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Associations Among Cultivar Cropping Sequence, 2,4-Diacetylphloroglucinol–Producing Pseudomonad Populations, and Take-All Disease of Winter Wheat in Oregon

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Abstract

Take-all of wheat (*Triticum aestivum* L.), caused by *Gaeumannomyces tritici* (syn. *G. graminis* var. *tritici*), is perhaps the most important soilborne disease of wheat globally and can cause substantial yield losses under several cropping scenarios in Oregon. Although resistance to take-all has not been identified in hexaploid wheat, continuous cropping of wheat for several years can reduce take-all severity through the development of suppressive soils, a process called "take-all decline" (TAD). Extensive work has shown that TAD is driven primarily by members of the *Pseudomonas fluorescens* complex that produce 2,4-diacetylphloroglucinol (DAPG), an antibiotic that is associated with antagonism and induced host resistance against multiple pathogens. Field experiments were conducted to determine the influence of agronomically relevant first-year wheat cultivars on take-all levels and ability to accumulate DAPG-producing pseudomonads within their rhizospheres in second-year field trials and in greenhouse trials. One first-year wheat cultivar consistently resulted in less take-all in second-year wheat and accumulated significantly more DAPG-producing pseudomonads than other cultivars, suggesting a potential mechanism for take-all reduction associated with that cultivar. An intermediate level of take-all suppression in other cultivars was not clearly associated with population size of DAPG-producing pseudomonads, however. The first-year cultivar effect on take-all dominated in subsequent plantings, and its impact was not specific to the first-year cultivar. Our results confirm that wheat cultivars may be used to suppress take-all when deployed appropriately over cropping seasons, an approach that is cost-effective, sustainable, and currently being used by some wheat growers in Oregon to reduce take-all.

Keywords: DAPG, *Gaeumannomyces tritici*, microbiome, microbiome-mediated genetic resistance, *Pseudomonas fluorescens*, take-all decline, take-all disease, wheat

Take-all disease of wheat (*Triticum aestivum* L.) is caused by the soilborne fungus *Gaeumannomyces tritici* (Arx & D.L. Olivier) (syn. *G. graminis* var. *tritici* Walker), 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. Take-all is considered to be one of the most destructive root diseases of wheat globally, with yield losses of 50% or greater having been reported (Christensen and Hart 2008; Cook 2003; Freeman and Ward 2004). 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.

Chemical and cultural controls for take-all are limited in effectiveness and/or practicality (Cook 2003). Genetic resistance to take-all has been researched extensively, but there are currently no known resistant cultivars within commercial hexaploid wheat (Cook 2003; Freeman and Ward 2004; Hornby et al. 1998; McMillan et al. 2014), despite evidence of it in related plant species (McMillan et al. 2014). Without highly effective chemical, cultural, or genetic controls, growers often rely on crop rotation to reduce take-all. By growing a nonhost or by leaving the land fallow for at least 1 year, the pathogen is deprived of a food source and substrate between growing seasons, effectively reducing inoculum potential to below economic thresholds (Christensen and Hart 2008; Cook 2003; Freeman and Ward 2004). However, some growers rely on natural disease suppression, mainly the development of suppressive soils (Cook 2003). Take-all suppressive soils are developed through the successive planting of susceptible hosts (Weller et al. 2007). Continued wheat monoculture results in increased disease for 3 to 5 years before declining to an economically acceptable level, known as take-all decline (TAD) (Smiley and Cook 1973; Weller et al. 2002, 2007). Extensive research has identified 2,4-diacetylphloroglucinol (DAPG)–producing *Pseudomonas fluorescens* as one of the key factors behind TAD (de Souza et al. 2003; Raaijmakers and Weller 2001; Weller et al. 2002). DAPG is a broad-spectrum polyketide antibiotic with known biocontrol properties against a range of root diseases, including take-all, as well as inducing resistance to foliar pathogens in its host (Weller et al. 2007). Although seed treatment with strains of fluorescent pseudomonads has been demonstrated to provide biological control of take-all (Weller and Cook 1983), we are unaware of currently available commercial biological control products.

An alternative approach to enhancing microbial suppression of plant disease is to grow crop genotypes that promote high populations of disease-suppressive microbes (i.e., microbiome-mediated genetic resistance). Plant genotypes that increase populations of disease-suppressive microbes, and/or plant response to those microbes, have been demonstrated or suggested for several host-pathogen systems (Bird 1982; Damerum et al. 2021; Jaiswal et al. 2020; Larkin et al. 1993; Lazcano et al. 2021; Mazzola et al. 2004; McMillan et al. 2011; Smith et al. 1997, 1999; Thaxton and El-Zik 2000). Wheat cultivars vary in their ability to increase the population size of DAPG-producing pseudomonads and may be a determinant of the length of time required to develop TAD (Kwak and Weller 2013; Mazzola et al. 2004). In the United Kingdom, the cultivar grown in the first year of wheat production has been reported to have a large impact on take-all severity and wheat yield in the

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second year (McMillan et al. 2011, 2018). The first-year wheat cultivar also selects for soil microbes antagonistic to the take-all pathogen (Mauchline and Malone 2017; Mauchline et al. 2015; Mehrabi et al. 2016; Osborne et al. 2018).

The take-all pathogen is widespread in Pacific Northwest wheat fields, even in dry production regions (Kwak et al. 2010; Smiley et al. 2013), and take-all can be a severe issue under several cropping scenarios in Oregon. In irrigated regions of central and north central Oregon, successive crops of wheat are sometimes planted to reduce inoculum of diseases for higher-value crops, such as potatoes. Under this scenario, growers assume 10 to 30% yield losses in the second-year wheat crop owing to take-all (C. C. Mundt, *unpublished data*). The Willamette Valley of Oregon has a high-rainfall maritime climate favorable to take-all, which reduces wheat yield by as much as 50% in the second and third years of continuous production (Christensen and Hart 2008). When grass seed prices are low and wheat prices relatively high, the wheat area planted increases, resulting in an increase of consecutive wheat crops and potential issues with take-all. Further, in the northern part of the Willamette Valley, a 2-year rotation of winter wheat and clover seed production has been common for decades. Take-all of wheat is an issue under this rotation, because volunteer wheat and susceptible, grassy weeds cannot always be adequately controlled in the clover crop.

Oregon wheat growers have expressed a strong need for host genetic control of take-all. Our initial approach to addressing this need focused on the Oregon winter wheat cultivar Bobtail because one of its parents, the U.K. cultivar Einstein (Nickerson (UK) Ltd. 2004/05), has been suggested to reduce take-all losses when grown as a second year of a wheat crop or as continuous wheat (Bateman et al. 2006; Nickerson (UK) Ltd. 2004/05). Preliminary data from observation plots in the Willamette Valley of Oregon suggested a positive impact of first-year Bobtail wheat on take-all levels and yield in the following wheat crop as compared with the historical check cultivar Stephens (C. C. Mundt, *unpublished data*). We thus undertook the research described here to include multiple commercially relevant cultivars grown as first-year wheat cultivars in adequately replicated and repeated trials to determine their impact on take-all severity and populations of DAPG-producing pseudomonads in subsequent field and greenhouse plantings.

Materials and Methods

Two field experiments were established, each conducted over two consecutive wheat seasons. Several wheat cultivars were planted in the first season of each experiment. One or two wheat cultivars were planted in the second season to test the selective impact of first-year cultivars on take-all disease and microbial populations. Take-all symptoms were evaluated in the field, and soil samples were collected to evaluate both take-all symptoms and dilution endpoints of DAPG-producing pseudomonads.

Field trial implementation

We evaluated the six highest-yielding wheat cultivars in the Willamette Valley of Oregon at the time the experiments were implemented based on data from the Oregon State University Wheat Variety Trials (https://cropandsoil.oregonstate.edu/wheat/osu-wheat-variety-trials). Cultivars Bobtail (Zemetra et al. 2013a), Kaseberg (Zemetra et al. 2012), and Rosalyn (Zemetra et al. 2013b) were developed by Oregon State University. Cultivars LCS Artdeco, LCS Biancor, and LCS Drive were developed by Limagrain Cereal Seeds (Limagrain Cereal Seeds 2023). The Oregon State University cultivar Stephens (Kronstad et al. 1978) that had dominated the Oregon wheat area for more than 20 years (Peterson 2001) was known to experience severe take-all disease under conducive conditions and served as a historical check in some of the studies described below. Seahawk (Pumphrey et al. 2014), a spring wheat cultivar, was developed by Washington State University and was used in one field trial in which flooding precluded the establishment of winter wheat.

Two winter wheat experiments were conducted in different fields at the Botany and Plant Pathology Field Laboratory in Corvallis, OR. Each experiment consisted of two consecutive wheat crops, with each consecutive wheat crop hereafter referred to as "year 1" and "year 2," respectively. Both experiments were in fields consisting of Chehalis silty clay loam soil, but fields did not have a history of take-all. Fields were fallow in the year before research initiation, with regular tillage and/or herbicide applications used to control weeds and volunteer wheat plants from a wheat crop 2 years prior. All fields received 28 kg/ha each of N, P_2O_5, and K_2O as a 16-16-16 formulation in the autumn, before planting. Winter wheat plots received 135 kg/ha of N as urea in late winter before jointing at Feeke's stage 6 (Large 1954). The 2017 spring wheat planting received 90 kg/ha of N as urea in the spring, before 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 plots 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 removed from year 1 plots with a 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 approximately 1 month, thus encouraging a natural build-up of the take-all pathogen. Fields were then sprayed with glyphosate herbicide and tilled with a disk and power harrow after the wheat volunteers and weeds died.

Experiment 1. The first experiment used six wheat cultivars as first-year treatments: Bobtail, Kaseberg, Rosalyn, LCS Drive, LCS Biancor, and 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 adjacent, 9.1-m-long passes of a 1.52-m-wide, Hege 500 series cone-type plot drill (H&N Manufacturing, Colwich, KS) with six double-disk openers 20 cm apart. Planting density was 270 seeds m^{-2}. To control foliar disease, plots were sprayed with a mixture of azoxystrobin (96.2 g/active ingredient [a.i.] ha^{-1}) as Quadris (Syngenta Crop Protection, LLC, Greensboro, NC) and propiconazole (117 g/a.i. ha^{-1}) as Tilt (Syngenta Crop Protection, LLC, Greensboro, NC) fungicides on 8 April 2016 and with propiconazole (117 g/a.i. ha^{-1}) (Syngenta Crop Protection, LLC, Greensboro, NC) only on 28 April 2016. At crop maturity, grain was removed from the entire field with a combine. The field was then irrigated intermittently to field capacity for 3 to 4 weeks to promote volunteer wheat growth and, hence, take-all severity in the following season.

Record rainfall and tillage decisions in October 2016 resulted in two failures to establish the second-year winter wheat treatments because of flooding. Instead, the entire field was planted to Seahawk spring wheat on 8 May 2017 at ~250 seeds m^{-2}, using a Great Plains 3P500 grain drill (Great Plains Ag, Salina, KS) with seven rows 19 cm apart for each 1.52-m planting pass. Planting passes were in the same direction and directly over planting passes of the year 1 plots.

Experiment 2. Design and planting methods for year 1 of the second experiment were identical to that of experiment 1 (Bobtail, Kaseberg, Rosalyn, LCS Drive, LCS Biancor, and LCS Art Deco), except that the historical 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 azoxystrobin (99 g/a.i. ha^{-1}) and propiconazole (86 g/a.i. ha^{-1}) combined in the product Trivapro A (Syngenta Crop Protection, LLC, Greensboro, NC) mixed with benzovindiflupyr (29 g/a.i. ha^{-1}) as the product Trivapro B (Syngenta Crop Protection, LLC, Greensboro, NC) on 10 May 2017. Grain was removed from the field, and volunteer wheat was encouraged with irrigation as described for experiment 1.

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 2/year 1 plots, using the same commercial grain drill and seeding rate as described for experiment 1/year 2. To control foliar diseases, plots were sprayed with a mixture of 80 g/a.i. ha^{-1} of trifloxystrobin and 80 g/a.i. ha^{-1} of propiconazole combined in the product Stratego (Bayer CropScience LP, St. Louis, MO) plus an additional 37 g/a.i. ha^{-1} of propiconazole as Tilt (Syngenta Crop Protection, LLC, Greensboro, NC) on 9 February 2018.

Field disease evaluations

Experiment 1. When seedheads were mature and beginning to dry in year 2, 10 plants were sampled at random from a 3.0 × 6.1–m section of each plot for disease assessment. Disease was assessed by digging up whole Seahawk plants, rinsing roots to remove soil, and assigning a rating of 0 to 5 based on the number of black lesions characteristic of take-all on the seminal roots.

Experiment 2. When seedheads were mature and beginning to dry in year 2, five plants from each of the three central planting rows of the two central subplots of each block were collected for disease assessment, totaling 15 plants from each subplot. 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. The percentage of 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 drill passes of each plot once per week for 3 weeks, beginning 11 May 2018. The mean percentage of whiteheads averaged over the 3 weeks was used to make treatment comparisons.

Field soil sampling

Experiment 1. A total of 7.6 liters of soil was obtained from subsamples collected in an "x" pattern from the interior 3.0 × 6.1 section of each plot after the harvest of year 1 plots. Subsamples were collected at the intersections of the "x" with planting rows to a depth of 15 to 20 cm using a hand-operated auger with an opening of 6.5 cm. The auger was angled underneath wheat rows to include rhizosphere soil. Subsamples were aggregated within plots, placed in polyethylene bags, and stored in a refrigerated room at 4 to 6°C for future testing.

Experiment 2. Plots were harvested and soil samples collected in year 2 for the two central subplots of each plot, as described above for experiment 1/year 1. Soil was collected at the end of year 2 to determine whether the year 2 subplot cultivar (Bobtail versus Rosalyn) had an impact on disease levels or pseudomonad populations. Plots were first irrigated to field capacity with overhead sprinklers after harvest to facilitate collection of the soil samples.

Greenhouse soil assay

Experiment 1. In the winter of 2016/2017, a soil bioassay using postharvest field soil from experiment 1/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 approximately 2.5 cm in diameter was placed at the bottom of Ray Leach Cone-tainers (ID code SC10U Stuewe and Sons, Tangent, OR) (cones) to prevent the soil mixture from exiting drainage holes at the bottoms of the cones. Cones were then filled with the soil mixture to within 5 cm of the top. Three seeds of the historical check cultivar Stephens were planted in each cone and topped with vermiculite, leaving approximately 1.3 cm of headroom, and grown in a greenhouse at 70% RH, 15°C/day, 10°C night, with 16-h days provided through supplementation with Sun System Digital 250/400W grow lights (Sunlight Supply, Inc., Vancouver, WA) operated at 400W. Five cones were planted from the 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 after germination, plants were thinned to one plant per cone. After 8 weeks, whole plants were collected, loose soil was removed, roots were lightly washed, and black lesions typical of take-all on the primary roots were counted. Roots were allowed to dry overnight before weighing and continued processing for rhizosphere sampling. The greenhouse experiment was conducted a total of four times during the period from 20 October 2016 through 5 January 2017.

Experiment 2. In the winter of 2018/2019, a soil bioassay using postharvest field soil from experiment 2/year 2 was used to assess the degree of take-all infection and soil suppression carried over from the previous two field seasons. Soil samples from each of the four field blocks were evaluated over time in four runs. Each of the 56 factor combinations (7 first-year cultivars × 2 second-year cultivars × 4 check cultivars) were replicated five times in each run. Extra cones 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. Soil preparation and greenhouse settings were identical to the previous year. Cultivars Bobtail, Rosalyn, LCS Artdeco, and Stephens were used as checks to determine how cultivars affect 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 check cultivars in the greenhouse. Resource limitations prevented us from using all seven cultivars as checks. 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 cones. The experiment was conducted during the period of 8 November 2018 through 1 April 2019.

Rhizosphere sampling for DAPG-producing pseudomonads

For both experiments, 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 and year 1 cultivar for experiment 1; field block, year 1 cultivar, year 2 cultivar, greenhouse cultivar, and greenhouse replication for experiment 2) were aggregated, and 5 g of root matter was placed into a Falcon 50 ml Conical Centrifuge Tube (Thermo Fisher Scientific, Waltham, MA) with 25 ml of sterile distilled water. Bacteria were dislodged by vortexing four times for 30 s each. One hundred µl of each sample was directly transferred to microtubes prefilled with 200 µl of sterile distilled water, and 50 µl of this was then transferred to a new microtube prefilled with 200 µl of 1/3× of the *Pseudomonas* selective King's medium B+++ (KMB+++ = King's medium B supplemented with ampicillin, chloramphenicol, and cycloheximide) (King et al. 1954; McSpadden Gardener et al. 2001) and incubated in the dark at a room temperature of approximately 18 to 24°C for 48 ± 4 h. One hundred µl of each sample was transferred to the first row of a 96-well microtiter (Thermo Scientific, Waltham, MA) plate prefilled with 200 µl of sterile distilled water and serial diluted down the plate. Fifty microliters from each cell was transferred to another plate prefilled with 200 µl of 1/3× KMB+++ and incubated in the dark at room temperature for 48 ± 4 h. 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 microtubes prefilled with 100 µl of 35% glycerol and frozen at −80°C (McSpadden Gardener et al. 2001). The result was a fivefold 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, thus allowing for a wide range of detection (McSpadden Gardener et al. (2001). The presence of a 629-bp band indicates the presence of *phlD*+ bacteria in the tested solution (Fig. 1). Root wash cultures were assessed for the presence of *phlD*+ *P. fluorescens* by PCR using gene-specific primers bpf2 + bpr4 and b2bf + bpr4 (McSpadden Gardener et al. 2001) (Table 1). *P. fluorescens* Pf-5, a

well-studied isolate and known producer of DAPG (Loper et al. 2007), was used as a positive control. Sterile broth alone was used as a negative control on each plate. Dilutions one through six of the root washes were chosen for amplifications in 25-µl reaction mixtures containing 2.5 µl of whole-cell template, 1× *DreamTaq* DNA polymerase buffer (Thermo Scientific, Waltham, MA), 200 µM solution dNTP (Qiagen, Inc., Germantown, MD), 25 pmoles of each primer, and 1.5 units *DreamTaq* DNA polymerase (Thermo Scientific, Waltham, 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 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and 72°C for 5 min, followed by a 4°C soak and stored at −20°C. 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 and compared with a 100-bp DNA ladder. Ninety-six–well plates were used to assess all possible combinations of year 1 field cultivar, year 2 field cultivar, and greenhouse check cultivar.

For experiment 1, detection of the PCR product was too low to be useful in the dilution series. However, there were obvious differences among cultivar sources in terms of the proportion of positive PCRs in the initial samples (the 1/3 dilution). Relative DAPG concentrations were thus estimated by the number of positive PCR reactions for samples derived from the 60 PCR reactions (5 cones × 4 greenhouse runs × 3 PCR replicates per sample) for each of the four field replicates of each cultivar. For experiment 2, there was a larger number of positive PCR reactions in the dilutions. Thus, the mean dilution factor of the final positive amplification was used to indicate the relative abundance of *phlD*+ pseudomonads.

Statistical analyses

Statistical analyses were carried out in R version 3.6.3 (R Core Team 2020) and RStudio version 1.2.5033 (RStudio Team 2020). 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 a graphical representation of results. Because the identities of the original field cultivars and experimental blocks were maintained and the samples analyzed separately during the greenhouse and PCR procedures, we were able to relate all results back to effects of the original cultivars in the field.

An analysis of variance was conducted as appropriate to the design of each data set. Tukey's honestly significant difference (HSD) (P = 0.05) 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, and all other collected data were treated as discrete variables. The SEM was calculated for each investigated experimental factor using the appropriate mean square error from each analysis of variance, so that variation associated with field blocking and greenhouse runs over time could first be removed.

The greenhouse portion of experiment 1 and experiment 2/year 1 was analyzed as a split-plot design, with the field replications assigned as blocks, the year 1 field cultivars as the main plots, and the greenhouse checks as subplots. The greenhouse portion of experiment 2/year 2 was analyzed as a split-split-plot design, with the field replications assigned as blocks, the year 1 field cultivars as the main plots, the year 2 field cultivars as the subplots, and the greenhouse checks as sub-subplots.

Results

Take-all severity

Experiment 1. Soil collected from the plots of six cultivars in the first year of experiment 1 differentially impacted the number of take-all lesions when tested against cultivar Stephens in the greenhouse (Fig. 2B; Table 2). Lesion numbers on greenhouse plants varied approximately fourfold 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, in which the spring wheat cultivar Seahawk was planted over the plots grown to the six cultivars of year 1 (Fig. 2A; Table 3). The effect of year 1 cultivars on the disease rating of Seahawk plants was significant (P = 0.0047), and there was more than a twofold 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, the 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 (Fig. 2A and B), and there was a significant positive correlation between field disease severity and greenhouse lesion numbers (R = 0.85; P = 0.032).

Experiment 2. In the trial comparing year 1 field cultivar effects on disease of subsequently planted cultivars, year 1 field cultivar

Table 1. Primer sequences used for PCR analysis of the *phlD* gene[a]

Primer name	Sequence (5′ to 3′)	Tm (°C)[b]
B2BF	ACCCACCGCAGCATCGTTTATGAGC	65.6
BPF2	ACATCGTGCACCGGTTTCATGATG	62.4
BPR4	CCGCCGGTATGGAAGATGAAAAAGTC	63.4

[a] Source: McSpadden Gardener et al. (2001).
[b] Melting temperature.

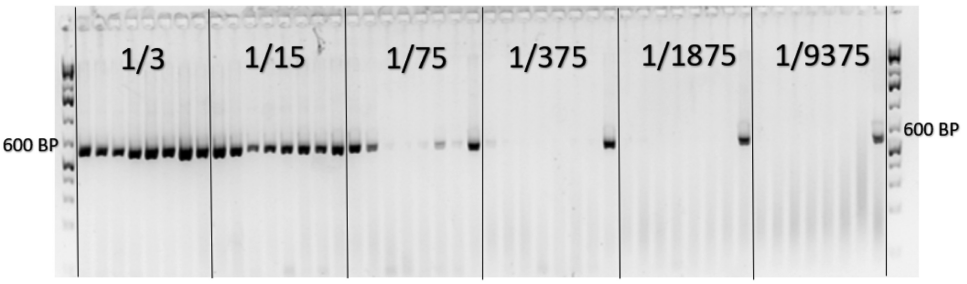

Fig. 1. Example ethidium bromide–stained agarose gel showing PCR amplification of the *phlD* gene in populations of *Pseudomonas* spp. derived from different wheat cultivars in the field. Fractions in the upper part of the figure indicate bacterial population dilutions in a dilution endpoint assay. Within each dilution, pseudomonad sources from left to right were from first-year wheat cultivars Bobtail, Rosalyn, LCS Drive, LCS Biancor, Kaseberg, LCS Artdeco, Stephens, and the nondiluted Pf-5 positive control. The figure shows a band for all cultivars at the 1/75 dilution, with Bobtail, Rosalyn, and the Pf-5 control showing the strongest bands. The 1/375 dilution shows a weak band for Bobtail at the 1/375 dilution and a barely perceptible band for Rosalyn. BP = base pair.

effects ranked similarly to those of experiment 1 (Fig. 3A). Averaged over second-year cultivars, plots that contained LCS Artdeco in the first year contributed to significantly more lesions than did plots that grew cultivar Bobtail based on Tukey's HSD (Fig. 3B). The year 1 field cultivar effect for whiteheads was significant only at $P = 0.078$ (Table 4), but the post hoc analysis using Tukey's HSD classified year 1 field Bobtail and year 1 field Stephens into different significance groups (Fig. 3C). Averaged over first-year cultivars, Rosalyn developed a mean of 10.3% whiteheads as the year 2 cultivar whereas Bobtail developed 9.04% (Fig. 3D), a small difference that was nonetheless significant at $P = 0.014$ (Table 4).

There was no significant interaction between year 1 and year 2 cultivars for either lesion numbers or whitehead percentage (Table 4), suggesting that year 2 cultivars responded similarly to the conditioning by year 1 field cultivars. A large and highly significant block effect was observed for both lesion numbers and whitehead percentage (Table 4), with northern blocks developing less disease and southern blocks developing more disease (data not presented). This was likely because of 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.

Year 1 field cultivar had a highly significant effect on lesion number averaged over the four greenhouse check cultivars in the soil bioassay (Table 5). Based on Tukey's HSD, cultivars planted into soil from year 1 field Stephens and LCS Artdeco plots developed significantly more disease than those planted into any other first-year cultivar soil, whereas cultivars planted into year 1 field Bobtail soil had approximately one-third the number of lesions as compared with plants grown to Stephens or LCS Artdeco in year 1 (Fig. 4A). There was not a significant difference for lesion numbers between the year 2 subplots of Bobtail versus Rosalyn or a significant interaction

Table 2. Analysis of variance for the number of take-all lesions on Stephens wheat planted in the greenhouse with soil from plots of six first-year cultivars of winter wheat grown in the field for experiment 1 and the mean positive amplification of *phlD* from greenhouse rhizosphere washes[a]

	Number of lesions				Number of positive amplifications of *phlD*			
	Df	MS	*F*	*P*	Df	MS	*F*	*P*
Year 1 cultivar	5	68.2	7.08	2.30e-6	5	1.19	82.7	2.0e-16
Block	1	45.6	4.73	0.030	1	0.242	16.9	1.2e-4
Greenhouse replicate (GHR)	1	610	63.3	1.69e-14	1	0.0208	1.45	0.233
V	5	5.90	0.614	0.689	5	0.0153	1.06	0.389
Residuals	416	9.60	–	–	59	0.0143	–	–

[a] Df = degrees of freedom; *F* = *F* statistic; MS = mean square; *P* = probability for *F* test.

Table 3. Analysis of variance for the mean effects of six first-year winter wheat cultivars on take-all severity of Seahawk spring wheat planted over the entire field the following season (experiment 1)[a]

	Df	MS	*F*	*P*
Year 1 cultivar	5	171.4	39.5	<2.2e-16
Block	3	0.21	0.243	0.622
Residuals	233	202	–	–

[a] Df = degrees of freedom; *F* = *F* statistic; MS = mean square; *P* = probability for *F* test.

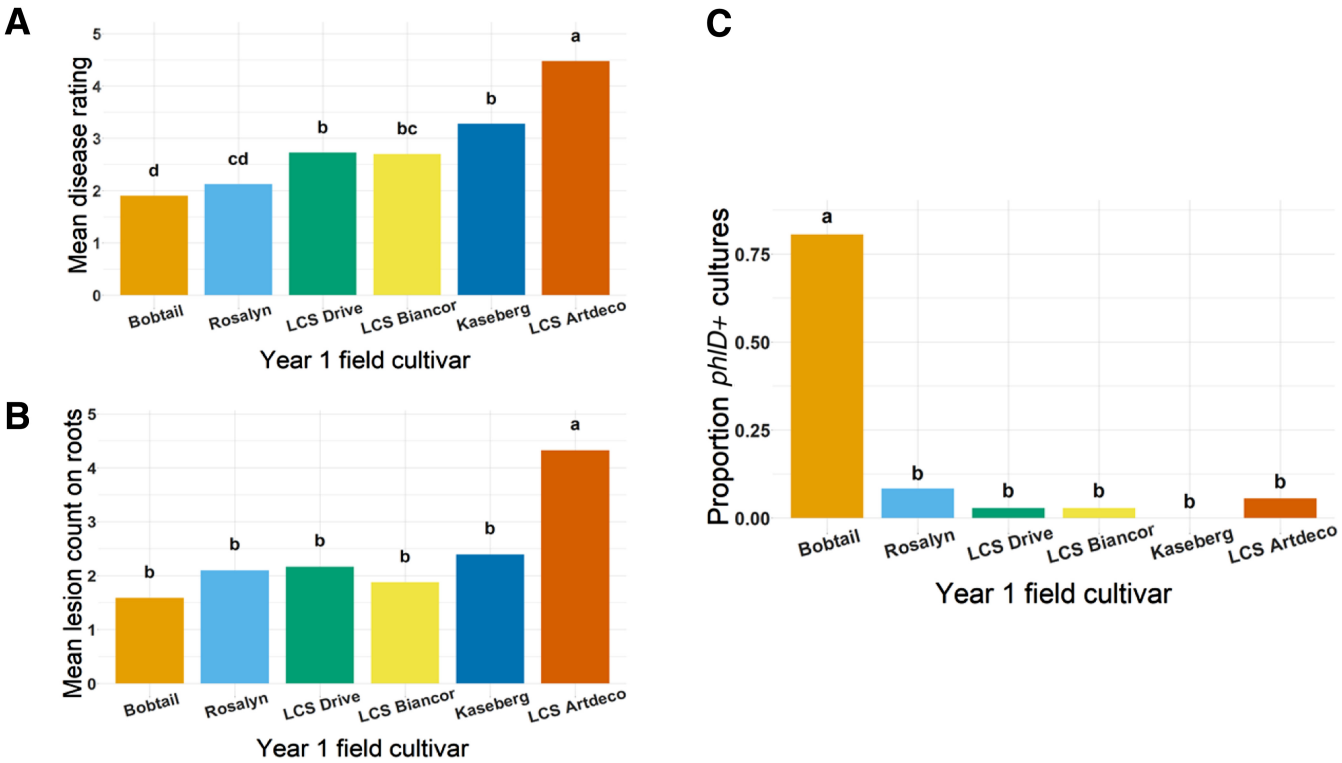

Fig. 2. Effect of first-year wheat cultivars on take-all disease severity and rhizosphere concentration of 2,4-diacetylphloroglucinol–producing *Pseudomonas* spp., measured by *phlD* amplification. **A,** Mean disease ratings of Seahawk spring wheat roots in the field planted after one of six winter wheat cultivars (SEM = 0.534). **B,** Number of take-all lesions on Stephens wheat roots in the greenhouse planted into postharvested soil from replicated field plots of six winter wheat cultivars. Data are means over four field replicates and six replicate plants per field replicate for each cultivar and four runs over time in the greenhouse (SEM = 0.0560). **C,** Mean proportion of positive amplifications of *phlD* from greenhouse rhizosphere washes. Data for each year 1 cultivar represents four field replicates, six replicate plants per field replicate for each cultivar, four greenhouse replicates, and three PCR replicates (SEM = 0.00225).

between year 1 field cultivar and year 2 cultivar (Fig. 4B; Table 5). The first four bars of Figure 4B show strong evidence for a lack of specificity of the year 1 field cultivar effect on year 2 cultivar disease. Although there was a highly significant effect of check cultivar on lesion number in the greenhouse soil assay (Table 5), only LCS Artdeco differed from the other three cultivars based on Tukey's HSD (Fig. 4C; Table 4).

DAPG-producing pseudomonad analyses

Experiment 1. PCR-based detection of *phlD*+ pseudomonads in rhizosphere samples from the greenhouse trial showed that year 1 Bobtail samples had a significantly larger proportion of *phlD* gene detection compared with the other cultivars (Fig. 2C; Table 2), indicating that it has a greater capacity for supporting DAPG-producing *Pseudomonas* bacteria. There was a weak correlation between greenhouse disease severity and detection of DAPG-producing *Pseudomonas* from the rhizosphere ($R = -0.191$; $P = 0.108$).

Experiment 2. Rhizosphere samples from the greenhouse assay indicated a highly significant year 1 field cultivar effect on the level of *phlD*+ pseudomonads (Table 5), although this effect was dominated by the impact of year 1 field Bobtail, which differed from all other year 1 field cultivars based on Tukey's HSD (Fig. 4D). Samples from year 1 Bobtail field plots had *phlD*+ pseudomonad populations more than fivefold greater than the next highest scoring year 1 field cultivar soil based on back calculations from the fivefold dilutions of the PCR assay. The second-year cultivar also had a significant (Table 5), though smaller, effect on *phlD*+ pseudomonads, with soils from Bobtail year 2 subplots consistently having a terminal dilution higher than that of Rosalyn, regardless of greenhouse check cultivar (Fig. 4E). The greenhouse check cultivars had a significant ($P = 0.0044$) effect on *phlD*+ pseudomonads (Table 5), with Bobtail having a higher terminal dilution than Stephens based on the HSD test (Fig. 4F). There were no significant interactions among the year 1 field cultivars, year 2 field cultivars, and the greenhouse check cultivars (Table 5). 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

Our study is consistent with research in the United Kingdom, 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

Table 4. Analysis of variance for the effects of seven first-year winter wheat cultivars on the percentage of whiteheads and number of take-all root lesions on two cultivars grown in the second field season of experiment 2[a]

	Percent whiteheads				Lesion counts		
	Df	MS	*F*	*P*	MS	*F*	*P*
Block	3	199	8.58	9.5e-4	191	13.1	8.8e-5
Year 1 cultivar	6	53.9	2.32	0.0776	64.0	4.39	6.7e-3
Error a	18	23.2	0.0635	1.00	14.6	7.01	<2.2e-16
Year 2 cultivar	1	17.4	7.10	0.0145	33.2	2.29	0.145
Year 1 cultivar: year 2 cultivar	6	1.33	0.544	0.769	5.51	0.380	0.883
Error b	21	2.44	0.0635	1.00	14.5	7.01	<2.2e-16

[a] Df = degrees of freedom; *F* = *F* statistic; MS = mean square; *P* = probability for *F* test.

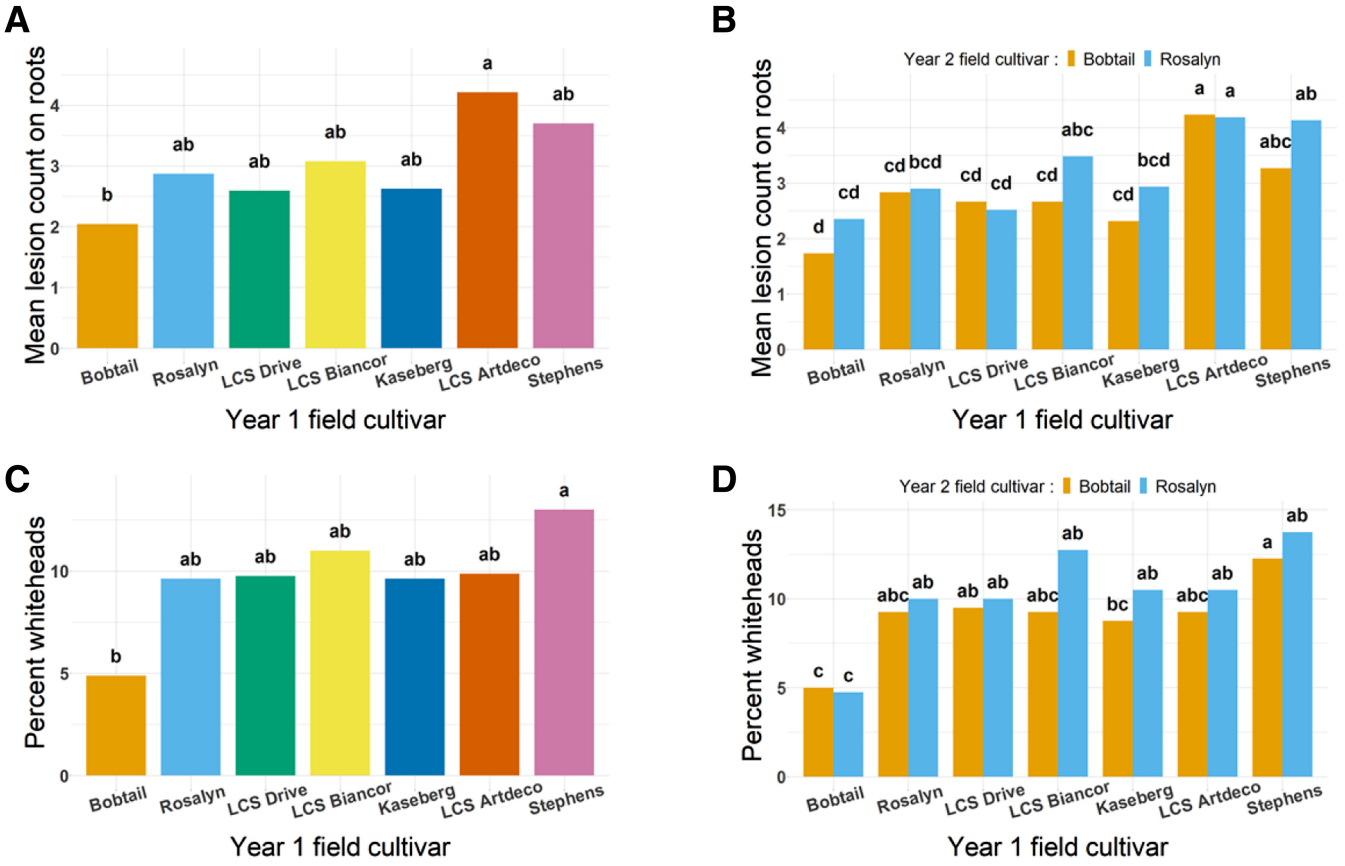

Fig. 3. Assessment of wheat take-all disease severity after the second season of experiment 2. **A,** Mean number of lesions on seminal roots of second-year wheat within plots of seven first-year cultivars, averaged over the two second-year cultivars (SEM = 0.779). **B,** Mean number of lesions on seminal roots of second-year wheat within each subplot, showing year 1 and year 2 cultivar effects (SEM = 3.2857). **C,** Percentage of whiteheads present within each plot (SEM = 1.7918). **D,** Percentage of whiteheads present within each subplot, showing year 1 and year 2 cultivar effects (SEM = 0.1886).

develops in the second year (McMillan et al. 2011, 2018). In both of our experiments, there was considerable variation for take-all impact among local cultivars that we evaluated, but the cultivar Bobtail always ranked best. In the second experiment, we also evaluated two second-year cultivars in the field, and a third exposure to four different cultivars in the greenhouse. The first-year cultivar had a strong effect, whereas the second-year cultivar showed no obvious trend and had no statistically significant effect. The third exposure of four check cultivars to the same rhizosphere soils had a statistically significant impact but was driven mostly by increased disease on check LCS Artdeco (Fig. 4C). However, even this difference was small relative to the impact of the first-year cultivar (Fig. 4A). This result suggests that the impact of the first-year cultivar is not simply a result of moderate disease resistance that reduced inoculum density for the subsequent crop. In experiment 2/year 2, it may have been more interesting to compare Bobtail with a cultivar not associated with take-all reduction, such as LCS Artdeco or Stephens. However, LCS Artdeco and Stephens are also 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 overall disease resistance spectrum among the seven cultivars evaluated and were the safest choices for evaluating take-all in the second year. We also had limited knowledge of the performance of the cultivars at the time experiment 2 was planted.

Take-all is well known to be spatially clustered within wheat fields (Palma-Guerrero et al. 2021). Efforts were thus made to reduce the effects of pathogen clumping within the field. The randomized complete block design that we used helped to account for spatial variation at a larger spatial scale (that of a block). In addition, we reduced variation at a smaller spatial scale by sampling at multiple sites with our experimental units and combining those samples to average out variation within experimental units.

We had initially assumed that the effect of first-year cultivar Bobtail on second-year take-all derived primarily from its Einstein parent. In U.K. field trials, however, Einstein was not among the best performing first-year cultivars (Osborne et al. 2018). Einstein was found to carry the favorable allele for take-all reduction at one of the two loci identified for the first-year cultivar Cadenza. However, it also was suggested that a favorable allele at only one of the loci may not be sufficient to have a substantial effect on take-all (McMillan 2012). The cultivars Bobtail and Rosalyn, the second-best cultivar in our study, share a parent in common, the cultivar Tubbs (Flowers et al. 2009). It is thus possible that Tubbs has contributed one or more favorable alleles for take-all impact to Bobtail and Rosalyn. Tubbs has contributed quantitative trait loci for resistance against several wheat diseases in the Einstein × Tubbs recombinant inbred line population, from which Bobtail was selected, and a second population with Tubbs as a parent, even when Tubbs itself has been susceptible to those diseases (Vazquez et al. 2015a, b; M. D. Vazquez and C. C. Mundt, *unpublished data*).

In addition to determining the effect of first-year wheat cultivars on second-year take-all severity, we studied 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. We focused on DAPG-producing *Pseudomonas* spp. because they have been consistently associated with take-all suppressive wheat fields in Washington state and the Netherlands (de Souza et al. 2003; Kwak et al. 2009; Mavrodi et al. 2007; Mazzola et al. 2004). In both of our experiments, the cultivar planted in the first year largely determined the concentration of DAPG-producing *Pseudomonas*, with first-year Bobtail supporting more than five times the amount of DAPG-producing *Pseudomonas* to subsequent plantings than any other first-year cultivar. Our results are consistent with those demonstrating a negative association between the diversity of *Pseudomonas* spp. and the ability of first-year wheat cultivars to suppress take-all in the second season (Mauchline et al. 2015, 2017; Mehrabi et al. 2016). If DAPG-producing species/genotypes are strongly selected within the *Pseudomonas* population, as shown in our study, this could result in a reduction in the total *Pseudomonas* diversity. Four of the seven wheat cultivars that we investigated had an intermediate effect on suppressing take-all in the second season when grown as first-year cultivars yet did not support elevated populations of DAPG-producing pseudomonads. Thus, if take-all suppression associated with these cultivars is controlled through the microbiome, there must be additional microbes involved (e.g., Osborne et al. 2018).

There is general agreement that genetic resistance, in the traditional sense, does not exist to take-all in hexaploid wheat (Cook 2003; Freeman and Ward 2004; Hornby et al. 1998; Palma-Guerrero et al. 2021). Further, the four check cultivars in experiment 2 showed similar levels of take-all when exposed to the same soil sources, despite having large differences in first-year wheat impacts on second-year wheat. Results of our study and those of others (Mauchline and Malone 2017; Mauchline et al. 2015; Mehrabi et al. 2016; Osborne et al. 2018) suggest that the first-year cultivar effect of wheat cultivars on second-year take-all may be microbiome-mediated genetic resistance. This resistance may result from the build-up of antagonistic organisms in the first year of culture to impact take-all in subsequent years. It is well known that some plant growth commonly promoting rhizobacteria can induce systemic resistance against a broad spectrum of plant diseases (Weller et al. 2012; Zhu et al. 2022). Thus, it is possible that wheat cultivars with microbiome-mediated resistance to take-all may provide resistance to additional diseases. In this regard, the cultivar Bobtail has the broadest spectrum disease resistance of any cultivar produced in Oregon to date and demonstrates at least partial resistance to eight different wheat diseases (C. C. Mundt, *unpublished data*).

There has long been an interest in understanding and managing disease-suppressive soils (Sagova-Mareckova et al. 2023). Although there certainly have been some successes (Collinge et al. 2022),

Table 5. Analysis of variance for the effect of first- and second-year wheat (*Triticum aestivum*) cultivars on the amount of take-all (*Gaeumannomyces tritici*) lesions that developed on greenhouse check cultivars and on the relative concentration of *phlD*+ pseudomonads within the greenhouse rhizospheres[a]

	Lesions				*phlD* dilution		
	Df	**MS**	***F***	***P***	**MS**	***F***	***P***
Block	3	35.1	1.20	0.337	57.6	44.8	1.5e-8
Year 1 cultivar	548	18.8	7.5e-7	–	11.3	8.77	1.5e-4
Error a	18	29.2	–	–	1.29	–	–
Year 2 cultivar	1	0.390	0.0324	0.859	32.3	46.1	1.0e-6
Year 1 cultivar: year 2 cultivar	6	8.56	0.704	0.650	0.491	0.700	0.653
Error b	21	12.2	–	–	0.701	–	–
Greenhouse cultivar	3	68.0	8.63	3.0e-5	6.32	4.59	4.4e-3
Year 1 cultivar: greenhouse cultivar	18	7.69	0.976	0.492	1.05	0.765	0.737
Year 2 cultivar: greenhouse cultivar	3	9.15	1.16	0.327	0.992	0.720	0.542
Year 1 cultivar: year 2 cultivar: greenhouse cultivar	18	8.46	1.07	0.387	0.594	0.431	0.979
Error c	–	126	1.38	–	–	7.88	–

[a] Df = degrees of freedom; *F* = *F* statistic; MS = mean square; *P* = probability for *F* test.

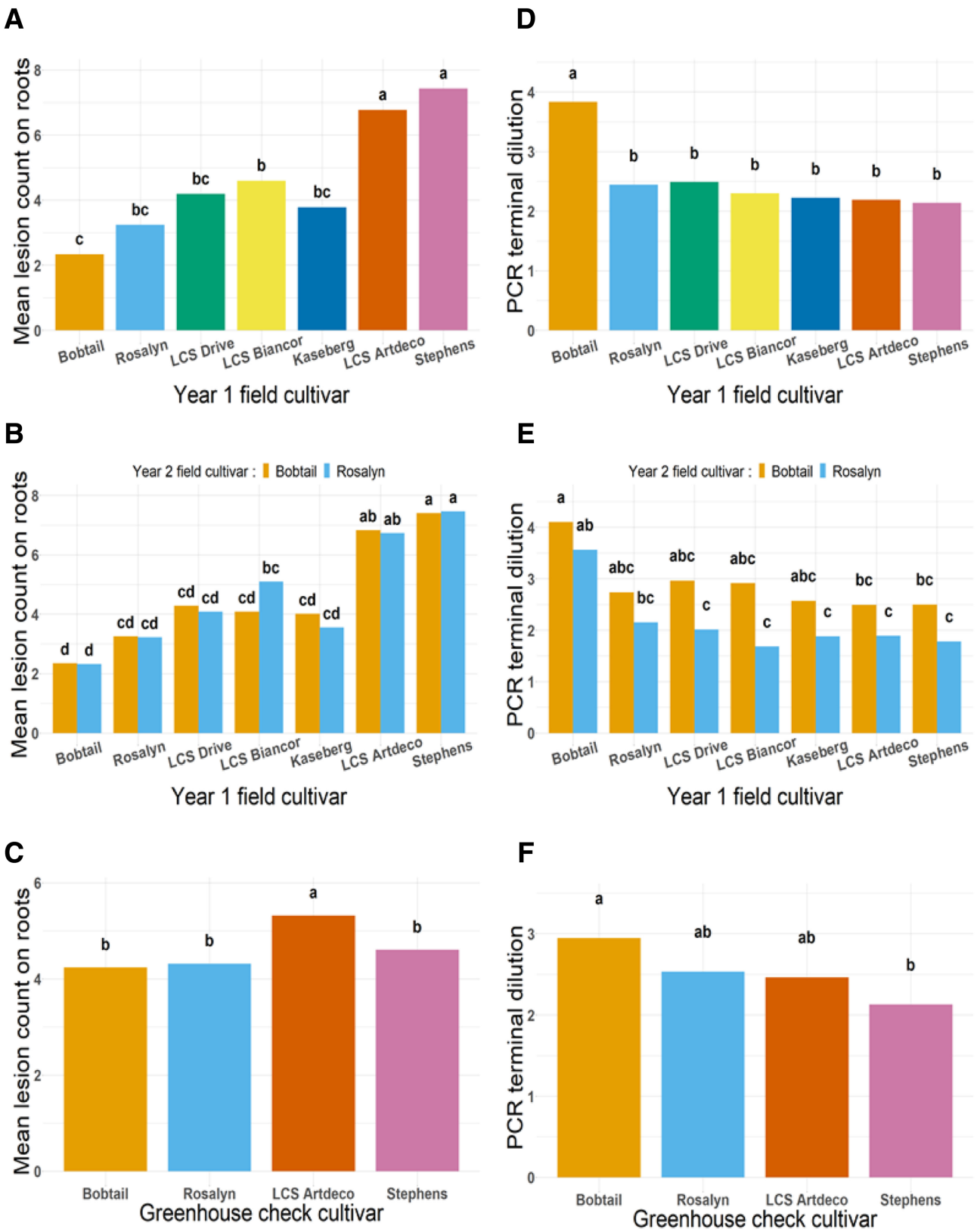

Fig. 4. Greenhouse disease assessment for the effect of first- and second-year field wheat cultivars on take-all disease severity of four greenhouse check cultivars (**A to C**) and corresponding PCR analysis for *phlD* + pseudomonads of rhizosphere root washes from greenhouse assessments (**D to F**). A, Take-all lesion counts on seminal roots averaged over both second-year cultivars and the four greenhouse checks (SEM = 0.872). B, Take-all lesion counts showing first- and second-year cultivar effects, averaged over all four check cultivars (SEM = 0.363). C, Take-all lesion counts on seminal roots of greenhouse checks, averaged over all first- and second-year field cultivars (SEM = 0.236; 817 days). D, Mean terminal dilution of positive amplification of *phlD* averaged over both second-year field cultivars and four greenhouse check cultivars (SEM = 0.0859). E, Mean terminal dilution of positive amplification of *phlD* of first- and second-year effects averaged over all four check cultivars (SEM = 0.0468). F, Mean terminal dilution of positive amplification of *phlD* for the four greenhouse checks averaged over all seven first-year cultivars and both second-year cultivars (SEM = 0.0920).

attempts to alter the microbiome through the use of inundative introduction of beneficial microbes to control plant disease has been challenging and often disappointing (Hawkes and Connor 2017; Kaminsky et al. 2019; Mazzola and Freilich 2017). These disappointing outcomes are likely because of the inability of introduced microbes to compete against diverse and dynamic indigenous microbial communities (Finkel et al. 2017; Mazzola and Freilich 2017). Altering the environment to encourage an effective microbiome is thus likely necessary to provide the desired ecosystem services. Although modifying the microbiome through large inputs of organic amendments may have potential for disease control in high-value crops (Lazarovits et al. 2001; Noble and Coventry 2005), it is likely not practical for extensively grown field crops. By contrast, growing new crop cultivars that provide an environment favorable to a disease-suppressive microbiome would be much easier to implement, a familiar application technology to growers, and cost-effective. In Oregon, growers have already used the wheat cultivars Bobtail and Rosalyn to suppress wheat take-all, and we are currently developing approaches to select for this trait in breeding programs (C. C. Mundt, *unpublished*).

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