**IMPACT OF MARINE EXTRACTS APPLICATIONS ON CV. SYRAH GRAPE (*VITIS
VINIFERA* L.) YIELD COMPONENTS, HARVEST JUICE QUALITY
PARAMETERS, AND NUTRIENT UPTAKE**

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Jonathan Martin
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COMMITTEE MEMBERSHIP

TITLE: Impact of marine extracts applications on cv. Syrah grape (*Vitis vinifera* L.) yield components, harvest juice quality parameters, and nutrient uptake

AUTHOR: Jonathan W. Martin

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COMMITTEE CHAIR: Lauren Garner, Ph.D.

COMMITTEE MEMBER: W. Keith Patterson, Ph.D.

COMMITTEE MEMBER: Terry Smith, Ph.D.

ABSTRACT

Impact of marine extracts applications on cv. Syrah grape (*Vitis vinifera* L.) yield components, harvest juice quality parameters, and nutrient uptake

Jonathan Martin

Vineyard management practices have an impact on grape berry development in ways that influence the quality of wine made from those grapes. The goal of this study is to determine whether exogenous applications of marine extracts on Syrah grapes can influence yield components, harvest juice quality parameters, and nutrient uptake. From 2009 to 2011, Syrah grape vines at the Trestle Vineyard on the California Polytechnic State University, San Luis Obispo campus received individual doses of marine extract via fertigation at berry set and veraison proportional to the amount they would receive on an annual per-acre basis. In 2011, marine extracts were also applied as foliar treatments. Treatments were analyzed for the effects on TSS, pH, TA, anthocyanins, tannins, fruit yield per vine, clusters per vine, average berry weight, cluster weight, berries per cluster, vegetative yield, and nutrient uptake. The marine extracts did not have any significant effects on yield components, harvest juice quality, or nutrient uptake at any point in this experiment. Therefore, there appears to be no benefit to applying these products in Syrah grapes growing in heavy clay soil in cool-climate conditions.

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CHAPTER 1

LITERATURE REVIEW

1.1. QUALITY PARAMETERS

Grape berry development. Grape berries follow a double-sigmoid pattern of growth with three phases. Phase I follows flowering with the formation of berries. During phase I, berries grow increasingly larger due to cell division and elongation (Figure 1.1). Phase I ends with bunch closure, when the clusters become more compact. Berry growth slows substantially during phase II, it is therefore also referred to as “lag phase.” During phase II, acidity peaks and then gradually declines as sugar development begins. At lag phase, berries are approximately half of their final harvest weight, though final harvest weights ranging from 1.7 to 2.6 times their lag phase weight have been observed [1]. Phase III starts at veraison, when the grape berries change color and soften. During phase III, grape berries increase in size due to cell elongation and start to accumulate color and flavor compounds [2, 3]. Between veraison and harvest, titratable acidity (TA) declines while total soluble solids (TSS) and pH increase.

TSS, pH, and TA. The three main berry chemistry parameters used to evaluate harvest juice quality are TSS, pH, and TA. All sugars are considered TSS and are expressed as °Brix. TSS accumulate most rapidly with daytime temperatures from 18 to 33 °C and are delayed by cool and hot daytime temperatures, high winds, high crop load, fruit zone shading, soil moisture, high soil nitrogen (N) [4-9], and virus pressure [10-12]. The TSS content at harvest will determine the alcohol content of the finished wine. Most red wine grapes are picked at approximately 24 °Brix, although wine grapes can be picked later if higher alcohol wines are desired. As TSS increase, the breakdown of malic acid during respiration results in decreased TA and increased pH [2]. Winemakers will typically

harvest at approximately 24-26 °Brix in order to maintain high TA and low pH. Wine with a pH above 3.6 and/or low TA will have a greater susceptibility to spoilage and less color stability than more acidic, lower pH wines [4]. Ideally, the TSS content preferred by the winemaker will coincide with low pH, high TA, high phenolics, and a strong flavor and aroma profile.

Phenolics. Phenolics are organic compounds metabolized by plants as a means of defending themselves against pests and environmental stress. All phenolics are either non-flavonoid or flavonoid, with anthocyanins, tannins, and flavonols classified as flavonoids. Anthocyanins are the pigments that give color to wine, while flavonols and tannins are responsible for bitterness and astringency, respectively [13]. Both tannins and flavonols bind to anthocyanins during the winemaking and aging process, resulting in color stability over time. Flavonoid synthesis is increased by exposure to light; however, red grapes will develop more anthocyanins with cool daytime temperatures. Flavonoid synthesis is hindered by high crop load, low-light conditions and high temperatures, with high night time temperatures causing flavonoid degradation [14].

Flavor and aroma compounds. Two groups of compounds that are strongly influenced by environmental conditions and management practices that have a strong impact on wine quality assessment are monoterpenes and methoxypyrazines. Free monoterpenes impart tropical fruit aromas and are encouraged by light in the fruit zone. Methoxypyrazines are responsible for green bell pepper and vegetal aromas, which are not considered favorable in red wines. Methoxypyrazines are degraded by exposure to light, while excess vigor causing shading in the fruit zone will aid in their formation [15]. **Yield.** In contrast to most fruit crops, high yields are usually not desired in premium wine grape production.

High crop loads can delay TSS accumulation, possibly resulting in grapes not reaching their target °Brix before the end of the growing season [4]. Since anthocyanins and flavonols accumulate in the skins, it is assumed that wine made from smaller grapes with a higher skin-to-pulp ratio will have better color, aging stability, and flavor potential than wine from larger, juicier berries [16]. Larger berries also pose the added danger of forming tighter, more compact clusters, increasing the risk of development of fungal diseases such as *Botrytis cinerea* [17]. However, researchers have found that wine quality parameters can remain unaffected by increasing yields and berry sizes [16], and in some cases improve with increasing yields up to a maximum yield specific to that vineyard [18]. Many of the desirable qualities of wine made from smaller berries are indirect effects of management practices common in premium wine grape vineyards, such as deficit irrigation and leaf pulling, which result in small, flavonoid-rich berries [13, 16, 19]. The fruit yield to vegetative yield ratio, known as the Ravaz Index, shows a stronger correlation to wine quality than fruit yields alone [18, 20]. Therefore, any management strategy should be focused on maintaining the fruit yield to vegetative yield ratio that is best suited to the cultivar and growing conditions in order to maximize wine quality and economic returns.

Fruit yields can vary from year-to-year and vineyard to vineyard based on environmental conditions and management practices. High rainfall in the previous winter can increase the availability of soil moisture during early vegetative growth, leading to higher cluster weights at fruit set [18]. Frost events after budbreak or extreme temperatures and precipitation during bloom can cause substantial yield losses by reducing the number and size of fruit clusters [21-23]. Fruit yield can also be influenced

by management practices such as irrigation strategy [13], canopy manipulation [24], pruning [24], fertilizers [25, 26], biostimulants [27-35], and rootstock selection [36]. Rootstock selection has a strong impact on yield components due to its role in regulating vigor and nutrient uptake (Table 1.1) [36]. Low vigor rootstocks, such as 420A and 3309C, will produce smaller clusters than medium and high vigor rootstocks [36]. Yields are also influenced by vine-yield compensation, the inverse relationship between cluster number and berry size and/or number. For example, when clusters numbers are reduced due to cluster thinning or pruning, cluster weights increase due to increased berry size and/or increased berries per cluster [24].

1.2. PLANT HORMONES IN GRAPEVINES

Introduction. Plant hormones are compounds produced and transported within plants that regulate growth and development. Changes in concentrations of auxins, cytokinins, gibberellins (GAs), and abscisic acid (ABA) control the timing of all growth stages in grapevines and can influence yield components, harvest juice quality parameters, and phenolics [37-39].

Auxins. Auxins are produced in grapevine shoot tips, leaves, and seeds. The primary roles of auxins are to promote cell elongation, cell division, nutrient transport, and lateral root formation [40], and to inhibit the ripening process until conditions are ideal for seed dispersal [37, 38]. Since zinc (Zn) is required for auxin formation, the tiny berries commonly referred to as “shot” that are observed when grape berries are deficient in Zn could be attributed to a lack of cell elongation and division as a result of insufficient auxin levels [41]. In reproductive structures, auxins are at their highest concentration from

flowering until the initiation of berry set, and decline in concentration throughout berry growth [42]. Synthetic auxin application delayed ripening by 10-14 days in Syrah grapes [37, 38] and suppressed sugar and anthocyanin accumulation in Cabernet Sauvignon [43]. This suggests that reductions in auxin concentration below a certain threshold or a low auxin to ABA ratio might be required in order to commence veraison [37, 38, 42].

GAs. GAs are synthesized early in the growing season in the roots, shoots and leaves and reach their maximum concentration in grape berries during phase I of berry growth [39]. Berry GAs decline in concentration to their lowest levels at the start of veraison. In grape berries, GAs primarily promote cell elongation, in addition to promoting cell division, seed germination, emergence from dormancy, and monoterpene biosynthesis [39, 44, 45]. Synthetic GAs are commonly used to enlarge seedless table grapes and loosen clusters [46]. GA application has been shown to increase berry weights in seedless grapes [27, 29, 47-49], with recorded increases in over 100% [27]. However, results are less favorable with seeded wine grapes. GA decreased cluster weights and berries per cluster while increasing berry shatter and the percentage of shot berries in White Riesling and Pinot Blanc [50] and delayed TSS accumulation in Thompson Seedless and Ruby Seedless grapes [27, 47, 49, 51]. Therefore, the use of exogenous GA on grapes is limited to seedless table grapes and is not recommended for wine grapes.

Cytokinins. Cytokinins are formed in grapevine root tips and seed embryos. Cytokinin synthesis is increased by warm temperatures and sunlight, signaling budburst and berry set. Cytokinins initiate cell division in grape berries during phase I of berry growth, leading to berry enlargement [40]. While cytokinins encourage cell division in berries, leaves, and lateral shoots, cytokinins inhibit cell division in roots [40]. The synthetic cytokinin,

forchlorfenuron (CPPU), can be used to increase grape berry size, resulting in compact clusters and berry weight increases of 52-100% relative to the control [35]. However, CPPU application can decrease TSS by as much as 4 °Brix, and is therefore not widely used in table or wine grape production [34, 35, 52].

ABA. ABA is produced in the roots and leaves of grapevines. During stressed conditions, ABA signals the closure of stomata in order to conserve water [43]. This effect has been observed with exogenous ABA applications to crops such as bentgrass, resulting in temporary reductions in net photosynthesis, stomatal conductance, and transpiration [53]. While auxins, cytokinins, and gibberellins decline in concentration towards veraison, berry ABA concentration is highest in the first few weeks of phase III [28]. The start of phase III begins when the auxin to ABA balance reaches a certain minimum threshold [39]. This results in an increase in anthocyanin production, causing rapid coloration of berries [43, 54].

The ability of exogenous ABA applications to rapidly increase grape color at the start of veraison is well demonstrated and might result in higher anthocyanins at harvest in some cultivars [28, 30-33, 55]. Synthetic ABA was approved for agricultural use in the state of California under the brand names “Contego” and “Protone” in 2010 [56]. Exogenous ABA applications have been shown to increase anthocyanins and flavonols in table and wine grapes [28, 30-33, 55]. Recently, ABA was shown to stimulate anthocyanin synthesis in grape cell cultures [54] and has been shown to increase the concentration of the regulatory gene and enzyme genes that control the anthocyanin biosynthetic pathway [43]. Exogenous ABA applied to Flame Seedless table grapes at veraison increased anthocyanin content by as much as 800% compared to the untreated control [32].

Exogenous ABA applied directly to Merlot clusters resulted in a 7% increase in anthocyanins as compared to those of untreated vines [55]. Exogenous ABA applications also resulted in increased anthocyanins in greenhouse-grown Pinot noir [31]. Exogenous ABA applications increased Cabernet Sauvignon berry flavonol concentration throughout ripening and resulted in significantly greater berry anthocyanin content at the start of veraison compared to non-treated grapes. However, at 80% red coloration, control and ABA treated berries were almost equal in anthocyanin content, with no significant differences in anthocyanin content at harvest. This suggests that exogenous ABA might not be able to increase anthocyanins at harvest in Cabernet Sauvignon [28]. Exogenous ABA applications did not affect TSS, pH, TA, fruit yield or berry weight in any of these studies [28, 30-33, 55].

1.3. GRAPEVINE NUTRITION

Introduction. Grapevines require the same 16 elements for growth as all other higher plants. The requirement for carbon, hydrogen and oxygen are satisfied by rainfall, irrigation water, and the atmosphere. The remaining nutrients must be absorbed from the soil by grapevine roots and/or applied directly to the grapevines in order to sustain growth and proper fruit development. When abundant, many elements will decrease or increase the availability of other elements, leading to nutrient deficiencies or excesses, which could have negative side effects on grapevine growth and wine quality [57-59].

N. N deficiency affects more acreage than any other nutritional element in California vineyards [58]. Grapevines require N for amino acid, lecithin, and chlorophyll synthesis,

making N crucial for growth and development. Soil microbes convert ammonium (NH_4^+) and other N compounds into nitrate (NO_3^-), a highly soluble form of N that moves through the soil via mass flow [57, 59]. N stored in roots is mobilized immediately after budbreak, with a sharp increase in soil N uptake by the roots during the first root flush that starts at bloom and continues until veraison [60]. No additional N uptake occurs between veraison and harvest [60]. At harvest time, approximately one-third to one-half of the vines' total N is stored in the fruit and subsequently removed from the vineyard [60]. Approximately one-third of the vine's total N at harvest is stored in the current season's leaves and shoots, while the remainder is retained by the trunk, canes, and roots [60]. N uptake resumes at harvest and halts at the end of leaf abscission during the second root flush, during which the majority of the remaining N is translocated into the roots and trunk [60]. Grapevines absorb a substantial portion of their annual N requirement post-harvest, resulting in N reserves that support the following season's growth [60].

Of the 13 plant essential nutrients found in soil, N is the only element that is not weathered from minerals [58]. Sources of N include decomposing organic matter, biologically-fixed N from leguminous ground cover, and dissolved NO_3^- in irrigation or rainwater, which might provide enough N so that supplemental N fertilization is unnecessary in some vineyards [58, 61]. To determine if applications of N are required, N status of grapevines is typically determined by taking petiole samples at full bloom. N deficiency symptoms often appear after the deficiency is established, and include reduced vigor, pale foliage, and reduced fruit yield. N excess accelerates vine vigor, causing the vine to form longer shoots and larger, darker green leaves [58]. Excess N can reduce fruit yield, TSS [5, 7-9, 62, 63], and anthocyanins [25], while increasing pH [5, 64-66].

Excessive N also leads to increased vigor and fruit zone shading, resulting in the formation of vegetal aromas [15, 67], while hindering the synthesis of anthocyanins and favorable fruity aromas that require filtered light [15]. The negative influence of excess N on yield and harvest juice quality parameters has resulted in relatively low fertilization recommendations (50-56 kg N/ha per year) as compared to other perennial fruit crops [58, 61].

N fertilization can alter vine uptake of nutrients. The effect of pre- and early season N fertilization on bloomtime petiole N is highly dependent on cultivar (Table 1.1) [62], rootstock [68, 69], irrigation [70], and canopy/crop manipulation [24]. Early season N fertilization resulted in increased bloom petiole N in all tested cultivars [6, 7, 26, 68]. Early season N fertilization resulted in decreased Phosphorus (P) status in Cabernet Sauvignon [71], Riesling [8], Merlot [7] and Chenin Blanc [61]. Riesling bloomtime leaf Magnesium (Mg) and calcium (Ca) increased with N fertilization rate during the spring [8], whereas Merlot petiole Mg and Ca were negatively correlated with N [7]. Increasing N fertilization in the spring resulted in increased bloomtime petiole sulfur (S) in Merlot [7] and decreased petiole S in Chenin Blanc [5].

Increasing N fertilization is strongly correlated with increased vegetative yields in grapevines, and can have stronger effects when applied during root flushes. Increases in vegetative yields and leaf area as a result of N application have been observed in many cultivars, including Riesling [26], Chenin blanc [61], Cabernet Sauvignon [71], and Merlot [7]. Cabernet Sauvignon shoot growth, leaf growth, and number of leaves per vine at harvest was positively correlated with N fertilization rates during bloom [25]. Increases in Merlot canopy density were observed when 40 kg N/ha was and post-harvest, as compared

to applying 40 or 80 kg N/ha at budbreak or bloom, respectively [63]. Fifty g N/vine, split between two applications at late budburst and two weeks after bloom, resulted in increased Cabernet Sauvignon vegetative yield, shoot length, vine density, leaf number, and the proportion of interior leaves as compared to the control. However, Neilsen (2010) found that the vegetative yields and shoot lengths of vines treated with 400 g N/vine were not significantly different from unfertilized control vines [71]. This suggests that increasing rates of N fertilization will rapidly increase vegetative yield in grapevines up to a certain point, after which vegetative yields plateau or possibly decline due to N toxicity [71].

Effects of N fertilization on fruit yield components are dependent on pre-fertilization N content and cultivar. Increasing N fertilization rates can substantially improve fruit yield components if there is a pre-existing N deficiency [25, 26]. Conversely, Thompson Seedless grapevines fertilized with 0, 112, 224, or 448 kg/ha N showed no significant differences in fruit yield per vine, total clusters, or cluster weight [64]. Bell et al. (1978) found that 112 kg N/ha resulted in 12-16% higher fruit yields as compared to the unfertilized control [72]. Merlot vines treated with 40 or 80 kg N/ha at budbreak had lower yields than vines receiving the same application rates at bloom, while the same N treatments had minimal effects on Cabernet Sauvignon vines in the same experiment [7, 63]. In that same study, applications of 80 kg N/ha resulted in reduced Merlot fruit yield as compared to 40 kg N/ha when applied at the same phonological stage [63]. Applications of 100 g N/vine split between budbreak and bloom resulted in an increased Ravaz Index of Cabernet Sauvignon by 56% compared to the control. No further increases in fruit yield were detected with applications of 200 g N/vine and 400 g N/vine resulted in decreased fruit yield as compared to all other treatments in 2 of the 3 study years [71], indicating that

excess N could decrease fruit yields. Fruit yield reductions from increased N fertilization rates have also been observed in Chardonnay [66] and Syrah [68].

The accumulation of TSS are often delayed by excessive N application. Hilbert found that Merlot juice from a “limited” N application treatment had 2.4 and 2.8 greater °Brix than the “mean” N treatment (2.5 times more applied N than the “limited” rate) and “excessive” N treatment (5 times more applied N than the “limited” rate), respectively [7]. In a 3 year study, Spayd (1994) determined that fertilizing White Riesling grapes with 56 kg N/ha/year applied in split applications between budbreak and fruit set delayed the accumulation of 21°Brix by an average of six days relative to the control, while applications of 224 kg N/ha/year delayed harvest by an average of 16 days and as much as 22 days in one year [5]. TSS reductions as a result of N fertilization have also been observed in Niagara, Concord [9], Grenache, Barbera, Chenin blanc, and French Colombard [6]. However, no significant N treatment effects on TSS were observed in similar studies of Chenin blanc [61], Cabernet Sauvignon [63] or Thompson Seedless [64].

N fertilization can have variable effects on pH and titratable acidity (TA). The timing of N fertilization of Riesling vines had no effect on must pH at application rates of up to 90 kg N/ha per year, and negligible effects on TA in Riesling [8], Cabernet Sauvignon, and Merlot [63]. Hilbert (2003) found that an “excessive” N application rate of Merlot resulted in the highest TA, while pH was unaffected as compared to the “mean” and “limited” N application rates [7]. Similar results were observed in a study of Grenache and Chenin Blanc, in which 112 kg N/ha resulted in increased TA in one year of a four year study, with no significant effect on pH [6]. However, in White Riesling grapes, increased N fertilizer rates ranging from 56 to 224 kg N/ha/year were positively correlated

with pH without having a significant effect on TA [5]. Both pH and TA increased as a result of 30 or 60 kg N/ha applied at bloom in Riesling [65], with the higher TA attributed to increased malate production.

While some N is required for phenolic synthesis, N excess can reduce wine quality by decreasing phenolics and encouraging the formation of less-desirable flavor compounds. Fermentation rate is positively correlated with must N content; therefore, insufficient N can lead to slower and incomplete fermentations [12], which could then hinder phenolic synthesis [64]. Unfertilized Thompson Seedless vines produced wine with lower aroma, flavor, and wine quality than vines receiving 112 kg N/ha/year, presumably due to a reduction in wine volatile formation caused by a slow fermentation rate [64]. Conversely, Cabernet Sauvignon berries from vines treated with 0.34 g N/vine applied at bloom had greater anthocyanins at veraison than berries from vines treated with 1.7 or 3.4 g N/vine [25]. Hilbert determined that Merlot berries from the “limited” N treatment had significantly higher berry skin anthocyanin content as compared to the “mean” and “excessive” N treatments [7]. Cabernet Franc wine from vines treated with 20 t cow manure/ha/year for 28 years had significantly higher herbaceous/vegetative and animal odors, in addition to significantly lower color intensity, anthocyanins, tannins, aroma intensity, and astringency than control wines [67].

P. P is a primary macronutrient that is immobile in the soil and moves through the soil by diffusion [57, 58]. Most soil P is derived from the breakdown of Ca, iron (Fe), and aluminum phosphates [59]. P uptake typically occurs in the orthophosphate (H_2PO_4^-) form, which is highly mobile in the vine. P stored in the root tissue is used for early season growth at the start of budbreak. Root uptake of P increases as the season progresses towards

veraison. Between veraison and harvest, root uptake of P stops, while P is translocated from the leaves into the clusters. Between harvest and leaf abscission, roots resume uptake of P, which is then stored during dormancy and used for the next season's growth [73].

P is required for energy transfer within the vine and is crucial for reproductive growth [74, 75]. P deficiency causes basal leaves to turn yellow and abscise prematurely, and appears as an interveinal reddening pattern starting from the edge of the leaves. P deficiency leads to severe yield losses by reducing canopy growth, berries per cluster, clusters per vine, cluster weights, and the number of initiated cluster primordia [74, 75]. Despite its importance, P deficiency in vineyards is extremely rare and has only been observed in a few isolated areas with poor, low pH soils and low cation exchange capacity [76]. P status is most accurately assessed by leaf analyses at bloom [74]. Since P fertilization is rarely needed, P excess is generally not a concern in California vineyards, although it could lead to Zn deficiency [59].

Potassium. Potassium (K) is a macronutrient that is commonly deficient in vineyards, especially those with sandy soils [58]. Vines grafted to rootstocks with *Vitis berlandieri* parentage such as 420A and 110R can have lower petiole K concentrations at bloom than vines with other rootstocks [77]. Soil K originates from the weathering of minerals such as micas and feldspars [59]. K moves through the soil by diffusion in less mobile forms and by mass flow when it becomes more soluble [57]. Root K uptake peaks between bloom and veraison. Grape clusters accumulate K between veraison and harvest as root K uptake decreases. K mobilization increases between harvest and leaf abscission, although there is no additional root K uptake in the post-harvest period [60].

K contributes to vine growth by facilitating cell division and aiding in the synthesis of carbohydrates and proteins. K is also important for frost protection, water relations and enzyme activity. K deficiency appears as a gradual yellowing of the leaves that is followed by leaf burn/curl and premature leaf abscission. Severe K deficiency could lead to reduced shoot growth that in combination with premature leaf abscission, causes severe yield reductions and delayed, uneven ripening [58]. K is typically applied to vineyards as K sulfate (K_2SO_4 , commonly known as ‘potash’) due to its low cost, ease of application via drip irrigation, low salt content, and rapid uptake. K status is most accurately predicted with petiole samples taken at bloom, with less than 1% dry weight K considered deficient [58].

K fertilization can alter the status of other nutrients in petioles and in the juice from the berries. Conradie (1989) found that 45 or 90 kg K/ha applied at post-harvest and budbreak to Chenin blanc resulted in increased foliar K and reduced petiole Ca and Mg concentrations, as compared to the control. Reductions in petiole Ca and Mg concentrations have also been observed in Concord vines with the application of 450 kg K/ha at budbreak [42]. Previous research has also demonstrated that increasing rates of K fertilization can lead to increased berry K [61, 78], which is associated with increased juice pH [61, 78]. Dundon and Smart (1984) determined that 1.62 kg K/vine applied 3 weeks after budbreak in a hot-climate vineyard only increased petiole K in one of the four years studied, and did not cause a corresponding increase in must K [79]. In that same study, K application in a cool-climate vineyard did not significantly increase petiole K concentrations in any of the 3 study years, despite soil exchangeable K concentrations as high as 1400% greater than the control [79]. While high harvest juice pH is often attributed to excess K fertilization,

the excessive K fertilization in this experiment did not influence Syrah harvest juice pH or TA as compared to the control [79].

Ca. Ca is weathered from limestone and FeCO_3 in the soil, and is the fifth-most common element in the earth's crust [57]. While Ca contents of different soils can vary widely, deficiency in vineyards is rarely observed due to their low Ca requirement [58]. Ca is applied to soils to improve water penetration and raise soil pH. The majority of Ca uptake occurs between bloom and veraison. Ca is relatively immobile in the vine and is stored in large quantities by vine bark [73]. Ca deficiency inhibits root and shoot growth, while excess Ca can reduce K and Mg uptake [57].

Mg. Mg is weathered from Mg-rich minerals such as Serpentine, Magnesite, Sulfite, Dolomite, and Olivine [57]. Mg is a component of chlorophyll molecules, making it indispensable for photosynthesis and vine growth [57]. Mg uptake in grapevines is unique compared to the macronutrients N, P, K, and Ca, in that grapevine roots absorb Mg continuously from early season growth until the start of leaf abscission [73]. Mg deficiency begins as leaf chlorosis at the margins that spreads into the areas between the smaller veins while the leaf tissue around the large veins remains green [58]. Mg deficiency can reduce yield and delay ripening if leaf chlorosis becomes severe enough to inhibit photosynthesis [58]. Mg deficiency is not common in California and is often isolated to a few vines or small areas in a vineyard [58]. While excess Mg can lead to K deficiency, the lack of Mg deficiency means that Mg fertilization is generally not required [58].

S. S is a constituent of proteins and enzymes [80]. S is present in soils as sulfates in soil solution, minerals, and organic matter [57]. Approximately 14 million kg S/year are applied to California vineyards as a foliar spray for powdery mildew control [81]. S deficiency

causes a uniform leaf chlorosis that slows vine growth, however it is rarely seen in vineyards [73]. Soil acidification can result from excess S levels in the soil [82].

Zn. Zn is the second-most common nutrient deficiency in California vineyards. All soils are very low in Zn content and over 90% of soil Zn is bound in mineral form [57]. Zn is required for the synthesis of auxins, chlorophyll, and starch. Grapevines require approximately 0.5 kg Zn/ha/year for sufficient internode elongation, leaf growth, pollen development, pollen tube growth, berry count, and berry enlargement, making Zn essential for fully developed clusters [58, 83]. Reduced leaf expansion, chlorotic leaf blades with dark green veins, stunted shoots, and withered clusters with tiny, under-ripe berries are all characteristics of Zn deficiency. The underdeveloped clusters and the reduced photosynthetic capacity of the shoot system can lead to substantial yield losses and delayed ripening in grapes. Soil Zn status is not a reliable indicator of vine Zn status, and tissue analysis is often not necessary due to the obvious fruit and canopy symptoms. If tissue analysis is used, Zn petiole dry weights lower than 13 ppm are deficient [83]. Soil Zn applications are usually unsuccessful due to rapid Zn fixation by most soils. Foliar application of ZnSO₄ between bud break and bloom or daubing fresh pruning cuts with ZnSO₄ are the only practical ways to correct Zn deficiency in most vineyards [58, 83]. Excess Zn can restrict root growth and induce chlorosis in young leaves [59].

Boron. Boron (B) is one of the micronutrients of greatest difficulty to correct due to the extremely narrow range between soil B deficiency and toxicity. B is more abundant in soils with sedimentary parent material rich in borosilicate minerals and in areas with B-rich irrigation water [58]. Vines absorb B as borate, which is used for new cell differentiation, carbohydrate metabolism, and pollen germination. Once in the vine, B is relatively

immobile and is not translocated from older to younger leaves [58]. The role of B in pollen germination makes sufficient B a requirement for fruit set and yield. Severely reduced internode and shoot length, shoot tip death, low to non-existent fruit set, and tiny berries are all common symptoms of B deficiency. B is deficient for grape production if concentrations are below 0.15 or 0.1 ppm B in soil extracts or irrigation water, respectively. However, B is toxic to grapevines with B concentrations greater than 1 ppm in soil extracts or irrigation water [83]. B excess can reduce yields by causing cupped downward growth of leaves and leaf necrosis. Foliar application can temporarily correct B deficiency, although the results are typically short-lived [58]. B accumulates in leaf blades more so than petioles, therefore leaf blades are the best indicator of B status, with 30-80 to ppm B petiole dry weight being the ideal range [84].

Fe. Fe is the third-most common micronutrient deficiency in California vineyards after Zn and B and is considered the most difficult nutrient deficiency to overcome [58]. Fe is found in a variety of minerals and composes 5.1% of the lithosphere. While Fe is the most common nutrient in soils, 90 to 99.98% of soil Fe is unavailable to plants [57]. Fe is needed for chlorophyll production, enzyme activation, and as a building block for organic compounds [58]. Fe's mobility is hindered in heavy soils or soils with high lime and phosphate, giving Fe deficiency leaf symptoms the name "lime-induced chlorosis." Fe is not mobile within the vine, therefore, it is not transported from older to younger leaves. Deficiency appears as a pale interveinal yellowing of the younger leaves, leading to reductions in shoot growth and yield. Fe toxicity can also reduce yield; however, Fe toxicity does not typically occur in vineyards. The high Fe fixing capacity of most soils means that the results of soil Fe fertilization are temporary, costly and impractical. Fe

deficiency is best diagnosed with visual criteria, due to inconsistencies between soil Fe, tissue Fe, and Fe deficiency symptoms. Fe deficiency can be corrected with foliar sprays of Fe chelates or Fe sulfates in 10-20 day intervals until symptoms disappear [58].

Manganese. Manganese (Mn) is weathered from ferromagnesian rocks in the soil and is used by vines for enzyme activation and chlorophyll formation [57]. Mn uptake occurs in the Mn ion form (Mn^{++}), after which it becomes immobile within the vine [58]. Mn deficiency is occasionally observed in vineyards and is most common in sandy, basic soils. Mn deficiency appears in mid-to-late summer as an interveinal chlorosis of the basal leaves. Since Mn deficiency only affects the less photosynthetically-active older leaves, yield losses from Mn deficiency are rarely a concern. Mn deficiency can be confirmed with petiole analysis, with deficiencies occurring below 20 to 25 ppm Mn. Mn deficiency can be corrected by foliar application of MnSO_4 , although excess Mn application can cause leaf burn and deficiencies in other nutrients [58].

Copper. Copper (Cu) is a micronutrient that is required by grapevines in trace amounts as a constituent of oxidation enzymes. Cu deficiencies have never been reported in California vineyards [58] and are considered to be rare worldwide [80]. However, they have been detected in a few vineyards in West Australia [80]. Cu deficiency can severely reduce productivity by reducing shoot length, internode length, leaf size, and leaf chlorophyll content. Cu toxicity can reduce vigor in the canopy and the root system, along with causing root system damage [80]. Cu is a common ingredient in fungicides applied in vineyards, the use of which could result in Cu toxicity in acidic soils, given that Cu availability is negatively correlated with soil pH [80, 85].

Molybdenum. Molybdenum (Mo) is a micronutrient that facilitates N metabolism [80]. Mo exists in very low quantities in the soil, and only a small handful of minerals are known to contain and weather Mo [57]. Plants require Mo in lower quantities than all other plant essential nutrients [57], and its deficiency has never been recorded in California vineyards [58]. Mo deficiency causes leaf chlorosis and necrosis, while toxicity leads to leaf malformation [59].

Chlorine. Chlorine (Cl) is involved in photosynthesis and stomatal regulation, and is provided to grapevines as chloride (Cl^-) in rain and irrigation water [57]. Due to its abundance in the environment, Cl deficiency has never been reported in vineyards [57, 58]. Leaf uptake of Cl^- progresses with the growing season, and might be toxic in concentrations as low as 0.5% of leaf blade dry weight. Cl^- toxicity first appears as leaf burn and can ultimately lead to reduction in vine growth and possibly death. Cl^- toxicity in irrigation water is a major concern for grape growers in areas with highly saline irrigation water and/or poor soil drainage characteristics [58].

Conclusion. All plant essential nutrients contribute to vine growth and development. However, unless correcting nutrient deficiencies, fertilizers generally do not improve harvest juice quality parameters or yield components in ways that can potentially increase wine quality. Increased vigor actually decreases wine quality, TSS and phenolics. Nutrient management in vineyards requires regular assessment of leaf, petiole and/or soil nutrient content and the application of fertilizers accordingly.

1.4. MARINE RESOURCE UTILIZATION IN AGRICULTURE

Origins of Fish-based Fertilizer in Agriculture. Nutrients suspended in aquatic environments are absorbed by fish throughout their life cycle [86]. Fish have, therefore, been used as fertilizer since the medieval period in France [87]. This use was first documented in an English publication from 1620. This tradition developed independently in Peru, where Incans used fish heads and guano as fertilizer [87]. At the present time, the non-edible remains and non-target species from the seafood processing and commercial fishing industries are converted into a variety of emulsions, extracts, powders, animal feeds, and potting soil amendments. Fish emulsion is made by boiling fish remains into a semi-soluble liquid fertilizer. Fish extracts are made by grinding fish remains and transferring them to a refrigerated holding tank for enzymatic hydrolysis, during which, the fish enzymes break down the remains into a liquid containing simpler proteins [88]. The extracts are then passed through a series of filter screens to remove bone particles [88]. Fish extracts are generally preferred to emulsions, due to the greater water-solubility and lesser smell of extracts. Most fish-based fertilizers contain between 2-4% N and 2-4% P by volume [88]. In a survey of 300 certified organic vegetable farmers, fish-based products were used by 20% of the respondents, and were ranked the fourth-most commonly used nutrient source after manure, compost, and leguminous cover crops [89]. Despite their relatively low nutrient value when compared to synthetic fertilizers, fish-based fertilizers play an important role in reducing the waste disposal demands of the seafood industry, while simultaneously providing a nutrient source for organic growers.

Effects of Fish-Based Fertilizer on Crop Yield. The nutrients found in fish have similar effects on yield as synthetic fertilizer when equal amounts of nutrients are applied. Radish, cucumber, potato, and tomato plants growing in a soil mix containing 1, 2, or 4% fish emulsion had yields that were not significantly different than those receiving synthetic fertilizer when equal amounts of N were applied [90, 91]. Similar yield responses were observed in millet and groundnut plantations in Ghana, where treatments of 2000, 4000, and 6000 kg fish remains/ha resulted in the same yield increases as synthetic fertilizers treatments when equal amounts of N were applied [92]. Yields of fish emulsion-fertilized tomatoes, chili peppers, and ornamental plants had yields that were not significantly different from yields of crops receiving synthetic fertilizers with the same amount of applied N [93].

Fish-based fertilizers also have the potential to increase marketable yields through disease suppression. Radishes and cucumbers planted into soil incorporated with fish emulsion had reduced *Rhizoctonia* and *Pythium* symptoms, resulting in increased marketable yields as compared to synthetic N treatments. *Verticillium* rot and scab symptoms were also reduced on potato plants fertilizer with fish emulsion, resulting in increased marketable yields as compared to those treated with synthetic fertilizers [91]. However, no improvements in disease suppression, fruit size, or number of fruits were observed with soil treatments of fish emulsion in organic fresh market tomato production [94].

Most fish-based fertilizers contain relatively small quantities of N, P, and K, and even smaller concentrations of other macro- and micronutrients, as compared to commonly used synthetic fertilizers. For example, N in the forms of NH_4NO_3 (33-0-0) and urea (45-

0-0) are 33 and 45% N by volume respectively [95], whereas a typical fish extract such as Neptune's Harvest (2-3-1) is only 2.4% N [88]. When used at manufacturer-recommended rates, the effectiveness of fish-based fertilizers to increase yields is limited due to the low amount of nutrients applied. For example, Brown (2004) found that foliar applications of Neptune's Harvest Fish Extract 2-4-1 or Fish/Seaweed Blend extract 2-3-1 applied at the manufacturer's recommended rate of 112 L/ha [88] did not result in significant changes in sweet pepper, broccoli, or lettuce yields [96]. Fish-based fertilizers also failed to increase yield or quality in tomatoes [94, 97], and wheat [98]. Yields of greenhouse-grown tomatoes were not significantly different when fertilized with fish emulsion or Hoagland's solution containing equal amounts of applied N. However the fish-emulsion treatment resulted in delayed ripening as compared to the Hoagland's treatment [99]. The low nutrient content per unit of cost and volume and general lack of yield increases or quality commodity improvement in response to fish-based fertilizers explains why their use is rare in conventional agriculture.

Origins of Seaweed-based Amendments in Agriculture. The use of seaweed for agricultural purposes is documented in 21 countries worldwide (Figure 1.2). At least 25 species of seaweed are used for fertilizer or animal feed across a wide variety of climates and cropping systems [100]. Along the Galician coast in northwest Spain, farmers have traditionally harvested drift seaweed and applied it to potatoes, grapevines, horticultural crops, and cereals [101]. Seaweed is especially useful in soils with low organic matter due to its ability to increase soil pore volume, aggregate stability, microbial biomass, and biological activity when incorporated into soil [102]. The use of seaweed extracts (SWEs) or concentrates is more common than using raw seaweed, due to its commercial availability

and ease of application [103]. SWEs are prepared by harvesting raw seaweed in intertidal zones and breaking them down by enzymatic hydrolysis [88]. This process raises the concentration of organic compounds and plant growth regulators (PGRs) that are credited for the various crop responses to SWE. Exact formulas for SWE preparation vary by producer, as each manufacturer has their own proprietary formula and seaweed source that might increase or decrease its efficacy relative to their competitors (H. Little, Acadian Seaplants, personal communication, February 28, 2011). Numerous PGRs have been found in seaweed and SWE, including cytokinins [104, 105], auxins [106, 107], GAs [108, 109], ABA [110], and betaines [111, 112]. It is hypothesized that seaweed synthesizes relatively high concentrations of PGRs because this confers an adaptive advantage that allows it to survive the extreme temperature ranges and levels of desiccation characteristic of intertidal zones [113]. Other organic compounds that are yet to be identified might also be responsible for the effects of SWEs on crop growth and yield [107, 114]. The amount of plant essential nutrients in seaweed and SWEs is too low to meet the legal definition of a fertilizer, therefore, seaweed products are marketed as soil amendments or biostimulants [115].

Impact of Seaweed-based Amendments on Nutrient Uptake. Changes in nutrient uptake from the application of SWE vary based on the crop being grown and the nutrient status of the plants and soil at the time of application. SWE from the species *Kappaphycus alvarezii* resulted in significant increases in N, P, K, and S uptake in soybeans when 49.75-97.5 L SWE/ha was applied foliarly at vegetative and flowering stages [116]. K, Fe, and Cu uptake increases were observed in olive trees treated with two wintertime applications of 17.5 ml SWE/tree [117]. In a study by Crouch (1990), 12 ml of foliar-applied SWE/plant resulted

in increases of lettuce leaf Ca, K, and Mg concentrations by 52%, 46%, and 37%, respectively, as compared to control plants receiving no SWE [112]. The concentrations of Ca, K, and Mg in the lettuce leaves were approximately 10 times greater than their respective concentrations in the SWE [112]. However, SWE had no influence on Ca, Mg, or K concentrations in leaves of nutrient-stressed lettuce [112]. Beckett (1990) observed that SWE resulted in increased Zn uptake of tomato seedlings grown in macronutrient deficient conditions, but did not significantly affect Zn uptake when macronutrients were sufficient [118]. Cu uptake was increased in nutrient deficient grapevines with four foliar applications of SWE at 15 day intervals during shoot growth [119]. Greenhouse-grown Sangiovese grapevines treated foliarly with a combination of 0.1% SWE and a 9-5-4 synthetic fertilizer twice a week for two months had increased N, P, K, and Mg leaf concentrations, N and P shoot concentrations, and P and K root concentrations as compared to fertilized and unfertilized vines receiving no SWE [120].

Impact of Seaweed on Yield and Quality. Seaweed-based amendments can increase crop yield and quality parameters. SWE application resulted in greater root-to-shoot ratios as compared to untreated control in maize [121], tomato [122], and wheat plants [123]. Koo (1988) found that 3 post-bloom foliar applications of 4.68 L SWE/ha resulted in greater orange yields in 2 out of 3 study years [124]. Additional crops for which increased yields from seaweed-based amendment application have been observed include lettuce [113], spinach [125], soybean [116], olives [117], potato [126], strawberries [127], watermelons [128], and beans [107, 129]. Orange trees receiving 3 post-bloom foliar applications of 4.68 L SWE/ha had increased rind color intensity and a decreased number of green fruits as compared to the untreated control [124]. Olive trees treated with two wintertime

applications of 17.5 ml SWE/tree developed color sooner than untreated trees [117]. Foliar SWE applications resulted in greater total phenolics in carrots [130] and cucumbers [131]. SWE typically does not influence TSS, pH, or TA of with strawberries [127], watermelons [128], oranges [132], or mandarins [124]. Thompson Seedless table grapes fertigated with 2.34 and 3.5 L/ha SWE pre-bloom, post-bloom, or at phase I of berry development had significantly greater fruit yield, berry size, and cluster weight than control grapes [133]. Eight foliar applications of 2 L/ha SWE between bloom and phase III of berry development resulted in increased table grape fruit yield, berry weight, cluster weight, rachis length, and percentage of first class graded clusters as compared to untreated control grapevines [134]. While SWE application did not result in changes in TSS, pH, or TA as compared to untreated fruit in several table grape experiments [133-135], Kok (2004) found that three foliar SWE applications between pre-bloom and phase I of berry development resulted in greater TA and higher tannin concentrations as compared to untreated control grapevines [136]. Tempranillo grapes fertigated with 20 L/ha of a grain-based biostimulant with a similar PGR profile as SWE resulted in juice with 22% greater total phenolics, 70% greater total anthocyanins, and higher chroma intensity than juice from untreated grapes [137]. However, in many studies, SWE applied at the manufacturers' recommended rates resulted in no significant effects on yield or quality parameters of crops, including onions [138], barley [139], wheat [140], tomatoes [94, 97], mandarins [124, 132], strawberries [141], sweet peppers, broccoli, and lettuce [96].

Impact of Seaweed-based Amendments in Stressed Conditions. The ability of seaweed to increase yields during times of drought stress might be related to their ability to induce drought and heat stress tolerance in crops [120, 123, 142]. Increased heat and drought

tolerance in creeping bentgrass as compared to untreated control plants was observed with foliar applications of 0.5 kg SWE/ha [142]. Soil drench applications of SWE applied at a rate of 1:250 resulted in increased wheat root growth during vegetative growth in drought-stressed and well-watered conditions, whereas SWE applied at a rate of 1:750 resulted in increased straw and grain yields, regardless of water status or growth stage [123]. Sangiovese grapevines treated with 4 applications of SWE during shoot growth had 25% greater vegetative yields, less-negative midday leaf water potential values, and faster recovery from drought stress to full photosynthetic capacity than non-treated grapevines upon rehydration [120].

Seaweed-based amendments can also increase yields during times of nutrient stress, but results are highly dependent on the crop and the nutrient status of the plant at the time of application. When nutrient-stressed and sufficiently-fertilized beans were applied foliarly with SWE, the treatment resulted in improved yields in all nutrient status treatments as compared to untreated beans, with greater yield increases observed in the more nutrient-stressed treatments [129]. Greenhouse cucumbers treated with SWE had greater yields and root-to-shoot ratios than untreated cucumbers [143]. 100 ml of 0.25% SWE solution applied as a root drench to soils with moderate and severe K deficiencies resulted in increased grain yield and root mass, though SWE had no effect on wheat grain yield or root mass in K sufficient soil [114]. However, yields of lettuce grown in nutrient-stressed conditions were not significantly affected by soil applications of 12 ml SWE/plant split between 3 applications [113].

SWE has also been shown to provide nematode and disease suppression. When comparing the effects of 4 soil-applied SWEs made from 4 different seaweed species on

chili peppers, all 4 SWEs resulted in reductions in nematode populations by over 50% within 24 to 48 hours as compared to untreated chili peppers, leading to an overall decrease in root galling and penetration by nematodes in the SWE treatments [144]. Sultana (2008) also found that several species of fungi that cause root rot were less prevalent in the SWE treated chili peppers than the untreated chili peppers [144]. Whapham (1994) observed that tomato plants receiving root drenches of SWE had 94% less nematode eggs after one generation as compared to control plants treated with water [145]. When surviving eggs were placed in a SWE solution, 34.9% fewer nematode eggs hatched as compared to eggs in untreated water [145]. Three foliar applications of 0.2% SWE reduced *Alternaria radicina* and *Botrytis cinerea* infections in carrots by 57% and 53.5%, respectively [130]. Greenhouse cucumbers treated with foliar or root drench applications of 0.15 or 0.3 ml of SWE at 3 10-day intervals had reductions in the severity of the fungal diseases *Alternaria cucumerinum*, *Didymella applanata*, *Fusarium oxysporum*, and *Botrytis cinerea* [146]. However, the SWE Marinure applied at the manufacturer's recommended rates had no impact on soil-borne diseases of tomatoes [94]. Six foliar applications of 1.68 L/ha SWE between early shoot growth and veraison resulted in a reduction in the number of powdery mildew infected table grape clusters by 41% as compared to untreated grapevines [135].

Conclusion. The use of marine extracts could have numerous potential benefits for grape production. Fish-based fertilizers could result in increased marketable yields by providing disease suppression. Both fish- and seaweed-based fertilizers could also improve nutrient uptake and provide small amounts of nutrients, which could then be used to improve minor nutrient deficiencies or reduce fertilizer inputs. By encouraging root growth, SWE could accelerate root system development in young vineyards. The endogenous GAs and

cytokinins in SWE could lead to increased yields and in grapes, improving economic returns. Considering that synthetic ABA application has resulted in improved grape phenolics in numerous studies, the endogenous ABA in SWE has the potential to improve phenolics in grapes. Given that SWE has been shown to assist in plant growth during times of heat, drought, nutrient, and pest stress, their use could be beneficial to wine grapes that frequently undergo periods of stress during the growing season. SWE has shown success in suppressing nematodes and fungal diseases that commonly plague grapes such as *Botrytis cinerea* and powdery mildew. SWE might prevent disease by directly encouraging systemic resistance, or indirectly by extending the rachis, which will loosen the cluster and reduce the probability of infection. Taken together, this suggests that fish- and seaweed-based amendments have the potential to affect yield, nutrient uptake, and wine quality. The objective of this study is to evaluate how fish and seaweed extracts impact Syrah grape nutrient uptake, yield components, and harvest juice quality parameters.

Figure 1.1. Grape berry development from flowering until harvest. From Kennedy (2002) [3].

Table 1. Characteristics of commonly used rootstocks for grape:						
Rootstock	Parentage	Relative scion vigor	Phylloxera protection	Root-knot nematode resistance	Dagger nematode resistance	Calcareous soil adaptation
St. George	<i>V. rupestris</i>	high	high	low	low	Low
Riparia	<i>V. riparia</i>	low	high	low	medium	low
Gloire 1616C	<i>V. riparia</i> x <i>V. solonis</i>	low	high	high	medium	low
101-14 Mgt	<i>V. riparia</i> x <i>V. rupestris</i>	medium	high	medium	medium	low
3309C	<i>V. riparia</i> x <i>V. rupestris</i>	Low to medium	high	low	low	low
Schwarzmann	<i>V. riparia</i> x <i>V. rupestris</i>	medium	high	high	high	medium
Freedom	<i>V. x champinii</i> , <i>V. riparia</i> , <i>V. labrusca</i> , <i>V. vinifera</i> , <i>V. solonis</i> complex hybrid	high	medium to high	high	high	medium
Ramsey	<i>V. x champinii</i>	high	high	high	low	medium
5C	<i>V. berlandieri</i> x <i>V. riparia</i>	medium	high	medium	medium	medium
5BB	<i>V. berlandieri</i> x <i>V. riparia</i>	medium	high	medium	low	medium
SO ₄	<i>V. berlandieri</i> x <i>V. riparia</i>	medium	high	medium	medium	medium
420A	<i>V. berlandieri</i> x <i>V. riparia</i>	low	high	medium	low	medium
110R	<i>V. berlandieri</i> x <i>V. rupestris</i>	medium	high	low	low	medium
1103P	<i>V. berlandieri</i> x <i>V. rupestris</i>	high	high	medium	low	medium
Boerner	<i>V. cinerea</i> x <i>V. riparia</i>	medium	high	high	high	low
Gravesac	<i>V. riparia</i> , <i>V. rupestris</i> , <i>V. berlandieri</i> complex hybrid	low	high	low	(information not available)	low
41	<i>V. vinifera</i> x <i>V. berlandieri</i>	medium	medium	low	low	very high
Millardet	<i>V. vinifera</i> , <i>V. berlandieri</i> complex hybrid	medium to high	medium	medium	(information not available)	very high
Fercal	<i>V. vinifera</i> x <i>V. rotundifolia</i>	high	high	low	high	Low

Table 1.1. Common grapevine rootstock parentage and characteristics. From Cousins (2005) [147].

Table 1.2. Total foliar N, P, K (% dry weight) and NH₄⁺, NO₃⁻, Zn, and B (ppm dry weight) of 12 wine grape cultivars. From Christensen et al. (1984) [54].

Cultivar	Total N(%)	NO ₃ ⁻ (ppm)	NH ₄ ⁺ ppm	P (%)	K (%)	Zn (ppm)	B (ppm)
Sauvignon blanc	3.51	913	2144	0.54	1.05	32.0	69.5
Petite Sirah	3.42	947	995	0.38	1.02	25.5	48.0
Chenin blanc	3.35	1130	1498	0.47	1.25	35.5	48.5
Zinfandel	3.31	480	1258	0.33	1.13	28.0	39.0
Rubired	3.25	627	1413	0.46	1.35	31.5	64.5
French Colombard	3.13	549	998	0.42	0.89	21.0	51.0
Barbera	3.06	460	544	0.34	1.02	23.5	42.0
Carignane	3.03	487	811	0.23	1.48	30.0	41.0
Grenache	3.00	1060	1637	0.36	0.90	25.5	61.5
Semillon	2.98	600	1452	0.39	0.78	18.0	63.5
Ruby Cabernet	2.86	410	691	0.27	1.30	27.5	48.0
Salvador	2.81	270	447	0.31	0.53	14.0	56.0

Figure 1.2. Countries where agricultural use of seaweed has been documented. Adapted from Zemke-White and Ohno (1999) [100].

CHAPTER 2

MATERIALS AND METHODS

Site description and cultural methods. All experiments were conducted at the Trestle Vineyard on the California Polytechnic State University campus in San Luis Obispo, CA (35°19'N, 120°41'W; elevation 100 m). All vines were of the cultivar Syrah (clone 877), trained to a Smart-Dyson trellis system with 1.52 m by 2.44 m row spacing in Montmorillinite clay soil. Vines were pruned to two buds per spur on 10 March 2009, 5 March 2010, and 13 February 2011. Shoots were thinned to 2 shoots per spur before bloom on 19 May 2009, 8 June 2010, and 28 May 2011.

Fertigation experiment. Vines were grafted on Schwarzmann, a medium-vigor rootstock [36]. Deionized water, fish extract (Neptune's Harvest, Gloucester, ME, USA; 2-4-1), seaweed extract (Neptune's Harvest; 0-0-1), or fish/seaweed extract blend (Neptune's Harvest; 2-3-1) was applied during irrigation at full berry set (12 June 2009, 6 July 2010, and 21 June 2011) and except in 2011, at the onset of veraison (3 August 2009, 20 August 2010) using a randomized complete block design (RCBD) with 4 blocks (Figure 2.1 and Table 2.1). Each experimental unit was composed of three treated data vines with a treated buffer vine on each side and an untreated buffer vine on each end. At each application time, each data vine and treated buffer vine received 420 ml per year of a 20:1 dilution of the fish extract, seaweed extract or fish/seaweed extract blend fertilizer in water (the per vine equivalent of the manufacturer's recommended 112 L/ha/year split into two doses) or 420 ml of water (control) underneath the drip emitter. In 2011, all data was collected prior to veraison; therefore, treatments were applied only at berry set, resulting in an overall application rate of (56 L/ha/year) (Table 2.1).

Foliar fertilizer experiment. Vines were grafted on 420A, a low-vigor rootstock [36]. A RCBD with 6 blocks and 4 treatments was utilized (Figure 2.2 and Table 2.1), with each block containing one treated data vine per treatment and an untreated buffer vine between each data vine. Using a calibrated manual backpack sprayer (SB415 Professional, Shindaiwa, Lake Zurich, IL, USA), each data vine was treated foliarly at berry set (21 June 2011), with 2121 ml of solution containing the same fish, seaweed or fish/seaweed blend fertilizers utilized in the soil fertigation experiment diluted with water at a rate of 100:1 (the per vine equivalent of the manufacturer's recommended 112 L/ha/year split into two doses) or 2121 ml of water (control). In 2011, all data was collected prior to veraison, therefore, only half of the manufacturer's recommended application rate was applied that year (Table 2.1).

Photosynthesis and stomatal conductance. To determine if the ABA in the seaweed extract or fish/seaweed extract blend applications had an effect on net photosynthesis and stomatal conductance, a portable photosynthesis system (LI-6200, Li-Cor, Inc., Lincoln, NE, USA) attached to an infrared gas analyzer (LI-6250, Li-Cor, Inc.) was used. A completely randomized design with 3 treatments and 6 replications was utilized. Vines were fertigated on 3 September 2009 with a 420 ml solution of a 20:1 dilution of seaweed extract or fish/seaweed extract blend, or 420 ml of water. Thirty minutes after application, 3 leaves from each vine were enclosed in a 4 L plastic chamber connected to the infrared gas analyzer that measured the change in carbon dioxide and relative humidity for 60 s. After all of the readings were completed, leaf areas were input into the portable photosynthesis system computer, which calculated net photosynthetic rate and stomatal conductance based on net carbon dioxide exchange and relative humidity, respectively.

Data collection. In 2009, all of the experimental units in the fertigation experiment were harvested on 21 September into separate bins and weighed with a digital scale (CD-11, Ohaus Corporation, Parsippany, NJ, USA). Berries were removed from 6 randomly selected clusters per bin, refrigerated overnight, and shipped on dry ice to ETS Laboratories (St. Helena, CA, USA) for phenolics testing. After the berries were slightly macerated, the grape must was heated and supplemented with 12% ethanol to simulate the extraction that occurs during the wine making process. Tannin and anthocyanin content were quantified using reverse phase analysis by a High Performance Liquid Chromatograph (1100, Agilent Technologies, Santa Clara, CA, USA). Twenty berries from 5 randomly selected clusters per bin were removed from each rachis. The total weight of the berries was determined with a digital gram scale (P-2002, Denver Instrument, Bohemia, NY, USA) and divided by 100 to determine the average berry weight of each experimental unit. The berries were then crushed to collect harvest juice samples for each experimental unit, from which TSS, pH, and TA were determined. TSS was measured with a manual refractometer (LR45227, Milton Roy Company, Ivyland, PA, USA). The pH was determined with a digital pH meter (Fisher Scientific, Pittsburgh, PA, USA). To determine TA, a 10 ml juice sample was diluted with 90 ml of deionized water and titrated with 0.1333 $\mu\text{mol/L}$ NaOH to a pH of 8.2. The amount of NaOH solution required to titrate to a pH of 8.2 was multiplied by 1000 to calculate TA.

It was not possible to obtain 2010 harvest data. On 5 March 2010, pruning weights of the data vines in each experimental unit were determined using a digital scale (CD-11, Ohaus Corporation). For each experimental unit, the total weight of the clusters measured

during the 2009 harvest was divided by the weight of the pruned canes to calculate the Ravaz Index.

For both the 2011 fertigation and foliar fertilizer experiments, all clusters were harvested upon full bunch closure on 1 August 2011 into separate bins by experimental unit. The clusters were weighed with a digital scale (UW4200 H, Shimadzu, Kyoto, Japan). Twenty berries from 5 randomly selected clusters from each experimental unit were weighed with the same digital scale and divided by 100 to calculate average berry weights. Rachis length was measured from the first berry-bearing stem to the end of the last pedicle. To estimate 2011 harvest yield, the 2011 fruit yields per vine and cluster weights were multiplied by 2, based on the widely used multiplier of “2” used to project harvest yields based on lag phase berry weights [1]. Sixty leaves per experimental unit that were opposite the first cluster on each shoot were removed from the vines, separated into petioles and leaf blades, and submitted to Fruit Growers Laboratory (Santa Paula, CA, USA) to determine nutrient concentration. All plant material was washed, oven-dried, and subjected to one of three analyses. Total leaf N and petiole NO_3^- were determined using the Dumas Combustion Method [148] and the Cadmium Reduction Method [149, 150], respectively (Appendix). Ash extractions [151] (Appendix) were used to determine all remaining measured macro- and micronutrients (P, K, Ca, Mg, Zn, Fe, Mn, B, and Cu).

Statistical analyses. All statistical procedures were performed with SAS statistical software (SAS 9.1, SAS Institute, Inc., Cary, NC, USA) and Minitab 16 statistical software (Minitab Inc., State College, PA, USA). The response variables and residuals of all datasets were analyzed using Levene’s test of equality of variance and the Shapiro-Wilk test for normality. The 2009 cluster weight dataset required a log transformation to pass Levene’s

test of equality of variance. Treatment differences were determined using the General Linear Model with post-hoc analyses conducted using Tukey's Studentized multiple-comparison procedure with a family error rate of $\alpha \leq 0.05$ or Dunnett's two-tailed t -test at $\alpha \leq 0.05$, which allows for comparisons of each treatment with the control.

To evaluate whether the disease presumed to be leaf-roll had a significant effect on 2009 harvest juice quality parameters and yield components in the fertigation blocks, t -tests for all harvest juice quality parameters and yield component variables were run between experimental units with no visual signs of disease (all of block 1 and block 4, experimental units 5 and 6 in block 2, and experimental units 11 and 12 in block 3), and those with signs of stunting (experimental units 7-10 in blocks 2 and 3). An ANOVA was run for all variables using only the non-diseased experimental units for analysis.

Figure 2.1. Map of the fertigation experiment conducted using Syrah vines on Schwarzmann rootstock at the Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Treatments were applied to 5 vines per treatment per block at berry set of 2009, 2010, and 2011, and at veraison in 2009 and 2010. Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Figure 2.2. Map of the foliar fertilizer experiment conducted using Syrah vines on 420A rootstock at the Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Treatments were applied by foliar application to individual vines at berry set in 2011. Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Table 2.1. Total amount of macronutrients (kg/ha/yr) applied in 2009 and 2010 by soil fertigation and in 2011 by soil fertigation or foliar application to Syrah vines at the Trestle Vineyard, California Polytechnic State University, CA. Control = water; Fish = fish extract, Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Nutrients (kg/ha/year)					
	Applied in 2009 or 2010			Applied in 2011		
	N	P	K	N	P	K
Control	0.00	0.00	0.00	0.00	0.00	0.00
Fish	2.40	3.10	0.15	1.20	1.55	0.08
Seaweed	0.00	0.00	0.15	0.00	0.00	0.08
Blend	1.81	2.32	0.15	0.91	1.16	0.08

CHAPTER 3

RESULTS

Harvest juice quality parameters and yield components. In the 2009 fertigation experiment, no significant treatment differences were detected between treatments in berry juice TSS (°Brix), pH, TA (g/100ml), sugar-to-acid ratio (TSS: TA), anthocyanin concentration (mg/L), tannin concentration (mg/L), or anthocyanin-to-tannin ratio (Table 3.1). Additionally, no significant differences were detected in clusters per vine, berries per cluster, cluster weight (g), average berry weight (g), fruit yield per vine (kg), vegetative yield per vine (kg), or Ravaz Index between soil fertigation treatments (Table 3.2). In 2011, soil fertigation (Table 3.3) and foliar fertilizer (Table 3.4) applications did not have significant effects on fruit yield per vine (kg), clusters per vine, berries per cluster, cluster weight (g), average berry weights (g), or rachis length (mm).

When soil fertigation was tested against time between 2009 and 2011, significant differences were found in yield components (Table 3.5). Clusters per vine and berries per cluster were significantly higher in 2009 than in 2011 ($p \leq 0.01$). There were no significant differences between fruit yield per vine (kg) in 2009 or estimated fruit yield per vine (kg) in 2011. However, 2011 estimated harvest cluster weights (g) and estimated average berry weights (g) were significantly greater than 2009 cluster weights (g) and average berry weights (g) at harvest ($p \leq 0.01$). The fish/seaweed extract blend treatments had significantly more clusters per vine than the fish extract treatment; however neither treatment resulted in a significantly different number of clusters per vine than seaweed extract or control (Tukey's Studentized multiple-comparison procedure at $\alpha \leq 0.05$ or Dunnett's two-tailed t -test at $\alpha \leq 0.05$). There were no significant time by treatment interactions for any yield component.

Nutrient uptake. The soil fertigation fish/seaweed extract blend treatment resulted in significantly greater leaf N (%) than the seaweed extract treatment (Tukey's Studentized multiple-comparison procedure at $\alpha \leq 0.05$; Table 3.6). The seaweed extract foliar treatment resulted in significantly greater leaf N (%) than the foliar fish/seaweed extract blend treatment (Tukey's Studentized multiple-comparison procedure at $\alpha \leq 0.05$; Table 3.7). However, none of the treatments resulted in significant differences in leaf N (%) as compared to the control (Dunnett's two-tailed t -test at $\alpha \leq 0.05$). There was a marginally significant increase in petiole Cu (ppm) ($p=0.07$) in the fertigation experiment; however, there were no significant differences detected by Tukey's Studentized multiple-comparison procedure at $\alpha \leq 0.05$ or Dunnett's two-tailed t -test at $\alpha \leq 0.05$. Marginally significant increases in Cu concentration in response to seaweed extract fertigation as compared to all other fertigation treatments were detected with Dunnett's two-tailed t -test at $\alpha \leq 0.15$. There were no other significant differences in petiole nutrient content in either the foliar or fertigation experiment (Tables 3.6 and 3.7). All of the experimental units in 2011 were found to be deficient in total leaf N (%) based on the critical values set forth by Christensen [58]. Some of the samples were deficient in petiole NO_3^- , P, and/or K [58]. Many of the experimental units were low or deficient in petiole Fe [58]. None of the experimental units were deficient in Ca, Mg, Zn, Mn, or Cu (Tables 3.6 and 3.7).

Net Photosynthesis and Stomatal Conductance. Seaweed extract resulted in lower rates of net photosynthesis than the control and fish/seaweed extract blend ($p \leq 0.01$; Table 3.8), whereas fish/seaweed extract blend resulted in higher rates of net photosynthesis than control and seaweed treatments ($p \leq 0.01$; Table 3.8). Marine extract treatment did not significantly affect stomatal conductance.

Impact of disease. Leaf roll virus appeared to be afflicting the Syrah block of the study site (personal observation, 21 September 2009). The symptoms included severely stunted shoots that were approximately 30-60 cm long and fruit that were still green at harvest. Fruit from vines showing no signs of disease had significantly higher ($p \leq 0.05$) mean TSS (mean = 23.16) than fruit from diseased/stunted vines (mean = 20.47). The diseased vines also had a significantly higher mean tannin-to-anthocyanin ratio. However, when data from putative diseased vines were removed from statistical analyses of the fertigation treatments, there were still no significant differences in harvest juice quality parameters or yield components.

Table 3.1. Effect of fertigation treatment on harvest juice TSS (°Brix), pH, TA (g/100ml) , sugar-to-acid ratio (TSS: TA), tannins (mg/L), anthocyanins (mg/L), and anthocyanin: tannin ratio of Syrah grapes harvested on 21 September 2009 from Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=16). Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	TSS (°Brix)	pH	TA (g/100ml)	TSS: TA	Tannins (mg/L)	Anthocyanins (mg/L)	Tannins: Anthocyanins
Control	23.55 $\pm$ 0.53	3.87 $\pm$ 0.13	0.52 $\pm$ 0.03	49.38 $\pm$ 5.11	639.5 $\pm$ 21.9	1475.3 $\pm$ 44.9	0.47 $\pm$ 0.02
Fish	24.35 $\pm$ 0.89	3.86 $\pm$ 0.03	0.57 $\pm$ 0.05	47.83 $\pm$ 5.23	642.5 $\pm$ 28.9	1485.5 $\pm$ 18.6	0.43 $\pm$ 0.02
Seaweed	23.33 $\pm$ 0.33	3.74 $\pm$ 0.09	0.53 $\pm$ 0.03	45.87 $\pm$ 2.56	563.3 $\pm$ 16.9	1515.0 $\pm$ 11.4	0.37 $\pm$ 0.01
Blend	24.18 $\pm$ 0.64	3.68 $\pm$ 0.13	0.61 $\pm$ 0.05	42.54 $\pm$ 3.26	759.8 $\pm$ 45.4	1570.5 $\pm$ 22.7	0.48 $\pm$ 0.02
<i>F</i>	0.20	1.41	0.72	0.36	1.57	0.61	1.79
<i>p</i>	0.89	0.30	0.57	0.78	0.26	0.62	0.20

Table 3.2. Effect of fertigation treatment on clusters per vine, berries per cluster, cluster weight (g), average berry weight (g), fruit yield per vine (kg), vegetative yield per vine (kg), and Ravaz Index of Syrah grapes harvested on 21 September 2009 from Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=16). Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Clusters/ Vine	Berries/ Cluster	Cluster Weight (g)	Average Berry Weight (g)	Fruit Yield/Vine (kg)	Vegetative Yield/Vine (kg)	Ravaz Index
Control	31.8 $\pm$ 1.4	70.6 $\pm$ 14.2	43.48 $\pm$ 3.64	0.67 $\pm$ 0.03	1.43 $\pm$ 0.17	0.95 $\pm$ 0.06	1.45 $\pm$ 0.12
Fish	27.1 $\pm$ 0.3	67.6 $\pm$ 11.0	42.28 $\pm$ 2.92	0.64 $\pm$ 0.05	1.18 $\pm$ 0.07	0.88 $\pm$ 0.05	1.36 $\pm$ 0.12
Seaweed	24.3 $\pm$ 0.9	84.6 $\pm$ 20.9	51.63 $\pm$ 0.81	0.63 $\pm$ 0.03	1.69 $\pm$ 0.13	1.02 $\pm$ 0.05	1.69 $\pm$ 0.12
Blend	34.9 $\pm$ 0.3	71.1 $\pm$ 14.1	37.04 $\pm$ 0.58	0.52 $\pm$ 0.01	1.29 $\pm$ 0.07	0.90 $\pm$ 0.03	1.47 $\pm$ 0.07
<i>F</i>	2.65	1.16	0.79	0.51	0.83	0.78	0.67
<i>p</i>	0.11	0.67	0.53	0.69	0.51	0.54	0.21

Table 3.3. Effect of fertigation treatment on fruit yield per vine (kg), clusters per vine, berries per cluster, cluster weight (g), average berry weight (g), and rachis length (mm) of Syrah grapes harvested on 1 August 2011 from Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=16). Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Fruit Yield/ Vine (kg)	Clusters/ Vine	Berries/ Cluster	Cluster Weight (g)	Average Berry Weight (g)	Rachis Length (mm)
Control	0.80 $\pm$ 0.14	21.0 $\pm$ 1.8	48.2 $\pm$ 3.7	35.46 $\pm$ 3.39	0.72 $\pm$ 0.02	130.0 $\pm$ 1.6
Fish	0.60 $\pm$ 0.06	19.8 $\pm$ 1.0	51.7 $\pm$ 2.7	29.60 $\pm$ 1.99	0.57 $\pm$ 0.02	133.5 $\pm$ 2.7
Seaweed	0.77 $\pm$ 0.06	23.6 $\pm$ 0.7	56.8 $\pm$ 3.0	36.27 $\pm$ 2.30	0.64 $\pm$ 0.02	133.4 $\pm$ 1.7
Blend	0.70 $\pm$ 0.04	22.0 $\pm$ 0.8	49.8 $\pm$ 1.2	30.42 $\pm$ 0.90	0.61 $\pm$ 0.02	137.9 $\pm$ 4.5
<i>F</i>	0.65	0.81	0.81	0.53	1.89	0.29
<i>p</i>	0.60	0.52	0.52	0.68	0.20	0.83

Table 3.4. Effect of foliar treatment on fruit yield per vine (kg), clusters per vine, berries per cluster, cluster weight (g), average berry weight (g), and rachis length (mm) of Syrah grapes harvested on 1 August 2011 from Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=24). Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Fruit Yield/ Vine (kg)	Clusters/ Vine	Berries/ Cluster	Cluster Weight (g)	Average Berry Weight (g)	Rachis Length (mm)
Control	0.94 $\pm$ 0.11	28.5 $\pm$ 2.0	61.6 $\pm$ 3.4	30.73 $\pm$ 1.67	0.50 $\pm$ 0.02	121.0 $\pm$ 2.3
Fish	0.55 $\pm$ 0.07	21.7 $\pm$ 2.0	55.6 $\pm$ 3.8	24.57 $\pm$ 1.09	0.46 $\pm$ 0.02	114.5 $\pm$ 4.5
Seaweed	0.76 $\pm$ 0.08	24.2 $\pm$ 1.9	71.3 $\pm$ 8.2	30.67 $\pm$ 1.86	0.49 $\pm$ 0.02	122.3 $\pm$ 1.9
Blend	0.76 $\pm$ 0.09	26.5 $\pm$ 2.3	67.3 $\pm$ 5.5	27.08 $\pm$ 1.25	0.44 $\pm$ 0.03	114.2 $\pm$ 3.1
<i>F</i>	1.14	0.68	0.57	1.17	0.76	0.92
<i>p</i>	0.37	0.58	0.64	0.35	0.53	0.45

Table 3.5. Effect of fertigation by treatment, year, and treatment*year interaction on fruit yield per vine (kg), clusters per vine, berries per cluster, cluster weight (g), and average berry weight (g) of Syrah grapes harvested on 21 September 2009 and 1 August 2011 from Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=32). Control = water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Fruit Yield/ Vine (kg)	Clusters/ Vine	Berries/ Cluster	Cluster Weight (g)	Average Berry Weight (g)
Control	1.38 $\pm$ 0.11	25.13 $\pm$ 1.48ab	59.40 $\pm$ 3.17	57.18 $\pm$ 4.41	1.03 $\pm$ 0.08
Fish	1.17 $\pm$ 0.06	23.44 $\pm$ 0.85b	59.64 $\pm$ 2.34	50.74 $\pm$ 2.79	0.89 $\pm$ 0.06
Seaweed	1.68 $\pm$ 0.07	28.02 $\pm$ 1.17ab	70.67 $\pm$ 3.83	62.05 $\pm$ 3.11	0.96 $\pm$ 0.07
Blend	1.35 $\pm$ 0.05	28.94 $\pm$ 1.20a	60.44 $\pm$ 2.65	48.94 $\pm$ 2.53	0.88 $\pm$ 0.07
F	1.73	3.27	1.29	1.25	1.49
p	0.19	0.04	0.30	0.32	0.24
Year					
2009	1.39 $\pm$ 0.08	31.54 $\pm$ 0.76a	73.45 $\pm$ 2.73a	43.60 $\pm$ 1.99a	0.60 $\pm$ 0.02a
2011	1.40 $\pm$ 0.08	21.22 $\pm$ 0.80b	51.63 $\pm$ 1.87b	65.86 $\pm$ 3.12b	1.27 $\pm$ 0.03b
F	0.00	53.27	20.81	17.00	144.93
p	0.96	<0.01	<0.01	<0.01	<0.01
Treatment*					
Year					
F	0.09	0.96	0.26	0.17	1.52
p	0.97	0.43	0.85	0.92	0.24

a, b: Treatment means within the same column with different letters indicate significance at $\alpha \leq 0.05$ using Tukey's Studentized multiple comparison procedure.

Table 3.6. Petiole NO₃⁻ (ppm dry weight), % dry weight leaf blade N, % dry weight petiole P, K, Ca, and Mg, and ppm dry weight petiole Zn, Mn, Fe, B, and Cu of petioles and leaves harvested from fertigation-treated Syrah vines on 1 August 2011 at Trestle Vineyard, California Polytechnic State University, CA. Values are means ± standard error (n=16). Control= water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	NO ₃ ⁻ (ppm)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Mn (ppm)	Fe (ppm)	B (ppm)	Cu (ppm)
Control	749 ± 94	2.36 ± 0.03ab	0.22 ± 0.01	3.32 ± 0.27	1.24 ± 0.06	0.57 ± 0.04	48.4 ± 1.4	49.0 ± 1.9	36.0 ± 0.7	34.1 ± 0.7	7.8 ± 0.1
Fish	593 ± 51	2.31 ± 0.05ab	0.25 ± 0.02	3.98 ± 0.05	1.10 ± 0.02	0.54 ± 0.01	43.1 ± 2.5	52.3 ± 2.9	35.5 ± 0.3	39.3 ± 1.1	8.0 ± 0.1
Seaweed	546 ± 51	2.19 ± 0.04a	0.27 ± 0.03	3.38 ± 0.27	1.13 ± 0.04	0.58 ± 0.02	48.9 ± 2.0	56.8 ± 2.9	38.5 ± 1.6	36.4 ± 1.2	9.5 ± 0.4*
Blend	987 ± 13	2.57 ± 0.04b	0.24 ± 0.01	3.47 ± 0.07	1.28 ± 0.04	0.65 ± 0.06	45.1 ± 2.7	52.0 ± 3.7	36.3 ± 1.0	36.6 ± 1.2	7.3 ± 0.2
<i>F</i>	2.0	3.75	0.23	0.85	1.58	0.72	0.34	0.83	0.37	1.18	3.29
<i>p</i>	0.18	0.05	0.87	0.50	0.26	0.57	0.79	0.51	0.78	0.37	0.07

a, b: Treatment means within the same column with different letters indicate significance at $\alpha \leq 0.05$ using Tukey's Studentized multiple comparison procedure.

*: Treatment means within the same column with a * are significantly different from control level using Dunnett's two- tailed t-test at $\alpha \leq 0.15$.

Table 3.7. Petiole NO₃⁻ (ppm dry weight), % dry weight leaf blade N, % dry weight petiole P, K, Ca, and Mg, and ppm dry weight petiole Zn, Mn, Fe, B, and Cu of petioles and leaves harvested from foliar-treated Syrah vines on 1 August 2011 at Trestle Vineyard, California Polytechnic State University, CA. Values are means ± standard error (n=24). Control= water; Fish = fish extract; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	NO ₃ ⁻ (ppm)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Mn (ppm)	Fe (ppm)	B (ppm)	Cu (ppm)
Control	753 ± 180	2.32 ± 0.04ab	0.16 ± 0.02	1.92 ± 0.16	1.35 ± 0.02	0.82 ± 0.03	44.2 ± 2.3	43.3 ± 2.5	30.0 ± 0.9	37.5 ± 0.9	8.3 ± 0.5
Fish	692 ± 162	2.29 ± 0.05ab	0.16 ± 0.02	2.62 ± 0.03	1.38 ± 0.04	0.70 ± 0.05	43.3 ± 1.8	42.3 ± 3.0	33.3 ± 1.4	36.2 ± 0.9	8.0 ± 0.4
Seaweed	904 ± 171	2.50 ± 0.04b	0.22 ± 0.04	3.15 ± 0.27	1.33 ± 0.04	0.70 ± 0.04	49.1 ± 1.2	51.2 ± 2.1	29.2 ± 0.2	41.4 ± 1.6	8.7 ± 0.5
Blend	519 ± 131	2.22 ± 0.02a	0.18 ± 0.01	2.16 ± 0.18	1.30 ± 0.02	0.77 ± 0.04	47.1 ± 2.6	44.8 ± 2.7	30.2 ± 0.4	34.6 ± 1.1	8.0 ± 0.3
<i>F</i>	1.76	3.55	0.92	1.92	0.12	0.49	0.67	0.61	1.03	1.76	0.13
<i>p</i>	0.20	0.04	0.45	0.17	0.95	0.70	0.58	0.62	0.41	0.20	0.94

a, b: Treatment means within the same column with different letters indicate significance at $\alpha \leq 0.05$ using Tukey's Studentized multiple comparison procedure.

Table 3.8. Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($\text{mol H}_2\text{O CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of Syrah grape leaves immediately after fertigation with water, seaweed extract, or fish/seaweed extract blend on 17 August 2009 at Trestle Vineyard, California Polytechnic State University, San Luis Obispo, CA. Values are means $\pm$ standard error (n=18). Control = water; Seaweed = seaweed extract; Blend = fish/seaweed extract blend.

Treatment	Net Photosynthesis ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	Stomatal Conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
Control	$3.12 \pm 0.19\text{b}$	0.055 ± 0.002
Seaweed	$1.76 \pm 0.28\text{a}$	0.045 ± 0.004
Blend	$4.83 \pm 0.29\text{c}$	0.060 ± 0.004
<i>F</i>	12.6	1.47
<i>p</i>	<0.01	0.26

a, b, c: Treatment means within the same column with different letters indicate significance at $p \leq 0.01$ using Tukey's Studentized multiple comparison procedure.

CHAPTER 4

DISCUSSION AND CONCLUSION

None of the marine extracts tested in the study reported herein resulted in significant differences in yield or harvest juice quality parameters of Syrah grapes as compared to the control. The only significant treatment difference for any yield component was that vines fertigated with fish/seaweed extract blend had significantly more clusters per vine than those fertigated with fish extract when the two years of yield data were combined. However, since fruit clusters were formed prior to fertigation in 2009, the treatments could not have affected the number of clusters per vine in that year. High variability in the number of clusters per vine could have been due to inconsistencies in training and pruning prior to the experiment. Whereas clusters per vine and berries per cluster decreased significantly from 2009 to 2011, cluster weight and average berry weight increased significantly without significant differences in total fruit yield per vine between the 2 years. While it is possible that these differences are an artifact of having estimated cluster weight, yield per vine, and average berry weight in 2011, they seem more likely due to vine-yield compensation. As clusters per vine and berries per cluster declined, cluster and average berry weights increased to compensate for the reduced number of clusters and berries. This is in agreement with Kleiwer et al. (1983), who found that cluster thinning reduced the number of clusters but did not reduce yield relative to the non-cluster thinning treatments. In that experiment, the average cluster weight of the cluster thinning treatments was heavier than the average cluster weight of the non-cluster thinning treatment.

Clusters per vine and berries per cluster were each significantly ($p \leq 0.01$) higher in 2009 than in 2011 of the soil fertigation experiment. These differences could have been a result of variations in canopy management, including pruning [24], and/or vine growth from year to year, despite attempts to maintain consistent conditions. Climatic conditions, including a possible late-spring frost in April 2011, could have reduced the cluster count. On 8 April 2011, a low of 32.7 °F was recorded at the California Irrigation System Management Information Service (CIMIS) weather station number 52, which is located 2.5 km from the study site [152]. Considering meso- and microclimatic variation between the study site and the weather station, it is possible that damaging sub-freezing temperatures occurred at the study site.

The lack of significant differences in yield components and harvest juice quality parameters could have been the result of the putative virus, which noticeably delayed ripening, leading to high variability in the means of these variables. The vines that were visually stunted, presumably due to a virus, had an average of 2.69 °Brix less than vines showing no visual signs of stunting. The reduced TSS in the fruit from the stunted vines is in agreement with the significantly greater tannin-to-anthocyanin ratio in the fruit from the stunted vines. Since the diseased fruit stayed green and under-ripe, there was a greater quantity of tannins relative to anthocyanins at harvest compared to properly ripened fruit that accumulated more anthocyanins. The greater tannin-to-anthocyanin ratio and reduced TSS in fruit from the stunted vines indicates that the virus may have significantly hindered fruit development in this experiment. The results of the study reported herein are consistent with results from previous studies in which virus pressures reduced the TSS of fruit from Albarino [10], Reisling [12], and Pinot Noir [11] grapevines. However, when diseased

samples were excluded from statistical analyses in the study reported herein, no significant differences in yield and harvest juice quality parameters were detected.

With the exception of an increase in leaf Cu concentration in response to fertigation with seaweed extract, none of the marine extract treatments resulted in significant differences in petiole or leaf nutrient concentrations as compared to the control. That fertigation with seaweed extract resulted in a significant increase in Cu concentration as compared to the control was consistent with the findings of Turan (2004). However, the lack of any other significant change in leaf nutrient concentration in the study reported herein are in contrast with the findings of Mancuso et al. (2006). They found that uptake of N, P, K, and Mg by Sangiovese grapevines increased significantly in response to foliar applications of SWE [120]. Fertigation with fish/seaweed extract blend resulted in significantly greater leaf N (%) than the seaweed extract, whereas foliar applications of seaweed extract resulted in significantly more leaf N (%) than the fish/seaweed extract blend. Since the fish/seaweed extract blend treatment added 1.81 more kg N/ha/yr compared to the seaweed extract alone, it is possible that the additional N applied with the fish/seaweed extract blend fertigation was the cause of the increase in leaf N concentration. had the highest total N application but did not result in leaf N concentrations that were significantly different from those of any other treatment. There was less applied N in the foliarly applied seaweed extract than the fish/seaweed extract blend and fish extract treatments; however, the seaweed extract treatment only had significantly greater leaf N concentrations than the fish/seaweed extract blend treatment. However, it is important to note that all of the foliar-treated vines were on 420A rootstock, whereas fertigated vines were on Schwarzmann rootstock. Different rootstocks vary in there nutrient uptake patterns

[36]. Therefore, it is not possible to determine whether if the treatment differences in leaf N concentration reported herein were due to differences in the compound applied, the application method (fertigation or foliar), or to the rootstock. Regardless, the effects of the marine extract treatments on leaf nutrient concentration were negligible.

The lack of significant effects on harvest juice quality parameters and yield components in response to marine extract applications in the study reported herein could have been due to the compounds low nutrient content. While TSS typically reduced as a result of N fertilization [4-9], some studies have reported no significant changes in TSS with higher N rates than were applied in the study reported herein [61, 63, 64]. Since pH and TA often have variable and inconsistent responses to N fertilizer application [5-8, 63, 64], it is not surprising that the small quantities of N applied during the study reported herein did not result in detectable changes in pH or TA. Several studies with wine or table grapes have demonstrated that N applications that were orders of magnitude higher than those utilized in the study reported herein also failed to significantly affect fruit or vegetative yield [63, 64, 71]. N treatment of grapes has also been reported to increase fruit yields [25, 26, 63, 72], while in other studies N treatment reduced fruit yields [7, 63, 66, 68]. However, the N application rates used in these studies were orders of magnitude higher than the N rates applied in the study reported herein. While working with the same marine extracts as those used in this study, Brown (2004) reported no significant effects on lettuce, broccoli or sweet pepper yields. Fish fertilizers also had no effect on crop yield in tomatoes [94, 97] or wheat [140]. Furthermore, SWE did not have a significant effect on yield or quality parameters of onions [138], barley [139], wheat [140], tomatoes [94, 97], mandarins [124, 132], or strawberries [141].

Marine extracts also have the potential to influence yield and harvest juice quality parameters due to their PGR content. Though ABA is not listed among the active ingredients in commercial marine extracts, Boyer [110] found that commercial SWEs contained 0.1 to 0.46 ppm ABA. Based on these estimates, each of the fertigated vines in the study reported herein would have received 4.2 to 9.66 μg ABA each year, with each foliar treated vine receiving approximately half of that amount. This amount of ABA is very small in comparison to the rates required to increase grape anthocyanin content. For example, 250 μg of ABA applied directly to each grape cluster was required to significantly increase the anthocyanin content of Pinot Noir grapes [31]. The ABA rates applied to grapevines in similar ABA studies [28, 30, 32, 33] were also much higher than the amount of ABA received from the SWE in the study reported herein. Other PGRs present in marine extracts containing SWE, such as cytokinins and GAs, could also be responsible for altering yield components in crops, however these PGRs are also present in negligible amounts in SWEs compared to the rates required to significantly affect grape yield components and harvest juice quality parameters [27, 29, 34, 35, 49, 52].

In previously published experiments, TSS, pH, and TA of grape berries have not been significantly affected by ABA applications [28, 30-33], which is consistent with the results of the study reported herein. Exogenously applied ABA can significantly increase color and anthocyanins in wine and table grapes [28, 30-33]. However, the most dramatic increases in color have been observed in color-poor table grape varieties such as Flame Seedless [32] and in grapes grown in hot climates [31-33] due to rapid anthocyanin biosynthesis early in phase III of berry growth [28]. While exogenous ABA applications can increase anthocyanin content in Flame Seedless table grapes by 800% compared to

untreated controls [32], ABA-treated Merlot grapes had only 7% more anthocyanins than untreated controls [55] and the anthocyanin content of ABA-treated Cabernet Sauvignon grapes was not significantly different that of control grapes when harvested late in phase III of berry growth [28]. Therefore, since ABA applications failed to increase anthocyanin content of Cabernet Sauvignon at harvest [28] and only marginally increased Merlot anthocyanin content [55], it would most likely have minimal effects on a high-anthocyanin cultivar such as Syrah.

Immediately after fertigation with seaweed extract or fish/seaweed extract blend, net photosynthesis changed significantly as compared to the control. Therefore, it is likely that the vine roots absorbed the marine extract treatments. These results are in contrast to Jeannin (1991), where foliarly applied SWE reduced stomatal conductance and transpiration rate in maize, but did not effect net photosynthesis [121]. The decreased rate of net photosynthesis as a result of seaweed extract fertigation could have occurred as a result of the product's putative ABA content. This is in agreement with Wang et al. (2004), who found that the application of ABA reduced net photosynthesis in creeping bentgrass. In the study reported herein, the fish/seaweed treatment resulted in increased net photosynthesis as compared to the control and seaweed treatments. This might have been due to the N in the fish/seaweed treatment. Increased net photosynthesis as a result of N application has been observed in Müller-Thurgau grapevines [23]. However, in the study reported herein, stomatal conductance was not significantly affected by either marine extract. The effects on net photosynthesis in the study reported herein were likely temporary, since yield and harvest juice quality parameters were not influenced by any fertigation treatment.

Seaweed extracts have been used to successfully improve yield and quality parameters in a wide variety of crops [107, 113, 116, 117, 121-132], including grapes [133-136]. However, there have been numerous studies where seaweed extracts had no effect on yield or quality components [94, 96, 97, 124, 132, 138-141]. The inconsistent findings could be due to multiple factors, including differences in species, cultivar, rootstock, application rate, seaweed source and species, soil characteristics, initial plant nutrient status, irrigation, and/or environmental conditions. For example, it is possible that Syrah could be less responsive to SWE application than Sangiovese or table grapes cultivars that are significantly affected by seaweed extract application, including increases in berry weight [133-135], fruit yield [133-135], TA [136], color [133], tannins [136], nutrient uptake [119, 120], vegetative yield [120], and drought stress tolerance [120]. To ascertain if the lack of effectiveness of the marine extracts tested in the study herein to increase the anthocyanin content is due to the extracts' low concentration of ABA, the cultivar used, or some other factor, it would be interesting to test the effectiveness of synthetic ABA applications to significantly increase anthocyanin content of Syrah fruit. More experiments with marine extracts are also needed in order to better understand how these products might be able to influence yield components, harvest juice quality parameters, and nutrient uptake.

Overall, there appears to be no beneficial or detrimental effects from applying marine extracts to Syrah grapes growing in cool-climates and heavy clay soils. While beneficial impacts have been observed in other experiments, the lack of significantly different results observed in this study suggest that the products utilized had little to no effect on harvest juice quality, yield components, or nutrient concentration. Conversely, if

a grower wanted to use these products for another purported benefits observed in previous experiments, such as disease and nematode suppression or to encourage root growth, it appears that there would be no negative side effects on harvest juice quality parameters or yield components.

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APPENDIX

NUTRIENT ANALYSIS PROCEDURES

Dumas Combustion Method (Total % Leaf N):

- 1) After drying plant tissue at 80 °C for 24 hours, leaf blades are ground into a fine powder (particle size < 250 µm) with a ball mill.
- 2) 5 X 9 tin capsules are filled with powder, weighed (µg), and recorded.
- 3) The leaf samples contained in tin capsules are dropped into a quartz combustion tube with an ambient temperature of 1200 °C.
- 4) The combusted N in the form of N gas (N₂) and N oxides is forced through a copper wire reduction column heated at 600 °C. The reduction column removes the oxygen from the N oxides, converting them into N₂.
- 5) The N₂ passes through 2 gas traps to remove water vapor and carbon dioxide.
- 6) The N₂ is channeled into an elemental analyzer, along with an isolated reference stream of Helium.
- 7) The difference in thermal conductivity is plugged into a linear regression model based on combustion values of known standard to materials to determine the mass of the N.
- 8) The total N value from the combustion is divided into the weight of the powder from step 2 to calculate total leaf N (%).

Cadmium Reduction Method (ppm petiole NO₃⁻):

- 1) Dry petiole samples for 24 hours at 50-55 °C.
- 2) Grind petiole samples into a fine powder with a ball mill.
- 3) Prepare 125 ml Erlenmeyer flask for each sample.
- 4) Prepare color reagent: Combine 50 ml phosphoric acid with 400 ml deionized water. Add 20 g sulfanilamide and swirl until dissolved. Then add 1 g N-1-naphthylenediamine dihydrochloride and swirl until dissolved. Filter solution with filter paper and set aside.
- 5) Measure approximately one gram of powdered plant material into each flask and record the weight of the sample.
- 6) Place 20 ml of 0.1 N KCl extraction solution (deionized water containing 0.932% KCl) into each bottle and shake for 15 minutes with a reciprocating shaker at a rate of 250 oscillations per minute.
- 7) Filter samples into test tubes with filter paper, and set aside the filtrate.
- 8) Send the filtrate through a copperized cadmium column to reduce the NO₃⁻ into nitrite.
- 9) Collect 15 ml leachate from the cadmium column and add 1 ml of color reagent.
- 10) Once the solution has developed a red color, analyze the light absorption of the sample solution with an electronic spectrophotometer at a 543 nm wavelength.
- 11) Plot the absorbance against a standard nitrate curve based on absorbance values of standard NO₃⁻ solutions (y-axis) with known NO₃⁻ concentrations (x-axis). The location of the absorbance value of the sample on the y-axis of the standard curve will correspond to a NO₃⁻ concentration value on the x-axis.

- 12) Take the NO_3^- concentration value from the x-axis and multiply it by the volume of the sample (leachate + reagent), then divide by the weight of the dried petioles from step 5 to calculate ppm NO_3^- .

Dry Ash Extraction (% petiole P, K, Ca, Mg; ppm petiole Zn, Fe, Mn, B, and Cu):

- 1) Dry petiole samples for 24 hours at 70 °C.
- 2) Grind petiole samples into a fine powder with a ball mill.
- 3) Weigh approximately 0.5 g of ground petiole material into a crucible, weigh the sample, and record the weight of the sample.
- 4) In a muffle furnace, gradually heat the plant material to 450 °C over the course of 90 minutes, then ash for 4 hours and allow to cool.
- 5) Place 10 ml of extraction acid into crucible, then transfer all contents into extraction vial.
- 6) Cap and shake extraction vial.
- 7) Measure the amount of each element with atomic absorption spectrophotometry. Divide that value into the weight of the sample in step 3 to determine % or ppm of nutrient/dry weight.