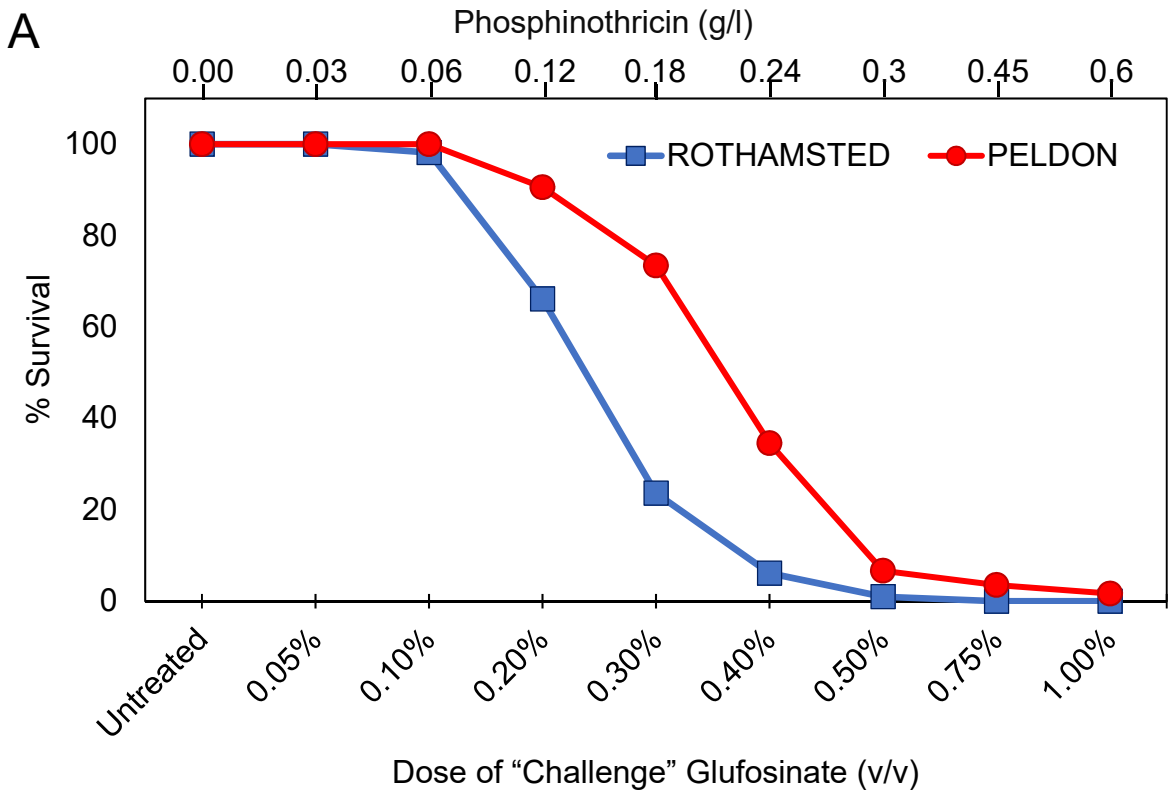

A
Phosphinothricin (g/l)
0.00
0.03
0.06
0.12
0.18
0.24
0.3
0.45
0.6
ROTHAMSTED
PELDON
% Survival
100
80
60
40
20
0
Untreated
0.05%
0.10%
0.20%
0.30%
0.40%
0.50%
0.75%
1.00%
Dose of “Challenge” Glufosinate (v/v)

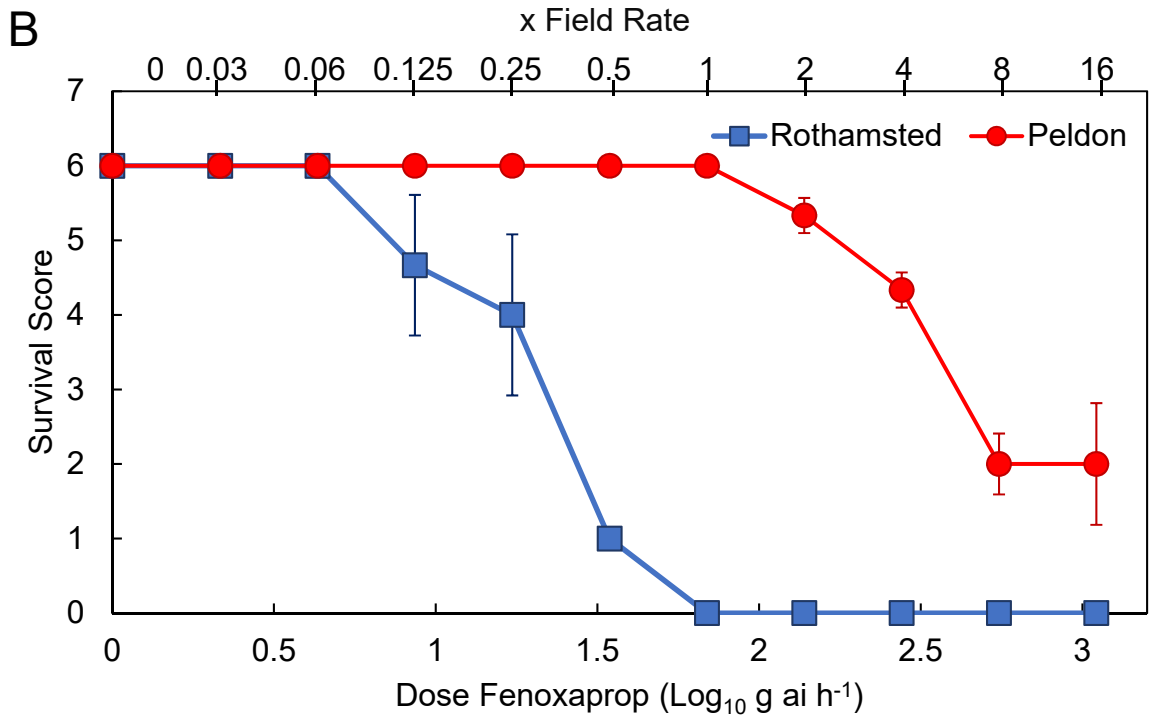

B
x Field Rate
0
0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
Rothamsted
Peldon
Survival Score
7
6
5
4
3
2
1
0
0
0.5
1
1.5
2
2.5
3
Dose Fenoxaprop (Log10 g ai h-1)

Figure S1: Dose-response curves for Rothamsted and Peldon biotypes when challenged with A) glufosinate (Challenge) or B) fenoxaprop. A) For glufosinate treatment, 0.5 grams of seed were sown within the top 0.5 cm of Weed Mix (80% Sterilised Screened Loam, 20% Grit (3-6mm Screened, Lime Free), and 2.0kg Osmocote Exact 5-6 month per m3) into containers and allowed to establish. This produced on average 108.7±6.2 plants for Rothamsted and 148.2±13.9 for plants Peldon per container. Each container was treated at the three-leaf stage with the indicated percentage of glufosinate in the form of “Challenge 60®”. 14 days after herbicide application survivors were counted. B) Three replicates of six plants of each biotype were transplanted into individual pots of Weed Mix and allowed to reach three-leaf stage under standard glasshouse conditions. These were screened for survival 4 weeks after dosing with the indicated level of fenoxaprop. Plants were scored as dead or alive. Averages of the three replicates ± standard error are shown. B) For fenoxaprop treatment, seeds were germinated in Petri dishes with filter paper and wetted with 3-4mL of 0.02M potassium nitrate; excess liquid was removed. Dishes were placed in incubators (Sanyo, MLR-350) for seven days at 14/10-hour day/night cycles at 17°C and 11°C, respectively. Seedlings that had produced a shoot and radicle at day 7 were transplanted into larger pots and grown in a glasshouse set to maintain 16°C/10°C day/night temperatures with supplementary lighting provided over a 14-hour day length. Germinated seedlings were sown into 8 cm pots containing a Kettering loam soil mixed with 2 kg m-2 Osmocote fertiliser. Six seedlings were sown per pot, with three replicate pots of each population at each herbicide dose. Plants were maintained in a glasshouse at approximately 16/10°C until the plants had reached the three-leaf stage. For herbicide application, pots were removed from the glasshouse and sprayed using a fixed track sprayer with a Teejet 110015VK nozzle mounted 50cm above the plants. Boom speed was 0.33 m s-1, delivering herbicide at a volume of 199 L ha-1. Herbicide doses are shown. Three weeks after herbicide application, sprayed and unsprayed plants were assessed for mortality using a standard visual score.

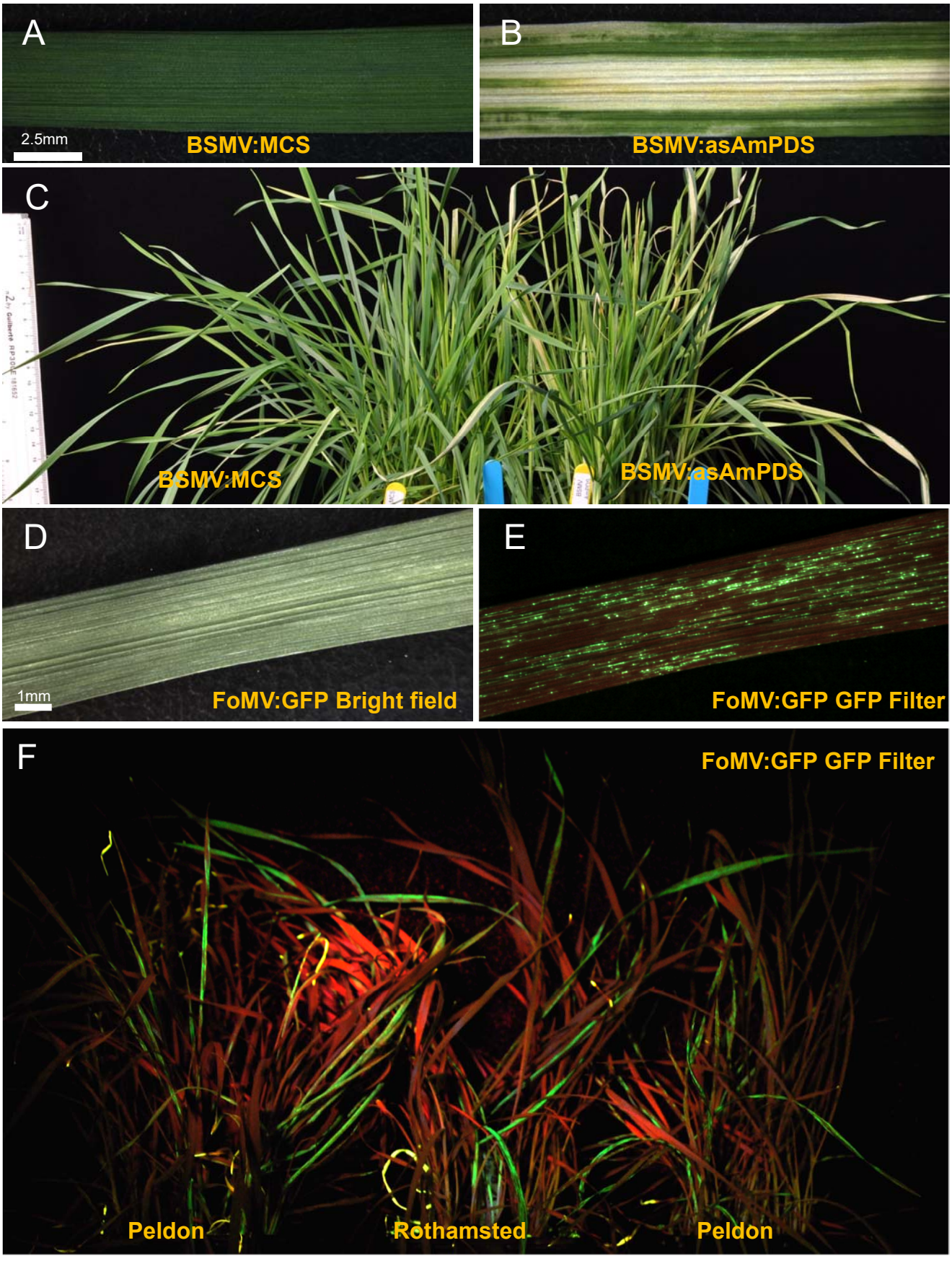
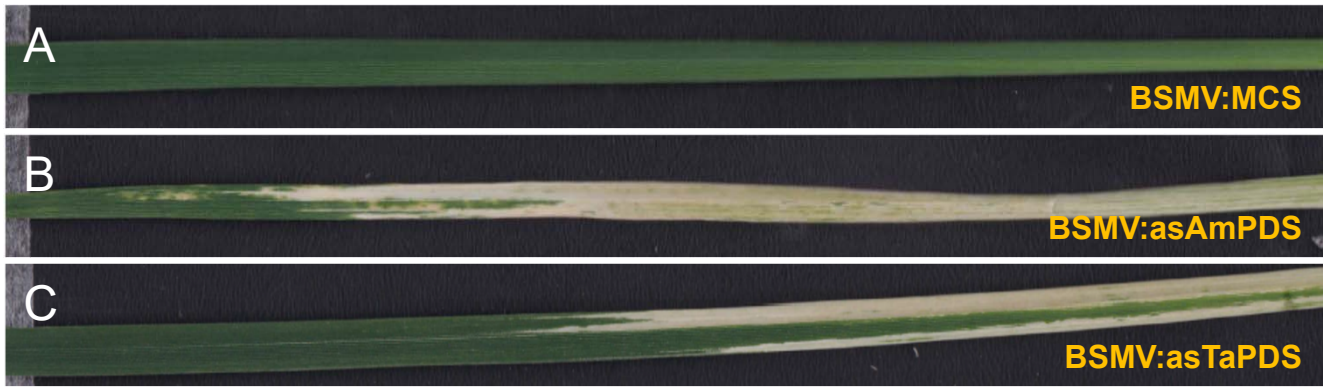

A
2.5mm
BSMV:MCS
B
BSMV:asAmPDS
C
BSMV:MCS
BSMV:asAmPDS
D
1mm
FoMV:GFP Bright field
E
FoMV:GFP GFP Filter
F
FoMV:GFP GFP Filter
Peldon
Rothamsted
Peldon

Figure S2: Virus-induced gene silencing (VIGS) and virus-mediated overexpression (VOX) are possible in black-grass in the herbicide-sensitive biotype Rothamsted equivalently to the herbicide-resistant biotype Peldon (Fig. 1). Data are representative of at least three independent replicates. A-C) Phenotypes of black-grass (Rothamsted) leaves that have been infected with *Barley stripe mosaic virus* (BSMV) carrying either A) an empty multiple cloning site (MCS), or B) the MCS with a portion of *PHYTOENE DESATURASE* (*PDS*) in antisense from black-grass (as*AmPDS*). C) Whole plant phenotypes of plants from A or B infected with BSMV:MCS or BSMV:asAmPDS as labelled. D-E) Phenotypes of black-grass (Peldon) leaves that have been infected with *Foxtail mosaic virus* (FoMV) carrying *GREEN FLUORESCENT PROTEIN* (*GFP*)-encoding gene from Bouton et al. (2018) under either D) bright field microscopy or E) using the GFP3 filter set. F) Phenotype of whole black-grass (Peldon, Rothamsted, Peldon) plants that have been infected with FoMV:GFP photographed using a Nikon D90 illuminated blue light using a Dual Fluorescent Protein flashlight through a long pass filter.


A
BSMV:MCS
B
BSMV:asAmPDS
C
BSMV:asTaPDS


D
BSMV:MCS
E
BSMV:asAmPDS
F
BSMV:asTaPDS

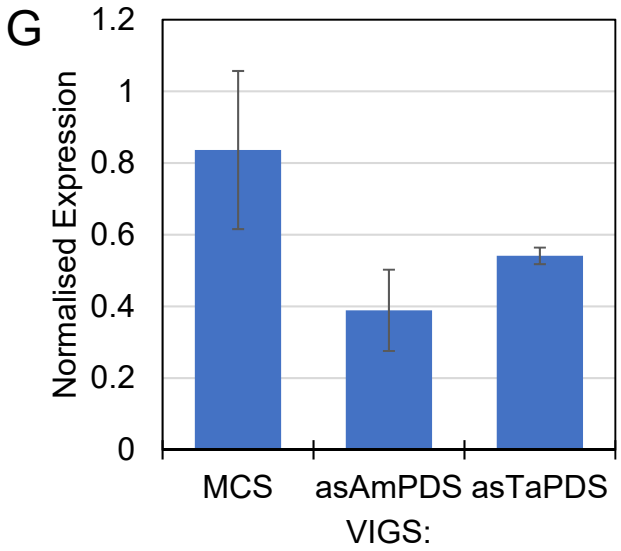
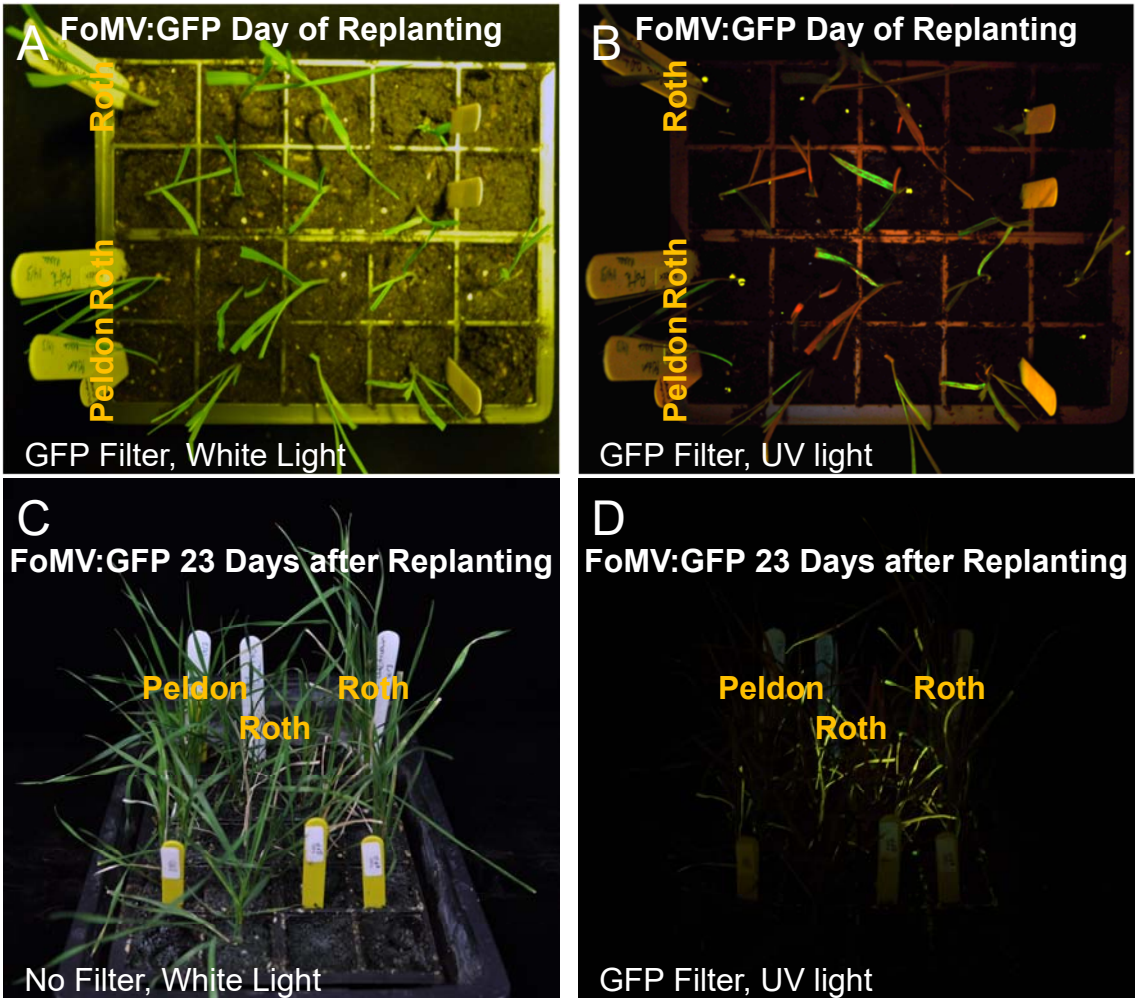

G
Normalised Expression
1.2
1
0.8
0.6
0.4
0.2
0
MCS
asAmPDS
asTaPDS
VIGS:

Figure S3: Leaf photobleaching is correlated to infection with BSMV:asTaPDS or BSMV:asAmPDS which is stable when the individual tillers are separated and rooted. A-C) Phenotypes of black-grass (Peldon) leaves that have been infected with *Barley stripe mosaic virus* (BSMV) carrying either A) an empty multiple cloning site (MCS), or the MCS with a portion of *PHYTOENE DESATURASE* (*PDS*) in antisense orientation from either B) wheat (*asTaPDS* from Lee et al., 2015) or C) black-grass (*asAmPDS*). D-F) Phenotypes of black-grass (Peldon) leaves that have been infected with *Barley stripe mosaic virus* (BSMV) carrying either D) an empty multiple cloning site (MCS), or the MCS with a 212 bp portion of *PHYTOENE DESATURASE* (*PDS*) in antisense orientation from either E) black-grass (*asAmPDS*) or F) wheat (*asTaPDS*) 59 days after individuals tillers were separated and rooted. G) qRT-PCR of *PDS* normalised against *UBIQUITIN* (*UBQ*) in Peldon plants inoculated with BSMV:MCS, BSMV:asAmPDS or BSMV:asTaPDS. The data are averages and standard errors from three independent biological replicates each.



N.B. Dead leaves photograph white under UV

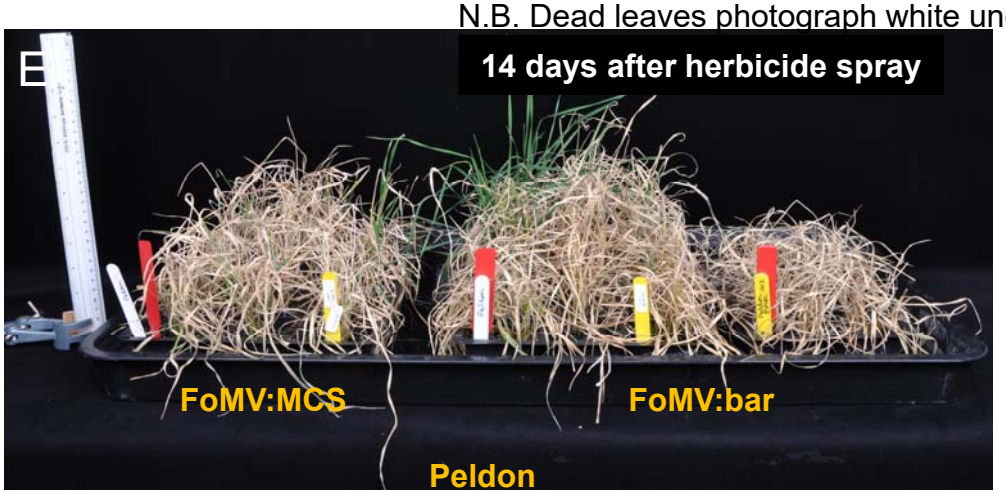


Figure S4: There is no evidence for stability of the FoMV VOX induced phenotypes when individual tillers are separated and rooted. Peldon and Rothamsted plants that were treated with FoMV:MCS, FoMV:GFP, or FoMV:bar were separated and the phenotype was challenged 14 days or 34 days after replanting. A-B) One Peldon plant and two Rothamsted plants treated with FoMV:GFP that exhibited high levels of GFP fluorescence under blue light (440-460 nm excitation) using a Dual Fluorescent Protein flashlight (Nightsea, Lexington, MA, USA) through a long pass (510 nm) filter (Midwest Optical Systems, Palatine, IL, USA) were separated into 4, 9 and 4 tillers respectively and replanted. These plants were healthy tillers (A) and the GFP was visible after replanting (B). C-D) No evidence of GFP was observed at 12, 15, 19, or 23 days after separation. Photographs were taken 23 days after separation with C) white light and no filter to demonstrate the tillers had recovered and were accumulating biomass and D) under blue light (440-460 nm excitation) using a Dual Fluorescent Protein flashlight (Nightsea, Lexington, MA, USA) through a long pass (510 nm) filter (Midwest Optical Systems, Palatine, IL, USA) to evidence the lack of GFP fluorescence. E) Peldon and F) Rothamsted plants infected with FoMV:MCS or FoMV:bar were separated and replanted. This gave FoMV:MCS 21 tillers, FoMV:bar 42 tillers, of Peldon and FoMV:MCS 20 tillers and FoMV:bar 30 tillers of Rothamsted. 34 days after replanting, plants were challenged with 0.5% Basta + 0.1% Tween. 14 days after herbicide application there were no obvious differences between FoMV:MCS and FoMV:bar for either biotype.

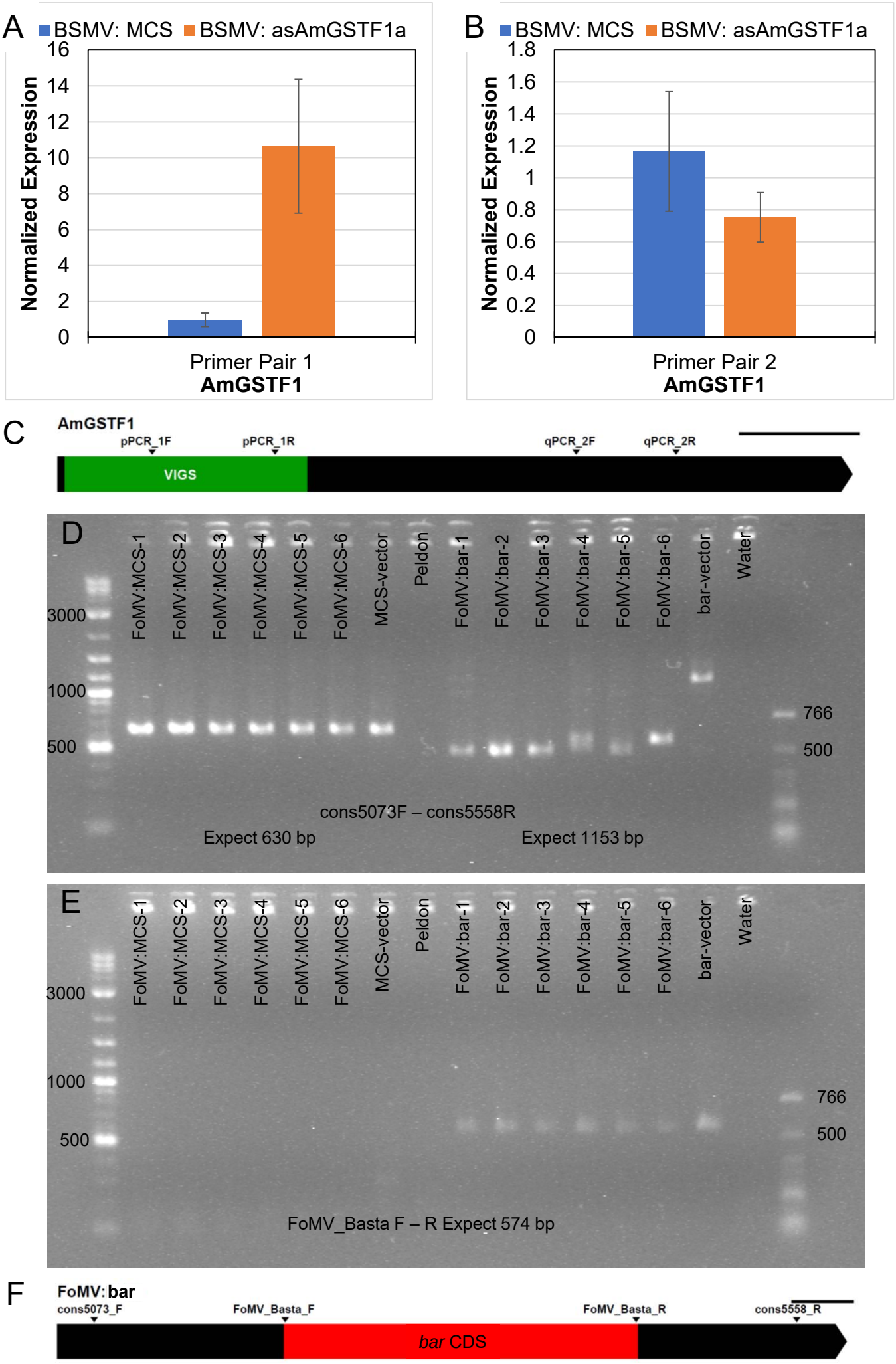

A
BSMV: MCS
BSMV: asAmGSTF1a
Normalized Expression
16
14
12
10
8
6
4
2
0
Primer Pair 1
AmGSTF1
B
BSMV: MCS
BSMV: asAmGSTF1a
Normalized Expression
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Primer Pair 2
AmGSTF1
C
AmGSTF1
pPCR_1F
pPCR_1R
qPCR_2F
qPCR_2R
VIGS
D
FoMV:MCS-1
FoMV:MCS-2
FoMV:MCS-3
FoMV:MCS-4
FoMV:MCS-5
FoMV:MCS-6
MCS-vector
Peldon
FoMV:bar-1
FoMV:bar-2
FoMV:bar-3
FoMV:bar-4
FoMV:bar-5
FoMV:bar-6
bar-vector
Water
3000
1000
500
766
500
cons5073F – cons5558R
Expect 630 bp
Expect 1153 bp
E
FoMV:MCS-1
FoMV:MCS-2
FoMV:MCS-3
FoMV:MCS-4
FoMV:MCS-5
FoMV:MCS-6
MCS-vector
Peldon
FoMV:bar-1
FoMV:bar-2
FoMV:bar-3
FoMV:bar-4
FoMV:bar-5
FoMV:bar-6
bar-vector
Water
3000
1000
500
766
500
FoMV_Basta F – R Expect 574 bp
F
FoMV:bar
cons5073_F
FoMV_Basta_F
FoMV_Basta_R
cons5558_R
bar CDS

Figure S5: Molecular data demonstrating the viruses are present in the virus-inoculated plants and alter RNA levels. These data are derived from plants equivalent to those shown in Figure 2. A-B) qRT-PCR of *AmGSTF1* normalised against *UBIQUITIN* (*UBQ*) in Peldon plants inoculated with BSMV:MCS or BSMV:asAmGSTF1. Two primer pairs used; one located within the portion of *AmGSTF1* that is expressed from the viral vector (Primer Pair 1) and a second at the 3' end of the coding sequence. Primer Pair 1 should amplify viral-derived and endogenous RNA whereas Primer Pair 2 will only amplify endogenous RNA. The data are averages and standard errors from four independent biological replicates each. C) Graphical representation of *AmGSTF1* coding sequence. Primers used (triangles) and region of *AmGSTF1* expressed in the VIGS vector (green) are identified. Black bar at the 3' end equals 100 bp. D-E) RT-PCR from plants harvested 14 days after inoculation infected with FoMV:MCS or FoMV:bar as indicated or appropriate vector and water controls. Total cDNA from uninfected Peldon plants was used as a negative control ("Peldon"). D) Primers (cons_5073F and cons_5558R) are specific to the viral vector and spanned the MCS region. PCR fragments smaller than the expected size are observed in the FoMV:bar samples indicating some insert instability as suggested previously (Bouton et al., 2018). These smaller DNA fragments will preferentially amplify during PCR. E) Primers used were specific to the *bar* coding sequence and were located at the start and stop codons of the gene. PCR fragments corresponding to the size of the full-length coding sequence of the bar gene were only seen in the FoMV:bar samples at equivalent levels to the vector control. F) Graphical representation of the *bar* gene coding sequence (red rectangle) inserted in the FoMV vector (black rectangles). Primers used (triangles) and *bar* coding sequence (red) expressed in the FoMV vector are identified. Black bar equals 100 bp.

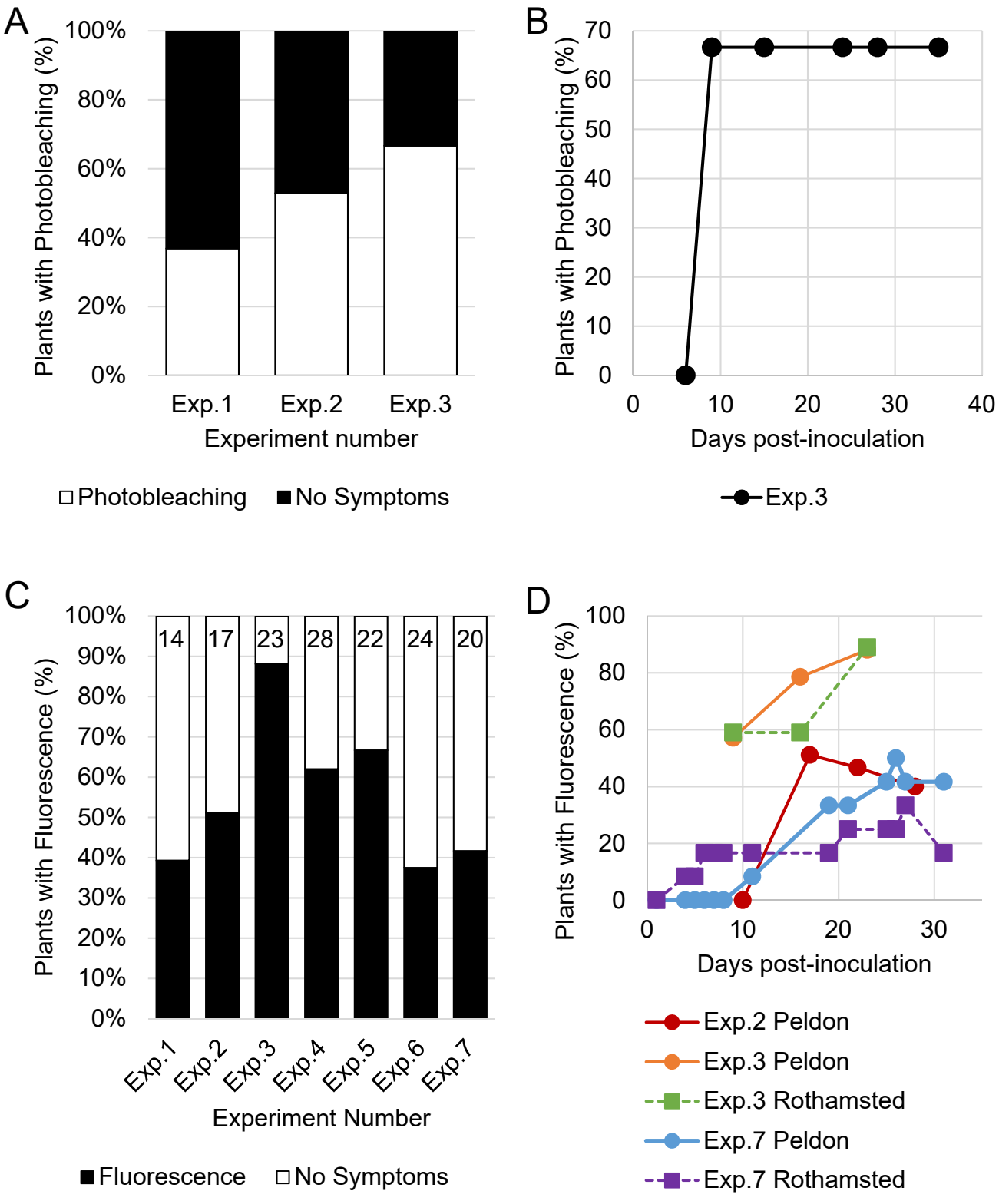

A
Plants with Photobleaching (%)
100%
80%
60%
40%
20%
0%
Exp.1
Exp.2
Exp.3
Experiment number
Photobleaching
No Symptoms
B
Plants with Photobleaching (%)
70
60
50
40
30
20
10
0
0
10
20
30
40
Days post-inoculation
Exp.3
C
Plants with Fluorescence (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
14
17
23
28
22
24
20
Exp.1
Exp.2
Exp.3
Exp.4
Exp.5
Exp.6
Exp.7
Experiment Number
Fluorescence
No Symptoms
D
Plants with Fluorescence (%)
100
80
60
40
20
0
0
10
20
30
Days post-inoculation
Exp.2 Peldon
Exp.3 Peldon
Exp.3 Rothamsted
Exp.7 Peldon
Exp.7 Rothamsted

Figure S6: The efficiency of BSMV VIGS and FoMV VOX in black-grass plants observed across experiments. A) The extent to which photobleaching is observed in plants infected with BSMV:asAmPDS in three different experiments. B) Photobleaching is stable and occurs between 6 and 9 days post-inoculation (DPI). C) The extent to which fluorescence is observed in plants infected with FoMV:GFP in seven different experiments. The values given at the top of the bars are the days post-inoculation on which these observations were taken. D) The appearance of GFP fluorescence depends on how long after inoculation the observation is taken. Similar trends were seen for both biotypes (Peldon circles connected with solid line, Rothamsted squares connected with dotted line). Therefore all sample collection for RNA and/or herbicide applications were done 14 days after inoculation when the level of GFP fluorescence was still rising. These data demonstrate that the protocols for VIGS and VOX can still be improved to increase efficiency of the infection. Very high infection levels can be obtained (upwards of 60% for VIGS-induced photobleaching and 80% for VOX-mediated GFP fluorescence) and all samples tested with RT-PCR in Fig. S5 show presence of the virus and full-length coding sequence of the insert.