7 Phosphate Buffering Aluminum Oxide Treatment in Peat and Peat-Free Potted Herbs, Amended with Mycorrhiza

7.1 Introduction

Arbuscular mychorrizal fungi (AMF) are able to solubilze Phosphorus-based soil resources through the extra cellular excretion of enzymes (Farahani et al. 2008; Grant et al. 2005; Parniske 2008), these resources, although abundant in form are otherwise inaccessible to plants due to various constraints demonstrated by substrate conditions (Richardson and Simpson 2011; Werner and Ami 2014). This ability shown by AMF and other soil microbes regarding Phosphorus solubilization can provide necessary nutrient exchanges for improved plant growth rates and resilience to biotic and abiotic factors (Poveda 2021).

Phosphorous (P) is a limited resource, predominately found in large quantities in bed rock and mineral resources (Pastore, Kernchen, and Spohn 2020). Phosphorous naturally occurring within soils is typically the result of rock weathering and the decaying organic matter (Cosgrove 1977). It is also applied to soil and various substrates as a fertilizing agent for improved crop yields (Syers, Johnston, and Curtin 2008; Veneklaas et al. 2012). Available P is rapidly adsorbed by both biotic and abiotic elements. Phosphate, for instance is a biologically available form of of P, but is rarely occurring in soil as the mechanics of P-resorption by soil constitutions maintains low levels of P by locking it away in mineral based components such as clay or via biological uptake through microbial factors (Werner and Ami 2014; Richardson and Simpson 2011). As a result typically low P level substrate conditions results in a substantial majority of plant species being reliant on AMF to provide P (Smith and Read 2010).

Within potted plants, this lack of P can have significant impacts on plant health if not replenished through fertilization. Potted plants have only the resources within the growing media. As such, the inability to extend root outside of the ‘locked’ environment of a pot in order to adsorb more nutrient resources makes potted plants significantly more vulnerable to deterioration through rapid nutrient depletion.

However, an abundance of Phosphorus may also negativity effect plant health. Increased P accumulation on agricultural landscapes can have devastating impacts on significant ecological resources such as freshwater resources, native flora and fauna (Sharpley and Withers 1994; Withers et al. 2014). Typical symptoms of excessive P accumulation on vegetation include decreased uptake of vital nutrients in plants such as zinc (Mousavi 2011), a critical component in plant enzyme regulation and proteins (Alloway 2009).

Increased nutrient rates in a medium without the means of regulating nutrient concentrations (such as the mechanism of P-absorption and P-desorption) can therefore have negative effects on plant health. Therefore, replicating phosphate buffering in artificial growing mediums such as Peat-Free and Peat substrates may enhance the ability for plants and mychorriza to co-operate more efficiently. This hypothesis is built on the the motion set by Koide (1991), wherein the idea of an environment abundance in Phosphorus may result in mycorrhizal symbiosis loosing resource efficiency due to “luxury consumption” (Koide 1991). Increased levels of substrate P has been shown to reduce colonisation effective of AMF (Motta et al. 2016).

Growing media or soil type can affect the level of P-sorption, Bloom (1981) demonstrated the ability for Phosphorous adsorption to be dramatically increased when introduced to an aluminium-peat complex, with increasing pH determining higher levels of P-sorbtion. Increased pH in soils promotes the fixation of phosphate ions (Hii et al. 2020), thereby reducing the availability of Phosphate resources for crop products.

This study aims to address the potential for increasing P-sorbtion through the use of an aluminium based buffering amendment. This buffering agent may therefore increase effective utilization of nutrient resources and increase host-plant growth and health when in symbiosis by AMF.

7.2 Compalox®

Compalox is a is a granular aluminium-oxide amendment, that acts as a Phosphate buffer, limiting plant available P. Compalox does not completely absorb Phosphate, but continuously adsorbs and de-absorbs the Phosphate, allowing for a low level output of the nutrient. This can be considered incredibly beneficially for substrates amended with mycorrhizal fungi as increased P levels have been demonstrated to reduce levels of colonisation (Motta et al. 2016).

Compalox is a unique granular form of aluminium oxide $[Al(OH)_3]$. Compalox was developed as an adsorption product for the removal of pollutants from liquid and gaseous products (such as the removal of heavy metals from drinking water, or the removal of fluorides from exhaust air). Compalox P-buffer was developed as a nutrient buffer for commercial plant production. The function of this product is to enable a steady availability of nutrients to croppings. In tandem with AMF, incorporating Compaloxmay enable higher functionality of AMF via deprivation of P resources, thereby increasing activity and ultimately, crop growth (Koide 1991).

7.3 Methods Materials

These series of trials were performed predominately on Basil (*Ocimum basilicum*). A series of treatments involving exponentially higher concentrations of compolox were used (0.5, 1, 2 %).

Design

The trials for this study were arranged in randomised blocks with between 4-6 replicates per treatment (*read* :pot). Pots were labelled with anonymised numbers for blind assessment.

Amendment

The treatment used in this study was Compalox. The Compalox was applied as a percentage of total substrate volume adapted from manufacturers instructions and applied as treatments in increasing increments; 0.5%, 1% and 2%. This was applied via hand and mixed in a bucket for each treatment. A control was established for each substrate with no Mycorrhizal innoculum or Compalox applied.

This was used in conjunction with AMF innoculum, a 6 species mixture of fungi developed by Plantworks. This product was added prior to seeding, following the manufacturer instructions.

Analysis and Assessment

Crop growth was assessed visually using typical phytometric techniques such as measuring crop height, leaf diameter and yield.

7.4 Results

7.4.1 Crop Height

Crop growth (see Figure 54 for early basil growth) as measured through height yielded significant results in regards to the effect of Compalox rates on crops. Peat growing media was relatively unaffected by increasing rates of Compalox. However, Peat-Free growing media demonstrated significant reduction of growth from the effects of increasing rates of Compalox: Control height at 32days growth was > 3cm than that of the highest rate (2%) of Compalox treated crop. A clear inverse reaction to increasing rates of Comaplox may be seen in Figure 55 with detailed results in Table 23. The impact of Mycorrhizal inoculum may also been demonstrated here, with increased growth rates in Peat growing media across each level of Compalox ($p < 0.05$), an average increase of 2.78 cm across AMF treatments compared to the Control values at 32days. The effect of AMF in Peat-Free substrates had no significant effects on overall height.


(a) (b)

Figure 54: Coteyldon growth stage of Basil *in situ*

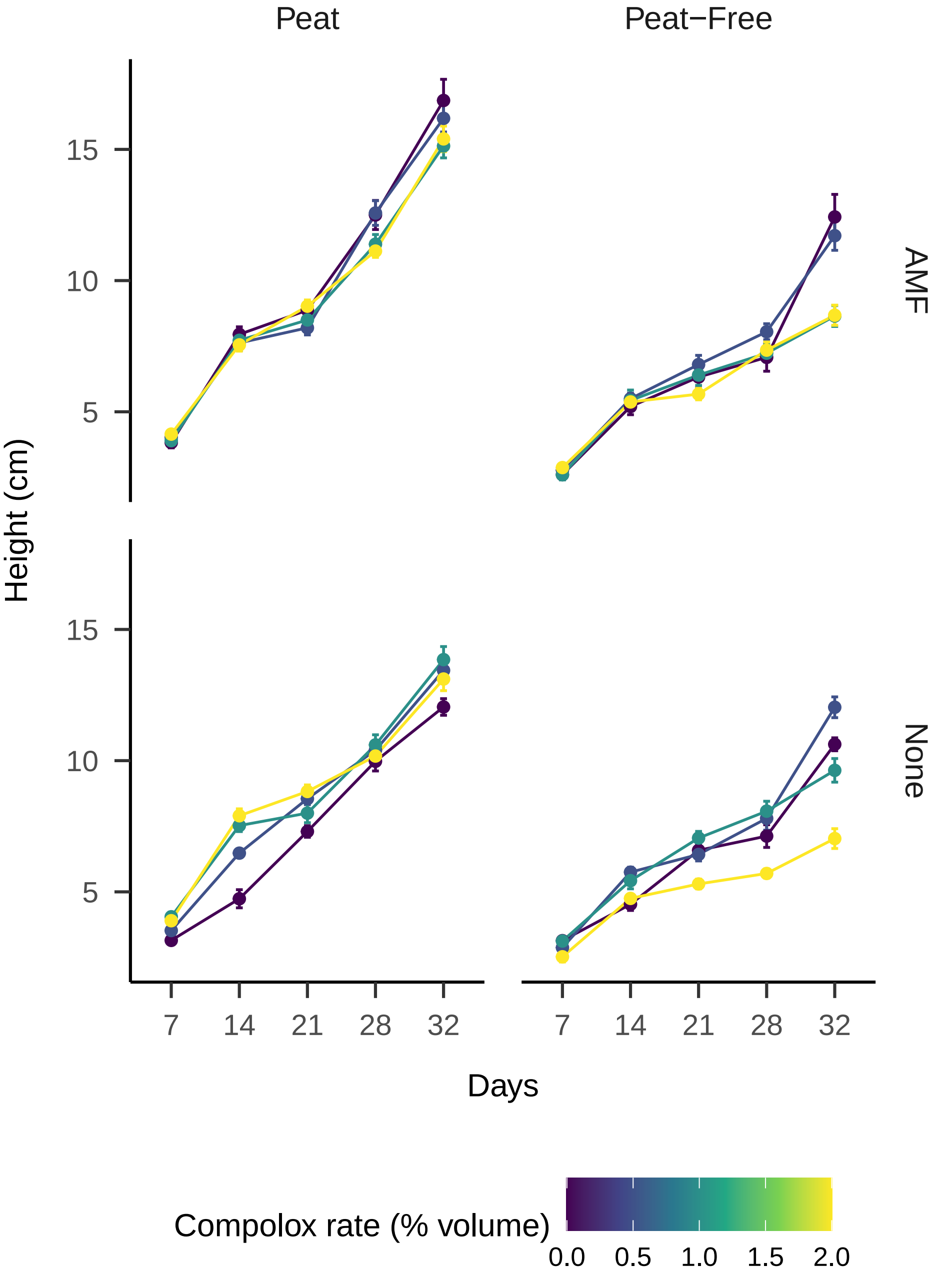

Figure 55: Height over the trial period for each treatment. Reduced growth in height can be observed with increasing Compalox concentrations, most notably in Peat-Free pots. The progressive, upwards trend in growth for each treatment is similar in pattern, however the increased height of Peat treatments suggests Compalox amendments do not effect growth in this substrate.

Table 23: Mean height for different Compalox rates

Substrate	AMF	Day	0	0.5	1	2
Peat	Control	7	3.15 (0.86)	3.52 (0.47)	4.05 (0.79)	3.90 (0.72)
-		14	4.74 (2.56)	6.47 (0.73)	7.53 (1.02)	7.90 (1.18)
-		21	7.30 (1.63)	8.55 (1.01)	8.00 (1.63)	8.82 (1.12)
-		28	9.97 (1.65)	10.40 (1.40)	10.60 (1.72)	10.18 (0.86)
-		32	12.05 (3.31)	13.44 (2.74)	13.85 (3.51)	13.11 (3.13)
Peat	AMF	7	3.83 (0.89)	4.03 (0.60)	3.90 (0.55)	4.15 (0.59)
-		14	7.95 (1.29)	7.62 (1.29)	7.72 (0.94)	7.55 (1.06)
-		21	8.88 (0.86)	8.20 (1.21)	8.50 (1.18)	9.03 (1.06)
-		28	12.50 (2.48)	12.57 (2.10)	11.39 (1.65)	11.12 (1.07)
-		32	16.86 (5.36)	16.18 (3.64)	15.13 (3.21)	15.40 (3.44)
Peat-Free	Control	7	3.14 (0.74)	2.88 (0.58)	3.12 (0.67)	2.52 (0.88)
-		14	4.53 (1.75)	5.75 (0.84)	5.42 (1.39)	4.75 (0.72)
-		21	6.58 (1.52)	6.42 (1.10)	7.05 (1.13)	5.30 (0.83)
-		28	7.12 (1.93)	7.80 (1.74)	8.07 (1.69)	5.70 (0.77)
-		32	10.62 (2.58)	12.03 (2.66)	9.63 (3.18)	7.03 (2.67)
Peat-Free	AMF	7	2.61 (0.81)	2.77 (0.72)	2.60 (0.76)	2.88 (0.60)
-		14	5.20 (1.39)	5.50 (0.95)	5.43 (1.53)	5.38 (1.09)
-		21	6.33 (1.84)	6.80 (1.57)	6.40 (1.53)	5.67 (0.98)
-		28	7.08 (2.36)	8.05 (1.37)	7.23 (1.31)	7.35 (1.39)
-		32	12.43 (5.89)	11.71 (3.74)	8.65 (2.52)	8.68 (2.72)

7.4.2 Crop Yield

Crop Yield was significantly affected by the presence of increased Compalox rates (see Figure 58). This was demonstrated solely in Peat-Free substrate wherein an inverse reaction to increasing rates of Compalox resulted in decreased yield for both Fresh (Figure 57 and Table 25) and Dry (Figure 56 and Table 24) yield. A one way ANOVA demonstrated this difference as significant between substrate yield output. There was no significant difference between control and AMF inoculated treatments. A Linear regression model highlighted the downwards progression of yield against increased Comaplox rates in Peat-Free substrate ($R^2 = 0.45$), see Figure 59. Peat substrate was relatively unaffected by increased Compalox rates with a marginally increased relationship between yield and rates ($R^2 = 0.53$).

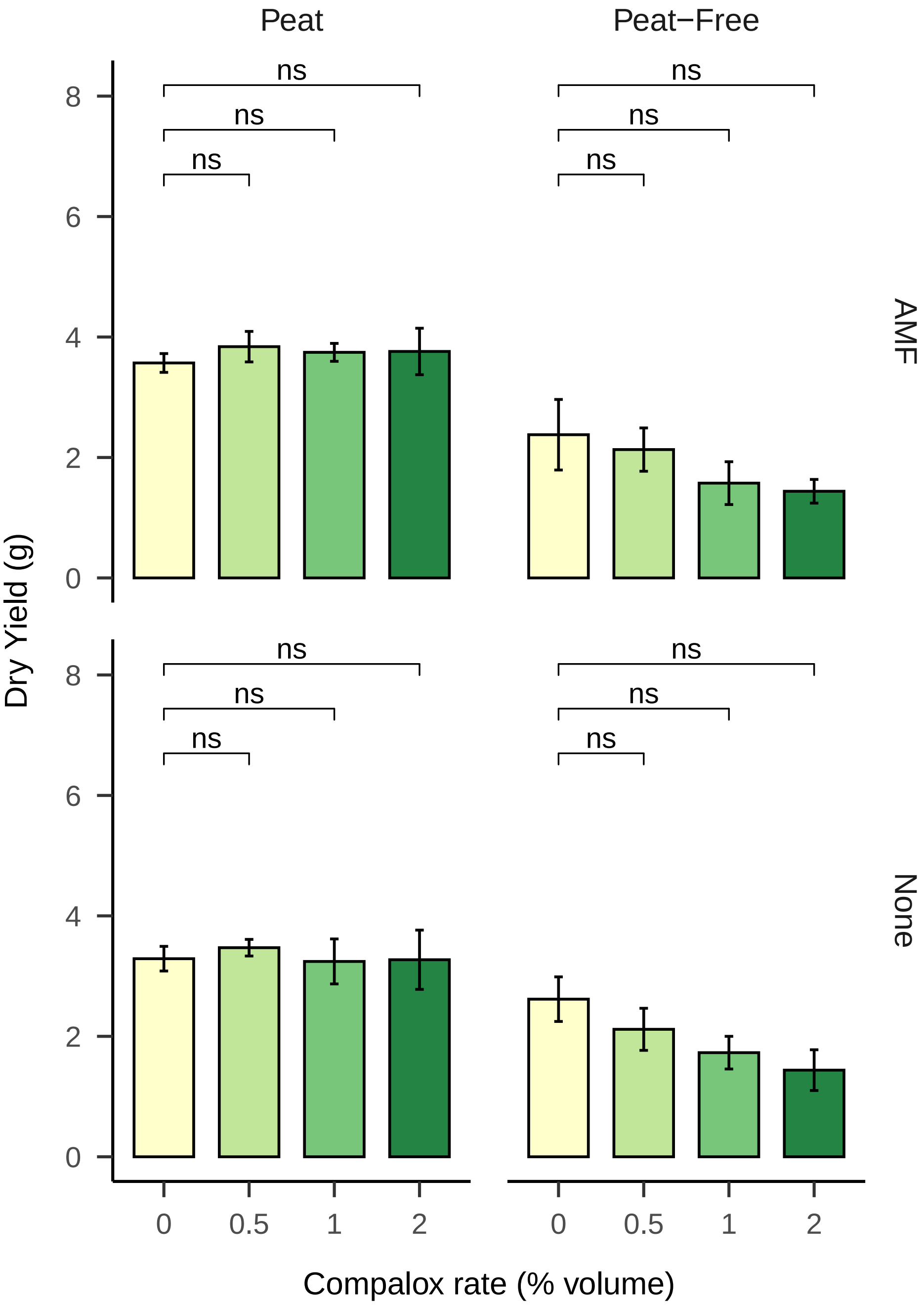

Figure 56: Dry Yield for Basil with means comparison (*ns* = not significant). Increasing concentrations of Compalox in the Peat-Free pots yeilds lower biomass. Peat pots were unaffected.

Table 24: Mean dry yield and standard deviation (*) for each substrate, crop and treatment at assessment intervals

Substrate	AMF	0%	0.5%	1%	2%
Peat	Control	3.15 (0.99)*	2.94 (0.86)	2.97 (0.77)	2.76 (1.12)
Peat	AMF	3.57 (0.49)	3.84 (0.80)	3.80 (0.44)	3.76 (1.16)
Peat-Free	Control	2.35 (1.74)	2.12 (1.05)	1.73 (0.81)	1.44 (1.07)
Peat-Free	AMF	2.38 (1.85)	2.13 (1.14)	1.57 (1.01)	1.44 (0.62)

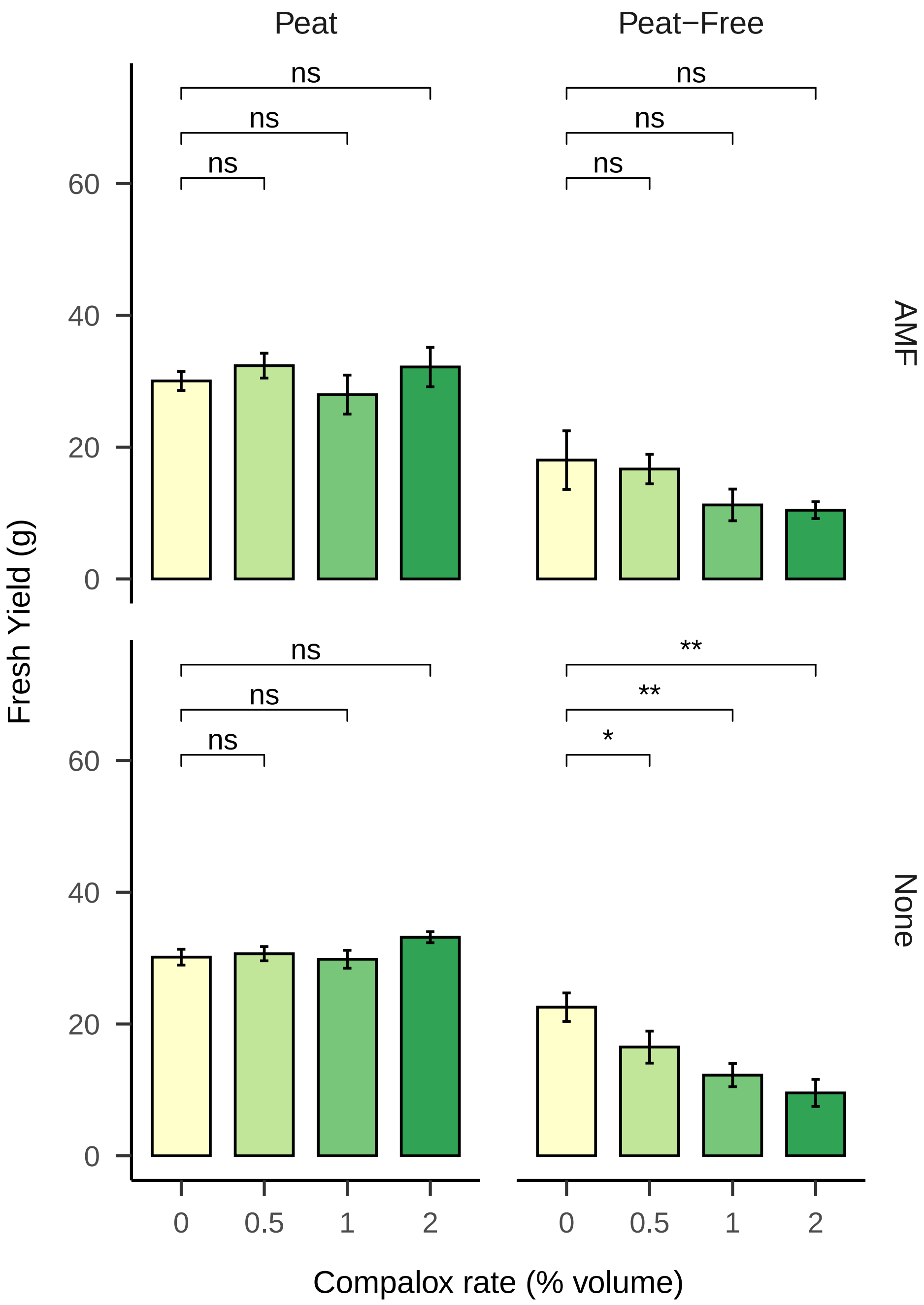

Figure 57: Fresh Yield for Basil with means comparison (*ns* = not significant, *, ** = significant) . Increasing concentrations of Compalox in the Peat-Free pots yeilds lower biomass. Peat treatments were unaffected by increased rates of Compalox.

Table 25: Mean fresh yield and standard deviation (*) for each substrate, crop and treatment at assessment intervals

Substrate	AMF	0%	0.5%	1%	2%
Peat	Control	28.22 (6.45)*	24.55 (9.31)	27.24 (4.40)	27.50 (7.84)
Peat	AMF	30.04 (4.60)	32.37 (5.96)	30.71 (3.77)	32.16 (9.48)
Peat-Free	Control	18.13 (12.68)	16.50 (7.29)	12.24 (5.58)	9.55 (6.50)
Peat-Free	AMF	18.02 (14.09)	16.67 (7.06)	11.22 (6.79)	10.43 (4.04)

(a) (b)

Figure 58: **Growth stage at *ca.* GS32**. Demonstration of differences in growth between substrate type and treatments. Significant discrepancies in crop quality are apparent between treatments.

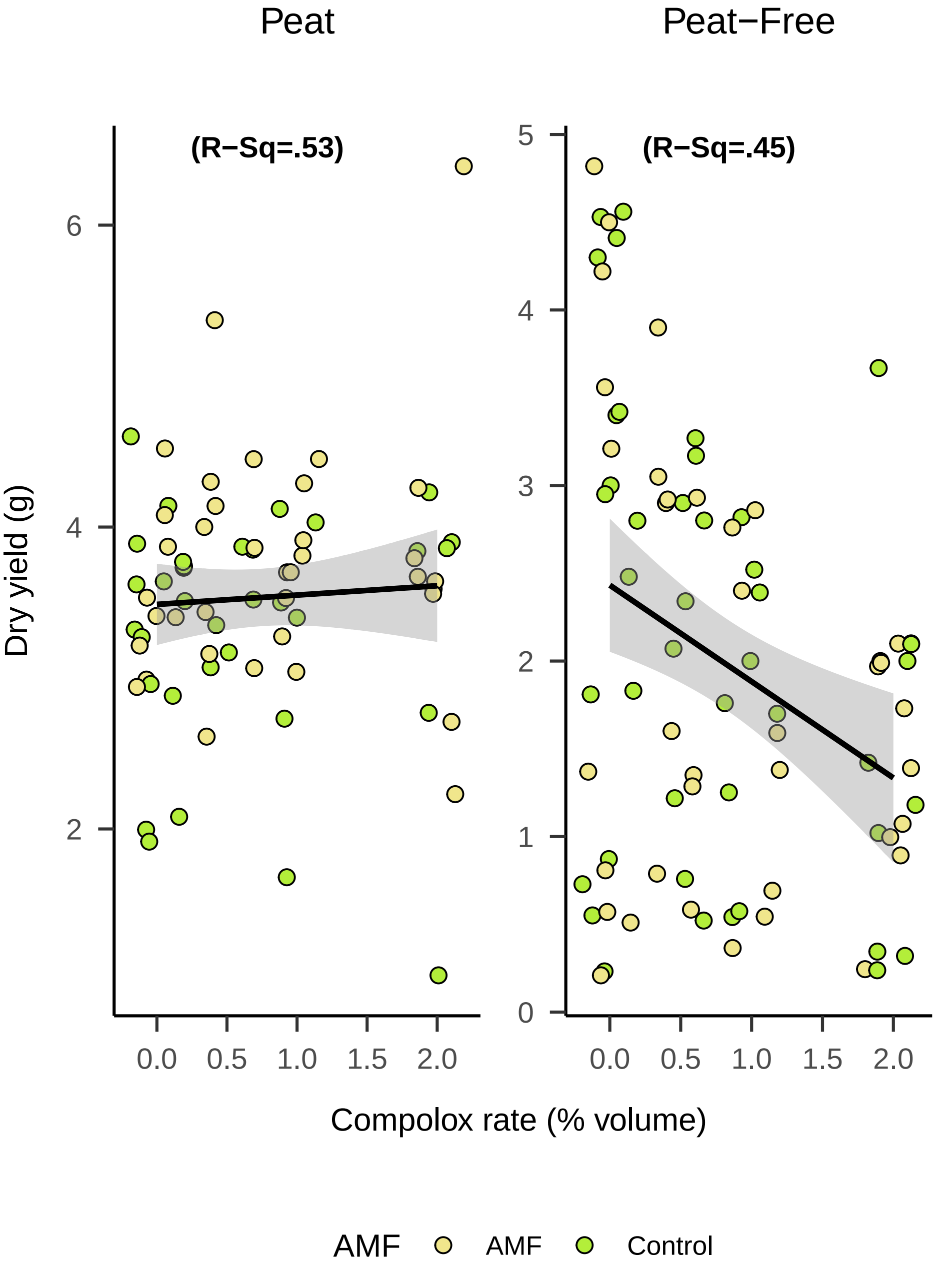

Figure 59: Dry yield distribution across each Substrate as a linear model. A horizontal performance of yield against compalox rates for Peat supports the data of crop height over time, wherein Peat substrate is unaffected by the introduction of Compalox, whereas Peat-Free substrates produce a decrease in crop quality with increasing concentrations of Compalox.

7.4.3 Leaf Diameter

Leaf Diameter (see Figure 60 and Table 26) was measured at *ca.* 32days growth. Peat substrates demonstrated significantly higher widths of leafs ($p < 0.05$) across each biological treatment (AMF) and Compalox rates compared to Peat-Free substrate. The addition of mycorrhizal inoculum increased mean leaf diameter in all Peat substrates under increasing Compalox levels. Peat-Free growing media only saw a significant increase in leaf diameter with mycorrhizal inoculum with a Compalox rate of 0.5% vol (2.3cm vs 1.83cm Control). An inverse reaction to Compalox in Peat-Free substrates can be seen in the Control (no AMF) at a Compalox rate of 1%, where a mean leaf diameter of 2.26cm is significantly higher than that of the Control value (1.85cm).

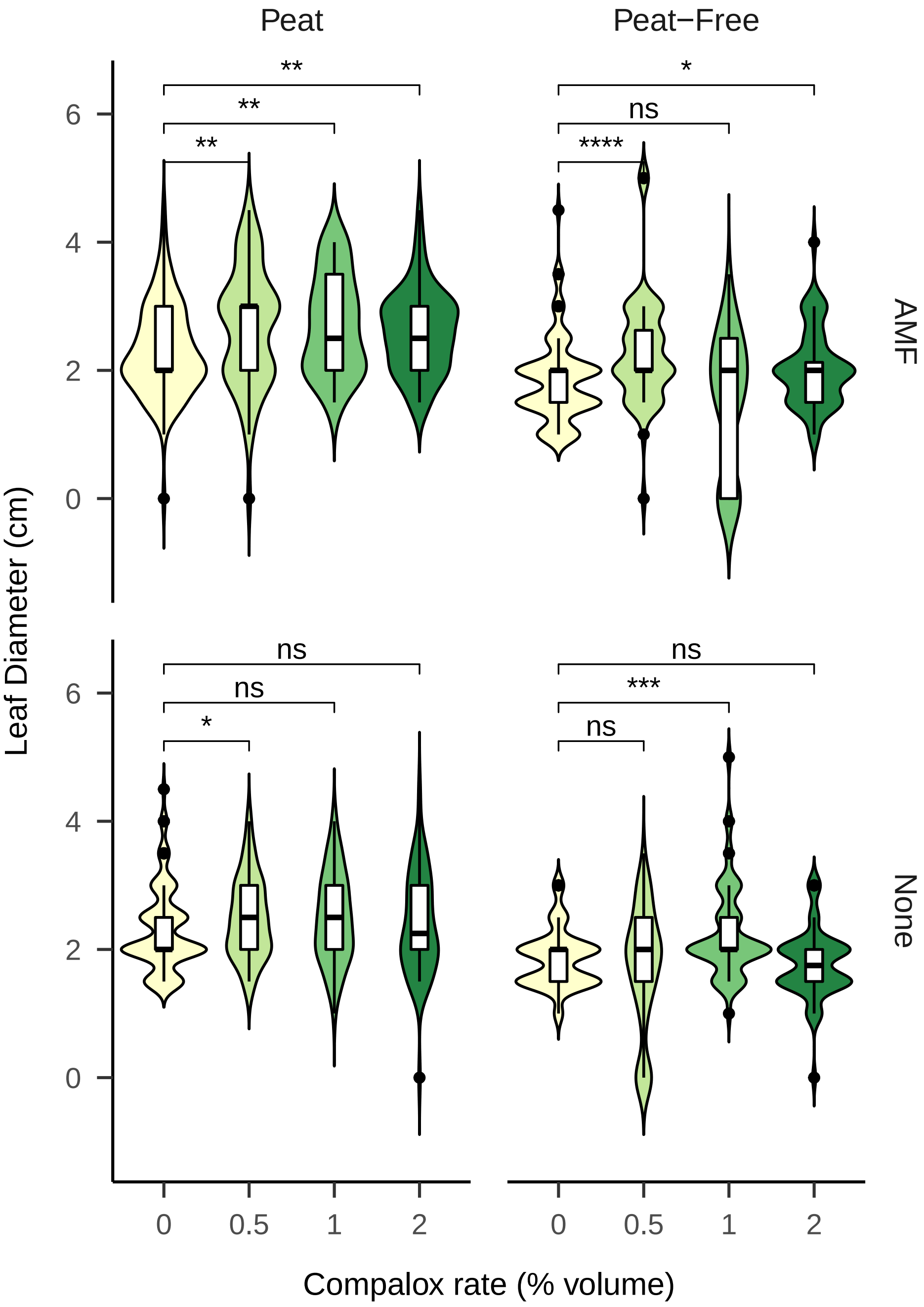

Figure 60: Leaf Diameter of potted Basil in Peat and Peat-Free substrates under various treatements. Means comparison (*ns* = not significant, *, **, *** = significant). Although the distribution of results is clearly different for each treatment, the pattern demonstrated in previous data is not replicated here.

7.5 Discussion

Peat-Free treatments performed poorly when treated with Compalox. Increased concentrations resulted in lower yield, reduced crop height and an ill-defined effect on mean leaf diam-

Table 26: Leaf Diameter Means for Comaplox rates, substrate and croppings.

Substrate	Treatment	0	0.5	1	2
Peat	AMF	2.31 (0.72)	2.67 (0.93)	2.72 (0.77)	2.61 (0.65)
Peat	Control	2.30 (0.66)	2.49 (0.62)	2.48 (0.69)	2.39 (0.79)
Peat-Free	AMF	1.84 (0.66)	2.30 (0.87)	1.52 (1.04)	2.02 (0.59)
Peat-Free	Control	1.85 (0.45)	1.73 (0.95)	2.26 (0.73)	1.77 (0.53)

eter. The addition of AMF on Peat-Free substrates had no significant effect.

However, analysis demonstrated a significant difference for the effect of AMF on crop height for Peat substrates (*p 0.02776* , F crit 5.0116). This can be seen in the mean height data wherein AMF treated pots in Peat substrates had increased average height across all Compalox treatments; 7 days +0.3225cm, 14days +1.05, 21 days +0.485cm, 28days +1.6075cm, 32days +2.78 cm. This increase in mean height did not translate into effecting Biomass (dry or fresh).

Overall, substrate type had significant effects on each measurement (height, biomass, leaf diameter $p < 0.001$), with Peat performing best. Compalox rates were also significant in affecting crop growth (height, dry yield $p < 0.005$) with a slightly less, yet significant effect on freshyield (*p 0.049*). The effect of Compalox had no significant beneficial effect on crop growth parameters for either substrate type.

The hypothesis of this study was to anticipate increased efficacy of AMF innoculum on crop growth in Peat and Peat-Free substrates while under the influence of a P buffering agent (Compalox). The effects of Compalox were detrimental for Peat-Free substrates for AMF treated, and untreated pots. Compalox had no effect on Peat based substrates, although pots treated with AMF performed better.

The potential for Compalox and it's ability to buffer P within substrates may be assumed as effective in Peat-Free substrates as demonstrated by the reduction in crop growth and quality which is directly correlated to increasing Compalox rates, and therefore lower P availability. Peat substrates appeared unaffected by Compalox, and may indicate a degree of increased nutrient regulation despite the presence of the buffering component.

Further research, utilising nutrient analysis of substrates may assist in demonstrating the level of buffering achieved in each substrate by assessing P concentrations across control and treated pots.