FINAL REPORT

TITLE: Evaluation of Biorational Methods for Control of Soilborne Pathogens of Apple (Project #AH-01-71)

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OVERALL OBJECTIVE:
Based on an understanding of apple replant disease etiology, develop practices that control the soilborne pathogens involved in disease development. Examine the use of cultural and biological management methods that enhance the development of plant beneficial soil microbial communities.

SIGNIFICANT FINDINGS 2000-2001:

I. Sequence of application significantly altered the benefits of wheat cultivation in conjunction with *Brassica napus* seed meal amendments to apple growth. The appropriate application sequence was also dependent on seed meal amendment rate.

II. Gala/M.26 established in *B. napus* seed meal amended soils continued to outperform controls in terms of vegetative growth at the Columbia View orchard.

III. Yield data for Gala/M.26 trees established at the Columbia View orchard suggest that, in certain instances, greenhouse experiments will reflect results obtained in the field. Specifically, metalaxyl/flutolanil fungicide applications at planting are effective for the control of replant disease and significantly enhance yields in the absence of a nematode component in the disease complex.

IV. Application of root exudates from certain wheat cultivars to orchard soil altered composition of the fluorescent pseudomonad community in a manner previously associated with development of a disease suppressive soil.

V. Cultivation of soils with wheat after bacterial application enhanced survival of the biocontrol bacterium *Pseudomonas putida* strain 2C8 in the presence of *B. napus* seed meal.

OTHER SIGNIFICANT FINDINGS:

VI. Enhanced growth of apple in response to prior cultivation of replant soils with wheat occurs in a wheat cultivar-specific manner and results from the suppression of fungal root pathogens, especially *Rhizoctonia solani.* Disease suppression functions through the wheat root-induced transformation of soil microbial communities, particularly the selection of antagonistic strains of fluorescent pseudomonads.

VII. *Brassica napus* seed meal amendments provide control of certain soilborne pathogens of apple, including *R. solani* and the lesion nematode, *Pratylenchus penetrans.* Control of these two organisms is achieved irrespective of glucosinolate content of the seed meal. However, *Pythium* spp. root infection increased when low-glucosinolate containing seed meal was utilized.

VIII. At high rates (>1% v/v) *B. napus* seed meal is phytotoxic to apple. Wheat cultivation of seed meal amended soils can eliminate phytotoxicity.

IX. Extended bare fallow periods, up to two years, did not significantly reduce the severity of apple replant disease at the Columbia View orchard.

OBJECTIVES (2000-2001):

1. Explore increased seeding rates, water management practices and spatial dynamics of replant disease in the orchard as a means to exploit the potential of a wheat cover crop to suppress apple replant disease.
2. Continue to monitor growth and yield of trees established in year 2000 field trials that examine the use of cover crops and seed meal amendments for control of replant disease, and establish new trials integrating promising management practices.
3. Evaluate the practice of wheat cultivation after rapeseed meal amendments as a means to re-establish populations of the disease suppressive bacterium *Pseudomonas putida* strain 2C8.
4. Examine the feasibility of wheat root exudate applications, in place of wheat cultivation, as a means to induce a disease suppressive microbial community.

PROCEDURES:

1 & 2.) Field trials were established at the Columbia View (CV) and Wenatchee Valley College (WVC) Auvil orchards in the fall of 2000 and spring of 2001. This included the establishment of Penawawa spring wheat in the orchard aisle, *B. napus* seed meal applications made in October 2000, May 2001 and October 2001, and establishment of solarization treatments alone or in combination with *B. napus* seed meal amendments. These field experiments will determine the efficacy of integrated strategies in the establishment of apple on old orchard sites. These trials will be planted to apple in the spring of 2002.

Vegetative growth data for Gala/M.26 (CV orchard) and Cameo/M.26 (WVC-sunnyslope orchard) were collected from field trials established in spring 2000. These trials are examining the impact of individual cultural practices including seed meal amendment, wheat cover crop and rapeseed cover crop on growth of apple. Yield data were collected from a trial established in 1998 at the CV orchard which is examining the impact of cultural practices and fungicides for the control of soilborne pathogens of apple.

3.) Greenhouse studies examined the impact of application sequence on the interactions between *Brassica napus* (rapeseed) seed meal amendment and wheat cultivation on growth of apple in replant orchard soils. Seed meal was applied to soil and cultivated to wheat for 8 weeks or soils were cultivated to wheat for 8 weeks prior to amending soils with seed meal at varying rates. Soils were planted to Gala seedlings, and plants were grown for 12 weeks in the greenhouse. Root weight, shoot weight and shoot length were determined at harvest. Root systems were sampled to assess the impact of treatments on the pathogen complex. Composition of the fungal community colonizing apple roots was determined by plating root segments on various selective and non-selective growth media. Populations of *Pratylenchus penetrans* were determined using standard laboratory procedures.

The impact of wheat cultivation on the establishment and maintenance of the biocontrol bacterium *Pseudomonas putida* strain 2C8 was examined in greenhouse trials. A rifampicin-resistant mutant of the strain was generated to allow for the study of population dynamics in natural soils. Soils were treated with *B. napus* seed meal at a rate of 2%, bacterial strains were introduced into soil as an atomized mist, and soils were planted to Penawawa wheat. Populations of the introduced bacterium were monitored over a 3-month period.

4.) Root exudates were generated by growing wheat under hydroponic conditions in controlled environment chambers. Exudates were collected, filtered sterilized and applied to orchard soil. The quantitative dynamics of the fluorescent *Pseudomonas* spp. population was monitored and the impact of exudate applications on qualitative attributes of this population were assessed through genetic and chemical analyses of isolates recovered from treated orchard soils.

RESULTS & DISCUSSION:

Enhanced growth of apple in response to a single application of *Brassica napus* seed meal (0.4% v/v) made in the autumn prior to planting was documented over a two year period at the Columbia View and Wenatchee Valley College Auvil orchards. The level of growth improvement was statistically significant relative to the control (Table 1), but was substantially less than that achieved via methyl bromide fumigation. *B. napus* seed meal was shown to effectively control *Rhizoctonia solani* and *Pratylenchus penetrans*, but did not effectively control *Pythium* spp. It is plausible that the application of methods to control *Pythium* spp., such as a Ridomil soil drench at planting, may provide an acceptable level of disease control.

Table 1. Impact of *Brassica napus* seed meal amendments and cover crops on 2-year diameter growth (cm) of Gala/M.26 and Cameo/M.26 at the CV and WVC-sunnyslope orchards, respectively

Treatment	CV orchard	WVC Orchard
Control	6.6	6.8
Methyl bromide fumigation	18.1	10.4
2-year fallow	8.2	-
B. napus seed meal	10.6	8.0
B. napus seed meal+ wheat	10.8	8.2
Wheat	9.8	6.0
B. napus cover crop (1 yr)	6.8	5.7
B. napus cover crop (2 yr)	11.8	-
LSD (P=0.05)	2.27	1.08

Wheat cultivation in concert with *B. napus* seed meal amendment eliminated apple seedling mortality that was observed when high rates of seed meal were applied. The benefit of wheat cultivation in conjunction with *B. napus* seed meal amendments was rate dependent, and also varied with the sequence in which treatments were applied. At lower rates (0.1%), wheat cultivation prior to seed meal amendment enhanced growth of apple in replant soil (Table 2) relative to seed meal amendment alone. However, at the same amendment rate, wheat cultivation of soils after application of *B. napus* seed meal resulted in a depression of apple seedling growth. This likely resulted from diminished nitrogen availability to apple seedlings due to uptake by the preceding wheat crop.

Table 2. Impact of application sequence and amendment rate on growth of apple in GC orchard soil (Manson, WA) after wheat cultivation/*Brassica napus* seedmeal treatments.

Treatment	Root weight (g)	Shoot weight (g)	Plant height (cm)
Control	0.75	1.74	9.9
0.1% seed meal	0.91	1.99	11.6
Wheat+0.1% seed meal	1.12	2.60	14.8
0.1% seed meal+wheat	0.52	1.57	10.2
2% seed meal	0.97	2.18	10.3
Wheat+2% seed meal	0.11	0.46	6.1
2% seed meal+wheat	1.43	4.45	17.6
LSD	0.15	0.74	2.9

At higher amendment rates (2% v/v), wheat cultivation after seed meal amendment dramatically enhanced growth of apple relative to that achieved when seed meal was applied alone. Wheat cultivation reduced phytotoxicity of the seed meal to apple, and eliminated seedling mortality. Unexpectedly, wheat cultivation prior to amendment of soil with *B. napus* seed meal at a rate of 2% dramatically reduced seedling biomass and increased seedling mortality. This suggests a microbial role, either directly or indirectly, in the phytotoxic nature of the seed meal, as wheat cultivation significantly increased biological activity in these soils.

Brassica napus seed meal amendments can have a negative influence on the survival of plant beneficial organisms, including the *Rhizoctonia*-suppressive bacterium *Pseudomonas putida* strain 2C8. Studies were conducted to determine whether the cultivation of wheat in conjunction with bacterial application to soil could promote survival of the bacterium when introduced into seed meal amended soils. Even when introduced at an initial population of 10^7 cfu per gram soil, strain 2C8 was not detected in seed meal amended soil within one week after application (Fig. 1). However, when wheat was cultivated in these same seed meal amended soils, the biocontrol bacterium survived maintained a population of approximately 10^5 cfu per gram soil even after 10 weeks. This suggests that enhanced survival of 2C8 and thus control of *Rhizoctonia solani* in nursery or orchard settings could be achieved through the use of a wheat cover crop in concert with application of this bacterial strain.

The growth benefits achieved through prior cultivation of orchard soils with specific wheat cultivars entails growing a cover crop for one or more years and is dependent upon the production of a wheat root system that effectively penetrates the soil profile to a depth of 30 cm or greater. We are conducting initial studies to determine whether the use of wheat root exudates or identifiable components of these exudates could be used as a soil treatment to achieve the same growth response in apple. Enhanced growth of apple in response to wheat cover crop is attained through the activity of specific microbial populations, including the fluorescent pseudomonad community. Thus, studies examined what impact application of a root exudate solution conferred on composition of the fluorescent pseudomonad population. Root exudates (7%) were applied to soil on 3 occasions at 4 week intervals, and the fluorescent pseudomonad community was characterized. Fluorescent pseudomonad communities recovered from replant soils treated with exudate from apple roots or the wheat cultivar Hill-81 were similar to the untreated control. The fluorescent pseudomonad population from soil treated with exudate from

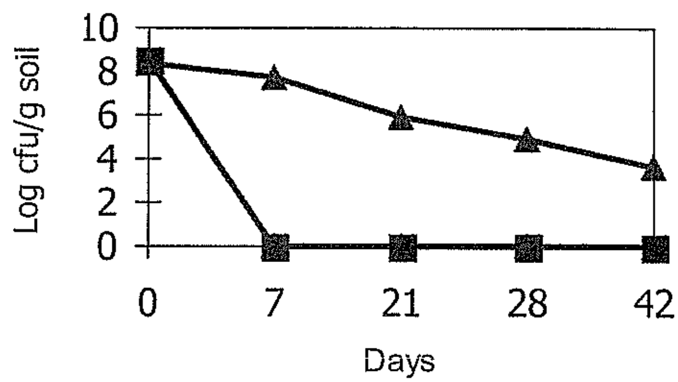

Figure 1. Impact of *Brassica napus* seed meal amendment (2% v/v) on survival of the biocontrol bacterium *Pseudomonas putida* strain 2C8 in WVC orchard soil with ( ) and without ( ) a wheat cover crop.

the wheat cultivar Penawawa was transformed in a manner similar to that obtained through wheat cultivation of these soils, and specifically stimulated populations of *Pseudomonas putida* btp A. We have shown that bacterial isolates of this species possess antagonistic activity towards many fungal pathogens of apple, including *R. solani* AG 5 and *Pythium ultimum*. The subsequent research program should include a determination as to whether the shift in microbial community structure induced simply via the application of root exudates to soil actually provides control of soilborne pathogens (e.g. *Rhizoctonia solani*) of apple.

CONCLUSIONS:

Based on results of this research program, it is apparent that, with the development of appropriate guidelines, integration of control techniques identified in these studies has significant potential as an alternative to pre-plant soil fumigation for control of apple replant disease. In particular, field trials demonstrated that altering spatial patterns in the orchard when replanting can have significant positive impacts on plant growth and yield (Table 3). Trees planted in the old drive row (aisle) provided yields that were equivalent to that achieved through soil fumigation. Trees gown in "excavated" soil continued to out perform controls in terms of vegetative growth, and yields were significantly greater than the control at the 2001 harvest. Although initially exhibiting apparent phytotoxicity, yields obtained for the 2001 crop from trees planted in soils treated with a combination of metalxyl (Ridomil) and flutolanil surpassed that obtained by the control, and were numerically, though not statistically, higher than the methyl bromide fumigation treatment. This finding suggests that the appropriate use of specific fungicides may be an effective alternative for management of replant disease on sites where lesion nematodes do not contribute to disease development.

Table 3. Effect of cultural, biological and chemical methods on growth and yield of 'Gala'/M.26 planted on replant ground in 1998 at Columbia View orchard, Orondo, WA

Treatment	2000 yields (kg/tree)	2001 yields (kg/tree)
Control	4.6	20.64
Methyl Bromide Fumigation	7.2*	27.12*
Soil Excavation	5.4	25.72*
Interplanting (aisle)	6.4*	-[y]
Pseudomonas putida 2C8	4.1	21.36
RootShield® (T. harzianum)	4.7	22.45
Difenconazole	3.4	23.71
Metalaxyl+flutolanil	4.5	29.1*
Humic acid	3.4	19.9

[z]Means in a column followed by (*) are significantly different (P=0.05) from the control.
[y]Trees removed from the aisle October 2000.

Results from field trials planted in the spring of 2000 demonstrate that a two-year cover crop of *Brassica napus* or wheat provides partial disease control and improved growth of apple on replant sites, but a two year bare fallow provides no detectable level of disease control. Of great significance is the positive impact of a single fall preplant application of *B. napus* seedmeal prior to planting on first-year growth of apple at two replant sites. The enhanced growth was in response to control of various elements of the pathogen complex that incites apple replant disease. Monitoring of these field trials will continue over the next three years. However, results from this research program already have provided significant insight into the use of potential alternatives for the control of apple replant disease, and provide the first significant alternative for control of this disease in organic production systems. Future studies will be formulated to develop guidelines for integration of promising methods into a systems approach for the management of this complex disease.

Fluorescent pseudomonads have long been the source of agents for the biological control of soilborne plant diseases. The primary impediment to commercial use of these agents has been the inability to ensure their long-term survival in competition with the indigenous soil microflora, and in particular the resident fluorescent pseudomonad community. We have identified a particular *Brassica napus* seed meal amendment that selectively eliminates the resident fluorescent pseudomonad population when applied at a specific concentration. This material may have several benefits including direct disease control as it is deleterious to *Rhizoctonia* spp. and *Pratylenchus penetrans*, and enhanced plant nutrition due to a fertilization effect. A novel use may be the application of the seed meal to eliminate potential rhizosphere competitors, in particular resident fluorescent pseudomonads, prior to application of biocontrol fluorescent pseudomonads as a means to enhance survival, and therefore efficacy, of the introduced agents.

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Table 4. Project Funding History.

Item	1999	2000	2001
Salaries	$9,600	$17,600	$18,900
Wages	0	5,000	4,200
Benefits	3,200	5,800	7,037
Laboratory supplies	10,000	10,000	10,000
Travel	0	0	0
Field supplies	5,000	5,000	5,000
Total	$27,000	$43,400	$45,137

Overall Project Cost: $115,537