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Brief Report

Impact of Water and Nutrient Supplementation on Yield of Prairie Plantings of Juneberry *Amelanchier alnifolia* Nutt., Cultivar and Windbreak Plantings

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Abstract: *Amelanchier alnifolia* Nutt. (Juneberry, Saskatoon berry or Serviceberry) fruit historically played an important role as fresh or dried food and as a medicinal staple in the Mandan, Hidatsa, and Arikara Tribal Nations. Natural Juneberry stands were lost during the creation of Sakakawea Reservoir on the Fort Berthold Reservation. Reintroduction of the Juneberry is important to the tribal communities. Therefore, the impact of water and fertilizer supplementation was explored in two mature Juneberry cultivar (Honeywood, Martin, and Smokey) plantings and a seedling windbreak planting. Yield was examined in three consecutive years with three treatments: (1) natural conditions (control; no additional water or fertilizer); (2) irrigation during flowering and fruit ripening period (irrigated); and (3) fertilization plus irrigation during flowering and fruit ripening period (fertilized). Yield varied from 5 to 258 g/0.03 m^{-3} across locations, treatments, and years. There was no difference in yield across locations and treatments in year one. Yield was greater in the second year than first year, but not different across locations or treatments. The fertilized treatment showed increased yield in the third year in contrast to irrigated treatment across locations. New plantings can be established more economically using seedling material and the yield increased if watered and fertilized during fruit development.

Keywords: juneberry; serviceberry; saskatoon berry; naakunaánu'; máacudabaa; mánabushaké

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1. Introduction

Native stands of *Amelanchier alnifolia* Nutt. (Juneberry, Saskatoon berry, or Serviceberry) in the bottom land next to the river and streambanks, draws, and coulees near the Missouri river were an important fruit source for the Mandan, Hidatsa, and Arikara Tribal Nations [1]. The fruit of this hardy shrub was a food staple, important for nutrition and medicine in the Native American diet [2,3]. The Juneberry is a small pome fruit and is typically the size of a blueberry (0.5 to 1.0 cm diameter), has many small seeds, a tender skin and is eaten fresh, steamed, mashed, or dried to a brick-like consistency for winter use [1,2,4]. Although lower in vitamin C than many berry fruits, the Juneberry is a good source of copper, manganese, magnesium, and carotenoids. Juneberry fruit is a rich source of polyphenols and has a high content of flavonoid antioxidants (anthocyanins, flavonols, procyadins, and phenolic acids) [5–11]. Historically, the fruit was used to treat diarrhea, sore eyes, and stomach and liver ailments [4,9,12]. In addition, leaves, twigs, bark and roots were used to create teas and tonics. Boiled bark was used as a disinfectant, to expel worms, and to promote discharge of the placenta after delivery [12].

Phytochemical analysis of the Juneberry fruit shows a high phenolic content and free-radical scavenging capacity [9,11,13]. The ability for crude fruit extracts to inhibit nitric oxide production in macrophage suggests a protective role against cardiovascular disease and chronic inflammation [14]. In addition, exploration of the biological activity of Juneberry extracts on cell viability indicates cell viability is maintained in the presence of free radicals [13]. Bioassays of Juneberry samples show improved glucose uptake and reduced expression of anti-inflammatory markers, suggesting an ability to mediate and reduce inflammation [7]. It is notable that the flavonoid compounds in fruit extracts can protect erythrocyte membranes against oxidation by interacting with the membrane surface [15]. Analysis of the antimicrobial, antihyperglycemic, and anti-obesity potential of the Juneberry antioxidants indicate antimicrobial activity. There is also a correlation with these fruit characteristics and its ability to inhibit pancreatic lipase, free amino acids, and antihyperglycemic activity [16]. Testing Juneberry powder in rats' diets indicated it regulates glucose and ameliorates cardiovascular and liver impacts of diet-induced metabolic syndrome [17]. Thus, the anti-inflammatory, anti-diabetic, and chemoprotective effects of this fruit emphasize the importance of improving the availability of this culturally relevant fruit to the Fort Berthold Reservation.

After the completion of Garrison Dam on the Missouri River in North Dakota, USA, by the United States Army Corps of Engineers, the Sakakawea reservoir flooded the bottom land on Fort Berthold Indian Reservation and members of the Mandan, Hidatsa, and Arikara Tribal Nations relocated to the arid plateau regions. This change also flooded the native habitat of the Juneberry and impacted the community members' ability to locate and harvest fruit from native stands that once grew along the Missouri River bottom [1]. In 2004, a project was developed to reintroduce this important fruit in the upland areas near the Sakakawea reservoir [1]. Currently, there are no standardized recommendations for Juneberry irrigation and fertilization. Therefore, the objective of this study was to evaluate whether supplemental water and/or fertilizer would improve the yield in an effort to develop baseline information for future detailed plant fertilization studies in this nutritious and culturally relevant fruit for the prairie regions.

2. Materials and Methods

The Juneberry irrigation and fertilization trial was conducted from 2019 to 2021 at three locations in North Dakota, USA: White Shield, War Coulee, and Elbowoods. All sites were located on the east side of the Sakakawea reservoir in North Dakota. The White Shield cultivar planting was located in McLean County, ND. The site was a glacial till moraine with a slope of $3 \pm 1\%$, soil pH 6.8, and 6.3/15/398 ppm (nitrogen/phosphorus/potassium) and 2–4% organic matter as determined by WARD Laboratories Inc. Kearney, NE, USA. This site had wind protection due to the typography of the site. The War Coulee cultivar planting was located in McLean County, ND. This site was a glacial till moraine with a slope of $3 \pm 1\%$ near the crest, soil pH 6.0, and 6/39/160 ppm (nitrogen/phosphorus/potassium) and 3–5% organic matter as determined by WARD Laboratories Inc. Kearney, NE, USA. The site was more exposed to wind than White Shield due to its elevated location. The Elbowoods seedling windbreak plot was in Mountrail County, ND, USA. The Elbowoods site was located on a glacial till moraine with a slope of $22 \pm 1\%$, soil pH 8.0, (nitrogen/phosphorus/potassium) and 5% organic matter as determined by WARD Laboratories Inc. Kearney, NE, USA.

White Shield and War Coulee plantings contained replicated blocks of clonally propagated Juneberry cultivars 'Martin', 'Honeywood', and 'Smokey' planted in 2004 [1]. The cultivars were replicated in groups of 30 or 33 bushes per cultivar and the three groups randomized in each of the four rows [1]. The plants were spaced at 0.9 m within rows and the rows were 3.66 m apart. Bushes at White Shield had a height and spread of 2.4 m $\times$ 0.9 m and War Coulee had a height and spread of 2.0 m $\times$ 0.9 m. The Elbowoods plot was established in 2016 with seedlings obtained from the Towner State Nursery (NDSU-North Dakota Forest Service, Towner, ND, USA). The seed was collected from fruit collected from wild stands, germinated, grown for one season and dormant bare root plants received from the nursery were planted in 2016. The Elbowoods planting planted in 2016 was composed of six rows (30 plants each), with plants spaced 0.9 m within the row and 3.66 m between rows. This planting was just beginning to bear fruit in 2019 and the height and spread of the bushes was 1.0 m × 0.5 m.

Three treatments were applied to ten consecutive plants/cultivar within each of the four rows: (1) no irrigation (control); (2) irrigation only (every third day at 18.93 l per bush using drip irrigation); (3) irrigation and fertilization (irrigation every third day at 18.93 l per bush and fertilization with the irrigation once per month. The irrigation treatment was applied every third day from April 15 to September 15 and the fertilizer treatment applied once per month at experimental sites in 2019, 2020, and 2021. A general-purpose fertilizer (Jack's 20-20-20) was delivered through the irrigation water via trickle irrigation, with each bush receiving 880 ppm N (46.5 ppm each liter) on or around the 15th of the month. All irrigation water was trucked to experimental sites, which were five to six miles from the Sakakawea Reservoir.

Harvest was completely random across the treatments and was conducted when Juneberry fruit reached full color on July 10th, 1st, and 11th in 2019, 2020, and 2021, respectively. Yield (g/0.03 m^{-3}) collected once per bush for seven randomly selected bushes in each treatment from across the 4 rows. A 0.03 m^3 cube was constructed using 2.5 cm diameter polyvinyl chloride plastic pipe to define a uniform harvest area since the bush size varied between the three locations. One measurement was carried out at a height of 1.5 m per bush for seven randomly selected bushes in each treatment (White Shields and War Coulee plots) and on seven seedling bushes (Elbowoods plot) in each treatment in years one and two. In year three, measurements were carried out on five bushes in each treatment.

Analysis of the Juneberry treatment yield data was conducted across cultivar, location, and year. The data were visualized using ggplot2 library in R (ggplot2) using box plots with the median across the box and the whiskers as lines extending from quartile1 and quartile3 to end points that are typically defined as the most extreme data points within Q1 − 1.5 × IQR and Q3 + 1.5 × IQR, respectively. Each outlier outside the whiskers is represented by an individual mark [18]. Dots outside the first and third quartile in figures are values that fall within the expected range of values are retained as a typical observation in agronomic experimentation. An analysis of variance (ANOVA) was performed for Juneberry yield data to study treatments (irrigation and fertilization), and its interactions with cultivar and the location and year. Interaction effects were further analyzed separately to compare each component using the Tukey adjusted mean separation method. The data were analyzed using the 2020 R statistical software packages (R version 4.2) [8].

3. Results

There was no precipitation during the fall and early spring in the three-year period (Table 1). Precipitation during the June flowering and fruiting period was less than 5 cm with the exception of June 2020.

Table 1. Monthly precipitation for McClean County (cm). Data from North Dakota Agricultural Weather Network.

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
2019	0.0	0.0	0.0	2.8	4.4	3.7	8.2	7.6	21.3	2.6	0.0	0.0	50.7
2020	0.0	0.0	0.0	0.2	2.4	14.4	2.9	2.8	0.6	0.4	0.0	0.0	23.6
2021	0.0	0.0	0.0	0.5	2.0	3.7	8.2	5.1	1.0	8.0	0.0	0.0	28.6

Figure 1 illustrates the variability in yield between the White Shield and War Coulee location and suggests the presence of cumulative treatment differences. In the 16-year-old cultivar plantings the yield main effects of treatment, location, and year were significant, but the cultivar effect was not (Table 2, Figure 2a). The yield was greater in year two and three than year one for all three treatments across all cultivars and locations, as shown in

Figure 2b. The fertilized treatment showed that the yield increased in year three in contrast to the irrigated treatment (Figure 2b). The interaction effects of treatment by cultivar, treatment by location, and treatment by year were significant (Table 2). At White Shield, the fertilized treatment increased yield in Honeywood and the irrigated and fertilized treatments increased yield in Smokey; however, these did not show a significant increase yield for Martin. At War Coulee, Martin and Smokey showed increased yield in irrigated and fertilized treatments in years two and three.

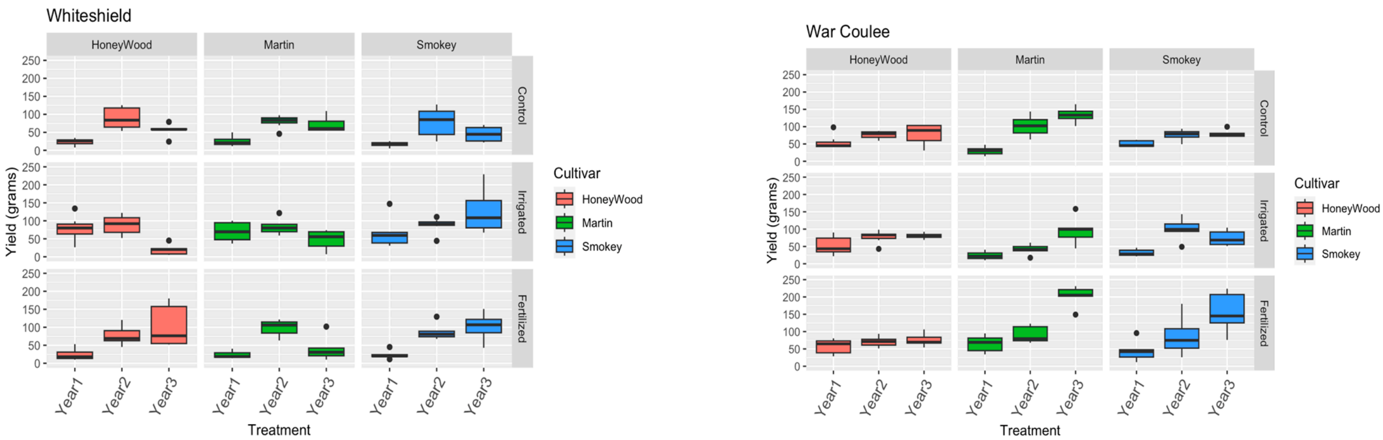
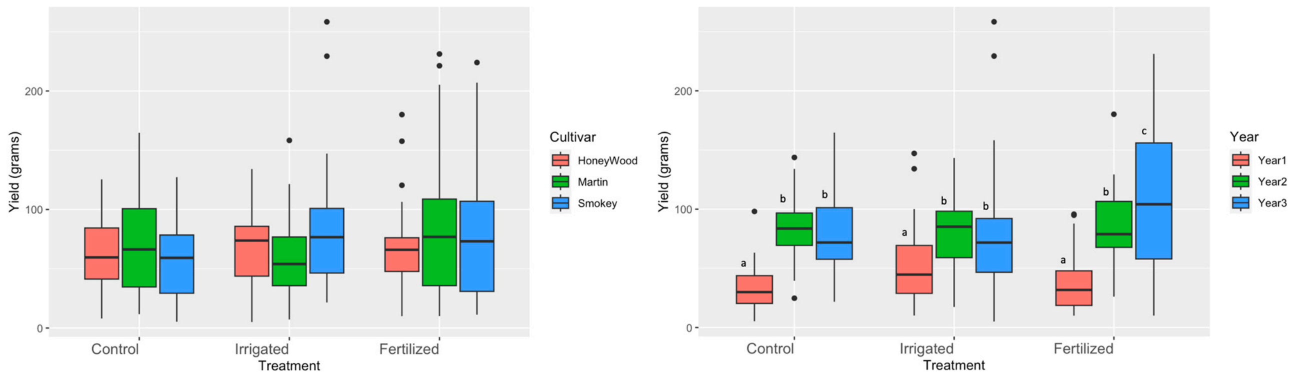


Figure 1. Visualization of treatment yield (g/0.03 m^{-3}) for three cultivars and three years in the 16-year-old Juneberry plantings at White Shield and War Coulee locations. Years 1, 2 and 3 are 2019, 2020, and 2021, respectively. Yield was measured for a constant bush volume (g/0.03 m^{-3}) in seven randomly selected bushes for each treatment in years one and two and five bushes in year three. Boxplots were constructed as described in the Methods section.

Table 2. Yield analysis of variance for main effects (cultivar, treatment, location and yield) and treatment by location, cultivar, and year interactions.

Factor	df	Mean sq	F-Value	*p*-Value
Location	1	9300	7.93	0.005
Cultivar	2	1561	1.33	0.266
Treatment	2	4162	3.55	0.030
Year	2	77,301	65.90	<0.001
Treatment–Location	2	13,884	11.84	<0.001
Treatment–Cultivar	4	4277	3.65	0.006
Treatment–Year	4	6563	5.59	<0.001
Residuals	322	1173		

The Elbowoods seedling plot was three years old at the beginning of this study and had limited fruit in year one (2019), (Figure 3). In 2020, the seedling had yields (g/0.03 m^{-3}) that were approaching those of the mature cultivar plantings and appeared to benefit from irrigation and fertilization (Figure 3). Conditions going into year three were very dry with only 12.9 cm of natural precipitation in the 12 months from July 2020 (harvest) to the end of June 2021. Under these conditions, birds were eating fruit before it was fully ripe and no fruit was available for harvest.



(**a**) (**b**)

Figure 2. Treatment yield (g/0.03 m^{-3}) for cultivars and years across locations. (**a**) Treatment yield across locations and years; (**b**) treatment yield across cultivars and locations. Yield was measured for a constant bush volume (g/0.03 m^{-3}) in seven randomly selected bushes for each treatment in year one and two and five bushes in year three. Boxplots were constructed as described in the Methods section. Significant differences within treatments and among years are noted using different letters. Treatment by year interaction *p*-value < 0.001; n = 21.

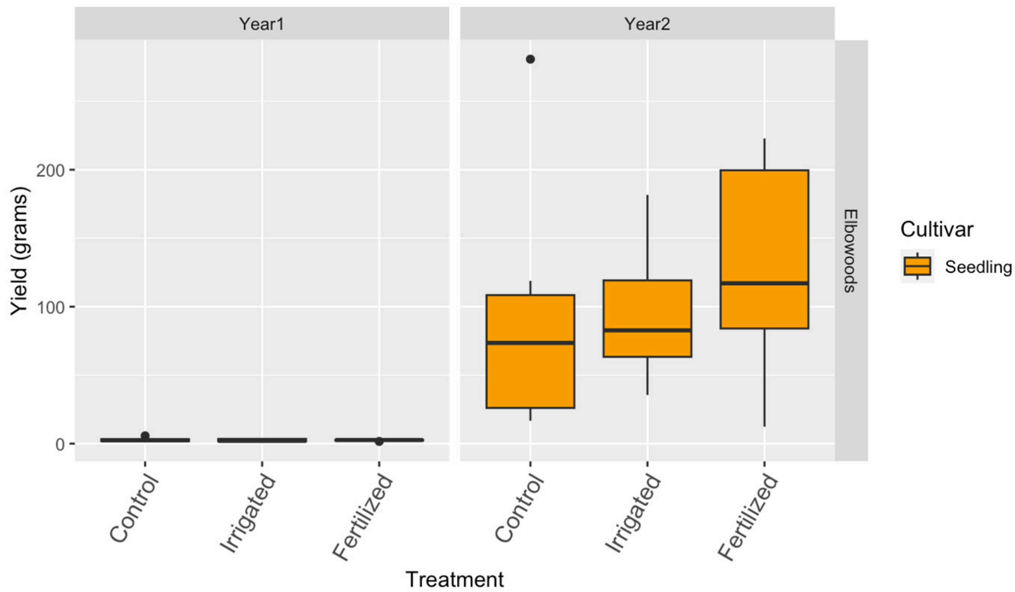


Figure 3. Elbowoods Turner State Nursery Juneberry seedling yields. The plants were three years old in year one (2019). Yield was measured for a constant bush volume (g/0.03 m^{-3}) in seven randomly selected bushes for each treatment in year one and two. Boxplots were constructed as described in methods. Year one was the first year of fruiting and yield was <5 g/0.03 m^{-3} (n = 7). Treatment effect was not significant in year two (n = 7), and in year three (2021) complete fruit predation eliminated data collection.

4. Discussion

Precipitation accumulation is limited between fall and spring in McClean County, on the Fort Berthold Reservation where the Juneberry cultivar trials were conducted. This restricted the soil water available for fruit development and the flower initiation for the next growing season in this perennial plant. Previous studies on the establishment of Juneberry in North Dakota have shown the importance of supplemental water to prevent young plant loss during the establishment of Juneberry plants [1,19]. Once the plants are established it is suggested that natural rainfall is adequate for the Juneberry maintenance [1,19]. The bushes at White Shield and War Coulee were the same age, had differing soil pH, but similar chemistry and the War Coulee upland and wind exposed site had smaller bushes. The War Coulee was exposed to more wind than the down slope White Shield site. The Elbowoods plants were just coming into production and were only 1.0 m tall. Therefore, all yields are presented for a uniform bush volume (0.03 m^3) rather than a total bush harvest as previously reported for non-irrigated Juneberry production [19]. It is noted that even though a consistent bush volume was used, the location effect was significant and differences in bush structure (i.e., canopy density) could be a contributing factor.

A previously published non-irrigated Juneberry cultivar evaluation indicates that yield varies widely between years and suggests that either precipitation or alternate year bearing (tendency to fruit heavily every other year) could promote this oscillation in yield [19]. There are many studies on Juneberry fruit chemical makeup but few studies on fruit yield in low rainfall conditions. A Canadian ecological study of fruit production in native stands of Juneberry and other native berry shrubs attempted to correlate rainfall and evapotranspiration in the summer with the following growing season's fruit production. A drought code was developed using rainfall and evapotranspiration during the fruiting period to provide an estimate of soil moisture availability, and correlation of the drought code with fruit production in the next growing season was explored [20]. A negative correlation was found between the summer drought code and the following season's production, suggesting the importance of the previous season's precipitation on fruit production in the following season. Although the native Canadian Juneberry fruit production was affected by dry summer weather, it is noted that the prior season fruit production level accounted for more of the annual production variation than the precipitation did. Thus, the previous season's production level and its impact on resource availability contributes to alternate year bearing patterns [20]. Juneberry inflorescence initiation occurs in the axillary buds towards the end of fruit ripening period. Resource competition (such as that related to water or nutrients) during fruiting period completion in the current season could reduce floral development for the next year, as suggested by previous studies [21]. Although precipitation was greater from July to September in 2019 (year 1) than in 2020 and 2021, the yield of the control treatment did not vary significantly across the years. Similarly, comparison of the control and irrigation treatment compared across locations and cultivars in years two and three indicated no significant difference in yield between the control and irrigated treatments. However, the fertilized treatment showed an increased yield with cumulative fertilization in the third year, suggesting that nutrient status is more limiting than the natural precipitation levels.

Despite being impacted by predation in the third year of data collection, a similar response appeared to be developing in the seedling plots. The native seedling materials were developed as an economically feasible alternative to the use of clonally propagated cultivars. Compared to the cost of clonally propagated cultivars, which comes to approximately USD 5/plant plus shipping, seedling plants can be purchased from the state nursery for a more affordable price of USD 1.5/plant. Although the plants are more variable in bush and berry size, the seedling plants from wild gathered berries may be more suitable to a given region. In addition, wild biotype fruits gathered in the same area of cultivar trials are richer in phenolic compounds and antioxidant activity than the fruit from cultivars. This suggests that the use of seedling material may provide a more functional food [8].

Currently there are no standardized recommendations for Juneberry fertilization. In this study, the fertilizer was delivered through the irrigation water to ensure incorporation into the soil since, because early spring precipitation is limited, a spring broadcast of granular fertilizer would not be easily incorporated into the soil. This study indicates that fertilization during the flowering and fruiting periods of 2019 to 2021 resulted in an increased yield in 2021. Future studies would be best served by using new seedling plantings, tracking fruit size and adding additional fertilization treatments, leaf foliar analysis and a fertilization-only treatment. Future studies should also consider pruning strategies to maintain an open sunlight canopy; as nutrient availability increases, it may result in shading within the bush interior and ultimately limit the productive area of the bush. This study suggested that the plants' nutrient availability may be a more limiting factor than that of water alone on the native *Amelanchier alnifolia.* Future studies should focus on exploring timing and amount of water and fertilization needed to develop recommendations for sustainable production. This will be especially important in arid regions where precipitation is limited and trucking water to plantings increases production costs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9060653/s1.

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