

PROJECT NO: GNK360, BJKU43

TITLE: Beneficial Endophytes for Winter Wheat

PERSONNEL: George Newcombe, Professor; Kurtis Schroeder, Assistant Professor; Mary Ridout, Postdoctoral Fellow; Shawna Faulkner, Graduate Student

LEAD PI: George Newcombe, 875 Perimeter Dr., MS1133, College of Natural Resources, Moscow, ID 83844-1133; Phone: 208-596-8271; E-mail: georgen@uidaho.edu **Correspondence and inquiries should be directed to:** Mary Ridout, E-mail: mridout@uidaho.edu

JUSTIFICATION: Wheat production in Idaho faces significant biotic and abiotic pressures, including serious pathogens (i.e., crown rot, *Fusarium* head blight, and rusts) and drought. Nutrient and water use efficiency are becoming increasingly important as costs for fertilizers escalate and a shifting climate changes precipitation patterns. Public perspectives on use of chemical products in agriculture are demanding alternatives for disease management. Even as agricultural production faces increasing biotic and abiotic pressures and escalating demand for production with burgeoning populations, calls are coming for reduction in the use of pesticides, commercial fertilizers, and water. Although significant progress has been made in breeding programs, the process is slow. In an arms race with pathogens and drought, multiple technologies should be examined.

Endophytic microbes colonize internal plant tissues and may confer substantial benefits to the host. Endophytic forest fungi from the dry, native environments of the PNW were found to improve yield by nearly 50% in drought-stresses wheat challenged by *Fusarium* crown rot and tripled survival of crown rot infect plants (1). Forest fungi have increased sweet corn yield by 25% to as much as 40% in our HM-Clause collaborative field plots in Nampa ID, USA and reduced *Fusarium* presence in the ears (unpublished). Forest fungi have also increased both above and below-ground biomass in greenhouse and field trials with wheat and sweet corn, respectively. Year 1 laboratory assays from the summer of 2015 identified an endophytic yeast capable of reducing the reproductive capacity of pathogenic *Fusarium* spp. by as much as 94%. In greenhouse testing, a forest fungus was found to parasitize *F. culmorum* and completely inhibit its ability to infect hard red winter wheat. As generalists, these same fungi may be efficacious not only against crown rot but also for biocontrol of *Fusarium* head blight (FHB) in the field.

Endophytes represent an emerging technology with potential for multiple applications. Preliminary data indicate more robust plants with greater drought and disease tolerance, earlier maturation and higher yield. Increased nutrient use efficiency from facilitated uptake and better root architecture and preemptive control of pathogens in inoculated plants could reduce chemical input costs. Microbially-mediated drought tolerance could be highly beneficial in regions where drought is becoming more limiting to productivity. Beneficial forest endophytes are locally common to the region, yet they represent species not usually found in agronomic crops. Once established, they also have the potential to remain in soil and residues to carry over to the next crop, making them cost-effective. If forest endophytes can be effectively incorporated into field cultural practices and enable reduced chemical applications without diminishing yield, they would not only reduce farm costs per acre, but also help meet societal demands for more sustainable crop production and fewer chemical inputs.

HYPOTHESIS & OBJECTIVES: Endophytes capable of mediating disease and stress tolerance in wheat and other cereals in greenhouse assays have yet to be tested in wheat production in the field. We hypothesize that endophyte applications to wheat under field conditions will improve disease resistance, stress tolerance, and yield. To test this hypothesis we will use a three-tiered experimental approach.

- A. Laboratory assays will be conducted to screen endophytes for tolerance to abiotic stresses and interactions with target pathogens.
- B. Greenhouse assays will be conducted in multiple wheat varieties and classes to test the efficacy of selected endophytes to mediate resistance to disease and abiotic stresses.
- C. Field studies will be conducted to verify laboratory & greenhouse results in the field.

PROCEDURES: Based on first year outcomes from laboratory and greenhouse assays, we would like to establish replicated field trials to test the efficacy of *Fusarium*-antagonizing fungi to reduce incidence and severity of FHB. Susceptible lines of hard white and hard red wheat will be sown in the spring trials in 5' by 20' plots. Plots will be replicated in five blocks. Each plot will be inoculated with a single endophyte applied to the seed prior to sowing and again during initial heading. Fungi currently being considered for testing include *Pichia membranifaciens* and *Clonostachys rosea*. Trials will be monitored for disease development and severity. Infected material will be tested for mycotoxin content. Other data will include yield, test weight, grain protein and water use efficiency. These trials would be established in S. Idaho.

Beneficial fungi providing positive outcomes in 2016 physiology and disease winter trials will be tested in a new set of replicated field trials in 2017 to determine repeatability. If possible, we would also like to expand physiology trials to include trials in S. Idaho. Additional fungi, including species of *Penicillium*, *Geopyxis*, and *Cladosporium*, with ability to solubilize nutrients for plant use will be incorporated into these trials. Trials will be inoculated via seed treatments or following emergence. Data collected will include spectral reflectance for crop stress analysis, porometry measurements for water use efficiency, time to joint formation and anthesis, and foliar nutrient analyses. Data will be geospatially correlated to microsite characteristics (ambient temperature, RH, soil water and nutrient content, and soil type) to better refine microbial selection and application technologies. At the conclusion of the experiments, seed yield, test weight and grain protein will be collected.

Simultaneously, new laboratory assays will be conducted to screen more endophytes collected from natural plant ecosystems within and adjacent to wheat production areas for ability to inhibit development of or reduce viability of selected wheat pathogens (e. g. *Fusarium* spp. and *Puccinia* spp.). Endophytes will also be screened for ability to confer drought tolerance, water use efficiency and nutrient use efficiency or nutrient fixing ability. Assays will include use of selective media (e.g. glycerol-based xerophile media) and detached plant tissues.

Laboratory assays will be supported by greenhouse assays to test promising endophytes in wheat seedlings. Pathogen-susceptible varieties of different classes will be inoculated with endophytes and challenged with FHB *Fusarium* spp., root and crown rot pathogens, or stripe rust (*Puccinia striiformis* f. sp. *tritici*). Plants will be scored for infection or rust pustule density and vegetative biomass data will also be analyzed. Contributions of endophytes to water use efficiency, drought tolerance, and nutrient use efficiency will also be examined.

Microbes showing high potential in greenhouse and field trials will undergo laboratory testing for product formulations for efficient field application methods (e.g. polymer coating, prills, soluble sprays).

DURATION: This proposal is for the second year of a continuing two-year project. A third year may be requested in order to test repeatability of field studies.

COOPERATION: HM Clause, a subsidiary of Limagrain Cereal Seeds, is providing partial support for a post-doctoral researcher for similar work in sweet corn seed production.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER:

If field applications are successful, we anticipate development of beneficial endophyte technologies for wheat production with potential to expand technology to other small grains. The long-term expected goal would be development of a cost-effective commercial product for application in wheat production. Peer-reviewed publications are expected and results will be presented to industry at state and regional meetings.

LITERATURE REVIEW: A number of endophytic fungi are known to mediate host resistance to pathogens and abiotic stresses (2). Forest microbes are known to suppress incidence of *Fusarium* spp. in soils and reduce severity of *Fusarium* crown rot in wheat (1, 3). Dingle and Mcgee (4) identified endophytes capable of reducing severity of the wheat leaf rust, *Puccinia recondita* f. sp. *tritici*. Endophytes have also been shown to mediate drought and heat tolerance in crop species (5). They may improve nutrient use efficiency while improving growth and yield (6, 7). These benefits may be more marked when novel relationships are formed between endophyte and hosts of differing origins (8), such as forest endophytes and wheat.

- (1) Ridout, M., and Newcombe, G. (2016). Disease suppression in winter wheat from novel symbiosis with forest fungi. *Fungal Ecology*, 20, 40-48.
- (2) Rodriguez, R., & Redman, R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany*, 59, 1109-1114.
- (3) Schisler, D. A., and Linderman, R. G. (1984). Evidence for the involvement of the soil microbiota in the exclusion of *Fusarium* from coniferous forest soils. *Canadian Journal of Microbiology*, 30, 142-150.
- (4) Dingle, J., & Mcgee, P. A. (2003). Some endophytic fungi reduce the density of pustules of *Puccinia recondite* f. sp. *tritici* in wheat. *Mycological Research*, 107, 310-316.
- (5) Hubbard, M., Germida, J., & Vujanovic, V. (2012). Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany*, 90, 137-149.
- (6) Kucey, R. M. N. (1987). Increased phosphorus uptake by wheat and field beans inoculated with a phosphorus-solubilizing *Penicillium bilaji* strain and with vesicular-arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology*, 53, 2699-2703.
- (7) Newsham, K. K. (2011). A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist*, 190, 783-793.
- (8) Baynes, M., Newcombe, G., Dixon, L., Castlebury, L., & O'Donnell, K. (2012). A novel plant-fungal mutualism associated with fire. *Fungal biology*, 116, 133-144.

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2015	\$	-
Allocated by	Idaho Wheat Commission	during FY 2016	\$	-

REQUESTED FY 2016 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	\$ 24,426	\$ 2,000	\$ 2,863	\$ 4,500	\$ 11,200	\$ 10,700	\$ 55,689

OTHER RESOURCES (not considered cost sharing or match):

TOTAL OTHER RESOURCES \$ -

TOTAL PROJECT ESTIMATE FOR FY 2017:	\$ 55,689	\$ -	\$ 55,689
	<i>(Requested)</i>	<i>(Other)</i>	<i>(Total)</i>

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	<i>George Newcombe</i>	<i>Kurt Schroeder</i>	<i>Mary Ridout(see Newcombe)</i>	<i>(PI name)</i>
Salary	\$ 24,426	\$ -	\$ -	-
Temporary Help	\$ 1,000	\$ 1,000	\$ -	-
Fringe Benefits	\$ 2,471	\$ 392	\$ -	-
Travel	\$ 3,500	\$ 1,000	\$ -	-
Operating Expenses	\$ 9,500	\$ 1,700	\$ -	-
Graduate Student Tuition/Fees	\$ 10,700	\$ -	\$ -	-
TOTALS	\$ 51,597	\$ 4,092	\$ -	-
Total Sub-budgets \$				55,689

ANNUAL REPORT

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Correspondence and inquiries should be directed to: Mary Ridout, Phone: 509-322-0485; E-mail: mridout@uidaho.edu

ACCOMPLISHMENTS: Endophytic fungi, or fungi that can colonize the internal tissues and organs of plants, can benefit host plants by mediating resistance to biotic and abiotic stress. We hypothesized that endophytes recovered from forest 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 endophytic fungi could improve yield in drought-stressed winter wheat infected with *Fusarium* crown-rot (Ridout and Newcombe 2016). Our objectives for 2015-2016 were to establish field trials for proof of concept that putative mutualist fungi could improve productivity and survival of winter wheat under biotic and abiotic stress and to identify other potential beneficial endophytes via laboratory and greenhouse assays.

In the late summer, laboratory assays were conducted using broth culture techniques for assessment of antagonism and mycoparasitism of endophytic fungi against pathogenic *Fusarium* spp. Test endophytes and *Fusarium* isolates were cultured singly or coinoculated into a high carbohydrate broth. After a three-day culturing period *Fusarium* propagules were quantified in a counting chamber for both coinoculated cultures and *Fusarium*-only controls. *In vitro*, several endophytic fungi were found to significantly reduce the reproductive capacity of *Fusarium* spp. that are pathogenic in cereals

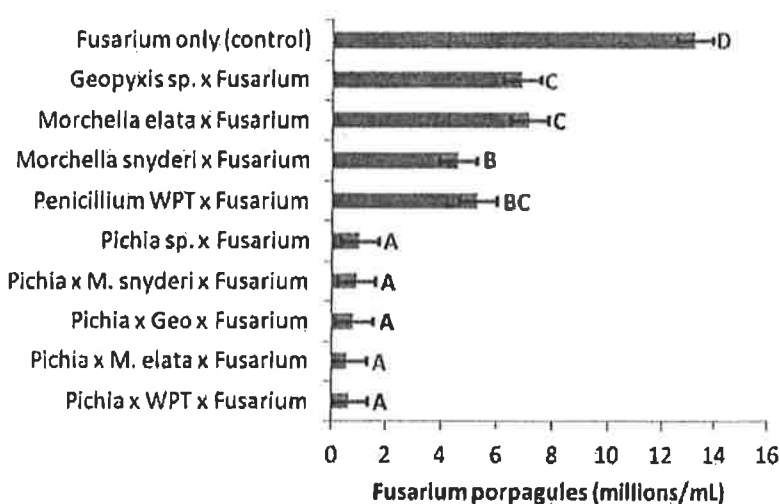


Fig 1. Several endophytic forest fungi decreased the overall fecundity of *Fusarium* spp. in broth culture

(Fig. 1). *Pichia membranifaciens*, a yeast associated with a number of plant tissues, was found to reduce the fecundity of *Fusarium* pathogens in broth cultures by over 90%. An isolate of the mycoparasite *Clonostachys rosea* recovered from Douglas-fir roots was also found to reduce the reproductive capacity of *Fusarium* spp. by nearly 87%. *In agar* competition assays designed by coinoculating agar plates with *Fusarium culmorum* and *C. rosea* revealed that *C. rosea* was able to reduce the fecundity and pathogenicity of the *F. culmorum* by physically parasitizing the pathogen and destroying the physical integrity of its conidia or asexual reproductive cells (Fig. 2).



Fig 2. *Clonostachys rosea* suppresses fecundity of *Fusarium culmorum* by destroying the physical integrity of the reproductive cells or conidia. Left: Healthy conidia from *F. culmorum*. Right: Conidia parasitized by *C. rosea* have been physically damaged, with the loss of the terminal cells that allow the pathogen to germinate and infect host plants.

To test the effects of *Clonostachys rosea* parasitism on the ability of *Fusarium culmorum* to infect winter wheat, we conducted a small greenhouse study. Forty four-week old seedlings were inoculated at the crown with either *C. rosea*-parasitized *F. culmorum*, wild-type *F. culmorum*, *C. rosea* only or sterile distilled water (n=10 per treatment). After eight weeks plants were assessed for *F. culmorum* crown rot infection and above-ground vegetative growth

harvested for dry biomass. *C. rosea*-parasitized *F. culmorum* failed to infect hard red winter wheat seedlings in greenhouse trials, while the non-parasitized pathogen caused disease in all treated seedlings. Moreover, vegetative biomass of plants treated with the parasitized pathogen were comparable to that of pathogen-free plants while *F. culmorum* alone significantly decreased vegetative biomass (Fig. 3).

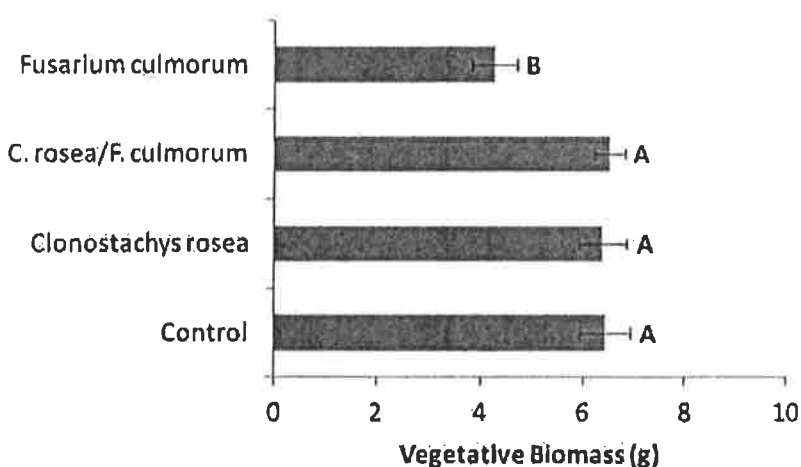


Fig 3. *Clonostachys*-infected *Fusarium culmorum* failed to cause crown rot disease in hard red winter wheat. Biomass of plants inoculated with this infected pathogen were comparable to plants inoculated only with *Clonostachys* or sterile distilled water (control).

Findings from both laboratory and greenhouse assays were exploited for the winter 2015-2016 field trials. As proposed in the FY2016 proposal, two paired trials of winter wheat were sown in replicated dryland 5' X 20' test plots at the University of Idaho Kambitsch Farm near Genesee, ID in October. One trial will assess the effects of fungal endophytes on the physiological status of winter wheat and potential contributions of these fungi to tolerance of abiotic stresses (ie. drought, cold, heat, etc.). The second will assess the ability of endophytic 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 endophyte inocula were generated by suspending fungal spores and/or finely ground fungal hyphae in distilled water. The physiology trial comprises four treatments: *Morchella snyderi*, *Phialocephala* sp., *Penicillium* sp. and a water control. The pathogen trial is composed of an eight-treatment factorial, including three endophyte treatments (*Clonostachys*

rosea, *Pichia membranifaciens*, and *Penicillium* sp.), a water control, and all four treatments in combination with the pathogen. Applications were made to emergent seedlings this fall and will be repeated in early spring. These trials will be monitored until harvest for physiological status (water use efficiency nutrient status, stress, and growth) and disease development.

Greenhouse assays for testing the efficacy of forest fungi against *Fusarium* head blight (FHB) and stripe rust are also under development and results from the first assays are expected early in 2016.

A PhD student was hired this fall to work on the project. Funding leveraged in part from summer laboratory and greenhouse assays was supplied from HM-Clause Inc. funding similar work in vegetable seed production with partial to full support for a post-doctoral scholar.

PROJECTIONS: Should results from laboratory and greenhouse assays be confirmed in the 2015-2016 field trials, we will confirm repeatability with another set of field trials in 2016-2017. Moreover, both fungi identified as possible candidates for reducing the fecundity of *Fusarium* spp. show strong potential for testing against *Fusarium* head blight (FHB). If activity against FHB by *Clonostachys rosea* and *Pichia membranifaciens* can be demonstrated in greenhouse assays, we will also conduct trials to test their efficacy against FHB in the field, possibly in a spring 2016 field trial. Species of both genera have been implicated in reductions in mycotoxin activity of toxic phytopathogenic fungi, so that assays for mycotoxin content in FHB infected seed following greenhouse and field trials will be implemented. Implications for mycotoxin control in cereals are of worldwide importance. Findings that identify fungi antagonistic to toxigenic fungal pathogens with potential to limit or reduce mycotoxin content in cereals and other crops such as pulses could be highly impactful to agriculture worldwide.

Future research need not be limited to a single cereal. Given the outcome of greenhouse and laboratory assays coupled with preliminary findings indicating several species of forest fungi are capable of reducing *Fusarium* ear and stalk rots in maize, we expect to leverage more funding from other agencies and industry for researching the efficacy of disease-suppressive endophytes in other important cereals. Moreover, potential of xerotolerant fungi to confer drought tolerance and increase water-use efficiency in inoculated wheat trials is also expected to lead to leveraging of further funding both for cereals and other dryland crops from other agencies and industry.

We have been examining the best approaches in timing of inoculations and formulation of inoculants as we have developed greenhouse assays. If results are consistent in replicated field trials, we can begin to move forward with formulation of inoculant products and improving application methods (eg. dry formulations in carriers, prills, encapsulated seed, etc.) for commercialization.

Further publications are expected.

PUBLICATIONS: Ridout, M.E. and G. Newcombe. 2016. Winter wheat benefits from novel symbioses with forest fungi. *Fungal Ecology* 20: 40-48.