates of Big bluestem have reported averages of 40- 50%, however some much lower rates were reported by Gibson and Sendor (2013) after similar dormancy breaking treatments (14-17%) (Keeler et al, 2004; Houseal, 2007; Espeland et al., 2017). Their tetrazolium viability assessments, like our results, showed much higher viable embryos then what germinated in the trials. Similarly, that a high variability existed within their viability ranges (23- 52%) (Gibson et al., 2013). The higher germination rates we observed in our study (M= 43.5% and 44.5% respectively) could reflect our use of Hydrogen Peroxide (H2O2) as a seed cleaning agent. H202 has been reported as a germination stimulant for agronomic and forestry species (Ching, 1959; Barba-Espin,

2012). The intent for its use in this study was a rapid disinfection to remove any pathogens prior to germination and viability testing but it is possible it could have positively influenced germination, reports of increased germination through solid matrix priming has been reported in greenhouse studies for Big bluestem (Kavak & Taylor, 2013). High viability (M=83 %) and low- moderate germination (M=47%) could reflect the fraction of seeds that are dormant in our tested seed lots. However, since we did not assess the viability of the ungerminated seed in our trial we cannot conclude that all ungerminated seed reflect dormant but intact embryos. This study suggests that Big bluestem seed quality measured through percent (%) germinable and viable seed is unaffected by whether a source population is remnant or restored, we estimate that this likely because of Big bluestems' robust genome and outcrossing nature.

2.6 Restoration Implications

Fitness traits between remnant and restored sites were similar for mean individual seed production, seed germination and seed viability rates. We are interpreting this as a positive account that points to Big bluestem being well adapted to the restored sites surveyed and resistant to deleterious effects of population fragmentation. Its inherent genetic and reproductive structure and wide ecological niche makes it less prone to fitness reductions than other species in endangered habitats. Considering the demographic fitness traits of plant size and reproductive tiller production, restored sites exhibited significantly higher levels. We posture that the management actions taking place in restored tallgrass prairies, specifically fire is contributing to higher fitness levels in these populations compared to remnant sites.

It is concerning that in our data, many plants in remnant stands were reduced in stature and size and likely not achieving positive population growth as there is well established correlation between height, biomass and seed production (Younginger et al., 2017). If not addressed through restoration management, disparities between seed production in remnant and restored stands will likely become significant and there is a risk of losing remnant stands as an efficient, high quality source of seed.Great progress has been made in tallgrass prairie conservation and restoration in Ontario and concern for the persistence of remnant sites has been previously noted. This study suggests that stewardship improves the fitness of Big bluestem, a keystone tallgrass prairie species. Future actions should prioritize invigorating small remnant tallgrass communities with fire management to improve seed production lest we lose these unique gene pools and ecosystem service forever (Tallgrass Ontario, 2019; Reznicek & Maycock, 1983).

Seedling fitness trait variation among remnant provenances of Big bluestem

In our study, seedlings were grown in a climate chamber experiment for 28 days to determine whether early life fitness traits differed between remnant populations of Big bluestem to understand if fitness traits were evolutionary in nature and to qualify populations utility as seed sources for restoration.

We observed that there was significant variation between early seedling growth traits, height, relative growth rate and biomass between populations. This was somewhat expected as ecotypic differentiation has been reported in Big bluestem in other studies and is a common phenomenon when habitats are heterogeneous and require local adaptations to environments or are spatially isolated from others and gene flow has been disrupted (Gray et al., 2014; Vogel et al., 2018)

It is interesting when considering population divergence in a relic species to think about the origins of Big bluestem in North America. According to dominant theory, Big bluestem and other prairie grasses recolonized post glaciation from three main refugia; the southern Rockies, which populate the northern great plains populations, a northern Texas refugia that colonized the southern plains and a south-eastern Gulf Coast area. Based on the phylogenetic work of C. McAllister, it seems likely that Ontario's tallgrass prairies were recolonized from the northern great plain's populations (McAllister & Miller, 2016). The post glacial and precolonial history of Ontario's prairie region certainly plays into current population differentiation. As opposed to the continuous prairies of the great plains in the States. Ontario prairies existed in large "inclusions" that were many dozens of hectares, evidenced by some prairie remnants in South-western Ontario. East of this region, prairie and Big bluestem communities were typified by more of a patchwork of irregular sized pockets that occurred on dry sand plains, gravel fluvial deposits, hill crests and were usually surrounded by open Oak woodlands. (Reznicek & Maycock, 1983. This population architecture, similar to the prairie communities in the North-Eastern States, likely contributes to higher differentiation between populations in the Northeast of Big bluestems' range, than in the continuous prairie regions (Price et al., 2012).

In several studies that investigate genetic trait variation between Big bluestem populations, precipitation is highlighted as a driver of divergence, which makes sense as precipitation is responsible for productivity in grass species (Moser & Vogel, 1995). Gray's study showed that regional climate, was strongly related to genome markers under divergent selection especially temperature and precipitation factors. Further, Price's study shows distinct genetic differences between Big bluestem populations in Wisconsin and New York state (approximately 1,000 km) suggesting that regional distances can represent distinct gene pools for this species. Vogel, also confirmed precipitation related ecotype divergence in midwestern Big bluestem prairies, but noted that within ecoregions it is widely adapted, and that latitude and longitude effects were more significant predictor of accession performance than proximity of collection site to restoration site when considering seed movement of this species (Gustafson et al., 1999; Gray., 2012; Price et al., 2012; Vogel et al., 2018).

Our furthest sites were 500 km apart and spanned two Ontario ecoregions, that are both characterized by mild climates with hot humid summers and cool winters. These ecoregions however differ in their mean temperature and precipitation ranges. With ecoregion 7E experiencing 17-69 mm more precipitation and an average 1.5 Celsius warmer than ecoregion 6E, as well as 677 more growing degree days. (Crins et al.,2009) (Climatedata.ca.,2022). This is a much milder environmental cline than in other parts of Big bluestem's range, but it could still be contributing to a degree of genotype divergence between the populations surveyed along our study's East to West gradient. Indeed, in Gibson's surveys of fitness traits from different seed provenances of Big bluestem in

Southern Illinois, he noticed high variability in reproductive traits among populations separated by only 16-80 km illuding to small scale differentiation (Gibson et al., 2013).

Our results showed that seedlings from the Ojibway Provincial Prairie site achieved significantly greater sizes than almost all other seedlings at a fast rate. This could indicate that the highly productive ecosystem at the Ojibway prairie site yields seedlings that have adapted to achieve rapid growth to gain access to photosynthesis in a short period of time to compete in a dense, light limited prairie. In Galliart's genotyping and correlation study of Single Nucleotide Polymorphisms (SNP's) under selection in Big bluestem's genome, their results showed that there is a "tall allele" that occurs in the greatest frequency in wet ecotypes of Big bluestem and lowest frequency in dry ecotypes (Gustafson et al., 2004; Gibson et al., 2013; Galliart et al., 2018). Which also supports our observations that in a competition free environment seedling height appears to be a function of genes rather than a phenotypic response to environmental conditions.

In terms of relative growth rate among provenances, seedlings from Ganaraska East had a significantly lower growth rate than half of the provenances sampled (3%). Depressed growth rates indicate that resource acquisition was low in these seedlings and suggests a potential adaptation to a less favourable, low resource environment (Lambers & Poorter, 2004). Low growth and vigour in seedlings could also be attributed to the effects of inbreeding depression (Espeland et al., 2017).

2.7 Conclusions and Restoration Implications

Early seedling growth traits are important to population establishment (Zirbel & Brudvig, 2020). Our results have shown that there are population level differences in early seedling growth traits; height, biomass, and growth rate for Big bluestem, with the strongest differences seen between seedlings from the far South-west in ecoregion 7E of our sample gradient and seedlings from ecoregion 6E.

Understanding how traits partition between populations of a heavily utilized keystone restoration species is beneficial to consider from a restoration perspective, this information can tool practitioners with information to guide seed provenance selection for different seed strategies. For example, ideal traits can be matched to compatible revegetation environments and unique ecotypes can be selected for seed increase strategies. Further research should involve a longer-term common garden experiment to understand how different seed provenances of Big bluestem in Ontario perform and to further identify ecologically relevant traits.

2.8 Tables and Figures

Table 2 : Site characterization of soil parameters: Carbon: Nitrogen ratio and Soil Organic Matter % for research sites.

Site	Population Status	% OM	C: N Ratio
Rondeau	Remnant	1.6	11.3
Pinery	Remnant	1.6	74.6
NCC	Restored	2.0	10.7
Hazel Bird	Restored	2.0	11.9
Kenesserie Prairie	Restored	3.1	12.7
Peters Woods	Restored	3.2	35.5
Red Cloud	Restored	3.6	23.8
Ganaraska East	Remnant	4.7	10.9
Mary’s Farm	Restored	5.8	68
Ducks Unlimited Property	Restored	6.3	34.2
Van Hove	Restored	7.5	17.4
Black Oak Heritage	Restored	7.6	14.3
Ojibway Prairie	Remnant	7.9	15.4
Delhi	Remnant	8.0	14.9
Dutton	Remnant	10.8	43.9

Table 3 : *S*ummarizes the relationships between soil parameters: Organic Matter %, Carbon: Nitrogen and field fitness measurements: Plant height and Seed production. There are moderate positive (r=0.485) but not significant (p=0.057) relationships between C:N ratios and seed production in the Big bluestem plants in this study. As well, Field Height has a significant, moderately positive correlation (r= 0.516) with Seed Production in the plants sampled (p =0.041).

Pearson's Correlations

Variable		**C:N ratio**	**OM%**	**Field Height**	**Seed Production**
1. C/N ratio	Pearson's r	—			
	p-value	—			
2. OM%	Pearson's r	0.065	—		
	p-value	0.812	—		
3. field height	Pearson's r	0.325	0.075	—	
	p-value	0.220	0.783	—	
4. seed production	Pearson's r	0.485	-0.144	0.516 *	—
	p-value	0.057		0.041	—

Table 4 : Summary of Nested ANOVA comparing mean fitness values between population types (Status) (Remnant versus Restored).

VARIABLE	SOURCE OF VARIATION	DF	MEAN SQUARES	F	SIG
HEIGHT	Status	1	516.198	1.286	0.027
	Population (Status)	14	401.350		
SEED WEIGHT	Status	1	13.727	1.213	0.223
	Population (Status)	14	11.318		
TILLER NO.	Status	1	368.63	4.886	0.043
	Population (Status)		75.449		
GERMINATION	Status	1	225.00	.796	.387
	Population (Status)	14	282.714		
VIABILITY	Status	1	330.286	2.82	.119
	Population (Status)	14	116.952		
SEEDLING BIOMASS	Status	1	10.688	.884	--
	Population (Status)	14	12.096		.362
SEEDLING RGR	Status	1	.324	.006	
	Population (Status)	14	116.952		.937

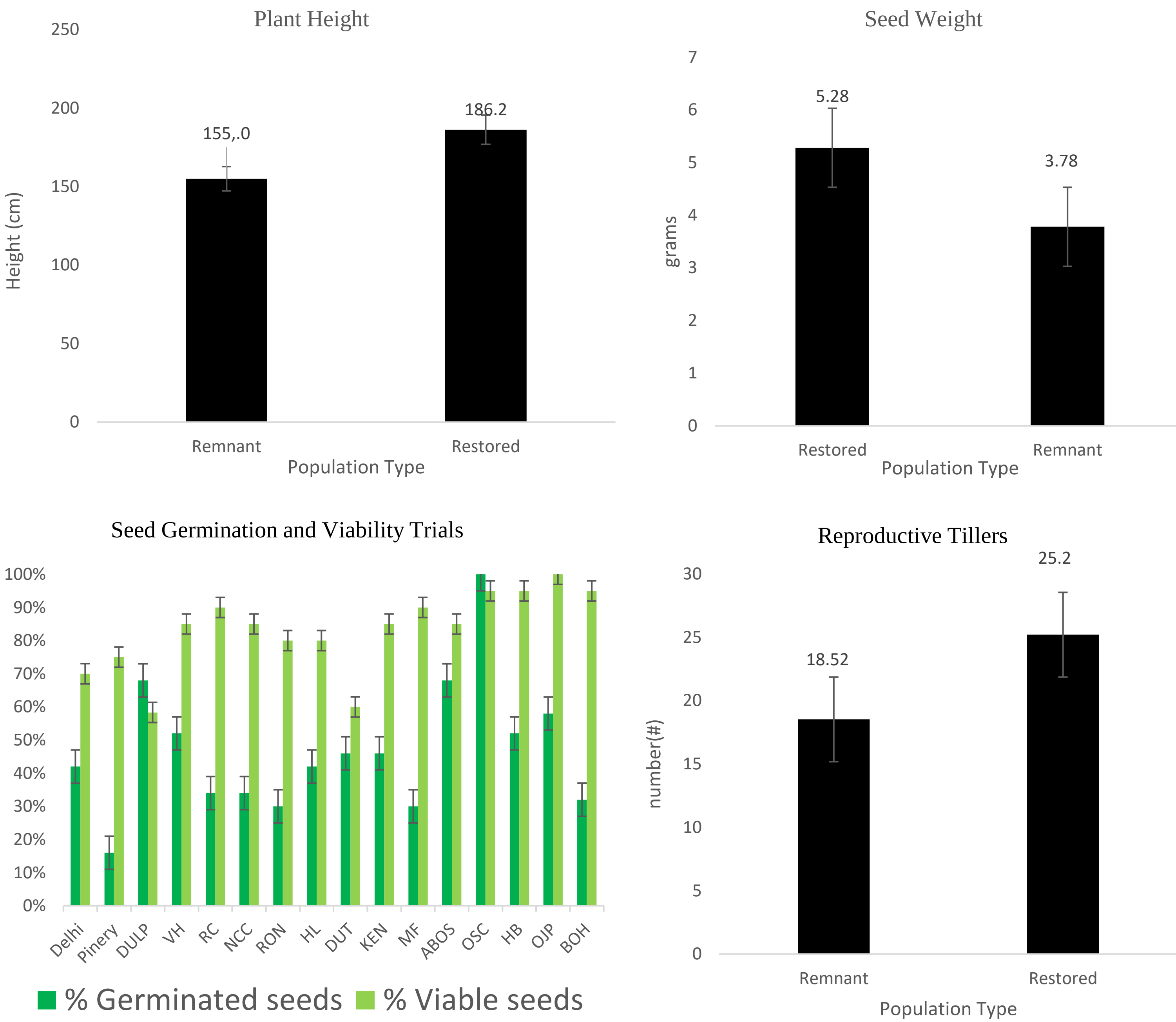


Figure 3: Bar charts displaying mean fitness parameters between population type. 1) Mean height (cm) of Big bluestem plants in remnant and restored sites in South - Central Ontario. 2) Mean inflorescence weight(g) in restored and remnant populations. 3) Mean germination and viability rates for all populations sampled. 4) Mean reproductive tiller numbers per plant between remnant and restored populations.

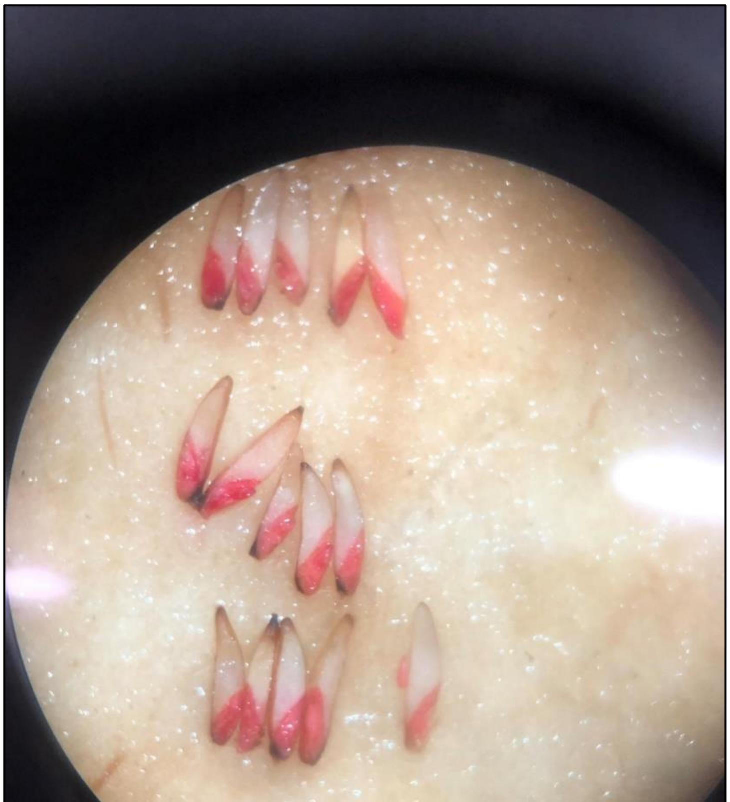


Figure 4: Big bluestem seed quality testing for seed viability. Tetrazolium dye indicates a respiring embryo. Opaque milky white endosperm indicates intact reserves.

Figure 5 : Big bluestem seed quality testing for germination. Coleoptile emergence from the seed indicates successful germination.

Table 5: Summary of ANOVA's detailing trait variation in seedling fitness parameters: Seedling height, relative growth rate, seedling biomass, % germination between remnant populations

ANOVA - Seedling Height

Cases	Sum of Squares	df	Mean Square	F	p
Site	189.036	7	27.005	6.730	< .001
Residuals	369.171	92	4.013		

Note. Type III Sum of Squares

ANOVA - RGR

Cases	Sum of Squares	df	Mean Square	F	p
Site	0.082	7	0.012	4.513	0.006
Residuals	0.042	16	0.003		

Note. Type III Sum of Squares

ANOVA - GERMINATION

Cases	Sum of Squares	df	Mean Square	F	p
SITE	0.117	6	0.020	2.236	0.101
Residuals	0.123	14	0.009		

Note. Type III Sum of Squares

ANOVA - seedling biomass

Cases	Sum of Squares	df	Mean Square	F	p
site	0.002	7	3.405×10^{-4}	6.504	< .001
Residuals	0.004	72	5.235×10^{-5}		

Note. Type III Sum of Squares

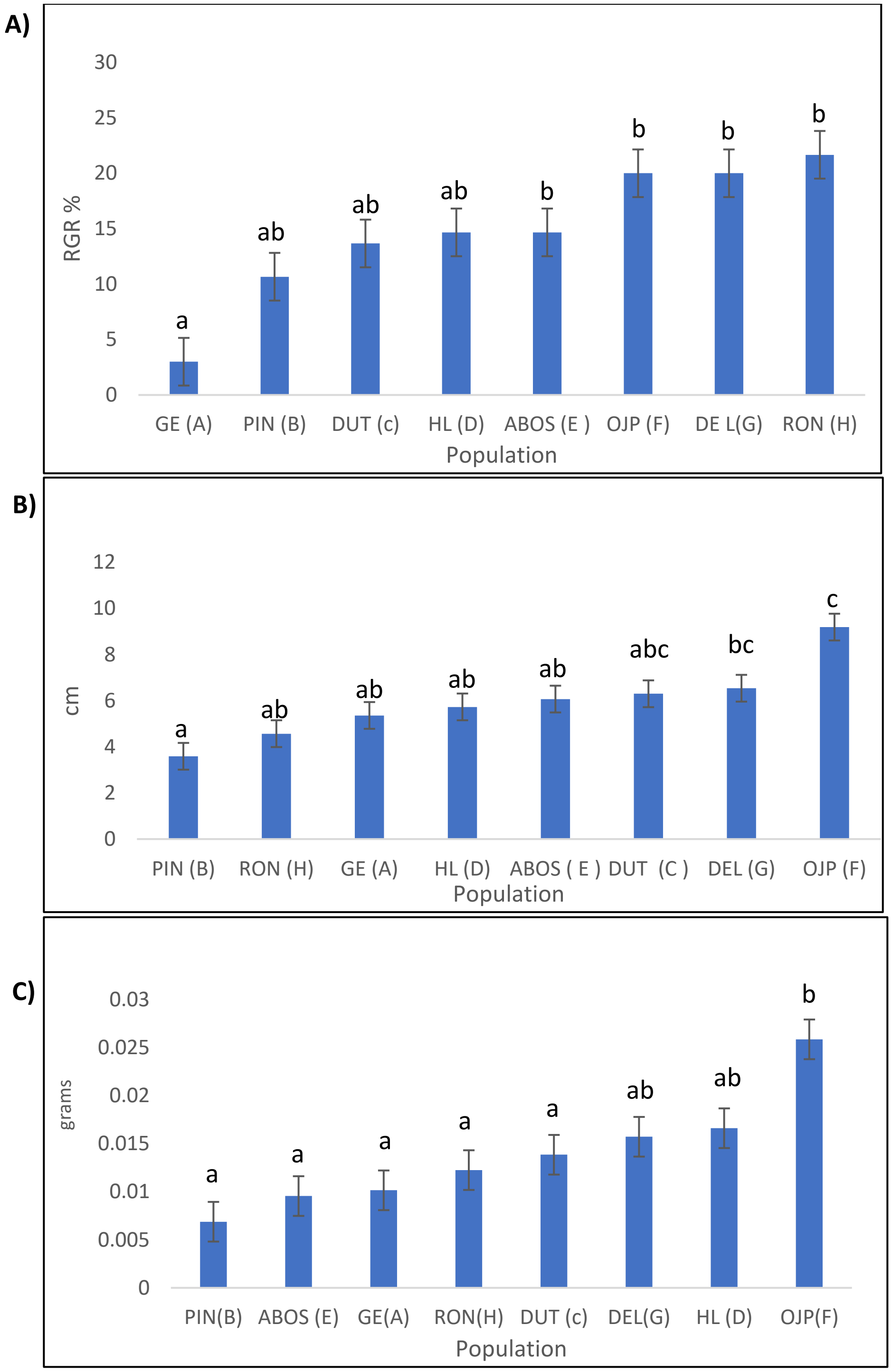


Figure 6: Bar charts displaying mean measured fitness traits of Big bluestem seedlings from 8 remnant populations grown for 28 days in a growth chamber under ideal (mesic) conditions. A) Relative Growth Rate (% RGR) of seedlings B) Seedling Height C) Seedling Biomass. Bars represent standard error. Letters indicate significant differences in the indicated morphological

Chapter 3: Practitioner Perspectives

3.1 Introduction

To be effective, scientific research requires input and direction from communities of practice. Without practitioner insight, scientific research risks being wasteful at worst and unhelpful at best. Practitioners are after all, on the front lines of land management and have valuable experiences and bodies of knowledge. Often, the demands of the task mean that best practices are not published, resulting in specialized, verbal knowledge systems. Practitioner perceptions of important ecological issues are place based and therefore have the power to catalyze appropriate scientific inquiries and facilitate meaningful collaborations between research and stakeholder organizations. (Jellinek et al., 2021).

Collaborations between researchers and land managers are especially pertinent in the field of restoration ecology, as it remains a developing branch of conservation. Its practice is as much of an art as it is a science and one size fits all approaches are ineffective given the multi variable nature of ecosystem restoration (Clewell & Rieger, 1997). In Jellinek et al's (2021) meta-analysis of practitioner and researcher engagement, the gap between practice and science was identified as a limiting factor to successful restoration and on the other hand, collaborations that contained engagement among other things, lead to improved conservation and restoration strategies.(Jellinek et al., 2021).

The roles of restoration practitioner and ecologist/researcher are often the same, Clewell defines the ecologists role as a person that uses "well-conceived restoration programs to serve as laboratories for deciphering the complexities of ecological dynamics that lead to the organization of biological communities" and a practitioners role as "individuals who are involved with the design and implementation of specific restoration efforts". In interviews designed to understand the values of restoration scientists and land managers Clark et al (2019) reported that restoration scientists more often mentioned concerns for the ecosystem as a whole or on a global scale than land managers, while managers mentioned human use and health concerns more frequently in their interviews. Practitioners and researchers overwhelmingly agree on many issues, a consistent topic identified by both is that organizational capacity and funding does not reflect the scope of the restoration task at hand. Under a review for the UN decade for ecosystem restoration, an organizational assessment was trialled for the restoration sector. Primary among many concerns, the framework identified that funding bodies typically do not provide a long enough commitment to achieve the outcomes restoration organizations are aiming for which can result in abandoned projects as well as umbrella organizations that are serving as funding subsidiaries often have unrealistic expectations of restoration projects which can lead to a loss of faith and credibility in the restoration team (Galatowitsch, 2022).

Methodologies for the restoration of different ecosystems are continuously being developed by practitioners in the field and when research and practice reduce the gap, innovations in methodologies can be made. Recently, a survey study was performed to factor in restoration practitioner perceptions into the design of mechanical direct seeders for native seeds; findings synthesized from a survey of 183 practitioners reported that mechanical direct seeders was the favored approach in comparison to nursery stock but that the seeders abilities were significantly limited in controlling for precise depth, especially with native seeds of varying morphologies. This research prompted Curtin university to develop precision seeding technology for tractor mounted seeders which is now being trialled for large scale applications of seeds on mining footprints in Australia (unpublished data, Pedrini, 2021).

These kinds of collaborations are especially critical for Indigenous scientists and practitioners, for whom Traditional Ecological Knowledge (TEK) which embodies long term connections and understandings of their homelands has been oppressed through colonization. Communities with TEK should be treated with the same respect and expert status as western scientific findings. Particularly in fire adapted landscapes where land burning practices are embedded into the cultures of many nations as an act of land management and restoration, western science has much to learn in this arena (Dickson-Hoyle,2022). Indeed, the 2019 principles and standards for ecological restoration requires we draw on many types of knowledges, including Traditional Ecological Knowledge. When we do incorporate many forms of knowledge, the benefits of restoration expand to include more than just ecological health but social and cultural health as well(Gann et al., 2019).

3.2 Prairies and People of South- Central Ontario

Following the last glacial maximum in southern half of Ontario, the combination of a warm climate in the Hypsithermal Interval and glacially deposited pockets of deep sandy soils along the shores of the post glacial Great Lakes, produced expansive sandy peninsula's where grassland vegetation dominated (Reznicek & Maycock, 1983) . A cooler, moister climate that followed aided the transition of many grassland communities into a forested landscape except for those that were maintained by the presence of Indigenous groups (Reznick.A 1980). Currently, these unique herbaceous communities compete for space in the most populated ecoregions of Ontario and following a century of conversion to agriculture and urbanization, 0.3% of is original extent remains (Farrell, 2004). Remnant patches of intact prairie can primarily be found underneath hydro corridors, in cemeteries and on private property (Farrell, 2004; Tallgrass Ontario, 2019). The largest tracts of intact prairies can be found on First Nation lands on Walpole Island, the Ojibway Prairie Provincial Park in Windsor and on the Rice Lake Plains at the Alderville Black Oak Savanna. Tallgrass communities large and small, are among the highest at-risk plant communities in Ontario and provide habitat for 20% of Ontario's at-risk species, but despite their significance they are under constant threat from development (Tallgrass Ontario, 2019). Even in their reduced state, tallgrass prairies in Ontario have invested communities of practice that are committed to their protection and restoration.

Objective

This chapter describes the results of a participatory workshop held with grassland conservation and restoration practitioners, researchers and restoration ecologists from Central and Southern Ontario. The purpose of the workshop was to present and ground truth the results of the biological studies on the keystone prairie species Big bluestem (*Andropogon gerardii*) presented in Chapters 1 and 2 of this document and to better understand the context of this work. Participatory mixed methods research, that blends both quantitative and qualitative data can bring a rich, grounded quality to the results that land in the real world.

3.2 Methods

Participants

Participants in the workshop were recruited based on their involvement with tallgrass prairie restoration and conservation in Ontario. I had already established a working relationship with most individuals through the course of my quantitative research, so I was able to reach out to them personally. Participants involved either represented an organization or had significant experience working in grassland ecosystems. Emails were sent out to participants a month before the workshop date; participants were encouraged to bring along other people within their organization they believed could contribute meaningfully or benefit from the workshop.

Workshop Background: Participatory Action Exercises

We utilized two main techniques from Chevalier and Buckles Handbook for Participatory Action Research (2013). The goal was to establish the greater story of engagement with Ontario's grasslands and to situate the quantitative research within it. To begin the workshops, I used a timeline visual exercise. Timeline exercises endeavour to tell stories of change over time and identify significant events and partnerships that have occurred on the topic. The topic was first defined as "The History of Tallgrass Prairies in Ontario" Participants were asked to add any milestones they felt were significant in the context of this main topic, with specific interest in conservation and restoration initiatives. Milestones were defined as being research projects, partnerships, actions, or achievements surrounding the main topic. This activity set the group up for further grounded explorations and discussion in the second part of the workshop. Following the timeline, the results of the biological studies in Chapter 1 and 2 were presented with discussion prompts to facilitate conversations on the impact -if any, of the results. The second structured exercise followed a "Blue Sky Thinking" approach. The group was prompted to imagine the future of tallgrass prairie conservation and restoration in Ontario, participants were asked to suggest an ideal endeavour that they would like to see come to fruition inspired by the topics of discussion.

3.3 Data Analysis and Results

All data was analyzed with NVIVO qualitative data analysis software. A thematic analysis was performed, and several main themes were identified and are discussed in detail in the discussion section below. Figures were generated in real time during from the participatory workshop using Power Point **(Figures 7 and 8).** Explanations of the results are elaborated on in the discussion.

Figure 7: Ontario Tallgrass Prairie Conservation and Restoration Timeline. This figure was generated from the participatory action exercises within a workshop aimed to situate the current study's research. Timeline begins in 1700, although Indigenous occupation of the Ontario prairie region spans well before this time. The timeline highlights important partnerships between organizations, and catalyzing actions for conservation and restoration of tallgrass prairies.

Figure 8: "The Future of Tall Grass Prairie Conservation and Restoration in Ontario" This figure depicts the results of a participatory action exercise that used a blue-sky thinking approach. Text bubbles hold quotes from practitioners that answer the question "What do you hope for the Future of tallgrass prairie conservation and restoration in Ontario". Abbreviations: Tallgrass Ontario (TgO) Traditional Ecological Knowledge (TEK)

3.4 Discussion

The Past, Present and Future of Tallgrass Prairie Restoration

A visual timeline was constructed from the workshop, it showed that there is a significant history of conservation and restoration of tallgrass prairies in Ontario and that there are several groups that understand their importance on the landscape. Significant events in the timeline include the establishment of protections for two of the largest tracts of remnant prairie at the Ojibway Prairie Provincial Park (1977) and at the Alderville Black Oak Savanna (1998). Momentum for restoration actions has been growing since the 1970's and has resulted in prescribed burning returning to the landscape as a stewardship tool (1980), a provincial recovery plan for the ecosystem (2019) and business initiatives like the prairie native plant nursery at the Alderville Black Oak Savanna (2021) **(Figure 7).** Globally, there is a grassland awareness disparity when it comes to restoration initiatives, but it appears that in the tallgrass prairie region in South -Central Ontario there is a group of people who are ensuring that grasslands are prioritized for restoration based on their ecological and intrinsic value (Tolgyesi et al, 2022).

During the future blue-sky thinking exercise, many unique ideas were generated by the group that spanned organizational, operational, and research sectors. From an organizational perspective an increase in the size and the scope of the non-profit Tallgrass Ontario was identified as well as increasing the diversity of voices in conservation and volunteer strategies. From a sustainable operations perspective, more volumes and more suppliers of native plants and seeds was identified by several participants. Optimistically "Native seed orchards everywhere!" was thought to be an ideal scenario **(Figure 8).** This desire for increased choices and availability in native seed and plants for grassland ecosystem restoration is paralleled at a global level. Seed production areas are increasingly highlighted as a solution to limited native seed supplies and delayed restoration (Nevill et al, 2016). It is true that wild stand native seed collecting, and processing is an expensive time consuming endeavour and is unsustainable when considering the amount of seed needed to supply the global restoration targets and the dwindling remnant populations of these plants (Broadhurst et al, 2008; Nevill et al 2016).

Workshop Themes and General Discussion

It was apparent from the workshop discussions that several practitioners held a deep concern for the sustainability of remnant populations and had seen a decline in their quality over the years. Specifically concerning the Delhi prairie remnant along an old rail corridor, Mary Gartshore added that the prairie vegetation was likely very old due to the presence of the endemic moth *Anacampsis lupinella* a species of conservation concern in the province, that was recently rediscovered on remnant populations of *Lupinus perennis* in this same tract of prairie (Otis et al, 2020). She also stated that prairie vegetation used to be continuous in 2010, but is now fragmented by cool season grasses. These observations corroborate the results reported in chapter 2 of this study which reported a reduced stature and size of Big bluestem plants in remnant prairie communities.

A somewhat surprising finding resonated by most participants in the group discussion was that baseline seed quality information was not often available for seeds being used in restoration projects. Provenance information was always available but baseline germination rates and viability estimates were not. One practitioner mentioned that having this type of data on seed accessions would be useful for calculating seeding rates and amounts in the field. It was also mentioned that the cost of testing wild type native seeds was expensive and that there is a knowledge gap in laboratories abilities and availability to test these seeds, but cut tests are often used by seed collectors in the region. The seed quality information provided in our study was of interest to some practitioners for use in seeding rate calculations and setting restoration target outcomes. According to a recently published paper outlining a set of international principles and standards for native seeds in ecological restoration, seed testing is imperative for heightened restoration results and can help determine the value of the seed lot and help set performance expectations for seed lots and subsequent restoration outcomes (Dixon and Pedrini, 2020). Future work should go into helping organizations adopt basic seed quality testing methods or establishing partnerships with local research institutions to test wild collected native grassland seed.

Another major theme that presented itself in the workshop discussion was the absence of seed movement guidelines for prairie species in Ontario. Although Season Snyder from Tallgrass Ontario mentioned how this issue has scientifically evolved in the past 7 or 8 years it is still an unsettled issue, especially as the scale of restoration in the province increases. It was echoed by several others during the workshop that we still don't know how far is too far to move for a lot of grassland species. General best practices were mentioned by Mary Gartshore that 100 kilometres of latitudinal movement and 300 km of longitudinal movement has often been used to guide herbaceous and non herbaceous seed movement. Although these rules may unintentionally restrict high quality seed provisioning, as we observed in thus study that some source populations have higher fitness traits and may be better candidates for restoration (Weber, 2021).

Species or clade specific seed strategies are increasing to provide confidence in seed transfer agreements and restoration plantings, further long term reciprocal transplant studies should be performed on Big bluestem to provide more information on the adaptive capacity of this keystone species (Nelson et al, 2017).

In the workshop discussion, polyploidy and its relationship to seed movement and compatibility was a new concept for restoration practitioners although population genetics and its importance in seed sourcing decisions was well understood. The fact that Big bluestem individuals surveyed were predominantly hexaploid received positive feedback as it reduces the chance for outbreeding depression when mixing seed from different provenances in Ontario.

3.5 Final Conclusions

Baseline genetic and demographic fitness trait data on seed sources can be a useful tool in a restorationists arsenal. Having this information could lead to increased restoration results at sites by avoiding maladaptation of seeds to site and genetic incompatibilities. Further, understanding how fitness traits vary across the landscape can help streamline collection programs and identify unique gene pools for genetic conservation and seed increase strategies.

The results of Chapter 1 of this study suggest that hexaploids are the dominant cytotype within Ontario populations and that the risk of encountering outbreeding depression as a result of combining ploidies at restoration sites is low. Still, cytotype is an important factor to consider for seed sourcing of Big bluestem, matching the ploidy type to the goals of the restoration project is warranted due to the reproductive differences between cytotypes. Screening seed and plant material from hotter and drier climates is justified as these are environmental indicators of enneaploid (9x) generation.

Our results also point to the need for an ecosystem-specific seed strategy to increase the sustainability of seed for tallgrass prairie restoration in Ontario. In this study's surveys, remnant sites of Big bluestem showed reduced stature and were trending towards less seed output at unmanaged sites; this is very concerning from both a biological and genetic diversity standpoint. Our learnings from the workshop detailed in Chapter 3 also corroborate that there is deep concern from the practitioner community for the survival of remnant tracts of prairie and that seed increase projects are central to the future of tallgrass prairie restoration in the province. Further, it is clear from our workshop there is significant momentum behind tallgrass prairie conservation and restoration here despite limited funding and a robust network with significant expertise in place.

In Chapter 2, our results showed fitness trait variation between seedlings grown in a common growth chamber from remnant populations along the 500 km study gradient which suggests ecotypic differentiation between populations, likely as a response to local climate factors. This information could provide foundations for specific seed increase goals and matching traits to restoration sites. Long term reciprocal transplant studies on Big bluestem would add more data to understand adaptive variation within this species.

A seed strategy entails increasing the diversity and availability of native plants and seeds available for restoration through sustainable collections and banking, made possible through collaborations between seed collectors, growers, storage facilities and ecologists. Seed strategies inherently require cross sector collaborations and as such are excellent platforms for bridging knowledge gaps between researchers and practitioners.(Mckim et al., 2019). As highlighted in Chapter 3, collaborations are paramount to success within ecological restoration. Our suggestion is to create an *ecosystem-based* seed strategy that centers grassland species across their historic range in Ontario. A useful template could be followed from the U.S Great Basin Native Plant Project whose goal is to increase the availability of genetically appropriate native plant materials in the Great Basin ecosystem through increasing seed and plant material and facilitating seed research on keystone species.

Appendix A

Table A1: DNA content of Big bluestem (*Andropogon* gerardii) samples. Calculated from the flow cytometry output and the DNA calculation: Sample DNA content= Ratio (between *Andropogon* mean and standard mean) x Standard DNA content (pg/2c) =0.39 x 16.19 pg/2c = 6.3 pg/2c

Sample ID	DNA Content	Sample ID	DNA Content	Sample ID	DNA Content
DUTB5	5.30	PWB1	6.38	ROB4	6.54
HBB1	5.34	PWB2	6.38	ROB5	6.54
HBB5	5.40	ABB7	6.40	BOHB3	6.55
HBB6	5.40	DUTB3	6.40	BOHB4	6.55
HBB3	5.41	DUTB4	6.40	OJPB7	6.56
HBB7	5.43	PIB5	6.41	DUTB7	6.58
NCCB5	5.44	PIB6	6.41	DUTB8	6.58
KPB1	5.50	PIB3	6.41	DULB3	6.58
NCCB3	5.51	PIB4	6.41	DULB4	6.58
OJPB3	5.53	MF B10	6.42	BOHB5	6.59
KPB5	5.55	HBB8	6.42	BOHB6	6.59
MF B7	5.56	RCB1	6.42	ABB8	6.62
MF B8	5.56	RCB2	6.42	OJPB5	6.68
MF B1	5.64	HBB2	6.44	BOHB1	6.69
NCCB4	6.16	ROB1	6.44	BOHB2	6.69
PIB1	6.20	GAB5	6.45	MF B3	6.74
PIB2	6.20	GAB6	6.45	MF B4	6.74
NCCB6	6.20	DULB1	6.45	GAB1	7.77
RCB3	6.22	DULB2	6.45	GAB2	7.77
RCB4	6.22	DEB1	6.45	MF B9	9.64
PIB7	6.24	DEB2	6.45		
PIB8	6.24	ROB2	6.45		
KPB7	6.26	ROB3	6.45		
KPB8	6.26	KPB6	6.45		
VH3	6.30	DUTB1	6.47		
VH4	6.30	DUTB2	6.47		
VH1	6.30	MF B5	6.47		
VH2	6.30	MF B6	6.47		
MF B2	6.30	ROB8	6.47		
ABB1	6.31	KPB2	6.47		
ABB2	6.31	OJPB4	6.48		
KPB3	6.31	ABB3	6.48		
KPB4	6.31	ABB4	6.48		
PWB3	6.31	GAB3	6.50		
PWB4	6.31	GAB4	6.50		
ROB6	6.32	HBB4	6.51		
ROB7	6.32	NCCB1	6.51		
VH5	6.34	NCCB2	6.51		
VH6	6.34	OJPB9	6.52		
DUTB6	6.34	OJPB10	6.52		

Table A2-1: Remnant seedling fitness trials. Tukey Post Hoc Test interaction effects between source populations and fitness trait. Seedling Height X Population

Post Hoc Comparisons - Site ▼

		Mean Difference	SE	t	p_{tukey}
ABOS	DELHI	−0.470	0.776	−0.606	0.999
	GE	0.710	0.776	0.915	0.984
	Holland Landing	0.340	0.633	0.537	0.999
	OJP	−3.120	0.776	−4.022	0.003**
	PINERY	2.480	0.776	3.197	0.039*
	Rondeau	1.500	0.776	1.933	0.532
	dutton	−0.230	0.776	−0.296	1.000
DELHI	GE	1.180	0.896	1.317	0.890
	Holland Landing	0.810	0.776	1.044	0.966
	OJP	−2.650	0.896	−2.958	0.073
	PINERY	2.950	0.896	3.293	0.029*
	Rondeau	1.970	0.896	2.199	0.362
	dutton	0.240	0.896	0.268	1.000
GE	Holland Landing	−0.370	0.776	−0.477	1.000
	OJP	−3.830	0.896	−4.275	0.001**
	PINERY	1.770	0.896	1.976	0.504
	Rondeau	0.790	0.896	0.882	0.987
	dutton	−0.940	0.896	−1.049	0.965
Holland Landing	OJP	−3.460	0.776	−4.460	< .001***
	PINERY	2.140	0.776	2.758	0.119
	Rondeau	1.160	0.776	1.495	0.808
	dutton	−0.570	0.776	−0.735	0.996
OJP	PINERY	5.600	0.896	6.251	< .001***
	Rondeau	4.620	0.896	5.157	< .001***
	dutton	2.890	0.896	3.226	0.036*
PINERY	Rondeau	−0.980	0.896	−1.094	0.957
	dutton	−2.710	0.896	−3.025	0.061
Rondeau	dutton	−1.730	0.896	−1.931	0.534

Note. P-value adjusted for comparing a family of 8

* p < .05, ** p < .01, *** p < .001

Table A2-2: Remnant seedling fitness trials. Tukey Post Hoc Test interaction effects between source populations and fitness trait. Relative Growth Rate X Population.

Post Hoc Comparisons - Site ▼

		Mean Difference	SE	t	p_{tukey}
ABOS	DEL	−0.010	0.042	−0.240	1.000
	DUT	0.053	0.042	1.280	0.894
	GE	0.160	0.042	3.840	0.024*
	HL	0.043	0.042	1.040	0.961
	OJP	−0.010	0.042	−0.240	1.000
	PIN	0.083	0.042	2.000	0.511
	RON	−0.027	0.042	−0.640	0.998
DEL	DUT	0.063	0.042	1.520	0.787
	GE	0.170	0.042	4.080	0.015*
	HL	0.053	0.042	1.280	0.894
	OJP	2.082×10^{-17}	0.042	4.996×10^{-16}	1.000
	PIN	0.093	0.042	2.240	0.380
	RON	−0.017	0.042	−0.400	1.000
DUT	GE	0.107	0.042	2.560	0.239
	HL	−0.010	0.042	−0.240	1.000
	OJP	−0.063	0.042	−1.520	0.787
	PIN	0.030	0.042	0.720	0.995
	RON	−0.080	0.042	−1.920	0.558
GE	HL	−0.117	0.042	−2.800	0.163
	OJP	−0.170	0.042	−4.080	0.015*
	PIN	−0.077	0.042	−1.840	0.605
	RON	−0.187	0.042	−4.480	0.007**
HL	OJP	−0.053	0.042	−1.280	0.894
	PIN	0.040	0.042	0.960	0.974
	RON	−0.070	0.042	−1.680	0.699
OJP	PIN	0.093	0.042	2.240	0.380
	RON	−0.017	0.042	−0.400	1.000
PIN	RON	−0.110	0.042	−2.640	0.211

* $p < .05$, ** $p < .01$

Note. P-value adjusted for comparing a family of 8

Table A2-3: Remnant seedling fitness trials. Tukey Post Hoc Test interaction effects between source populations and fitness trait. Seedling Biomass X Population

Post Hoc Comparisons - site						
		Mean Difference	**SE**	**t**	**p_{tukey}**	
ABOS	DELHI	-0.006	0.003	-1.857	0.584	
	Dutton	-0.004	0.003	-1.359	0.872	
	GE	-6.010×10^{-4}	0.003	-0.186	1.000	
	Holland Landing	-0.007	0.003	-2.182	0.375	
	OJP	-0.016	0.003	-5.041	< .001	***
	PINERY	0.003	0.003	0.825	0.991	
	Rondeau	-0.003	0.003	-0.836	0.990	
DELHI	Dutton	0.002	0.003	0.577	0.999	
	GE	0.006	0.003	1.676	0.702	
	Holland Landing	-8.878×10^{-4}	0.003	-0.267	1.000	
	OJP	-0.010	0.003	-3.050	0.060	
	PINERY	0.009	0.003	2.660	0.152	
	Rondeau	0.003	0.003	1.043	0.966	
Dutton	GE	0.004	0.003	1.169	0.938	
	Holland Landing	-0.003	0.003	-0.875	0.987	
	OJP	-0.012	0.003	-3.801	0.007	**
	PINERY	0.007	0.003	2.203	0.362	
	Rondeau	0.002	0.003	0.503	1.000	
GE	Holland Landing	-0.006	0.003	-1.997	0.491	
	OJP	-0.016	0.003	-4.855	< .001	***
	PINERY	0.003	0.003	1.011	0.971	
	Rondeau	-0.002	0.003	-0.650	0.998	
Holland Landing	OJP	-0.009	0.003	-2.859	0.097	
	PINERY	0.010	0.003	3.007	0.067	
	Rondeau	0.004	0.003	1.346	0.878	
OJP	PINERY	0.019	0.003	5.866	< .001	***
	Rondeau	0.014	0.003	4.205	0.002	**
PINERY	Rondeau	-0.005	0.003	-1.661	0.712	

* p < .05, ** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 8

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