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The Effect of Yard Trimmings as a Mulch on Growth of Avocado and Avocado Root Rot Caused by *Phytophthora cinnamomi*

A.J. Downer J.A. Menge H.D. Ohr B.A. Faber B.S. McKee E.G. Pond M.G. Crowley S.D. Campbell

University of California Cooperative Extension, Ventura County
Department of Plant Pathology, University of California, Riverside

SUMMARY

Raw yard-trimmings (wood, leaves and grass) were applied to newly planted avocado trees on three different rootstocks at the rate of 0.3 cuM/tree/year in two commercial avocado groves infested with the avocado root rot fungus - *Phytophthora cinnamomi.* Mulched trees were compared with unmulched trees in randomized, replicated experiments. Mulch increased yield by 13% and canopy volume by 43% in one orchard. In the second orchard mulch initially reduced yields and canopy volume, but by the end of the experiment canopy volume was no different in mulched trees than in unmulched trees. Root growth was greatly stimulated by mulch in both orchards and was increased as much as 184% compared to the unmulched trees. Mulches were found to change the depth of rooting of avocado trees, since most roots in mulched trees were found in the interface between the mulch and the soil. Effects of the mulch were more dramatic on rootstocks less tolerant to avocado root rot.

Root infections caused by *P. cinnamomi* were very low in the mulch and at the mulch/soil interface but significantly higher in the soil, whether it was mulched or not. Populations of *P. cinnamomi* in the soil were unaffected by mulch applications. Zoospore production by *P. cinnamomi* at the mulch/soil interface was significantly *lower* than at depths deeper in the soil. Hyphal lysis of *P. cinnamomi* was at a maximum at the mulch/soil interface and it was significantly higher than lysis in unmulched soil. Enzymes that lysed the hyphae of *P. cinnamomi* were found to be abundant in mulches but not at 7.5 and 15 cm below the mulch or in unmulched soil. Two enzymes which were shown to damage hyphae of *P. cinnamomi,* cellulase and laminarinase, were very abundant in the mulches but were much less abundant in the soil below the mulch and in unmulched soil.

KEYWORDS

Cellulase, Laminarinase.

INTRODUCTION

Mulches have long been known to inhibit avocado root rot caused by *Phytophthora*

cinnamomi Rands and stimulate growth of avocado trees in soil infested with *P. cinnamomi* (Broadbent and Baker, 1974; Pegg, 1976; Rosas *et al.,* 1986; Wolstenholme *et al*., 1996; Zentmyer, 1953). Wolstenholme *et al.,* (1996), however, reported that the benefits of mulch are often overcome by wet or shallow soils. The mechanisms responsible for the beneficial effects of mulches toward avocado in soil with root rot have never been fully explained. The legendary Ashburner mulch method from Australia was thought to function by inhibiting zoosporangia and lysing hyphae of *P. cinnamomi.* It was believed the effect was mediated by soil microorganisms, but no specific microorganisms were ever correlated with the suppressive effect (Broadbent and Baker, 1974). The purpose of this study was: 1) to determine if yard trimming mulch would reduce avocado root rot in California soils and 2) to determine the mechanism by which mulch was antagonistic to root rot or stimulatory to avocado.

MATERIALS AND METHODS

Plot establishment

Two field plots were established to assess the affects of mulch on avocado trees growing in *Phytophthora* infested soil. The Sprinkling plot consisted of nursery-grown Hass avocado on three rootstocks (Duke 7, Thomas, and UC2011) which were planted into soil infested with Phytophthora root rot. The trial was located in Somis, California in Ventura County. The trees were treated annually, during the spring from 1994-1997, with raw yard trimmings (chipped wood, leaves and grass) obtained from Agromin Co. in Ventura County. Mulch was spread under the tree canopy out to the drip line but was kept away from the trunk. Twenty trees of each root-stock received 1/3 cu yd of mulch annually. Twenty trees of each rootstock received no yard trimming mulch. The trial was irrigated via mini-sprinklers. The plot was designed as a randomized block factorial, with rootstock, and mulch being the two factors. There were 20 blocks with one replicate per block for a total of 120 trees.

The Vanoni plot consisted of nursery-grown, Hass avocado on three types of rootstocks (Duke 7, Thomas, and Toro Canyon) which were planted into soil infested with Phytophthora root rot. The trial was located in Somis, California in Ventura County. The trees were treated annually during the summers from 1994-1997 with mulch derived by processing the wood, bark, leaves and fruit *of Eucalyptus globulus* Labill. through a commercial brush chipper. Mulch was spread under the tree canopy out to the drip line but was kept away from the trunk. Sixteen trees of each rootstock received 1/3 cu yd of the raw mulch annually. Sixteen trees of each rootstock received no mulch. The trial was irrigated via mini-sprinklers. The plot was designed as a completely randomized factorial, with rootstock and mulch being the two factors. There were 16 replications per treatment for a total of 96 trees.

Trees from both plots were irrigated and fertilized by the growers in a manner consistent with commercial avocado production. After three years canopy volume (cu M) and yield (kg/tree) were measured.

Root length

Root samples were taken from soil cores which were collected from the east and west

sides of each trees midway from the trunk to the edge of the mulch treatment in June of 1997. In the Sprinkling plot, two 6.25 x 15 cm soil cores, one from each side, were taken only from the Duke 7 rootstock trees. In the Vanoni plot, four 15 x 10cm soil cores, two from each side, were taken from all rootstocks. Roots were removed from the soil and root length (cm/100 cc of soil) was calculated using the line intersect method (Newman, 1966).

To determine if rooting depth was altered by mulching, 0.5 L soil and mulch samples were gathered from both the east and west sides of five mulched Thomas trees in the Vanoni plot at the following depths: mulch surface, mid mulch, soil/mulch interface, 7.5 cm and 15 cm deep in the soil. Similar soil samples were gathered from unmulched trees at the soil surface and 7.5 and 15 cm deep. Roots were removed from the samples and the root length was calculated as above. Data were averaged for the two sub-samples per tree and expressed as root length/100 g of soil.

Phytophthora infection and soil populations

To determine if the location of root infections by *P. cinnamomi* was affected by mulching, 0.5 L soil and mulch samples were gathered from both the east and west sides of five mulched Thomas trees in the Vanoni plot at the following depths: mulch surface, mid mulch, soil/mulch interface, 7.5 cm and 15 cm deep in the soil. Similar soil samples were gathered from unmulched trees at the soil surface and 7.5 and 15 cm deep. Roots were extracted from these samples and one-cm samples (10 per sample) were placed on PARPH medium for the detection off! *cinnamomi* (Kellam and Coffey, 1985). Data were averaged for the two sub-samples per tree and expressed as number of infections/10 1-cm root pieces.

Soil populations of *Phytophthora cinnamomi* were determined using leaf baits. For the Sprinkling plot, two 6.25 x 15 cm soil samples, one from each side midway between the trunk and the edge of the mulch, were taken only from the Duke 7 rootstock trees. From these combined samples, a sub-sample was taken from near identifiable roots. One gram of this soil was placed in 9 ml of water in a 15 x 60 mm petri dish. Two and four - fold dilutions of the soil were established into other petri dishes. Five 8 mm leaf disks of *Eucalyptus globulus* were floated on each plate. This procedure was replicated three times for each tree. After three days the leaf disks were pressed into PARPH agar (Kellam and Coffey, 1985) for detection *of P. cinnamomi.* Using the number of plates in each dilution with positive recovery of *P. cinnamomi,* the population of *P. cinnamomi* can be calculated for the soil sample from tables designed for the most probable number method (Alexander, 1965). For the Vanoni plot, four 1.25 x 15 cm soil cores from each of the four compass points were taken midway between the trunk and the edge of the mulch from each tree. These samples were bulked for each tree and a one-gram sample was placed in a standard petri plate with 9 ml of water. Ten 8 mm leaf disks from *Persea indica* (L.) Spreg. were floated on each plate. After three days the disks were pressed into PARPH agar (Kellam and Coffey, 1985) for detection of *P. cinnamomi.* Data were recorded as the number of leaf disks colonized by *P. cinnamomi* per gram soil.

Zoospore production and hyphal lysis from buried mats

Phytophthora cinnamomi was cultured on V8-c agar (per liter: V8 juice, 200 ml; $CaCO_3$,

2 g, agar, 15 g, deionized water, 800 ml) in the dark at 24C. Five mm disks of an actively growing culture of *P. cinnamomi* were transferred, one each to 15 x 60 mm petri plates, covered with 7 ml ° strength V8-c broth and placed in the dark for three days. Mycelia were washed three times with deionized water, the agar disk was removed, the mycelium was weighed and mycelial mats were placed in 100 mm Miracloth (Behring Diagnostics, La Jolla, CA) envelopes that were secured in plastic 35 mm slide mounts. Slides bearing mycelium were placed under five, mulched Thomas trees in the Vanoni plot at the following depths: mulch surface, mid mulch, soil/mulch interface, 7.5 cm and 15 cm deep in the soil. Slides bearing mycelium were also placed under five, unmulched Thomas trees at the soil surface and 7.5 and 15 cm deep. After three days the mycelia were removed, washed with deionized water and placed in 15 x 60 mm petri dishes with deionized water at 24C for 30 min for release of zoospores. Zoospores were counted with a counting chamber (Hawksley Co., England) and data was expressed as zoospores/ mg of mycelium. The mycelium was then examined under the microscope and rated for lysis using a scale from 1 to 5. One = no lysis, healthy mycelium; 5=mycelium degraded, only residual fragments remained.

Enzyme assays

Soil and mulch samples (0.5 L) were gathered from both the east and west sides of five mulched Thomas trees in the Vanoni plot at the following depths: mulch surface, mid mulch, soil/mulch interface, 7.5 cm and 15 cm deep in the soil. Similar soil samples were gathered from unmulched trees at the soil surface and 7.5 and 15 cm deep. Enzymes were extracted from these samples as described by Alef and Nannipieri (1995) with the following modifications. Ten gram (dry wt.) of each sample was measured into a 125 Erlenmeyer flask. To each flask, 15 ml of 0.05M acetate buffer was added. The appropriate substrate to be tested was dissolved in acetate buffer and 15 ml was added to the reaction mixture. Substrates were carboxym-ethyl cellulose (0.7% w/v, Sigma Medium viscosity), laminarin (0.1% w/v Sigma) and *P. cinnamomi* cell walls (0.1% w/v) for detection of total cellulase activity, b-1,3 glucanase and *Phytophthora* degrading enzymes, respectively. Flasks were constantly agitated on a rotary shaker at 100 rpm for 4 hr at 36º C. Controls were prepared by adding 15 ml substrate-buffer solution after incubation but immediately before centrifugation. Aliquots of 1.5 ml were removed into micro-centrifuge tubes and spun at 13 Krpm for 3 min. The supernatant was assayed for reducing sugars according to the methods of Schinner and Von Mersi (1990) with modifications made to analyze smaller volumes. The optical density was measured at 690 nm after the one hour for color development. Treatments were read after calibrating against the 0-time control samples with substrate added. All assays were run in duplicate and averaged before further statistical analysis. Liquid cultures of *P. cinnamomi* were grown in 500 ml of ° strength V8-c broth in one L Erlenmeyer flasks for 2 wk in the dark at 24º C in stationary culture. Mycelium was harvested from the cultures and chopped in a Servall omnimixer for one min and centrifuged at 3000 rpm for 20 min. The pellet was re-suspended in deionized water and the process was repeated three times to remove most of the broth. The hyphae were further broken with glass beads according to the method used by Lippman *et al.*, (1974). The resulting preparation was rinsed and centrifuged as above, lyophilized and stored at -20º C. This crude preparation which contained cell walls free of cytoplasm was used as a substrate for enzyme assays.

Statistical analysis

Mean separation was done Waller's K-ratio t test, Fishers Protected LSD test, or LSD test if the *F* values were significant using a standard ANOVA analysis. Transformations were sometimes used to homogenize the variences and produce normally distributed data. Statistical tests were computed with SAS (SAS Institute, Gary, NC).

RESULTS AND DISCUSSION

Growth and yield

In the Sprinkling plot the mulch significantly increased the growth of trees on Duke 7 rootstock by 117% (Table 1). Similar results documenting increased growth of avocado in mulched soils infested with *P. cinnamomi* have been reported (Broadbent and Baker, 1974; Pegg, 1976; Rosas *et al.,* 1986; Wolstenholme *et al.,* 1996; Zentmyer, 1953). Growth of trees on the other rootstocks, which are more resistant to avocado root rot, were not significantly increased in growth by mulch, although the overall increase in canopy growth for the three rootstocks was 43%. Yield was not significantly increased by the mulch in the Sprinkling plot, but the overall increase in yield was 13% (Table 1). In the Vanoni plot growth was not significantly increased by the mulch (Table 2), and in the first two years growth appeared to be depressed by the mulch. Yield was significantly decreased by 39% by mulch at the Vanoni plot. Where mulches increased the growth, appearance or yield of avocado, it is believed that mulches reduced avocado root rot and improved the ability of avocado to grow in the presence of avocado root rot. Where mulches reduced growth and yields of avocado it is believed that the mulches prevented the soil from drying out. Avocado root rot is favored by wet soils. At the Sprinkling ranch, irrigation was monitored by tensiometer so that the trees were only watered when the soil became dry. In the Vanoni trial, trees were watered every two weeks by the calendar, and this apparently kept the soil too wet and the inhibitory effects of the mulch on avocado root rot was decreased. As the trees became older and used more water, the trees were no longer too wet and the mulches no longer inhibited growth.

Root length

Mulching increased avocado root length by 184% in the Sprinkling plot and by 43% in the Vanoni plot (Table 3). In the mulched plots, roots proliferated greatly in the soil/mulch interface and decreased in numbers further down in the soil (Table 4). In unmulched soil maximum number of roots were found at the 7.5 cm depth. Avocado, which is a shallow rooted tree, naturally prefers to concentrate its roots at the mulch/soil interface, when mulch is present. This preference is exaggerated by the fact that this area is suppressive to *Phytophthora* and therefore roots survive better in this region. In soil without mulch, the avocado is forced to root at deeper depths where *Phytophthora* is often more abundant (Table 4).

Phytophthora infections and soil populations

Root infections due to *P. cinnamomi* were significantly more prevalent in the soil than in the mulch or at the soil/mulch interface despite the fact that there were far more roots at the much/soil interface than in other regions (Table 4). However, *Phytophthora*

populations in the soil below the mulch were unaffected by mulch treatments in both the Sprinkling and Vanoni plots (Table 5).

Zoospore production and hyphal lysis from buried mats

Zoospore production was greatest 7.5 cm below the mulch and was significantly less at the mulch surface and at the mulch/soil interface (Table 6). Hyphal lysis of *P. cinnamomi* was significantly greater at the mulch/soil interface than at depths deeper in the soil (Table 6). It appears that the mulch is inhibitory to *Phytophthora,* but the effect does not extend into the soil.

Enzyme assays

Cellulase and laminarinase, known to degrade cell walls of *P. cinnamomi* hyphae and damage the encystment of zoospores (Downer, 1998; El-Tarabily *et al.,* 1996), were prevalent in the mulches and in the mulch/soil interface but not in the soil (Table 7). These and/or other enzymes, which degraded cell walls of P. cinnamomi, were also present in the mulch but not in the soil (Table 7). It is believed that the cellulase and glucanase enzymes produced in mulches by many cellulose-decomposing fungi, but particularly Basidiomycete fungi which were prevalent in the mulch (Downer, 1998), are responsible for the inhibitory effects of the mulch toward *P. cinnamomi.* These enzymes could readily explain the hyphal lysis and zoosporangia inhibition reported by Broadbent and Baker (1974) from suppressive soil and the suppression of *P. cinnamomi* by cellulase producing bacteria (El-Tarabily, 1996). These enzymes are readily bound and inactivated by montmorillonitic clay soils (Harter and Stolzy, 1971) which explains why these enzymes and the inhibitory effect of the mulches were not found in soil below the mulches. It also may explain the preference of *P. cinnamomi* for clay soils.

CONCLUSIONS

Mulches may be used to promote growth and yield of avocado in *Phytophthora* -infested soils, but care must be taken not to over-irrigate, because mulches increase soil moisture by reducing soil evaporation (Downer, 1998; Wolstenholme *et al.,* 1996). Since *P. cinnamomi* prefers wet soil, over-irrigating mulched soil can exacerbate *P. cinnamomi* and prevent avocados from realizing the full disease-reducing benefits of the mulch. Under these conditions avocados can be stunted.

Yard trimming mulches appear to stimulate abundant root development in the vicinity of the mulches. Cellulase and glucanase are produced abundantly by cellulose decomposing fungi in the mulch. These enzymes are known to lyse cell walls and prevent sporulation of *Phytophthora,* because the cell walls of *Phytophthora* are com-

posed of cellulose and glucans (Downer, 1998). These enzymes are thought to be the inhibitory factor toward *Phytophthora* which has been reported in mulch, many suppressive soils and from the legendary Ashburner mulch from Australia (Broadbent and Baker, 1974; Pegg, 1976; Rosas *et al*., 1986; Wolstenholme *et al*., 1996). The presence of these enzymes in the mulch protects the roots produced there. The enzymes unfortunately do not affect roots produced deeper in the soil, because the enzymes are absorbed and deactivated on clay particles (Harter and Stolzy, 1971).

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Table 1.

The effect of raw yard trimming mulch on growth and yield of avocado on three rootstocks in *Phytophthora* infested soil after three years at the Sprinkling Ranch.

	No Mulch		Mulch	
Rootstocks	Canopy volume Cu M	Yield Kg/tree	Canopy volume Cu M	Yield Kg/tree
Duke 7	1.56B[Z]	1.11B	3.39A	2.01AB
UC2011	2.40A	1.91B	3.05A	1.45B
Thomas	3.96A	2.64A	4.91A	2.94A

[Z]A11 means with the same units not followed by identical letters are significantly different P=0.05 according to ANOVA and mean separation by Waller's K-ratio t test.

Table 2.

The effect of raw yard trimming mulch on growth and yield of avocado on three rootstocks in *Phytophthora* infested soil after three years at the Vanoni Ranch.

Treatment	Canopy volume Cu M	Yield Kg/tree
Mulch		
+	25.1A[Z]	0.81B
-	25.7A	1.33A
Rootstock		
Thomas	24.2B	0.85B
Duke 7	21. 7C	0.67B
Toro Canyon	30.4A	1.80A

[Z]Means in each column not followed by identical letters are significantly different P=0.05 according to ANOVA and mean separation by Fishers Protected LSD test

Table 3.

The effect of raw yard trimming mulch on avocado root length in *Phytophthora* infested soil in two trials.

	Trial	
	Vanoni[y]	Sprinkling[y]
Treatment	Root length (cm/100cc) in mulch and top 15 cm soil	
No mulch	13.3B[z]	5. IB
Mulch	19.0A	14.5A

[y]Avocado rootstock in the Vanoni trial was Thomas. In the Sprinkling trial the rootstock was Duke 7.
[z]Means in each column not followed by identical letters are significantly different P=0.05 according to ANOVA and mean separation by Waller's K-ratio t test.

Table 4.

The effect of raw yard trimming mulch on root length and root infection of avocado at different depths in a *Phytophthora* infested soil.

Sample location	Root length (cm)/100g soil	Number of root infections by *Phytophthora cinnamomi/10 1-cm root pieces*
Mulched tree		
Mulch surface	0.00C[z]	0.0B
Mid -mulch	0.16C	0.0B
Interface	10.24A	0.4B
Soil 7.5 cm	3.78BC	1.2A
Soil 15 cm	3.14BC	0.8AB
Unmulched tree		
Soil surface	0.47C	0.0B
Soil 7.5 cm	6.11 AB	0.4B
Soil 15 cm	3.70BC	1.0A

[z]All values were transformed via Tangent before analysis. Means in each column not followed by identical letters are statistically different P=0.05 according to ANOVA and mean separation by LSD.

Table 5.

The effect of yard trimming mulch on populations of *Phytophthora cinnamomi* in soil under the mulch in two avocado trials.

	Trial	
	Vanoni	Sprinkling
Treatment	Number of leaf baits[y] colonized/g soil	Propagule/g rhizospherel soil
No mulch	0.8A[z]	5.3A
Mulch	1.0A	3.0A

[y]Ten 8mm leaf discs cut from leaves of *Persea indica* were floated on 9 ml water containing 1 g test soil.
[z]Means in each column not followed by identical letters are significantly different according to ANOVA and mean separation by Waller's k-ratio t test.

Table 6.

The effect of yard trimming mulch on zoospore production by and hyphal lysis of *Phytophthora cinnamomi* at different depths in avocado soil.

Sample location	Zoospores/mg mycelium[y]	Lysis rating (0-5)[x]
Mulched tree		
Mulch surface	603C	1.4BC
Mid-mulch	3090AB	2.8ABC
Interface	2065B	4.5A
Soil 7.5 cm	11 426 A	3.6AB
Soil 15 cm	6493AB	3.1ABC
Unmulched tree		
Soil surface	121C	0.8C
Soil 7.5 cm	3640AB	0.9BC
Soil 15 cm	2269B	1.0BC

[x]Visual rating of the integrity of recovered mycelia of *P. cinnamomi:* 0=no hyphal lysis; 5=mycelium completely dissolved.
[y]Zoospores released from mycelial mats of *P cinnamomi* which were placed at the sample locations for three days.
[z]Means in each column not followed by identical letters are significantly different P=0.05 according to ANOVA and mean separation by LSD.

Table 7.

The effect of yard trimming mulch on enzyme activities at different depths in avocado soil.

	Enzyme activity mg reduci ng sugars/g/hr		
Sampling location	CMCase[x]	Laminarinase	"P. cinnase"[y]
Mulched tree			
Mulch surface	4.22A[Z]	11.83A	2.63A
Mid-mulch	3.28A	9.63A	1.53A
Interface	1.44B	3.33B	0.38B
Soil 7.5 cm	0.30C	0.02B	0.13B
Soil 15 cm	0.15C	0.00B	0.00B
Unmulched tree			
Soil surface	0.02C	0.04B	0.00B
Soil 7.5 cm	0.02C	0.00B	0.13B
Soil 15 cm	0.00C	0.08B	0.00B

[x]CMCase is carboxymethyl cellulase.

[y]"P. cinnase" is the enzyme activity detected against cell walls of *Phytophthora cinnamomi.*

[z]Means in each column not followed by identical letters are significantly different P=0.05 according to ANOVA and Mean separation by LSD.