Article (refereed) - postprint

Blake, Robin J.; Westbury, Duncan B.; Woodcock, Ben A.; Sutton, Peter; Potts, Simon G.. 2012 Investigating the phytotoxicity of the graminicide fluazifop-P-butyl against native UK wildflower species. *Pest Management Science*, 68 (3). 412-421. 10.1002/ps.2282

Investigating the phytotoxicity of the graminicide fluazifop-P-butyl against native UK wildflower species

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Running Title: Investigating fluazifop-P-butyl against native UK wildflower species

Abstract

BACKGROUND: The selective graminicide fluazifop-P-butyl is used for the control of grass weeds in dicotyledonous crops, and commonly applied in amenity areas to reduce grass productivity, and promote wildflower establishment. However, evidence suggests that

fluazifop-P-butyl might also have phytotoxic effects on some non-target plants. This study investigates the effects of fluazifop-P-butyl on the emergence, phytotoxicity and above-ground biomass of nine perennial wildflower species, and two grass species, following pre and post-emergent applications at half, full and double label rates in a series of glasshouse experiments.

RESULTS: Whilst pre- and post-emergent applications of fluazifop-P-butyl caused reductions in seedling emergence and increased phytotoxicity on native wildflower and grass species, these effects were temporary for the majority of wildflower species tested, and generally only occurred at the double application rate. No differences in biomass were observed at any of the rates, suggesting good selectivity and no long-term effects of fluazifop-P-butyl application on the wildflower species from either pre-emergent or post-emergent applications.

CONCLUSION: These results have direct relevance to the management of amenity areas for biodiversity as they confirm the suitability of these wildflower species for inclusion in seed mixtures where fluazifop-P-butyl is to be applied to control grass productivity.

Keywords: Buffer strips; Fusilade Max; grasses; management

1 INTRODUCTION

Graminicides are selective herbicides that are widely used for the post-emergent control of annual and perennial grasses (Poaceae) in dicotyledonous crops.[1] Non-crop applications of graminicides are becoming increasingly common to reduce grass productivity and control problematic or invasive grasses such as *Avena fatua* Linnaeus and *Elytrigia repens* (L.) Desv. ex Nevski that can threaten native biodiversity in forestry,[2] moorland

restoration,[3] pastures,[4] and urban parks and meadows.[5,6] Previous studies have also demonstrated that sown wildflower development in buffer strips can be promoted through a reduced application rate of graminicide, with the objective of suppressing competitive grasses rather than eliminating them.[7,8] Sowing wildflowers in agricultural landscapes is increasingly being used to boost pollen and nectar sources for insect pollinators, and enhancing existing habitats such as buffers strips represents a potentially important method of achieving this.[9] Buffer strips are non-cropped areas of perennial vegetation, and whilst they can be an important habitat for generalist predatory invertebrates such as beetles and spiders,[10] they are typically established with only a few grass species, and as such the plant diversity tends to be limited.[8] Introducing wildflowers into these strips can increase their biodiversity value, for example by providing foraging resources for bumblebees.[9] However, the existing vegetation tends to be highly competitive and suppression of the grass component is necessary to promote the establishment and development of the introduced wildflowers.[11] Graminicides including cycloxydim, alloxydim and fluazifop-P-butyl,[12] and growth retardants such as paclobutrazol,[13] have all been investigated for use in buffer strips. However, much of the subsequent work has focussed on fluazifop-P-butyl, and its predecessor fluazifop-butyl, for which there is a large dataset available.[14] In addition, fluazifop-P-butyl has a label use for non-cropped buffer strips.[2] Following application, fluazifop-P-butyl, and fluazifop-butyl, are rapidly absorbed into the leaves of target grasses, hydrolysed to the active form fluazifop-P, and translocated to the site of active growth where they disrupt lipid synthesis through the inhibition of acetyl coenzyme A carboxylase [15].

Whilst fluazifop-P-butyl has been observed to promote wildflowers[7-9], evidence suggests that fluazifop-P-butyl might also be having a negative impact on the species being introduced. For example, glasshouse trials with fluazifop-P-butyl showed adverse effects on germination, emergence and establishment of native woodland dicotyledons (*Banksia* spp.

(Family Proteaceae)).[5] Similarly, following laboratory applications of fluazifop-butyl, wilting and necrosis has been demonstrated in the dicotyledonous weed *Acanthospermum hispidum* DC. (Family Asteraceae).[16]

Given the increased use of fluazifop-P-butyl in amenity areas, investigating the effects of fluazifop-P-butyl against native wildflower species will have direct relevance to the management of areas for biodiversity. For example, this may allow recommendations on which species should be sown in future wildflower areas that are likely to be exposed to the graminicide. The aim of this study was to investigate potential detrimental effects of both pre- and post-emergent applications of fluazifop-P-butyl on wildflower and grass species commonly sown into buffer strips. In addition, we attempted to elucidate whether any observed effects are due to the active ingredient or the presence of another compound within the formulation. While commercial herbicides are routinely formulated with adjuvants such as surfactants and wetting agents, that enhance the effectiveness of the active ingredient,[1] these compounds can cause increased phytotoxicity.[17] We tested the following predictions: (1) Pre- and post-emergent applications of fluazifop-P-butyl will not have a detrimental effect on the emergence, phytotoxicity or above-ground biomass of the wildflower species investigated; (2) Any observed effects will be due to the presence of the active ingredient and not due to another component of the formulation.

2 EXPERIMENTAL METHODS

All experiments were performed in climate-controlled glasshouses with artificial lighting (set to 20 ºC; 60 % relative humidity; 16 hour photoperiod) at Syngenta, Jealott's Hill International Research Centre, Bracknell, UK between October and December 2009. A light meter (Skye Quantum Sensor, Skye Instruments, UK) reading taken under the artificial

lights was in the range 180-230 μ mol m^{-2} sec^{-1}. Fertiliser was applied to all plants at every watering using a 30 % stock solution of NPK (18-11-18) fertiliser, with a Dosatron diluter, set at 0.4 %, and producing a final dilution of 1.2 g L^{-1}.

2.1 Plant species

The study species consisted of nine perennial native wildflower species commonly included in buffer strip seed mixtures: *Achillea millefolium* L. (Am), *Centaurea nigra* L. (Cn), *Galium verum* L. (Gv), *Leucanthemum vulgare* Lamarck (Lv), *Lotus corniculatus* L. (Lc), *Plantago lanceolata* L. (Pl), *Rumex acetosa* L. (Ra), *Silene dioica* L. (Sd) and *Trifolium pratense* L (Tp). In addition, the responses of two perennial native grasses, *Dactylis glomerata* (Dg) and *Festuca rubra* (Fr), commonly sown in grass seed mixtures were investigated. Whilst *F. rubra* is resistant to fluazifop-P-butyl,[18] *D. glomerata* is susceptible,[4] thus the inclusion of *D. glomerata* was to verify the performance of fluazifop-P-butyl. No seeds were pre-treated prior to sowing.

2.2 Test design and application details

The study consisted of three experiments. Experiments 1 and 2 investigated the pre-emergent (Experiment 1) and post-emergent (Experiment 2) activity of formulated fluazifop-P-butyl on the nine wildflower species and two grasses. The formulation used was Fusilade Max 125 g L^{-1} EC, an emulsifiable concentrate containing 12.5 % w/v fluazifop-P-butyl and wetting agents (Syngenta Crop Protection Ltd.). It was diluted in deionised water and applied at rates of 93.75, 187.5 and 750 g a.i. ha^{-1}. The application rates of 93.75 and 187.5 g a.i. ha^{-1}

correspond to the half and full label rate permitted for use in non-cropped buffer strips, and 750 g a.i. ha^{-1} represents double the maximum application rate of 375 g a.i. ha^{-1}.[2] Hereafter, these rates will be referred to as half (93.75 g a.i. ha^{-1}), full (187.5 g a.i. ha^{-1}) and double (750 g a.i. ha^{-1}) rate. Experiment 3 investigated if the observed effects in Experiments 1 and 2 were due to the active ingredient or another component of the formulation such as a wetting agent. Formulated fluazifop-P-butyl was applied post-emergence, together with a blank formulation of fluazifop-P-butyl containing the wetting agents without the active ingredient, and at the full and double rates. Both the formulation and blank were diluted in deionised water. All applications were conducted with a laboratory sprayer fitted with a Tee-Jet flat-fan nozzle, operating at a pressure of 2.0 bars, and a water volume of 200 L ha^{-1}. The sprayer speed was 81 cm per second, with a target height of 30 cm.

2.3 Experiment 1: Pre-emergent activity

Seeds of all species were sown to a depth of approximately 0.5 cm in a sandy loam soil (Trough Mix A) contained in 9 cm diameter plastic pots. Each pot was sown with a single species at a density of 20 seeds per pot. The soil characteristics were 75 % unsterilized Surrey loam and 25 % sharp sand (organic matter 1.4 % Walker Black, pH 7.2, pH $CaCl_2$ 6.6, sand 53 %, silt 31 %, clay 16 %). The procedure was repeated so that there were nine replicate pots per species and application rate. This was the maximum replication possible based on the glasshouse space available. Following sowing the soil surface was firmed down to ensure seed to soil contact. Additional unsown controls were used to check if the study plant species were also found in the soil seed bank. Fluazifop-P-butyl was applied to the soil surface at the half, full and double application rates using the laboratory sprayer. The control plants were left untreated. Following application the pots were moved back to the glasshouse bay and arranged

in three randomised blocks, with three replicate pots of each species and application rate per block. The pots were watered as necessary.

Plants were visually assessed for leaf damage relative to the control treatment at 7, 14, 21 and 28 days after treatment (DAT) using a percentage scale (0 = healthy; 100 = dead). Typical leaf damage symptoms included chlorosis, necrosis, stunting and reduced vigour. The number of plants emerged in each replicate pot was recorded on each assessment day. Following the 28 DAT assessment, all above-ground vegetation from each treatment replicate was harvested and placed into a labelled paper bag. The bags were placed into a drying oven (Termaks TS 8430 Drying Oven, Bergen, Norway) at 60 ºC for 48 hours, and the dry matter contents weighed using a balance (Sartorius ME2545, Germany) and recorded.

2.4 Experiment 2: Post-emergent activity

The plant species were sown as for Experiment 1, with nine replicate pots per species and application rate. Following sowing all pots were placed into the glasshouse bay and watered as necessary. Planting dates were staggered so that the growth stage of all species had reached two to four leaves by the application date (approximately three to four weeks after sowing). Prior to application the plants were thinned to five plants per pot.

Fluazifop-P-butyl was applied to the plants at the half, full and double application rates using the laboratory sprayer. The control plants were left untreated. Following application the pots were moved back to the glasshouse bay, randomised and watered as per Experiment 1.

Plants were visually assessed for leaf damage relative to the control treatment at 3, 7, 14 and 21 days after treatment (DAT) using a percentage scale (0 = healthy; 100 = dead). Following the 21 DAT assessment, the dry matter content was determined as per Experiment 1.

2.5 Experiment 3: Adjuvant activity

Based on the results of Experiments 1 and 2 only *G. verum*, *P. lanceolata*, *R. acetosa*, *S. dioica*, *T. pratense*, and *D. glomerata* were tested in Experiment 3, with four replicates per species and application rate. The plants were set up and sprayed as per Experiment 2. Following application the pots were moved back to the glasshouse bay and arranged in four randomised blocks, with one replicate pot of each species and application rate per block. The pots were watered as necessary. Assessments were conducted relative to the untreated controls as per Experiment 2. Following the 21 DAT assessment, the dry matter content was determined as per Experiment 1.

2.6 Data analysis

Percentage plant emergence, percentage phytotoxicity and above-ground biomass were determined for each treatment block to reduce the impacts of variation associated with different locations in the glasshouse. Repeated-measures analysis using general linear mixed models in SAS 9.2[19] were used to analyse the response of the individual plant species to the treatment effects and categorical environmental variables. Response variables were the plant emergence (arcsine square root transformation), phytotoxicity (arcsine square root) and biomass ($\log_e n + 1$). The analysis was divided into three separate models. Models 1 and 2 tested for the responses of the individual plant species to treatment effects from Experiments 1 and 2 respectively. Both models included the fixed effects of explanatory variables of time (Time), treatment (Treat), and their interactions. Model 3 tested for the responses of the individual plant species (*G. verum*, *P. lanceolata*, *R. acetosa*, *S. dioica*, *T. pratense* and *D.*

glomerata) to treatment effects from Experiment 3 (adjuvant activity), and included the explanatory variables of time, rate (Rate), treatment, and their interactions. All models used an autoregressive covariance structure to account for increased similarity between repeated measures in subsequent assessment dates. Block was used as a random effect. Solutions for both fixed explanatory and random effects were estimated using the residual maximum likelihood approach, with denominator degrees of freedom calculated using Kenward Rogers approximation. All models were simplified by deletion of non significant terms (except where they were part of a significant interaction). Significance values were derived from F-ratios of fixed effects, calculated using adjusted sums of squares where the final minimum adequate model contained only those parameters that had significant F-values, or were part of significant interaction terms. Between-treatment differences in response variables were tested using *post hoc* Tukey's multiple comparison test ($P = 0.05$). All comparisons of statistical difference due to the experimental treatments were made relative to the untreated control treatment of that species.

3 RESULTS

3.1 Experiment 1: Pre-emergent activity

The emergence of all species responded to time, and Tukey's test revealed significant differences between the emergence at seven days, and at 15, 21 and 28 days ($P < 0.05$) (Table 1). *Achillea millefolium*, *C. nigra*, *P. lanceolata*, *R. acetosa*, *D. glomerata* and *F. rubra* responded to the graminicide treatment, however clear patterns were only observed with *R. acetosa* and *D. glomerata*. The emergence of the *R. acetosa* control plants was higher than

for the three graminicide treatments ($P < 0.05$). For *D. glomerata*, Tukey's test revealed a trend of decreasing emergence with increasing treatment rate (control > half > full > double). *Dactylis glomerata* did not emerge at the double rate. No significant interactions between treatment and time were observed for any species.

Interactions between treatment and time were identified for the response of phytotoxicity to *D. glomerata* and *F. rubra* with symptoms of chlorosis, necrosis, stunting and reduced vigour. Differences between the control, and half and full rates, existed in *D. glomerata* at all assessment days ($P < 0.05$), with a significant difference between the half and full rates at 21 days. For *F. rubra*, the double rate produced a significantly higher phytotoxicity compared to the control, half and full rates at 15, 21 and 28 days. *Trifolium pratense* responded weakly to treatment. Whilst Tukey's test revealed a difference between the control and double rate, this equated to an average of < 5 % phytotoxicity damage (chlorosis and necrosis of leaves) (RJ Blake, *unpublished data*).

None of the wildflower species differed in growth stage between the control and graminicide treatments at day 28. There were differences between the *D. glomerata* control and half rate (two leaves to one tiller), and full rate (two leaves to 0.5 tillers). For *F. rubra*, the growth stage of the double rate treatment was emerging to two leaves, with the control, half and full rate treatments identical at cotyledons to one tiller.

Significant treatment effects on biomass were observed for *L. corniculatus*, *D. glomerata* and *F. rubra*. *Lotus corniculatus* responded positively with a higher biomass at the half rate ($P < 0.05$). In contrast, *D. glomerata* biomass was severely affected by the graminicide, with significant differences between the control and all rates, and also between the half and full rate, and double rate. For *F. rubra* there was a significantly lower biomass at the double rate.

3.2 Experiment 2: Post-emergent activity

Significant interactions between treatment and time were identified for the response of phytotoxicity to *G. verum*, *L. vulgare*, *L. corniculatus*, *P. lanceolata*, *R. acetosa*, *T. pratense*, and *D. glomerata* (Table 2, Figure 1). In *G. verum*, chlorosis and damage to the growing points caused significantly higher phytotoxicity values for all graminicide treatments at day three, although by day seven only the double rate treatment was significantly different, and no difference was observed at days 14 and 21 ($P < 0.05$). Phytotoxicity was only observed at the double rate in *L. vulgare* (day three) and *L. corniculatus* (days three and seven) with symptoms of leaf curl/distortion, chlorosis, and damage to growing points. The double rate produced a significantly higher phytotoxicity at all assessment days with *P. lanceolata* and *R. acetosa*, with symptoms of leaf curl/distortion, chlorosis, reduced vigour and leaf necrosis. There were also differences between the half and full rates, and the double rate, at all assessment days (*R. acetosa*) and day three (*P. lanceolata*). For *T. pratense*, differences were observed between the control, and the full and double rates at days three and 21, and between the control and all three graminicide rates at days seven and 14 (chlorosis, reduced vigour, leaf necrosis). As expected there were significant differences in phytotoxicity (chlorosis, necrosis, stunting and reduced vigour) between the control and all rates for *D. glomerata* at all assessment days. There was also a higher phytotoxicity at the double rate compared to the half and full rates from days seven to 21. Despite no interaction between treatment and time for *S. dioica* and *F. rubra*, both species did respond to treatment, with chlorosis, leaf curl and leaf necrosis symptoms being greater at the double rate. *Achillea millefolium* and *C. nigra* showed no phytotoxic responses.

None of the wildflower species, or *F. rubra*, differed in growth stage between the control and graminicide treatments at day 21. However, *D. glomerata* was severely affected

with differences between the control (one to three tillers), half rate (three leaves to one tiller), and full and double rates (three to four leaves).

Significant treatment effects on biomass were observed in *T. pratense*, *D. glomerata* and *F. rubra*. There was a lower biomass at the double rate, compared with the half and full rates in both *T. pratense* and *F. rubra* ($P < 0.05$), and a higher biomass in the *D. glomerata* control compared to all three graminicide rates.

3.2 Experiment 3: Adjuvant activity

Significant interactions were observed between treatment, rate and time for *G. verum*, *R. acetosa*, *S. dioica* and *T. pratense* (Table 3, Figure 2). For *G. verum*, the control, and full rate formulation and blank (187.5 g a.i. ha^{-1}) were significantly different to the double rate formulation and blank (750 g a.i. ha^{-1}) (days three and 7) ($P < 0.05$). The double rate formulation also produced a higher phytotoxicity compared to the double rate blank at day three. For *R. acetosa*, the control and full rate blank were different to the full and double rate formulation, and double rate blank (days three and seven) with the highest phytotoxicity as follows: double formulation > double blank > full rate formulation. In addition, the double rate formulation produced a significantly higher phytotoxicity at day 21. For *S. dioica*, the double rate formulation and blank were significantly different from the control and full rate formulation and blank from days three to 21. In addition the full rate formulation differed from the control at day three, and the double rate formulation produced a higher phytotoxicity than the double rate blank at day seven. For *T. pratense*, the double rate formulation and blank produced consistently higher phytotoxicity values from days three to 21 relative to the control and full rate formulation and blank. In addition, the full rate formulation differed from the control at days seven to 21, whilst the full rate blank differed at day seven only.

There were significant interactions between treatment and rate for *P. lanceolata* and *D. glomerata* (Table 3, Figure 3). For *P. lanceolata* all treatments were different from the control with the highest phytotoxicity observed at the highest rate (double formulation > double blank > full rate formulation and blank) ($P < 0.05$). All treatments caused significant phytotoxicity in *D. glomerata*, with the highest values for the formulation (double and full rate formulation > double rate blank > full rate blank).

Leaf curl, necrosis and reduced vigour symptoms were observed in all species regardless of treatment, with chlorosis also apparent in *G. verum*, *T. pratense* and *D. glomerata*. None of the wildflower species differed in growth stage between the control and any treatments at day 21. The control and blank treatment plants of *D. glomerata* were at three to four tillers at day 21, with the formulation plants only at one tiller.

For the biomass, *T. pratense* responded weakly to treatment with a significant difference between the control and blank treatment ($P < 0.05$). Significant treatment effects were observed with *D. glomerata*, with a lower biomass in the formulation treatments, relative to the control and blank formulation treatments.

4 DISCUSSION

Our study has demonstrated that whilst application of fluazifop-P-butyl can have some effects on native wildflower and grass species, these effects were temporary for the majority of species tested, and generally only occurred at the double application rate of 750 g a.i. ha^{-1}.

No effects on seedling emergence, phytotoxicity or above-ground biomass were observed in *A. millefolium* and *C. nigra* following pre- and post-emergent fluazifop-P-butyl

applications confirming their suitability for use in amenity areas where fluazifop-P-butyl is applied.

Lower seedling emergence was observed in *R. acetosa* following pre-emergent applications of fluazifop-P-butyl at all rates (93.75, 187.5 and 750 g a.i. ha^{-1}). These results support a previous study that showed reductions in seedling emergence of over 70 % in *Veronica persica* Poiret following fluazifop-butyl application to soil, albeit at a much higher rate of 1600 g a.i. ha^{-1} (over four times the maximum field rate).[20] However, the emergence of *R. acetosa* seedlings in the control was only 44 % on average (RJ Blake, *unpublished data*), suggesting that other factors such as growth requirements may also be important, although this was not investigated in this study. For example, the importance of burial depth has been previously demonstrated, with fatal germination occurring in untreated soil when *R. acetosa* seeds were either sown on the surface or at a depth of one centimetre.[21] Furthermore, field experiments have demonstrated no emergence of *R. acetosa* seeds sown into existing buffer strips, even when no graminicide was applied.[8]

Phytotoxicity was observed in the majority of wildflower species following post-emergent applications, and also at low levels (< 5 %) in *T. pratense* following pre-emergent application. As expected, the greatest levels of post-emergent phytotoxicity (< 25 %) were observed at the double rate, with symptoms persisting until the end of the experiment (21 days) in *G. verum*, *P. lanceolata*, *R. acetosa* and *T. pratense*. These effects demonstrate the importance of following label application rates to minimise adverse effects to non-target species such as wildflowers. The full (187.5 g a.i. ha^{-1}) and half (93.75 g a.i. ha^{-1}) rates represent more realistic application rates, and have relevance for many amenity uses including non-cropped buffer strips and pastures.[2] Application at the full rate produced phytotoxicity in *R. acetosa* and *T. pratense*, and although this persisted throughout the experiment, the average effects were less than 10 %. Temporary phytotoxicity was observed

in *G. verum* and *P. lanceolata*, with no effects in *L. corniculatus*, *L. vulgare* or *S. dioica* confirming their suitability for use in amenity areas where fluazifop-P-butyl is applied. Lower levels of phytotoxicity were observed at the half rate with temporary effects of less than 10 % in *G. verum*, *P. lanceolata* and *T. pratense* only. Our results support previous studies that have demonstrated approximately 15 % phytotoxicity in *Solanum lycopersicum* L. (Tomato) six weeks after application of fluazifop-butyl at 100 g a.i. ha^{-1},[22] and 18 % phytotoxicity in *Rhododendron obtusum* Planch. (Azalea) following fluazifop-P application at 280 g a.i. ha^{-1}.[23] The effects observed in *R. obtusum* were not apparent after six weeks, suggesting that if our study was continued for longer than 21 days, then the observed symptoms in *R. acetosa* and *T. pratense* following the full rate application may have disappeared.[23] These responses suggest that herbicide selectivity is important with some wildflower species affected more than others. Factors affecting selectivity include differences in metabolism rate by plant species, differences in uptake and transport, and most importantly, differences at the enzyme active site.[24] Although the level of phytotoxicity observed in the wildflowers at the half and full rates was less than 10 %, such effects might be expected to reduce the ability of these species to compete with other plants in the short-term for nutrients and light, and ultimately, reduce photosynthetic capacity;[25] in turn, affecting plant community composition. However, no effects on above-ground biomass at the half and full application rates were observed, and field experiments have demonstrated that a full rate application of fluazifop-P-butyl actually increased the cover of the sown wildflowers investigated in this study.[26] Thus, the ability of the wildflower species to photosynthesise or compete with other species is unlikely to be affected. A further consideration is the translation of phytotoxic effects from the glasshouse to the field. Previous studies have demonstrated that plants can show greater susceptibility to herbicides when grown under glasshouse conditions,[27,28]

therefore the effects noted in our study might be expected to be further diluted under field conditions.

The performance of *D. glomerata* and *F. rubra* may have implications for fluazifop-P-butyl application where non-target grasses are present. In our study, *D. glomerata* was susceptible to pre- and post-emergent fluazifop-P-butyl applications, whereas *F. rubra* was generally resistant except at the double rate. Although *F. rubra* has been shown to be resistant to fluazifop-P-butyl at a rate of 250 g a.i. ha^{-1},[18] the rate used in our study was three times higher and probably accounted for the observed effects. Fluazifop-P-butyl does not show selectivity between problematic and non-target, native grasses,[2,5] thus control requirements should be balanced against biodiversity objectives in areas where non-target, native species are present.

Prediction two attempted to elucidate whether any observed post-emergent effects were due to the active ingredient or adjuvant. Adjuvants including wetting agents are added to herbicide formulations to enhance activity by improving spreading and retention on leaf surfaces, thereby increasing uptake or translocation within plants.[29,30] As expected, the double rate of formulation and blank produced consistently higher phytotoxicity for all the wildflower species, and these observations support previous studies that have demonstrated that adjuvants can cause phytotoxicity damage in plants.[17,23,31] However, given the increased phytotoxicity observed with the formulation, other factors such as the activity of the active ingredient, or environmental conditions should be considered.[31] Formulation type and the presence of adjuvants can also affect selectivity, and the use of different adjuvants or formulation types may help to reduce the levels of phytotoxicity observed in the wildflower species;[17,24] however, this was not investigated in this study. The biomass of *G. verum*, *P. lanceolata*, *R. acetosa* and *S. dioica* was not reduced by any of the treatments suggesting no long-term consequences. However, the blank treatment, together with the double rate of the

formulation in Experiment 2, did produce a weakly significant reduction in *T. pratense* biomass. Whilst the presence of wetting agents may have caused the observed effects, fluazifop-P-butyl has been demonstrated to reduce nodulation, and therefore biomass, in the nitrogen-fixing legume *T. repens*.[32] As nitrogen fixation can be an important determinant when choosing to grow legumes, for example, in pastures, further work to investigate fluazifop-P-butyl activity on the nodulation of *T. pratense* and other legumes such as *L. corniculatus* would be beneficial.

5 CONCLUSIONS

The application of graminicides is increasingly common in amenity areas to reduce grass productivity and control problematic and invasive grasses, and promote desirable species such as wildflowers. Whilst annual mowing offers an alternative to graminicides, it tends to be labour intensive and encourages tillering of the grasses which can shade desirable species, essentially creating a grass-dominated sward.[25] The dicotyledon *Rhinanthus minor* L. has also been used successfully in grassland restoration to reduce sward productivity;[7] however studies have highlighted its inability to persist long-term.[33] Furthermore, the creation of gaps is considered to be important for its successful establishment, therefore a combined approach using *R. minor* and fluazifop-P-butyl may help suppress grass growth and promote desirable species, both in the short and long-term.[7] This study has demonstrated that whilst pre- and post-emergent applications of fluazifop-P-butyl can affect the emergence, phytotoxicity and biomass of wildflowers, these effects were mainly observed at double the maximum application rate (750 g a.i. ha^{-1}). No long-term effects on the perennial wildflowers tested were observed at the recommended rates for buffer strips, parks, meadows and pastures (93.75 and 187.5 g a.i. ha^{-1}). As wildflower establishment in existing vegetation

is unlikely to be successful in the absence of graminicide due to the competitive dominance of the grasses,[8] short-term effects from fluazifop-P-butyl application on wildflowers could be tolerated as the net benefit for pollinators and other invertebrates from having wildflowers present is likely to outweigh the risks. Our results, therefore, have direct relevance to the management of these areas for biodiversity as they confirm the suitability of these wildflower species for inclusion in seed mixtures where fluazifop-P-butyl is to be applied to control grass productivity or problematic species.

6 ACKNOWLEDGEMENTS

This project was funded by the Biotechnology and Biological Sciences Research Council and Syngenta. Thanks to Matt Cordingley, Kay Fullick and James Murdock from Syngenta for their assistance with the project.

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Table 1 Summary of plant emergence, phytotoxicity and biomass model outputs to pre-emergent treatments (Model 1, Experiment 1)

Species	Percentage emergence (Arcsine square-root)	Percentage phytotoxicity (Arcsine square-root)	Above ground biomass ($\log_e n + 1$)
Achillea millefolium	Treat: $F_{3,39} = 6.59$** Time: $F_{3,39} = 10.44$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Centaurea nigra	Treat: $F_{3,41} = 7.79$*** Time: $F_{3,41} = 53.01$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Galium verum	Treat: NS Time: $F_{3,42} = 57.33$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Leucanthemum vulgare	Treat: NS Time: $F_{3,42} = 84.98$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Lotus corniculatus	Treat: NS Time: $F_{3,42} = 8.53$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: $F_{3,8} = 12.28$**
Plantago lanceolata	Treat: $F_{3,39} = 19.23$*** Time: $F_{3,39} = 26.83$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Rumex acetosa	Treat: $F_{3,39} = 5.69$** Time: $F_{3,39} = 77.06$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Silene dioica	Treat: NS Time: $F_{3,42} = 186.03$*** Treat × Time: NS	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Trifolium pratense	Treat: NS Time: $F_{3,42} = 3.04$* Treat × Time: NS	Treat: $F_{3,42} = 3.71$* Time: NS Treat × Time: NS	Treat: NS
Dactylis glomerata	Treat: $F_{3,39} = 807.33$*** Time: $F_{3,39} = 9.34$*** Treat × Time: NS	Treat: $F_{2,22} = 230.78$*** Time: $F_{3,22} = 61.25$*** Treat × Time: $F_{6,22} = 18.54$***	Treat: $F_{3,8} = 29.43$***
Festuca rubra	Treat: $F_{3,39} = 40.72$*** Time: $F_{3,39} = 8.99$*** Treat × Time: NS	Treat: $F_{3,32} = 594.83$*** Time: $F_{3,32} = 84.75$*** Treat × Time: $F_{9,32} = 84.75$**	Treat: $F_{3,6} = 24.97$***

NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Coding for environmental variables is given in Section 2.6. Non-significant terms removed from models by stepwise deletion.

Table 2 Summary of plant phytotoxicity and biomass model outputs to post-emergent treatments (Model 2, Experiment 2)

Species	Percentage phytotoxicity (Arcsine square-root)	Above ground biomass ($\log_e n + 1$)
Achillea millefolium	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Centaurea nigra	Treat: NS Time: NS Treat × Time: NS	Treat: NS
Galium verum	Treat: $F_{3,30} = 41.20$*** Time: $F_{3,30} = 23.31$*** Treat × Time: $F_{9,30} = 3.44$**	Treat: NS
Leucanthemum vulgare	Treat: $F_{3,30} = 3.67$* Time: $F_{3,30} = 3.67$* Treat × Time: $F_{9,30} = 3.67$**	Treat: NS
Lotus corniculatus	Treat: $F_{3,32} = 7.77$*** Time: NS Treat × Time: $F_{9,32} = 2.63$*	Treat: NS
Plantago lanceolata	Treat: $F_{3,32} = 95.16$*** Time: $F_{3,32} = 6.69$** Treat × Time: $F_{9,32} = 3.08$**	Treat: NS
Rumex acetosa	Treat: $F_{3,30} = 394.49$*** Time: $F_{3,30} = 8.67$*** Treat × Time: $F_{9,30} = 5.48$***	Treat: NS
Silene dioica	Treat: $F_{3,44} = 65.87$*** Time: NS Treat × Time: NS	Treat: NS
Trifolium pratense	Treat: $F_{3,32} = 201.70$*** Time: $F_{3,32} = 12.22$*** Treat × Time: $F_{9,32} = 3.58$**	Treat: $F_{3,8} = 5.69$*
Dactylis glomerata	Treat: $F_{3,30} = 1548.98$*** Time: $F_{3,30} = 682.98$*** Treat × Time: $F_{9,30} = 80.50$***	Treat: $F_{3,8} = 472.93$***
Festuca rubra	Treat: $F_{3,42} = 223.93$*** Time: NS Treat × Time: NS	Treat: $F_{3,8} = 8.83$**

NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Coding for environmental variables is given in Section 2.6. Non-significant terms removed from models by stepwise deletion.

1 **Table 3** Summary of plant phytotoxicity and biomass model outputs to post-emergent
2 treatments (Model 3, Experiment 3)

Species	Percentage phytotoxicity (Arcsine square-root)	Above ground biomass ($\log_e n + 1$)
Galium verum	Treat: NS Time: $F_{3,57} = 14.68$*** Rate: $F_{1,57} = 74.59$*** Treat × Time: $F_{3,57} = 3.46$* Treat × Rate: NS Time × Rate: $F_{3,57} = 24.87$*** Treat × Rate × Time: $F_{4,57} = 3.33$*	Treat: NS Rate: NS Treat × Rate: NS
Plantago lanceolata	Treat: $F_{1,63} = 28.74$*** Time: $F_{3,63} = 3.51$* Rate: $F_{1,63} = 128.82$*** Treat × Time: $F_{6,63} = 8.42$*** Treat × Rate: $F_{1,63} = 37.16$*** Time × Rate: NS Treat × Rate × Time: NS	Treat: NS Rate: NS Treat × Rate: NS
Rumex acetosa	Treat: $F_{1,57} = 31.43$*** Time: $F_{3,57} = 21.45$*** Rate: $F_{1,57} = 45.17$*** Treat × Time: NS Treat × Rate: NS Time × Rate: $F_{6,57} = 10.16$*** Treat × Rate × Time: $F_{7,57} = 3.38$**	Treat: NS Rate: NS Treat × Rate: NS
Silene dioica	Treat: $F_{1,57} = 8.46$** Time: $F_{3,57} = 8.58$*** Rate: $F_{1,57} = 431.40$*** Treat × Time: NS Treat × Rate: NS Time × Rate: $F_{6,57} = 6.11$*** Treat × Rate × Time: $F_{7,57} = 2.34$*	Treat: NS Rate: NS Treat × Rate: NS
Trifolium pratense	Treat: $F_{1,57} = 14.36$*** Time: $F_{3,57} = 10.65$*** Rate: $F_{1,57} = 207.74$*** Treat × Time: $F_{3,57} = 4.78$** Treat × Rate: $F_{1,57} = 6.59$* Time × Rate: $F_{3,57} = 3.80$* Treat × Rate × Time: $F_{3,57} = 18.46$***	Treat: $F_{2,17} = 3.83$* Rate: NS Treat × Rate: NS
Dactylis glomerata	Treat: $F_{1,63} = 1273.40$*** Time: $F_{3,63} = 143.16$*** Rate: $F_{1,63} = 71.00$*** Treat × Time: $F_{3,63} = 217.21$*** Treat × Rate: $F_{1,63} = 5.96$* Time × Rate: $F_{3,63} = 5.15$** Treat × Rate × Time: NS	Treat: $F_{2,17} = 45.85$*** Rate: NS Treat × Rate: NS

3 NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Coding for environmental variables is given in Section 2.6.
4 Non-significant terms removed from models by stepwise deletion.

Figure captions

Figure 1. Mean percentage phytotoxicity of wildflowers (± SE) showing treatment × time interaction relative to the control treatment following post-emergent application of formulated fluazifop-P-butyl (Experiment 2) at half (93.75 g a.i. ha^{-1}), full (187.5 g a.i. ha^{-1}) and double (750 g a.i. ha^{-1}) application rates. Time corresponds to days after treatment (3, 7, 14 and 21). Graphs show untransformed data.

Figure 2. Mean percentage phytotoxicity of *G. verum* (± SE), *R. acetosa* (± SE), *S. dioica* (± SE) and *T. pratense* (± SE) showing treatment × rate × time interaction relative to the control treatment following post-emergent applications of the blank formulation (Full blank = 187.5 g a.i. ha^{-1}; Double blank = 750 g a.i. ha^{-1}) and formulated fluazifop-P-butyl (Full form = 187.5 g a.i. ha^{-1}; Double form = 750 g a.i. ha^{-1}) treatments (Experiment 3). Time corresponds to days after treatment (3, 7, 14 and 21). Graphs show untransformed data.

Figure 3. Mean percentage phytotoxicity of *P. lanceolata* (± SE) and *D. glomerata* (± SE) showing treatment × rate interaction relative to the control treatment following post-emergent applications of the blank formulation (Full blank = 187.5 g a.i. ha^{-1}; Double blank = 750 g a.i. ha^{-1}) and formulated fluazifop-P-butyl (Full form = 187.5 g a.i. ha^{-1}; Double form = 750 g a.i. ha^{-1}) treatments (Experiment 3). Time corresponds to days after treatment (3, 7, 14 and 21). Graphs show untransformed data. Treatment means which do not differ significantly ($P < 0.05$) share the same letter.

Figure 1.

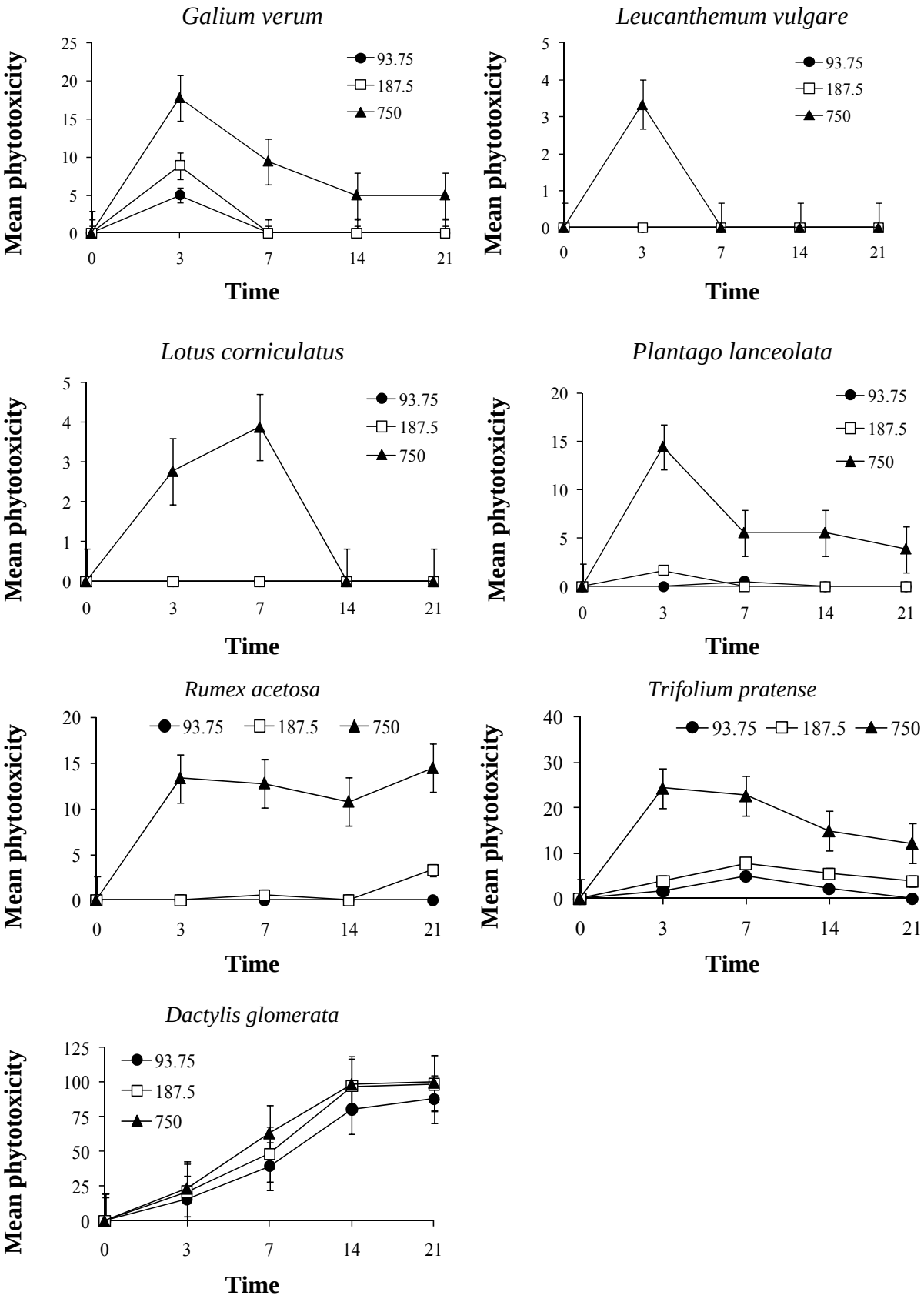

Galium verum
Leucanthemum vulgare
Lotus corniculatus
Plantago lanceolata
Rumex acetosa
Trifolium pratense
Dactylis glomerata
Mean phytotoxicity
Time
93.75
187.5
750

Figure 2.

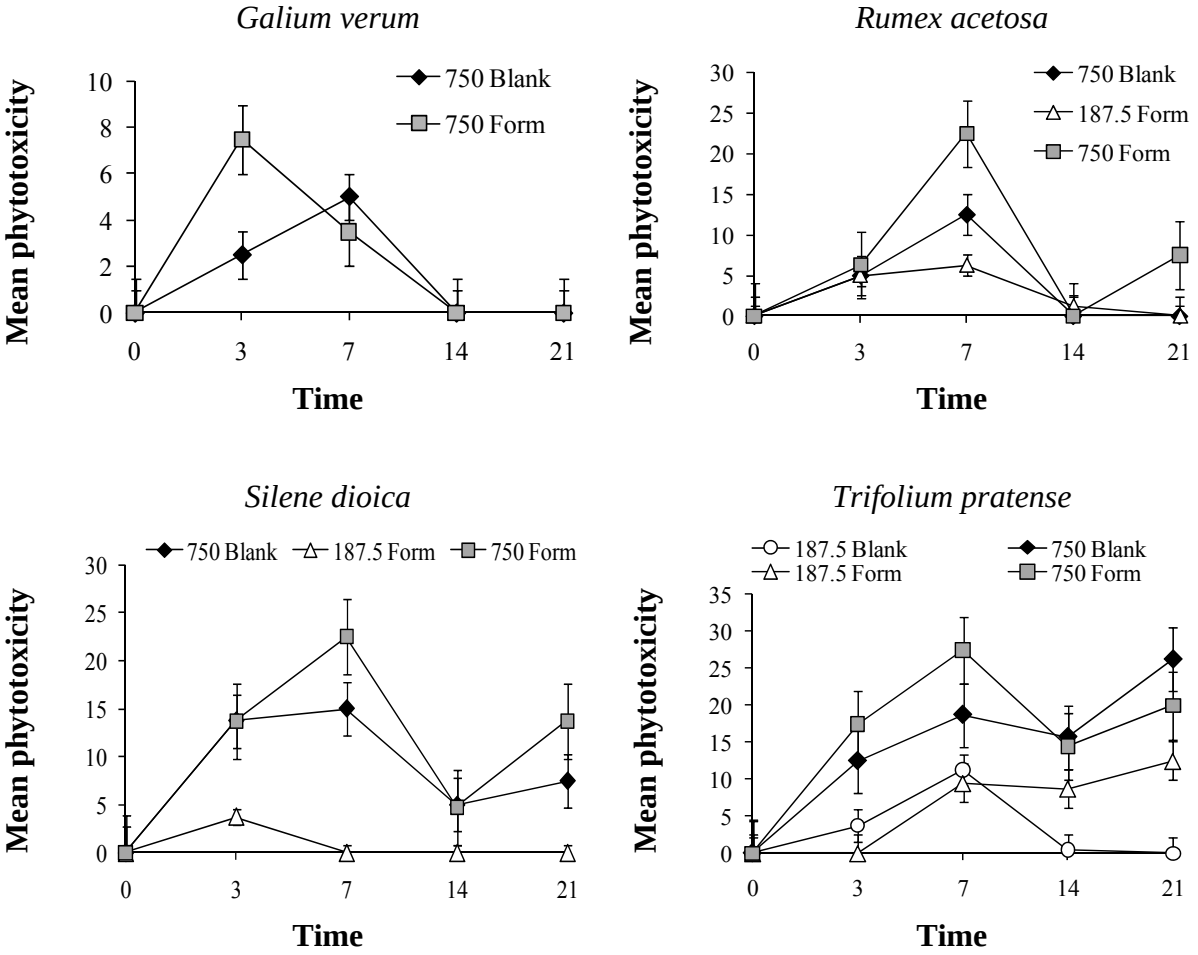
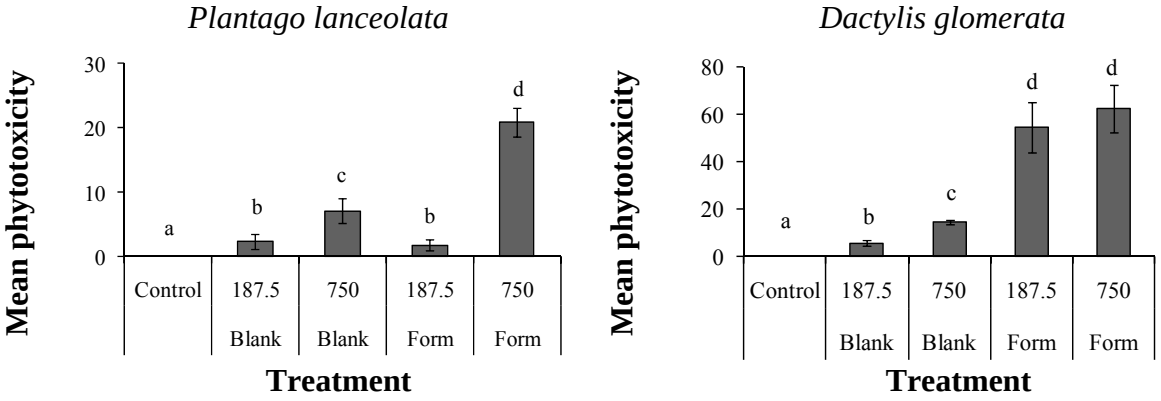

Galium verum
750 Blank
750 Form
Mean phytotoxicity
Time
Rumex acetosa
750 Blank
187.5 Form
750 Form
Mean phytotoxicity
Time
Silene dioica
750 Blank
187.5 Form
750 Form
Mean phytotoxicity
Time
Trifolium pratense
187.5 Blank
187.5 Form
750 Blank
750 Form
Mean phytotoxicity
Time

Figure 3.


Plantago lanceolata
Mean phytotoxicity
30
20
10
0
a
b
c
b
d
Control
187.5 Blank
750 Blank
187.5 Form
750 Form
Treatment
Dactylis glomerata
Mean phytotoxicity
80
60
40
20
0
a
b
c
d
d
Control
187.5 Blank
750 Blank
187.5 Form
750 Form
Treatment