

ANNUAL REPORT

PROJECT NO: GNK361, BJKT43

TITLE: Beneficial Endophytes of Winter Wheat

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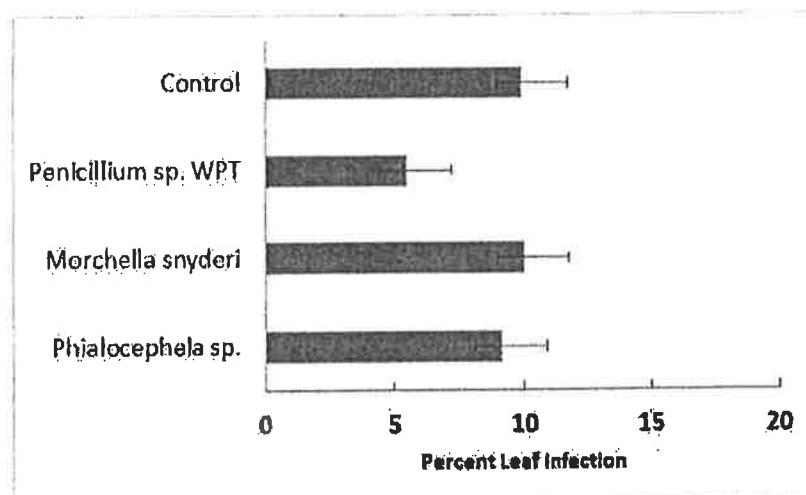
ACCOMPLISHMENTS: Endophytic fungi, or fungi that can colonize the internal tissues and organs of plants, form an integral part of a plant's microbiome and can benefit host plants by mediating resistance to biotic and abiotic stress. We hypothesized that endophytic microbes recovered from plants in the intermountain Pacific Northwest may provide such benefits to wheat production via novel symbioses. Preliminary findings in greenhouse and laboratory assays indicated that some fungi could improve yield and vegetative biomass in drought-stressed winter wheat infected with *Fusarium* crown rot (Ridout and Newcombe 2016), suppress spore viability of *Fusarium* spp. in direct competition, and alter myotoxin production by *Fusarium* spp. To examine effects at a field level and determine whether microbes effective in laboratory and greenhouse trials might be equally effective in the field, we established replicated field trials of winter wheat in October of 2015.

Two paired trials of winter wheat were sown in replicated dryland 5' X 20' test plots at the University of Idaho Kambitsch Farm near Genessee, ID. One trial assessed the effects of fungal microbes on the physiological status of winter wheat and potential contributions of these fungi to tolerance of abiotic stresses. The second assessed the ability of fungi to mediate resistance to *Fusarium* crown rot. For each trial, two classes of winter wheat were sown: hard red (306 UI SRG) and soft white (Stephens). For the pathogen resistance trial, sterile millet inoculated with *Fusarium culmorum* was mixed with the wheat seed in half the test plots at planting. Viable microbial inocula were generated by suspending fungal spores and/or finely ground fungal hyphae in distilled water. The physiology trial comprised four treatments: *Morchella snyderi*, *Phialocephala* sp., *Penicillium* sp. WPT and a water control. The pathogen trial was composed of an eight-treatment factorial, including three microbial treatments (*Clonostachys rosea*, *Pichia membranifaciens*, and *Penicillium* sp. WPT), a water control, and all four treatments in combination with the pathogen. Applications were made to emergent seedlings in late fall and again in early spring.

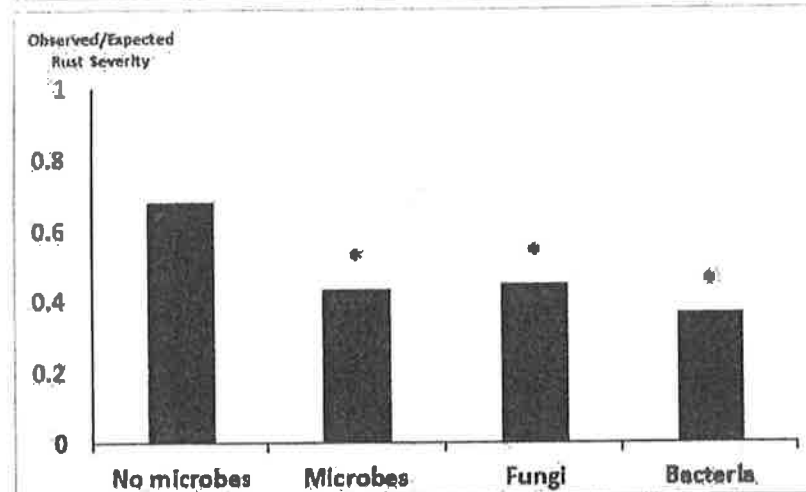
Data were collected throughout the growing season beginning with stand counts and included crown rot scoring, stripe rust coverage in the Stephens plots (from a naturally occurring outbreak), infrared plot temperature readings, stomatal conductance, foliar nutrient analyses, yield, and test weights.

Due to a wet late spring, the severity of *Fusarium* crown rot was strongly reduced. The crop suffered little stress throughout the growing season, which likely reduced the efficiency of potential symbioses between the microbes and the novel host. Average stomatal conductance indicated the plants were not under stress. Conductance declined over the course of the season with the maturation of the crop, but there were no significant effects of microbial treatments on gas exchange. Crop canopy temperatures were not affected by treatment even over time as confirmed by both infrared temperatures and leaf surface temperatures. Foliar nutrient content was also not significantly influenced by microbial treatment. Although the presence of *Fusarium* inoculum reduced yield slightly, these reductions were not significant and crown

rot scoring failed to reveal any significant increase in severity compared to untreated controls. Regardless of treatment, fungi failed to significantly increase yields or test weights in either the pathogen resistance or physiology trials, although trends indicated small increases in yield compared to the controls with the addition of microbes, particularly in the presence of *Fusarium* inoculum. Stand counts were neither significantly improved by microbial treatments compared to controls nor significantly reduced by the presence of the *Fusarium* inoculum.



Expression of rust infection in the Stephens trials was highly variable across plots and within plots leading to a lack of significant differences among treatments. However, in Stephens plots from the physiology trials, percent coverage of infected leaves by stripe rust was reduced almost by half in plots treated with *Penicillium* WPT (left).



Above: Seedlings from wheat seed that contained no culturable microbes had a significantly higher leaf rust severity ratio than those from seed that had microbes. Of these, two groups were dominant—fungi and bacteria. When compared to seedlings from seeds that had no microbes, seedlings from seeds containing bacteria had the lowest severity ratio. The asterisks indicate significant decreases in leaf rust severity compared to the controls (no microbes).

One problem faced in the research of microbiome management for exploiting microbial technologies and understanding thresholds of efficacy from laboratory to field is understanding microbiome assembly and the role it might play in the function of individual microbes as beneficial, neutral, or negative. Essentially, microbiome assembly in a plant starts in the seed. In laboratory experiments, it was determined that 45% of wheat seeds contain no culturable microbes and 96% of those seeds colonized contained only one microbe. In a follow-up greenhouse assay, plants from seed naturally containing one endophytic microbe had a 25% lower severity ($P=0.003$) of leaf rust infection than plants that lacked any culturable microbial associate at planting. Both bacterial and fungal microbes reduced severity of infection; however, bacterial seed-borne microbes were most effective.

Laboratory assays also indicated that microbes associated with the seed could improve the vigor of the seed and subsequent seedling. In replicated laboratory germination assays, spores of four *Penicillium* spp. isolates were applied to seeds of hard red winter wheat cultivar UI SRG Three of the *Penicillium* spp.

increased germination compared to untreated controls and all four *Penicillium* isolates stimulated significantly greater seedling growth ($P=0.00002$) than untreated control seedlings.

Since microbes associated with seed appear to have effects on plant health and development, microbes applied in the 2016-2017 winter wheat field trials were applied to seed prior to planting. Two replicated factorial disease trials were sown. The first trial repeated the 2015-2016 *Fusarium* crown rot trial in the hard red winter wheat 306 UI SRG. Based on the summer 2016 stripe rust data, a second trial was sown with the susceptible soft white wheat Stephens. Four microbial treatments were applied in each trial based on selections from laboratory, greenhouse and winter 2015-2016 field trials: *Clonostachys rosea*, *Penicillium* sp. WPT, *Pichia membranifaciens*, and a control were applied in a HRWW/crown rot trial and *Cladosporium* sp. POP1, *Penicillium* WPT, *P. membranifaciens*, and a control were applied in a SWWW/stripe rust trial. Microbial inocula were suspended in the 0.5% methylcellulose solution which was then coated onto seed in a tumbler. After some experimentation, methylcellulose was selected for seed coating due to its lack of phytotoxicity. *Fusarium* was applied to the crown rot trial at planting encapsulated on millet with methylcellulose. Stripe rust will be applied in the spring to emergent seedlings in the rust trial.

PROJECTIONS: Outcomes from the coming 2016-2017 field trials may be key to determining if endophytic microbes applied to wheat systems can benefit the crop under adverse conditions. Given that the presence of microbes within seed may improve plant health and performance, seed treatment in this winter's field trial may bring more effective responses—particularly if environmental conditions induce greater stress on the plants. Data from laboratory, greenhouse, and field experiments in both the wheat program and supporting corn project clearly show a strong biotic and abiotic context dependency in plant response to microbial inoculation making microbial exploitation challenging. However, data indicate that endophytes transferred vertically in the seed may provide positive benefits to host plants, including increased resistance to rust pathogens. Before microbes can be effectively utilized in the field, more basic research must be done to determine which biotic and abiotic parameters most shape a plant's microbiome.

Outcomes from this project and the supporting corn project are expected to be used to leverage more basic research funding. At present, we will not be pursuing a third year of funding from the IWC. However, if further testing indicates improved resistance to rust and improved biotic and/or abiotic stress resistance from the presence of natural seed endophytes, we will begin to move forward with translating positive effects to the field by management of seed and seedling microbiome development and microbial seed treatments for crop improvement.

Research from this project will be used to leverage funding through the NSF/USDA joint Plant Biotic Interactions program to examine the scope and significance of vertically transmitted seed endophytes to plant health and development.

At least two publications are expected this year directly from this research and a second from similar research in corn.

PUBLICATIONS: Ridout, M.E., Newcombe, G. and Godfrey, B., 2016. First report of *Fusarium temperatum* in diseased sweet corn ears in the western United States. *Plant Disease*. 100(12):2527. <http://dx.doi.org/10.1094/PDIS-04-16-0518-PDN>