AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Microbiome-Mediated Resistance of Wheat to the Take-All Disease

RESEARCH LEADER: Christopher C. Mundt. Note: Work summarized in this report was conducted primarily by graduate student Evan Perkins.

COOPERATORS: none

EXECUTIVE SUMMARY: Microbial antagonist populations were collected from two previously conducted field experiments. In both of the two-year experiments, different wheat cultivars were planted in year 1 to determine if the cultivar grown in the first year would influence the degree of take-all disease in the subsequent wheat season. In both cases, take-all severity in year 2 was reduced by about 50% in plots grown to cultivar 'Bobtail' the previous year. Soil samples were collected at the end of the first season for the 2015-2017 field experiment, and at the end of the second season for the 2016-18 experiment. In both cases, greenhouse tests showed differences in take-all severity among the different soil sources to be similar to that found in the field. In the current study, root washes from these greenhouse experiments showed that 2,4-diacetylphloroglucinol (DAPG)-producing isolates of the bacterium *Pseudomonas* were 6 to 25-fold higher in soils collected from the cultivar Bobtail in comparison with six other cultivars tested. Such bacteria have previously been shown to strongly suppress the take-all disease. The wheat cultivar grown in year 1 had a much greater impact than did the cultivar grown in year 2 or the cultivar used to assay the soil samples in the greenhouse. As wheat cultivars resistant to take-all have been difficult or impossible to find, our results may provide an avenue for identifying wheat genotypes that may suppress this important disease. Further, 2,4 DAPG-producing *Pseudomonads* have been shown to induce systemic resistance against a broad spectrum of diseases, may explain why the cultivar Bobtail possesses a very broad spectrum of disease resistance, and may provide a new mechanism to select for multiple disease resistance in wheat.

OBJECTIVE: Determine if microbial populations known to be antagonistic to the take-all pathogen are higher in soils sown to wheat varieties that suppress the build-up of the wheat take-all disease.

PROCEDURES: Microbial antagonist populations were collected from two previously conducted field experiments. In both of these two-year experiments, different wheat cultivars were planted in year 1 to determine if the cultivar grown in the first year would influence the degree of take-all disease in year 2 when one (first experiment) or two (second experiment) common tester cultivars were grown. In both cases, take-all severity in year 2 was reduced by about 50% in plots grown to cultivar 'Bobtail' the previous year, though there were also some differences among the other cultivars. Soil samples were collected at the end of the first season for the

2015-2017 experiment, and at the end of the second season for the 2016-18 experiment. In both cases, greenhouse tests (using a single cultivar for the first experiment and four cultivars for the second experiment) showed differences in take-all severity among the different soil sources to be similar to that found in the field.

Soil samples from the above greenhouse tests were obtained after growing plants in a 1:3 mixture of sand and field-collected soil in cone-shaped plastic "conetainers". After 8-12 weeks of growth, plants were removed from the containers, shaken, and the adhering soil washed into screw-top centrifuge tubes. These washes were then concentrated in a centrifuge and frozen for future analysis of microbial antagonist populations. It has long been known that certain strains of soil-borne *Pseudomonas* spp. that produce the phenolic compound 2,4-diacetylphloroglucinol (2, 4 DAPG) can reduce the severity of take-all (Weller et al. 2002. Annu. Rev. Phytopathol. 40:309- 348). We used the methodology developed by McSpadden et al. (2001. Phytopathology 91:44-54) to determine the relative population sizes of 2,4 DAPG-producing *Pseudomonads* in the concentrated root washes. We utilized a PCR primer that recognizes a gene in the biochemical pathway that produces 2,4 DAPG. The root washes were first pipetted onto *Pseudomonas*-specific liquid growth media. PCR reactions were then conducted on serial dilutions of the cultures in microtiter plates. The initial samples were diluted three times, followed by subsequent 5X dilutions. The last dilution at which the PCR product is recognized provides an estimate of the relative concentration of 2,4 DAPG-producing *Pseudomonas* in a given sample. We also back-calculated from the dilution endpoints to obtain estimates of the relative numbers of 2,4 DAPG-producing *Pseudomonads* for each main effect and normalized them to the level of Bobtail for each main effect. Initial effort was focused on samples from the 2017-18 field experiment, as these samples were collected after two years of host selection in the field and from two different cultivars in year 2, and were tested against four different cultivars in the greenhouse. Samples from the first experiment were collected at the end of year 1 and had lower *Pseudomonad* population sizes. Thus, we simply recorded the number of positive PCR hits in the first dilution as a rough measure of *Pseudomonad* population size.

SIGNIFICANT ACCOMPLISHMENTS:

Samples from the second field experiment showed substantial impacts on levels of 2,4 DAPG-producing *Pseudomonads*. Of the three factorial main effects incorporated within these samples, the wheat cultivar grown in year 1 had the largest impact on 2,4 DAPG-producing *Pseudomonas* population size (Fig. 1). In particular, samples from plots that were initially sown to cultivar Bobtail in year 1 had 6 to 25-fold more 2,4 DAPG-producing *Pseudomonads* as compared to the other cultivars. Cultivar Rosalyn had the next highest level of 2,4 DAPG-producing *Pseudomonads* after Bobtail and was the second most successful cultivar reducing take-all severity in the previous field and greenhouse experiments. Cultivars LCS Art Deco and Stephens had the lowest *Pseudomonad* populations and the highest levels of take-all disease. The cultivar grown in the second year of wheat production (Fig. 2) and the tester cultivar used in the greenhouse (Fig. 3) had much smaller impacts on the size of *Pseudomonad* populations than did the effect of the cultivar grown in year 1. These results suggest that 2,4 DAPG-

producing *Pseudomonas* populations attain dominance early when favorable wheat cultivars are grown, and maintain this dominance in subsequent crops.

Populations of 2,4 DAPG-producing *Pseudomonads* in soil samples collected after year 1 of the first experiment were overall lower than those collected after year 2 of the second experiment. As a result, it was not possible to conduct dilution endpoint analyses. Nonetheless, there clearly were more PCR hits for 2,4 DAPG-producing *Pseudomonads* in soils collected from Bobtail wheat in comparison to the other five cultivars evaluated (Fig. 4). This result suggests that the favorable impact of cultivar Bobtail on the 2,4 DAPG-producing *Pseudomonad* populations may be a consistent result.

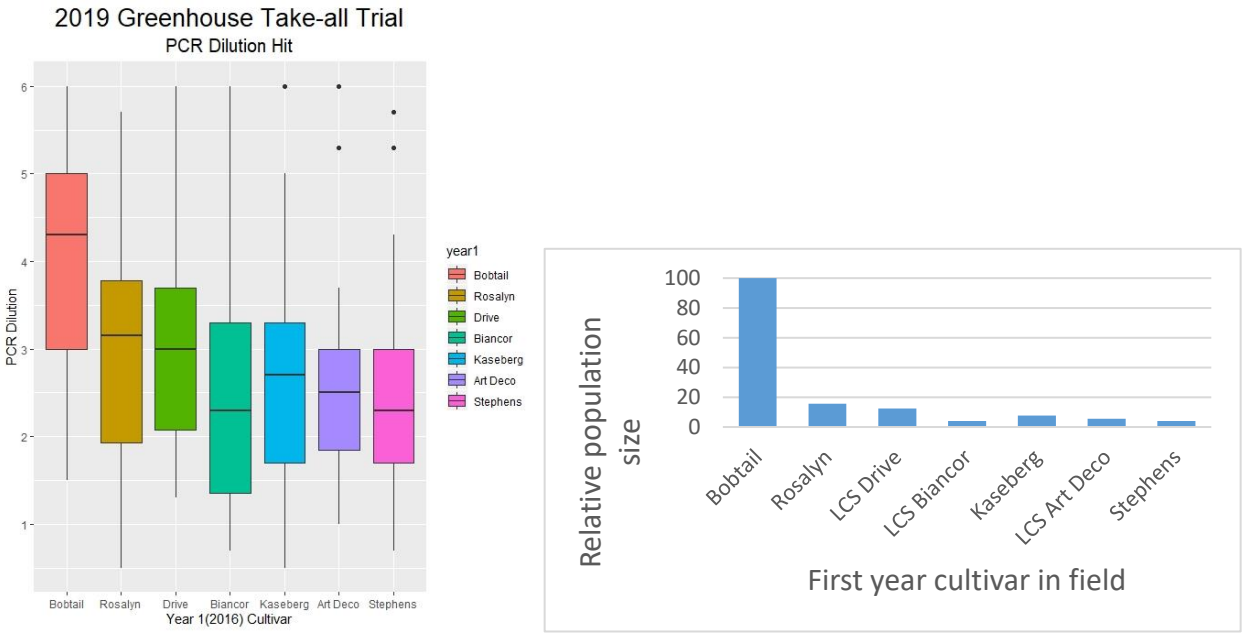

Figure 1. Effect of the wheat cultivar grown in the first year of a field rotation on levels of 2,4 DAPG-producing *Pseudomonas* bacteria in root washes collected in subsequent greenhouse tests. Each bar is the mean over both cultivars grown in year 2 and the four tester cultivars utilized in four greenhouse trials. Left panel shows dilution endoint data, which were used to calculate population sizes relative to the cultivar Bobtail, shown in the right panel.

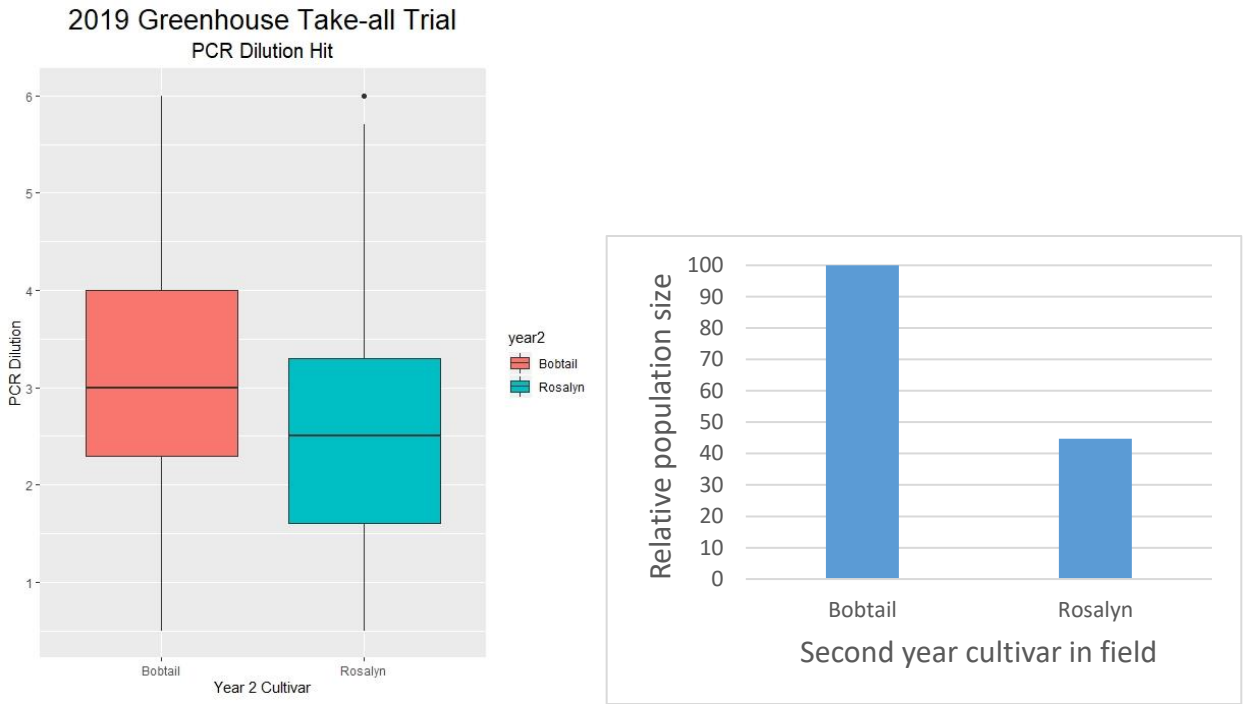

Figure 2. Effect of the wheat cultivar grown in the second year of a field rotation on levels of 2,4 DAPG-producing *Pseudomonas* bacteria in root washes collected in subsequent greenhouse tests. Each bar is the mean over all seven cultivars grown in year 1 and the four tester cultivars utilized in four greenhouse trials. Left panel shows dilution endpoint data, which were used to calculate population sizes relative to the cultivar Bobtail, shown in the right panel.

Figure 3. Effect of the wheat cultivar used as a tester in greenhouse studies on levels of 2,4 DAPG producing *Pseudomonas* bacteria in root washes collected in subsequent greenhouse tests. Each bar is

the mean over seven cultivars grown in the field in year 1, two cultivars grown in in the field in year 2, and four greenhouse trials. Left panel shows dilution endoint data, which were used to calculate population sizes relative to the cultivar Bobtail, shown in the right panel.

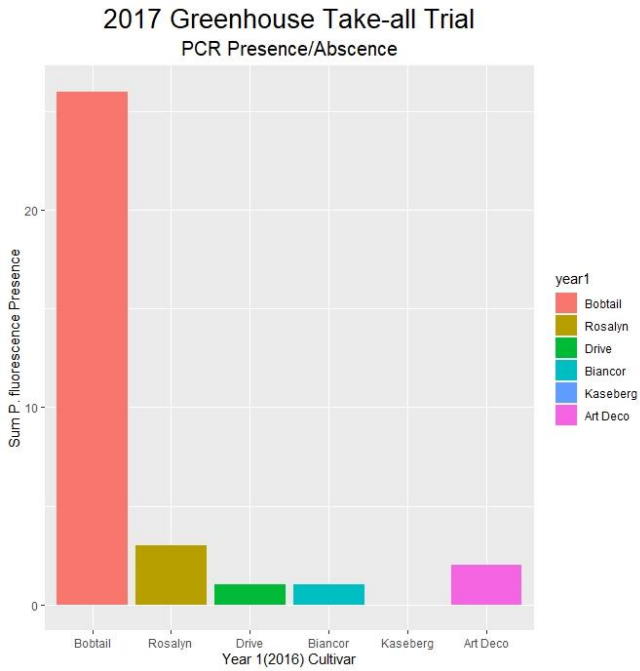

Figure 4. Effect of six wheat cultivar grown in the field on levels of 2,4 DAPG producing *Pseudomonas* bacteria in root washes collected in subsequent greenhouse tests. Each bar represents the number of positive PCR hits out of a possible 36.

BENEFITS & IMPACT: Results to date suggest that we may have, for the first time, the opportunity to suppress the devastating take-all disease through genetic resistance of wheat cultivars. Work proposed herein will help to understand the underlying mechanism such that the trait can more easily be identified in breeding programs to produce future cultivars that suppress take-all development; determining antagonistic microbial populations is a much faster process than field experiments, which require large plots and two years to conduct. Further, the cultivar Bobtail has been shown to have resistance to a very broad range of diseases. Genetic and other evidence suggests that there may be a common mechanism impacting multiple diseases. As the cultivar Bobtail was originally selected from a molecular mapping population, we may be able to eventually develop DNA-based markers in wheat that are associated with enhanced populations of bacterial antagonists against take-all and perhaps other diseases. This would make the take-all disease an ideal experimental system for studying microbiome-mediated disease resistance. Though there has been substantial interest in manipulating microbiomes to control disease, our take-all example appears to be one of very few where this is actually being accomplished in the “real world”. This would make the take-all disease an ideal model system for pursuing federal funding in this currently very “hot” area of studying plant microbiomes.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: none

FUTURE FUNDING POSSIBILITIES: I was invited by the Idaho Wheat Commission to submit a proposal to further advance this area of research. A proposal of $52,052 for year one has been submitted, with anticipation of two subsequent years if the first year is funded and progress is adequate. I am also considering potential application to the NSF/USDA-NIFA Plant Biotic Interactions Program.