RESEARCH REPORT

BY

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ON THE

Effect of *Ascophyllum nodosum* seaweed extract and powder on vegetative growth characteristics and foliar nutrient composition of *Vitis spp.* hybrid cv. L'Acadie Blanc

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Conflict of Interest Statement

The researcher and advisors disclose that they do not have a special interest, financial or otherwise, in the pursuit or outcome of this research.

Importance

This research will be of particular interest to grape growers in Nova Scotia and New Brunswick, who are seeking soil amendments to improve vegetative growth of grape vines, especially during the establishment phase of a vineyard, where locally available rockweed (*Ascophyllum nodosum*) is available for purchase or harvest. Grape growers in other regions with similar climates, such as British Columbia may also be interested due to the wide availability of seaweed meal and extract products, either of *A. nodosum* or other species that are thought to provide similar benefits.

Objectives

- Test for effects of seaweed species *Ascophyllum nodosum* (AN) on vegetative growth characteristics and foliar nutrient composition of *Vitis spp*. hybrid cv. L'Acadie Blanc (LB).
- Compare extract and powder forms of AN in terms of effects on cv. LB.

Abbreviations

AN – *Ascophyllum nodosum*; **ANE –** AN extract; **EC –** electrical conductivity; **LSE –** liquid seaweed extract; **NS –** Nova Scotia; **NB –** New Brunswick; **LB –** L'Acadie Blanc; **MA** - *Mycophycias ascophylli*; **VV –** *Vitis vinifera*; **cv. –** species cultivar

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Abstract

Seaweeds are used in agriculture to provide nutrients, biostimulation and soil conditioning, among other benefits if harvested sustainably. Wine grape (*Vitis vinifera*) varietals grown in coastal regions may benefit from a locally available seaweed amendment. Existing studies show mixed response from the use of seaweed extracts on wine grapes. This research studies the effect of *Ascophyllum nodosum*, a North Atlantic seaweed species on *Vitis spp*. hybrid cv. L'Acadie Blanc white wine grapes. We conducted a two factor, eight treatment experiment, with *A. nodosum* powder and extract at four levels, to compare efficacy by measuring vegetative growth characteristics and foliar nutrient concentration of grape vines (*V. vinifera* cv. 'L'Acadie Blanc'). Total number of nodes, number of growing points, length of the longest shoot, and many macro and micro nutrients in leaves were measures. This research will assess whether amending vineyard soil before establishment with seaweed powder will result in potential benefits to wine grape plant growth.

Introduction

Seaweed is a well-known marine organism that has been used by humans for centuries. It can be an effective amendment to improve yield in many crops (Sharp 1986). Today, 221 species of seaweed have commercial value and are extensively harvested for foods, pharmaceuticals, biofuels, animal feed, and fertilizer, representing an industry worth more than eight billion dollars per year (Ferdouse et al. 2018). Global seaweed harvests in 2015 yielded 29 and 1.1 million tonnes of farmed and wild seaweed, respectively (Ibid). Canada harvests wild seaweed only, yielding 11,573 t (Ibid); other figures suggest nearly double that with yields in British Columbia and Atlantic Canada at ~400 t and ~20,000 t, respectively (Ugarte and Sharp 2012, Lindop 2017). Almost all of the seaweed harvested in Atlantic Canada is *Ascophyllum nodosum* (AN), one of the species central to this research (Ugarte and Sharp 2012, Ugarte and Sharp 2001, Ferdouse et al. 2018, Sharp 1986).

Ascophyllum nodosum (AN)

AN is a marine species of Phaeophyta, a division of brown algae native to the Atlantic Ocean from the Arctic Circle to New Jersey and Europe. It is photosynthetic, contains carotenoids, carbohydrates, sterols, tannins, and in some cases, pheromones (Ibid). Commonly called rockweed, this name is also applied to other seaweeds species (Ibid). Morphologically, it has dichotomously branching shoots originating from a holdfast that extends to the surface by air filled bladders, forming a floating canopy (Cousens 1984, Sharp 1986). It grows in partially to fully sheltered shoreline beds where wave action is limited (Ibid). In Nova Scotia (NS) and New Brunswick (NB) AN is harvested in government regulated zones in intertidal areas using a cutter rake from a fishing boat (Ugarte and Sharp 2001, Ugarte and Sharp 2012).

L'Acadie Blanc

The other player at the centre of this study, *Vitis spp*. cv. L'Acadie Blanc (LB), is a domesticated white wine grape cultivar that has become Nova Scotia's specialty wine grape varietal (Peters and Pihney 2016). *Vitis spp.* are rambling vines with multiple growing points that rely on other structures for support. They tend to be vigorous, requiring little nutrition in comparison to other plant species (White 2009). Maximizing the quality of grape production requires reducing plant vegetative vigour using canopy management and training vines on a trellis system to maximize sun exposure in vineyards (Ibid). Vigorous vines may lead to quickly established vineyards but once a vine matures, reduction in vine vigour makes for better wine grapes and better wine (Ibid).

NS is relatively unknown in the wine world, though a wine industry has emerged with plantings of French-American hybrid *Vitis spp.* and the hardiest of less cold-tolerant VV varieties (Peters and Phiney 2016, Shaw 1999). NS has a long history with wine grapes, the first having been planted in LaHave in the mid 1600's. The modern industry didn't begin until the 1980's with hybrid vines. French-American hybrids are a cross between European *Vitis vinifera* (VV) and North American species *Vitis riparia* and *Vitis labrusca* (Peters and Phiney 2016). They are cool climate wine grapes that ripen earlier (Ibid). LB is a complex hybrid with parentage of 8 *Vitis* generas (Ibid). Originally known as V53261, LB was hybridized at the Vineland Research Station in Niagara, Ontario, where it didn't perform well in the hot summers of Southern Ontario (Ibid). It was brought to NS in the 1980's and renamed by the Kentville Research Station (Ibid). Resistant to botrytis bunch rot (due to loose grape clustering), providing consistent yield, and producing high quality white wine grapes, LB represents more than 50% of vineyard plantings in NS (Ibid). LB provides for a full bodied wine with floral and honey aromas in still or, more notably, traditional method sparkling wines (Robinson et al. 2012). Some compare growing conditions of Nova Scotia's Annapolis Valley to that of Champagne, and LB, in traditional method sparking form, has been able to complete globally, winning prestigious wine awards (Peters and Phiney 2016).

The NS vineyard industry, of which LB makes up more than 50% of plantings, has grown 12% per year, from 56 t in 1980 to 1,146 t in 2013. In 2014 there were 658 acres planted and only 10 farms larger than 10 acres (Nova Scotia Department of Agriculture 2014). The Wine Association of Nova Scotia's goal is for 1,000 acres under production by 2020 (Grape Growers

Association of Nova Scotia 2020). The industry is supporting the growth of small scale vineyards, which bolsters the economic health of communities in which they are located and successful production of LB is an important contributor to the socioeconomic viability of rural NS. LB is an important grape variety to the burgeoning NS wine industry. As the industry expands, finding locally-available, sustainably-sourced amendments that encourage vegetative growth of vines will help producers.

Sustainable marine harvest?

Considering the potential economic value of AN, it is important to assess the sustainability of AN harvest. Harvesting a marine species in an area where past overexploitation of marine species has caused ecological and economic crises is cause for scrutiny. NS and NB regulate harvest of AN and evidence suggests annual harvests have reached a consistent yield per hectare of AN and that standing crop biomass has remained stable. During peak harvest year 2010, harvests ranged from 8.6% to 23.5% of standing stock in management zones, far below the rate of regrowth (Sharp 1986). Environmental monitoring shows AN associated species have not been deleteriously affected; limits on harvesting are determined using previous-year stock surveys (Ugarte and Sharp 2012). Ecosystem, not species specific, marine management plans addressing potential ecological issues have resulted in a relatively sustainable harvest (Ibid). As such, AN appears to be a good candidate for study as a locally available, sustainably sourced amendment for LB. It is available as an extract and in powder form.

Effect of *Ascophyllum nodosum* seaweed extract and powder on vegetative growth characteristics and foliar nutrient composition of *Vitis spp.* hybrid cv. L'Acadie Blanc

Known Benefits & Formulations

Seaweed meal and extracts have long been known to contain nutrients essential to crop plants including N, P, K, Cu, Co, Zn, Mn, Fe, Ni, Mo and B (Craigie 2011). In raw forms, such as meal, seaweed acts as a soil conditioner that helps improve water retention capacity and soil texture (Ibid). Research on the effect of seaweed on plants focuses on liquid seaweed extract (LSE), a concentration of compounds found in seaweed, produced through fermentation, pressure, or alkaline or acid hydrolysis (Arioli et al. 2015). LSE prepared from AN is commonly referred to as ANE (De Saeger et al. 2019).

Hormones & Biostimulant Properties

Extracts from brown seaweeds, such as AN, contain auxins, cytokinins, gibberellins, abscisic acid, and brassinosteroids (Stirk et al. 2014). The presence of these substances suggest that brown seaweed may also enhance or alter metabolic processes of the plants to which they are applied. De Saeger et al. state that "ANEs affect the endogenous balance of plant hormones by modulating the hormonal homeostasis, regulate the transcription of a few relevant transporters to alter nutrient uptake and assimilation, stimulate and protect photosynthesis, and dampen stress-induced responses" (2019). The exact mechanisms by which ANE operates are not well-known due to the likelihood of polygenic effects of ANE; research is ongoing (Ibid). Though, Rayorath et al. found evidence for ANE modulating concentration and localisation of auxin (2008). The presence of auxins may result in quicker germination of seeds. Application of ANE on beet seed resulted in 84% germination compared to 0 in the control (Senn and Skelton 1966). Yields tended to increase among most crop plants with ANE applied in a range of studies including those of tomato, tobacco, peas, turnip, cotton, white pine, holly, among others. (Ibid).

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Microbial Associate

AN forms fungal associations including with fungal symbiont *Mycophycias ascophylli* (MA), an endophyte belonging to phylum and class *Ascomycota Eurotiomycetes* (Xu et al. 2008). AN forms the physical structure and the fungus colonizes the plant. Researchers contemplated calling this symbiosis a lichen but instead decided on mycophycobiosis between a marcoalga and endophyte fungi (Ibid). Interestingly, AN is always found in association with MA (Garbary and Deckert 2001). This is an important consideration in assessing the growth of vines treated with AN since it is well-known that mycorrhizal relationships aid in the transfer of water, nutrients, and provide for improved soil structure (Lowenfels 2017). In Northern Italy a study on vineyard microbes found the most common phyla of fungi identified to be Ascomycota, at 77% (Longa et al. 2017); results in a South African study found the same result (Carmichael et al 2017). Further, research on vineyard microbial communities in Washington State found association between fungal presence and both lower chlorosis and higher leaf nutrient levels. Even though it is unclear which mycobionts are responsible, members of the *Eurotiomycete* class of *Ascomycota*, to which MA belongs, were present (Lewis et al. 2018).

Effects of seaweed on plant growth

ANE has benefited a wide variety of plants but relatively few studies involve wine grapes. Research on the effects of seaweed on crop plants is well-established, showing evidence of supporting pest and disease resistance (Jayaraman et al. 2011, Ali et al. 2019, Frioni et al. 2019, Wite et al. 2015), improved yield (Ali et al. 2019, Arthur et al. 2003, Trejo et al. 2018, Kumar and Sahoo 2011, Jannin et al. 2013, Pramanick et al. 2017), reduced drought stress (Santaniello et al. 2017, Zhang and Ervin 2004), a source of nutrients Ca, K, P, N, Fe, Cu, Zn, B, Mn, Co, and Mo (Ferdouse et al. 2018, Khan et al. 2009), improved quality (Frioni et al. 2019, Pramanick 2017), increased germination rates (Economou et al. 2007, Kumar and Sahoo 2011, Hong et al. 2007, Moller and Smith 1999), stimulation of shoot growth and branching (Temple and Bomke 1989), improved bacterial symbiont colonization of roots (Khan et al. 2012), frost resistance (Wilson 2001, Senn and Skeleton 1966), and biostimulation (Battacharyya et al. 2015, Stirk et al. 2014, De Saegar 2019, Rayorath et al. 2008).

Effects of seaweed on vine growth

Few studies have assessed effects of seaweed on vegetative growth of wine grape (Taskos et al. 2019); and the available results are mixed. Studies examining seaweed effects on fruit yield, disease resistance, grape quality, and phenolic composition of grapes and wines are discussed below (Gutiérrez-Gamboa et al. 2020). At the time of writing, no research on the effects of LSE or meal of any seaweed species on LB had occurred.

Disease: Foliar application of ANE reduced spread of grey mold in VV cv. Sangiovese (Frioni 2019).

Yield Measurements: Seaweed foliar extract application had a positive impact on vine size among 6 yield components when used on hybrid cv. Baco Noir in Niagara-on-the-Lake (Wiens and Reynolds 2008). Pinot Noir and Cabernet Franc had greater dry biomass with ANE application in Michigan (Frioni et al. 2019). However, fertigation application of LSE had no significant impact on yield components or uptake of Syrah in California (Martin 2012). A two-year study in Turkey found no effects on dry biomass of VV cv. Narince (Sabir et al. 2014).

Nutrition: In the same study referenced above leaf analysis showed chlorophyll content and nutrients Ca, Zn, and S increased while Mn, Fe, and Cu decreased in both years (Ibid). Thompson seedless varieties responded to LSE with increases in leaf area and chlorophyll; unlike Sabir et al., leaves of Thompson seedless found increases in N, P, and K with foliar applications (Abou-Zaid et al. 2019). Pereira de Carvalho et al. also found mixed results with ANE foliar extracts on VV cv. Niágara Rosada table grapes with an increase in K, B, and Zn (2019). LSE from three seaweed species resulted in increase in foliar Cu (Ibid). Yet, Nagy and Pinter found no effect on leaf nutrient concentration among VV cv. Blaufrankish, even though yield increased; field observations noted larger, greener leaves among vines (2015). ANE applied to VV cv. Tempranillo did not increase ammonium levels in wine musts over two seasons (Gutiérrez-Gamboa et al. 2020). Of potted VV provided foliar treatments, seaweed extract showed improved growth and Ca and K uptake (Mancuso et al. 2006) Mugnai et al. found LSE to enhance nutrient absorption of ammonium and K between the meristem and elongating root region in VV, with some extracts resulting in increase in dry root weight and total dry weight; they hypothesize that auxin from LSE is responsible for improved nutrient absorption (2008).

Liquid Extract Versus Meal

Another factor to consider in the use of AN for viticulture is whether refining seaweed to LSE is necessary. Solid, dry forms known as meal *could* provide the same or similar benefit. Harvesting, desalinating, drying, and crushing methods are available to the low-tech viticulturist looking to provide a quality product. Further, organic material from unprocessed seaweed could bring benefits to the soil such as moisture retention, slow release of nutrients, and improved soil structure, among others. A lack of literature points to a gap in research on the use of seaweed meal and the comparison of meal versus extract in their effect of vine growth. Most literature discusses meal in the context of animal feed (Evans et al. 2014). In the few studies evaluating meal against a control, Haslam and Hopkins found that chopped pieces of a brown seaweed, *Laminaria digitate*, increased pore volume and soil stability (2006); seaweed composts have aided in improving yield and disease resistance in tomatoes (Eyras et al. 2008); and, raw forms of seaweed have increased potato yields (Lopez-Mosquera and Pazos 1997). Milton reports that LSE application results in more immediate growth of plants (when compared to meal), which he explains by the lack of nitrogen due to decomposition and therefore nitrogen immobilization of the carbon-rich meal form (1964). Further, he observed a 15 week period of seed germination inhibition and presence of toxic sulfhdryl compounds after applying solid seaweed, indicating that the solid form may not be suitable for seedlings (Ibid).

One of the reasons seaweed extract is studied more frequently than meal might be the difficulty of working amendments into the soil of established vineyards and the reported high capacity for ion exchange of grape leaves when sprayed, since seaweeds have chelating properties (Pereira de Carvalho et al. 2019); further, several researchers claim that LSE, when combined with minerals, can result in more permeable leaf membranes and therefore improved nutrient uptake (Craigie 2011, Mugnai et al. 2008, Mancuso et al. 2006). Seaweed meal effects on vine growth are not well documented.

Objective

The study was conducted to determine the effects of AN on vegetative growth characteristics and foliar nutrient composition of one year-old *Vitis spp*. hybrid cv. LB. In particular, this research investigated whether an extract or meal (powder) form had different effects on LB.

Methods & Materials

Study Area & Plant Material

The study took place at the Kwantlen Polytechnic University (KPU) Research and Teaching Farm in Richmond, BC, Canada (49.175° N, 123.12° W) at 1.0 m above sea level (Environment Canada 2019). Mean January and July temperatures are 4.1°C and 18.0 °C, respectively, and the area receives approximately 1191 mm of precipitation annually with the majority falling from October to March when monthly totals average at least 100 mm (Ibid). One-year old bare-root LB vines were used.

Experimental Design

The experimental design consists of 48 potted vines distributed in a completely randomized design with two factors, seaweed form (powder and extract) and quantitative application rate (none, low, medium, high), resulting in eight treatments of six replicates. The vines were planted in 18 L pots in a medium consisting of peat and an additional 1 L of compost. The extract and powder was applied with guidance from manufacturer's specifications.

Based on manufacture specifications, the mean application rate for extract was calculated on a per plant basis and found to be 2 ml per 2 to 4 week period. This amount is low enough to be questionable as to whether it would have any impact and so 2 ml per 2 week period was selected as the low amount, 4 ml per 2 week period the medium amount and 8 ml per 2 week period the high amount. Extract was applied every two weeks between June 22 and August 29 and mixed with 4 parts water to ensure translocation to the rhizosphere. The powder application rate was also calculated on a per plant basis ,with a low amount of 300g, medium of 600g, and high of 1200g. The powder was incorporated into the medium before planting. The plants were transplanted into pots and provided drip irrigation and were allowed to drain freely.

Biometrics

On a bi-weekly basis beginning June 22 and August 29 the total number of nodes on the longest vine, length of longest vine, number of growing points, and SPAD reading of the most mature leaf was recorded. Measurement of foliar nutrient content occurred on the days between August 29 and September 10. Methods for foliar analysis were adapted from conifer foliar sampling from Domes et al. (2018). Petioles were dried in an oven at 70°C, milled to less than 1mm and analyzed at the BC Ministry of Environment and Climate Change Strategy's Analytical Chemistry Services Laboratory for mg/Kg of B, Ca, Cu, K, Mg, Mn, P, S, and Zn via VHP closed vessel microwave acid digestion followed by inductively coupled plasma pass spectroscopy and for % C, S and N via combustion elemental analysis.

Data Analysis

Repeated measures data was analyzed using a mixed model analysis and means were separated by Holm's test when significant effects are detected. EC and nutrient data were analyzed via ANOVA and means were separated by Holm's test. EC was transformed via log to reach normal distribution. All analyses were conducted using the jamovi interface for the R statistical computing environment, with a critical threshold of $\alpha = 0.05$ maintained throughout.

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Results

Length of longest vine

Date and concentration showed an effect on the length of longest vine length, form nearly showed an effect and other interaction effects were or were nearly significant (Table 1). The concentrations within each form shows differences among powder treatments but not extract treatments (Figures 1a and 1b). Vines receiving low and medium powder treatments generally caught up to the best performing vines that received no powder by the end of the growing season, whereas those that received high powder treatments showed the least growth (Figure 1a). Due to pest and disease damage and weather damage, two of the vines that received high powder treatments snapped, leading to other vines on the same plant becoming the longest and therefore being measured for length – this explains the drop in length of the longest vine between August 16 and 29 among vines receiving high powder treatments (Figure 1a).

Table 1. Results of mixed model analysis on mean length of longest vine.

	F	Num df	Den df	*p*
Form	3.42	1	40	0.072
Date	153.20	5	200	**< .001**
Concentration	3.57	3	40	**0.022**
form ✻ date	1.76	5	200	0.122
form ✻ concentration	2.69	3	40	0.059
date ✻ concentration	3.01	15	200	**< .001**
form ✻ date ✻ concentration	3.18	15	200	**< .001**

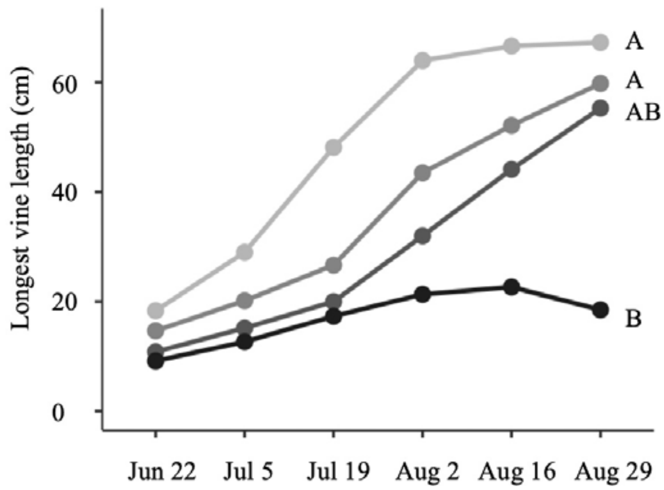


Figure 1a. Longest vine length trend over time by powder treatment concentration. August 29 means labelled with the same letter do not differ significantly.

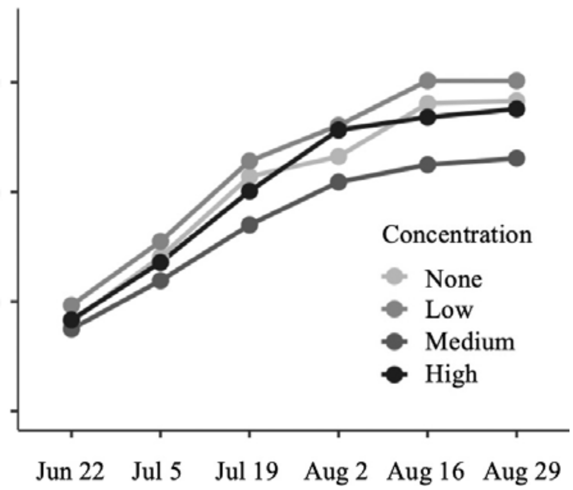


Figure 1b. Longest vine length trend over time by extract treatment concentration. No significant concentration effect was detected for August 29.

SPAD Reading

Date showed an effect on SPAD reading, form nearly did and concentration did not; other interaction effects show varying results (Table 2). The concentrations within each form did not show any differences; SPAD readings declined slightly over the growing season and no readings were recorded on June 22 as the leaves were not large enough to measure (Figures 2a and 2b).

Table 2. Results of mixed model analysis on SPAD reading.

	F	**Num df**	**Den df**	***p***
form	3.807	1	40	0.058
date	25.489	4	200	**< .001**
concentration	2.251	3	40	0.097
form ✻ date	4.825	4	200	**< .001**
form ✻ concentration	0.493	3	40	0.689
date ✻ concentration	1.735	12	200	0.064
form ✻ date ✻ concentration	2.367	12	200	**0.008**

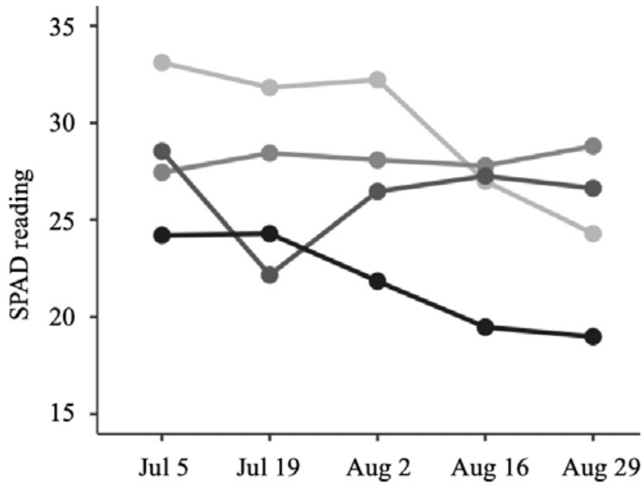


Figure 2a. SPAD reading trend over time by powder treatment concentration. No significant effect of concentration was detected for August 29.

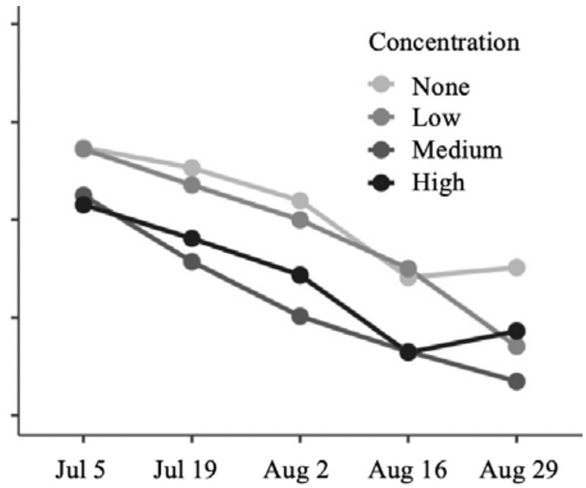


Figure 2b. SPAD reading trend over time by extract treatment concentration. No significant effect of concentration was detected for August 29.

Mean internode length on longest vine

Date, form and concentration affected mean internode length on the longest vines (Table 3). The concentrations within each form did not show any differences (Figure 3a). However, difference between high powder and no powder treated vines is almost significant with a *p-value* of 0.64; and, powder treated vines has observably shorter mean internode length as powder concentration increased (Figures 3a and 3b).

Table 3. Results of mixed model analysis on mean internode length on longest vine.

	F	Num df	Den df	*p*
form	4.662	1	40	**0.037**
date	18.505	5	200	**< .001**
concentration	3.785	3	40	**0.018**
form ✻ date	1.019	5	200	0.407
form ✻ concentration	2.018	3	40	0.127
date ✻ concentration	0.605	15	200	0.869

Table 3. Results of mixed model analysis on mean internode length on longest vine.

	F	Num df	Den df	*p*
form ✻ date ✻ concentration	1.113	15	200	0.347

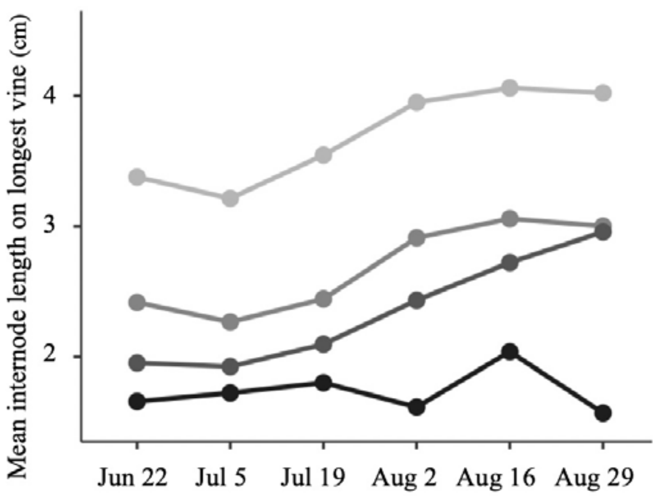


Figure 3a. Internode length on longest vines over time by powder treatment concentration. No significant effect was detected for August 29.

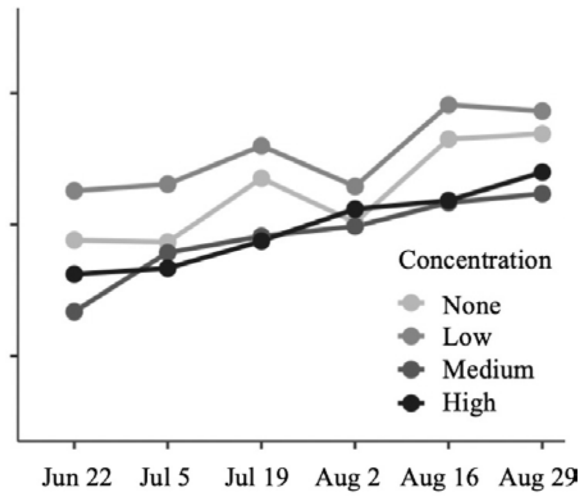


Figure 3b. Internode length on longest vines over time by extract treatment concentration. No significant effect was detected for August 29.

Soil EC

Form and concentration showed an effect on soil EC (Table 4). Powder treatments has higher soil EC than extract treatments (Figure 4a). EC trended lower with lower concentrations (Figure 4b).

Table 4. Results of ANOVA on log (dS/m) of soil EC on July 7.

	Sum of Squares	df	Mean Square	F	p
form	0.931	1	0.931	7.78	**0.008**
concentration	2.412	3	0.804	6.72	**< .001**
form ✻ concentration	0.600	3	0.200	1.67	0.189

Table 4. Results of ANOVA on log (dS/m) of soil EC on July 7.

	Sum of Squares	df	Mean Square	F	p
Residuals	4.666	39	0.120		

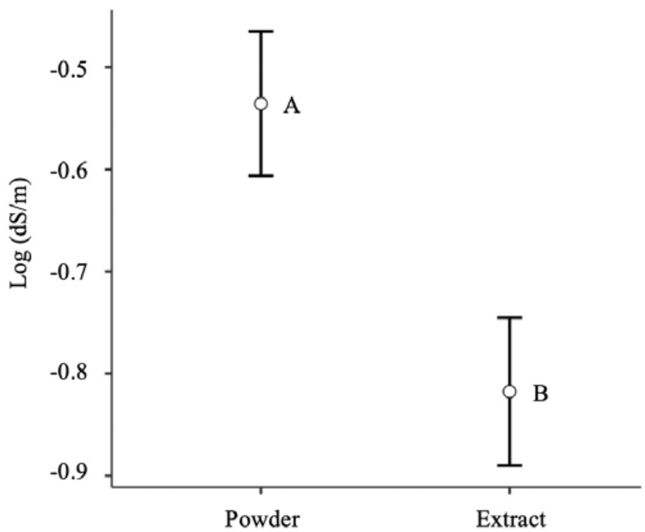


Figure 4a. Log_{10}-transformed soil EC by seaweed form on July 7. Bars denote standard error of each mean. Means labelled with the same letter are not significantly different.

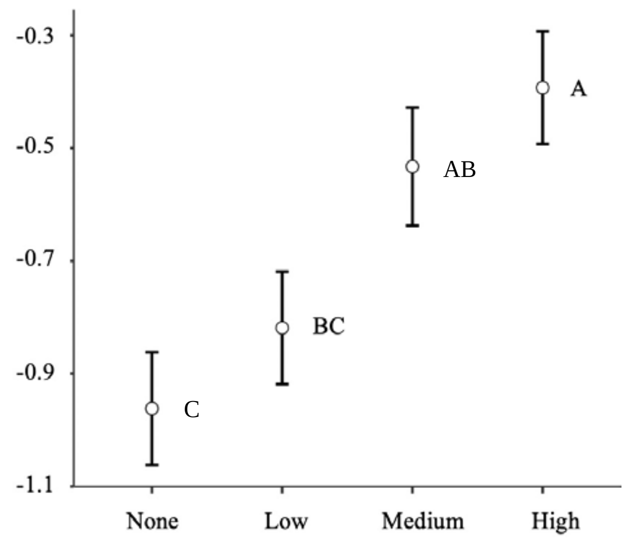
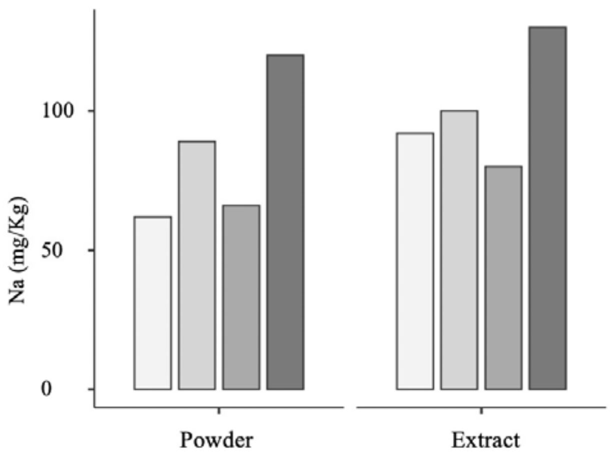


Figure 4b. Log_{10}-transformed soil EC by seaweed concentration on July 7. Bars denote standard error of each mean. Means labelled with the same letter are not significantly different.

Nutrient & Foliar Analysis

Nutrient analysis of a single sample of both the extract and the powder showed no differences for all nutrients except for Na and S (Table 5). Na levels in mg/Kg were elevated in the powder and S levels in mg/Kg were elevated in S. Foliar analysis showed no differences for most nutrients between forms and among concentrations, except for that of Na and N (Table 6).

N levels in percent mass were elevated in powder treated vines over extract treated vines (Figure 6b). Na levels in mg/Kg were elevated in extract treated vines and in vines treated with higher concentrations (Figure 6a).



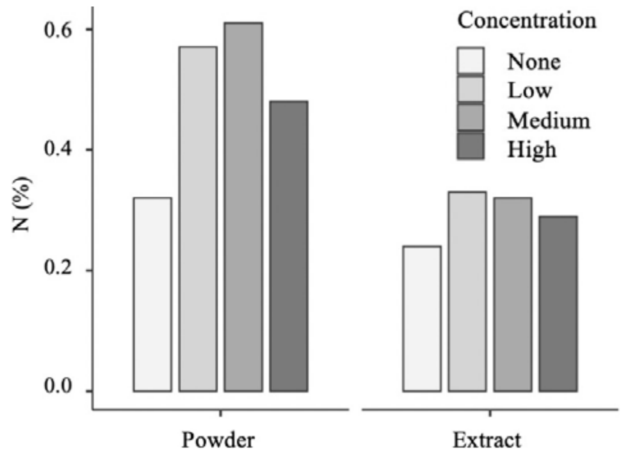


Figure 6a. Na (mg/Kg) of leaves showing higher mass of Na in both extract and high concentration.

Figure 6b. N (%) of leaves showing higher percentage in powder treated vines.

Table 5: Results of students' t-test showing p-values between powder and extract samples for nutrients.

	Statistic	df	*p*
Al (mg/Kg)	2.38	1.00	254
B (mg/Kg)	12.00	1.00	053
Ca (mg/Kg)	2.24	1.00	267
Cu (mg/Kg)	1.00	1.00	500
Fe (mg/Kg)	2.13	1.00	279
K (mg/Kg)	1.25	1.00	431
Mg (mg/Kg)	4.31	1.00	145
Mn (mg/Kg)	6.25	1.00	101
Mo (mg/Kg)	1.00	1.00	500
Na (mg/Kg)	30.00	1.00	**021**
P (mg/Kg)	6.00	1.00	105
S (mg/Kg)	51.00	1.00	**012**
Zn (mg/Kg)	1.25	1.00	430
N (%)	1.46	1.00	382
C (%)	2.50	1.00	242
S (%)	3.33	1.00	186

Table 6: Results of ANOVA showing p-values of differences between forms and among concentrations for nutrients among treated vines.

	p (Form)	*p* (Concentration)
Al (mg/Kg)	0.417	0.777
B (mg/Kg)	0.098	0.230
Ca (mg/Kg)	0.781	0.340
Cu (mg/Kg)	0.609	0.820
Fe (mg/Kg)	0.083	0.396
K (mg/Kg)	0.750	0.702
Mg (mg/Kg)	0.251	0.854
Mn (mg/Kg)	0.083	0.446
Mo (mg/Kg)	-	-
Na (mg/Kg)	**0.040**	**0.012**
P (mg/Kg)	0.335	0.282
S (mg/Kg)	0.382	0.392
Zn (mg/Kg)	0.954	0.907
N (%)	**0.021**	0.165
C (%)	0.182	0.292
S (%)	0.087	0.206

Discussion

Synopsis of Results

The application of powder and extract, and higher concentrations of either, generally coincided with reduced vine length and higher electrical conductivity - an index for soil salinity. Na concentrations, as mg/Kg, showed to be higher in leaves treated with extract and in leaves treated with higher concentrations of either powder or extract; N concentrations, as percent mass, were higher in powder treated vines. Control treated vines tended to perform best, based on longer vine length; SPAD readings and mean internode length of longest vine did not indicate any differences. Powder treated vines preformed worse than extract treated vines based on longer vine length.

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Potential Explanation

Vines that did poorly in terms of vine length show virtually no difference in foliar nutrition except for increased N and Na. This is an interesting finding considering that the same vines (those receiving high concentrations and powder) received the most nutrients, including both N and Na. Based on analysis of the raw powder and extract we can see that there is virtually no difference in nutrient content except for elevated Na levels in the powder and elevated S levels in the extract. Further, if we assume that one mL of extract corresponds to roughly one gram of material we could say that extract treated vines received approximately 0, 12, 24 and 48 grams of AN whereas the powder treated vines received 0, 300, 600 and 1200 grams of AN. Therefore the powder treated vines received much more nutrition, potentially far too much.

It may be that there was no limiting nutrient, but too many nutrients, preventing the vines that performed poorly from imbibing water and nutrients that would have otherwise been carried in soil-water solution – the vines may have been suffering from osmotic stress and/or salt stress. Powder treated vines that received 300 and 600 grams eventually caught-up in vine length to the powder control over the growing season: perhaps this is an indication of nutrient leeching and volatilization.

Osmotic Stress

Grapevines are typically grown in conditions where an appropriate level of water-stress contributes to improvements in compact growth of both vegetative and fruiting structures alongside improvements in desirable characteristics of fruit. Vines are adapted to manage water-stress (Downtown et al. 1990). However, found in a review of photosynthetic response of plants under different abiotic stresses that drought stress caused increased stomatal closure, reduced

CO_2 intake, reduced photosynthesis and reduced ATP production (Sharma et al. 2020 and Shahid et al. 2020). Further, high levels of electrical conductivity and, what may have been excessive nutrient loading per the prescribed rates of powder application, point directly to the potential for salt stress on these vines.

Though, grapevines are considered to be moderately salt-sensitive (Downton et al. 1990). Downton et al. found the same results as Sharma et al. in that as salt concentration increased among treatments, intercellular CO_2 decreased and photosynthesis decreased. Interestingly, they found that while salt inhibited respiration and photosynthesis, the effect was short-term, suggesting that excessive nutrient-loaded fertilizer applications, may not have long-term, irreversible impacts on grapevines.

If this is the case then why would N levels be higher in salt effected vines, such as those treated with powder and higher levels of extract? Shahid et al. found that N containing compounds accumulate in plants in response to saline environments, which may help to explain why N levels were higher (2020). N containing compounds "include amino acids, amides, quaternary ammonium compounds, and polyamines. Their reported functions are osmoprotection, osmotic adjustment, ROS scavenging, nitrogen provision, and maintenance of pH (Ibid)." Though, it may be because so much more N was applied, the plants were able to take more up. It may have been a mixture of both factors. Mansour also found N containing compounds in elevated levels in response to osmotic stress but was unable to determine the exact mechanisms of action considering that N is involved in many plant chemical processes (2000).

A study on the effects of salt stress on germination and growth of pistachio (*Pistacia vera* L.) in Algeria showed that an increase in salt concentration resulted in reduced leaf growth, resulting from what the researchers believe is an adaptive strategy of pistachio to overcome water stress from excess salt (Benmahioul, et al. 2009). While leaf area index was not quantified the researchers anecdotally observed smaller leaf size of vines with lower longest vine length. Given that SPAD readings and mean internode length showed no different there may be an application for using AN, at lower rates than used in this study, to counter highly-vigorous vines on fertile soils during the establishment of new vineyards

Study Limitations

Clearly, this study over a single growing season doesn't reflect the long term implications of using AN powder and extract on vegetative growth characteristics or nutrient foliar concentrations of grape vines. And, this study occurred in pots with drainage, not field growing conditions. Further, this study does not begin to suggest how AN may impact grape quality and quantity.

Future Research Directions

Future research on the effect of AN on grapevines should calibrate, via chemical analysis, any materials for nutrient content so that other differences that may have been masked by the overwhelming nutrient load provided by powder and higher concentrations are visible in the results. Further, the initial intent of the research was to study AN meal and extract, not powder and extract. Gaining access to already available meal and a raw, dried form of AN would complement a multi-year, nutrient-calibrated study. It would be interesting to corroborate the hypothesis that excess Na caused elevated N levels as part of a stress response; conducting further research where Na concentration is applied would assist answering that question.

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Conclusion

From a sustainability perspective, using no AN product results in the healthiest vines, as the addition of AN did not show any positive effects on growth. Though, AN provided a wide-variety of nutrients to the soil and potentially to the plant. However, Na and potentially S are serious concerns that can increase soil salinity and application rates should be considered before using AN as an amendment. If applying AN do so only in well-draining soil, without existing salinity issues and in climates without drought stress; apply no more than a maximum of 300 grams of AN per plant per year in either powder or extract. Further research is required to quantify whether AN in any form confers positive quantifiable benefits to grapevines.

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