Final Report

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Project Title:	Employing Biological Elements of Orchard Ecosystems for Enhanced Tree Health
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Prior research identified cultural and biologically active methods, which when applied independently, provide partial control of the pathogen complex that incites apple replant disease and enhanced yield of Gala on M.26 rootstock in orchard trials. In previous studies (Mazzola et al., 2001. Phytopathology 91:673-679), soil amendments were identified that effectively controlled certain elements of the causal pathogen complex and have demonstrated the ability to enhance growth of apple on replant sites. During the three year funding period of this program, studies were conducted to integrate multiple methods identified in previous studies into a systems approach with the goal of achieving levels of disease control and initial tree growth and yield on replant sites comparable to that obtained via pre-plant soil fumigation.

Objectives: The objective of this research program is to develop management programs that will ensure the successful establishment of new trees on old orchard sites and sustain productivity of these orchards through the use of biological resources resident to the site and/or with the use of minimal inputs. The specific objectives of this research program are to:

1.) Evaluate the capacity of commercially available rootstocks, and elements of the *Malus* germplasm, to support plant beneficial microbial communities.
2.) Assess relative growth of *Prunus* spp. in apple or pear replant soils and determine whether biological impediments exist to successful tree establishment on such sites.
3.) Evaluate the relative tolerance/resistance of commercial rootstocks to fungal components of the pathogen complex which incites apple replant disease.
4.) Implement and evaluate orchard trials on the use of integrated strategies for the control of apple replant disease.

Significant findings:

- *Brassica napus* (rapeseed) seed meal (RSM) in conjunction with a Ridomil soil drench was as effective as TeloneC17 fumigation in enhancing growth and yield of Gala/M26 at the CV Orchard. Preliminary studies suggest additional benefits from this alternative control method including weed control and plant nutrition.

- The same alternative treatment provided disease control and enhanced growth of Golden/M7 at the WVC-Airport Orchard, however the growth response was inferior to pre-plant fumigation, and appears to have resulted from re-infestation of RSM-amended soils by *P. penetrans*.

- Field and greenhouse trials indicate significant differences among apple rootstocks in relative tolerance toward pathogen complexes which incite replant disease. Comparable results were obtained for an individual rootstock in field and greenhouse trials conducted in the same orchard soil. This finding demonstrates the value of greenhouse trials for predicting the impact of treatment or rootstock genotype on growth of apple in replant soils in the field.

- Although horticulturally “challenged”, G16 exhibited consistent tolerance to replant disease in the absence of significant lesion nematode populations.

- In the presence of a lesion nematode component, growth of all apple, cherry and pear rootstocks in native soils was suppressed significantly relative to that achieved in sterilized soils. Old HomexFarmindale97, Giesla and Mahaleb rootstocks performed poorly even in the absence of the lesion nematode.

- Peach rootstocks did not sustain populations of the lesion nematode and plant biomass was not significantly diminished in apple orchard replant soils. This finding implies that establishment of peach may not be negatively impacted by the pathogen/parasite complex resident to sites previously planted to apple.

- Cherry rootstocks planted in apple replant orchard soils exhibited a significant increase in susceptibility to freeze damage relative to the same rootstock established in pasteurized replant soil.

- Genetic composition and fungal inhibition potential of the fluorescent pseudomonad population isolated from apple roots was highly variable among rootstocks.

- Apple rootstocks differ in ability to support populations of resident *Streptomyces* spp. in RSM amended soils, which may result in enhanced or diminished RSM-induced disease control.

Results and discussion:

Alternative Control Practices-Field Trials: *B. napus* seed meal (RSM) amendment in the fall prior to planting in conjunction with a post-plant Ridomil® soil drench, and Telone-C17 fumigation were effective in controlling replant disease at the CV and WVC Orchards. Over the 3-year life of the CV orchard, growth of Gala/M26 planted in RSM-Ridomil treated soil has been equivalent to that of trees established in fumigated soil (Figure 1). Yields from trees receiving this alternative treatment have been comparable to or better than that obtained in response to soil fumigation (Table 1). Wheat cropping followed by seed meal amendment and seed meal in conjunction with solarization also significantly enhanced tree growth and yield relative to the control, but were inferior to soil fumigation.

At the WVC orchard, growth of Golden Delicious/M7 has been enhanced relative to the non-treated control by the RSM-Ridomil and RSM-solarization treatments. After three growing seasons, alternative treatments have provided a growth response that is inferior to that obtained with pre-plant soil fumigation. An analysis of pathogens and parasites resident to the root systems of Golden/M7 was conducted. All treatments, with the exception of solarization, were effective in reducing root infection by resident populations of *Rhizoctonia solani* (Table 2). Likewise, all treatments were similar in the capacity to control *Pythium sylvaticum*, the dominant plant-pathogenic species of *Pythium* resident to this orchard soil.

However, our data demonstrate that although alternative treatments substantially reduced initial colonization of M7 roots by *P. penetrans*, by the end of the second growing season these populations had increased dramatically in all but the Telone-C17 treatment (Table 2). The recolonization of RSM-amended soils by *P.* penetrans could be attributed to the distribution of the seedmeal through the soil profile. As the amendment was rotovated into soil to a depth of 6-8 inches, incorporation by alternative means to attain greater soil depths has potential to resolve this problem.

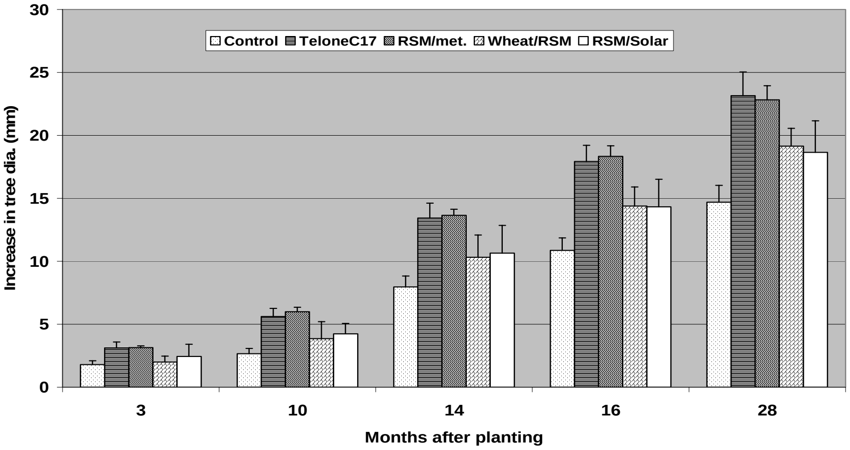

Fig. 1. Increase in trunk diameter of Golden/M7 over three growing seasons at the CV orchard, Orondo, WA.

Table 1. Impact of soil treatments on 2003 and 2004 yield of Gala/M26 at the Columbia View Orchard.

Treatment^z	2003 Yield (kg/tree)	2004 Yield (kg/tree)
Control	0.0a	5.67a
Telone C17	0.37b	13.29c
RSM-Ridomil	1.32c	13.40c
Wheat + RSM	0.19ab	8.98b
RSM+Solarization	0.11ab	8.46b

^{z}Means in the same column followed by the same letter are not significantly (*P*=0.05) different.

Table 2. Impact of soil treatments on populations of *Pratylenchus penetrans* (lesion nematode) and 16-month growth of Golden/M7 at Wenatchee Valley College-Airport Orchard.

Treatment^z	% *R. solani* infection	%*P. sylvaticum* infection	*P. penetrans* 2002 #/g root	*P. penetrans* 2003 #/g root
Control	12.8c	9.4b	279c	621c
Telone C17	4.5a	0.0a	51a	84a
RSM-Ridomil	3.8a	0.8a	56a	304b
Wheat + RSM	0.0a	1.9a	125b	567bc
RSM + Solarization	1.6a	0.0a	73a	260b
Solarization	8.4b	1.3a	252c	1135d

^{z}Means in the same column followed by the same letter are not significantly (*P*=0.05) different.

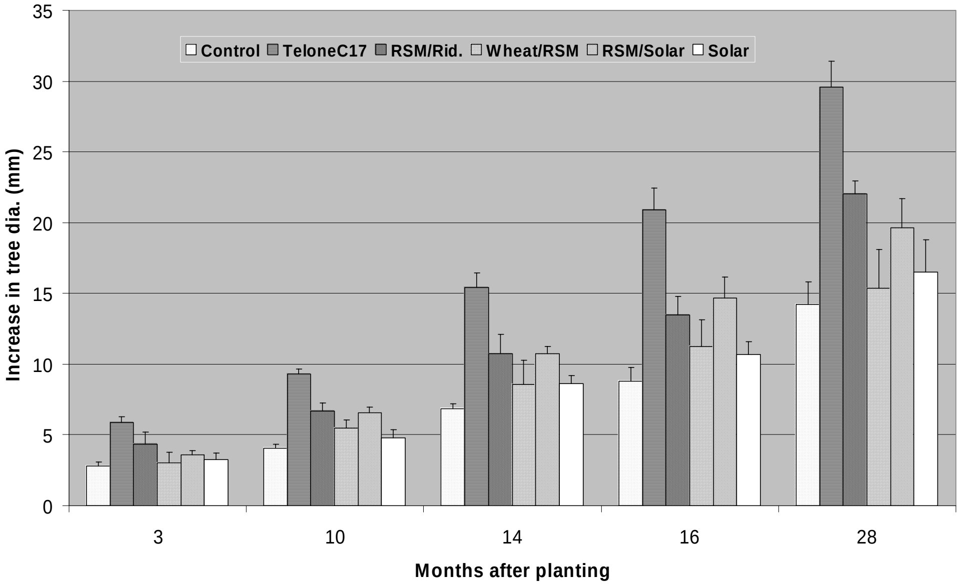

Figure. 2. Increase in trunk diameter of Golden/M7 over three growing seasons at the WVC-airport orchard, E. Wenatchee, WA.

In addition to its disease control properties, the alternative method may provide additional benefits in terms of plant nutrition and initial weed control. As RSM contains 6% N as well as 1-2% S, P, and K, application of this material will also have the potential to significantly reduce costs for fertilization during the initial growing season. Initial trials also suggest that the amendment may reduce the need for weed control during the first growing season (Figure 3). These elements will be the subject of future study.

Figure 3. Impact of RSM amendment on weed biomass production at the CV orchard. Treatments were applied 24 March 2004, figure represents weed biomass harvested from individual block on 21 July 2004.

Apple Rootstock Tolerance to Replant Disease and Response to Alternative Treatments: In two independent studies conducted at the CV Orchard, the apple rootstocks Bud9, G16, Maruba and 5935 exhibited significant, though at times variable, levels of tolerance to replant disease. For the 2003 field trial, growth of these rootstocks was comparable or superior in non-treated soil relative to that obtained in fumigated soil (Table 3). Growth of G11, M7, M9, M26, M111 and 7707 was enhanced significantly by soil fumigation, indicating significant tree damage incited by the resident pathogen complex. The same relative growth patterns for rootstocks were comparable in greenhouse trials conducted in CV orchard soil.

Table 3. Comparative growth (%) of apple rootstocks in non-treated soil relative to that obtained in TeloneC17 fumigated (field) or pasteurized CV Orchard soil.

	Bud9	G11	G16	M7	M9	M26	M111	Maruba	7707	5935	Seed.
2003 Field	101	42	122	67	81	72	86	123	54	101	106
2003 Greenhouse	98	-	104	71	66	56	80	-	-	-	-
2004 Field	83	45	92	71	81	54	61	-	-	95	67

Trials were repeated during the 2004 growing season at an adjacent CV orchard block. For most rootstocks, relative growth in the field during the 2004 trial was similar to that obtained at the orchard in 2003. "Seedling" rootstock exhibited the greatest year-to-year deviation, and this may be attributed to the genetic diversity associated with this material due to its non-clonal nature. As observed in 2003, Rootstock 5935 from the USDA-ARS/Cornell University breeding program once again demonstrated disease tolerance based upon growth performance. Relative growth of G11 was consistent among the two field trials and exhibited the poorest growth among those materials evaluated in both years at the CV orchard.

Additional rootstocks from the USDA-ARS/Cornell University breeding program were evaluated in the field at the CV orchard in 2004. To date, based on initial-year growth, all rootstocks performed poorly in non-treated soil and appear susceptible to the disease complex resident to CV orchard soil (Table 4)

Table 4. Comparative growth (%) of apple rootstocks in non-treated soil relative to that obtained in TeloneC17 fumigated (field) or pasteurized CV Orchard soil

	3041	4003	4213	4214	4814	5046	5087	5179	5860	6143	6253	6589	6879
2004 Field	44	50	49	71	68	55	46	73	56	61	38	61	40

When conducted in GC orchard soil, a soil system containing a significant lesion nematode population, growth of all apple rootstocks was significantly enhanced by soil pasteurization indicating susceptibility to the causal pathogen complex (Table 5). This was observed irrespective of rootstock tolerance, as indicated by root populations, toward the lesion nematode. For instance, growth of both Bud9 and M9 in non-treated soil was approximately 50% of that achieved in pasteurized soil, yet Bud9 obviously supported significantly lower lesion nematode populations than did M9. These findings suggest that lesion nematode root populations alone may not be of value for predicting tolerance to apple replant disease.

Table 5. Comparative growth (%) and root populations of *Pratylenchus penetrans* for apple rootstocks grown in GC orchard soil.

Rootstock	% of Pasteurized Check	# *P. penetrans*/g root
Bud9	50	76ab
G16	50	23a
M7	55	366c
M9	52	362c
M26	41	262bc
MM106	38	621d
MM111	64	484cd
Seedling	69	151b

In field trials apple rootstocks responded differentially to pre-plant application of *B. napus* seedmeal in concert with a post-plant application of Ridomil or soil solarization (Table 6). As observed previously in the absence of significant lesion nematode populations, growth performance of G16 was not significantly influenced by Telone-C17 pre-plant fumigation. Pre-plant fumigation did not enhance growth of Bud9 in 2003, but significant growth promotion was observed for this rootstock in the 2004 trial. Growth of M7, M9, M26 and MM111 rootstocks was enhanced significantly by pre-plant fumigation in both trials. *B. napus* seedmeal amendment in the spring of 2003 in concert with a Ridomil soil drench at planting was superior to fumigation in promoting initial growth of all apple rootstocks tested. This study was repeated in the spring of 2004 at the same site. Typically, in 2003, the RSM-Ridomil treatment provided a growth response which was superior to that obtained with RSM-solarization, however the results with M7 were the exception. When compared to pre-plant fumigation, the RSM-Ridomil treatment afforded the greatest % growth increase in concert with the rootstock G16 (561%) in the 2003 trial. In the 2004 trial, with the exception of M9 and M26, all rootstocks grown in RSM-Ridomil treated soils attained an increase in tree diameter which was equivalent to 150-185% of that attained in fumigated soil and 200-300% of that achieved in non-treated (control) soils.

Table 6. Increase in caliper (mm) of apple rootstocks 4-months after planting at the CV orchard in separate trials established in 2003 and 2004.

	Bud9		G16		M7		M9		M26		MM111	
Treatment	2003	2004	2003	2004	‘03	‘04	‘03	‘04	‘03	‘04	‘03	‘04
Control	0.61	1.05	1.19	1.94	0.29	0.62	0.47	1.16	0.76	0.87	0.49	0.59
TeloneC17	0.62	1.47	0.80	2.13	0.43	0.87	0.58	1.61	0.95	1.59	0.61	0.96
RSM/Ridomil	1.66	2.37	4.49	3.15	1.13	1.31	2.02	1.74	2.52	1.88	1.82	1.78
RSM/Solar	1.31	2.50	2.54	2.97	1.74	1.17	1.58	1.36	2.27	2.53	1.20	2.30

Growth of alternative tree fruit rootstocks in apple replant soils: In general, growth of cherry rootstocks was suppressed when planted in soils collected from apple replant sites, though growth depression typically was more evident when assays were conducted in soils possessing significant lesion nematode populations (GC orchard; Table 7). However, even in the absence of *P. penetrans* the cherry rootstocks Mazzard and Mahaleb were more susceptible to winter injury when established in non-treated soils than when grown in pasteurized soil. Mortality of Mazzard rootstock in control soil after over-wintering was 80% and 100% for the 2002 and 2003 trials, respectively. In addition, 40 and 60% tree death was observed for Mahaleb rootstock during the 2003-04 winter, in non-treated GC and WVC soil, respectively. Growth of peach rootstocks in either GC or WVC orchard replant soil was not significantly impacted by the disease complex resident to either of these orchard soils. The pear rootstock Old HomexFarmindale97 performed poorly in both soils tested; growth was suppressed to a greater degree and in both the 2002-03 and 2003-04 trial nematode populations were equivalent to or higher than observed for the apple rootstocks G16 and M26 grown in the respective soils. Again, in the absence of *P. penetrans* (WVC soil), G16 grew similarly in natural and pasteurized soil (Table 8). These preliminary findings indicate that pre-plant treatments will be imperative for the re-establishment of pear or cherry on old apple sites; to date the data do not suggest a comparable level of risk when establishing peach on similar sites.

Table 7. Lesion nematode populations supported by tree fruit rootstocks when grown GC orchard soils.

Rootstock	Experiment 2002-03	Experiment 2003-04
G16	123b[a]	116b
M26	672d	93b
Giesla	115b	190bc
Mahaleb	261bc	213c
Mazzard	182b	ND[b]
OldHome	573d	401d
Citation	26a	6a
Nemaguard	7a	5a

[a] Means in the same column followed by the same letter are not significantly (*P*=0.05) different.

[b] ND=not determined; all trees failed to survive overwintering.

Table 8. Relative growth (% of growth in pasteurized soil) of fruit tree rootstocks in apple orchard replant soils.

	Experiment 2002-03		Experiment 2003-04	
Rootstock	% Past. Ck-WVC	% Past. Ck-GC	% Past. Ck-WVC	% Past. Ck-GC
G16	162**	64*	86 NS	83 NS
M26	74 NS	73*	84 NS	72*
Giesla	54*	49**	69*	52**
Mahaleb	53*	74*	60*	58*
Mazzard	106 NS	30**	ND	ND
OldHome	61*	66**	73*	77*
Citation	98 NS	75 NS	117 NS	101 NS
Nemaguard	82 NS	73 NS	105 NS	109 NS

[a] *= $P \leq 0.05$; **= $P \leq 0.01$; NS=no significant difference in growth of rootstock in natural soil when compared to growth of the same rootstock in pasteurized soil; ND=not determined; all trees failed to survive overwintering.

Rootstock-dependent composition of microbial populations: Genetic composition of the fluorescent pseudomonad community recovered from apple rootstocks prior to and post planting in WVC orchard soil was assessed. These analyses demonstrated that the structure of this bacterial community varied significantly between rootstocks (Table 9). Genotype 1 is typically the dominant genotype recovered from apple planted on replant sites. Whereas the population recovered from Seedling and Geneva16 rootstock was dominated by genotype 13, the population from the rhizosphere of Bud9, MM106, M26, and M7grown in the same orchard soil consisted primarily of isolates defined as genotype 1. Isolates belonging to genotype 1 do not exhibit in vitro antagonism toward the apple pathogen Rhizoctonia solani AG-5, while a preponderance of genotype 13 isolates inhibit growth of this pathogen. Interestingly, when planted into soil artificially infested with *R. solani* AG 5, seedling rootstock appeared tolerant to this pathogen while Bud9, which supported a population dominated by non-suppressive genotypes (G1 and G36), was susceptible to this pathogen. The data demonstrate a major role for soil type in determining the genetic composition of these populations as G41, which dominated the population from seedling rootstock prior to planting, was not detected in the population recovered from the rootstock after 20-weeks growth in WVC orchard soil. Only the seedling rootstock possessed isolates of belonging to G9 in both the pre-and post-plant samples. G9 isolates produce the antibiotic 2,4-diacetylphloroglucinol, which has activity against a broad range of plant pathogenic fungi. These findings suggest that selection of the appropriate rootstock will impact the capacity of apple to exploit the microbial resources resident to orchard soils.

Table 9. Genetic composition of the fluorescent pseudomonad population recovered from the rhizosphere of apple rootstocks obtained from a single nursery (N), the population recovered from the same trees 20 weeks after planting in WVC orchard soil (O), and growth of these rootstocks in the presence of *Rhizoctonia solani* AG-5.

Rootstock	G1		G4*		G9*		G13*		G36		G37		G40		G41*		G42		Growth in presence of R. solani^b
	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O	
Seedling	8	25	2	0	8	13	27	55	2	3	2	0	0	0	43	0	0	0	113
Bud9	33	88	7	0	0	0	0	8	51	3	0	0	7	0	0	0	0	0	51
G16	52	23	2	0	0	5	0	68	30	2	0	0	2	0	0	0	2	0	103
M7	45	62	4	0	0	2	9	27	30	5	4	0	9	2	0	0	0	0	55
M9	40	57	10	0	0	0	7	35	31	35	2	0	0	0	0	0	9	0	100
M26	52	70	2	0	0	7	11	23	21	0	0	0	13	0	0	0	0	0	41
MM106	71	76	2	0	0	0	0	14	10	5	2	0	0	0	0	0	2	2	67
MM111	38	48	5	0	0	5	0	47	2	0	0	0	48	0	0	0	7	0	72

[a] Genotypes designated with (*) are fungal antagonists possessing the capacity to inhibit *in vitro* growth of *Rhizoctonia solani* AG-5. Values are percent data with 60 isolates analyzed for each

rootstock at each sampling period. Only major genotypes are presented, thus values in a in a row for a given sample may not sum to 100%.

[b] Values represent the increase in cross-sectional area of trees grown in the presence of *Rhizoctonia solani* AG-5 relative to that obtained for trees grown in non-infested soil. Data were collected 20 weeks after planting.

Recent findings from this laboratory indicate that suppression of *Rhizoctonia solani* observed in response to *B. napus* seed meal amendment is a plant-mediated phenomenon rather than resulting directly from the application of RSM. Furthermore, our data suggest that the plant response is directed by soil microorganisms, and specifically bacteria belonging to the genus *Streptomyces*, which increase 2-3 orders of magnitude in response to RSM-amendment. Therefore, studies were initiated to assess the relative capacity of apple rootstocks to support resident populations of *Streptomyces* spp. when grown in RSM amended soil.

Significant differences were observed in the recovery of *Streptomyces* spp. from apple rootstocks grown in RSM amended soil at the CV orchard. G16 supported the largest populations of *Streptomyces* spp., which were significantly higher than that recovered from the roots of Bud9, M7 and MM111 rootstocks. Populations of *Streptomyces* recovered from M7 and MM111 were significantly smaller than that recovered from all other rootstocks evaluated.

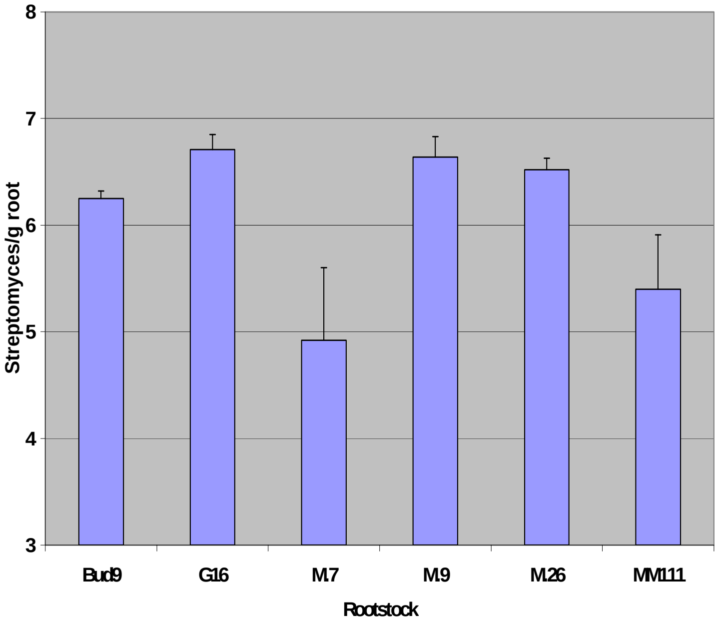

Figure 4. Populations of *Streptomyces* spp. recovered from the roots of apple rootstocks grown in RSM-amended replant soil at the CV orchard.

In greenhouse assays, the vast majority (>95%) of *Streptomyces* recovered from the roots of Gala seedlings had the capacity to produce nitric oxide (NO) and possessed a nitric oxide synthase (NOS$^+$) homolog. Likewise, production of nitric oxide in the rhizosphere of apple was confirmed through confocal image analysis. These data are significant as exogenous sources of NO can prime plant defenses by stimulating the production of phenylpropanoids including salycilic acid. One focus of future studies outlined in a new proposal will aim to further define the role of bacterial-derived NO in the suppression of *R. solani*, the importance of host genotype in eliciting the response, and the impact of seed meal source and cultivar in promoting biologically active populations of *Streptomyces*.

Project Funding History: 2002-2004

Item	2002	2003	2004
[1]Salaries	19,000	19,760	20,550
Benefits (33%)	6,270	6,520	6,780
[2]Wages	6,800	11,000	11,000
Benefits (16%)	1,088	1,760	1,760
Equipment	0	0	0
[3]Supplies	10,000	10,000	10,000
[4]Travel	800	800	800
Miscellaneous	0	0	0
Total Proposed	$43,958	$49,840	$50,890