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Capítulo 1

Efecto de las prácticas agronómicas sobre las bacterias deletéreas rizosféricas y endofítas

Effect of agronomic practices on deleterious rhizosphere and endophytic bacteria

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Introduction

Agronomic practices are well known to affect soil microorganisms (Lupwayi et al. 1998). The effects of tillage (Ceja-Navaro et al. 2010), cover crops (Carrera et al. 2007), crop rotation (Buyer et al. 2010), and applications of pesticides (Allison et al. 2007), fertilizers (Jangid et al. 2008), and soil disinfectants (Roux-Michollet et al. 2008) on soil microorganisms have been documented on many crops. For example, positive effects have been shown in agricultural systems with reduced tillage, cover crops or crop rotation, indicating that these practices increased soil microbial diversity and activity (Ceja-Navarro et al. 2010; Yin et al. 2010; Lupwayi et al. 1998). Likewise, green manures and crop rotations can enhance soil bacterial population density and reduce plant disease incidence through stimulation of antagonists (Wiggins, Kinkel, 2005). Soil amended with poultry litter has higher bacterial diversity than inorganically fertilized soils (Carrera et al. 2007). The effect of some agronomic practices on soil microorganisms can also have negative consequences for plant growth. Deleterious rhizobacteria (DRB), for instance, have been linked with reduced plant growth in systems with continuous pesticide use and for monoculture crops (Schippers et al. 1987; Stroo et al. 1988). Rhizosphere and root-colonizing microorganisms represent a highly diverse microbial assemblage that metabolizes the nutrients exuded by roots and interacts closely with plants (Kluepfel, 1993). Only a minority of rhizosphere microbes have been examined thoroughly for their relative importance in mediating beneficial plant-microbial interactions (Compant et al. 2010). Several rhizobacterial isolates are used as biological control agents or plant growth promoters (Raaijmakers et al. 2009). However, the plant-rhizobacteria interaction does not always result in enhanced plant growth or health, as some rhizobacteria cause deleterious effects on plant growth (Kremer, 2007).

Given the importance of soil microorganisms for soil health, quality, and nutrient cycling, it is important to determine the effect of agronomic practices on soil microbial ecology. This manuscript reviews literature and presents new data on the response of the rhizosphere and endophytic bacteria to two common agronomic practices used to manage pathogens: the application of systemic fungicides and soil steaming.

Rhizosphere bacteria: deleterious and beneficial

The concept of deleterious rhizobacteria (DRB) initially emerged as a way to explain possible mechanisms of growth promotion by plant growth promoting rhizobacteria (PGPR). The original concept was that plant growth is promoted by PGPR and reduced by DRB, and it is the balance between the two that affects the overall plant growth. PGPR and DRB are both described as non-infective rhizobacteria, because they do not parasitize plants nor colonize vascular tissues at high populations, but are aggressive root colonizers able to antagonize other rhizosphere microorganisms (Kremer, 2007).

DRB have been isolated from a variety of crops such chrysanthemum (Burkett-Cadena et al. unpublished data), potatoes (Sturz et al. 2000), carrots (Surette et al. 2003), citrus (Gardner et al. 1985), peas (Berggren et al. 2001), and peach (Benizri et al. 2005). These crops exhibited detrimental responses associated with the presence of specific bacterial strains, while no other explanation such as disease symptoms, presence of pathogens, or nutritional deficiency could be identified. Several bacterial groups have the potential to induce deleterious plant effects, and among soil bacteria the fluorescent pseudomonads are the most commonly reported DRB in the literature (Kremer, 2007).

The positive effects of PGPR on plants occur through multiple mechanisms (Bottini et al. 2004; Compant et al. 2010; Patten, Glick, 2002; Rodríguez, Fraga, 1999). Similarly, multiple mechanisms are involved in the inhibition of plant growth by DRB. Production of cyanide, plant growth regulators (auxins), antibiotics, and/or phytotoxic metabolites by DRB can induce detrimental effects in plants (Bakker, Schippers, 1987; Boel et al. 1993; Kremer, Souissi, 2001). For example, indole acetic acid (IAA) is a metabolite involved in mediating the deleterious effect(s) of DRB. IAA is a phytohormone that can be synthesized by different bacterial groups, including plant pathogenic and beneficial bacteria (Omer et al. 2004). The effect of IAA on root growth is highly dependent on the concentration to which roots are exposed. Promotion of root growth occurred at low IAA levels (i.e., 5 µg/ml), whereas inhibition of root growth occurred when IAA levels were high (i.e., 72 µg/ml) (Barazani, Friedman, 1999). In another study, inhibition of sugar beet root growth was correlated with IAA concentrations produced by *P. fluorescens* and *P. putida* rhizobacteria (Loper and Schroth, 1986). Similarly, studies by Sarwar and Kremer (1995) and Xie et al. (1996) found a correlation between concentration of IAA produced by rhizobacteria and root growth in different plant models, with high levels resulting in deleterious effects on plant growth.

Soil steaming and its effects on soil and rhizosphere bacteria

Soil steaming is a disinfestation method to reduce soil-borne pathogens and weeds in open fields and greenhouses. Steaming was developed in Germany in the early 1800s and in 1890s it was first commercially used in the United States. Baker and Olsen (1960) conducted some of the first research on pasteurization via soil steaming for the elimination of soil-borne plant pathogens (Baker, 1962). Their studies included using steamed soil in combination with a «retarding organism» (antagonist) to prevent damping off of bedding plants caused by *Rhizoctonia solani*.

The temporal dynamics of soil microbial communities following steam disinfestation have been studied. Roux-Michollet et al. (2008) showed that over a 60-day period steaming caused significant decreases in the activity and culturable counts of the bulk soil microbial community, and induced changes in its composition with nitrifying bacteria being the most negatively affected (Roux-Michollet et al. 2008). A study of DRB in a commercial flower monoculture in Colombia found that the primary bacterial groups associated with a reduction in chrysanthemum growth in a steamed soil were the fluorescent pseudomonads. Steaming was used to disinfest the soil prior to cultivation and, therefore, to manage soil-borne pathogen populations. In addition, tests in a greenhouse pot assay revealed that consecutive steaming reduces the microbial populations post-steaming, and that after planting the populations of fluorescent pseudomonads increase rapidly in the chrysanthemum rhizosphere (Burkett-Cadena et al. unpublished data).

Effects of fungicide application on rhizosphere and endophytic bacteria- A field study case

Leatherleaf fern (*Rumohra adiantiformis*) is an important commodity used in flower arrangements because fronds are highly symmetrical and have a long shelf-life after harvest. Distortions in frond shapes were reported in Florida during the 1980s, coinciding with widespread use of the systemic fungicide Benlate DF (Mills et al. 1996). Benlate was also reported to increase populations of phytotoxic fluorescent pseudomonads in the leatherleaf fern rhizosphere (Kremer et al. 1996). The name «fern distortion syndrome (FDS)» was proposed in 2010 for the problem of distorted fronds (Kloepper et al. 2010).

FDS was found to be present in farms where leatherleaf fern was successively cultivated in the same field, which is in agreement with Schippers et al. (1987) who pointed out that allelopathic bacteria may arise in areas where one crop is grown successively. In addition, the incidence and severity of FDS in Costa Rica were found to relate to endophytic populations of fluorescent pseudomonads inside fern rhizomes (Kloepper et al. 2010). These results and observations made in Costa Rica suggested «a model of latent infections of allelopathic or deleterious bacteria that induce damage when a threshold population is reached» (Kloepper et al. 2010). Those deleterious or allelopathic bacteria

could reduce or alter normal plant growth via production of allelochemicals such as IAA, which was reported by Barazani and Friedman, (1999) to be related to allelopathic effects of some rhizosphere bacteria. The strong association between FDS and fluorescent pseudomonads observed in Costa Rican ferns suggested that future work should characterize these fluorescent pseudomonads for production of IAA and other allelopathic effects in a plant bioassay.

The overall aim of the current study was to examine the relationship of Benlate to FDS and to its effect on pseudomonad populations. The objectives of the study were 1) to determine if leatherleaf fern grown in Florida with a history of Benlate use had greater populations of fluorescent pseudomonads than ferns grown without Benlate use; 2) if Benlate treatment of rhizomes affects fluorescent pseudomonad populations; and 3) if the fluorescent pseudomonads associated with FDS produce IAA and exhibit allelopathic effects in a cucumber seed bioassay.

Ratings of FDS, and sampling for fluorescent pseudomonads

Two ferneries were selected for the study, KHD with a history of Benlate use and Floricrops without Benlate use (Mills et al. 1996). Both ferneries were near Deland, Florida and were less than 500 m apart. In October of 2005 each fernery was rated to determine the incidence and severity of FDS using the 4-point rating system developed in Costa Rica (Kloepper et al. 2010). The results of FDS ratings (Table 1) indicated that all four quadrants at Floricrops had significantly lower fern deformation incidence and severity than all four blocks of KHD. The average incidence at Floricrops was 11%, while it was 90.25% at KHD.

Table 1.
Incidence and severity of fern deformation at KHD and Floricrops.

Fernery	History of Benlate use	Quadrant Location	Incidence	Mean Severity*
Floricrops	No	Southeast	21%	0.21^{c}
		Northeast	7%	0.07^{c}
		Northwest	9%	0.10^{c}
		Southwest	7%	0.07^{c}
KHD	Yes	Northwest	86%	1.34^{b}
		Southwest	92%	1.58^{a}
		Southeast	90%	1.79^{a}
		Northeast	93%	1.40^{b}
			$LSD_{0.01}$	0.22

*Means followed by different letters are significantly different at P = 0.01.

Bacterial populations were determined in the rhizosphere, rhizomes, and petioles from asymptomatic plants at Floricrops and from asymptomatic and damaged plants at KHD. Results from these isolations in 2005 during the months of September (Table 2) and October (Table 3) generally indicated higher populations of total bacteria and fluorescent pseudomonads (FPs) from ferns with damage in the Benlate-treated fernery (KHD) than from healthy ferns in the control fernery (Floricrops). Total culturable bacteria counts (on 10% TSA) from the rhizosphere of plants from Floricrops were significantly lower than those from plants from KHD. Similarly, total bacteria within the rhizomes or petioles of plants at Floricrops were significantly lower than those from at least one of the treatments from KHD. Populations of fluorescent pseudomonads (on King's B) followed the same trend as the total bacterial populations. At the 95% probability level, the population in the rhizosphere was highest in deformed plants from KHD, next highest in normal-appearing plants at KHD, and lowest with plants from Floricrops. Within rhizomes and petioles the population of fluorescent pseudomonads was significantly lower (99% probability) in plants from Floricrops than from KHD.

Table 2.
Bacterial populations from Floricrops and KHD ferneries, September, 2005*.

	Rhizosphere		Intra-rhizome		Intra-petiole	
Sample no.	**Total bacteria (log cfu/g)**	**Fl. Pseudo. (log cfu/g)**	**Total bacteria (log cfu/g)**	**Fl. Pseudo. (log cfu/g)**	**Total bacteria (log cfu/g)**	**Fl. Pseudo. (log cfu/g)**
1. Floricrops asymptomatic	6.16 B	5.25 B,c	4.15 b	0.14 B	4.58 b	0.00 b
2. KHD asymptomatic	6.66 A	5.75 A,b	4.50 a	2.25 A	5.45 a	0.91 a
3. KHD deformed	6.93 A	6.03 A,a	4.27 ab	2.02 A	4.43 b	1.32 a
$LSD_{0.01}$	0.45	0.32	0.31	0.37	1.06	0.93
$LSD_{0.05}$	0.33	0.24	0.23	0.27	0.79	0.69

* Means followed by different letters are significantly different. Capital letters indicate significance at $P \leq 0.01$; lower case letters indicate significance at $P \leq 0.05$.

Table 3.
Bacterial populations from Floricrops and KHD ferneries, October, 2005*.

	Rhizosphere		Surface of rhizomes	
Sample no.	Total bacteria (log cfu/g)	Fl. Pseudo. (log cfu/g)	Total bacteria (log cfu/g)	Fl. Pseudo. (log cfu/g)
1. Floricrops asymptomatic	6.31 B*	5.12 B	2.81 B	1.35 B
2. KHD asymptomatic	6.64 A	6.08 A	3.33 A	2.33 A
3. KHD deformed	6.72 A	6.19 A	3.36 A	2.53 A
$LSD_{0.01}$	0.45	0.32	0.31	0.37

* Means followed by different letters are significantly different at $P \leq 0.01$.

In addition to culturable bacterial counts, culture-independent PCR-DGGE analysis was conducted using DNA extracted from rhizosphere samples and from the surface of the rhizomes. Total community DNA was extracted using the Ultraclean™ Soil DNA isolation kit (MoBio Laboratories,USA). Bacterial 16S rRNA gene amplicons were amplified using the primers 968-GC and 1401 R (Smalla et al. 2001). For amplification of *Pseudomonas* spp. the primer system 311Ps and R1459Ps described by Milling et al. (2004) was used. For amplification of *Bacillus* spp. and related taxa the primer BacF described by Garbeva et al. (2003) was used in combination with the universal bacterial primer 1401R. In all cases the recommended thermalcycling conditions for the respective primer sets was used, and equal amounts of genomic DNA template (50 ng) were added to each reaction. The presence of amplicons was verified by agarose gel electrophoresis prior to resolution on a DGGE gel. A 45% to 60% denaturing gradient within an 8% polyacrylamide gel was prepared. The DGGE was performed in 1X TAE at 60 °C and a constant voltage of 100 V for 16 h. Individual DGGE bands were quantified and compared by densitometry using SigmaStat program. The DGGE analyses generally supported the enumeration results, with no significant changes observed using bacteria domain-level or *Bacillus* genus-level primer sets (data not shown). In contrast, *Pseudomonas*-specific amplicons from KHD were increased 132% compared to samples from Floricrops (Figure 1), but there were no differences observed in *Pseudomonas* taxa composition based on DGGE (data not shown). This supports the conclusion that overall numbers of FPs were greater at KHD, but the relative abundance of different *Pseudomonas* spp. was not significantly altered.

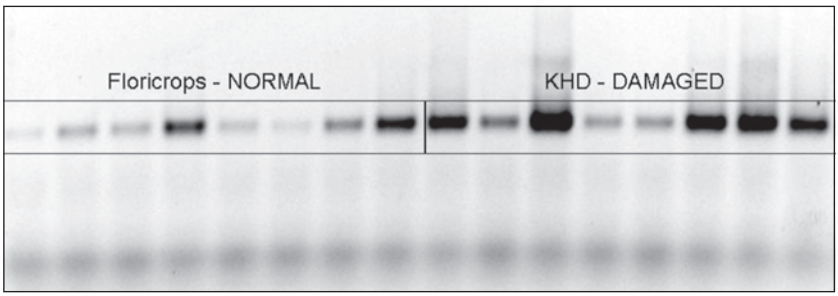

Figure 1: Intensity of *Pseudomonas* 16S rRNA amplicons in 8 replicate samples of rhizospheres from healthy plants at Floricrops (left) and from damaged plants at KHD (right).

Rhizome diameter, root weight, and microscopy of rhizomes checking for natural openings

It was previously reported that ferns with symptoms of FDS in Costa Rica had smaller rhizome diameters and reduced root weight (Kloepper et al. 2010). Results from sampling in Florida revealed the same trend (Table 4). The largest rhizome

diameters were observed in asymptomatic plants from Floricrops, with a mean rhizome diameter significantly greater than that of rhizomes from asymptomatic or deformed plants from KHD. Deformed plants at KHD had a significantly smaller rhizome diameter than asymptomatic plants at KHD (Figure 2). Asymptomatic plants at Floricrops had a much greater root weight compared to plants at KHD, especially deformed KHD plants that had rhizomes with irregularly shaped growing tips compared to rhizomes from asymptomatic plants (Figure 2).

Table 4.
Rhizome and Root effects in Florida Ferns sampled October, 2005.

Treatment	Rhizome diameter (mm)[a]	Root weight from a5-cm section of rhizome
1. Floricrops asymptomatic	10.8 A*	10.39 A
2. KHD asymptomatic	9.5 B	4.54 B
3. KHD deformed	8.55 C	2.89 BC
$LSD_{0.01}$	0.75	1.81

*Means followed by different letters are significantly different at $P \leq 0.01$.
[a]Diameter was measured 3 cm from the growing tip of 20 samples from each treatment.

Figure 2: Rhizome diameter of asymptomatic plants from Floricrops (left) and deformed plants at KHD (right). Note the irregularly-shaped growing tips of rhizomes from KHD.

Microscopic analysis of rhizomes revealed the presence of natural openings in all rhizome tips. The growing tip of the rhizome was not observed to have the «scales» that cover mature parts of the rhizome (Figure 3). A series of photographs with increasing magnification of the rhizome tip is shown in Figure 3. Here, one can clearly see the presence of uncovered regions of the rhizome that would allow bacteria in the root zone to enter and establish endophytically during normal rhizome development (Figure 3).

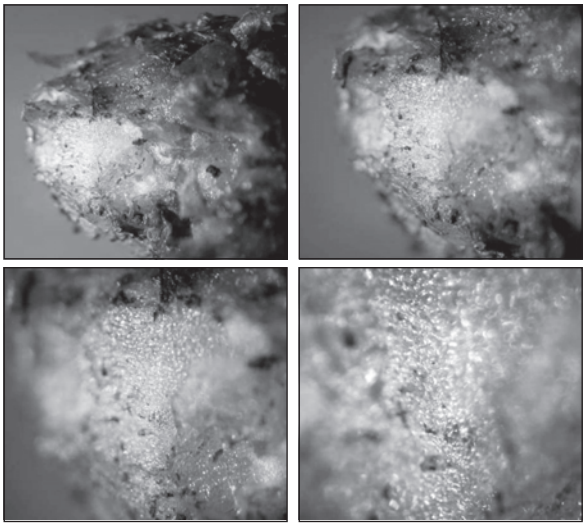

Figure 3: Photomicrograph of a growing tip of a typical rhizome of an asymptomatic fern plant. Lowest magnification (10x) is in the upper left panel and the highest magnification (63x) is in the lower right panel. Note the presence of unprotected cells (not covered by scales) at the rhizome tip that allows bacterial access to the inside of the growing rhizome.

Effects of fungicide application on rhizosphere pseudomonads: A greenhouse study

Greenhouse experiments were designed to determine the effect of Benlate on populations of fluorescent pseudomonads. Rhizomes, collected from asymptomatic plants at Floricrops fernery, were dipped into water (control), Benlate DF, or Benlate WP at the label rate of 1 lb/100 gal (1.2 g/L) and were then planted in field soil in a greenhouse. Ferns were grown at 23°C and artificially shaded with polypropylene shade fabric. Ferns were watered daily and fertilized with 20-10-20 twice per week. Eight replicate pots (plants) were used for each treatment. Genomic DNA extraction and PCR-DGGE analyses were conducted from rhizosphere soil at 6 weeks after treatment, as described above. Based on the previous results with PCR-DGGE from field samples, the analysis was performed using primers for total bacteria, *Bacillus* spp., and *Pseudomonas* spp.

PCR-DGGE analyses indicated that Benlate altered the composition of the rhizosphere community, including the pseudomonads. With the bacterial domain-level primer set, 5 ribotypes (bands) were significantly more abundant (greater gel densitometry reading) with Benlate treatment (Figure 4). An analysis of the *Bacillus* community indicated that one ribotype significantly increased with Benlate treatment (Figure 5). Using a *Pseudomonas*-specific primer set, three ribotypes exhibited a significant increase with Benlate treatment (Figure 6).

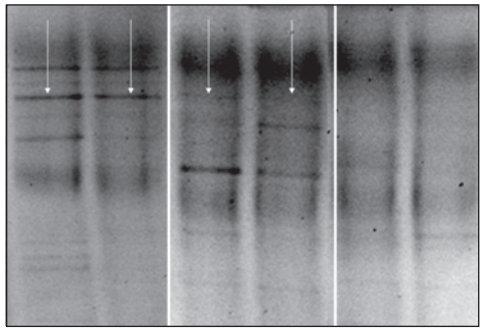

Figure 4: PCR-DGGE analysis of the rhizosphere total bacteria community 6 weeks after dipping rhizomes in Benlate DF (left), Benlate WP (center), or water (right). Arrows indicate bands present in the Benlate treatment but not in control samples.

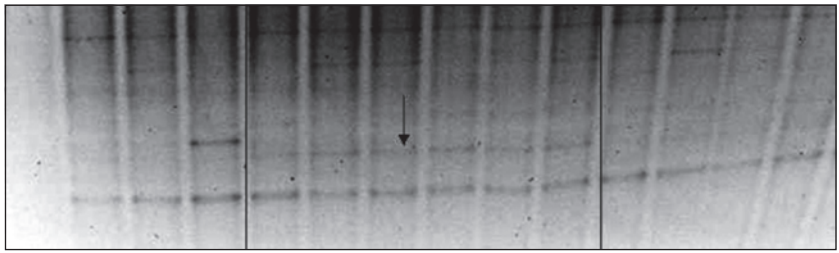

Figure 5: CR-DGGE analysis of *Bacillus* spp. from rhizosphere samples 6 weeks after dipping rhizomes in Benlate DF (left), Benlate WP (center), or water (right). The arrow indicates a band not present (or abundant) in the control samples.

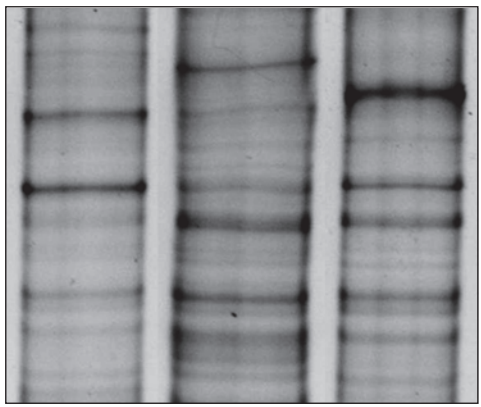

Figure 6: PCR-DGGE analysis of *Pseudomonas* spp. from rhizosphere samples 6 weeks after dipping rhizomes in Benlate DF (center), Benlate WP (right), or water (left). Note the increased number of bands with both formulations of Benlate.

Production of IAA by fluorescent pseudomonads isolated from fern's rhizosphere and from Benlate-treated ferns

A collection of 100 strains of fluorescent pseudomonads isolated in September of 2005 from the rhizosphere of asymptomatic ferns at Floricrops and 100 strains from the rhizosphere of deformed ferns at KHD were examined for IAA production. Development of a red color within 5 min after adding Reagent A was scored as ++ (and equated to an IAA standard of 20-40 µg/ml), while development of a pink color by 30 min was scored as + (and equated to an IAA standard of 10-20 µg/ml).

Two experiments were conducted to determine if Benlate treatment affects IAA production by fluorescent pseudomonads colonizing leatherleaf fern. Rhizomes containing fronds were collected from asymptomatic plants at Floricrops fernery and were then planted in field soil in the greenhouse. In the first experiment, Benlate was applied as a foliar spray while the second experiment used rhizome dips. Treatments in both experiments included Benlate DF and Benlate WP at the label rate of 1 lb/100 gal (1.2 g/L) and a water control. Eight replicate pots (plants) were used for each treatment. In the foliar spray experiment, at four weeks after treatment endophytic fluorescent pseudomonads were isolated and 50 strains per treatment were tested for IAA. In the rhizome dip experiment, rhizosphere pseudomonads were isolated at four weeks after treatment and 50 strains per treatment were examined for IAA production.

A marked difference in the frequency of IAA production by fluorescent pseudomonad strains from the rhizosphere of deformed plants at KHD and asymptomatic plants at Floricrops was observed (Table 6). There were 6-fold more isolates from deformed plants at KHD that were capable of producing IAA compared to the isolates from asymptomatic plants. The IAA reaction developed rapidly for strains isolated from KHD, with 30% having a red color within 5 min. Based on IAA standards, this rapid color change indicates a high IAA concentration of >20 µg/ml. Hence, while 30% of the KHD strains were high producers of IAA, none of the strains from Floricrops were observed to have high IAA producers.

Table 6.
IAA production frequency of *Pseudomonas* isolates from rhizospheres of deformed ferns at KHD and asymptomatic ferns at Floricrops*.

Fernery and appearance of ferns	% of strains ++ for IAA[a]	% of strains + for IAA[b]	% of strains + or ++ for IAA	% of strains negative for IAA
Floricrops, asymptomatic	0	8	8	92
KHD, deformed	30	19	49	51

* 100 isolates from each fernery were tested.
[a] Development of red or pink color within 5 min on nitrocellulose membrane (Bric et al., 1991).
[b] Development of red or pink color by 30 min on nitrocellulose membrane.

These results indicated a functional change within the population of rhizosphere fluorescent pseudomonads that is related to the use of Benlate, and this change persists long after Benlate applications have ceased.

Results of Benlate applications to fern on the frequency of IAA production by fluorescent pseudomonads indicated a profound shift in the percentage of endophytic bacteria inside the rhizomes capable of producing IAA (Table 7). The frequency of strains that strongly produced IAA (red color in 5 min) after spray with Benlate DF was 70% compared to 6% from the control. Benlate WP did not change the frequency of IAA production, relative to the control. An increase in the frequency of IAA production by rhizosphere fluorescent pseudomonads was observed in the rhizome-dip test (Table 8). The percentage of strains producing high levels of IAA increased 4- to 6-fold, with both formulations of Benlate stimulating this trait.

Table 7.
Effect of foliar sprays of Benlate on the frequency of IAA production by endophytic fluorescent pseudomonads within rhizomes.

Treatment (Foliar spray)	% of strains ++ for IAA	% of strains + for IAA	% of strains + or ++ for IAA	% of strains negative for IAA
Benlate DF	70	10	80	20
Benlate WP	6	18	24	76
Water control	6	19	25	75

* 50 isolates from each fernery were tested.
[a] Development of red or pink color within 5 min on nitrocellulose membrane (Bric et al. 1991).
[b] Development of red or pink color by 30 min on nitrocellulose membrane.

Table 8.
Effect of rhizome dips in Benlate on the frequency of IAA production by fluorescent pseudomonads in the rhizosphere.

Treatment (Rhizome dip)	% of strains ++ for IAA	% of strains + for IAA	% of strains + or ++ for IAA	% of strains negative for IAA
Benlate DF	43	22	65	35
Benlate WP	32	26	58	42
Water control	7	17	24	76

* 50 isolates from each fernery were tested.
[a] Development of red or pink color within 5 min on nitrocellulose membrane (Bric et al. 1991).
[b] Development of red or pink color by 30 min on nitrocellulose membrane.

Allelopathic effects in cucumber seed bioassays

Two cucumber seed bioassays were developed to evaluate allelopathic or deleterious potential of fluorescent pseudomonads isolated from fern rhizospheres. The assays were modifications of a lettuce seed bioassay that was used in previous studies of deleterious rhizobacteria (Alström et al. 1989; Barazani, Friedman 1999; Kremer, 2007; Kremer et al. 1996).

The first bioassay was a whole-cell assay in which bacterial isolates were plated onto 50% King's B agar and incubated at 28ºC for 24 hr. One small loopful of each strain was agitated in 9 ml sterile water, and two 10-fold serial dilutions were prepared. Three replicate cucumber seeds were placed onto a water agar plate, and 50 µl of a 10^{-2} dilution for each tested strain was applied to each cucumber seed, resulting in an average inoculum density of log 4.3 cfu/seed. We estimated that since populations enumerated from damaged ferns in the field were observed as high as log 5.5 cfu/g in the rhizosphere, the inoculum density used in the cucumber assay would be conservative. After inoculation, seeds were placed at room temperature in the light for 4 days. Plates with bacterial inoculations were compared visually to control plates and rated as + (better root growth than the control), - (less root growth than the control), or 0 (no change from the control). In addition, the length of each radical was measured, and the mean length for each bacterial treatment was calculated and compared to the mean length of the controls.

The second bioassay was a cell-free metabolite assay in which bacterial isolates were grown 48 hr in 9 ml tryptic soy broth. The resulting suspension was filter-sterilized through a 0.45 µm filter, and 50 µl of the sterile filtrate containing bacterial metabolites was inoculated onto each of 3 cucumber seeds per plate. Controls were inoculated with 50 µl of sterile tryptic soy broth. After inoculation, seeds were grown and roots were measured as in the first bioassay.

A collection of 100 fluorescent pseudomonad strains was isolated in September of 2005 from the rhizosphere of asymptomatic ferns at Floricrops and an additional 100 strains were isolated from the rhizosphere of deformed ferns at KHD. All isolates were tested in both bioassays.

Visual differences in the root systems of inoculated and control cucumber were apparent with some bacteria and some metabolites in all tests. A higher percentage of fluorescent pseudomonads from deformed ferns at KHD induced damage in both bioassays than did strains from asymptomatic ferns at Floricrops (Table 9). Hence, more pseudomonad isolates from deformed ferns at KHD with a history of Benlate use demonstrated allelopathic potential than did pseudomonads from ferns at Floricrops without a history of Benlate use. Some representative examples of reduced cucumber root growth in response to pseudomonad metabolites is presented in Figure 7.

Table 9.
Phytotoxic potential of fluorescent pseudomonads from rhizospheres of ferns at KHD and Floricrops.*

	% of strains exhibiting indicated effect		
Effect in cucumber bioassay	**Fernery and appearance of ferns**	**Whole-cell bioassay**	**Cell-free metabolite bioassay**
Reduced overall growth of cucumber roots compared to control[a]	KHD, deformed	44	22
	Floricrops, asymptomatic	7	7
Increased overall growth of cucumber roots compared to control[a]	KHD, deformed	0	0
	Floricrops, asymptomatic	5	16
Decreased average root length by over 50% compared to control	KHD, deformed	13	3
	Floricrops, asymptomatic	0	0

* A collection of 100 strains from normal-appearing ferns at Floricrops and 100 strains from damaged plants at KHD was tested in two separate bioassays on cucumber.

[a] Based on visual comparison of cucumber seeds inoculated with cells or supernatants compared to controls treated with water.

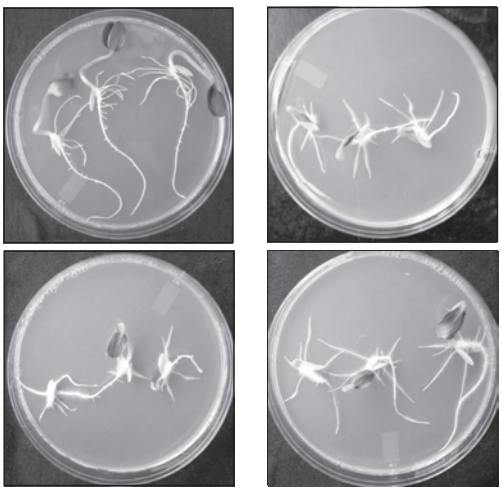

Figure 7: Representative negative visual ratings (reduced growth of cucumber root systems) with inoculation with fluorescent pseudomonads in whole cell bioassay. Upper left = control; other three images are different strains from the rhizosphere of deformed ferns at KHD nursery with a history of Benlate use.

In this study we determined the effect of Benlate® application on populations of fluorescent pseudomonads in ferns. Previously a strong correlation was observed between fern distortion and fungicide application. Areas with a history of routine Benlate application had a higher incidence and severity of frond growth distortion. The results of this study indicated that bacterial population densities in different fern microhabitats were increased due to Benlate application. Greater total bacterial populations and especially fluorescent pseudomonads associated with Leatherleaf fern increased following Benlate application. Increases in pseudomonads were observed by culture-based and DNA-based methods, and were still detected in soils years after the last Benlate application. The finding that higher endophytic populations of fluorescent pseudomonads are associated with symptomatic plants in the Benlate-treated fernery suggests that fluorescent pseudomonads contribute to fern deformation following a shift in the endophytic micro flora caused by the systemic fungicide. Additionally, Benlate application is not only related to changes in bacterial populations but also with functional traits. Isolation and characterization of fluorescent pseudomonad strains from symptomatic ferns indicated that Benlate application resulted in a higher frequency of IAA producing strains and deleterious effects in cucumber seedlings. These findings are relevant because they show that agronomic practices can adversely change the dynamics of the bacterial populations inside and outside the plant host. Those changes are associated with alteration in the production of bacterial metabolites and ultimately those metabolites can be deleterious for the plant.

Conclusions and remarks

In this manuscript the effects of agronomic practices on plant-bacterial interactions were investigated. We show that fern growth distortion is correlated with changes in bacterial populations, specifically with increased populations of fluorescent pseudomonads. The alteration of the bacterial community structure in relation to agronomic practices can be of particular interest in perennial crops like fern and vegetatively propagated crops like chrysanthemum. To maintain the commercial value and productivity ferns and chrysanthemums are cultivated in high density and continuously in the same field. Thus residues from the previous crops provide resources for the survival of DRB, which will persist as a consequence of little modifications in their habitat. The application of products such as Benlate, which can cause changes in bacterial populations and their associated functional traits, can have profound effects on plant health, through a cascade of ecological interactions in the rhizosphere. Changes in the microbial community lead to the over-production of bacterial metabolites, which are ultimately deleterious for the plant.

It is important to consider that common mechanisms can be shared by beneficial and deleterious microorganisms, for example in the plant-microbe

interactions that exist in rhizobia-legume and plant-pathogens. In the same way, similar strategies may be used by plant growth promoting rhizobacteria and by allelopathic bacteria. Consequently, agricultural practices may fundamentally alter soil microbial communities, leading to increases in populations of certain functional bacterial groups. Those bacterial groups such as the fluorescent pseudomonads and their metabolites are integral to the processes that result in detrimental effects in cultivated plants. The role of microorganisms in the soil agro ecosystem has been very well documented. Now the tripartite interaction, including the plant host, should be further addressed.

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