Effect of glyphosate application to grass weeds on levels of *Gaeumannomyces graminis* var. *tritici* inoculum

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Summary
The cereal pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*) that causes the disease take-all infects *Elytrigia repens* (couch) and a number of other grass weed species. Soil *Ggt* DNA levels 14 months after couch had been planted into a naturally *Ggt* infected site, in which *Bromus willdenowii* (prairie grass) was also present, indicated that the presence of couch maintained rather than increased *Ggt* levels. Following an early glyphosate application soil *Ggt* levels increased three and eight fold, one and two months respectively after application (86 days before wheat sowing) to plots with couch present. A later glyphosate application (38 days before wheat sowing) also resulted in rapid increases in *Ggt* inoculum levels in plots with couch and to a lesser extent in plots without couch. Results confirm that couch is an important host of *Ggt* with more roots per wheat plant infected, more plants infected and a greater take-all severity in plots with couch present. Early or late glyphosate applications did not affect subsequent levels of take-all in wheat. Prairie grass seedlings were also identified as a significant *Ggt* inoculum source, but this effect was independent of glyphosate and couch treatments. Overall, increases in *Ggt* inoculum levels following glyphosate application are large and relatively rapid. *Ggt* inoculum associated with couch rhizomes is persistent. Finally, very early glyphosate applications will be required to allow time for the break down of couch rhizomes and decline in *Ggt* inoculum levels thus reducing the risk of take-all in subsequent wheat crops.

Key words: *Gaeumannomyces graminis* var. *tritici*, take-all, inoculum, couch, *Elytrigia repens*, Prairie grass, *Bromus willdenowii*, glyphosate, grass weeds.

Introduction
A key component of the management of two root pathogens, *Gaeumannomyces graminis* (Sacc.) von Arx and Oliver var. *tritici* Walker (*Ggt*) and *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *avenae* (Turner) Dennis (*Gga*), which cause the disease take-all in cereals, is inoculum management. In the absence of a host, *Ggt* inoculum declines to low levels in the soil because *Ggt* does not produce resting spores capable of long-term survival (Shipton 1981). Hosts of *Ggt* are restricted to species in the Graminacae, so crop rotations can be used to restrict the build up of *Ggt* by alternating periods of susceptible and non-susceptible crops. However, if non-crop host species, such as *Ggt*-susceptible grass weeds, are present within resistant crops then the ability to manage *Ggt* levels by crop rotation is reduced (Cotterill and Sivasithamparam 1988, Nilsson and Smith 1981) and the challenge becomes one of managing weed species.

Elytrigia repens (L.) Nevski (couch) is a rhizomatous grass weed whose roots are highly susceptible to *Ggt.* It has long been a host of *Ggt* in wheat fields in New Zealand (Waters 1920). Couch as a host of *Ggt* has also been extensively reported worldwide (Nilsson and Smith 1981). *Bromus willdenowii* Kunth (prairie grass), a non-rhizomatous weed, is also a host of *Ggt* (Chng *et al.* 2005). Prairie grass and other brome grasses are emerging as problem weeds in cropping systems, including because they host *Ggt* (Chancellor and Froud-Williams 1986, Chng *et al.* 2005, Gutteridge *et al.* 2005). Glyphosate is an effective herbicide on couch due to its ability to kill rhizomes and is used to control couch in cereal cropping (Harvey *et al.* 1981, Leroux 1993). Although glyphosate does not directly affect *Ggt* in culture (Grossbard and Harris 1976), it can facilitate infection by decreasing the numbers of micro-flora antagonistic to *Ggt* (Mekwatanakarn and Sivasithamparam 1987).

Knowledge of the effects of herbicides on hosts and associated inoculum levels of soil-borne pathogens is important when developing effective management strategies for these pathogens. It has been proposed that glyphosate reduces the resistance of couch rhizomes to *Ggt*, leading to increased colonization and increased take-all risk in susceptible crops (Harvey and Braithwaite 1986, Yarham *et al.* 1989). One study has reported increased levels of take-all in wheat planted into glyphosate-treated couch compared to mechanically treated couch (Mielke 1983). Studies of *Pythium* and *Fusarium* species have identified that increases in inoculum levels following herbicide application to hosts are often short term, a matter of days or weeks (Descalzo *et al.* 1996, Levesque *et al.* 1987). However, dead couch rhizome material appears to be persistent as on average 70% of the total biomass of couch rhizomes were present as dead material in an established couch sod over four months of sampling (Johnson and Bucholtz 1962).

We hypothesize that applying glyphosate to couch increases *Ggt* inoculum, and that, due to the persistence of couch rhizome material, these increases in *Ggt* inoculum levels would be maintained for 1–3 months. This study investigated the effects of glyphosate application on *Ggt* inoculum in couch by comparing two herbicide application timings, and recording subsequent take-all levels in a spring wheat crop at a field site also infested with prairie grass.

Materials and methods
Description of trial
The trial was carried out at the Plant and Food Research farm in Lincoln, Canterbury, in 2005–07. The site had previously been sown to wheat, which was harvested on 24/2/2005. The area was disced and shallow cultivated (maxi-till), then sprayed with glyphosate (360 g ha^{-1}) on 11/4/2005. Six replicates of five treatments were laid out in three rows by five columns of plots, with the layout comprising two adjacent incomplete 3 × 5 Latin squares. Individual plots were 3 × 3 m with an internal 0.5 m buffer area within each plot, providing a 2 m^2 sample area. A 0.5 m wide lane was left between plots and 1 m wide lane between blocks.

Treatments consisted of two factors, presence (+C) or absence (–C) of couch, and an early (EG) or late (LG) glyphosate application, with the fifth treatment being a duplicate of +C EG that was allocated for destructive sampling during the trial (check plots). For the +C plots, lengths of couch rhizome (five node pieces, ~70–90 mm long) were planted 15–20 mm deep at a density of 12 m^{-2} on 18 and 19/4/2005 (Figure 1). The grasses in plots were periodically mechanically cut to a height of 200 mm to prevent seed development. Glyphosate (a.i. 2.4 g L^{-1}) treatments were applied at the recommended rate for couch using a backpack sprayer (Anon. 2005). The early glyphosate treatment was made on 26/6/2006 and the late glyphosate treatment on 13/8/2006.

Wheat cv. 'Vanquish', treated with triadimenol 150 g L^{-1}, imazalil 50 g L^{-1},

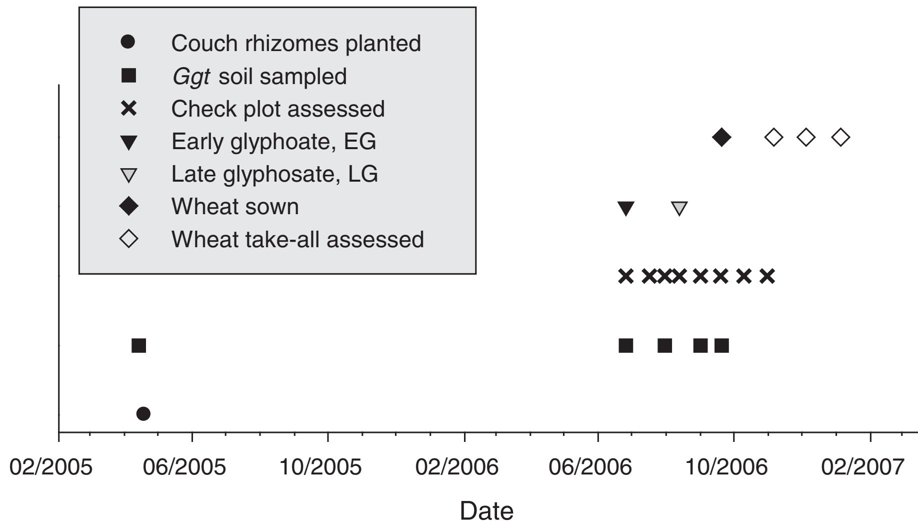

Figure 1. Timeline of field trial establishment, treatments and sampling.

fuberidazole 100 g L^{-1}, imidacloprid 600 g L^{-1} (Baytan Combi) at 90 mL t^{-1}, was sown into plots on 20/9/06 using a Hill plot seeder. Wheat was sown into 16 sub plots per plot, with 10 seeds and 6 g of fertilizer per sub plot (50:50, di-ammonium phosphate and ammonium sulphate, N 19.3, P 10.0, S 12.5). The 16 subplots were spaced approximately 0.65 m apart on the square, and the area sown in each subplot was 0.0225 m^2. The remainder of the plot was mechanically weeded during the trial.

Assessments

Couch, prairie grass and wheat volunteer populations, prior to glyphosate. An estimate of percentage cover by couch was made by eye on 17/5/2006. Counts of couch and large prairie grass plants were made across each plot on 25/5/2006. For several plots, young prairie grass seedlings were observed and a separate estimate of the numbers of these per plot was made by randomly placing four 0.25 × 0.25 m quadrats in the plot, and counting the seedlings within these.

Couch and prairie grass dry matter on check plots. Population estimates of couch and prairie grass were made on eight occasions (26/6/2006, 17/7/2006, 31/7/2006, 13/8/2006, 1/9/2006, 19/9/2006, 10/10/2006 and 31/10/2006) (Figure 1). Quadrats of 0.26 × 0.26 m were randomly placed in each check plot. The plants and the soil below the quadrats (a block measuring 25 × 25 × 10 cm) were removed and taken to the laboratory. Here, the plant material was separated and removed by washing over sieves. The roots and rhizomes of couch and roots of prairie grass were inspected for take-all symptoms – dark stellar lesions in conjunction with black runner hyphae on roots, and dark lesions in conjunction with black runner hyphae on rhizomes. For couch, an estimate was made of the percentage of internodes with take-all lesions and the percentage of rhizome area covered by these lesions. The roots and rhizome material and all above ground plant material of couch and prairie grass were dried for 48 h at 65°C in a forced air oven then weighed.

***Ggt* isolation.** On two occasions (26/6/2006 and 10/10/2006), roots and rhizomes identified as having take-all symptoms were used in *Ggt* isolation assays. Two methods, 'Direct' plating and 'Baiting', were used for both species at both dates, except that couch rhizomes were not measured by Direct plating on 26/6/2006.

The Baiting method was based on Chng *et al*. (2005). Three ~15 mm sections of root or rhizome material per plot were divided into three pieces (~5 mm each) and placed beside the roots of wheat seedlings. After 10 days the roots of the seedlings were inspected using a binocular microscope (7X) for the presence of runner hyphae and black stellar lesions. Four ~5 mm sections per seedling of wheat root with these symptoms from each infected seedling were surface sterilized (using a 1% sodium hypochlorite solution for 1 min. prior to rinsing twice in sterile water) and plated on to semi-selective potato dextrose agar (PDA) containing L-3-4 dihydroxyphenylalanine (0.5 g L^{-1}), streptomycin sulphate (100 mg L^{-1}), metalaxyl (10 mg L^{-1}), dichloran, 10 mg L^{-1} (Botran), vinchlozolin 10 mg L^{-1} (Ronilan) fludioxonil 1 mg L^{-1} (Maxim) and rifampicin (1 mL L^{-1}). *Gaeumannomyces*-like cultures were cultured as necessary on PDA or antibiotic PDA (containing streptomycin sulphate (1.2 g L^{-1}), chlortetracycline (0.5 g L^{-1}) and chloramphenicol (0.5 g L^{-1})). The resulting cultures were then stored in sterile water at 5°C in agar discs.

For the 'Direct' plating method 4 × ~15 mm root or rhizome sections were surface sterilized (as described above) and two (3–4 mm) sections from each of these four pieces were plated on to the semi-selective agar (as described above), giving eight sections per sample. Sections that provided cultures indicative of *Ggt* or *Gga* were subcultured on to PDA or antibiotic PDA as necessary, and then stored in sterile water at 5°C in agar discs.

The resulting isolates from both methods were examined for pathogenicity using the bioassay described by Chng *et al*. (2005). Agar plugs (8 mm diameter) were taken from the growing edge of a 7–8 day-old PDA culture of each isolate. The plug was placed above the root tip of a 5–6 day-old wheat seedling (controls used cultureless PDA agar plugs). Each replicate consisted of five wheat seedlings and each bioassay used three replicates. The wheat seedlings were assessed as described for the baiting method after 10 days. This procedure was then repeated also using oat seedlings for isolates from 26/6/06. Pathogenicity to wheat or oats was confirmed by the presence of runner hyphae and black stellar lesions.

Soil *Ggt*. Soil cores were taken from each plot on five occasions (14/4/2005, 26/6/2006, 31/7/2006, 1/9/2006 and 20/9/2006) (Figure 1). Fifteen soil cores (14 mm diameter, 100 mm depth) per plot were taken from the 2 m^2 sample area. The soil corer was cleaned between each plot. Following collection, the samples were dried in a glasshouse (~ 25°C) for 48 h, then further dried at 40°C for 48 h in a drier. Samples were frozen at −20°C for two weeks after packaging in two layers of sealed plastic bags. The samples were then sent to the Root Disease Testing Service (SARDI, Adelaide) for a combined *Ggt*–*Gga* DNA content analysis (Ophel-Keller *et al*. 2008). Real-time PCR was performed using TaqMan® MGB(tm) probes and Qiagen Quanti Tect Probe Master Mix in 10 µL reactions on an ABI PRISM® 7900HT Sequence Detection System. Thermal cycling conditions were: an initial temperature of 95°C for 15 min. to activate Taq Polymerase, followed by melting step of 95°C for 15 s and annealing/extension step (combined) of 60°C for 1 min. for 40 cycles. Standards were produced using DNA extracted from aseptically grown *Gga* diluted through 10-fold dilution series from 200 to 2 pg μL^{-1}. Results were supplied as pg DNA g^{-1} soil. Samples collected on 14/4/2005 were also tested for the presence of *Gga* with a separate analysis (Ophel-Keller *et al*. 2008).

Infection of wheat and couch roots. Wheat was sampled by removing plants from four of the 16 sub plots in each plot on 6/11/2006, 5/12/2006 and 5/1/2007 (Figure 1). The four samples from each plot were kept separate and the roots washed and assessed under water against a white

background. The number of roots with take-all symptoms (stellar lesions and the presence of black runner hyphae) on each plant was counted. Each plant was also assessed for the total area of lesions covering the root system and categorized as having 0, 1–10, 11–30, 31–60 or 61–100% of root area infected and a take-all index (TAI) calculated (Hornby and Beale 2000).

Methods of analysis
Prairie grass and wheat volunteer populations, prior to glyphosate treatment, were analysed as described in the results.

Couch and prairie grass dry matter data from check plots were analysed with analysis of variance after first transforming with ln(data + 0.1) to stabilize the variance. Checks were made to determine whether any adjustment needed to be made for the repeated measuring of plots: no adjustment was found to be necessary.

Soil *Ggt* DNA data were analysed for each of the four dates separately using analysis of variance, after first log transforming the data to stabilize the variance. Further analyses explored possible relationships between *Ggt* levels and couch or prairie grass levels. The relationships were explored graphically, and also by including numbers of couch plants, and numbers of small and large prairie grass plants at the 25/5/2006 assessment in a mixed model analysis, along with the interaction between these variables and the treatments. Prairie grass numbers were included in the analysis both before and after the Treatment main effect to allow the prairie grass effects to be assessed both before and after adjusting for treatment effects. Couch effects were only assessed after the inclusion of the Treatment main effect since couch was only present due to planting. The effects were assessed using the F-tests produced by the analysis (GenStat Committee 2008, section 5.3.6). Only prairie grass or couch effects that were important were included in the final analysis, and the model was refitted to produce the results presented.

Numbers of infected roots, total plants and infected roots per plant were totalled over the four sub-plots before analysis. Each was then analysed using a Poisson generalized linear model with a logarithmic link (GLM, McCullagh and Nelder 1989). The percentage of infected plants and TAI were calculated on a plot basis, summarizing over the four sub-samples. These were then analysed with a binomial generalized linear model with a logit link (GLM, McCullagh and Nelder 1989) using the numbers of infected plants of the total plants and the calculated TAI score out of 100 respectively.

Comparisons between treatments were made as for an analysis of variance, using contrasts between treatments fitted as part of the analysis of deviance done. The contrasts were assessed with F-tests using the estimated dispersion (McCullagh and Nelder 1989). In the results, the percentages for each treatment are presented along with 95% confidence limits, which were calculated on the transformed (logarithmic or logit) scale and then back-transformed to the original scale.

The above analyses ignore the structure (blocks, plots, samples within plots) of the experiment. The effect of this was explored using a Generalized Linear Mixed Model approach (Engel and Keen 1994, details not shown). The variation associated with the blocks was negligible, but there was more variation between plots than within plots. Thus, the data were summarized on a plot basis over sub-samples before analysis (as above) to allow the more straightforward presentation of the GLM analysis and avoid difficulties in presenting results from the more complex GLMM analysis.

Data for the check plots were included in the analyses, but results are not presented since for most variables there was little evidence of a difference between the check plots and the corresponding +C early glyphosate plots.

Results

Plant populations in all plots prior to herbicide application
Couch and prairie grass populations were assessed immediately prior to the first herbicide application. No couch was found in any of the −C plots. In the +C plots, percentage cover ranged from 3% (in one plot) to 90% with a median of 57.5%, and numbers of shoots ranged from 4–1244 shoots m^{-2}, with a median of 276.

Prairie grass was not evenly distributed across the trial, being more prevalent in one block, and absent in another. Prairie grass was initially present as a few large established plants; less than nine large plants per square metre occurred in any plot. But in the autumn (April 2006) prairie grass seedlings emerged in some areas – in the most heavily infested plots there were up to 104 seedlings m^{-2}. Prairie grass was not associated with the couch treatment (P >0.4 for t-tests).

Grass weed dry matter, take-all symptoms and Ggt isolation from check plots
Couch rhizome and root densities were high in check plots, with an average dry matter of 95 g m^{-2} at the 26/6/2006 pre-glyphosate assessment. This declined to 28 g m^{-2} at 10/10/2006 (back transformed values). The couch aboveground dry matter declined from ~38 g m^{-2} in July to ~ 2 g m^{-2} at 10/10/2006. There was no evidence of a decline in prairie grass root dry matter over the sampling period (P = 0.262); the minimum and maximum dry matter values ranged from 0.6 to 39 g m^{-2}.

Take-all-like lesions were observed on couch roots. Dark stellar lesions occurred in conjunction with black runner hyphae on the surrounding non-lesioned tissue. Lesions on couch rhizomes did not extend to the stele but occurred in conjunction with black runner hyphae.

Lesion incidence on rhizomes was particularly low (<1% of internodes with lesions) at the first two inspections (26/6/2006 and 17/7/2006). Rhizomes changed colour from white to yellow by the 31/7/2006 inspection, when growing points had started to decay. By ~100 d after the early glyphosate application (10/10/2006) internode lesion incidence had increased to 41.7% and finally by 31/10/2006 it was 77.5%. The severity of lesions, as indicated by the total area of lesions, followed a similar trend to that of incidence, with values not exceeding 3% until 10/10/2006 and exceeding 15% by 31/10/2006. Couch roots were inspected for lesions, with lesions consistently observed, but damage to roots during sampling and root breakdown following glyphosate application limited the ability to quantify visual infection levels throughout the sampling period.

Table 1. Numbers of *Ggt* cultures per tissue sample from check plots (+C EG) isolated using two methods, baiting (B) and direct (D), from couch and prairie grass samples at two sampling periods: pre-glyphosate application (26/6/2006); post-glyphosate application (10/10/2006).

	No. of plots sampled and method of isolation	No. of *Ggt* isolates per tissue sample	
		Direct method	Baiting method
Pre-glyphosate			
Couch rhizomes	0D and 5B	–	0.1
Couch roots	5D and 2B	0.4	3.2
Prairie grass roots	2D and 1B	0.5	2.0
Post-glyphosate			
Couch rhizomes	5D and 6B	0.5	3.9
Couch roots	6D and 6B	0.2	1.3
Prairie grass roots	3D and 6B	0.3	2.0

The isolation of *Ggt* from check plot samples with take-all-like lesions was successful prior to glyphosate and following glyphosate application (Table 1). For the pre-glyphosate assessment, isolation from couch and prairie grass roots consistently yielded *Ggt*, while couch rhizomes had a low isolation success (0.1 *Ggt* isolates per tissue sample). Comparisons between isolate species at this date are limited as prairie grass only occurred in two samples. A pathogenicity test of isolates on wheat (8.9–22.7% of the root system infected) and oat seedlings (0% infection) indicated that all isolates were *Ggt* rather than *Gga*. For isolations after glyphosate application (10/10/2006) baited couch rhizomes provided more *Ggt* isolates than couch or prairie grass roots. Results indicated an increase in *Ggt* in couch rhizomes between the two dates.

Soil Ggt measurements from all plots
At the April 2005 assessment, *Ggt* was predominant with 96.6% of the 489.7 *Ggt* DNA (pg g^{-1} soil) per plot attributable to *Ggt* after subtraction of the combined *Ggt*-*Gga* result (507.2 *Ggt*–*Gga* DNA (pg g^{-1} soil) per plot from the separate *Gga* analysis (17.4 *Gga* DNA (pg g^{-1} soil) per plot. Results are discussed in terms of *Ggt*.

As expected, *Ggt* DNA levels did not vary significantly before couch was planted and glyphosate applied (P = 0.89) in April 2005 (Table 2). *Ggt* DNA levels had declined by the 26/6/2006 sample, 14 months after the couch plots had been planted, and did not vary significantly between +C and −C (P = 0.150). By 31/7/2006, after the early glyphosate application, the levels of *Ggt* between treatments were noticeably different (P = 0.005). Both couch (P = 0.009) and glyphosate (P = 0.019) were significant factors at that date with +C and EG having higher *Ggt* DNA levels. At the 1/9/2006 assessment, couch was a significant factor, with the +C plots having the highest values. At the 20/9/2006 assessment, *Ggt* DNA levels for the +C LG and −C LG had increased. By contrast there was no increase in level for the −C EG treatment. This was the primary cause of differences (P = 0.009) between treatments at this date.

Analysis of the effects of prairie grass and couch populations on *Ggt* DNA levels indicated that the numbers of large, established prairie grass plants or couch shoots were not significantly associated with *Ggt* DNA levels at any of the five sampling dates (P >0.1). Prairie grass seedling numbers, however, were significantly associated with *Ggt* DNA levels at all but the pre-sowing April assessment (P = 0.311, 0.004, 0.002, 0.004, 0.012 after adjusting for treatment effects, for the five assessments respectively). The relationships did not vary significantly with the treatment, except for the 1/9/2006 assessment (P = 0.876, 0.498, 0.200, 0.015, 0.294). The analysis was repeated with prairie grass seedling numbers alone. The September interaction was not present when the large established prairie grass and couch shoot numbers were excluded from the analysis. Prairie grass seedlings contributed to *Ggt* DNA levels at the site − log10(DNA) levels increased by about 6×10^{-3} for every prairie grass seedling at later assessments (Table 3). This is equivalent to a 10–20% increase in DNA level per 10 prairie grass seedlings.

Wheat take-all assessments
Numbers of infected wheat roots per plot varied significantly between the treatments at the December and January assessments, but not at the November assessment (P = 0.131, P = 0.045, P = 0.054 for overall treatment differences for November, December and January respectively) (Table 4). In November the number of infected roots per plot was ~45, apart from −C EG which had only 11 roots infected. This trend (P = 0.05) for lower infection in −C EG continued in the following months, although differences were not significant (P >0.1). For the December and January assessments couch was a significant factor (P = 0.007, P = 0.006, respectively) with more infected roots in +C treatments. The number of plants did not vary significantly between treatments (P >0.05), so the numbers of infected roots per plant (Figure 2) followed a similar pattern to numbers of infected roots per plot.

The percentage of infected plants varied for the couch treatment (P <0.002 at all assessments, Figure 3a), with infection lower for −C. Glyphosate also had an effect on take-all incidence in November and January (P = 0.004, P = 0.034), but not in December (P = 0.107), with infection levels generally lower for the EG application. The effect of glyphosate timing varied only slightly between the −C and +C plots in November, and there was no significant couch by glyphosate interaction (P >0.05). Assessments of severity using TAI were not made for the November assessment as take-all infection was limited to small areas of initial infection (data not presented). At the following assessments

Table 2. Mean *Ggt* DNA levels (pg g^{-1} soil) for treatments of + and − couch (C) and early (EG) or late glyphosate (LG) at five assessment dates (values presented are back-transformed means).

Treatment	14/4/05	26/6/06	31/7/06	1/9/06	20/9/06
−C LG	333.7	72.5	87.1	161.9	558.0
−C EG	332.5	40.2	98.1	87.1	90.1
+C LG	318.0	88.0	94.8	234.0	710.3
+C EG	429.3	60.6	225.4	507.5	411.0
LSR[A] (df = 20)	2.83	2.39	2.53	3.79	3.14

[A] Least Significant Ratio between two means at the 5% level. If the ratio of two means (larger mean/smaller mean) is greater than the LSR, then the larger mean is significantly greater than the smaller mean at the 5% level.

Table 3. Coefficients for prairie grass seedlings (PGS): log(DNA) = Mean0 + (b × 10^{-3})*PGS.

Assessment	b	(SE b)
Pre-sow	4.19	(2.41)
June	6.67	(2.53)
July	6.15	(2.53)
E. Sept	5.32	(2.59)
L. Sept	7.25	(2.66)

*Mean0: Treatment mean, adjusted to 0 PGS

Table 4. Mean numbers of infected wheat roots per plot for treatments of + and − couch (C) and early (EG) or late glyphosate (LG) at three assessments, for each treatment (95% confidence limits, df = 25).

	Infected roots per plot					
Treatment	November		December		January	
−C LG	46.5	(34.8,62.0)	127.2	(91.5,176.8)	576.2	(469.4,707.2)
−C EG	11.2	(6.2,20.1)	52.2	(31.3,87.1)	370.3	(286.8,478.2)
+C LG	43.2	(32.0,58.2)	199.8	(153.6,259.9)	796.5	(669.1,948.2)
+C EG	45.2	(33.7,60.5)	199.2	(153.1,259.2)	839.8	(708.7,995.2)

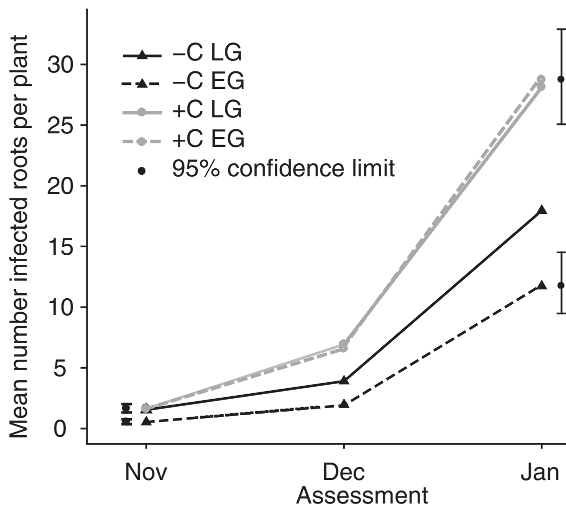

Figure 2. Mean numbers of infected roots per plant, for treatments of + and − couch (C) and early (EG) or late glyphosate (LG) at three assessments. Error bars are the 95% confidence intervals for selected points.

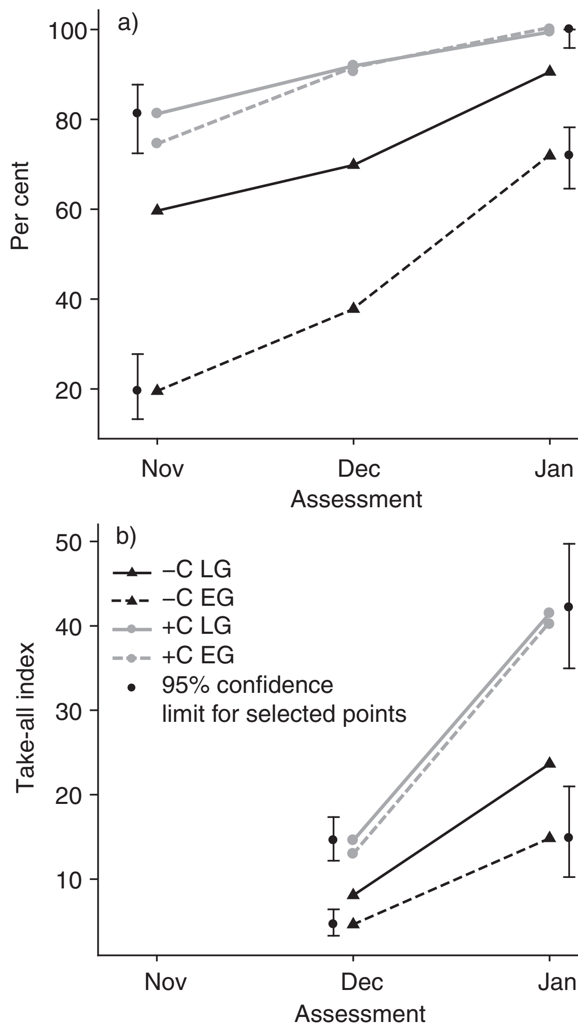

Figure 3. (a) Mean percentage infected wheat plants and (b) Take-all index, for treatments of + and − couch (C) and early (EG) or late glyphosate (LG). Error bars are the 95% confidence intervals for selected points.

TAI varied principally for the factor couch ($P \leq 0.002$), with the +C treatments associated with more severe take-all than −C (Figure 3b). Glyphosate timing did not have a strong effect on TAI at either date ($P > 0.3$ for the glyphosate main effect and glyphosate by couch interaction).

Discussion

Glyphosate effects on grass weeds

Couch rhizome dry matter in check plots, prior to glyphosate application, exceeded 100 g m^{-2}. This rhizome density could be described as moderate to high in comparison to previous couch studies (Leroux 1993). The dry matter assessments confirmed that rhizomes persist for at least several months following glyphosate application. Prairie grass, while less intensively sampled due to its uneven distribution in the trial, appeared to have a durable root system following glyphosate application.

Grass weeds as hosts of Ggt

The pathogenicity testing on wheat and oats of isolates confirmed the identity of *Ggt* isolated from lesions on couch roots and rhizomes. The inability of these isolates to infect oat seedlings indicated that, although *Gga* DNA was present at low levels at the site prior to the establishment of the trial, the *Gga* did not compete well with *Ggt*. Other reports indicate that *Gga* usually predominates when oats are the main cereal crop (Yeates *et al.* 1986).

Couch has been identified as an important host for *Ggt* (Kirby 1922, Nilsson 1969, Padwick 1935). However, *Ggt* DNA levels 14 months after couch had been planted suggested that the presence of couch maintained rather than increased *Ggt* because *Ggt* inoculum levels in couch plots were no greater than in other plots. Although prairie grass seedling counts were associated with increased *Ggt* DNA levels, this effect was independent of glyphosate and couch treatment effects. This also suggested that prairie grass seedlings may be more susceptible to take-all than mature prairie grass plants.

Glyphosate effects on Ggt inoculum

Glyphosate application has been associated with an increase in the extent of dark lesions, thought to be caused by *Ggt* (Harvey and Braithwaite 1986), but decay processes and other pathogens could also be responsible for colour changes in rhizomes (Penn and Lynch 1981). However, the greater incidence of *Ggt* isolation from baited couch rhizomes after glyphosate application in this study indicated increased *Ggt* infection. In contrast, *Ggt* isolation from couch roots declined following glyphosate application. Dead couch roots decompose rapidly, with an average 50% loss in dry matter over a six-week burial period (Steen 1985). Non-glyphosate sprayed couch roots also have a higher species diversity of fungi than non-glyphosate sprayed couch rhizomes (Chng *et al.* 2004). Greater competition between fungal colonizers on roots than rhizomes may have contributed to reduced isolation levels of *Ggt* from couch roots than from rhizomes.

The *Ggt* DNA results demonstrate a clear positive association between glyphosate application to couch and increased *Ggt* inoculum levels. For example, one month after the early glyphosate application *Ggt* inoculum levels had increased only in sprayed plots with couch present. The rapid increase in *Ggt* DNA levels from early glyphosate plots with couch was reflected in a substantial increase in *Ggt* isolation from rhizomes following glyphosate application, but lesion incidence and severity on rhizomes only increased slightly, suggesting that the host was not responding to the invasion. The presence of *Ggt* in wheat roots without visible disease symptoms has been reported using DNA analysis (Irzykowska 2007). The increase in *Ggt* levels may be due to pre-symptomatic *Ggt* infection of rhizomes or non-symptomatic colonization following host death.

Glyphosate appears to facilitate increases in *Ggt* inoculum levels by providing susceptible host material because *Ggt* is known to be a poor soil saprophyte (Shipton 1981). Kawate *et al.* (1997), in comparing the colonization of *Pythium ultimum* Trow and *Fusarium solani* (Mart) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans., observed that the action of glyphosate in pathogen-host interactions was more effective for pathogens that are not effective soil saprophytes. Infection prior to host death may also provide an advantage to *Ggt* over other colonists, facilitating the rapid colonization of dying tissue (Levesque and Rahe 1992). *Ggt* is a root pathogen; infection by *Ggt* of living couch rhizomes often results in a restricted area of infection in comparison to couch roots (data not presented). The loss of partial resistance to *Ggt* of couch rhizomes following herbicide application and the volume of rhizome material available for colonization, in addition to pre-existing infection providing an advantage,

appear to be the major factors responsible for increases in *Ggt* inoculum levels following glyphosate application.

Increases in *Ggt* DNA levels following late glyphosate application provided some results that could not be fully explained by increased host susceptibility of couch because plots without couch to which a late glyphosate application was made also had large increases in *Ggt* DNA levels. Two other indirect mechanisms independent of host susceptibility effects have been proposed for herbicide-pathogen interactions (Katan and Eshel 1973). These are that the herbicide affects the pathogen's virulence or, secondly, that other micro-organisms interacting with the pathogen are affected. For example, the cause of an increase in pathogenicity and saprophytic survival of *Ggt* following glyphosate application to non-sterile soil was identified as being due to glyphosate decreasing the competitive effects of secondary micro-organisms with *Ggt* (Mekwatanakarn and Sivasithamparam 1987). This mechanism may partially explain the increases in *Ggt* DNA in late glyphosate plots without couch. In addition, prairie grass was present as an alternative host in some plots, but prairie grass populations as a factor did not explain the observed changes in *Ggt* inoculum levels. Although speculative, one explanation may be that prairie grass was more important as a secondary host for the late glyphosate applications made in spring than for the early glyphosate applications made at the end of winter due to the effects of greater root growth providing increased tissue for infection over the early spring period.

Glyphosate timing effects on Ggt inoculum

The timing of glyphosate application affects inoculum levels of some pathogens, with applications in general leading to increased inoculum levels that then decline (Levesque *et al.* 1987, Smiley *et al.* 1992). This inoculum flux following herbicide application has been demonstrated in work with *Ggt*. For example, the application of a mixture of herbicides to grass break crops following wheat gave subsequent temporary peaks in *Ggt* inoculum levels (Sivasithamparam and Bolland 1985). The temporary increase was probably due to the subsequent breakdown of the host residues. It would therefore be expected that glyphosate applications that allow for subsequent host breakdown (early applications) would result in lower *Ggt* inoculum levels than late glyphosate applications. Work comparing early and late, winter and spring herbicide applications to grasses in break crops demonstrated this effect, with both early and late winter applications reducing *Ggt* inoculum levels in comparison to late spring herbicide applications (Kidd *et al.* 2002). However, in our trial inoculum levels at the final sample date following early and late glyphosate treatments with couch present did not differ, although there was some indication that inoculum levels had started to decline in couch plots with early glyphosate applications. The continuation of *Ggt* inoculum sampling following the sowing of wheat may have revealed subsequent changes in inoculum levels, but sampling was discontinued to prevent damage to the wheat.

Couch, glyphosate timing and Ggt inoculum levels – effects on take-all

The timing of herbicide application can affect the disease risk of soil-borne pathogens to crops by affecting inoculum levels. For example, the application of herbicide to weeds or volunteers close to the sowing date of a crop was demonstrated to lead to increased cereal diseases (Pittaway 1995, Smiley *et al.* 1992). Kidd *et al.* (2002) also demonstrated a clear relationship between the timing of herbicide application and subsequent *Ggt* inoculum levels, levels of take-all and yield losses due to take-all in wheat. In that work, the time between spraying of annual non-rhizomatous grass hosts and sowing of wheat ranged from six to 11 months. In our trial early and late glyphosate applications were made at 86 and 38 days, respectively, before the sowing of spring wheat. This was a comparatively short period given that couch rhizomes decay slowly. Take-all infection levels in spring wheat primarily reflected the importance of couch as a host of *Ggt*. The timing of glyphosate application was a secondary effect, with no clear separation of effects for plots with couch present. But for plots without couch, the early glyphosate application was associated with a lower incidence of take-all in November and December than a late glyphosate application. That take-all incidence was lower for the early application of glyphosate in the absence of couch demonstrated that *Ggt* can decline following an early glyphosate application when durable inoculum sources such as couch are not present.

Conclusion

Results supported the hypothesis that glyphosate application increases *Ggt* levels in treated hosts. Glyphosate application may increase the susceptibility of rhizomes and so provide prior *Ggt* infection with a colonization advantage. This work evaluated glyphosate as it is commonly used to manage couch in cereals, other rhizome killing herbicides may have similar effects. Take-all levels increased in the presence of couch, but not following the application time of glyphosate, indicating that couch rhizomes provide a durable *Ggt* inoculum source. The hypothesis that couch maintained *Ggt* inoculum for up to three months after herbicide application was supported. As couch rhizomes are such a durable reservoir of *Ggt*, periods longer than three months between glyphosate application and wheat sowing will be required to reduce take-all infection levels when couch is present.

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