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BIOLOGICAL CONTROL OF *PHYTOPHTHORA* ROOT ROT OF AVOCADO WITH MICROORGANISMS GROWN IN ORGANIC MULCHES

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ABSTRACT

Organic mulches colonized with microbial biocontrol agents, termed bioenhanced mulches, were tested for their ability to reduce *Phytophthora* root rot of avocado (*Persea americana* Mill.). Benomyl-resistant mutants of *Gliocladium virens* (KA 230-1) and *Trichoderma harzianum* (KA 159.2) isolated from suppressive soils and selected as efficient antagonists of *P. cinnamomi* were evaluated for their ability to colonize different mulches under controlled laboratory conditions. Sudangrass and a coarse yardwaste were found to be better substrates than a fine yardwaste, woodwaste or rice hulls for biocontrol agents propagules production. The most suitable conditions for colonization were an optimum temperature of 24°C, a moisture content of 20% for sudangrass and 30% for the coarse yardwaste, and a continuous light exposure during a 15-day incubation period. In the greenhouse, fresh sudangrass and a coarse yardwaste colonized with *G. virens* and used as a surface mulch proved to be the best combination for reducing the population of *P. cinnamomi* in 4-liter pots containing artificially-infested soil. Healthy avocado roots made up 31-37% of the roots in the *G. virens*-mulch combinations compared to 0% healthy in infested controls after two months.

Key words: *Phytophthora cinnamoni, Gliocladium virens, Trichoderma harzianum,* avocado

INTRODUCTION

In California 60-75% of the avocado (Persea Americana Mill.) acreage is infested with *Phitophathora cinnamomi* causing an estimated annual loss of $44 million (14). The soils used for avocado culture in California are generally very conducive to *Phytophthora* root rot (PRR) (44). According to Coffey (13) the absence of any mulching practice in most groves in California may also be an important factor in disease development.

Although *Phytophthora cinnamomi* induces severe root rot of avocados in subtropical environments, there are some locations such as in Australia, where the disease occurs but does not induce severe losses due to soil suppressiveness induced most likely by the soil microbiota (6,7). The biological basis of PRR suppression in Australia has been intensively investigated, but the specific mechanisms for it are still debated.

Prior to the Australian example, only Pratt (37) and Morquer and Touvet (30), conducted any relevent assessment of antagonists to *P. cinnamomi*. Mycorrhizal Fungi were suggested as playing a role in the suppression of *P. cinnamomi* on pines and conifers (38,41). Avocado roots, however, do not develop such mycorrhizal associations (14). In early studies, *Trichoderma* spp. were reported to be ineffective against *P. cinnamomi* (25), possibly due to their poor performance in wet soils (35). Chakraborty *et al.*, (11) found that some species of amoebae might have the ability to perforate and lyse melanized propagules of *P. cinnamomi*.

Rhizobium isolates (29), *Penicillium funiculosum* (40) *P. spinulosum* (31) *Myrothecium roridium* (17), *Epicoccum purpurescens* (= *E. nigrum*) (8,26) have also been characterized as potential *P. cinnamomi* antagonists.

Developing suitable technologies for using microbial antagonists in the field requires the development of appropriate

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delivery technologies. In this sense the avocado system offers some opportunities, since the potting mix is fumigated or treated. Colonization of such a mix by biocontrol agents is then rendered much easier. In addition, the incorporation of a biocontrol agent growing on a suitable substrate into a planting mound or later as a mulch may be feasible (12,20,21,22).

In the case of PRR of avocado, the use of organic and inorganic soil amendments has been widely reported in attempts to encourage microbial activity antagonistic to the germination and growth of *P. cinnamomi* (36,42). Zentmyer (43) was the first to report reasonable control of PRR based on these principles. At that time, the great increase in microbial population with addition of alfalfa meal was considered a factor in the biological control of PRR. Zentmyer and Thompson (45) also suggested the control might be related to the fungitoxicity of the saponin fraction of alfalfa meal. However, Gilpatrick (18) later observed that control in avocado groves was not satisfactory when alfalfa meal was applied to the soil surface under trees, and some phytotoxicity was also noticed. Since many different organic soil amendments produce phytotoxic products in soil (9), the effect of such products on plant growth and disease control may be more important than most workers suspect.

The key for large-scale utilization of disease suppressive composts is the development of composts with defined and consistent properties. Technology available for some composts, such as tree barks which are largely used in greenhouse crops (4,5), needs to be developed for other solid wastes. This material could potentially be incorporated into California avocado groves as a mulch and as a suitable substrate for delivering biocontrol agents.

The objectives of this research were to determine the optimum temperature, moisture content, and light necessary to colonize potential composts with fungal biocontrol agents (BCA), such as *Trichoderma harzianum* and *Gliocladium virens* and define the best mulch-BCA combination for reducing avocado PRR under greenhouse conditions.

MATERIALS AND METHODS

In a previous study, Casale (10) compared 18 different organic mulches for their ability to be colonized in vitro by some potential biocontrol agents (BCAs). Those mulches which gave the best results (rice hulls, sudan grass, wood compost, and coarse or fine yardwaste) were selected for use in this research.

Two potential biocontrol agents isolated from avocado soils at the root zone and indicating suppressiveness to PRR (10) were selected for these tests. Benomyl-resistant *Gliocladium virens* mutant KA230-1 and *Trichoderma harzianum* mutant KA159-2 were developed from these BCAs, allowing recovery from soils and mulches using a benomyl-containing medium. The assays of mulches as a substrate for growth of antagonistic microorganisms were conducted as follows. *Trichoderma harzianum* KA159-2 and *G. virens* KA230-1 were grown separately on PDA-benomyl plates for 7 days. Conidia were harvested by flooding the plates with sterile water containing 0.01% Tween 20 and dislodging with a sterile paint brush No. 14. Spore suspensions containing 1 x 10^6 conidia were used to inoculate 100 ml fresh mulch samples (dried at 80°C for 24 hr and fumigated with methyl bromide 72 hours prior to inoculation). These were placed in 33 x 33 cm colorless autoclaved polyethylene bags containing a 35-mm diameter microporus filter patch to allow air exchange.

A volume of water amended with V8c broth (20%, v/v) was added to the mulch samples to reach 10, 20, 30, and 40% of moisture content (v/v). After 15 days incubation at 20, 22, 24, 28 and 32°C, equivalents of 1.0 gm dry weight samples were blended in 100 ml sterile water for 10 sec., using a Betty Crocker handheld blender. Serial dilutions of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, were made in 5 test tubes, plating out 5 plates each of 10^{-5} and 10^{-7} dilution for each mulch sample. Samples inoculated with *Trichoderma* were plated out on PDA + 50 ppm benomyl + 80 ppm ampicillin. Those inoculated with *Gliocladium* were plated out on modified Rose Bengal medium consisting of K_2HPO_4, 0.5 g; peptone, 0.5 g; yeast extract, 0.5 g; dextrose, 10 g; rose bengal, 0.05 g; agar, 17 g; and distilled water to make 1 liter. Streptomycin sulfate was added to the above cooled agar medium just before pouring at a concentration of 30 mg/liter. Oxygall (1000 ppm) was added to both media to limit colony size and enhance colony counting accuracy. The plates were incubated in the dark. Final data was expressed in number of propagules per volume (ppv) of mulch.

After the selection of the most suitable temperature and moisture for each mulch-BCA association, the effect of light on colonization was investigated by incubating the bags for 15 days under 15w cool-white lamps (distant 30 cm). The bags were turned over daily to homogenize inoculum production. All experimental procedures were repeated twice, and each treatment submitted to three replications. Data were analyzed by LSD and Waller-Duncan K-ratio T test, using PROC GLM of the SAS computer program (SAS Institute, Cary, NC).

Inducing suppressiveness to PRR using mulches colonized with potential biocontrol agents.

Seeds of a susceptible cultivar of avocado (Topa-Topa) were planted in plastic seedling bags containing UC mix, pH 5.8, consisting of 50% fine sand, 50% peat moss plus 2.2 kg of dolomite, 1.5 kg of super phosphate, 138 g of KNO_3, 148 g K_2SO_4 m^{-3} (2). Two-month-old seedlings were then transplanted, root ball intact, into one gallon pots containing natural soil (clay loam) from Barr Ranch in Fallbrook, CA, amended with millet seed cultures of *P. cinnamomi*. Millet seeds (300 g) were autoclaved for 1 hr on two consecutive days and inoculated with mycelium from two 5-day-old PDA

cultures of *P. cinnamomi*. After 10-14 days of incubation at 24°C, the millet cultures were blended twice (1 min at high speed) in a blender and mixed into the soil (0.1% v/v) at the transplanting date.

The treatments, consisting of 500 ml of different mulches colonized or not colonized with the biocontrol agents, were spread on the soil surface in each pot at the planting date. In each experiment, 6 replicates were used per treatment. Control treatments contained soil infested or not infested with *P. cinnamomi*. Pots were laid out in a randomized complete block design on benches in the greenhouse. The daily temperatures under such conditions ranged from 18.5-30°C (Mean = 24°C). The plants were sprinkler-type irrigated as needed. The experiments were terminated after 2 months.

The BCAs population on mulches and soil were determined at the end of each experiment by the same dilution technique described above. The *P. cinnamomi* populations were also determined in the soil at the same time by the dilution plate technique on cornmeal-PARPH medium selective for *Phytophthora*. This medium consisted of 1.8% Difco cornmeal agar (Difco Laboratories, Detroit, MI) with (per milliliter) 133 mg of pentachloronitro-benzene, 125 mg of ampicillin, 10 mg of rifampicin, 10 mg of pimaricin, and 50 mg of hymexazol. Sampling was conducted by collecting 10 g of infested soil from each experimental unit, suspending in 100 ml sterile water, and blending with a Betty Crocker hand-held blender for 20 sec, to give 10^{-1} dilution. One milliliter of the soil slurry was placed on each of five plates of cornmeal-PARPH agar. After 48 hr, the soil was washed off the plates and colonies of *P. cinnamomi*, recognizable by their distinctive growth with clusters of hyphal swellings, were counted. Population densities were expressed as propagules per gram of soil.

Other data collected consisted of plant heights (measured from pot edges) at the beginning and end of each experiment, dry weight of roots, roots lengths determined by the root intersect method (34), and visual determination of percentage of root rot in comparison to the controls inoculated and non-inoculated with *P. cinnamomi*. The experiments were repeated three times. All data were analyzed by Waller-Duncan K-Ratio test using PROC GLM of the PC SAS pragram (SAS Institute, Cary, NC). Proportional values were transformed to arcsin and numerical values to $\log_{10}$ before analysis.

RESULTS AND DISCUSSION

Suitable conditions to bioenhance mulches with biocontrol agents

Both biocontrol agents, *Gliocladium virens* (Table 1) and *Trichoderma harzianum* (Table 2), successfully colonized all organic composts, producing a compact mass of mycelium and

Table 1. Population of *Gliocladium virens* in organic mulches after 15 days as influenced by temperature and moisture.

Temperature[x] (°C)	Moisture[y] (%)	No. of propagules (x10^{-4}) per gram of mulch		
		Sudangrass	Coarse yardwaste	Woodwaste
20	10	1.00 Bc[z]	< 0.01 Aa	< 0.01 Aa
	20	2.08 Be	< 0.01 Ac	< 0.01 Aa
	30	7.13 Ad	0.08 Ad	< 0.01 Ac
	40	6.98 Ac	0.10 Ac	< 0.01 Ab
22	10	3.11 Dbc	0.01 Ba	< 0.01 Ba
	20	19.30 Ad	0.50 Bc	0.01 Ba
	30	12.60 Bd	13.00 Ac	0.03 Ba
	40	7.45 Cc	10.80 Ab	0.08 Ab
24	10	5.78 Db	0.01 Ca	0.01 Ba
	20	85.10 Aa	17.30 Ba	0.08 Ba
	30	47.80 Ba	41.10 Aa	0.78 Ba
	40	26.90 Cb	23.20 Ba	3.82 Aa
28	10	8.88 Da	< 0.01 Ca	< 0.01 Ba
	20	48.60 Ab	4.05 Bb	0.07 Ba
	30	34.60 Bc	27.10 Ab	0.18 Ab
	40	23.20 Cb	28.10 Aa	0.16 Ab
32	10	8.87 Ca	<0.01 Ba	0.01 Ba
	20	28.00 ABc	0.53 Bc	0.01 Ba
	30	26.60 Bc	8.08 Ac	0.11 Ab
	40	31.40 Aa	11.90 Ab	0.13 Ab

[x] Temperature of incubation
[y] Moisture content of mulches (w/w)
[z] Means in a column followed by upper case letters denotes significant differences between moisture tratments within each temperature and lower case denotes significant differences between temperatures each moisture tratment according to Fisher's LSD at $P < 0.05$.

Table 2. Population of *Trichoderma harzianum* in organic mulches after 15 days as influenced by temperature and moisture.

Temperature[x] (°C)	Moisture[y] (%)	No. of propagules (x10^{-4}) per gram of mulch		
		Sudangrass	Coarse yardwaste	Woodwaste
20	10	0.03 Cez	< 0.01 Cb	< 0.01 Aa
	20	0.07 Be	< 0.01 Cd	< 0.01 Ac
	30	0.24 Ad	0.10 Bd	< 0.01 Ad
	40	0.05 Bcd	0.18 Ad	< 0.01 Ad
22	10	0.88 Dbc	0.01 Cb	< 0.01 Ba
	20	8.13 Ad	07.37 Bb	0.01 Bc
	30	3.86 Bc	15.10 Ac	0.53 Ac
	40	1.22 Cc	14.90 Ac	0.50 Ac
24	10	8.61 Ca	0.13 Ca	0.01 Ca
	20	35.60 Aa	21.00 Ba	0.18 Ca
	30	13.90 Ba	52.00 Aa	4.85 Ba
	40	4.57 Db	23.20 Bb	6.64 Aa
28	10	5.13 Cb	0.01 Db	0.05 Da
	20	27.90 Ab	4.57 Cbc	0.13 Cb
	30	11.10 Bb	42.70 Ab	1.29 Bc
	40	7.82 Bcb	27.30 Ba	3.87 Ab
32	10	3.07 Cc	0.03 Cb	0.01 Ba
	20	13.10 Ac	1.57 Cc	0.04 Bc
	30	14.00 Aa	13.20 Bc	0.86 Abc
	40	8.58 Ba	17.30 Ac	0.97 Ac

[x] Temperature of incubation
[y] Moisture content of mulches (w/w)
[z] Means in a column followed by upper case letters denotes significant differences between moisture tratments within each temperature and lower case denotes significant differences between temperatures each moisture tratment according to Fisher's LSD at $P < 0.05$.

a slight conidiation. However the efficiency varied among composts and temperature-moisture combinations, presenting a wide range of propagules per volume of mulch (ppv) (Tables 1 and 2).

The less favorable mulches for colonization with *G. virens* were the fine yardwaste and the rice hulls, since they didn't produce more than 5 ppv and no statistical difference could be detected at the various temperature-moisture combinations. The wood compost was a slightly better substrate, with a temperature of 24°C and 40% moisture content as most conducive for colonization. Sudan grass and the coarse yard waste compost were by far the best substrates for *G. virens* colonization. Both gave best results at 24°C, but sudan grass was better colonized at 20% (v/v) moisture content and coarse yardwaste at 30% (v/v) moisture content. Under such conditions these composts could produce, respectively, 8,500 and 4,100 ppv, indicating the superiority of sudan grass over coarse yardwaste (Table 1).

On the other hand, *T. harzianum* colonized better the coarse yardwaste (5,200 ppv) than sudan grass (3,500 ppv). The most suitable conditions for *T. harzianum* to colonize coarse yardwaste were at either 24°C or 28°C when the mulch moisture content was maintained at 30% (v/v). Sudan grass was better colonized at 24°C, with 20% moisture content (Table 1). The optimum conditions for colonization of sudan grass with *T. harzianum* and *G. virens* were the same; however, the latter was able to produce almost 2.5 as much inoculum than the former. *Trichoderma harzianum*, when using wood compost as a substrate, was able to consistently produce higher amounts of inoculum with 40% of moisture content and 24°C incubation. (Tables 1 and 2) In this study, whenever the ideal moisture content for each mulch was achieved, any additional increase in moisture level tended to reduce production of ppv, the mycelia were, in general, less compact, and the visual conidiation (greenish color of fungi growth) was reduced. In previous studies, *Trichoderma* spp. have not been recorded as effective against *P. cinnamomi* (25), due to their reputed poor performance in soils with high moisture content (35).

The temperature played a minor effect on inoculum production. Sometimes, in this study an ideal single temperature for mulch colonization could not be determined. High amounts of inoculum of both BCAs were produced either at 24 or 28°C as shown with coarse yardwaste (Table 2).

It has been known for years that fresh organic matter can serve as a direct food base to fungi (16, 24). However, there is a lack of reports in the literature regarding on the use of organic matter as an artificial substrate to enhance the inoculum potential of BCAs for use against *P. cinnamomi*. Apparently, the association of organic composts with BCAs to control a plant disease have found in the *Rhizoctonia solani* a better

research model (32). Hyperparasitic activity of *Trichoderma* against *R. solani* eradicates the pathogen in mature compost. In fresh compost, on the other hand, the pathogen survives in spite of the higher *Trichoderma* population (33). The *Trichoderma* strain is largely active as a saprophyte in fresh organic matter. Cellulose concentration and compost stability, therefore, regulate the hyperparasitic activity of biocontrol agents such as *Trichoderma* spp. (24,15) suggested that the mechanism could involve repression of chitinase activity in fresh compost high in cellulose.

In the present study only fresh composts were used and, since both *G. virens* and *T. harzianum* have the ability to colonize cellulolytic material (35), that may be considered an important factor for colonization of mulches that contain higher content of cellulose like sudan grass.

Most species of *Trichoderma* and *Gliocladium* are photosensitive sporulating readily with more conidia being produced during the light period (3, 35). In this research the effect of light on the bioenhancement of mulches with BCAs was significant only for sudan grass and the coarse yardwaste. The production of ppv of *G. virens* and *T. harzianum* was increased by about 20% due to the phialoconidiogenesis induced by the continued exposure to light. Conidiogenesis was observed mostly at the mulch/incubation plastic bag interface; the inner 'ball' of mulches tended to produce a dense mycelia, especially in mulches that tend to clump together like sudan grass. The usefulness of light for the enhancement of *G. virens* and *T. harzianum* inoculum potential through conidiogenesis has been discussed. A relationship seems to exist between the kind or size of the propagules of these organisms and their sensitivity to soil fungistasis; conidia are expected to be more sensitive than hyphae or chlamydospores (28,35;39,27) showed that *Gliocladium*, *Trichoderma* and other potential biocontrol fungi proliferate abundantly in various natural soils when added as young mycelium in intimate contact with a food base, but not as conidia with or without a food base. For this reason, in the subsequent greenhouse studies, the mulches were all incubated under dark conditions, since the amount of inoculum produced was already maximized into acceptable levels for deliver into the soil.

Suppressiveness to PRR using mulches colonized with potential biocontrol agents.

The potential of *G. virens* and *T. harzianum* as biocontrol agents to suppress *P. cinnamomi* when delivered by organic mulches were evaluated in a number of two-month-period greenhouse experiments. The initial inoculum level of *P. cinnamomi* in the inoculated soil ranged from 24 to 30 ppg, while the non-inoculated control had a natural infestation of 0-5 ppg. None of the treatments in this research was able to reduce the initial population of the pathogen. However, some mulch-BCA combinations were able to reduce the final population (110-126 ppg) by more than 50%. In a two-month-period experiment, *P. cinnamomi* increased 3-4 fold its initial population into the soil. During the same period, sudan grass and a coarse yardwaste efficiently delivered the BCAs in the soil to the point that consistent populations of *G. virens* (1,200-1,500 ppg) and *T. harzianum* (1000-1080 ppg) were established. Because with this system the final population of *P. cinnamomi* increased in only 0.5-1 fold, we assume that these organisms were antagonizing the pathogen. The antagonism was also reflected on the root health index. Using sudan grass and coarse yardwaste, *G. virens* treatments improved root health by 31-37% (Table 3 and Fig. 1), while *T. harzianum* improved root health 22-25% (Table 4). The efficacy of these organisms as biocontrol agents suggests that they were able to colonize the rhizoplane of avocado roots. The strong cellulolytic activity of these fungi (1, 35), which probably played a strong role colonizing the mulches, might also be acting to favor their competitiveness in soil and result in their ability to colonize the rhizosphere of *Persea americana*. In so doing, they might antagonize the pathogen before it can damage the new feeder roots, resulting in a increase in root health over the inoculated control plants.

A two-months experiment cannot, however, detect wether the BCAs would be able to continuously proliferate in the plant rhizoplane. Further studies are necessary since the lack of proper nutrients, the presence of toxic substances in root exudation, or the presence of antagonistic or competing organisms may eventually be an obstacle for massive augmentation in the soil (6, 23, 35).

Overall in this study, wood compost was not a promising substrate for delivering the biocontrol agents. Consequently it will not be used in further studies. These results indicate the effectiveness of sudan grass and coarse yardwaste in delivering *G. virens* or *T. harzianum*. However the general data did not significantly differentiate sudan grass from coarse yardwaste, except that coarse yardwaste was able to support the BCAs longer than sudan grass. By the end of two months, 410-480 ppv of BCAs could still be recovered from he coarse yardwaste, while only 80-130 ppv were recovered from sudan grass (Tables 3 and 4). These results suggest that for the control of a long-term disease like PRR, extended periods of delivery of the BCAs might be more successful.

Among the other independent variables observed in the greenhouse studies, root dry weight and root length were useful only in the *G. virens* experiments for differentiating between treatments with or without the BCA. However no statistical differences were detected among the types of mulch (Table 3). Also no differences were detected in root dry weight or root length (34) among treatments containing the organic mulches alone or associated with *T. harzianum* (Table 4). The lack of differences with these parameters, and also with plant height (data not presented), may be attributed to the short length of

the experiments associated the high genetic heterogeneity of Topa Topa seedlings used in the study. Seedlings with higher genetic homogeneity (clones) in longer term experiments might result in more control of these extraneous variables.

The use of fresh organic composts as an effective and economic food base for introducing specific biocontrol agents into avocado system to suppress PRR has been shown before (17). However the technique for mixing the colonized substrate into the soil, can be achieved only at the initial planting mound. Once the avocado grove is established, the substrate cannot be continuously mixed into the soil. The new avocado feeder roots that are continuously produced concentrate at the top 10-cm layer of the soil, and any manipulation in soil structure can damage the roots, thereby stressing the plants, and sometimes causing death (19). As mentioned elsewhere, because PRR of avocado is a typical long-term disease problem, long-term strategies for delivering the potential biocontrol agents have to be developed. Drenching the soil with conidial suspensions or mycelial preparations has proved to be not effective for controlling PRR (17) and many other diseases (35).

Figure 1. Sudan Grass associated with *Gliocladium virens* can improve root health and the root length of avocado under greenhouse condition.

Table 3. Effect of mulches and *Gliocladium virens* on the control of Phytophthora root rot of avocado in a 2- month greenhouse experiment.

Treatments	% of healthy roots	Root dry weight (gm)	Root length (cm)	*P. cinnamomi* in the soil (ppg)[x]	*G. virens* in the soil (ppg)	*G. virens* in the mulches (ppg)
Non infested control	100.0 A[y]	13.6 A	459.2 A	2 C	0 D	__
Sudangrass + *G. virens*	37.9 B	6.9 B	108.2 BC	69 B	1450 A	140 B
Coarse yardwaste + *G. virens*	31.7 B	6.3 B	115.1 B	66 B	1190 B	410 A
Woodwaste + *G. virens*	19.1 BC	5.9 BC	96.2 BC	87 AB	72 C	38 C
Sudangrass	7.9 C	5.4 C	71.1 CD	90 AB	0 D	0 C
Coarse yardwaste	8.3 C	5.3 C	75.7 CD	93 A	0 D	0 C
Woodwaste	6.2 C	4.0 C	77.3 BC	101 A	0 D	0 C
Infested control	0.0 C	4.1 C	59.2 D	113 A	0 D	__

x ppg = propagules per gram.
y Means within a column followed by the same letter are not significantly different at P = 0.05 according to Waller-Duncan's k-t-test.

Table 4. Effect of mulches and *Trichoderma harzianum* on the control of *Phytophthora* root rot of avocado in a 2-month greenhouse experiment.

Treatments	% of healthy roots	Root dry weight (gm)	Root length (cm)	*P. cinnamomi* in the soil (ppg)[x]	*G. virens* in the soil (ppg)	*G. virens* in the mulches (ppg)
Non infested control	100.0 Az	11.7 A	571.0 A	6 E	0 C	__
Sudangrass + *G. virens*	25.8 B	6.8 B	8.2 BC	83 D	1030 A	80 B
Coarse yardwaste + *G. virens*	22.9 B	5.7 BC	84.6 B	86 D	890 A	480 A
Woodwaste + *G. virens*	10.8 C	5.5 BC	71.8 BC	102 C	120 B	92 B
Sudangrass	9.2 CD	4.5 CD	58.8 BC	106 BC	0 A	0 C
Coarse yardwaste	7.3 CD	4.8 CD	60.2 BC	115 AB	0 C	0 C
Woodwaste	8.6 CD	4.2 D	51.2 C	114 AB	0 C	0 C
Infested control	0.0 C	3.1 D	43.6 C	126 A	0 C	__

[x] ppg = propagules per gram.
[y] Means within a column followed by the same letter are not significantly different at P = 0.05 according to Waller-Duncan's k-t-test.

The results in this work suggest that appropriate organic mulches can be efficiently colonized by some biocontrol agents, and also that at least for a short period the mulches could be used to deliver BCAs into soils. Future research is encouraged to confirm the role of bioenhanced mulches as an appropriate measure for the control of *Phytophthora* root rot in avocados and determine which possible mechanisms of suppressiveness are involved.

RESUMO

Controle biológico da podridão radicular de *Phytophthora* no abacateiro utilizando substratos orgânicos colonizados

Compostos orgânicos colonizados com agentes de controle microbiológico, então denominados compostos bioativados, foram testados quanto a sua habilidadade controlar à podridão radicular de *Phytophtora* no abacateiro (*Persea americana* Mill.) Mutantes de *Gliocladium virens* (KA 230-1) e *Trichoderma harzianun* (KA 159-2) resistentes a benomyl recuperados de solos supressivos e selecionados como eficientes antagonistas à *P. cinnamoni* foram avaliados quanto à sua capacidade de colonizar diversos compostos orgânicos em condições de laboratório. O Capim Sudão e um Composto de Jardim de alta granulação demonstraram quanto à sua capacidade de multiplicar propágulos de agentes de biocontrole, serem superiores à um composto de Jardim de granulação fina, à um composto de madeira e à um composto de casca de arroz. A condição ideal de colonização destes compostos foi encontrada à 24°C sob teor de umidade de 20% para Capim Sudão e 30% para o composto de Jardim de alta granulação, desde que incubados por 15 dias. Em casa de vegetação Capim Sudão e o Composto de Jardim de alta granulação quando colonizados por *G. virens* e utilizados como cobertura superficial do solo demonstraram serem as melhores combinações para redução da população de *P. cinnamomi.* Em vasos contendo solos artificialmente infestados e cultivados com abacateiro por dois meses, a combinação destes compostos com *G. virens* produziu de 31 à 37% de raízes de abacateiro sadias contra 0% de sanidade no controle.

Palavras-chave: *Phytophthora cinnamoni, Gliocladium virens, Trichoderma harzianum,* abacateiro

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