

CELLULASE PRODUCTION BY VARIOUS SOURCES OF MULCH

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SUMMARY

Organic mulches are reported to control root rot of avocado, caused by *Phytophthora cinnamomi*. A possible mechanism for this control is enzyme (specifically cellulase and glucanase) production by the large microbial population supported by the mulch, which can degrade the cell walls of the pathogen. Samples of a wide range of organic mulches were set out in plots at two sites for two years. Rate of decomposition, measured as change in depth, and cellulase activity in the mulch, at the mulch-soil interface and in the soil were measured. Mulches with a low rate of decomposition tended to have low levels of cellulase activity, whereas mulches that decomposed rapidly varied in their cellulase activity. Manuka and eucalyptus mulches had the highest activities. Cellulase activity in the soil 5cm below the mulches was not increased by any of the mulches. Mulches taken from an avocado orchard had similar cellulase activities to mulches from the trial plots, but fresher samples (four months cf. two years) had lower activities. These findings need to be extended by establishing the level of cellulase necessary in the mulch to give control of the root rot fungus.

Key Words: cellulase, *Phytophthora*, mulch

INTRODUCTION

Biological control of *Phytophthora cinnamomi* in avocado through the use of mulches was identified by an Australian grower and later described as the "Ashburner Method" by Broadbent and Baker (1974a). The technique uses large amounts of organic matter as a mulch along with a source of calcium. Control of avocado root rot in the Ashburner method was attributed to the presence of *Pseudomonas* bacteria and actinomycetes (Broadbent and Baker 1974b). Multiple antagonists are more likely the cause of biological control, since no single organism has been found to be consistently associated with soils suppressive to *P. cinnamomi* (Cook and Baker, 1983).

The use of organic mulches has multiple effects, such as altered soil nutrient and water status (Robinson, 1988) and improved physical structure (Merwin *et al*, 1994). Any improvements in plant

status resulting from improvements in the growing environment can improve plant health. The effect of organic amendments on soil physical and chemical properties can vary considerably depending on soil texture and the environment. One of the most consistent effects of organic amendments is an increase in biological activity (Perucci, 1992). Increases in organic substrate lead to increased fungal and bacterial populations. In numerous cases, this increase in biomass has been associated with disease suppression (Shea and Broadbent, 1983; Malajzuk, 1983; Erwin and Ribiero, 1996). This biological control can be ascribed to several mechanisms: competition, antibiosis, parasitism, predation and induced resistance in the plant (Hornby, 1990).

The microbial biomass is responsible for release of enzyme products and polysaccharides in soils (Martens and Frankenburgh, 1991). The microbially-produced enzymes cellulase and glucanase have been demonstrated to have a significant effect on *Phytophthora* populations (Downer *et al*, 2001a; Downer *et al*, 2001b; and Downer *et al*, 2002). This mechanism of antibiosis is possible because the microbes are releasing these enzymes to solubilize organic matter. Unlike other fungi, *Phytophthora* have cell walls that are comprised of cellulose and in the process of decomposing organic matter with enzymes, an environment is created that is also hostile to the pathogen.

This study was designed to evaluate whether there are differences in the production of cellulase from different mulching materials in a field setting. The fields were established in two different environments to determine the impact of climate on cellulase production.

MATERIALS AND METHODS

Two sites with different climatic regimes and soil types were used. Te Puke Research Station (sandy loam) situated on the east coast of New Zealand's North Island has an annual rainfall average of 1691 mm and mean annual temperature of 14.5° C. Ruakura Research Center (loam soil texture) is an inland site with mean annual rainfall of 1190 mm and mean annual temperature of 13.7° C. At both stations, areas were cleared and cultivated and 1.5 meter by one meter plots of mulch materials were laid out in the open. Materials were spread to a depth of 100 mm in side-by-side plots and covered with polypropylene bird netting to prevent wind dispersion. Materials included those easily available from windbreak plantings, such as poplar, willow and casuarina, as well as materials from the forest and greenwaste industries. These materials were also selected for their range in texture and potential rate of decay. Each material was replicated two times at each site.

Windbreak materials were chipped in place and applied as fresh materials at the test sites. One of the mulches was inoculated with *Trichoderma*, a beneficial fungus. At Ruakura, 21 materials were evaluated and 23 at Te Puke (Table 1). Weeds were controlled by occasional glyphosate sprays. Depths of the mulches were measured at the end of one and two years. Cellulase activity was measured in samples of the mulch, the mulch-soil interface and soil at a depth of 5cm at Ruakura and in samples of three mulches only at Te Puke at the end of year two.

Duplicate sub-samples (100 mg) were taken from each mulch or soil sample and incubated for four hours at room temperature with 2 ml of 2 mmol/L p.nitrophenyl-cellobioside in 50 mmol/L acetate buffer (pH 5.0). Cellulase activity was determined by absorbance at 410 nm and expressed as mmoles of substrate hydrolysed per hour per gram dry weight. This is a modification of the method of Tank and Webster (1988).

Least significant differences (Lsd) between means at 5% significance were calculated using Genstat.

Table 1. Materials applied as mulches at Te Puke and Ruakura

Willow <i>Salix matsudana</i>	Cryptomeria <i>Cryptomeria japonica</i>	Poplar <i>Populus nigra</i>	Avocado <i>Persea americana</i>
Pine bark <i>Pinus radiata</i>	Eucalyptus <i>Eucalyptus globulus</i>	Casuarina <i>Casuarina cunninghamiana</i>	Bamboo* <i>Phyllostachys bambusoides</i>
Cypress * <i>Cupressus empervirens</i>	Manuka <i>Leptospermum scoparium</i>	Pine sawdust	Pine wood chips + cow manure
Greenwaste + chicken manure compost	Biosolids compost	Recycled wood chips, fine	Recycled wood chips, coarse
Greenwaste compost screenings, coarse	Greenwaste compost screenings, fine (1)	Greenwaste compost screenings, fine (2)	Composted pine bark
Composted pine bark + <i>Trichoderma</i>	Composted greenwaste	Wool waste	*@Te Puke only

RESULTS AND DISCUSSION

Since cellulase production is part of the decomposition process, the rate of decomposition should be a partial indicator of the amount of cellulase present. Graph 1 shows the depths of various materials at the Ruakura site after one and two years of decomposition. After a mulch application there is generally settling due to rainfall-caused compaction, but much of the decline by the second year is due exclusively to decomposition. The more recalcitrant materials, such as bark, wood chips and sawdust have barely lost half their depth after two years, while others such as shredded eucalyptus, manuka, avocado and willow are less than 20% of their initial depth. Much of the shredded/chipped material, such as eucalyptus had a significant fraction of leaves in the mulch. The wool disappeared a little after one year. The greenwaste + chicken manure compost is nearly the same depth as the wood chips, since it is a material that had gone through a decomposition process prior to its application and much of the easily digestible materials had already been decomposed. Decomposition at Te Puke followed a similar pattern (data not shown).

The rate of decomposition has some bearing on the rate of cellulase production (Graph 2). Eucalyptus and manuka had the two greatest rates of decomposition and show the highest levels of cellulase production. However, poplar, willow and avocado had high rates of decomposition, but their cellulase activities were half those of manuka and eucalyptus. Cellulase level regressed against the two year loss of the materials gave an equation of :

Cellulase = -19.64 + 13.72 (dry matter loss). The P-value in the ANOVA tables is less than 0.10 so there is a 90% confidence in the fitted model. The correlation coefficient (R^2) equals 0.388 which indicates a weak relationship between the variables.

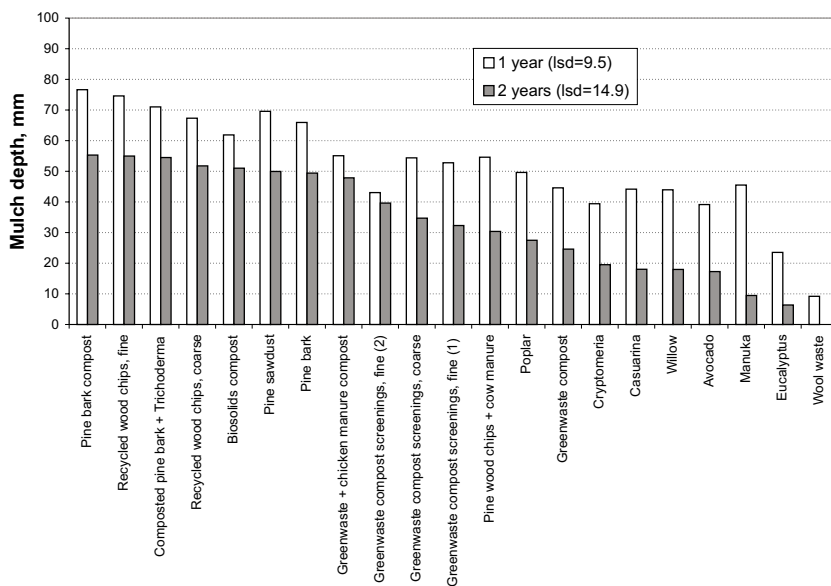
Cellulase activities of the manuka samples from Ruakura and Te Puke were similar (Graph 2). It is clear that the cellulase effect is limited to the layer of mulch and not to depth within the soil. There is some effect at the soil surface, but at 5 cm. cellulase activity drops to background levels (Graph 2). There is earthworm activity at these test sites and one idea was that earthworm incorporation of organic matter would move the cellulase production into the soil. Maybe with further time this would occur. As it is, when mulches are applied to avocado, the roots tend to proliferate in the mulch, out of the soil where the cellulase activity is the least.

We do not know what levels of cellulase are necessary to control the root rot fungus. It may be that levels seen with pine bark are more than adequate. Also we have measured cellulase pro-

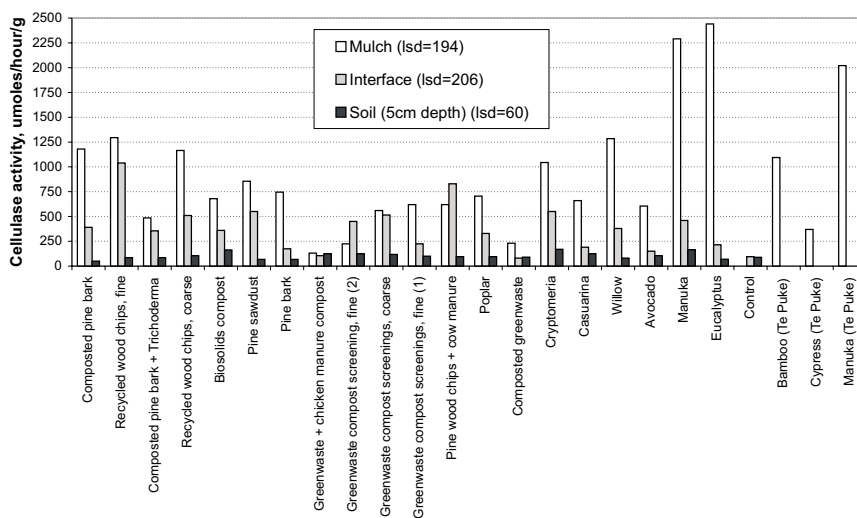
duction at only one time in a two-year period and it is quite likely that this does not fully represent what is happening before and after. A further reminder is that cellulase is only one of the many by-products associated with decomposition and many of the antagonistic properties that are associated with the microbial biomass are not being measured in this trial. Having developed this screening procedure, what needs to be done next is to take high, medium and low cellulase producing mulches and challenge the fungus to verify that this is a good way to evaluate mulches.

REFERENCES

- BROADBENT, P. AND K.F. BAKER. 1974a. Behavior of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Aust. J. Agric. Res.* 25:121-137.
- BROADBENT, P. AND K.F. BAKER. 1974b. Association of bacteria with sporangium formation and breakdown in *Phytophthora* spp. *Aust. J. Agric. Res.* 25:139-145.
- COOK, R.J. AND K.F. BAKER. 1983. The nature and practice of biological control of plant pathogens. *Amer. Phytopathol. Soc. Press, St. Paul, MN.* 539 pp.
- DOWNER, A.J., J.A. MENGE AND E. POND. 2001a. Effects of cellulytic enzymes on *Phytophthora cinnamomi* Rands. *Phytopath.* 91:839-846.
- DOWNER, A.J., J.A. MENGE AND E. POND. 2001b. Association of cellulytic enzyme activities in eucalyptus mulches with biological control of *Phytophthora cinnamomi*. *Phytopath.* 91:847-855.
- DOWNER, J., B. FABER AND J. MENGE. 2002. Factors affecting root rot control in mulched avocado orchards. *HortTech.* 12(4): 601-605.
- ERWIN, D.C. AND O.K. RIBIERO. 1996. *Phytophthora Diseases Worldwide.* *Amer. Phytopathol. Soc. Press. St. Paul, MN.* 562. pp.
- HORNBY, D. 1990. *Biological control of soil-borne plant pathogens.* *CAB Int'l. Wallingford, U.K.* 479 pp.
- MALAJZUK, N. 1983. Microbial antagonism to *Phytophthora*. Pps. 197-218. In: *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology.* D.C. Erwin, S. Bartinicki-Garcia, P.H. Tsao, Eds. *APS Press, St. Paul, MN.* 392 pp.
- MARTENS, D.A. AND W.T. FRANKENBURGH. 1991. Saccharide composition of extracellular polymers produced by soil microorganisms. *Soil Biol. Biochem.* 23:731-736.
- MERWIN, I.A., W.C. STILES AND H.M. VAN ES. 1994. Orchard groundcover management impacts on soil physical properties. *J. Amer. Soc. Hort. Sci.* 119:222.
- PERUCCI, P. 1992. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. *Biol. Fertil. Soils* 14:54-60.
- ROBINSON, D.W. 1988. Mulches and herbicides in ornamental plantings. *HortSci.* 23:547-551.
- SHEA, S.R. AND P. BROADBENT. 1983. Developments in cultural and biological control of *Phytophthora* diseases. Pps. 335-350. In: *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology.* D.C. Erwin, S. Bartinicki-Garcia, P.H. Tsao, Eds. *APS Press, St. Paul, MN.* 392 pp.
- TANK, J.L. AND J.R. WEBSTER. 1988. Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* 79 (6): 2168-2179.



Graph 1. Depths of applied mulches at Ruakura, one and two years after application. Initial depth was 100 mm.



Graph 2. Cellulase activity within the mulch, at the soil-mulch interface and at a depth of 5 cm in the soil below the mulch