The effects of nanobubble enriched water on grapevine performance in the field

Mark Krasnow, Georgia Lee, Stewart Field

Executive summary

A trial investigating potential benefits of nanobubble enriched drip irrigation water in a Marlborough Sauvignon blanc vineyard was undertaken in the 2021-22 season. Oxygen and air nanobubble enriched water was delivered through the dripline to plants and was compared with irrigation with nonaerated water directly from the bore. Nanobubble enrichment dramatically increased the dissolved oxygen in the treated water, and this elevated oxygen was maintained through the lines and out the drippers. There was no consistent effect of water treatment on stem water potential, stomatal conductance, or SPAD measurements of leaf chlorophyll. Berry weight was consistently lower from the air nanobubble treatment compared with the control and the oxygen nanobubble treatment. However at harvest there was no effect on yield due to treatment. It is possible that earlier provision of oxygen enriched water than in this study (treatments started 6 December, 2021), might have had a more profound effect on vine growth, water relations, and productivity.

Introduction

All plant roots need oxygen to function. Soils generally have about 50% air space, which allows the roots access to oxygen from the atmosphere. However, some soils, either due to their particle size (i.e. high clay and silt content), physical structure, or to a high water table lack sufficient oxygen for roots to thrive, and could potentially lead to reduced productivity or to diseases such as *cylindrocarpon* or *verticillium*, which can kill the vine (Mundy, 2015). Drip irrigation is the most common method for watering vines, but this water doesn't add any oxygen to the soil, and can actually fill the air spaces in the soil, making it more anoxic around roots.

Enriching the water around roots with oxygen is a common practice in hydroponics and aquaponics to ensure that roots have adequate oxygen for optimal growth (Ebina et al., 2013). In order to test whether adding oxygen to drip irrigation water in the field could improve vine health, productivity, or fruit quality, a trial was set up in a Sauvignon blanc vineyard in Marlborough, New Zealand. This vineyard is located in the lower Wairau valley, near the coast, and the soil type is a Motukarara f series, a silty, deep, very poorly drained soil prone to anoxia.

Nanobubbles are bubbles so small that they are neutrally buoyant and have a high zeta potential, slowing their coalescence into larger bubbles that can easily leave solution. Enriching irrigation with nanobubbles has been successfully trailed in several crops, including lettuce (Park et al., 2009), Brassica campestris (Ebina, et al., 2013), avocados, blueberries, cucumber and peppers (https://www.moleaer.com/case-studies). Nanobubbles can be created from any gas, including pure oxygen or air. Pure oxygen nanobubbles would obviously provide more oxygen to roots than air nanobubbles, which would only contain about 18% oxygen.

Materials and methods

Trial setup-The vineyard is Sauvignon blanc (MS clone) on S04 rootstock, with -2.8 m between rows and 1.8 m between vines. The vineyard was planted in 2016. Irrigation was undertaken

every other night. For each irrigation, vines received 7.8 L of water which was either not aerated and direct from the bore (control), was nanobubble aerated with pure oxygen before pumping (O2), or was nanobubble aerated with compressed air before pumping (air). Water for the air and oxygen nanobubble treatment was aerated in a 5000 liter tank before pumping into the driplines. Treatments were imposed on entire rows of vines, which received either water direct from the well, water enriched with air nanobubbles, or water enriched with oxygen nanobubbles. Six replicates of each treatment were set up in the trial vineyard in a Latin square block design.

Oxygen measurement in irrigation water-Water was collected directly from the drippers in each row of the trial (six replicates per treatment). Dissolved oxygen was measured with a RDO-X sensor (In Situ inc.).

Midday stem water potential-Midday stem water potential (SWP) was measured from two vines in each replicate (12 vines per treatment). Reflective bags were placed over leaves, which were allowed to equilibrate for at least 15 minutes before measurement. After equilibration, water potential was measured with a Scholander pressure chamber (PMS model 610).

Stomatal conductance-Midday stomatal conductance was measured with a porometer (SC 1 leaf porometer, Meter Inc.). Measurements were made from either one or two vines in each replicate (6 and 12 vines per treatment, respectively).

SPAD measurements-Leaf chlorophyll content was measured with a SPAD meter (SPAD-502, Konica-Minolta Sensing Inc.). For each vine leaves were measured in triplicate and averaged to generate a single value per leaf. Five leaves per vine were measured on 12 vines per treatment (60 leaves per treatment).

Berry samples-100 berry samples were collected weekly and at harvest from each treatment replicate. Samples were collected from 22 February, 2022 until harvest on 25 March, 2022. Samples were weighed to determine berry weight, and composition measured using a FTIR analysis (FOSS Winescan SO_2) to assess juice soluble solids, pH, tartaric and malic acids, potassium, and total yeast assimilable nitrogen (YAN).

Harvest-At harvest bunch number and yield per vine were recorded. Triplicate 17 L wine ferments of the oxygen and control treatments were made at the NMIT winery using temperature controlled fermenters.

Wine composition- One composite wine was made from the oxygen nanobubble treatment and one from the control. Approximately 25 kg of fruit was crushed and destemmed, and ferments according to standard protocols. Fermentation took place in controlled-temperature fermenters, held at 15°C during fermentation. After fermentation wines were measured for methoxypyrazines, alcohols, terpenes, norisoprenoids, and esters by the method of Parr et al. (2007). Wine pH, titratable acidity, glucose, fructose, malic acid, and percent alcohol were analysed by FTIR analysis (FOSS wineascan).

Pruning weight-At dormancy, the weight of prunings from each vine was collected and weighed. Two vines per replicate (12 vines per treatment) were assessed.

Results and discussion

As a proof of concept, the dissolved oxygen in the irrigation output was assessed using a dissolved oxygen meter on water collected from a dripper at the end of the drip line. The oxygen nanobubble treatment had extremely elevated oxygen compared with the control (almost four times saturation levels) (Table 1). Nanobubble aeration with compressed air had dissolved oxygen elevated above the control, but dramatically less than the oxygen nanobubble treatment (Table 1). This finding shows that the bubbles (both oxygen and air) are maintained from the feed tank, through the irrigation lines, and out the drippers without coalescing and leaving solution.

Table 1: Dissolved oxygen in drip irrigation output. Values are averages of six replicates per treatment.

Treatment	**Dissolved O_2 (mg/L)**
Control	**8.56 c**
Air	**10.82 b**
Oxygen	**32.75 a**
P value	**<0.00000**

There were no consistent effects of water treatment on midday stem water potential (SWP) (Table 2). Towards the end of the season (17 and 31 March), the control vines had the least negative SWP, however which treatment had the most negative SWP differed on these dates (Table 2). Before harvest (on the 17th March), the oxygen treatment had the most negative SWP, whereas after harvest (31st March), the air treatment had the most negative SWP. It's unclear what was leading to these differences, and their inconsistency makes interpretation of these data challenging. In any case, the differences in SWP were not so extreme as to lead to stomatal closure, since the stomatal conductance did not vary between treatments at any sampling date (Table 3).

Table 2: Midday SWP (in bars) from the trial. Values in bold with different lower case letters on the same date indicate significant differences at the p=0.05 level.

	22/02/22	1/03/22	3/03/22	8/03/22	15/03/22	**17/03/22**	**31/03/22**
Control	-4.0	-3.5	-4.0	-3.8	-5.4	**-5.8 a**	**-4.3 a**
Air	-4.5	-3.6	-4.1	-4.7	-6.2	**-7.0 ab**	**-5.4 b**
Oxygen	-4.3	-3.7	-3.9	-4.6	-6.2	**-7.5 b**	**-4.3 a**
P value	0.2308	0.8581	0.8347	0.0955	0.1368	**0.0135**	**0.0070**

Table 3: Stomatal conductance (mmol/m^2*s) from the trial. There were no significant differences in this parameter at any point between treatments.

	9/02/22	11/02/22	18/02/22	1/03/22	8/03/22	15/03/22
control	456.4	487.1	703.0	525.5	605.6	615.5
oxygen	461.1	487.6	708.4	523.8	591.2	591.5
air	444.3	471.1	674.8	533.9	589.9	582.0
p value	0.6533	0.6439	0.238	0.9422	0.7336	0.4255

In order to assess whether the water treatment had any effect on leaf chlorophyll levels, SPAD measurements were undertaken in the trial on 25th March, one day before harvest. No differences in SPAD measurements were seen (Table 4), indicating that the water treatment did not influence leaf chlorophyll levels later in the season. Taken together these data indicate that the aeration of the irrigation water with either air or oxygen did not negatively or positively influence vine performance or water relations.

Table 4: SPAD measurement of leaves from the trial on 25 March, the day before harvest. Values are averages of three measurements per leaf and five leaves per vine. Two vines per replicate (12 vines per treatment) were measured. No significant differences were seen.

	SPAD measurement
control	34.6
oxygen	33.9
air	34.8
ANOVA	0.2782

Berry development was followed by measuring berry weight and composition weekly from shortly after veraison to harvest. Berries from the air treatment were consistently smaller than the other two treatments (Table 5). There was a trend, which became more significant as the season progressed, for the air treatment to have lower juice malate than the other two treatments (Table 5). On two dates (1/3 and 22/3), the air treatment also had higher potassium than the oxygen treatment. This could possibly be due to the smaller berries from the air treatment, as the potassium is associated with hypodermal cells, and smaller berries have a higher skin-to-pulp ratio (Roby et al, 2004). It's unclear why the air treatment reduced berry growth and decreased malate levels compared with either of the other two treatments, but this finding has been seen in other crops, where air nanobubbles reduced growth compared with the control (Leon Power, personal communication). The oxygen nanobubble treatment did not offer any obvious benefit in terms of berry growth or composition compared with the control.

Table 5: Berry weight and juice composition over ripening. Values are means of six 100 berry samples per treatment. Values with different lower case letters for the same date denote significant differences at the p=0.05 level.

Date	Treatment	Berry weight (g)	Brix	TA (g/L)	pH	Tartrate (g/L)	Malate (g/L)	K (mg/L)	YAN (mg/L)
22/02/22	Control	**1.46 a**	12.4	17.4	2.74	9.0	9.6	1242	238.8
	Oxygen	**1.47 a**	12.5	17.4	2.74	9.0	9.5	1240	245.5
	Air	**1.34 b**	12.3	17.1	2.75	9.3	9.0	1333	242.8
1/03/22	Control	**1.49 ab**	13.8	15.1	2.80	8.3	7.8	**1362 ab**	188.7
	Oxygen	**1.51 a**	13.9	15.2	2.81	8.3	7.9	**1348 b**	197.3
	Air	**1.41 b**	13.7	14.6	2.82	8.4	7.3	**1404 a**	212.0
8/03/22	Control	**1.54 ab**	15.2	12.6	2.91	7.7	6.2	1397	236.3
	Oxygen	**1.62 a**	15.7	12.4	2.93	7.7	6.1	1407	265.7
	Air	**1.49 b**	15.3	12.2	2.93	7.7	5.8	1430	254.3
17/03/22	Control	1.62	16.6	9.4	3.02	6.8	3.8	1418	160.7
	Oxygen	1.64	16.5	9.4	3.03	6.7	3.9	1414	172.8
	Air	1.56	16.5	9.0	3.04	6.8	3.5	1430	181.3
22/03/22	Control	1.74	17.5	9.2	3.03	6.8	**3.9 ab**	**1296 ab**	235.7
	Oxygen	1.76	17.5	9.6	3.01	6.8	**4.2 a**	**1254 b**	244.5
	Air	1.67	17.5	8.8	3.05	6.7	**3.5 b**	**1334 a**	252.5
26/03/22	Control	**1.83 a**	17.1	8.9	3.03	6.8	**3.3 a**	1373	169.6
	Oxygen	**1.82 a**	17.0	8.8	3.05	6.7	**3.4 a**	1391	177.2
	Air	**1.70 b**	17.1	8.1	3.05	6.5	**2.8 b**	1386	167.4

At harvest, there were no significant differences in bunch number, yield per vine, or bunch weight between treatments (Table 6). It is a bit surprising that the air treatment, which had consistently smaller berries during development and at harvest (Table 5), did not have lower yield per vine. This suggest that the air treatment might have had more berries per bunch, but this parameter was not directly assessed in this trial. In any case, there was no benefit to overall yield from the oxygen nanobubble treatment versus the control, contrary to findings in other crops (Ebina et al., 2013; case studies found on: https://www.moleaer.com/case-studies). Many of the case studies involve hydroponics and aquaponics, where root anoxia can be a larger problem than in actual vineyard soils. However, many vineyards have poorly drained soils, and especially in the early spring, can have standing water and anoxic roots.

Table 6: Bunch number, yield per vine, and calculated bunch weight at harvest (26/3/22). No significant differences in any parameter was seen.

	bunch #	Yield (kg/vine)	bunch weight (g)
control	89.8	11.64	130.1
oxygen	83.8	11.19	132.9
air	86.8	11.28	130.1

These lack of effects of oxygen nanobubbles could possibly be due to the fact that the treated water only started being applied on 6 December, 2021, after full canopy would have been established and flowering and set had already occurred. More benefit of the oxygenated water would likely have been seen had the vines received a full season of treated water. Given the soil type of the vineyard, which is very poorly drained, the most benefit might be seen from oxygenated water in the early season, when the soil is saturated, and roots could potentially be in an anoxic environment, however treatments could not be applied that early in the 2021-22 season due to delays in getting the nanobubble generators installed. It is hoped that this trial will be continued next season, with the application of the treated water starting much earlier than in the 2021-22 season.

Table 7: Wine pH, TA, hexoses, malic acid, tartaric acid, and alcohol percent from the control and oxygen nanobubble treatments. No wines were made from the air nanobubble treatment.

Wine	pH	TA (g/L)	Glucose (g/L)	Fructose (g/L)	Sum (G+F)	Malic acid (g/L)	Ethanol (v/v%)
Control	2.87	8.64	0.2	4.24	4.44	3.29	12.59
Oxygen	2.87	8.51	0.08	1.04	1.12	3.19	12.53

Literature cited

Ebina, K., Shi, K., Hirao, M., Hashimoto, J., Kawato, Y., Kaneshiro, S., ... & Yoshikawa, H. (2013). Oxygen and air nanobubble water solution promote the growth of plants, fishes, and mice. *PLoS One*, *8*(6), e65339.

Mundy, D. C. (2015). Ecology and control of grapevine root diseases in New Zealand a review. *New Zealand Plant Protection*, *68*, 396-404.

Park JS, Kurata K (2009) Application of Microbubbles to Hydroponics Solution Promotes Lettuce Growth. Horttechnology 19: 212–215

Roby, G., Harbertson, J. F., Adams, D. A., & Matthews, M. A. (2004). Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. *Australian Journal of Grape and Wine Research*, *10*(2), 100-107.