

ANNUAL REPORT

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Title: Determining genetic control of dwarf bunt resistance in *Bt8* differential lines

Personnel: J.M. Marshall, Professor, Cereals Pathology and Agronomy
D. Hole, Professor, Wheat Breeding, Utah State University
J. Chen, Associate Professor, Wheat Breeder
Dr. Phillip Wharton, Associate Professor, EPPN
Rachel Patterson, student

Address: J.M. Marshall, 1776 Science Center Drive, Suite 205, Idaho Falls, ID 83402;
208-529-8376; jmarshall@uidaho.edu

Accomplishments: While dwarf bunt (DB) and common bunt (CB) can cause significant yield losses, the most significant impact is usually in grain quality. The fungi infect and replace the grain kernel with a "sorus" or bunt ball harboring millions of spores. During harvest, the bunt balls release the spores, covering healthy seed with a dark dust that carries a significant odor resulting from fungal production of trimethylamine (TMA). Such grain, while not known to be toxic to humans or animals, will render end-use products undesirable for consumption.

Host resistance is critical in areas of dryland winter wheat production where the fungus is endemic. Seed treatments containing Dividend (difenoconazole) effectively control dwarf bunt when seed treatments are properly applied but are not effective on volunteer wheat seeds that remain after harvest. With the importance of a few heavily utilized resistance genes (such as *Bt8*) in protecting Idaho wheat, understanding the modes of resistance in protecting against infection will contribute to the ability to stack for resistance in newly developed cultivars and determine the potential contribution of quantitative resistance. We utilized partially resistant variety 'Yayla' (PI178210 with (*Bt8*) and susceptible variety 'Heines VII' (*Bt0*) to study the progression of the common bunt fungus within the host tissue using a qPCR assay and microscopic techniques. The ability to track infection in conjunction with the possibility of non-destructive leaf-based molecular diagnostics could create a rapid process for resistance breeding without the employment of expensive field assays. As host resistance for dwarf and common bunt often rely on the same genetics, we used the common bunt-wheat pathosystem due to easier experimental manipulation.

Our efforts have been focused on developing and adapting existing microscopic *in planta* fungal diagnostic assays for common bunt in wheat. We are also working towards developing a qPCR assay to detect bunt DNA in infected wheat tissue; and attempt to correlate fungal biomass in host tissue with resistance level at heading.

We tested bunt reactions of two *Bt8* differentials with appropriate races of common bunt to verify we are using appropriate inoculation techniques. The differentials were inoculated and infection results are presented in Chart 1. The susceptible *Bt0* (Heines VII) line was successfully infected by all inoculated bunt races, as expected. The two resistant lines (IDO444 and 'UI Sparrow') showed no infection, as expected. We expected PI178210 to be infected with races L-18 (CB) (confirmed) and D-19 (DB) (in testing) both of which have virulence on *Bt8*. We also expected PI554120 to be infected with races L-18 (confirmed) and D-19 (in testing) that has

virulence on *Bt8*. The inoculation protocol has been improved with the use of methyl cellulose to adhere spores to seed, as suggested in the work of Blair Goates (Chart 2).

1) In current experiments, we are determining the host plant response to artificial common bunt inoculation throughout the wheat plant's lifespan, using both developed methods to track the growth or lack of growth in incompatible and compatible reactions in Yayla (PI178210) and Heines VII, respectively.

With the help of Dr. Phillip Wharton, a number of existing methods and stains were reviewed. Ultimately, Toluidine blue O (TBO) in phosphate-buffered saline (PBS) was selected for its ability to differentially stain lignified (plant) and unlignified (fungal) cell walls (Figure 1). This staining method, used with an ethanol-based clearing of plant tissue, relies only on affordable and non-toxic materials. The accessibility of the method and lack of required specialized equipment shows promise for diagnostic purposes in areas without access to more expensive technology.

So far thin sections of young crown, coleoptile, and leaf tissue have been hand-cut from the first three growth stages of the first of three trials and observed with the developed microscopy method. In observing plants 13-15 days post-inoculation, there seems to be infection as expected;

- 80% of the susceptible Heines VII infected with CB race L-18 appeared infected,
- 40% of the susceptible Heines VII infected with CB race T-34 appeared infected,
- 80% of the T-34 susceptible Yayla infected with CB race T-34 appeared infected, and
- 50% of the L-18 resistant Yayla infected with CB race L-18 appeared resistant.

It remains of interest that the remaining 50% of the L-18 inoculated Yayla did show likely colonization by the common bunt fungus at the base of the wheat growing point (Figure 3), as has been recorded in earlier work with different wheat varieties.

The second trial has been planted, with data forthcoming in February 2021. Complementary qPCR data will be available February 2021.

2) Progress towards developing a qPCR assay to detect fungal DNA in infected wheat tissue.

Four DNA extraction methods have been compared for DNA extraction of fungal cultures and wheat samples. Of these, the Norgen Biotek Plant/Fungi DNA Isolation Kit has proven the most successful.

A number of published primers for both *Tilletia controversa/caries/laevis* and wheat reference genes have been tested for performance in standard qPCR conditions. Of these, those *Tilletia* primers and probes published by Zgraja et al. (2016), and wheat beta-actin primers published by Wei et al. (2015) with probes designed by the Marshall lab have shown success in amplifying their respective targets (Table 2, Figure 2).

Table 2. Primers used in this work					
Name	Gene Accession #	Sequence	bp	Target	Source
AB181991-F	AB181991	F: AGCGGTCTGAACAACCTGGTA	101	wheat ACT	Wei et al. (2015)
AB181991-R		R: AAACGAAGGATAGCATGAGGAAGC		wheat ACT	Wei et al. (2015)
AB181991-Pr		AB181991-Pr: [HEX]-TGAGCCACACTGTCCAATC-[BHQ1]		wheat ACT	this work
Til122-F	unreported	Til122-F: ACCCATGTCTTCGGACTTG	140	<i>Tilletia</i> spp. ITS region	Zgraja et al. (2016)
Til122-R		Til122-R: GGTGCGTTCAAAGATTCGAT		<i>Tilletia</i> spp. ITS region	Zgraja et al. (2016)
Til175-P		Til175-P: [FAM]-CTTGTTCTCCCATCGATGAAGA-[BHQ1]		<i>Tilletia</i> spp. ITS region	Zgraja et al. (2016)

These primers will be tested for sensitivity and multiplex efficacy in January-February 2021.

Projections: The microscopy and qPCR assay methods may provide efficient and accurate method of assessing resistance of wheat to DB and CB and may permit the evaluation of wheat varieties for bunt resistance at an earlier growth stage instead of waiting until maturity. This would significantly improve the efficiency and speed of bunt resistance breeding in wheat. This work will be published in scientific journals and presented at commodity schools.

Publications: None as yet.

Chart 1. Inoculation results of various wheat genotypes with 1) no bunt spores 2) bunt races D-19, L-18, T-13, T-33 and T-35. There were no infected heads on the control plants. On the susceptible line *Bt0*, infection occurred with each race of the bunt. There was no infection of UI Sparrow or IDO444, as expected. Rio Blanco was not infected, with the expectation of full infection.

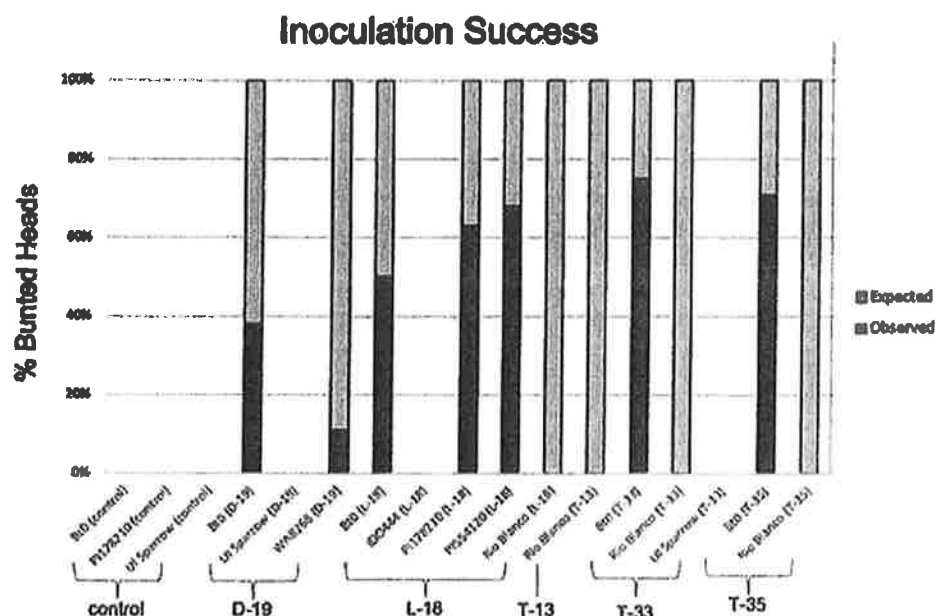


Chart 2. Inoculation success as expressed by percent infected heads. Yellow patterned bars indicate the inoculations were performed with the addition of methyl cellulose, and blue without.

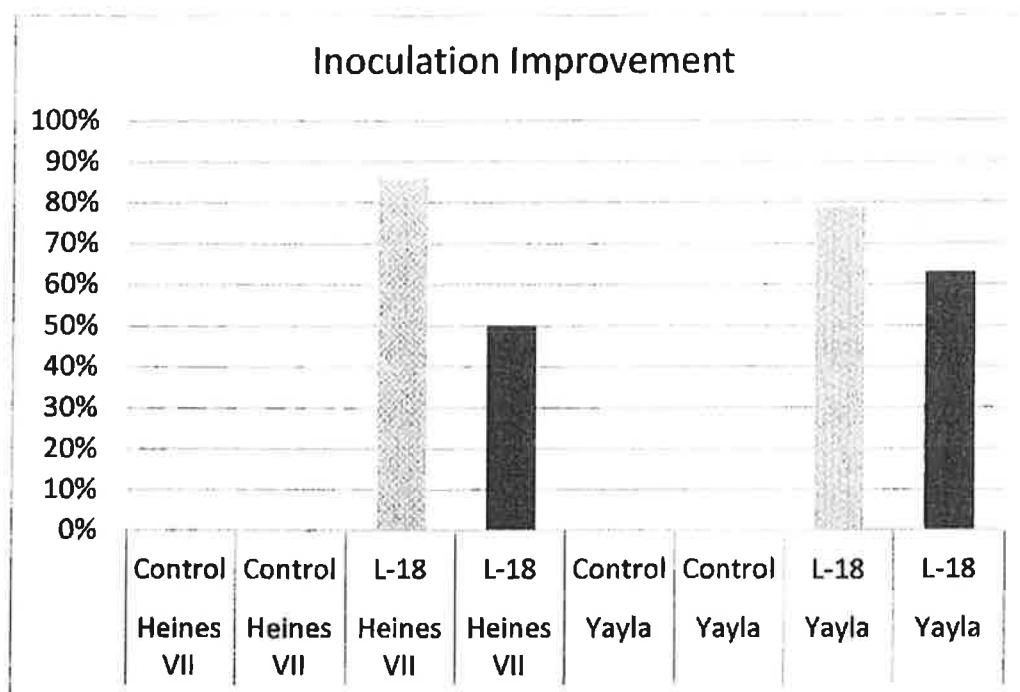


Figure 1. Magenta-stained hyphae in a section of young wheat crown tissue.

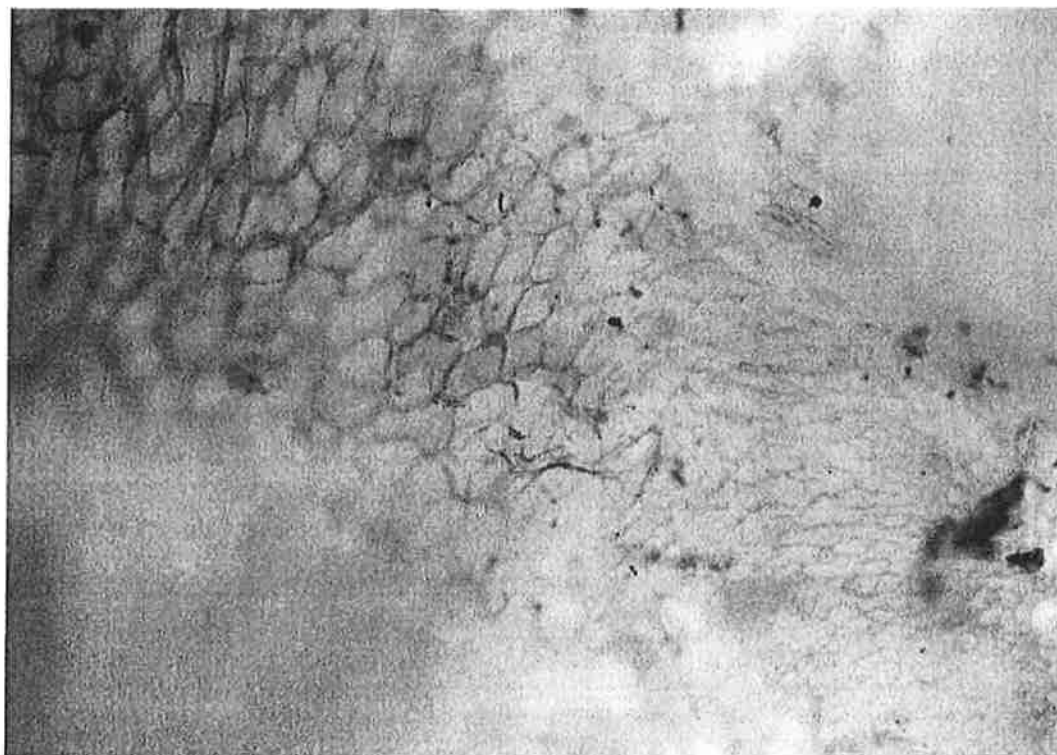


Figure 2. Amplification of fungal and wheat DNA using primer-probe combinations of *T. spp.* and wheat.

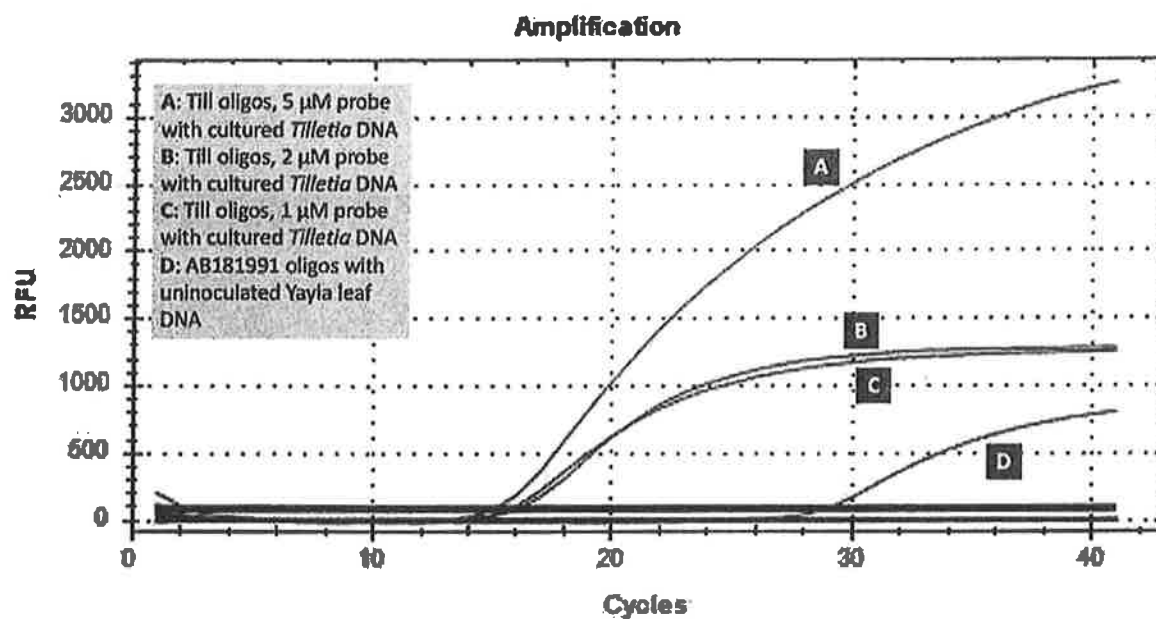


Figure 3. Magenta-stained CB mycelium colonizing the base of the growing point in a resistant cultivar ('Yayla') 15 days post-inoculation.

