Fragaria species grown in a Greenhouse Cropping System chemigated by Phosfite® and Bacillus® in subsidence of *Phytophthora fragariae* and *Verticillium dahliae*

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Abstract

Fragaria species grown in a Greenhouse Cropping System chemigated by Phosfite®, and Bacillus® in subsidence of _Phytophthora fragariae_ and _Verticillium dahliae_

Russell L. Morgan

Strawberry yields depend directly on infestation *of Phytophthora fragariae and Verticillium dahliae* incorrespondance to the use of Phosfite and Bacillus. The objective is to find Understanding of newly administered techniques in prevention of *Phytophthora fragariae* and *Verticillium dahliae* and prohibiting the use of *Methyl Bromide.* Application of Phosfite and Bacillus on patented *Fragariae* species to identify utilization of P and ZN forms and their aid in prevention of *Phytophthora* and *Verticillium* species. In the Plots on the western bench the developmental symptoms of PRR disease were absent within all infested soil containers containing the FHS-1 crop. In the plots on the eastern bench the developmental symptoms of VVD was present among a select few plants within the study and caused necrosis and death to one plant within this particular study, Can # 95 a container confirmed to have residual V. dahliae fungus. Our results demonstrated that the suppressive effect of Phosphite against PRR in the FHS-1 was not evident in the study. Throughout the experiment large quantities of strawberries showed imperfect shape and structure due to unsatisfactory in complete pollination by greenhouse bees. Overall fruit quality was satisfactory. Throughout the study The UTC, and the strawberries tested with Phosfite had no evidence of any disease symptoms present. Adding gypsum to the soil helped aid in water penetration solving the irrigation problem and could have been the catalyst in suppressing the PRR disease as correlated in previous studies found on riddance *of Phytophthora Cinamoni* in Avocado Orchards. In future studies plant leaf analysis in comparison of P and Zn species will be conducted to monitor a closer balance among the treatments used. Phosphoric Acid solutions will be provided to study at specific concentrations (ppm).

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Hypothesis presentation: In correspondence to concerning issues of *Methyl Bromide* and its effects to soil structure, Macro and Micro soil fauna and the environment, overall objective is to evaluate chemigated materials and fertilizers to surface evidence of suppression or subsidence of *Phytophthora fragariae,* a water borne fungal disease and *Verticillium dahliae*, a vascular wilt disease that prevents the xylem from transporting water through the strawberry plant.

Introduction

California is the fifth largest supplier of food and agricultural commodities in the world. California's agricultural contribution to the United States and to other countries has annual makings of slightly 2% of the states annual gross of 1.55 Trillion. Airborne exports of perishable fruits and vegetables amounted to approximately $685 million in 2007, with the production and sales of berries accounting for the majority grown within Watsonville-Salinas Valley (USDA, 2006). Carolyn O'Donnell, spokeswoman for the California Strawberry Commission of Watsonville monitored the growers in Watsonville-Salinas and Oxnard areas counting a harvest of more than 5 million trays a week from April through June 21. Strawberry acreage continues to climb in the state, from 34,642 acres in 2007 to 35,696. In the past twenty years devastating issues have cause concern in health and environmental risks due to the use of soil fumigate *Methyl Bromide*. *Methyl Bromide* is used to eradicate pests around a large amount of agricultural acreage and was recorded to be phased out of use as of January 1, 2005 (EPA, 2005). *Chloropicrin*, an organic molecule that spreads with rapid diffusion within the soil pores provides selective removal of common root rot fungus. This agricultural fumigant is a staple in the use of agricultural production all over the nation today and the future (USEPA, 2005). European countries, including England, mandatory use of certified strawberries and strawberry material mandated by the OEPP/EPPO was enforced to provide sufficient guarantees (OEPP/EPPO, 1994). Legislation controlling the production of strawberry plants and the prevention of red stele core, if any disease is found within a wholesale grower's acreage, the disease is notifiable to authorities. Scotland requires an official certificate of health, a condition that is evaluated to be absent of the red stele core disease or the strawberry runners cannot be sold to any grower, but destroyed. Most countries feel due to the severity of the disease that a

zero tolerance policy should be placed on the disease because of it's sufficient life cycle of remaining within infested soil for long periods of time, although many have agreed to enforce this policy some countries allow small percentages of the root rot in certified stock (Navatel and Fournier, 1986).

San Luis Obispo County located in the central coast of California possesses a xeric moisture regime, a weather classification that ensures an average temperature of 18 – 23 C° throughout the entire year. Throughout Grover Beach, Huasna Valley and the Nipomo region, increased precipitation and continual nightly fog encourage *Phytophthora fragariae* and *Verticillium dahliae*, diseases that are of dangerous concerns to the local farmers that have been growing for over 50 years. Many studies have been conducted on providing information that cultivars of strawberries and specific races of Phytophthora fragariae, specifically the variety fragariae interact in a gene for gene (GFG) manner as described by Flor (1956) for flax and flax rust (*Malamspora lini*), (Wilcox, 1993). Phosphate is one of the major plant nutrients that influences virtually all the biochemical processes and developmental phases of plants. (Raghothama, 1999). (HPO_3^{2-}), referred to as phosphoric acid or phosphonate, is an isostere of the Phosphate anion in which one of the oxygen's bound to the P atom is replaced by hydrogen. Phosphoric acid exhibits various biological activities on fungi and plants allowing the plant to become more stable for several months deterring the attack of the fungus. *Phytophthora citricola* and *Phytophthora cinnamomi* in past studies have provided results of being sensitive to Phosphoric acid (Guest and Grant, 1991). Very little is known about soil amendments compost fertilizers, and limestone influence Phytopthora Root Rot (*PRR*) in strawberry and raspberry production. In recent years studies have provided significant growth and yields by the application of Gypsum ($CaSO_4$) and reduced yields of *P. citricola* in avocado plantings, (Menge et al.,

1994). Cultural techniques in suppression of *Phytophthora* and *Verticillium* vary by cropping system (Maloney and Pritts, 2005). Conducted tests on Apple trees grown in either grass sod or crown vetch ground cover vegetation management, which remained free of the crown and root rot symptoms (Merwin et al., 1992). While adjacent trees in other cropping systems were surrounded by straw mulch, the crops became diseased (Heiberg 1995).

Methyl Bromide is a commonly used fumigation practice to ensure the riddance of fungal diseases after cropping and harvesting occurs generally in the fall. Other chemicals that are listed to be used by growers throughout the Watsonville-Salinas Valley use the list as follows; Mefenoxam (Ridomil Gold, Sygenta Crop Protection, Inc., Greensboro, N.C.), this product is used for control of PRR. Metalaxyl has been used as a beneficial component of integrated pest management systems including raised beds or genetic host resistance. Inline, a soil fumigant used in subterranean dispersal amongst raised beds. Tricon (57:43), a combination of 57 % Methyl Bromide and 43 % Chloropicrin at 350 lbs / acre. Although with the insurance of ridding the diseases to the crops, these chemicals are known to diminish macro and micro biological life within the soil, not only eliminating harmful bacteria, killing off beneficial microbes that ensure stimulation of elemental fixing products provided in a symbiosis relationship between the microbes and the plants themselves. The Persistence of *Phytophthora Fragariae* is due to its wide host; with the fungus remaining present in nurseries used for runner plant propagation and fruit production fields (Gordon et al., 2002). In the absence of effective fumigates the difficulty to maintain soil populations of harmful fungus below damaging levels to large quantities of strawberries produced (Harris and Yang, 1996). With breeding for resistances among new hybrids of strawberry plants and starts, Drischolls is part of the fore front in testing

new hybridized varieties in search of resisting harmful fungus that wipe out large production of harvestable strawberries each year.

The objective of the experiment is to provide validity in the use of Phosfite fertilizer to suppress or prevent the growth of *Phytophthora fragariae* and find any beneficial results in the quality and growth of the tested plants. Relationships between soil fertility on strawberry starts treated with Bacillus, an inoculant for root formation in soils infected by *Verticillium dahliae* will also be further reviewed. Fruit and soil analysis of specified plants conducted within the study will provide relationships and trends that could help in future studies in the subsidence of *Phytophthora fragariae* and *Verticillium dahliae.*

Materials and Methods

Redman and Vasquez Ranch Sites

In the experiment there are two types of soils from two different locations. The soil from the Vasquez Ranch in Watsonville California is a clay loam used in strawberry production. This soil was evaluated by a local plant pathologist from the USDA extension service and tested with a positive confirmation of residual *Verticillium dahliae* within the topsoil (Table 1).

Table 1. Dominant physical properties of Redman and Vasquez Ranch located in Watsonville, CA.

Sample Location	Soil Series	Texture	Bulk Density (g/cm^3)	% water (Mass / Volume)
Vasquez Ranch	Santa Ynez	Fine Sandy Loam (48.5-23.1-28.4)	1.32	18.6% / 24.55%
Redman Ranch	Conejo	Clay Loam (35.8-34-30.20)	1.2	18.1% / 21.7%

The Redman Ranch soil, a fine sandy loam, was derived from a nearby location that was from agriculture production of strawberries and occasionally used for raspberries and blackberry

Figure 1. Soil Sample locations derived from Redman and Vasquez ranch located near Watsonville, CA.

production. This soil showed a positive confirmation for the pathogen of *Phytophthora fragariae* (figure 1).

Soil Sampling and Greenhouse Operation

With the quantity of two soils that were confirmed to have pathogens, untreated soil that is free of both pathogens being observed to the best of our knowledge was gathered for further studies within the experiment. The soils were gathered and contained within steel oil drums

from the ranches located near Watsonville, California and were relocated to the soil science greenhouse located on Via Carta next to the horticultural unit at California Polytechnic State University, San Luis Obispo California. Within the greenhouse, two benches were designated to have the *V. dahlia* infested soil occupy one bench and the *P. Fragariae* infested soil occupy the other bench (Figure 2). Each Soil was placed within a horticultural 1 gallon black container and was marked if the soil was clean from all pathogens or if it was from the containers that were respectively marked dirty. Numerous studies and treatments are being conducted within the experiments as one pertained to this study with be labeled separately. Analysis of treatments and observations will be conducted on fresh transplants of fragariae hybrid species. These species are patented by Drischolls®, a world leader in berry production and agricultural farming of strawberries. Drischolls is a facility that is located in Watsonville and in the Salina's Valley of California, and donated the transplants for research on alternatives for prevention of fungal diseases and the riddance of soil fumigant Methyl Bromide and other harmful chemicals used in agricultural production to date. This species will be referred as FHS-1 variety.

When the transplants were planted a fibrous barrier was placed at the bottom of each pot to help contain excess soil runoff and to help keep the soil moisture at a heightened level to aid in germination of possible fungal spores from both species of pathogens. Each plant is connected to its own drip line for proper watering by drip irrigation. The watering cycle will come on twice a day on the *phytophthora* bench and once a day on the *Verticillium* bench. Differences in water regimens are due to texture of soil and their physical properties (table 1). The Redman soil confirmed with Phytophthora was treated with gypsum to help disperse alkaline properties within the soil and open pore space to allow water infiltration in the containers. Soil Pores were sealed by increased cementation of chemical properties within the

soil from previous farming cycles. Each container was tagged with a number ranging from 1-360 where 320 are sampled and the other 40 are reserves for other tests if desired. And was placed into blocks where thirty two containers occupy each block. Tests will only be conducted on selected species from both tables and are blocked yellow (figure 2). Starting March 1st boxed honey bees will be placed within the greenhouse to ensure symmetrical and perfect ripened fruit during the study and to help alleviate *Phyllody*, a disease known to distort fruit from genetics issues of fruit production.

Infested
Clean

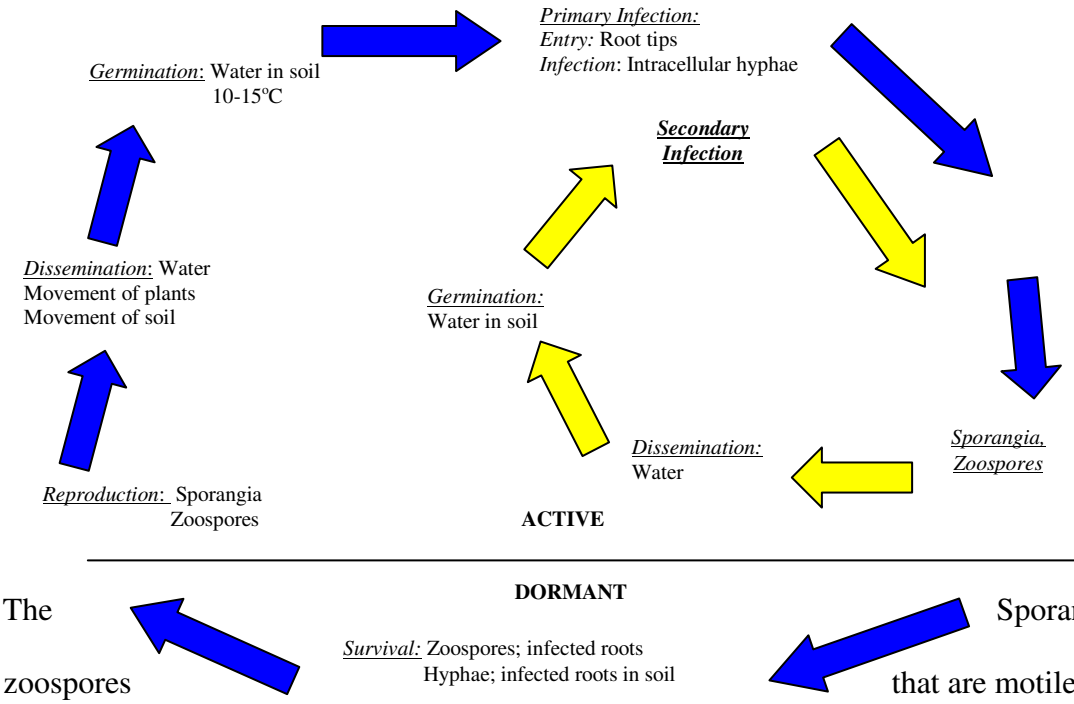
Sampled (Yellow)
360 Total Plants FHS-1

	Verticillium dahliae expt.				**Door**	*Phytophthora fragariae expt.*			
Row 1	**1 buffer row**					**1 buffer row**			
	Block 1					Block 1			
Row 2	Prom	Bacillus	Ozone (M)	2_scan		Prom	Bacillus	UTC	Ozone (L)
Row 3	Ozone (M)	2_scan	Bacillus	Prom		2_scan	MB:CP	Mycor	Ozone (M)
Row 4	Filler-M-P	Filler-P	UTC	Filler		Phos	1_scan	3_scan	CP:Tel
Row 5	1_scan	Mycor	Ozone (H)	CP:Tel		Bacillus	Ozone (L)	UTC Phos	Prom
Row 6	1_scan	Mycor	CP:Tel	Ozone (H)		Ozone (H)	InLine	Filler-M-P	Filler-P
Row 7	UTC	Filler-M-P	Filler-P	Filler		InLine	Filler-M-P	Filler-P	Ozone (H)
Row 8	MB:CP	3_scan	Ozone (L)	InLine		Ozone (M)	2_scan	Mycor	MB:CP
Row 9	MB:CP	Ozone (L)	InLine	3_scan		1_scan	CP:Tel	3_scan	Phos clean
	Block 2					Block 2			
Row 10	Filler-P	Filler-M-P	Filler	UTC		Filler-P	Filler-M-P	Ozone (H)	InLine
Row 11	InLine	3_scan	MB:CP	Ozone (L)		MB:CP	Mycor	Ozone (M)	2_scan
Row 12	Ozone (H)	1_scan	Mycor	CP:Tel		3_scan	CP:Tel	Phos Dirty	1_scan
Row 13	Ozone (H)	Mycor	CP:Tel	1_scan		CP:Tel	3_scan	Phos	1_scan
Row 14	InLine	MB:CP	3_scan	Ozone (L)		Mycor	Ozone (M)	2_scan	MB:CP
Row 15	2_scan	Filler m-p	Ozone (M)	Prom		Filler-M-P	Filler-P	Ozone (H)	InLine
Row 16	2_scan	Prom	Bacillus	Ozone (M)		UTC	Ozone (L)	Prom	Bacillus
Row 17	Bacillus clean	Filler-P	UTC	Filler		Ozone (L)	UTC	Prom	Bacillus
	Block 3					Block 3			
Row 18	Filler-P	Filler	Bacillus clean	UTC		1_scan	3_scan	CP:Tel	Phos
Row 19	Mycor	CP:Tel	Ozone (H)	1_scan		Ozone (M)	2_scan	MB:CP	Mycor
Row 20	CP:Tel	1_scan	Ozone (H)	Mycor		Filler-M-P	Filler-P	InLine	Ozone (H)
Row 21	Ozone (L)	InLine	3_scan	MB:CP		Ozone (H)	InLine	Filler-M-P	Filler-P
Row 22	2_scan	Prom	Ozone (M)	Bacillus		2_scan	Mycor	MB:CP	Ozone (M)
Row 23	Ozone (M)	2_scan	Bacillus	Prom		Prom	Bacillus	Ozone (L)	UTC
Row 24	3_scan	InLine	Ozone (L)	MB:CP		Bacillus	UTC	Ozone (L)	Prom
Row 25	UTC	Filler	BcillusDirty (VVD) #95	Filler-P		Phos Clean	1_scan	CP:Tel	3_scan
Row 26									
Row 27	**3 buffer rows b/c plumbing**					**3 buffer rows b/c plumbing**			
Row 28									
	Block 4					Block 4			
Row 29	Bacillus	2_scan	Prom	Ozone (M)		Mycor	Ozone (M)	2_scan	MB:CP
Row 30	CP:Tel	Ozone (H)	Mycor	1_scan		Ozone (L)	Prom	Bacillus	UTC
Row 31	3_scan	InLine	MB:CP	Ozone (L)		CP:Tel	Phos	1_scan	3_scan
Row 32	Prom	Bacillus	2_scan	Ozone (M)		MB:CP	2_scan	Ozone (M)	Mycor
Row 33	UTC	Filler	Filler-P	Bacillus Dirty		Ozone (L)	Prom	Bacillus	UTC
Row 34	Ozone (L)	InLine	3_scan	MB:CP		InLine	Ozone (H)	Filler-P	Filler-M-P
Row 35	CP:Tel	Ozone (H)	1_scan	Mycor		Filler-P	Ozone (H)	InLine	Filler-M-P
Row 36	UTC	Filler	Filler-P	Filler-M-P		CP:Tel	Phos Dirty	1_scan	3_scan
	Block 5					Block 5			
Row 37	Filler-M-P	UTC	Filler	Filler-P		3_scan	Phos	1_scan	CP:Tel
Row 38	MB:CP	Ozone (L)	InLine	3_scan		UTC	Ozone (L)	Prom	Bacillus
Row 39	Bacillus	Ozone (M)	Prom	2_scan		Filler-M-P	InLine	Filler-P	Ozone (H)
Row 40	Prom	Ozone (M)	2_scan	Filler-m-p		Filler-P	Filler-M-P	Ozone (H)	InLine
Row 41	Mycor	CP:Tel	1_scan	Ozone (H)		MB:CP	2_scan	Mycor	Ozone (M)
Row 42	Filler-P	UTC	Filler-M-P	Filler		3_scan	CP:Tel	Phos	1_scan
Row 43	3_scan	MB:CP	Ozone (L)	InLine		UTC Phos	Bacillus	Prom	Ozone (L)
Row 44	1_scan	CP:Tel	Ozone (H)	Mycor		Mycor	MB:CP	Ozone (M)	2_scan
Row 45	1 buffer row					1 buffer row			
					Barn				

Figure 2. Greenhouse design for *Verticillium dahliae* and *Phytophthora fragariae* experiment with specified species used within study (Yellow Blocks).

Phytophthora fragariae

The genus *Phytophthora* means "plant destroyer" (Agrios, 2007). Species of this genus are the casual agents of devastating events in agricultural history, late blight on potato, the cause for the famine in Ireland (*Phytophthora infestans*), *Phytophthora cinnamoni*, avocado root rot fungus and *Phytophthora Fragariae*, Species of funguses that infect fragaria x ananassa, varieties susceptible to red stele root rot. The roots of the strawberry plant show red color when affected and "damping off" of the adventitious roots (Agrios, 2007). Only one other host, loganberries (Rubus hybrid), have been found to naturally host P (Mckeen, 1958) fragariae and numerous species in the rosaceae family have been infected artificially (Pepin, 1967). Survival of Phytophthora fragariae occur by Oospores produced during the ------stage in the cycle of reproduction. Oospores germinate to form one or several sporangia and the optimal temperature range for germination is approximately 10-15 ^{o}C although experiments have been conducted where Oospores have germinated at 20 ^{o}C and very slowly at 5^{o}C (Figure 2).



The Sporangia release zoospores that are motile in

Figure 3. *Phytophthora fragariae;* Lifecycle of Strawberry Root Rot Fungus

water where they can come into contact with root tips of host species where the spores with cyst onto the plant and form germ tubes. These germ tubes attach within the plant and penetrate within the root. The fungus will transverse the cortex and inter and intracellularly attack the stele, colonizing the phloem and the pericycle of the plant. Non-papiilate secondary sporangia can be produced within a few days and the fungus can produce many cycles of infection over the course of a winter (Kennedy and Duncan, 1993).

The life cycle can survive harsh cold winters and dry hot summers by dormant spores and , these spores can remain in the ground (Quote) and other believe for up to 15 years (figure 3). Symptoms of the plant generally occur in the upper parts of the plant during the spring and early summer when the plant starts to become under stress. This is a faster occurrence in low lying wet areas. The roots when cut into reveals steles wine red to brick red in color and the adventitious roots appear grey and brown in color referring to their rotting appearance.

Verticillium dahliae

A worldwide known disease, *Verticillium*, Wilt develops in seedlings and induces its host plants at much lower temperatures than other diseases. A soil borne pathogen in the Deuteromycetes group, *Verticillium* is a vascular disease infecting the xylem prohibiting water to circulate into the plants leaves producing a "Wilty" look. *Verticillium* produces conidia and can produce microsclerotia in dying tissue; these sclerotia produce masses of hyphae that over winters in the soil and this mycelium can have the potential to survive up to 15 years (Agrios, 2007). Conidia are single celled ovoids that surround the conidiophores by a verticillate arrangement or whorled pattern (Figure 4).

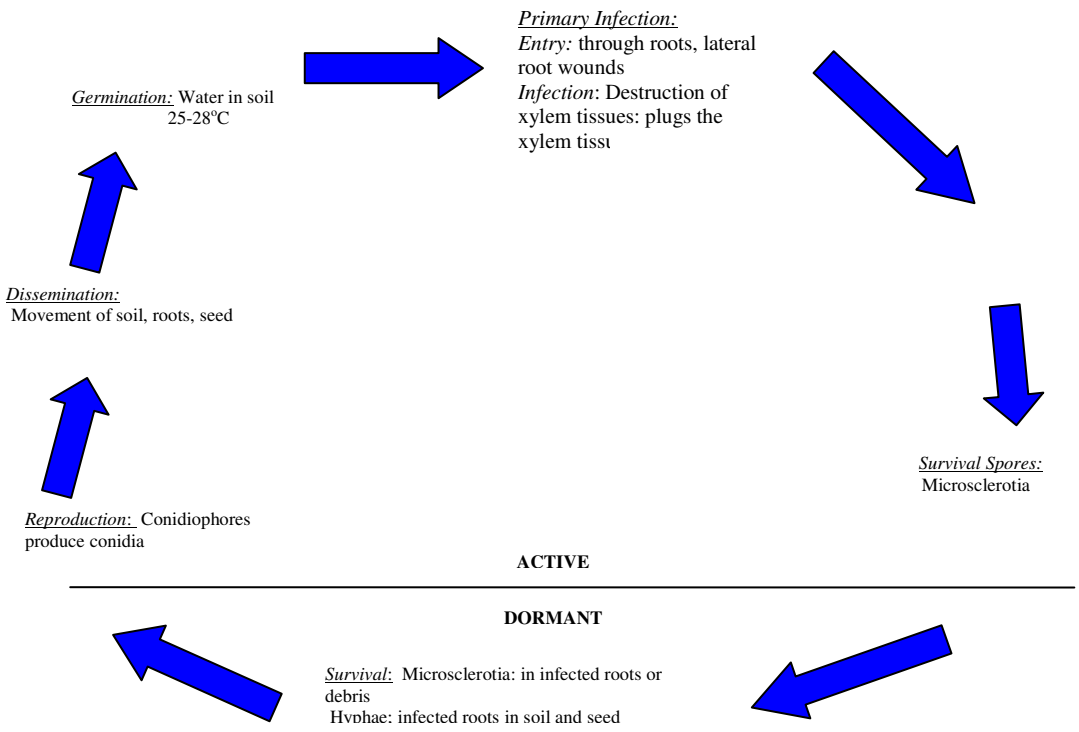


Figure 4. *Verticillium dahliae;* Lifecycle of Strawberry Wilt.

Verticillium produces better in temperatures around 25-28 ^{0}C. Consistent cropping of numerous varieties susceptible to the fungus are tomatoes, strawberries, raspberries, potatoes increase risks of plant infestation. *Verticillium Dahliae* is considered to be one of the most important pathogens affected commercial strawberry productions in California. *Verticillium* wilt is controlled by pre-plant soil fumigation practices, but the soil population of Conidia is only partially eradicated by each soil treatment, repeated fumigation treatments may be needed to reduce infection to acceptable thresholds (Wilhelm, 1980). The disease continues to be a major concern along with *Phytophthora fragariae* diseases being monitored for their issues to farmers in California that practice perennial planting systems (Shaw and Gubler, 1997).

Phosfite, Bacillus, and Maintenance Treatments

In attempt to monitor the suppression or degradation of the diagnosed pathogen diluted rates of Phosfite and Bacillus will be applied every other week (appendix B), throughout the experiment to provide understanding in utilization of the P forms compared to necessity of the Phosphate form (PO_4^{3-}) and zinc species within the soil. The Phosfite is within a five gallon jug and 2 mL of Phosfite will be pippetted into beaker and filled with water to 600 mL total volume. 30 mL of solution will be provided to the ten total plants receiving the Phosfite solution. Bacillus is poured at 150 mL from a five gallon jug into a solution of 2500 mL total volume. 100 mL of solution will be applied to each plant labeled Bacillus totaling to twenty plants. There are two jugs of Bacillus a blue jug and a yellow jug. The Bacillus (Yellow) will be applied two weeks alone at the beginning of the experiment and applied two weeks alone for the Bacillus (Blue) jug. Following those additions of Bacillus the yellow and blue jug will be mixed and provided on a weekly schedule.

The Phosfite solution will only be provided to 10 plants occupying the *Phytophthora* bench and the bacillus will be provided to 20 containers, 10 from each bench. Fertility tests in evaluation of the Bacillus solution will be only measured and compared by the samples from the Verticillium bench as the Fosphite will be tested only on the Phytophthora bench. Values obtained will be compared of 2 soils infested (Inf.) with the disease and two soils not infested (non-Inf) with the disease and 2 soils that were used as Untreated Controls (UTC) for both benches infested with both of the prescribed diseases. Throughout the experiment infestation of *Tetranychus urticae (*2 spotted mite), Aphid, and fungal pathogen Powdery Mildew have accumulated and was treated.

Determination of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and Micro Nutrients of FHS-1 Fruit

Identification of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Conservative cations that contribute to total Alkalinity of solutions are determined by Atomic absorption Spectrophotometry (AAS), This method precisely determines Ca^{2+} and Mg^{2+}, and K^{+} and Na^{+} are determined by Atomic Emission Spectrophotometry (AES). These methods are followed under prescribed calibrations using standard solutions according to manufacturer guidelines of Quality Assurance and Quality Control measures. (Appel, Stubler, 2009). Determination of all cations will be performed in individual samples, selected samples every tenth measurement will be used as a replicate during the experiment. Samples will be measured at noted ratios of 1X, 10X, 50X, and 500X dilution factors (appendix A). Fruit production and harvest starting January 31st and will be conducted 2X a week until July 22, 2009. All strawberries are picked, weighed as fresh fruit and recorded as specified plant identification number. The fruit are placed within zip-lock bags and stored into two freezers. As all specimens of fragariae species have produced desired amounts of fruit for analysis, the harvests will be recorded as 1st fruit harvest of the season and second fruit harvest of the season. The fruit will be weighed before the fruit is oven dried to dehydrate excess water and allow for analysis. After the fruit has dried for a 14-18 hour period at an internal oven temp of 105 ^{o}C the fruit will be ground with a mortar and pestle.

Determination of Carbon and Nitrogen of FHS-1 Fruit and Soil Analysis

Analysis of Anions NO_3^- and Carbon conducted by the Colorimetric and ISE processes will only be conducted on fruit analysis. The Soil samples were placed into bags and brought to

the Earth and Soil's Department to allow air drying and was sent for complete physical and chemical analysis (% Soil Organic Matter, CEC ($Cmol_C/kg^{-1}$), ECe (dS/m^{-1}), pH, N, Olsen Method P, K, S, Mg^{2+}, Ca^{2+}, Na^{+}, Al^{3+}, Mn^{4+}, Cu^{2+}, Zn^{2+}, B^{3+}). (Data provided by A and L Laboratories), (Craig Stubler, Personal communication).

Results

Phytophthora fragariae Experiment

In the Plots on the western bench the developmental symptoms of PRR disease were absent within all infested soil containers containing the FHS-1 crop. Throughout the experiment no can FHS-1 species showed any characteristics or signs of red stele disease or unsatisfactory growth and production of fruit throughout the experiment. In the early stages of the experiment petiole cuttings were taken and tested on V-8 blended agar containing essential nutrients and vitamins to ensure growth of the PRR disease and if signs were present, and there was a negative indication of any disease present from germination of zoospores and growth of sporangia. (Figure 5) Plant shoot weight were recorded and provided an average of weight of 129.85 for UTC samples, 135.50 g for the Phosphite (Inf) samples and 135.80 for the Phosphite (Non-Inf). The roots of the Phosphite (Inf) had sufficient production of fibrous adventitious roots with healthy white to eggshell white coloration and no visible indication of rot or excess contact with water forming anaerobic conditions within the containers as shown as infected roots from Red stele disease in (Figure 5).

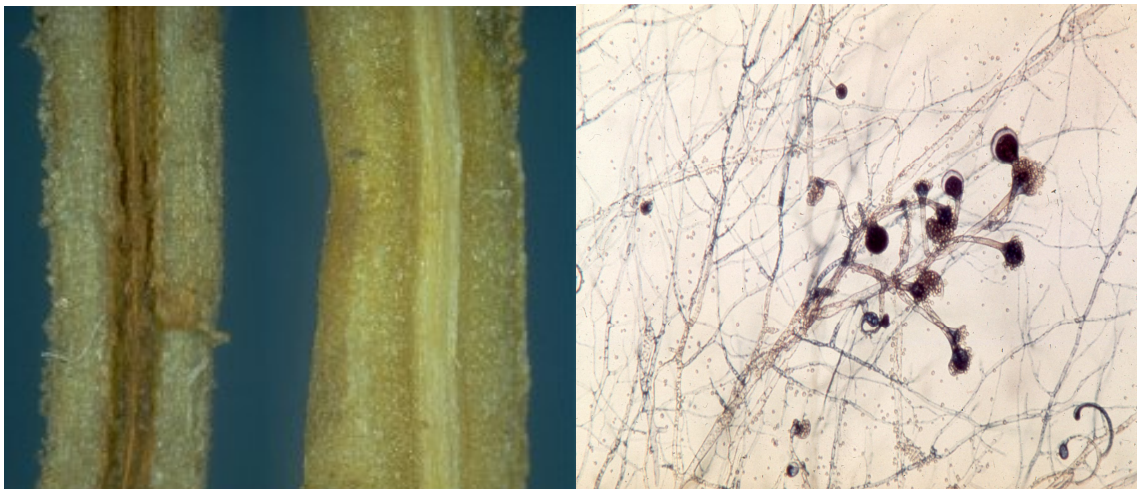


Figure 5. Petiole discoloration from PRR on the left; a healthy petiole of *Fragaria species* on the right; (Left Photo). Sporangia produced on sporangiaphores incubated on V-8 enriched agar (right Photo), (Millholland, Cline and Daykin, 1989).

Verticillium dahliae Experiment

In the plots on the eastern bench the developmental symptoms of VVD was present among a select few plants within the study and caused necrosis and death to one plant within this particular study, Can # 95 a container confirmed to have residual V. dahliae fungus. In the early stages of the disease the plants showed new growth and vital signs of fighting of the disease. As the study continued the plants turgor pressure decreased and cause a wilty affect with no transpiration present until the plant was completely dead. In the early stages of the experiment petiole cuttings were taken and tested on V-8 blended agar containing essential nutrients and vitamins to ensure growth of the VVD disease and if signs were present, and there was a positive indication of disease present from germination of conidiophores and growth of conidia surrounding the tips. Conidiophores are slender, branched, and at least some of the branches are verticillate (in whorls); the conidia are ovoid shaped and some may have an ellipsoid shape. All are hyaline and single celled when born in small moist clusters apically (figure 5, right photo)

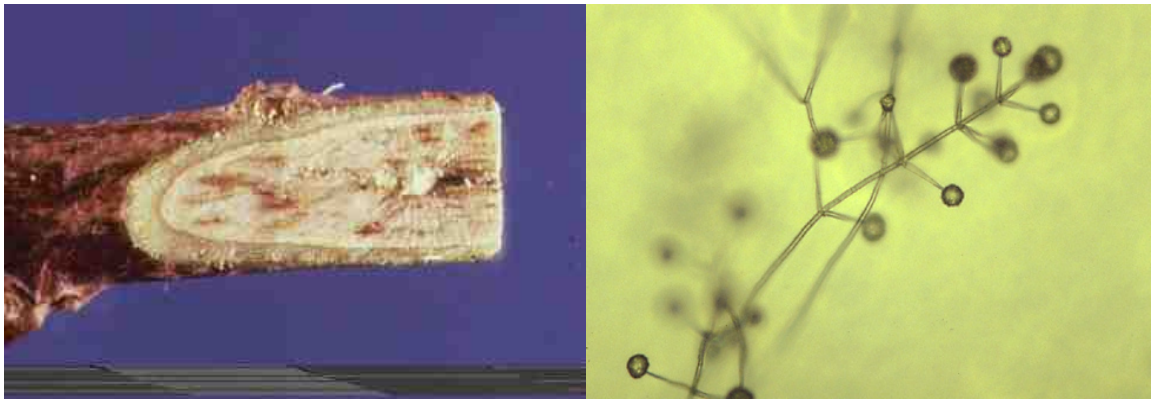


Figure 6. Vascular discoloration of Xylem on cotton (left photo) and Verticillate branching of conidia bearing conidiophores on water agar (right photo) (Tjamos et al., 2000).

These conidia are known as vascular parasites causing wilt to the plant and grow saprophytically until death as in the case of specimen sample # 95 (infested FHS-1 treated with Bacillus). Plant shoot weight were recorded and provided an average of weight of 80.05 g for UTC samples, 67.95 g for the Bacillus (Inf) sample because the average is low due to death of can #95 in early stages of the experiment and 88.5 for the Bacillus (Non-Inf).

Redman and Vasquez **Chemical Property Soil Analysis**

From the analysis of the soils tested the pH levels were relatively close in comparison with the Redman (D) and (C) and the Vasquez (D) around 6.9. The Vasquez (C) soil was the only soil among the four that had a tremendous value difference with a pH level tested around 7.7 (Table 2). From the analysis of the soils the electrical conductivity provided values in the following order, Redman (D) and Vasquez (D), around 0.65 mMols / cm, and the Redman (C) and Vasquez (C) 0.69 and 0.77 mMols / cm (Table 2). The CEC due to mineral fraction was estimated and calculated by the ECEC method and all values are reported (cmolc/kg) and reported as follows, The Vasquez (C) soil recorded the lowest value of 7.6, the Vasquez (D) soil

had a value around 8.8 and the Redman (D) and (C) soils had values of 13.4 and 21.9 respectively with minimal amounts contributed due to soil organic matter (SOM) (Table 2).

Table 2. Dominant chemical properties of Redman and Vasquez Ranch located in Watsonville, CA.

Soil I.D.	pH	CEC due to SOM cmolc/kg	CEC due to mineral fraction cmolc/kg	%BCS	%O.M.	EC mMol/cm
Redman D	6.9	2.90	13.39	98.51	2.91	0.6
Redman C	6.8	3.44	21.84	96.80	3.44	2.0
Vasquez D	6.9	1.54	8.77	98.70	1.55	0.7
Vasquez C	7.7	3.23	7.60	100	3.23	1.4

PRR and VVD Analysis

With results of soil analysis on the Redman and the Vasquez soils confirmed dirty and clean some elemental comparison are provided. The red (C) reported the highest nitrate values with 51 ppm recorded and the lowest was the vas (C) with a value of 39 ppm. The Redman (D) and (C) soils had relatively high analysis of Phosphorus with 78 ppm and 74 ppm in comparison to extremely low phosphorus values recorded within the Vasquez (C) and (D) with 23 ppm and 18 ppm respectively. Potassium was extremely high in the red (C) soil with a value of 406 ppm and the lowest potassium levels was 96 ppm in the Vasquez (C) soil. In respect to micro nutrients Iron levels were distributed among the soil quite unevenly. The red (C) soil had a very high recording of Iron levels with 36 ppm. The Vasquez (C) soil was the lowest Iron concentration with 12 ppm and both the Redman and Vasquez (D) soils had equal amounts of Iron Concentration recorded with 22 ppm each (Figure 6 (A & B)).

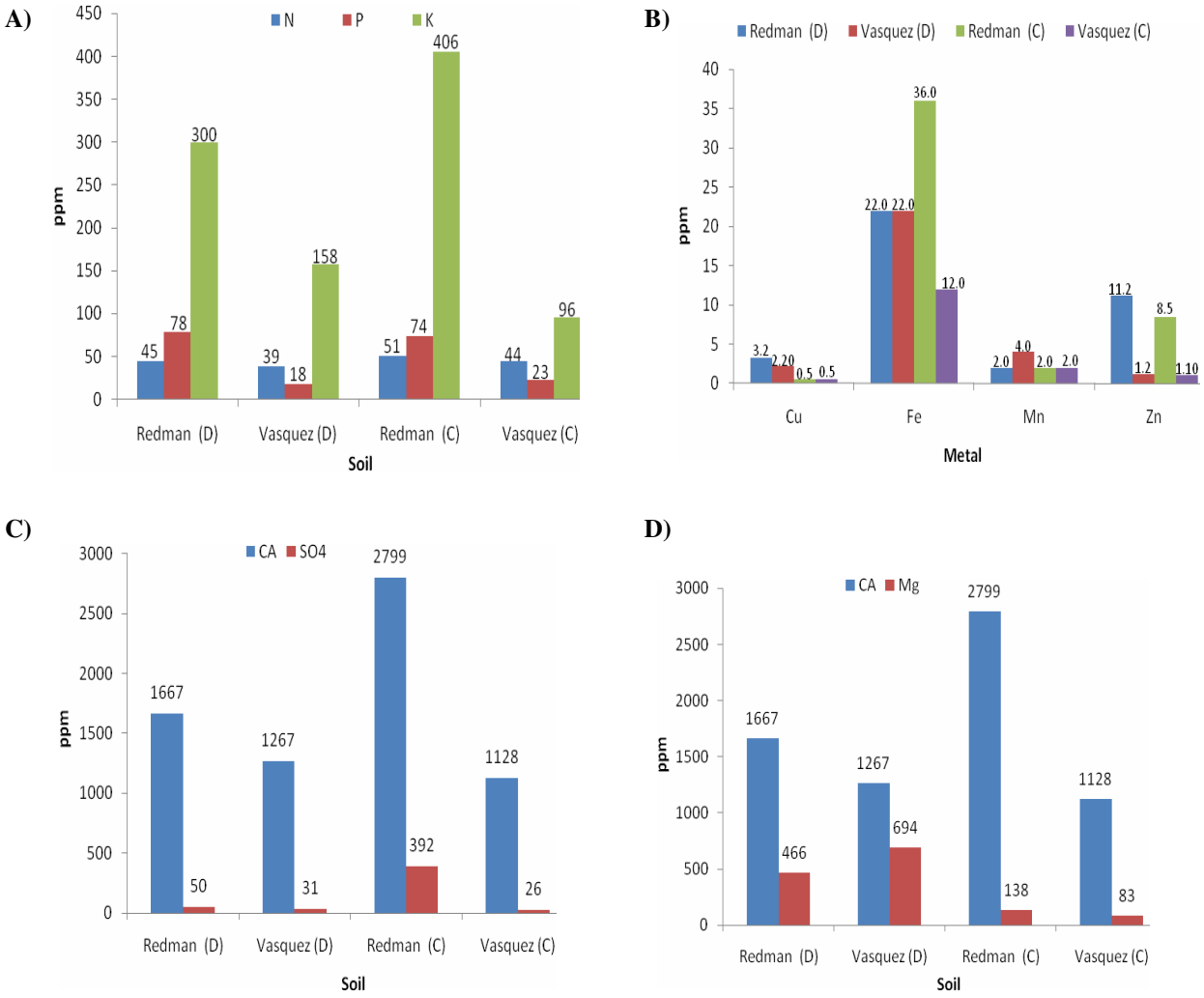


Figure 6. A) N,P,K relationship, B) Cu, Fe, Mn, Zn relationship, C) Ca & SO_4 comparisons, and D), CA & Mg ratios amongst Redman and Vasquez infested and non-infested soils.

The Calcium and sulfate ions within the experiment showed determination of the Redman (C) soil having the highest amount of Calcium with 2799 ppm and 392 ppm of sulfate recorded (fig 6 (C)). The Redman (C) soil had a Ca: Mg ratio of around 20:1 and the Vasquez (D) soil reported the lowest Ca: Mg ratio with a 2:1 comparison. (Figure 6, (D)). Within the study Phosphorous comparison with zinc and copper were evaluated. The Redman (D) soil concluded

the highest P value with 78 ppm, a P: Zn ratio of 7:1 and P: Cu ratio of 24:1. The Vasquez (D) soil had a P value of 74 ppm, a P: Zn ratio of about 9:1 and P: Cu ratio of 33:1. Both the Redman and Vasquez (C) non-infested soil's, not treated with Bacillus or Phosphite had lower P values recorded and very minimal values for Copper and Zinc (Figure 7).

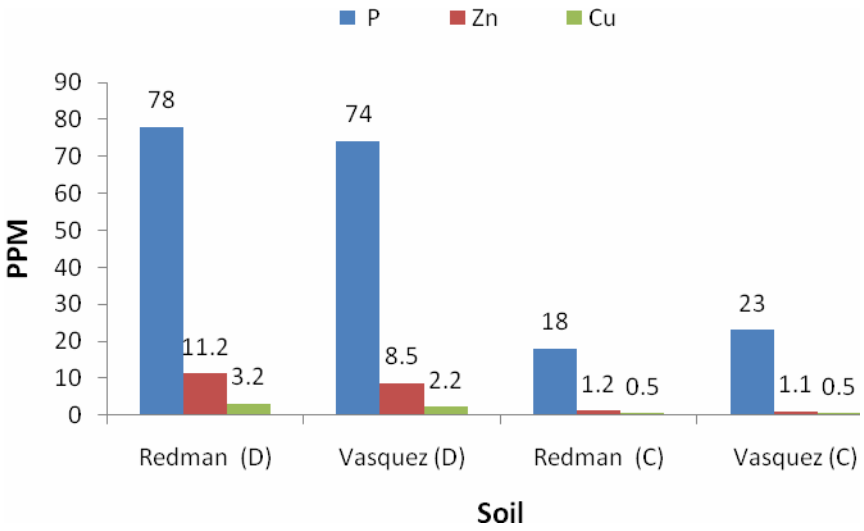


Figure 7. Phosphorous, Zinc and Copper amongst Redman and Vasquez infested and non-infested soils.

Nutrient Analysis of VVD Fruit 1st bearing and 2nd bearing

In analysis of the the 1st bearing fruit from the FHS-1 crop planted in clean and dirty soil containing VDD, the clean, dirty bacillus treatment and the UTC crops were compared for characteristics and relationships (Table 3). The average Ca: Mg ratio among the treatments determined values of .64:1 ppm for crops 116 and 67 (Clean Bacillus), .68:1 ppm for crops 71 and 125 (UTC), and .58:1 ppm for crop 128 only with crop 95 deceased from VDD in early stages of the experiment. Micro metals among the different treatments within the study showed consistent relationships throughout the crops in the 1st bearing fruit harvest with all

concentrations showing < .50 ppm respectively and consistent trends in Nitrogen: Carbon ratios with values around a .30:1 consistently through experimental plants (Table 3).

Table 3. Nutrient analysis of 1st bearing strawberry fruit on VVD on FHS-1.

I d #	Type	Ca	Mg	K	Fe	Mn	Zn	N	C
		----------ppm----------							
116	Clean Bacillus	9.02	13.23	161.77	0.46	0.19	0.14	11945.27	427823.40
67	Clean Bacillus	6.54	10.79	169.48	0.36	0.16	0.13	13945.82	433221.40
71	UTC	7	11.5	154.03	0.38	0.17	0.13	12529.78	440157.30
125	UTC	7.88	11.62	162.92	0.32	0.2	0.14	14834.76	444537.80
128	Dirty Bacillus	6.94	11.81	168.92	0.34	0.22	0.13	11944.12	414473.20
95	Dirty Bacillus	0	0	0	0	0	0	0	0

In analysis of the the 2nd bearing fruit from the FHS-1 crop planted in clean and dirty soil containing VDD, the clean, dirty bacillus treatment and the UTC crops were compared for characteristics and relationships (Table 3). The average Calcium: Magnesium ratio among the treatments determined values of .64:1 ppm for crops 116 and 67 (Clean Bacillus), .75:1 ppm for crops 71 and 125 (UTC), and roughly 1:1 ppm for crop 128 only with crop 95 deceased from VDD in early stages of the experiment. Micro metals among the different treatments within the study showed consistent relationships throughout the crops in the 2nd bearing fruit harvest with all concentrations showing < .50 ppm respectively and consistent trends in Nitrogen: Carbon ratios with values around a .30:1 consistently through experimental plants (Table 3). In comparison of fruit from 1st harvest and 2nd harvest provided increased Ca levels and decreased Mg levels from each fruiting cycle and the micro metals pertained similarities between each

harvest during the experiment with both showing <.50 ppm. The C: N ratios were consistent amongst each harvest in the study (Table 3).

Table 4. Nutrient analysis of 2nd bearing strawberry fruit on VDD on FHS-1.

I d #	Type	Ca	Mg	K	Fe	Mn	Zn	N	C
		----------ppm----------							
116	Clean Bacillus	7.72	12.61	147.28	0.28	0.19	0.11	12103.64	436914.71
67	Clean Bacillus	8.29	10.61	139.17	0.25	0.15	0.15	14435.46	442942.01
71	UTC	7.99	11.74	182.37	0.38	0.19	0.13	12720.51	440021.82
125	UTC	7.13	8.93	155.25	0.33	0.19	0.1	15039.30	442105.79
128	Dirty Bacillus	8.87	8.93	159.85	0.33	0.25	0.12	12055.19	434386.63
95	Dirty Bacillus	0	0	0	0	0	0	0.00	0.00

Discussion

***Phytophthora fragariae* Root Rot Disease**

In correspondence to the FHS-1 there was no detection of PRR present in the crops treated with Phosphite and UTC's within the experiment. External environmental factors, internal greenhouse temperatures within the glass greenhouse cropping system established poor ventilation and air circulation causing increased temperatures. The heightened increase in temperatures possibly could be a catalyst for un-sufficient germination of sporangia or Oospores within the soil contained containers. The FHS-1 starts were planted into their individual containers and the first sign of fruit was acknowledged late February, the annual precipitation was considered a draught year in San Luis Obispo County. The draught caused more dry seasonal days with lower percent humidity, another negative impact on germination of *Phytophthora fragariae*. The Disease outbreak starts with small foci of infected strawberry

plants and as the plants generally increase in size, Down slope where water build up increases due to saturated field capacity conditions a large number of plants can become infected more readily.

Species of the FHS-1 in the Greenhouse cropping system used within the study, each individual plant was contained within their own individual quart sized container that singlely isolate their own rhizosphere when plants grow to mature size. The space allowed was very minimal and limited the detection area of any noticeable confirmation of PRR in selected plant roots or crowns studied. Outdoor cropping systems have infinite amounts of pore space with the capacity to hold infinite amounts of sporangia, root tips infected by Hyphae or Zoospores contained with water. Symptoms usually appear on the upper parts of the plants that become stressed in later spring or early summer and are especially noticeable in low lying wet areas within the fields. Adjustments that could be of benefit in establishing disease is collecting the water from all plant containers subjected to the experiment and recycle the irrigation water that has the potential to hold pathogenic Zoospores. The fungus can spread in surface water and/or drainage water in higher percentages in locations of very wet, mild winter environments (Menge, 2004).

Early in the experiment soil pores within individual containers containing the Redman Soil (Conejo; Clay Loam-35.8-34-30.20) showed chemical and physical properties of cementation due to high lime distribution within the field and an abundance of available sodium within the soil, all factors contributing to hydrophobic soil properties. The plant containers also contained black mat like structures placed at the bottom to help maintain sufficient soil levels and loss of media within the pots.

Decisions of adding Gypsum to allow dispersion of soil particulates fixed the cementation of the soil pores and allowed for proper irrigation throughout the experiment. In reference to the addition of Gypsum the suppression of PRR is a possibility by slowing the growth characteristics of *the Phytophthora fragariae* or *Phytophthora cinnamonii* according to studies conducted by the university of California integrated pest management (Marais, 2008). The studies showed that with the addition of $CaSO_4$ near the crowns of strawberries and underneath the canopies of avocados, along with organic mulches suppress PRR. Mulches will help stimulate the development of antagonistic microorganisms towards the PRR disease and reduce the adverse effects of saline soil and water (Menge, 2008). In Figure 6 the comparison of Ca^{2+} and $SO4^{2-}$ ions within the soil showed higher values and ratio's in the Redman soils that were provided the gypsum in relations to the Vasquez soils that didn't receive any Gypsum within the monitored study.

Element Concentration Comparison

Control of *Zoopsoric* pathogens has long been associated with various forms and uses of copper (Kennedy and Erwin, 1961, Smith, 1979 and Slade and Pegg 1993). Production of potted plants in systems with recirculation of nutrient solutions is known to provide conditions conducive to dispersal of *Zoosporic* and *Oosporic* pathogens like *Phytophthora spp*. And Pithium spp. (Toppe and Thinggaard, 1998). In past studies of Prevention of PRR on *Gerbera jamesonii* production continual ratios among treatments provided a P to Zn ppm ratio of 64:11 and a P to Cu ppm ratio of 63:1. The results of their experiment suggested that increased copper ion concentration in nutrient solutions could be a component of disease management in greenhouse grown Gerbera's via Ebb and Flow systems®, and is similar to our study conducted

on PRR in greenhouse grown FHS-1 (Toppe and Thinggaard, 1998). Our results demonstrated that the suppressive effect of Fosphite against PRR in the FHS-1 was not evident in the study. The UTC within the study provided zero percent infected specimens and provided no comparison explanation of treated to non-treated specimens. The P: Cu ratios conducted within this particular study had half the values as provided previously by Toppe and Thiagaard in 1998 for greenhouse grown gerbera's. Only future studies will provide data will a more sound comparison of restricted PRR.

V. Dahliae Vascular Disease

In respect to VVD a visual symptom of the disease were present and developed faster in Plant # 95 and was confirmed dead before any fruit production analysis was completed. In the study other plants were confirmed with VDD and the disease was plated on water agar with positive confirmation of the specified studied disease. Due to internal greenhouse temperatures the plants confirmed with VDD showed intense stress stages, that had drooping of the leaves during peak heat periods in the middle of the day and confirmed erect natural plant physical properties during evening and morning time stages of growth. Relative humidity has provided reason to believe that greenhouse temperatures provided unsatisfactory growth of VDD by slowing down the diseases developmental stages within the Xylem of the plant. With the temperatures continually fluctuating within the glass structure and consistency in irrigation, the plants need for water were on different cycles in human staged environment and instead of the natural cycle of weather outside in a field cropping system. Cool nights can extremely discourage the growth of a plant's immunity to VVD causing complete vascular destruction in a matter of hours.

In the south eastern bloc, rows 1 – 17 of the VDD bench (Fig 2) the plants in early pre flowering stages had numerous amounts of flushes of green growth and had increased sizes of canopy and crowns than any other specimens occupying the bench. Reasoning for this is still under investigation for answers concerning differences in growth patterns and could be related to better internal conditions in specified location. Within this particular bloc there was no significant growth difference between the *Bacillus* treated FHS-1 subject in comparison to other treated species in the study. With the test plants that did show signs to VVD the wilty under watered appearance of the plant followed similar to the support of classification of *V. dahliae* as a "turgor reducer" (Bowden, 1990) associated with the decline in leaf water potential, reduced stomatal conductance leading to a slower rate of carbon assimilation in the symptomless leaves of infected plants (Bowden and Rouse, 1991).

FHS-1 Fruit Analysis

During the experiment strawberry production increased through the month of May and started decreasing until about mid July. Throughout the experiment large quantities of strawberries showed imperfect shape and structure due to unsatisfactory in complete pollination by greenhouse bees. The greenhouse used for the experiment showed inconsistency in building materials with large holes and missing glass sheets and when the side walls would raise for air circulation, no nets were placed to ensure proper safe keeping of bees within the structure itself. In future studies mosquito netting will be placed around each bench ensuring satisfactory pollination of fruit and ensuring each disease studied will have its own colony throughout FHS-1 flowering. The bacillus treatment provided had no significant visual impact on strawberry growth and maturity in fruit production and this was conceived during the experiment, On the

other hand the treatment provided healthy flushes of roots stimulated by the bacillus and provided stability for healthy flushes of green foliage growth. Roots among the samples tested all proved to have healthy root structures and showed no difference in size and shape with respect to the use of the Bacillus treatment, with the exception of test subject #95 that showed intense rotting and necrosis of adventitious and larger tap roots as VVD progressed throughout the vascular system of the plant.

Conclusion

In correspondence to concerning issues of *Methyl Bromide* and its effects to soil structure, Macro and Micro soil fauna and the environment, overall objective was the evaluation of chemigated solutions Fosphite and Bacillus and their surfacing evidence of suppression or subsidence of *Phytophthora fragariae,* a water borne fungal disease and *Verticillium dahliae*, a vascular wilt disease. The FHS-1 hybrid used in the experiment was a patented selected variety from Drischolls and was planted and tested on two different soils, The Redman Ranch soil and The Vasquez Ranch Soil derived from Watsonville, CA. Throughout the study The UTC, and The strawberries tested with Fosphite had no evidence of any disease symptoms present. Understanding the issue with adding gypsum to the soils to help aid in water penetration solved the irrigation problem and could have been the catalyst in suppressing the PRR disease as correlated in previous studies found on riddance *of Phytophthora Cinamoni* in Avocado Orchards. Other external issues could be changed In future studies as conducting the experiment outdoors in a true agricultural environment and with the use of recycled irrigation water might help the Zoospores induce PRR by water movement and penetration through adventitious roots.

The plants tested with the Bacillus root inoculants showed more visual symptoms of *Verticillium dahliae* within the experiment. visual symptom of the disease were present and developed faster in Plant # 95 and was confirmed dead before any fruit production analysis was completed. Relative humidity has provided reason to believe that greenhouse temperatures provided unsatisfactory growth of VDD by slowing down the diseases developmental stages within the Xylem of the plant. The plants showed stress and wilt like symptoms, but as internal greenhouse temperatures shifted throughout day and night the plant showed improved vital signs in growth. In future studies plant leaf analysis in comparison of P and Zn species will be conducted to monitor a closer balance among the treatments used. Phosphoric Acid solutions will be provided to study at specific concentrations (ppm) to show trends and relationships among plant immunity and overall fertility among *Fragariae* species.

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