Wheat volunteers in *Lolium perenne* – effects on *Gaeumannomyces graminis* var. *tritici* carry-over and take-all

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Abstract Crop rotations can be used to manage the build-up of inoculum of *Gaeumannomyces graminis* var. *tritici* (Ggt), an important pathogen that causes take-all in wheat crops. This study investigated whether the value of a perennial ryegrass (*Lolium perenne*) break crop is impaired by the presence of wheat volunteers. Soil Ggt concentrations after perennial ryegrass without wheat volunteers were approximately 20% of those with wheat, but perennial ryegrass was less effective than fallow at reducing soil Ggt. Wheat volunteers reduced the value of a perennial ryegrass break when they were present at densities of 40 volunteers/m²; below this density soil Ggt concentrations and take-all incidence levels were similar to the perennial ryegrass only treatment. After the volunteer season, under low volunteer densities (1-15 plants m²) Ggt concentrations tended to be below pre-sowing levels. In the 40 volunteer/m² and full wheat treatment, Ggt was greater than pre-sowing levels. In the fallow treatment, Ggt concentrations declined to below the detection level (< 5 pg DNA) within 1 year.

INTRODUCTION

Crop rotations can be used to manage the build-up of inoculum of *Gaeumannomyces graminis* var. *tritici* (Ggt), an important pathogen that causes take-all in wheat crops, by following the susceptible crop with a resistant (break) crop (Yarham 1981). However, the effectiveness of the break crop may be affected by wheat volunteers from the previous crop (Yarham & Gladders 1993; Jenkyn et al. 1998).

Crop rotations in cereal growing areas in New Zealand are often wheat-intensive because of that crop's high economic returns. Grass seed crops such as perennial ryegrass (*Lolium perenne*) are important in arable cropping rotations in New Zealand (McKenzie et al. 1999). In a New Zealand survey (Cromey et al.

2006), it was found that only a small proportion of wheat crops following perennial ryegrass breaks had significant levels of take-all. Wheat volunteers can be problematic when a ryegrass break crop follows wheat because they provide a host on which Ggt can survive, contributing to the risk of take-all in a subsequent wheat crop (Brassett & Gilligan 1990; Jenkyn et al. 1998; Bateman et al. 2005).

The present study used a field trial to determine whether the value of ryegrass as a break crop to reduce the risk of take-all in a subsequent wheat crop was reduced by the presence of wheat volunteers that may maintain Ggt concentrations in the soil. Further, it examined the influence of volunteer numbers on

the effectiveness of perennial ryegrass as a break crop.

METHODS

The trial was carried out at the Plant & Food Research farm in Lincoln, Canterbury, from 2005–07. The site had previously been in a wheat crop, which was harvested on 24 February 2005. The area was sprayed with glyphosate (360 g ai/litre, 1 litre/ha) then cultivated after harvest.

Trial layout and management

The trial comprised five replicates of eight treatments laid out in an incomplete Latin Square (five rows by eight columns). There was a complete replicate in each row of plots, and each column contained all treatments except three, with a different set of treatments omitted from each column. Plots were 3×6 m, with 0.3 m between rows and 6 m between columns. The trial was bordered by a 1.75 m buffer. Within each plot a 0.5 m wide buffer area around the outside edge of each plot was not sampled.

The eight treatments were: perennial ryegrass (cv. Grasslands Nui) in six treatments, with wheat (cv. Claire) sown at target rates of 0, 1, 5, 10, 15 and 40 volunteers/m²; a positive control (full wheat at 160 seeds m²) and a negative control (chemical fallow). Fallow plots were sprayed with glyphosate at 2- to 3-month intervals.

In the 2005–06 season the wheat control was sown at a rate of 62 kg/ha and the perennial ryegrass was sown at a rate of 15 kg/ha on 1 April 2005. The grass plots were cut in January 2006 (height of ~150 mm) and the residues baled and removed. Grasses were mown again in February and March 2006. For the 2006–07 season all plots were sprayed with glyphosate (360 g ai/litre) at 1 litre/ha and tribenuron (750 g ai/kg) at 40 g/ha on 27 March 2006. Wheat (cv. Claire) was sown in all plots at a rate of 76 kg/ha (equivalent to 146 seeds/m²) with a direct drill on 1 May 2006.

Trial assessments

Counts of wheat plants were made on 3 August 2005 and 5 October 2006 using three 0.13 m² quadrats per plot; perennial ryegrass cover was estimated on 25 August 2005.

To sample soil for *Ggt*, twenty-five soil cores (14 mm diameter, 100 mm length) were collected from each plot. Samples were collected on: 1 April 2005 prior to the sowing of grass treatments; 24 April 2006 (after a year of grass treatments); and on 15 March 2007 following the final wheat crop. Samples were dried and sent to the Root Disease Testing Service (SARDI, Adelaide, Australia) for quantitative assessment of soil *Ggt* concentrations (Ophel-Keller et al. 2008). Results were supplied as pg *Ggt* DNA/g soil.

Assessments of take-all and *Ggt* isolations in grasses were undertaken from three turf blocks (115×115 mm, 100 mm deep) sampled from the perennial ryegrass-only plots using a spade. The soil was washed from roots of plants and the root systems were inspected for take-all-like lesions and assessed as percentage area of lesions on the root system. Root segments were inspected under a binocular microscope for the presence of black dark runner hyphae and take-all lesions. Fungi were isolated from roots (with runner hyphae or black lesions where possible) using a selective medium. Pathogenicity of *Ggt*-like isolates to wheat was assessed using the seedling bioassay described by Chng et al. (2005).

Take-all in wheat was assessed in the initial wheat crop on 3 December 2005 (early grain fill growth stage) by digging plants from a 200 mm row length in each of three areas of the plot. Plots were also assessed visually for the percentage area in take-all patches. The numbers of plants per sample were counted, and three measures of take-all were made using washed root systems assessed under water against a white background. Each plant was assessed for the presence or absence of take-all lesions (incidence), the percentage area of take-all lesions (stellar lesions and the presence of black runner hyphae) covering the root system of each plant (lesion area) and the severity of take-all using a take-all index (TAI). The TAI was calculated from the numbers of plants in five categories (0 no take all; 1: > 0 and ≤ 10%; 2: > 10 and ≤ 30%; 3: > 30 and ≤ 60%; 4: > 60% root system affected):

$$TAI = \frac{(10 \times N_1 + 30 \times N_2 + 60 \times N_3 + 100 \times N_4)}{(N_0 + N_1 + N_2 + N_3 + N_4)}$$

where N_i is the number of plants in category i (Hornby & Beale 2000). The incidence was the number of plants with take-all lesions as a percentage of the number of plants in the sample. In the second year of the trial, take-all was assessed in all plots, again during early grain fill (15-18 December 2006). Three quadrats (0.13 m² each, covering three 360 mm row lengths) were sampled in each plot. Take-all was assessed on wheat plants as in the previous year. Yields were assessed in all plots in the second year of the trial using three quadrats (0.13 m²) from each plot (each quadrat sampling three rows, each 360 mm in length). The grain was weighed, the number of seeds in each sample counted and the thousand seed weight calculated.

Data analyses

Data were explored using simple graphical techniques and correlations. Where multiple measurements had been taken per plot, correlations were calculated using mean plot values. Prior to the main analyses, mixed model analyses of each variable were carried out using REML (restricted maximum likelihood (Patterson & Thompson 1971)) to explore whether there were any spatial trends in measurements not associated with the complete replicate blocks. For all the data presented here, such trends were found not to be substantial. Thus, all data could be appropriately analysed with analysis of variance, and details of the spatial analyses are not presented here.

For formal analyses, all data were analysed with analysis of variance, using an appropriate transformation prior to the analysis, as below, to stabilise the variances. Where more than one measurement had been taken from a plot, data were summarised to plot means before analysis (and transformation). Lesion area, TAI and take-all incidence were transformed using a logit transformation or, if 0 or 100% were present, using a modified logit transformation:

$$\text{logit}(TA) = \log\left(\frac{TA}{100 - TA}\right)$$

$$\text{mod.logit}(TA) = \log\left(\frac{TA + 0.5}{100.5 - TA}\right)$$

Ggt values were log transformed ($\ln(Ggt)$); values of zero were substituted with 0.5 before *Ggt* was transformed.

A subset of variables was used in two multivariate analyses: Principal Components analysis (PCA) and Canonical Variates Analysis (CVA) (Legendre & Legendre 1998). Where a variable was transformed for the ANOVA analyses, it was also transformed prior to the multivariate analyses. PCA is a tool for exploring relationships between variables, and takes no account of the treatments. CVA, in contrast, identifies which variables most separate the treatments. For both of these analyses, the results are presented in biplots – two-dimensional graphical representations that include data (and treatment means) plotted as points, and the variables (measurements) as vectors. The variable vectors are plotted such that their relative lengths show the importance of each variable within the plot, with the longest vectors being most important. Variables that follow similar angles within the plots are correlated. Vectors that are close to perpendicular are for variates that are essentially uncorrelated.

All analyses were carried out with GenStat (GenStat Committee 2009).

RESULTS

Year one: 2005-2006 season

The density of wheat volunteer plants established at a range close to the experimental design (Table 1), with the exception that the 25 and 50 volunteers/m² plots had lower densities – 16 and 40/m², respectively. The ryegrass established evenly across plots.

At 11 months after sowing 52% of perennial ryegrass roots sampled had runner hyphae present. A small proportion (<1%) of isolates from root samples with runner hyphae were morphologically similar to *Ggt*, but none was pathogenic to wheat seedlings in a subsequent bioassay.

All wheat plants assessed in the 2005-2006 season were infected, with an average TAI of 97. Take-all patches covered most of the area of the plots and severe stunting occurred in the most heavily infected areas.

Table 1 Results for Ggt concentrations (pg Ggt DNA/g soil), take-all incidence (%), take-all lesion area (area %) and take-all indices (TAI) and wheat densities (plants/m²), yield (t/ha) and thousand seed weight (TSW; g) of wheat for the eight break treatments. Values are back-transformed means with transformed means in parentheses.

Ggt April 2005	Break treatment 2005	Volunteer wheat density Aug 2005	Ggt April 2006	Wheat density Nov 2006	Take-all incidence Dec 2006	Lesion area Dec 2006	TAI Dec 2006	Yield Feb 2007	TSW Feb 2007	Ggt Mar 2007
184.0 (5.22)	0	-	30.6 (3.42)	77.8	75.4 (1.11)	17.9 (-1.53)	25.6 (-1.07)	4.7	42.2	49.8 (3.91)
73.0 (4.29)	1	1.4	43.0 (3.76)	70.0	82.8 (1.55)	21.7 (-1.29)	28.5 (-0.92)	5.1	41.4	65.0 (4.17)
64.1 (4.16)	5	5.6	17.5 (2.86)	71.2	72.0 (0.93)	15.6 (-1.69)	22.3 (-1.25)	5.2	42.7	56.2 (4.03)
43.5 (3.77)	10	10.5	32.9 (3.49)	75.3	85.7 (1.76)	17.7 (-1.53)	25.3 (-1.09)	5.0	42.6	56.2 (4.03)
75.7 (4.33)	25	16.0	38.5 (3.65)	69.5	92.6 (2.46)	26.4 (-1.03)	34.5 (-0.64)	4.5	42.9	77.4 (4.35)
31.0 (3.43)	50	40.4	64.4 (4.17)	78.6	90.6 (2.22)	38.5 (-0.47)	49.8 (-0.01)	5.0	42.9	69.4 (4.24)
67.3 (4.21)	full wheat	-	152.7 (5.03)	67.9	98.4 (3.87)	37.3 (-0.52)	49.3 (-0.03)	5.5	40.7	69.1 (4.23)
52.2 (3.96)	fallow	-	3.8 (1.33)	86.8	30.6 (-0.81)	0.8 (-4.83)	3.1 (-3.44)	6.7	45.4	59.2 (4.08)
LSD (0.59)		2.2	(0.78)	9.0	(0.97)	(0.81)	(0.71)	0.7	1.6	(0.63)
SED (1.20)		4.7	(1.59)	18.4	(2.00)	(1.65)	(1.46)	1.5	3.3	(1.29)
d.f.		16	28	28	28	28	28	28	28	28

Year two: 2006–2007 season

In November 2006 sown wheat populations did not vary significantly between treatments ($P=0.462$), but populations did range from the lowest for the wheat treatment ($68/\text{m}^2$) to the highest for the fallow treatment ($87/\text{m}^2$).

Take-all indices (TAI) were highest in the full wheat and 40 volunteer/ m^2 treatments, being higher than those in the full ryegrass and fallow treatments. The TAI in the fallow was approximately one-tenth of that of the full wheat and 50 volunteer/ m^2 treatments, and one-fifth to one-sixth of that of the ryegrass and volunteer treatments. The take-all incidence generally reflected the ranking of TAI values, with the exception of the fallow treatment, where 34% of plants were infected but severity was low (TAI of 4).

There were significant differences between the treatments ($P<0.001$, $P=0.002$, $P=0.005$) for TAI, lesion area and incidence, respectively. All three take-all parameters were substantially lower in the fallow treatments than for all other treatments. Lesion area and TAI tended to increase with increasing numbers of wheat volunteers, but this trend was not significant ($P=0.95$, $P=0.58$) for lesion area and TAI respectively. Incidence was higher for wheat than for all other treatments, although not significantly so for the two highest volunteer rates (16 and 40).

Grain yields did not vary significantly between the treatments ($P=0.177$). However, yield was noticeably higher for the fallow treatment than for the other treatments (6.7 t/ha cf. other yields below 6 t/ha). Thousand seed weights (TSW) did not vary significantly between the treatments ($P=0.246$). However, TSW was highest for fallow (45 g) and lowest for the full wheat treatment (41 g).

Prior to sowing in 2005, *Ggt* concentrations did not vary significantly between the treatment plots ($P=0.190$). At the second assessment, *Ggt* concentrations varied between treatments, with the highest values found for the full wheat (152 pg *Ggt* DNA/g soil). This was significantly greater ($P<0.05$) than for either the fallow or the five volunteer/ m^2 treatments, and greater (but not significantly, $P>0.05$) for other volunteer treatments. These ranged from 31 to 64 pg *Ggt* DNA/g soil. Thus, *Ggt* concentrations for all non-

full wheat volunteer treatments were less than half those found for full wheat. *Ggt* concentrations tended to increase with the numbers of wheat volunteers. Ten months after sowing wheat, *Ggt* concentrations had increased in all treatments except the year one full wheat treatment. Concentrations in these plots were less than one-third of the concentration of the prior year. The largest increase was in the fallow plots.

Principal components and canonical variates analyses

These analyses were carried out using all three *Ggt* measurements, TAI, plant density in 2006, yield and TSW (seven variables in total). Where variables were transformed for the analyses of variance, they were first transformed in the same way before being used for these multivariate analyses.

Figure 1 shows the biplot for a principal components analysis (PCA). This plot displays the first two components, which accounted for only 57% of the variation in the data, indicating that the components did not account for the majority of the variance. The next two components accounted for a further 16 and 12%, and the fifth, a further 6.6%. Five components would be needed to describe over 90% of the variation in the data, and thus, PCA did not provide a useful reduction from the seven original variables. However, the analysis did demonstrate a correlation between yield and plant density and a high correlation between *Ggt2* and TAI. The first component is primarily associated with yield, density and *Ggt1*, and the second with *Ggt2* and TAI. Amongst the plot data, points for fallow are somewhat separated from the rest of the data. However, there is no clear separation between the other treatments.

Figure 2 shows a biplot of the results from the canonical variates analysis. The first two components accounted for over 85% of the variation between the crop means, with the third component accounting for a further 9%. Thus, three components accounted for most of the differences between the crops. The majority of the variation between the means is associated with the first component, which separates the crops into two groups: fallow on the left with

Figure 1 Principal components biplot, with (top) data for individual plots (hollow symbols), means for each crop (solid symbols) and convex hulls showing the grouping of plots for the same crop. The variables are shown at the bottom, with standard length, indicating the importance of each variable within the plot. *Ggt1*, *Ggt2* and *Ggt3* are *Ggt* for April 2005, April 2006 and March 2007 respectively. Density2 is wheat plants/m² in November 2006.

low TAI and *Ggt2*, and ryegrass/wheat on the right with higher TAI and *Ggt2*. The second axis accounted for much less of the variation between the means, and was most strongly associated with *Ggt1*, yield and TSW. That axis primarily separated out wheat slightly further from the treatments, but also separated other treatments to a lesser extent, with an approximate trend from low numbers of sown volunteers at the bottom to higher numbers moving up the graph.

DISCUSSION

In this trial perennial ryegrass was considerably less effective than the fallow treatment for reducing soil *Ggt* concentrations and take-all

Figure 2 Canonical variates analysis biplot, with (top) data for individual plots (hollow symbols), means for each crop (solid symbols) and convex hulls showing the grouping of plots for the same crop. The variables are shown at the bottom, with $2 \times$ standard length, indicating the importance of each variable within the plot. *Ggt1*, *Ggt2* and *Ggt3* are *Ggt* for April 2005, April 2006 and March 2007 respectively. Density2 is wheat plants/m² in November 2006.

in a following wheat crop even when free of wheat volunteers. While post-ryegrass soil *Ggt* concentrations were about 20% of those where wheat had been sown, they were eight times those following fallow. Perennial ryegrass has been shown to be a host of *Ggt* in laboratory studies (Chambers 1971; Schultz 1981; Chng et al. 2005) but field studies report that it is not an effective host (Schultz 1981). Results of the present isolations from roots suggest that ryegrass is a poor host of *Ggt*, but soil tests indicate that it is capable of maintaining inoculum, albeit at a lower level than wheat.

Take-all incidence and severity in the following wheat also indicated that perennial ryegrass

reduced the risk of take-all in comparison with no break, but it was not as effective as a fallow. Gutteridge et al. (2006) also found that the TAI of wheat after ryegrass was greater than after fallow, and also that the ryegrass-only had a lower TAI than a high-density mixture of wheat (100 seeds/m²) and ryegrass.

The value of ryegrass as a break crop was reduced by the presence of wheat volunteers. Post-break *Ggt* concentrations at 5 volunteers/m² were similar to those of the perennial ryegrass-only treatment, which were both lower than the full wheat treatment. The largest increase in *Ggt* concentrations occurred between the 16 and 40 volunteer/m² treatments.

Despite the wide range of volunteer densities and TAI values differing by a factor of two between extreme treatments, volunteer density provided no significant effect on take-all severity when wheat was sown following the break. There were, however, notable differences in the components of the disease epidemic between treatments. The incidence of take-all in the post-break wheat from treatments of 1-10 volunteers/m² was lower than that of the full wheat, which approached maximal values (98%). The canonical variate analysis also identified take-all incidence and severity as variables effective for separating treatments. Other trials, using much higher densities, have shown a clearer effect of volunteers (Jenkyn et al. 1998; Gutteridge & Hornby 2003; Ennaifar et al. 2005) than the present study. The distribution of volunteer wheat populations in New Zealand ryegrass crops that follow wheat is not regular, usually being concentrated particularly in lines where straw lies on the soil surface following harvest (S.L. Bithell, Plant & Food Research, unpublished data). Results of the current trial suggest that the presence of wheat volunteers in ryegrass crops may be responsible for the relatively high take-all severity seen in some New Zealand wheat crops that have followed a ryegrass break (Cromey et al. 2006). In particular, take-all patches in first wheat crops following a ryegrass break may be associated with concentrated areas of wheat volunteers in break crops (Dulout et al. 1997).

In spite of substantial differences in take-all severity between treatments in 2006, there were

few differences in grain yields. The large yield difference between the fallow treatment and the other treatments may be due in part to the low take-all severity, but may also be due to improved nutrient availability following fallow.

Particular phases of take-all epidemics of consecutive wheats following a break are associated with changes in the population of *Ggt* (Bailey et al. 2009). This study showed substantial changes in *Ggt* concentrations with differing densities of susceptible host (wheat) or absence of a host in a break year. There were clear patterns of response between the break treatments ranging from a substantial decline to the build-up of soil *Ggt* concentrations. Under low volunteer densities (1-15 plants/m²) *Ggt* tended to remain below pre-sowing concentrations, whereas in the 40 volunteer/m² and full wheat treatment *Ggt* built up to greater than pre-sowing concentrations. In contrast, soil *Ggt* concentrations in the fallow treatment declined to below detection levels (< 5 pg DNA) within 1 year.

Break crop effects on *Ggt* concentrations are transitory. In the principal components analysis, although *Ggt* concentrations after the break crop were related to take-all concentrations in the following wheat, the post-wheat harvest *Ggt* concentrations were similar for all treatments. Even the fallow treatment resulted in post-wheat *Ggt* concentrations that were no different from the other treatments, indicating that the presence of a host crop can result in a rapid build-up of inoculum, even from a very low base. It has been shown that populations of take-all suppressive microorganisms can be lost following a single year away from wheat (Bailey et al. 2009). It is possible that such a loss in suppressiveness in the fallow treatment enabled the rapid build-up of *Ggt* in wheat following fallow in spite of low take-all severity in the crop.

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