EVALUATION OF THE SUSCEPTIBILITY OF VARIOUS GRASS SPECIES TO *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*

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ABSTRACT

Take-all, caused by the soil-borne pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is a devastating root disease of wheat. As well as infected host residues from previous wheat crops, grass crop or weed species also play an important role in the carry-over of inoculum to the next wheat crop. However, the survival and spread of inoculum on different grasses differs considerably depending on their susceptibility to the pathogen. Using *Triticum aestivum* (wheat) and *Avena sativa* (oat) as susceptible and resistant standards, the susceptibility to *Ggt* of 24 grass species commonly found within wheat crops in New Zealand was examined in a simple laboratory assay. Of all the grass species evaluated, 83% were susceptible to *Ggt* with *Bromus diandrus*, *Bromus willdenowii*, *Bromus inermis* and *Pennisetum clandestinum* being highly susceptible, while *Cynosurus cristatus*, *Cynodon dactylon* and *Paspalum dilatatum* were highly resistant to the pathogen.

Keywords: take-all, *Gaeumannomyces graminis* var. *tritici*, susceptibility, grass species, hosts.

INTRODUCTION

Take-all disease, caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* (*Ggt*), is a devastating root disease of autumn-sown wheat. Wheat infected by take-all generally shows above ground symptoms such as yellowing of the lower leaves on tillers, stunting of growth, small heads and early maturation of heads. Heads may appear bleached and are usually sterile due to restriction of water flow to the tops (Cook 2003).

Ggt infects living susceptible roots. It persists in infected root residues (roots and stem bases) and by being a pathogen on other susceptible grass hosts. Control of take-all has been difficult, as resistant wheat cultivars are not yet available, and chemical controls are impractical and at best provide limited control (Hornby 1979). Well planned crop rotations can control the disease effectively if fields are relatively free from susceptible grasses (Nilsson 1969). If susceptible grasses are present, the fungus can survive and multiply in the living and decomposing roots or rhizomes of these grasses, carrying over inoculum to a following cereal crop (Kidd et al. 2002). The range of grasses that can host and carry the pathogen is extensive; 402 grass species have been listed as hosts of *Ggt* by Nilsson (1969).

This paper reports a laboratory assay that evaluated the relative susceptibility to *Ggt* of grass species common in New Zealand arable farms and examined the effects of the disease on the development of the roots of these grasses. This information can be used to target the elimination of susceptible grass weeds and plan better crop rotations in order to minimise *Ggt* carry-over from and to wheat crops.

MATERIALS AND METHODS

This assay was similar to the one described by Hunger et al. (2002) for testing resistance to *Sclerotium cepivorum* in *Allium* species.

Grass species

Twenty-four grass species commonly found within wheat crops or as break crops were selected for this study (for a list of the species see Table 2). As *Ggt* attacks *Triticum aestivum* (wheat) but not *Avena sativa* (oats) (Deacon 1997), *T. aestivum* cv. Regency and *A. sativa* cv. Stampede were used as susceptible and resistant standards respectively.

Seeds were germinated by spreading them in a row 3 cm from the top, across three moist, non-sterilised buff germination papers (32 x 45 cm) (Anchor Paper Limited, Minnesota, USA) stacked together. Buff germination papers are widely used in germination tests by the New Zealand Seed Testing Institute. Germination rates varied between grass species. For species with low germination rates, extra seeds were germinated. Papers were then rolled up firmly, placed in plastic bags (one roll per bag) and sealed with twist-ties at the top to prevent the papers from drying up. All the paper rolls were positioned upright to promote geotropic growth and placed in an incubator maintained at 25°C with 12:12 h light:dark.

Inoculation

Due to varying growth rates of different grass species, these were categorised into three groups based on the number of days after sowing when seedlings were suitable for inoculation prior to the experiment. The three groups were 'Slow' (20-30 days after sowing), 'Medium' (10-15 days after sowing) and 'Fast' (5-7 days after sowing).

Assays for the three groups were carried out separately using *T. aestivum* cv. Regency and *A. sativa* cv. Stampede standards each time. Five healthy seedlings of similar sizes from each tested host on each paper roll were randomly selected for the assay, while non-germinated seeds and extra seedlings were discarded. Each paper roll was considered as one replicate. Three replicates of each grass species were inoculated with *Ggt*, and one replicate was the uninoculated control. *Ggt* strain A3SL4, which was isolated in 2002 from a rhizome of *Elytrigia repens* sampled from Canterbury, New Zealand, was used as the inoculum. Inoculation was carried out by placing an 8 mm diameter potato dextrose agar (PDA) disc (obtained from the actively growing edges of an 8-day-old *Ggt* culture), 2 mm above the growing tip of one selected root radical of each seedling. Controls were inoculated with clean PDA discs. Each paper roll was then placed into its original plastic bag, sealed and incubated for a further 10 days.

Infection assessment and data analysis

Inoculated seedlings were visually scored according to the symptoms observed (Table 1) 10 days after inoculation. The susceptibility of each grass species was determined by calculating the percentage of seedlings under each visual score using the formula: % seedlings = 100 * (Number of infected seedlings with each visual score / Number of inoculated seedlings).

TABLE 1: Disease scores used for visual root assessment 10 days after inoculation.

Score	Susceptibility	Observable symptoms
0	Highly resistant	Turgid roots with no visible lesions.
1	Resistant	Turgid roots with light take-all lesions less than or equal to the size of inoculum (diameter = 8 mm).
2	Slightly susceptible	Roots with light take-all lesion extending beyond the size of inoculum (diameter = 8 mm).
3	Susceptible	Roots with dark take-all lesions greater than the size of inoculum.
4	Highly susceptible	Roots with extensive dark lesioning and necrosis.

The root length of all seedlings was measured. For infected seedlings, the severity of disease was examined by measuring lengths of all lesions. The percentage of the root area affected (with lesion) was also calculated using the formula: % root area affected = $100 \times (\text{lesion length} / \text{total root length})$.

Root and lesion length data were analysed with analysis of variance, after logarithm transformation to stabilise the variance across the range of the data. The percentage root area covered by the lesion was also analysed with analysis of variance. The analyses included contrasts between the three groups (Slow, Medium, Fast) as well as between each species. For root length data, contrasts between inoculated and uninoculated and the interaction between species and inoculation were also included. After the analysis, the change in root length between the uninoculated and inoculated roots was calculated using the formula: % change = $100 \times [(\text{Uninoculated Mean Length} - \text{Inoculated Mean Length}) / \text{Uninoculated Mean Length}]$. Analyses were carried out with GenStat (GenStat, Eighth Edition (2005), VSN International Ltd, Oxford).

RESULTS

Susceptibility of grass species to *Gaeumannomyces graminis* var. *tritici*

Using *T. aestivum* (highly susceptible) and *A. sativa* (highly resistant) as standards, the susceptibilities of all the tested grass species to *Ggt* were arranged in order of median disease scores, and then by the percentage with higher scores (score 4 and 3 respectively) and then by the percentage for each lower score (score 2, 1 and 0 respectively) (Fig. 1). Take-all lesions developed on the roots of 83% of the grass species with *Bromus diandrus*, *B. willdenowii*, *B. inermis* and *Pennisetum clandestinum* being highly susceptible to the fungus (100% of seedlings being infected). *Avena fatua*, *Cynosurus cristatus*, *Cynodon dactylon* and *Paspalum dilatatum* were all highly resistant to the fungus with none of the seedlings being infected. None of the uninoculated seedlings showed any symptoms of take-all infection, hence the disease scores of these controls are not included in Figure 1.

Effect of inoculation on the roots

There were overall differences ($P < 0.001$) between the three groups in root length, with 'Slow' group being generally shorter than the 'Medium' and 'Fast' groups (mean lengths of 28.4 mm, 41.7 mm and 151.8 mm respectively). Roots of inoculated seedlings were generally shorter than uninoculated seedlings (71.4 mm and 76.9 mm respectively averaged across all three groups) ($P < 0.001$). Root lengths for the 'Slow' and 'Fast' groups were generally reduced by inoculation, although this effect varied between the species ($P < 0.001$). However, there were few differences between the root lengths of inoculated and uninoculated seedlings of grass species in the 'Medium' group (mean length of 43.3 mm and 41.2 mm respectively).

There were no strong relationships between changes in root lengths and disease scores following inoculation. Grass species that were highly susceptible (disease score 4) to the fungus in general showed a decrease in root length with inoculation whereas the root length of inoculated grass seedlings that were either highly resistant or resistant (scores 0 and 1) to *Ggt* did not differ significantly, except for *Agrostis capillaris* (root length reduced by 55.2%) (Fig. 1 and Table 2). Grass species in the groups 'Slow' and 'Fast' showed large variations (-55.2 to +23.7% for 'Slow' and -31.1 to +32.4% for 'Fast') in the root length with inoculation, whereas grass species in group 'Medium' gave smaller variations (-18.8% to +16.5%) in root length with inoculation (Table 2).

FIGURE 1: Susceptibility of grass species to *Gaeumannomyces graminis* var. *tritici* in their order of median disease score, then by the percentage for scores 4 to 0.

Total lesion lengths (Fig. 2a) varied between the groups ($P < 0.001$) (mean of 11.5, 26.9 and 56.1 mm for 'Slow', 'Medium' and 'Fast' respectively) and varied substantially within each group ($P < 0.001$). The percent root area with lesion (Fig. 2b) varied between the grass species in the 'Slow' and 'Fast' groups ($P < 0.001$) (mean of 22.0% and 38.3% respectively), but did not vary substantially between the species in the 'Medium' group (mean of 66.0%). This effect was similar to that observed on infected root length (Fig. 2a). There was also no strong relationship between percent root area with lesion and disease scores for most grass species (Fig. 2c).

TABLE 2: Mean total root lengths (back-transformed from means of logarithm lengths) of inoculated and uninoculated grass species and their percent changes in root length arranged in order of decreasing susceptibility to *Ggt* as seen in Figure 1.

Grass species	Group	Root length		
		Uninoculated (mm)	Inoculated (mm)	% change
<i>Triticum aestivum</i> cv. Regency	Fast	234.6	199.2	-15.1
<i>Bromus diandrus</i>	Fast	218.7	216.5	-1.0
<i>Bromus willdenowii</i>	Fast	147.2	101.4	-31.1
<i>Bromus inermis</i>	Fast	99.5	78.3	-21.4
<i>Pennisetum clandestinum</i>	Fast	94.9	72.9	-23.2
<i>Eleusine indica</i>	Slow	49.5	50.9	2.9
<i>Elytrigia repens</i>	Medium	58.0	58.4	0.6
<i>Echinochloa crus-galli</i>	Medium	52.9	46.4	-12.3
<i>Lolium perenne</i>	Fast	107.0	100.5	-6.1
<i>Poa annua</i>	Medium	30.4	28.5	-6.1
<i>Phleum pratense</i>	Medium	29.5	29.8	1.2
<i>Phalaris minor</i>	Medium	42.0	34.1	-18.8
<i>Setaria pumila</i>	Medium	73.7	66.3	-10.0
<i>Festuca rubra</i> subsp. <i>rubra</i>	Medium	37.2	34.8	-6.5
<i>Bromus hordeaceus</i>	Fast	87.3	100.6	15.3
<i>Festuca rubra</i> subsp. <i>commutata</i>	Slow	39.1	38.3	-2.2
<i>Phalaris canariensis</i>	Fast	150.4	153.9	2.3
<i>Lolium multiflorum</i>	Fast	93.7	124.0	32.4
<i>Dactylis glomerata</i>	Medium	39.2	45.7	16.5
<i>Holcus lanatus</i>	Slow	31.0	31.8	2.5
<i>Agrostis capillaris</i>	Slow	22.8	10.2	-55.2
<i>Avena fatua</i>	Fast	229.6	181.5	-20.9
<i>Cynosurus cristatus</i>	Slow	22.2	21.0	-5.4
<i>Cynodon dactylon</i>	Slow	17.8	22.1	23.7
<i>Paspalum dilatatum</i>	Slow	43.3	44.0	1.7
<i>Avena sativa</i> cv. Stampede	Fast	221.1	223.6	1.1

Least significant ratio¹ (df=68) to compare

Inoculated with uninoculated for:

Wheat or oats	1.2
All other species	1.3

Between species for inoculated or uninoculated for:

Wheat or oats	1.2	1.1
Wheat or oats with any other species	1.3	1.2
Species other than wheat or oats	1.3	1.2

¹Least significant ratio refers to the smallest ratio of the larger mean over the smaller mean that is significantly different at P=0.05.

DISCUSSION

The laboratory assay has demonstrated that many grass species that are common weeds or crops grown in New Zealand arable sites are potential hosts of *Ggt*, and they vary in their susceptibility to the pathogen. Highly susceptible grass species, such as *Bromus diandrus*, *B. willdenowii*, *B. inermis* and *Pennisetum clandestinum*, when grown as break crops or are present as weeds in break crops, may get infected and assist in the

FIGURE 2: (a) Total infected root and lesion length (mm) obtained from the back-transformed means (LSR = 1.15, df = 68); (b) Percent root area with lesion (LSD ($P=0.05$) = 19.2%, df = 68); (c) Median disease scores of grass species from the visual root assessment, of the three groups of inoculated grass species.

survival of *Ggt* (on both living and decomposing roots) and contribute to the increase in *Ggt* inoculum and take-all incidence in subsequent wheat crops. *Elytrigia repens*, a common grass weed that is susceptible to *Ggt*, must be controlled well in advance of sowing wheat, mainly because this grass not only carries *Ggt* through break crops but also because it may prevent or delay the natural development of take-all decline (decrease in take-all incidence when wheat is grown continuously for many seasons) (Hornby et al. 1998).

In contrast, some of the less susceptible grass species, such as *Holcus lanatus*, *Dactylis glomerata* and *Lolium multiflorum*, may not support growth of the pathogen and therefore may not contribute to take-all incidence in a subsequent wheat crop.

Take-all is best managed by crop rotation away from wheat, barley or other susceptible grasses for one or two years, depending on the soil and climatic conditions (Cook 2003). This laboratory assay has identified suitable non-host pasture species for use as break crops from wheat. For instance, *L. multiflorum* and *D. glomerata* may be more suitable break crops than *L. perenne* since they are more resistant to *Ggt*. Although *Agrostis capillaris*, *Avena fatua*, *Cynosurus cristatus*, *Cynodon dactylon* and *Paspalum dilatatum*

are not commonly grown as pastures, their presence will not contribute to an increase in take-all incidence.

A decrease in root length due to infection was mostly observed in grass species that were highly susceptible (disease score 4) to *Ggt*. However, because growth characteristics differed between the grass species, comparing root length and percent change in root length will not be useful in determining the resistance or susceptibility of a grass species to *Ggt*. For instance, a small change in root length (10 mm) of a slow-growing species such as *A. capillaris*, which was resistant to *Ggt*, will result in a larger proportional change in root length than a faster growing species.

Since all the susceptible grass species in the three groups have different growth rates and seedling sizes, the varying root radical sizes might have allowed the runner hyphae of *Ggt* to grow in the vascular systems at different rates. Take-all lesions in smaller root radicals could have been severe and have extended up to the crowns of the seedlings, stunting their growth completely by the end of incubation (10 days) (Fig. 2a). In this case, the extent of lesions may sometimes give a misleading impression of the effects of disease on the hosts (Fig. 2a). Therefore, the percent root area with lesions cannot be used as an indicator of susceptibility because most tested hosts have different growth habits and characteristics (Fig. 2b). This explains why there were no strong relationships between change in root length, percent of root area with lesions, and disease scores from the visual root assessment.

In conclusion, this laboratory assay provided a rapid preliminary assessment of the susceptibility of various grasses to *Ggt*. It could also be used to test the pathogenicity of *Ggt* isolates to grass hosts. Further work will be required to determine the role of these grass species in maintaining *Ggt* inoculum under field conditions.

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