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The effect of herbicides and permanent swards on soil microbial populations in the vineyard.


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Abstract

This paper reports on an investigation of the effects of herbicide versus site adapted permanent swards on soil microbial populations in two vineyards: one in a warm climate and the other in a cooler climate. The population dynamics and diversity of the soil fungi, bacteria and actinomycete community were monitored for 2.5 years. Preliminary results indicate that herbicide use caused changes to the microbial community in the inter-row soil. Soil from plots that had been repeatedly treated with herbicide contained lower populations of cellulolytic bacteria, *Pseudomonas* spp. and fungi. The decline in the soil pseudomonad population was most severe in the cooler climate vineyard.

Key Words

Soil microbiology, *Pseudomonas* spp., grapevine, cellulolytic

Introduction

Soil health and microbial diversity have become vital issues for the grape growing industry. This study examines the effect on soil microbial biodiversity of vineyard floor management practices similar to those found in many organic vineyards (i.e. low input systems with continuous plant cover) as compared with conventional systems which maintain weed free under-vine and/or inter-rows by herbicide application.

Weed free vineyards have been described as essentially monocultures with high input and output characterised by a lack of stability due to their inherent ecological simplicity (Tedders 1983). Vineyards with few ecological niches for predators are susceptible to pests, pathogens and weed competition. On the other hand, natural ecosystems are characterised by high biological diversity so that mass multiplication of any one organism is impossible due to competition, predators and parasites. This enables disturbances and balance shifts to be self-regulated internally. Continuous plant cover also results in increases in the soil organic matter, leading to improved vineyard soil structure, nutrient storage capacity, water infiltration, water holding capacity and microbial density (Gulick *et al* 1994, Bugg and van Horn 1997, Pinamonti *et al* 1996). Soil health has been described as implying “ecosystem sustainability, diversity, functional connectedness, and resilience in response to a...
disturbance or stress” (van Bruggen and Semenov 1999) with soils with low microbial biodiversity being less sustainable and resilient.

The effect of herbicides on vineyard soil microbial populations is likely to involve not only the direct effect of the herbicide but also the indirect effect of reduction in rhizosphere exudates and organic material due to the lack of vegetation after repeated herbicide applications. Glyphosate, a widely used broad spectrum, non-selective, post-emergence herbicide in Australian viticulture, has been shown to reduce soil bacterial populations in peach orchard and soybean field soils (Araújo et al 2003) and in wheat soils (Mekwatanakarn and Sivasithamparam 1987) and to reduce bacterial, actinomycete and fungal populations in forest soil (Gorlach-Lira et al 1997) and vineyard soil (Encheva and Rankov 1990). On the other hand, some studies report increased populations of soil actinomycetes and fungi with glyphosate (Araújo et al 2003), increased soil microbial biomass (Haney et al, 2002) or no long-term change in microbial populations (Busse et al 2001). Another herbicide widely used in Australian viticulture consists of a mixture of diquat and paraquat. Both glyphosate and paraquat were reported to cause activation in soil urease and invertase soil enzymes but suppression of phosphatase enzymes (Sannino and Gianfreda 2001) whilst diquat and paraquat increased fungal populations, decreased the population of some fungal antagonists to take-all and increased the take-all incidence in wheat soil (Mekwatanakarn and Sivasithamparam 1987). In a cropping field study, paraquat increased populations of bacteria, fungi and actinomycetes at normal application rates but at higher application rates were toxic to fungi (Camper et al, 1973). Few studies have demonstrated long-term impacts on soil microbial populations of herbicide applications. It appears that in viticulture, only Encheva and Rankov (1990) have studied the long-term effect of glyphosate and none have reported on the long-term effect of diquat and paraquat.

This study investigated the effects of herbicide application versus site adapted permanent swards in two vineyards: one in a warm climate and the other in a cooler climate. Soil fungi, cellulolytic bacteria and Pseudomonas spp. populations were monitored for 2.5 years and population differences in winter 2004 are reported here.

**Methods**

**Vineyard field trial**

There were three floor management treatments: a complete spray out with herbicide both in the undervine and inter-row regions (C), slash only with no herbicides applied (S) and under-vine herbicide spray only (U). These approaches represent a broad range of floor management techniques being utilised in Australian viticulture today. The treatments were applied at two field sites located in New South Wales vineyards (*Vitis vinifera* cv. Chardonnay) in regions representing warm climate and cooler climate viticulture.

The warm climate site was located at the Charles Sturt University vineyard at Wagga Wagga. The region has an average annual rainfall of 522 mm with most of the rain received through winter, although rainfall was below average throughout the trial
period (Table 1). Summer temperatures can often reach 40°C. Canopy management at this site was a simple single cordon wire and one foliage wire. A cover crop of cocksfoot and subterranean clover mix was sown in June 2002 but severe rainfall deficiencies during winter and spring resulted in poor establishment, so that the non-herbiced plots contained a diversity of plant species with a mixture of grasses such as Cynodon, Stellaria, Eragrostis, Digtaria and Panicum, and other herbaceous species. Twelve bulked soil cores (10 cm deep, 60mm diameter) were used to describe the soil. The soil was a brown sandy loam and NaHCO₃-extractable P was 36 mg/kg soil in the inter-row (Colwell 1963). Soil pH (1:5, 0.01 M of CaCl₂) was 7.1 and oxidisable organic carbon (dichromate oxidation method) was 0.91%. Vine rows were 3 m apart and each panel contained 3 grapevines spaced 2 m apart. The experimental plots were 3 panels long and 4 mid-rows wide with a buffer row between each plot. Four replicates of the 3 treatments were arranged in a randomised block design.

The cooler climate site was located in Tumbarumba. This region has an annual rainfall of 968 mm although rainfall was below average throughout the trial period (Table 1). Frost is often experienced in the early part of the growing season. Excessive vine vigour can be a problem and canopy management relies on split canopy systems. A subterranean clover pasture dominated the non-herbiced inter-row area (23% to 25% of plant species). The soil was a brown clay-loam and NaHCO₃-extractable P was 20 mg/kg soil in the inter-row (Colwell 1963). Soil pH (1:5, 0.01 M of CaCl₂) was 5.9 and oxidisable organic carbon (dichromate oxidation method) was 1.7%. Vine rows were 3m apart and each panel contained 8 grapevines spaced 1m apart. The experimental plots were 2 panels long and 4 mid-rows wide with a buffer row between each plot.

### Table 1. Rainfall for Wagga Wagga and Tumbarumba, 2002 to 2004.

<table>
<thead>
<tr>
<th></th>
<th>Long term average rainfall (mm)</th>
<th>Rainfall in 2002 (mm)</th>
<th>Rainfall in 2003 (mm)</th>
<th>Rainfall from 1st January to 1st July, 2004. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wagga Wagga</td>
<td>533</td>
<td>373</td>
<td>438</td>
<td>130</td>
</tr>
<tr>
<td>Tumbarumba</td>
<td>968</td>
<td>752</td>
<td>872</td>
<td>271</td>
</tr>
</tbody>
</table>

**Herbicide applications**

At the Wagga Wagga trial site, a diquat and paraquat mixture (2.78 L/ha) was applied to the appropriate treatment plots in April, September, October and November of 2002 and in June, November and December of 2003. A mixture of carfentrazone-ethyl and glyphosate (1.85 L/ha) was applied in March and October of 2003.

At the Tumbarumba trial site, glyphosate (1.85 L/ha) was applied to the appropriate treatment plots in July 2002. A diquat and paraquat mixture (2.78 L/ha) was applied in October and November of 2002; and in January, June, November and December of 2003; and in January of 2004. A mixture of carfentrazone-ethyl and glyphosate herbicide was applied in September and October of 2003.
**Dilution plating**

Six soil cores (10 cm deep, 60 mm diameter) were collected from the inter-row region at a position 50 cm from grapevine trunk and bulked. The bulked soil samples were vortex mixed with phosphate buffered saline (pH 7.2) for 10 s, sonicated at 260W/cm² for 15 s and orbitally shaken at 290 rpm for 30 min on ice. Culturable bacteria and actinomycetes were then isolated by dilution plating (with three plate replicates) on Pseudomonas Agar CCF (Oxoid) (PS) which is selective for pseudomonads, and Cellulose Bacterial Agar (CBA) (Tuitert et al 1998). Fungi were isolated by dilution plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Pitt and Hocking 1985). All plates were placed in plastic bags loosely wrapped so that there was ample air supply and incubated at 25°C in darkness for 1 week before colonies were counted.

**Statistical analysis**

Data were subjected to a 2-way analysis of variance (ANOVA) test using Genstat for Windows, 7th Edition. Least significant differences (l.s.d.) were calculated at $P < 0.05$.

**Results and Discussion**

Table 2. Cellulolytic bacteria, *Pseudomonas* spp. and fungi isolated from non-herbicide and herbicide treated inter-row soil at Wagga Wagga and Tumbarumba field sites, July 2004.

<table>
<thead>
<tr>
<th></th>
<th>Cellulolytic bacteria $(10^5 \text{ cfu} / \text{cm}^3 \text{ dry soil})$</th>
<th><em>Pseudomonas</em> spp. $(10^4 \text{ cfu} / \text{cm}^3 \text{ dry soil})$</th>
<th>Fungi $(10^3 \text{ cfu} / \text{cm}^3 \text{ dry soil})$</th>
<th>% Soil water content in July 2003</th>
<th>% Soil water content in July 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wagga Wagga, nil herbicide²</strong></td>
<td>349*</td>
<td>28*</td>
<td>170*</td>
<td>12.1</td>
<td>10.9</td>
</tr>
<tr>
<td><strong>Wagga Wagga, herbicide³</strong></td>
<td>168</td>
<td>7</td>
<td>117</td>
<td>11.1</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>l.s.d (P&lt;0.05)</strong></td>
<td>171</td>
<td>14</td>
<td>45</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Tumbarumba, nil herbicide²</strong></td>
<td>489*</td>
<td>110*</td>
<td>253*</td>
<td>13.7*</td>
<td>18.6</td>
</tr>
<tr>
<td><strong>Tumbarumba, herbicide³</strong></td>
<td>191</td>
<td>3</td>
<td>154</td>
<td>16.7</td>
<td>18.4</td>
</tr>
<tr>
<td><strong>l.s.d (P&lt;0.05)</strong></td>
<td>228</td>
<td>92</td>
<td>63</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

¹ colony forming units  
² Treatments S and U (slash only and undervine spray-out)  
³ Treatment C (complete spray-out)  
* Treatments without herbicide are significantly different from treatments with herbicide ($P < 0.05$)

In July 2004, the populations of soil cellulolytic bacteria, *Pseudomonas* spp. and fungi were significantly lower ($P < 0.05$) in the inter-rows that had been treated with
herbicide, indicating a significant effect of the herbicide treatment on soil microbial biodiversity. The herbicide treatment decreased the pseudomonad population by 75% in Wagga Wagga and by 97% in Tumbarumba. It also decreased the population of cellulolytic bacteria by 52% in Wagga Wagga and by 61% in Tumbarumba. The fungal population was decreased by 31% in Wagga Wagga and by 39% in Tumbarumba.

The major herbicides used in this trial were glyphosate, diquat, paraquat and carfentrazone-ethyl. These herbicides effectively decreased the vegetation in the inter-row to low levels so it was not possible to determine whether the changes in soil microbial population were due to the direct effect of the herbicides on the soil microbes alone or in combination with the indirect effect of the elimination of inter-row plant growth and therefore reduction in rhizosphere exudates and organic material after repeated herbicide applications. Interestingly, in Tumbarumba the decrease in microbial population occurred despite the fact that the soil moisture level had been significantly higher in the bare herbicided soil in 2002 (data not supplied) and in winter 2003.

The decline in the population of soil cellulolytic bacteria after herbicide treatment was in agreement with Voets et al (1974) who found that cellulolytic bacteria were permanently reduced in an apple orchard kept free from direct vegetative cover by the long term application of atrazine herbicide. Cellulolytic bacteria and fungi have a key role in the decomposition and transformation of organic matter in soil. As many as 90% of soil fungi are capable of utilizing cellulose (Sims 1990).

Dry conditions in 2002 and 2003 caused low soil moisture levels at the trial sites of this study (Table 2). Pseudomonads, being less resilient to dessication than spore-forming bacteria or bacteria capable of forming a non-multiplying resting state (Labeda et al 1976) did not survive well in the herbicide treated inter-row in the absence of plant roots. The Pseudomonas genus contains species that are beneficial (Bavaresco and Fogher 1996) and other species that are pathogenic to grapevines (Waschkies et al 1994). Pseudomonads are generally R-strategists (adapted to grow and multiply in high nutrient environments) so they are usually much more numerous on plant roots rather than in bulk soil (Smit et al 2001). Marilley and Aragno (1999) found that plant roots were selective towards members of the Pseudomonas genus to the detriment of Gram positive bacteria and those belonging to Acidobacterium division. Różycki et al (1999) reported that most of the bacteria isolated from roots of pine and oak trees belonged to the genera Pseudomonas and Bacillus and that the majority of these bacteria had nitrogenase activity (i.e. they were diazotrophs, or nitrogen fixers). Similar studies of grapevine roots have not been made although Bavaresco and Fogher (1996) reported that the inoculation of grapevine roots with fluorescent pseudomonads improved the establishment of arbuscular mycorrhizal fungi. Barka et al (2000) also showed that inoculation of grapevine plantlets with Pseudomonas sp. (strain PsJN) induced a significant growth promotion which made them more hardy and vigorous and induced resistance to Botrytis cinerea.

Loss of microbial biodiversity can affect the functional stability of the soil microbial community.
Griffiths et al (2001) reported that soils of reduced biodiversity due to low plant species diversity were less ‘resistant’ (i.e. able to withstand the immediate effects of) and ‘resilient’ (i.e. able to recover from) to perturbations such as copper sulfate application and heat stress. Soil contains significant populations of microorganisms with the ability to attack or suppress plant pathogenic fungi. Fungistasis has been shown to be strongest in resistant soils with high organic matter content and microbial activity, with soil bacteria such as cellulolytic bacteria and pseudomonads, but also fungi such as *Trichoderma* spp. implicated as major causes of such fungistasis (Sturz et al 1997, Whipps 2001).

Further work will investigate the effect on inter-row microbial populations of the above commonly used viticultural herbicides. The effect of the herbicides on the grapevine rhizosphere and undervine soil microbial populations will also be examined.

**Acknowledgments**

This project is funded by the GWRDC which is a partnership between and jointly funded by the Commonwealth and the Australian winegrape and wine industry. NWGIC staff Rhonda Smith, Andrew Smith and Sylvie Sicard have provided valuable assistance in the field and laboratory. Thanks must also go to Charles Sturt University winemaker Greg Gallagher and Stuart Barclay of Tumbarumba Wine Estates who have provided the experimental sites at Wagga Wagga and Tumbarumba respectively.

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