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RESEARCH NOTE

Acetohydroxyacid synthase (AHAS)-inhibitor herbicide resistance detected in recent *Alopecurus myosuroides* (black-grass) incursions into New Zealand

Christopher E. Buddenhagen[1,4*], Matilda Gunnarsson[2], Deborah L. Hackell[1], Ivan Lawrie[2], Zachary Ngow[1], Ashley Mills[2] and Tim Ryder[3]

[1] Bioeconomy Science Institute, Private Bag 3123, Hamilton 3240, New Zealand
[2] Foundation for Arable Research, PO Box 23133, Christchurch 8441, New Zealand
[3] Readiness and Response Services Directorate, Ministry for Primary Industries, Private Bag 4765, Christchurch, New Zealand
[4] Better Border Biosecurity (B3) research collaboration

**Corresponding author:* Chris.Buddenhagen@agresearch.co.nz

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Summary

Alopecurus myosuroides Huds. (black-grass) has become problematic globally due to its repeated evolution of herbicide resistance. Three incursions via contaminated seed lots have occurred in the past 20 years despite strict border biosecurity in New Zealand. However, it is not known if the black-grass populations linked to these incursions carried herbicide resistance. Enough seed was obtained from contaminated seed lots (ryegrass-linked population UK2007; linseed-linked population FR2021) for a dose–response assay to test resistance to a commercial herbicide product containing both iodosulfuron and mesosulfuron, used for black-grass control in Europe, but not enough for repetition of the experiment or to test multiple herbicides. Seed lot FR2021 showed a high level of resistance, requiring 250 g ai/ha to achieve 90% control (LD_{90}) more than ten times the label rate whereas seed lot UK2007 had an LD_{90} of 19.0 g ai/ha almost twice the label rate (10 g ai/ha). Acetohydroxyacid synthase (AHAS)-inhibitor herbicides should not be used against black-grass populations during incursion responses in New Zealand. Any future black-grass incursions will be increasingly likely to carry herbicide resistance traits. Strong border measures, certified seed use, avoiding seed sourced from infested areas and screening for resistance are critical for prevention and effective control of black-grass incursions.

Keywords black-grass, iodosulfuron-methyl, seed contaminant, linseed, ryegrass

INTRODUCTION

Alopecurus myosuroides Huds. (black-grass) is a major weed of arable systems in western Europe (and increasingly in China) where it has evolved herbicide resistance to multiple modes of action through a range of both target-site and non-target-site mechanisms (Moss 2017, Lan et al. 2022). Due to this evolved resistance, black-grass populations are becoming more difficult to manage and have been responsible for significant losses in profit (Gerhards et al. 2016). European black-grass populations are already resistant to many of the herbicides widely used in New Zealand arable systems and therefore pose a high risk to New Zealand agriculture.

To prevent regulated weeds like black-grass from being introduced to New Zealand, the Ministry for Primary Industries (MPI) requires that seed importers inspect seed lots prior to, and on arrival, into New Zealand (Buddenhagen et al. 2022). Prior to 2010, black-grass was regarded as rare or absent in New Zealand (Edgar & Connor 2010) but, between 2014 and 2018, MPI documented at least four instances of black-grass detections at the border in perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.) and pea (*Pisum sativum* L.) seed lots from France and the Netherlands (Rubenstein et al. 2021). Aside from seed contamination, black-grass has also been detected on imported spray equipment (Bullians 2015). Also, imported

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red fescue (*Festuca rubra* L.) seed containing black-grass was spilt along the Methven Highway (Canterbury) in 2013, but a black-grass population failed to establish. While these incursions (and potentially many others) were prevented because of strict biosecurity measures, three incursions have occurred in the last twenty years that have resulted in the establishment of black-grass on New Zealand farms and triggered incursion responses managed by Biosecurity New Zealand (MPI) and the Foundation for Arable Research (FAR).

1. The first of the three incursions (UK2007) was detected when ryegrass seed multiplied in New Zealand was inspected for certification prior to export in 2015, and again in 2016 and 2020 (Foundation for Arable Research, 2020). The lineage of the black-grass contamination was traced to two ryegrass seed lots imported from the United Kingdom (UK) in early 2007. This incursion was managed with the objective of eradication on several Canterbury farms, and in June 2025, eradication proved successful, and the response was formally closed.

2. The second incursion (FR2021) was detected in December 2021, when an inspector involved in seed certification found live black-grass plants on a Canterbury farm. The origin of that population was traced back to a contaminated seed consignment of linseed (*Linum usitatissimum* L.) imported from France in August 2021. That consignment had been sown on four mid-Canterbury farms in December 2021 (Foundation for Arable Research 2022, Bleach 2023). Later in 2021, an additional five properties were subject to monitoring after a 'sister line' linked to the contaminated linseed lots was sown. By 2022, five further properties had sown seed harvested from one of those properties. Then, another mid-Canterbury farm linked to the linseed from the 'sister line' had black-grass detected in a subsequent wheat crop in January 2023 (Bleach 2023). Farms linked to this French linseed consignment are still being monitored, but as of January 2026, have had no more black-grass detected.

3. In May 2025, a third incursion of black-grass was detected in a south Canterbury perennial ryegrass seed crop (Foundation for Arable Research 2026). It remains unclear if this incursion was, or was not, linked to a perennial ryegrass seed lot imported from the United States in December 2023. As of March 2026, there have been no more black-grass plants detected in farms linked to this third incursion.

Apart from the inherent risk of black-grass establishment from recent incursions we were concerned that they could also be herbicide resistant, thus limiting control options in the incursion response. Using seed supplied by staff involved in managing these previous incursions, we set out to determine if two black-grass populations (one originating from ryegrass (UK2007) and the other from linseed (FR2021) seed lots) were also resistant to a common herbicide used for its control.

MATERIALS AND METHODS

Plant materials

Permissions were obtained under Sections 52(d) and 53(2) of the Biosecurity Act 1993 to work with black-grass, and a small number (<200) of seeds of variable viability were obtained that had been collected from plants in the field by MPI contractors and FAR staff involved in incursion responses initiated in 2015 and 2021, respectively. Seeds from both incursion responses were refrigerated under physical containment level 1 (PC1) as part of the permission agreement.

Dose-response with Othello® OD

In late April 2024, these two seed 'populations' (UK2007; FR2021) were tested for resistance in a dose-response assay using Othello® OD (Bayer Crop Science New Zealand) but no (sensitive) susceptible black-grass control was available for comparison. To break dormancy, black-grass seeds were treated with a solution of 0.2% KNO_3 for 24 hours. There were at least eight replicates (pots) assigned to each herbicide treatment. Five seeds were sown into each 9 cm × 9 cm × 10 cm (depth) plastic pot containing potting mix (Dalton's grass and clover mix; 33.3% coco-coir, 33.3% pumice, 33.3% bark). Pots were kept moist (watered every 2–3 days) in a temperature-regulated PC1-containment glasshouse at Ruakura maintained between 18 and 25°C in natural light conditions. At the time of spraying, pots contained between one and five plants pot (mean number of plants in each pot was 1.1 for UK2007 and 1.3 seedlings for FR2021). Overall, there were a total of 71 plants for the UK2007 population and 83 plants for FR2021.

When plants reached the 3-4 leaf stage on 1 May 2024, they were counted and the pots sprayed with a range of doses of Othello® OD. Othello® OD contains 7.5g/litre mesosulfuron methyl (Group 2, AHAS inhibitor), 2.5g/litre iodosulfuron methyl sodium (Group 2, AHAS inhibitor), 50g/litre diflufenican (Group 12, phytoene desaturase inhibitor) and 22.5g/litre mefenpyr-diethyl (safener). Black-grass has been sprayed with similar products in Europe (i.e., Othello®; Bayer Crop Science) that contain iodosulfuron + mesosulfuron at the same ratio (but not diflufenican) (Moss 2017). The "grams per hectare" rates applied here were calculated as the sum of the two active ingredients - mesosulfuron + iodosulfuron (Othello); these were 5, 10, 20, 40, 80, 160, and 320 g ai/ha (the label rate is 10 g ai/ha, similar to doses used in European studies (Hamouzová et al. 2023). A water-only spray treatment was included as a control.

Treatments were applied with a moving belt sprayer, fitted with a single TeeJet TT11002 flat fan nozzle at 200 kPa, and calibrated to apply a water rate of 200 L/ha. This equipment has been tested and is known to give repeatable reliable rates across treatments. The height of the nozzle relative to the moving belt, means the plants are positioned in the central third of the nozzle's fan 'swath' and this provides even droplet coverage across the treatment area. Calibration involves passing a known surface area container at plant height through the sprayer and weighing the spray droplets deposited on it.

Mortality counts were taken on the 21 May 2024, three weeks after treatment. Plants were classified as alive if they had active growth and no necrosis at the base. Biomass harvests were also taken on the 21 May 2024. Individual plants were cut at the soil surface and oven dried at 80°C for 24 hours before being weighed.

Data analysis

Dose response curves for the two populations were fit using the drc package's drm function model in R with biomass and mortality data (of individual plants) to estimate the GR_{50} (the dose producing Growth Rate 50% inhibition), the LD_{50} or the LD_{90} (Lethal Dose for 50% or 90% individuals) respectively. The GR_{50} and LD_{50} can both can be described as the ED_{50} (Effective Dose producing 50% of maximal effect) while the LD_{90} can be described as the ED_{90} (Effective Dose producing 90% of maximal effect) (Ritz et al. 2015). Specific options selected for model fitting were a four-parameter model (fct=LL.4) with the lower limit *c* parameter set to zero for the biomass measurements and a two-parameter model (fct=LL.2, type= "binomial") for the mortality. Model fit was confirmed with a lack-of-fit test for biomass measurements and goodness-of-fit test for mortality (*modelFit* function from the *drc* package). The EDcomp function was used to estimate the response ratios (resistance index), calculated as RI= ED_{50} of the 'resistant' population FR2021 divided by the ED_{50} of the 'susceptible' population UK2007. Post-hoc comparison of the ED_{50} values of populations were done using the *compParm* function (*drc* package).

RESULTS and DISCUSSION

Dose-response with Othello® OD

Effective doses for Othello® OD differed between the two black-grass populations (Table 1; Figure 1). There was no overlap in confidence intervals between populations for the mortality model and all parameters in that model were statistically significant (p-value < 0.01). The response ratio between FR2021 and UK2007 was 7.1× for LD_{50} (50% mortality), and the comparison of LD_{50} values between populations was statistically significant (p-value < 0.01). The LD_{50} (and LD_{90} and GR_{50}) estimates for FR2021 have wide confidence intervals due to the relatively high level of resistance up to the dose of 160 g ai/ha – although full mortality was still observed at 320 g ai/ha (32 times the label rate). If additional seeds become available, this study could be repeated by adjusting the rates such that two to four doses were above the LD_{50} estimate. Nonetheless, this result is indicative of the high level of resistance in that population. Photos show the responses of the plants to the treatments (Supplementary Figure 1).

While the LD_{50} showed significant differences between populations, there were no such differences for biomass (GR_{50}; p-value = 0.4498). There was high variability for FR2021, with error estimates overlapping with zero and no statistical significance for the GR_{50} estimate (p-value = 0.39032). UK2007 had a comparatively low error range and had a statistically significant estimated GR_{50} (p-value < 0.01). The biomass of untreated plants from UK2007 (mean 0.50 ± standard error 0.03 g) was twice that of FR2021 (0.23±0.01 g).

Resistance status of black-grass incursions

The black-grass seed populations examined here differed markedly in their response to Othello® OD. The black-grass from contaminated linseed (FR2021) showed clear herbicide resistance, while that from contaminated ryegrass seed (UK2007) remained sensitive at substantially lower application rates. The estimated LD_{90} values of 250 g ai/ha (France) and 19 g ai/ha (UK) indicate that both populations required rates above the New Zealand label recommendation of 10 g ai/ha (25× and 1.9×, respectively), highlighting a much stronger resistance phenotype in the French population (Table 1; Figure 1). It is worth noting that label-recommended rates vary internationally (e.g. 15 g ai/ha in France). the two incursions represent distinct risk profiles in terms of herbicide sensitivity.

Herbicide sensitivity varies significantly among European black-grass populations. This can reflect herbicide use histories (selection for resistance), as well as stochastic differences in their genetics in unexposed populations. Such variability also complicates the distinction between susceptible and resistant populations. Reported GR_{50} values for populations characterised as susceptible range from 0.4 g ai/ha to 2.4 g ai/ha in different European arable systems (Kaiser & Gerhards 2015, Keshtkar et al. 2015, Hull & Moss 2017), which are all lower than the GR_{50} estimated for

Table 1. The effective dose (ED) responses at a given level of effect are shown either for the ED_{50} (expressed as GR_{50} or LD_{50}), or for the ED_{90} expressed as LD_{90} (with upper and lower estimates) and with response ratios (ED_{50} of the more-sensitive population compared to the less-sensitive one) after spraying with the herbicide containing mesosulfuron. The dose is the sum of 7.5 g ai/ha + 2.5 iodosulfuron g ai/ha for the black-grass populations (UK2007, FR2021) linked to contaminated seed lots.

Population	Type of response	Effective dose response (g ai/ha)	SE	95% CI Lower	95% CI Upper	Response Ratio (95% CI)
FR2021	LD_{50}	85.4	15.1	55.7	115.1	7.1 (4.1, 10.2)
UK2007	LD_{50}	12.0	1.5	9.0	14.9	-
FR2021	LD_{90}	250.3	78.5	96.3	404.3	13.2 (3.5, 22.8)
UK2007	LD_{90}	19.0	3.9	11.4	26.6	-
FR2021	GR_{50}	48.9	56.8	-63.3	161.1	9.1 (-12.0, 30.1)
UK2007	GR_{50}	5.4	1.0	3.5	7.3	-

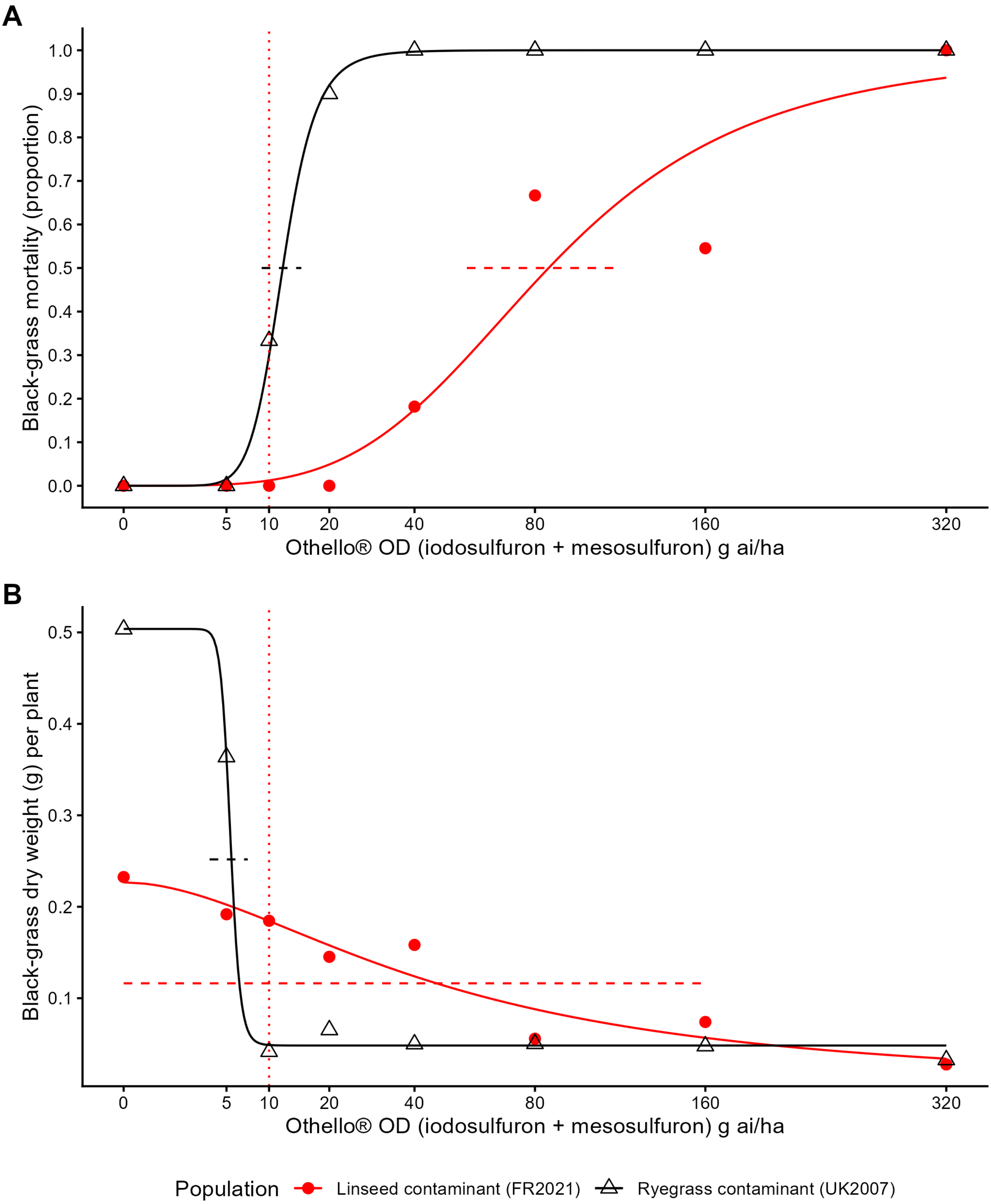

Figure 1. (A) Dose response curves for the mortality of black-grass populations (ryegrass-associated UK2007, linseed-associated FR2021) treated with Othello® OD. The vertical dashed line shows the label rate. The 95% confidence intervals (CI; horizontal error bars) are presented for LD_{50} values of each population **(note all plants died at the highest dose for the FR2021 population).** (B) Dose response curves for the dry weight of black-grass populations (ryegrass-associated UK2007, linseed-associated FR2021) The 95% CI (horizontal error bars) are presented for GR_{50} values of each population **(note for FR2021 population this extends below zero; see Table 1).**

UK2007 (5.4 g ai/ha). However, higher GR_{50} values have also been reported for populations still classified as susceptible, such as 8 g ai/ha in the Czech Republic (Hamouzová et al. 2023). Comparisons with overseas studies are difficult due to differences in the conditions in which plants were grown and treated across studies, and in the herbicide product used (for example our study used a product that also contains diflufenican). Nonetheless, the current study does show that these introduced populations are not adequately controlled at the label rate.

Against this background, contrasting herbicide sensitivities of the two New Zealand incursions we tested align with broader temporal trends of increasing resistance in Europe. Herbicide resistance in black-grass has been documented in the UK since the 1980s, but was initially rare, by the early 2000s, approximately 80% of populations were resistant to one or more modes of action, and resistance is now widespread across England (Moss et al. 2011, Hull et al. 2014). Resistance frequencies have since increased further across Europe, particularly in France (Moss 2017). This trend suggests that seed contaminants of arable seed for sowing from Europe will have an increasing likelihood of carrying resistant seeds as resistance levels increase.

Biosecurity implications

These findings have important implications for biosecurity risk management. Despite stringent inspection and border controls, three black-grass incursions have occurred in New Zealand within two decades, indicating that occasional introductions linked to seed for sowing trade networks are likely to continue. Our study provides preliminary evidence that such incursions can involve populations with 'pre-evolved' resistance. There is precedence for this in another species: *Abutilon theophrasti* Medik. (velvetleaf; butterprint) may have arrived in New Zealand already with atrazine resistance (James & Cooper 2012).

Herbicide resistance should be explicitly considered when deciding to import seeds, in seed certification, border protection, and incursion response decision-making. Determining the resistance status of newly detected incursions, such as the one detected in 2025 and potentially linked to a perennial ryegrass seed lot from the United States of America, could improve response effectiveness. Further investigation into the effectiveness of other herbicides with different modes of action in the two populations in this study was limited by seed availability but, if seed becomes available, further studies could reveal more issues as black-grass resistance has been reported previously for five different herbicidal modes of action (Heap 2025). Similar risks apply to other globally problematic weeds with widespread resistance including species not yet present in New Zealand, such as *Amaranthus palmeri* S.Watson (Palmer amaranth), as well as species present but not regarded as major issues yet, such as *Lolium rigidum* Gaudin (rigid ryegrass). Populations of both these species carrying herbicide resistance traits are known to have spread internationally via contaminated seed and grain (Shimono et al. 2010, 2020, Torra et al. 2020, Rubenstein et al. 2025).

Seed companies play an important role in reducing weed contamination and herbicide resistance risks. Introducing herbicide-resistant weeds through contaminated seed can impose high costs on growers and seed producers through increased weed management, seed cleaning, certification issues, and potential market-access restrictions. To reduce risks, the industry should use seed that is locally produced, cleaned, and certified, or sourced from low-risk regions, with stringent inspection standards. Ongoing research and surveillance of herbicide resistance in seed contaminants will further help protect New Zealand's agricultural systems.

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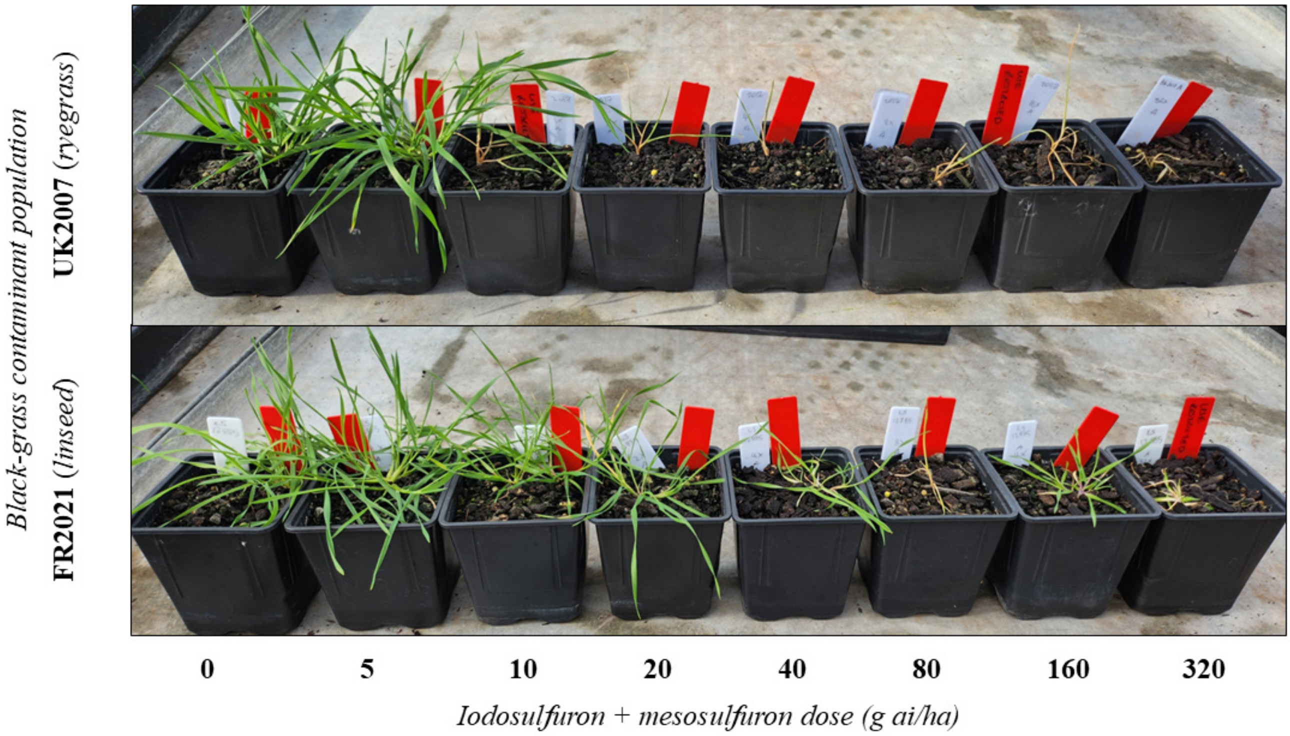
SUPPLEMENTAL INFORMATION



Supplementary Figure 1. Photos of black-grass plants (*above* = ryegrass-associated population UK2007; *below* = linseed-associated population FR2021) twenty days after treatment (21 May 2024) with increasing rates of Othello® OD. This is the same day that plants were measured for mortality and biomass.