

Annual Vogel Progress Report

Project #: 6947

Progress Report Year: 2008

Title: Loss of Disease Suppressiveness in Washington Take-All Decline (TAD) Soils

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Results:

Take-all, caused by *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker (*Ggt*), generally develops at a soil pH of 5.5 to 8.5 and where wheat is grown under moist conditions. As a result, take-all is common throughout the world and is considered the most important root disease of wheat. Wheat is highly susceptible to take-all, but other Gramineae (i.e., barley, rye, and triticale) are also infected. Take-all is controlled by crop rotation but many growers control the disease by continuous wheat monoculture. Take-all decline (TAD) is a natural biological control of take-all that develops when wheat or barley is grown in continuous monoculture following a severe outbreak of disease. The onset of decline occurs in the 4th - 6th year of monoculture and take-all remains suppressed as long as the monoculture is not broken. TAD develops because of the build up of 2,4-diacetylphloroglucinol (2,4-DAPG)-producing *Pseudomonas fluorescens* and the accumulation of 2,4-DAPG in the rhizosphere.

We have isolated over 400 putative isolates of *Ggt* using a baiting technique and the semi-selective and diagnostic medium R-PDA during a survey of spring and winter wheat fields in central and eastern Washington during 2007 and 2008. Fields sampled included TAD fields near Quincy, Moses Lake, Lind, Ritzville, Pullman, Colfax and the Port of Almota, as well as nearby fields without a history of continuous wheat or barley (non-TAD), and non-cropped (virgin) sites where the fungus lives on the roots of native grasses. Samples were also sent to us from Idaho and Montana. In non-TAD (crop rotation) and virgin soils, DAPG-producing *P. fluorescens* are below the limit of detection, 2,4-DAPG is not isolated from the roots, and the soils are not suppressive.

After many years of monoculture, some TAD fields show a reduction in the robustness of their take-all suppressiveness. In the first part of the study, we determined whether isolates of *Ggt* become less sensitive to 2,4-DAPG during wheat monoculture as a result of exposure to 2,4-DAPG-producing *P. fluorescens* and the antibiotic over multiple growing seasons. We compared 177 isolates from non-cropped, TAD and non-TAD fields near Lind, Ritzville, Pullman and Almota. Isolates were characterized using morphological traits, *G. graminis* variety-specific PCR (**Fig. 1**) and pathogenicity tests. The sensitivity of *Ggt* isolates to 2,4-DAPG was determined by measuring radial growth of each isolate on media amended with 2,4-DAPG (**Fig. 2**). The 90%

effective does value (ED_{90}) was 3.1 to 4.4 $\mu\text{g ml}^{-1}$ for 2,4-DAPG sensitive isolates, 4.5 to 6.1 $\mu\text{g ml}^{-1}$ for moderately sensitive isolates, and 6.2 to 11.1 $\mu\text{g ml}^{-1}$ for less sensitive isolates. Sensitivity of *Ggt* isolates to 2,4-DAPG was normally distributed in all fields and was not correlated with geographic origin or cropping history of the field (**Fig. 3**). Furthermore, there was no correlation between virulence on wheat and geographical origin (**Fig. 4**), or virulence and sensitivity to 2,4-DAPG (**Fig. 5**). These results indicate that *Ggt* does not become less sensitive to 2,4-DAPG during extended wheat monoculture in TAD soils. Thus, loss of the robustness of the suppressiveness in some TAD fields does not appear to result from the pathogen becoming insensitive to the antibiotic.

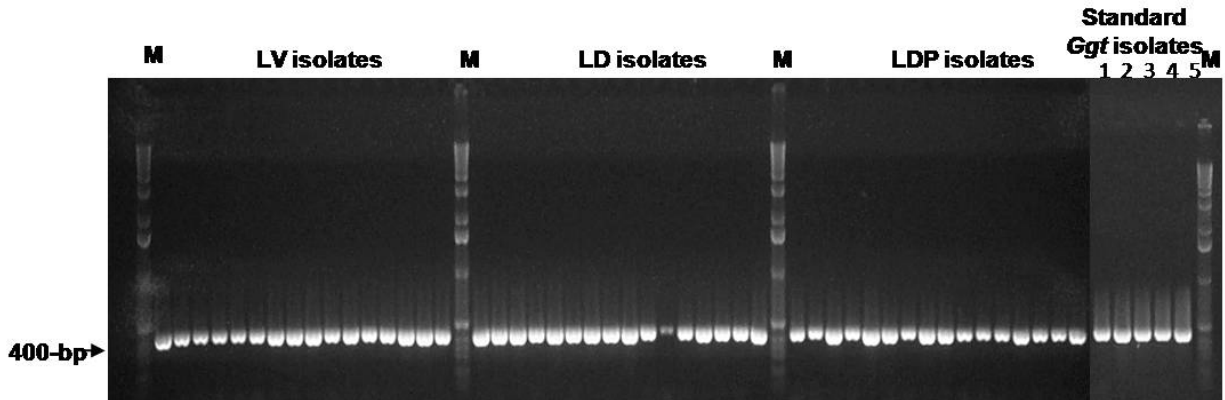


Fig. 1. Amplification of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) DNA with NS5 and GGT-RP primers. All PCR amplicons were approximately 410-bp in size (characteristic of *Ggt*) and were visualized by gel electrophoresis in 1.5% agarose. Lane M contained a 1-kb DNA ladder as a molecular weight standard. LV, isolates were from Lind virgin soil; LD and LDP isolates were from the Lind TAD field (LD from healthy wheat; LDP from within a take-all patch). The TAD field was initiated on the WSU Lind Dryland Research Station by R. James Cook and it has been in continuous irrigated wheat since 1968; in the last 9 years, there has been a reduction in the suppressiveness of the field. The Lind virgin site is a non-cropped area on the edge of the station about 100 m from the TAD field. Standard *Ggt* isolates: 1, MV-116; 2, MV-119; 3, L-109; 4, ARS-A1; 5, R3-111a-1.

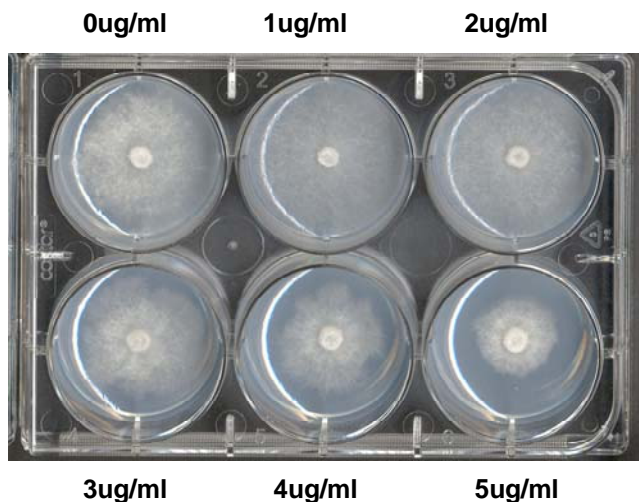


Fig. 2. Six-well plate assay used to test the sensitivity of *Ggt* and *Phialophora* isolates to 2,4-DAPG. 1/5× potato dextrose agar was amended with either 0, 1, 2, 3, 4, or 5 µg of 2,4-DAPG ml⁻¹. A plug of the fungus was placed in the center of the well. Radial growth of each isolate was measured over a period of 3 to 7 days after inoculation. The picture was taken at 6 days after inoculation.

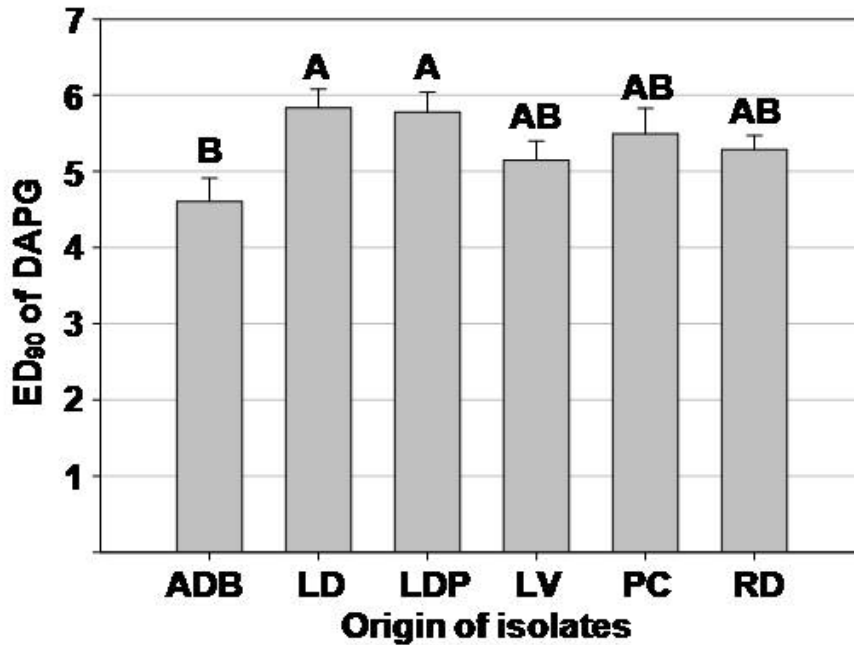


Fig. 3. ED₉₀ of 2,4-DAPG against *G. graminis* var. *tritici* isolates from fields in Washington State with different cropping histories. The ED₉₀ of 2,4-DAPG was calculated on the basis of the inhibition of radial growth of *Ggt* on 1/5× PDA amended with a range of concentrations of 2,4-DAPG. Bars with the same letters are not significantly different according to Tukey’s HSD ($P = 0.05$). ADB, Almotat TAD field; LD, Lind TAD field; LDP, Lind TAD field-patch; LV, Lind virgin field; PC, Pullman non-TAD field; and RD, Ritzville TAD field.

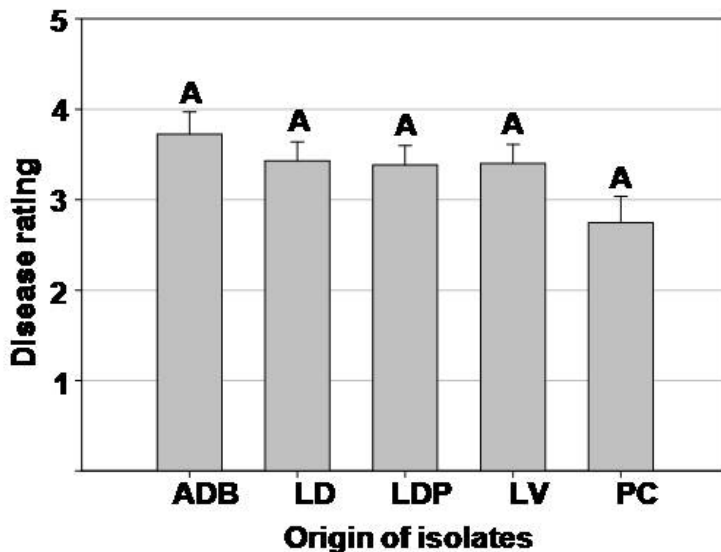


Fig. 4. Severity of take-all caused by *G. graminis* var. *tritici* isolates from fields in central and eastern Washington State. Disease was measured using the tube assay and plants were incubated in a growth room (16 °C, 16 h photoperiod). Take-all was rated at 4 weeks using a 0 to 8 scale where 0 = healthy plant and 8 = dead or nearly so. Bars with the same letters are not significantly different according to the Least Significant Difference Test (LSD) ($P = 0.05$). ADB, Almota TAD field; LD, Lind TAD field; LDP, Lind TAD field-patch; LV, Lind virgin field; and PC, Pullman non-TAD field.

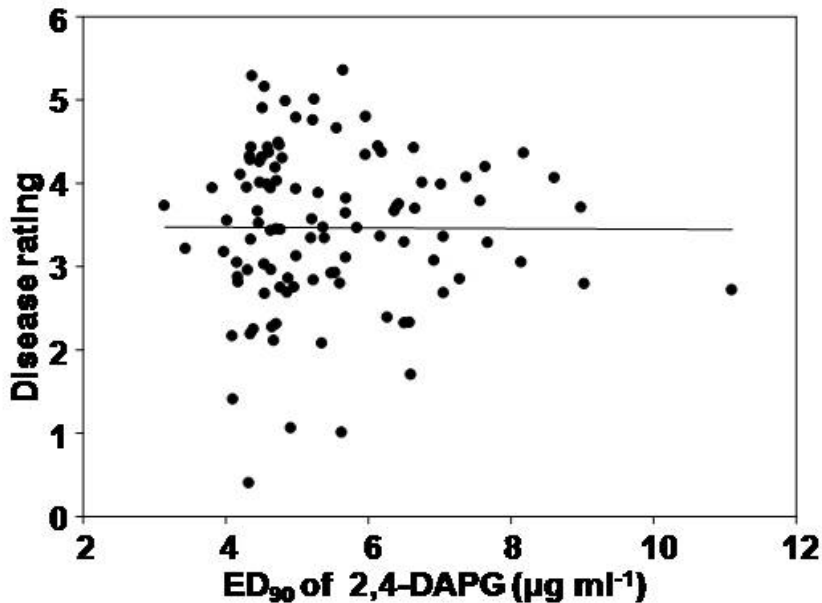


Fig. 5. Relationship between disease ratings on wheat (cv. Penawawa) and sensitivity to 2,4-DAPG of *G. graminis* var. *tritici* isolates from fields in central and eastern Washington State. Isolates were tested in the ‘tube assay’ and take-all severity was rated on a 0-8 scale. *Ggt* isolates were grown on 1/5× PDA amended with a range of concentrations of 2,4-DAPG (0-5 µg ml⁻¹) and radial growth was measured daily. ED₉₀ was calculated using the Probit analysis function in SigmaPlot 8.0. Correlation coefficient ($P = 0.9573$; $r^2 = 0.00498$).

Ggt causes take-all on wheat, barley, triticale and rye; *G. graminis* var. *avenae* (*Gga*) causes similar symptoms on wheat as *Ggt*, but *Gga* also causes take-all of oats and take-all patch of turf-grasses. In our 2007 progress report, we reported the occurrence of *Gga* isolates, based upon variety-specific primers developed by Rachdawong et al. (2002, Plant Dis. 86:652-660), in direct-seeded TAD fields near Almota and Pullman, but not in other conventionally cultivated fields sampled. Further analysis has shown that the putative *Gga* isolates are in fact *Phialophora* spp., a fungus closely related to *Gaeumannomyces*, and sometimes an anamorph of *Gaeumannomyces*. *Phialophora* isolates comprised 95% and 25%, respectively, of the *Ggt*-like isolates from the Pullman and Almota TAD fields. *Phialophora* isolates are significantly less virulent on wheat and barley than *Ggt* and produce almost no disease on oat. Figure 6 shows the amount of disease produced by *Ggt* isolate R3-111a-1 and several *Phialophora* isolates on wheat, barley and oat. Lesions caused by the *Phialophora* generally remained confined to the root and did not spread to the crown or stems as is typical of *Ggt*. The lack of virulence on oats

(Fig. 6), the production of lobed hyphopodia (Fig. 7), and the production of phialides and phialospores collectively indicated that the *Ggt*-like fungi are *Phialophora* sp.

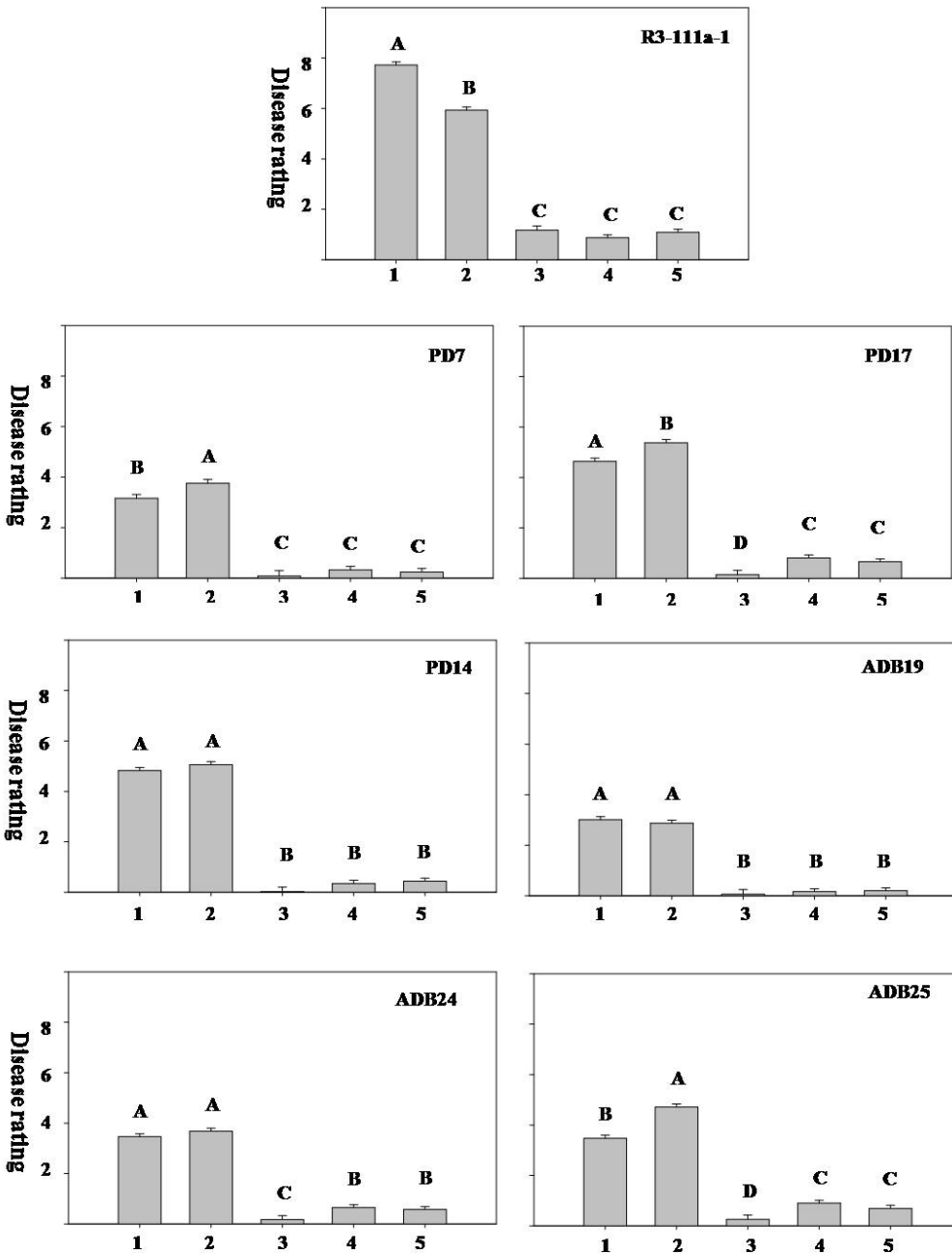


Fig. 6. Severity of take-all symptom caused by *Ggt* (R3-111a-1) and *Phialophora* isolates (PD7, PD17, PD14, ADB19, ADB24, and ADB25). Take-all symptoms were rated 4 weeks after planting using a 0 to 8 scale. Bars with the same letters are not significantly different according to LSD ($P=0.05$). 1, wheat (cv. Penawawa); 2, barley (cv. Baronesse); 3, wild oat; 4, oat (cv. Mondia); 5, oat (cv. Otana).

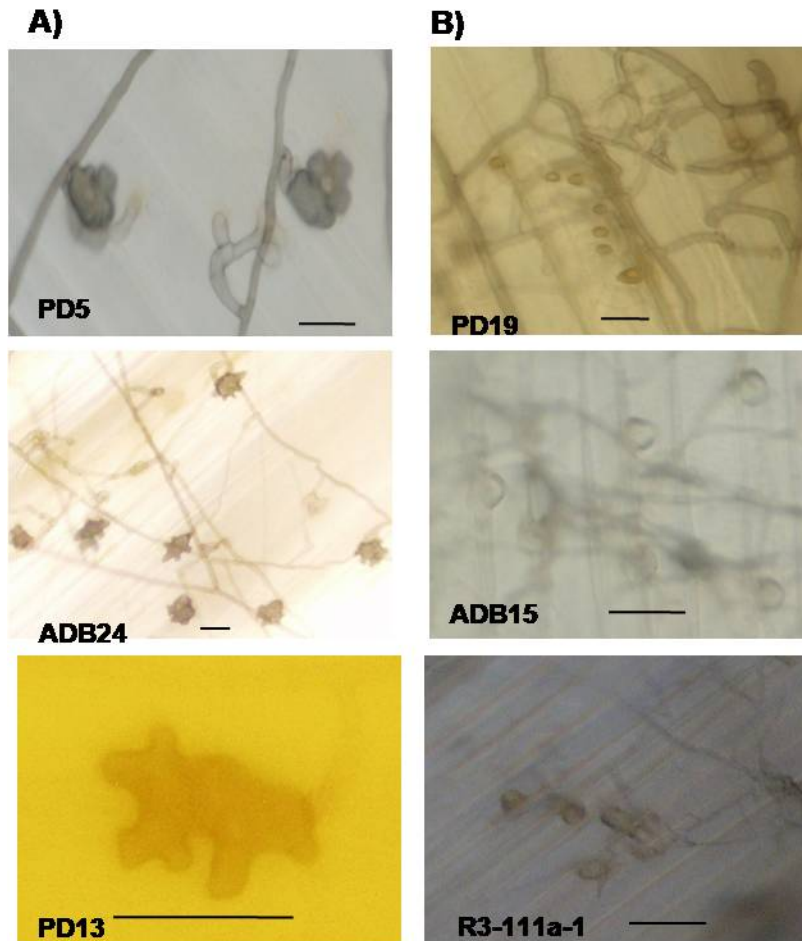


Fig. 7. Hyphopodia produced by A) *Phialophora* (lobed) and B) *Ggt* (simple, unlobed) isolates on wheat coleoptiles. Bars = 20 μm .

Eighteen *Phialophora* sp. isolates were tested for sensitivity to 2,4-DAPG in vitro using 6-well cell culture plates. *Phialophora* sp. isolates showed a wide variation in sensitivity to 2,4-DAPG. For example, the ED_{90} for ADB isolates ranged from 20.31 to 48.19 $\mu\text{g ml}^{-1}$. For PD isolates, the ED_{90} ranged from 11.94 to 30.47 $\mu\text{g ml}^{-1}$. The average ED_{90} was 29.4 $\mu\text{g ml}^{-1}$ for ADB isolates; and 19.8 $\mu\text{g ml}^{-1}$ for PD isolates, and this difference between fields was significant (LSD $P = 0.05$). The average ED_{90} for PD and ADB isolates were significantly greater than ED_{90} values for all *Ggt* isolates. We now speculate that the *Phialophora* isolates may enhance the take-all suppressiveness in the two direct-seeded TAD fields by providing cross protection against *Ggt*, which is already weakened by 2,4-DAPG produced by *P. fluorescens*. *Phialophora* is well known in the literature as a biocontrol agent of *Ggt*.

Accomplishments (Outputs, Outcomes, Impacts):

1) Two plant pathologists from The Netherlands on sabbatical with Dr. Weller and a plant pathology Ph.D. student (Youn-Sig Kwak, supported on this grant) initiated this project in June 2007. In 2008, Mr. Kwak increased the size of the collection of isolates, and now it contains

over 400 isolates of *Ggt* and *Phialophora*. Isolates have been characterized and tested for virulence against wheat and sensitivity to 2,4-DAPG (antibiotic responsible for TAD). **Impact:** This is only the 4th survey of *Ggt*, and the first for *Phialophora*, in the history of Washington State. It is the first study to fully characterize Washington *Ggt* isolates using both morphological characteristics and molecular approaches. This study has demonstrated that the pathogen continues to be very widespread throughout the state, even in dry areas, and that isolates vary widely in virulence and morphology within the same field. *Ggt* is also common on wild grasses in non-cropped sites, thus providing the inoculum when wheat is grown. Take-all can be an acute problem in irrigated wheat and a chronic problem in areas of moderate and low rainfall. This work is providing practical information for growers to manage take-all disease and fundamental new information about the ecology of this important soilborne pathogen.

2) Since our last report, we tested a new set of *Ggt*-specific primers (NS5 and GGT-RP), which amplify PCR amplicons of approximately 410-bp in size. These primers appear to consistently and specifically amplify *Ggt* DNA from a wide range of isolates collected across the Pacific Northwest. **Impact:** Distinguishing *Ggt* from other *Ggt*-like organisms can be difficult without pathogenicity tests. PCR using these primers allows fungal isolates to be rapidly identified as *Ggt* and eliminates the need for pathogenicity tests. Primers specific for the *Phialophora* isolates are now being tested.

3) *Ggt* causes take-all on wheat, barley, triticale and rye, and *Phialophora* attacks wheat and barley but is much less virulent than *Ggt*. Previously it was thought that *Phialophora* was rare in Washington wheat fields. Throughout the world, *Phialophora* is known as a biocontrol agent of *Ggt*. We introduced *Phialophora* sp. and *Ggt* into the same soil and *Phialophora* reduced the severity of take-all as compared to *Ggt* introduced alone. **Impact:** We have shown for the first time that *Phialophora* is abundant in direct-seeded monoculture wheat and barley fields in Washington. The basis of the enrichment of *Phialophora* in these fields will be the subject of future research. We will be exploring further the interaction of *Phialophora* with *Ggt* and the role of *Phialophora* in the epidemiology of take-all. This study is also the first to implicate *Phialophora* as a potential additional mechanism of take-all suppression in Washington directed-seeded TAD fields, working in concert with 2,4-DAPG producers.

Publications:

Kwak, Y.-S., Bakker, P. A. H. M., Glandorf, D. C. M., Rice, J. T., Paulitz, T. C., and Weller, D. M. 2008. Diversity, virulence and 2,4-diacetylphloroglucinol sensitivity of *Gaeumannomyces graminis* var. *tritici* isolates from Washington State. *Phytopathology*. *in press*.

Presentations and Reports:

Kwak, Y., Bakker, P.A., Glandorf, D. C., Paulitz, T., and Weller, D. M. 2008. Diversity, virulence and 2,4-diacetylphloroglucinol sensitivity of *Gaeumannomyces graminis* var. *tritici* isolates from Washington State. *Phytopathology* 98:S85.

The above poster was presented at the Annual Meeting of the American Phytopathological Society, Minneapolis, MN, July 26-30, 2008.