ENHANCING ECOSYSTEM SERVICES IN VINEYARDS TO IMPROVE THE MANAGEMENT OF *BOTRYTIS CINEREA*

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Organic mulches and cover crops mulched *in situ* were assessed for their effects on *B. cinerea* primary inoculum and disease levels in inflorescences at flowering and/or bunches at harvest.

Organic mulches were used to enhance biological degradation of vine debris to reduce levels of *B. cinerea* primary inoculum the following season. Four mulch types (anaerobically and aerobically fermented marc (grape pressings), inter-row grass clippings and shredded office paper) were applied under ten-year-old Riesling vines in a ten-replicate randomized block design in New Zealand over two consecutive years. Plastic mesh bags, each containing naturally infected vine debris, were placed under vines on bare ground (control) and at the soil-mulch interface, in winter (July) 2003 and 2004. In each year, half the bags were recovered at flowering (December) and the remainder at leaf plucking (February), for assessment of *B. cinerea* sporulation from the vine debris and debris degradation rate. Bait lamina probes, which measure soil biological activity, were placed in the soil-mulch interface three weeks before each of the two bag-recovery dates in both years and were then removed and assessed at the same times as were the bags. All mulches led to a reduction in *B. cinerea* sporulation. This reduction was significantly correlated with elevated rates of vine debris decomposition and increased soil biological activity. Over both years, compared with the controls, all treatments gave a 3-20-fold reduction in *B. cinerea* sporulation, a 1.6-2.6-fold increase in vine debris degradation and in the two *marc* and the paper treatments, a 1.8-4-fold increase in activity of soil organisms.

The mulches also altered vine characteristics and elevated their resistance to *B. cinerea* through changes to the soil environment. Functional soil biological activity, as measured by

Biolog Ecoplates and bait lamina probes, was increased 2-4 times in the two *marc* and paper treatments, compared with the control, an effect relating to the elevated soil moisture and reduced temperature fluctuations under these mulches. Soil nutrient levels and the C:N ratios were also affected in these treatments. The mulched paper lowered vine canopy density by up to 1.4 times that of the other treatments, an effect which probably led to elevated light penetration into the canopy and consequent increased canopy temperature, photosynthesis and lowered canopy humidity. These changes to soil and vine characteristics increased grape skin strength by up to 10% in the paper treatment and sugar concentrations by 1.2-1.4 ºBrix in the two *marc* and paper treatments. The severity of *B. cinerea* infections in the anaerobic *marc*, aerobic *marc* and paper treatments were reduced to 12%, 3% and 2.2% of the control, respectively, in field assessments averaged over two consecutive harvests.

Cover crops mulched *in situ* had similar effects to those of the organic mulches, increasing soil biological activity and reducing *B. cinerea* primary inoculum and the severity of *B. cinerea* infection in grapes at harvest (2006). Inter-row phacelia and ryegrass were mulched in winter 2005 and compared with a bare ground control, under 10-year-old Chardonnay vines in a ten-replicate randomized block design. Functional soil biological activity increased by 1.5-4.5 times in the two cover crop treatments compared with the control, an effect possibly related to elevated soil moisture in these treatments. This increase in soil moisture and soil biological activity increased vine debris degradation, reduced *B. cinerea* primary inoculum on the debris and decreased *B. cinerea* severity at flowering (December 2005) and harvest (April 2006).

These results show the potential of organic mulches and cover crops mulched *in situ* to enhance soil ecosystem services and improve the sustainability of viticultural practices.

Key words: Botrytis cinerea, cover crop, mulch, conservation biological control, soil biological activity, vine debris degradation, primary inoculum, grape vines, vine resistance, yield, grape quality.

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In part adapted from:

Jacometti, M. A., Wratten, S. D. and Wilcox, W. F., unpublished. Conservation biological control of *Botrytis cinerea* in vines: progress and prospects. Biological Control, *awaiting submission.*

1.1 Western agricultural practices

World-wide modern agricultural systems during the last 50-60 years have been characterised by high inputs and high yields. These systems follow 'substitution agriculture', where soil fertility maintenance and weed, pest and disease control was often achieved by the addition of agrichemicals, especially the use of herbicides, insecticides and fungicides that caused severe environmental harm, including a decrease in biodiversity. This loss in biodiversity has caused reductions in ecosystem-service contributions, making these agricultural systems increasingly dependent on the chemical inputs they received and raising public concern about the unsustainability of current practices. Ecosystem services provide more services to humanity than only those associated with food production and quality. They include climate, soil and environment regulation, nutrient cycling, genetic diversity/resources and recreation (Table 1.1). Costanza *et al.* (1997) estimated the total value of these services to humanity to be worth US 33×10^{12} p.a. world-wide in 1997.

The world population is expected to grow from the current 6.3 billion, of which over 10% are undernourished (UN, 2005), to 9 billion in 50 years time (Pimentel and Wilson, 2004). To accommodate this growth, sustainable practices need to be adopted whereby biological systems and the ecosystem services they provide are maintained or enhanced in agriculture (Tilman *et al.*, 2002). The Millennium Ecosystem Assessment Synthesis Report (2005), which represents the views of 1300 experts from 95 countries concluded that "any progress achieved in addressing the goals of poverty and hunger eradication, improved health, and environmental protection is unlikely to be sustained if most of the ecosystem services on which humanity relies continue to be degraded".

Table 1.1. Ecosystem services provided by nature (source: Costanza *et al.*, 1997). Arrows depict categories targeted in vineyards by the research programme outlined in this thesis.

Table 1 Ecosystem services and functions used in this study

Number	Ecosystem service*	Ecosystem functions	Examples
1	Gas regulation	Regulation of atmospheric chemical composition.	CO_2/O_2 balance, O_3 for UVB protection, and SO_x levels.
2	Climate regulation	Regulation of global temperature, precipitation, and other biologically mediated climatic processes at global or local levels.	Greenhouse gas regulation, DMS production affecting cloud formation.
3	Disturbance regulation	Capacitance, damping and integrity of ecosystem response to environmental fluctuations.	Storm protection, flood control, drought recovery and other aspects of habitat response to environmental variability mainly controlled by vegetation structure.
4	Water regulation	Regulation of hydrological flows.	Provisioning of water for agricultural (such as irrigation) or industrial (such as milling) processes or transportation.
5	Water supply	Storage and retention of water.	Provisioning of water by watersheds, reservoirs and aquifers.
6	Erosion control and sediment retention	Retention of soil within an ecosystem.	Prevention of loss of soil by wind, runoff, or other removal processes, storage of silt in lakes and wetlands.
7	Soil formation	Soil formation processes.	Weathering of rock and the accumulation of organic material.
8	Nutrient cycling	Storage, internal cycling, processing and acquisition of nutrients.	Nitrogen fixation, N, P and other elemental or nutrient cycles.
9	Waste treatment	Recovery of mobile nutrients and removal or breakdown of excess or xenic nutrients and compounds.	Waste treatment, pollution control, detoxification.
10	Pollination	Movement of floral gametes.	Provisioning of pollinators for the reproduction of plant populations.
11	Biological control	Trophic-dynamic regulations of populations.	Keystone predator control of prey species, reduction of herbivory by top predators.
12	Refugia	Habitat for resident and transient populations.	Nurseries, habitat for migratory species, regional habitats for locally harvested species, or overwintering grounds.
13	Food production	That portion of gross primary production extractable as food.	Production of fish, game, crops, nuts, fruits by hunting, gathering, subsistence farming or fishing.
14	Raw materials	That portion of gross primary production extractable as raw materials.	The production of lumber, fuel or fodder.
15	Genetic resources	Sources of unique biological materials and products.	Medicine, products for materials science, genes for resistance to plant pathogens and crop pests, ornamental species (pets and horticultural varieties of plants).
16	Recreation	Providing opportunities for recreational activities.	Eco-tourism, sport fishing, and other outdoor recreational activities.
17	Cultural	Providing opportunities for non-commercial uses.	Aesthetic, artistic, educational, spiritual, and/or scientific values of ecosystems.

* We include ecosystem 'goods' along with ecosystem services.

In 2004, a New Zealand-wide report was published by the Parliamentary Commissioner for the Environment (2004), called "Growing for good: Intensive farming, sustainability and New Zealand's environment". This publication reported on how New Zealand's increasingly intensive methods in agriculture, horticulture and viticulture were polluting the environment and damaging ecosystem services. They also reported that key export markets in Europe and Asia were increasingly rejecting products sourced from farms that operated in such a unsustainable way (Parliamentary Commissioner for the Environment, 2004).

Pressure for sustainable practices, both through market pressure (Manson, 2000) and regulatory bodies, such as the European Union (EU) is particularly evident in viticulture. In 2003, the EU limited the use of copper-based fungicides in organic (BIO-GRO registered) systems to 8 kg/hectare/year (Spera *et al.*, 2003), but in 2006 this ceiling is to be halved to 4 kg/ha/yr (www.europa.eu.int/). In addition to this pressure from international markets and regulatory bodies, some fungicides are becoming increasing ineffective as a result of fungicide resistance (Pearson & Goheen, 1988) which is reducing reliance on chemical control (Manson, 2000) and leading to initiatives to find alternative methods of control (Crosse, 1998; Gaskin *et al.*, 2002), such as use of plant-derived essential oils, plant defence stimulants, introduced biological control agents and conservation biological control techniques.

1.2   Viticulture in New Zealand

Viticulture has had an increasingly important role in the New Zealand economy. Wine exports increased seven-fold from 97.6 to 246.5 million dollars per annum from 1998 to 2003 and there was an increase from 7,580 to 15,800 ha in grapes over the same time period (nzwine.com). During the last 10-15 years, there has been increasing emphasis on use of sustainable viticulture and wine-making methods to justify the marketing slogan "New Zealand wine, the riches of a clean green land".

New Zealand vineyards can be infected with a variety of temperate-climate fungal diseases, many of which are facilitated by each other or other vineyard pests. Downy mildew (caused by *Plasmopara viticola* Berliner & de Toni.), powdery mildew (caused by *Uncinula necator* (Schw.) Burr.) and botrytis rot (caused by *Botrytis cinerea* Persoon ex Fries) are the most common diseases, each of which can cause total crop loss in the absence of control (Nicholas *et al.*, 1994). To a lesser extent, phomopsis, (*Phomopsis viticola* Sacc.) and black spot (*Elsinoe ampelina* de Bary) can also be important (Nicholas *et al.*, 1994). The lightbrown apple moth (*Epiphyas postvittana* Walker) and other tortricids are particularly important

insect pests, as well as various mite species including grapeleaf blister and bud mite (*Colomerus vitis* Pagenstecher) and rust mite (*Calepitrimerus vitis* Nalepa). These species not only cause damage on vines and berries, but can also facilitate secondary fungal infections (Nicholas *et al.*, 1994). The two predominant bird pests are the introduced, species silvereyes (*Zosterops lateralis* Latham) and starlings (*Sturnus vulgaris* L.), both of which can cause crop loss through berry damage/removal and subsequent secondary infections (Nicholas *et al.*, 1994).

1.3 *Botrytis cinerea*

Botrytis rot is one of the most important diseases of grapes in cool temperate climates, such as New Zealand. The susceptible grape varieties grown and long period of time that berries take to ripen mean they are harvested in autumn when frequent rain can enhance botrytis rot. Control of the pathogen is becoming increasingly difficult due to fungicide resistance, latent infection and the limited number of fungicides used due to wine residues and public perception (Howell, 2001).

1.3.1 Taxonomy

The genus *Botrytis* is the conidial state of *Botryotinia* (Appendix 1) of which there are 22 species. The species of this genus are found globally in subtropical, temperate and cool climates (Hennebert, 1973) and contain pathogens of agricultural, horticultural and floricultural crops, many of which are economically important (Hennebert, 1973; Maude, 1980). Unlike the rest of the genus *B. cinerea* has many hosts, with over 230 hosts world wide (Jarvis, 1977), of which more than 100 occur in New Zealand (Pennycook, 1989). In many hosts, the pathogen may infect flowers, leaves, buds, shoots, stems and/or fruits, often limiting plant development, fruit-set, yield and fruit quality in fruit crops (Maude, 1980; Nicholas *et al.*, 1994), and yield and crop quality in vegetables (Maude, 1980; Alfonso *et al.*, 2000). It may also attack seedlings, reducing establishment and so plant density in a new crop (Burgess *et al.*, 1997). In fruits such as grape, pear, apple, strawberry, raspberry, blackcurrant and eggplant, infection at flowering can cause latent infections which become active, causing rot as the fruits ripen (Jarvis, 1977).

B. cinerea is the anamorph of *Botryotinia fuckeliana* (de Bary) Whetzel (Pearson & Goheen, 1988), but this teleomorph stage is rarely observed in the field (Faretra *et al.*, 1988). The high genetic and morphological variation, found among *B. cinerea* populations is believed to be caused by frequent mutations, aneuploidy (Buttner *et al.*, 1994; Baraldi *et al.*, 2002) and heterokaryosis (Backhouse *et al.*, 1984). However molecular studies have also shown that

sexual reproduction is a likely source of the genetic diversity in this fungus. Genetic variability within the species in Champagne, France, has now led to the species being divided into two sympatric siblings, *B. vacuma* and *B. transposa*, based on their differences in a range of attributes, including pathogenicity and resistance to fungicides (Albertini *et al.*, 2002).

1.3.2 Morphology

B. cinerea is primarily identified through its macroconidia and conidiophore structures (Figure 1.1). Macroconidia, usually called conidia, are produced in clusters from enlarged apical cells at the end of branched, slender, conidiophores (1-3 mm long) (Pearson & Goheen, 1988), which originate from enlarged basal cells (Jarvis, 1977, 1980). They are smooth, single-celled, faintly ash-coloured structures, quite large (8-14 × 6-9 μm) and oval in shape (Willetts, 1997). The mycelium of *B. cinerea* is olive brown in colour with cylindrical, septate hyphae, 11-23 μm in diameter (Pearson & Goheen, 1988). In unfavourable environmental conditions, *B. cinerea* can produce sclerotia (2-4 x 1-3 mm) which are extremely resistant, hard, grey-black structures, which securely adhere to the plant surfaces (Pearson & Goheen, 1988) and can survive years of burial in the soil (Fokkema, 1993). With the return of favourable conditions, sclerotia usually produce conidiophores, but if fertilized by microconidia (phialospores), single-celled spores, 2-3 μm in diameter, that form in chains on old aerial mycelium (Pearson & Goheen, 1988), they may also germinate to form apothecia. Apothecia are cupulate, stalked, brownish structures, with a 4-5 mm-long stipe. Ascospores are smooth, oval and single-celled measuring 7 × 5.5 μm (Pearson & Goheen, 1988).

Figure 1.1. Conidia of *B. cinerea* (source (Jarvis, 1980))

1.3.3 Disease cycle of *B. cinerea* on grapevines (*Vitis vinifera* L.)

B. cinerea over-winters as mycelium or sclerotia on dead canes, or as mycelium in cane inner bark, dormant buds, mummified berries, rachides, tendrils, or other plant debris on the vineyard floor, as well as on necrotic tissue of other plant species (Pearson & Goheen, 1988; Mullins *et al.*, 1992; Nicholas *et al.*, 1994). In spring, under appropriate environmental conditions, large numbers of conidia are produced on sclerotia and infested debris and are dispersed by wind and water (Pearson & Goheen, 1988). If environmental conditions are favourable (15-20°C, 95% RH) the conidia germinate (Pearson & Goheen, 1988) produce appressoria (Tenberge, 2004) and secrete enzymes which facilitate germ tube penetration (Sirjusingh *et al.*, 1996) and infection, causing necrosis of the plant tissue (Pearson & Goheen, 1988). Conidia can be released either singly or in clusters (Coertze *et al.*, 2001). The quantity of enzymes secreted is much higher from a cluster than from a single spore, so likelihood of an infection also increases (Elad *et al.*, 2004). This was demonstrated by Coertze & Holz (1999) who observed that single conidia were unable to infect ripe table grapes (cv. Dauphine), yet clusters are able to create infection on ripe berries (Nair & Allen, 1993; Broome *et al.*, 1995). Similarly, when primary inoculum levels are high, singular spores and clusters can accumulate on plant surfaces and increase the chance of infection (Coertze *et al.*, 2001). For this reason, secondary cycles, which can produce high numbers of spores, usually cause more disease than do primary ones (Nicholas *et al.*, 1994) (Figure 1.2). Although *B. cinerea* conidia can travel long distances, the majority of them are deposited within a few meters from the source. In vineyards, 95% of spores travelled less than a meter from the ground source (Seyb, 2004). Conidia can also be dispersed on body parts or in faeces of various insect species (Fermaud & Menn-le, 1992; Fermaud & Gaunt, 1995; Louis *et al.*, 1996) and these insects can also facilitate infection through feeding damage (Mullins *et al.*, 1992). Conidia in faeces occur in clusters of 10-50 spores, of which 60% were found to germinate in 56% relative humidity (RH) (Holz *et al.*, 2004).

Figure 1.2. The disease cycle of botrytis rot on grapevines. Boxes indicate where mulches could disrupt the disease cycle (adapted from (Nicholas *et al.*, 1994)).
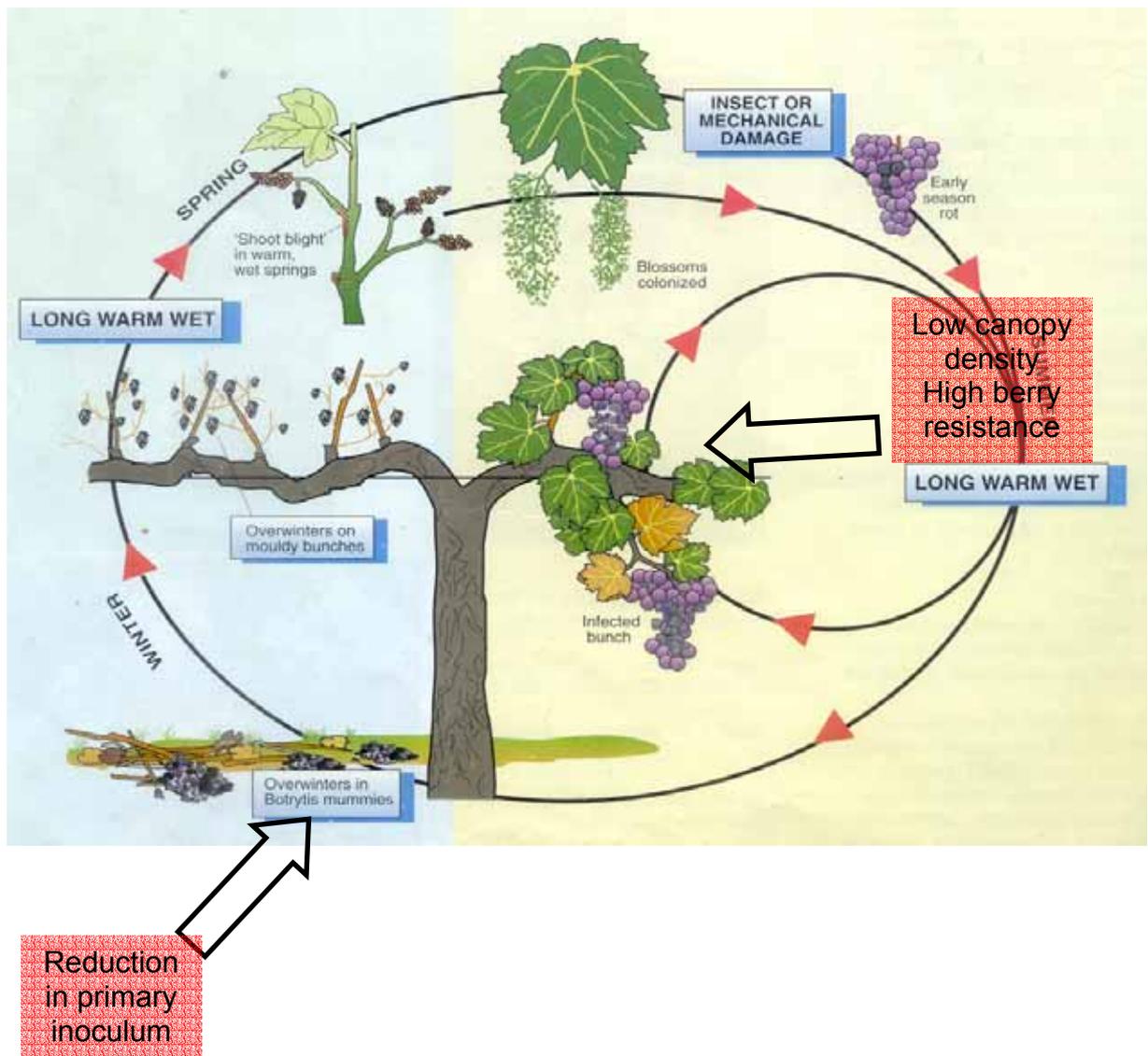
1.3.4   Symptoms of *B. cinerea* in grapes.

When environmental conditions are favourable, *B. cinerea* can become active in early spring, causing 'shoot blight', a soft brown rot in buds and young shoots (Figure 1.3.). The infected shoots tend to break at their nodes to expose internal brown discolouration (Nicholas *et al.*, 1994). Shoots are often girdled by the rot, resulting in their wilting, dying and breaking off (Mullins *et al.*, 1992; Nicholas *et al.*, 1994). Leaves may also develop large, wedge-shaped, necrotic patches, often appearing from leaf edges (Pearson & Goheen, 1988), or as irregular areas of dead tissue where hail damage or other physical leaf damage has provided access points for the pathogen (Nicholas *et al.*, 1994). At this time, the pathogen can infect inflorescences, causing them to rot, dry out and fall off (Mullins *et al.*, 1992). The infected flowers may either host latent infections, which cause bunch rot later in the season (McClellan & Hewitt, 1973; Nair & Parker, 1985) or continue to develop and spread to the pedicels or rachides, first appearing as brown patches, which later turn black (Pearson & Goheen, 1988).

Figure 1.3. Botrytis blight on grape vine shoots (source Dion Mundy, HortResearch, Blenheim, NZ)

As summer progresses, girdling of the pedicels or rachides can occur, causing withering of the bunches below the infected areas (Pearson & Goheen, 1988). Immature, green bunches can be infected by soft brown rot in early summer, which is known as 'mid-season rot' (Nicholas *et al.*, 1994); however, infection over this period usually occurs only if the berries have prior damage, as immature berries are resistant to *B. cinerea* infection. This is because of high skin strength (Nicholas *et al.*, 1994), low concentrations of soluble solids such as sugars (Huang *et al.*, 2001) and high concentrations of inhibitory compounds such as phytoalexins (Bavaresco

et al., 1997; Schouten *et al.*, 2002), tannins, organic acids and phenolic compounds (Pearson & Goheen, 1988; Coertze *et al.*, 2001). After *veraison*, berry skins soften, sugar levels increase and levels of many inhibitory compounds decrease, making them more susceptible to infection.

Characteristic symptoms of botrytis rot on ripe berries include small, circular water-soaked spots that appear brown on white grape varieties and slightly clear on red grape varieties. At this stage of infection, rubbing causes the skin to slip over the inner pulp, a condition known as 'slip skin' (Pearson & Goheen, 1988). Berries then soften and the pulp turns brown. After periods of mild weather and high humidity, berries develop grey fluffy spores, initially in cracks in the skin (Figure 1.4.), then over the entire infected area (Nicholas *et al.*, 1994). Infection can move from berry to berry either via spore dispersal or mycelium growth. If humidity is low, the infected berries dry to raisins, which usually remain attached to the vine (Nicholas *et al.*, 1994). In addition to this damage, the botrytis rot lesions can act as entry points for secondary fungi, yeasts, bacteria and vinegar flies (*Drosophila melanogaster* L.) (Nicholas *et al.*, 1994).

Figure 1.4. Botrytis bunch rot in grape berries

1.4 Control methods for botrytis rot in grapes

1.4.1 Botrytis bunch rot – current control practices

Currently, *B. cinerea* is primarily managed with synthetic fungicides (Rosslenbroich & Stuebler, 2000) and a variety of cultural techniques, predominantly that reduce canopy humidity. These include canopy defoliation (English *et al.*, 1993), trellis design (Savage & Sall, 1984), managing vine vigour through rootstock selection (Pearson & Goheen, 1988), growth of cover crops, and by limiting irrigation and fertilisers, especially with respect to

nitrogen (Mullins *et al.*, 1992). Vineyard sanitation is also practiced (Nair *et al.*, 1995), where vine debris is removed and either buried, burnt or composted. Leaf litter is generally left on the ground, a portion of which, blows away and rachides are either removed with the prunings, when mechanically harvested, or removed by hand at harvest, when hand picked. Mummies (old bunches) are generally left on the vine and then removed with the prunings, as are tendrils. In regards to vineyard sanitation, organic vineyards tend to compost prunings then return them to the vineyard, where conventional operations tend to bury their debris. In general, organic and conventional viticulture practice similar cultural techniques. Conventional operations also use agrichemicals to manage *B. cinerea*, many of which still apply them prophylactically. More progressive growers now use forcasting techniques/software to minimise spray applications, tend to use 'softer' fungicides and apply sprays from many fungicide groups to minimise resistance. These fungicide groups affect various aspects of pathogen development and can be categorised into two main groups according to their method of protection, contact and systemic. The contact fungicides are often broad-spectrum agrichemicals that act predominately on plant surfaces, often affecting respiration of the pathogen, and require uniform application for efficacy. They include the chemical groups, chlorophenyls (Chlorocarb®, Greenguard®), cyclic imides (Captan®, Merpan®), dicarboximides (Iprodione®, Defence® 500), hydroxyanilides (Teldor®), sulphamides (Euparen® Multi) and triazines (Botrysan®). The systemic fungicides are often narrow-spectrum compounds that are absorbed through plant surfaces, so complete spray coverage is not required and their efficacy is not compromised by much later rainfall events. These fungicides act at the site of pathogen entry or effect specific metabolic processes. They can be applied at lower rates than most broad-spectrum fungicides. Chemical groups in this category include the anilopyrimidines (Pyrus® 400SC, Scala®), benzimidazoles (Carbendazim®, Topsin® M-4 A) dicarboximides (Ronilan®, Rovral®, Sumisclex®) and phenylcarbamates (Sumico®) (Young, 2005). Fungicides are currently applied during the high-risk periods associated with particular vine growth stages and when weather conditions are conducive for infection and sporulation (e.g. more than 48 h of >95% RH and 15-25ºC). Under high disease pressure fungicides are commonly applied when shoots are 10-15 cm long twice over flowering, pre-bunch closure, and then as required over *veraison* according to the weather conditions (Nicholas *et al.*, 1994).

1.5 Alternatives to fungicide use

1.5.1 Plant-based oils

Plant-based oils can be effective in managing *B. cinerea* but high concentrations can be toxic to the plant and applications may not be appropriate after *veraison*, when they could significantly alter the flavour of the wine. However, these oils may have a place in an integrated disease management program when wine flavour would be unlikely to be affected, such as at flowering and pre-bunch closure.

Research has shown that *B. cinerea* disease of other crops can be managed by plant-based oils. Tomato fruit dipped in oils of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) had reduced *B. cinerea* disease severity, and pathogen growth was reduced in agar amended with the oils. Lemon grass (*Cymbopogon* sp.,) oil also inhibited *B. cinerea* growth when added to agar or when applied as a vapour, as did oils of oregano, thyme and coriander (*Coriandrum sativum* L.) (Plotto *et al.*, 2003). Thyme and massoi (*Cryptocarya massoia* R.Br.) oils have been effective at controlling *B. cinerea* on necrotic grape leaf discs in the laboratory and on grape berries at *veraison*, with no phytotoxic effects; however, multiple applications from flowering to harvest caused abortion of inflorescences (Walter *et al.*, 2001). *B. cinerea* has also been suppressed in agar amended with oils from other plant species, including dictamnus (*Origanum dictamnus* L.) (Daferera *et al.*, 2003), marjoram (*Origanum majorana* L.) (Daferera *et al.*, 2003), oregano (*Origanum compactum* Benth.) (Bouchra *et al.*, 2003), *Thymus glandulosus* (Req.) (Bouchra *et al.*, 2003) and pennyroyal (*Mentha pulegium* L.) (Bouchra *et al.*, 2003).

1.5.2 Plant defence stimulants

Compounds that directly or indirectly stimulate plant defence mechanisms include plant growth regulators extracted from plant material or microbial communities and inhibitory metabolites extracted from composts or microorganisms (Elmer & Reglinski, 2006). Plant growth regulators such as salicylic acid (SA), ß-aminobutyric acid (BABA) and jasmonic acid can induce a direct physiological vine defence response to disease (Elmer & Reglinski, 2006), whereas chemical components of the extracts from composts and microorganisms, either induce a direct response through elicitor-active chemicals (compounds naturally released at the plant/pathogen interface during initiation of infection), or through simulation of microbial attack (Elmer & Reglinski, 2006). The microbial activity of the extracts can also be important,

as shown by a reduction in this plant response by pasteurisation of the extracts (Elad & Shtienberg, 1994) or its nullification by extract sterilization (Hoitink *et al.*, 1997).

There are limitations to the use of these compounds as all plant defence stimulants change vine physiology, an affect which can have negative effects on vine development, including early berry development, reduced berry weight, lower concentrations of berry phenolics and reduced wine quality (Elmer & Reglinski, 2006), as well as leading to other negative qualities such as detectable residues in wines (Elmer & Reglinski, 2006). These compounds are therefore not suitable at all stages of vine development and appropriate application rates/concentrations, and their timings, need to be carefully considered.

1.5.3 Biological control

There are three main types of biological control; classical, inundative and conservation. Due to the widespread nature of *B. cinerea* (Pearson & Goheen, 1988), its rapid growth rate and the low rates of parasitism observed (Card, 2006), biological agents have not been extensively researched for classical biological control, but inundative and conservation biological control show great potential to control *B. cinerea* in grapes.

1.5.3.1 <u>Inundative biological control – "Biofungicides"</u>

This method uses living organisms released *en masse* to control the pathogens, where the agent does not persist in high numbers in the crop environment. These biocontrol organisms are being researched extensively and are being adopted as commercial formulations become available, a review of which has been recently been published by Elmer & Reglinski (2006). Bio-fungicides suppress the pathogen through a variety of mechanisms, including competition for space and nutrients, parasitism or inhibitory metabolites (Elmer & Reglinski, 2006). These agents include such fungi as *Trichoderma* spp., *Ulocladium* spp., *Alternaria* spp., *Cladosporium* spp and a variety of bacterial, yeast and mycovirus agents. All bio-fungicides act as preventative treatments and are generally not effective post-infection (Whipps & Lumsden, 2001). Therefore to be effective in grape vines, for example, they need to be applied before periods of high vine vulnerability, such as flowering, leaf plucking/trimming and after *veraison*, in climatic conditions conducive to vine colonisation by the biocontrol agent (Whipps & Lumsden, 2001). For these reasons, biocontrol agents have low persistence and are less consistent in their efficacy than are synthetic fungicides. In grape bunches, penetration by any sprayed product is greatly reduced after bunch closure, and a biocontrol agent is unlikely to persist on the inner surfaces of the bunch until harvest, when fruit become

most susceptible to infection. This lack of consistency and persistence of bio-fungicides has been a major hurdle in the adoption of this technology, but it may be effectively used in conjunction with other techniques.

1.5.3.2 Conservation biological control

Conservation biological control has been defined by Eilenberg *et al.* (2001) as 'the modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms, to reduce the effect of pests'. In soil-borne plant pathogens, conservation biological control is a management-oriented development of the 'disease-suppressive soils' concept, in which soils manipulated with amendments and crop rotations increase the activity and/or diversity of existing soil biota. Crops grown on such soils can become more resistant to plant pathogens (Lumsden *et al.*, 1983; Hornby, 1990; Whipps, 1997; Alabouvette, 1999) including root pathogens which are primarily controlled through competition and parasitism, but also shoot pathogens which are controlled through induced resistance (Termorshuizen *et al.*, 2004). Peters *et al.* (2003), reported the effects of two-year crop rotations of spring barley and potato (*Solanum tuberosum* L.) (cv. 'Russet Burbank') compared to a three-year rotation of barley (*Hordeum vulgare* L.) (undersown with red clover (*Trifolium pratense* L.)), red clover and potato. Potatoes harvests from the three year rotation had significantly less ($P<0.05$) canker and black scurf caused by *Rhizoctonia solani* Kuhn., in potato stem, stolon, and tuber tissues, dry rot caused by (*Fusarium* spp.) and silver scurf caused by (*Helminthosporium solani* Dur.). Tubers also showed significantly less pink rot caused by (*Phytophthora erythroseptica* Pethybr.) after inoculation with the pathogen. Similarly, *Thielaviopsis basicola* (Berk. and Broome), the causal agent of black root rot in many economically important crops, can be reduced by 90-100% by mulches of oat straw (*Avena sativa* L.), corn stover (*Zea mays* L.) or lucerne hay (*Medicago sativa* L.), in tobacco (*Nicotiana tabacum* L.), sesame (*Sesamum indicum* L.) and beans (*Phaseolus vulgaris* L.) grown in greenhouse conditions (Papavizas, 1968).

Mulch and other such amendments have the potential to enhance ecosystem services in vineyards and to wider society, and these potential services have been indicated in Table 1.1. To date, some vineyard research projects have focussed on the effects of the mulches on a number of soil and plant parameters, such as soil biological activity (Mundy & Agnew, 2002), soil nutrient status and structure, improved plant nutrient status, yield and fruit quality (Biala *et al.*, 2000). However, the effects of mulches on disease levels within vineyard systems have been reported on only a few occasions. For example, Mundy & Agnew (2002) applied four

organic mulches comprising a combination of vineyard waste, and other waste-stream products (Table 1.2) under vines in four vineyards in Marlborough, New Zealand. Overall, one mulch treatment led to a significant ($P<0.05$) increase in the number of fungal colony-forming units found from soil below the mulch in six of the 12 assessments made in three years. *B. cinerea* incidence was reduced ($P<0.05$) in 10 of the 48 assessments made in all mulch treatments and vineyards over three years, with Mulch 2 being most effective (Table 1.2). In boysenberries (*Rubus loganobaccus*. Bailey), in laboratory conditions, a bark and sewerage sludge compost increased ($P<0.05$) degradation of boysenberry leaf debris by 1.4 times and reduced ($P<0.01$) *B. cinerea* sporulation from 0.25% leaf coverage in the control to no sporulation under mulch (Walter *et al.*, 2004).

Table 1.2. Percentage (by volume) of waste-stream components in each mulch treatment applied to vineyard soils (sourced from Mundy & Agnew (2002)).

Mulch	Vineyard prunings	Grape *marc*	Green waste	Pine bark	Animal manure	Mussel shells
Mulch 1	36%	18%	46%	0	0	0
Mulch 2	27%	13%	34%	26%	0	0
Mulch 3	25%	12%	32%	25%	6%	0
Mulch 4	23%	12%	30%	23%	6%	6%

In vineyards, a change from current disease management practices which are dependent on synthetic fungicides is inevitable. Fungicide resistance, market and regulatory pressure in regards to residues, environmental and human-health effects of fungicides are increasing. This will change the face of viticulture, causing adoption of new control techniques which may replace or be integrated with current practices to effectively and safely control fungal pathogens, of which *B. cinerea* is one of the most damaging.

1.6   Aims and objectives of this thesis

The aim of the present work was to investigate use of mulches, that were comprised of vineyard wastes and other waste-stream products or cover crops mulched *in situ*, to enhance ecosystem services (Costanza *et al.*, 1997), disturb the *B. cinerea* lifecycle (Figure 1.2.) and improve vine resistance to *B. cinerea*.

Key objectives of this work were to evaluate the:

1. effects of mulches on soil physical, chemical and biological properties.

2. effect of the mulches on degradation of overwintering vine debris and on its ability to generate inoculum of *B. cinerea*, the following season.

3. the impact of the mulches on berry resistance to *B. cinerea*.

4. the impact of the mulches on yield and various aspects of berry quality.

5. the effects of the mulches on the severity of botrytis rot in flowers and bunches at harvest.

The thesis is structured as follows:

CHAPTER 1: Introduction. This outlines the New Zealand viticultural industry, the disease cycle of *B. cinerea* and its effect on wine production as well as current and prospective disease management techniques.

CHAPTER 2: The effects of organic mulches on soil biological activity, debris degradation and *Botrytis cinerea* primary inoculum. This assesses the effects of organic mulch on soil biological activity and their impact on vine debris degradation and how these factors affect *B. cinerea* primary inoculum.

CHAPTER 3: The impacts of organic mulches on resistance to *Botrytis cinerea,* grape quality and yield. This examines the effects of organic mulches on soil physical, chemical and biological properties, vine and berry attributes including canopy density, berry sugar accumulation and yield and *B. cinerea* disease severity in bunches at harvest.

CHAPTER 4: The effects of mulched cover crops on *Botrytis cinerea* primary inoculum and severity of infection in inflorescences and grapes. This evaluates the effects of cover crops mulched *in situ* on various soil physical and biological attributes, the effects of these changed

properties on degradation of vine debris and *B. cinerea* primary inoculum, as well as the impact of the mulches on *B. cinerea* severity in inflorescences at flowering and grapes at harvest.

<u>CHAPTER 5: Concluding discussion.</u> This discusses the potential use of mulch, for management of botrytis rot, as well as the prospects for its integration into current and new disease and pest management techniques. The probable factors influencing industry adoption and future development of these protocols are also discussed.

CHAPTER 2: The effects of organic mulches on soil biological activity, debris degradation and *Botrytis cinerea* primary inoculum.

2.1   Abstract

Botrytis bunch rot, caused by *Botrytis cinerea* (Pers.: Fr) is an important disease of grapevines that causes world-wide crop losses and reductions in wine quality. The pathogen predominantly overwinters on vine debris on the vineyard floor. In the current work, organic mulches were used to enhance biological degradation of vine debris to reduce levels of *B. cinerea* primary inoculum the following season. Four mulch types (anaerobically and aerobically fermented marc (grape pressings), inter-row grass clippings and shredded office paper) were applied under ten-year-old Riesling vines in a ten-replicate randomized block design in New Zealand over two consecutive years. Plastic mesh bags, each containing naturally infected vine debris, were placed under vines on bare ground (control) and at the soil-mulch interface, in winter (July) 2003 and 2004. In each year, half the bags were recovered at flowering (December) and the remainder at leaf plucking (February), for assessment of *B. cinerea* sporulation from the vine debris and debris degradation rate. Bait lamina probes, which measure soil biological activity, were placed in the soil-mulch interface three weeks before each of the two bag-recovery dates in both years and were then removed and assessed at the same times as were the bags. All mulches led to a reduction in *B. cinerea* sporulation. This reduction was significantly correlated with elevated rates of vine debris decomposition and increased soil biological activity. Over both years, compared with the controls, all treatments gave a 3-20-fold reduction in *B. cinerea* sporulation, a 1.6-2.6-fold increase in vine debris degradation and in the two *marc* and the paper treatments, a 1.8-4-fold increase in activity of soil organisms. These results show the potential of enhanced soil organism activity in the soil for disrupting the *B. cinerea* life cycle. The implications of these results for infection levels of grapes are currently being investigated.

2.2 Introduction

Botrytis cinerea, the causal agent of botrytis bunch rot, or grey mould, is an important disease world-wide, affecting a wide range of economically important crops (Mullins *et al.*, 1992). In grapes, *B. cinerea* infection leads to a reduction in wine quality by causing off-flavours, increased sensitivity to oxidation and other biochemical changes (Pearson & Goheen, 1988), and can cause total crop loss under cool, moist conditions (Nicholas *et al.*, 1994). Traditionally, this disease has been managed through a combination of cultural and chemical practices (Pearson & Goheen, 1988) but grape growers are now increasingly seeking more sustainable, environmentally-aware disease control (Howell, 2001).

In grapevines, *B. cinerea* typically overwinters on vine debris, as mycelia and sclerotia (Pearson & Goheen, 1988). This debris can subsequently contribute up to 70% of disease levels on the vine at flowering and 28% at harvest (Nair *et al.*, 1995). The aim of this chapter was to use organic mulches (grape marc, inter-row grass clippings and shredded office paper) to enhance ecosystem services (Costanza *et al.*, 1997; Daily, 1997) associated with the decomposition of organic matter on the vineyard floor, to reduce levels of primary inoculum of *B. cinerea*. The hypothesized mechanism was expected to be biological control of the disease through enhanced microbial degradation of vine debris. Although mulches have been used in vineyards and other horticultural systems, the research to date has mainly focused on the effects of the mulches on various soil and plant parameters, such as soil biological activity (Mundy & Agnew, 2002), soil nutrient status and structure, improved plant nutrient status, yield and fruit quality (Biala *et al.*, 2000), as well as reduced soil erosion (Sauvage, 1995). The effects of mulch on disease within these horticultural systems have been reported on only a few occasions. Organic mulches comprising a combination of vineyard waste, and other waste stream products had an inconsistent effect on the severity of *B. cinerea* in four vineyards in Blenheim, New Zealand (Mundy & Agnew, 2002). In laboratory conditions, a bark and sewerage sludge compost enhanced degradation of boysenberry debris and reduced levels of *B. cinerea* sporulation (Walter *et al.*, 2004). The aim of the present work was to use conservation biological control through mulch applications, to enhance degradation of vine debris, via elevated soil biological activity, to reduce *B. cinerea* primary inoculum.

2.3   Materials and methods

2.3.1   The field site

Field work was conducted over two consecutive years starting in the New Zealand summer of 2003/04 at Seresin Estate Ltd, a vineyard situated on clay, overlaying free-draining river gravels, near Blenheim, New Zealand (41.31°S, 173.48°E; 62 m above sea level). The winter temperatures typically range from –5 to 16°C (5°C daytime average) with 5 to 30°C (20°C daytime average) over summer. Sunshine hours are approximately 2500/year and rainfall is usually between 650 and 700 mm/year. The vineyard used certified organic practices, based on the standard of BIO-GRO NZ (www.bio-gro.co.nz/), a section of IFOAM (the International Federation of Organic Agricultural Movements). *B. cinerea,* and powdery mildew caused by *Uncinula necator* (Schw.) Burr. have occasionally caused crop losses in the vineyard and have been managed through the use of fish oil (6-10 l/ha) and sulphur (3-6 l/ha) sprays. The current experiment was conducted under 10-year-old Riesling vines (Clone TK05209) grafted to variety 3309, a phylloxera-resistant rootstock, which was drip irrigated. Prior to the trial, the vines were pruned to two canes, and subsequent leaf plucking, leaf trimming and other practices followed normal procedures in the area. The inter-row spaces are sown with a ryegrass, clover mix (15:85% ratio) which is mown 3-4 times per year.

2.3.2   Experimental design

Five understorey treatments were used in a completely randomized block design. The treatments were: aerobically and anaerobically fermented grape marc, inter-row grass clippings and shredded office paper, all in a 0.4 m wide strip directly under the vines, as well as a bare ground control. The nutrient analyses of these materials are shown in Table 2.1. There were 10 blocks, each 12.8 m long, within two adjacent rows. Plots within rows were separated by one bay of vines (3.4 m). Single replicates of each of the five treatments (2 m by 0.4 m) were randomly distributed in each block. Both the marc and the grass materials were sourced on-site, and the shredded office paper was sourced from a local company. Mulches were applied by hand on 24 July 2003 and 25 June 2004 at an initial thickness of approximately 100 mm. The corresponding bulk densities are presented in Table 2.1.

In year one, grapevine debris was enclosed in 250 × 350 mm bags, constructed from 15 mm-aperture plastic mesh. After the first year it was observed that smaller fragments of vine debris could fall through the mesh, so the following year bags with 7 mm-aperture mesh were used.

In year one, each bag contained weighed sub-samples (three items each) of cane (25 cm long), whole rachis, tendril (5-8 cm long) and whole leaf, which were collected 1-2 days earlier from

Table 2.1 Bulk density and nutrient analysis after synthetic precipitation leaching procedure (SPLP) (US Environmental Protection Agency, 1996) of mulch treatments at time of application (July 2003)

	Mulch treatments			
Analysis	Anaerobic marc	Aerobic marc	Shredded office paper	Mulched inter-row grass
Bulk density - fresh (g/L)	664	676	58.4	63.3
Bulk density – dry (g/L)	315	364.9	55.1	21.4
pH	7.9	8.2	7.2	7.8
Electrical conductivity (mS/cm)	4.4	3.4	0.24	2.2
Total ammonia-N (mg/L)	2	<1	0.02	101
Dissolved phosphorus (mg/L)	10	9	0.39	16.2
Sulphate (mg/L)	481	370	5.2	92
Dissolved potassium (mg/L)	1420	1110	1.22	337
Dissolved calcium (mg/L)	40	29	9.1	24.6
Dissolved magnesium (mg/L)	13	7	0.38	13.9
Dissolved sodium (mg/L)	43	41	27.7	11.0

the vineyard floor, within the trial area. The same types of vine debris were placed in the vine debris bags in year two, except that leaf material was omitted. In addition, a second bag was made up containing fragmented vine debris of approximately 40 mm, a size that resembled debris that had been mulched. The second bag was placed under each mulch beside the first bag. At the time when the vine debris bags were buried under the freshly applied mulch, 20 bags of vine debris were assessed for levels of *B. cinerea* (see Assessment and analysis,

below) and dry weights recorded. Each year, half of the 100 bags were collected when the vines reached 50% flowering (9 December 2003 and 12 December 2004) and the remainder at leaf plucking (3 February 2004 and 14 February 2005). In year two, there were too few rachides on the vines to fill the vine debris bags for both the 50% flowering and the leaf plucking assessments. Based on the results from year one, which showed higher levels of *B. cinerea* at 50% flowering than leaf plucking, rachides were put in the vine debris bags for assessment at 50% flowering only.

On both recovery dates in each year, soil biological activity was measured under each mulch using bait lamina probes (Figure 2.1) (based on the design used by Torne, (1990)) that had been inserted in between drip irrigators, 140 mm into the mulch 3 weeks earlier, one into each treatment replicate. The probes were strips of rigid plastic, 6 × 160 mm, each having sixteen 2-mm holes drilled into the lower half of the strip. The holes had been filled with bait comprising cellulose, agar, bentonite and bran, constituents which were intended to mimic dead plant material in the soil. Soil organisms utilize this substrate such that the number of holes that are intact, or partially or completely removed, gives a measure of the activity of these organisms in the soil (Toerne, 1990; Kratz, 1998). On Friday 3 December 2004, the plots were accidentally weeded with an underground weeder which disturbed the soil profile under all blocks.

Figure 2.1 Bait lamina probes

2.3.3 Assessment and analysis

2.3.3.1 Bait lamina probes

The number of baits that were intact, partially or completely removed was recorded by eye on the two assessment dates (see above).

2.3.3.2 Vine-debris bags

Vine debris was removed from the bags and placed on polystyrene trays ($353 \times 280 \times 20$ mm) lined with three sheets of moist paper towel. The trays were then enclosed individually in new polythene bags (300×400 mm), and incubated on a bench top under ambient conditions. Under these conditions, conidiophores of *B. cinerea* are readily produced from infected grape debris (Seyb, 2004).

2.3.3.3 *B. cinerea* sporulation

After 4-10 days incubation, depending on the peak in primary sporulation on each litter type (rachides, 4-6 days; canes, tendrils and leaves, 8-10 days), the percentage area covered by *B. cinerea* conidiophores was calculated from the mean of all three plant parts (sub-samples) from each debris type. Each plant part was assessed once by eye on all debris surfaces for conidiophore coverage, under 10-40 $\times$ magnification using a stereo microscope, and then categorized into the following coverage classes: 0, 1, 5, 10, 25, 50, 75 and 100%. [1]

2.3.3.4 Degradation of vine material

After sporulation had been assessed on all items of vine debris, the latter were dried at 70ºC for 20 days and biomass reduction was calculated, using the dry/fresh-weight ratio for each type of vine debris.

2.3.3.5 Potential of mulch to harbour *B. cinerea*

In winter 2005, all four mulches and soil from the bare ground control were collected from 0.5 m^2 areas within four randomly selected blocks. A 50 g sub-sample was taken from each of these and were compared with a necrotic grape leaf (positive control) for their ability to harbour *B. cinerea*. Each of the five treatments and the positive control were placed in a Petri dish and inoculated with a mycelium plug of *B. cinerea*, 30 mm in diameter and also through dry spore inoculation. All material in Petri dishes were then sprayed with water from a hand mister, placed on a tray, lined with moist tissue paper, enclosed in a plastic bag and incubated at room temperature.

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[1] In a pilot study, spores were washed off vine debris with a 0.2% triton®-X100 solution then diluted 1, 5, 10 and 100-fold. Spore numbers were then attempted to be estimated using a haemocytometer, however, due to the high concentrations of dirt and contaminants washed off with the sample, this method proved unreliable and the method above was developed.

2.3.3.5.1 Mycelium plug

At 4, 6, 8 and 10 days the vegetative growth radiating out into the mulch from the edge of the mycelium plug was measured twice, the largest and the smallest distance. The mean value was taken and then analysed with repeated measures ANOVA.

2.3.3.5.2 Dry spore inoculation

The material in Petri dishes was checked at 4, 6, 8 and 10 days for spore production, assessed by eye into the following categories (0, 5, 10, 25, 50, 75 and 100% coverage), and analysed with repeated measures ANOVA.

2.3.3.6   <u>Statistical analysis</u>

All data were analyzed using analysis of variance (ANOVA) with Fisher's Protected least significant differences (LSD), to assess differences between treatments. Debris types were analyzed separately as there was high variation amongst debris types. Conidiophore-coverage data did not conform to a normal distribution so data were log-transformed before analysis to restore normality, then back-transformed for Table 2.5 and Table 2.6. Correlations were tested using Spearman's correlation coefficient. The statistical package used for all analyses was Genstat 7.

2.4   Results

2.4.1   Bait lamina probes

Overall, in both years, averaged over both assessment dates, activity of soil organisms was elevated (*P*<0.001) by the two marc and the paper treatments, by 1.6 to 9 times that of the control (Table 2.2). Grass had a lesser effect increasing (*P*<0.05) soil biological activity by 1.3 times at leaf plucking in year one only In the second year, soil biological activity was 1.5 times lower at leaf plucking compared with 50% flowering. An interaction between treatments and assessment dates (*P*=0.011) was observed in year two, where control, grass and paper had significantly lower levels of soil biological activity at leaf plucking compared with 50% flowering, when the two *marc* treatments did not. Soil biological activity was higher in year one than year two, yet the comparable effects of the mulch treatments were similar.

Table 2.2. Mean percentage bait removed by soil organisms from bait lamina probes in two consecutive years.

Year	Assessment date	Mulch treatments					Mean
		Control	Grass	Anaerobic Marc	Aerobic marc	Paper	
1	50% flower	39[a]	45[a]	82[b]	96[b]	80[b]	68[x]
1	Leaf pluck	48[a]	63[b]	86[c]	83[c]	75[bc]	71[x]
1	Both date	43[a]	54[a]	84[b]	90[b]	77[b]	*70*
2	50% flower	21[a]	32[a]	50[b]	36[b]	70[b]	42[x]
2	Leaf pluck	6[a]	7[a]	41[bc]	56[c]	34[b]	29[y]
2	Both date	13[a]	20[a]	46[b]	46[b]	52[b]	*35*

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). [xy] Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$). Italics denote data for which where statistical comparisons were not possible.

2.4.2 Vine debris degradation

Overall in both years, averaged over both assessment dates and debris types, all mulch treatments caused a 2.1-2.7-fold increase in debris degradation compared with the control (Table 2.3 and Table 2.4). Overall, weight loss of vine debris was increased ($P<0.01$) by the two *marc* treatments by 1.4 to 7.4 times compared with the control, in both years, in all debris types, at both assessment dates, except for cane at 50% flowering in year 2. Paper had a similar effect, significantly reducing ($P<0.01$) debris weight by up to 1.4 to 4.8 times compared with the control, overall, in both years, in all debris types in both assessment dates, except for rachis at leaf plucking and tendril at 50% flowering in year 1, and cane at 50% flowering in year 2. Grass clippings also reduced ($P<0.05$) the weight of all debris types, overall, in both assessment dates, in each year by 1.3 to 3 times, except for leaf and rachis at 50% flowering and rachis at leaf plucking in year one, and cane at 50% flowering in year 2.

Of all debris types, leaf material degraded most rapidly, followed by rachis, tendril and cane debris. In all debris types, in both years, the levels of degradation were significantly higher ($P<0.001$) at leaf plucking than at 50% flowering, except for rachis in year one. The degradation rate increased ($P<0.01$) by 1.1 to 1.3 times over all debris types in the fragmented debris treatment compared with the whole debris treatment (Table 2.4). In both years, the

mulches had similar effects on levels of debris degradation in all debris types and overall levels of debris degradation were also similar (Table 2.3 and Table 2.4).

A photograph showing typical differences in debris degradation rates between treatments for all debris types at leaf plucking in year 1 is presented in Figure 2.2.

Table 2.3. Mean percentage debris degradation at two assessment dates in year one.

Debris type	Assessment date	Mulch treatments					
		Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	Mean date
Cane	50% flower	11^a	16^b	23^c	28^d	22^c	19^x
Cane	Leaf pluck	18^a	23^b	32^c	32^c	26^b	26^y
Leaf	50% flower	27^a	40^a	100^b	100^b	92^b	71^x
Leaf	Leaf pluck	50^a	76^b	100^c	100^c	99^c	85^y
Rachis	50% flower	36^a	43^{ab}	56^{bc}	60^c	58^{bc}	50^x
Rachis	Leaf pluck	37^a	53^a	72^b	83^b	48^a	59^x
Tendril	50% flower	13^a	29^b	52^c	38^b	13^a	28^x
Tendril	Leaf pluck	10^a	30^b	59^c	65^c	28^b	39^y
Mean cane	Both dates	14^a	20^b	27^d	30^d	23^c	*23*
Mean leaf	Both dates	38^a	58^b	100^c	100^c	95^c	*78*
Mean rachis	Both dates	37^a	48^b	64^c	72^c	53^b	*55*
Mean tendril	Both dates	12^a	30^b	56^c	51^c	20^{ab}	*34*
All debris	*50% flower*	*27*	*40*	*56*	*40*	*46*	*42*
All debris	*Leaf pluck*	*19*	*36*	*42*	*46*	*45*	*38*
All debris	*Both dates*	*25*	*39*	*62*	*64*	*48*	

^{abc} Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). ^{xy} Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$). Italics denote data for which statistical comparisons were not possible.

Table 2.4. Mean percentage debris degradation and of fragmented and whole vine debris at two assessment dates in year two.

Debris type	Assessment date	Fragment size	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	Mean date
Cane	50% flower	Both sizes	17^a	17^a	19^a	15^a	17^a	17^x
Cane	Leaf plucking	Both sizes	20^a	29^b	29^b	29^b	29^b	27^y
Tendril	50% flower	Both sizes	8^a	24^b	59^c	31^b	38^b	33^x
Tendril	Leaf plucking	Both sizes	18^a	42^b	56^{bc}	63^c	62^c	49^y
Rachis	50% flower	Both sizes	55^a	79^{bc}	90^c	75^b	82^{bc}	*76*
Mean cane	Both dates	Both sizes	18^a	24^a	24^a	22^a	24^a	*22*
Mean tendril	Both dates	Both sizes	14^a	35^b	58^c	47^c	52^c	*41*
Cane & tendril^2	*50% flower*	*Both sizes*	*13*	*21*	*39*	*23*	*27*	*25*
Cane & tendril^2	*Leaf pluck*	*Both sizes*	*19*	*36*	*42*	*46*	*45*	*38*
All debris	*Both dates*	*Both sizes*	*24*	*38*	*50*	*43*	*46*	
Cane	Both dates	Fragment	20^a	27^a	26^a	26^a	25^a	25^x
Cane	Both dates	Whole	17^a	21^a	23^a	18^a	23^a	20^y
Rachis	50% flower	Fragment	57^a	83^{bc}	94^c	78^b	84^{bc}	79^x
Rachis	50% flower	Whole	53^a	74^b	86^b	73^b	79^b	73^y
Tendril	Both dates	Fragment	18^a	40^b	63^c	51^c	58^c	47^x
Tendril	Both dates	Whole	10^a	30^b	52^c	43^{bc}	45^{bc}	37^y
All debris	*Both dates*	*Fragment*	*31*	*49*	*61*	*52*	*55*	*50*
All debris	*Both dates*	*Whole*	*26*	*41*	*54*	*44*	*48*	*43*

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). [xy] Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$). Italics denote data for which where statistical comparisons were not possible.

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^{2} *Insufficient rachis material available.*

Figure 2.2 Degradation of under-vine debris: cane (a), tendril (b), rachis (c) and leaf debris (d) over seven months from pruning to leaf plucking, under four organic plant-based mulch treatments and a bare-ground control, in an organic vineyard, near Blenheim, New Zealand. C = bare ground control, G = mulched grass, P = shredded office paper, Ma = aerobic *marc* and Man = anaerobic *marc*.
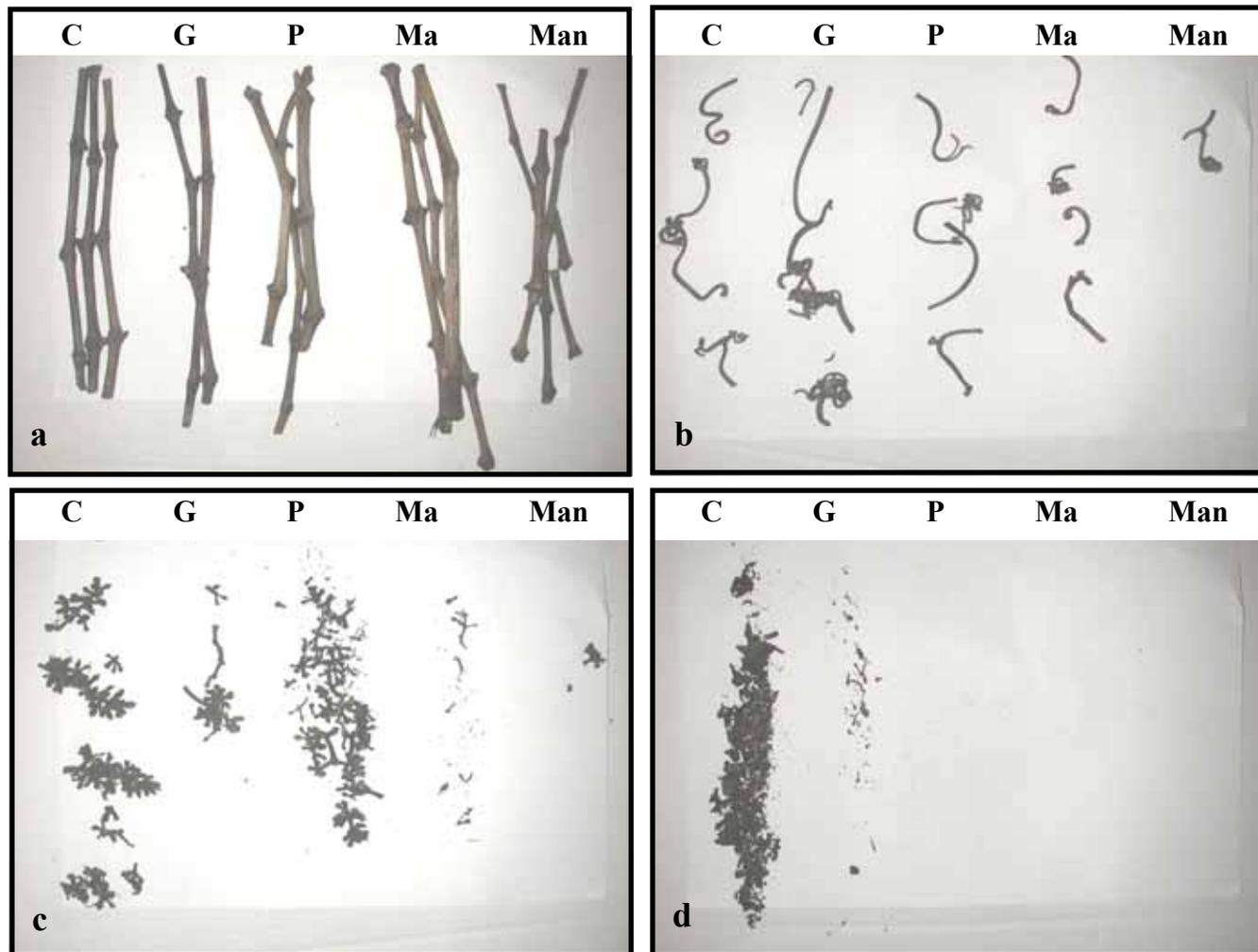
2.4.3 *B. cinerea* sporulation

Overall, in both years, averaged over both assessment dates and debris types, all mulch treatments caused a 3-20-fold reduction in *B. cinerea* conidiophore coverage on the vine debris compared with the control (Table 2.5 and Table 2.6). Overall, the two *marc* treatments reduced ($P<0.05$) *B. cinerea* sporulation on vine debris, in all debris types, in both years and at both assessment dates, by 2 to 65 times compared with the control, except for leaf debris at leaf plucking in year one and cane and tendril at leaf plucking in year two. Paper also reduced ($P<0.05$) *B. cinerea* sporulation on vine debris overall by 4 to 33 times compared with the control, in all debris types, in both years and at both assessment dates, except for leaf debris at leaf plucking in year one and cane and tendril at leaf plucking in year two. Similarly, grass led to a reduction ($P<0.01$) in *B. cinerea* sporulation levels of 2.4 to 11 times overall compared to the control on all vine debris types, in both years, in both assessment dates, except for leaf and rachis at leaf plucking in year one and cane and tendril at leaf plucking in year two. In both years at both assessment dates, rachides supported the highest density of *B. cinerea* conidiophores per unit area of vine debris, followed by canes and tendrils, with leaf material generating the fewest conidiophores. Each year, *B. cinerea* conidiophore coverage on vine debris was significantly ($P<0.001$) higher at 50% flowering than it was at leaf plucking, except for leaves in year one. There was a treatment*date interaction ($P<0.01$) for tendrils in year one, because *B. cinerea* levels were reduced from 21% to 4.6% at 50% flowering and leaf plucking respectively in the control, whereas levels in aerobic *marc* and paper treatments ranged from 0.7 to 0.3% (Table 2.5). A similar interaction was observed in year 2 where the control treatments dropped 10 and 100 fold from about 3% at 50% flowering to 0.27% and 0.03% at leaf plucking in cane ($P<0.001$) and tendrils ($P<0.001$) respectively, while levels in the two *marc* and paper treatments ranged from 0.4% to 0.0% over both assessment dates (Table 2.6). Fragment size had no effect on *B. cinerea* sporulation levels on vine debris. In year one there was generally more disease in all debris types under all treatments in comparison to year two. The effectiveness of the mulches for reducing *B. cinerea* sporulation were similar between years, with the exception of rachis data, which showed aerobic *marc* to be highly effective at reducing *B. cinerea* sporulation in year one but only moderately effective in year two, whereas anaerobic *marc* was only moderately effective in year one, but highly effective in year two (Table 2.5) and (Table 2.6).

Table 2.5. Mean percentage conidiophore coverage of *B. cinerea* on vine debris, retrieved and moist-incubated at two assessment dates in year one (back-transformed data).

Debris type	Assessment date	Mulch treatments					Mean date
		Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	
Cane	50% flower	18^a	5.4^b	1.4^c	0.5^c	1.4^{bc}	5.4^x
Cane	Leaf pluck	6.5^a	0.6^b	0.3^{bc}	0.1^c	0.2^{bc}	1.5^y
Leaf	50% flower	0.5^a	0^b	0^b	0^b	0.1^b	0.1^x
Leaf	Leaf pluck	0.2^a	0.02^a	0^a	0^a	0.1^a	0.1^x
Rachis	50% flower	62^a	26^b	31^b	16^b	5^c	28^x
Rachis	Leaf pluck	7.3^a	6.8^{ab}	2.4^{bc}	1.6^c	1.8^{bc}	4^y
Tendril	50% flower	21^a	5.8^b	1.6^c	0.5^c	0.7^c	5.9^x
Tendril	Leaf pluck	4.6^a	1.2^b	0.2^c	0.3^{bc}	0.6^{bc}	1.4^y
Mean cane	Both dates	12^a	3^b	0.8^c	0.3^c	0.8^{bc}	*3.4*
Mean leaf	Both dates	0.33^a	0.01^b	0^b	0^b	0.08^b	*0.08*
Mean rachis	Both dates	34^a	16.8^{ab}	16.6^{bc}	8.9^{cd}	3.6^d	*16*
Mean tendril	Both dates	13^a	3.5^b	0.9^c	0.4^c	0.6^c	*3.6*
All debris	*50% flower*	*25*	*9.4*	*8.4*	*4.3*	*1.9*	*9.9*
All debris	*Leaf pluck*	*4.5*	*2.3*	*0.7*	*0.5*	*0.7*	*1.7*
All debris	*Both dates*	*15*	*5.8*	*4.6*	*2.4*	*1.3*	

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD (*P*<0.05).[xy] Letters denote significant differences between assessment dates calculated through Fisher's Protected LSD (*P*<0.05). Italics denote data for which statistical comparisons were not possible.

Table 2.6. Mean percentage conidiophore coverage of *B. cinerea* on vine debris, retrieved and moist-incubated at two assessment dates and of fragmented and whole vine debris under each mulch treatment in year two (back-transformed data).

Debris type	Assessment date	Fragment size	Mulch treatments					
			Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	Mean date
Cane	50% flower	Both sizes	3.2[a]	0.4[b]	0.3[b]	0.3[b]	0.3[b]	0.9[x]
Cane	Leaf pluck	Both sizes	0.27[a]	0.06[a]	0[a]	0[a]	0[a]	0.06[y]
Tendril	50% flower	Both sizes	3.7[a]	1.1[b]	0.1[b]	0.2[b]	0.2[b]	1.1[x]
Tendril	Leaf pluck	Both sizes	0.03[a]	0.09[a]	0[a]	0.4[a]	0[a]	0.1[y]
Rachis	50% flower	Both sizes	57[a]	20[bc]	6.3[cd]	23[b]	3.2[d]	*22*
Mean cane	Both dates	Both sizes	1.7[a]	0.2[b]	0.13[b]	0.15[b]	0.14[b]	*0.48*
Mean tendril	Both dates	Both sizes	1.8[a]	0.6[b]	0.06[b]	0.3[b]	0.08[b]	*0.6*
Cane & tendril[3]	*50% flower*	*Both sizes*	*3.4*	*0.8*	*0.2*	*0.3*	*0.2*	*1.0*
Cane & tendril[3]	*Leaf pluck*	*Both sizes*	*0.2*	*0.1*	*0*	*0.2*	*0*	*0.1*
All debris	*Both dates*	Both sizes	*12*	*3.1*	*0.9*	*4.6*	*0.6*	
Cane	Both dates	Fragment	1.5[a]	0.19[b]	0.26[b]	0.20[b]	0.10[b]	0.44[x]
Cane	Both dates	Whole	2.0[a]	0.28[b]	0.01[b]	0.10[b]	0.18[b]	0.51[x]
Rachis	Both dates	Fragment	47[a]	17[b]	2.6[c]	23[a]	5[bc]	19[x]
Rachis	Both dates	Whole	67[a]	24[b]	10[b]	22[b]	1[c]	25[x]
Tendril	Both dates	Fragment	3.66[a]	1.11[ab]	0.11[c]	0.20[bc]	0.16[bc]	1.05[x]
Tendril	Both dates	Whole	1.43[a]	0.14[b]	0.08[b]	0.49[b]	0.02[b]	0.43[x]
All debris	*Both dates*	*Fragment*	*9*	*2.8*	*0.5*	*4.4*	*0.91*	*3.6*
All debris	*Both dates*	*Whole*	*14*	*3.3*	*1.3*	*4.7*	*0.2*	*4.6*

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). [xy] Letters denote significant differences between assessment dates calculated through Fisher's Protected LSD ($P<0.05$). Italics denote data for which statistical comparisons were not possible.

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[3] *Insufficient rachis material available.*

2.4.4 Potential of mulch to harbour *B. cinerea*

No significant differences were found between soil from the bare ground control, grass, aerobic and anaerobic *marc* in their ability to harbour *B. cinerea* from mycelium (Table 2.7). *B. cinerea* spread significantly ($P<0.05$) more on the paper than the other mulch treatments, but on the paper, mycelium was sparse and grew on the surface of the substrate suggesting that it might be using it as a medium of spread rather than a nutrient source. Mulches showed no differences between each other when inoculated with dry spores. In both assessments, the positive control had high levels of disease.

Table 2.7. The mulches' ability to host *B. cinerea* from mycelium spread from a mycelium plug or infection from dry spore inoculation, in comparison with a necrotic leaf (positive control).

		Assessment times				All times
	Mulch type	1	2	3	4	
Mycelium plug	Control (soil)	0.5	0.5	0.6	0.6	0.5[a]
	Mulched grass	0.2	0.5	0.6	0.6	0.5[a]
	Anaerobic *marc*	0.0	0.0	0.0	0.0	0.0[a]
	Aerobic *marc*	0.3	0.4	1.2	1.3	0.8[a]
	Shredded paper	2.2	5.3	7.1	7.6	5.5[b]
	Positive control (grapevine leaf)	24.4	25.0	25.0	25.0	24.8[c]
Dry spore	Control (soil)	0.0	0.0	0.6	0.6	0.3[a]
	Mulched grass	0.0	0.0	0.0	0.0	0.0[a]
	Anaerobic *marc*	0.0	0.0	0.0	0.0	0.0[a]
	Aerobic *marc*	0.0	0.6	4.4	5.6	2.7[a]
	Shredded paper	0.0	0.6	8.8	22.5	8.0[a]
	Positive control (grapevine leaf)	21.2	78.8	100.0	100.0	75.0[b]

2.4.5 Correlations

In both years, levels of *B. cinerea* on vine debris were inversely correlated with both debris degradation rates and biological activity of the soil. In year one, levels of *B. cinerea* on vine debris were inversely correlated with degradation rates of vine debris in all debris types, with the respective r values for cane, leaf, tendril and rachis being -0.482; $P<0.001$, -0.45; $P<0.001$, -0.352; $P<0.001$ and -0.23; $P<0.01$. A similar inverse correlation occurred between *B. cinerea* levels and soil biological activity, with the respective r values for cane, tendril and

leaf material being -0.412; $P<0.001$, -0.385; $P<0.001$ and -0.245; $P<0.01$. The debris degradation rates of leaf, cane and tendril were positively correlated with soil biological activity, with respective r values of 0.466; $P<0.001$, 0.382; $P<0.001$ and 0.220; $P<0.01$. In year two, *B. cinerea* sporulation was inversely correlated with degradation of debris types with the respective r values for rachis, tendril and cane being -0.558; $P<0.001$, -0.160; $P<0.05$ and -0.159; $P<0.05$). *B. cinerea* sporulation on vine debris was also inversely correlated with soil biological activity in rachides and tendrils with respective r values of -0.273; $P<0.01$ and -0.194; $P<0.05$. Debris degradation rates of rachides were also correlated with soil biological activity ($r = 0.203$; $P<0.001$).

2.5   Discussion

2.5.1   Bait lamina probes

In this trial, the two *marc* and paper treatments significantly increased soil biological activity. This was probably because they enhanced the soil environment for existing soil organisms by improving moisture retention (Pickering *et al*., 1998), soil nutrient status or nutrient availability (Biala *et al*., 2000) and reducing soil temperature fluctuations (Pickering *et al*., 1998). In year two the soil profile was disturbed by the unplanned underground weeding of all treatment replicates on Friday 3 December 2004. This disturbance may have been responsible for the lowered biological activity and elevated variability of all treatments and resulted in a significant treatment*date interaction in this year. This work supports that of Mundy & Agnew (2002), who applied four mulch treatments (containing varying ratios of vine prunings, grape marc, green waste, pine bark, animal waste and mussel (*Mytilus* sp.) shells) under vines in four vineyards in Marlborough, New Zealand. They found that one treatment, consisting of 23% vine prunings, 12% marc, 30% green waste, 23% pine bark, 6% animal manure and 6% mussel shells caused a significant ($P<0.05$) increase in the number of fungal colony-forming units plated out from soil washings below the mulch in six of the 12 assessments made in all vineyards over three years.

2.5.2   Vine debris degradation

The two *marc* and paper treatments caused the highest levels of debris degradation in all vine debris types, over both assessment dates, in both years, except for cane in year two, and these effects were correlated with soil biological activity. Fragment size of vine debris also had an effect on the degradation rate. Smaller fragments degraded faster, which could be attributed to the increase in both surface area and the exposure of internal, less lignified plant tissue, which

are more readily degraded by soil organisms (Moore-Landecker, 1972). For this reason vine debris with the lowest lignin levels degraded fastest namely, leaf material, rachis and then tendril and cane. At leaf plucking, the degradation rates were higher than at 50% flowering because the debris had been under the mulches for a longer period.

2.5.3 *B. cinerea* sporulation

The two *marc* and paper treatments caused the largest reduction in *B. cinerea* levels on vine debris compared with the control. At 50% flowering, all debris types reduced *B. cinerea* levels on vine debris compared with the control. At leaf plucking levels of *B. cinerea* were often so low across all treatments that no significant differences were seen between treatments. The reductions in *B. cinerea* sporulation attributable to the mulch at 50% flowering are more important than at leaf plucking because at this time, when the mulches caused the largest reductions in spore numbers, the grapevine inflorescence is very vulnerable to infection by *B. cinerea* primary inoculum (Nicholas *et al.*, 1994). Interactions between treatment and assessment date occurred when some of the treatments, particularly the control and grass, differed greatly in levels of *B. cinerea* between 50% flowering and leaf plucking; the effects of other treatments, particularly the two *marc* and paper treatments, did not. Of the debris types, rachis supported the highest levels of *B. cinerea* conidiophores probably because this tissue had the highest spore loading; tendril and cane were next, followed by leaf material. The latter supported the lowest levels of *B. cinerea* conidiophores, probably because it degraded fastest and it supported such high levels of other organisms that *B. cinerea* was out-competed. In this work, *B. cinerea* primary inoculum was measured as percentage area coverage, so was independent of reductions in debris surface area and therefore underestimated the effects of mulches on *B. cinerea* sporulation.

The findings of this research support other work in this area. Leaf litter mulch reduced the survival of *Phytophthora palmivora* (Butl.) on cocoa debris under cocoa trees in Papua New Guinea, a finding also related to increased degradation of host/crop debris (Konam & Guest, 2002). A similar study on the effects of compost placed below boysenberry (*Rubus* hybrid) vines also found a similar relationship between *B. cinerea* inoculum from host/crop debris and the compost treatments (Walter *et al.*, 2004).

2.6 Conclusions

In this study, organic mulches under grape vines, especially the two *marc* and paper treatments, elevated the activity of soil organisms, causing indirect biological control, which led to a reduction in *B. cinerea* inoculum via accelerated decomposition of vine debris and

higher levels of competition with *B. cinerea* for the vine material. This is the first time that a relationship between *B. cinerea* primary inoculum, soil biological activity and degradation rate of vine debris has been shown under mulches in a vineyard environment and these effects could have a direct impact on *B. cinerea* levels in grapes at harvest. Nair *et al.* (1995) attributed up to 70% of *B. cinerea* inoculum levels on the vine at flowering and 28% at harvest to primary inoculum and demonstrated that flowering and latent infections are inoculum driven, while secondary cycles later in the season are driven by environmental factors. Since most spores land near their source, the mulches are likely to have an impact on *B. cinerea* levels in the nearby vines. This was shown by Seyb (2004) who found that 95% of *B. cinerea* air-borne spores landed within 1.6 m of the source. The trials of Mundy & Agnew (2002) also showed that use of a multi-component mulch reduced *B. cinerea* incidence ($P<0.05$) in five of 12 assessments.

The mulches used in this work are likely to operate through a progression of different mechanisms in the vineyard environment, as regards *B. cinerea* primary inoculum. Initially, the mulch may act primarily as a barrier to spore dispersal. The mulches may also start to alter the soil environment at this time, enhancing soil biological activity, degrading vine debris and competing with *B. cinerea* for resources. Over time, as the mulches decay or are disturbed through wind, animal activity or viticultural practices, the thickness of the mulch will diminish and may need to be reapplied to maintain its function. At this time, it is unknown what the minimum depth of mulch is required to function in this way, or how regularly the mulch will need to be reapplied to maintain this thickness. The types of materials available for use as organic mulches will depend on the regional industries and so waste stream materials, but before use they should be carefully evaluated for potential nutrient and toxin effects. In this study, the ink on the office paper was known to be safe as modern inks are produced with vegetable and mineral oils, unlike older formulations which contained lead (Heichel *et al.*, 1974). In the organic vineyard where this experiment was conducted, the use of printed paper as a mulch was permitted under the New Zealand BIO-GRO organic certificate scheme (Anonymous, 1994).

The biological mechanisms that operate in the vineyard system could potentially have an impact on the population dynamics of other vine diseases such as downy mildew (*Plasmopara viticola* (Burk. and Curt.) Berl. and de Toni, as it also overwinters on vine debris on the vineyard floor (Nicholas *et al.*, 1994) and could be affected by high soil biological activity and elevated degradation of the debris. Other vineyard understorey manipulation techniques, such as the provision of flowering plants between vine rows to provide nectar and shelter for

beneficial insects (Berndt *et al.*, 2002) could potentially be adapted to reduce survival of pathogens in the vine debris by mulching these plants into the below-vine area after their flowering has ended.

CHAPTER 3: The impacts of organic mulches on resistance to *Botrytis cinerea*, grape quality and yield.

Adapted from

Jacometti, M. A., Wratten, S. D. and Walter, M., unpublished. Understorey management increases, grape quality, yield and resistance to *Botrytis cinerea*. Submitted to Agriculture, Ecosystems and Environment 10 July, 2006.

3.1 Abstract

In the current work, the same organic mulches described in Chapter 2 altered vine characteristics and elevated their resistance to *B. cinerea* through changes to the soil environment. Functional soil biological activity, as measured by Biolog Ecoplates and bait lamina probes, was increased 2-4 times in the two *marc* and paper treatments, compared with the control, an effect relating to the elevated soil moisture and reduced temperature fluctuations under these mulches. Nutrient levels and the C:N ratios were also affected in these treatments. The mulched paper lowered vine canopy density by up to 1.4 times that of the other treatments, an effect which probably led to elevated light penetration into the canopy, increased canopy temperature, photosynthesis and lowered canopy humidity. In the anaerobic *marc* and paper treatments, grape skin strength was increased by up to 10% and in the two *marc* and paper treatments sugar concentrations were increased by 1.2-1.4 °Brix. The severity of *B. cinerea* infections in the anaerobic *marc*, aerobic *marc* and paper treatments were reduced to 12%, 3% and 2.2% of the control, respectively, in field assessments averaged over two consecutive harvests. These results show the potential of mulches in enhancing ecosystem services, including the ability of the soil to buffer hydrological fluctuations, and to improve vine health and bunch resistance to *B. cinerea*.

3.2 Introduction

The chemical control strategies for botrytis bunch rot discussed in Chapter 1 are increasingly regarded as unsustainable, and alternative methods of control are being investigated. Conservation biological control can disturb the *B. cinerea* life cycle as discussed in Chapter 2, but can potentially also alter other vine attributes which have been shown to increase vine resistance to pathogens (Mullins *et al.*, 1992; Bavaresco *et al.*, 1997; Goetz *et al.*, 1999).

Characteristics of vines that promote resistance to *B. cinerea* infection are low canopy density (Duncan *et al.*, 1995), open bunch structure (Mullins *et al.*, 1992), berry skin strength (Chardonnet & Doneche, 1995) and phenolic content (Bavaresco *et al.*, 1997). Vines with these characteristics can potentially be managed with less chemical input and the wine from them marketed as a more sustainable product, an increasingly important aspect of wine consumer requirements.

3.3 Materials and methods

3.3.1 The field site and experimental design

This work was a continuation of the research on the effects of the mulches on soil biological activity, vine debris degradation and *B. cinerea* primary inoculum levels that were assessed in 2003/04 and reported in Chapter 2. The mulch treatments were first applied to plots in 24 July 2003 and replenished annually (25 June 2004 and 21 June 2005) to a total thickness of 100 mm. The current research was conducted in 2005/06 and investigated mulch effects on soil and vine characteristics, yield and *B. cinerea* severity on vines.

3.3.2 Soil attributes

Soil biological activity was assessed monthly with bait lamina probes and Biolog Ecoplates, as was the antibiotic activity of soil filtrates and soil moisture (by weight) from February 2005 to February 2006. Soil temperature was measured continuously from 19 November 2005 to 21 March 2006, soil nutrients were assessed in May 2005 and carbon:nitrogen (C:N) ratios in November 2005. Bait lamina probes and soil moisture were assessed in all replicates, while Biolog Ecoplates, antibiotic exudates, soil temperature, soil nutrients and C:N ratio assessments were all conducted in replicates 3, 4 and 8, which were randomly selected at the start of the trial.

3.3.2.1 Biolog Ecoplates

This technique indirectly measures the microbial diversity and activity of soil microbial communities (Gagliardi *et al.*, 2001; Buyer *et al.*, 2002; Girvan *et al.*, 2003). The plates had 96 wells, with three replicates each of the 31 carbon sources commonly used by soil microbial organisms and three control wells of water only. In each well, there was a reducible tetrazolium dye that changed from colourless to red when the carbon source was utilised by oxidative metabolic processes. At monthly intervals, five 50 g soil samples were taken from the soil/mulch interface from each treatment in three randomly selected replicates. These five

samples were combined, shaken by hand for 15 seconds and then sieved (5 mm mesh). A 2.5 g sample was taken from each of these, shaken for 30 minutes on a rotary shaker in a 22.5 ml 0.85% NaCl solution, then settled for 10 minutes to clear the supernatant. The supernatant was then decanted and pressure-filtered through 'Whatman No. 40' filter paper. The resulting filtrate was diluted by 10^3 and a 140 µl aliquot of the resulting suspension was put in each well of the Ecoplate, which was incubated at 20-25°C for 2 weeks. Measurements of the dye reduction were made with a micro-plate reader (FluroStar, BMG Labtechnologies, Germany) using a 590 nm filter (BMG 241A Abs), on day zero and day one, then 12 hourly from day two to day four, and 24 hourly from day five to day eight, with the last measurement on day 14. The mean percentage of wells that gave an absorbance reading of over 0.8, a colour change easily seen by eye, was then recorded. The largest differences between treatments in both rate of change and proportion of wells coloured, was seen between day one and four, so only these data were used for analysis in the current work.

3.3.2.2 Antibiotic activity of filtrates from soil under the mulch

The undiluted supernatants (post filtration through 'Whatman no. 40' filter paper) from the Biolog Ecoplate assessment were tested for antibiotic activity against *B. cinerea* growth. These supernatants were filtered through lens tissue to remove small soil particles and then filter sterilised (0.22 µm) to remove all micro-fauna (Elad *et al.*, 1994). Five 1 ml wells were cut near the perimeters of 10 replicate PDA (Potato Dextrose Agar) plates, each well filled with 1 ml of each treatment's supernatant. A 10 mm diameter plug of *B. cinerea* mycelium was placed in the centre of each plate and incubated at 20ºC. At day 2, 4, 6 and 8, the closest point of mycelium growth was measured from the treatment wells (Figure 3.1).

3.3.2.3 Bait lamina probes

Three to four weeks before each soil assessment date (see Section 3.3.2), one probe was placed in each treatment replicate, inserted 40 mm below the soil/mulch interface, then removed and visually assessed at each soil assessment date.

3.3.2.4 Soil moisture

Five 50 g samples were taken from the soil/mulch interface in between drip irrigators, in each treatment replicate and pooled for each replicate, weighed, dried for 14 days at 70ºC, reweighed and the initial percent moisture calculated.

Figure 3.1. A diagrammatic example of the antibiotic filtrate test. Five wells were cut into the perimeter of a PDA plate and filled with filtrates from the soils under mulches to test their effects on *B. cinerea* growth. All mulches except treatment four expressed antibiotic activities in this example.
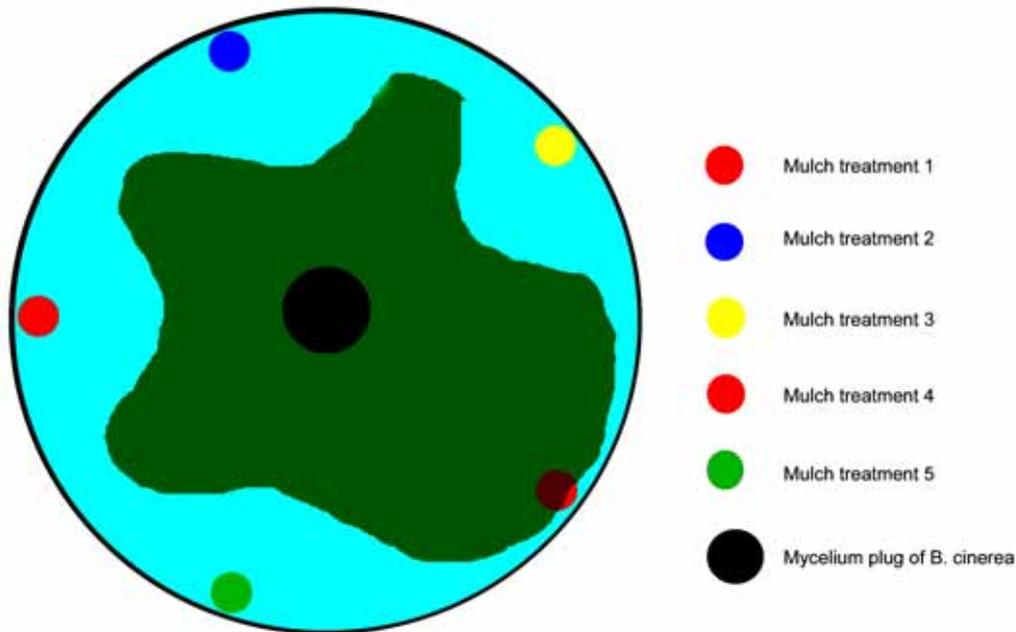
3.3.2.5 Soil temperature

Mean soil temperature and maximum/minimum temperatures were measured 30 mm below the soil mulch interface from each treatment in three randomly selected blocks, at 6-minute intervals using data loggers (Hobo H08-004-02, 4 channel external; -20ºC - 70ºC, 0 - 95% RH; Onset Computer Corporation, USA), running TMC – HD water/soil temperature probes, accuracy ± 0.5ºC, the data from which were retrieved monthly.

3.3.2.6 Soil nutrients

Five 50 g soil samples were taken from the soil/mulch interface in each treatment and pooled in three randomly selected blocks in May 2005, then sent to Hill Laboratories, Hamilton, New Zealand to be assessed for pH, Olsen P, potassium, calcium, magnesium, sodium, cation exchange capacity (CEC), base saturation and bulk density.

3.3.2.7 Soil C:N ratio

The C:N ratio of soil was assessed from soil samples taken from each treatment in three randomly selected blocks in November 2005. Soil samples were taken from 30 mm below the soil/mulch interface, minimising the amount of mulch included in the sample. The soil was dried at 70°C for 14 days, sieved through a 250 μm sieve and then analysed for carbon and nitrogen (LECO CNS-2000 Elemental Analyser, Leco Corporation, USA). Despite efforts to exclude mulch from the sample, fine particles of vine debris, predominantly fragments of rachis, were still visibly present in two *marc* samples. These particles were thought to have artificially raised the C:N ratio compared with the other mulch treatments.

3.3.3 Plant attributes

3.3.3.1 Canopy density

Canopy density was measured in all replicates in winter (24 June 2005) by measuring the pruning weights of all the trial vines, which were pruned to two canes by professional pruners, according to the standard set for the whole vineyard[4].

3.3.3.2 Leaf nutrients

In May 2005, 15 leaves were randomly selected from each of three randomly selected vines per treatment, then pooled by treatment type, in three randomly selected blocks. These leaves were sent to Hill Laboratories, Hamilton, New Zealand for a whole leaf analysis of nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, sodium, iron, manganese, zinc, copper and boron.

3.3.4 Berry attributes

Sugar levels (°Brix) and *B. cinerea* levels in the grape berries were assessed at harvest 2005 and 2006. All other berry attributes were measured in 2005 only.

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[4] An attempt was made to measure canopy density by the point quadrat system (Smart and Robinson, 1991), in which a metal rod is by inserted horizontally into the canopy and the number of contacts (with leaves and shoots) and the gaps is recorded. Rods were inserted ten times, each 200 mm apart and 1300 mm off the ground before leaf plucking (late December 2004). This technique proved unreliable and heavily influenced by human error, so was discarded.

3.3.4.1 Berry skin strength

At harvest 2005, five grape berries of similar size were taken from the outside of one randomly selected bunch from each treatment replicate. Each berry was weighed and placed in a 15 mL conical tipped Vulcan tube for serial centrifugation to determine the speed at which berries ruptured. The randomly selected tubes were centrifuged in batches for 2 minutes, starting from 1500 rpm and increasing in 250 rpm increments until all the berries had ruptured. The centrifuge speed at which the individual berries had ruptured was then recorded and converted to relative centrifugation force (g). Measurement of skin thickness by cross section (Gabler et $al.$, 2003) and penetrometer measurements (Mitcham et $al.$, 1998) were attempted in conjunction with this technique. Skin thickness through cross section proved problematic due to irregularities in the cut surface when cut by hand or freezing microtome, high variability in skin thickness around the berry section and the inherent indistinct boundary between the cuticle, exocarp (skin) and mesocarp cells (pulp) (Jackson, 1994). Similarly, the penetrometer was not useful as the berry ruptured before a measurement was registered.

3.3.4.2 Sugar concentration

Sugar content was assessed on the same five grape berries from each treatment replicate that were assessed for skin strength. These five berries were pooled and squeezed by hand in a new polythene bag. A sample of juice was taken from this and sugar concentration ºBrix was measured using a digital refractometer (Atago PR-101, 0-45% ºBrix ±0.1% @ 5-40ºC; Atago Company, Ltd, Japan). At harvest 2006, one entire bunch per replicate was placed in a new polythene bag, squeezed by hand and a sample assessed as above.

3.3.4.3 Total phenolics

Five grape berries were randomly selected from the outside of one randomly selected bunch from each treatment replicate. These berries were frozen and squashed in a standard Petri dish with a new Vulcan tube lid. The seeds were then removed and the remaining sample was homogenised (Polytron PT3100, 0-25,000 rpm; Kinematica Ag, Switzerland) for 1 minute at 25,000 rpm, in a 13 ml round-bottomed centrifuge tube, capped, then placed in crushed ice to keep cool for no more than 20 minutes. Total phenolics were then extracted according to the protocol described by Iland et al. (2000) and the absorbance was read at 700 nm, 520 nm and 280 nm in a spectrophotometer (Heliois Alpha, 190-1100 nm ± 1 nm; Unicam, United Kingdom).

3.3.4.4 Berry nutrients

At harvest 2005, ten randomly selected grape berries were taken from the inside and ten from the outside of one randomly selected bunch, from each treatment in three randomly selected blocks. Each group of ten berries was pooled, then sent to Hill Laboratories, Hamilton, New Zealand for assessment of ammonium, phosphorus, potassium, calcium, magnesium, sodium and boron content.

3.3.4.5 Resistance of berries to *B. cinerea*

Two sets of four grape berries were taken from one randomly selected bunch from each treatment in eight randomly selected replicates at harvest 2005. These berries were surface-sterilised with 50% ethanol for two minutes and rinsed twice in sterile water for two minutes, then air-dried in a lamina flow cabinet. A drop of *B. cinerea* spore suspension (2.5×10^5 conidia/mL) was then put on the skin surface of the first set of berries. The second set of berries was punctured with a sterilised needle and a drop of same *B. cinerea* spore suspension was placed on the wound. They were then placed on racks in trays, lined with moist paper towels, enclosed in new polythene bags and incubated at room temperature. The grape berries were assessed for presence of sporulating *B. cinerea* at 7, 11 and 15 days. Berries that were infected with *B. cinerea* were removed at each assessment date to avoid cross contamination. Another four grape berries were taken from a subset of the five of the same bunches as above. These berries were surface-sterilised in the same way, frozen for 4 hours, incubated using the same incubation and assessment methods as before. This procedure assessed latent infections in the different treatments and allowed the data to be adjusted for the levels of prior infection in the both sets of inoculated berries.

3.3.4.6 Bunch yield

At harvest 2005 and 2006, all bunches were counted from the single treatment vine, from each replicate. In 2006, the weights were recorded for five randomly selected bunches from each replicate.

3.3.4.7 *B. cinerea* infection of berries at harvest

At harvest 2005 and 2006, a survey of sporulating *B. cinerea* was done for all bunches in the single treatment vine for each replicate. The proportion of grape berries with sporulating *B. cinerea* (severity) was estimated per bunch and from this disease severity was calculated per vine.

3.3.5 Statistical analysis

All data were analysed by analysis of variance (ANOVA), with the exception of Biolog Ecoplates, antibiosis activity, soil temperature and resistance of berries to *B. cinerea*, which were analysed by repeated measures ANOVA. Correlations were tested using Spearman's correlation coefficient. All data sets conformed to a normal distribution, so no transformations were made. The statistical package used for all analyses was Genstat 7.

3.4 Results

3.4.1 Soil characteristics

3.4.1.1 Biolog Ecoplates

The number of carbon compounds utilised by soil organisms in the Biolog Ecoplates was 1.9 to 2.3 times higher for the two *marc* and paper treatments than for the control ($P<0.05$) (Table 3.1). There was also a strong seasonal effect ($P<0.001$), in which the utilisation of carbon materials in the Ecoplates varied up to six-fold over the year. A treatment*month interaction ($P<0.01$) was also present, where little differences were seen between treatments in low activity months and large differences were seen between the more effective treatments and the control in the high activity months[5].

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[5] In addition to the proportion of carbon compounds used, the mean absorbance for each plate was also assessed for each treatment replicate for each month, the data of which is presented in Table 3.2.

Table 3.1. Mean percentage of Biolog Ecoplate wells that had an absorbance reading above the threshold of 0.8, for soils under each mulch, with monthly assessments.

Assessment time	Mulches					Mean date
	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	
February 2005	9.7	9.0	23.1	16.2	30.2	17.6[b]
March 2005	9.0	9.9	22.2	30.6	30.7	20.5[bc]
April 2005	12.3	28.0	24.1	39.2	27.4	26.2[c]
May 2005	6.1	3.0	5.7	8.9	9.9	6.7[a]
June 2005	13.7	22.7	22.1	23.1	13.4	19.0[bc]
August 2005	28.7	18.1	34.4	20.7	38.9	28.1[c]
September 2005	16.5	28.0	30.2	16.3	13.4	20.9[bc]
October 2005	0.0	3.8	16.3	17.5	1.9	7.9[a]
November 2005	6.6	39.2	43.6	33.9	42.0	33.1[c]
December 2005	26.7	36.3	59.0	35.4	57.3	43.0[d]
January 2006	16.5	28.0	30.2	16.3	13.4	20.9[bc]
February 2006	9.9	17.5	39.6	32.5	41.3	28.2[c]
Mean months	13.0[a]	20.3[ab]	29.2[b]	24.2[b]	26.7[b]	

[abc] Letters denote significant differences calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by month LSD ($P<0.05$) = 18.5; significant differences between months by treatment LSD ($P<0.05$) = 17.3.

Table 3.2. Mean absorbance value of Biolog Ecoplate wells, for soils under each mulch, with monthly assessments.

| Assessment time | Mulches | | | | | Mean treatment |
	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	
February 2005	0.50	0.50	0.61	0.54	0.65	0.56[b]
March 2005	0.47	0.50	0.58	0.63	0.66	0.57[bc]
April 2005	0.52	0.64	0.61	0.72	0.64	0.63[bc]
May 2005	0.48	0.44	0.46	0.48	0.50	0.47[a]
June 2005	0.54	0.64	0.62	0.65	0.58	0.61[bc]
August 2005	0.64	0.51	0.70	0.56	0.71	0.62[bc]
September 2005	0.56	0.63	0.64	0.51	0.47	0.56[b]
October 2005	0.32	0.39	0.48	0.54	0.39	0.43[a]
November 2005	0.47	0.73	0.76	0.68	0.75	0.68[c]
December 2005	0.67	0.71	0.92	0.71	0.91	0.78[d]
January 2006	0.56	0.63	0.64	0.51	0.47	0.56[b]
February 2006	0.49	0.54	0.72	0.67	0.75	0.63[c]
Mean months	0.52[a]	0.57[ab]	0.64[b]	0.60[b]	0.62[b]	

[abc] Letters denote significant differences calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by month LSD ($P<0.05$) =0.17; significant differences between months by treatment LSD ($P<0.05$) = 0.16.

3.4.1.2   Antibiotic activity of filtrates from soil under the mulch

No significant differences were seen between treatments.

3.4.1.3   Bait lamina probes

Overall, the soil micro-fauna under the two *marc* and paper treatments removed 3.4 to 4.1 times as many baits as in the control ($P<0.001$) (Table 3.3). Most baits disappeared ($P<0.001$) in the top 13-56 mm, with 35% of baits removed averaged over these depths, whereas 29% were removed from 63-100 mm (Table 3.4). The lowest rates of activity were in the top 10 mm ($P<0.001$) where only 25% of baits were removed. There was a treatment*depth interaction ($P<0.001$) were control and grass had large differences in biological activity throughout the soil profile, with high activity in shallow soil compared with that of deep soil, with coefficients of variation of 148 and 127 for control and grass, respectively. In contrast,

Table 3.3. Percentage of bait used in the bait lamina probes by soils under the different mulch treatments, over all soil depths by assessment date and over all months combined.

Assessment time	Mulches					Mean treatments
	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	
February 2005	6.1	7.4	41.2	55.9	33.8	29.6[b]
March 2005	4.7	11.7	51.3	50.3	41.7	33.3[bc]
April 2005	7.5	13.4	53.1	50.9	41.6	33.3[bc]
May 2005	7.8	29.1	47.2	57.2	38.4	35.9[bc]
June 2005	5.3	8.1	15.6	12.5	14.7	11.3[a]
October 2005	5.9	15.9	40.3	20.3	13.8	19.3[a]
November 2005	19.4	33.4	58.1	39.1	34.7	36.9[bc]
December 2005	20.6	31.9	50.0	35.6	70.0	41.6[c]
January 2006	20.9	16.6	57.5	30.3	70.9	39.3[c]
February 2006	13.4	12.2	49.4	27.5	49.1	30.3[b]
Mean months	11.2[a]	18.0[a]	46.4[c]	38.0[c]	40.9[c]	

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by month LSD ($P<0.05$) = 21.1; significant differences between months by treatment LSD ($P<0.05$) = 19.6.

Table 3.4. Percentage of bait used in the bait lamina probes at different soil depths under the different mulch treatments.

Depth (mm)	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	All treatments
6	18	14	34	33	27	24.8[a]
13	22	25	52	42	35	35.2[de]
19	18	26	61	46	37	37.3[e]
25	16	16	52	45	47	34.7[de]
31	09	21	49	46	47	34.4[de]
38	08	17	49	39	47	31.8[bcd]
44	12	19	37	46	52	33.0[de]
50	14	20	53	47	42	35.0[de]
56	09	22	54	35	42	32.2[cd]
63	07	19	40	33	42	28.0[abc]
69	06	16	51	42	46	31.7[bcd]
75	04	12	50	30	35	25.9[a]
81	10	17	42	30	38	27.2[ab]
88	11	13	43	28	38	26.6[a]
94	10	17	40	31	38	26.9[a]
100	13	21	44	40	45	32.5[cd]

[abc] Letters denote significant differences calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by depth LSD ($P<0.05$) = 14.4; significant differences between depths by treatment LSD ($P<0.05$) = 10.6.

anaerobic *marc*, aerobic *marc* and paper were much more uniform with coefficients of variation of 63, 79 and 73, respectively (Table 3.5). There was also a month*depth interaction ($P<0.001$), with June and October having high variation through the soil profile with coefficients of variance (CVs) of 279 and 195, respectively, compared with December 05 and January 06 which had CVs of 117 and 122, respectively.

Table 3.5. Percentage of bait used in bait lamina probes at different depths in each month, averaged over all mulch treatments.

| Depth (mm) | 2005 | | | | | | | | 2006 | |
	Feb	Mar	Apr	May	Jun	Oct	Nov	Dec	Jan	Feb
6	48	54	54	18	1	5	13	18	22	15
13	49	45	45	46	16	14	26	34	47	30
19	39	39	39	31	20	25	48	48	49	35
25	34	41	41	24	22	17	47	36	40	45
31	37	34	34	33	16	19	45	41	50	35
38	33	40	40	29	19	20	35	41	39	22
44	38	37	37	35	13	15	38	37	46	34
50	27	40	40	43	12	24	36	39	51	38
56	31	29	29	35	12	22	41	48	42	33
63	25	23	23	40	9	30	39	41	26	24
69	17	39	39	39	8	24	40	42	39	30
75	22	23	23	36	2	18	38	36	31	30
81	21	20	20	41	4	14	39	52	35	26
88	19	26	26	39	6	17	31	41	35	26
94	18	17	17	38	8	20	35	52	32	32
100	15	26	26	48	12	24	40	60	44	30

Significant differences between months by depth LSD ($P<0.05$) =15.5; significant differences between depths by month LSD ($P<0.05$) = 15.6.

3.4.1.4 Soil moisture

All mulch treatments significantly increased soil moisture compared to the control ($P<0.001$) (Table 3.6). The paper and anaerobic *marc* had the greatest effect, raising soil moisture to 27%, followed by aerobic marc, grass and control with 26%, 21% and 17%, respectively. There was a seasonal effect on soil moisture ($P<0.001$), with 28-29% soil moisture in winter

months and 17-23% in summer months, averaged over all treatments. There was also a treatment*month interaction (P<0.001), with soil moisture in the control having a range of 18% between the wettest and the driest months, whereas in the two marcs and paper treatments it was more stable, with a range of 12-13%.

Table 3.6. Mean percentage soil moisture under the different mulch treatments, in all treatments and assessment dates.

| | Mulches | | | | | Mean |
Assessment time	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	treatments
February 2005	11.9	13.8	28.4	30.3	33.1	23.5
March 2005	15.0	17.2	23.6	22.1	26.4	20.8
April 2005	25.5	27.2	29.8	29.8	29.8	28.4
May 2005	25.3	28.1	30.0	31.4	29.3	28.8
June 2005	27.0	27.5	31.6	31.0	29.0	29.2
September 2005	22.2	24.5	25.3	25.2	24.0	24.2
October 2005	18.9	26.9	34.2	30.8	27.2	27.6
November 2005	9.1	19.5	18.7	18.1	30.8	19.3
December 2005	14.6	16.9	30.8	25.2	27.0	22.9
January 2006	10.7	11.4	25.3	20.8	21.8	18.0
February 2006	11.8	12.9	21.1	17.7	20.7	16.8
Mean months	17.4	20.5	27.1	25.6	27.1	

Significant differences between treatments by month LSD (*P*<0.05) = 4.1; significant differences between months by treatment LSD (*P*<0.05) = 3.8.

3.4.1.5 Soil temperature

No significant differences were seen between treatments in mean daily soil temperature. There was a treatment*date interaction (*P*<0.05) in which the bare-ground control had higher mean soil temperature than the other mulch treatments on warm days, especially after a period of cooler weather. The maximum daily soil temperature was highest (*P*<0.05) in the control, followed by grass, anaerobic *marc*, aerobic *marc* and paper, with respective values of 28, 25, 25, 24 and 22ºC, over all treatments, replicates and days. The minimum daily soil temperature was lowest (*P*<0.01) in the control with a mean of 13.8ºC; for all other treatments it was 15.2-15.6ºC.

3.4.1.6 Soil nutrients

The mulches altered the soil nutrient/mineral status, except under grass it was generally similar to that of the control. However, most of the values from the two *marc* and paper treatments were significantly different to the control, especially the CEC and calcium values (Table 3.7).

3.4.1.7 C:N ratio

The mulch treatments had a significant ($P<0.01$) effect on the mean C:N ratio of the soil, with ratios of 13, 12, 11, 10 and 10, for aerobic marc, paper, anaerobic marc, control and grass, respectively (Table 3.7).

Table 3.7. Mean nutrient concentrations in soil under mulch treatments from three randomly selected blocks in May 2005 (Water extraction, SPLP (US Environmental Protection Agency, 1996)).

Measurement	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper
pH	7.00^{ab}	6.87^{ab}	7.10^{bc}	6.67^{a}	7.47^{c}
CEC cmole^{c} kg^{-1}	16^{a}	18^{a}	29^{b}	25^{b}	24^{b}
Calcium cmole^{c} kg^{-1}	12^{a}	12^{a}	23^{b}	17^{ab}	21^{b}
Magnesium cmole^{c} kg^{-1}	1.68^{a}	2.08^{b}	2.55^{c}	3.60^{d}	1.52^{a}
Olsen P ug mL^{-1}	11^{a}	18^{a}	47^{b}	20^{a}	14^{a}
Potassium cmole^{c} kg^{-1}	0.65^{a}	1.69^{bc}	2.61^{c}	1.89^{bc}	1.11^{ab}
Volume weight	0.85^{b}	0.84^{b}	0.68^{a}	0.75^{a}	0.85^{b}
Carbon:nitrogen	10.45^{ab}	9.80^{a}	11.29^{bc}	13.06^{d}	11.79^{cd}

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$).

3.4.2   Plant attributes

3.4.2.1   <u>Canopy density</u>

Mean pruning weights were reduced ($P<0.05$) by 25-32% in vines under paper (396 g) compared with all other treatments. The pruning weights of all other treatments were 530-564 g of which there were no significant differences (Table 3.8).

3.4.2.2   <u>Leaf nutrients.</u>

Although there were no significant differences in mean total leaf nitrogen levels between treatments, paper had lowest, followed by the control, the two marcs and grass treatments. There were significant differences ($P<0.01$) in copper levels between treatments with the paper treatment having the highest value of 5.3 mg kg^{-1}, followed by aerobic marc, grass, control and anaerobic *marc* with 4.3, 4.3, 4 and 3.3 mg kg^{-1}, respectively (Table 3.8). These differences were unlikely to have any biological significance as normal levels of copper range between 0.5 and 10 mg kg^{-1} in grape leaves (Raath & Schutte, 2001).

3.4.3   Berry attributes

3.4.3.1   <u>Berry skin strength</u>

Vines from the anaerobic *marc* and paper mulches produced berries whose skins were 10% stronger than those from bare ground ($P<0.05$). The berries from these mulch treatments broke under centrifugation of about $1185 \times g$, whereas those from the aerobic *marc*, grass and control treatments broke at 1051, 998 and $962 \times g$, respectively (Table 3.8).

3.4.3.2   <u>Sugar concentration</u>

In 2005 and 2006, mean sugar levels in the berries were elevated from 18.1 in the control, to 19.3-19.5 ºBrix in the two *marc* and the paper mulch treatments ($P<0.01$). Berries from vines under grass had mean sugar levels of 18.7 ºBrix but this was not significantly different from the control. The mean sugar concentrations were higher in 2006 than in 2005 ($P<0.001$), with 20.7 and 17.3 ºBrix, respectively, possibly because the berries were sampled two weeks before harvest in 2005 and at harvest in 2006 (Table 3.8).

Table 3.8. Mean values of plant and berry attributes for grapevines with the different mulch treatments.

		Time/year	Control	Grass	Anaerobic *marc*	Aerobic *marc*	Paper	All treatments
					Mulches			
Plant attributes	Pruning wt (kg/vine)	2005	0.53^a	0.55^a	0.56^a	0.54^a	0.40^b	
	Nitrogen (%)	2005	1.1^a	1.3^a	1.4^a	1.1^a	1.0^a	
	Leaf copper (mg/kg)	2005	4.0^{ab}	4.3^b	3.3^a	4.3^b	5.3^c	
	Carbon:nitrogen	2005	10.5^{ab}	9.8^a	11.3^{bc}	13.1^d	11.8^{cd}	
Berry attributes	Skin strength (g force)	2005	962^a	998^a	1180^b	1051^{ab}	1192^b	
	°Brix	2005	15.9^a	17.1^{ab}	17.9^b	18.2^b	17.6^b	17.3^x
		2006	20.4^a	20.2^a	21.1^b	20.7^{ab}	21.0^b	20.7^y
		Both years	18.1^a	18.7^{ab}	19.5^c	19.5^c	19.3^{bc}	
	Phenolics (g/100ml)	2005	0.17^a	0.20^a	0.19^a	0.19^a	0.20^a	
	B. cinerea challenge (% infection of non punctured inoculated berries)	Day 7	3	0	0	0	0	0.6^x
		Day 11	16	2	2	5	0	5^y
		Day 15	33	9	13	15	5	15^z
		Mean of all days	17^a	4^b	5^b	7^{ab}	1.6^b	
	Latent infection (%)	Day 7	2.9^a	1.8^a	2.3^a	2.6^a	1.4^a	
	Bunch number/vine	2005	29.4^a	32.4^a	31.8^a	32.9^a	30.8^a	31.5^x
		2006	14.8^a	15.8^{ab}	21.0^{bc}	21.9^c	21.2^c	18.9^y
		Both years	22.1^a	24.1^{ab}	26.4^b	27.4^b	26.0^b	
	Yield/vine	2006	806^a	849^a	1249^b	1298^b	1354^b	

^{abc} Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). ^{xyz} Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$). Significant differences in *B. cinerea* challenge between treatments by day LSD ($P<0.05$) = 0.104; significant differences in *B. cinerea* challenge between days by treatment LSD ($P<0.05$) = 0.587.

3.4.3.3 Total phenolics

The mulch treatments caused differences (P=0.07) in total berry phenolics, with the mean lowest level in the control treatment (0.164 g/100 ml), followed by anaerobic marc, aerobic marc, paper and grass treatments with values of 0.187, 0.192, 0.196 and 0.201 g/100 ml respectively (Table 3.8).

3.4.3.4 Berry nutrients

No significant differences in berry nutrients occurred between treatments.

3.4.3.5 Resistance of berries to *B. cinerea*

3.4.3.5.1 Non-wounded berries

All mulches significantly (P<0.05) increased the ability of non-wounded berries to resist infection by *B. cinerea*, with berries from shredded paper, grass, anaerobic *marc* and aerobic *marc* treatments having 10, 21, 29 and 38% the disease incidence of the control berries (Table 3.8). As expected there was a time effect (P<0.001) where disease increased over time. There was also a time*treatment interaction (P<0.001) as the berries from the control treatment showed a steady increase of *B. cinerea* over time, whereas all other treatments had low rates of infection at day 7 and 11 after inoculation, but a marked increase on day 15.

3.4.3.5.2 Wounded berries

There were no significant differences between treatments.

3.4.3.5.3 Latent infection

There were no significant (*P*=0.11) differences between treatments, but a trend was observed where the control had the highest infection incidence followed by aerobic *marc*, anaerobic *marc*, grass and paper (Table 3.8).

3.4.3.6 Bunch yield

In 2005, there were no significant differences between treatments, although vines from all mulch treatments had higher mean bunch numbers than the control (Table 3.8). In 2006, vines from the two *marc* and paper treatments had significantly more (P<0.05) bunches per vine, with means of 21-22, compared to 15 in the control. The mean number of bunches per vine was significantly higher (P<0.001) in 2005 than in 2006. In 2006, bunch weight per vine was 1.5 times higher (P<0.01) in the two *marc* and paper treatments than in the control and grass

treatments. Total bunch weight increased (P<0.01) from 806 and 849 g/vine in the control and grass treatments, respectively, to 1249, 1298 and 1354 g/vine in the anaerobic marc, aerobic *marc* and paper treatments, respectively.

3.4.3.7 *B. cinerea* infection of berries at harvest

The two *marc* and paper treatments significantly (P<0.05) reduced the severity of *B. cinerea* (infected berries/vine) from 1.2 in the control, to 0.75, 0.28, 0.07 and 0.04% in the grass, anaerobic marc, aerobic *marc* and paper, respectively, in 2005. In 2006, respective data were 11, 8.8, 5.1, 5 and 5 (Figure 3.2). Disease severity was greater (P<0.001) in 2006 than 2005, with infection rates of 6.96% and 0.47% respectively per vine, averaged over all treatments.

Figure 3.2. Percentage of grapes with visible signs of *B. cinerea* sporulation on vines at harvest 2005 and 2006 and the economic threshold for control for botrytis rot in Riesling.
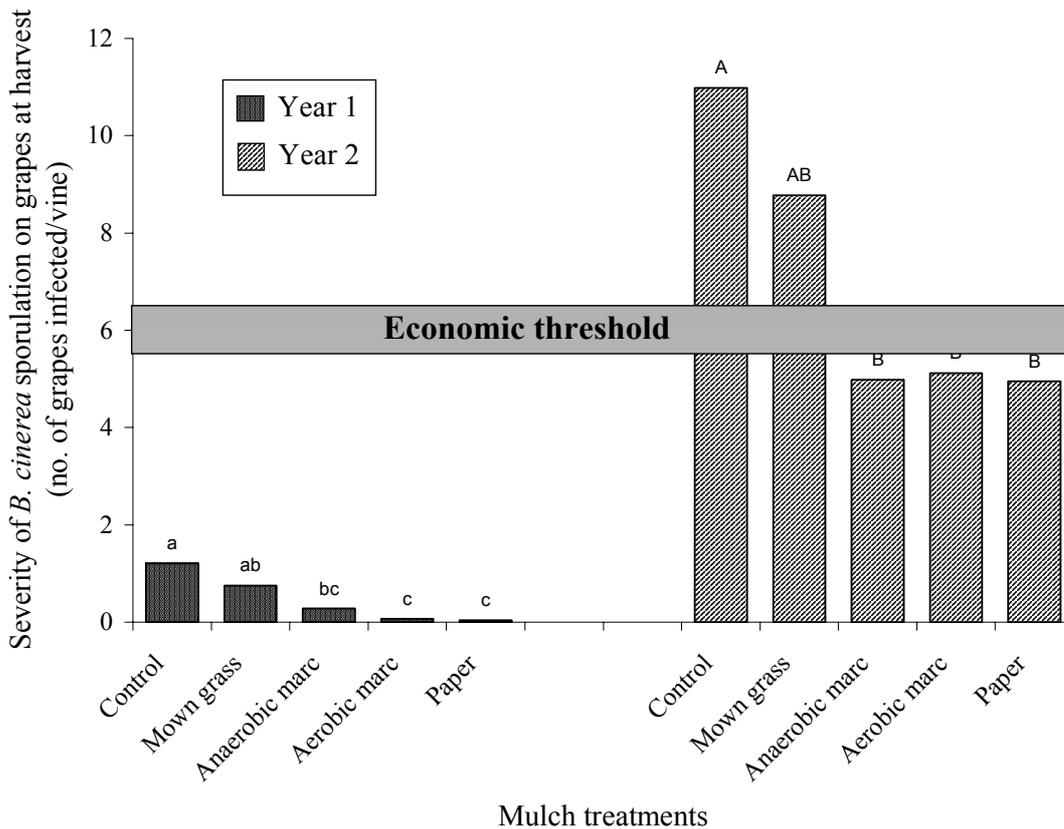
[abc] Letters denote significant differences calculated through Fisher's Protected LSD (*P*<0.05).

3.4.4 Correlations

Correlation analysis (Table 3.9) demonstrated the links between various soil and vine parameters in 2005 and 2006.

Table 3.9. Correlation values, showing relationships and their significance between soil and vine characteristics in 2005 and 2006.

Year			Bait lamina probes	Daily soil temperature range	Berry resistance to *B. cinerea*	Soil calcium	Soil CEC	Total vine yield	Bunch number per vine
2005	Mean soil temperature	R value	-0.431						
		P value	(*P*<0.01)						
	Daily soil temperature range	R value	-0.644						
		P value	(*P*<0.001)						
	Soil moisture	R value	0.499	-0.546	-0.374				
		P value	(*P*<0.001)	(*P*<0.001)	(*P*<0.05)				
	Berry skin strength	R value	0.412			0.500	0.418		
		P value	(*P*<0.05)			*P*=0.058	*P*=0.12		
	B. cinerea levels at harvest	R value			0.330				
		P value			(*P*<0.05)				
2006	Berry sugar (ºBrix)	R value						0.292	
		P value						(*P*<0.05)	
	B. cinerea levels at harvest	R value						-0.320	-0.335
		P value						(*P*<0.05)	(*P*<0.05)

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3.5 Discussion

3.5.1 Soil characteristics

The results from the tests with Biolog Ecoplates and bait lamina probes indicated that the two *marc* and the paper mulches increased soil biological activity in a commercial vineyard. The increased activity may have been due to the greater diversity and numbers of organisms in the soil microfauna communities. The increase in biological activity under these three treatments was probably largely due changes in various physical and chemical properties of the soil. Soil temperature was stabilised under the mulches, an effect likely to be related to their insulative properties, and in paper (white) the high degree of reflected radiation energy. Soil moisture was higher under the mulches, an affect partially attributed to insulation and lower soil temperatures, but also to the high water-holding capacity of the mulches because of their high organic matter content (Li *et al*., 2004) and low bulk densities (Table 1.1). An increase in soil moisture of 34% was also found under straw, another low bulk density organic mulch, in South Australia (Buckerfield & Webster, 1996). The C:N ratio of the soil was also elevated under paper as it was a high carbon source, and as soil organisms incorporated this carbon into the soil, nitrogen was removed (Frey *et al*., 2003).

The C:N ratio of these soils were all at or below 13, a ratio that commonly indicates the soil is well aerated, microbially active and conducive to healthy vine growth (Raath & Schutte, 2001). Low soil nitrogen reduces vine vigour (Mullins *et al*., 1992) and this was reflected by the lower pruning weights from the paper treatment. On sites with high vigour, mulched paper could be used to devigorate vines and reduce pruning costs.

Of the two soil biological activity assessments, analysis of data from bait lamina probes was simplest to perform. Data from Biolog Ecoplates were much more complex and would be more suitable for identifying functional groups of organisms, especially when used alongside denaturing gradient gel electrophoresis (DGGE) (Heuer *et al*., 1997) or other molecular techniques.

3.5.2 Grapevine characteristics

The two *marc* and paper treatments increased canopy aeration, grape skin strength, ºBrix, resistance to infection, grape yield and reduced *B. cinerea* levels on the vines at harvest. These changes were probably effected by changes in soil physical and chemical properties, soil biological activity and vine characteristics such as canopy density. Low canopy density is important in regards to disease and fruit quality as it allows higher light penetration and fruit

temperature, and lower canopy humidity (Mullins *et al.*, 1992). These changes can reduce the incidence of diseases, including botrytis bunch rot, downy mildew and powdery mildew (Pearson & Goheen, 1988). High light penetration into the canopy elevates grape yield (Mullins *et al.*, 1992) as well as quality parameters such as sugar content (Mullins *et al.*, 1992) and skin to pulp ratio (Keller *et al.*, 1998). Increased skin quality, measured as thickness (Karadimcheva, 1981) and elasticity (Matthews *et al.*, 1987), can increase grape resistance to disease, an effect correlated with berry development (Matthews *et al.*, 1987). Skin strength/resistance to disease is also improved by vine nutrition, especially in regards to calcium (Chardonnet & Doneche, 1995; Huang *et al.*, 2005). These effects of light and calcium on skin strength are supported by the current work in which berries which had the highest skin strength came from treatments with either low canopy density, in the case of paper, or from soils with high calcium and CEC values, which increases the efficiency of nutrient uptake (Li *et al.*, 2004) as with the two *marcs* and paper treatments.

Phenolics and other antifungal compounds also protect the grape berries from infection (Bavaresco *et al.*, 1997). They are found in the berry skin and pulp (Bavaresco *et al.*, 1997) and vary in concentration and constitution by variety, the stage of development of the berry (Bavaresco *et al.*, 1997) and environmental conditions. Research has shown that excessive light exposure (Haselgrove *et al.*, 2000) and nitrogen fertiliser (200 g/vine) (Delgado *et al.*, 2004) reduced concentrations of anti-fungal compounds in grapes, while moderate levels of light (Bergqvist *et al.*, 2001) and nitrogen (50 g/vine) (Delgado *et al.*, 2004) increased their concentrations. High soil moisture also reduces the concentration of phenolic compounds in grape berries (Nadal & Arola, 1995; Ginestar *et al.*, 1998; Sivilotti *et al.*, 2005) due to rapid increases in berry size (Ginestar *et al.*, 1998). In this trial, mulched grass had the highest levels of soil nitrogen, low soil moisture, the lowest bunch weight, high concentrations of phenolic compounds, low latent infection and high disease resistance to *B. cinerea* infection in inoculated berries, all characteristics which support previous research in the relationships between these variables. In this trial, the mulch treatments which had high berry phenolic concentrations and/or high skin strength also had high levels of disease resistance to *B. cinerea*. When the physical and chemical barriers were removed (punctured berries), no significant differences were seen between treatments, which demonstrates the importance of these functions in berry resistance to *B. cinerea* infection.

Sugar content of berries was highest with the two *marc* and paper treatments. These findings support previous research which found water stress (Wang *et al.*, 2003; Rubio *et al.*, 2004), low levels of light penetration (Mullins *et al.*, 1992) and canopy temperature (Mullins *et al.*,

1992) to cause a decrease in sugar accumulation in the fruit. The degree of these effects differ, according to the grape variety and is probably also related to the origin of the vine (Schultz, 1997). These three mulches also increased vine yield, which is influenced by environmental conditions at flowering, fruit set and the early stages of grape expansion (Wang *et al.*, 2003). Flowering and fruit set in grapevines is increased by light exposure (Mullins *et al.*, 1992; Ferree *et al.*, 2001), periods of high canopy temperature (Mullins *et al.*, 1992; Ebadi *et al.*, 1995), adequate nutrition (Mullins *et al.*, 1992; Bravdo, 2000) and soil moisture (Hardie & Considine, 1976; Matthews & Anderson, 1989; Mullins *et al.*, 1992). Similarly, berry size and bunch weight are positively correlated with soil moisture (Smithyman *et al.*, 2001), predominantly during the early stages of berry development when they grow rapidly (Wang *et al.*, 2003). Similarly, adequate light exposure (Kliewer & Lider, 1968), canopy temperature (Klenert *et al.*, 1978) and soil nutrient levels (Mullins *et al.*, 1992) also increase berry size and bunch weight.

In this experiment, the two *marc* and paper mulches increased soil moisture and soil nutrient availability through an elevated CEC. The paper treatment also increased light penetration into the canopy and possibly increased inner canopy and berry temperature (Bergqvist *et al.*, 2001). These changes to the soil and canopy environment may have led to the observed increases in bunch number, yield and *B. cinerea* severity as shown with the use of straw and mulched cover crops in a vineyard in South Australia, where a 46% increase in yield was linked to elevated soil moisture and earthworm presence (Buckerfield & Webster, 1996).

In both years of the current work, the two *marc* and paper treatments caused significant reductions in *B. cinerea* severity on bunches at harvest. In 2006, pathogen sporulation was reduced from 11% bunch coverage in the control to 5% in these treatments, a severity which lies below the economic threshold of between 5.5 and 6.5% in this grape variety (Trought, M., 2006. *pers. comm.*; Creasy, G., 2006. *pers. comm.*; Balasubramaniam, R., 2006. *pers. comm.*). In addition, the physiological changes to grape disease resistance, such as skin strength and possible chemical responses to disease could also be applicable to insect pests, either directly or indirectly, through a lower rate of disease facilitated by insect-herbivore feeding.

CHAPTER 4: The effects of mulched cover crops on *Botrytis cinerea* primary inoculum and severity of infection in inflorescences and grapes.

4.1 Abstract

In the current work, cover crops mulched *in situ* increased soil biological activity, reduced *B. cinerea* primary inoculum and reduced the severity of *B. cinerea* infection in grapes at harvest 2006. The mulched clippings from inter-row phacelia and ryegrass were spread under 10-year-old Chardonnay vines in winter 2005. The effects of the mulches were compared with a bare ground control, in a five-replicate randomized block design. Functional soil biological activity increased by 1.5-4.5 times in the two cover crop treatments compared with the control, an effect possibly related to elevated soil moisture in these treatments. This increase in soil moisture and soil biological activity increased vine debris degradation, reduced *B. cinerea* primary inoculum on the debris and decreased *B. cinerea* severity at flowering (December 2005) and harvest (April 2006). These results show the potential of mulched cover crops to enhance soil ecosystem services and improve the sustainability of viticultural practices. Other possible mechanisms operating in this system are also discussed.

4.2 Introduction

Control of *B. cinerea* with the prophylactic application of agrichemicals is increasingly being seen as unsustainable, and alternative methods of control are being sought. Conservation biological control techniques may be integrated into current control regimes to achieve this. The technology can reduce *B. cinerea* inoculum, increase vine disease resistance and reduce botrytis bunch rot in vines as shown in Chapter 2. In continuation of this work, the mulches increased vine resistance to botrytis bunch rot and subsequently reduced severity of the

disease in bunches under high and low disease pressure, as well as increasing grape quality and yield (Chapter 3).

The aim of the current work was to investigate the effects of inter-row cover crops grown in the vineyard and subsequently mulched on soil biological activity and moisture, vine debris degradation and *B. cinerea* primary inoculum and severity in the vines at flowering and harvest. The extent to which these parameters were correlated were investigated and the possible mechanisms causing the observed effects are discussed.

4.3 Materials and methods

4.3.1 The field site

The research site was in a block of 10-year-old Chardonnay vines (Clone mvig-1) grafted to a phylloxera-resistant rootstock (variety 5C) in Seresin Estate Ltd (Section 2.3.1 - Chapter 2). Vines were grown on vertical shoot position (VSP) trellis, with drip irrigation and pruned to two canes.

4.3.2 Experimental design

Phacelia (*Phacelia tanacetifolia* Benth., cv. Balo) and perennial ryegrass (*Lolium perenne* L., cv. Kingston)[6] were drilled 10-15 mm deep in the inter-row spaces at a rate of 5 and 25 kg/ha, respectively, into a lightly cultivated seedbed on 16 March 2005 in a 5-block, randomised block design. Each of the treatments comprised a 40 m long strip (phacelia, ryegrass and control), in each of two adjacent inter-row spaces. These spaces were 2 m wide and the strip occupied the central 1.6 m wide area of each inter-row space. These three treatments were randomly positioned with respect to each other for each of the five blocks. Each block was 30 m long and 40 m wide, comprising a 15 row area (Figure 4.1).

On 7 July 2005, the cover crops were mowed with a rotary lawn mower, that caught the clippings. The clippings were then applied as a mulch by hand at an initial thickness of approximately 100 mm under two randomly-selected vines in the vine row between the pair of strips (Figure 4.1). These formed two pseudo-replicate plots 0.4 × 2 m, 16.2 m apart and 11.9 m from the edge of the block, whose results were averaged for data analysis. At monthly

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[6] In addition to phacelia and perennial ryegrass, Forage brassica (*Brassica napus* L spp. *biennis* cv. Winfred), Italian Ryegrass (*Lolium multiflorum* Lam. cv. Crusader) and Lucerne (*Medicago sativa* L. subsp. *Sativa*) were also sown (Figure 4.2). These three latter crops failed to establish so were excluded from this trial and as by chance each of the three crops that established, were always either on the northern or southern end of each block, the experimental design could be simplified (Figure 4.1).

Figure 4.1. The experimental design of a block (1 of 5) adapted for the current work after three cover crops failed.
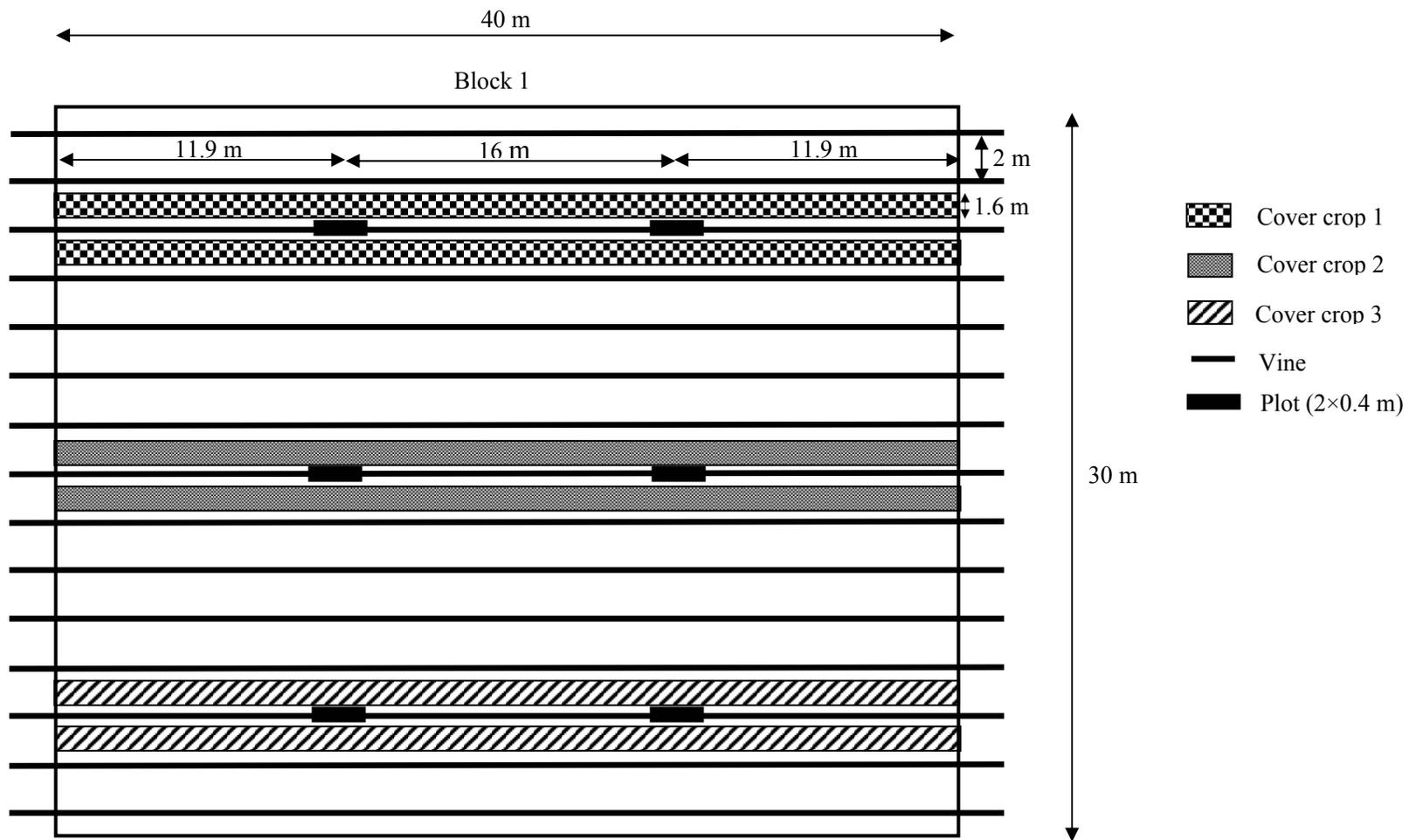
Figure 4.2. Original experimental design, with five cover crops and a bare-ground, under-vine (existing sward inter-row) control.
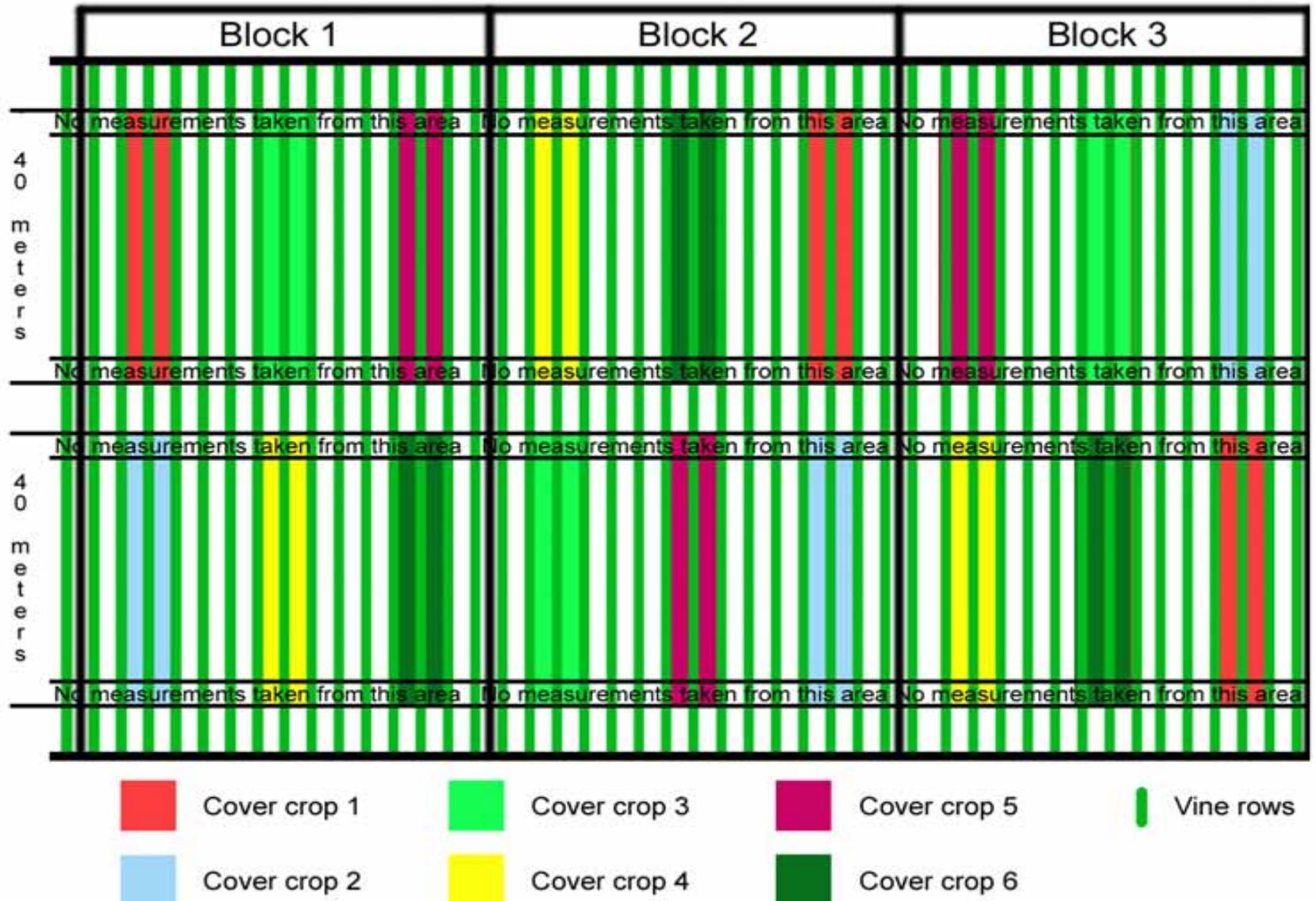
intervals, the cover crops were re-mown and the catchings were reapplied to maintain the thickness of the mulch at 100 mm.

4.3.3   Vine debris bags

The same vine debris types, fragment sizes and numbers as in year 2 (2004), Section 2.3.3.2, Chapter 2 for cane and rachis only, were enclosed in the same types of vine debris bags. Two bags with intact debris and two with fragmented material were placed under the mulch in each pseudo-replicate vine. At the time of application, assessment of initial *B. cinerea* levels on vine debris was done as described in Chapter 2. Half the 120 bags placed in the vineyard were collected for assessment when the vines reached 50% flowering (8 December 2005) and the remainder were collected at leaf plucking (8 February 2006).

4.3.3.1   *B. cinerea* sporulation from vine debris

Vine debris was incubated and assessed for conidiophore coverage as described in Section 2.3.3.3, Chapter 2.[7]

4.3.3.2   Degradation of vine material

After sporulation had been assessed on all debris items, they were dried at 70ºC for 20 days and the dry-fresh-weight ratio for each debris type was calculated (as in Section 2.3.3.4, Chapter 2).

4.3.3.3   Potential of mulch to harbour *B. cinerea*

In January 2005, the two mulched cover crops, soil from the bare ground control and a necrotic Chardonnay leaf (positive control) were inoculated with *B. cinerea* mycelium and conidia, as described in Section 2.3.3.5, Chapter 2.

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[7] The degree to which spores released from debris would be trapped by the standing cover crops was also assessed in a wind tunnel and field experiment. *B. cinerea* conidia were released from sporulating PDA plates in a wind tunnel, then caught on trap plates, incubated and assessed. This was found to not accurately simulate field conditions. A field trial was then conducted following a modified version of the methodology used by Seyb (2004) with Nit mutants caught on selective agars. These plates had high levels of biological contaminants that outgrew *B. cinerea* on various selective agars and the technique was abandoned. A full description of both methods and results are presented in Appendix 2 (wind tunnel) and Appendix 3 (field trial).

4.3.4 Soil attributes

Bait lamina probes, Biolog Ecoplates, antibiotic activity of soil filtrates and soil moisture were assessed monthly from August 2005 to February 2006. Biolog Ecoplate and antibiotic activity assessments were made for blocks 1, 2 and 3, which were randomly selected at the start of the trial; bait lamina probes and moisture level assessments were made for all replicates[8].

4.3.4.1 Biolog Ecoplates

Five 50 g soil samples were taken monthly from the soil/mulch interface from each treatment in three randomly selected replicates and assessed as described in Section 3.3.2.1, Chapter 3.

4.3.4.2 Antibiotic activity of filtrates from soil under the mulch

The antibiotic activity of soil filtrates from the soil/mulch interface were assessed as described in Section 3.3.2.2, Chapter 3

4.3.4.3 Bait lamina probes

Bait lamina probes were assessed monthly as described in Section 3.3.2.3, Chapter 3.

4.3.4.4 Soil moisture

Soil moisture was assessed as described in Section 3.3.2.4, Chapter. 3

4.3.5 Vine attributes

4.3.5.1 Bunch number per vine

At harvest, all bunches were counted in one randomly-selected vine from each replicate.

4.3.5.2 *B. cinerea* infection of flowers

At 50% flowering (8 December 2005), two randomly selected inflorescences were removed from each of the two pseudo-replicate vines in each replicate. These inflorescences were immediately

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[8] Soil temperature was also measured continually from 19 November 2005 to 20 March 2006 at 15 minute intervals by a Campbell CR10 data logger. Due to time constraints, a Seresin staff member undertook the installation of soil probes and the underground, inter-row pipe in which the probe wire was contained. All probes were verbally verified to be at 30 mm below the soil/mulch interface and were then tested to be operational and accurate. However, on analysis of data collected from these probes, temperatures were highly variable. On further inspection, several probes were found to have been put under rocks and so the data had to be discarded.

inserted into growth medium blocks (GMB) (Oasis, $200 \times 100 \times 70$ mm) previously saturated with water and were then transported to the laboratory in closed polystyrene boxes; they were then incubated at room temperature in the boxes for 4 days. The proportion of the inflorescence area with sporulating *B. cinerea* was then assessed by eye.

4.3.5.3 *B. cinerea* infection of bunches at harvest

  At harvest 2006, a survey of sporulating *B. cinerea* (percent bunch covered) was done in all bunches in each of the two pseudo-replicate vines from each treatment replicate.

4.3.6 Statistical analysis

All data were analysed by ANOVA, except data from Biolog Ecoplates and antibiosis of filtrate which were analysed by repeated measures ANOVA. Correlations were tested using Spearman's correlation coefficient. All data sets conformed to a normal distribution, so no transformations were made. The statistical package used for all analyses was Genstat 7.

4.4 Results

4.4.1 Vine debris bags

4.4.1.1 *B. cinerea* sporulation from vine debris

Compared with the control, mean *B. cinerea* conidiophore coverage on vine debris from under ryegrass and phacelia mulch was reduced on canes by 6 and 18 fold, respectively ($P<0.001$) and on rachides by 6 and 7 fold, respectively ($P<0.001$) (Table 4.1). On canes, *B. cinerea* levels were 98 times higher ($P<0.001$) at 50% flowering than at leaf plucking. On rachides, infection rates were 17 times higher ($P<0.001$) at 50% flowering than at leaf plucking. In both debris types there was a treatment*date interaction ($P<0.001$) as the mulches significantly reduced *B. cinerea* coverage at 50% flowering but not at leaf plucking, due to low disease levels overall. Debris fragment size had no effect on *B. cinerea* sporulation.

Table 4.1. Mean percentage conidiophore coverage of fragmented and whole vine debris at 50% flowering and leaf plucking.

Debris type	Assessment date	Debris size	Mulched cover crops			Mean treatments
			Control	Phacelia	Ryegrass	
Cane	Both dates	Fragmented	1.8	0.1	0.1	0.7
Cane	Both dates	Whole	0.9	0.0	0.3	0.4
Cane	50% flowering	Both sizes	2.3[a][x]	0.1[b][x]	0.4[b][x]	0.9[x]
Cane	Leaf plucking	Both sizes	0.0[a][y]	0.0[a][x]	0.0[a][x]	0.0[y]
Cane	50% flowering	Fragmented	3.2	0.2	0.3	
Cane	50% flowering	Whole	1.5	0.1	0.5	
Cane	Leaf plucking	Fragmented	0.1	0.0	0.0	
Cane	Leaf plucking	Whole	0.0	0.0	0.0	
Cane	Both dates	Both sizes	1.3[a]	0.1[b]	0.2[b]	
Rachis	Both dates	Fragmented	19.8	2.7	3.0	8.1
Rachis	Both dates	Whole	20.8	3.2	3.8	8.9
Rachis	50% flowering	Both sizes	33.9[a][x]	4.8[b][x]	6.1[b][x]	14.7[x]
Rachis	Leaf plucking	Both sizes	1.9[a][y]	0.9[a][x]	0.0[a][y]	0.9[y]
Rachis	50% flowering	Fragmented	32.5	5.0	5.3	
Rachis	50% flowering	Whole	35.1	4.5	6.8	
Rachis	Leaf plucking	Fragmented	3.3	0.0	0.0	
Rachis	Leaf plucking	Whole	0.4	1.7	0.0	
Rachis	Both dates	Both sizes	20.3[a]	2.9[b]	3.4[b]	

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$).[xy] Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$).

4.4.1.2 Degradation of vine material

Both mulch treatments caused a 1.1-1.4-fold increase ($P<0.05$) in mean debris degradation in canes compared with the control, with rates of 27%, 31% and 38% in the control, phacelia and ryegrass, respectively (Table 4.2). The mulches increased ($P<0.001$) debris degradation in

rachides from 79% in the control to 91% in phacelia and 92% in ryegrass. Degradation was higher ($P<0.05$) in fragmented debris than whole debris for cane, being 36% and 28%, respectively, whereas the increase was not statistically significant in rachides. The degradation rate was lower ($P<0.001$) at 50% flowering than at leaf plucking for canes, being 14% and 50%, respectively, and for rachis ($P<0.001$), which had rates of 80% and 94%, respectively.

Table 4.2. Mean percentage debris degradation of fragmented and whole vine debris at 50% flowering and leaf plucking.

Debris type	Assessment date	Debris size	Mulched cover crops			All treatments
			Control	Phacelia	Ryegrass	
Cane	Both dates	Fragmented	31	31	46	36[x]
Cane	Both dates	Whole	23	30	31	28[y]
Cane	50% flowering	Both sizes	9	14	19	14[x]
Cane	Leaf plucking	Both sizes	45	47	57	50[y]
Cane	50% flowering	Fragmented	11	15	29	18
Cane	50% flowering	Whole	7	13	9	10
Cane	Leaf plucking	Fragmented	51	47	62	54
Cane	Leaf plucking	Whole	38	47	52	46
Cane	Both dates	Both sizes	27[a]	31[b]	38[b]	
Rachis	Both dates	Fragmented	76	92	96	88
Rachis	Both dates	Whole	81	90	88	86
Rachis	50% flowering	Both sizes	68	86	87	80[x]
Rachis	Leaf plucking	Both sizes	89	96	98	94[y]
Rachis	50% flowering	Fragmented	66	86	92	81
Rachis	50% flowering	Whole	70	86	81	79
Rachis	Leaf plucking	Fragmented	87	98	100	95
Rachis	Leaf plucking	Whole	91	94	96	94
Rachis	Both dates	Both sizes	79[a]	91[b]	92[b]	

[abc] Letters denote significant differences within rows calculated through Fisher's Protected LSD ($P<0.05$). [xy] Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$)

4.4.1.3 Potential of mulch to harbour *B. cinerea*

No significant differences were seen between mulch treatments (Table 4.3).

Table 4.3. The mulches' ability to host *B. cinerea* from mycelium spread from a mycelium plug (mm growth from plug) or infection from dry spore inoculation (% coverage), in comparison with a necrotic leaf (positive control).

	Mulch type	Assessment times				All times
		1	2	3	4	
Mycelium plug	Control (soil)	0.4	2.2	4.3	4.8	2.9^x
	Phacelia	0.0	1.4	2.5	3.2	1.8^x
	Ryegrass	0.0	1.5	1.7	1.8	1.3^x
	Positive control (Chardonnay)	22.8	25.0	25.0	25.0	24.5^y
Dry spore	Control (soil)	0.0	0.0	0.0	0.0	0.0^x
	Phacelia	0.6	5.0	5.6	8.1	4.8^x
	Ryegrass	0.6	1.3	1.3	1.3	1.1^x
	Positive control (Chardonnay)	36.3	97.5	100.0	100.0	83.4^y

^{xy} Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$)

4.4.2 Soil attributes

4.4.2.1 Biolog Ecoplates

Soil organisms under mulch used 3.6-3.9 times the number of carbon compounds in the Biolog Ecoplates than that in the control treatment ($P<0.001$) (Table 4.4). There was a strong seasonal effect ($P<0.001$), in which the utilisation of carbon materials in the Ecoplates varied six fold over the year. A treatment*month interaction ($P<0.01$) was also present, with increasing activity in

mulch treatments and more differences between the two mulch treatments at the beginning of the trial than the end, but bare ground followed a different trend (Table 4.4)[9].

Table 4.4. Mean percentage of Biolog Ecoplate wells that had an absorbance reading above the threshold of 0.8 and percentage of bait used in bait lamina probes in the different mulch treatments, mean of all soil depths.

Assessment	Month	Control	Phacelia	Ryegrass	All treatments
Biolog Ecoplates	August 2005	7	4	11	7^w
	September 2005	17	29	17	21^x
	October 2005	4	16	12	11^w
	November 2005	11	45	37	31^y
	December 2005	13	68	56	46^z
	January 2006	9	56	60	42^{yz}
	February 2006	6	48	52	36^y
	All months	10^a	38^b	35^b	
Bait lamina probes	August 2005	0	4	0	1^x
	October 2005	14	27	38	26^z
	November 2005	3	12	26	13^y
	December 2005	9	39	35	28^z
	January 2006	9	42	24	25^z
	February 2006	3	33	46	27^z
	All months	6^a	26^b	28^b	

[abc] Letters denote significant differences within row calculated using Fisher's Protected LSD ($P<0.05$). [xy] Letters denote significant differences within columns calculated through Fisher's Protected LSD ($P<0.05$)

4.4.2.2   Antibiotic activity of filtrates from soil under the mulch

No significant differences were seen between treatments.

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[9] In addition to the proportion of carbon compounds used, the mean absorbance for each plate was also assessed for each treatment replicate for each month, the data of which is presented in Table 4.5

4.4.2.3   Bait lamina probes

Over all assessment months, the soil biota under the two mulch treatments used 4.3 to 4.7 more bait than did those in the control ($P<0.05$) (Table 4.4) and there was a strong seasonal effect ($P<0.001$). A treatment*month interaction ($P<0.001$) occurred where small differences were seen between treatments in the initial stages of the trial, while large differences were seen at the end.

Table 4.5. Mean absorbance value of Biolog Ecoplate wells, for soils under each mulch, with monthly assessments.

Assessment	Month	Control	Phacelia	Ryegrass	All treatments
Biolog Ecoplates	August 2005	0.48	0.45	0.46	0.47^a
	September 2005	0.57	0.65	0.57	0.60^b
	October 2005	0.40	0.54	0.49	0.48^a
	November 2005	0.56	0.78	0.71	0.68^b
	December 2005	0.59	0.96	0.90	0.82^d
	January 2006	0.53	0.87	0.91	0.77^{cd}
	February 2006	0.48	0.80	0.84	0.71^{bc}
	All months	0.52^a	0.72^b	0.70^b	

^{abc} Letters denote significant differences calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by month LSD ($P<0.05$) = 0.06; significant differences between months by treatment LSD ($P<0.05$) = 0.04.

4.4.2.4   Soil moisture

Over all assessment months, mean soil moisture increased from 13% in the control to 21% in the two mulch treatments ($P<0.001$) (Table 4.6). There was a seasonal effect, with winter months having higher ($P<0.001$) soil moisture (19-24%) than summer months (15-17%). A treatment*month interaction ($P<0.001$) occurred, with the control having higher fluctuations in soil moisture throughout the season (8-24%) than the two mulch treatments (16-25%).

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Table 4.6. Mean percent soil moisture under each mulch, with monthly assessments.

Month	Control	Phacelia	Ryegrass	All treatments
August 2005	24.3	23.6	25.5	24.5^e
September 2005	15.1	20.9	20.5	18.8^c
October 2005	18.7	22.6	23.1	21.5^d
November 2005	7.6	19.6	19.3	15.5^{ab}
December 2005	13.0	16.3	17.7	15.6^{ab}
January 2006	9.5	20.7	21.7	17.3^{bc}
February 2006	8.5	21.1	15.5	15.0^a
All months	13.8^a	20.7^b	20.5^b	

^{abc} Letters denote significant differences calculated through Fisher's Protected LSD ($P<0.05$). Significant differences between treatments by month LSD ($P<0.05$) = 4.5; significant differences between months by treatment LSD ($P<0.05$) = 3.9.

4.4.3 Vine attributes

4.4.3.1 Bunch number per vine

There was no significant difference between treatments in bunch number per vine.

4.4.3.2 *B. cinerea* infection of flowers

Both mulch treatments decreased ($P<0.001$) mean *B. cinerea* conidiophore coverage of flowers by up to 10 times, from 15% in the control to 1.5% with ryegrass and 5.5% with phacelia (Figure 4.3).

4.4.3.3 *B. cinerea* infection of bunches at harvest

The mulch treatments significantly reduced ($P<0.05$) *B. cinerea* severity (% bunch area covered with sporulating *B. cinerea* per vine), from 2% in the control to 0.4% and 0.09% in the ryegrass and phacelia treatments, respectively (Figure 4.3).

Figure 4.3. Mean percent *B. cinerea* conidiophore coverage visible per vine inflorescence, incubated under laboratory conditions, and per vine on grape bunches at harvest. The industry economic threshold for *B. cinerea* on Chardonnay grape bunches at harvest in New Zealand is shown.
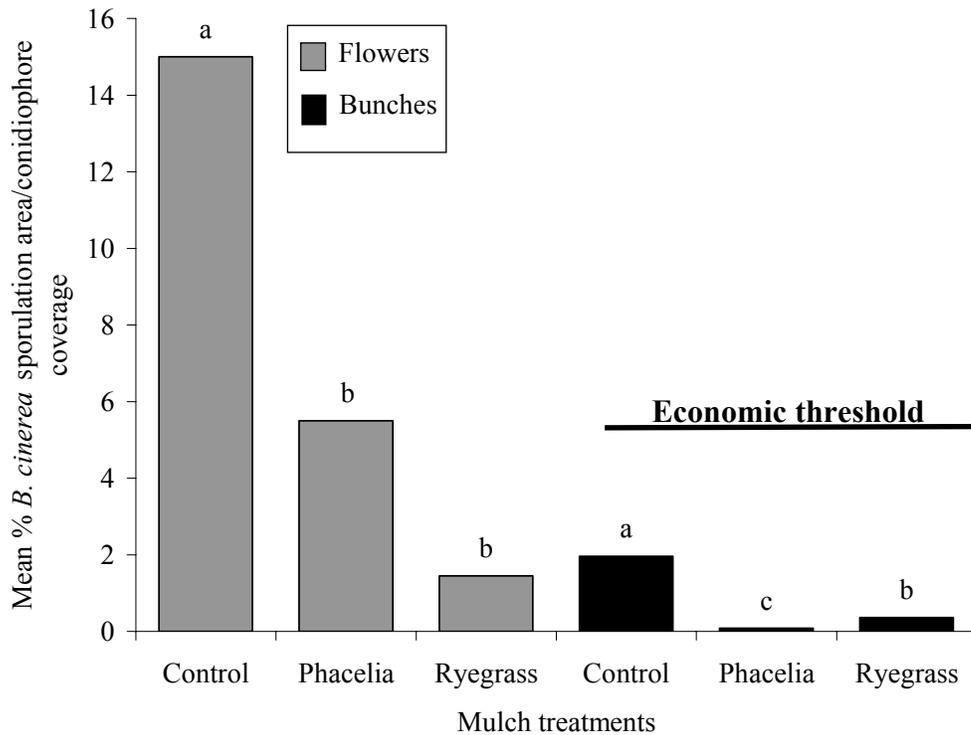
[abc] Letters denote significant differences calculated through Fisher's Protected LSD (*P<0.05*).

4.4.4 Correlations

B. cinerea sporulation rates on fragmented cane were negatively correlated with results from bait lamina probes (*P<0.01*, r = -0.302). *B. cinerea* sporulation rates and debris degradation were correlated on fragmented cane (*P<0.05*, r = -0.275), whole cane (*P=0.054*, r = -0.271), fragmented rachis (*P<0.001*, r = -0.726) and whole rachis (*P<0.001*, r = -0.688). Debris degradation rates for fragmented rachides were also correlated with soil moisture (*P<0.01*, r = 0.384). Results from bait lamina probes and Biolog Ecoplates were positively correlated (*P<0.01*, r = 0.400). *B. cinerea* severity in bunches at harvest were also correlated with *B. cinerea* sporulation rates on vine debris (*P<0.01*, r = 0.514).

4.5 Discussion

Mulched phacelia and ryegrass reduced *B. cinerea* inoculum produced from vine debris compared with the control. These differences were most pronounced at flowering when primary inoculum is very important, as it can affect fruit set and cause latent infection (Mullins *et al.*, 1992). The correlation between *B. cinerea* infection at flowering and *B. cinerea* conidiophore coverage on whole cane, demonstrates the importance of the interventional use of mulches early in the season. The reduction in primary inoculum is likely to be related to high biological activity under the experimental treatments, through soil biota competing with *B. cinerea* for space and resources, which also resulted in increased debris degradation rates. Debris degradation was highest when the material was fragmented, as the increased surface area provided soil biota with greater access to internal, less lignified plant materials, which are more readily degraded by soil organisms (Moore-Landecker, 1972). This effect was most pronounced in cane as differences in lignin levels between internal and external material are proportionally much greater in this plant material (Mullins *et al.*, 1992). In this debris type, the degradation rate for all treatments was similar between 50% flowering and leaf plucking, as the availability of readily digestible low-lignin material was probably sufficiently constant over the two periods. For rachides, significant differences in degradation rates occurred between the two dates under the mulch treatments, although not in the control. This suggests that below mulch, most of the low-lignin material would have been used before 50% flowering, but after that the remaining rachides had a higher proportion of lignified material which was slow to decompose.

The relationship between *B. cinerea* sporulation on vine debris and debris degradation rates is similar under mulched cover crops and organic mulches (Chapter 2) and is supported by previous work in grape vines and other crops (Konam & Guest, 2002; Mundy & Agnew, 2002; Walter *et al.*, 2004). This is likely to be driven by elevated soil biological activity under the mulches which may relate to the reductions in soil temperature fluctuations, increased availability of soil minerals observed under organic mulch (Chapter 2) and the elevated soil moisture. As with organic mulches, the soil moisture could be attributed to the mulches' insulative effect and possibly and higher organic matter. The findings of the current work agree with that of Buckerfield & Webster (1996) who also found increases in soil moisture and earthworm abundance under straw and various mulched cover crops, including triticale, ryegrass and clover, oats, barley and faba beans, all of which increased grape yield at harvest. It is possible that the mulched cover crops in this trial could have also increased vine resistance to *B. cinerea* as

measured under organic mulch (Chapter 2) coupled with the decrease in *B. cinerea* primary inoculum, may have led to the decrease in *B. cinerea* infection in flowers and bunches/berries.

In New Zealand grape production, fungicide-spray scheduling in conventional viticulture throughout the season is largely based on weather forecasting and vine physiology. Post-veraison, *B. cinerea* monitoring is also used to initiate spraying, with a severity of about 5% bunch coverage used as the industry threshold in Chardonnay (Figure 4.3) (Trought, M., Marlborough Wine Research Centre, *pers. comm*., 2006.; Creasy, G., Lincoln University, *pers. comm.*, 2006; Balasubramaniam, R., Delegat's Wine Estate New Zealand, *pers. comm.*, 2006.). Disease pressure in the year of this trial was sufficiently low that all treatments were below the economic threshold and the mulch treatments may not have reduced fungicide applications in a commercial vineyard. However, given the similarities between mulched cover crops and organic mulches in regards to *B. cinerea* severity reductions, it is likely that the mulches in this work would achieve similar levels of control at the high levels of disease pressure observed in year 2 (Chapter 2).

CHAPTER 5: Concluding discussion

In part adapted from:

Jacometti, M. A., Wratten, S. D. and Wilcox, W. F., unpublished. Conservation biological control of *Botrytis cinerea* in vines: progress and prospects. Biological control, *awaiting submission*.

5.1 Key objectives

Key objectives (Section 1.6, Chapter 1) of this work were to evaluate the:

1. effects of mulches on soil physical, chemical and biological properties.

2. effects of the mulches on degradation of overwintering vine debris and on its ability to generate inoculum of *B. cinerea*, the following season.

3. the impact of the mulches on berry resistance to *B. cinerea*.

4. the impact of the mulches on yield and various aspects of berry quality.

5. the effects of the mulches on the severity of botrytis rot in flowers and bunches at harvest.

The key results were:

1. Mulches increased soil biological activity, elevated soil moisture, reduced temperature fluctuations and altered soil nutrient composition, C:N ratio and CEC.

2. These changes increased vine debris degradation and reduced *B. cinerea* primary inoculum.

3. Mulches increased phenolic concentration and berry skin strength, which increased resistance of non wounded grapes to *B. cinerea* infection, reduced canopy density and, therefore within-canopy humidity.

4. In this work, mulches increased yield, berry sugar concentrations, possibly skin thickness and therefore skin:pulp ratio, berry phenolic concentration and possibly the uniformity of berry ripening. These changes to berry characteristics are likely to increase the quality of juice and wine.

5. Mulches also reduced the severity of *B. cinerea* infection in flowers and bunches at harvest to such an extent that infection rates on those plant parts in the unmulched controls were 45 and 10 times as high, respectively

5.2 Impacts of conservation biological control on *B. cinerea* management

In the current work, mulches were effective at controlling *B. cinerea* in an organic vineyard, 2-3 years after application. In conventional viticulture, where the vines and soils will typically be more 'dependent' on synthetic chemicals for disease control, mulches and cover crops may take longer to achieve this effect. For mulches and cover crops to be effective, agrichemicals and heavy soil management programmes should be avoided as they could disturb the biology of the system. Over-worked soil has poor soil structure, reduced organic matter and biological activity (McLaren & Cameron, 1994) and, as a consequence, a decline in soil moisture and nutrient concentrations and availability (Li *et al.*, 2004) are likely. It is therefore recommended that cover crops be direct drilled, or drilled with minimum tillage (McLaren & Cameron, 1994).

5.3 The likelihood of the technologies developed in this thesis being adopted by viticulturalists

Market and regulatory pressures and public concern regarding residues, environment and consumer health are increasing (Elmer & Reglinski, 2006), putting pressure on viticulturalists to use more sustainable disease management practices. New Zealand will be increasingly influenced by this pressure as the exported proportion of the country's wine increases (nzwine.com). This economic pressure, coupled with education about the advantages of more sustainable agronomic practices, is essential for persuading viticulturalists to adopt such practices. However, they may resist change because biological control techniques, such as bio-fungicides, have a reputation for being unreliable, often relying on specific environmental conditions for efficacy (Whipps & Lumsden, 2001). If viticulturalists were educated to understand the potential of habitat manipulation with mulches, their perception of conservation biological control may improve sufficiently for these practices to be adopted on a trial basis or in a proportion of their vineyards. Adoption would also depend on the cost and availability of mulch materials, the perceived risk of the new practices and the ease with which they could be integrated with existing control techniques that are perceived to be reliable.

The New Zealand grape-growing community represents a wide range of vineyards, sizes and growing styles, but, in general, fungicide use is static or decreasing (Mantelow *et al.*, 2004), and grape growers are beginning to respond to pressures from New Zealand environmental groups and overseas markets. The increasing awareness of grape growers to environmental issues is demonstrated by the success of the 'Greening Waipara' programme, a recent environmentally-driven initiative, which has had an exceptionally high level of grower adoption. This collaborative venture between Lincoln University and members of the Waipara Winegrowers' Association (waiparawine.co.nz) has led to native plants being returned to the Waipara Valley vineyard region, with the aim of reducing pesticide inputs, enhancing vineyard biocontrol and giving the district a marketing advantage. The work in this thesis supports that initiative, and some Waipara vineyards have already begun to use under-vine mulches.

5.4   Potential future research

Future research needs to address further the impact of mulches and cover crops on wine quality. The current work suggests that wine quality would not be compromised and may even be enhanced by the mulch treatments (increased berry sugar concentration, possible increased skin:pulp ratio and possible increased uniformity of ripening). Wine production is, however, considered by some to be as much an art as it is a science and the limited berry quality parameters measured in this study may not have reflected quality changes in the berries that could have an impact on the wine they produce. It is therefore recommended that a large-scale field trial is conducted, the juice from the specific mulches being made into separate wines and assessed for quality. The large-scale trials should be conducted in commercial vineyards so that the data might instil confidence in the industry and set a precedent for the future of conservation biological control of vine pathogens.

The mechanisms by which soil biota reduced *B. cinerea* sporulation on vine debris were not fully investigated in this trial and warrant further work. Possible mechanisms include antibiosis, as observed in many pathogen systems including damping off (*Aphanomyces cochlioides* Drechsler.) (Tofazzal-Islam *et al.*, 2005), bacterial wilt (*Ralstonia solanacearum* L.) (Ran *et al.*, 2005) and common potato scab (*Streptomyces* spp) (Sturz *et al.*, 2004), competition and parasitism (Elmer & Reglinski, 2006), as well as allelopathy which has been found to occur under cover crop treatments (Bending & Lincoln, 1999).

The use of other types of waste-stream materials could be investigated, either separately or incorporated into the mulches used in this trial. Waste-stream products that constitute a disposal problem, such as green waste from cities, are of particular interest, as these products could benefit ecosystems rather than damage them (Probert *et al.*, 2005). Alternatives to the cover crops used here, which may operate through different mechanisms could also be further developed. A range of brassicas release bio-fumigants (usually sulphur-based) into the soil (Bending & Lincoln, 1999) which inhibit some soil-borne fungal pathogens (Sarwar *et al.*, 1998) if used in this way, with potential benefits for disease management and vine health.

The use of mulches cover crops described in this work could also be investigated for reduction of other vine diseases, such as powdery mildew and downy mildew which also overwinter on vine debris. Cover crop species could also be selected to fulfil other requirements, such as the provision of flowering plants, to supply the nectar and pollen food resources needed by beneficial insects of insect pests in vines and other crops (Gurr & Wratten, 2000; Landis *et al.*, 2000; Wade *et al.*, in press; Zehnder *et al.*, in press).

5.5   Environmental Impacts

As stated in Section 1.1, Chapter 1, western agriculture needs to change its practices in relation to pest, disease and weed management to sustain future food production through maintaining or enhancing ecosystem services (Costanza *et al.*, 1997). At present, alternatives to synthetic chemicals, such as essential oils and plant defence stimulants, are unreliable and can have adverse affects on vine growth and development or wine production (Elmer & Reglinski, 2006). New Zealand wine is increasingly being sold for export (nzwine.com) and these markets are demanding a product that is being produced as far as possible in a 'sustainable' way. Current disease-management practices do not reflect that demand (Howell, 2001). Conservation biological control of *B. cinerea* has been shown to be effective, low risk and easily integrated into current practice. The technology has high potential if the industry can be given confidence that proposed protocols are robust, reliable, economic and compatible with other vineyard practices, as well as acceptable to markets and lead to appropriate levels of disease suppression.

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APPENDIX 1: Taxonomy of *Botrytis cinerea* and *Vitis vinifera*

Botrytis cinerea

Kingdom | Fungi

Phylum | Ascomycota

Subphylum | Pezizomycotina

Class | Leotiomycetes

Order | Helotiales

Family | Sclerotiniaceae

Genus | *Botryotinia*

Species | *Botryotinia fuckeliana*

Type species | *Botrytis cinerea* (Pers. : Fr)

Vitis vinifera

Kingdom | Plantae

Phylum | Magnoliophyta

Class | Magnoliopsida

Order | Vitales

Family | Vitaceae

Genus | *Vitis*

Species | *V. vinifera* L.

APPENDIX 2: *B. cinerea* spore dispersal from under crop species (a laboratory wind tunnel experiment)

Experimental design and methods

In September 2005, cover crops (Table 5.1) and a potting-mix-only control were sown in trays 400 × 290 × 80 mm in a 5 block, randomised block design in a glasshouse, Lincoln University. Trays were filled with potting mix consisting, 400 L bark, 100 L pumice, 1000 g Osmocote plus (15-4.8-10.8, N-P-K), 500 g agricultural lime and 500 g hydraflo (wetting agent) and sown at densities of 8, 15, 10 and 30 kg/ha for forage brassica, lucerne, phacelia and ryegrass respectively. These plants were grown for 20 days to a height of approximately 300 mm then transferred to a laboratory, in which there was a wind tunnel (1400 mm × 350 mm × 550 mm) creating air movement (negative pressure) of 7 m/s (pump – Tipo CA 150 50/60 Hz, 85 W, T55). A single tray of plants was then placed at the upwind end of the wind tunnel and five 85 mm Petri dishes containing potato dextrose agar (PDA) were placed at the other, following the configuration shown in Figure 5.1. Ten 35 mm Petri dishes, with fully sporulating *B. cinerea* (source plates) were then placed evenly under the crop, the wind tunnel was switched on and lids of the source plates removed. Each source plate was sprayed once with water using a hand mister to help release the spores. The wind tunnel was allowed to run for two minutes. The trap plates were then removed and incubated for two days at 20ºC, in a 12 h light dark photoperiod, when colonies of *B. cinerea* were counted.

Figure 5.1 The layout of trap plates 85 mm (PDA) in the down wind end of the wind tunnel.
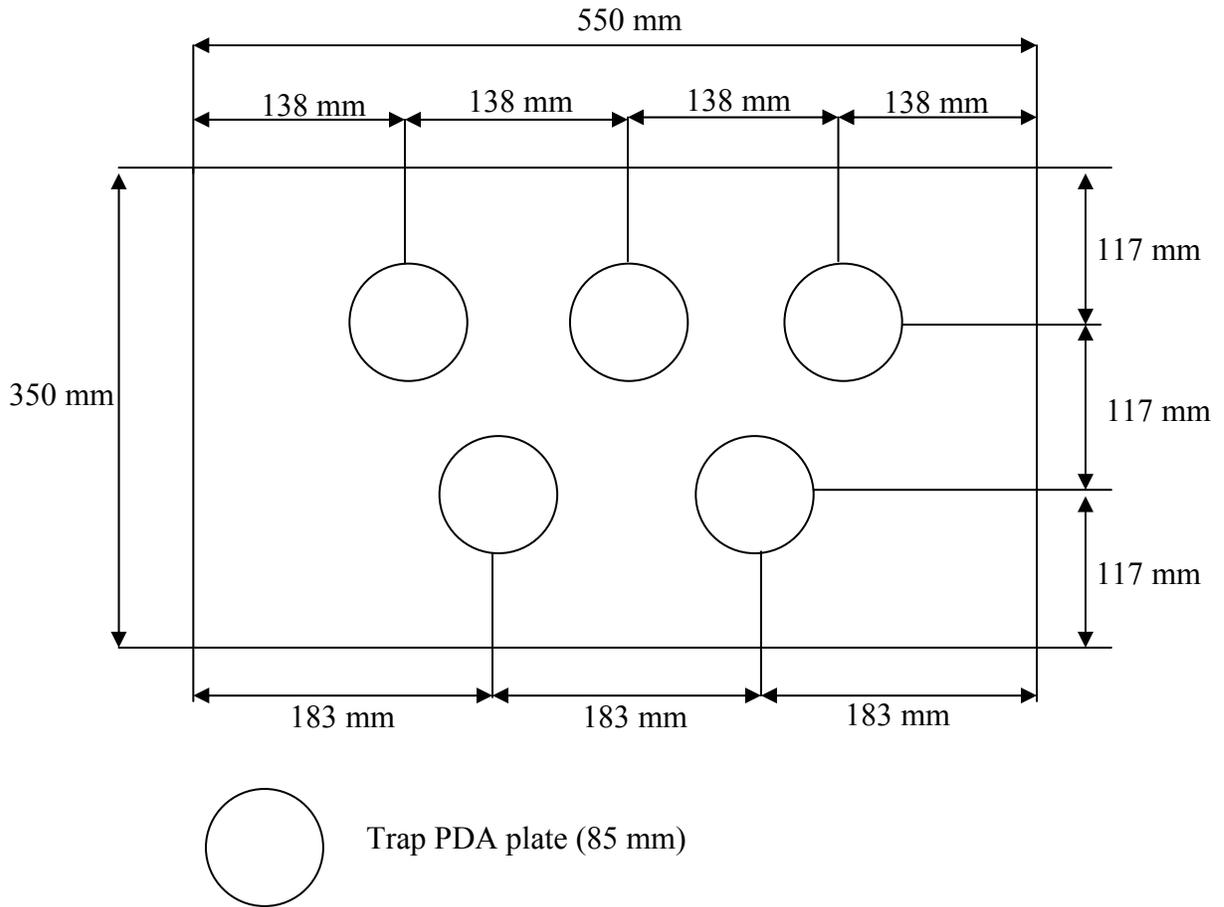
Results and discussion

All cover crop species significantly reduced spore dispersal compared with the bare surface control, but no significant differences were seen between plant species (Table 5.1). Phacelia and ryegrass tended to have the lowest number of spores dispersed from the crop, but this was due to these cover crops collapsing due to their height and abnormal growth in glasshouse conditions. Most conidia were caught on the lower two trap plates, as densities of airborne conidia would be highest in this section of the wind tunnel due to gravity.

Table 5.1. Mean number of *B. cinerea* colonies counted on trap plates when conidia were released under plant species in a wind tunnel experiment.

Trap plate location	Control	Forage brassica	Lucerne	Phacelia	Ryegrass	Mean
		Cover crops				
Top left	181	37	65	12	40	67^a
Top middle	182	86	74	10	39	78^a
Top right	269	109	67	13	76	107^b
Bottom left	249	105	140	7	93	119^{bc}
Bottom right	323	156	123	12	99	143^c
Mean	241^a	98^b	94^b	11^b	69^b	

APPENDIX 3: *B. cinerea* spore dispersal from under crop species (Field experiment).

This experiment used single isolates of nitrate non-utilising mutants, or more specifically, a) isolates of *B. cinerea* defective in the nitrate reductase apoenzyme (*nit1*) and b) isolates of *B. cinerea* defective in the molybdenum-containing cofactor pathway (NitM) as the marked source of inoculum (Weeds *et al.*, 1998; Beever & Parkes, 2003; Seyb, 2004). These isolates were used as they have been shown to be equally pathogenic and fit in the short term as wild-type isolates (Weeds *et al.*, 1998) and because they occur naturally only at very low levels. This means that they can be tracked, thereby eliminating the confounding effect of background pathogen levels. As *nit1* and NitM are generated from specific parent ascospore isolates, complementation assessments can be made to determine if trapped spores originate from released isolates or are naturally occurring isolates. The *nit1* and NitM isolates (sourced from Ross E. Beever, Landcare Research Auckland, New Zealand) were stored and maintained according to the protocol described in Weeds *et al.* (1998). Chlorate agar[10] plates were used as trap plates as these have been found to be effective in the isolation of *B. cinerea* (*nit1*) previously released into field experiments (Seyb, 2004).
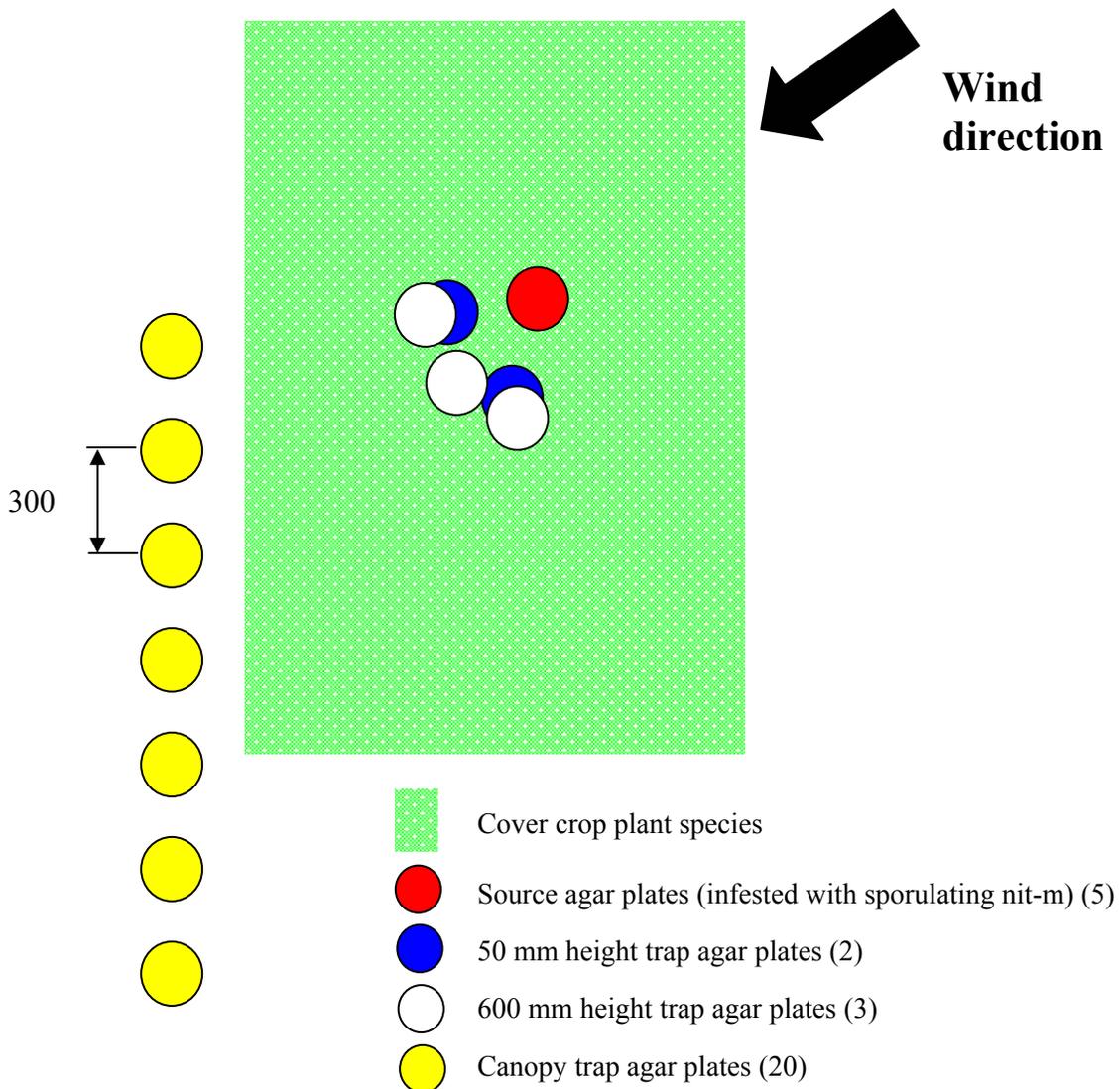
Experimental design and methods

This experiment was conducted in three randomly selected blocks from the field trial in Chapter 4, using methodology of a spatial distribution study by Seyb (2004). The experiment was to be conducted, monthly, for four months, in winter/spring 2004, mid morning, in light winds (<5 m/s) with an anemometer placed in the row to measure local wind speed. Five horizontally placed (face up) agar plates (85 mm diameter) with sporulating *nit1* mutants (20°C, 12 hr photoperiod, 5 days) (source plates) were placed on the ground in a group, 20 mm apart, under each cover crop treatment. Two selective chlorate agar plates (trap plates) were then suspended horizontally (face down), 50 mm above the sward, (with a minimum of 150 mm above ground level) and an additional three (horizontally placed, face down), 600 mm above the source plates in the configuration shown in Figure 5.2. A further 20 trap plates were placed vertically at 300 mm intervals in the canopy (along the fruiting wire) on the

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[10] Refer Appendix 4

down-wind side of the source plates (Figure 5.2). The source` plates were left to release spores for three hours (more time leads to problems associated with agar dehydration, spore damage and microbial contamination (Seyb, 2004)) and then the trap plates were collected, lids replaced and transported to the laboratory in cool polystyrene boxes. During each assay, 10 trap plates were also placed in a separate row, 3 rows downwind from the most downwind of the cover crop treatments, to determine the levels of cross contamination between treatments.

Figure 5.2. The spatial configuration of source agar plates (infested with sporulating *nit1*) and trap plates in a single treatment replicate of the field spore dispersal experiment.

Cover crop plant species

Source agar plates (infested with sporulating nit-m) (5)

50 mm height trap agar plates (2)

600 mm height trap agar plates (3)

Canopy trap agar plates (20)

B. cinerea spore recovery

Trap plates were incubated at 19-20°C in a 12 h light/dark photoperiod, for five days and an attempt made to identify *B. cinerea* colonies and count them. Thin mycelial growth was to be assessed as potentially being from *nit1* isolates, which were then to be confirmed with a complementation assessment with NitM. The resulting data were to be analysed through a one-way ANOVA and the trial was to be repeated four times throughout winter 2004.

Results and discussion

Due to high levels of biological contamination and high levels of competition with *B. cinerea* on the trap plates, *B. cinerea* was successfully identified in only 4 of the 180 plates. A contributing factor to this was that *B. cinerea* does not sporulate on chlorate medium so mycelium was transferred to PDA where it would sporulate and could be identified. This extra step proved to be unreliable as high levels of contamination were evident on the PDA and the process was complicated and time consuming. Other selective media were then considered. *Botrytis cinerea* selective media (BSM)[11] (Edmunds & Seddon, 2001) was considered but was deemed to compromise the organic status of Seresin Estate. PDA amended with low concentrations of Triton ®-X100 and chloramphenicol was then considered and approved by the vineyard. A pilot study was then conducted in field conditions testing the ability of *B. cinerea* to compete with other organism on PDA (38 g/L) amended with all concentrations of ®-X100 (1, 2 and 4 ml/L) and chloramphenicol, (0.04, 0.08 and 0.16 g/L). The most selective agar (2 ml Triton®-X100 and 0.08g chloramphenicol) was then used in the spore dispersal experiment. Again, contamination and competition were high and *B. cinerea* was observed in 7 of the 180 plates. No significant differences were seen between treatments.

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[11] 2g glucose
0.1 g $NaNO_3$
0.1 g K_2HPO_2
0.2 g MgSO4.7H2O
0.1 g KCl
0.2 g chloramphenicol
0.02 g pentachloronitrobenzene (Terraclor® 75W)
0.02 g 80% manganese ethylene bisdithiocarbamat (Maneb 80)
0.05 g rose Bengal
5 g tannic acid
adjust pH to 4.5 with 1 mol/L NaOH prior to addition of the agar.

20g agar

The selective agars used in this experiment were unsuitable for this type of experiment. High levels of spores are dispersed within one meter of the source (Seyb, 2004) but they were unable to be identified using the media and techniques used in this experiment.

APPENDIX 4: Chlorate agar (from Seyb (2004))

Minimal media + ClO$_3$ (chlorate agar)

MM	1 L
Potassium chlorate	30 g

Preparation: Ingredients were combined, except Vogel's medium N, and autoclaved at 121°C and 15 psi for 15 min. Vogel's medium N-was filter sterilised using 0.22 μm sterile Millex®-GS filters and then added to the medium once it had cooled to *ca* 45-50°C.

Minimal media (MM) (Weeds, *et al.,* 1998)

This media is based on Vogel's medium N (Vogel, 1964).

Vogel's medium N	20 mL
Sucrose	15 g
Agar	15 g
Distilled water	980 mL

Vogel's medium N

Sterile distilled water	98 mL
Vogel's concentrate N	2 mL

Vogel's concentrate N

Vogel's salts N	1 L
Trace element solution	5 mL
Vogel's biotin	2.5 mL
Chloroform (preservative)	1 mL

Preparation: Ingredients were combined. If necessary, the final solution, at a 50x concentration, was adjusted to 1 L.

Vogel's salts N

Distilled water	775 mL
Trisodium citrate	125 g
Potassium dihydrogen orthophosphate	250 g
Magnesium sulphate	10 g
Calcium chloride	5 g
Ammonium nitrate	100 g

Preparation: Salts were added to distilled water in order; before each addition the previous salt was dissolved completely by stirring the solution on a gentle heat with a magnetic stirrer. The calcium chloride was added 1 g at a time.

Vogel's trace elements (makes 100 mL)

Distilled water	95 mL
Citric acid	5 g
Zinc sulphate	5 g
Ferrous ammonium sulphate	1 g
Cupric sulphate	0.25 g
Manganese sulphate	50 mg
Boric acid	50 mg
Sodium molybdate	50 mg

Preparation: Dissolve in distilled water and adjust volume to 100 mL.

Vogel's biotin (makes 100 mL)

50% ethanol	100 mL
Biotin	5 mg

Preparation: Biotin was dissolved in ethanol and stored in a refrigerator.

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PUBLICATIONS/PRESENTATIONS FROM THIS THESIS
AS OF THESIS SUBMISSION DATE

Publications

Gurr, G. M., Scarratt, S. L., Jacometti, M. and Wratten, S. D. Management of pests and diseases in vineyards in New Zealand and Australia. In: Biological control: case studies from around the world. (Eds C. Vincent, M. Goettel and G. Lazarovits), CABI Publishing, U.K. (in press)

Jacometti, M. A., Wratten, S. D. and Walter, M., 2007. Management of understorey to reduce the primary inoculum of *Botrytis cinerea*: Enhancing ecosystem services in vineyards. Biological Control 40, 57-64.

Jacometti, M. A., Wratten, S. D. and Walter, M., *submitted* 10 July 2006. Enhancing ecosystem services in vineyards: understorey management increases resistance to *Botrytis cinerea*, grape quality and yield. Agriculture, Ecosystems and Environment.

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Jacometti, M.A., Wratten, S.D., Walter, M. and Jaspers, M.V. (2005) Conservation Biological Control: Using Mulches to Reduce *Botrytis cinerea* in Vineyards. Proceedings of the Romeo Bragato Conference, Gisborne 2005, Section7.

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Posters

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Presentations

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