

Wheat cultivar susceptibility to *Gaeumannomyces graminis* var. *tritici* and influence on *Pseudomonas fluorescens* numbers in the rhizosphere

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Abstract Yields from wheat (*Triticum aestivum* L.) cultivars grown in natural soils and soils inoculated with *Gaeumannomyces graminis* var. *tritici* (*Ggt*), the fungus causing take-all, were obtained in separate second-year wheat fields over three successive growing seasons in Canterbury, New Zealand. Cultivars ‘Empress’, ‘Oakley’, ‘Phoenix’ and ‘Sage’ were compared with the standard, ‘Wakanui’, over three seasons, ‘Conquest’, ‘Consort’, ‘Einstein’ and ‘Starfire’ over three seasons, and ‘Raffles’, ‘Savannah’ and ‘Torch’ in one season. Root take-all severity averaged 44 % in *Ggt*-inoculated soils compared with 20 % in natural soils. Adding *Ggt* inoculum to soil at sowing decreased mean grain yields for all cultivars by 0.78 t ha⁻¹ (9.5 %). ‘Starfire’ had the greatest yield (9.36 t ha⁻¹) and ‘Consort’ the least (6.51 t ha⁻¹) compared to ‘Wakanui’ at 8.24 t/ha. No cultivar exhibited resistance to take-all, but the variation in tolerance provided growers with management options. ‘Empress’, ‘Starfire’ and ‘Raffles’ produced high yields in soils initially containing large amounts of *Ggt* inoculum, and would be suitable as second year wheat crops where the risk of take-all is high. Soils sown with ‘Starfire’, ‘Oakley’ or ‘Sage’ resulted in the least build-up of inoculum after harvest, so these cultivars

would be suitable as first-year wheat and less likely to induce *Ggt* inoculum in soil for subsequent wheat crops. The numbers of *Pseudomonas fluorescens* on wheat roots appeared not to be associated with any cultivar. Only in the first growing season were high numbers of these bacteria associated with high *Ggt* inoculum, which is a prelude to take-all decline.

Keywords Take-all · Pathogen DNA · Disease susceptibility

Introduction

Take-all, caused by the fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is one of the most important root diseases of wheat (*Triticum aestivum* L.) worldwide (Hornby et al. 1998; Cook 2003). The pathogen infects susceptible hosts, resulting in blackened roots, severe stunting of plants and premature death (Cook 2003). Between crops, *Ggt* survives saprophytically on crop debris (Asher and Shipton 1981). The concentration of *Ggt* inoculum in soil declines as the crop debris decays (Hornby 1981; Bithell et al. 2009), and the rate of inoculum decline is more rapid where warm wet conditions follow harvest (Cook 2003) than in dry environments (MacNish and Dodman 1973).

The amount of *Ggt* inoculum present in soils at crop establishment affects the extent of primary infection of susceptible cereal hosts (Bailey and Gilligan 1999). *Ggt* DNA concentrations of less than 6 pg before sowing have been associated with low risk of take-all in second wheat crops, and more than 80 pg *Ggt* DNA g⁻¹ soil with high risk, when environmental conditions favour disease development (Ophel-Keller et al. 2008; Bithell et al. 2012; Bithell et al. 2015).

Wheat cultivars differ in resistance to take-all irrespective of *Ggt* inoculum concentrations (Gao et al. 2004; Liatukas et al. 2010), as measured by the amount of disease. Host

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resistance thus influences the capacity of cultivars to contribute to pathogen inoculum in soil after harvest. Resistance may also be related to associations with microbial antagonists in root rhizospheres. *Pseudomonas fluorescens* has been implicated in triggering host resistance and suppression of *Ggt* and take-all (Cook 2007). This bacterium may also modulate defence/stress gene expression in cereal roots (Okubara et al. 2010). For instance, wheat genes TiERF1 and RC7 associated with inhibition of *Ggt* hyphae growth on roots (Liu et al. 2013a), and myeloblastosis transcription factors (Liu et al. 2013b), have been associated with wheat resistance to take-all. Genes conferring resistance, from the diploid wheat species *T. monococcum*, could also be utilised to improve the performance of *T. aestivum* in high take-all risk situations (McMillan et al. 2014). Wheat cultivars may also differ in their tolerance to particular amounts of take-all, such that tolerant cultivars have less yield loss than less tolerant ones (Krzyszynska et al. 2006; Rachova and Kancheva 2015).

While there are currently no commercial wheat cultivars classified in New Zealand as resistant to take-all, some cultivars may have some degree of resistance or tolerance to the disease and still produce high grain yields irrespective of the presence of *Ggt* inoculum in the soil. This paper presents results from field trials in each of three successive growing seasons in Canterbury in the South Island of New Zealand. The aim of this research was to quantify the degrees of resistance or tolerance of commercially available wheat cultivars to take-all as assessed by the relationship between take-all severity and yields. Five cultivars were evaluated in all years, with seven others included in, or removed from, trial evaluations in response to industry needs. All the cultivars are registered and grown in New Zealand, although three of the 12 ('Consort', 'Einstein' and 'Savannah') are not commonly grown here now. Seven ('Consort', 'Einstein', 'Oakley', 'Phoenix', 'Raffles', 'Savannah' and 'Torch') are derived from the United Kingdom but only 'Oakley' is grown there currently. The cultivars were sown into soil of fields grown for wheat in the previous year, which allowed for the build-up of *Ggt* inoculum, and also into soil further inoculated with *Ggt*. This was to ensure sufficient inoculum was present for comparisons. The trials were managed by the Foundation for Arable Research and New Zealand Arable. Wheat cultivar yields and grain quality were related to take-all severity and *Ggt* DNA concentrations in roots, and *P. fluorescens* populations in root rhizospheres, for each cultivar.

Materials and methods

Field trials

A trial was set up in second-year wheat field located at separate sites in each of 3 years. The 2012-sown trial was

at Makikihi (44°38'23.1"S; 171°08'35.0"E) on a Ytoh1z(50 %) soil type, the 2013-sown trial was at Makikihi (44°37'01.2"S; 171°08'49.9"E) on a Temp2z(60 %) soil type and the 2014-sown trial was at Lismore (43°53'14.4"S; 171°29'50.3"E) on a Lism3z(60 %) soil type (Landcare Research 2016). Soils were prepared within 6 weeks prior to sowing by burning existing stubble, and cultivation by rotary tiller (2012), deep-tyne and rotary tiller (2013), or by ploughing, levelling and grubbing (2014). The wheat cultivars 'Empress', 'Oakley', 'Phoenix', 'Sage' and 'Wakanui' were sown in the three trials, while 'Conquest', 'Consort', 'Einstein', 'Starfire' (KWW46), 'Raffles', 'Savannah' and 'Torch' were evaluated in one or two trials.

For the 2012, 2013 and 2014 trials, the crops were sown in autumn, on 3 May 2012, 9 May 2013 and 3 June 2014, respectively. The trial plots were each 10 m long and 1.65 m wide, comprising nine rows from one drill-width (15 cm row spacing). The seeds were sown at 3–4 cm depth at 150 kg ha⁻¹ using a disc type plot drill. A 1.7 m wide buffer of 'Consort' wheat was sown around the trial and between the ends of each plot. In total for each year, there were 72 plots, with eight plots for each cultivar, four of which were inoculated with *Ggt*-and four uninoculated (natural). The plots were arranged according to a resolvable Latinised block design in a 36-plot by two-plot array, generated using CycDesign (CycDesign v4.1, VSN International).

Ggt inoculum was produced on sterilised oat grains (Weller and Cook 1983). The grains were inoculated with five *Ggt* isolates (Biomill SC3, A3SL4, H9T3 R1/1.2, H11T3 R1/3 and Bio3) collected from couch grass (*Elymus repens*) or wheat in Canterbury. Following colonisation by each *Ggt* isolate in individual sterile 2.5 L flasks for 3 weeks, the oat grains colonised by the five isolates were pooled, mixed and air-dried at room temperature (20 °C). The dried inoculum was stored at 4 °C until use. The *Ggt*-infested grains were mixed with the wheat seed for each cultivar and drilled in the inoculum-designated plots at the rate of 211 *Ggt*-infested seeds m⁻².

Crop management

The crops were managed in accordance with best practice. Fertilisers urea (46 % N) at 100 kg/ha at Zadoks (Zadoks et al. 1974) growth stage (GS) 26, Ammo® 31 (30.7 % N, 14.4 % S) at 193 kg/ha and muriate of potash at 57 kg/ha at GS 31, urea at 150 kg ha⁻¹ at GS 37 and again at 200 kg ha⁻¹ at GS 39 were applied in 2012; 65 kg urea ha⁻¹ at GS 26 and 163 kg urea ha⁻¹ at GS 31 and GS 37 in 2013; 15 % potash sulphur super at 300 kg/ha prior to sowing and Ammo® 31 at 100 kg ha⁻¹ at GS 39 in 2014.

The pre-emergence herbicide Firebird® SC (400 g L⁻¹ flufenacet and 200 g L⁻¹ diflufenican) was applied 500 mL ha⁻¹ alone (2014) or at 300 mL ha⁻¹ and terbuthylazine at 1.5 kg ha⁻¹ (2012), or at 500 g ha⁻¹ (2013), were sprayed within 1 week of sowing, and with the post emergence fluroxypyr at 249 g ha⁻¹ in 2013, and terbuthylazine at 375 g ha⁻¹ and Image® (120 g L⁻¹ bromoxynil, 120 g L⁻¹ ioxynil and 360 g L⁻¹ mecoprop-p) at 1.75 L ha⁻¹ in 2014, for the selective control of broad-leaved weeds.

The insecticide tau-fluvalinate was sprayed at 24 g ha⁻¹ at GS 21, GS 32 and GS 35 to control aphids that could transmit *Yellow Dwarf Virus* in 2014. Fungicides for disease control were epoxiconazole at 50 g ha⁻¹ at GS 35, epoxiconazole at 87.5 g ha⁻¹ plus pyraclostrobin at 100 g ha⁻¹ at GS 51 and epoxiconazole at 35 g ha⁻¹ plus azoxystrobin at 70 g ha⁻¹ at GS 73 applied in 2012; epoxiconazole at 37.5 g ha⁻¹ at GS 30 and GS 32, and at 94 g ha⁻¹ alone at GS 33 and with pyraclostrobin at 150 g ha⁻¹ at GS 39 and again with 100 g ha⁻¹ at GS 59 in 2013; tebuconazole at 172 g ha⁻¹ at GS 32 and GS 35 in 2014.

The plant growth regulator chlormequat chloride was applied at 1.5 kg ha⁻¹ in the trial sown in 2012 and at 1.3 kg ha⁻¹ in 2014, and at 1.1 kg/ha⁻¹ with 50 g ha⁻¹ trinexapac-ethyl in 2013 at approx. GS 32.

No irrigation was applied in the 2012 trial. During summer, the trial sown in 2013 had two applications of 40 mm water and the trial in 2014 one application of 130 mm water.

Assessments

Plant numbers

Plant emergence was assessed at approx. 8 weeks after sowing (30 June 2012, 23 June 2013 and 25 July 2014) by counting the number of plants in a 1 m length pair of drill rows (0.3 m²) at two sites in each plot. The number of harvestable ears (above the harvester cutting bar) was assessed in a 0.6 m length of a drill row pair (0.18 m²) immediately prior to harvest on the same day.

Take-all above ground

Above-ground take-all symptoms were assessed from the middle 8.0 m length of the seven middle rows in each plot. Since *Ggt* can restrict the growth of plants, the degree of unevenness or “patchiness” in the crop canopy, that is height variation, was visually estimated in each plot on 20 November and 13 December after the trial was sown in 2013, and 21 October, 18 November and 17 December after the trial was sown in 2014. Crop patchiness (%) was the average score from two independent assessments, where 0 % = even growth, with all plants having similar heights, 100 % = uneven

growth, with variable heights. Symptoms of take-all include white heads. Thus, for the 2012 and 2013 trials, the percentage of white heads per plot was estimated on 7 and 13 January, about 6 weeks before harvest.

Root sampling for *Gaeumannomyces* and *Pseudomonas fluorescens* analyses

At grain fill (GS 75), on 13 to 18 December, approx. 20 wheat plants were sampled with a garden fork from a 1 m² zone 30 cm away from the edges at both ends of each plot. Most of the plants were assessed for take-all *Ggt* in roots, and the remaining three plants used for determining *P. fluorescens* populations.

Take-all in roots

The roots of the plants were washed to remove adhering soil and assessed for severity of take-all lesions (Schoeny and Lucas 1999) on a scale of 0–4 (0 = no take-all; 1 = 1–10 % of the root system affected; 2 = 11–30 %; 3 = 31–60 %; 4 = 61–100 %). A take-all index (TAI) was then calculated as (0a + 10b + 30c + 60d + 100e)/T where a, b, c, d and e are the number of plants in each infection category and T = total number of plants (a + b + c + d + e) (Hornby and Beale 2000). This calculation leads to a maximum take-all index of 100 %.

A sub-sample of the roots of plants from the trial sown in 2012, which had been re-mixed after the take-all assessment and a sub-sample from categories 2 and 3 from the trial sown in 2013, were retained for quantification of *Ggt* DNA concentrations. The roots were air-dried at 40 °C for 4 days and stored at –18 °C until DNA extraction.

Root *Ggt* DNA quantification

Roots were prepared for Quantitative polymerase chain reaction (qPCR). Each dried root sample was ground in liquid nitrogen (N) using a mortar and pestle and stored at –20 °C. DNA was extracted from a ≈ 20 mg sub-sample of the ground roots using a DNeasy Plant Minikit (Qiagen), following the manufacturer’s instructions. The extracted DNA was suspended in 400 μL of TE manufacturer’s buffer (pH 8.0). Samples were diluted 1:20 (0.05) with TE buffer (pH 8.0) to reduce inhibitor effects.

PCR amplifications were carried out in a StepOnePlus qPCR machine (Applied Biosciences) using the methods of Keenan et al. (2015), to quantify the amount of *Ggt* DNA in each duplicate PCR sample. A Sybr green-based qPCR with primers Pot25SF and Pot25SR identified the samples containing inhibitors likely to affect detection of *Ggt* DNA, and these samples were omitted from the *Ggt* DNA analyses. To measure *Ggt* DNA concentrations in the cereal roots, a qPCR assay was used that targeted the translation elongation factor

1-alpha (EF1- α) gene from *Ggt*, using GgtProbe 1 and primers GgtEF1 and GgtEFR1. All quantities of target DNA were estimated using a standard curve constructed using standard regression of the cycle threshold (Ct) values against the \log_{10} of the initial concentration. Each qPCR plate contained duplicate or triplicate *Ggt* standards at 10, 1, 0.1, 0.01 and 0.001 ng *Ggt* DNA/ μ L buffer. The parameters of the standard curve were used to convert Ct values into estimated amounts of *Ggt* in the sample. An undetected Ct value was assumed to indicate that the sample contained no *Ggt* DNA (0).

Pseudomonas fluorescens Population analysis

Excess soil was shaken from the roots of test plants collected at the same time as for take-all and *Ggt* assessments in roots. The method described in van Toor et al. (2005) was used to obtain numbers of fluorescent colonies forming units (CFU) per g of root tissues in each sample.

Grain yield and quality

The grain yields were assessed in each of the 3 years, from a 8.0 m or 8.8 m length \times 1.65 m width area in the middle portion of each trial plot. Plots were harvested with a '2035 Plot combine harvester' (Sampo Rosenlew, Finland) containing weighing and electronic moisture meter equipment. Weights of grain were adjusted to 14 % moisture. The grain quality, determined by percent of screenings from a 2.0 mm Rota-screen, grain density as defined by the specific weight of the grain (kg hL^{-1}) and thousand grain weight (TGW), was assessed by New Zealand Seedlab, Christchurch.

At comparable cereal performance trials (CPT2) at 11 sites in south, mid and central Canterbury, wheat cultivars were sown into soil out of pasture or non-cereal crops in autumn of 2013 (Craigie 2014) and 2014 (Craigie 2015). This enabled yields from cultivars from the take-all trials sown as second-year wheat into soils containing *Ggt* inoculum to be compared with those sown as first-year wheat, into soils likely to have no *Ggt* inoculum. Thus, the 3-year mean yields from nine cultivars sown as second-year wheat were compared with yields from the same cultivars sown as first-year wheat in six to 20 comparable trials over the 2 years.

Soil *Ggt* DNA quantification

Within 1 week prior to sowing of the trials in 2013 and 2014, four groups of 20 soil cores, 10 mm diameter by 100 mm deep (AccucoreTM), were collected along a 'W' sampling pattern in the area of the field intended for the trial to obtain the background soil *Ggt* inoculum concentrations.

Within 2 weeks of harvest, 20 soil cores were collected along a 'W' sampling pattern between rows from each plot in the trials sown in 2012 (sampled 1 March 2013) and 2013

(sampled 22 February 2014). Soil *Ggt* DNA in the trial sown in 2014 could not be determined as the trial was ploughed immediately after harvest.

Following collection, the samples were air-dried at 40 °C for 3 days, giving 350–550 g dried soil samples per plot. These were sent to the South Australian Research & Development Institute (SARDI) for DNA analysis. Results were supplied as pg *Ggt* DNA g^{-1} soil.

Statistical analyses

Analysis for data from individual years

Yields, TGW, Test weights and soil *Ggt* DNA concentrations were analysed with analysis of variance. Soil *Ggt* DNA concentrations were \log_{10} transformed before analysis (to stabilise the variances). Percent white heads, patchiness, TAI and % screenings were analysed with a Binomial-beta hierarchical generalised linear model approach (HGLM, Lee et al. 2006), or, where random effects were found not to be important, with a binomial generalised linear model (GLM, McCullagh and Nelder 1989). Random effects (e.g. replicates, plot rows) were included with a beta distribution, and fixed effects (cultivar, inoculation and the interaction) with a binomial distribution, using logit links in both cases.

Numbers of plants and ears, root *Ggt* DNA concentrations and *P. fluorescens* CFU were analysed with a Poisson-gamma HGLM approach, or (where no random effects were found to be important) with a standard Poisson GLM. Logarithmic links were used for both fixed and random effects. In addition to the random effects listed above, these analyses included random components for the qPCR run and also plot, since more than one well per qPCR plate was inoculated for each plot. For the analyses of *Ggt* DNA quantities and *P. fluorescens* counts, offsets (McCullagh and Nelder 1989) were included to adjust results from the raw scale of the measurements to ng *Ggt* DNA mg^{-1} root, and *P. fluorescens* CFU g^{-1} root.

For the binomial and Poisson analyses, random effects were assessed using X^2 tests of the change in deviance on dropping the effect, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2014). Where random effects were required in the final analysis, fixed effects were assessed similarly to the random effects, but using GenStat's HGFTEST procedure. For analysis of variance and Poisson or binomial data not requiring random effects, fixed effects were assessed with F-tests.

Cross-year analyses

A cross-year analysis determined the mean yields and TAI for each cultivar over the years they were sown. Yields were analysed with a mixed-model approach, fitted with restricted

maximum likelihood (REML, Payne et al. 2014), where a different residual variance ('error') was estimated for each trial, and replicate blocks for each trial were included as separate random effects. The importance of the fixed effects were assessed with F-tests of Wald statistics, using the Kenward-Roger estimated for the denominator degrees of freedom (Kenward and Roger 1997). TAI data were analysed with a binomial-beta HGLM. For each year, separate dispersions were estimated, and random effects comprised a separate adjustment for replicates. For both analyses, cultivars, inoculation and their interaction, plus a main effect for years, were fitted. This approach gives weighted means across the years, but ignores the strong interactions between year and cultivar and/or inoculation.

For the analyses of data within and cross years, means are presented along with 95 % confidence limits. For the Poisson and binomial data and amounts of soil *Ggt*, the means and confidence limits were obtained on the link or transformed scale, and back-transformed for presentation. Where an offset had been included, the means were predicted for an offset of 0 (=log(1)), to give means on the required scale for ng *Ggt* DNA mg⁻¹ root and *P. fluorescens* CFU g⁻¹ root.

Relationships among variables were primarily explored graphically, and with calculation of simple Pearson's correlation co-efficients (*r*). All analyses were carried out with GenStat (GenStat Committee 2014).

Results

Plant emergence

Although numbers of plants m⁻² varied among the cultivars ($P < 0.004$) in each of the 3 years of assessment, plant numbers were not strongly influenced by the *Ggt*-inoculum application in any year ($P > 0.1$, Inoculation and Inoculation × Cultivar). In 2012, plant numbers were least for 'Phoenix' (mean = 101) and greatest for 'Empress' (129); in 2013 least for 'Sage' (mean = 139) and greatest for 'Starfire' (166); and in 2014 least for 'Wakanui' (mean = 122) and greatest for 'Starfire' (151).

Crop patchiness

No lodging was observed in any trial. *Ggt* infection influenced plant growth, which was reflected in the unevenness of plant heights. In the 2 years when the uniformity of crop height was assessed, crop height was less even in the inoculated plots than in natural plots, with some differences in evenness among cultivars. For the trial sown in 2013, crop height uniformity varied substantially, both among cultivars ($P < 0.001$) and with inoculation ($P < 0.001$), although the effect of inoculation was similar for all cultivars ($P = 0.522$ Inoculation ×

Cultivar). On average, patchiness for inoculated plots was 22 % and for natural plots was 7 %. Patchiness was greatest for 'Consort' (mean = 21 %), followed by 'Einstein', 'Empress' and 'Wakanui' (16–18 %), and least for 'Sage' and 'Starfire' (both <10 %). For the trial sown in 2014, patchiness was greater for inoculated than natural plots in November and December ($P < 0.001$), but variation among cultivars was less pronounced ($P = 0.098$ Cultivar and $P = 0.661$ Inoculation × Cultivar) in the early assessment. In the later assessment the differences among cultivars ($P = 0.001$ Cultivar) were similar, regardless of the presence of inoculation ($P = 0.450$ Inoculation × Cultivar). Patchiness estimates from November were moderately related to those for December ($r = 0.62$). Patchiness was greatest for 'Sage' (mean = 23 %), followed by 'Oakley', 'Phoenix', 'Empress' and 'Wakanui' (16–19 %), and least for 'Raffles' and 'Starfire' (both ≈ 10 %).

Ear density

For the trials sown in 2012 and 2014, the numbers of ears m⁻² varied among the cultivars ($P < 0.001$ and $P = 0.028$, respectively for the two trials) and also with *Ggt* inoculation ($P < 0.001$ for both trials), and these effects were similar for each cultivar ($P = 0.515$ for the Cultivar main effect and $P = 0.451$ for the Inoculation × Cultivar interaction). In 2012 there were 50 % more ears m⁻² in natural plots than in the *Ggt*-inoculated plots. There were much fewer ears for 'Oakley' (mean = 168) than for the other cultivars; 'Sage' had the most ears m⁻² (401) followed by 'Wakanui' and 'Consort' (365 and 360), and 283 to 346 plants m⁻² for the other cultivars. In 2014, there were about 12 % more ears m⁻² in natural plots than in the *Ggt*-inoculated plots. There were fewer ears for 'Wakanui' (mean = 284) than for the other cultivars, and the most ears m⁻² for 'Empress' (355) followed by 'Conquest' and 'Starfire' (both 328), and 300 to 314 plants m⁻² for the other cultivars.

For the trial sown in 2013, there were 13 % more ears m⁻² in natural than *Ggt*-inoculated plots ($P < 0.001$), with the effect fairly similar for all cultivars ($P = 0.107$ for the main effect, $P = 0.111$ for the Inoculation × Cultivar interaction).

White heads

Ear senescence (white heads), which reflects crop maturity and effects of *Ggt*, varied strongly among cultivars ($P < 0.001$) in both years where assessments were carried out. In the trial sown in 2012, mean senescence was affected by inoculation ($P < 0.001$), but the effect varied among the cultivars ($P < 0.001$ Inoculation × Cultivar). Senescence was least for the late maturing 'Savannah' (4 %) and greatest for the early maturing 'Conquest' (73 %), and ranged from 7 to 31 % for the other cultivars. The effects of inoculation on

senescence varied from negligible ('Conquest', 'Oakley') to substantial ('Sage' increased by 34 % and 'Einstein' by 23 %). In the 2013 trial, senescence was not strongly affected by inoculation ($P = 0.315$ Inoculation; $P = 0.256$ Inoculation \times Cultivar). It was least for 'Consort' (16 %), and greatest for 'Sage' (56 %) and 28–37 % for the other cultivars.

Take-all in roots

In all 3 years, there was little variation in TAI among cultivars ($P > 0.1$ Cultivar and Inoculation \times Cultivar) (Table 1). However, in 2012 and in 2014, TAI was 44 % and 42 % on average for the *Ggt*-inoculated plots and greater ($P < 0.001$) than the 13 % in 2012 and 14 % in 2014, for the natural plots. In 2013, inoculating plots with *Ggt* did not substantially increase ($P = 0.159$) take-all severity in roots.

Over the 3 years, TAI did not vary substantially with cultivar ($P = 0.222$ Cultivar; $P = 0.578$ Inoculation \times Cultivar), but was greater on average over the 3 years in *Ggt*-inoculated plots (44 %) than in natural plots (20 %; $P < 0.001$). On average, the TAIs were similar for the three seasons ($P = 0.325$ Year).

Ggt DNA in roots

The analysis of wheat roots with take-all confirmed the existence of *Ggt*. For plant roots sampled from the trial sown in 2012, the concentration of *Ggt* DNA in roots was 870 times greater on average in inoculated plots (7.3 ng mg⁻¹ root) than in natural plots (1.0 ng mg⁻¹ root) ($P < 0.001$), but this varied among cultivars ($P < 0.001$ Inoculation \times Cultivar). No *Ggt* DNA was detected in the roots of uninoculated 'Savannah', Phoenix' or 'Consort'. *Ggt* DNA concentration (95 % confidence limit) in roots of 'Oakley' was similar for the inoculated plots (4.8_(0.9, 25.3) ng mg⁻¹ root) and natural plots (5.8_(1.1, 31.2) ng mg⁻¹ root). For the remaining cultivars, *Ggt* concentrations in wheat roots in inoculated plots were four to 833 times greater than those in wheat roots from natural plots.

For the trial sown in 2013, the concentration of *Ggt* DNA was estimated separately for plant roots from take-all categories 2 and 3. For both categories, the differences among cultivars were small ($P > 0.1$ Cultivar and Inoculation \times Cultivar). However, average root *Ggt* DNA concentrations in the natural plots from roots for category 2 (12.6 ng mg⁻¹ root) were 22 % greater ($P = 0.046$), and for category 3 (18.2 ng mg⁻¹ root), 36 % greater ($P = 0.007$), than for those from the inoculated plots for category 2 (10.3 ng mg⁻¹ root) and category 3 (13.6 ng mg⁻¹ root).

Rhizosphere *Pseudomonas fluorescens*

There was little consistency in the treatment effects on the numbers of *P. fluorescens* in the rhizosphere among years

(Fig. 1). In 2012, the *P. fluorescens* numbers overall were similar for the cultivars ($P = 0.114$), but were 4.3 times greater ($P < 0.001$) in inoculated plots (mean = 1.7×10^{-6} CFU g⁻¹ roots) than in natural plots (4.0×10^{-5} CFU g⁻¹ roots). The inoculation effect varied slightly among the cultivars ($P = 0.070$), with CFUs from inoculated 'Savannah' plots 0.7 times, and in 'Conquest' over 30 times greater, as from the natural plots. In contrast, CFUs in 2013 varied substantially among the cultivars ($P = 0.007$), where CFUs in the rhizosphere of 'Consort', which had the greatest numbers, were 2.8 times greater than for 'Starfire', which had the smallest populations. There was little effect of Inoculation ($P = 0.269$) and Inoculation \times Cultivar ($P = 0.309$) on *P. fluorescens* numbers. In 2014, the populations varied substantially among the cultivars ($P < 0.001$) and with inoculation ($P = 0.014$) but with no significant interaction between the two factors ($P = 0.159$). *P. fluorescens* numbers were substantially greater in the rhizospheres of 'Wakanui' (9×10^7 CFU g⁻¹ roots) than for those of all the other cultivars (5.5×10^6 – 1.8×10^7 CFU g⁻¹ roots), and were almost three times greater in natural plots than the *Ggt*-inoculated plots.

Amounts of *Gaeumannomyces graminis* DNA in soil

For the trial sown in 2012, the concentration of *Ggt* DNA in soil after harvest was 25 times greater on average in *Ggt*-inoculated plots (mean = 149 pg g⁻¹ soil) than in natural plots (6 pg g⁻¹ soil, $P < 0.001$), but this did not vary substantially among cultivars ($P = 0.368$ Cultivar; $P = 0.749$ Inoculation \times Cultivar) (Table 2). In 2013, soil *Ggt* concentrations at the time of sowing were moderate, averaging 103 pg *Ggt* DNA g⁻¹ soil. At time of harvest, soil *Ggt* DNA concentrations varied among the cultivars ($P = 0.021$). They were least for 'Starfire' (mean = 46 pg g⁻¹ soil) and 'Oakley' (50 pg g⁻¹ soil), which were classed as medium risk for take-all. They were greatest for 'Phoenix' (110 pg g⁻¹ soil) and 'Wakanui' (148 pg g⁻¹ soil), which were classed as high risk. Soil *Ggt* did not vary substantially with *Ggt* inoculation ($P = 0.438$ Inoculation; $P = 0.491$ Inoculation \times Cultivar). In 2014, soil *Ggt* concentration at time of sowing was low, averaging 6 pg *Ggt* DNA g⁻¹ soil. *Ggt* concentrations were not assessed at harvest.

Grain yields

Yields varied substantially among the wheat cultivars ($P < 0.001$) and with *Ggt* inoculation ($P < 0.001$) in each of the 3 years (Table 3). In wheat sown in 2012, the effect of *Ggt* inoculation was similar for all cultivars ($P = 0.791$ Inoculation \times Cultivar), and mean yield was 1.47 t ha⁻¹ greater in uninoculated plots than in inoculated plots. 'Wakanui' followed by 'Empress' had the greatest yields; 'Oakley' followed by 'Conquest' the smallest yields. For crops sown in 2013,

Table 1 Mean take-all percentage indices (95 % Confidence limits) for different wheat cultivars sown in *Gaeumannomyces graminis* var. *tritici* (*Ggt*)-inoculated soil and natural soil at one new site in three growing seasons in Canterbury, New Zealand

Cultivar	2012		2013		2014		2012–2014 mean	
	Inoculated	Natural	Inoculated	Natural	Inoculated	Natural	Inoculated	Natural
'Conquest'	50.2 (32.2,68.2)	14.5 (5.9,31.3)	-	-	-	-	49.2 (32.2,66.4)	15.9 (7.8,29.7)
'Consort'	34.6 (19.5,53.6)	8.6 (2.8,23.8)	47.3 (36.9,57.9)	42.8 (32.7,53.6)	47.4 (35.8,59.2)	16.2 (9.2,27.1)	39.6 (22.6,59.5)	26.5 (13.6,45.3)
'Einstein'	37.4 (21.7,56.4)	6.1 (1.6,20.6)	56.5 (45.7,66.7)	49.2 (38.7,59.8)	-	-	46.4 (27.9,65.9)	29.2 (15.4,48.5)
'Empress'	40.6 (24.1,59.4)	21.3 (10.1,39.3)	50.9 (40.4,61.5)	40.4 (30.5,51.2)	38.4 (27.6,50.5)	12.1 (6.2,22.3)	42.9 (28.9,58.2)	22.4 (13.1,35.6)
'Oakley'	49.9 (31.9,67.9)	22.4 (10.8,40.6)	50.3 (39.7,60.8)	51.4 (40.8,61.8)	41.8 (30.7,53.9)	11.9 (6.1,22.1)	46.4 (31.9,61.6)	26.1 (15.8,40.1)
'Phoenix'	43.7 (26.7,62.3)	12.7 (4.9,29.1)	33.8 (24.5,44.6)	41.5 (31.5,52.3)	40.2 (29.2,52.3)	17.9 (10.5,29.0)	38.7 (25.4,54.0)	23.6 (13.9,37.0)
'Raffles'	-	-	-	-	51.9 (40.0,63.5)	18.8 (11.1,29.9)	51.9 (29.8,73.3)	18.3 (7.6,38.1)
'Sage'	53.3 (34.9,70.8)	15.9 (6.7,33.0)	47.3 (36.9,57.9)	40.1 (30.2,50.9)	36.7 (26.1,48.8)	11.7 (5.9,21.9)	43.7 (29.5,58.9)	20.9 (12.1,33.7)
'Savannah'	39.4 (23.2,58.3)	7.3 (2.2,22.2)	-	-	-	-	39.5 (17.4,66.8)	7.4 (1.6,27.9)
'Starfire'	-	-	50.9 (40.3,61.4)	44.9 (34.6,55.6)	41.4 (30.3,53.4)	10.3 (4.9,20.1)	46.4 (29.1,64.5)	24.2 (12.8,40.9)
'Torch'	-	-	-	-	45.3 (33.9,57.3)	17.9 (10.4,28.9)	45.2 (24.5,67.8)	17.4 (7.1,36.8)
'Wakanui'	51.6 (33.4,69.4)	15.4 (6.5,32.4)	46.1 (35.7,56.8)	42.3 (32.2,53.1)	34.2 (23.9,46.3)	13.2 (6.9,23.5)	41.7 (27.8,57.0)	22.2 (13.0,35.3)

however, the Inoculation \times Cultivar interaction was statistically significant ($P = 0.05$), indicating a difference among cultivars in tolerance to take-all. Yield decreases from inoculation varied from small ('Consort' by 0.46 t ha^{-1}) to large ('Phoenix' by 1.55 , 'Einstein' by 1.68 and 'Wakanui' 1.85 t ha^{-1}). For crops sown in 2014, the Inoculation \times Cultivar interaction was not significant ($P = 0.507$), with the mean difference of 0.5 t ha^{-1} between most *Ggt*-inoculated and natural plots.

Over the 3 years, yields differed among the cultivars ($P < 0.001$) and *Ggt* inoculation ($P < 0.001$), but the inoculation effect was similar for all cultivars ($P = 0.516$ Inoculation \times Cultivar) (Table 4). The mean yields were similar for the 3 years ($P = 0.963$ Year). *Ggt* inoculation decreased yields by 0.78 t ha^{-1} , equivalent to a 9.5 % reduction averaged over all of the cultivars. 'Starfire' and 'Raffles' were ranked in the three top yielding cultivars both in the *Ggt*-inoculated and natural plots.

The 3-year mean yields from nine cultivars sown as second year wheat (high inoculum) in the current study were compared with the 2-year mean yields from the same cultivars sown as first-year wheat (nil inoculum) in six–20 comparable trials (Table 4). Overall, compared with 10.59 t ha^{-1} for the cultivars grown as first-year wheat in natural soil, yields for cultivars grown as second year wheat in *Ggt*-inoculated soils were 8.41 t ha^{-1} (21 % less) and in natural soils were 9.15 t ha^{-1} (14 % less). The reduced yields for the cultivars grown as second-year crops indicated the potential yield loss associated with soil-borne *Ggt* inoculum.

Grain quality

Ggt inoculation affected screenings only for the trial sown in 2012 ($P < 0.05$), which were 0.42 % in grain harvested from the *Ggt*-inoculated plots compared with 0.28 % from natural plots. This effect was similar for all the cultivars ($P = 0.334$ Inoculation \times Cultivar). Percent screenings varied among the cultivars in all years ($P < 0.001$). Screenings in 2012 were least for 'Wakanui' and 'Sage' (0.23 and 0.26 %), and greatest for 'Phoenix' (0.49 %) and 'Oakley' (0.44 %); in 2013 least for 'Phoenix' (0.83 %) and 'Wakanui' (0.84 %) and greatest for 'Consort' (1.91 %) and 'Empress' (1.55 %); in 2014 least for 'Conquest' (0.29 %) and 'Raffles' (0.32 %) and greatest for 'Torch' (3.41 %) and 'Starfire' (1.64 %).

TGW was strongly affected by *Ggt*-inoculation in trials sown in 2012 ($P < 0.001$) and 2013 ($P = 0.002$), but more weakly ($P = 0.067$) in 2014. TGW for 2012 was on average 3.0 g (6 %) less, for 2013 1.0 g (2 %) greater, and for 2014 0.5 g (1 %) greater in inoculated plots than in natural plots. Mean TGW also varied among the cultivars ($P < 0.001$ in each year), with the inoculation effect similar for all cultivars in each of the 3 years ($P = 0.174$ – 0.694 Inoculation \times Cultivar). TGW in 2012 was least for 'Conquest' (44 g)

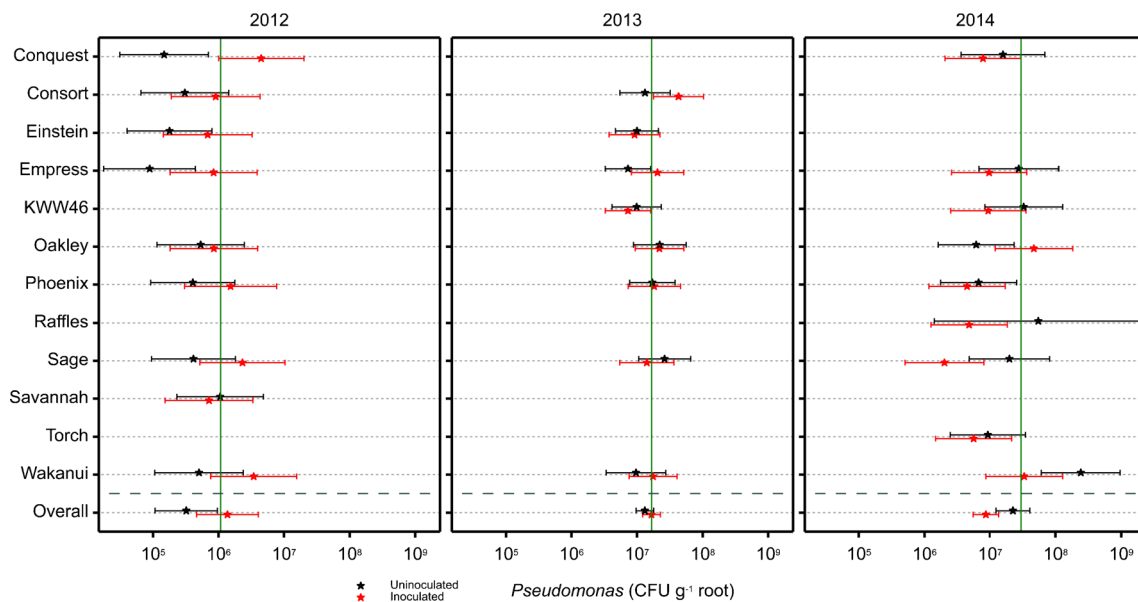


Fig. 1 Mean numbers of fluorescent *Pseudomonas* colony forming units (CFU) g^{-1} root for different wheat cultivars and the mean CFU over cultivars with and without soil inoculation of *Gaeumannomyces graminis* var. *tritici* in each of three growing seasons in Canterbury, Lincoln, New

Zealand. Grey vertical lines indicate the overall mean (mean over all plot values). Error bars are 95 % confidence limits for the means. Cultivars are in order of increasing overall mean within each year

followed by ‘Empress’ (45 g), and greatest for ‘Sage’ (53 g) followed by ‘Oakley’ (51 g) and ‘Phoenix’ (51 g); in 2013 least for ‘Consort’ (34 g) and ‘Empress’ (36 g), and greatest for ‘Phoenix’ (47 g) followed by ‘Sage’ (44 g), ‘Starfire’ (42 g) and ‘Einstein’ (42 g); in 2014 least for ‘Empress’ (36 g), ‘Torch’ (37 g) and ‘Oakley’ (38 g) and greatest for ‘Phoenix’ (42 g) and ‘Raffles’ (42 g).

The density of grain harvested from each of the trials was also affected by Inoculation ($P < 0.001$ to $P = 0.005$), with test weights averaging 1.9 (2 %) kg hL^{-1} in 2012, 1.3 kg hL^{-1} (2 %) in 2013 and 0.7 kg hL^{-1} (1 %) in 2014 less from *Ggt*-inoculated plots than from the natural plots. Test weight of grain also varied among the cultivars ($P < 0.001$), with the inoculation effect similar for all cultivars in each of the 3 years

($P = 0.108$ – 0.610). In 2012, test weights averaged 78.5 kg hL^{-1} for ‘Conquest’ and ‘Sage’, and between 75 and 76.3 kg hL^{-1} for the other cultivars. In 2013, they were least for ‘Consort’ (60 kg hL^{-1}), ‘Empress’ (62 kg hL^{-1}) and ‘Oakley’ (63 kg hL^{-1}), and greatest for ‘Starfire’ and ‘Sage’ (both 71 kg hL^{-1}). In 2014, mean grain density was least for ‘Oakley’ (66 kg hL^{-1}), and greatest for ‘Conquest’ (79 kg hL^{-1}).

Relationships among variables

Correlations (r) were determined for each of the 3 years between mean plot values for each of the measurements, including the *Ggt*-inoculated and natural plots. Correlations with $|r|$

Table 2 Mean concentrations of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) (back-transformed \log_{10} (pg *Ggt* g^{-1} soil))* immediately after harvest in *Ggt*-inoculated soil and natural soil sown with different wheat cultivars in two growing seasons in Canterbury, New Zealand

Cultivar	2012		2013	
	Inoculated	Natural	Inoculated	Natural
‘Conquest’	118.8 (29.5478.7)	7.1 (1.8,28.6)	-	-
‘Consort’	242.0 (60.1975.2)	11.3 (2.8,45.6)	86.4 (43.1173.2)	44.8 (22.3,89.8)
‘Einstein’	123.1 (30.6496.2)	5.5 (1.4,22.0)	62.5 (31.2125.4)	68.5 (34.2137.4)
‘Empress’	181.4 (45.0,730.8)	9.7 (2.4,39.2)	100.5 (50.1201.6)	67.9 (33.9136.2)
‘Oakley’	85.9 (21.3346.1)	2.7 (0.7,10.9)	45.7 (22.8,91.6)	55.6 (27.7111.4)
‘Phoenix’	276.2 (68.6,1113.0)	5.3 (1.3,21.5)	83.9 (41.8168.2)	145.0 (72.3290.7)
‘Sage’	52.4 (13.0,211.1)	6.0 (1.5,24.0)	56.6 (28.2113.4)	61.8 (30.8124.0)
‘Savannah’	179.7 (44.6724.0)	1.4 (0.3,5.5)	-	-
‘Starfire’	-	-	31.9 (15.9,63.9)	66.9 (33.3134.1)
‘Wakanui’	250.1 (62.1,1007.9)	14.6 (3.6,58.8)	117.5 (58.6235.6)	187.2 (93.4375.5)

* 95 % confidence limits

Table 3 Mean yields grain (t ha⁻¹) for different wheat cultivars sown in *Gaeumannomyces graminis* var. *tritici* (*Ggt*)-inoculated soil and natural soil at one new site in each of three growing seasons in Canterbury, New Zealand

Cultivar	2012		2013		2014	
	<i>Ggt</i> -inoculated	Natural	<i>Ggt</i> -inoculated	Natural	<i>Ggt</i> -inoculated	Natural
'Conquest'	5.39	6.93	-	-	8.43	8.98
'Consort'	8.61	9.30	6.25	6.71	-	-
'Einstein'	8.23	10.28	7.82	9.50	-	-
'Empress'	8.61	10.22	7.63	8.87	9.18	9.34
'Oakley'	5.03	6.35	8.63	9.48	8.66	9.45
'Phoenix'	6.92	8.76	8.29	9.84	8.90	9.32
'Raffles'	-	-	-	-	8.82	9.46
'Sage'	8.55	10.13	7.72	8.73	7.76	8.45
'Savannah'	8.38	9.82	-	-	-	-
'Starfire'	-	-	9.96	10.91	9.37	9.78
'Torch'	-	-	-	-	8.28	8.96
'Wakanui'	9.01	10.19	7.88	9.73	8.42	8.96
Df	51		45		51	
LSD(<i>P</i> = 0.05)	1.04		0.59		0.40	
CI*	0.73		0.41		0.21	

*95 % confidence interval (CI) is given by a mean +/- this value

(absolute size of *r*) less than 0.6 are classed here as weak, between 0.6 and 0.8 as moderate, and above 0.8 as strong.

For the trial sown in 2012, grain yield was not strongly related to any of the assessed measurements. For the trial sown in 2013, yield was negatively correlated with estimates of patchiness (*r* = -0.67), but not for the trial in 2014 where all correlations between yield and the other variables were weak.

TAI was moderately related to root *Ggt* DNA concentration in the trial sown in 2012 (*r* = 0.61), when TAI was estimated from roots of all severity categories. TAI was weakly related

to numbers of CFUs of *P. fluorescens* on roots of wheat sown in 2012 (*r* = 0.41) and in the two subsequent trials (*r* ≤ 0.13). Patchiness in the trial sown in 2014 was weakly related to TAI (*r* = 0.54), but patchiness scores at neither of the two assessments were related to any other variable.

Correlations between yield and TAI in roots or soilborne *Ggt* concentrations varied for each cultivar in the years they were sown during the 3 years of the trials (Table 5). Yields of 'Consort' (*r* = -0.835) and 'Savannah' (*r* = -0.654) were most influenced by take-all. Yields of 'Conquest' (*r* = -0.767),

Table 4 Mean grain yields (t ha⁻¹)* for different wheat cultivars sown in *Gaeumannomyces graminis* var. *tritici* (*Ggt*)-inoculated soil and natural soil over the years (N) in Canterbury, New Zealand. The cultivars are ranked in order of greatest yielding in *Ggt*-inoculated plots. They are compared with yields (min-max; number of trials) from first-year wheat in comparable crop performance trials

Cultivar	N years	2nd year wheat (present study)		1st year wheat (auxiliary study) ^a	
		Yield Inoculated	Yield Natural	% increase	Yield Natural
'Starfire'	2	9.36 (8.36,10.36)	9.85 (8.85,10.85)	5.2	11.50 (6.6-16.4; 13)
'Empress'	3	8.83 (7.84,9.83)	9.18 (8.19,10.18)	4.0	10.88 (6.6-15.6; 13)
'Raffles'	1	8.70 (7.70,9.71)	9.35 (8.34,10.35)	7.4	9.96 (7.8-11.9; 7)
'Phoenix'	3	8.67 (7.67,9.66)	9.29 (8.30,10.29)	7.2	10.42 (6-15.7; 20)
'Savannah'	1	8.54 (6.40,10.68)	9.98 (7.85,12.12)	16.9	-
'Oakley'	3	8.48 (7.49,9.47)	9.29 (8.30,10.29)	9.6	-
'Wakanui'	3	8.24 (7.25,9.24)	9.00 (8.01,10.00)	9.2	11.62 (7-16.5; 13)
'Conquest'	2	8.24 (7.24,9.25)	8.82 (7.81,9.82)	7.0	9.11 (6.7-11.3; 7)
'Torch'	1	8.16 (7.15,9.17)	8.84 (7.84,9.85)	8.4	11.62 (6.6-16.1; 13)
'Einstein'	2	7.85 (6.70,9.01)	9.57 (8.42,10.73)	21.9	10.38 (8.1-12.7; 6)
'Sage'	3	7.67 (6.68,8.67)	8.43 (7.43,9.42)	9.8	9.86 (7.7-11.1; 7)
'Consort'	2	6.51 (5.35,7.66)	6.99 (5.84,8.15)	7.4	-

* 95 % confidence limits

^a Mean yields from cultivars sown as first-year wheat in six-20 trials in mid- and central-Canterbury during autumn in 2013 (Craigie 2014) and 2014 (Craigie 2015)

Table 5 Correlations (r) between wheat yields, take-all indices (TAI) in roots, and soilborne \log_{10} (Ggt) concentrations shortly after harvest for different wheat cultivars over the three growing seasons of the trials

Cultivar	Yield vs TAI	Yield vs soilborne Ggt^a	N years	N values
'Conquest'	-0.275	-0.767	2	16
'Consort'	-0.835	-0.202	2	16
'Einstein'	-0.578	-0.529	2	16
'Empress'	-0.509	-0.483	3	24
'Oakley'	-0.104	0.023	3	24
'Phoenix'	-0.198	-0.318	3	24
'Raffles'	-0.557	*	1	8
'Sage'	-0.358	-0.745	3	24
'Savannah'	-0.654	-0.769	1	8
'Starfire'	0.137	0.415	2	16
'Torch'	-0.454	*	1	8
'Wakanui'	-0.521	-0.403	3	24
Overall	-0.278	-0.231		

^a Relationship between yield and soilborne Ggt for crops sown in 2012 and 2013 only, as soilborne Ggt was only sampled after harvest of wheat from those growing seasons

'Sage' ($r = -0.745$) and 'Savannah' ($r = -0.769$) were most influenced post-harvest Ggt concentrations in soil. The weaker relationships for the other cultivars inferred influences by other factors.

Discussion

Selection of wheat cultivars to mitigate take-all

Of the 12 cultivars tested within the 3 years of the trials on soils with second-year wheat, 'Starfire' consistently produced the greatest yields in Ggt -inoculated plots and the second greatest highest yield in the natural plots. 'Starfire' was also one of the greatest yielding cultivars as a first-year wheat with likely nil to low soil inoculum, indicating its ability to yield well under nil and high inoculum pressures. 'Empress' produced the second greatest yield in Ggt -inoculated plots. With less intense pressure from Ggt in natural soils, 'Savannah' and 'Einstein' also yielded well. 'Wakanui' and 'Torch' were also ranked as great yielding first-year wheats, but were 5th and 6th ranked of the cultivars as second-year wheats in inoculated soil, indicating that these cultivars tolerated Ggt less than 'Starfire'. Nevertheless, all of the cultivars were susceptible to take-all. Adding Ggt inoculum to soil at sowing influenced plant growth by increasing crop patchiness and the onset of senescence. This was reflected in decreased grain yields for all cultivars by 0.78 t ha^{-1} on average for all three trials. The decrease was similar for all cultivars, although tending to be greater for 'Einstein' and 'Savannah'. The weak correlation among yields of most cultivars (except 'Consort' and

'Savannah') and TAI suggested they produced these yields in spite of take-all or the amount of Ggt inoculum in the soil, rather than any inherent resistance to Ggt .

There was evidence that the cultivars influenced the build-up of soil Ggt inoculum, which supports the work by McMillan et al. (2011). There was a tendency for 'Phoenix', 'Savannah' and 'Wakanui' to have relatively high post-harvest soil Ggt concentrations, which would result in a greater residual inoculum than the other cultivars. Using these three cultivars in first wheat crops is therefore likely to lead to greater risk of severe take-all in following second wheat crops. In contrast, 'Starfire', 'Oakley' and 'Sage' tended to give relatively low post-harvest soil Ggt concentrations and were thus less likely to cause build-up of soil Ggt inoculum than the other cultivars. Used in first wheat crops, these cultivars could therefore lead to a lower risk of take-all in following second wheat crops than the other cultivars.

Take-all tolerance

Given that the cultivars evaluated did not show evidence of differences in take-all severity, the relative tolerance among cultivars was indicated by comparing yields from plots with or without Ggt inoculum. Cultivars with the least difference are likely to be the most tolerant to the disease. These were 'Starfire' and 'Empress' (Table 4). The large differences in yields among the cultivars both in Ggt -inoculated and natural soils inferred that the greatest yielding cultivars had better tolerance to the disease pressure. Within any year, grain yields and quality for each cultivar could not be explained by the severity of take-all, Ggt DNA concentration in roots or numbers of *P. fluorescens* on the root rhizospheres. Yet for all cultivars, grain yields and quality were influenced by Ggt inoculation; yield reductions of up to 1.47 t ha^{-1} , % screenings doubled, TGW $0.5\text{--}3 \text{ g}$ lighter, and test weights $0.7\text{--}1.8 \text{ kg hL}^{-1}$ less, from second-year wheat grown in Ggt -inoculated soil than in natural soil.

The weak relationships between grain yields and quality with TAI provides further evidence that the yields were associated with most cultivars having a tolerance to take-all rather than any intrinsic resistance to the disease. In the presence of Ggt inoculum in all the 3 years of the trials, these more tolerant cultivars had weaker relationships between yield and take-all. Whereas 'Consort' and 'Savannah' showed moderate negative correlations between yield and TAI in roots and soilborne Ggt concentrations (Table 5), these are interpreted as having less tolerance to take-all. This implies that plots for those cultivars with the lower yields had greater postharvest Ggt than plots where the other cultivars had grown, and the yields were influenced by the soilborne Ggt towards harvest.

In all three growing seasons, the addition of Ggt inoculum decreased yield on average in all cultivars, but only resulted in an increase of TAI in wheat roots in two of those seasons. In

those 2 years, the addition of *Ggt* to soil reduced yields via increased disease. However, for the trial sown in 2013, there was no difference in root TAI or in postharvest soilborne *Ggt* between inoculated and natural plots, but greatest reductions in yields occurred with the addition of *Ggt*. Therefore, if take-all severity was not increased by *Ggt*-inoculation, the inoculum must have reduced yields by some other means. Soil temperature and rainfall interacting with the inoculum may have contributed to the expression of take-all and its effects on yield. Poole et al. (2015) showed that in Western Australia, risk from *Ggt* was influenced by autumn (April, May and June) and summer (January, February and March) rainfall, summer (February and March) soil temperatures and other soil-borne pathogens.

Pseudomonas fluorescens

P. fluorescens bacteria are known antagonists of *Ggt* (Kwak and Weller 2013) and as a consequence their presence in the rhizospheres of wheat plants may partially explain a cultivar's ability to produce high yields in *Ggt*-infested soil. However, this appeared to be not the case in the present study. For the trial sown in 2012, the numbers of *P. fluorescens* on roots from all cultivars were 4.3 times greater in *Ggt*-inoculated plots than in natural plots, when the differences in the soilborne *Ggt*-DNA concentrations at harvest and root take-all between the two inoculation treatments were large. In 2013, there was little difference in the population sizes of *P. fluorescens* between the inoculum treatments, when there was also little difference between them in soilborne *Ggt*-DNA concentrations and root take-all. However, in 2014, take-all severity was again much greater in inoculated than natural treatments, but the *P. fluorescens* populations were least in the inoculated plots. This trend follows that for yield and inoculation over the three growing seasons. The bacteria possibly favoured the rhizospheres in the presence of *Ggt* (van Toor et al. 2015) in only the first of the 3 years. There were also no consistent trends in the cultivars between years, with the bacteria showing no affinity to any particular cultivar.

The poor relationship between populations of fluorescent *Pseudomonas* and concentrations of *Ggt* in the roots in the trials suggests that these bacteria were not sufficiently attracted or antagonistic to *Ggt* in sufficient quantities to influence the population of *Ggt* or expression of take-all symptoms. However, *P. fluorescens* bacteria accumulating in roots containing *Ggt* may induce take-all decline, thereby affecting take-all in following years. This phenomenon is known to develop after 3–7 years of growing wheat in the same field, depending on local environmental and soil conditions (Hornby 1983; Cook 1994; Weller et al. 2002). Like-wise, the build-up of *P. fluorescens* populations or take-all decline might have

been at an early developmental stage as the plants were all in their second year of cropping.

Variation in root colonisation by different strains of *P. fluorescens* on specific cultivars reported by Okubara et al. (2004) suggested that host genes could be involved in wheat-rhizosphere interactions. A biocontrol *P. fluorescens* strain has been shown to modulate defence/stress gene expression in wheat roots (Okubara et al. 2010). The build-up of soil populations of *P. fluorescens* that produce antibiotics capable of suppression of *Ggt* has also been reported to be cultivar-specific (Mazzola et al. 2004; Okubara and Bonsall 2008). These reports emphasise the complexity in the proliferation of beneficial soil *P. fluorescens* for different wheat cultivars. Furthermore, only *P. fluorescens* strains producing the antibiotic 2,4-diacetylphoroglucinol (DAPG) at populations greater than 10^5 CFU/g root are reported to be inhibit *Ggt* growth (Kwak and Weller 2013). Although in some instances in the present study *P. fluorescens* populations in the rhizospheres of some cultivars ('Consort' and 'Wakanui') were high, there may not have been enough cropping seasons to allow for the selection and proliferation of DAPG-producing *P. fluorescens* associated with take-all decline. DAPG-producing Pseudomonads may not common in Canterbury soils. Of the 43 isolates of *P. fluorescens* extracted from roots of wheat grown as first, second or continuous year wheat, none contained the *phlD* gene responsible for production of DAPG (Warren et al. 2016).

Future studies should include defining the antagonistic properties of the rhizosphere *P. fluorescens* from different cultivars against *Ggt* and benchmarking/developing a threshold level of *Pseudomonas* population against root *Ggt* DNA concentrations, where take-all severity has become constant.

Take-all management strategies

Based on the results from the cultivars presented here, a plausible take-all management strategy would be to sow 'Starfire', 'Oakley' or 'Sage' as first wheat crops where it is likely a particular field will be used for a following second wheat crop. The build-up of *Ggt* inoculum in the soil is likely to be less with these cultivars than for the others. The cultivars 'Empress', 'Starfire' or 'Raffles' should be used for second wheat crops where inoculum is likely to be high, as these cultivars will produce greater yields in soils with high amounts inoculum than do the poorer yielding cultivars such as 'Consort', 'Einstein' or 'Sage' when sown in soils containing low amounts of inoculum. Soils considered to have large amounts of inoculum are those that contain more than 80 pg *Ggt* DNA g^{-1} soil before sowing (Bithell et al. 2012).

Based on mean yields from cultivars common in trials in first-year crops, where soilborne *Ggt* inoculum is likely to be

negligible, a yield loss of 1.44 t ha⁻¹ associated with sowing second-year wheat can be expected (Table 4), irrespective of the cultivar selected. Furthermore, a yield loss of approx. 0.78 t ha⁻¹ can be expected from wheat crops sown in soils with high inoculum, where the TAI is 44 %, compared with that of soils producing a low TAI of 20 %. These losses should be factored into crop budgeting.

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Compliance with ethical standards

Conflict of interests The authors declare that there is no conflict of interest regarding the publication of this article.

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