Final Report: Biological Mediation of Apple Replant Disease in Organic Apple Orchards

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Project Summary: Apple replant disease is a major impediment to organic orchard production systems. This disease is thought to result from soil-borne pathogens and parasites that build in soil over the life of an orchard. Apple replant disease does not negatively impact existing orchard plantings, however, when new trees are planted in an existing orchard, the yield and vigor are substantially reduced and in some cases results in tree death. Because of the severity of these impacts, orchardists have removed land from organic certification and used pre-plant fumigation to remediate soil-borne pathogens prior to planting new apple trees. Pre-plant fumigation negatively impacts on the health and diversity of soil biological communities. Alternatively, orchardists can remediate apple replant disease by planting wheat. Results of our trials indicate that planting wheat in orchard soils increases populations of beneficial soil microbial communities that are well known for their antagonistic activity against soil-borne pathogens. Correspondingly, we observed decreased populations of soil-borne pathogens and parasites, reduced apple root infection by pathogens, and increased apple seedling vigor following wheat cultivation. Furthermore, our results indicate that wheat varietal selection conditions influence the potential for wheat varieties to enhance beneficial microbial antagonists and suppress soil-borne pathogens. Wheat varieties bred under organic conditions had the greatest potential to increase beneficial soil microbial communities and suppress pathogens and parasites in our trials. Similar pathogen complexes affect various crop plants, thus findings from these studies are likely

to be of value across multiple systems. In addition, our trials provide evidence that plant breeders can select for beneficial plant root-microbial relationships.

Introduction to the topic: During the life of an orchard, soil-borne pathogens belonging to the genera *Rhizoctonia*, *Pythium*, *Phytophthora, Cylindrocarpon* and *Pratylenchus* become prevalent in the tree root zone, but they generally do not appreciably affect the health or productivity of mature trees (Mazzola, 1998; Mazzola, 1999). However, when orchardists remove old trees and plant new ones in response to the age of an orchard or market pressure for a different variety, the establishment and productivity of new trees are adversely impacted by these pathogens. This phenomenon is commonly termed apple replant disease. Digging holes and filling them with imported soil that is free of the causal pathogens is not cost-effective in a large orchard, and will generally only control disease symptoms for the first year or two (Anonymous, 2001). Leaving soil fallow for an extended period of time has also been ineffective in controlling the causal pathogen complex (Mazzola and Mullinix, 2005; Fuller, personnel communication). Presently the only effective method to control these pathogens and ensure the long-term productivity of an orchard is pre-plant soil fumigation. This practice not only reduces the integrity of organic production systems, but also alters the structure and function of the resident soil biology, including both plant beneficial and potentially pathogenic organisms. For example metam sodium, a fumigant commonly used to treat apple replant disease, has been found to disrupt beneficial free-living nematodes, mycorrhizae, and beneficial bacteria and fungi that cycle nitrogen (Cox, 2006). Additionally, this chemical is a carcinogen that can negatively impact farm worker health (Cox, 2006). Organic orchardists need non-chemical treatments that simultaneously build soil quality and enhance orchard tree health.

A more viable strategy to suppress apple replant pathogens and enhance tree health is to plant a cover crop prior to planting new apple trees. Cover crop cultivation has been shown to reduce disease incidence in multiple crop systems (Janvier et al., 2007). In many cases, reductions in pathogen populations have been correlated with measurable changes in the composition of the entire soil microbial community (Benitez et al., 2007; Janvier et al., 2007; Larkin et al., 2011). Plants commonly release signaling molecules and carbohydrates from their roots to attract and support beneficial microbial species in exchange for protection from pathogens. For example, long-term wheat monoculture is well known for enrichment of *Pseudomonas fluorescens* which have the ability to produce 2,4-diacetylphoroglucinol (DAPG), a compound that suppresses the wheat root pathogen *Gaeumannomyces graminis* (Weller et al., 2002*)*. However, plant varieties differ in the amount and composition of these exudates, which can influence pathogen dynamics in soil (Rengel, 2002). In apple replant systems, wheat varieties were previously found to differ in their capacity to enrich beneficial *Pseudomonas spp.* and suppress root infection by *Pythium spp.* and *Rhizoctonia solani* (Gu and Mazzola, 2003). Wheat varieties that did not significantly alter this bacterial community from that initially resident to the replant orchard soil did not yield subsequent disease control (Gu and Mazzola, 2003). Under field conditions, the wheat cropping strategy was as effective as soil fumigation in controlling infection of Gala/M26 roots by *R. solani* (Mazzola and Mullinix, 2005). However, although *Pratelyencus penetrans* populations were effectively suppressed by wheat cultivation in greenhouse experiments, nematode suppression in response to wheat cultivation was not observed in field trials. The inability of wheat cultivation to suppress nematodes in field trials may have resulted from an inability of the wheat roots to fully explore the soil profile.

Selecting plant varieties under organic and low-input production systems is important for identification of varieties possessing traits uniquely suited to these systems (Murphy et al., 2007). The composition of the resident soil microbial is the biggest factor regulating the composition of microbes inhabiting plant roots. The benefits and costs that plants incur by associating with individual microbial species is likely to alter how they selectively signal and support, or actively exclude individual species after successive generations in a given soil. When sugar cane breeders selected for high yield under low-input conditions, they inadvertently selected for cultivars capable of interaction with native diazotrophic bacteria, which help facilitate nutrient uptake (Baldini et al., 2002). In contrast, in comparison to historic soybean cultivars, modern cultivars selected under high input conditions have reduced ability to discriminate between rhizobial strains that actively fix atmospheric nitrogen in exchange for plant resources and those that obtain resources from the plant but do not actively fix or contribute N to the plant (Kiers et al., 2007). Smith et al. (1999) discovered that a genetic basis exists for the ability of tomato cultivars to support rhizosphere populations of *Bacillus cereus* leading to the suppression of *Pythium torulosum.* These studies indicate that breeding program could be designed to exploit beneficial plant-microbe interactions, and that direct selection for these traits under organic and low-input conditions has potential to increase selection for this beneficial trait.

Breeding specifically for resistance to soil-borne pathogens in annual crops has not been common (Wissuwa et al., 2008). However, wheatgrasses like *Thinopyrum spp.*, the relatives of modern wheat, and perennial wheat cultivars derived from crosses with *Thinopyrum spp.*, are resistant to a number of diseases that impact annual wheat (Cox et al., 2005) including tolerance to *Rhizoctonia* and *Pythium* (Hoagland, unpublished). Wheatgrass and perennial wheat cultivars have deeper and more extensive root systems than annual wheat, which may increase their potential to exploit beneficial plant-microbe interactions and enhance microbial transformations in soil to facilitate disease suppression. Perennial wheat cultivars might also have the added benefit of reducing erosion in steep orchards, and reducing nitrate loss.

Objectives Statement:

The objectives of our proposal were to:

1) Determine the ability of annual and perennial wheat cultivars to selectively support resident microbial antagonists of apple root pathogens and enhance apple seedling health.

2) Determine whether selection conditions and introgression of genes from wild relatives impact the ability of wheat cultivars to facilitate beneficial plant-microbial interactions

3) Determine the ability of an annual or perennial wheat-apple system to mediate apple replant disease and enhance the health of newly established organic apple orchards.

We did not make any changes and were able to meet our stated objectives.

Materials and Methods:

Objectives 1 and 2: Determine the ability of annual and perennial wheat cultivars to selectively support resident microbial antagonists of apple root pathogens and enhance apple seedling health; and determine whether selection conditions and introgression of genes from wild relatives impact this plant-microbial interaction. Soil was collected from the root zone of areas previously planted to apple at Ray Fuller's Stormy Mountain Orchard, Chelan, WA and the WSU Tukey Farm, Pullman, WA. Field soil from each location was mixed, distributed into 3.8L pots, and planted to the following treatments in a complete randomized block design in the greenhouse: 1) Control, 2) Pasteurized, 3) one of four modern annual wheat varieties, 4) one of four historic wheat varieties, 5) one of four wheat varieties from our advanced organic breeding programs, 6) two wheat relatives (*Thinopyrum elongatum* and *T. intermedium*) 7) a model annual wheat cultivar (Chinese Spring) and two perennial wheat lines developed from crossing the wheat relatives to this model cultivar (CS4E and CS4J) 8) one of four perennial wheat cultivars from our advanced perennial breeding program, and 9) annual ryegrass (all, 5 reps per treatment); resulting in a total of 24 treatments. After 28 days, the aboveground biomass for annual wheat cultivars was cut and discarded, and soil was mixed to simulate cultivation, returned to the same pot and replanted to the same cultivar; aboveground biomass was cut and allowed to regrow in perennial treatments. This process was repeated for a total of three growth cycles.

At completion of the wheat growth cycles, five six-week-old Gala apple seedlings were planted in each pot. After 10 weeks, apple seedlings were harvested, root systems were shaken to remove loosely adhering soil, and a 0.5 g root sample was collected for rhizosphere microbial analyses described below. Remaining seedling root systems were rinsed under a stream of tap water, and plant height, shoot weight, and root weight were determined. *P. penetrans* root populations were determined by placing a 0.5 g composite root sample per pot into a 125-ml flask containing 80 ml sterile distilled water and shaken at 150 rpm for six days. Nematodes were collected by passing the suspension twice through a 350-mesh sieve and backwashing into a counting dish, and *P. penetrans* counted using a light microscope (40X). For each seedling, 10 root segments (0.5-1.0 cm) were plated onto PSSM agar plates for detection of *Pythium spp.* and *Phytophthora spp.* and water agar amended with ampicillin (100 μg ml^{-1}) for detection of *Cylindrocarpon* and *Rhizoctonia spp.* Plates were incubated at room temperature and examined using a light microscope (x100) at 48-72 h for the presence of hyphal growth and reproductive structures indicative of these genera. Soil microbial communities associated with the apple rhizosphere were analyzed for organisms commonly thought to be associated with disease suppression in apple (*Pseudomonas* (7,15,17), *Streptomyces* (16,18) and non-pathogenic *Fusarium* spp. Briefly, roots with closely adhering soil were placed in sterile water, vortexed and serial dilutions plated on media selective for the respective microbial groups. All statistical analyses were carried out using SAS software. Analysis of variance and subsequent separation of treatment means were used to assess plant biomass, root infection by pathogens, and individual rhizosphere microbial species populations.

Objective 3: Determine the ability of an annual or perennial wheat-apple system to mediate apple replant disease and enhance the health of newly established organic apple orchards. These studies were carried out at Stormy Mountain Orchard, Chelan, WA and the WSU Tukey

Orchard, Pullman, WA. At each location, mature apple were removed in summer 2009 and soils were subject to the following treatments: 1) an unamended control, 2) annual wheat variety cv. Penewawa, 3) an advanced perennial wheat accession, and 4) wheatgrass cv. Rush. The treatments were arranged in a randomized complete block design with four replicates per treatment. Each plot was 13.5m^2 in length and 2.5 m in width. Seed beds were prepared for planting by cultivating soils with a rotovator to a depth of 15 to 20 cm. Wheat was seeded at a rate of 140 kg ha^{-1} and grown under irrigation. In fall, annual wheat biomass was cut and removed and perennial wheat and wheatgrass biomass was cut and removed in spring 2010. Six Gala/M9 apple trees were planted in summer 2010 in each treatment plot with a spacing of 1.5 m within row, and 4 m between rows. Tree health and productivity were assessed using tree cross sectional area and lateral tree branch extension.

Project Results: Annual ryegrass cultivation did not improve apple root biomass relative to control treatments in greenhouse trials using soil collected from two orchards with apple replant disease (Fig. 1). In contrast, wheat cultivation improved apple root biomass relative to the control, but the magnitude of the impact was dependent on the wheat variety and conditions under which the varieties were selected. When averaged across the four entries representing each breeding category, cultivation of wheat varieties from the advanced organic breeding program significantly increased apple root biomass relative to historic varieties. In contrast, differences in apple root biomass following cultivation of varieties selected under high-input conditions did not differ from historic varieties. Cultivation of perennial wheat also increased apple root biomass relative to the control, annual ryegrass and historic wheat varieties, however, there was wide variability in the performance among the four perennial wheat entries tested. One perennial wheat accession (P-0006), increased apple root biomass equal to the pasteurized treatment, while the other perennial accessions did not positively impact apple seedling health (data not shown).

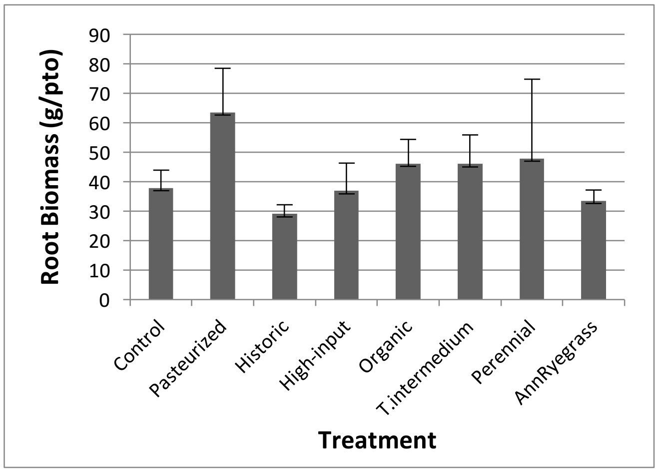

Figure 1. Impact of cover crop cultivation on apple root biomass in two greenhouse trials

Improvements in apple seedling health following wheat cultivation were correlated with modification of the soil microbial community (Table 1). The top entries representing organic (Onas/Madsen) and high-input (cv. Penewawa) breeding programs, a perennial wheat variety under development (P-0006), and a wheat ancestor (T. *intermedium* cv. Rush), all increased populations of beneficial microbial species and decreased soil-borne pathogens belonging to the complex that is widely recognized to incite apple replant disease.

Table 1. Rhizosphere microbial populations in greenhouse trials (CFU per 0.5g apple root)

Treatment	Beneficials *Fluorescent Pseudomonas*	 *Fusarium*	Total pathogens[†]
Control	31.6 X 10^8 bc[*]	9 X 10^4 c	58 X 10^4 a
Pasteurized	6.05 X 10^8 c	62 X 10^4 a	15 X 10^4 c
T. intermedium (cv. Rush)	93.5 X 10^8 a	5 X 10^4 c	14 X 10^4 c
Perennial (P-0006)	94.4 X 10^8 a	28 X 10^4 b	32 X 10^4 b
Organic (Onas/Madsen)	93.9 X 10^8 a	13 X 10^4 c	32 X 10^4 b
High-input (cv. Penewawa)	70.7 X 10^8 ab	27 X 10^4 b	15 X 10^4 c

[*] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; n=10);
[†] *Cylindrocarpon, Pythium, Rhizoctonia*, and *Verticillium*

Soil samples collected from field trials also indicated that wheat cultivation modified soil microbial community structure and improved apple seedling health. For example, cultivation of the annual wheat variety, Penewawa, and the perennial wheat entry (P-0006) significantly reduced lesion nematode populations (Figure 2) in soil collected from field trials conducted at the Stormy Mountain Orchard. Stem diameter of apple seedlings grown in soil collected from the field trial conducted at Stormy Mountain Orchard was greater in plots cultivated with wheat cv. Penewawa than control treatments. Despite poor establishment and productivity in field trials, stem diameter of apple seedlings grown in soil collected from plots cultivated with the perennial wheat entry (P-0006) was also greater than the control treatment.

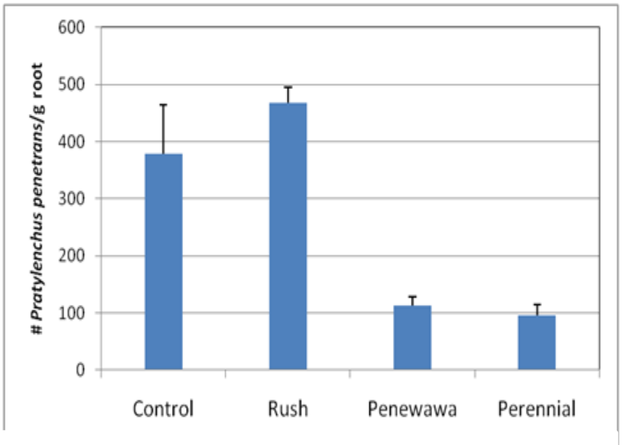

Figure 2. Impact of wheat cultivation on *Pratylenchus penetrans* at the Stormy Mountain Orchard, Chelan, WA.

Conclusions and Discussion: The results of our trials indicate that including only one year of annual wheat cultivation after removal of an existing apple orchard can increase beneficial soil microbial species, suppress soil-borne pathogens and parasites, and improve the establishment and productivity of newly apple

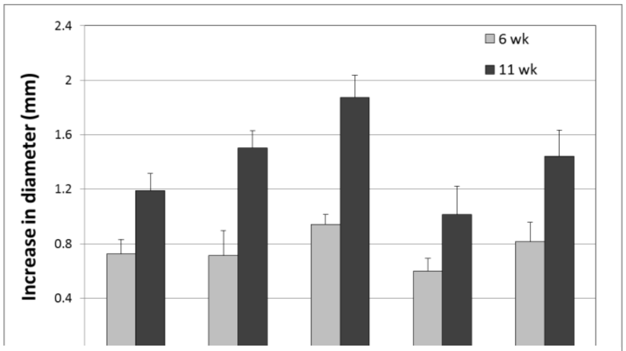

Figure 3. Impact of wheat cultivation on apple stem diameter in soil collected from the Fuller field trial

planted trees. These results support the hypothesis that modification of soil microbial community composition likely plays a role in disease suppression following cover crop cultivation. However, benefits depended on the wheat variety grown, as well as the management conditions under which the variety was selected. This supports previous studies demonstrating differences among wheat genotypes and their ability to enhance populations of biocontrol *Pseudomonas* spp. that suppress soil-borne pathogens. (Gu and Mazzola, 2003; Meyer et al., 2010). As a group, wheat varieties selected under organic management had the greatest beneficial impacts on soil ecology and apple health. These results indicate that recent efforts to select wheat with improved yield under organic conditions has inadvertently selected varieties with greater capacity to modify soil microbial community structure and suppress soil-borne pathogens and parasites. These results also indicate that plant breeders can select for beneficial plant-microbial relationships that will enhance the productivity of organic production systems. Cultivation of perennial wheat also increased beneficial soil microbial species, suppressed pathogens and increased apple seedling health. However, results were highly variable among accessions indicating that continued selection is needed before perennial wheat can be used to reliably suppress pathogens and parasites and increase the health of newly planted trees in existing orchards. Planting perennial wheat it the fall rather than in spring like our trial, has potential to improve establishment and increase the benefits of perennial wheat cultivation.

Useful Tools, Information, and Resources for Farmers: Prior to replanting new apple trees in an existing apple orchard, growers are advised to collect a soil sample and determine the abundance of soil-borne pathogens and parasites. Growing a vigorous annual wheat variety at a high density in old orchard rows infested with soil-borne pathogens and parasites has potential to suppress these pathogens and improve the performance of newly planted apple trees. Obtaining a wheat variety that was developed in an organic breeding program is suggested once they are commercially available. Related pathogens can be found in a variety of crop production systems. Therefore, including wheat in rotation with other crop species has potential to reduce disease severity and increase crop performance. For example, results-to-date of on-going trials indicate that cultivation of wheat cv. Penewawa increases beneficial microbial species and reduces root infection of *Phytophthora capsici* in pepper production systems (Hoagland, unpublished data).

Outreach: An overview of this project, including pertinent results, have been presented at several meetings, including the 2011 Organic Seed Alliance Conference in Port Townsend, WA, and the 2014 Wisconsin Fruit and Vegetable Growers Conference in The Delles, WI. Results were also summarized in the 2011 Organic Seed Alliance Conference Proceedings (Hoagland et al., 2011), and the presentation at this conference was recorded and archived on the eOrganic website. The webinar can be viewed at the following website http://www.youtube.com/watch?v=XD9EMPkHiMg

Financial accounting: Grant funds were allocated to Dr. Hoagland's salary while she was a post-doctoral research assistant at WSU. Funds were also allocated to salary for undergraduate research assistants who assisted with the project, to purchase field and lab supplied to conduct the research, and to compensate the orchard managers for their time and land resources used in the project.

Leveraged resources: Dr. Hoagland has leveraged results from these trials to obtain grant funding to support research trials investigating wheat cover crops and other soil amendments for their potential to suppress soil-borne pathogens in vegetable cropping systems in Indiana. This has included an internal grant from Purdue University Agriculture Research Programs, and an Indiana Specialty Crop Block Grant.

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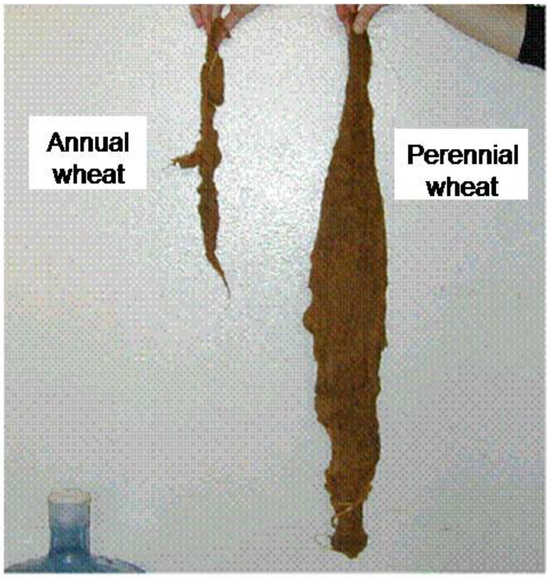
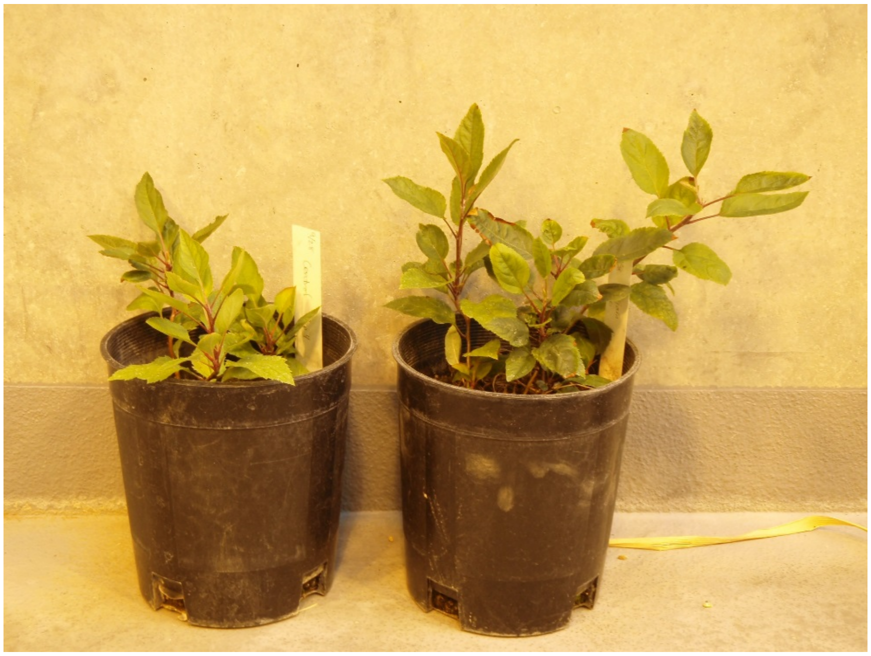
Photos and other addenda:



Root systems of annual and perennial wheat grown in hydroponics

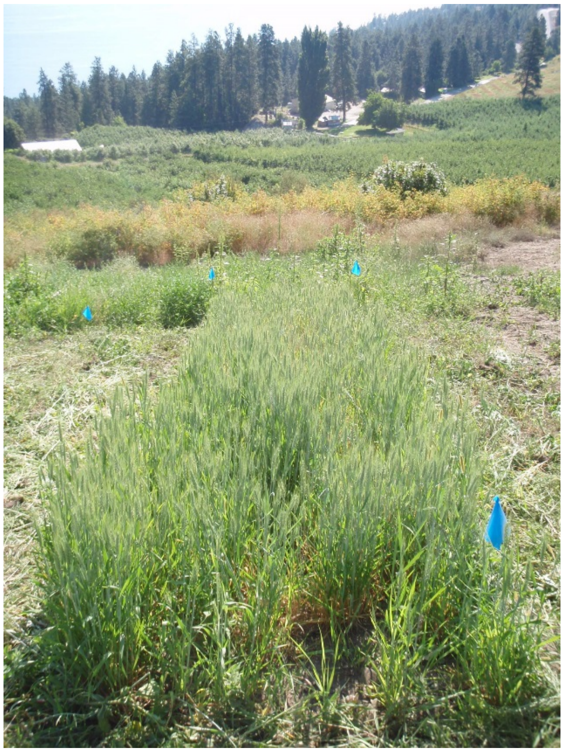
Pots in greenhouse with perennial wheat that has just been cut, and pots with annual wheat cut to soil surface, mixed to simulate cultivation and replanted (April, 2009).



Apple growth in pot previously planted to wheat (control left, organic wheat cultivar right) (July, 2009).

Cutting branches off trees in preparation for stump removal at Tukey Orchard, Pullman, WA (June, 2009).

Ripping stumps out with a backhoe at Tukey Orchard, Pullman, WA (June, 2009).



Annual wheat growth at Stormy Mountain Orchard, Chelan, WA (July, 2009).

Perennial wheat regrowth in autumn at Story Mountain Orchard, Chelan, WA (September, 2009).

Penewawa wheat just prior to harvest at Story Mountain Orchard, Chelan, WA (September, 2009).

Newly planted apple trees in plots previously planted to wheat at WSU Tukey Orchard, Pullman, WA (June, 2010).