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Urban and agricultural wastes for use as mulches on avocado and citrus and for delivery of microbial biocontrol agents

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SUMMARY

Urban and agricultural waste products generally available to avocado and citrus growers in southern California were analyzed for their suitability for use as bioenhanced mulches on citrus and avocado. Of the mulches tested only yard waste (consisting of wood chips, grass and leaves), rice hulls and rice hulls-and-paper were not harmful to any growth parameter of citrus or avocado and were also adequate substrates for the growth of three biocontrol agents: *Trichoderma harzianum*, *Gliocladium virens*, and *Pseudomonas fluorescens*. Several mulches such as milled peanut hulls, milled almond hulls, chicken manure, a horse/cow manure mixture, cow manure and alfalfa hay were poor substrates for growth of the biocontrol agents and were damaging to at least one growth parameter of avocado and citrus. These mulches released toxic amounts of ammonia upon degradation, some in excess of 1000 $\mu g\ NH_3^{-1}$ dry wt. The percentage of healthy citrus roots, percentage of healthy avocado roots and growth of *T. harzianum* and *P. fluorescens* were negatively correlated with both ammonia evolved and total nitrogen content of the mulches. Citrus grew better with mulches having lower carbon/nitrogen ratios than did avocado; organic matter and organic matter/nitrogen ratio were negatively correlated with citrus shoot weight and the percentage of healthy citrus roots but were not negatively correlated with avocado growth parameters. Growth of all three biocontrol agents was positively correlated with the organic matter/nitrogen ratio and negatively correlated with the pH of the mulches. Growth of *G. virens* was negatively affected by the sodium concentration of the mulches. For the various mulches, the percentage of healthy citrus roots, percentage of healthy avocado roots, avocado root weight, avocado height increase and avocado shoot weight were positively correlated with the growth of *P. fluorescens*. In addition, the growth of *T. harzianum* on mulches was correlated positively with the percentage of healthy avocado roots mulched with the same material. This indicates that mulch characteristics which favour healthy roots of citrus and avocado also favour the growth of *P. fluorescens* and *T. harzianum*.

THE benefits of applying organic mulches to crops are well documented (Gallardo-Laro and Nogales, 1987; Lanini *et al.*, 1988; Opitz, 1974; Stephensen and Schuster, 1945) and include improving soil structure and reducing soil temperature, resulting in improved root growth and more efficient use of water and nutrients. By providing a slow release of nitrogen and other nutrients and increasing the nutrient-holding properties of the soil, organic mulches reduce the need for heavy applications of nitrogen fertilizer and hence decrease possible nitrate contamination of ground water. Organic mulches also help to control weeds, nematodes and plant diseases.

Environmental and human health concerns

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have led to increasing restrictions on the use of chemical fungicides and to the current emphasis in agriculture on adopting alternatives to chemical disease control practices. In response to this situation, we are attempting to develop alternative methods to control Phytophthora root rots of avocado and citrus. It has been suggested that organic material added to soil can reduce Phytophthora root rots of avocado and citrus by (i) increasing the activity of the indigenous microflora resulting in suppression of the pathogen population through competition or specific inhibition (Broadbent and Baker, 1974a; 1974b), (ii) releasing degradation compounds such as carbon dioxide, ammonia, nitrites or saponins that are generally toxic to *Phytophthora* (Zentmyer and Thompson, 1967; Tsao and Zentmyer, 1979), (iii) acting as a trap, since *Phytophthora* zoospores will be attracted to and encyst on organic matter (Grant *et al.*, 1985), (iv) inducing plant defence mechanisms (Gilpatrick, 1969), or (v) creating an environment that stimulates root development but physically inhibits *Phytophthora* (Turney and Menge, 1993a; 1994).

Biocontrol through the use of specific microbial antagonists of pathogens is an attractive approach to disease control. Strains of the common soil bacterium, *Pseudomonas fluorescens*, and the ubiquitous soil fungi, *Gliocladium virens* and *Trichoderma harzianum* (Papavizas, 1985), have received much attention as biocontrol agents for a variety of soilborne plant diseases. In greenhouse experiments, selected isolates of these organisms showed potential for suppressing Phytophthora root rots of avocado and citrus (Casale, 1990). To use microbial biocontrol agents on avocado and citrus in the field, we must (i) apply microbial biocontrol agents without damaging existing roots and (ii) provide soil conditions that maintain the populations and activities of biocontrol agents at effective levels. Organic mulch materials colonized with biocontrol agents (i.e. bioenhanced mulches) allow application of the biocontrol agents as frequently as necessary without mechanical damage to existing roots. In addition, an appropriate mulch and the organic matter added to the soil upon its decomposition could provide a substrate for sustaining the biocontrol agents.

Current laws in California and other states severely inhibit the disposal of urban and agricultural plant waste products into landfills. Sixty-three million tons of this waste material is available in California alone (Anon., 1993) and alternative methods of its disposal currently are being sought. Use of these materials as mulches and substrates for microbial biocontrol agents would be both useful for growers and an innovative method of disposal.

We report here on analyses of various urban and agricultural waste products generally available to avocado and citrus growers in southern and central California. The effects of these materials on plant growth and health, and their ability to support the growth of microbial biocontrol agents is examined. In addition, biological parameters are correlated with the chemical composition of the mulch materials to determine which mulch characteristics are important in predicting their suitability for use on avocado and citrus, and for supporting the growth of microbial biocontrol agents.

MATERIALS AND METHODS

Mulch materials and soils

Fresh alfalfa hay, non-composted cow manure (from a dairy farm), fresh grass clippings and fresh sudangrass hay were obtained locally in southern California. A commercially-available composted chicken manure, called E-Z Green, was obtained from Mountain Spring Ranch, Carlsbad, CA. Fresh milled almond hulls and milled peanut hulls were purchased from C. V. Organic Fertilizers, Thermal, CA. Fresh orange peels were obtained as a waste product from the Sunkist processing plant, Ontario, CA. A heat-treated, composted sewage sludge product, called Thermicompost, and an earthworm-composted sludge product, called Vermicompost (produced by culturing earthworms in Thermicompost) were obtained from the Fallbrook, CA Sanitary District. Mushroom compost (horse manure and straw previously used in commercial mushroom production), fresh rice hulls, fresh rice hulls-and-paper, wood compost and fresh yard waste (V)

(leaves, grass and wood chips) were purchased from Agromin Horticultural Soils, Camarillo, CA. A composted horse/cow manure [1/1] mixture called Avocado Mulch, and fresh yard waste (Ag) (leaves, grass and wood chips) were obtained from Aguinaga Fertilizer, Irvine, CA. Composted yard waste (SB) (composted leaves, grass and wood chips) was obtained from Santa Barbara Parks Department. Approximately one cubic meter of each mulch material was carefully mixed and kept in large plastic bags at 5°C until used in all of the following experiments.

The soil used with citrus was a Vista coarse sandy loam collected from a citrus orchard at Maddock Ranch Nursery, Fallbrook, CA. It had the following characteristics: pH 7.1; organic matter, 0.85%; EC, 2.2 mmhos cm^{-1}; NH_4–N, 3.8 ppm; NO_3–N, 25.9 ppm; P, 11 ppm; K, 82 ppm; Ca, 9.2 meq l^{-1}; Mg, 8.6 meq l^{-1} Na, 6.2 meq l^{-1}; Zn, 4.9 ppm; Mn, 18.5 ppm; Cu, 0.75 ppm; and Fe, 11.5 ppm.

The soil used for avocado was a Placenta sandy loam from an avocado orchard at Barr Ranch, Fallbrook, CA. It had the following characteristics: pH 5.4; organic matter, 3.16%; EC, 2.5 mmhos cm^{-1}; NH_4–N, 5.1 ppm; NO_3, 84 ppm; P, 75 ppm; K, 85 ppm; Ca, 13.6 meq l^{-1}; Mg, 6.9 meq l^{-1}; Na, 6.5 meq l^{-1}; Zn, 95 ppm, Mn, 21 ppm; Cu, 0.86 ppm; and Fe, 60 ppm. These soils were conducive to Phytophthora root rot of citrus and avocado, respectively.

Analyses for standard soil variates

All analyses for standard soil variates were performed by the University of California, Department of Agriculture and Natural Resources Analytical Laboratory except for ammonium (NH_4^+) that was analyzed in our laboratory as described below. Electrical conductivity (EC), a measure of salinity, and pH were determined from a water saturation solution and water interface electrodes (Chapman and Pratt, 1961). Organic matter (OM) was determined by the ignition method (Ball, 1964). Total nitrogen was determined using a semi micro-Kjeldahl procedure (Black *et al.*, 1965). For elemental analysis the soil was digested in 97% potassium sulfate and 3% copper sulfate. Phosphorous, potassium, sodium, calcium, magnesium, manganese, zinc, iron and copper were extracted with acid and quantified on an atomic adsorption spectrophotometer as described as Labanauskas *et al.*, (1967). Chlorine was extracted with acid and detected with a chlorodometer as described by Chapman and Platt (Chapman and Pratt, 1961). Carbonate (CO_3^{2-}) analysis was performed by gravimetric determination after reaction with hydrochloric acid as described by Richards (1954). Cation exchange capacity (CEC) was determined by the modified barium chloride-triethanolamine procedure of Chapman and Pratt (1961).

Determination of cellulose

For determination of cellulose content, samples of mulch materials were dried overnight at 100°C. Two-gramme samples of dried material were ground in 100 ml of distilled water with an Omnimixer for 10 min. The container was cooled in an ice bath during mixing. Ten milliliters of the ground mulch suspension was transferred to a 15 ml Corex tube. Cellulose was determined by the anthrone method described by Updegraff (1969) with the following minor modifications. 2.0 ml of anthrone reagent was added dropwise with mixing to 1.0 ml of sample diluted 100-fold in distilled water. Tubes containing samples were placed in a boiling water bath for 10 min, then cooled in an ice bath. The absorbance at 630 nm was measured and the concentration of glucose from cellulose was estimated from a D-glucose (10 to 100 μg) standard curve. The amount of cellulose in each sample was calculated by multiplying the estimated glucose concentration by 0.9 to account for the addition of water during hydrolysis. To test the accuracy of the cellulose determination method, 20 mg of cellulose powder (prepared from Whatman chromatography paper) in 10 ml distilled water were extracted and assayed as described.

Determination of soluble carbohydrate and amino acids

Air-dried samples of mulch materials were ground in a Wiley Mill with a 2 mm screen. Mulch extracts to be assayed for soluble carbohydrates and amino acids were prepared

as follows. 5 g of ground mulch was extracted four times with 100 ml of 80% ethanol in a closed 250 ml polypropylene centrifuge bottle with rotary shaking at 75°C for 10 min each time. The bottles containing mulch extractions were centrifuged at 4000 × g for 5 min. Next the supernatant was decanted and filtered through Whatman No. 1 paper. After filtration, the filter paper was rinsed with 10 ml of 80% ethanol. The four extracts and washes were pooled and concentrated by evaporation *in vacuo* at 37°C to a volume of less than 50 ml. The volume of each sample was adjusted to 50 ml with water and extracted with 50 ml of chloroform. The phases were separated by centrifugation at 3000 × g for 5 min. The aqueous phase was collected and assayed for carbohydrates and amino acids as described below.

"Total carbohydrates" in mulch extracts was determined by the phenol method (Herbert *et al.*, 1971). The absorbance at 488 nm was measured and the concentration of carbohydrate, expressed as "μg glucose equivalents", was estimated from a D-glucose standard curve. This assay will estimate the concentration of all types of sugars (Herbert *et al.*, 1971).

"Total amino acids" in mulch extracts was determined by the ninhydrin method (Herbert *et al.*, 1971). The absorbance at 570 nm was measured and the α-amino nitrogen concentration estimated from an L-alanine standard curve. Ammonia and other amines also give colours with ninhydrin and, therefore, were included in the estimate of "total amino acids".

Determination of total ammonium

To estimate the total ammonium concentration, 1 g of mulch material was extracted with 10 ml of 10% trichloroacetic acid using a tissue homogenizer. The mixture was centrifuged at 3000 × g for 5 min and the supernatant filtered through Whatman No. 1 paper. The ammonium concentration of the extract was determined with a Wescan Model 360 Ammonia Analyzer (Alltech Associates) (Carlson, 1978) using a KOH-DTPA solution containing 113.4 g of potassium hydroxide and 10 g of diethylenetriaminepentaacetic acid (DTPA) per litre, and an absorbing solution containing 10 g of boric acid and 5 ml of 0.02 M ammonium hydroxide per litre.

Ammonia released during decomposition

Approximately 100 ml of each mulch material was weighed, soaked with distilled water to field capacity and placed in a 900 ml air-tight plastic container. Air was bubbled through water and then via Tygon tubing through the mulch in each container. Ammonia released from the mulch left each container through Tygon tubing and was bubbled into 20 ml of 10% trichloroacetic acid in a 50 ml Erlenmeyer flask. The samples of trapped ammonia were collected every 48 h and analyzed as ammonium as described above. The experiment was continued for 20 d. The experiment was arranged in a randomized complete block design with four replicates on a greenhouse bench. For a control, 10 ml of ammonium chloride (50 ppm) in 10% trichloroacetic acid was added to 10 ml of KOH-DTPA solution in a 50 ml Erlenmeyer flask placed inside a plastic container.

Mulching citrus and avocado seedlings

Greenhouse experiments were used to examine the effects of mulches on plant growth and root health. No experiments included infestation of soil with any pathogens. Mulches were applied to 'Topa Topa' avocado and 'Troyer' citrange seedlings in a greenhouse as follows. Six to twelve month old citrange seedlings grown in flats containing peat moss:sand (1:1) were transplanted into individual 3 litre plastic pots containing an autoclaved citrus orchard (Maddock Ranch) soil. Avocado seeds were germinated and the young seedlings grown in individual 5 × 7 × 30 cm black poly planting sleeves with holes (Norplex Inc., Kent, WA). Three- to four-month old avocado seedlings were transplanted with root ball intact into individual 3 litre plastic pots containing autoclaved avocado orchard (Barr Ranch) soil.

Two days after transplanting, mulch material (500 ml, loosely packed) was layered on the soil surface in each pot. The diversity in the types of mulches used resulted in densities that varied considerably among the mulches. Five replicate plants were used per treatment and

TABLE 1

Cellulose, total soluble carbohydrate and soluble amino acid (as α-amino nitrogen) contents of mulch materials. Also indicated is whether the mulch materials used throughout this work are composted or not composted

Mulch material	Cellulose concentration (mg g^{-1} dry wt)	Soluble carbohydrate concentration (mg glucose eq g^{-1} dry wt)	α-amino nitrogen concentration (μg g^{-1} dry wt)
Rice hulls & paper (not composted)	446.9 ± 13.9*	3.54 ± 0.04	40.74 ± 1.57
Wood compost	409.6 ± 12.9	4.57 ± 0.07	59.25 ± 4.21
Yard waste (V) (not composted)	334.3 ± 2.1	11.90 ± 0.04	75.98 ± 1.81
Grass clippings (not composted)	328.1 ± 14.5	3.38 ± 0.05	5499.30 ± 31.54
Sudangrass hay (not composted)	322.8 ± 1.9	83.80 ± 3.32	1233.26 ± 31.14
Horse/cow manure (composted)	225.1 ± 9.1	2.37 ± 0.09	57.73 ± 2.99
Alfalfa hay (not composted)	219.6 ± 2.3	27.10 ± 0.20	727.05 ± 0.82
Milled peanut hulls (not composted)	199.5 ± 2.4	116.40 ± 2.11	1513.73 ± 54.10
Composted yard waste (SB)	177.4 ± 9.2	2.20 ± 0.06	265.84 ± 11.82
Rice hulls (not composted)	160.3 ± 2.3	8.30 ± 0.62	80.22 ± 2.36
Chicken manure (composted)	148.4 ± 5.3	1.19 ± 0.03	370.45 ± 10.24
Cow manure (not composted)	137.9 ± 4.4	1.10 ± 0.03	33.19 ± 0.94
Composted sewage sludge	131.4 ± 1.4	2.52 ± 0.05	435.10 ± 6.86
Yard waste (Ag) (not composted)	121.4 ± 7.9	6.84 ± 0.05	592.70 ± 6.60
Mushroom composted	88.4 ± 7.6	1.35 ± 0.08	71.26 ± 2.20
Orange peels (not composted)	86.9 ± 6.5	320.54 ± 2.67	771.89 ± 13.79
Milled almond hulls (not composted)	84.1 ± 12.2	108.05 ± 2.69	653.90 ± 11.80
Earthworm-composted sludge	64.2 ± 0.8	0.99 ± 0.03	63.86 ± 1.55
Maddock soil	54.6 ± 4.0	not assayed	not assayed

*Mean ± standard deviation of at least 3 replicate samples.

plants were arranged in a randomized complete block design on greenhouse benches. The experiment was repeated and the resulting data combined for analyses. Daily temperatures fluctuated from 18.5 to 30°C with a mean of 24°C. Plants were irrigated as needed with 14% Hoagland's solution minus phosphorus. The experiments were terminated three months after transplanting.

Growth of microbial biocontrol agents on mulch materials

Three biocontrol agents, *Trichoderma harzianum* benomyl-resistant mutant KA159-2, *Gliocladium virens* isolate KA230 and *Pseudomonas fluorescens* kanamycin-resistant mutant C8-2, were included in this study. *T. harzianum* and *G. virens* were grown separately on potato dextrose agar (PDA) plates for 7 d. Conidia were harvested by shaking the contents of a plate, including the sporulating fungus and agar medium, in a sterile 1-litre beaker containing 300 ml of sterile water plus a drop of Tween 20. The suspension was filtered through several layers of sterile cheese cloth to remove the agar and hyphal fragments. The spore suspensions were adjusted to 3×10^3 conidia ml^{-1}. *P. fluorescens* was grown on Difco Pseudomonas Agar F (PAF) containing kanamycin (50 μg ml^{-1}) and incubated at room temperature for 24 h. The bacteria were harvested in sterile water and the concentration adjusted to 1.2×10^5 cfu ml^{-1}.

Air-dried mulch material was placed in sterile 18 × 150 mm culture tubes (10 ml of mulch material per tube) and fumigated with methyl bromide (454 g in a 112 l chamber for 48 h). The samples were removed from the chamber 48 h prior to inoculation with biocontrol agents. Each tube was inoculated with 4 ml of conidial or bacterial suspension and incubated at room temperature under ambient light conditions.

Populations of biocontrol agents colonizin the mulch materials were estimated by dilutio plating. *T. harzianum* and *G. virens* cultures were assayed 10 d after inoculation by blending 0.2 g dry wt samples in 200 ml sterile water using a hand-held blender (Hamilton Beach) for 10 sec. Serial dilutions in sterile water were plated on PDA containing benomyl (50 ppm) and ampicillin (80 ppm) for *T. harzianum*, or on rose bengal medium (RB-M2) (Tsao, 1964) for *G. virens*. Populations of *P. fluorescens* were determined at 4 and 30 d after inoculation by shaking 0.2 g dry wt samples in 20 ml sterile water; serial dilutions were plated on PAF containing kanamycin (50 ppm).

TABLE IIA
Analysis of mulch materials for standard soil characteristics

Mulch material	pH	EC (mmhos cm $^{-1}$)	OM (%)	N (%)	NH_4^+ (μg g^{-1} dry wt)
Alfalfa hay	5.9	12.88	91.9	2.360	390
Horse/cow manure	8.4	21.10	54.5	1.136	440
Cow manure	8.0	27.10	42.1	1.371	2470
Chicken manure	6.4	34.60	32.2	1.349	5780
Grass clippings	5.8	32.10	n.d.*	4.215	2130
Milled almond hulls	5.7	7.80	93.3	5.470	380
Milled peanut hulls	6.2	14.44	94.9	6.600	7200
Mushroom compost	7.3	34.00	40.0	1.363	910
Orange peels	3.4	6.32	92.8	1.079	33
Rice hulls	6.0	5.38	76.5	0.231	100
Rice hulls & paper	6.2	3.14	n.d.	0.673	18
Soil (Maddock)	7.9	2.75	2.7	0.072	5
Sudangrass hay	5.7	11.76	94.5	1.011	765
Composted sewage sludge	6.2	11.74	46.8	1.535	248
Earthworm-composted sludge	6.7	17.86	35.8	1.116	271
Wood compost	5.5	4.46	n.d.	0.880	162
Yard waste (Ag)	5.6	15.10	60.3	1.529	3130
Composted yard waste (SB)	7.6	13.79	49.0	1.577	2660
Yard waste (V)	4.8	10.11	n.d.	1.562	125

*n.d. = not determined.

RESULTS

Chemical assays

The cellulose contents of mulch materials determined as described above are presented in Table I. The cellulose content of three 20 mg samples of cellulose powder used to test the accuracy of the cellulose assay was estimated at 19.69 mg (s = 0.65 mg).

The total soluble carbohydrate and amino acid concentrations of mulch materials as determined by these assays are shown in Table I. In preliminary experiments, the soluble carbohydrates and amino acids in the fourth 80% ethanol extraction represented less than 10% of the total amounts from the four extractions. Extraction for 25 or 60 min did not yield more detectable amino acids or carbohydrate than did the 10 min extraction. Whatman No. 1 paper used for filtering aqueous ethanol extracts did not absorb significant amounts of the assayed material based on comparisons with extracts filtered through glass wool.

Other chemical analyses of mulch materials

TABLE IIB
Analysis of mulch materials for standard soil characteristics (continued)

Mulch material	P (ppm)	K (ppm)	Na (meq l^{-1})	Ca (meq l^{-1})	Mg (meq l^{-1})	Mn (ppm)
Alfalfa hay	283	21,500	12.7	67.9	28.0	21
Horse/cow manure	201	21,000	46.6	6.2	7.2	9
Cow manure	812	27,200	59.4	3.9	11.0	5
Chicken manure	868	22,700	66.6	11.1	64.5	65
Grass clippings	14,000	52,800	32.8	6.5	39.1	71
Milled almond hulls	n.d.*	14,800	1.4	8.7	46.5	11
Milled peanut hulls	264	13,500	1.5	4.8	50.5	13
Mushroom compost	150	21,600	69.1	72.5	38.9	44
Orange peels	700	12,700	10.7	6.5	5.5	50
Rice hulls	63	5,560	1.8	0.6	5.3	123
Rice hulls & paper	1,600	3,210	6.6	3.0	4.8	115
Soil (Maddock)	38	820	7.2	13.2	8.9	5
Sudangrass hay	469	16,200	2.6	11.0	18.0	14
Composted sewage sludge	265	6,840	31.6	8.0	11.8	85
Earthworm-composted sludge	250	8,570	62.8	15.3	20.9	50
Wood compost	900	5,910	9.0	4.4	8.7	51
Yard waste (Ag)	196	14,000	25.6	10.4	23.8	56
Composted yard waste (SB)	224	13,200	12.3	19.0	20.3	32
Yard waste (V)	1,400	10,500	14.8	62.0	36.5	20

*n.d. = not determined.

TABLE IIc
Analysis of mulch materials for standard soil characteristics (continued)

Mulch material	Zn (ppm)	Fe (ppm)	Cu (ppm)	Cl (meq l^{-1})	CO_3^{2-} (meq l^{-1})	CEC (meq 100^{-1} g)
Alfalfa hay	19	21	7	38.3	<0.1	25.5
Horse/cow manure	15	5	2	118.8	<0.1	44.5
Cow manure	20	59	3	150.3	<0.1	41.0
Chicken manure	204	145	16	90.9	<0.1	31.5
Grass clippings	32	44	5	135.9	<0.1	27.0
Milled almond hulls	20	24	7	4.1	<0.1	<0.5
Milled peanut hulls	10	27	9	5.4	<0.1	<0.5
Mushroom compost	34	310	3	149.4	<0.1	44.5
Orange peels	3	19	2	15.0	<0.1	<0.5
Rice hulls	6	14	1	16.7	<0.1	<0.5
Rice hulls & paper	16	81	3	9.7	<0.1	18.5
Soil (Maddock)	19	58	3	7.7	<0.1	14.5
Sudangrass hay	10	7	3	49.5	<0.1	<0.5
Composted sewage sludge	112	477	48	55.3	<0.1	48.5
Earthworm-composted sludge	121	373	108	91.2	<0.1	39.5
Wood compost	47	27	2	18.5	<0.1	23.5
Yard waste (Ag)	73	290	6	66.6	<0.1	45.5
Composted yard waste (SB)	190	147	4	63.8	<0.1	47.0
Yard waste (V)	14	4	2	27.4	<0.1	45.5

for standard soil parameters are shown in Tables IIa, IIb, and IIc.

Ammonia released during decomposition

The greatest amounts of ammonia were released from alfalfa hay, grass clippings, milled almond hulls and milled peanut hulls (Table III). Alfalfa hay and grass clippings showed a peak of ammonia production early, between two and eight days (Figure 1). Ammonia release from alfalfa hay dropped to a low level by 14 d, whereas grass clippings still had moderately high ammonia release after 14 d. Peak release of ammonia from decomposition of milled almond hulls occurred later, between 14 and 20 d (Figure 1). Milled peanut hulls had a gradually increasing release of ammonia over the course of the experiment (Figure 1). Ammonia release as a function of time for mulch materials with lower total ammonia release are not shown. Recovery of ammonia from the ammonium chloride control ranged from 87–98%. The pH of the mulch materials did not change significantly during incubation (data not shown).

TABLE III
Total ammonia released during 20 d of decomposition of mulch materials

Mulch material	Total ammonia released (μg g^{-1} dry wt)
Grass clippings	13,403.8 ± 1830.4[x]
Milled almond hulls	10,943.2 ± 1380.9
Alfalfa hay	8,485.5 ± 978.1
Milled peanut hulls	2,546.3 ± 1169.6
Composted sewage sludge	1,121.4 ± 145.3
Chicken manure	943.0 ± 166.1
Horse/cow manure	923.2 ± 157.5
Sudangrass hay	705.6 ± 229.6
Cow manure	558.3 ± 37.3
Mushroom compost	546.3 ± 118.4
Composted yard waste (SB)	545.4 ± 131.1
Yard waste (Ag)	181.6 ± 118.2
Rice hulls	59.8 ± 22.0
Rice hulls & paper	31.9 ± 12.6
Earthworm-composted sludge	19.8 ± 6.5
Wood compost	18.2 ± 6.8
Orange peels	7.9 ± 3.3
Soil (Maddock)	0.3 ± 0.2
Yard waste (V)	0.2 ± 0.1

[x]Mean ± standard deviation of four replicate samples.

Effect of mulches on citrus and avocado seedlings in the greenhouse

None of the mulches significantly increased growth of citrange seedlings compared to the control (no mulch) (Table IV). Some mulches clearly were detrimental to the plants (Table IV). Almond and peanut hulls were most damaging with less than 10% of the roots in these treatments appearing healthy. Other mulches which significantly ($\alpha = 0.05$) reduced at least one growth parameter of citrus included alfalfa hay, sudangrass hay, orange peels, grass clippings, chicken manure, wood compost and yard waste (Ag). Soils were autoclaved prior to use in greenhouse experiments and we were unable to isolate *Phytophthora* from roots at the end of the

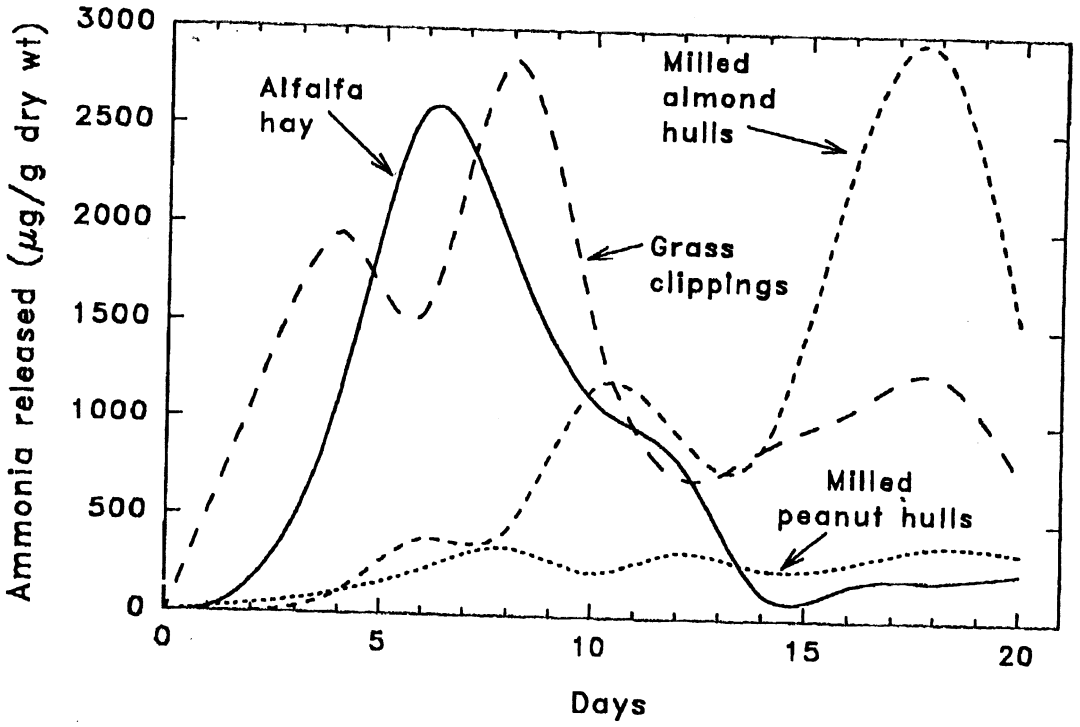

FIG. 1.
Ammonia released by alfalfa hay, grass clippings, milled almond hulls and milled peanut hulls during decomposition. Measurements include ammonia released and trapped as described over 48 h periods.

TABLE IV
Effect of mulches on growth and health of Troyer citrange seedlings in the greenhouse. Six to twelve week old seedlings were transplanted into three-litre plastic pots containing sterile soil which was then overlayed with various mulches; data were taken after three months. Means presented below are the average of two experiments

Mulch material	Healthy roots (%)	Fresh weight (g)		Height increase (cm)
		Root	Shoot	
Yard waste (V)	91.0 a[x]	2.63 abc	7.88 a	11.6 ab
Control	89.6 ab	4.16 a	8.75 a	6.2 ab
Horse/cow manure	84.6 abc	3.46 abc	5.97 abcd	8.0 ab
Cow manure	82.6 abcd	3.41 abc	7.36 abc	11.4 ab
Rice hulls	79.4 abcd	4.13 ab	5.70 abcd	5.6 b
Composted sewage sludge	78.0 abcd	4.25 a	8.31 a	14.8 a
Composted yard waste (SB)	77.8 abcde	4.41 a	7.71 ab	9.0 ab
Earthworm-composted sludge	75.8 abcde	3.41 abc	6.09 abcd	12.0 ab
Yard waste (Ag)	74.0 abcde	2.14 bc	3.18 cd	7.8 ab
Rice hulls & paper	73.6 abcde	2.91 abc	7.15 abcd	11.4 ab
Mushroom compost	60.4 bcdef	3.18 abc	5.35 abcd	6.6 ab
Alfalfa hay	58.4 cdef	3.56 abc	5.42 abcd	8.6 ab
Sudangrass hay	56.8 cdef	2.97 abc	3.51 bcd	8.4 ab
Orange peels	53.6 def	2.71 abc	6.30 abcd	9.6 ab
Grass clippings	47.4 efg	2.57 abc	4.96 abcd	9.0 ab
Chicken manure	43.0 fg	1.65 c	3.27 cd	5.0 b
Wood compost	22.8 gh	1.75 c	3.15 cd	9.5 ab
Milled almond hulls	8.6 h	3.14 abc	4.48 abcd	7.0 ab
Milled peanut hulls	2.6 h	3.24 abc	3.05 d	—

[x]Means in a column with the same letter are not significantly different by LSD ($\alpha = 0.05$).

TABLE V

Effect of mulches on growth and health of avocado cv. Topa Topa seedlings in the greenhouse. Three-to four month old seedlings were transplanted into three-litre plastic pots containing sterile soil which was then overlayed with various mulches; data were taken after three months. Means presented below are the average of two experiments

Mulch material	Health roots (%)	Fresh weight		Height increase (cm)
		Root	Shoot	
Yard waste (Ag)	99.1 a[x]	58.3 a	71.3 ab	8.6 bcd
Earthworm-composted sludge	96.3 ab	43.0 ab	68.1 abc	11.0 abc
Wood compost	90.4 abc	35.5 bc	41.9 de	5.2 defgh
Grass clippings	90.0 abc	30.1 bcd	71.4 ab	15.0 a
Composted yard waste	88.7 abc	35.0 bc	77.4 a	13.2 ab
Rice hulls & paper	87.5 abc	27.9 bcde	44.3 de	3.4 defgh
Yard waste (V)	76.2 abc	35.3 bc	45.9 de	3.8 defgh
Rice hulls	74.9 abc	36.6 bc	52.7 bcde	6.0 cdef
Sudangrass hay	65.2 abcd	19.3 cdef	61.0 abcd	7.2 cde
Orange peels	59.0 bcd	25.2 bcde	50.2 cde	4.0 defgh
Control	58.9 bcd	18.3 cdef	45.9 de	4.0 defgh
Alfalfa hay	58.4 cd	27.7 bcde	47.0 de	5.8 cdefg
Mushroom compost	57.8 cd	28.2 bcde	44.9 de	8.2 bcd
Composted sewage sludge	32.2 de	16.0 def	47.0 de	4.2 defgh
Horse/cow manure	22.6 e	11.3 ef	38.7 ef	2.4 efgh
Cow manure	10.4 e	6.3 f	23.0 fg	1.2 fgh
Chicken manure	9.8 e	5.0 f	17.2 g	0.2 gh
Milled almond hulls	0.1 e	4.5 f	11.5 g	0.0 h
Milled peanut hulls	0.1 e	3.5 f	8.7 g	0.0 h

[x]Means in a column with the same letter are not significantly different by LSD ($\alpha = 0.05$).

experiment, so the damage was due to effects of the mulches.

In contrast, avocado seedlings did show a beneficial effect from some mulches (Table V). Yard waste (Ag)-treated avocados had the greatest percentage of healthy roots and highest root fresh weight (Table V). Other mulches which significantly ($\alpha = 0.05$) improved at least one growth parameter of avocado included earthworm-composted sludge (Vermicompost), grass clippings and composted yard waste (SB). The plant materi-

TABLE VI

Populations of biocontrol agents after growth on 10 ml of mulch materials in culture tubes; Trichoderma harzianum *KA159-2 and* Gliocladium virens *KA230 after 14 d;* Pseudomonas fluorescens *C8-2 after 4 and 30 d*

Mulch material	Population (log cfu ml^{-1})			
	Trichoderma harzianum	*Gliocladium virens*	*Pseudomonas fluorescens*	
			4 d	30 d
Sudangrass	9.43 a[x]	9.17 a	9.41 b	8.38 cd
Wood compost	9.28 a	7.06 b	8.70 b	7.88 cd
Orange peels	8.91 b	7.04 b	0.00 b	0.00 d
Rice hulls	7.70 b	7.01 b	9.48 b	8.77 b
Yard waste (V)	7.35 b	9.09 a	6.68 b	7.92 cd
Rice hulls & paper	7.32 b	7.75 b	9.39 b	8.47 c
Composted yard waste (SB)	6.54 b	4.00 b	7.46 b	0.00 d
Yard waste (Ag)	6.42 b	8.97 ab	8.98 b	8.93 a
Composted sewage sludge	6.12 b	5.34 b	10.40 a	6.26 d
Earthworm-composted sludge	5.45 b	4.30 b	7.72 b	6.41 d
Milled peanut hulls	4.95 b	6.12 b	0.00 b	8.17 cd
Alfalfa hay	0.00 b	0.00 b	9.49 b	6.95 d
Horse/cow manure	0.00 b	0.00 b	6.64 b	0.00 d
Cow manure	0.00 b	0.00 b	7.62 b	0.00 d
Chicken manure	0.00 b	5.86 b	0.00 b	5.60 d
Grass clippings	0.00 b	4.70 b	9.93 b	5.00 d
Milled almond hulls	0.00 b	5.74 b	0.00 b	0.00 d
Mushroom compost	0.00 b	0.00 b	7.16 b	5.00 d
Soil (Maddock)	0.00 b	0.00 b	6.78 b	6.18 d

[x]Means with a common letter are not significantly different by LSD ($\alpha = 0.05$).

TABLE VII
Significant correlations ($\alpha = 0.05$) among growth parameters by Pearson's correlation test. Data are shown in other tables

Parameter measured	Correlation	Significantly correlated parameters
Citrus (Troyer citrange) growth		
Shoot fresh weight	negative	OM, OM/N, NH_4^+, Mg
Root fresh weight	positive	pH
Healthy roots (%)	positive	CEC, *P. fluorescens*
Healthy roots (%)	negative	OM, OM/N, N, Mg, NH_3
Avocado (Topa Topa) growth		
Seedling height increase	positive	*P. fluorescens*
Shoot fresh weight	positive	*P. fluorescens*
Root fresh weight	positive	*P. fluorescens*
Healthy roots (%)	positive	*T. harzianum*, *P. fluorescens*
Healthy roots (%)	negative	N
Growth of biocontrol agents		
Trichoderma harzianum	positive	OM/N, avocado healthy roots, *G. virens*
Trichoderma harzianum	negative	pH, EC, K, NH_3
Gliocladium virens	positive	OM/N, *T. harzianum*
Gliocladium virens	negative	pH, Na
Pseudomonas fluorescens	positive	Mn, OM/N, citrus healthy roots, avocado height increase, avocado shoot weight, avocado root weight, avocado healthy roots
Pseudomonas fluorescens	negative	soluble carbohydrate, pH, Mg, N

als generally had the most beneficial effects on avocado seedling growth and root health, whereas the manure-based materials (with the exception of the well composted earthworm-composted sludge product) were generally detrimental to avocado (Table V). Mulches which significantly (α = 0.05) decreased at least one growth parameter of avocado were horse/cow manure, cow manure, chicken manure, milled almond hulls and milled peanut hulls.

Growth of microbial biocontrol agents on mulch materials

T. harzianum grew best on sudan hay and wood compost (Table VI), whereas *G. virens* grew best on sudan hay and yard waste (V) (Table VI). These mulches were cellulose-rich with a low nitrogen content. *T. harzianum* did grow on the yard waste materials, but the populations were not as high as they were on sudan grass hay or wood compost. No colonies of either *T. harzianum* or *G. virens* were detected in alfalfa hay, horse/cow manure, cow manure, mushroom compost or soil. *T. harzianum* was not detected in grass clippings, chicken manure or milled almond hulls, whereas *G. virens* was recovered at relatively low populations from these materials. *G. virens* grew very well on yard waste (V) and yard waste (Ag), but the mean population of *G. virens* on the composted yard waste (SB) was considerably lower than the other yard wastes, although the differences between growth on yard waste (Ag) and composted yard waste (SB) were not judged statistically significant (Table VI).

Populations of *P. fluorescens* were measured after 4 and 30 d of growth (Table VI). Composted sewage sludge (Thermicompost) had the highest populations of *P. fluorescens* after 4 d, but the levels dropped 10^4-fold by 30 d. The change in *P. fluorescens* populations on most mulch materials was not so dramatic, typically less than 30-fold (Table VI). Yard waste (Ag) supported the highest populations of *P. fluorescens* after 30 d. Rice hulls and rice hulls-and-paper also had significantly higher populations of *P. fluorescens* than the control soil. Although at 4 d moderate populations of *P. fluorescens* were measured in cow manure, composted yard waste (SB) and horse/cow manure, no *P. fluorescens* was detected in these materials after 30 d. No *P. fluorescens* was detected in milled almond hulls or orange peels at either 4 or 30 d.

Statistical correlations among parameters

Statistical correlations were used to identify characteristics of mulch materials useful for

predicting their performance as bioenhanced mulches, although these correlations do not prove a cause and effect relationship. Table VII shows the correlation ($\alpha = 0.05$) of physical and chemical variates of mulch materials with growth parameters of avocado and citrus seedlings and the growth of biocontrol agents in those mulches. Shoot fresh weight of citrus seedlings treated with mulches was negatively correlated with organic matter content, organic matter/nitrogen ratio, magnesium concentration and ammonium concentration of the mulches. Fresh weights of citrus roots was positively correlated only with the pH of the mulches. The percentage of healthy citrus roots growing in the mulch treatments was positively correlated with the cation exchange capacity of the mulches and the populations of *P. fluorescens* supported by the mulches. The percentage of healthy citrus roots was negatively correlated with organic matter content, organic matter/nitrogen ratio, magnesium concentration, total nitrogen, and released ammonia of the mulches. All of the avocado parameters (seedling height increase, shoot weight, root weight and the percentage of healthy roots) were positively correlated with the growth of *P. fluorescens* in the mulches. This means that mulch materials which were beneficial to avocado growth and health also were able to support high populations of *P. fluorescens*. In addition, avocado seedling height increase was positively correlated with phosphorous concentrations of the mulch; the percentage of healthy avocado roots was positively correlated with the growth of *T. harzianum* on the mulches. The only factor judged to be detrimental to avocado was total nitrogen content of the mulches which reduced the percentage of healthy avocado roots.

Trichoderma harzianum and *G. virens* responded similarly ($\alpha = 0.05$) to the mulches. The organic matter/nitrogen ratio of the mulches was positively correlated with the growth of these two fungi in the mulches, whereas the pH of the mulches was negatively correlated with fungal growth. In addition, growth of *T. harzianum* in the mulches was positively correlated with the percentage of healthy avocado roots and negatively correlated with EC, potassium concentration and the released ammonia of the mulches. Growth of *G. virens* in the mulches was negatively correlated with the sodium concentration of the mulch. As noted above, *P. fluorescens* population levels in the mulches was correlated with all the avocado growth parameters as well as with the percentage of citrus healthy roots. In addition, growth of *P. fluorescens* was positively correlated with the organic matter/nitrogen ratio and the manganese concentration of the mulches. *P. fluorescens* growth was negatively correlated with pH, the concentration of magnesium, total nitrogen and soluble carbohydrate.

DISCUSSION

Citrus and avocado are well suited to benefit from mulches and the introduction of specific biocontrol agents in the form of bioenhanced mulches has potential for further improving root health. Both citrus and avocado have very shallow root systems. Citrus normally produce nearly 80% of their roots in the top 50 cm of soil (Cahoon *et al.*, 1956), whereas avocados produce nearly 60% of their roots in the top 40 cm of soil (Salazar-Garcia and Cortes-Flores, 1986). The wild ancestors of citrus and avocado compete in forest ecosystems and have evolved with a distinct subtropical litter layer covering their shallow root systems. It has been observed (Broadbent and Baker, 1974a; 1974b) that these trees grow best with a mulch that mimics a forest litter layer. We have observed that avocado roots will often grow up directly into a mulch layer (which may explain why avocado appears to respond more positively to mulching (Tables IV, V and VII) then does citrus).

Among the mulches examined, yard waste should most closely resemble a forest litter layer in structure and chemical composition. Several types of yard wastes composed of wood chips, grass clippings and leaves are among the materials which did not damage citrus and which stimulated the growth of avocado (Table IV and V). Green manures such as mustard, clover, vetch, barley, alfalfa, peas and bean straw have been used as mulches on citrus in the past with positive results (Craig, 1916; Hodgson, 1925; Vaile,

1922; McNees, 1916; Lefferts, 1919; Kelly, 1922; Mertz, 1918). Avocados also seem to respond to green manures such as *Dolichos*, corn, lupine, oats, barley, tree prunings (including leaves) or even weed species piled around the crown as mulches (Broadbent and Baker, 1974a; 1974b; Turney and Menge, 1994). These materials, especially tree prunings, are not unlike the fresh yard waste materials reported here, and after some decomposition would approximate composted yard waste. In the field, with time, these mulches would come to resemble a forest litter layer.

Wood compost was not beneficial to citrus (Tables IV and V), which agrees with the results of McNees (1916) with wood shavings in the field. A popular explanation for the negative affects of wood chips on citrus is that they include *Eucalyptus* wood which contains oils that damage plant roots. This apparently is not the case as shown by Hardy and Sivasithamparam (1989) who use composted *Eucalyptus* bark for a container medium in which to grow plants. Also, much of the wood chips in the yard waste material used in this study was from *Eucalyptus* and this material did not damage citrus or avocado. In light of these results, a better explanation might be that decay of a mulch with a high carbon/nitrogen ratio immobilizes nitrogen and results in a temporary nitrogen shortage. A mulch layer will typically decompose during a three-month greenhouse experiment (with adequate watering) to approximately 1/5 to 1/10 its initial thickness. This explanation is consistent with the results of McNees (1916), since he did not add supplemental nitrogen. The benefits of yard wastes observed in our study can be explained by the high nitrogen concentration grass that offsets the high C/N composition of wood chips and greatly reduced any temporary nitrogen shortage. Grass clippings typically have the highest nitrogen content (and lower C/N ratio than wood) of available plant materials because of the extensive use of nitrogen fertilizers on lawns. While nitrogen is an important fertilizer for both citrus and avocado, citrus responds to nitrogen deficiencies far more rapidly than does avocado. This may explain why citrus preferred higher nitrogen and lower carbon mulches than did avocado. High organic matter and organic matter/nitrogen ratio of the mulches was correlated with reduced citrus shoot growth and the percentage of healthy citrus roots, but avocado growth was not negatively affected (Table VII).

Rice hulls or rice hulls-and-paper, while not outstanding, also appeared to be adequate as a mulch material for citrus and avocado. Other strictly plant materials, such as sudangrass hay, alfalfa hay, grass clippings, and orange peels all inhibited growth or reduced root health of citrus or avocado under our conditions.

The sewage sludge materials, Thermicompost and Vermicompost, contained straw collected from stables and also appeared to be acceptable as mulch materials (Tables IV and V). Mushroom compost, an animal manure mixed with straw and which had previously been used to grow mushrooms and contained mushroom refuse, was a benign mulch material for citrus and avocado, even though it contained high levels of sodium.

Several of the mulches such as milled peanut hulls, milled almond hulls, chicken manure, horse/cow manure, cow manure, and alfalfa hay appeared to be unsuitable since they reduced avocado or citrus growth or root health and resulted in undetectable populations of at least two of the biocontrol agents. These mulches all released large amounts of ammonia upon degradation (Table III); several released the ammonia in peaks that were in excess of 1000 $\mu g\ NH_3\ g^{-1}$ dry wt (Figure 1). However, several mulches such as composted sewage sludge, sudangrass hay and grass clippings all produced more ammonia upon degradation than did cow manure and did not have as adverse an effect on the growth of plants or biocontrol microorganisms, although two of these mulches were detrimental to citrus root health. Although ammonia may be important in limiting the use of certain materials as mulches for citrus and avocado for carriers of biocontrol agents, it is likely that many factors contributed to waste materials being unsuitable. The significance of each of these factors may also vary among specific mulches, so that no single mulch

characteristic is of primary importance in all cases.

The observation that animal waste materials such as chicken manure, cow manure and horse/cow manure, as a group, are generally unsuited for use as mulches and as carriers for microbial biocontrol agents is important since they are among the most common amendments to citrus and avocado orchards in California. However, it is possible that these materials, when applied infrequently at lower rates, are beneficial since they provide nutrients for growth. In light of the discussion above on immobilization of nitrogen by high C/N ratio mulches, well composted animal manures which do not release ammonia may be acceptable for use on citrus.

Root damage after applying animal manures to citrus orchards is common (personal observation) and the difficulty in judging the stability of these waste materials after composting and in identifying application rates makes the use of animal manures as mulches a problem for citrus and avocado growers. We have observed that high ammonia releasing mulches often cause obvious damage to young citrus and avocado trees in the field, whereas established trees do not seem to be as severely damaged. Young trees are also more sensitive to *Phytophthora* infection. The reason for this is not clear. Rather than any increase in resistance of feeder roots with tree age, however, we believe the explanation may reside in the higher foliage-to-root ratio of young compared with older trees. Since young trees require a greater proportion of their root system to support the plant than do older trees, young trees have a lower threshold for tolerating root damage. Therefore, some of the high ammonia releasing mulches that were phytotoxic to young plants in the greenhouse may be used on established trees in the field with little or no noticeable damage. However, even larger trees will suffer some root loss even though above-ground symptoms are negligible.

Caution should be used when extending the results of these greenhouse and laboratory experiments to effects of mulching in the field. The confines of pots and the short duration of the experiments probably exaggerated the harmful effects of phytotoxic compounds. Also, many of the long-term beneficial effects such as improved soil structure are not measured adequately. However, these results represent a rapid screening method which allows us to concentrate on the most promising mulches for further field studies. The results also correlate well with historical mulching experiments with citrus and avocado. Vaile (1922), in a series of large experiments, determined that manures enhanced the growth of citrus trees when compared with non-fertilized trees, but commercial fertilizers and green manures often were superior. Benefits were delayed for several years after manure treatments were initiated and the best results were seen 1–2 years after manurial treatments were discontinued. Parker and Jones (1951) also stated that green manures often enhanced citrus productivity more than did animal manures. Mertz (1918) found that green manured trees were superior in every way (tree size, yield and fruit size) to trees similarly treated with animal manures.

In our greenhouse experiments, although seeds were planted into sterilized potting medium and seedlings transplanted into sterilized soil, subsequent contamination with microorganisms present in the greenhouse environment was inevitable. Moreover, mulch materials used in greenhouse experiments were not sterilized and therefore contributed a significant microflora. Apart from performing isolations from damaged roots to detect pathogens (which were not found), we made no identifications of microorganisms present in the system. The activity of microorganisms was, of course, an important component of the system as it was responsible for the decomposition of the mulch materials and release of nutrients. In this initial screening of mulch materials, however, we made no attempt to identify or monitor microbial populations. We acknowledge the significance of microbial activity to the system, of course, and are addressing this in subsequent, particularly field, experiments. Therefore, we are unable to comment on the relationship of the observed plant responses to actual measure-

ments of microbial activity or specific microorganisms in the present study.

The biocontrol agents, *G. virens*, *T. harzianum* and *P. fluorescens*, as a group grew best in yard waste, sudangrass hay, wood compost, rice hulls and rice hulls-and-paper (Table VI). Of these, only yard waste, rice hulls and rice hulls-and-paper were completely acceptable as mulches for avocado and citrus. The sewage sludge materials, Thermicompost and Vermicompost, supported growth of the biocontrol agents, although they were not outstanding in this regard (Table VI), but could be considered as mulches for the tree crops, especially citrus. All other mulch materials either did not adequately support growth of the biocontrol organisms or were not suitable as mulch materials for avocado and citrus.

G. virens and *T. harzianum* have similar growth requirements and their growth in the mulches was strongly correlated (Table VII). These fungi generally are categorized as litter/soil fungi. They have been reported from a wide variety of plant material including wood, peat, grass, leaves, straw, tubers, roots, moss, seeds, cotton, and paper (Papavizas, 1985; Domsch *et al.*, 1980). Like avocado and citrus they appear to flourish on mulches which mimic litter.

T. harzianum is capable of growing on a wide range of carbohydrates including cellulose (Domsch *et al.*, 1980; Danielson and Davey, 1973). It apparently thrives on substrates with a high carbon/nitrogen ratio since its growth was correlated positively with the organic matter/nitrogen ratio and negatively with ammonia release (Table VII). Although Eastburn and Butler (1988) classified *T. harzianum* as a ruderal, a fungus closely associated with nutrient substrates and not able to colonize soil far from the substrate, they were not able to correlate populations of *T. harzianum* with organic matter in an alfalfa field. It is interesting that *T. harzianum* did not colonize alfalfa readily in our study, perhaps because of the low C/N ratio. Under the conditions in their alfalfa field, Eastburn and Butler (1988) did correlate *T. harzianum* populations with competing microorganisms which may be more competitive under high-nitrogen conditions.

Less is known about the ecology of *G. virens*, but it too apparently grows best on substrates with a high carbon/nitrogen ratio since its growth was correlated positively with the organic matter/nitrogen ratio (Table VII). *G. virens* is apparently strongly inhibited by sodium (Table VII; Gindrat, 1977), and this could be a problem if it is cultured in certain waste materials.

P. fluorescens is a widespread, soilborne bacterium frequently associated with plant roots (Weller, 1984; 1988). It is capable of decomposing a wide variety of substrates, but the species generally is not considered to decompose cellulose or woody materials. However, *P. fluorescens* isolate C8-2 used in our study is cellulolytic (unpublished data) and grows well on materials composed almost entirely of complex carbohydrates such as rice hulls and wood compost (Table VI). *P. fluorescens* isolate C8-2 was recovered by baiting citrus rhizospheres with *Phytophthora* hyphae (Turney and Menge, 1993b) which contain cellulose as a cell wall component (Bartnicki-Garcia and Wang, 1983). These characteristics are unusual for *P. fluorescens*, and they indicate that isolate C8-2 may be *P. fluorescens* var. *cellulosa* (Mullings and Parish, 1984). This strain may not be well suited for competition in high sugar substrates, which would explain why its growth is negatively correlated with soluble carbohydrate (Table VII).

The percentage of healthy citrus roots, the percentage of healthy avocado roots, avocado root weight, avocado height increase and avocado shoot weight in mulch treatments were all correlated ($\alpha = 0.05$) with the growth of *P. fluorescens* in the mulches (Table VII). It is not unexpected that conditions favouring avocado and citrus roots would favour *P. fluorescens*, since this bacterium was isolated from the rhizosphere of citrus and has been shown to reduce pathogen populations in the rhizosphere of citrus (Turney and Menge, 1993b). Elevated levels of microorganisms including *Pseudomonas* spp. have been associated with the so called suppressive soils which promote root health of avocado in Australia (Broadbent and Baker, 1974a; 1974b). Whereas these organisms may well

be responsible for the beneficial effects of suppressive soils on avocado roots, our data suggest that it is simply a matter of the conditions favouring avocado root health may also favour *Pseudomonas* populations; Broadbent and Baker (1974a; 1974b) could not find specific bacteria that resulted in suppressive soil. The growth of *T. harzianum* in the mulches was also correlated with the percentage of healthy avocado roots treated with those mulches. This is extremely useful information since it indicates that mulches most beneficial to citrus and avocado roots are also efficient substrates for these biocontrol agents. Therefore, these materials have tremendous potential as bioenhanced mulches for improving the root health of avocado and citrus.

As discussed above, mulches such as milled peanut and almond hulls, animal manures and alfalfa hay released high amounts of ammonia upon decomposition. These materials resulted in undetectable populations of at least two of the biocontrol agents (Table VI). Ammonia can be toxic to soil microbes as well as plant roots, and the release of ammonia from these waste materials may limit their use as mulches or carriers of microbial biocontrol agents (Gilpatrick, 1969; Tsao and Oster, 1981; Schippers *et al.*, 1982). *Gliocladium* and *Trichoderma* are particularly sensitive to ammonia and as little as 1 $\mu g\ NH_3\ g^{-1}$ of air is inhibitory to growth of some *Trichoderma* spp. (Schippers *et al.*, 1982).

Substrate pH is considered to be very important for biocontrol agents (Eastburn and Butler, 1988; Weller, 1988). All of our biocontrol agents appear to have requirements for low pH since high pH was correlated with poor growth (Table VII). For instance, all of our mulch materials exceeded the reported optimal pH range of 3.7–4.7 for growth of *T. harzianum* (Domsch *et al.*, 1980). *P. fluorescens*, a bacterium, might be expected to prefer high pH conditions, but being a rhizosphere colonizer it grows best between pH 6.0 and 6.5 (Weller, 1988), still well below the pH of some of the animal manure mulches. As indicated above, ammonia is extremely toxic to many microorganisms including *Trichoderma* and *Gliocladium* and this effect is exacerbated by high pH (Schippers *et al.*, 1982; Tsao and Oster, 1981). This fact may help explain the poor growth of the biocontrol agents in mulch materials of high pH.

None of the biocontrol agents are coprophilic and hence are not adapted for growth in high-nitrogen animal wastes (Table VI). High EC and concentrations of magnesium, potassium and sodium, which were all negatively correlated with at least one of the biocontrol microorganisms, are typically associated with animal manures and high levels of nitrogen.

The several types of yard waste seem ideally suited for use as carriers for biocontrol agents as they did for mulches for avocado and citrus. The proportions of yard waste components with either high or low nitrogen content can be adjusted to optimize the efficiency of the mulches. However, perhaps the most compelling reason to use yard waste as a bioenhanced mulch is that it solves a huge urban problem of waste disposal. The disposal of 63 million tons of yard waste annually in California (Anon., 1993) is extremely costly and yard waste will no longer be allowed in landfills. Use of yard waste as a bioenhanced mulch may prove beneficial to both the agricultural and urban community and solve farm root health problems as well as urban waste disposal problems.

CONCLUSIONS

The use of urban and agricultural wastes as bioenhanced mulches for citrus and avocado has the potential to solve a major waste disposal problem and contribute to root health of these crops. Materials that mimicked a forest litter layer (e.g. urban yard wastes containing grass, leaves and wood) were the best performers with respect to citrus and avocado growth and health and also colonization by biocontrol agents. Materials which released high amounts of ammonia upon decomposition (e.g. animal manures, milled peanut or almond hulls, and alfalfa hay) damaged plants and were detrimental to the growth of biocontrol agents. However, phytotoxicity of these high ammonia releasing materials may be less of a problem in field situations, particularly when used on established trees. Since mulches most beneficial to avocado and citrus roots are also efficient

substrates for biocontrol agents (especially *P. fluorescens* and *T. harzianum*) these materials have tremendous potential as bioenhanced mulches for improving the root health of avocado and citrus.

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