Author Contributions

DRM conceived the original idea and formulated the research plan. DRM designed the experiments with input from MM-S and FM. MM-S, FM and DRM performed the experiments. VC and/or DRM developed the black-grass specific VIGS and VOX constructs. KK provided general guidance and support regarding BSMV and FoMV biology and VIGS and VOX methodology. DRM wrote the article with contributions from KK and all the authors. DRM agrees to serve as the author responsible for contact and ensures communication.

Materials and Methods

Plants and growth conditions

Nicotiana benthamiana plants were grown using protocols detailed in Lee et al. (2015).

For VIGS and VOX Black-grass (*Alopecurus myosuroides*) plants were grown under conditions detailed in Bouton et al. (2018). Seeds from purified blackgrass biotypes Rothamsted “herbicide sensitive” and Peldon “herbicide resistant” were used. These were pre-germinated on two filter-papers in Petri dishes. The filter papers were wetted with 2 g/L potassium nitrate. 5-7 days later, 2 or 5 germinated seeds that had a similar sized radicle were chosen to be transplanted to square 11 cm pots filled with Rothamsted Standard Compost Mix (75% Medium grade (L&P) peat, 12% Screened sterilised loam, 3% Medium grade vermiculite, 10% Grit (5 mm screened, lime free), 3.5 kg per m^3 Osmocote® Exact Standard 3-4 M, 0.5 kg per m^3 PG mix, ~ 3kg lime pH 5.5-6.0 and 200 ml per m^3 Vitax Ultrawet). To aid establishment, propagator lids covered seedlings for 2 days following transplantation.

For glufosinate treatment in Fig. S1, 0.5 grams of seed were sown within the top 5 cm of Weed Mix (80% Sterilised Screened Loam, 20% Grit (3-6 mm Screened, Lime Free), and 2.0 kg Osmocote Exact 5-6 month per m^3) into containers and allowed to grow to three-leaf stage before application of glufosinate.

The seed lines used were from “purified populations”; this is defined as the population that is created when plants specifically selected for the phenotype are allowed to bulk cross in isolation. For Peldon, these individuals exhibited strong NTSR herbicide resistance but did not carry any known TSR mutations; for Rothamsted, they were the clones from plants confirmed to be sensitive to all herbicides tested.

Images of leaves and/or plants

Individual leaves were scanned using a Cannon LiDE110 flatbed scanner. Whole plants were photographed with a Nikon NRK-D90(B) camera (serial number 7051046) with elinca sa CH-1020 D-Lite 2 softbox lamps (serial number e/M2 003658 Renes Switzerland) and Velour Vinyl black backdrop (Superior Seamless 234312).

For microscopy, a Leica M205 FA stereomicroscope with Leica DFC 310FX digital camera using LAS AF software (Leica Microsystems, Milton Keynes, UK) was used with white light and no filter or UV

illumination and a GFP3 filter set (excitation filter: 470 ± 40nm; emission filter: 525±50 nm) as indicated. Pictures were taken and quantified using Leica LAS AF software (Leica Microsystems Ltd).

For photographs and monitoring of whole plants for GFP fluorescence, a Dual Fluorescent Protein flashlight (Nightsea, Lexington, MA, USA) was used for illumination and visualisation was done through a long-pass (510 nm) filter (Midwest Optical Systems, Palatine, IL, USA).

Extraction and Quantification of RNA

To obtain the cDNA, the entire plant was frozen and ground in liquid nitrogen from which 100 mg used for total RNA extraction using E.Z.N.A.® Plant RNA Kit (Omega Bio-tek). The RNA was converted into cDNA using SuperScript IV RT (Invitrogen cat# 18090010) and Oligo$(dT)_{20}$ Primer (Invitrogen cat# 18418020) with RNaseOUT™ Recombinant Ribonuclease Inhibitor (Invitrogen cat# 10777-019). Carried over DNA was removed by off-column treatment with RQ1 RNase-free DNase (Promega cat# M610140). qPCR was done with diluted cDNA using an Applied Biosystems 7500 Fast Instrument with Quantitation - Standard Curve experimental type and Takyon Low ROX SYBR 2X MasterMix blue dTTP (Eurogentec cat# UF-LSMT-B0710) using a three-step protocol for optimal sensitivity and 45 cycles in total. Data were normalised to two different control genes: *UBQ* validated by Petit et al. (2012) and against the *UBQ10* (AT4G05320) homologue with primers designed for this study. As similar results were seen, only one control gene is shown. Primers used herein are listed in Supplemental Table S1.

Extraction and Quantification of GFP Protein

Protein extraction was done using protocols detailed in Gould et al. (2013) and quantification using protocols in Bouton et al. (2018) using BioRad's ChemiDoc V3 Western Workflow. Detection of chemiluminescence was accomplished using ChemiDoc Imaging System (cat# 12003153) and the on-board image-acquisition software with auto-exposure settings appropriate to the Clarity ECL.

Cloning of *BSMV* and *FoMV* vectors

The BSMV:MCS and BSMV:asTaPDS vectors are published (Lee et al., 2012, Lee et al., 2015). The methods required to create the black-grass BSMV:asAmPDS and BSMV:asAmGSTF1 variants are described in detail in Lee et al. (2015) with the following changes. Phusion ® High-Fidelity DNA polymerase (NEB cat# M0530) was used on cDNA libraries made from Peldon plants described above (Extraction and Quantification of RNA) with the primers detailed in Supplemental Table S1. PCR products were gel purified with either Wizard® SV Gel and PCR Clean-Up System (Promega cat# A9281) or Isolate II PCR and Gel Kit (Bioline cat# BIO-52059) according to the manufacturer's protocols. The target sequences were then cloned into the BSMV RNAγ vector pCa-γbLIC (BSMV:MCS) vector from Yuan et al. (2011) via the ligation-independent cloning protocols exactly as described in Lee et al. (2015). They were fully sequenced using primers in the viral backbone to verify the products. For BSMV:asAmPDS, both the Peldon and Rothamsted sequences were cloned. Both were shown to induce the white leaf phenotype; the results shown here are all from the Peldon sequence. The black-grass sequence is the same portion of *PDS* that was used in Lee et al. (2012).

This vector was also able to induce loss of green colour in black-grass and the loss of colour was stable when the individual tillers were separated and rooted (Supplemental Fig. S5).

The FoMV:MCS and FoMV:GFP vectors are published (Bouton et al., 2018). The primers used for the creation of FoMV:bar are in Supplemental Table 2. These introduce a NotI site at the 5' end, mutated TGA stop codons to TAA, and introduced an XbaI site at the 3' end of the sequence. The *bar* gene was amplified with Phusion ® High-Fidelity DNA polymerase (NEB cat# M0530) from the pAL156 vector (Amoah et al., 2001) and was subcloned into Zero Blunt ® TOPO PCR cloning kit for sequencing (Invitrogen cat# 450159). Once sequencing confirmed there were no PCR-induced mutations, the *bar* gene was removed with the NotI and XbaI restriction sites created via the PCR primers and inserted into the FoMV:MCS via traditional cut and paste cloning with T4 DNA ligase (Fisher cat# EL0014) following the manufacturer's protocols, and the ligated products were transformed into JM109 Competent Cells (Promega cat# L2005).

Once the BSMV and FoMV vectors were confirmed by sequencing, they were transformed into *Agrobacterium tumefaciens* strain GV3101 through standard electroporation techniques and recombinants selected based on survival of dual selection with kanamycin and gentamycin. Individual colonies were selected, multiplied, and verified by colony PCR with the appropriate primers (Supplemental Table S2).

Preparation of the virus inoculum from *Nicotiana benthamiana*

The recombinant BSMV and FoMV viruses were propagated via agroinfiltration in *Nicotiana benthamiana* using protocols detailed in Lee et al. (2015b). The leaf that was infiltrated was harvested 3-5 days after infiltration for BSMV vectors and 5-7 days after infiltration for FoMV vectors. One leaf from three different *N. benthamiana* plants were weighed into foil packets and plunged into liquid nitrogen before being stored at -80°C.

Rub-inoculation of black-grass

These protocols are based on those published in Lee et al. (2015b) and Bouton et al. (2018) with minor changes. Black-grass seedlings were grown at 27°C day / 21°C night with 16 hours of daylight for 18-29 days or until the 2 or 3 tiller stages. The second leaf on a thick tiller of each plant was chosen for rub-inoculation. To facilitate inoculation, each leaf was marked with a paint-pen, then Carborundum (Technical, SLR, Extra Fine Powder, ~ 36 µm (300 Grit), Fisher Chemical cat 10345170) was applied through a cheesecloth to evenly coat the adaxial side of the leaf. The inoculum was prepared by grinding the three agroinfiltrated *N. benthamiana* leaves in a 2:1 (w/v) ratio in 10 mM potassium phosphate buffer pH 7. The thumb and forefinger of a gloved hand were dipped in the inoculum and rubbed the length of the leaf 10 times firmly. The plants were incubated in the controlled environment room overnight (covered to create low light conditions) and were returned to standard growth conditions the following day.

Herbicide Applications and Assessments

Herbicides were applied 14 days after rub-inoculation. The herbicides applied, Fenoxaprop (Foxtrot) or Glufosinate (Challenge), are both commercially available. Treatments were fenoxaprop (Foxtrot, 69 g/l (6.9 % w/w) fenoxaprop-p-ethyl, Headland Agrochemicals) and glufosinate (Challenge-60™, 200 g/L glufosinate-ammonium, Bayer). Fenoxaprop was applied at 1.5x field rate (103.5 g/l of fenoxaprop-p-ethyl) diluted in distilled water. For Figure 4, glufosinate was applied with 0.1% Tween in distilled water at 0.5% (0.3 g/l of glufosinate-ammonium). For Supplemental Figure S1, 8 doses of Challenge-60™ and 1 Untreated were used (0.0%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% and 1.0%) in 0.1% Tween in distilled water and applied using the same methods. The herbicide was diluted to the chosen concentration then transferred into a Cooper Pegler CP 1.5 Mini Pro Sprayer bottle which was used to saturate black-grass plants. After application of fenoxaprop and glufosinate, at 28 days and 14 days later respectively, all plants had observations and photographs taken and were harvested for fresh weights of aboveground tissue.

References

Amoah, B. K., Wu, H., Sparks, C. & Jones, H. D. 2001. Factors influencing Agrobacterium-mediated transient expression of uidA in wheat inflorescence tissue. *Journal of Experimental Botany,* 52**,** 1135-1142.

Bouton, C., King, R., Chen, H., Azhakanandam, K., Bieri, S., Hammond-Kosack, K. & Kanyuka, K. 2018. Foxtail mosaic virus: A Viral Vector for Protein Expression in Cereals. *Plant Physiology,* 177**,** 1352-1367.

Gould, P., Ugarte, N., Domijan, M., Costa, M., Foreman, J., Macgregor, D., Rose, K., Griffiths, J., Millar, A., Finkenstädt, B., Penfield, S., Rand, D., Halliday, K. & Hall, A. 2013. Network balance via CRY signalling controls the Arabidopsis circadian clock over ambient temperatures. *Molecular systems biology,* 9.

Lee, W.-S., Hammond-Kosack, K. & Kanyuka, K. 2012. Barley stripe mosaic virus-mediated tools for investigating gene function in cereal plants and their pathogens: VIGS, HIGS and VOX. *Plant Physiology***,** pp.112.203489.

Lee, W.-S., Rudd, J. & Kanyuka, K. 2015. Virus induced gene silencing (VIGS) for functional analysis of wheat genes involved in Zymoseptoria tritici susceptibility and resistance. *Fungal Genetics and Biology,* 79**,** 84-88.