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REGULAR ARTICLE

Selection for and evaluation of an avocado orchard soil microbially suppressive to *Phytophthora cinnamomi*

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Abstract A survey was undertaken in southern California in 1999–2000 to search for an avocado soil that exhibits natural suppression to avocado root rot caused by *Phytophthora cinnamomi*. The Somis-1 soil, which had consistent massive degradation of *P. cinnamomi* hyphal mats, low *P. cinnamomi* populations and good tree health, was shown to be a soil with a high level of microbial suppression to *P. cinnamomi* in greenhouse studies. Other soils chosen for study, which seemed to demonstrate some type of suppression in the field as evidenced by high *P. cinnamomi* soil populations yet with good tree health, did not demonstrate microbial suppression in greenhouse studies. These soils may represent a type of suppression which is ephemeral or highly dependent on specific environmental factors. The suppression of the Somis-1 soil was transferable to a conducive soil with as little as 1% natural soil mixed with 99% fumigated soil. The suppression was gradually eliminated in soil pre-treated at various temperatures from 25° to 90°C. The suppression in the Somis-1 soil did not correspond with cellulase or laminarinase activity or soil microbial activity. The suppression appeared to correspond with moderately well-drained soils as found in the Somis-1 soil, which drained at a rate that might be conducive to the growth and activity of microorganisms antagonistic to *P. cinnamomi*.

Keywords Biocontrol · Phosphonate · Water potential

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Introduction

Avocado root rot caused by *Phytophthora cinnamomi* Rands is one of the limiting factors to worldwide avocado production and occurs in most countries where avocados are cultivated. The first report of avocado decline in California occurred in San Diego County in the 1920s. In 1942 *P. cinnamomi* was determined to be the cause of this decline and it was estimated in 1989 that 60–75% of the orchards in California were infected with an annual loss of $44 million (Coffey 1987, 1992). At the present time, it is estimated that about 10% of the trees in California are infected with *P. cinnamomi* (J.A. Menge, personal communication) which translates into an annual loss of approximately $36 million (G. Witney, California Avocado Commission, personal communication).

Standard management practices in California consist of an integrated approach of resistant rootstocks, sanitation, cultural practices, and chemical control

with phosphonate fungicides (Menge and Ploetz 2003). One of the most important components of this approach is the use of phosphonate fungicide (fosetyl-Al). However, because of its extensive use by growers, concerns have been raised over the possibility of resistance developing in *P. cinnamomi*. These concerns are justified as tolerance to phosphonate has been demonstrated in vitro in *P. capsici* and *P. cinnamomi* (Bower and Coffey 1985; Wilkinson et al. 2001). In addition, insensitivity to phosphonate has been documented in the field in *Bremia lactucae* (downy mildew) which is in the same order as *P. cinnamomi* (Brown et al. 2004). Because of these concerns, efforts to find an effective biological control agent of *P. cinnamomi* to supplement the integrated management of this disease are important.

Soils naturally suppressive to avocado root rot are a potential source of biocontrol agents. A naturally suppressive soil to *P. cinnamomi* was documented in Australia in 1971 (Broadbent and Baker 1974). This soil occurred in an avocado grove in Queensland and the suppression was induced by the addition of large amounts of organic matter for a period of time before planting. The increased organic matter stimulated the microbial activity of the upper soil layer, which led to suppression of *P. cinnamomi*. This type of cultural control came to be known as the Ashburner method and was adopted by other growers in Australia with some success but it has not been as effective on avocado root rot in other parts of the world (Downer 1998). The complex Ashburner method has been modified in Australia to become a simpler mulching method and application of a woody mulch and gypsum has shown some success in California (Menge et al. 1994). However, these "manufactured" suppressive soils require constant application of organic matter and the beneficial results are usually restricted only to the mulch area itself (Downer et al. 2001a). The soil below the mulch still harbors high populations of viable *P. cinnamomi*. Other naturally occurring suppressive soils have been discovered (Halsall 1982a, b; Malajczuk 1979), which do not depend on the continual application of organic matter. The mechanisms of suppression occurring in these soils may allow us new insights on how to control *P. cinnamomi*.

A distinction is made between two types of microbial suppression – general and specific (Weller et al. 2002). General suppression is related to the total microbial biomass of the soil, is enhanced by the addition of organic matter and is not transferable between soils. The Ashburner method noted above could be an example of general suppression (Cook and Baker 1983). Specific suppression is related to individual or specific groups of microbes active against certain stages of the pathogen life cycle. However, the key characteristic of specific suppression is its transferability to a conducive soil (Weller et al. 2002). A well-known example of specific suppression is the take-all decline (TAD) phenomenon in wheat monoculture (Weller et al. 2002).

A survey was undertaken in southern California in 1999–2000 to search for an avocado soil that exhibits natural suppression to avocado root rot. Out of 20 groves surveyed, four were chosen for study over a 2 year period. The purpose of this study was to identify a natural, microbially suppressive soil to *P. cinnamomi* and to evaluate the mechanisms responsible for the suppressive effect. This could lead to an economical and effective biological control agent of avocado root rot as part of an integrated control strategy, which would help to decrease the reliance on phosphonate fungicides. Such a biological control agent would lessen the chance of the development of *P. cinnamomi* resistance to phosphonate.

Materials and methods

Field survey of avocado groves

Twenty avocado (*Persea americana* Mill.) groves in S. California were surveyed for suppression of avocado root rot between 1999 and 2000. Twenty trees from each grove were systematically chosen. After removing the leaf litter layer, five soil core samples were taken from around each tree, near the drip line, to a depth of about 15 cm, and mixed together. In the laboratory, the soil was passed through a 1 cm sieve to remove large debris. This soil was used to assess *Phytophthora cinnamomi* soil populations and *P. cinnamomi* hyphal mat degradation. Trees in all groves were also evaluated for tree health.

Determination of Soil Population of *P. cinnamomi*

Soil populations of *P. cinnamomi* from under each tree were determined using the Most Probable Number (MPN) technique based on the work of

Cochran (1950) with modifications. Ten grams (dry weight) avocado soil were placed into a beaker and brought up to 40 ml with deionized (DI) water, resulting in a 1:4 soil/water suspension. Aliquots of 1 ml (1:4), 250 µl (1:16) and 62.5 µl (1:64) of the suspension were dispensed onto 60×15 mm plastic petri dishes and brought up to 10 ml of DI water. There were five replicates per dilution. Five 2-mm blue gum (*Eucalyptus globulus* Labill.) disks were cut from fresh leaves and placed on the soil slurry in each petri dish. Petri dishes were placed in the dark for 3 days at 25°C to allow zoospores to infect the disks. Leaf disks from each dilution were then dipped briefly into DI water and blotted on a paper towel to remove excess water. Leaf disks were then plated onto PARPH medium (Kellam and Coffey 1985) and placed in the dark for 4–5 days at 25°C. Any *P. cinnamomi* infection per plate was considered a positive and the MPN of propagules per gram of soil was calculated using Most Probable Number tables (Cochran 1950).

Isolate description and inoculum preparation

The *P. cinnamomi* isolate used was isolated from avocado roots from a Somis, CA avocado grove in 1996. *P. cinnamomi* inoculum was maintained on cleared V8 agar medium (per liter: V8 juice 200 ml; $CaCO_3$ 2 g; agar 15 g; DI water 800 ml; cleared by centrifugation). To produce hyphal mats, a 5 mm agar plug with *P. cinnamomi* hyphae was transferred aseptically into a 60×15 mm plastic petri dish and covered with one-half strength cleared V-8 broth and placed in the dark for 3 days at 25°C. Three-day-old hyphal mats were then rinsed three times with DI water. If sporangia formation on hyphal mats was desired, 1% soil filtrate was used to just cover the mats after the broth was rinsed off. Mats were then placed under lights (Cool White F20T12/CW, 20W) for 3 days for sporangia formation. Soil filtrate (1%) was produced by taking 10 g field soil and thoroughly mixing with 990 ml DI water for 1 min. Suspension was allowed to settle overnight on the bench and then filtered through a #1 Whatman filter.

Assessment of *P. cinnamomi* hyphal mat degradation

Soil was collected as described in the field survey above. Approximately 80 ml of each avocado field soil was placed into a 100×25 mm glass petri dish and brought to field capacity with DI water. A 3-day-old *P. cinnamomi* hyphal mat (about 3 cm in diameter) inserted into a Nitex nylon 100 µm mesh envelope was buried in each petri dish for 8 days. Using a Zeiss compound microscope (40×), hyphae from each mat were then rated on a scale of 0–5, with 0 being healthy and 5 being completely degraded. Hyphal degradation was defined as wall disintegration and/or loss of cytoplasm.

Tree health evaluation

Avocado tree health was evaluated visually. Trees were rated on a scale of 0–5 with 0 being healthy and 5 being completely dead. Tree characteristics that were evaluated included dieback of branches, size and color of leaves, and wilting symptoms (Menge et al. 1992).

Selection of four soils

Based on the assessment of *P. cinnamomi* soil populations, hyphal mat degradation and overall tree health, three soils were chosen for further evaluation of possible microbial suppression. Grove sites chosen were located in Somis, Thousand Oaks and Tustin, California. One avocado soil, from Escondido, CA, was chosen as a susceptible control. All further tests were done on soil selected from one tree in each grove. For laboratory analyses, soil was collected from each tree in each grove as described in the initial field survey.

Soil analyses of four selected soils

The Somis-1 avocado soil was a Rincon silty clay loam with the characteristics noted in Table 1. There was an epidemic of avocado root rot prior to 1994 at the Somis-1 avocado grove. Many trees died and yield was extremely low. Most of the trees were destroyed in 1994 and new trees were planted on Duke 7, Thomas and Toro Canyon rootstocks. The original trees that died were Haas on Topa Topa, which is a very susceptible rootstock. A group of about 40 trees that were not completely dead were not removed. These trees were treated with a foliar spray of phosphorus acid (2.27 kg/acre sprayed to wet) once per year for 4 years and only periodically after that. Trees on Topa

Table 1 Soil analysis values of four avocado orchard soils in southern California, collected between 1999 and 2000

Units		Location			
		Somis	TO	Tustin	Escondido
pH		7.73	7.43	7.33	5.85
EC	µS/cm	818	932	752	512
OM	mg/g dry soil	28.5	37.3	17.1	21.3
NO_3-N	µg/g dry soil	13.1	19.1	19.1	27.4
Olsen P	µg/g dry soil	27	9.8	7.1	21
K	µg/g dry soil	243	250	196	76
Ca	µmol/g dry soil	168	106	107	92
Zn	µg/g dry soil	175	501	169	171
Cu	µg/g dry soil	36.2	34.9	9.2	9.6
Mn	µg/g dry soil	290	299	233	269
Fe	µg/g dry soil	19,900	13,100	10,700	11,800
Cl	meq/l	1.6	5.1	4.8	3.4
Mg	meq/l	1.7	5.1	6.3	3.7
Na	meq/l	3.4	5.0	7.3	5.8
SO_4-S	µg/g dry soil	40	52	188	65

TO Thousand Oaks, *OM* organic matter

Topa rootstocks will usually not recover from avocado root rot by using minimal foliar sprays of phosphorus acid. A grove can often regain health when treated with phosphorus acid injections, but treatments must be frequent. These trees went from nearly dead to a high yielding grove in about 6 years with a foliar spray applied less than once per year. Because of the unusual and rapid recovery, it was hypothesized that there was some other factor contributing to the health of the trees besides the phosphorus acid application. At the time of the soil survey in 1999–2000, these trees were approximately 20 years old.

The Thousand Oaks avocado soil was a Garretson loam with characteristics noted in Table 1. Prior to 1994 an epidemic of avocado root rot decimated the grove but most of the trees (Haas on Topa Topa rootstock) did not die. Instead, after treatment with phosphorus acid, the trees recovered significantly with a rating of approximately 1–2 on a scale of 0–5, with 0=healthy and 5=dead. It was thought that the soil might be suppressive. At the time of the soil survey in 1999–2000, these trees were approximately 18 years old.

The Tustin avocado soil was a San Emigdio sandy loam with the characteristics noted in Table 1. Prior to 1994 an epidemic of avocado root rot decimated the grove and the original trees were removed. The grove was replanted with experimental resistant rootstocks (Duke 7, Thomas and Toro Canyon) as well as susceptible controls (Topa Topa rootstock). Avocado root rot never developed to a high degree on the replanted trees. Even the susceptible controls remained healthy with a rating of approximately 1–2. The susceptible control trees were reinoculated with infested millet or hyphal mats on three different occasions. Epidemic root rot never developed which led to the conclusion that the soil was now suppressive to root rot. At the time of the soil survey in 1999–2000, these trees were approximately 7 years old. All soil analyses were done on a susceptible control tree only.

The Escondido-1, California avocado soil was an Arlington coarse sandy loam with the characteristics noted in Table 1. Prior to 1994, an epidemic of avocado root rot decimated this grove (Haas on Topa Topa rootstock). Despite heavy treatments with phosphorus acid and other control measures, trees continued to die and showed symptoms of avocado root rot. For this reason, the plot was considered to be a typical root rot-infested soil, which would serve as the control for this study. At the time of the soil survey in 1999–2000, these trees were approximately 21 years old.

Soil enzyme assays

The Somis-1, Escondido-1, Thousand Oaks and Tustin soils were evaluated for cellulase, laminarinase and *Phytophthora*-degrading enzymatic activity in January (winter), April (spring), July (summer) and October (fall) of both 2001 and 2002. These are

enzymes that degrade hyphae and chlamydospores of *P. cinnamomi* (Downer et al. 2001b). The enzyme assay is based on the method of Alef and Nannipieri (1995) with modifications. Assays were conducted using the substrates of carboxymethyl cellulose (0.7% w/v, Sigma, medium viscosity), laminarin (0.1% w/v Sigma) and *P. cinnamomi* cell walls. Soil was collected as noted above in the selection of four soils and kept at −20°C until ready for use. The day before evaluation, the soil was allowed to come to room temperature overnight. There were three replicates for each treatment. Ten grams (dry weight) soil was placed into 125 ml Erlenmeyer flasks. To each flask, 15 ml of 0.5 M acetate buffer was added. Each treatment had 15 ml of substrate added. Flasks were placed on a rotary shaker for 4 h at 60 rpm at 36°C. After removing from the shaker, 15 ml substrate was added to each of the control flasks. Aliquots of 1.8 ml were removed into microcentrifuge tubes and spun at 15,700×*g* for 3 min. The supernatant was assayed for reducing sugars according to the methods of Schinner and Von Mersi (1990). The optical density was measured at 690 nm after 1 h for color development. All assays were run in duplicate and averaged prior to statistical analysis.

In order to produce *P. cinnamomi* cell walls for the enzyme assays, liquid cultures of *P. cinnamomi* were grown in 500 ml 1/2-strength cleared V8 broth in 1 l Erlenmeyer flasks for 2 weeks in the dark at 24°C. The cultures in the flasks were initiated with cleared V8 agar disks (5 mm) cut from the growing margin of a colony of *P. cinnamomi*. Hyphae were harvested and chopped in a Servall Omnimixer for 1 min and centrifuged at 850 g for 20 min. The pellet was re-suspended in DI water and the process repeated three times to remove all broth. The hyphae were further broken down 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 −20°C. This preparation of *P. cinnamomi* cell walls free of cytoplasm was used as a substrate for enzyme assays.

Microbial activity assays

The Somis-1, Escondido-1, Thousand Oaks and Tustin soils were evaluated for microbial activity in April, 2005. Microbial activity was determined by the hydrolysis of fluorescein diacetate (FDA) as described by Gamliel and Stapleton (1993), with modifications. Soil was collected as noted in the selection of four soils and processed the same day. Five grams of soil sample were added to 20 ml of 60 mM phosphate buffer (KH_2PO_4+K_2HPO_4), pH 7.6. Samples were agitated on a rotary shaker at 150 rpm for 30 min. FDA (Sigma Chemical, St. Louis) was added to the samples (0.2 ml of 2 mg/ml FDA stock solution) which were then agitated on a rotary shaker at 150 rpm for 20 min. Acetone (20 ml) was added to stop the reaction and samples were again agitated for 5 min. Aliquots of 15 ml were taken from each sample and centrifuged at 8000 g for 10 min. Absorbance was measured at 490 nm with an LKB spectrophotometer (Spectronic Unicam, model Genesys 10vis, Rochester, NY). Standard curves for fluorescein hydrolysis were prepared for each soil sample to avoid errors caused by adsorption of fluorescein to organic matter. A range of fluorescein concentrations (0, 100, 200, 300, 400 μg) was added to 5 ml buffer and completely hydrolyzed in a boiling water bath for 60 min. The fluorescein and 15 ml additional buffer were added to soil samples as described previously, shaken, and acetone was added and analyzed as described previously. There were five replicates of each soil sampled and the experiment was conducted twice.

Water penetration in suppressive soils

The Somis-1, Escondido-1, Thousand Oaks and Tustin soils were evaluated for speed of water penetration in April, 2005. Metal cylinders, 15 cm in diameter and 61 cm in length, were driven into each experimental field soil to a depth of approximately 7.6 cm. Water was then poured into the metal cylinder, filling the cylinder to the top, and then allowed to soak into the soil. The rapidity with which the water was absorbed by the soil was measured in ml/cm^2 soil surface/min. There were three replicates for each of the four soils.

Evaluation of the four soils in the greenhouse

In July 2001, field soil was obtained from around the tree labeled #1, which had shown suppressiveness in the original soil survey, in the Somis-1, Thousand Oaks, and Tustin groves. In the Escondido-1 grove, soil from the chosen susceptible control tree was also obtained. Leaf litter was removed before the soil samples were taken.

Soil samples were taken from two to three sites around each tree to a depth of about 15 cm, underneath the drip line, and bulked together. Soil was taken back to the greenhouse and passed through a 1-cm sieve to remove large debris. Each soil had two treatments consisting of nonautoclaved and autoclaved soil, with 10 replicates per treatment. Autoclaved soil was treated twice at 121° C at 7.7-kg pressure with a 24-h-period of cooling between treatments, to ensure elimination of heat resistant, spore-forming bacteria. Each soil was planted with 3-month-old Topa Topa avocado seedlings in 20.3 cm clay pots. Each pot was inoculated with two *P. cinnamomi* hyphal mats bearing sporangia (see inoculum preparation above), about 1.5 cm below the surface, equidistant from each other, between the pot edge and the plant. The soil was kept continually moist for 24 h to allow for good infection by zoospores. Thereafter, the soil was watered as necessary and allowed to dry out moderately between waterings. Greenhouse temperatures ranged from 21 to 37.8°C during the day and from 15.5 to 32.2°C during the night. After 3 months, seedlings were removed from the pots and the soil washed off the roots. Roots were rated visually for percent healthy roots on a scale from 1 to 100. Roots were rated separately by three individuals and the results were combined and averaged. Roots and shoots were then dried thoroughly and weighed. This experiment was repeated in September of 2001.

Somis-1 fumigation gradient

The Somis-1 soil was chosen for further greenhouse analysis based on significantly greater root health than the other three soils. In October, 2001, field soil was collected from tree #1 at the Somis-1 avocado grove in the same manner as the previous greenhouse analysis. Fumigated soil was covered with a tarp for 24 h and treated with 0.68 kg of 100% methyl bromide (Meth-O-Gas, Great Lakes Chemical Corp., West Lafayette, IN). The tarp was removed for 48 h prior to soil use to allow methyl bromide to dissipate. Treatments consisted of 100% fumigated soil, 100% natural soil, 50% fumigated mixed with 50% natural, 90% fumigated mixed with 10% natural and 99% fumigated mixed with 1% natural soil. There were 10 replicates for each treatment. Starting with the 100% fumigated treatment, each treatment was thoroughly mixed in a sterilized cement mixer for 15 min. Planting of seedlings, inoculation method, duration of experiment, watering regimen, greenhouse temperatures, and takedown procedure same as above.

Somis-1 temperature gradient

In July, 2002, field soil was obtained from tree #1 at the Somis-1 avocado grove in the same manner as the previous greenhouse analysis. Soil was slightly moistened and mixed well by hand to distribute the moisture. Soil was then placed into double-bagged, 3.8 l Ziploc bags, about 3 l per bag. Then the double-bagged soil was placed into a third, much larger plastic bag to ensure against water leakage into the inner bags. Using temperature-controlled water baths, the water was brought up to the treatment temperature. Then the triple-bagged soil was placed into the water bath, ensuring that the surface of the soil was beneath the water line. Thermometers were inserted into the center of the soil. When the center of the soil reached the water temperature, the inner bag was partly closed around the thermometer to keep the heat in. Soil was heated for 1 h after it had reached the treatment temperature. The five temperature treatments were 25, 45, 60, 75 and 90°C with 10 replicates for each treatment. Soil was allowed to cool to room temperature in plastic bags overnight. Planting of seedlings, inoculation method, duration of experiment, watering regimen, greenhouse temperatures, and takedown procedure same as above.

Statistical analysis

Statistical analysis was performed using SAS statistical software (SAS Institute, Cary, NC). Means were separated by Waller's *k*-ratio *t* test. The experimental design of all greenhouse tests was a randomized block.

Results

Field survey of avocado groves

The Somis-1 soil was the only one with the majority of mats showing high degradation (18 of 20 mats≥ 2.5) together with low *P. cinnamomi* populations and good tree health (Table 2). Many other sites, such as Thousand Oaks, Tustin, Pala, Escondido-3, Somis-2, Carpinteria-1, Carpinteria-2, Somis-3 and Oxnard, had

Table 2 Grove location, *Phytophthora cinnamomi* populations, hyphal mat ratings and avocado tree health from a survey of 20 avocado groves in southern California for soil suppressive to *P. cinnamomi*, 1999–2000[a,b]

Location	*P. cinnamomi*[c] (propagules/g soil)	Mat rating[d] (0 to 5)	Tree health[e] (0 to 5)
Somis-1	0.12	3.5 (18 mats ≥2.5)[f]	0.15
Escondido-1	0.34	0.13	1.88
Thousand Oaks	16.30	0.71 (1 mat >2.5)	1.0
Tustin	2.30	0.5 (3 mats =2.5)	0.2
Valley Center	0	0.48	0
Pala	0	0.79 (2 mats ≥2.5)	1.15
Ramona	0	0.1	0.75
San Pasqual	0	0.19	0
Escondido-2	0	0.05	0
Fallbrook-1	1.68	0.16	3.13
El Cajon	0	0.3	0.93
Fallbrook-2	0	0.075	0.97
Escondido-3	0	1.70 (4 mats ≥2.5)	0.13
Escondido-4	3.24	0	2.5
Somis-2 (16 trees only)	1.02	1.43 (4 mats =2.5)	1.5
Hidden Hills	0	1.64	2.7
Carpinteria-1	1.08	0.63 (2 mats =2.5)	1.45
Carpinteria-2	0.12	0.5 (1 mat =3.0)	1.6
Somis-3	0	1.95 (7 mats ≥2.5)	0.5
Oxnard	0.54	0.9 (1 mat =2.5)	2.3

[a] Soils are considered suppressive because of low *P. cinnamomi* populations, high mat degradation ratings and/or healthy trees. All groves tested positive for *P. cinnamomi* at one time, but perhaps not the sections we sampled in this study.

[b] All measurements based on the average of 20 trees or soil samples from each grove.

[c] *P. cinnamomi* soil populations determined by Most Probable Number technique (propagules baited by leaf disks floated on three soil dilutions of each soil sample and then plated on selective media).

[d] Mat rating includes hyphal degradation measured by wall disintegration and/or loss of cytoplasm. 0=healthy, 5=completely degraded.

[e] 0=healthy trees, 5=dead trees

[f] Number of mats out of 20 with rating of 2.5 or higher indicating high degree of suppressive activity

soil from only a few of the 20 trees sampled causing heavily degraded *Phytophthora* mats (Table 2). Soil from sampled trees in the Thousand Oaks and Tustin sites showed very high *P. cinnamomi* populations and caused only a small amount of hyphal mat degradation, but the trees were relatively healthy (Table 2). Other sites from which no *P. cinnamomi* could be isolated such as Valley Center, Pala, Ramona, San Pasqual, Escondido-2, El Cajon, Fallbrook-2, Escondido-3, Hidden Hills and Somis-3, may have been good sources of suppressive soil but were ultimately dismissed as suppressive to *P. cinnamomi* as it could not be proven that *P. cinnamomi* caused tree damage at these sites. These sites also did not have high levels of mat degradation. Degraded hyphal mats from the soil survey were rarely associated with soil fungi. However, degraded mats were frequently associated with bacteria that clustered around the hyphae. Many of these bacteria were rod-shaped and motile. The Somis-1, Thousand Oaks, and Tustin soils were selected for further studies with the Escondido-1 soil chosen as a susceptible control.

Soil enzyme assays

In both 2001 and 2002, the control soil from Escondido-1 had significantly high levels of CMCase, laminarinase and P. cinn-ase, while in 2001, the Somis-1 soil also exhibited significantly high levels of CMCase and P. cinn-ase (Table 3). The other two soils, Thousand Oaks and Tustin, exhibited lower levels of these soil enzymes in both 2001 and 2002 (Table 3).

Microbial activity assays

The mean microbial activity was significantly higher in the Tustin soil and significantly lower in the Somis-1 soil (237 and 65 μg fluorescein diacetate hydrolyzed/g sample/h, respectively). The Escondido-1 and Thousand Oaks soils were not significantly different from each other and their values fell between the Tustin and the Somis-1 soils (133 and 93 μg fluorescein diacetate hydrolyzed/g sample/hr, respectively).

Water penetration in suppressive soils

Water penetration was significantly faster in the Escondido-1 soil compared to the Somis-1, Thousand Oaks or Tustin soils (10.35, 4.18, 1.35 and 0.88 ml/cm^2 soil surface/min, respectively). The Tustin and Thousand Oaks soils were the slowest draining soils, with the Somis-1 soil showing water penetration which was approximately in the middle range.

Table 3 Average soil effect of Somis-1, Escondido-1, Thousand Oaks and Tustin avocado orchard soils on cellulase, laminarinase and "P. cinn-ase" enzyme activity, 2001–2002[a,b,c]

Sampling location	CMCase		Laminarinase		"P.cinnase"	
	2001	2002	2001	2002	2001	2002
Somis-1	0.6550 a	0.2886 b	0.6572 b	0.4230 c	0.6138 a	0.2154 b
Escondido-1	0.6431 a	0.3479 a	1.5484 a	1.4640 a	0.5949 a	0.3158 a
T. Oaks	0.2244 c	0.1759 c	0.5940 b	0.5929 b	0.3691 b	0.1461 c
Tustin	0.3625 b	0.2698 b	0.2009 c	0.5665 b	0.3652 b	0.1112 c

[a] Data is average soil effect of soil × month interactions.

[b] Enzyme activity expressed as μg reducing sugars g^{-1} sample h^{-1}. CMC is carboxymethyl cellulose. "P.cinnase" is the activity detected against cell walls of *P. cinnamomi*. There were three replicates for each soil-substrate treatment.

[c] Mean values in each column followed by identical letters are not statistically different according to Waller's *k*-ratio *t* test.

Evaluation of four soils in the greenhouse

Because results were similar in the July and September experiments, data were combined in Table 4. Only in the Somis-1 soil was root health in the natural soil significantly higher than root health in the autoclaved soil. In the Escondido-1, Thousand Oaks and Tustin soils, root health in the natural soil was not significantly different from root health in the autoclaved soil (Table 4). There were a significantly greater percentage of healthy roots in the natural Somis-1 soil than the natural Escondido-1, Thousand Oaks or Tustin soils (Table 4).

Somis-1 fumigation gradient 2001

There were a significantly greater percentage of healthy avocado roots in all levels of Somis-1 natural soil mixed with Somis-1 fumigated soil as compared to the 100% fumigated control (Table 5). Even at 1% Somis-1 soil and 99% fumigated soil, the root health ratings were significantly higher than root health ratings from 100% fumigated soil. Root weight was also significantly greater in mixtures of Somis-1 natural and fumigated soil than in the 100% fumigated control, except for the 50/50 mixture (Table 5).

Somis-1 temperature gradient 2002

There were a significantly greater percentage of healthy avocado roots in the 21°C (room temperature) and 45°C soil treatments than in the 90°C soil treatment (Table 6). There appeared to be a gradient of poorer root health and less root weight as the suppressive soil was subjected to higher temperatures

Table 4 Effect of natural and autoclaved soils of four southern California avocado groves infested with *Phytophthora cinnamomi* on plant root health, 2001[a,b]

Soil	Natural	Autoclaved
	(% healthy roots)	
Somis-1	35.13 a	16.58 b
Escondido-1	1.07 c	5.30 bc
Thousand Oaks	1.25 c	7.23 bc
Tustin	3.98 c	4.15 c

[a] Data combined from two experiments, July and Sept. 2001

[b] Mean values in each column followed by identical letters are not statistically different according to Waller's *k*-ratio *t* test.

Table 5 The effect of mixtures of natural and fumigated Somis-1 soil, infested with *Phytophthora cinnamomi*, on avocado root health and root dry weights, 2001[a]

Somis-1 soil treatment	Mixture (%)	Root health (% healthy)	Root dry weight (g)
Fumigated	100	10.58 d	3.85 d
Natural/ fumigated	1/99	74.85 a	9.88 a
Natural/ fumigated	10/90	77.08 a	8.24 ab
Natural/ fumigated	50/50	49.13 bc	5.68 cd
Natural	100	60.45 ab	6.51 bc

[a] Mean values in each column followed by identical letters are not statistically different according to Waller's *k*-ratio *t* test.

Table 6 Effect of temperature treatment of Somis-1 soil infested with *Phytophthora cinnamomi* on avocado root health and root weight, 2002[a]

Somis-1 soil treatment (°C)	Root health (% healthy)	Root dry weight (g)
21	60.38 a	6.13 a
45	44.00 ab	5.45 a
60	31.50 bc	5.24 a
75	24.25 bc	4.53 a
90	14.48 c	3.54 a

[a] Mean values in each column followed by identical letters are not statistically different according to Waller's *k*-ratio *t* test

(Table 6). There was no significant difference of dry root weights between treatments.

Discussion

From the results of the avocado grove field survey, it appears that there may be several types of soil suppressive to *P. cinnamomi*. The Somis-1 soil, which showed high levels of *P. cinnamomi* hyphal mat degradation and low populations of *P. cinnamomi*, was shown to be microbially suppressive to *P. cinnamomi* since it was the only soil to have suppressiveness removed by autoclaving and fumigation and was able to be transferred into a conducive soil. Other soils, which showed high levels of *P. cinnamomi* but low levels of disease, such as soil from Tustin or Thousand Oaks, may have been suppressive in the field but not in greenhouse studies where microbial suppression was not sustained. The fact that suppressive soils, as indicated by high *Phytophthora* hyphal mat degradation, appeared sporadically and in widely-separated trees within a grove indicates that some types of suppression may be very sensitive to environmental factors and may fluctuate spatially from season to season in avocado groves. Suppression in some of these locations may not be related to microbes, but may be the result of gaseous, chemical or physical aspects of soil drainage which are easily destroyed by physically moving the soil.

Microbial suppression of avocado root rot was first noted in Australia over 30 years ago (Broadbent and Baker 1974, 1975). This suppression was induced by large amounts of organic matter incorporated into the soil which stimulated microbial activity, leading to suppression of *P. cinnamomi*. This method was developed to simulate rain forest soils that were noted to be naturally suppressive to *P. cinnamomi*. In addition, other areas of native forest in Australia with low levels of fertility also showed natural microbial suppression against *P. cinnamomi* (Halsall 1982a, b).

The Somis-1 soil, which did not have large amounts of organic matter incorporated into the soil, demonstrates a key characteristic of specific suppression in that it was transferable to a conducive soil by very small amounts (1%). This was similar to results found with TAD suppressive soils (Weller et al. 2002). In our study, the root health rating and root dry weights were actually higher when the smaller amounts of suppressive soil (1%, 10%) were added to fumigated soil. This effect might be due to the greater biological vacuum created in the treatments with a higher percentage of fumigated soil. The biological vacuum created by fumigation leads to rapid recolonization by introduced antagonists because of the lack of competition from other soil organisms (Baker and Cook 1974; Cook and Baker 1983). However, the exact mechanism of the suppressive effect in this soil still needs to be elucidated.

When our suppressive Somis-1 soil was treated at increasingly higher temperatures, there was a gradual decrease in root health, suggesting suppressive microorganisms were being progressively eliminated. This gradual elimination of suppression over a range of temperatures, instead of a more sudden elimination of suppression from one temperature to another, as is true of the TAD model (Weller et al. 2002), suggests that the suppressive microorganisms may consist of more than one genus. If this is the case, then this could indicate a more general type of suppression which is due to the total microbial activity in a soil. However, general suppression is usually not transferable in greenhouse tests (Weller et al. 2002) and our results indicate that total microbial activity alone is not responsible for suppression of avocado root rot since the Somis-1 soil had the lowest level of microbial activity. If, according to Weller et al. (2002), transferability of suppression is the key characteristic of specific suppression, then the Somis-1 soil would seem to be exhibiting specific suppression, however further studies would be necessary to confirm this.

Enzymatic activity in the soil has been shown to play an important role in suppression of *P. cinnamomi* in mulched avocado soils (Downer et al. 2001b). Oomycete cell walls contain cellulose (β-1,4 glucans) and laminarin (β-1,3 glucans) and thus are sensitive

to the enzymes cellulase and laminarinase (Bartnicki-Garcia and Wang 1983). These enzymes are produced by microorganisms to degrade organic matter, which is composed largely of cellulose. Soils low in organic matter have low levels of enzyme activity (Tateno 1988). However, our results did not support the hypothesis that these soil enzymes were responsible for soil suppression of *P. cinnamomi* since they did not correspond well with the findings in the greenhouse trials. The control Escondido-1 soil often had very high levels of soil enzymes suppressive to *P. cinnamomi* and yet there is no evidence that the Escondido-1 soil is suppressive to *P. cinnamomi*. The high levels of soil cellulase and laminarinase in the Escondido-1 soil may be because, as a coarse sandy loam, it has only small amounts of clay. Clay adsorbs enzymes, taking them out of the soil solution (Paul and Clark 1996).

Downer et al. (2001a), evaluated the effect of cellulolytic enzymes in woody mulch layers on *P. cinnamomi* in an avocado grove. Their results showed high levels of cellulase activity associated with the upper mulch layers which gradually decreased toward the soil/mulch interface and continued to decrease to low levels to a depth of 15 cm into the soil (Downer et al. 2001a). The presence of the cellulase enzyme correlated very well with the presence of healthy roots indicating that the cellulase may be destroying hyphae and sporangia of *P. cinnamomi*.

In this current study, there was no woody mulch applied to the soil, yet there was significant degradation of the *P. cinnamomi* hyphal mat in the Somis-1 soil. The cellulase activity measured in the Somis-1 soil was relatively low (0.29–0.66 μg reducing sugars/g sample/h), which is consistent with the findings of Downer et al. (2001a), who found low levels of cellulase activity in the soil (0.11–0.30 μg reducing sugars/g sample/h) as compared to the mulch layers (1.44–4.29 μg reducing sugars/g sample/h). In the Downer et al. (2001a) study, very little degradation of *P. cinnamomi* hyphae was found at these lower levels of soil cellulase. By these standards, our highest levels of cellulase enzymes would be considered medium to low.

Despite the low levels of cellulase activity in the Somis-1 soil, compared to those found in mulch layers in the Downer et al. (2001a) study above, the *P. cinnamomi* hyphae were still highly degraded. This lower level of enzymatic activity could be attributed to cellulase-secreting bacteria in the soil (Paul and Clark 1996) which, due to their smaller size, would secrete considerably less cellulase than the wood-decay fungi (Downer et al. 2001a). Various strains of cellulolytic bacteria have been identified (El-Tarabily et al. 1996; Lednicka et al. 2000; Lynd et al. 2002), however, the exact nature of the degradation of *P. cinnamomi* hyphal cell walls in the Somis-1 soil, which demonstrates low concentrations of cellulolytic enzymes, would require further analysis.

Of all the abiotic factors affecting soil suppressiveness, the water potential of the soil is particularly important because of its effect on the pathogen, the other microorganisms in the soil and the host. Most root diseases are more severe in wet soils, especially those caused by *Pythium, Phytophthora, Aphanomyces* and other oomycetes (Cook and Papendick 1972). Factors which affect the water potential changes in the soil include the soil texture and structure. At a given moisture potential, clay holds more water and holds it more tightly than a loam or sandy soil. In regards to structure, if a soil is well granulated, there are larger pore spaces which lead to better drainage of soil water. In the water penetration study, the Escondido-1 soil was the fastest draining by far of the four soils, indicating that it would dry out quickly and any antagonistic microorganisms present would have less chance to become established before dessication. *P. cinnamomi*, on the other hand, would do well in such an environment as it can survive a wide range of water potentials in the soil (Menge and Ploetz 2003). During periods of moisture, such as irrigation or rain, *P. cinnamomi* can take advantage of this brief wet period by releasing zoospores which can infect the feeder roots within a matter of hours (Menge and Steddom 2000). This could explain why the Escondido-1 soil is such a conducive soil. The Thousand Oaks and Tustin soils were significantly slower draining than the other two soils which could lead to water-logged conditions which, in turn, can rapidly lead to anaerobiosis. Anaerobic conditions would be unfavorable to aerobic antagonists and could explain why these soils were not suppressive to *P. cinnamomi*. Perhaps the Somis-1 soil was the only soil that drained at a rate that would be conducive to microorganisms antagonistic to *P. cinnamomi*. Water did not sit for prolonged periods of time or drain out of the soil rapidly, but drained at a steady rate as compared to the other three soils. This would give microbial antagonists enough moisture for a long enough period of time to build up their numbers.

Once a certain critical mass of antagonistic microorganisms was reached, then suppression of *P. cinnamomi* would be noticeable. These observations support those of Zentmyer (1980) who clearly indicated that certain well-drained soils were far less at risk for avocado root rot than poorly-drained soils.

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