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Evan M. Perkins for the degree of Master of Science in Botany and Plant Pathology presented on July 2, 2020

Title: Differential Effects of First Year Wheat Genotype on the Suppression of Take-All Disease and the Establishment of Suppressive Microbial Agents in the Rhizosphere

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Christopher C. Mundt

Recent research in the UK has found that the wheat cultivar grown in the first year can have a significant impact on the amount of take-all that develops in the second year, regardless of the cultivar planted in year two. 'Einstein' is one such cultivar that reduces take-all disease (reduced take-all buildup or TAB) and may possess a gene that encourages favorable populations of *Pseudomonas* to colonize the rhizosphere. Cultivar Einstein is a parent to the wheat cultivar 'Bobtail' that was released by Oregon State University in 2012. This study aims to determine if the low TAB trait has been inherited by Bobtail, if the trait is robust across the UK and US field environments, and how first year Bobtail compares to other cultivars commonly grown in the PNW in regard to take-all development. The secondary objective was to determine if DAPG-producing pseudomonad prevalence is associated with reduced take-all disease, and whether these pseudomonads might play a role in establishing a priority effect by the first year cultivar. Field experiments were conducted to determine the influence of different first year wheat cultivars on take-all levels in subsequent field and greenhouse studies. PCR targeting an essential gene in the biosynthetic pathway of the antibiotic 2,4-diacetylphloroglucinon (DAPG) was run on serial dilutions of *Pseudomonas fluorescens* populations derived from rhizosphere washes of wheat planted in soil previously exposed to different wheat cultivars.

We successfully identified that soil from first year Bobtail wheat consistently resulted in less take-all and accumulated significantly more 2,4-DAPG producing *P.*

fluorescens than soil from other cultivars ($p<0.001$), and that the two effects were correlated ($r = -0.25$, $p<0.001$), suggesting that Bobtail may have inherited one or more genes associated with take-all resistance and *Pseudomonas* accumulation from its parents. An intermediate level of take-all suppression in some other cultivars may be regulated by a different mechanism. The first-year cultivar effect dominated the response in subsequent plantings ($p<0.001$), and its impact was not specific to the first year cultivar. These results suggest that wheat genetics may be used to produce an environment favorable to soil microbiome components that suppress take-all, an approach that is cost effective and sustainable over conventional chemical control. This microbiome effect may be determined, in part, by populations of 2,4 DAPG-producing pseudomonads, which have also been shown to induce resistance to a diversity of other important wheat diseases.

Differential Effects of First Year Wheat Genotype on the Suppression of Take-All Disease and the Establishment of Suppressive Microbial Agents in the Rhizosphere

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Evan M. Perkins, Author

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DEDICATION

This work is dedicated to my mother, Barbara, who's memory will always give me strength and comfort, and my father, Richard who kept me on the right path, despite my best efforts.

Differential Effects of First Year Wheat Genotype on the Suppression of Take-All Disease and the Establishment of Suppressive Microbial Agents in the Rhizosphere

Chapter 1: Literature Review

Introduction

Wheat is the second most important crop worldwide, making up 20% of the daily calorie and protein consumption for 4.5 billion people, coming second only to rice (Lucas, 2012). It is grown on every continent except Antarctica, with 219 Mha, planted in the 2018 harvest year, making it the most widely cultivated cereal in the world, with more than 60% of global production coming from developing countries. Wheat yield is predicted to have to increase by 60% by 2050 to meet the needs of a growing population, but rising global temperatures project a yield decrease of as much as 27% (FAO, 2018; Ordon *et al.*, 2019). In 2019 the United States harvested 15M ha, with the Pacific Northwest accounting for 11% of production (USDA, 2020). Even with modern pesticide use, disease and pests cause an average global yield loss of 20% (Serfling *et al.*, 2017), but the rising costs of treatment combined with environmental concerns highlight the need to develop stable, affordable, non-chemical disease control (Ordon *et al.*, 2019).

Soil-borne Plant Pathogens

Simplistically, plants can be broken down into their above ground and below ground portions. The above ground portions, including the leaves, stems, and fruit, are what is most easily observed, harvested, and studied, so it is what has historically received the greatest scientific scrutiny. However, it is the root system that is responsible for the uptake of water and nutrients required for healthy plant growth, as well as the anchoring and support of the entire organism. Because of these important functions, pathogens that target the root system routinely cause significant damage to the plant, resulting in reduced quality and crop yield (Weller *et al.*, 2002; Cook, 2003; Coninck *et al.*, 2015). Soil-borne diseases can affect plants from seedlings through adulthood, and are capable of causing significant yield losses through partial to total disruption of water and nutrient uptake (Mihajlovic *et al.*, 2017). The economic impact of soil-borne diseases is expected to increase, as more farmers convert to no-tillage field strategies

(UNEP, 2013) to conserve water and top-soil (Manici *et al.*, 2014), giving the pathogens more reservoirs in which to survive (Moore & Cook, 1984; Rothrock, 1987; Panth *et al.*, 2020).

Soil-borne pathogens are difficult to control due to the formation of tough survival structures that persist in the soil (Coninck et al., 2015) and the difficulty of thorough application of effective pesticides (Cook, 2003; Coninck *et al.*, 2015). Soil solarization shows promise in some disease systems as a relatively inexpensive control, but its effectiveness is reduced relative to soil depth (Katan *et al.*, 1976). Tillage practices and irrigation schedule also play a major role in the development, severity, and persistence of soil-borne pathogens (Rothrock, 1987; Mavrodi *et al.*, 2012; Yin *et al.*, 2017).

Early research into root disease and soil-borne pathogens can be attributed to S.D Garrett in the early and mid 20th century, largely by his research into take-all (Cook, 2003). Biological control of soil-borne pathogens has been an area of interest since the early 1920's, but it was not until 1965 when it began to be seen as a commercially viable alternative to pesticides, stimulating a rapid expansion in research efforts (Weller, 1988). Since then numerous studies have shown that highly complex plant-microbe interactions play a significant role in plant growth, development, and disease resistance in a variety of systems (Smiley, 1973; Weller, 1988; Mazzola & Cook, 1991; Kowalchuk *et al.*, 2002; Raaijmakers *et al.*, 2009; Vorholt, 2012; Mauchline & Malone, 2017).

Microbiome Effect on Disease Suppression and Plant Health

The most basic understanding of plant pathogens focuses on the interaction between host and pathogen, ignoring the numerous other factors affecting plant health and pathogen virulence, including the interaction between the soil-borne microbial community, which represents the greatest biological diversity currently known (Berendsen et al., 2012). The plant microbiome is made of all microbiota within the rhizosphere, phyllosphere, and endosphere of the plant (Compant *et al.*, 2019), and is highly specific to plant genotype, even when grown in the same environment (Lindow & Brandl, 2003; Landa *et al.*, 2006; Berendsen *et al.*, 2012). Most plants have developed strategies to support specific groups of microorganisms within the microbiome, to act as a

defense against potential pathogen threats (Weller *et al.*, 2007; Niu *et al.*, 2017). A survey of different *Sphingomonas* strains revealed that isolates obtained from the phyllosphere granted their host increased disease resistance, while those isolated from the air, dust, or water lacked this quality, regardless of their density. This suggests that plant protective qualities are not purely density dependent, and that strains that live on or within plants are more likely to possess traits that enhance the health of their host (Innerebner *et al.*, 2011). Furthermore, there is evidence of potential keystone species within the microbiome, that aid in maintaining stable population structure once established (Niu *et al.*, 2017). The endosphere population is correlated with the soil population, and thus can be manipulated by growing plants that enrich the soil with the desired microbiota (Conn & Franco, 2004) or by inoculation of roots with specific microbiota to enhance plant health and disease tolerance. The same effect can be obtained by appropriate agricultural management strategies that promote the growth and diversity of desired microbiota (Compant *et al.*, 2019).

The microbiome and its effects on plant health and disease suppression have been studied extensively, but its mechanisms are poorly understood, beyond general observations. Plant growth promoting bacteria exist in the rhizosphere of nearly all studied plant systems and are known to enhance plant growth and nutrient uptake (Compant *et al.*, 2019). Rhizobial microbiota can also act to increase their host's resistance to disease, either through competitive inhibition, production of anti-microbial compounds, or though the induction of systemic resistance in the host (Noble & Coventry, 2005; Compant *et al.*, 2005; Busby *et al.*, 2017). Microbiome populations are largely defined by geography first, and then on host genotype and environmental factors (Gdanetz & Trail, 2017; Compant *et al.*, 2019). The diversity of the plant community within a given plot has little effect on the bulk soil microbial community, but significant changes within the rhizosphere communities have been detected between different plant species when grown in the same field (Kowalchuk *et al.*, 2002).

The most obvious effect of the soil microbiome is the development of disease suppressive soils after continued cropping (Weller, 1988; Noble & Coventry, 2005; Cook, 2014; Schlatter *et al.*, 2017). Suppressive soil are "soils in which the pathogen

does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." (Baker & Cook, 1974; Weller, 1988; Weller *et al.*, 2007). Suppressive soils are categorized as either long-standing, where the suppression is a natural characteristic of the soil and persists in the absence of plants, or induced by the transfer of a small amount of suppressive soil to a conducive soil or through the continued monoculture of an introduced crop (Weller *et al.*, 2007). Disease suppression can be either general and non-transferable, bearing to the cumulative effect of multiple organisms to reduce the severity of disease, or specific and transferable, with one or few organisms reducing the growth and development of a specific pathogen by a significant degree (Weller *et al.*, 2002).

Pseudomonas fluorescens complex

The *Pseudomonas fluorescens* group has long been identified as housing multiple species capable of producing anti-microbial compounds, and is used as a general catchall term to denote pseudomonads with biocontrol properties (Mauchline *et al.*, 2015; Almario *et al.*, 2017). The *Pseudomonas* genus is home to many species that have been identified as potential biocontrol agents, primarily through the production of antibiotic compounds. To date pseudomonads have been found to produce amphisin, 2,4-diacetylphloroglucinol (2,4-DAPG), oomycin A, hydrogen cyanide, pyoluteorin, cyclic lipopeptides, phenazine, pyrrolnitrin, tensin, and tropolone, depending on the species. The production of these chemicals can be turned on and off in the presence or absence of environmental triggers such as the abundance of glucose, root exudates, and plant host genotype (Compant *et al.*, 2005). The complex is large and highly heterogenous, but nearly all genes responsible for multitrophic interactions are unique to a subset of strains, or even a single specific strain (Fuente *et al.*, 2006; Loper *et al.*, 2012).

P. fluorescens has been consistently isolated from both conducive and suppressive soils in various agricultural systems, with 2,4-DAPG producing strains thought to be the primary agent in disease suppressive soils (Weller, 1988; McSpadden Gardener *et al.*, 2001; Weller *et al.*, 2002; de Souza *et al.*, 2003; Maria *et al.*, 2005). The phlACBD genes are responsible for the production of 2,4-DAPG (Bangera & Thomashow, 1999), genetic

analysis of this operon showed evidence of multiple independent acquisitions of the trait, rather than a vertical inheritance from a single source. This suggests that DAPG biosynthesis may not be restricted to *P. fluorescens*, and that rhizosphere analyses may be underrepresenting the amount of 2-4 DAPG producing bacteria present in suppressive soils (Almario *et al.*, 2017). Studies in the United States have identified at least 22 different genotypes, with more reported in Europe, and likely still more undetected. Within take-all suppressive fields in the Pacific Northwest genotype D, now called *Pseudomonas brassicacearum,* dominates, making up 60-95% of the DAPG producing pseudomonads isolated from wheat and barley roots (Schlatter *et al.*, 2017; Yang *et al.*, 2018).

P. fluorescens exists in high quantities in both the bulk soil and the rhizosphere, but there is clear separation of rhizosphere and bulk soil genotypes. Of the rhizosphere genotypes, host plant genotype plays a key role in the abundance and diversity of 2,4-DAPG producing pseudomonads. Soil type also plays a significant role, with greater 2,4-DAPG producing pseudomonad density and diversity within take-all suppressive soil, than in conducive soil (Maria *et al.*, 2005). But, it should be noted that increased diversity can result in decreased disease suppression, if the primary agents are reduced below a critical threshold (Mehrabi *et al.*, 2016). In take-all suppressive soils in the Netherlands 2,4-DAPG producing *P. fluorescens* were also a key factor in inhibiting the disease, but the genotypic composition differed from that from the Pacific Northwest (de Souza *et al.*, 2003). Furthermore, the populations and colonization frequencies of suppressive *Pseudomonas* varies based on moisture regime, with irrigated fields and high-rainfall environments being dominated by 2,4-DAPG producing *Pseudomonas,* and non-irrigated fields by phenazine producing *Pseudomonas* (Mavrodi *et al.*, 2012).

Take-all of Wheat as a Model System

The Disease: Take-all Disease of Wheat

Take-all, caused by the soil-borne fungus *Gaeumannomyces graminis* var *tritici,* was recognized as a disease as early as 1852, in Australia (Hornby, 1998), where the yield loss was so substantial that there was no grain to harvest. In the mid to late 20th century, two leading rhizosphere microbiologists, A.D. Rovira and C.A. Parker

transitioned from general rhizosphere microbiology to root pathology, with a focus on studying take-all in southern and western Australia, respectively. There, Parker identified "dryland take-all" in the drier wheat growing regions of western Australia as distinct from the "wetland take-all" more commonly seen in irrigated fields. Dryland take-all has since been confirmed in other parts of the world, where soils are too dry for what is characteristically a moisture driven disease (Cook, 2003). Because of its ability to infect and grow in both wet and dry conditions it is one of the most important soil-borne diseases of wheat in the world (Kwak & Weller, 2013).

Take-all is a soil-borne disease that causes stunting, lack of grain fill, chlorosis, and eventually death as it spreads throughout the root system and into the crown, disrupting water transportation and destroying the root system (Mathre, 2000; Cook, 2003; Christensen & Hart, 2008). Classic symptomatology occurs under wet conditions in patches, due to the ability of the fungus to travel across the root system via runner hype, originating from a single source of inoculum. Roots are infected superficially at first, showing coal black lesions with dark runner hyphae growing along the surface of the roots, then are penetrated by the fungus, making roots black to the core and brittle. New infections develop throughout the growing season and are favored by moist conditions and soil temperatures of 10-20°C. Early infections will spread to the root crown, and often increase exponentially when conditions are favorable (Mathre, 2000; Christensen & Hart, 2008; Kwak & Weller, 2013). In dryland systems with as little as 250-300mm of annual precipitation the diagnostic lesions and blackened stem do not typically form. This is because the growth of take-all is substantially reduced at soil matric potentials of -35 to -40 bars and halved at -15 to -20 bars. These levels are easily achieved near the soil surface in non-irrigated fields. In these conditions the roots will grow up to 180cm deep, while the pathogen is confined to the top 20cm of soil, which dries out over the growing season. This allows the crop to continue obtaining water and nutrients, while the diseased roots are restricted to the surface, and infection is slowed. The matric potential within the root system remains higher, which could explain the continued development of take-all upwards to the stem, when no lesions are present, and for the lack of the characteristic patchiness of 'wetland' take-all, since the pathogen

cannot grow along the surface of the roots to new hosts (Cook, 2003; Loper *et al.*, 2012; Kwak & Weller, 2013). The pathogen survives between seasons as mycelium on stubble and dead roots, as well as on the roots of grassy weeds and volunteer wheat, which then serves as the inoculum source for the next year, if not properly controlled (Rothrock, 1987; Yin *et al.*, 2017; Schlatter *et al.*, 2017).

The Pathogen: *Gaeumannomyces graminis* var tritici

Gaeumannomyces is an ascomycete that was originally classified in the order *Diaporthales*, before being identified as a member of the *Magnaporthaceae*, within the order *Magnaporthales*. There are seven known species within the genus: *G. graminis* , *G. cylindrosporus,*, *G. caricis*, *G. incrustans*, *G. medullaris*, *G. amomi*, and *G. wongoonoo* (Hornby, 1998; Wong, 2002; Cook, 2003). Host specificity varies within the genus, with each typically only being isolated from a specific genus, or family; *G. graminis* and *G. cylindrosporus* infect cereals and grasses, *G. caricis* sedges, *G. incrustans* turf-grass, *G. medullaris* the rush *Juncus roemerianus*, *G. amomi* wild ginger, and *G. wongoonoo* buffalo grass (Freeman & Ward, 2004). Within *G. graminis* there are four varieties, var. *tritici* that causes take-all of wheat, rye, barley, and triticale, *var avenae* that causes take-all of wheat, rye, barley, triticale, oats, and turf-grass, var *graminis* that causes sheath blight of rice and dieback of Bermuda grass (Walker, 1981; Hornby, 1998), and var. *maydis* that causes take-all of maize (Hornby, 1998). Modern phylogenetic tools are being used to more closely assess the genus, and suggest that the *G. graminis* var *tritici* and var *avenae* may in fact be separate species, which may explain their increased virulence when compared to other *G. graminis* strains (Hernández-Restrepo *et al.*, 2016).

G. graminis var. *tritici* was originally given the name *Ophiobolus graminis* and placed in the family *Magnaporthaceae* in 1881 by early mycologists and was not given its current name or designation as an ascomycete until 1952. In 1972, Walker differentiated the *tritici* variant that was known to infect wheat from other *G. graminis* variants that infect other Poaceae (Cook, 2003). *G. graminis* var *tritici* is homothallic with an optimal growth range of between 20°C and 25°C, but growth is possible between 4°C and 30°C, within pH 3 and 10, with severity reported to increase with pH. It

produces hyaline hyphae in culture followed by darkly pigmented macrohyphae that curl back at the edges, as the culture ages (Cook, 2003; Kwak & Weller, 2013). Simple round hyphopodia are produced on the coleoptile, while perithecia produced on the lower leaf sheath and stem have uninucleate asci with slightly curved ascospores (Hornby, 1998; Kwak & Weller, 2013).

Take-all Control Methods

Chemical Control

Effective chemical control is rare and is largely restricted to the use of soil fumigants such as methyl bromide and chloropicrin, but economic cost and legislation restricting their use for environmental reasons make them unusable at an agronomic scale. The granular fungicides benzimidazole and triazole gave significant control of take-all, but the rates needed to achieve this are uneconomical for wheat (Cook, 2003). Several seed treatments are effective when applied at high rates (Schoeny & Lucas, 1999) and are cost effective alternatives to direct soil treatment (Bateman *et al.*, 2004). Commercially available seed treatments include difenoconazole and silthiofam based formulations (Schoeny & Lucas, 1999; Dyer *et al.*, 2012; Pscheidt & Ocamb, 2015; Karow, 2020), however, 27 of 66 tested isolates of *G. graminis* from China showed resistance to the latter, indicating that broad resistance to silthiofam is likely with continued use (Yun *et al.*, 2012).

Cultural Control

Due to the poor saprophytic nature of take-all, it is primarily managed through crop rotation away from cereal crops and other susceptible grasses for one to two years to reduce inoculum levels. This method of treatment requires rigorous field scouting to remove grass hosts in off-seasons, to deprive the pathogen of a food source and growth medium. Other methods include soil amendments to stimulate antagonistic microbiota (Cook, 2003; Christensen & Hart, 2008), delayed seeding to avoid optimal infection periods, fertilizer application timed to plant uptake to reduce pathogen growth, lowering the soil pH with ammoniacal nitrogen applications to slow pathogen growth, maintaining adequate levels of trace nutrients to boost plant response to disease, increased row

spacing to promote soil drying (Cook, 2003; Christensen & Hart, 2008), and using the Chamberlain system, the establishment of late season trefoil and ryegrass in the wheat understory to deprive the pathogen of nitrogen during its saprophytic stage, but releasing it in time for the next cereal crop (Garrett & Mann, 1948; Cook, 2003). In comparing the relationship been suppressive soils and pathogens in Switzerland, Imperiali et al. (2017) showed that the degree of disease resistance imparted by the soil depends on the type of pathogen, as well as soil type, and available nutrients.

Numerous studies have been performed to assess the degree to which seeding method affects take-all disease severity year to year. An analysis of adjacent plots that were either direct seeded or cultivated before seeding showed a significant increase in severity for the direct seeded crop, leading to the hypothesis that direct seeding left more infested residue in the soil, resulting in more disease than cultivation that disturbed the debris (Moore & Cook, 1984; Cook *et al.*, 2002a). Conversely, a three year survey of 270 wheat fields in the Pacific Northwest found no difference in disease severity between direct seeding and cultivation prior to planting (Ramsey, 2001). Delayed planting of winter wheat into October also reduces the risk of early take-all infections by planting into colder soil, where the pathogen is less active. However, delaying planting into October increases the chance of rain that can reduce stand development and, in fields with poorly drained soils, can cause significant yield losses. This highlights the variability in treatment approaches and the need to properly understand environmental conditions before appropriate action can be taken (Christensen & Hart, 2008).

Fertilizer application, timing, placement, and formulation has been extensively studied (Hornby, 1998). The most obvious effect being that plants infected with take-all have rotted, missing, or damaged roots, and thus become less efficient in the uptake of key nutrients. Application of fertilizer at key plant growth stages helps to mitigate these symptoms (Cook, 2003; Christensen & Hart, 2008). In a study comparing fertilization regimes, it was observed that plots fertilized with a complete fertilizer with micro-nutrients had less infected plants and a lower disease severity than plots fertilized with just nitrogen and sulfur (Moore & Cook, 1984). Application timing and rate having a significant effect on yield, but overapplication of fertilizer to compensate for take-all

effects can result excess nitrogen in the soil and nitrogen leaching that can damage water supplies (Lucas *et al.*, 1997; Christensen & Hart, 2008; Efretuei *et al.*, 2016). Properly timed fertilization also has the potential to enable crops to 'outgrow' take-all (Hornby, 1998). It has been further shown that ammoniacal nitrogen can reduce the severity of take-all while also lowering the pH, but it not clear whether the ammonium, lower pH, or an interaction between them is responsible for the effect, but the practice of liming to raise soil pH increases disease severity (Smiley, 1973; Christensen & Hart, 2008). Chloride amendment has shown some effect on take-all, but the underlying mechanism is unclear, and results have been inconsistent. Some proposed mechanisms include lowering the osmotic potential of cells to a level that is suppressive to take-all, or that Chloride amendment delays nitrification by lowering the soil pH, keeping nitrogen in the ammonium form later into the season (Thomason *et al.*, 2001; Christensen & Hart, 2008).

Biological Control

An intersection between cultural and biological control is the phenomenon of take-all decline (TAD), which develops in response to a prolonged monoculture of susceptible species. Over the course of a susceptible monoculture, usually between 3-5 years, take-all severity will continually increase for several years, before spontaneously declining to low to moderate levels, and persisting in that state for the remainder of the monoculture. This control method does not eliminate the disease, but instead compensates the low level of sustained yield loss due to disease with the reduced input costs of not applying chemical treatments and increased profits by continually growing higher value crops (Cook, 2003; Christensen & Hart, 2008; Schlatter *et al.*, 2017).

Take-all decline occurs worldwide and has been studied extensively, and many different microorganisms have been investigated in the attempt to find effective biocontrol agents. It is currently believed that antibiotic producing *Pseudomonas fluorescens* are the primary agents responsible for TAD, but the mechanisms are still under investigation. Phenazine-1-carboxylic acid (PCA) was the first antibiotic to be consistently associated with TAD soil, but it has since been hypothesized that the production of 2,4-diacetlyphloroglucinol (DAPG) is the more important component in take-all suppression (Weller *et al.*, 2002, 2007; Cook, 2003; Schlatter *et al.*, 2017). 2,4-

DAPG acts on multiple basic cellular functions in the pathogen (Kwak *et al.*, 2011), possibly explaining why *G. graminis* remains sensitive to 2,4-DAPG throughout monoculture, making TAD a robust form of control, with little if any selection for resistant pathogen strains (Kwak *et al.*, 2009).

Other factors that may also impact the speed of onset and robustness of suppressive soils are the differential effects of wheat cultivars to attract and support 2,4-DAPG producing *P. fluorescens* at an adequate density and population structure (Pierson & Weller, 1994; Mazzola *et al.*, 2004; Kwak & Weller, 2013; Osborne *et al.*, 2018), the sensitivity of the pathogen and the host plant to 2,4-DAPG (Kwak *et al.*, 2012), and the expression of plant genes in response to colonization by 2,4-DAPG producing *P. fluorescens* (Haas & Défago, 2005; Maketon *et al.*, 2012). Extensive sampling in the Pacific Northwest has identified the D genotype as the dominant DAPG producing pseudomonad in the rhizosphere of wheat, and may be explained by way of a mutual affinity between genotype D and wheat that allows for extensive colonization, that other pseudomonads lack (Raaijmakers & Weller, 2001; Weller *et al.*, 2002, 2007; Landa *et al.*, 2006).

The same pseudomonads suspected to cause TAD can be applied as a seed treatment to reduce the severity of take-all infection. The pseudomonads aggressively colonize the roots of take-all infected plants and persist throughout the growing season. If treated seeds are not challenged by take-all, the pseudomonads only partially colonize the roots and then decline. This interaction suggests that there is a unique antagonistic interaction between take-all suppressive *pseudomonas* and take-all (Weller, 1983, 1988; Raaijmakers & Weller, 2001; Cook *et al.*, 2002b).

Genetic Control

Full genetic resistance to take-all has been a long-time goal of wheat breeding programs (Hornby, 1998), but no modern cultivars have displayed a high degree of resistance (McMillan *et al.*, 2014). The lack of resistance genes in wheat supports the theory that other factors are primarily responsible for the suppression of take-all at their centers of origin, and thus there is little selection pressure to develop resistance naturally (Cook, 2003). One potential genetic factor is hinted at by the observation that the speed

of TAD establishment is correlated with the cultivar grown (Mazzola *et al.*, 2004; Maria *et al.*, 2005; Meyer *et al.*, 2010; Maketon *et al.*, 2012). Even within TAD soils, wheat cultivars differ in their abilities to sustain the effect over time, indicating that this is likely a genetic trait in need of further exploration (Yang *et al.*, 2018). The related species oat, rye, barley, and triticale all exhibit considerably more tolerance to take-all than hexaploid wheat, suggesting that they could be used as potential sources of resistance in future breeding programs. However, the difference in take-all resistance between hexaploid and octoploid triticale, greater and lesser resistance respectively, suggests that the resistance in rye is genetically complex, and likely difficult to transfer (Cook, 2003). *Triticum monococcum,* an ancestral relative of hexaploid wheat, also exhibits resistance to take-all in some of its genotypes, suggesting that resistance genes may already exist within modern hexaploid wheat, and should continue to be explored (McMillan *et al.*, 2014).

Though lacking true genetic resistance, modern wheat cultivars possess a novel genetic trait termed take-all inoculum build-up (TAB), which can be used to categorize cultivars based on how much take-all inoculum they build up in the soil, when grown as the first wheat crop in a rotation (Mauchline *et al.*, 2015; McMillan *et al.*, 2018). High TAB cultivars encourage the growth of antagonistic *P. fluorescens* genotypes, while low TAB cultivars encourage *P. fluorescens* genotypes with greater siderophore production, which can reduce fungal growth my limiting available iron (Mauchline *et al.,* 2015), but there is still much debate as to whether low or high TAB cultivars are the better choice for inducing suppressive soils (Mauchline *et al.*, 2015; Mauchline & Malone, 2017; McMillan *et al.*, 2018).

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Chapter 2: Differential Effects of First Year Wheat Genotype on the Suppression of Take-All Disease and the Establishment of Suppressive Microbial Agents in the Rhizosphere

Evan Perkins, Chris Mundt

Summary

- We studied the effectiveness and mechanistic basis of microbiome-mediated genetic resistance of wheat to take-all, an important soil-borne disease.
- Field experiments were conducted to determine the influence of different first year wheat cultivars on take-all levels in subsequent field and greenhouse studies. PCR targeting an essential gene in the biosynthetic pathway of the antibiotic 2,4-diacetlyphloroglucinon (DAPG), which is associated with antagonism and induced host resistance against the take-all pathogen, was used to evaluate communities of *Pseudomonas* spp. derived from root washes.
- Soil from one first year wheat cultivar consistently resulted in less take-all and accumulated significantly more 2,4-DAPG producing pseudomonads than soil from other cultivars. An intermediate level of take-all suppression in some other cultivars may be regulated by a different mechanism. The first year cultivar effect dominated the response in subsequent plantings, and its impact was not specific to the first year cultivar.
- Wheat genetics may be used to produce an environment favorable to soil microbiome components that suppress take-all, an approach that is cost effective and sustainable. This microbiome effect may be determined, in part, by populations of 2,4 DAPG-producing pseudomonads, which have also been shown to induce resistance to a diversity of other important wheat diseases.

Key Words

Gaeumannomyces graminis var. *tritici*, microbiome, *Pseudomonas fluorescens*, take-all disease, take-all decline, take-all build-up (TAB), 2,4 DAPG, *Triticum aestivum* (wheat)

Introduction

The use of microbiota to increase plant and soil health dates back to the 1800's, with the inoculation of legumes with rhizobium bacteria to enhance nitrogen fixation (Busby et al., 2017). More recently, interest in biological control has increased, especially in the area of pathogen suppressive soils, which have been defined as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." (Baker & Cook, 1974). A review of the rhizospheres from a disease suppressive sugar beet field identified over 33,000 bacterial and archaeal operational taxonomic units (OTU), highlighting how diverse the root microbiome is (Mendes *et al.*, 2011). Suppressive soils can be either general, supporting a reduction in disease severity via the collective effects of soil microbiota, or specific, being driven by a specific microbial agent or group of agents against a specific pathogen on a specific crop. Both forms of pathogen suppression are naturally occurring and have a range of effectiveness, but specific suppression can be transferred with 0.1%-10% w/w addition to conducive soil (Weller, 1988; Weller *et al.*, 2002; Cook, 2014). In a thorough review of three suppressive soil systems, ranging from the specific disease suppression of take-all, to the broadly suppressive *Streptomyces*, each system was found to have unique soil microbial populations and mechanisms by which they develop (Schlatter *et al.*, 2017). These vast differences highlight the need to better understand the unique attributes of each system so they can be better managed and applied effectively in production environments.

Historically, plant disease has been managed primarily via environmental modification through pesticide application and crop rotation, and by breeding for disease resistant crops. However, pesticides are expensive and can have negative effects on human health and the environment, while resistant crops may select over time for pathogen genotypes with increased virulence (Panth *et al.*, 2020). Utilizing the microbiome holds potential solutions to all of these problems, allowing for a more environmentally benign form of agriculture, naturally increasing plant health and nutrient acquisition, and increased disease resistance (Mauchline & Malone, 2017). This points to

the continued need to investigate natural means of disease control, including microbiome-mediated disease suppression (Busby *et al.*, 2017).

Large, coordinated research efforts into better understanding the human microbiome are underway (Turnbaugh *et al.*, 2007; Qin *et al.*, 2010), but no such coalition exists for the consolidation of information gathered in plant systems, despite a growing wealth of information across many staple crops and model research species, stemming from the segregation of academic and industry researchers, and their segregation from growers (Busby *et al.*, 2017). The application of microbiome research to the problems of modern agriculture is essential for the advancement of the industry, but the best method of microbiome improvement remains unclear (Compant *et al.*, 2019). Advances in nucleotide sequencing technologies enables rapid DNA analysis of rhizosphere samples to explore potential plant-microbe interactions and characterization of the microbial communities, and how the microbiome influences metabolic activities (Weller *et al.*, 2007; Mavrodi *et al.*, 2007; Mauchline *et al.*, 2015). Recent research into the maize phytobiome has indicated that a subset of commonly isolated microbiota show heritability within a genome, supporting the argument that the microbiome is, at least in part, driven by host genotype (Wallace *et al.*, 2018). The development of microbiome focused crop breeding, amendment procedures, and farming strategies are key to enhancement of the agricultural industry (Compant *et al.*, 2019). Of equal importance is how to apply these academic findings in a way that is both beneficial and understandable to growers (Busby *et al.*, 2017).

Soil-borne diseases are a major limiting factor in modern agriculture, with some pathogens causing yield losses as high as 75%, and making up about 90% of the 2000 major diseases faced by commercial agriculture (Panth *et al.*, 2020) Take-all disease of wheat is caused by the soil-borne fungus *Gaeumannomyces graminis* var. *tritici,* a root necrotrophic ascomycete that causes stunting, lack of grain fill, chlorosis, and eventually death as it spreads throughout the root system and into the crown, disrupting water transportation and destroying the root system. It is considered to be one of the most destructive root diseases of wheat around the world, with yield losses of 50 percent or greater having been reported (Cook, 2003; Freeman & Ward, 2004; Christensen & Hart,

2008). Take-all is most severe under irrigated and high-rainfall conditions, where it occurs in patches, spreading root-to-root from a central source of inoculum via runner hyphae. New infections develop throughout the growing season and are favored by moist conditions and soil temperatures of 10-20°C. Early infections will spread through the root system to the crown, and can increase exponentially while conditions are favorable (Mathre, 2000; Christensen & Hart, 2008; Kwak & Weller, 2013)

Soil fumigation with chemicals is effective for the control of take-all, but is too expensive to use at an agronomic scale for wheat; the same can be said of granular fungicides applied alongside planting (Cook, 2003).There are relatively few seed treatments available that have proven to be viable against take-all, all using either difenoconazole or silthiofam formulations. However, 27 of 66 tested isolates from China showed resistance to the latter, indicating that resistance to silthiofam is likely with continued use (Schoeny & Lucas, 1999; Dyer *et al.*, 2012; Pscheidt & Ocamb, 2015). Without effective chemical controls most growers must rely on crop rotation to reduce inoculum levels between crops of the same species. By growing a non-host or by leaving land fallow for at least one year, the pathogen is deprived of a food source and substrate to survive in between growing season, effectively reducing inoculum potential to below economic thresholds (Cook, 2003; Freeman & Ward, 2004; Christensen & Hart, 2008). Unfortunately, the global trend is an increase in wheat monoculture to meet the growing population demands and does not allow for enough time between wheat harvest and planting to adequately decrease inoculum potential (Cook, 2003).

Genetic resistance to take-all has been researched extensively, but there are currently no known cultivars within commercial hexaploid wheat that bear this trait, despite evidence of it in related species (Hornby, 1998; Cook, 2003; Freeman & Ward, 2004; McMillan *et al.*, 2014). In lieu of resistant cultivars, some farmers choose to rely on natural disease suppression, mainly the development of suppressive soils (Cook, 2003). Suppressive soils have been described for many different pathogens and cropping systems throughout the world, including *G. graminis* var. *tritici*, but the mechanisms by which they function are not fully understood. Specific suppressive soils are developed through the successive planting of susceptible cultivars (Weller *et al.*, 2007). Continued

wheat monoculture results in increased disease for 3-5 years before declining to an economically acceptable level, known as take-all decline (TAD) (Smiley, 1973; Weller *et al.*, 2002, 2007).

Extensive research has identified 2,4-diacetylphloroglucinol (2,4-DAPG)-producing *Pseudomonas fluorescens* as one of the key factors behind TAD (Raaijmakers & Weller, 2001; Weller *et al.*, 2002; de Souza *et al.*, 2003). 2,4-DAPG is a broad-spectrum polyketide antibiotic with known biocontrol properties against a range of root disease, including take-all, as well as inducing resistance to foliar pathogens in its host (Weller *et al.*, 2007). Factors that likely impact the speed of onset and robustness of the suppressive soil are the differential effects of wheat cultivars to attract and support 2,4-DAPG producing *P. fluorescens* at an adequate density and population structure (Pierson & Weller, 1994; Mazzola *et al.*, 2004; Kwak & Weller, 2013; Osborne *et al.*, 2018; Zadjali, 2019), the sensitivity of the pathogen and the host plant to 2,4-DAPG (Kwak *et al.*, 2012, p. 20), and the expression of plant genes in response to colonization by 2,4-DAPG producing *P. fluorescens* (Haas & Défago, 2005; Maketon *et al.*, 2012). Extensive sampling in the Pacific Northwest has identified the D genotype as the dominant DAPG producing pseudomonad in the rhizosphere of wheat, and may be explained by way of a mutual affinity between genotype D and wheat that allows for extensive colonization, that other pseudomonads lack (Raaijmakers & Weller, 2001; Weller *et al.*, 2002, 2007; Landa *et al.*, 2006).

Even within TAD environments, cultivars differ in their ability to support the suppression of take-all over time, adding further evidence for a genetic component to the development of suppressive soils (McMillan, 2012; Osborne *et al.*, 2018; Yang *et al.*, 2018). This phenomenon is the result of a novel genetic trait termed take-all inoculum build-up (TAB), which can be used to categorize cultivars based on how much take-all inoculum they develop in the soil when grown as the first cultivar in a new field. The trait is usually measured indirectly, however, by quantifying the amount of take-all disease that develops in soils exposed to different wheat cultivars in a sequence (McMillan *et al.*, 2011; McMillan, 2012; Mauchline & Malone, 2017). It has been observed that the growth of high TAB cultivars encourages the growth of *P. fluorescens* genotypes better

adapted to microbial competition, whereas the growth of low TAB cultivars encourages genotypes with greater siderophore production, which could reduce fungal establishment (Mauchline *et al.*, 2015).

Recent research in the UK has found that the wheat cultivar grown in the first year can have a significant impact on the amount of take-all that develops in the second year, regardless of the cultivar planted in year two (McMillan *et al.*, 2011). 'Einstein' is one such cultivar that reduces take-all disease and may possess a gene or genes that encourages favorable populations of *Pseudomonas* to colonize the rhizosphere (McMillan, 2012; Liu *et al.*, 2019). The cultivar Einstein is a parent in the wheat cultivar 'Bobtail' that was released by Oregon State University in 2012. This study aims to determine if the low TAB trait has been inherited by Bobtail, if the trait is robust across the UK and US field environments, and how first year Bobtail compares to other cultivars commonly grown in the PNW in regard to take-all development. We also designed experiments to determine if DAPG-producing pseudomonad prevalence is associated with reduced take-all disease, and whether these pseudomonads might play a role in establishing a priority effect by the first year cultivar.

Materials and Methods

Host Cultivar Selection

To assess the degree of TAB present in modern wheat production, we studied the six highest yielding wheat cultivars in the Willamette Valley of Oregon at the time the experiments were implemented, based on data from wheat variety trials conducted by the Oregon State University Cereal Extension Program. Cultivars 'Bobtail' (Zemetra *et al.*, 2013a), 'Kaseberg' (Zemetra *et al.*, 2012), and 'Rosalyn' (Zemetra *et al.*, 2013b) were developed by Oregon State University. 'LCS Artdeco' 'LCS Biancor', and 'LCS Drive' were developed by Limagrain Cereal Seeds (Limagrain, 2020). The Oregon State University cultivar 'Stephens' (Kronstad *et al.*, 1978) dominated the Oregon wheat area for over 20 years (Peterson, 2001), was known to experience severe take-all disease under conducive conditions, and served as a historical check and as a greenhouse check cultivar in some of the studies described below. 'Seahawk' (Pumphrey *et al.*, 2018), a

spring wheat, was developed by Washington State University and was used in one field trial in which flooding precluded establishment of winter wheat.

Disease Identification

Take-all disease was diagnosed visually. All plants identified as having take-all had one or more characteristic symptoms, depending on the degree of infection. Observed symptoms included black and brittle roots from surface to core, dark growth under leaf sheath, dark runner hyphae along the surface of the roots, stunting, and pre-mature grainfill. Severely infected plants were stunted, and white heads were observed. Plants identified as being infected with take-all lacked the eye shaped lesions of strawbreaker foot rot and sharp eyespot, the brown roots and stem streak of fusarium root rot, and the brown root, leaf spots, and black head spots of common root rot.

Pseudomonads were isolated from rhizosphere root washes using *Pseudomonas* selective media Kings medium B^{+++} (KMB^{+++}) (King *et al.*, 1954; McSpadden Gardener *et al.*, 2001), and identified morphologically and with allele specific primers as members of the *Pseudomonas fluorescens* complex capable of producing 2,4-DAPG.

Field Assessment and Sample Collection

Three winter wheat experiments were conducted in different fields at the Botany and Plant Pathology Field Laboratory in Corvallis, OR USA [44.568260, -123.242765] to determine if a cultivar grown in the first year of wheat production influenced the level of take-all disease on one or more cultivars grown over the same plots in the second year of production. All experiments were grown in fields consisting of Chehalis silty clay loam soil. Fields were fallow in the previous year, with regular tillage and/or herbicide applications used to control weeds and volunteer wheat plants. All fields received 28 kg/ha of N, P_2O_5, and K_2O in the autumn, prior to planting. Winter wheat plots received 135 kg/ha of N as urea in late winter, prior to the jointing growth stage, Feeke's stage 6 (Large, 1954). The 2017 spring wheat planting received 90 kg/ha of N as urea in the spring, prior to jointing. Weed control and tillage practices used to grow the experimental plots were standard for wheat production in the Willamette Valley of Oregon. Winter wheat plantings were not irrigated during the crop year, but the spring wheat planting in 2017 was irrigated regularly to maintain a healthy crop. Metal stakes of known distance

from the corners of the Year 1 experiments were established in non-tilled ground surrounding the fields so that wheat plots could accurately be established in areas known to contain a given cultivar in the previous year. Grain was harvested from Year 1 plots with a plot combine in late July to remove grain from the field. The experimental areas were subsequently irrigated intermittently to allow volunteer wheat seedlings to grow for about a month, thus encouraging natural build-up of the take-all pathogen. The field was then sprayed with glyphosate herbicide and tilled after the volunteers and weeds died.

Experiment 1

The first experiment was a preliminary one to determine if there was potential for the cultivar Bobtail grown in the first year to reduce take-all disease in the second year of winter wheat production. There were two Year 1 treatments: the cultivars Bobtail and Stephens. Two 12.2 ×12.2 m plots of each cultivar were planted linearly (east-west) in a randomized complete block design on 13 October 2014. Plots were planted with eight 1.52 m passes using a cone-type experimental plot drill with six double-disc openers 20 cm apart. Planting density was 270 seeds m^{-2}.

For year 2, the entire experimental area was planted on 7 October 2015 to the winter wheat cultivar ‘Rosalyn’. Rosalyn was seeded perpendicular to the 2014 planting, using a commercial grain drill with seven rows 19 cm apart for each 1.52 m planting pass and a planting density of 270 seeds m^{-2}. To control foliar disease, plots were sprayed with a mixture of azoxystrobin (96.2 g a.i. ha^{-1}) and propiconazole (117 g a.i. ha^{-1}) fungicides on 8 April 2016 and with propiconazole (117 g a.i. ha^{-1}) only on 28 April 2016. Plots were observed for symptoms of take-all during the season, and four 1.52 m wide strips were harvested across the north half of each plot with a plot combine on 26 July 2016 to evaluate yield differences.

Experiment 2

The second experiment utilized six wheat cultivars as first year treatments: Bobtail, Kaseberg, Rosalyn, LCS Drive, LCS Biancor, LCS Art Deco. The six cultivars were planted in a randomized complete block design with four blocks on 9 October 2015. Each experimental unit (plot) was 6.1 × 9.1 m, resulting from six 6.1 passes of the experimental plot drill described for Experiment 1. To control foliar disease, plots were

sprayed with the same fungicides and at the same rates and dates as described above for Year 2 of Experiment 1. A total of 7.6 L of soil was obtained from subsamples collected in an × pattern from the interior 3.0 × 6.1 section of each plot. Subsamples were collected at the intersections of the "×" with planting rows, to a depth of 15-20 cm, using a hand-operated auger. Subsamples were aggregated within plots and stored in a refrigerated room for future testing.

Record rainfall in October 2016 resulted in two failures to establish the second year winter wheat treatments. Instead, the entire field was planted to Seahawk spring wheat on 8 May 2017 at ~250 seeds m^{-2}, using the same commercial grain drill described for Year 2 of Experiment 1, with planting passes the same direction and planted directly over planting passes of the Year 1 plots. When seed heads were mature and beginning to dry, 10 plants were sampled at random from 3.0 ×6.1m section of each plot for disease assessment. Disease was assessed by digging up whole plants, rinsing roots to remove soil, and assigning a rating of 0-5 based on the number of black lesions characteristic of take-all on the seminal roots. The innermost two planting passes of each plot were harvested with an experimental plot drill and this grain was used to evaluate the yield, test weight, and % protein of grain from each plot. Seeds were allowed to dry for three weeks, and then tested with an Infratec 1241 Grain Analyzer (FOSS, Eden Prairie Minnesota).

Experiment 3

Design and planting methods for Year 1 of the third experiment were identical to that of Experiment 2, except that the cultivar Stephens was added, providing a total of seven cultivars. Heavy rainfall delayed planting of this experiment until 10 November 2016. To control foliar diseases, plots were sprayed with propiconazole (117 g a.i. ha^{-1}) on 12 March 2017, and with a mixture of azoxystrobin (99 g a.i. ha^{-1}), benzovindiflupyr (29 g a.i. ha^{-1}), and propiconazole (86 g a.i. ha^{-1}) on 10 May 2017.

In Year 2, a split plot experiment was established by planting alternating 1.52 m strips of the cultivars Bobtail and Rosalyn within each of the 6.1 × 9.1 m plots on 6 October 2017. Planting was in the same row direction as in Experiment 3/Year 1 plots, using the same commercial grain drill and seeding rate as described for Experiment

1/Year 2. To control foliar disease, plots were sprayed with a mixture of trifloxystrobin (80 g a.i. ha^{-1}) and propiconazole (117 g a.i. ha^{-1}) 5 February 2018.

When seedheads were mature and beginning to dry, five plants from each of the three central planting rows of the two central sub-plots of each block were collected for disease assessment, totaling 15 plants from each sub-plot. Disease was assessed by digging up whole plants, rinsing roots to remove soil, and counting the number of black lesions characteristic of take-all on the seminal roots. Percent whiteheads (seedheads that turned white prematurely and were largely empty of grain), a common symptom of take-all disease, was estimated for the two central sub-plots of each plot once per week for three weeks, beginning 11 May 2018. Plots were harvested and soil samples collected for the two central sub-plots of each block as described for Experiment 2/Year 1. Plots were first irrigated after harvest to facilitate collection of the soil samples.

Greenhouse Soil Assay

In the winter of 2016-2017 a soil bioassay using post-harvest field soil from Experiment 2/Year 1 was used to assess the degree of take-all inoculum and soil suppression carried over from the previous field season. Field soil was mixed 3:1 with sand to allow for easier separation of media from roots at the end of the study. A cotton ball was placed at the bottom of Ray Leach Cone-tainers ID code SC10U (Stuewe & Sons, Tangent, OR) which were then filled to within 5 cm of the top with soil. The containers had a volume of 164 ml, a diameter of 3.81 cm, and a depth of 21.0 cm. Three seeds of Stephens wheat were planted in each Cone-tainer and topped with vermiculite, leaving ½" of headroom, and grown in a greenhouse at ~70% RH, 15/10 °C (day/night), with 16-hour days provided by artificial lighting. Foliar insect pest control was applied at the discretion of the greenhouse staff. Five pots were planted from soil of each field plot, watered every other day, and fertilized every other week with Miracle-Gro All-Purpose Plant Food (24-8-16, Scotts Miracle-Gro Products, Marysville, OH). At 1 week post-germination, plants were thinned to one plant per pot. After 8 weeks, whole plants were collected, loose soil removed, roots lightly washed, and black lesions typical of take-all on the primary roots counted. Roots were allowed to dry overnight before weighing and continued processing for rhizosphere sampling (see below). The experiment was

conducted a total of four times during the period from October 20, 2016 through January 5, 2017.

In the winter of 2018-2019, a soil bioassay using post-harvest field soil from Experiment 3/Year 2 was used to assess the degree of take-all inoculum and soil suppression carried over from the previous two field seasons. Seven extra pots were planted for each combination of field soil and greenhouse cultivar, to be destructively sampled throughout the trial to determine the appropriate time for disease assessment, for a total of 12 pots for each combination. Soil preparation and greenhouse settings were identical to the previous year. Cultivars Bobtail, Rosalyn, LCS Artdeco, and Stephens were used to determine how tester cultivar affects disease development in this third cycle of exposure of soil to wheat cultivars. This experiment thus became a split-split plot design, with main plots being the cultivars grown in the field in Year 1, subplots being the Bobtail and Rosalyn planting strips in the field in Year 2, and sub-subplots being the four tester cultivars in the greenhouse. Resource limitations prevented us from using all seven cultivars as testers. Plants were managed, harvested, and processed identically to the previous year, except that plants were evaluated at 12 weeks after planting based on destructive sampling of the extra pots. The evaluations included one run for each block of the experiment during the time period of 8 November2018 through 1 April 2019, totaling four runs.

Rhizosphere Sampling

For Experiment 2, dry roots from the greenhouse assay were separated from the remainder of the plant with a clean razor blade, treatments with all like factors(field block, year 1 cultivar, year 2 cultivar, greenhouse cultivar, and greenhouse replication) were aggregated and 5g of root matter was placed into a Falcon 50mL Conical Centrifuge Tubes (Thermo Fisher Scientific, Walltham, MA) with 25ml of sterile distilled water. Bacteria were dislodged by vortexing four times for 30 seconds each. 100 ul of each sample was transferred to microtubes prefilled with 200 ul of sterile distilled water, 50ul of this was then transferred to a new microtube prefilled with 200 ul of 1/3x Kings medium $B^{+++,}$ and incubated in the dark at room temperature for 48 +/- 4 hours. Replicate plates were made by transferring 100 µl of each culture to racked micro-tubes pre-filled

with 100 µl 35% glycerol and frozen at -80°C (McSpadden Gardener et al., 2001). This was performed three times per sample, resulting in three replications of each factor combination.

For Experiment 3, year 2, dry roots from the greenhouse assay were separated from the remainder of the plant with a clean razor, treatments with all like factors(field block, year 1 cultivar, year 2 cultivar, greenhouse cultivar, and greenhouse replication) were aggregated, and 5g of root matter was placed into a Falcon 50mL Conical Centrifuge Tubes (Thermo Fisher Scientific, Wallthem, MA) with 25ml of sterile distilled water. Bacteria were dislodged by vortexing four times for 30 seconds each. One-hundred microliters of each sample was transferred to the first row of a 96-well microtiter (Thermo Scientific, Wallthem, MA) plate prefilled with 200 µl of sterile distilled water and serial diluted down the plate. 50 µl from each cell was transferred to another plate pre-filled with 200 µl of 1/3x KMB^{+++} and incubated in the dark at room temperature for 48 +/- 4 hours. Bacterial growth was assessed spectrophotometrically with a Spectramax 190 microplate reader (Molecular Devices, San Jose, CA) with a reading of ≥ 0.05 recorded as positive. Replica plates were made by transferring 100 µl of each culture to racked micro-tubes pre-filled with 100 µl 35% glycerol and frozen at -80°C. The end result is a five-fold dilution from well to well, ranging from relative concentrations of 3.33×10^{-2} to 2.13×10^{-5} at the final dilution. This was performed three times per sample, resulting in three replications of each dilution series.

Root Wash Analysis by PCR Amplification

Primers were chosen based on their ability to detect *phlD*+ pseudomonads from all 13 genotypes that have been defined by BOX-PCR, allowing for a wide range of detection. Primers to detect *phlD* were designed and tested by the Weller lab (McSpadden Gardener *et al.*, 2001). The presence of a 629-bp band indicates the presence of *phlD*+ bacteria in the tested solution. The dilution factor of the final positive amplification is used to estimate the relative abundance of *phlD*+ pseudomonads.

Root wash cultures were assessed for the presence of *phlD*+ *Pseudomonas fluorescens* by PCR using gene specific primers bpf2 + bpr4 and b2bf + bpr4, and using the dilution factor of the last positive florescence to estimate bacterial abundance in the

rhizosphere. *P. fluorescens* Pf-5 was used as a positive control. Dilutions one through six were chosen for amplifications in 25 ul reaction mixtures containing 2.5ul of whole cell template, 1x *DreamTaq* DNA polymerase buffer (Thermo Scientific, Walltham, MA), 200uM solution dNTP (Qiagen, Inc., Germantown, MD), 25 pmoles each primer, and 1.5 units *DreamTaq* DNA polymerase (Thermo Scientific, Walltham, MA) .

Amplifications were performed in a PTC-200 DNA Engine Thermal Cycler (Bio-Rad Laboratories, Inc, Hercules, CA) with the following settings: 95°C for 3 min, 35 × 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, 72°C for 5 min, followed by a 4°C soak and –20°C storage. After amplification, 8 µl of each reaction was loaded onto 1.5% agarose gels in 0.5× Tris-borate-EDTA and electrophoresed for 1 h at 125 V. Gel images were visualized by ethidium bromide staining, measured with 100-bp DNA ladder, and saved as JPG files. 96 well plates were used to assess all possible combinations of year 1, 2, and 3 cultivars.

Statistical Analysis

Statistical analyses were carried out in R version 3.6.3 (R Core Team 2020, Vienna, Austria). R Studio version 1.2.5033 (RStudio Team 2019,Boston, MA). R packages Agricolae version 1.3-2 and emmeans version 1.4.7 were used to analyze split-plot, and split-split-plot data. R package ggplot2 version 3.3.1 and scales version 1.1.0 were used to generate graphical representation of results.

Statistical analyses were performed using analyses of variance appropriate to the design of each data set. Tukey's honest significance difference was used to test for differences among treatment means. Residual plots for each data set indicated that data transformation was not necessary. Correlation coefficients were calculated using Pearson's correlation test. In all experiments cultivars were treated as categorical variables, all other collected data was treated as a discrete variable. Disease development and *P. fluorescens* population density were assessed as proxies for disease suppressiveness soil.

The greenhouse portion of experiment 2 and experiment 3 year one was analyzed as a split-plot design, with the field replications assigned as blocks, the year one field cultivars as the main plots, and the greenhouse checks as sub-plots.

The greenhouse portion of experiment 3 year two was analyzed as a split-split-plot design, with the field replications assigned as blocks, the year one field cultivars as the main plots, the year two field cultivars as the sub-plot, and the greenhouse checks as sub-sub-plots.

Results

Experiment 1

Severe take-all disease developed in the preliminary trial comparing the yield of second year Rosalyn after first year wheat cultivars Bobtails and Stephens, with a strong disease gradient from north to south and a visually obvious difference in plots planted to Bobtail versus Stephens in the previous year. Poor stands caused by severe take-all in the south halves of the plots resulted in heavy weed growth, making harvest infeasible. Yield in the north halves of the first year Bobtail treatment yielded 4.12 Mg/ha, while the first year Stephens treatment yielded 2.13 Mg/ha. With only two replicates per treatment, this difference was significant only at P=0.095 in the analysis of variance (Table 1), but suggests a potentially strong impact of first year cultivar on wheat yield in the subsequent year.

Table 1 ANOVA table for Experiment 1 yield data, comparing the effect of year one Bobtail wheat versus year one Stephens wheat on second year yield of cultivar Rosalyn

Mean yield

	Df	Mean Sq	F value	Pr(>F)
year1	1	3.935	44.22	0.095
block	1	0.903	10.15	0.194
Residuals	1	0.089		

Experiment 2

Soil collected from plots of six cultivars in the first year of Experiment 2 differentially impacted the number of take-all lesions when tested against cultivar

Stephens in the greenhouse (Fig. 1A, Table 2). Lesion numbers on greenhouse plants varied approximately four-fold among soils collected from the six Year 1 cultivars. Disease resulting from soil collected in Bobtail and LCS Artdeco plots differed significantly based on Tukey's HSD; the other four cultivars were intermediate in lesion number and overlapped statistically with Bobtail and LCS Artdeco. These results are consistent with the Year 2 field trial, where the spring wheat cultivar Seahawk was planted over the plots grown to the six cultivars of Year 1 (Fig. 2B, Table 2). The effect of Year 1 cultivar on the disease rating of Seahawk plants was significant (P = 0.0047), and there was more than a two-fold range from the lowest to the highest treatment mean. Year 1 treatments Bobtail and Rosalyn showed significantly less disease than LCS Artdeco, with the remaining Year 1 cultivars being intermediate and overlapping statistically with the lowest and highest treatments. Overall, ranking of first year cultivar effects were very similar for the greenhouse test with Year 1 soil, and plants evaluated in the Year 2 field trial (Figs. 1A and B), and there was a significant, positive correlation between field disease severity and greenhouse disease lesion numbers (R=0.85, p=0.032).

PCR tests for the presence of *phlD*+ *P. fluorescens* in rhizosphere samples from the greenhouse trial showed that Year 1 Bobtail samples had significantly more cultures that rated positive for phlD presence that any of the other Year 1 cultivars (Fig. 1C), indicating it has a greater capacity for attracting and supporting 2,4-DAPG producing *Pseudomonas* bacteria. There was a weak correlation between greenhouse disease severity and the incidence of *phlD*+ pseudomonads recovered from the rhizosphere (R = -0.191, p=0.108).

There was visually less severe take-all disease in Experiment 2 than in the preliminary Experiment 1 trial. In addition, flooding conditions imparted substantial stand variability in the experiment. As a result, the coefficient of variation in the ANOVA for yield was 22% and the significance for a year one cultivar effect on yield was P=0.671.

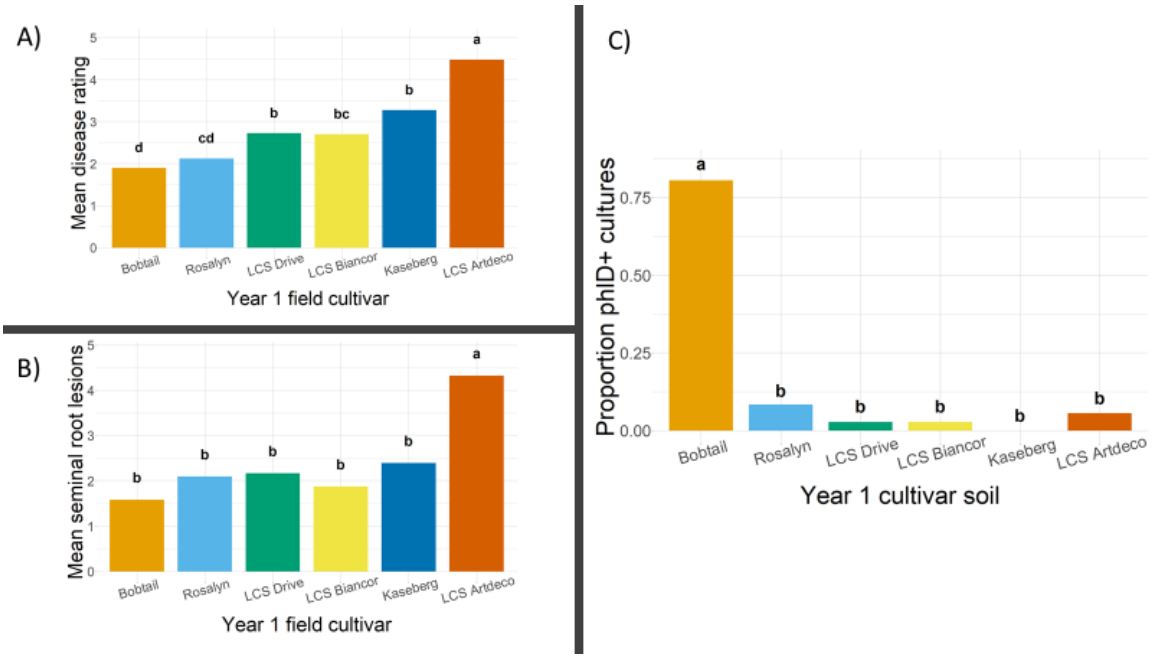

Fig. 1 A) Mean disease rating of root system of Seahawk spring wheat in the field when planted after one of six winter wheat cultivars. Roots were assessed on a 0-5 scale for take-all severity when heads were beginning to ripen. SEM = 0.534. B) Number of take-all lesions on root systems of Stephens wheat in the greenhouse when planted into soil sampled post-harvest from replicated field plots of six winter wheat cultivars. Roots were evaluated eight weeks after sowing in the greenhouse. Data are means over four field replicates and six replicate plants per field replicate for each variety, and four runs over time in the greenhouse. SEM = 0.0560 C) Mean positive hits from greenhouse rhizosphere washes using *phlD* specific PCR primers. Data for each year one cultivar represents four field replicates, six replicate plants per field replicate for each variety, four greenhouse replicates, and three PCR replicates. SEM = 0.00225.

Table 2 ANOVA table for experiment two greenhouse and field disease assessment comparing the mean effects of seven different first year cultivars on second year disease development, and rhizosphere analysis of the effect of seven first year cultivars on the prevalence of *phlD+* pseudomonads in second year wheat rhizosphere.

Mean take-all disease rating (field)

	Df	Mean Sq	F value	Pr(>F)
Year 1 cultivar	5	171.43	39.531	<2e-16
Block	3	0.21	0.243	0.622
Residuals	233	202.09		

Mean Lesion count on seminal roots (greenhouse)

	Df	Mean Sq	F value	Pr(>F)
Year 1 cultivar	5	68.2	7.075	2.30E-06
Block	1	45.6	4.726	0.0303
GH rep	1	610.4	63.313	1.69E-14
Year 1 cultivar:GH rep	5	5.9	0.614	0.6889
Residuals	416	9.6		

Proportion of positive P. fluorescens PCR hits

	Df	Mean Sq	F value	Pr(>F)
Year 1 cultivar	5	1.1852	82.656	< 2e-16
Block	1	0.242	16.876	0.000125
GH rep	1	0.0208	1.453	0.232870
Year 1 cultivar:GH rep	5	0.0153	1.065	0.388697
Residuals	59	0.0143		

Experiment 3

In the trial comparing year one field cultivar effects on disease of subsequently planted cultivars, year one cultivar effects ranked similarly to those of Experiment 2 (Fig. 2A). Averaged over second year cultivars, plots that grew LCS Artdeco in the first year developed significantly more lesions than plots that grew cultivar Bobtail based on Tukey's HSD (Fig. 2A). The year one cultivar effect for whiteheads was significant only at P=0.078 (Table 3), but the posthoc analysis using Tukey's HSD classified year one Bobtail and year one Stephens into different significance groups (Fig. 2C). Averaged over first year cultivars, Rosalyn developed a mean of 10.32% whiteheads as the year two cultivar while Bobtail developed 9.04% (Fig. 2D), a small difference that was nonetheless significant at P=0.014 (Table 3).

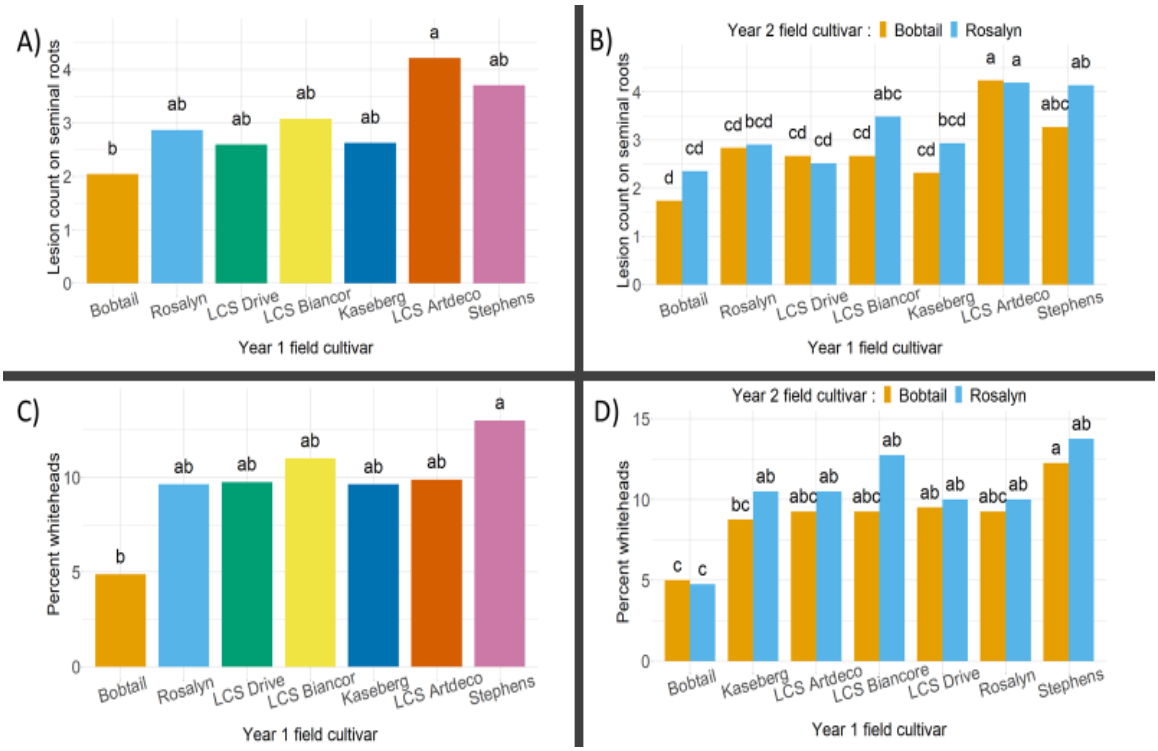

Fig. 2 Field assessment for take-all after two seasons. Year one consisted of four blocks of seven cultivars, year two consisted of alternating passes of two cultivars across the whole main plots. A) Mean number of lesions on seminal roots of second year wheat within each plot, ignoring second year effect. Data represents three sampling dates consisting of five samples per day across four blocks, SEM = 0.779. B) Mean number of lesions on seminal roots of second year wheat within each subplot, showing year one and two cultivar effect from three sampling dates consisting of five samples per day across four blocks, SEM = 3.2857 C) Percentage of whiteheads present within each plot, SEM = 1.7918 D) Percentage of whiteheads present within each sub-plot, showing year one and two cultivar effects, SEM = 0.1886.

Table 3 ANOVA table for Experiment 3 field disease assessment of the effect of seven first year and two second year wheat cultivars on the percentage of whiteheads developed by second year wheat, and the amount of lesion development on second year wheat roots

	Percent whiteheads (field)				Mean take-all lesion count on seminal roots (field)		
	Df	Mean Sq	F value	Pr(>F)	Mean Sq	F value	Pr(>F)
Block	3	199.262	8.5797	0.0009463	191.023	13.111	8.84E-05
Year 1 cultivar	6	53.913	2.3213	0.0776071	63.951	4.3893	0.00666
Error a	18	23.225	0.0635	1	14.57	7.0117	< 2.2e-16
Year 2 cultivar	1	17.357	7.1006	0.0144991	33.201	2.2921	0.14494
Year 1 cultivar : Year 2 cultivar	6	1.329	0.5438	0.7690507	5.507	0.3802	0.88338
Error b	21	2.444	0.0635	1	14.485	7.0117	< 2.2e-16

There was no significant interaction between first year and second year cultivars for either lesion numbers or percent whiteheads (Table 3), suggesting a lack of cultivar specificity for cultivar effects on take-all. A large and highly significant block effect was observed for both lesion numbers and percent whiteheads, with northern blocks developing less disease and southern blocks developing more disease (Table 3). This was likely due to field topography, with the field sloping downward slightly, from north to south, causing the northern blocks to dry quicker than the southern blocks, thus slowing disease development.

Rainfall during planting season of both years of the experiment resulted in significant yield variation among plots. As a result the coefficient of variation in the ANOVA for the field was 35%, and there were no significant differences based on the cultivars planted in year one (data not shown). Rosalyn yielded more than Bobtail in year two of the experiment (P= 0.00044), though this is likely due to differences in yield potential between the two cultivars.

Year one cultivar had a highly significant effect on lesion number averaged over the four greenhouse tester cultivars in the soil bioassay (Table 4). Based on Tukey's HSD, cultivars planted into soil from year one Stephens and LCS Artdeco plots developed significantly more disease than those planted into any other first year cultivar

soil, while year one Bobtail soil had only about one-third the number of lesions of Stephens and LCS Artdeco and had significantly less disease than any other first year cultivar soil (Fig. 3A). There was not a significant difference for lesion numbers between the year two sub-plots of Bobtail versus Rosalyn, nor a significant interaction between year one and year two cultivars (Fig. 3B, Table 4). The first four bars of Fig. 2B show strong evidence for lack of specificity of the year one cultivar effect on year two cultivar disease. Though there was a highly significant effect of tester cultivar in the greenhouse soil assay (Table 4), only LCS Artdeco differed from the other three cultivars based on Tukey's HSD (Fig. 2, Table 4).

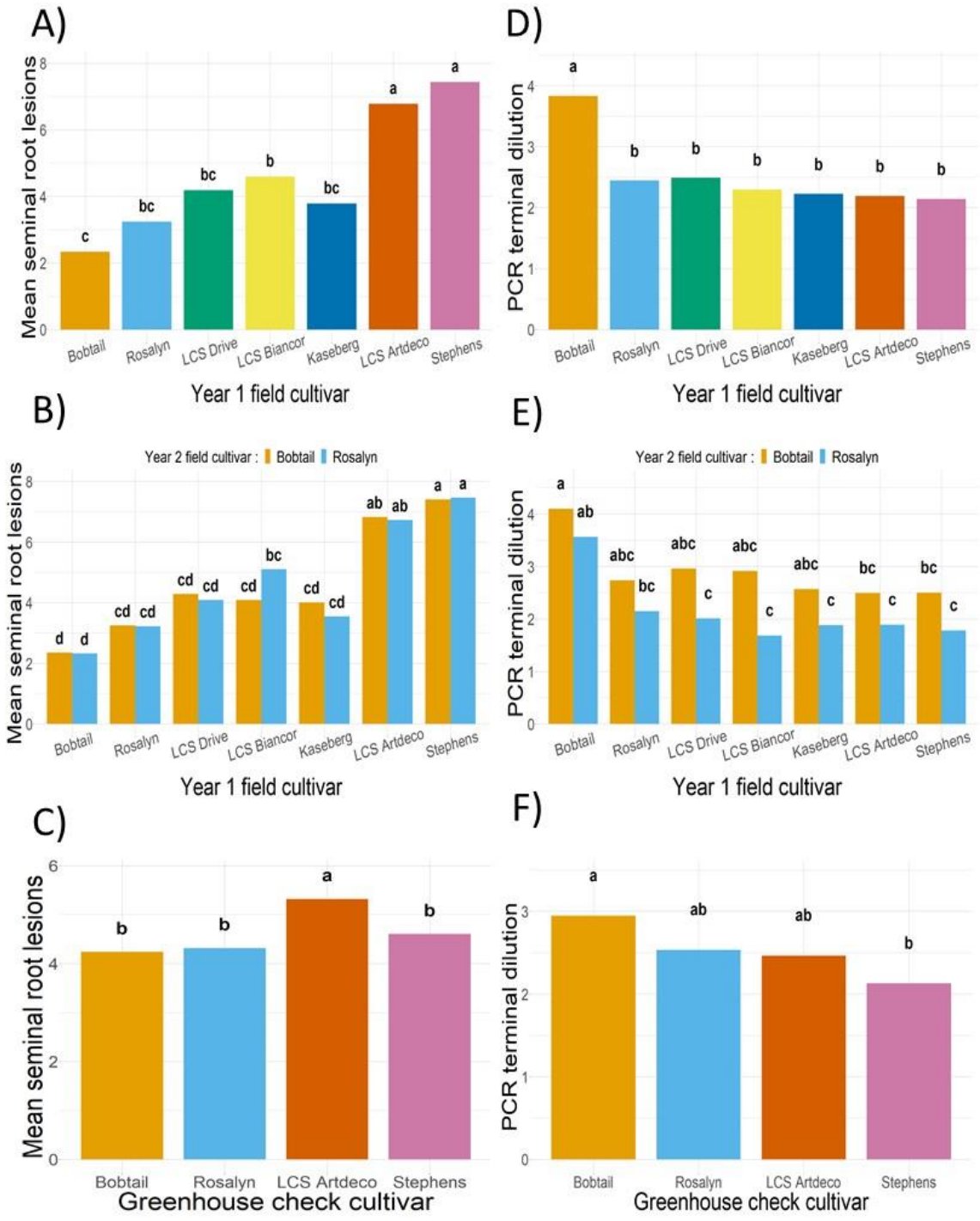

Fig. 3 Greenhouse disease assessment for the effect of first and second year field cultivars on the disease severity of four greenhouse test cultivars (A-C), and PCR analysis for *phlD+ pseudomonas* using log5 serial dilutions of the rhizosphere root

washes from the greenhouse assessment (D-F). A) Take-all lesion count on seminal roots of greenhouse testers, showing first year effect, SEM = 0.872. B) Take-all lesion count on seminal roots of greenhouse testers, showing first and second year effect, SEM = 0.363. C) Take-all lesion count on seminal roots of greenhouse check, showing third year effect, SEM = 0.236. D) Mean terminal dilution that scored positive for *phlD* presence, first year effect, SEM = 0.0859. E) Mean terminal dilution that scored positive for *phlD* presence, first and second year effect, SEM = 0.0468 . F) Mean terminal dilution that scored positive for *phlD* presence, greenhouse tester effect, SEM = 0.0920.

Table 4 ANOVA table for Experiment 3 assessment of the effect of seven first year cultivars, two third year cultivars, and four greenhouse test cultivars on the relative concentration of *phlD+* pseudomonads within the greenhouse test cultivar rhizosphere and the amount of take-all lesions that develop on greenhouse test cultivar.

	Mean P. fluorescens concentration within the rhizosphere				**Mean take-all lesion count on seminal roots**		
	Df	Mean Sq	F value	Pr(>F)	Mean Sq	F value	Pr(>F)
Block	3	57.625	44.817	1.486E-08	35.11	1.2033	0.3369
Year 1 cultivar	6	11.282	8.7745	1.48E-04	548.25	18.792	7.50E-07
Error a	18	1.286			29.18		
Year 2 cultivar	1	32.33	46.119	1.024E-06	0.39	0.0324	0.8589
Year 1 cultivar : year 2 cultivar	6	0.491	0.7	0.653	8.56	0.704	0.6497
Error b	21	0.701			12.16		
GH cultivar	3	6.316	4.587	4.40E-03	67.99	8.6253	2.99E-05
GH cultivar : year 1 cultivar	18	1.053	0.765	0.737	7.69	0.9757	0.4916
GH cultivar : year 2 cultivar	3	0.992	0.7203	0.542	9.15	1.1611	0.3273
GH cultivar : year 1 cultivar : year 2 cultivar	18	0.594	0.4314	0.979	8.46	1.0726	0.3872
Error c	126	1.377			7.88		

Rhizospheres from the greenhouse assay indicated a highly significant year one cultivar effect on level of *phlD+* pseudomonads (Table 4), though this effect was dominated by the impact of year one Bobtail, which differed from all other year one cultivars based on Tukey's HSD (Fig. 3D). Samples from year one Bobtail plots had *phlD+* pseudomonad populations more than five-fold greater the next highest scoring year one cultivar soil based on back calculations from the 5-fold dilutions of the PCR assay. The second year cultivar also had a significant (Table 4), though smaller, effect on

phlD+ pseudomonads, with soils from Bobtail year two subplots consistently having a terminal dilution higher than that of Rosalyn, regardless of year 3 tester cultivar (Fig. 3E). The greenhouse tester cultivars had a significant (P= 0.0044) effect on *phlD*+ pseudomonads (Table 4), with Bobtail having a higher terminal dilution than Stephens based on the HSD test (Fig 3E). There were no significant interactions among the year one, two, and three cultivar effects (Table 4). Lesion numbers in the greenhouse were negatively correlated with the estimated concentration of *phlD*+ pseudomonads recovered from the rhizosphere as measured by terminal dilution (R = -0.248, p=0.00017).

Discussion

The importance of the soil microbiome to biological processes is well appreciated (Berendsen *et al.*, 2012; Gdanetz & Trail, 2017; Busby *et al.*, 2017; Mauchline & Malone, 2017; Compant *et al.*, 2019), and there is currently a strong desire to manipulate these microbiomes to positive effect in agroecosystems (Noble & Coventry, 2005; Compant *et al.*, 2005; Mazzola & Freilich, 2016; Gdanetz & Trail, 2017). Because introduced microbiomes must face strong competition from the native microbiome (Mazzola & Freilich, 2016; Compant *et al.*, 2019), a change in the soil environment is often required to attain a substantial, lasting change of the microbiome, e.g., through addition of soil amendments, a process that is often not economically feasible for low value per hectare, extensively grown crops such as wheat. However, a direct, potentially long-lasting, and economical way to alter the soil environment would be to grow crop genotypes that support a favorable microbiome (Mazzola *et al.*, 2004, p. 200; Maria *et al.*, 2005; Landa *et al.*, 2006; Meyer *et al.*, 2010; McMillan *et al.*, 2011; Mauchline *et al.*, 2015; Wagner *et al.*, 2016; Osborne *et al.*, 2018).

Our study was prompted by research in the UK, which indicated that the wheat cultivar grown in the first year of a sequence can have a significant impact on the amount of take-all that develops in the second year, regardless of the cultivar planted in year two (McMillan *et al.*, 2011, 2018), and that the cultivar Einstein is one wheat genotype that possesses that trait (McMillan, 2012). Einstein is a parent of the Oregon wheat cultivar Bobtail, which expressed the low TAB trait in our experiments. This suggests that the TAB trait is heritable and robust across the UK and US field environments. Effects of

take-all levels and first year cultivar treatments on grain yield can vary among years from very large to negligible (McMillan *et al.*, 2018). We had the additional complication of high rainfall in consecutive Octobers (2015 and 2016) that resulted in variable plant stands, high coefficients of variation for grain yield, and no significant yield effects. We were still able to identify significant differences for disease variables, however, these were based on the plants that successfully established. In our pilot study, which was conducted under more normal rainfall regimes, there was evidence for a very substantial difference in yield between the two different first-year cultivars (table 1). In all three experiments there was considerable variation in the TAB trait among local cultivars that we evaluated, but Bobtail always ranked best for the TAB trait. In the third experiment, we also evaluated two second year cultivars in the field, and a third exposure, to four different cultivars, in the greenhouse. First, second, and third year cultivars all had an impact on take-all, though the first year effect always dominated. It may have been more interesting to compare Bobtail to a cultivar with a high TAB phenotype in year two, such as LCS Artdeco or Stephens. However, these two cultivars are susceptible to two lower stem diseases (eyespot and sharp eyespot), which had the potential to interfere with our measurement of take-all. Bobtail and Rosalyn had the best disease resistance spectrum among the seven cultivars evaluated and were the safest choices for second year cultivars. A comparison of second year effects with Bobtail and Rosalyn did demonstrate a lack of cultivar specificity of the TAB trait, however.

A second goal was to study the potential role that DAPG-producing pseudomonads play in the control of take-all during continuous wheat production, and how the cultivars planted each year impact their abundance within the rhizosphere. DAPG-producing *Pseudomonas* have been consistently isolated from take-all suppressive wheat fields in Washington state and The Netherlands (de Souza *et al.*, 2003; Mazzola *et al.*, 2004; Mavrodi *et al.*, 2007; Kwak *et al.*, 2009), and DAPG-producing *Pseudomonas* have been shown to aid in the defense of plant roots from pathogens, as well as inducing resistance to foliar pathogens (Weller *et al.*, 2007, 2012; Kwak *et al.*, 2009; Imperiali *et al.*, 2017). Given the diversity of wheat genotypes available, it is important to understand their individual effects on microbiome development (Mazzola *et al.*, 2004; Landa *et al.*,

2006; Yang *et al.*, 2018), and how they interact with TAD soils (Yang *et al.*, 2018), if they are to be used as an effective management strategy. In all three experiments the cultivar planted in the first year largely determined the concentration of DAPG-producing *Pseudomonas*, with first year Bobtail granting more than five times the amount of DAPG-producing *P. fluorescens* to subsequent plantings than any other first year cultivar. Subsequent cultivars had an impact on the rhizosphere microbial population, but not on the amount of disease observed, and Bobtail soils consistently had greater concentrations of DAPG-producing *Pseudomonas*, regardless of what year it was planted. This suggests that the population structure of the soil, while initially defined by the first year cultivar, is amenable to change over time if DAPG enriching cultivars are grown.

Studies exploring the diversity of the TAB trait in UK wheat germplasm revealed a large degree of heterogeneity across commonly grown wheat varieties, with no clear single source of resistance for the trait, but rather a range of genetically diverse sources that express low TAB (McMillan *et al.*, 2011). In field trials, Einstein was evaluated to be susceptible to take-all and to be a higher TAB variety, but that it shares an allele suspected to be associated with the low TAB trait of Cadenza, as well as another associated with the high TAB trait of Avalon (McMillan, 2012). Cadenza has consistently demonstrated the ability to reduce take-all inoculum buildup in the soil when planted as the first cultivar in a new monoculture, regardless of the subsequent cultivar (McMillan *et al.*, 2011, 2018). Similarly, comparisons of low TAB cultivar Cadenza and high TAB cultivar Hereward have shown a correlation between the species diversity, concentration, and nature of the rhizosphere *Pseudomonas*, with the high TAB trait being associated with an increase in the total abundance of saprophytic *Pseudomonas*, while low TAB cultivars encouraged the development of *Pseudomonas* species that communicate with the plant host to increase total health and disease resistance (Mauchline *et al.*, 2015). Our results show that Bobtail, a low TAB cultivar, significantly reduced the level of disease experienced by subsequent cultivars planted into the same field, which significantly increase the concentration of DAPG-producing *Pseudomonas* species. It is possible that if we had tested the rhizosphere for all *Pseudomonas* and not just those capable of producing antimicrobial compounds, we may have seen a similar

increase in abundance, which could illuminate further factors involved in take-all suppression.

Taking all three experiments into consideration, we see that disease severity and *phlD+ Pseudomonas* concentrations are negatively correlated, signifying that they play at least some part in the suppression of take-all. This trend is true for all of the cultivars tested in the first year, e.g. a plot that contained a high TAB cultivar like Stephens continued to experience higher levels of disease in subsequent years and had lower concentrations of DAPG-producing *Pseudomonas* present in the rhizosphere. This suggests that DAPG-producing *Pseudomonas fluorescens* act as a sort of keystone species, shaping and stabilizing the microbial population for as long as a conducive environment is maintained. Without a full survey of the bulk soil, rhizosphere, and endosphere populations it is impossible to say how broad of an impact *P. fluorescens* has on community structures, but it is clear to see that it has an interaction with *G. graminis* var *tritici*, and acts to suppress its growth while above a critical threshold. Significantly, growers are already using the cultivar Bobtail to control take-all and have reported positive outcomes (Mundt, C.C., unpublished).

The two lowest TAB cultivars, Bobtail and Rosalyn, exhibited similar levels of take-all in some cases, but Bobtail consistently had significantly greater concentrations of DAPG-producing *Pseudomonas*, suggesting that there are other factors impacting the level of TAB. We had assumed that the low TAB of Bobtail derived primarily from Einstein. Interestingly, Einstein is not among the best of the low TAB cultivars studied in the UK (McMillan, 2012). One possibility is that the low TAB trait in Bobtail derived totally from Einstein, and we simply have fewer cultivars with low TAB than is the case in the UK. The cultivars Bobtail and Rosalyn (the second best low TAB cultivar in our study) share a parent in common, the cultivar Tubbs. It is thus possible that Tubbs has contributed one or more genes to the TAB trait that is not mediated through the DAPG-producing pseudomonads. In fact Tubbs, has contributed some QTL for resistance against several other wheat diseases in the Einstein x Tubbs recombinant inbred line population from which Bobtail was derived (Vazquez *et al.*, 2015b,a, Vazquez et al., unpublished).

Substantial evidence has accumulated suggesting that resistance genes may often impact multiple diseases, especially genes that contribute to quantitative resistance (Wiesner-Hanks & Nelson, 2016). The cultivar Bobtail has the broadest level of disease resistance of any wheat cultivar ever released by Oregon State University and expresses at least some level of resistance to eight different diseases that have been studied (Mundt, C.C., unpublished). Multiple disease resistance has been studied in two molecular mapping populations in Oregon, each a cross of a European wheat variety with the Oregon variety Tubbs, including the Einstein x Tubbs population that contained the recombinant inbred line that became the cultivar Bobtail. The populations were initially evaluated for resistance to Cephalosporium stripe (Vazquez *et al.,* 2015b) and stripe rust (Vazquez *et al.,* 2015a). Subsequently, these populations were tested against several other diseases, showing that several DNA markers are associated with quantitative trait loci (QTL) that provide some measure of resistance to multiple diseases (Vazquez et al. in preparation). Of particular interest is a marker on wheat chromosome 5AL that was found to be associated with reduced levels of stripe rust, Cephalosporium stripe, Fusarium crown rot, strawbreaker foot rot, and perhaps barley yellow dwarf virus. Others have identified markers on chromosome 5AL that condition resistance to stripe rust, powdery mildew, and a biotype of Hessian fly (Liu *et al.*, 2016) and to Fusarium crown rot, Rhizoctonia root rot, and two nematode species (Thompson *et al.*, 2017). Interestingly, it has also recently been shown (Liu *et al.*, 2019) that a wheat QTL on chromosome 5AL promotes the growth of a phosphorous-solubilizing strain of *Pseudomonas*, resulting in increased plant growth. This QTL is located close to the 5AL QTL discussed above that we found to promote multiple disease resistance, providing further evidence for a role of wheat chromosome 5AL in encouraging the population of favorable *Pseudomonas* bacteria. Further, a gene controlling the effect of Einstein wheat on the take-all disease also appears to be present on chromosome 5A (McMillan, 2012). Previous works have shown that increased presence of *P. fluorescens* in the rhizosphere has induced resistance to other diseases, including foliar pathogens (Iavicoli *et al.,* 2003; Meyer *et al.,* 2010; Weller *et al.,* 2012) , and that *P. fluorescens* modulates multiple stress or defense pathways in wheat roots (Maketon *et al.,* 2012).

While there were clear differences in the severity of disease experienced by cultivars in our trials, it is well documented that there is no true genetic resistance to take-all in hexaploid wheat genotypes when evaluated within a single season under artificial inoculation or in naturally infested fields (Hornby, 1998; Cook, 2003; Freeman & Ward, 2004). Our results thus corroborate those conducted in the UK (McMillan *et al.*, 2011, 2018; McMillan, 2012; Mauchline *et al.*, 2015), and suggest that trials must be conducted over multiple years to identify wheat genotypes that encourage development of suppressive soils, perhaps partly due to increase in concentration of DAPG-producing *Pseudomonas* spp. The possibility that an increase in concentration of these pseudomonads could be related to an increase in multiple disease resistance provides increased incentive for focusing on this trait in wheat breeding programs, and a search for similar mechanisms in other plant species.

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Author Contributions

EP and CM planned and designed the research. EP performed the measurements, analysis, and wrote the manuscript. CM provided technical expertise and editing.

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Chapter 3: General Conclusion

Take-all of wheat is one of the most important soil-borne diseases of wheat globally, and frequently causes yield losses of 50 percent or more (Cook, 2003; Christensen & Hart, 2008; Kwak & Weller, 2013). It is most severe under continuous wheat production in irrigated and high-rainfall environments like those found in the Pacific Northwest of the U.S. (Mathre, 2000; Cook, 2003; Kwak & Weller, 2013). Current cultivars of hexaploid wheat lack genetic resistance to take-all (Hornby, 1998; Cook, 2003; McMillan *et al.*, 2014), and there are few effective chemical treatments on the market (Schoeny & Lucas, 1999; Dyer *et al.*, 2012; Yun *et al.*, 2012), forcing growers to rely on cultural means for disease control. The primary form of cultural control for continuous wheat production is through the development of specific suppressive soils, called take-all decline (TAD). Take-all decline occurs after several years of continuous wheat production, where disease severity steadily increases before declining to a stable and economically sustainable level. It is thought that TAD is primarily driven by the concentration of antibiotic producing *Pseudomonas fluorescens* present in the bulk soil and rhizosphere, which competes against and inhibits *G. graminis* var *tritici*, the causal agent of take-all(Smiley, 1973; Raaijmakers & Weller, 2001; Weller *et al.*, 2002, 2007). Work from the UK has shown that specific wheat cultivars grown in the first year of monoculture can substantially impact the amount of take-all that develops in subsequent years(McMillan *et al.*, 2011; Mauchline & Malone, 2017), while other research has identified the differential effect wheat cultivars have on the development of take-all suppressive microbes in the soil (Mazzola *et al.*, 2004; Mavrodi *et al.*, 2007; Kwak *et al.*, 2012; Mauchline *et al.*, 2015; Yang *et al.*, 2018). It has been shown that Einstein, a parent of the soft white winter wheat cultivar Bobtail, has a gene responsible for reducing take-all severity, while the other parent, Tubbs, has genes that promotes the growth of beneficial *Pseudomonas* that confer an increased level of resistance to several common diseases of wheat (McMillan *et al.*, 2011; Liu *et al.*, 2016).

In this study we attempted to determine if the wheat variety Bobtail inherited the genetic traits of its parents that enhance take-all resistance and encourage favorable populations of *Pseudomonas* to colonize the rhizosphere, and if this trait is shared by a selection of other top-yield cultivars. If present, we also sought to quantify the impact this trait has on the severity of take-all in subsequent years, and whether the first year cultivar drives the structure of the

microbiome in subsequent years. Finally, we compared disease severity results from both field and greenhouse assays to rhizosphere *Pseudomonas* concentrations to determine if and how strong their interactions were.

The work done by this study builds upon previous research and shows that Bobtail wheat likely possess a genetic trait that allows for the rapid accumulation of DAPG producing *Pseudomonas fluorescens* within the soil, and that this correlates with a reduction in the disease severity experienced by subsequent wheat plantings. This trait was not observed in any other tested cultivar. The strength of this effect is largely defined by the cultivar that is grown in the first year of a new monoculture and is only affected to a minor degree by the subsequent cultivars grown. These results suggest that crop genetics play a major role in the development of take-all suppressive soils, and that future breeding with microbiome structure in mind could provide a new avenue of disease resistance research.

This work is limited in that it seeks to investigate the cultivar effect on a single component of the microbiome, rather than the whole community structure. This could mask other significant factors that impact the suppression of take-all and the development of take-all decline. It also does not quantify the amount of DAPG present in the rhizosphere, and instead only identifies if pseudomonads capable to producing DAPG are present. Thus, it is not possible to conclusively say whether this chemical is responsible for the control of take-all or if some other mechanism unique to DAPG-producing *P. fluorescens* is acting to suppress the disease or induce resistance to it.

Future studies can expand on this work by identifying the genes responsible for the *Pseudomonas* accumulation trait in Bobtail and searching for similar sources of genetic resistance within its parental line and their offspring. Expanding the rhizosphere analysis to a full survey of the rhizosphere and bulk soil microbial communities developed by first year Bobtail should also be a goal, to identify other potential factors involved in suppressing take-all beyond the accumulation of 2,4-DAPG producing *P. fluorescens.*

In summary, this research demonstrated the presence of previously unavailable genetic resistance to take-all of wheat via rhizosphere microbiome manipulation. The cultivar Bobtail was capable of accumulating significantly more DAPG producing *P. fluorescens* than any other tested cultivar of hexaploid wheat, with a corollary reduction in take-all disease. The impact of growing Bobtail in the first year significantly increased the concentration of DAPG producing P.

fluorescens in the rhizosphere of cultivars planted in subsequent years, which in turn significantly decreased the severity of take-all that they experience. This effect was durable over the course of the study, regardless of what cultivar was chosen in the subsequent years. The demonstrated ability of the wheat cultivar Bobtail to manipulate the microbiome and suppress take-all provides growers with a new and novel method with which to control a devastating disease without additional amendments, something that was until now, unavailable.

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