1	Impact of management regimes on fruit quality of sweet cherry (Prunus avium
2	L.)
3	
4	Running title: Comparing management regimes in cherry orchards
5	

6 Abstract

7	The impact of alternative farming systems on fruit quality of orchard crops has been rarely
8	examined. This study compared the effect of two management regimes with or without
9	effective microorganisms (EM) on fruit quality of sweet cherry (Prunus avium L. cultivar
10	Sweetheart). After four years, EM had no effect on quality however, fruit from regime 2 based
11	on organic amendments and no herbicide had higher TSS and malic acid concentration but
12	reduced size, compression firmness and stem retention force than fruit from regime 1 which
13	was based on current conventional fertigation practices combined with herbicide application.
14	All quality attributes reached 'export finest' standard for all treatments, providing evidence that
15	organic amendment regimes can supply fruit acceptable for export markets.
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20	KEYWORDS: Effective Microorganism TM ; soil amendment; sustainable; humates; nutrients
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INTRODUCTION

23 In recent years, the commercial focus of the Australian sweet cherry (Prunus avium L.) 24 industry has been on fruit size and quality rather than total yield per tree. Consequently, 25 management practices have aimed to produce superior quality fruit for a premium price to 26 enable access to niche markets in Asia (James et al. 2011). Conventional management 27 systems involve extensive use of chemical fertilisers, herbicides and pesticides. For example, 28 nitrogen (N) application rates in Australia range from 75-200 kg N ha⁻¹, which is consistent 29 with usage reported in United States and Turkey, the two largest global producers of sweet 30 cherry. The standard method of weed control in perennial orchards is to maintain a bare earth 31 strip along the tree row using herbicides (Bound 2014); commonly used herbicides include 32 glyphosate, simazine, paraquat and diquat. According to Radcliffe (2002), glyphosate usage 33 in Australia is approaching 15,000 tonnes per annum. 34 Reliance on synthetic fertilisers and pesticides/herbicides can have serious 35 environmental impacts (Radcliffe 2002; Pimentel et al. 2005; Zhang et al. 2011). As such, 36 interest in alternative farming systems including organic and integrated farming (a 37 combination of organic and conventional techniques) has increased in recent years. However, 38 some uncertainty remains as to whether fruit quality in alternative systems can be maintained. 39 There is some evidence indicating that for apples, organic and integrated systems increase 40 fruit quality characteristics without compromising yield (Reganold et al. 2001). However, 41 very few studies have examined the impact of alternative systems in sweet cherry, which has 42 a maximum storage life of four-six weeks (Padilla-Zakour et al. 2007), and hence is 43 substantially more perishable than apples. 44 Alternative management systems are distinguished from conventional systems by the

45 use of organic based fertilisers such as manures, bio-fertilisers and humic based fertilisers
46 (Rigby and Cáceres 2001; Pettit 2004). Bio-fertilisers are also referred to as microbial

47 amendments or microbial fertilisers, and one that is receiving increased attention is effective micro-organisms (EM) (Privadi et al. 2005; Javaid 2006; Hu and Qi 2013). EM are a mixture 48 49 of beneficial microorganisms, predominantly lactic acid bacteria and yeasts (Higa and 50 Wididana 1991) that are purported to promote the microbial decomposition of complex 51 organic compounds, detoxification of harmful gasses, production of anti- microbial or fungal 52 compounds, and production of plant hormones (Higa and Wididana 1991; Gourlay, 2015). 53 Although EM have been studied in a wide range of crops (Xu 2001; Xu et al. 2001; Javaid 54 2006; Hu and Qi 2013), very few studies have been conducted on perennial fruit crops, 55 particularly sweet cherry. This study compared the impact of two management regimes and soil applied EM on fruit quality in a commercial sweet cherry orchard. The management 56 57 regimes were based on either current conventional practices with fertigation and herbicide 58 application for weed control or organic amendments and mowing for weed control.

59 MATERIALS AND METHODS

60 A field trial was established on a commercial orchard in the Derwent Valley (42.7077° S, 146.9458° E) in southern Tasmania. Mean annual rainfall at the site is 573 mm, and mean 61 pan evaporation is in excess of 949 mm (Australian Bureau of Meteorology). Mean daily 62 63 maximum and minimum temperatures are 23.4 and 9.7 °C in summer and 11.9 and 1.9 °C in 64 winter. The soil is dolerite clay (Isbell 1996). Uniform, mature, regular bearing eight-year-old 65 sweet cherry trees, cultivar 'Sweetheart' were selected in the spring (October) of 2012. Trees 66 were on 'Colt' rootstock and pruned to a Kym Green Bush system (Green 2005) with a 67 planting spacing of 4.0 x 1.8 m and an east-west row orientation. All trees received daily 68 irrigation via micro-sprinklers at a rate of 48 L/h for 3 h (a total of 12.5 mm) per week 69 throughout the growing season (November to February).

70 Trial design

71 Treatments were allocated at random to five-tree plots within each block in a complete 72 randomised block design to give four replicates per treatment. The treatments were a factorial 73 arrangement of two management regimes and soil applied EM (nil [EM-] or recommended 74 label rate [EM+]). Regime 1 was based on current conventional fertigation practices with trees fertigated weekly during the growing season, and receiving a total of 80, 15 and 60 kg ha⁻¹ N, 75 76 P and K, respectively for the season. Weeds were controlled with a herbicide program using 77 glyphosate and BastaTM (200 g/L glufosinate-ammonium, Bayer Crop Science Pty Ltd) every 78 3-4 months. Regime 2 used organic amendments and targeted minerals based on annual soil 79 analysis; weeds were controlled by mowing. The organic amendments were applied in spring 80 and autumn. FerbonTM (lignite-based soil conditioner, Interstate Energy Group, Bacchus Marsh, Australia) was applied at 300 kg ha⁻¹ in the first two seasons, and humified compost 81 (Foundation Aerobic Compost, Pure Living Soils, Tasmania, Australia) at 800 kg ha⁻¹ 82 combined with soluble humate granules (Nutri-Tech Solutions, Australia) at 20 kg ha⁻¹ in the 83 84 following two seasons. The chemical properties of both organic amendments are presented in 85 Table 1. Targeted minerals applied to regime 2 in September 2012 and 2013 were sulphates of ammonia (30 kg ha⁻¹), potassium (20 kg ha⁻¹), manganese (25 kg ha⁻¹), zinc (2 kg ha⁻¹) and 86 copper (2 kg ha⁻¹), and sodium borate (8 kg ha⁻¹). In 2014 and 2015, only manganese sulphate 87 88 was applied at rate of 15 and 25 kg ha⁻¹, respectively. Weeds in the regime 2 were controlled 89 by mowing.

Soil EM amendment (EM1, VRM Pty Ltd) was applied monthly at the recommended
rate (15 L activated EM ha⁻¹) throughout the experimental period, commencing in October
2012. EM amendment was prepared by adding 75 mL of activated EM solution and 5 g of
Acadian soluble seaweed extract (SSE) in 10 L of non-chlorinated water for each plot.

94 Sampling and assessments

Fruit were harvested on 18th January 2016 at normal commercial harvest time. Harvested fruit
was weighed and five samples of 30 unblemished fruit with a skin colour rating of at least 3
(Table 2) were collected from each replicate for fruit quality assessment. Fruit assessments
were conducted at 0, 14, 28, 42 and 56 days post-harvest. Samples stored at 14, 28, 42 and 56
days post-harvest were placed in PeakFresh® bags and stored at 0 °C.

100 At each assessment date, quality assessments included fruit weight, diameter, pedicel 101 diameter, skin colour, compression firmness, flesh firmness, skin puncture force, flesh colour, 102 stem retention force, total soluble solids content (TSS), malic acid (MA) concentration and 103 juice pH.

104 Both fruit and pedicel diameters were measured with digital callipers (DigiMax, Wiha-105 41101, Wiha Switzerland). Skin and flesh colour rating were measured using the Australian 106 Cherry Colour Guide (Cherry Growers Australia). Fruit compression firmness was measured with a FirmTech 2 (Bioworks Inc, USA). Flesh firmness and skin puncture force were both 107 108 measured with a fruit texture analyser (Guss model GS-20, South Africa). Due to equipment 109 failure, data for skin puncture force and flesh firmness at 14 days post-harvest was not obtained. 110 Stem retention force was measured using a stand mounted Mark-10 Series 5 force gauge 111 (Mark-10, USA).

Fruit from each replicate were juiced collectively and duplicate samples taken for measurement of TSS, pH and MA concentration. TSS content, expressed as °Brix, was measured with an Atago PR-1 digital refractometer (Atago Co. Ltd., Japan). Juice pH was the initial pH value of 10 mL juice samples measured using a Mettler Toledo G20 compact titrator (Mettler Toledo, Australia). MA concentration was calculated as g L⁻¹. Australian sweet cherry industry standards which classifies fruits into 'domestic' and

118 'export finest' were used as a reference for fruit quality (Table 2).

119 Statistical Analysis

All data was analysed by three-way analysis of variance (ANOVA). Block was treated as a random factor, while management regime, soil EM application and storage time were considered fixed factors. The assumptions of ANOVA such as homogeneity of variance and the Gaussian distribution were evaluated by the use of quantile–quantile plots and residual plots for all variables. Fisher's protected least significant difference post hoc tests were used to determine significant differences among treatment means. All analyses were performed using IBM SPSS statistics 23. Significance was calculated at p = 0.05.

127 The focus of this study was to examine the effects of management regime and soil128 applied EM and their interactions on fruit quality from harvest up to 56 days post-harvest.

129 **Results**

130 Management regime significantly influenced seven out of the thirteen physical and chemical 131 fruit quality attributes during storage (Table 3). Fruit size, in terms of weight and diameter, were significantly higher in regime 1 than regime 2. Fruit from regime 1 was 0.9 g (5%) 132 heavier than from regime 2, and 1.1 mm (4%) greater in diameter. Fruit compression 133 134 firmness was also 11% higher in the regime 1 compared with regime 2. There was no difference in flesh firmness or skin puncture force. Stem retention force was (10%) greater in 135 136 regime 1 than regime 2. There was no difference in skin colour between regimes, but flesh 137 colour was darker in regime 2 (Table 4). TSS and MA concentration were higher (~5%) in 138 regime 2 than regime 1 (Table 4).

There were no interactions between management regime, EM and storage period for fruit quality attributes, with the exception of stem retention force (Table 3). There was a significant interaction between management regime and storage period. From harvest to 14 days post-harvest, stem retention force was higher in regime 1 compared with regime 2,
however these differences disappeared from 28 days post-harvest in both regimes (Figure 1).
Compared with untreated trees (EM⁻), adding EM to the soil had no effect on fruit
quality attributes (Table 5). Similarly, neither management regime nor storage period
interactions modified this response (Table 3).

147 **Discussion**

Four years after the initial imposition of treatments, over half of the thirteen fruit qualityattributes tested were found to be significantly influenced by management regime.

Specifically, fruit size, stem retention force and compression firmness were higher in regime 1 which followed conventional nutrition and herbicide practices. However, fruit from regime 2 based on organic amendments had higher TSS and MA concentrations. Despite differences between management regimes, all quality attributes measured were above the cherry 'export finest' standards, as described in Table 1. Although we only have 1 year of fruit quality data, it suggests management regimes using organic amendments and no herbicide can produce fruit high quality fruit. However, further research will be required to confirm this.

157 As previously mentioned, the impact of alternative management systems on fruit quality has been rarely studied for sweet cherry. However, the findings in this study are 158 159 similar to those reported for apple (*Malus domestica* L.) species. For example, similar studies 160 on organic apples were reported to have higher TSS than conventional apples. This was 161 achieved five years after converting a 5-year-old orchard from conventional to organic 162 management (Amarante et al. 2008). Similarly, in a 10-year study, Peck et al. (2006) found 163 the organic system produced apples of higher TSS than the conventional system. In this study, the cherries produced in regime 2 using organic amendments had increased MA 164 165 concentration as reported for organic apples (Velimirov 2004). Alternatively, Reganold et al.

166 (2001) noted that while the sugar-acid ratio was highest in organic apples, there was no167 differences in TSS or MA.

Although compression firmness differed between the two management regimes 168 (regime 1, 346 ± 5 g mm⁻² and regime 2, 311 ± 5 g mm⁻²), both were above the industry 169 standard of > 300 g mm⁻². Hence the lower compression firmness in regime 2 did not impact 170 171 'export finest' quality. It is also worth noting that while there were differences in 172 compression firmness between the management regimes, flesh firmness was not affected. 173 Normally these two attributes are closely correlated (Bound, unpublished data). In contrast, 174 fruit firmness has been shown to be higher in organic than conventional apples (Amarante et 175 al. 2008), though this response may vary with season (Reganold et al. 2001; Peck et al. 2006). 176 Currently, there is insufficient understanding of the factors affecting fruit size and firmness of 177 sweet cherry, but Neilsen et al. (2007) and Jönssson et al. (2009) suggest that the lack of 178 mineral nutrition or slow release of nutrients from compost and manure could lead to lower 179 nutrient availability for fruit development, hence producing smaller and less firm fruit. 180 Stem retention is an important attribute in the market, with a minimum force of 500 g 181 being the benchmark in Australia for both domestic and export fruit. In this study, stem 182 retention force was significantly lower in regime 2 at harvest and 14 days post-harvest, but it 183 was still above the industry standard from harvest through to 28 days post-harvest. Although 184 the stem retention force value did drop below the industry threshold in either regime after 42 185 days in storage, the stem retention force observed in this study would be considered 186 acceptable at 28 days post-harvest as the shelf life of sweet cherries typically ranges from 30 187 to 40 days (Padilla-Zakour et al. 2007).

After four years of monthly application of EM, results from this study indicate that EM had no effect on fruit quality of sweet cherry. There is currently a lack of information regarding the impact of EM on sweet cherry fruit quality, but in other studies examining 191 microbial inoculants such as plant growth-promoting rhizobacteria (PGPR), Esitken et al. 192 (2006) reported positive impacts on fruit diameter and titratable acidity of sweet cherry. 193 Karakurt et al. (2011) reported improvement in sour cherry fruit set, plant growth (shoot 194 thickness and leaf area) and fruit quality (TSS, MA, fruit size) with EM application. In 195 addition, Karlidag et al. (2007) reported a positive impact on apple fruit size and foliar nutrition. The lack of consistency in results from different studies with EM may be due to the 196 197 soil types and soil nutrient levels. Microorganisms in the bio-fertiliser product interact 198 differently with soil of different properties (Bossio et al. 1998). Furthermore, the orchard 199 used had received high levels of inorganic inputs for eight years prior to the trial. As such, the 200 effect of EM may have been masked in both management regimes.

201 Conclusion

202 This study suggests that management regimes using organic based amendments and 203 exclusion of herbicide can produce sweet cherry fruit of comparable quality to more 204 conventional regimes based on inorganic fertilisers and herbicide use. However, soil 205 applications of EM had no impact on fruit quality. Further studies are required to examine 206 how soil type effects the efficacy of bio-fertiliser such as EM in perennial orchards. More 207 importantly, quality attributes of all fruit met 'export finest' standards of the Australian sweet 208 cherry industry. Therefore, the management regime based on organic amendments as used in 209 this study should be considered as a feasible management option for producing high quality 210 sweet cherries more sustainably.

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