Cultural Management of Microbial Community Structure to Enhance Growth of Apple in Replant Soils

Mark Mazzola, David M. Granatstein, Don C. Elfving, Kent Mullinix, and Yu-Huan Gu

ABSTRACT


Apple replant disease typically is managed through pre-plant application of broad-spectrum soil fumigants including methyl bromide. The impending loss or restricted use of soil fumigants and the needs of an expanding organic tree fruit industry necessitate the development of alternative control measures. The microbial community resident in a wheat field soil was shown to suppress components of the microbial complex that incites apple replant disease. Pseudomonas putida was the primary fluorescent pseudomonad recovered from suppressive soil, whereas Pseudomonas fluorescens bv. III was dominant in a conducive soil; the latter developed within 3 years of orchard establishment at the same site. In greenhouse studies, cultivation of wheat in replant orchard soils prior to planting apple suppressed disease development. Disease suppression was induced in a wheat cultivar-specific manner. Wheat cultivars that enhanced apple seedling growth altered the dominant fluorescent pseudomonad from Pseudomonas fluorescens bv. III to Pseudomonas putida. The microbial community resident in replant orchard soils after growing wheat also was suppressive to an introduced isolate of Rhizoctonia solani anastomosis group 5, which causes root rot of apple. Incorporation of high glucosinolate containing rapeseed ('Dwarf Essex') meal also enhanced growth of apple in replant soils through suppression of Rhizoctonia spp., Cylindrocarpon spp., and Pratylenchus penetrans. Integration of these methods will require knowledge of the impact of the biofumigant component on the wheat-induced disease-suppressive microbial community. Implementation of these control strategies for management of apple replant disease awaits confirmation from ongoing field validation trials.

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result in development of a soil microbial community that is both conducive to and capable of inciting apple replant disease. Knowledge of the microbial community resident in soils that support optimal tree growth could also provide clues as to the relative importance of specific microorganisms in disease suppression.

Changes in microbial community structure in response to planting apple were documented at a site that had been cropped to dryland wheat prior to orchard establishment (17). A soil microbial community capable of inciting symptoms of apple replant disease developed within 3 years of orchard establishment, and in contrast to non-replant soil from this site, growth of apple was significantly enhanced by pasteurization of replant soil. The relatively poor growth of apple was associated with increased recovery of fungi belonging to the genera *Cylindrocarpon*, *Phytophthora*, *Pythium*, and *Rhizoctonia*. Significant changes in rhizosphere bacterial communities were also observed. These included dramatic reductions in relative recovery of *Burkholderia cepacia* with prolonged orchard establishment, and transformation of the fluorescent *Pseudomonas* population from one dominated by *Pseudomonas putida* to one comprised almost exclusively of *Pseudomonas fluorescens* bv. III and *Pseudomonas syringae*.

Interestingly, the microbial community from non-replant soil suppressed root rot caused by an introduced isolate of *Rhizoctonia solani* anastomosis group 5 (AG-5), but soil from the same site that had been in apple production for three or more years was conducive to disease development (17). The majority of isolates of *Pseudomonas putida* from this site suppress in vitro growth of each element of the fungal complex that incites replant disease and provide biological control of *Rhizoctonia* root rot of apple (6, 16, 19). In contrast, the overwhelming majority of isolates of *Pseudomonas fluorescens* bv. III did not exhibit in vitro inhibitory activity toward any of the target fungi. These findings suggest a role for certain fluorescent pseudomonads in the suppression of *R. solani* AG-5 observed in the non-replant soil and that establishment of a wheat cover crop during orchard renovation could benefit apple growth on replant sites.

**Stimulation of a disease suppressive microbial community.**

Capturing the essence of naturally occurring disease suppressive soils has long been a goal of plant pathologists. Based on the *Rhizoctonia*-suppressive nature of former “wheat-field” soil prior to orchard establishment, studies were conducted to determine the feasibility of a phyto-remediation approach for the control of apple replant disease. The intent was to use short-term wheat cultivation of replant soils as a means to enhance populations and activity of resident microbial antagonists. In greenhouse experiments, replant soils were cultivated to three successive 28-day cycles of wheat and planted to ‘Gala’ apple seedlings. Prior cultivation with any of three wheat cultivars substantially improved the growth of apple in orchard replant soils (19). Although the relative growth response of apple was consistent among multiple replant soils, the magnitude of the response varied among the three wheat cultivars examined (‘Penawawa’ > ‘Rely’ > ‘Eltan’). Enhanced growth of apple in response to prior growth of wheat in replant soils was associated with significant reductions or complete elimination of apple root infection by species of *Pythium* and *Rhizoctonia*. In addition, while *Pratylenchus penetrans* populations were far below damage threshold levels (10), prior growth of wheat further and substantially reduced populations of this nematode in apple seedling roots.

Control of these soilborne fungal pathogens and *Pratylenchus penetrans* in response to wheat cultivation of orchard replant soils was associated with significant changes in composition of the fluorescent *Pseudomonas* community (19). Prior to wheat cultivation, *Pseudomonas fluorescens* bv. III and *Pseudomonas syringae* were dominant, respectively, in replant soils and the rhizosphere of apple grown in these soils. In contrast, *Pseudomonas putida* dominated the population recovered from replant soils following wheat cultivation, and this species represented a significant component of the *Pseudomonas* population isolated from the rhizosphere of apple grown in the same soil. Among the three wheat cultivars, ‘Penawawa’ was superior, not only in the ability to promote growth of apple in replant soils but also in the ability to enhance populations of *Pseudomonas putida* that were capable of colonizing the rhizosphere of apple. Relative recovery of *Burkholderia cepacia* from soil or the apple rhizosphere was not altered by prior cultivation of replant soils with wheat, but recovery of actinomycetes from apple roots increased significantly.

The microbial community derived through wheat cultivation of an apple orchard replant soil in the greenhouse was assessed for its ability to suppress an introduced isolate of *R. solani* AG-5. Soil from the Columbia View Research and Demonstration (CV) Orchard, WA, was cultivated to three successive 28-day cycles of ‘Eltan’, ‘Penawawa’, or ‘Rely’ wheat. Wheat-cultivated, non-treated and pasteurized orchard soils were infested with oat-bran inoculum of *R. solani* AG-5 strain 5-103 (14) at a rate of 0.1% (vol/vol). These artificially infested, treated and nontreated orchard soils were planted with 6-week-old ‘Gala’ apple seedlings. Plants were harvested after 12 weeks. The CV orchard replant soil contained a resident population of *Rhizoctonia* spp., and amendment of this soil with *R. solani* AG-5 resulted in a significant increase in recovery of *Rhizoctonia* spp. from apple (Table 1). The microbial community that developed in response to cultivation of CV orchard soil with any of three wheat cultivars suppressed root infection by the introduced isolate of *R. solani*. As was observed for suppression of indigenous *Rhizoctonia* spp. (19), prior cultivation of orchard soil with ‘Penawawa’ appeared superior to either ‘Eltan’ or ‘Rely’ wheat for suppression of the introduced isolate of *R. solani* AG-5 (Table 1).

In subsequent studies, we have demonstrated that the ability to enhance apple growth in replant soils is not universal among wheat cultivars. Likewise, cultivation of the same orchard soils with annual ryegrass had no effect on subsequent growth of apple (M. Mazzola and Y.-H. Gu, unpublished data). Wheat cultivars that enhanced subsequent growth of apple in replant soils produce root exudates that support growth of the biocontrol bacterium *Pseudomonas putida* strain 2C8 (16) when used as a sole carbon

**TABLE 1. Impact of prior wheat cultivation on growth of ‘Gala’ apple seedlings in Columbia View Research and Demonstration orchard replant soil artificially infested with *Rhizoctonia solani* anastomosis group 5 (AG-5) strain 5-103a**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root weight (g)</th>
<th>Shoot weight (g)</th>
<th>Shoot height (cm)</th>
<th>% Root infectionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (−)</td>
<td>1.14 a</td>
<td>1.13 a</td>
<td>9.8 a</td>
<td>17.3 b</td>
</tr>
<tr>
<td>Control (+)</td>
<td>0.91 a</td>
<td>0.98 a</td>
<td>9.1 a</td>
<td>29.4 c</td>
</tr>
<tr>
<td>Pasteurization (+)</td>
<td>1.37 ab</td>
<td>2.28 b</td>
<td>12.2 a</td>
<td>44.0 d</td>
</tr>
<tr>
<td>‘Eltan’</td>
<td>1.84 bc</td>
<td>3.01 c</td>
<td>16.4 b</td>
<td>13.3 b</td>
</tr>
<tr>
<td>‘Penawawa’</td>
<td>2.16 c</td>
<td>3.38 c</td>
<td>17.8 b</td>
<td>2.2 a</td>
</tr>
<tr>
<td>‘Rely’</td>
<td>1.42 ab</td>
<td>2.93 bc</td>
<td>16.8 b</td>
<td>17.7 b</td>
</tr>
</tbody>
</table>

a Means in a column followed by the same letter are not significantly (P = 0.05) different based on the Student-Newman-Keuls procedure.

b Soil was cultivated to three successive 28-day cycles with one of the three wheat cultivars listed. Wheat-cultivated, control (+), and pasteurized (+) soils were infested with *R. solani* at a rate of 0.1% (vol/vol). Inoculum of *R. solani* AG-5 strain 5-103 was not added to control (−) soil. Soils were planted to 6-week-old apple seedlings with five seedlings in each of three replicates. Plants were harvested after 12 weeks.

c Root infection by *Rhizoctonia* spp. was assessed by plating 10 root segments from each seedling onto 1.5% water agar amended with ampicillin at 100 µg ml⁻¹.
source in minimal media. In contrast, root exudate from a wheat cultivar that did not exhibit the ability to enhance subsequent growth of apple, or that provided a negligible growth response, exhibited a reduced or no ability to support growth of *Pseudomonas putida* strain 2C8 (M. Mazzola and Y.-H. Gu, unpublished data). These data strongly suggest that alterations in composition of the fluorescent pseudomonad community, at least in part, contribute to the reduction in disease severity achieved through cultivation of replant soils with wheat prior to planting apple.

Cover cropping and crop rotation systems have long been used to manage soilborne plant pathogens. They are perceived to function, in part, by denying the pathogen substrate necessary for survival, growth, and reproduction. Increasingly, these systems have included species of the Brassicaceae that suppress pathogens through production of allelochemicals, such as glucosinolate hydrolysis products including isothiocyanates (3). Previous studies have shown that a wheat cover crop can suppress lesion nematode populations (22) and increase inoculum potential of vesicular-arbuscular mycorrhizal fungi (2) in annual cropping systems. The scheme employed in our studies suggest that wheat cultivation can suppress disease not simply through denying a suitable host to the pathogen but also by stimulating of microbial communities that are suppressive to these pathogens and beneficial to apple growth.

**Application in orchard ecosystems.** Although crop rotation is not an economically viable pest control strategy in the management of perennial tree fruit crops, habitat manipulation through the maintenance of certain cover crops in orchard ecosystems has been examined extensively as a means to manage various insect pests (4,25). Various cover crops have been used to harbor distinctive complexes of natural enemies of orchard insect pests (7,11).

Cultivation of cover crops during orchard renovation has been suggested as a means to enhance the growth of newly established apple trees on old orchard sites. However, beyond insect pest management, these attempts have primarily examined use of cover cropping systems as a means to enhance nutrient availability (1) or suppress populations of the lesion nematode (12,20). The wheat cultivation system we have employed appears to function, in part, through enrichment of a microbial community that is suppressive toward the fungal complex that incites apple replant disease. As such, a significant time interval is likely to be required in a field setting to induce such a response, and the duration of this interval will determine the feasibility of such an approach in managing apple replant disease. Current field studies are attempting to answer just this question. In a preliminary step, soils were collected from orchard blocks within a replant site that had been left fallow or cultivated to ‘Penawawa’ wheat for 1 year. These soils, and soil collected from the same site where trees had not been removed (control), were planted to ‘Gala’ apple seedlings in the greenhouse. Based on these bioassays, the distinctive shift in composition of the fluorescent pseudomonad population observed in greenhouse trials (19) was initiated in the field after 1 year of wheat cultivation. Likewise, growth of apple seedlings in the greenhouse was greater in soils cultivated to wheat in the field relative to that obtained in the bare fallow treatment (Table 2). The 1-year bare fallow provided no benefit to seedling growth, nor was a shift in composition of the fluorescent pseudomonad population detected in this treatment.

**Integration of cultural practices.** Although prior cropping to wheat provides a significant benefit to growth of apple in replant soils, this treatment often fails to induce a growth response equivalent to that achieved by soil pasteurization. The frequency of infection of apple seedling roots by *Pythium* spp., *Rhizoctonia* spp., and *Pratylenchus penetrans* are suppressed by wheat cultivation, whereas frequency of infection by *Cylindrocarpon* spp. is typically not altered by this treatment (19). In contrast, soil pasteurization effectively eliminates apple root infection by all of these agents. This demonstrates the continued need to develop a system for the management of apple replant disease that targets all components of this pathogen complex.

One such effort is the use of a biofumigant cover crop or plant by-product in conjunction with wheat cultivation of replant orchard soils. While the products of glucosinolate hydrolysis have action against a broad spectrum of microorganisms, *Brassica* plant residues incorporated into soil also can be phytotoxic to subsequent crops (21,27). Application of *Brassica napus* cv. Dwarf Essex seed meal caused extensive damage and death of ‘Gala’ apple on M.26 rootstock when applied directly into the tree hole at the time of planting (D. M. Granatstein, unpublished data). Thus, effective use of this material will require information concerning appropriate rates, duration of incubation period between application and planting, and the evaluation of other practices that could limit toxicity toward apple.

Glucosinolate hydrolysis products resulting from degradation of *Brassica* plant residues have been cited as the primary mechanism of pathogen suppression. However, studies using *Brassica napus* seed meal amendments varying in glucosinolate content but not composition indicate that the role of these compounds may be pathogen-dependent (18). Application of seed meal to orchard replant soils suppressed apple root infection by *Rhizoctonia* spp., and the level of disease suppression was independent of glucosinolate content. In contrast, root infection by *Pythium* spp. remained static in soils treated with the high glucosinolate seed meal but increased dramatically in response to soil amendment with low glucosinolate seed meal. Control of *Rhizoctonia* spp. may have operated through a microbial mechanism because both seed meals stimulated soil populations of total bacteria, fluorescent *Pseudomonas* spp., and actinomycetes in an equivalent manner.

Effective integration of wheat cultivation with this allelopathic plant residue for control of apple replant disease will be influenced by the impact of glucosinolate hydrolysis products on plant beneficial microbial communities. In greenhouse trials, the benefit of rapeseed meal amendments to subsequent growth of apple in replant soils was rate dependent, with higher rates being phytotoxic to apple (Table 3). Cultivation of wheat subsequent to rapeseed meal amendment of replant soils significantly reduced

### Table 2. Growth of ‘Gala’ apple seedlings in greenhouse trials in replant soils cultivated to ‘Penawawa’ wheat in the field at the Columbia View Research and Demonstration orchard, WA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root weight (g)</th>
<th>Shoot weight (g)</th>
<th>Shoot height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.30 a</td>
<td>0.58 a</td>
<td>9.1 a</td>
</tr>
<tr>
<td>Pasteurization</td>
<td>0.59 c</td>
<td>0.88 b</td>
<td>13.6 a</td>
</tr>
<tr>
<td>1-year fallow</td>
<td>0.29 a</td>
<td>0.61 a</td>
<td>9.3 a</td>
</tr>
<tr>
<td>1-year ‘Penawawa’</td>
<td>0.44 b</td>
<td>0.82 b</td>
<td>11.9 a</td>
</tr>
</tbody>
</table>

*Soils were planted to 6-week-old ‘Gala’ apple seedlings, with five seedlings in each of three replicates. Plants were harvested after 6 weeks. Means in the same column followed by the same letter are not significantly different (*P* = 0.05) based on the Student-Newman-Keuls test.

### Table 3. Impact of ‘Dwarf Essex’ rapeseed meal amendments on growth of ‘Gala’ apple seedlings in replant soils from the Columbia View Research orchard, WA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
<th>Root weight (g)</th>
<th>Shoot weight (g)</th>
<th>Shoot height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 a</td>
<td>1.01 b</td>
<td>0.96 a</td>
<td>8.5 a</td>
</tr>
<tr>
<td>Pasteurization</td>
<td>0 a</td>
<td>1.72 c</td>
<td>3.52 b</td>
<td>17.0 c</td>
</tr>
<tr>
<td>0.1% rapeseed meal</td>
<td>0 a</td>
<td>2.13 d</td>
<td>3.19 b</td>
<td>15.1 bc</td>
</tr>
<tr>
<td>1.0% rapeseed meal</td>
<td>7 a</td>
<td>1.92 cd</td>
<td>5.64 c</td>
<td>21.6 d</td>
</tr>
<tr>
<td>2.0% rapeseed meal</td>
<td>77 b</td>
<td>0.43 a</td>
<td>1.56 a</td>
<td>11.2 ab</td>
</tr>
</tbody>
</table>

*Soils were planted to 6-week-old apple seedlings with five seedlings in each of three replicates. Plants were harvested after 12 weeks. Means in the same column followed by the same letter are not significantly different (*P* = 0.05) based on the Student-Newman-Keuls test.
phytotoxicity in apple, and was effective in reestablishing the fluorescent pseudomonad population that was eliminated by such an amendment. In greenhouse trials, wheat cultivation of rape-seed-amended replant soils provided a level of disease suppression that was superior to either treatment alone.

CONCLUSION

An important component of sustainable agricultural production systems is the optimization of internal biological resources of the agroecosystem. The development of a systems approach that will harness the genetic and biological resources inherent to resident microbial communities in orchard soil as a means to manage apple replant disease is the goal of this research program. Wheat cultivation during orchard renovation appears to hold promise as a means to suppress most but possibly not all components of the pathogen complex that incites replant disease. Without question, application of such a system awaits field validation of the results obtained in greenhouse studies conducted to date. While this practice is unlikely to serve as a stand alone alternative to methyl bromide or other soil fumigants for the control of apple replant disease, integration of this approach with biocidal plant products or cultural practices such as soil disturbance (16) may provide effective and economically feasible disease control on replant sites.

LITERATURE CITED