

Effects of glyphosate on flower production in three entomophilous herbaceous plant species
(*Rudbeckia hirta* L., *Centaurea cyanus* L. and *Trifolium pratense* L.)

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Abstract

Reproductive endpoints are generally not considered in regulatory risk assessments used to inform registration decisions for pesticides, and relatively few studies have examined effects of herbicides on reproduction in non-target plants. In two sets of greenhouse experiments using three wild species (*Rudbeckia hirta* L., *Centaurea cyanus* L. and *Trifolium pratense* L), effects on flowering phenology and inflorescence characteristics were investigated following low, drift-equivalent glyphosate exposure at an early bud stage. Weekly post-spray observations included the number of inflorescences, aborted buds and malformed inflorescences. In the experiment focusing on inflorescence characteristics (*C. cyanus* and *T. pratense* only), inflorescences and pollen were collected at five weeks post-spray to measure inflorescence dry weight, count the number of reproductive florets, estimate the amount of pollen per floret, and assess pollen germination *in vitro*. Flower production was adversely affected in all three species, including delays in flowering, significant increases in the number of aborted buds and malformed inflorescences, an overall reduction in the number of inflorescences produced, as well as a reduction in the duration of individual inflorescence bloom time (*R. hirta* and *T. pratense* assessed only). Inflorescence dry weight and *in vitro* pollen germination were significantly reduced for *C. cyanus* exposed to glyphosate, but not for *T. pratense*. However, both species experienced a significant reduction in the number of reproductive florets produced per inflorescence in response to glyphosate exposure. Neither species was observed to have significant reductions in the amount of pollen produced per reproductive floret. These results have important implications for risk assessment, demonstrating that current glyphosate use in Canada and elsewhere could be adversely affecting non-target flowering plants in field margins, as well as other taxa that rely on them, particularly pollinators.

Résumé

Les effets sur la reproduction des plantes ne sont généralement pas pris en ligne de compte dans les évaluations réglementaires sur les risques des pesticides lors de leur homologation. De plus, relativement peu d'études ont examiné les effets des herbicides sur la reproduction des plantes non ciblées. Dans deux séries d'expériences en serres avec trois espèces sauvages (*Rudbeckia hirta* L., *Centaurea cyanus* L. et *Trifolium pratense* L), les effets sur la phénologie florale et les caractéristiques des inflorescences ont été étudiés après une faible exposition au glyphosate équivalente à la dérive durant la pulvérisation lorsque les plantes sont au début des boutons floraux. Des observations hebdomadaires post-pulvérisation ont été effectuées sur le nombre d'inflorescences, de bourgeons avortés et d'inflorescences malformées. Dans l'expérience portant sur les caractéristiques des inflorescences (*C. cyanus* et *T. pratense* seulement), les inflorescences et le pollen ont été recueillis cinq semaines après la pulvérisation pour mesurer le poids sec des inflorescences, compter le nombre de fleurons reproducteurs, estimer la quantité de pollen par fleur et évaluer la germination du pollen *in vitro*. La production de fleurs a été affectée chez les trois espèces, y compris des retards de floraison, des augmentations significatives du nombre de bourgeons avortés et d'inflorescences malformées, une réduction globale du nombre d'inflorescences produites et une diminution de la durée de floraison par inflorescence. (*R. hirta* et *T. pratense* évalués seulement). Le poids sec des inflorescences et la germination *in vitro* du pollen ont été significativement réduits chez *C. cyanus* exposé au glyphosate, mais pas chez *T. pratense*. Cependant, les deux espèces ont subi une réduction significative du nombre de fleurons reproducteurs produits par inflorescence en réponse à l'exposition au glyphosate. Aucune des deux espèces n'a montré de réduction significative de la quantité de pollen produit par fleur. Ces résultats ont des implications importantes pour l'évaluation de risques, démontrant que le

glyphosate, tel qu'utilisé présentement au Canada et ailleurs, pourrait nuire aux plantes à fleurs non ciblées retrouvées en bordure de champs, ainsi qu'à d'autres taxons qui en dépendent, particulièrement les pollinisateurs.

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1 Introduction

Both plants and animals have been negatively impacted by the expansion and intensification of agriculture and urbanization over the past two centuries. Herbicide use is ubiquitous in conventional farming (Cooper and Dobson 2007) and poses risks to organisms inhabiting agroecosystems. Plant species can be adversely affected by relatively low rates of herbicide exposure, and effects have been associated with plant population declines in agroecosystems (de Snoo and van der Poll 1999; Boutin et al. 2014; Schmitz et al. 2014).

For regulatory risk management in North America and Europe, pesticide registrants are required to submit reports of standard non-target plant toxicity tests which evaluate effects on seedling emergence as well as on vegetative vigour at early (vegetative) growth stages (U.S. EPA 2012a,b; OECD 2006a,b). These tests are typically carried out with crop species. Currently there are no known standard protocols for testing effects in non-target plants exposed to pesticides at later life stages (e.g., reproductive); and reproductive endpoints (measures of effects) are generally not considered in regulatory risk assessment. Yet, several studies have demonstrated that plants can be more sensitive when reproductive, and in some cases reproductive endpoints (e.g., seed production) are more sensitive than measures of vegetative vigour (i.e., height, above ground dry weight; Fletcher et al., 1996; Riemens et al. 2008; Rotchés-Ribalta et al. 2012; Strandberg et al. 2012; Carpenter et al. 2013; Boutin et al. 2014). Current regulations for pesticide registration and use may not be sufficient for protecting non-target plants and other taxa that rely on them (e.g., pollinators).

Beyond being vital to angiosperm reproduction and population persistence, flowers represent a crucial food resource for pollen- and nectar-eating species. In turn, most flowering plants rely on

animals for pollination (Klein et al. 2007; Thomann et al. 2013). Evidence of declining bee diversity in Britain and the Netherlands has been associated with the parallel decline of plant species that depend on pollinators (Biesmeijer et al. 2006). A national scale study, also conducted in Britain, found evidence of a decline in bumblebee forage plants over the course of the 20th century (Carvell et al. 2006). Pesticide and fertilizer use have been directly implicated in shifts in non-target plant community structure, as well as in plant and pollinator declines (Carreck and Williams 2002; Chagnon 2008; Pleasants and Oberhauser 2012; Potts et al. 2010; Schmitz et al. 2013). Bee losses have been attributed in part by some beekeepers to poor foraging conditions and starvation (Naug 2009, Huang 2012).

Herbicide exposure can reduce the number of flowers produced by plants (Boutin et al. 2000; Erickson 2006; Kruger et al. 2012; Bohnenblust et al. 2016; Strandberg et al., 2018). Also, herbicides at non-lethal rates have been found to delay and shorten the duration of flowering (Boutin et al. 2014; Londo et al. 2014; Bohnenblust et al. 2016; Yu et al. 2017), cause floral malformation, and lead to more subtle effects including malformed pollen grains (Pline et al. 2002a,b, 2003; Thomas et al. 2004; Erickson 2006; Shimada and Kimura 2006; Yasour, 2007; Baucom et al. 2008; Guo 2009; Mikkelsen and Lym 2013; Qian et al. 2015; Yu et al. 2017). Such effects may reduce overall seed production at relatively low rates of herbicide exposure in several species (Boutin et al., 2014; Strandberg et al. 2018); and may also be associated with reduced seed viability (Strandberg et al. 2018). Effects on seed production and seed viability have obvious consequences for plant reproduction. For pollinators, however, flower production may be of greater immediate consequence. Bohnenblust et al. (2016) recently demonstrated a causative relationship between herbicide treatment and reduced plant visitation by pollinators.

These authors investigated the effects of low rates of dicamba (a benzoic acid herbicide) on the floral production, resource quality (pollen protein) and rates of visitation by honeybees and other pollinators of alfalfa (*Medicago sativa* L.) and common boneset (*Eupatorium perfoliatum* L.) in a field experiment. Dicamba adversely affected flowering in both species (both timing and number of flowers), and also affected the number of honey bee visitations. Thus, non-lethal effects of herbicides on flowering can affect pollinator foraging and may affect food availability and quality.

Glyphosate (N-(phosphonomethyl)glycine) is probably the most widely used pesticide globally (Benbrook 2016). It is a broad spectrum, non-selective systemic herbicide that was commercialized in the mid-1970s for pre-plant weed burndown. It is the active ingredient found in the well-known RoundUp[®] suite of herbicides. With the advent of glyphosate-resistant (“RoundUp[®] Ready”) crop varieties in the mid-1990s its use has rapidly increased; in the US, from less than 10 million kilograms per year to over 100 million kilograms (Duke, 2018).

Glyphosate inhibits plant growth by impeding the synthesis of aromatic amino acids. It competitively blocks 5-enolpyruvyl-shikimate-3 phosphate-synthase which initiates the pathway to chorismate, which is the substrate for the synthesis of the amino acids phenylalanine, tyrosine and tryptophan. Subsequently, protein synthesis is inhibited. Glyphosate is an acid but is manufactured in various salt formulations for packaging and handling (Franz et al. 1997).

Terrestrial plant toxicity data are therefore typically expressed in acid equivalent (a.e.) rates.

There have been numerous greenhouse studies conducted to determine the toxicity of glyphosate to non-target plant emergence and vegetative vigour at early life stage (see collated data

presented in PMRA (2015) and more recently Strandberg et al. 2018). These studies have shown that vegetative vigour (measured as biomass) is typically a more sensitive measure of adverse effects of exposure than seedling emergence. The estimated rates causing 25% effect (ER25s) for seedling emergence reportedly range from 1,570 to >11,210 g a.e./ha, with ER25s >4,480 g a.e./ha for most species. Whereas the ER25 values for biomass from vegetative vigour studies range from 3 to 2,136 g a.e./ha, with ER25s >100 g a.e./ha for most species (PMRA 2015).

Fewer studies have examined reproductive effects (Table 1), or the effects of exposure at later life stages (Marrs et al. 1989; Walker and Oliver, 2008; Guo et al. 2009; Olszyk et al. 2009; Panigo et al. 2012; Kruger et al. 2012; Strandberg et al. 2012; Londo et al. 2014; Olszyk et al. 2017; Strandberg et al. 2018). Several studies with glyphosate have demonstrated that it can cause floral and pollen malformations, as well as male-sterility (Pline et al. 2002a,b, 2003; Thomas et al. 2004; Shimada and Kimura 2006; Shimada and Kimura 2007; Yasour, 2007; Baucom et al. 2008; Guo 2009; Londo et al. 2014). Many studies examining effects on flowers and flower parts have either been conducted with glyphosate resistant crops (Pline et al. 2002a,b, 2003; Thomas et al. 2004; Londo et al. 2014) or relatively tolerant weeds (in the context of the studies; Walker and Oliver, 2008, Guo et al. 2009; Baucom et al. 2008; Panigo et al. 2012) and were concerned with crop protection or herbicidal efficacy.

At least two studies have examined effects on pollen germination with glyphosate applied to germination medium. *In vitro* inhibition of pollen germination in the presence of glyphosate solution has been demonstrated in *Nicotiana sylvestris* Speg. & Comes (7.81 to 500 mg/L; Grabe and Kristen 1997). In this same model species, it was also demonstrated that addition of key

amino acids (phenylalanine, tyrosine and tryptophan) to the germination medium reduced the inhibitory effects (Grabe and Kristen 1997), suggesting that glyphosate may have induced a deficit. In a subsequent *in vitro* pollen germination experiment with *Solidago canadensis* L. glyphosate was added to standard germination medium at 220 to 1000 mg/L (Guo et al. 2009). Pollen germination and tube length declined almost linearly with increasing glyphosate concentration (Guo et al. 2009).

In glyphosate resistant cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.), reproductive effects of glyphosate applications in excess of 700 g a.e./ha have included abnormal flowers, inhibited elongation of the staminal column, (resulting in reduced pollen deposition to stigma), non-dehiscent anthers, malformed/immature pollen, non-viable pollen, and in some cases these effects were negatively correlated with seed production. Effects to female parts (i.e., pistil) were less pronounced or absent (Table 1). Subtle effects on shape of anthers in *Brassica napus* L. of resistant transgenic origin have also been reported at rates as low as 85 g a.e./ha (Londo et al. 2014). Adverse effects in the flowers of glyphosate resistant crops may be due to a lower expression of the CP4 EPSPS (the gene that gives glyphosate resistance to glyphosate) in the male reproductive tissue, which has been observed in cotton (Yasuor et al. 2007).

Similar adverse effects on flowers have been reported in *Ipomoea purpurea* L. Roth and *Commelina erecta* L., considered relatively tolerant weed species, exposed to glyphosate at rates ≥ 900 g a.e./ha (Baucom et al. 2008; Panigo et al. 2012, Table 1). Baucom et al. (2008) also reported that glyphosate-exposed *I. purpurea* anthers lacked obvious pollen grains. The corollas of flowers with shrunken anthers were sometimes stunted, but some flowers with stunted corollas

did not have malformed anthers. Gynoecium in flowers with malformed anthers were normal in appearance. Flowers that had sterile male parts were still able to produce seeds at a comparable rate to normal flowers when hand pollinated with pollen from untreated plants. However, in the field, seed production was negatively correlated with anther malformation. In both the field and greenhouse experiments anther deformation was transient, with malformations declining over time through the census period of several weeks (Baucom et al. 2008). *C. erecta* exposed to glyphosate were observed to abort flowers (Panigo et al. 2012).

Londo et al. (2014) investigated the effects of glyphosate (RoundUp® formulation) exposure on the flowering phenology of seven different *Brassica* varieties, including a *B. napus* cv. RoundUp Ready® variety, and four wild varieties (Table 1). Greenhouse-grown plants were exposed to 0.177 or 0.234 L/ha of formulation (at 480 g a.e./L, exposure rates would be 85 or 112 g a.e./ha respectively) four weeks after planting at which time most varieties were pre-bolt or bolting. Glyphosate caused delays in flowering (except in the transgenic variety), and in some varieties shortened the duration of flowering. Flowers that formed following treatment were malformed, sometimes lacking stamens, and having pale petals. Closer examination of male reproductive parts revealed that treatment resulted in malformed anthers that did not normally dehisce (with the exception of the transgenic variety). Pistils were observed to be normal; however, their overall functioning in non-transgenic varieties was found to be sensitive to glyphosate in that hand pollination of exposed plants was not as successful as it was in control plants. Self-fertility was also significantly reduced in three varieties examined (Londo et al. 2014).

Effects on reproductive measures including flower production, seed production and seed germination have been observed at much lower glyphosate application rates (in the range of

14.4-720 g a.e./ha) in greenhouse studies with non-target plants (Table 1). *Petunia hybrida* D. Don ex Loudon experienced significant changes in floral symmetry as a result of exposure to 0.5 mM solution (~42 g/ha) of glyphosate sprayed onto leaves and buds three times, at 2-day intervals, until 45 days after planting (Shimada and Kimura 2007). The symmetry of flowers was reportedly the zygomorphic type in treated plants as opposed to actinomorphic in untreated plants (Shimada and Kimura 2006). In a subsequent study by the same authors with the same model organism, equivalent exposures also resulted in increased free amino acid content of corollas but had no effect on aromatic amino acids (Shimada and Kimura 2007). Glyphosate reduced the nitrate content by 45% and RNA by 63% of controls. The authors suggested that glyphosate at low concentrations may alter the regulation of flower symmetry via effects on RNA biosynthesis (Shimada and Kimura 2007).

Strandberg et al. (2012) compared effects on seed production of two annuals (*Silene noctiflora* L., *Geranium molle* L.) and two perennials (*Silene vulgaris* (Moench) Garcke, *Geranium robertianum* L.) exposed to glyphosate at a vegetative life stage versus a reproductive (early bud) stage (Table 1). Reported ER50s for seed production were lower for annual plants exposed at the early bud stage. ER50s for the perennial plants were similar when treated at the vegetative and early bud stages. Seed germination was not significantly affected by treatment when tested in two of the species, and no correlation was found between treatment and seed mass, though relative seed mass did seem to decline with treatment in both perennial species. ER50s for seed production of species sprayed at an early bud stage (*S. noctiflora*, *S. vulgaris*, *G. molle*) were less than 45 g a.e./ha. Most recently Strandberg et al. (2018) carried out a series of experiments that investigated effects of glyphosate and other herbicides on biomass, competition, seed production

and germinability, and flower production in a suit of non-target and non-crop herbaceous plants. In paired experiments in Denmark and Canada, significant effects on flowering and seed production and viability were observed at both test rates (14.4 and 72 g a.e./ha) depending on species/test combination (Table 1).

Many field experiments have examined the effects of glyphosate on reproductive endpoints (Table 1). Generally, adverse effects on growth and reproduction were noted in field studies only at rates ≥ 83 g a.e./ha (Walker and Oliver 2008; Guo et al. 2009; Olszyk et al. 2017). The exception to this was two field studies conducted to determine the response in tomato (*Solanum lycopersicum* L.) flowering and yield following exposure to glyphosate at a vegetative or early bloom stage, in which effects were observed at considerably lower rates (%5 effect on flowering and yield predicted at <5 g .a.e./ha for early bloom application; Kruger et al. 2012; Table 1). Walker and Oliver (2008) examined weed seed production in a suite of species. Glyphosate (formulation not reported) was applied 2-3 weeks after transplant, and then also one month later. Glyphosate induced higher yield losses when sprayed at the early bloom stage. At 43.9 and 8.5 g a.e./ha a 25% reduction in yield was reported for applications at a vegetative stage and early bloom stage, respectively. Trends in the estimated number of flowers at 48-days post treatment were in line with these results with ER25s of 51.1 and 7.5 g a.e./ha, respectively (Kruger et al. 2012). Field studies were also carried out over three years to investigate effects of late-season glyphosate applications on seed production of several weed species, including barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), Palmer amaranth (*Amaranthus palmeri* S. Watson), pitted morning glory (*Ipomoea lacunosa* L.), prickly sida (*Sida spinosa* L.), and sicklepod (*Senna obtusifolia* (L.) Irwin & Barneby; AR, USA; Walker and Oliver 2008). Growth stages of the test

species varied at time of application, among species and across years, but all applications were made when at least one of the species had begun to flower. When only a single application was used (as opposed to sequential reapplications every 10 days), seed production and plant weight were generally significantly lower than controls at 840 g a.e./ha, sometimes lower at 420 g a.e./ha (depending on timing of application), and rarely lower at 210 g a.e./ha (sicklepod). In the case of sicklepod, plant biomass was not influenced by glyphosate applications. Barnyard grass did have reductions in seed weight at and above 210 g a.e./ha, while glyphosate applications did not elicit changes in seed mass in other species tested. Barnyard grass seeds of treated plant were reported as being small and not entirely filled. Sequential applications until harvest typically resulted in significant effects on seed production and plant biomass at the lowest treatment rate of 110 g a.e./ha (Walker and Oliver 2008).

In China, where *Solidago canadensis* is considered an invasive weed, researchers investigated the effects of glyphosate on seed quality in field experiments. Glyphosate was applied at rates between 300 and 1100 g/ha (formulation not reported) to plants at an early bud stage, or at a full bloom stage. One month later seeds were collected and used in seed germination tests. Plants sprayed at an early bud stage were unable to produce viable seeds, even at the lowest glyphosate application rate. Plants sprayed in full bloom were still able to produce viable seeds; however, viability decreased with increasing application rates. Subsequently, Olszyk et al. (2017) used constructed plant communities of grassland perennials (*Camassia leichtlinii* (Baker) S. Watson, *Elymus glaucus* Buckley, *Eriophyllum lanatum* (Pursh) Forbes, *Festuca idahoensis* Elmer subsp. *roemerii* (Pavlick) S. Aiken, *Fragaria virginiana* Duchesne, *Iris tenax* Douglas ex Lindl., *Potentilla gracilis* Douglas ex Hook., *Prunella vulgaris* L. subsp. *lanceolata* (W. Bartram)

Hultén, and *Ranunculus occidentalis* Nutt.) to investigate the effects of glyphosate on growth and reproduction of these species. Communities were transplanted into two sites and assessed over two years and measures of land cover, reproductive structures, seed production, and vegetative biomass were acquired. Herbicide applications were made in late April, when plants were growing on sites (OR, USA). Significant effects on seed weight and the number of seed heads were seen in most species that had produced sufficient seeds for assessment, generally at or above 83 g a.e./ha; effects were not observed at the next lowest rate of 8.3 g a.e./ha. In *E. lanatum* an increase in immature seeds was also seen at 83 g a.e./ha glyphosate exposure. In contrast to the results for reproduction stated above, most species did not experience a significant effect on either percent cover or vegetative biomass.

Based on downwind drift deposition models for aerial and ground spray applications of pesticides used in regulatory risk assessment (e.g., AgDrift v2.1.1; Teske et al. 2003), drift equivalent rates may be considered anything less than approximately 50% of the applied field rate of a pesticide (estimated deposition at less than 30 cm off-field for both ground and aerial applications). However, in general, drift declines exponentially with distance from the treated field. Higher downwind deposition is expected for aerial applications (Teske et al. 2003). Based on the Canadian label for Roundup WeatherMax[®] With Transorb 2 Technology Liquid Herbicide (Monsanto, 2015), glyphosate can be applied at a maximum aerial application rate of 1798 g a.e./ha, and a maximum ground application of 4320 g a.e./ha. In Canada, buffers of 15 m and 40-70 m are prescribed for sensitive terrestrial habitat, for ground and aerial applications (Monsanto 2015).

In an early drift field study conducted by Marrs et al. (1989), effects of glyphosate sprayed in adjacent fields were tested on a variety of herbaceous plants following fall and/or spring/summer ground applications. The application rates assessed were 500 and 2200 g a.i./ha, and both rates were assessed at average windspeeds of 2.5 and 3.5 m/s with four upwind swaths. Plants of unreported age were placed at setback distances from 0 to 50 m (fall) or 0 to 8 m (spring/summer). Through observations of the plants following applications (once, but timing not specified), distances protective of lethal effects, plant damage and flower suppression were determined to range from 0 to 20 m and varied considerably among species. Protective distances were also established based on yield and seed production (0-8 m; assessed at harvest; Marrs et al. 1989). At the time the application rates assessed were representative of low and high rates for glyphosate applied with a ground sprayer. More recently, Strandberg et al. (2018) conducted a drift experiment in Denmark with a variety of perennial species that had been sown in the field a year prior to exposure. They examined the effects of downwind glyphosate exposure with a field application rate of 1440 g a.e./ha. Percent cover did not appear affected by glyphosate application; however, glyphosate spray drift had an adverse effect on the cumulative number of flowers produced by *Trifolium pratense* L. and *Lotus corniculatus* L., the species with the greatest covers in the test plot. No significant effects on timing of flowering were detected.

Given glyphosate's mode of action, and effects observed in the male parts of some resistant and tolerant species, key amino acids (phenylalanine, tyrosine and tryptophan) may be reduced in the pollen of plants exposed to sub-lethal levels of glyphosate. In addition to adverse effects on reproduction, there could be negative consequences for bee nutrition, given that two of the three amino acids inhibited, phenylalanine and tryptophan, are among the ten amino acids that are

essential to honey bees (deGroot 1953). Pollen is the primary source of proteins, free amino acids, starch, sterols, lipids, vitamins and minerals for some pollinators (Alaux et al. 2011; Forcone et al. 2011; Stanley and Linsken 1974), and for honey bees, is particularly important in brood rearing (see Brodschneider and Crailsheim 2010).

2 Hypotheses and Objectives

The purpose of this study was to quantify effects of early bud stage application of glyphosate on flowering of a small assortment of entomophilous wildflowers that are found in agroecosystems of North America and to determine if effects include reduced pollen quality or quantity.

Specifically, we aimed to answer two principal questions: (1) to what extent do environmentally relevant (i.e., drift-equivalent) rates of glyphosate affect flowering when exposure occurs at an early bud stage? (2) Do such exposures affect pollen quantity or quality? I hypothesized that glyphosate would delay and inhibit flowering, as has been documented in other species; and, that pollen quantity and quality would be reduced if effects to male parts manifested in these species, as has been observed in other species. Addressing these questions is important in supporting ecological risk assessment for flowering plants and the animals that rely upon them.

3 Methods

All experiments were carried out at the National Wildlife Research Centre, Environment and Climate Change Canada, located on Carleton University Campus, Ottawa, Canada. The greenhouse phases of the definitive experiments were conducted between December 6, 2016 and August 2, 2017.

Preliminary assessment of local native or naturalized herbaceous plants was carried out to determine suitability based on flowering characteristics and value to pollinators as a food source (qualitative assessment of timing, consistency, number of inflorescences, pollen production; Appendix A). Three species were selected based on this assessment: black-eyed Susan (*Rudbeckia hirta* L.), bachelor's button (*Centaurea cyanus* L.), and red clover (*Trifolium pratense* L.). These species produced flowers within a suitable time frame from planting, were relatively consistent in their growth among individuals, and produced sufficient pollen (Appendix A). All seeds were acquired from Richters Herbs (Goodwood, ON; *R. hirta* lot #18653; *C. cyanus* lot # 19653; *T. pratense* lot #18388).

Rudbeckia hirta is a native forb from the Asteraceae family that can behave as an annual, biennial or perennial. In Ontario it is generally found in disturbed upland habitats but can also be found in wetlands (Oldham et al. 1995). Insects, in particular bees, flies and butterflies, feed on pollen and nectar from this species (Nuffer 2007, Girard et al. 2012). *Centaurea cyanus* is a non-native, but naturalized forb in Ontario that grows in upland habitats. It also belongs to the Asteraceae family. It is primarily pollinated by bees (Carreck and Williams 2002), and both its pollen and nectar are consumed by them (Hintermier 2011). *Trifolium pratense* is a member of the Fabaceae family. It is a non-native but naturalized perennial forb found in fields and roadsides, occurring in both upland and wetland habitats in Ontario (Oldham et al. 1995). It is frequently grown for forage and as a cover crop. It is entirely entomophilous, requiring pollinators for seed set and its nectar and pollen are known to be readily collected by bees (Palmer-Jones et al. 1966).

Two sets of experiments were performed. The first set was conducted to investigate effects of glyphosate on the timing and extent of flowering, or flowering phenology, in three test species (the PHEN experiments hereafter). All PHEN experiments had five treatment levels plus controls. For *R. hirta*, each treatment group had four replicates of six pots each with two plants per pot (48 experimental plants per treatment group). For *C. cyanus* and *T. pratense*, each treatment and control had six replicates of six pots each with one plant per pot (36 experimental plants per treatment group). All species received the same nominal application rates (Table 2). These rates were selected based on preliminary test results which revealed only subtle effects on *R. hirta* and *T. pratense* at 45 g a.e./ha (Rodney et al. 2015), and no significant effects on phenylalanine or tyrosine in pollen at these rates (Appendix B). Phenology data were gathered weekly for eight weeks following spray application.

Data gathered in the PHEN experiments were used to determine appropriate application rates for subsequent experiments focused on inflorescence characteristics and pollen in two of the test species (*C. cyanus* and *T. pratense*; hereafter referred to as the INFLO experiments). The INFLO experiments were designed to investigate effects of glyphosate on the extent and mass of individual inflorescences, as well as the quantity and quality of pollen produced by inflorescences. The initial intent was to sacrifice 1-2 plants weekly from each replicate to: (1) measure inflorescence dry weight, (2) count reproductive florets, harvest pollen for (3) glyphosate analysis, (4) amino acid analysis, (5) in vitro pollen germination testing, and (6) pollen count. However, shortly after treatment, it was apparent that effects on treated experimental plants were greater than anticipated based on the preliminary testing and the PHEN experiment. It was apparent that there would not be sufficient pollen for glyphosate or amino

acid analyses. For this reason, plants were sacrificed concurrently at 5-weeks post spray. A component of the phenology experiments was implemented (as described below), so that outcomes could be compared between the two sets of experiments.

3.1 General Methods

Experimental plants were grown in a mixture of 80% (w/w) sand (Humid Sand, S. Boudrias Inc, Laval, Québec), and 20% (w/w) clay (EPK Pulverized Kaolin, CAS 1332-5807, Edgar Minerals Inc., Edgar, Florida). A mixture of equal parts (by volume) peat (Premier® Sphagnum Peat Moss, Canada), sheep manure compost (Ritchie Composted Sheep Manure), and shrimp-peat compost (Fafard, Canada) was added to achieve 3% (w/w) organic matter, in accordance with guidelines established by the Organization for Economic Cooperation and Development (OECD, 2006) and the United States Environmental Protection Agency (USEPA, 2012). Organic matter in these components was determined by muffle furnace (loss on ignition), and density of all soil components was estimated to allow for mixing by volumes. At planting Plant-Prod SmartCote® Annual Flower (12-14-12) controlled release fertilizer was applied to the soil surface at a nominal rate of 0.024 mL/cm², following label directions.

All plants were sown and grown in 2.84 L cylindrical polypropylene pots. In the day following test solution application, replicates of six pots each were placed in white weaved polypropylene Demo Bags®, to accommodate bottom watering. Bags were cut so that the top of a bag would reach beyond the top of the pots, but not shade the experimental plants. Small holes were made in the bottoms of the bags to allow for drainage.

Plants were gradually thinned to two per pot for *R. hirta*, and one per pot for *C. cyanus* and *T. pratense* in the weeks after emergence and prior to spray. Experimental plants were sprayed with test solution when $\geq 25\%$ of plants had buds, but no florets had opened (Table 2). One exception to this was the *T. pratense* INFLO experiment, in which five experimental plants did have 1-2 blooming inflorescences at the time of spray. These inflorescences were tagged and excluded from all analyses. Plants were coarsely sorted by size and reproductive status (buds/no buds). Extremely small and large plants were excluded, and remaining plants were evenly distributed to replicates. Replicates were then randomly assigned to treatment groups. Post-spray application replicates were placed randomly in a greenhouse unit. Weekly, locations for replicates were re-randomized to reduce environmental variability (i.e., light level) due to placement within the greenhouse. The exception to this was the INFLO experiment for *C. cyanus*, where movement of blooming plants could result in loss of pollen and cross-contamination. Accordingly, *C. cyanus* were moved only once after spray in the INFLO experiment but were not rotated thereafter.

Test solutions were prepared either the day prior, or on the day of application. Following preparation, and prior to application, test solutions were stored in a refrigerator at $\sim 4^{\circ}\text{C}$. The herbicide formulation used in all experiments was Roundup WeatherMax[®] With Transorb 2 Technology Liquid Herbicide (Monsanto Canada Inc., Winnipeg, Manitoba) containing 540 g acid equivalent (a.e.) glyphosate per litre, as a potassium salt. Test solutions were prepared with tap water in 500 mL polypropylene bottles. Following the herbicide label recommendations to enhance efficacy of the herbicide, a surfactant was added to all test solutions. Agral 90[®] (Norac

Concepts Inc., Guelph, ON, Canada), a non-ionic solution containing nonyl phenol ethoxylate, was added at a rate of 4.17 mL per 500 mL spray solution bottle.

Test solutions were applied to experimental plants using a track-spray booth (de Vries Manufacturing, Hollandale, MN, USA) with TeeJet 8002E flat-fan spray nozzle (Spraying Systems, Wheaton, IL, USA; as in Dalton and Boutin 2010, Carpenter et al. 2013; Boutin et al. 2014). Three to eight experimental plants were treated with each active pass of the sprayer. Timing and rates are provided in Table 2. Rates were selected based on subtle effects observed in *R. hirta* and *T. pratense* in preliminary testing at 10 and 45 g a.e./ha (Rodney et al. 2015).

To verify sprayer application rates, test solution was captured on 11 cm-diameter filter papers placed on clean over-turned pots in the spray-booth, alongside experimental plants, during glyphosate application. Three filter paper samples were collected for each treatment level, for each experiment. Samples were placed in polypropylene test tubes and stored in a freezer at approximately -21°C, until they were analysed by liquid chromatography-mass spectrometry (LC-MS) in the National Wildlife Research Centre laboratory.

Throughout all experiments a 16:8 h day: night light cycle was maintained with sunlight and supplementary greenhouse lighting. All plants were top-watered (over canopy) as needed until test substance application. Following application plants were not watered for at least 24 hours in accordance with label instructions (i.e., “Do not apply if rainfall is forecast for the time of application.”). The first watering after test substance application was a top watering performed at the base of the soil (below canopy). Plants were bottom-watered as needed for the remainder

of experiments to avoid test substance wash-off. Minimum and maximum temperature, and humidity were recorded daily. Photosynthetic active radiation (PAR) was recorded every 3-5 days, between 10 and 2 pm using a Li-Cor LI 6400XT portable photosynthesis system.

To control thrips the predatory mites *Neoseiulus cucumeris* Oudemans and *Hypoaspis miles* Berlese (Applied Bio-Nomics Ltd., B.C., Canada) were applied as needed. Convergent lady beetles (*Hippodamia convergens* Guérin-Ménéville) were also released in the greenhouse units containing *T. pratense* experiments to control aphids and spider mites. *T. pratense* plants were treated with Green Earth Garden Sulphur Fungicide-Miticide, 15 mL/L spray, one week prior to glyphosate treatment in order to inhibit the growth of powdery mildew, as well as to aid in spider mite control. In the INFLO experiment, another application was made the day before termination to remove spider mites prior to harvest.

Weekly observations in all experiments included phytotoxicity scoring and counting of inflorescences on plants from each replicate. The phytotoxicity scoring scale was 0-5, with 0 being no signs of toxicity, 1 indicating slight adverse effect effects restricted to one part of the plant (e.g. one leaf), 2 indicating moderate adverse effects not restricted to one area (e.g., mild chlorosis), 3 indicating a severe adverse effect (e.g., severe leaf desiccation), 4 indicating severe adverse effects to the entire plant, and 5 being mortality. Inflorescences were counted if at least one reproductive floret was open (or in the case of *T. pratense*, could be opened). At termination of the experiments, all above ground biomass of each replicate was placed into paper bags and oven dried at ~70°C for a minimum of one week. Dried bags with and without contents were

weighed in triplicate on a Mettler PC4400 scale to the nearest 0.001 g. Average bag mass was subtracted from average total mass to produce a measure of replicate above ground biomass.

3.2 Phenology Experiments (PHEN)

In addition to phytotoxicity score, and newly identified inflorescences, each week the following was recorded for each plant in the PHEN experiment: the number of inflorescences in bloom (at least one open reproductive floret, with no indication of senescence (drying, browning or wilting)) and the number of observed malformed inflorescences (observed marked asymmetry, auxiliary parts, underdeveloped parts). One pot of each replicate was used to track bloom duration for *R. hirta* and *T. pratense*. Newly identified inflorescences were tagged and their status was updated weekly (blooming or senescing). *C. cyanus* inflorescences rarely lasted more than one week, and therefore was not assessed for this measure of effect.

3.3 Inflorescence and Pollen Experiments (INFLO)

At five weeks post spray, three inflorescences from each replicate bearing sufficient blooms were taken for dry weight measurement. The number of reproductive florets on each inflorescence was counted, and the inflorescences were placed in a brown paper bag for oven drying at ~70°C.

Following methods developed by the Plant Research Team at the National Wildlife Research Centre *in vitro* pollen germination tests were carried out at 5-weeks post-spray in the INFLO experiment (based on Brewbaker and Kwack 1963). Information on the pollen germination media are provided in Appendix C. Pollen was collected from *C. cyanus* and *T. pratense* blooming inflorescences. Reproductive florets were removed, one from each *C. cyanus* replicate,

two from each *T. pratense* replicate, and introduced to germination medium (15 μ L droplet on gridded microscope slides). The slides were placed in petri dishes on moistened filter paper, covered with a lid to maintain humidity, and left at room temperature for five hours to allow for pollen germination. After five hours slides were removed from the petri dishes. Coverslips were then placed on top of the droplets and photos were taken with a Zeiss microscope (Zeiss Axio Imager.A2). Four photos of 4 mm² grid squares were taken per droplet. Subsequently, germinated and ungerminated pollen grains were counted. A germinated grain was one that clearly had an attached pollen tube that exceeded its own width in length (following Pline et al. 2002a).

In the INFLO experiment, at 5-week post spray, pollen was also collected to estimate the number of grains available from reproductive florets. From *C. cyanus* and *T. pratense* blooming inflorescences, 10 and 20 florets were collected, respectively per replicate. For *T. pratense*, the wings and keel of the floret were removed at collection to ensure no trapping of pollen. Samples were placed in 2 mL microcentrifuge tubes. Tubes were left open and were placed in an incubator (Precision Scientific Mechanical Conventional Incubator Economy Model 4EM) at ~30°C for 24 hours to allow for complete pollen release. After 24 hours in the incubator 100 and 50 μ L of 90% lactic acid solution (Acros Organics) was added per floret, for *C. cyanus* and *T. pratense* respectively, to preserve the samples for subsequent photographing and counting. Tubes were closed and stored in a standard refrigerator at ~4°C. Prior to sampling the mixture tubes were shaken to distribute pollen in the solution. Two 10 mL aliquots were taken from each sample tube with a micropipette and introduced to each side of a gridded Neubauer counting chamber (following Albuquerque et al. 2010 and Silva 2015). Photos were taken of each of nine 1 mm²

square grids on each side of the chamber (18 photos per replicate). Each square represented 0.1 μL of sample solution. Accordingly, the number of grains per floret was estimated.

3.4 Data Processing and Statistical Methods

3.4.1 *Binary Measures of Effects*

Binary response variables included: plant mortality, the proportion of surviving plants producing inflorescences, the proportion of aborted buds, the proportion of malformed inflorescences, and the proportion of ungerminated pollen grains.

If monotonic rate-response was suggested by examination of the plotted data, the PROBIT procedure in SAS Software[®] (SAS 9.3, SAS/STAT 12.1) was used to estimate rate-response curves and estimated rates causing 10, 25 and 50% effect (ER10, ER25 and ER50) assuming normal/probit, logisitic or Gompertz cumulative distribution functions, the latter of which is sigmoidal like the probit and logistic but is not restricted to being symmetrical (SAS Institute 2010). A Pearson scaling parameter was calculated to account for any over-dispersion of assumed binomial response. In addition, where control response was non-zero, a threshold response was estimated. If the model with the highest Pearson-Chi-square goodness of fit value, had an associated p value > 0.10 , the model was accepted as best-fitting. Otherwise, non-parametric estimates for ERx values were generated using the GAM procedure in SAS with spline interpolation over rate.

If rate-response was not marked in the observation of the plotted data, a step-down Cochran-Armitage test for trend was carried out. In advance of this, data were tested for binomial

response with Tarone's test (Tarone 1979). If the binary response data were found to not follow a binomial distribution (typically due to over-dispersion), they were adjusted for design effect following Rao and Scott (1992). These calculations were carried out in MS Excel[®] 2016. The Cochran-Armitage test was conducted on adjusted, or unadjusted data using the FREQ procedure in SAS Software[®]. If a trend was detected, rate-response was modeled as described above.

Where data were observed to be non-monotone, Fisher exact test was conducted with pairwise comparisons of control and treatment groups (with Bonferroni correction for the family-wise error rate), using the FREQ procedure in SAS.

3.4.1.1 Continuous Measures of Effects

Continuous response variables included: replicate average above ground biomass; average time to first bloom; average bloom-weeks per plant (calculated as the count of total number of inflorescences in bloom over all weeks of observation divided by the number of plants – serves as an indicator of floral resource availability); average number of blooms per plant; and average number of reproductive florets and pollen count (treated as continuous). Where appropriate both (1) experimental and surviving plants, or (2) experimental and reproductive plants (that survived) were considered separately in the measures of effects. Here experimental plants are all the plants that were treated, whereas surviving, and reproductive plants may be a subset of the experimental unit. This treatment of the data was done to account for both aggregated and isolated effects on individuals (e.g., the sum of mortality and reproductive effects on overall reproductive endpoint, versus strictly effects of mortality or reproduction). These differences have important

implications for risk assessment (i.e., whether or not zeros are included in rate-response curves; Rodney et al. 2013).

The average cumulative number of inflorescences in the PHEN experiment was analysed at both five weeks and 8 weeks (termination), so that the five-week data could be compared qualitatively with the results of the INFLO experiment (which was terminated five weeks after applications).

For average time to first bloom, which is not appropriately expressed as a percentage of controls, PROC GLM was used to estimate rate-response, if appropriate, following Bailer and Oris (1997). For all other continuous variables the statistical methods are described below.

If monotonic rate-response was observed in the plotted data, linear and nonlinear models were fit in the NLMIXED procedure in SAS Software[®]. This procedure estimates model parameters by maximum likelihood estimation methods and provides information criterion to compare goodness of fit across models. In addition, this procedure allows for both fitting and specification of the error distribution, providing a means of accounting for collapse in variance that is often seen in non-target plant testing, and other toxicity tests since variables such as mass and height approach zero (Bruce and Versteeg, 1992). Here an error distribution was specified, such that it could be a function of the response variable. Error distributions were specified as normal ($Y, Y^{\theta} \cdot \text{VAR}$) following (Dmitrienko et al. 2007) where Y is the response and θ and VAR are error distribution parameters that are estimated. If θ is 0 then VAR is estimated homogenous and is the common variance through the model range of the data, otherwise the error distribution changes with Y . The models fit to the continuous data included: linear,

exponential, lognormal, log logistic and log Gompertz models based on approaches to estimating ER_x covered in the open literature and available in standard toxicity testing statistical software (Bruce and Versteeg, 1992; Stephenson et al. 2000; Environment Canada 2005; Tidepool 2013).

The models that were used are specified in Equations 1 through 5.

Linear (Environment Canada 2005):

$$y = y_0 + \left(\frac{-y_0 * p}{ER_x} \right) rate$$

Equation 1

where:

- y = The dependent variable (units of measure)
- y_0 = The y-intercept (estimated control response; units of measure)
- ER_x = The rate at which x% effect is estimated (g a.e./ha)
- p = Proportion associated with x%
- $rate$ = Application rate (g a.e./ha)

Exponential (Stephenson et al. 2000):

$$y = y_0 * e^{\left(\ln(1-p) * \frac{rate}{ER_x} \right)}$$

Equation 2

where:

- y = The dependent variable (units of measure)
- y_0 = The y-intercept (estimated control response; units of measure)
- ER_x = The rate at which x% effect is estimated (g a.e./ha)
- p = Proportion associated with x%
- $rate$ = Application rate (g a.e./ha)

Log logistic (Stephenson et al. 2000):

$$y = \frac{y_0}{1 + \left(\frac{p}{1-p}\right) \left(\frac{rate}{ER_x}\right)^\sigma}$$

Equation 3

where:

- y = The dependent variable (units of measure)
- y_0 = The y-intercept (estimated control response; units of measure)
- ER_x = The rate at which x% effect is estimated (g a.e./ha)
- p = Proportion associated with x%
- $rate$ = Application rate (g a.e./ha)
- σ = scale parameter

Log Gompertz (Environment Canada 2005):

$$y = y_0 * e^{\left[\ln(1-p) * \left(\frac{rate}{ER_x}\right)^\sigma\right]}$$

Equation 4

where:

- y = The dependent variable (units of measure)
- y_0 = The y-intercept (estimated control response; units of measure)
- ER_x = The rate at which x% effect is estimated (g a.e./ha)
- p = Proportion associated with x%
- $rate$ = Application rate (g a.e./ha)
- σ = scale parameter

Lognormal (Bruce and Versteeg 1992):

$$y = y_0 * \Phi \left[\frac{\log(ER_x) - \log(rate)}{\sigma} + Z_x \right]$$

Equation 5

where:

- y = The dependent variable (units of measure)
- y_0 = The y-intercept (estimated control response; units of measure)
- ER_x = The rate at which x% effect is estimated (g a.e./ha)
- p = Proportion associated with x%
- $rate$ = Application rate (g a.e./ha)
- σ = scale parameter
- Z_x = The standard normal deviate above which the area under the normal distribution is x.

Normality (Shapiro-Wilk test) and homogeneity of variance (Levene and Brown-Forsythe tests) were assessed using the UNIVARIATE and GLM procedures in SAS[®]. Residual plots were examined to rule out any systematic departures from the model. The converging model that conformed to model assumptions with the lowest AIC was selected as best-fitting. If there were substantial departures from model assumptions, or models did not converge, or convergence was questionable, even with adequate starting parameter values, a nonparametric approach was taken using the GAM procedure in SAS[®] with spline interpolation over rate to estimate ERx values.

If data were non-monotonic based on observation of the plotted data, or rate-response was not evident in the plotted data, ANOVAs were carried out. If ANOVA assumptions were met, and the F-test was significant, Williams (monotone) or Dunnett's test (non-monotone) were conducted. If the data were monotone but did not meet the ANOVA assumptions Jonckheere-Terpestra step-down tests were conducted using the FREQ procedure in SAS[®]. If rate-response was significant, it was modeled as specified above. If the data were non-monotonic and ANOVA

assumptions were not met, the Wilcoxon (Mann-Whitney U) test was conducted on pairwise comparisons with controls (with Bonferroni correction for the family-wise error rate) using the NPAR1WAY procedure in SAS[®]. Example SAS code for all procedures is provided in Appendix D.

4 Results

4.1 Measured Application Rates

The method detection limit for glyphosate was 0.61 g/ha, and the method reporting limit was 3.0 g/ha. Measured application rates ranged from 92.0 to 138.9% of the anticipated nominal rates on individual filter samples. In the *R. hirta* PHEN experiment average measured rates were 114.0 (± 6.6) % of nominal. In the *C. cyanus* experiments, the average measured rates were 110.3 (± 5.4) and 101.5 (± 4.0) %, for the PHEN and INFLO experiments respectively. In the *T. pratense* experiments, the average measured rates were 109.2 (± 3.3) and 120.8 (± 4.9) % of nominal rates for the PHEN and INFLO experiments, respectively. All subsequent analyses were carried out with measured application rates. Chemical results are presented in Appendix E.

4.2 Environmental Conditions

Temperature, humidity and PAR data are summarized in Appendix F. Average maximum daily temperature and maximum daily humidity was markedly higher during the INFLO experiments than during the PHEN experiments. In the PHEN experiments average maximum temperatures ranged from 29.4 to 31.1°C; average maximum relative humidity ranged from 57.7 to 60.4%. In the INFLO experiments average maximum temperatures ranged from 40.0 to 41.8°C; average maximum relative humidity ranged from 81.6 to 81.8%.

4.3 Effects on Test Species

The following sections describe results by species, beginning with effects to survival and growth, and then presenting effects on flowering phenology and inflorescence characteristics. Details of the statistical analyses performed on each dataset are provided in Appendix G.

4.3.1 *Rudbeckia hirta* Survival and Growth

There were no *R. hirta* mortalities in controls or treatment groups up to and including the 52.8 g a.e./ha dose. However, there were significant mortalities at and above the 74.2 g a.e./ha treatment group (Cochran-Armitage step-down trend test, $p \leq 0.0254$; Figure 1). The highest observed mortality was 41.7% in a replicate at the 74.2 g a.e./ha treatment group. Average mortality in affected treatment groups was less than 20%. Efforts to model rate-response were unsuccessful. Application rate was a significant predictor of above ground biomass at test termination for all experimental plants ($p < 0.0001$), and also for surviving plants ($p < 0.0001$; Figure 2; Table 3), with biomass reduced with increasing glyphosate exposure.

4.3.2 *Rudbeckia hirta* Flowering Phenology

For *R. hirta*, application rate was a significant predictor of average time to first bloom (which increased with increasing application rate; $p < 0.0001$; Figure 3) and the proportion of plants producing inflorescences (which decreased with increasing rate; $p < 0.0001$; Figure 4 and Table 3). There were significant trends ($p \leq 0.0464$) of increasing proportion of aborted buds with increasing application rate which were sustained in the Cochran-Armitage step-down test until the highest treatment level included was 52.8 g a.e./ha (Figures 5). None of the parametric

models applied fit the data well (Pearson Chi-Square, $p < 0.0001$). Accordingly, a nonparametric model was fit, and used to estimate ER_x values (Figure 6, Table 3; $p < 0.0001$ for rate as predictor). Glyphosate application rate was a significant predictor of average bloom-weeks per experimental plant, and also per surviving and reproductive plant ($p < 0.0001$; Figure 7 and Table 3). Rate was also a significant predictor of the average number of inflorescences per experimental plant ($p = 0.006$; Figure 8 and Table 3); however, there was no significant difference in the average number of inflorescences per surviving and reproductive plant ($p = 0.4055$). Glyphosate application rate was a significant predictor of bloom duration ($p < 0.0001$; Figure 9 and Table 3).

4.3.3 *Rudbeckia hirta* Inflorescence Characteristics

Observed malformations in *R. hirta* inflorescences included missing sterile ray floret petals, curled sterile floret petals, rumpled/bumpy surface to the inflorescence head and sterile ray floret petals emerging from near centre of the inflorescence head (Figure 10). There was a significant increasing trend of proportion of malformed inflorescences with glyphosate rate at all treatment levels using the Cochran-Armitage step-down trend test ($p \leq 0.0068$; Figure 11). Since the parametric models applied did not provide good fit (Pearson- Chi-Square; $p = 0.0037$), a nonparametric model was fit by spline interpolation over rate to estimate ER_x values (Figure 12 and Table 3).

4.3.4 *Centaurea cyanus* Survival and Growth

In both the PHEN experiment and the INFLO experiment there were no *C. cyanus* mortalities in the controls. In both experiments application rate was a significant predictor of mortality (with mortality increasing with rate; $p < 0.0001$ and $p = 0.008$, respectively; Figure 13). In the PHEN

experiment, a single mortality occurred at the 31.4 g a.e./ha treatment level, otherwise all mortalities occurred at or above 77.9 g a.e./ha, with four whole replicates lost at 167.6 g a.e./ha. In the INFLO experiment, mortalities occurred at all treatment levels, with up to five of six plants being lost from one replicate in the highest treatment group (74.7 g a.e./ha). Predicted ER10, ER25 values for mortality were higher for the shorter INFLO experiment, with no overlap of 95% confidence limits (Table 3). In both experiments rate was also a significant predictor of above ground biomass, for experimental plants and surviving plants ($p < 0.0001$; Figures 14 and 15, and Table 3). In the INFLO experiment, ER10, ER25 and ER50 estimates exceeded those from the PHEN experiment (Table 3).

4.3.5 *Centaurea cyanus* Flowering Phenology

For *C. cyanus*, application rate was a significant predictor of the average time to first bloom ($p < 0.0001$; Figure 16), with duration to first bloom increasing with glyphosate application rate. The proportion of surviving plants producing inflorescences was 100% in controls and all treatment groups except the 47.4 and 117.2 g a.e./ha treatments. Only the latter was significantly different from controls (Fisher exact $p < 0.0001$; Figure 17). With no clear indication of rate-response, no models were fit to these data. The proportion of buds aborted increased with increasing application rate. Parametric models applied to these data did not provide good fit (Pearson Chi-Square; $p < 0.0001$). Accordingly, a nonparametric model was fit (Figure 18). In this model rate was a significant predictor of the proportion of aborted buds ($p < 0.0001$). Glyphosate application rate was also a significant predictor of average bloom-weeks per experimental plant ($p < 0.0001$), and surviving and reproductive plant ($p < 0.0001$; Figure 19). In the PHEN experiment rate was again found to be a significant predictor of cumulative average number of blooms per experimental plant ($p < 0.0001$), and per surviving and reproductive plant

at both 5- and 8-weeks ($p < 0.0001$; Figures 20 and 21). In the INFLO experiment, at 5-weeks the cumulative average number of inflorescence per experiment and surviving and reproductive plants were also predicted by rate ($p < 0.0001$; Figure 22). However, in the INFLO experiment ER10, 25 and 50 values for these measures of effects were all approximately three to eight times greater than in the PHEN experiment, with no overlap of confidence limit (Table 3).

4.3.6 *Centaurea cyanus* Inflorescence Characteristics

Observed malformations in *C. cyanus* inflorescences included primarily incomplete opening and absent reproductive florets (Figure 23). There was an increasing trend in the proportion of malformed *C. cyanus* inflorescences with rate in the PHEN experiment that was sustained through all steps down in the Cochran-Armitage test ($p \leq 0.0063$; Figure 24). Parametric models applied did not fit the data well (Pearson Chi-Square; $p \leq 0.0042$). Accordingly, a nonparametric model was fit to estimated ERx values (Figure 25; Table 3; $p < 0.0001$ for rate as a predictor in this model). The average mass of *C. cyanus* inflorescences decreased with increasing application rate (Figure 26). Due to some apparent attenuation of effects, the parametric models applied did not provide good fit. Accordingly, a nonparametric model was fit by spline interpolation over rate to estimated ERx values (Figure 27; Table 3; $p < 0.0001$ for rate as a predictor in this model). There was a significant decreasing trend in the average number of reproductive florets found on *C. cyanus* inflorescences in the INFLO experiment (Jonckheere-Terpestra; $p \leq 0.0145$; Figure 28) that was sustained between controls and the lowest treatment level. Parametric models did not fit the data well, and accordingly, again a nonparametric model was fit by spline interpolation over rate in order to estimated ERx values (Figure 29; Table 3; $p = 0.0005$ for rate as a predictor in the model). There was no significant trend in pollen count per reproductive floret across treatment

groups (Jonckheere-Terpstra; $p=0.4387$). However, there was a trend of decreasing *in vitro* pollen germination with increasing rate (Cochran-Armitage $p=0.0425$). This trend extended to the 54.2 g a.e./ha treatment group ($p=0.0333$; Figures 30 and 31, and Table 3) but was no longer significant when this group was excluded. A nonparametric model was fit to the data, with rate as a significant predictor of germination rate ($p<0.0001$).

4.3.7 *Trifolium pratense* Survival and Growth

In both the PHEN and INFLO experiments, there were no mortalities in the control groups. Despite mortalities at all treatment levels in the PHEN experiment, there was no significant trend in mortality with application rate (Cochran-Armitage; $p=0.3679$). In all but one case, replicates experiencing mortality had one of six plants die; one replicate at the 51.6 g a.e./ha treatment level had two mortalities. In the INFLO experiment there were no *T. pratense* mortalities. In both the PHEN and INFLO experiments, glyphosate application rate was a significant predictor of above-ground biomass at test termination for both experimental plants and surviving plants ($p<0.0001$; Figures 32 and 33; Table 3).

4.3.8 *Trifolium pratense* Flowering Phenology

Glyphosate application rate was a significant predictor of the proportion of surviving *T. pratense* plants producing inflorescences ($p<0.0001$; Figures 34; Table 3). The average time to first bloom was longer than controls for all treatment groups except the highest treatment; however, only the first treatment group was significantly different from controls (Exact Wilcoxon $p=0.0043$; Figure 35). Application rate was also a significant predictor of the number of aborted buds, average-bloom weeks, the cumulative average number of inflorescences produced by *T. pratense* experimental plants and surviving and reproductive plants at 8-weeks and 5-weeks

($p < 0.0001$; Figures 36 through 39; Table 3). Glyphosate application rate was also a significant predictor of cumulative average number of inflorescences in the INFLO experiment ($p < 0.0001$; Figure 40), with more pronounced effects and no overlap of the 95% confidence limits on ER50 estimates (Table 3).

4.3.9 *Trifolium pratense* Inflorescence Characteristics

Glyphosate application rate was a significant predictor of bloom duration for *T. pratense* in the PHEN experiment ($p < 0.0001$; Figure 41 and Table 3). Observed malformations in *T. pratense* inflorescences included apparent lack of florets, and stunted petals with protruding filaments and anthers (Figure 42). The proportion of malformed inflorescences generally seemed to increase from controls to the 108.3 g a.e./ha treatment group. However, there were only three inflorescences at the highest treatment group, none of which were observed to be malformed. All treatment levels had significantly higher proportions of malformed inflorescences than the control, other than this highest treatment group ($p < 0.0001$; Figure 43). In the INFLO experiment the average dry weight of inflorescences decreased with application rate up to and including the 35.7 g a.e./ha treatment level. At the next and highest treatment level (50.7 g a.e./ha) there was only one replicate available and this mean fell within the range of controls: The F-test from the associated ANOVA was not significant ($p = 0.517$). The average number of florets per inflorescence decreased linearly with glyphosate application rate ($p < 0.0001$; Figure 44). However, there was no significant trend in pollen count per floret (Jonckheere-Terpestra; $p = 0.2875$), or the proportion of ungerminated pollen grains (Cochran-Armitage; $p = 0.1669$) with glyphosate application rate.

5 Discussion

Glyphosate at drift-equivalent rates had severe effects on flowering in three entomophilous plants when applied at an early bud stage. These effects included delayed flowering, reduced bloom duration, inhibition of flower production and increased bud abortion and floral malformations. With respect to the degree of effects, there were apparent differences between species, endpoints, and experiments. Although the amount of pollen per floret was not significantly reduced by glyphosate exposure, the number of reproductive florets per inflorescence was, in both *C. cyanus* and *T. pratense*. In *C. cyanus*, inflorescence dry weight was also affected. *In vitro* pollen germination was also impacted in, *C. cyanus*, but not in *T. pratense*.

A recent study (Strandberg et al. 2018) has also demonstrated effects on reproductive measures, including flowering, in *C. cyanus* and *T. pratense* exposed to glyphosate (Table 1). However, the present study was more extensive in its examination of effects on flowering in these species (e.g., plants producing inflorescences, aborted buds, bloom-weeks, duration of blooms). More than two (five) treatment levels were assessed, rate-response relationships were established, and importantly, inflorescence characteristics were examined including pollen quantity and quality, malformations, and number of reproductive florets.

5.1 Survival and Growth

With respect to survival, *C. cyanus* was the most sensitive in both the PHEN and INFLO experiments, followed by *R. hirta* (PHEN), while *T. pratense* did not experience any significant effects on survival in either experiment (Tables 3 and 4). However, with respect to above ground

biomass the PHEN experiments suggest that *T. pratense* was the most sensitive species for this endpoint followed by *R. hirta* and *C. cyanus*. In the shorter INFLO experiments *C. cyanus* was considerably more sensitive, with the lowest ERx values across all species and experiments for above ground biomass, while *T. pratense* had a response that was comparable to the PHEN experiment (Tables 3 and 4).

Boutin et al. (2004) reported ER50 values for 21-day above ground biomass for *R. hirta* and *C. cyanus* of 24.7 (95% CI: 15.86-29.98) and 29.2 (95% CI: 23.3-27.3) g a.e./ha, respectively with glyphosate applications at the 4-8 leaf stage in those experiments. Results for *C. cyanus* were replicated later by Strandberg et al. (2012), who reported an ER50 of 32.7 (26.5, 38.8) for plants exposed at the 4-leaf stage. These ER50s are lower than those derived in the present study for plants eight weeks following application at an early bud-stage (Table 3). However, the ER50s for biomass of *C. cyanus* are comparable with results presented here for plants harvested five weeks after spray at an early bud stage (INFLO experiment; Table 3). Strandberg et al. (2018) recently investigated the effects of glyphosate on above ground biomass of *C. cyanus* and *T. pratense* exposed at a 6-8 leaf stage or at an early bud stage to 14.4 or 72 g a.e./ha. Significant effects were observed when plants were sprayed at 72 g a.e./ha at the 6-8 leaf stage (in the Canadian experiment only), and at both 14.4 and 72 g a.e./ha at the early bud stage (Danish experiment only). Differences in results may be attributed in part to differences in greenhouse conditions, and herbicide application system (Strandberg et al. 2018). By comparison in this study ER50s for above ground biomass of 27.2 (95% CI:16.6, 44.6; INFLO) and 77.6 (95% CI: 67.4, 89.4; PHEN) g a.e./ha for experimental plants were estimated, which is similar to effects on biomass observed in the Danish experiments.

In Strandberg et al. (2018) *T. pratense* above ground biomass was significantly affected at the highest of two treatment levels, 72 g a.e./ha, with both early and late stage applications, but not at 14.4 g a.e./ha. By comparison, in the current study the ER10s of above ground biomass of experimental plants were close to the lower treatment level in Strandberg et al. (2018), and a decreasing trend was observed through 72 g a.e./ha. In an early greenhouse study Sergura et al. (1978) applied 100-400 g a.e./ha of glyphosate on two-month-old red clover plants (stage not specified, but likely close to bud or early flowering). Glyphosate significantly lowered above ground dry weight at 200 g/ha and higher at the time of the first harvest (15 days post treatment); for the second harvest at 30 days post-treatment, significant reductions were only detected in the highest treatment group of 400 g/ha. No significant effects were found at 100 g a.e./ha. In contrast the present study predicts effects on biomass at concentrations almost an order of magnitude lower (12.5 g a.e./ha PHEN ER10 for *T. pratense* above ground biomass per experimental plant, Table 3). Unfortunately, environmental conditions were not reported by Sergura et al. (1978).

Above ground biomass results for other species exposed to glyphosate suggest that early life stages are generally more sensitive to effects on above ground biomass. Strandberg et al. (2012) in another set of experiments compared effects of glyphosate on above ground biomass in three annuals (*Tripleurosperum inodorum* (L.) Sch.Bip., *S. noctiflora*, *G. molle*) and three perennials (*Achillea millefolium* L., *Silene vulgaris* (Moench) Garcke, *G. robertianum*) exposed at a vegetative stage versus a reproductive stage. In all cases, ER50 values were greater for plants

treated at a reproductive stage, and there was no trend in terms of annuals or perennials being more or less sensitive (Strandberg et al. 2012).

5.2 Effects on Flowering Phenology

For measures of effects to flowering, including the time to first bloom in surviving plants, the percent of aborted buds, the average bloom-weeks and average cumulative number of inflorescences (per experimental and surviving and reproductive plant), and the average bloom duration (assessed for *T. pratense* and *R. hirta* only), *T. pratense* was generally more sensitive than *C. cyanus*, which was more sensitive than *R. hirta* (Table 4). That *T. pratense* was more sensitive to effects on flowering may in part be a function of its life cycle and associated differential responses to stress. *C. cyanus* is a strict determinate annual. Under stress, such short-lived species may increase reproductive efforts to improve the likelihood of establishing offspring (Grime 2006). *T. pratense* is more flexible in its life cycle, and generally behaves as a perennial, and as such perhaps can afford the time to recover from exposure before significant reproductive efforts are made. *R. hirta* was least sensitive to effects on flowering. Although this species is also flexible in its life cycle, and is known to behave as an annual, biennial or perennial, it is possible that *R. hirta* was simply less sensitive than the other species to exposure possibly due to the presence of trichomes over the surface of its leaves and stems. These structures may suspend spray droplets above the leaf surface, limiting absorption (Whitford et al. 2014). When assessing seed production in annuals and perennials exposed to various herbicides, Strandberg et al. (2018) found that annuals were generally more sensitive.

Effects on the average cumulative number of flowers produced at five weeks was much more pronounced in the INFLO experiments than the PHEN experiments for *C. cyanus* and *T. pratense*. This could be due to variable environmental conditions, though for *T. pratense* pest pressures may have also been a factor (see discussion of study limitations further below).

In the recent Canadian experiments from Strandberg et al. (2018), *T. pratense* experienced approximately a 50% reduction in flower production when treated with 72 g a.e./ha at an early bud stage, with no significant effects of application at 14.4 or 72 g a.e./ha when application occurred at the 6-8 leaf stage. For *C. cyanus* two seed lots were tested in Canada (one lot sourced in Canada, and the other in Denmark). The Danish *C. cyanus* seed lot was also tested in Denmark, while the Canadian seed lot was not tested in Denmark. Results for *C. cyanus* in the Danish and Canadian experiments of Strandberg et al. (2018) were mixed, with no effects in the Canadian experiment with Danish seed stock, and at or close to 100% reduction in flower production at 72 g a.e./ha when applications occurred at both the 6-8 leaf stage and early bud stage in the Danish experiment with Danish seed stock. Canadian seed stock was tested in Canada, and effects were only seen with application at the 6-8 leaf stage, and 72 g a.e./ha (approximately 40% reduction in flower production). For *C. cyanus* these results bracket the current PHEN experiment, from which ER50s of 70.9 and 77.3 are reported for five and eight weeks post spray, respectively (Table 3). Differences may in part be attributed to seed sources, glyphosate formulation, differences in growing conditions and pest pressures. Notably, differences between Danish and Canadian experiments in Strandberg et al. (2018) were attributed to differences in growing conditions and methods of herbicide application (seeds were from the same source). Flower production for *T. pratense* was also assessed in a drift study

conducted by Strandberg et al. (2018). Flower production was found to be significantly affected in a 20x100 m plot that had been established with a mix of perennials one year prior to conducting the drift exposure with glyphosate (application rate of 1440 g a.e./ha). The authors reported that 2.8% of the application rate was the highest measured in the plot, however, this deposition was measured on plastic hair curlers. Thus, the estimates in this case reflects deposition on this particular shape, as opposed to vertical deposition on a flat horizontal surface. The latter is used to determine exposure in standard toxicity studies and to estimate drift exposure (e.g., units of g/ha or lb/A).

For both *R. hirta* and *C. cyanus* (PHEN) glyphosate rate was found to be a significant predictor of average time to first bloom. By comparison, Strandberg et al. (2018) found that peak bloom was also delayed in *C. cyanus* treated with 72 g a.e./ha both at the 6-8 leaf stage and at the early bud stage, in both the Canadian and Danish experiments. In the present study *T. pratense* only showed a significant delay in flowering at the lowest treatment level; an anomalous result. In Strandberg et al. (2018), *T. pratense* did not experience any significant delay in peak flowering when exposed at the early bud stage, though plants exposed at the 6-8 leaf stage to 72 g a.e./ha did experience a delay.

No other studies were found examining flowering in the same test species used in the present study. However, significant effects of glyphosate on flower production have been reported for *Solanum lycopersicum* (field study; Kruger et al. 2012), *E. montanum*, *T. officinale*, *V. arvensis*., *Cerastium arvense* and *K. arvensis* (greenhouse studies; Strandberg et al. 2018), at drift-equivalent rates; also, in the glyphosate-tolerant *C. erecta* (Panigo et al. 2012). Delays in

flowering (measured as either time to first bloom, or time of peak bloom) have been observed in *Brassica spp.* (greenhouse; Londo et al. 2014), *E. montanum*, *S. notiflora*, *Cirsium arvense*, *T. officinale* and *Ceratium arvense* (greenhouse; Strandberg et al. 2018), and in glyphosate-resistant cotton (Pline et al. 2003).

In the current study both cumulative average number of inflorescences as well as average bloom-weeks (average of sum of flower in bloom, counted weekly) were measured. Bloom-weeks is considered here an indicator of overall floral resource availability, because in theory the same number of flowers can be produced, but if treated flowers are not lasting as long, then resources are limited for pollination and pollinators. For *C. cyanus* rate-response for average-bloom weeks per experimental plant and average cumulative number of inflorescences were comparable (Table 3), suggesting a lack of reduced bloom duration (not assessed in this species) and or negligible reduction in the number of living experimental plants not producing flowers, which was demonstrated (except at 117.2 g a.e./ha; Figure 17). In both *R. hirta* and *T. pratense* average bloom-weeks was slightly more sensitive than the average cumulative number of inflorescences per experimental plant (Table 3), suggesting reduced bloom durations and/or reduced number of surviving plants producing blooms, both of which were demonstrated to some extent in both species (Table 3). When considering surviving and reproductive plants only, response of average bloom-weeks was similar to average cumulative number of inflorescences for again, *C. cyanus*, but also for *T. pratense*. This suggests that bloom duration was perhaps a less important factor in overall floral availability than surviving plants not producing flowers for *T. pratense*. Correspondingly the ER25 and ER50 were both higher for bloom duration than for the number of living plants not producing inflorescences. Interestingly, for *R. hirta* there was no

significant effect on average cumulative number of inflorescences, but there was a rate-response relationship for average bloom-weeks for surviving and reproductive plants. Thus, we can conclude that the observed reductions in bloom duration in *R. hirta* (Table 3) has a considerable impact on floral resource availability, despite no significant reduction in overall flower production per reproducing plant.

Aborted buds were observed in all test species and increased with glyphosate rate. Bud abortion is seen as a mechanism for adjusting reproductive output to meet the resources available (see Mooney et al. 1991 and references therein). Aborted buds have not been reported for these test species elsewhere; however, aborted buds have been reported in at least one other species exposed to glyphosate, *C. erecta* (considered a weed in some agricultural contexts; Panigo et al. 2012). It is perhaps a common effect that is not typically documented in toxicity tests.

5.3 Effects on Inflorescence Characteristics

Malformation of *R. hirta*, *C. cyanus* and *T. pratense* inflorescences were documented as a result of drift-equivalent exposure rates of glyphosate application. Malformations in these test species were seen in the preliminary testing with *R. hirta* and *T. pratense* (Rodney et al. 2015) but have not been reported elsewhere. These changes to floral morphology are consistent with reports of floral malformation in other species including *P. hybrida* treated three times with approximately 42 g/ha at two-day intervals when budding (Shimada and Kimura 2007), glyphosate-resistant annual *I. purpurea* treated with 1121 g a.e./ha 1-2 months from planting (Baucom et al. 2008), and *Brassica spp.* treated with estimated rates of 85 or 112 g a.e./ha exposed four weeks after planting (Londo et al. 2014). A common observed malformity in the INFLO experiments was

clearly reduced number of reproductive florets in both *C. cyanus* and *T. pratense*. This translated into significant effects in the number of reproductive florets in both species; however, inflorescence dry weight was only significantly reduced for *C. cyanus*. No studies appear to have examined effects of glyphosate on floral mass.

5.4 Pollen

Pollen quantity was not significantly reduced for either *C. cyanus* or *T. pratense* on a per reproductive floret basis. However, given the effect to the number of reproductive florets we can deduce that inflorescences of plants treated with glyphosate produced less pollen. *In vitro* pollen germination tests were used to assess potential effects of glyphosate exposure on pollen germination. Significant negative effects on germination were found for *C. cyanus* only. Effects of glyphosate on pollen have not been reported for these test species elsewhere; however, non-viable (or non-germinating) pollen has been reported in glyphosate-resistant cotton and corn treated with glyphosate (Thomas et al. 2004; Yasour et al. 2007); and in *in vitro* experiments with *N. sylvestris* (Grabe and Kristen 1997) and *S. canadensis* (Guo et al. 2009). Baucom et al. (2014) reported decreased pollen production in *Ipomoea purpurea* treated with glyphosate.

With respect to the aromatic amino acids inhibited by glyphosate (phenylalanine, tyrosine and tryptophan), I found no effects on the phenylalanine or tyrosine concentrations in pollen of *R. hirta* and *T. pratense* treated at 10 and 45 g a.e./ha (see Appendix B) in preliminary testing. Tryptophan requires a separate analysis that we intended on employing in the INFLO experiment. Unfortunately, flower production was not sufficient to collect enough pollen for amino acid analyses. Although to my knowledge there are no published results examining amino

acids in pollen of glyphosate treated plants, Shimada and Kimura (2007) did investigate the aromatic amino acid content of corollas of glyphosate treated *P. hybrida*. They found no significant effects of glyphosate treatment but did report an increase in the amino acid proline.

5.5 Implications for Agroecosystems

In the current study all tested rates can be considered drift-equivalent, in that they are less than 50% of the highest recommended label rates. The highest measured application rate in the current study was 167.6 g a.e./ha which is approximately 62% of the lowest recommended application rate on the Roundup WeatherMax[®] With Transorb 2 Technology Liquid Herbicide label of 270 g a.e./ha (Monsanto, 2015). Using AgDrift v 2.1.1 I estimated downwind deposition (Teske et al. 2003); using the Tier I aerial model with the maximum aerial application rate (1798 g a.e./ha), and assuming coarse to very coarse droplet size, gives a deposition of 740 g a.e./ha at 1 m downwind, and 35 g a.e./ha at 50 m downwind. With the lowest aerial application rate of 300 g/ha I estimated a deposition of 125 g a.e./ha at 1 m downwind, and 5.8 g a.e./ha at 50 m downwind. For ground application the maximum allowable application rate is 4320 g a.e./ha (Monsanto 2015). For this rate by ground spray with a low boom (0.5 m) I estimated a median deposition of 361 g a.e./ha at 1 m downwind and 6.8 g a.e./ha at 50 m downwind. With a high boom (1.27 m) the estimates increase to 665 and 12.1 g a.e./ha, respectively. In these rate ranges severe effects to survival, growth and flowering would be anticipated given the results of this study. Potential effects on nontarget plant reproduction may extend beyond 50 m downwind from application based on ER10 estimates (Table 3).

As reviewed by Boutin and Jobin (1998), islands and corridors of uncultivated land in agroecosystems are recognized as being important to the conservation of plant species. These refuges are vulnerable to and affected by their proximity to managed agricultural land. Plant species distribution and richness in hedgerows have been found to be best predicted by adjacent land use, environmental conditions and management practices (de Blois et al. 2002, Decker et al. 2004). Herbaceous plants associated with forests, and a tolerance for shade, have been positively correlated with less intensive agricultural practices (de Bois et al. 2002, Bratli et al. 2006). Short-lived grasses, introduced and weedy species have been associated with managed agricultural land (Boutin and Jobin 1998; Bratli et al. 2006). Freemark et al. (2002) found that native plant species richness was higher in eastern Ontario agricultural landscapes that contained marshes, woodlots and wooded fence rows, than those that did not. In southeastern Norway, researchers found that plant species frequencies were strongly right skewed in patches within agroecosystems, suggesting that rareness was common (Bratli et al. 2006). In many agroecosystems, the relatively small margins between fields are practically the only unmanaged fractions of the landscape.

The width of many hedgerows is small enough that protection from effects of glyphosate may not be attainable for some species given current application methods. Aude et al. (2003) measured 26 hedgerows in agricultural regions of Denmark, and the documented mean hedgerow width was 5.2 ± 0.19 m. In the St. Lawrence lowlands ecoregion of Quebec, Roy and de Blois (2008) measured the width of 117 hedgerows and reported a range of 1.5 to 32.7 m. These authors also found that hedgerow width was the principal predictor of plant richness, abundance and diversity at the study sites, with these variables increasing with width. In Germany, of 130 studied hedgerows, Wehling and Diekmann (2009) reported widths of 3.5 to 5 m. Even a large

hedgerow of 35 m, based on the samples described above, with aerial application and a 70 m buffer, at the far downwind edge we still might expect approximately 13.7 g a.e./ha deposition (not accounting for interception), which exceeds many of the ER10s estimated here for *T. pratense* and *C. cyanus* for both growth and reproductive measures of effects.

These findings are important because non-target plants are already under considerable pressures in agroecosystems due to loss of habitat, and other elements of intensive agriculture including the use of fertilizers, which in combination with herbicide and other pesticide exposures can lead to greater adverse outcomes (e.g., Gove et al. 2007; Schmidt et al., 2013). With climate change projections estimating increasing summer mean and high temperatures (IPCC, 2014), effects of glyphosate on non-target plants may be exacerbated (see discussion of study limitations below). Further, effects on flowering in plants are likely to lead to effects on pollinators, which for entomophilous plants could incite an adverse positive feedback loop of degraded floral resources → low pollination → low reproduction → further degraded floral resources, and so on. In such a scenario, pollinators may be adversely affected if alternative adequate forage is scarce (see Bohnenblust et al. 2016).

Further, the malformation of inflorescences observed in the current study, and by others (Gove et al. 2007; Baucom et al. 2008), may also affect pollination. Flowers lure pollinators with sensory stimuli that include their shape. Flower shape has been demonstrated to affect pollinator visitation (McCall and Primack 1992; Dafni and Kevan 1997; Cepero et al. 2015).

Malformations may obscure intended signals, and impede successful foraging by pollinators, and reproduction of affected plants. This warrants further investigation.

5.6 Regulatory Implications

Generally, ER25 or ER50 values for growth (e.g., height or above ground biomass) are used in regulatory risk assessment to inform risk management decisions regarding pesticide registrations. Results presented here are in accordance with those presented in the literature (Rotchés-Ribalta et al. 2012; Strandberg et al., 2012; Carpenter et al. 2013): ERx values for reproductive measures may be lower or higher than standard ERx values for growth depending on the species, herbicide and test conditions. The current regulatory paradigm does not account for the full life-cycle of potentially exposed plants. Enough data has been amassed to demonstrate that no one assessment endpoint or toxicity endpoint can be reliably used to ensure protection of non-target plants. Effects of herbicide exposure are aggregated in individuals (e.g., an exposed plant does not only experience an ERx for biomass, it also experiences concurrently an ERx for survival, reproduction, and other potential adverse effects), and these effects are modified by both biotic and abiotic factors in the surrounding environment. Clearly defined protection goals should lead to suitable assessment endpoints. However, regulatory protection goals are generally equivocal (e.g., no unacceptable risks to the environment; U.S. Federal Insecticide, Fungicide, and Rodenticide Act), leading to correspondingly equivocal risk assessments.

If risks to plants are to be considered, in accordance with standard assessment of effects to wildlife, survival, growth *and reproduction* should be assessed. Reproductive endpoints, whether lower or higher than vegetative growth endpoints, should be considered in the context of protection goals in risk assessment and risk management decision-making.

5.7 Limitations of the Current Study

In this study it was not possible to completely eliminate pests, which can be difficult to control in the greenhouse. Effects of aphids and spider mites on *T. pratense* could not be precluded, particularly in the INFLO experiment. The presence of these pests was noted in many replicates, with no apparent trend with treatment. It is possible that glyphosate exposure with concurrent pest pressure led to more severe effects than would have been predicted with glyphosate exposure alone, and this could in part explain the lower ERx values obtained in this experiment, compared to other (e.g., Strandberg et al. 2018).

Although statistical comparisons were not appropriate due to differences in timing of application and sizes of experimental units, sensitivity of both *C. cyanus* and *T. pratense* average cumulative flower production by five weeks post-spray was greater in the INFLO experiment than in the PHEN experiment. *C. cyanus* survival at five-weeks was also more sensitive in the INFLO experiment than at 8-weeks in the PHEN experiment. These differences may be attributed in part to differences in absorption and translocation of glyphosate under different environmental conditions, given the higher average maximum temperature and humidity in the INFLO sets of experiments. With Bermuda grass (*Cynodon dactylon* L.), it was found that 24-hours after treatment with 560 g/ha, greater translocation of radiolabelled glyphosate was observed with higher temperature and humidity. Fresh weight data 8-weeks post-treatment indicated that at 40% relative humidity and 32°C glyphosate (at 560 g/ha) was more toxic to Bermuda grass than at 22°C; the greatest effects to fresh weight were observed at 100% humidity (Jordan 1977). Effects on above ground biomass, node viability and translocation of glyphosate in *Elymus repens* L. generally increased with light, temperature and humidity treatments in the 48 hours

following application, demonstrating the importance of environmental conditions following applications (Coupland 1983). It is in this time frame immediately after application that the herbicide is making direct contact with the plant and it has the potential for absorption and translocation to sites of toxic action (Coupland 1983). Similarly, Ganie et al. (2017) found that efficacy (represented by 21-day above ground dry weight) was improved with high temperature (29°C day/17°C night) over low temperature (20°C day/11°C night) in *Ambrosia* spp. with the difference attributed to greater translocation. In field studies, time of day of glyphosate application appears to influence efficacy (measured as % weed control), with midday application being most efficacious. This result has been attributed to increased air temperature and may also be related to presence of dew and diurnal leaf movement (Stopps et al. 2013). The environmental conditions in the greenhouse, particularly during the INFLO experiment, were likely not representative of expected conditions in many agroecosystems of the northern hemisphere. In particular, an average maximum temperature of 40°C or more may only be comparable to growing conditions in some locations in the southwestern United States, North Africa and South Asia.

Another limitation of this study is that glyphosate exposures were not exactly reflective of drift exposures in the field. In this experiment plants were sprayed directly (from above) in a spray booth. However, actual exposure (i.e., the true dose and pattern of interception of that dose) in the field due to drift from treated fields will differ even at equivalent deposition rates to the spray booth. This may be attributed to changes in droplet size spectrum (due to evaporation and deposition), the orientation of the plant relative to the drift cloud, as well as interception by surrounding vegetation. In field drift studies with test organisms, researchers have found that

effects are less than expected based on direct spray test results (e.g., Strandberg et al. 2012; Brain et al. 2017); however, it has been hypothesized that differences may also be attributed in part to the physical properties of the pots in which plants were exposed (Strandberg et al. 2012). Nevertheless, exposure will evidently differ between direct overhead spray and primarily horizontal drift. Thus, a limitation of the current study, is that effects in test species may not be representative of field exposures due to differences in actual dosing and administration among spray booth and drift exposures at the same deposition rates.

The effects of plant species interactions were not accounted for in this study. Test organisms were grown with one (*T. pratense* and *C. cyanus*) or two (*R. hirta*) per pot. However, under real world exposure conditions exposed plants will be part of a community with interspecies competition. It has been demonstrated that these more realistic exposure scenarios can alter the outcome for individuals exposed to glyphosate (Dalton and Boutin 2010; Strandberg et al. 2018).

5.8 Conclusion

Glyphosate is extensively used globally to control weeds in agricultural fields. In this study, I demonstrated that drift-equivalent deposition rates can cause severe effects to flowering of entomophilous herbaceous plants when applied at an early bud stage. If effects of this degree occur in field margins, other adverse repercussions may ensue. Inhibition of flowering can reduce pollinator visits (Bohnenblust et al. 2016), and is expected to reduce seed production, and limit contributions to the seed bank (reserve of seeds stored in soil for future propagation). In turn this may alter plant community structure and composition, and further reduce forage for higher trophic levels. Demonstrating the potential long-term effects of herbicide use in

agroecosystems is constructive to the discussion of acceptable risk. If risks from herbicides to plant species (and the taxa that rely on them) are to be appropriately managed, risk managers require inclusive risk characterizations that include reproductive assessment endpoints. Future research should continue to examine effects to floral resources, potential indirect effects on mutualistic species, population- and community-level endpoints.

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7 Tables

Table 1. Summary of reviewed studies reporting on adverse effects of direct application of glyphosate on reproductive measures in terrestrial plants^a

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Pea, <i>Pisum sativum</i> L.	Crop	Non-tolerant	Non-target	Green-house	RoundUp Original, Monsanto	Direct application	0, 0.833, 1.666, 8.33, 83.3	14 or 15 days after emergence or at opening of first flowers(18 to 21 days after emergence)	Single species	Seed dry weight	NOEL = 8.33 g a.i./ha; ER25=8 ± 1.06 (SE) g a.i./ha	Olszyk et al. 2009
Tomato, <i>Lycopersicon esculentum</i> L.	Crop	Non-tolerant	Non-target	Field	Not reported	Direct application	0, 0.64, 2.1, 6.4, 21, 64, 210	vegetative or early bloom stage	Single species	Flower production	Early vegetative application: ER25 = 51.1 g a.e./ha, ER50 = 67.0 g a.e./ha; Early bloom application: ER25 = 7.5 g a.e./ha, ER50 = 13.6 g a.e./ha	Kruger et al. 2012
Tomato, <i>Lycopersicon esculentum</i> L.	Crop	Non-tolerant	Non-target	Field	Not reported	Direct application	0, 0.64, 2.1, 6.4, 21, 64, 210	vegetative or early bloom stage	Single species	Fruit production	Early vegetative application: ER25 = 43.9 g a.e./ha; Early bloom application: ER25 = 8.5 g a.e./ha	Kruger et al. 2012
Corn, <i>Zea mays</i> L.	Crop	Tolerant (Resistant cultivar: DK 662RR and DKC 6410RR)	Non-target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 1120	DK 662RR: V6, V10, and V6 and V10; DKC 6410RR: V4, V8, V4 and V8, V4 and V10	Single species	Pollen viability	Significant reductions with applications after V6 for both varieties.	Thomas et al. 2004

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Corn, <i>Zea mays</i> L.	Crop	Tolerant (Resistant cultivar: DK 662RR and DKC 6410RR)	Non-target	Greenhouse	Roundup UltraMax, Monsanto	Direct application	0, 1120	DK 662RR: V6, V10, and V6 and V10; DKC 6410RR: V4, V8, V4 and V8, V4 and V10	Single species	Pollen and anther production	Pollen and anther production were significantly adversely affected by glyphosate application.	Thomas et al. 2004
Corn, <i>Zea mays</i> L.	Crop	Tolerant (Resistant cultivar: DK 662RR and DKC 6410RR)	Non-target	Greenhouse	Roundup UltraMax, Monsanto	Direct application	0, 1120	DK 662RR: V6, V10, and V6 and V10; DKC 6410RR: V4, V8, V4 and V8, V4 and V10	Single species	Kernel set and weight	No significant adverse effects of treatment.	Thomas et al. 2004
Corn, <i>Zea mays</i> L.	Crop	Tolerant (Resistant cultivar: DK 687RR and DKC 6410RR)	Non-target	Field	Roundup UltraMax, Monsanto	Direct application	0, 1120	V4, V8, V4 and V8, V4 and V10	Single species	Pollen viability	Significantly reduced when application occurred after V4	Thomas et al. 2004
Corn, <i>Zea mays</i> L.	Crop	Tolerant (Resistant cultivar: DK 687RR and DKC 6410RR)	Non-target	Field	Roundup UltraMax, Monsanto	Direct application	0, 1120	V4, V8, V4 and V8, V4 and V10	Single species	grain yield	No significant effects.	Thomas et al. 2004
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivar: DP 5415RR)	Non-target	Field	Roundup Ultra, Monsanto	Direct application	0, 720, 1080, 1444	2-3 leaf stage (early), 4-5 leaf stage (medium), 8-10 leaf stage (late), 12-13 leaf stage (very late) or early and late (split)	Single species	Abnormal flowers	Detected in plants treated at 8 leaf stage and older. Abnormalities included: partially developed anthers (non-dehiscent), undeveloped pollen, stigma protruding above stament, some style elongation	Yasuor et al. 2007

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivar: DP 5415RR)	Non-target	Field	Roundup Ultra, Monsanto	Direct application	0, 720, 1080, 1444	2-3 leaf stage (early), 4-5 leaf stage (medium), 8-10 leaf stage (late), 12-13 leaf stage (very late) or early and late (split)	Single species	Pollen viability	All pollen grains from treated plants collapsed, were deemed non-viable with staining method.	Yasuor et al. 2007
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivar: DP 5415RR)	Non-target	Field	Roundup Ultra, Monsanto	Direct application	0, 720, 1080, 1444	2-3 leaf stage (early), 4-5 leaf stage (medium), 8-10 leaf stage (late), 12-13 leaf stage (very late) or early and late (split)	Single species	Boll weight	Significantly reduced with split application in one trial of four at 1444 g a.e./ha.	Yasuor et al. 2007
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivar: DP 5415RR)	Non-target	Field	Roundup Ultra, Monsanto	Direct application	0, 720, 1080, 1444	2-3 leaf stage (early), 4-5 leaf stage (medium), 8-10 leaf stage (late), 12-13 leaf stage (very late) or early and late (split)	Single species	Seed yield	No significant effect of glyphosate application	Yasuor et al. 2007
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Greenhouse	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	pollen viability (Alexander's stain, fluorochromatic reaction, Brewbacker & Kwack, Brewbacker and Kwack with	Significant reductions in viability for three of four methods of assessing viability with glyphosate treatment.	Pline et al. 2002a

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
										aniline blue)		
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	pollen abnormalities (fluorochromatic reaction)	Significant increase in abnormal pollen with glyphosate treatment. Pollen of treated plants was malformed and presumed to be at an immature developmental stage or aborted at anthesis.	Pline et al. 2002a
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	seeds per boll	Significant decrease in seeds per boll for DP 5415RR, but not for SG 125RR with glyphosate treatment.	Pline et al. 2002a
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Staminal column height	Significant decrease in staminal column height in first two weeks of flowering, but not in weeks three or four.	Pline et al. 2002b
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Stigma height	Stigma height significantly greater in SG 125RR treated plants. No significant difference for DP 5415RR.	Pline et al. 2002b
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Anther length	Significant decrease in anther length with glyphosate	Pline et al. 2002b

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		5415RR and SG 125RR)									treatment in both cultivars	
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Anther to stigma distance	Significant increase in anther to stigma distance in first three weeks of flowering of both cultivars. In fourth week distance remained on average greater in flowers of treated plants, but was only significantly different for DP 5415RR.	Pline et al. 2002b
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Loose pollen grains per stigma	Significant reduction in loose pollen grains per stigma with glyphosate treatment of both cultivars.	Pline et al. 2002b
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Days to first bloom	Greater average time to first bloom in treated cultivars. Difference significant for SG 125RR only.	Pline et al. 2003
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non- target	Green- house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Bolls	Lower average number of bolls for treated cultivars, but difference not statistically significant.	Pline et al. 2003

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Green-house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Squares	Lower average number of squares for treated cultivars, but difference not statistically significant.	Pline et al. 2003
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Green-house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Aborted positions	Increased average number of aborted positions for treated cultivars, but difference not statistically significant.	Pline et al. 2003
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Green-house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Attached dead bolls/squares	None.	Pline et al. 2003
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Green-house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Boll starch	No significant effect in either cultivar.	Pline et al. 2003
Cotton, <i>Gossypium hirsutum</i> L.	Crop	Tolerant (Resistant cultivars: DP 5415RR and SG 125RR)	Non-target	Green-house	Roundup Ultra, Monsanto	Direct application	0, 1120	4- and 8-leaf stages	Single species	Boll sugars	Significant reduction in boll tissue fructose of treated SG 125RR only.	Pline et al. 2003
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR)	Non-target	Green-house	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Days to flowering	Significant effects (delay) on non-transgenic varieties at both treatment levels.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		(resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))										
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Flower attempt	Significant increase in non-transgenic varieties at low rate.	Londo et al. 2014
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Duration of flowering	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther length	Significant decrease in all but the resistant variety.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		transgenic), cv. Sponsor (non-transgenic))										
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther width	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther ratio	Significant change.	Londo et al. 2014
Brassica, <i>Brassica napus</i> L.	Crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Pistil function	Significant decrease. Only null variety assessed.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		(non-transgenic))										
Bachelor's button, <i>Centaurea cyanus</i> L.	Non-crop	Non-tolerant	Non-target	Green-house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: significant reductions at 72 g a.e./ha for both 6-8 leaf and early bud stage application; Canada: significant reduction at 14.4 g a.e./ha at 6-8 leaf stage application	Strandberg et al. 2018
Bachelor's button, <i>Centaurea cyanus</i> L.	Non-crop	Non-tolerant	Non-target	Green-house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Denmark: significant reductions at 72 g a.e./ha for both 6-8 leaf and early bud stage application; Canada: significant reduction at 14.4 g a.e./ha at 6-8 leaf stage application	Strandberg et al. 2018
Bachelor's button (sourced in Canada), <i>Centaurea cyanus</i> L.	Non-crop	Non-tolerant	Non-target	Green-house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Canada: Significant effects at 72 g a.e./ha for 6-8 leaf stage application only.	Strandberg et al. 2018
Bachelor's button (sourced in Denmark), <i>Centaurea cyanus</i> L.	Non-crop	Non-tolerant	Non-target	Green-house	Denmark experiments: Glyphogan, Adama; Canadian	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf and early	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
					experiments: Glyphos® Cheminova, Canada						bud stage application; Canada: No significant effects.	
Bladder campion, <i>Silene vulgaris</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Roundup Bio, Monsanto	Direct application	0, 22.5, 45, 90, 180, 360, 720	6-10 leaf or early bud stage	Single species	Seed production	6-10 leaf ER50 = 37.6 (17.7-57.4) g a.i./ha; early bud ER50 approximately 11-45 g a.i./ha	Strandberg et al. 2012
Blue wildrye, <i>Elymus glaucus</i> Buckley	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover, reproductive structures, total seed dry weight	No significant effects.	Olszyk et al. 2017
Blue wildrye, <i>Elymus glaucus</i> Buckley	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% immature seed dry weight	Significant effects at 83.0 g/ha	Olszyk et al. 2017
Blue wildrye, <i>Elymus glaucus</i> Buckley	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	Reproductive structures	Significant effects at 166 g/ha across locations.	Olszyk et al. 2017
Common woolly sunflower, <i>Eriophyllum lanatum</i> (Pursh) Forbes	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
Common woolly sunflower, <i>Eriophyllum lanatum</i> (Pursh) Forbes	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% immature seed dry weight	Significant effects at 83.0 g/ha at one of two sites.	Olszyk et al. 2017
Common woolly sunflower, <i>Eriophyllum</i>	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Reproductive structures	Significant effects at 83.0 g/ha at one of two sites.	Olszyk et al. 2017

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
<i>lanatum</i> (Pursh) Forbes												
Common woolly sunflower, <i>Eriophyllum lanatum</i> (Pursh) Forbes	Non- crop	Non- tolerant	Non- target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Total seed dry weight	Significant effects at 83.0 g/ha across sites.	Olszyk et al. 2017
Common woolly sunflower, <i>Eriophyllum lanatum</i> (Pursh) Forbes	Non- crop	Non- tolerant	Non- target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% cover, reproducti ve structures, total seed dry weight	No significant effects of glyphosate across sites.	Olszyk et al. 2017
Common woolly sunflower, <i>Eriophyllum lanatum</i> (Pursh) Forbes	Non- crop	Non- tolerant	Non- target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% immature seed dry weight	Significant effects at 166 g/ha across locations.	Olszyk et al. 2017
Creeping thistle, <i>Cirsium arvense</i>	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: No significant effects.	Strandberg et al. 2018
Creeping thistle, <i>Cirsium arvense</i>	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinabl e seeds	Denmark: No significant effects.	Strandberg et al. 2018
Creeping thistle, <i>Cirsium arvense</i>	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama;	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for the 6-	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
					Canadian experiments: Glyphos® Cheminova, Canada						8 leaf stage application only.	
Dandelion, <i>Taraxacum officinale</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: significant reduction at 72 g a.e./ha for the 6-8 leaf and early bud stage applications only; Canada: no significant effects.	Strandberg et al. 2018
Dandelion, <i>Taraxacum officinale</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Denmark: significant reduction at 72 g a.e./ha for the 6-8 leaf only; Canada: no significant effects.	Strandberg et al. 2018
Dandelion, <i>Taraxacum officinale</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf and early bud stage application; Canada: No significant effects.	Strandberg et al. 2018
Dovesfoot Geranium, <i>Geranium molle</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Roundup Bio, Monsanto	Direct application	0, 22.5, 45, 90, 180, 360, 720	6-10 leaf or early bud stage	Single species	Seed production	6-10 leaf ER50 approximately 22.0-45.0 g a.i./ha; early bud ER50 approximately 0-22.0 g a.i./ha	Strandberg et al. 2012

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
European field pansy, <i>Viola arvensis</i> Murray	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: significant reductions at 72 g a.e./ha for both 6-8 leaf and early bud stage application, and at 14.4 g a.e./ha for the early bud stage application; Canada: no significant effects	Strandberg et al. 2018
European field pansy, <i>Viola arvensis</i> Murray	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Denmark: significant reductions at 72 g a.e./ha for both 6-8 leaf and early bud stage application; Canada: no significant effects	Strandberg et al. 2018
European field pansy, <i>Viola arvensis</i> Murray	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf and early bud stage application, also at 14.4 g a.e./ha for early bud stage application; Canada: No significant effects.	Strandberg et al. 2018
field chickweed, <i>Cerastium arvense</i> L.	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments:	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: No significant effects.	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
					Glyphos® Cheminova, Canada							
field chickweed, <i>Cerastium arvense</i> L.	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinabl e seeds	Denmark: No significant effects.	Strandberg et al. 2018
field chickweed, <i>Cerastium arvense</i> L.	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf and early bud stage application; Canada: No significant effects.	Strandberg et al. 2018
field scabiosa, <i>Knautia arvensis</i>	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: significant reduction at 72 g a.e./ha for the 6- 8 leaf stage application only	Strandberg et al. 2018
field scabiosa, <i>Knautia arvensis</i>	Non- crop	Non- tolerant	Non- target	Green- house	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinabl e seeds	Denmark: significant reduction at 72 g a.e./ha for the 6- 8 leaf stage application only	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
field scabiosa, <i>Knautia arvensis</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf stage application only.	Strandberg et al. 2018
Lance selfheal, <i>Prunella vulgaris</i> L. subsp. lanceolata (W. Bartram) Hultén	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover, reproductive structures, total seed dry weight	No significant effects across locations.	Olszyk et al. 2017
Lance selfheal, <i>Prunella vulgaris</i> L. subsp. lanceolata (W. Bartram) Hultén	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% cover	No significant effects	Olszyk et al. 2017
Lance selfheal, <i>Prunella vulgaris</i> L. subsp. lanceolata (W. Bartram) Hultén	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	Reproductive structures	No significant effects	Olszyk et al. 2017
Large camas, <i>Camassia leichtlinii</i> (Baker) S. Watson	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover, % immature seed dry weight, reproductive structures, total seed dry weight	No significant effects.	Olszyk et al. 2017
Large camas, <i>Camassia leichtlinii</i> (Baker) S. Watson	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	Significant effects at 166 g/ha across locations.	Olszyk et al. 2017

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Large camas, <i>Camassia leichtlinii</i> (Baker) S. Watson	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Reproductive structures and total seed dry weight	Significant effects at 83.0 and 166 g/ha across sites.	Olszyk et al. 2017
Nightflowering silene, <i>Silene noctiflora</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Roundup Bio, Monsanto	Direct application	0, 22.5, 45, 90, 180, 360, 720	6-10 leaf or early bud stage	Single species	Seed production	6-10 leaf ER50 = 87.2 (51.7-122.7) g a.i./ha; early bud ER50 = 43.1 (20.9-65.4) g a.i./ha	Strandberg et al. 2012
nightflowering silene, <i>Silene noctiflora</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: No significant effects.	Strandberg et al. 2018
nightflowering silene, <i>Silene noctiflora</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Denmark: No significant effects.	Strandberg et al. 2018
nightflowering silene, <i>Silene noctiflora</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: No significant effects.	Strandberg et al. 2018
Petunia, <i>Petunia hybrida</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	Free amino acids in corollas	Not significant different for all but two amino	Shimada and Kimura 2007

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
D. Don ex Loudon											acids (Proline increased, Methionine decreased).	
Petunia, <i>Petunia hybrida</i> D. Don ex Loudon	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	Soluble protein, soluble phenols, lignin, indole-3-acetic acid in corollas	No significant difference	Shimada and Kimura 2007
Petunia, <i>Petunia hybrida</i> D. Don ex Loudon	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	Nitrate in corollas	Significantly reduced	Shimada and Kimura 2007
Petunia, <i>Petunia hybrida</i> D. Don ex Loudon	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	RNA in corollas	Significantly reduced	Shimada and Kimura 2007
Petunia, <i>Petunia hybrida</i> D. Don ex Loudon	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	DNA in corollas	No significantly different	Shimada and Kimura 2007
Petunia, <i>Petunia hybrida</i> D. Don ex Loudon	Non-crop	Non-tolerant	Non-target	Greenhouse	Not reported	Direct application	0, 42	45 days from planting	Single species	Number of flower buds	Not significantly different	Shimada and Kimura 2006
Red clover, <i>Trifolium pratense</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos@ Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Canada: Significant effects at 72 g a.e./ha for the early bud stage application only.	Strandberg et al. 2018
Red clover, <i>Trifolium pratense</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Canada: Significant effects at 72 g a.e./ha for the	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
					experiments: Glyphos® Cheminova, Canada						early bud stage application only.	
Red clover, <i>Trifolium pratense</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Canada: Significant effects at 72 g a.e./ha for the early bud stage application only.	Strandberg et al. 2018
Robert geranium, <i>Geranium robertianum</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Roundup Bio, Monsanto	Direct application	0, 22.5, 45, 90, 180, 360, 720	6-10 leaf or early bud stage	Single species	Seed production	6-10 leaf ER50 approximately 110 g a.i./ha; early bud ER50 approximately 120 g a.i./ha	Strandberg et al. 2012
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% immature seed dry weight	Significant effects at 83.0 g/ha across sites.	Olszyk et al. 2017
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Reproductive structures	No significant effects across locations.	Olszyk et al. 2017

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Total seed dry weight	No significant effects across locations.	Olszyk et al. 2017
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% cover, reproductive structures, total seed dry weight	No significant effects of glyphosate across sites.	Olszyk et al. 2017
Roemer's fescue, <i>Festuca idahoensis</i> Elmer subsp. roemerii (Pavlick) S. Aiken	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% immature seed dry weight	Significant effects at 166 g/ha at one of two sites only.	Olszyk et al. 2017
Slender cinquefoil, <i>Potentilla gracilis</i> Douglas ex Hook.	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
Slender cinquefoil, <i>Potentilla gracilis</i> Douglas ex Hook.	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% cover, reproductive structures, total seed dry weight	No significant effects across locations.	Olszyk et al. 2017
Toughleaf iris, <i>Iris tenax</i> Douglas ex Lindl.	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
Toughleaf iris, <i>Iris tenax</i> Douglas ex Lindl.	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Reproductive structures	No significant effects across locations.	Olszyk et al. 2017

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Toughleaf iris, <i>Iris tenax</i> Douglas ex Lindl	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	Total seed dry weight	No significant effects across locations.	Olszyk et al. 2017
Toughleaf iris, <i>Iris tenax</i> Douglas ex Lindl	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	Total seed dry weight	Significant effects at 83.0 and 166 g/ha across sites.	Olszyk et al. 2017
Virginia strawberry, <i>Fragaria virginiana</i> Duchesne	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 8.3, 83.0	Late April of 2010	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
Virginia strawberry, <i>Fragaria virginiana</i> Duchesne	Non-crop	Non-tolerant	Non-target	Field	Roundup Original	Direct application	0, 83.0, 166	Late April of 2011	Complex	% cover	No significant effects across locations.	Olszyk et al. 2017
willowherb, <i>Epilobium montanum</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Seed production	Denmark: significant reduction at 72 g a.e./ha for the 6- 8 leaf stage application only; Canada: no significant effects.	Strandberg et al. 2018
willowherb, <i>Epilobium montanum</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments: Glyphos® Cheminova, Canada	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Germinable seeds	Denmark: significant reduction at 72 g a.e./ha for the 6- 8 leaf stage application only; Canada: no significant effects.	Strandberg et al. 2018
willowherb, <i>Epilobium montanum</i>	Non-crop	Non-tolerant	Non-target	Greenhouse	Denmark experiments: Glyphogan, Adama; Canadian experiments:	Direct application	0, 14.4, 72.0	6-8 leaf or early bud stage	Single species	Flower production	Denmark: Significant effects at 72 g a.e./ha for 6-8 leaf and early bud stage	Strandberg et al. 2018

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
					Glyphos® Cheminova, Canada						application; Canada: No significant effects.	
Barnyard grass, <i>Echinochloa crus-galli</i> L. Beauv.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"	Complex	Seed production	Significant reduction for all timings of application at 840 g a.e./ha, as well as for sequential applications from 210 g a.e./ha.	Walker and Oliver 2008
Barnyard grass, <i>Echinochloa crus-galli</i> L. Beauv.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod	Complex	Seed weight	Significant reduction for all timings of application at 210, 420 and 840 g a.e./ha.	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"				
Palmer amaranth , <i>Amaranthus palmeri</i> S. Watson	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued	Complex	Seed production	Significant reduction for all timings of application at 210, 420 and 840 g a.e./ha.	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								until maturation or desiccation of all species in the plot when no further reproductive growth occurred"				
Palmer amaranth , <i>Amaranthus palmeri</i> S. Watson	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"	Complex	Seed weight	No significant effect.	Walker and Oliver 2008
Pitted morning glory, <i>Ipomoea lacunosa</i> L.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer	Complex	Seed production	Significant effects at all timings at 840 g	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"			a.e./ha, and at all rates for sequential application. Significant reductions at 420 g a.e./ha for applications at pitted morning glory and prickly sida flowering.	
Pitted morning glory, <i>Ipomoea lacunosa</i> L.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when	Complex	Seed weight	No significant effect.	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"				
Prickly sida, <i>Sida spinosa</i> L.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive	Complex	Seed production	For most application timings 840 g a.e./ha resulted in significant reductions in seed production. 420 g a.e./ha resulted in significant reductions when application occurred when prickly sida was flowering. Sequential applications at and above 110 g a.e./ha significantly reduced seed production.	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								growth occurred"				
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	Flowers per plant	Significant effects of split and 12 leaf stage applications.	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	No. pods	Significant adverse effects at 280 g/ha	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	No. seeds	Significant adverse effects at 280 g/ha	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	Total seed weight	Significant adverse effects at 280 g/ha	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	Pod length	No significant effects.	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	50-seed weight	No significant effects.	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Green-house	Roundup UltraMax, Monsanto	Direct application	0, 112, 280	4 leaf stage, 8 leaf stage, 4 and 8 leaf stage, 12 leaf stage	Single species	Seed germination	No significant effects.	Thomas et al. 2005
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted	Complex	Seed production	Significant reductions across all rates when averaged across	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/ drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"			application timings.	
Sicklepod, <i>Senna obtusifolia</i> L.	Non-crop	Non-tolerant	Target	Field	Not reported	Direct application	0, 110, 210, 420, 840	"once when barnyardgrass, Palmer amaranth, pitted morningglory, prickly sida, or sicklepod flowered, determined by the appearance of open blooms, or sequentially every 10 d, initiated when the first weed species in the	Complex	Seed weight	Significant reductions across all rates.	Walker and Oliver 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
								complex flowered, and continued until maturation or desiccation of all species in the plot when no further reproductive growth occurred"				
Canada goldenrod, <i>Solidago canadensis</i>	Non-crop	Non-tolerant	Target ^b	Field	Not reported	Direct application	0, 300, 500, 560, 780, 900, 1100	flower bud stage or flowering	Single species	Seed germination	Both application timings LOEL = 300 g/ha	Guo et al. 2009
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Days to flowering	Significant effects (delay) at both treatment levels.	Londo et al. 2014
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Flower attempt	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Duration of flowering	Significant decrease for OR variety at both treatment levels.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther length	Significant decrease.	Londo et al. 2014
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther width	Significantly increased for OR variety.	Londo et al. 2014
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther ratio	Significant change.	Londo et al. 2014
Brassica, <i>Brassica rapa</i> L.	Non-crop	Non-tolerant (Two wild varieties: OR, CA; not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Pistil function	Significant decrease. Only OR variety assessed.	Londo et al. 2014
Brassica, <i>Brassica nigra</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Days to flowering	Significant effects (delay) at both treatment levels.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Days to flowering	Significant effects (delay) at both treatment levels.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		known to be tolerant)										
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Flower attempt	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica nigra</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Duration of flowering	Significant decrease at highest treatment level only.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Duration of flowering	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica nigra</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther length	Significant decrease.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther length	Significant decrease.	Londo et al. 2014
Brassica, <i>Brassica nigra</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther width	No significant effect.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther width	No significant effect.	Londo et al. 2014

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
		known to be tolerant)										
Brassica, <i>Brassica nigra</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther ratio	Significant change.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Anther ratio	Significant change.	Londo et al. 2014
Brassica, <i>Brassica juncea</i> L.	Non-crop	Non-tolerant (Wild variety not known to be tolerant)	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Self fertility	Significantly reduced at both treatment levels.	Londo et al. 2014
Dayflower, <i>Commelina erecta</i> L.	Non-crop	Tolerant	Target	Growth room	Zamba, NideraS.A.	Direct application	0, 900, 1800	6-7 leaf	Single species	Flower production	Significantly reduced.	Panigo et al. 2012
Morning glory, <i>Ipomoea purpurea</i> L. Roth	Non-crop	Tolerant and non-tolerant (Mixed origins of tolerant and susceptible individuals)	Target	Greenhouse	Not reported	Direct application	0, 1121	2 months from planting	Single species	Pollen production	Significantly reduced.	Baucom et al. 2008
Morning glory, <i>Ipomoea purpurea</i> L. Roth	Non-crop	Tolerant and non-tolerant (Mixed origins of tolerant and susceptible individuals)	Target	Greenhouse	Not reported	Direct application	0, 1121	2 months from planting	Single species	Pistil function	No significant effects.	Baucom et al. 2008
Morning glory, <i>Ipomoea</i>	Non-crop	Tolerant and non-tolerant	Target	Field	Not reported	Direct application	0, 1121	--	Single species	Anther deformation.	Significant increase.	Baucom et al. 2008

Species	Crop/ Non-crop	Tolerant/ Non-tolerant	Target or Non-target	Location	End Use Product	Direct Application/drift	Rates (g/ha)	Timing of Application	Single species/ complex	Measure of Effects	Observations/ Toxicity Endpoints	Study
<i>purpurea</i> L. Roth		(Mixed origins of tolerant and susceptible individuals)										
Morning glory, <i>Ipomoea purpurea</i> L. Roth	Non-crop	Tolerant and non-tolerant (Mixed origins of tolerant and susceptible individuals)	Target	Field	Not reported	Direct application	0, 1121	--	Single species	Pollen production	Significantly reduced.	Baucom et al. 2008
Morning glory, <i>Ipomoea purpurea</i> L. Roth	Non-crop	Tolerant and non-tolerant (Mixed origins of tolerant and susceptible individuals)	Target	Field	Not reported	Direct application	0, 1121	--	Single species	Seed production	Negatively correlated with anther deformation.	Baucom et al. 2008
Morning glory, <i>Ipomoea purpurea</i> L. Roth	Non-crop	Tolerant and non-tolerant (Mixed origins of tolerant and susceptible individuals)	Target	Field	Not reported	Direct application	0, 1121	--	Single species	Flower production	Negatively correlated with anther deformation.	Baucom et al. 2008
Brassica, <i>Brassica napus</i> L.	Non-crop	Tolerant and non-tolerant (Three crop varieties: cv. RR (resistant), cv. Null (non-transgenic), cv. Sponsor (non-transgenic))	Non-target	Greenhouse	RoundUp	Direct application	0, 85, 112	Pre-bolt/bolting	Single species	Self fertility	Significantly reduced at both treatment levels in non-transgenic varieties (resistant variety not assessed).	Londo et al. 2014

Table 2. Experiment schedule for experiments focusing on flowering phenology (PHEN) and inflorescence characteristics (INFLO)

Species	Experiment	Replicates per Treatment Level	Plants per Pot ^b	Total Plants per Treatment Level	Plant Date	Spray Date (weeks from planting)	Termination Date (weeks from spray)	Nominal/Targeted Glyphosate Application Rates (g a.e./ha) ^a
<i>Rudbeckia hirta</i>	PHEN	4	2	48	06/12/2016	14/02/2017 (10)	11/04/17 (8)	30, 45, 67.5, 101, 152
<i>Centaurea cyanus</i>	PHEN	6	1	36	14/12/2016	01/02/2017 (7)	29/03/17 (8)	
<i>Trifolium pratense</i>	PHEN	6	1	36	08/12/2016	16/02/2017 (10)	13/04/17 (8)	
<i>Centaurea cyanus</i>	INFLO	9	1	54	12/05/2017	23/06/2017 (6)	28/07/17 (5)	40, 47.5, 55, 62.5, 70
<i>Trifolium pratense</i>	INFLO	6	1	36	27/04/2017	29/06/2017 (9)	03/08/17 (5)	10, 20, 30, 40, 50

^a For all experiments untreated controls were included with the same number of replicates as treatment levels.

^b In all experiments there were six pots per replicate.

Table 3. Estimated glyphosate rates (g a.e./ha) associated with 10, 25 and 50% adverse effect on survival, growth and reproductive measures in experiments focused on flowering phenology (PHEN) and inflorescence characteristics (INFLO)

Species, Experiment, and Measures of Effects				ER10	ER25	ER50	Selected Model	Associated Figure
<i>Survival</i>	<i>C. cyanus</i>	PHEN	Mortality	51.7 (39.0, 61.7)	73.4 (61.4, 82.9)	99.8 (89.3, 109.7)	Log Gompertz	13
		INFLO	Mortality	19.6 (0.9, 29.9)	42.8 (17.6, 51.3)	>74.7	Log Gompertz	
<i>Growth</i>	<i>R. hirta</i>	PHEN	Average above ground biomass per experimental plant	16.6 (11.5, 21.7)	45.3 (31.4, 59.2)	109.2 (75.8, 142.6)	Exponential	2
			Average above ground biomass per surviving plant	19.6 (13.6, 25.6)	53.5 (37.3, 69.8)	129.0 (89.8, 168.2)	Exponential	
	<i>C. cyanus</i>	PHEN	Average above ground biomass per experimental plant	44.2 (33.1, 59.1)	57.7 (46.9, 71.2)	77.6 (67.4, 89.4)	Lognormal	14
			Average above ground biomass per surviving plant	34.5 (19.2, 49.9)	86.4 (48.0, 124.7)	>167.6	Linear	
		INFLO	Average above ground biomass per experimental plant	4.7 (1.0, 22.2)	10.8 (3.8, 30.6)	27.2 (16.6, 44.6)	Lognormal	15
			Average above ground biomass per surviving plant	3.0	9.0	32.7	Non-parametric	
	<i>T. pratense</i>	PHEN	Average above ground biomass per experimental plant	12.5 (6.4, 18.6)	24.1 (15.5, 32.6)	46.5 (35.3, 57.7)	Log Logistic	32
			Average above ground biomass per surviving plant	13.3 (7.6, 19.1)	25.4 (17.5, 33.3)	48.3 (38.2, 58.3)	Log Logistic	
		INFLO	Average above ground biomass per experimental plant ^a	12.8 (7.8, 17.9)	35.1 (21.3, 48.8)	>58.1	Exponential	33
	<i>Reproduction</i>	<i>R. hirta</i>	PHEN	Percent of surviving plants not producing inflorescences	103.2 (79.8, 128.0)	163.1 (131.0, 259.8)	>162.1	Log Probit
Aborted buds/total buds				99.9	>162.1	>162.1	Non-parametric	5, 6
Average bloom-weeks per experimental plant				45.9 (26.1, 80.7)	71.7 (48.7, 105.6)	117.6 (87.6, 157.8)	Lognormal	7
Average bloom-weeks per surviving and reproductive plant				50.5 (27.8, 91.8)	82.7 (55.8, 122.5)	142.9 (104.2, 196.0)	Lognormal	
Average number of bloomed inflorescences per experimental plant				41.9 (13.2, 70.6)	104.7 (33.0, 176.4)	>162.1	Linear	8
Average bloom duration				24.5 (18.1, 30.8)	61.2 (45.3, 77.1)	122.3 (90.5, 154.1)	Linear	9
Number of malformed inflorescences per total bloomed inflorescences				49	64.1	(>162.1)	Non-parametric	11,12

Species, Experiment, and Measures of Effects			ER10	ER25	ER50	Selected Model	Associated Figure	
Reproduction	<i>C. cyanus</i>	PHEN	Aborted buds/total buds	70.5	103.6	134.1	Non-parametric	18
			Average bloom-weeks per experimental plants	53.7 (38.6, 68.7)	63.3 (52.8, 73.7)	73.1 (67.4, 78.8)	Log Gompertz	19
			Average bloom-weeks per surviving and reproductive plant	50.5 (36.8, 69.5)	65.3 (52.5, 81.2)	86.8 (75.5, 99.7)	Lognormal	
			Average number of bloomed inflorescences per experimental plant at 8 weeks	43.7 (30.5, 57.0)	59.3 (48.0, 70.5)	77.3 (68.7, 86.0)	Log Gompertz	20
			Average number of bloomed inflorescences per surviving and reproductive plants at 8 weeks	60.7 (46.2, 79.8)	75.1 (61.9, 91.1)	95.3 (84.1, 108.1)	Lognormal	
			Average number of bloomed inflorescences per experimental plant at 5 weeks	50.8 (42.4, 59.2)	60.0 (52.4, 67.7113)	70.9 (64.2, 77.6)	Log Logistic	21
			Average number of bloomed inflorescences per surviving and reproductive plants at 5 weeks	45.6 (30.4, 68.5)	59.9 (45.5, 78.8)	81.0 (67.8, 96.8)	Lognormal	
		INFLO	Average number of bloomed inflorescences per experimental plant	2.6 (2.2, 2.9)	7.1 (6.1, 8.1)	17.1 (14.7, 19.6)	Exponential	22
			Average number of bloomed inflorescences per surviving and reproductive plant	3.4 (2.9, 3.9)	9.2 (7.8, 10.6)	22.1 (18.8, 25.4)	Exponential	
		PHEN	Number of malformed inflorescences per total bloomed inflorescences	122.9	>167.6	>167.6	Non-parametric	24, 25
		INFLO	Average inflorescence dry weight	17.3	33.7	>74.7	Non-parametric	27
			Average number of reproductive florets per inflorescence	29.3	≥43.7	>74.7	Non-parametric	28, 29
			Pollen germination	3.0	8.2	≥23.5	Non-parametric	30
		<i>T. pratense</i>	PHEN	Percent of surviving plants producing inflorescences	25.9 (16.5, 33.3)	37.9 (28.2, 45.4)	55.4 (46.4, 64.0)	Log Logistic
	Aborted buds/total buds			34.0	43.7	59.6	Non-parametric	36
	Average bloom-weeks per experimental plants			9.2 (3.7, 14.8)	17.1 (10.4, 23.8)	29.0 (22.3, 35.8)	Log Gompertz	37
	Average bloom-weeks per surviving and reproductive plant			11.4 (5.0, 17.9)	19.7 (12.7, 26.7)	31.7 (25.5, 37.9)	Log Gompertz	
	Average number of bloomed inflorescences per experimental plant 8 weeks			20.2 (13.9, 29.2)	26.7 (20.0, 35.6)	36.5 (29.4, 45.2)	Lognormal	38
	Average number of bloomed inflorescences per surviving and reproductive plant 8 weeks			12.5 (5.2, 19.7)	21.3 (13.5, 29.0)	34.0 (27.0, 41.0)	Log Gompertz	
	Average number of bloomed inflorescences per experimental plant 5 weeks			7.8 (1.9, 13.6)	15.2 (7.9, 22.6)	27.5 (19.7, 35.3)	Log Gompertz	39

Species, Experiment, and Measures of Effects				ER10	ER25	ER50	Selected Model	Associated Figure
<i>Reproduction</i>	<i>T. pratense</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant 5 weeks	9.0 (1.7, 16.3)	17.1 (8.4, 25.8)	30.1 (21.9, 38.4)	Log Gompertz	40
		INFLO	Average number of bloomed inflorescences per experimental plant	2.7 (0.47, 5.0)	4.7 (1.7, 7.8)	8.2 (4.4, 12.0)	Log Logistic	
		INFLO	Average number of bloomed inflorescences per surviving and reproductive plant)	3.3 (0.5, 6.1)	5.6 (2.2, 9.0)	9.6 (5.9, 13.3)	Log Logistic	
		PHEN	Average bloom duration	18.3 (14.3, 22.4)	45.8 (35.6, 56.0)	91.7 (71.3, 112.0)	Linear	41
		INFLO	Average number of reproductive florets per inflorescence	6.7 (3.8, 9.5)	16.6 (9.5, 23.8)	33.3 (19.1, 47.5)	Linear	44

^a No mortalities.

Table 4 Relative sensitivity of test species in experiments focused on flowering phenology (PHEN) and inflorescence characteristics (INFLO)

Measure of Effects	Experiment	Sensitivity ^a
Mortality/Survival	PHEN	CC> RH>TP
	INFLO	CC>TP (no mortalities)
Average plant above ground biomass ^b	PHEN	TP>CC>RH
	INFLO	CC>TP
Percent of surviving plants not producing inflorescences	PHEN	TP>CC>RH
Percent of buds aborted	PHEN	TP>CC>RH
Average bloom duration	PHEN	TP>RH
Average bloom-weeks per plant ^d	PHEN	TP>CC>RH
Average cumulative number of inflorescences per plant ^e	PHEN	TP>CC>RH
	INFLO	TP>CC
Percent of inflorescences malformed	PHEN	TP ^c >RH>CC
Average inflorescence dry weight	INFLO	CC>TP
Number of reproductive florets	INFLO	TP>CC
Pollen germination	INFLO	CC>TP

^a Based on order of ER50 estimates (or lower ECx values if censored) or NOEL/LOEL estimates. CC = *C. cyanus*, TP = *T. pratense*, RH = *R. hirta*. In some cases, rate-response curves overlapped such that the order of sensitivity varied with ERx value, although this was rare. For simplicity here ER50 values, or median responses were considered first.

^b For both experimental plant averages, and averages of surviving plants.

^c *T. pratense* had no significant effect at the highest treatment level, however there were only three inflorescences considered – insufficient for assessment of monotonicity.

^d For experimental plants and surviving and reproductive plants.

^e For experimental plants and surviving and reproductive plants, and for 5-week and 8-week assessment in the PHEN experiments

8 Figures

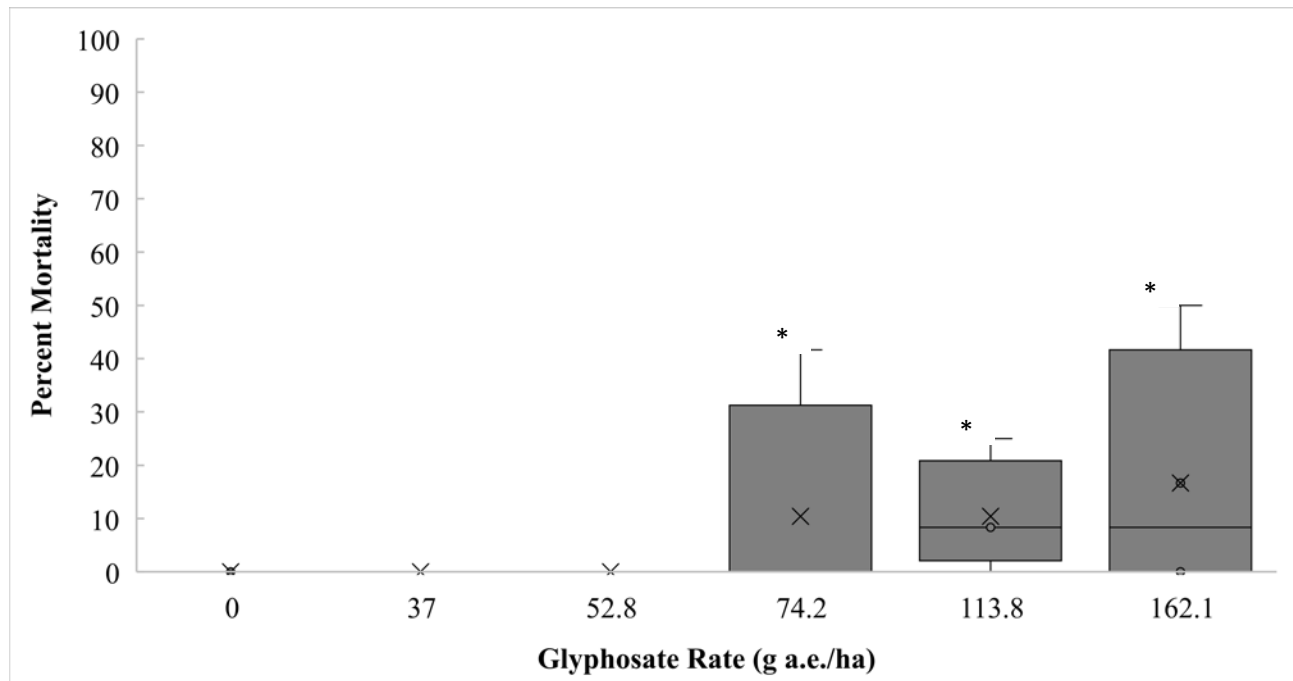


Figure 1. *R. hirta* mortality in PHEN experiment. Asterisks indicate $p < 0.05$ when treatment included in Cochran-Armitage step-down trend test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range.

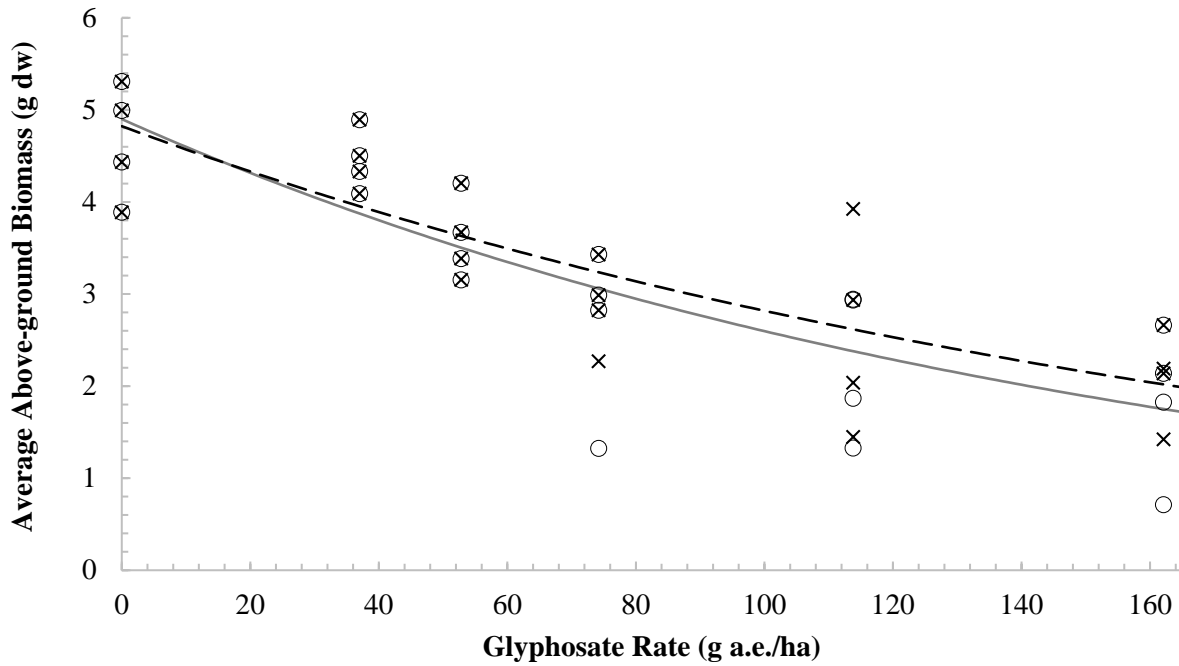


Figure 2. *R. hirta* average above ground biomass at 8-weeks post spray for PHEN experimental plants (o) and PHEN surviving plants (x) with best fitting models (exponential); solid line is for experimental plants; dashed line is for surviving plants.

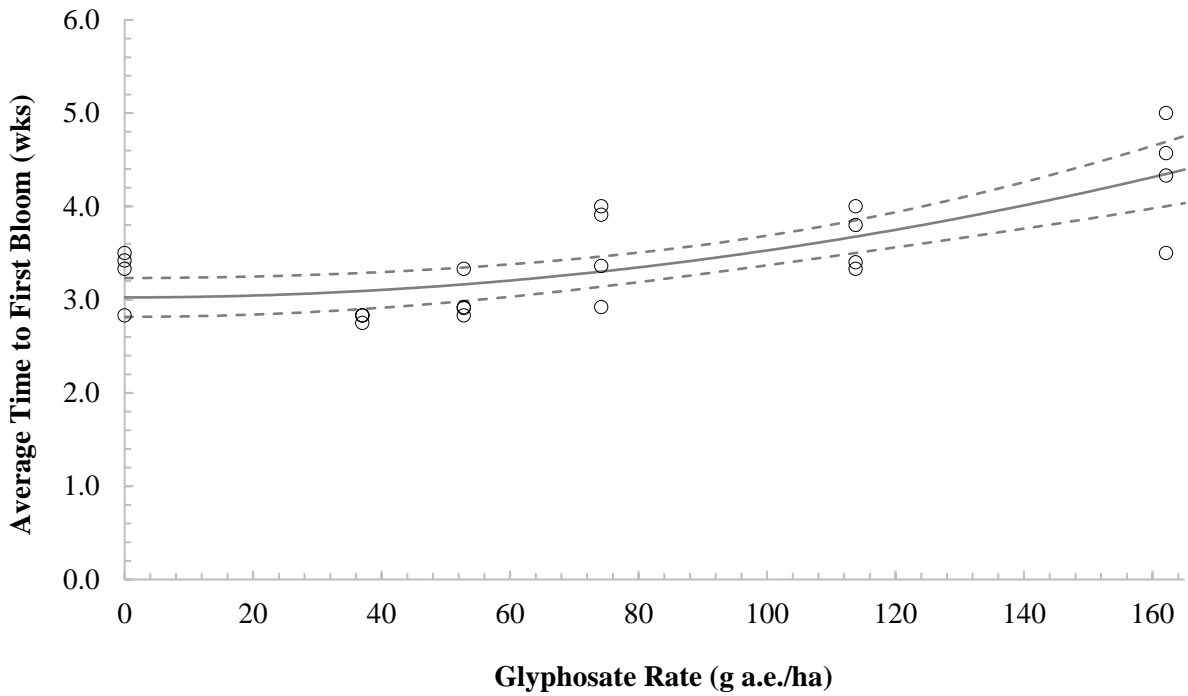


Figure 3. *R. hirta* average time to first bloom from glyphosate application in PHEN experiment. Polynomial model with 95% confidence limits.

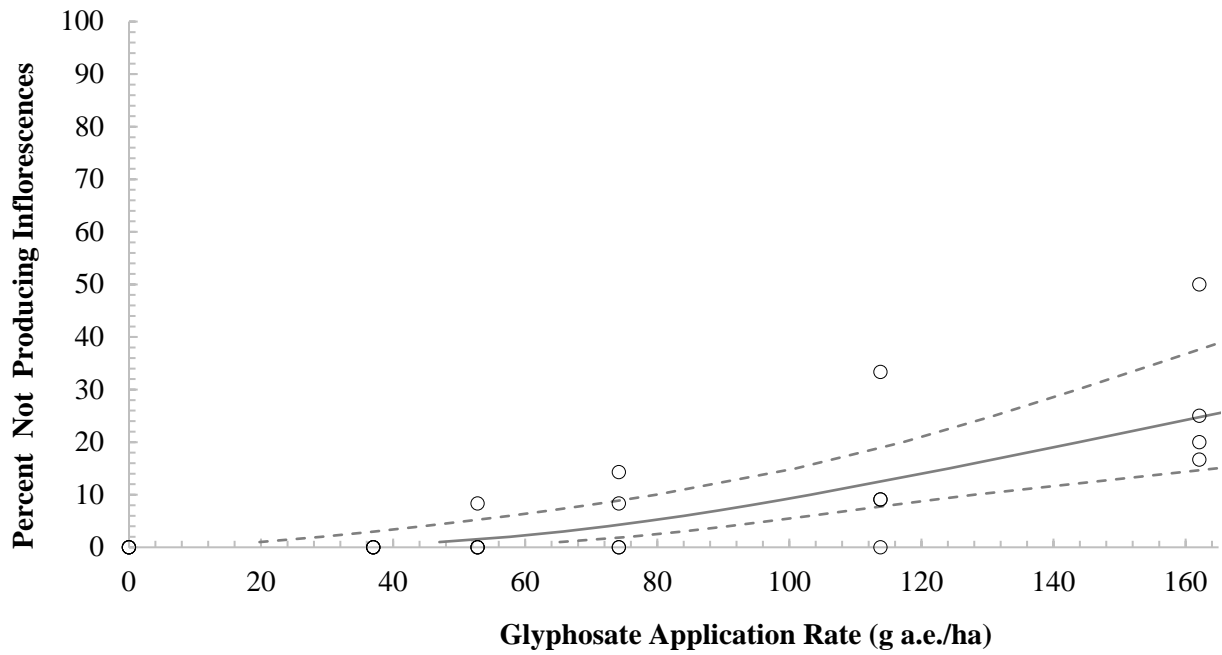


Figure 4. Percent of surviving *R. hirta* plants not producing inflorescences in PHEN experiment. Probit model with 95% confidence limits.

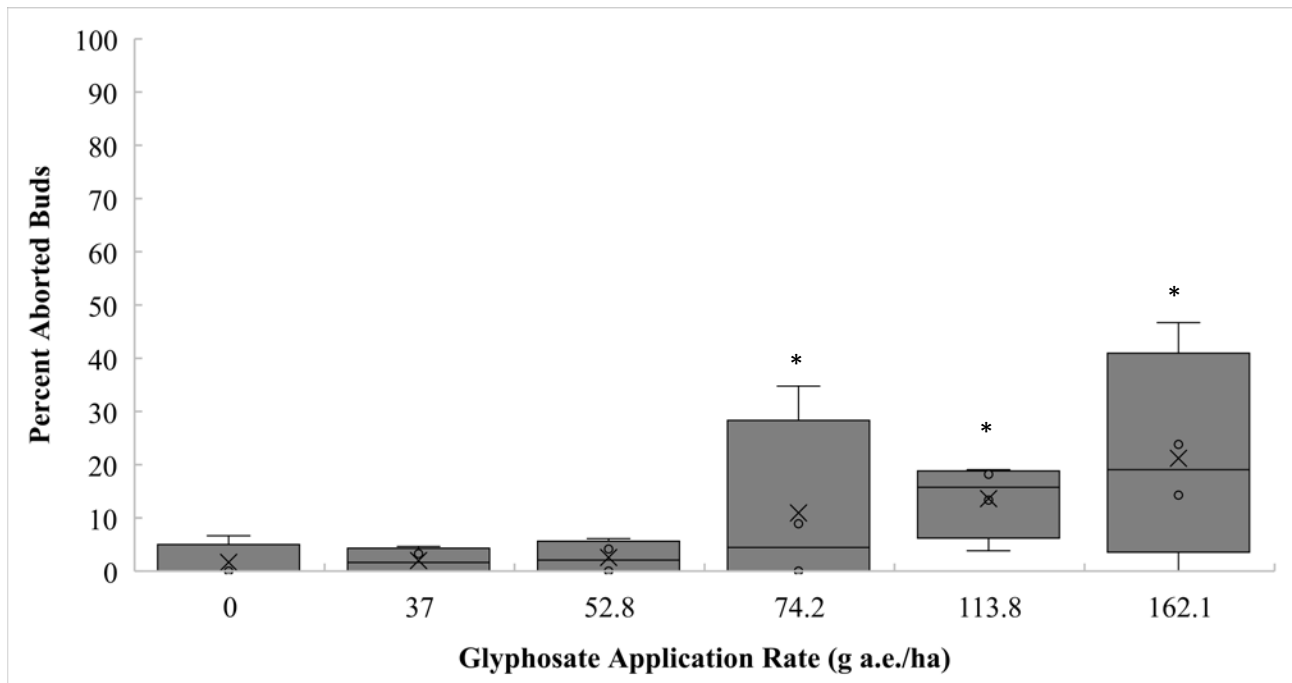


Figure 5. Percent *R. hirta* buds aborted in PHEN experiment. Asterisks indicate $p < 0.05$ when included in Cochran-Armitage step-down trend test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range..

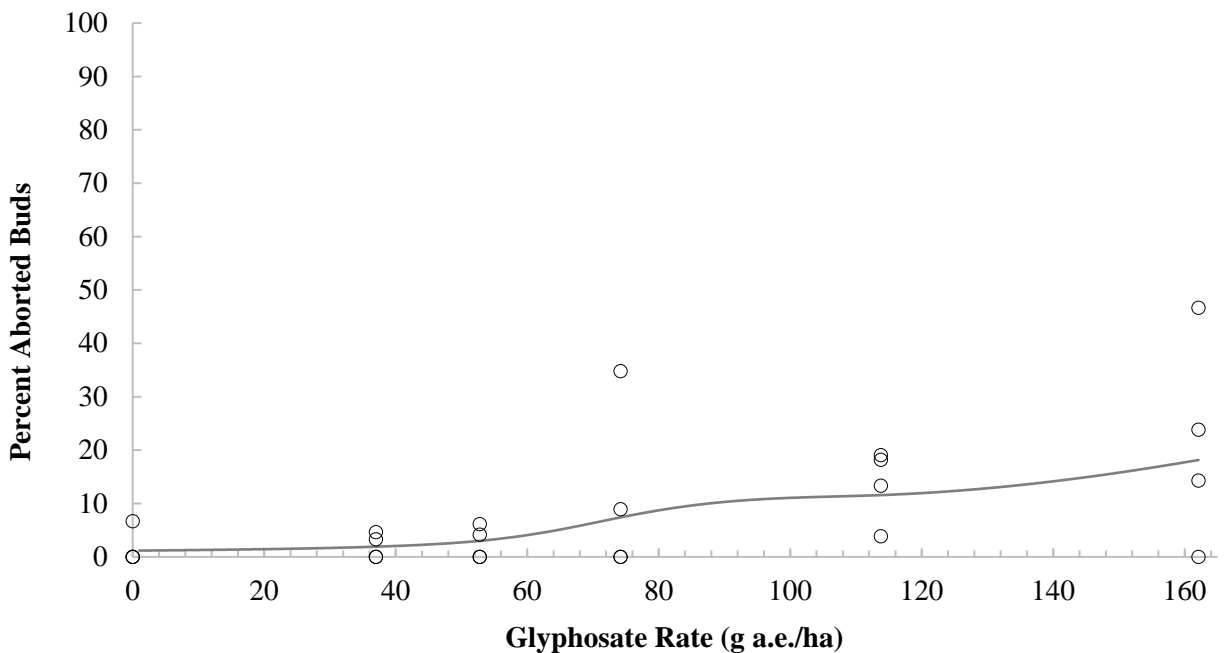


Figure 6. Percent of *R. hirta* buds aborted in PHEN experiment with nonparametric model fit by spline interpolation over rate.

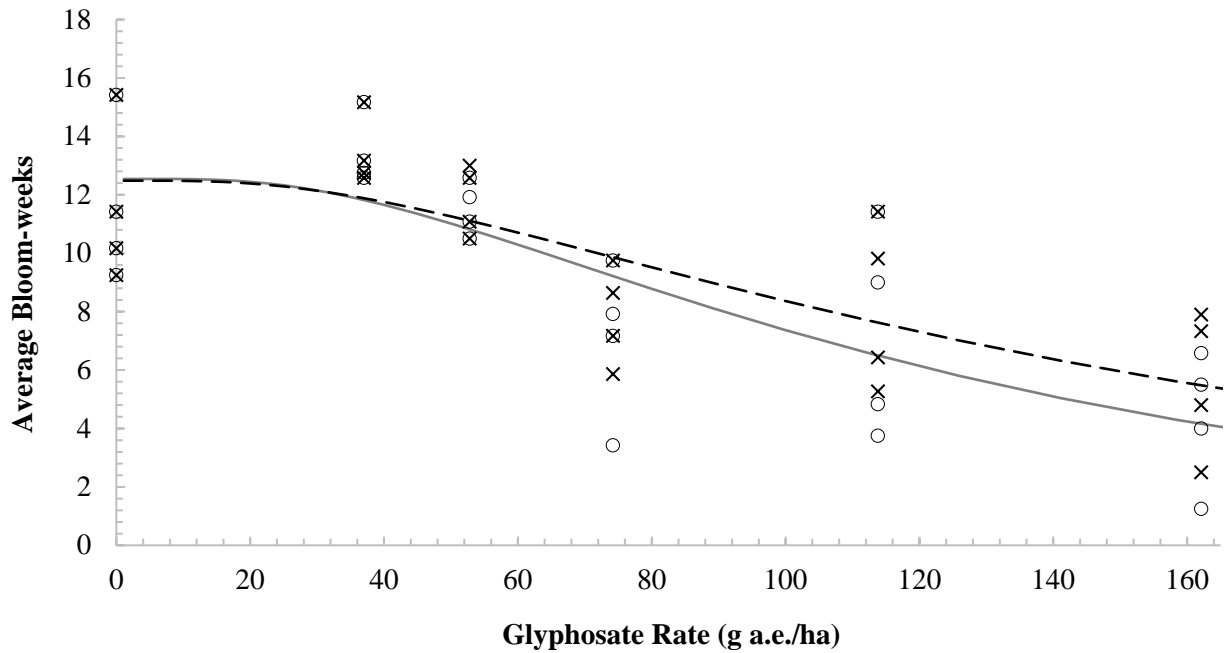


Figure 7. Average bloom-weeks of *R. hirta* experimental plant (o) and surviving and reproductive plants (x) with best-fitting models. Solid line is model fit to experimental plant data, dashed line is fit to surviving and reproductive plant data.

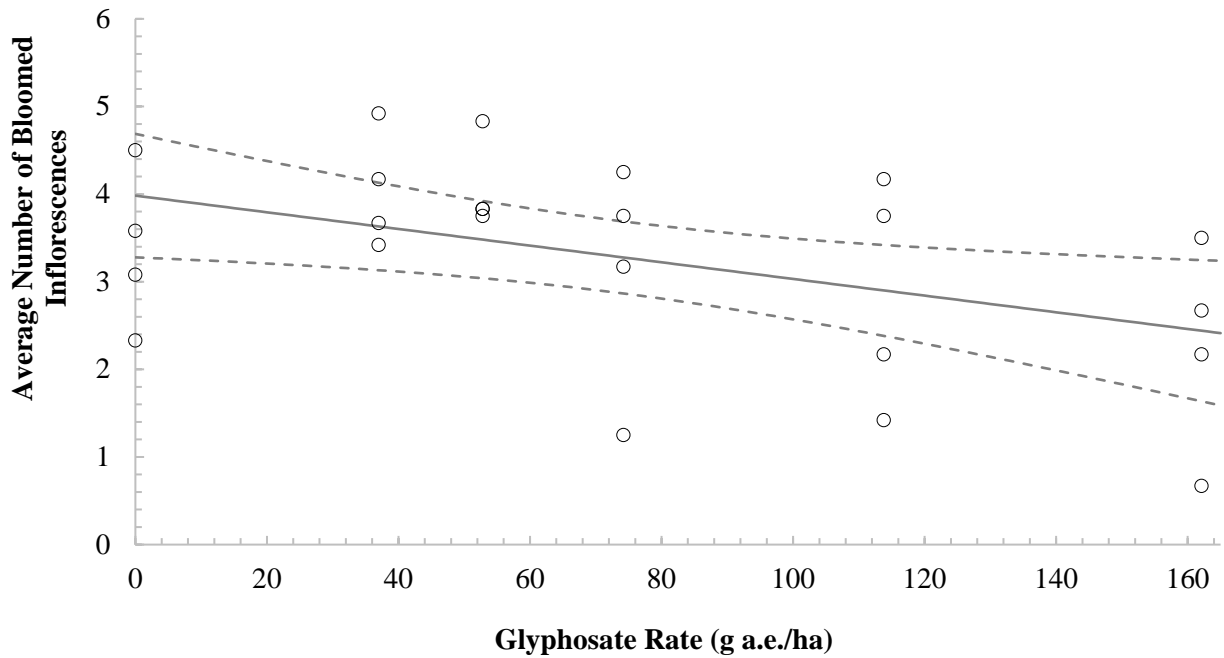


Figure 8. Average number of bloomed inflorescences per *R. hirta* experimental plant with best-fitting (linear model) and 95% confidence limits.

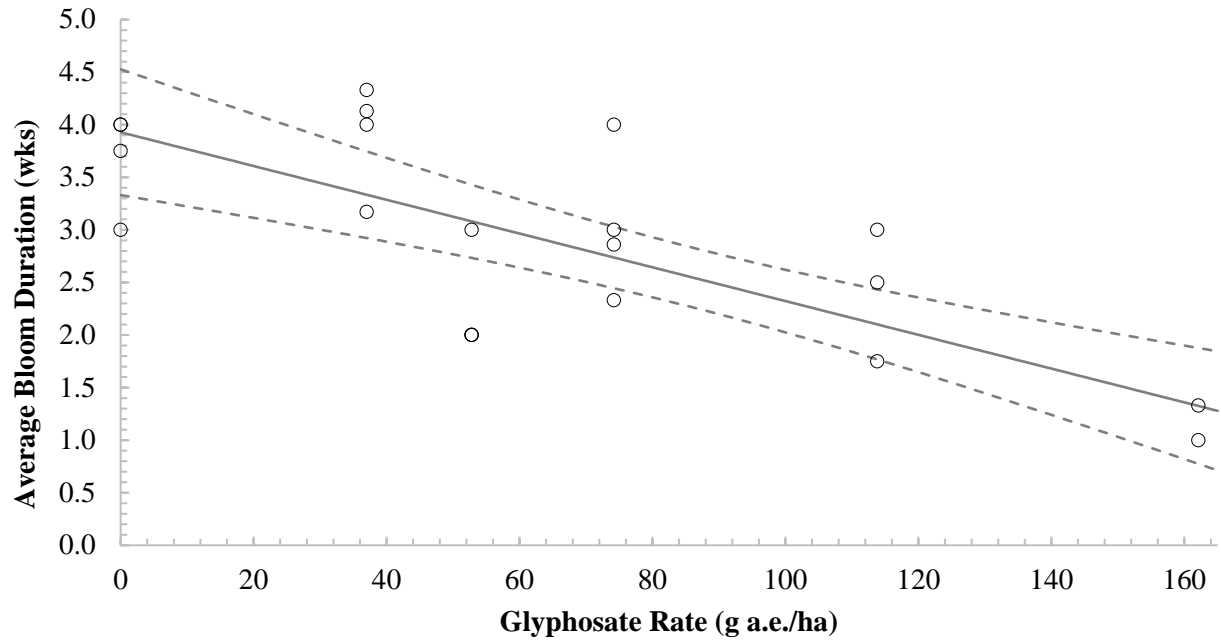


Figure 9. Average *R. hirta* bloom duration of inflorescences that senesced before termination with best-fitting model (linear) and 95% confidence limits.



Figure 10. Malformations observed in *R. hirta* inflorescences.

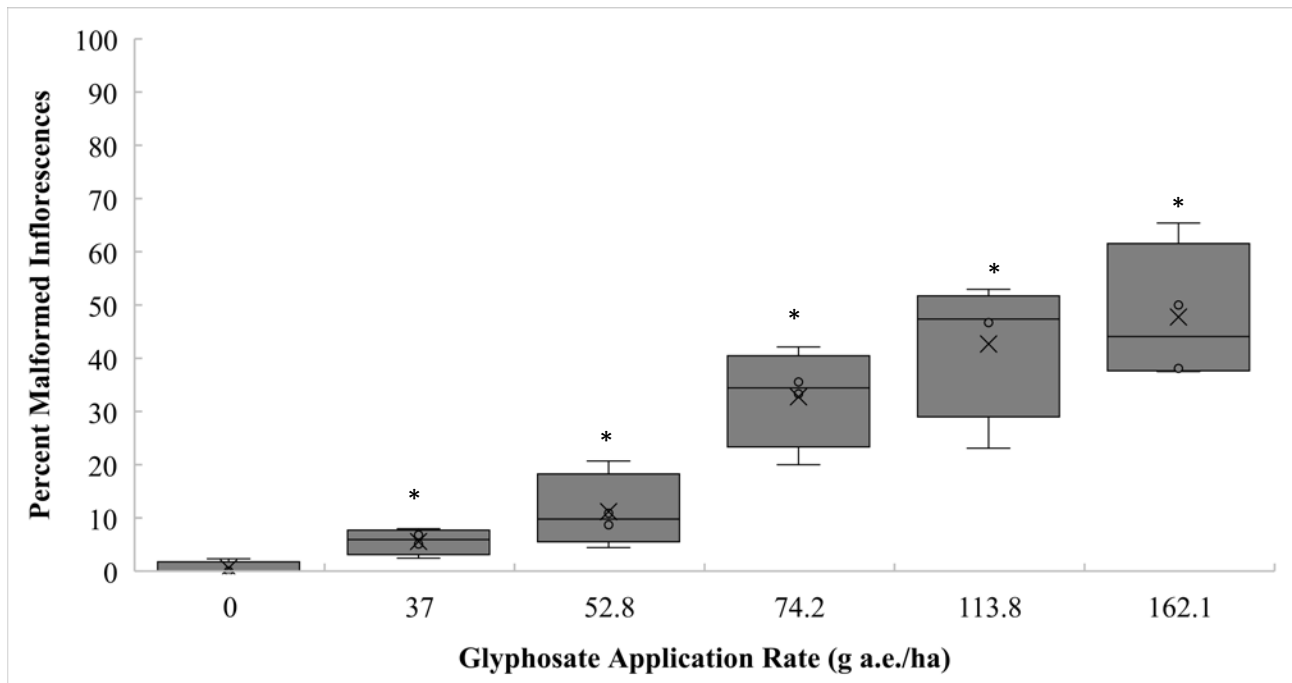


Figure 11. Percent of *R. hirta* inflorescences observed to be malformed in PHEN experiment. Asterisks indicate $p < 0.05$ when included in the step-down Cochran-Armitage test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (- - - - |) extend to furthest data points from the median falling within 1.5 times the interquartile range..

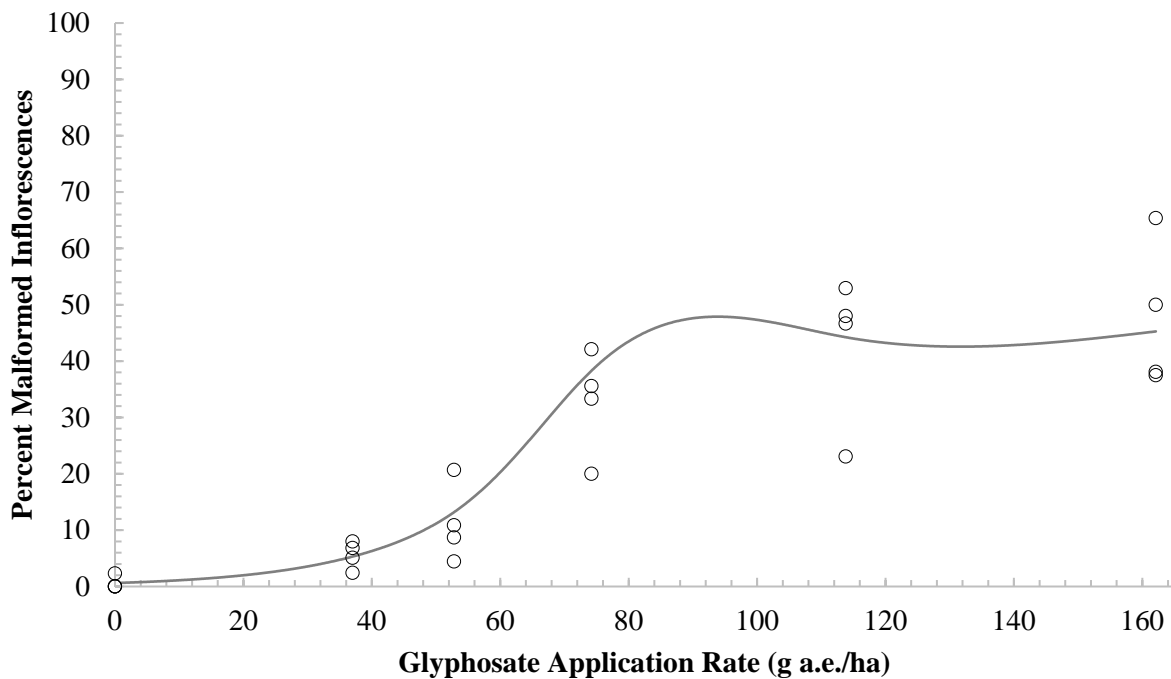


Figure 12. Percent of *R. hirta* inflorescences observed to be malformed in PHEN experiment with a nonparametric regression model fit by spline interpolation over rate.

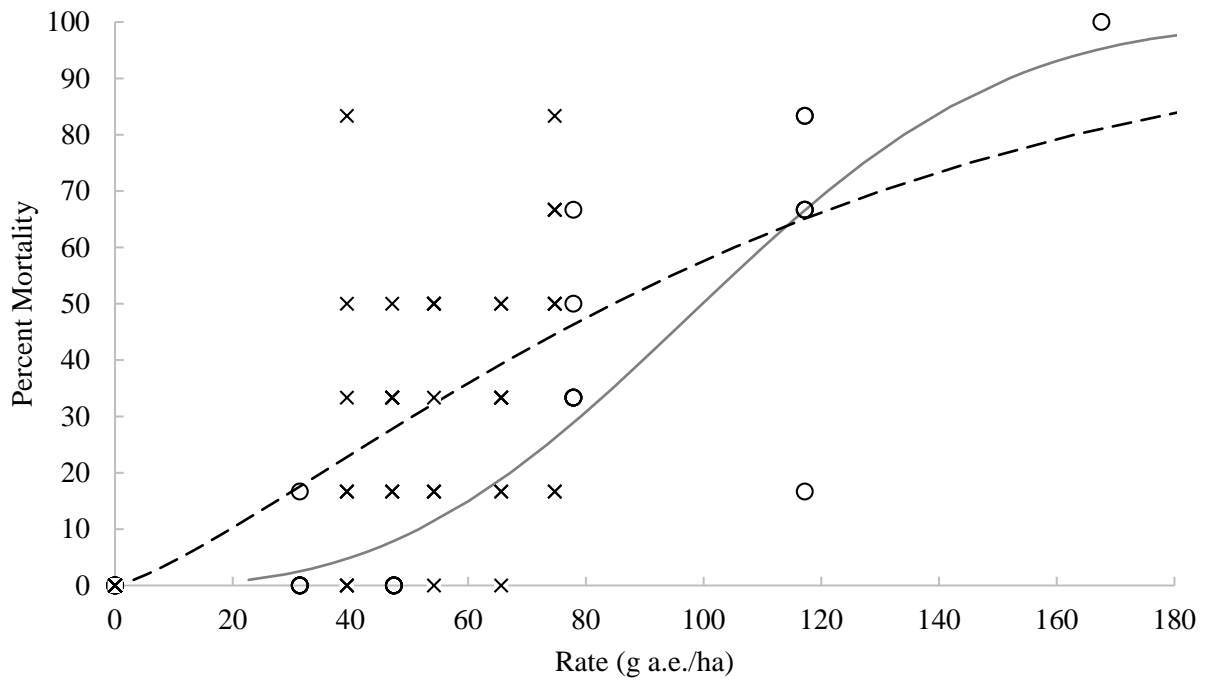


Figure 13. *C. cyanus* mortality in the PHEN (o) and INFLO (x) experiments with best-fitting models log Gompertz, solid and dashed lines, respectively.

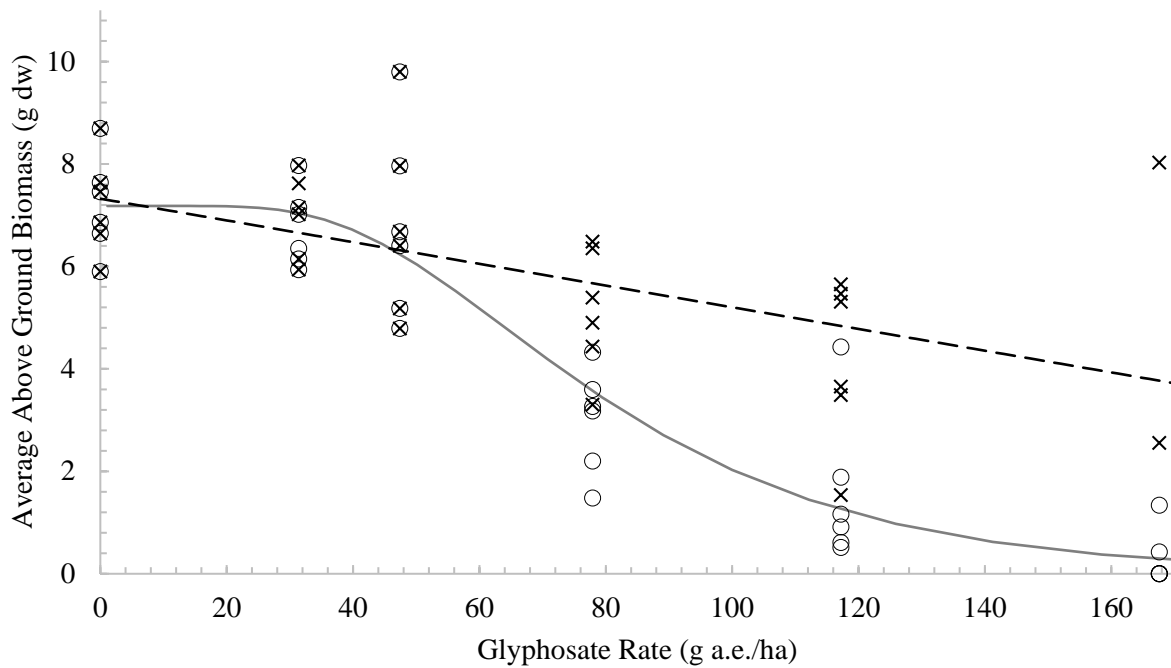


Figure 14. *C. cyanus* average above ground biomass per PHEN experimental plant (o) and per surviving plant (x), with best-fitting models, lognormal and linear, respectively.

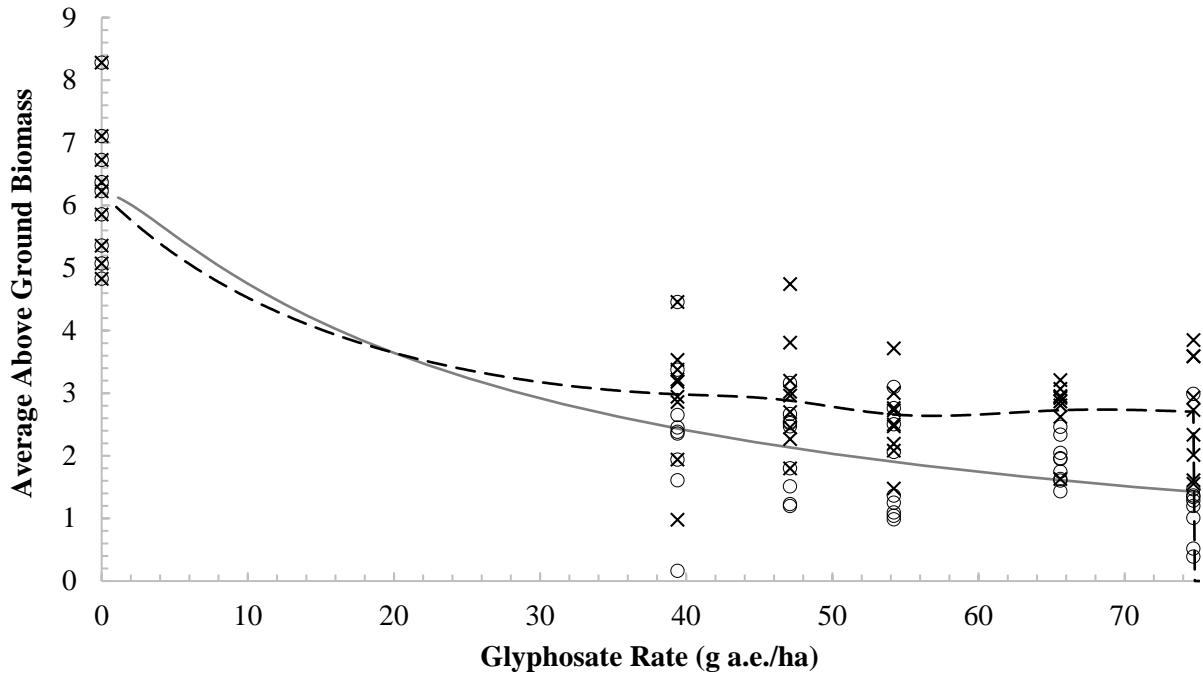


Figure 15. *C. cyanus* average above ground biomass per INFLO experimental plant (o) and per surviving plant (x), with best-fitting models (log-normal and nonparametric, respectively).

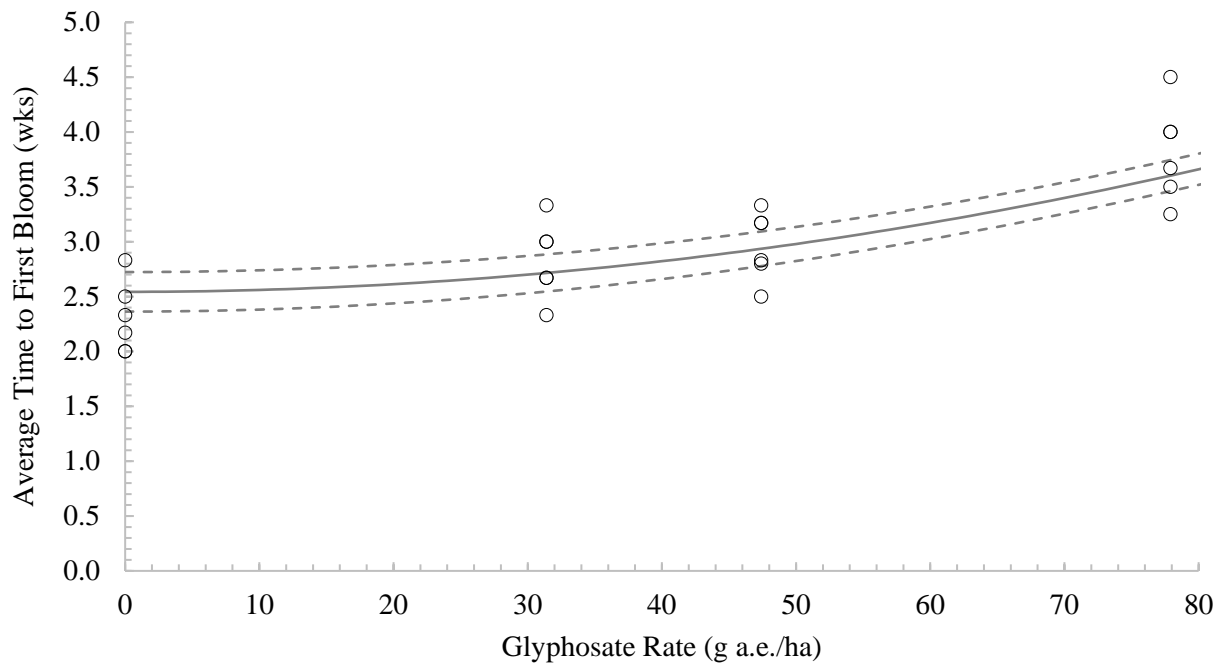


Figure 16. *C. cyanus* average time to first bloom in PHEN experiment with polynomial model and 95% confidence limits.

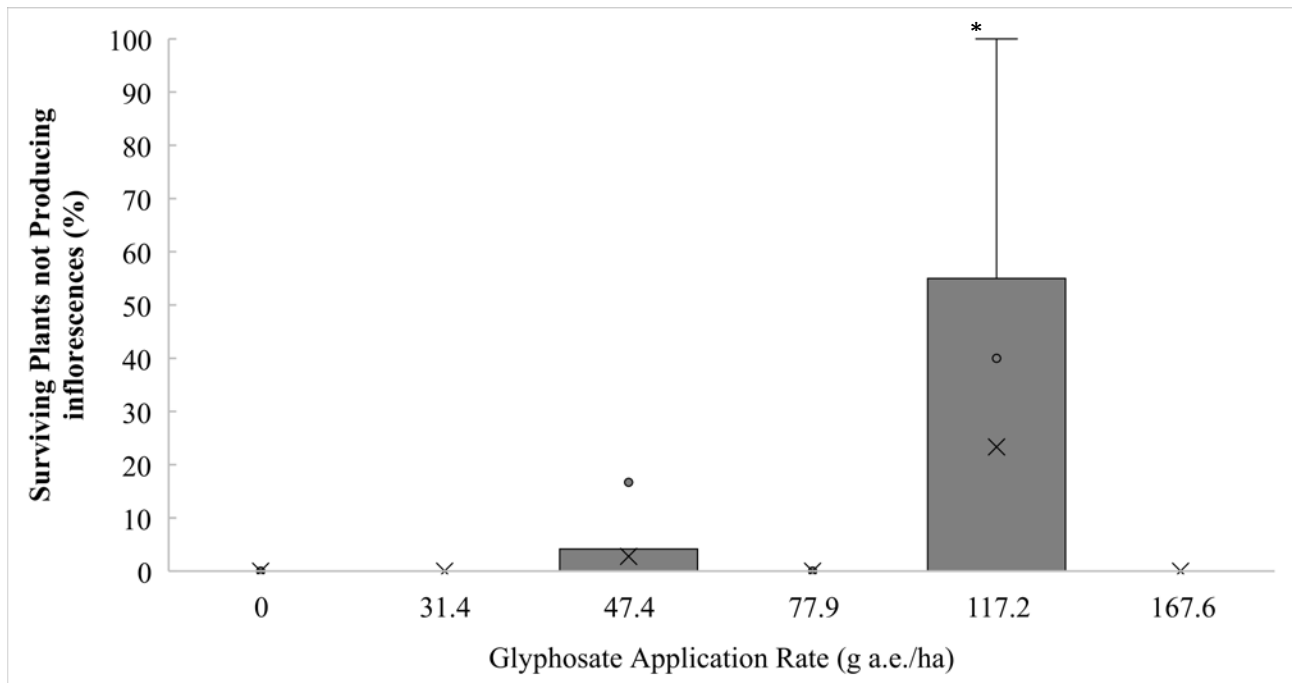


Figure 17. Percentage of surviving *C. cyanus* plants not producing inflorescences. Asterisk indicates $p < 0.01$ for Fisher exact comparison to controls. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range..

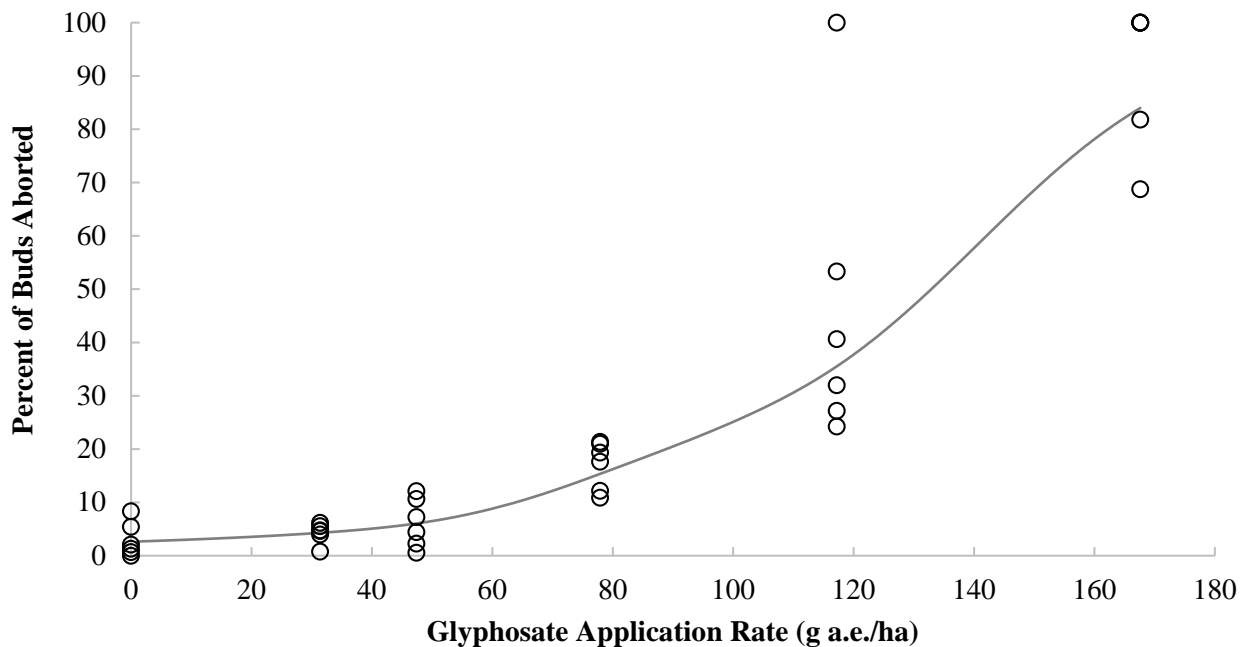


Figure 18. Percent of *C. cyanus* buds aborted with nonparametric model fit by spline interpolation over rate.

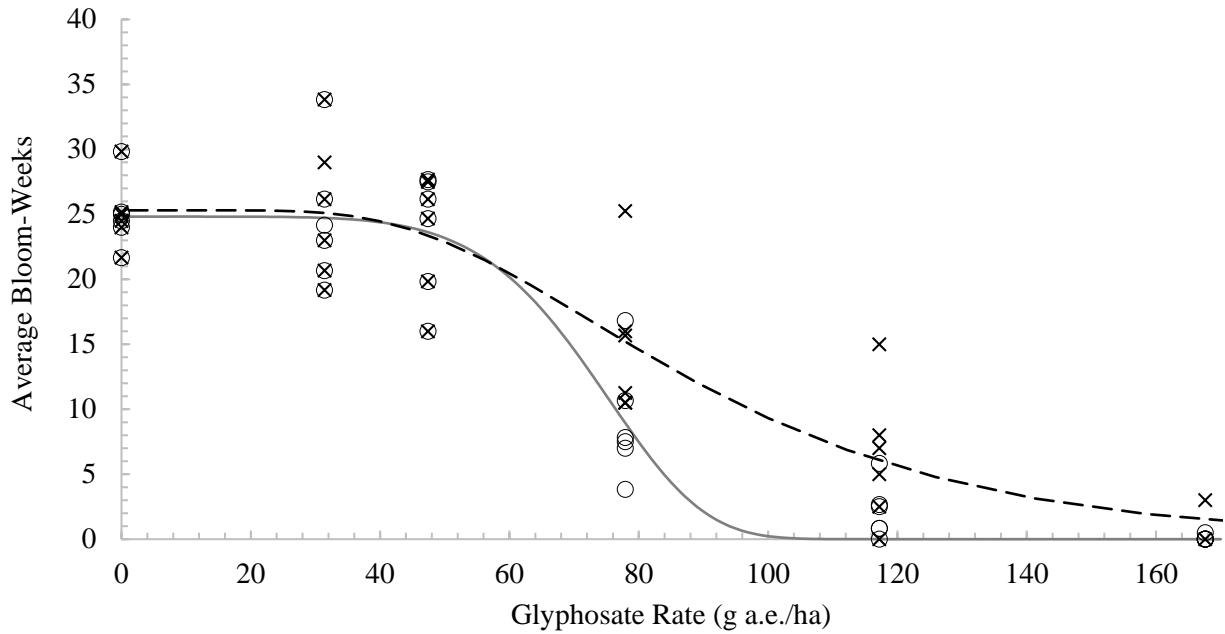


Figure 19. Average bloom-weeks of *C. cyanus* experimental plants (o) and surviving and reproductive plants (x) with best-fitting models, log Gompertz (solid line) and lognormal (dashed line), respectively.

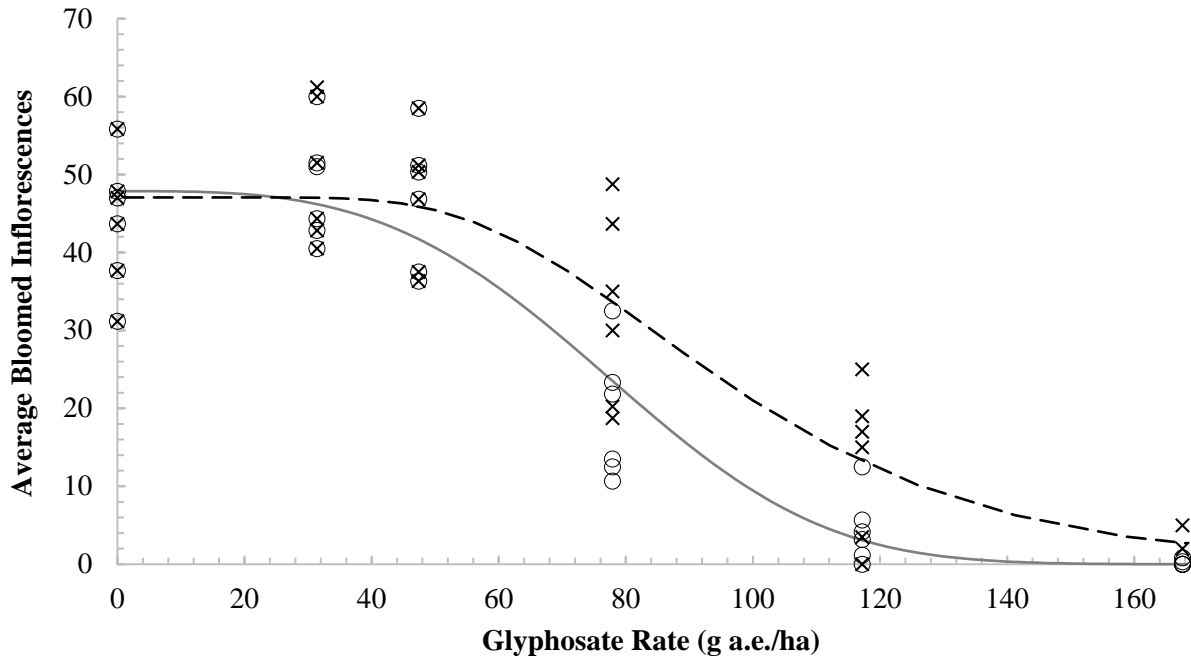


Figure 20. Average number of bloomed *C. cyanus* inflorescences per experimental plant (o), and per surviving and reproductive plant (x) in the PHEN experiment with best-fitting models, log Gompertz (solid line) and lognormal (dashed line), respectively.

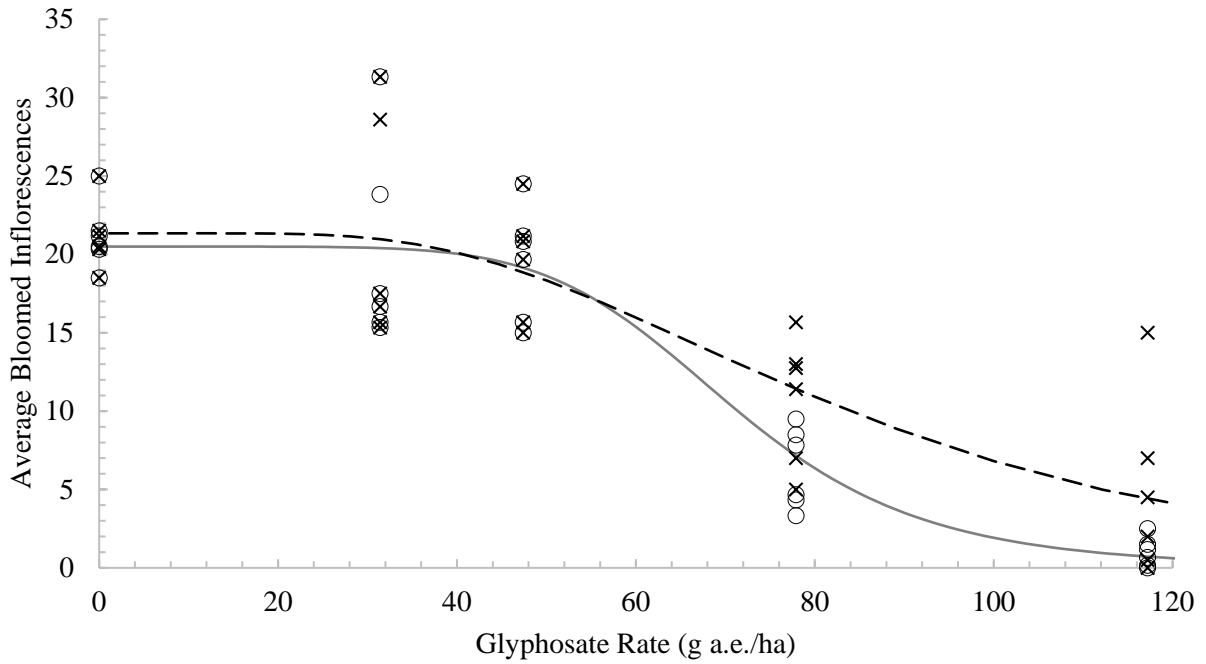


Figure 21. Average number of bloomed *C. cyanus* inflorescences per experimental plant (o), and per surviving reproductive plant (x), in the PHEN experiment at 5-weeks, with best-fitting models, log logistic (solid line) and lognormal (dashed line), respectively.

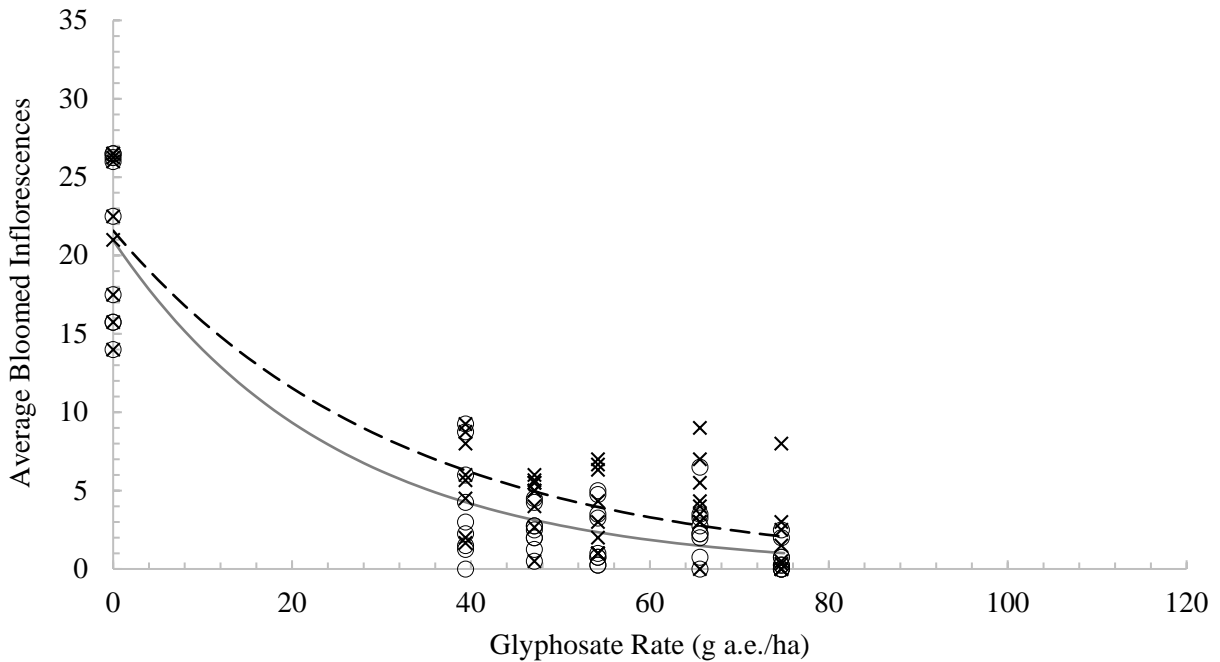


Figure 22. Average number of bloomed *C. cyanus* inflorescences per experimental plant (o), and per surviving reproductive plant (x) in the INFLO experiment with best fitting models (exponential); solid line is the model for experimental plants, dashed line is the model for



Figure 23. Malformations observed in *C. cyanus* inflorescences.

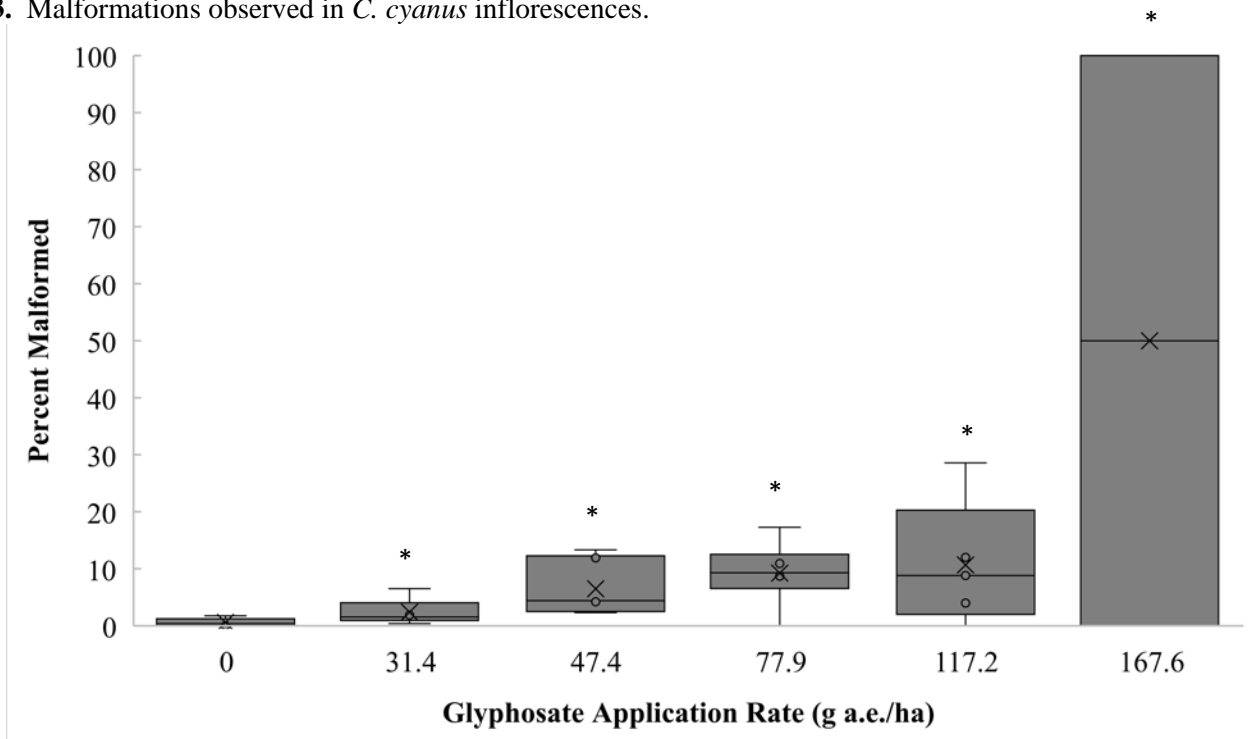


Figure 24. Percent malformed *C. cyanus* inflorescences in the PHEN experiment. Asterisks indicate significant trend when included in step-down test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range..

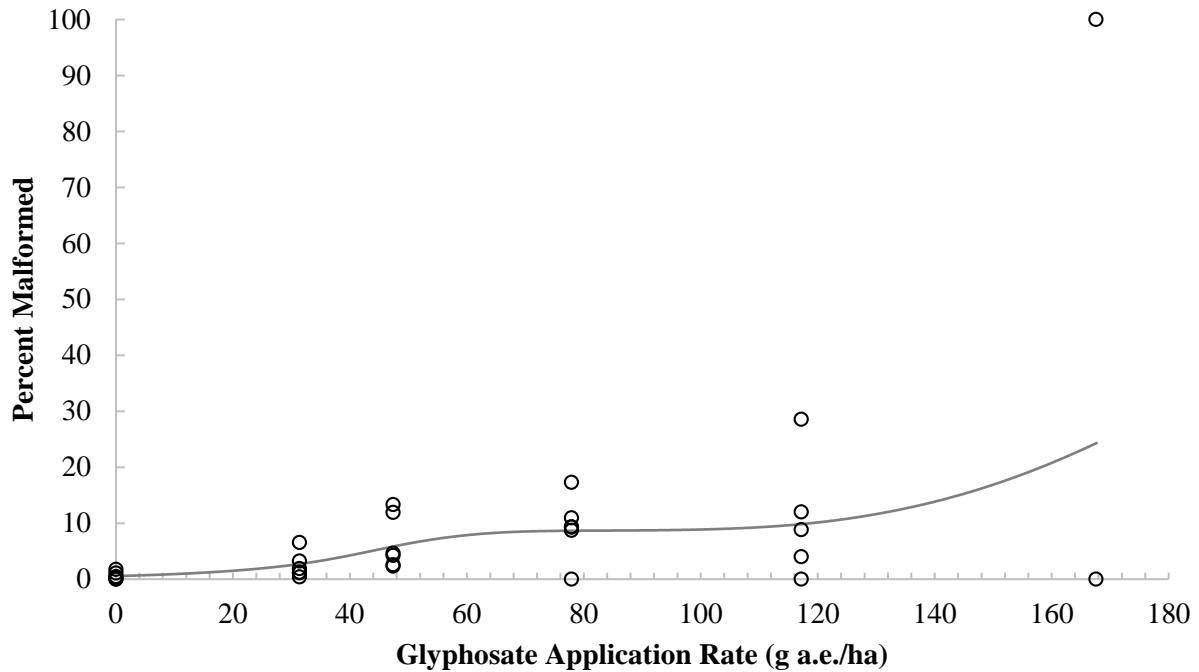


Figure 25. Percent malformed *C. cyanus* inflorescences in the PHEN experiment with nonparametric model fit to data by spline interpolation over rate.

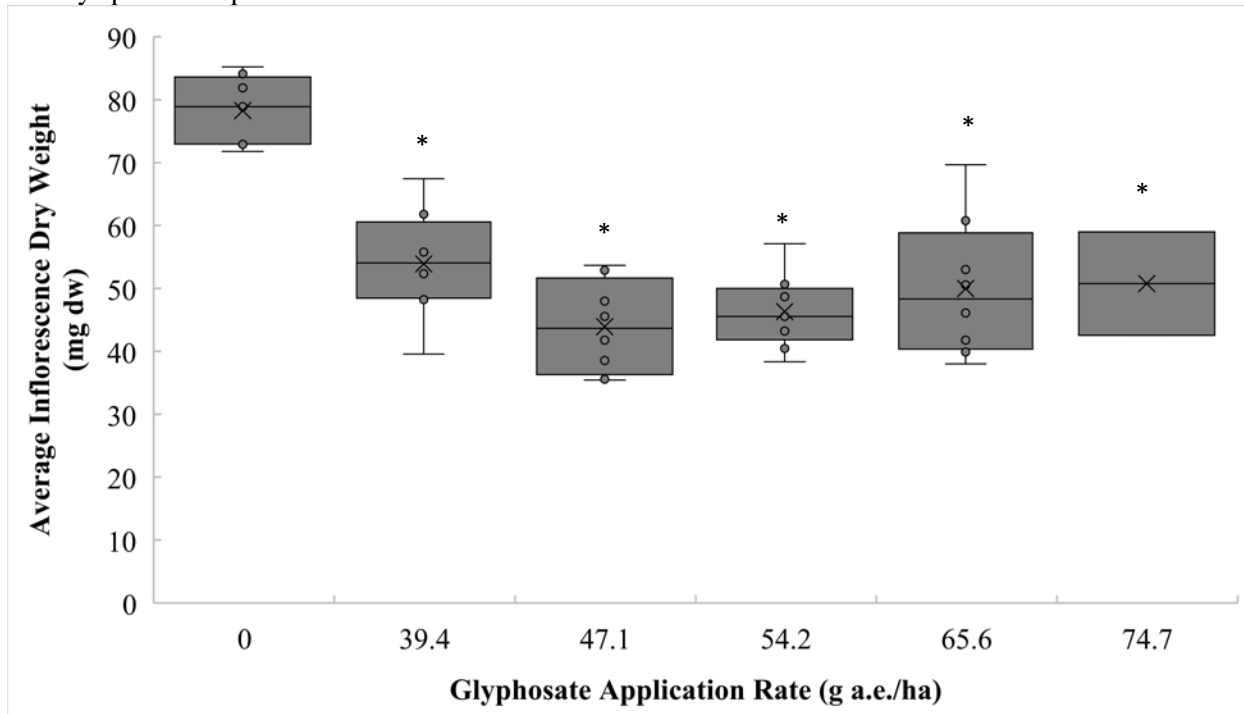


Figure 26. Average *C. cyanus* inflorescence dry weight. Asterisks indicate significant trend when included in Williams step-down test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range..

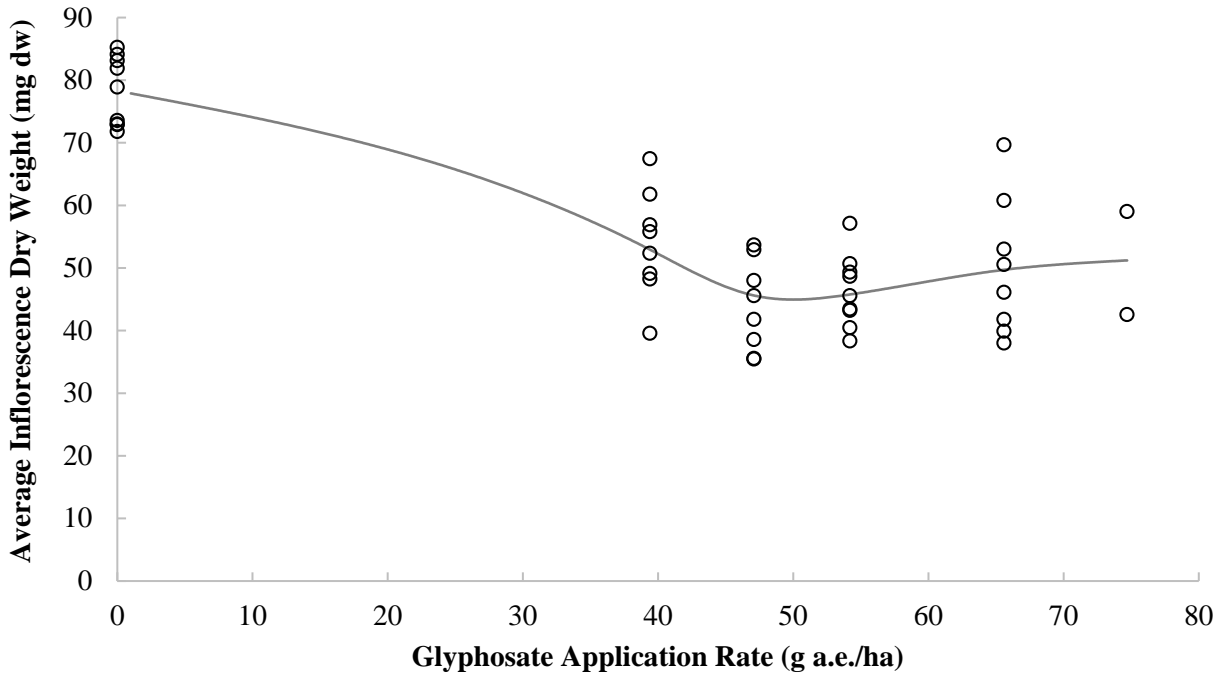


Figure 27. Average *C. cyanus* inflorescence dry weight and nonparametric model fit by spline interpolation over rate.

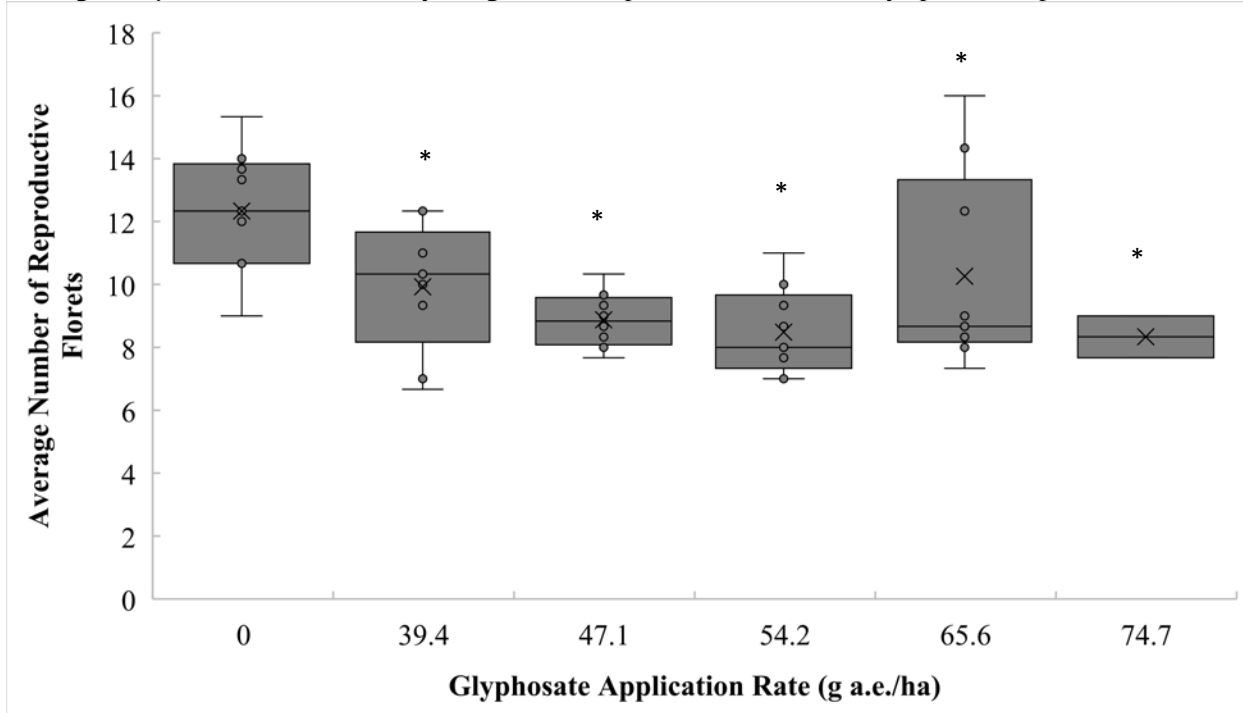


Figure 28. Average number of reproductive florets per *C. cyanus* inflorescence. Asterisks indicate $p < 0.05$ for Jonckheere-Terpestra step-down test when included. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range.

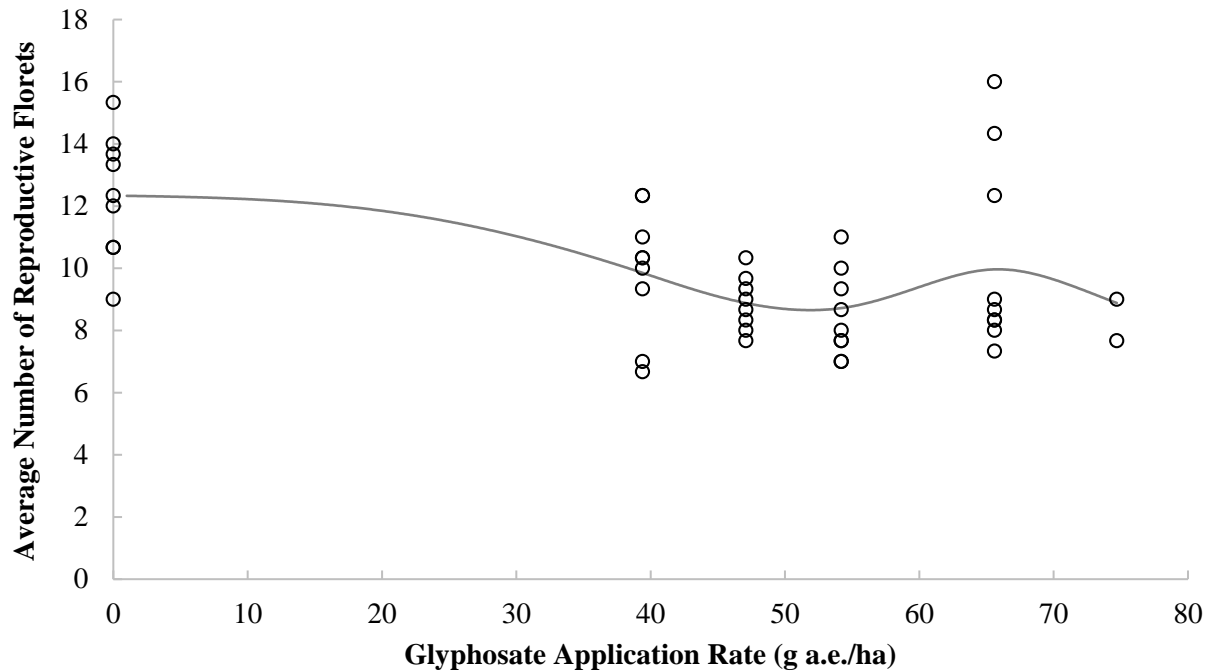


Figure 29. Average number of reproductive florets per *C. cyanus* inflorescence with nonparametric model by spline interpolation over rate.

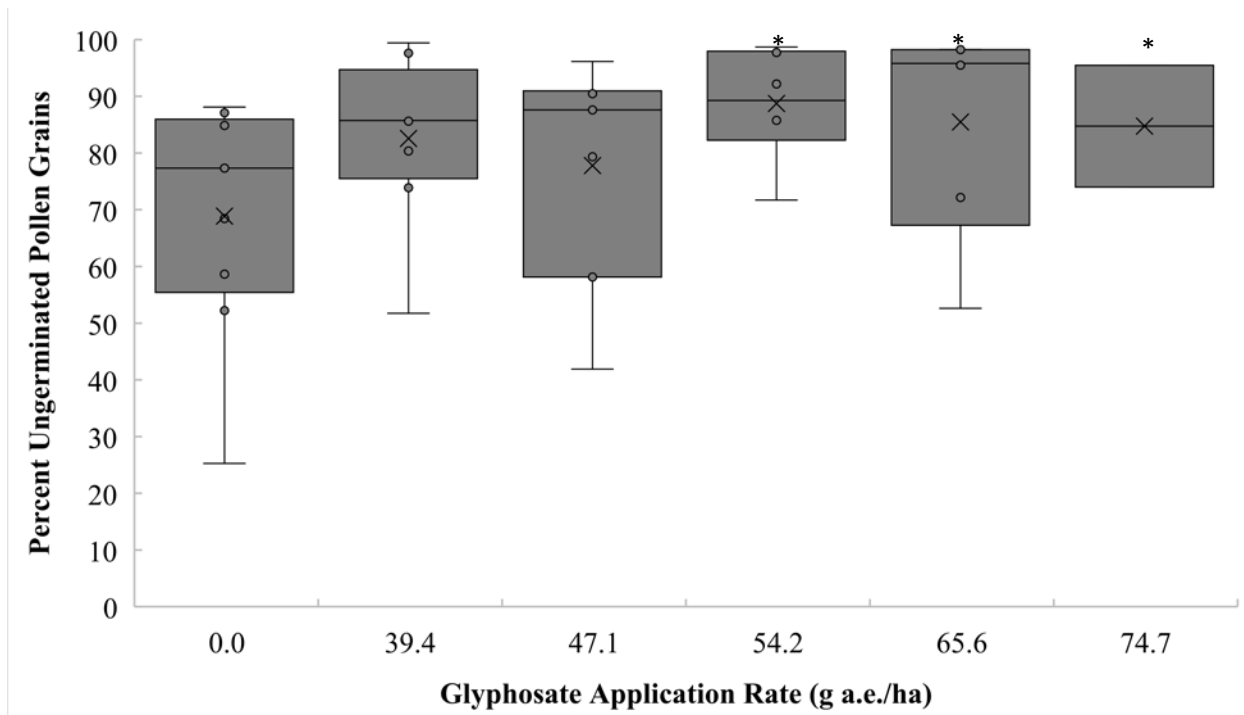


Figure 30. Percent ungerminated *C. cyanus* pollen grains in INFLO experiment. Asterisks indicate significant trend when included in Cochran-Armitage step-down test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range.

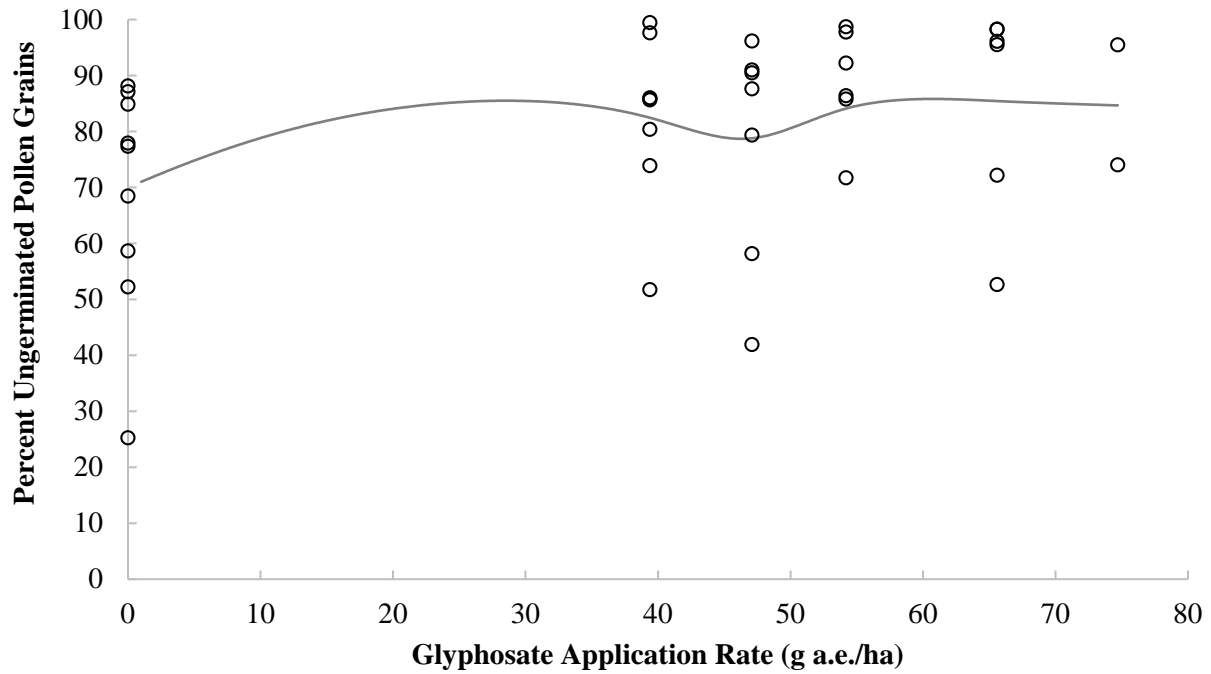


Figure 31. Percent ungerminated *C. cyanus* pollen grains in INFLO experiment with nonparametric model fit by spline interpolation over rate.

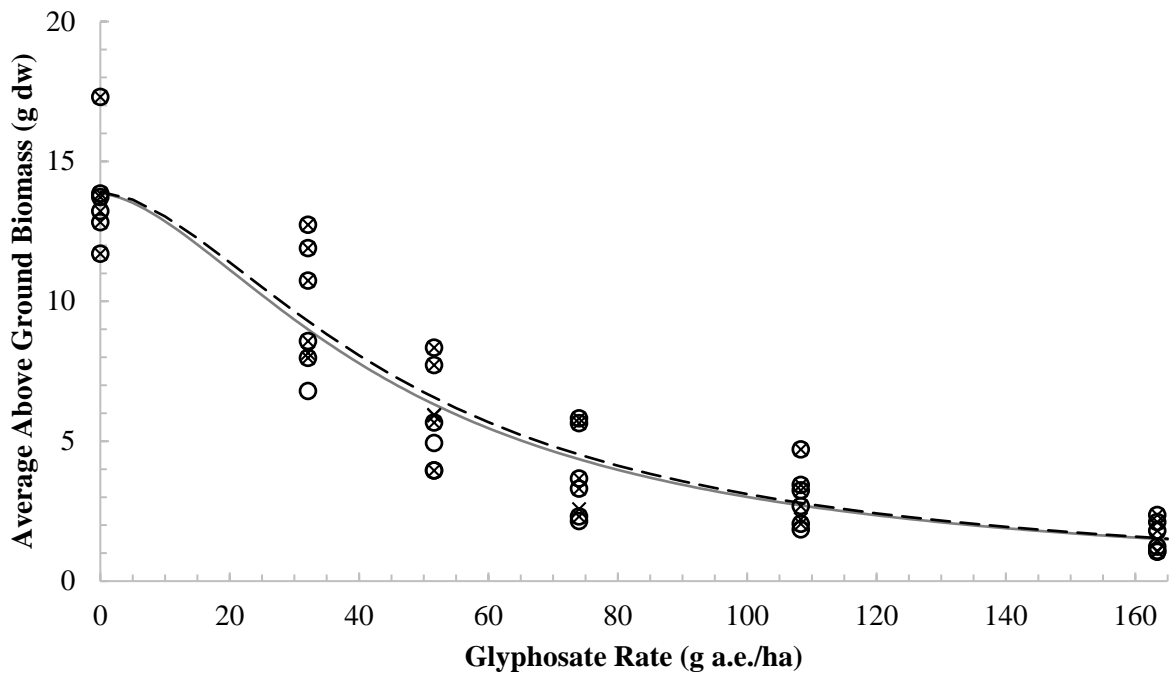


Figure 32. Average above ground biomass of *T. pratense* PHEN experimental plants (o) and surviving plants (x) with best-fitting models (log logistic), solid line and dashed line, respectively.

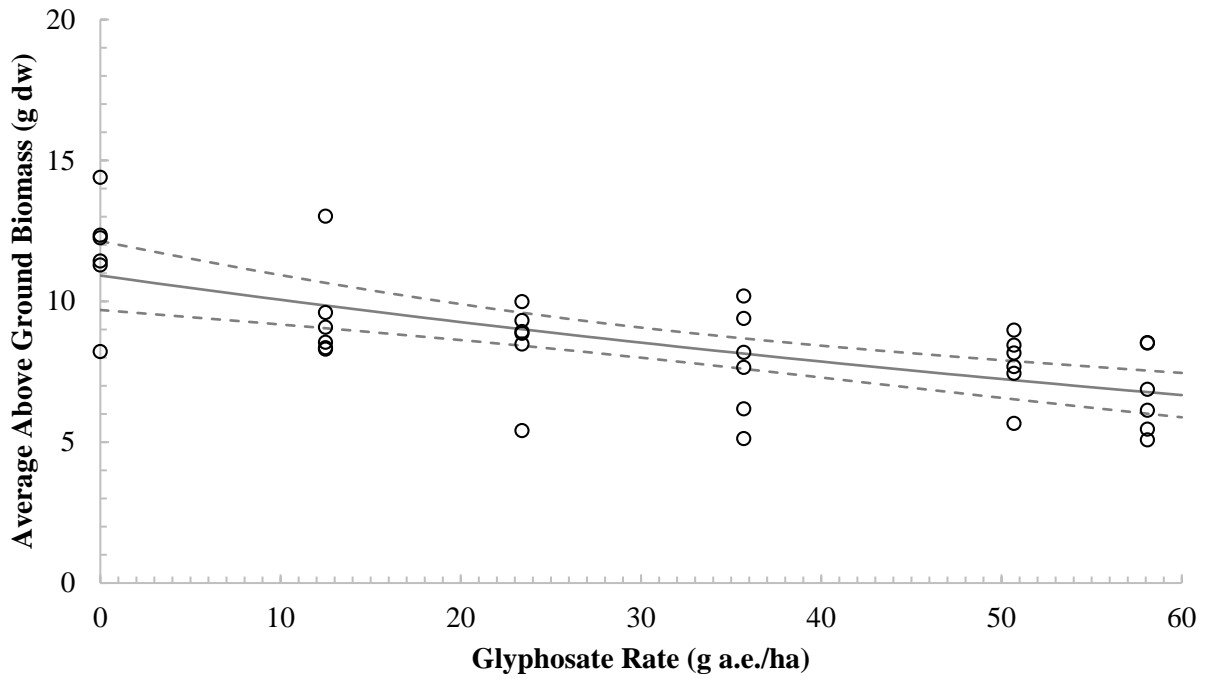


Figure 33. Average above ground biomass of *T. pratense* INFL0 experimental plants with best-fitting linear model and 95% confidence limits (note: no mortalities in this experiment).

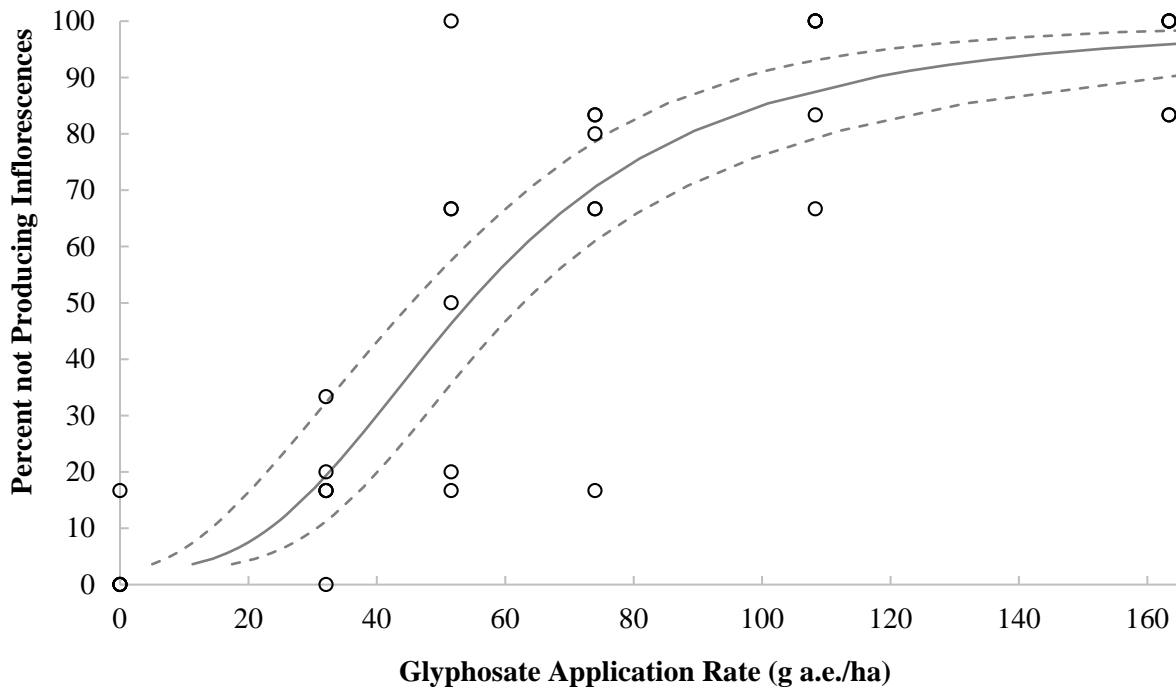


Figure 34. Percent of surviving *T. pratense* plants not producing inflorescences in the PHEN experiment with log logistic model and 95% confidence limits.

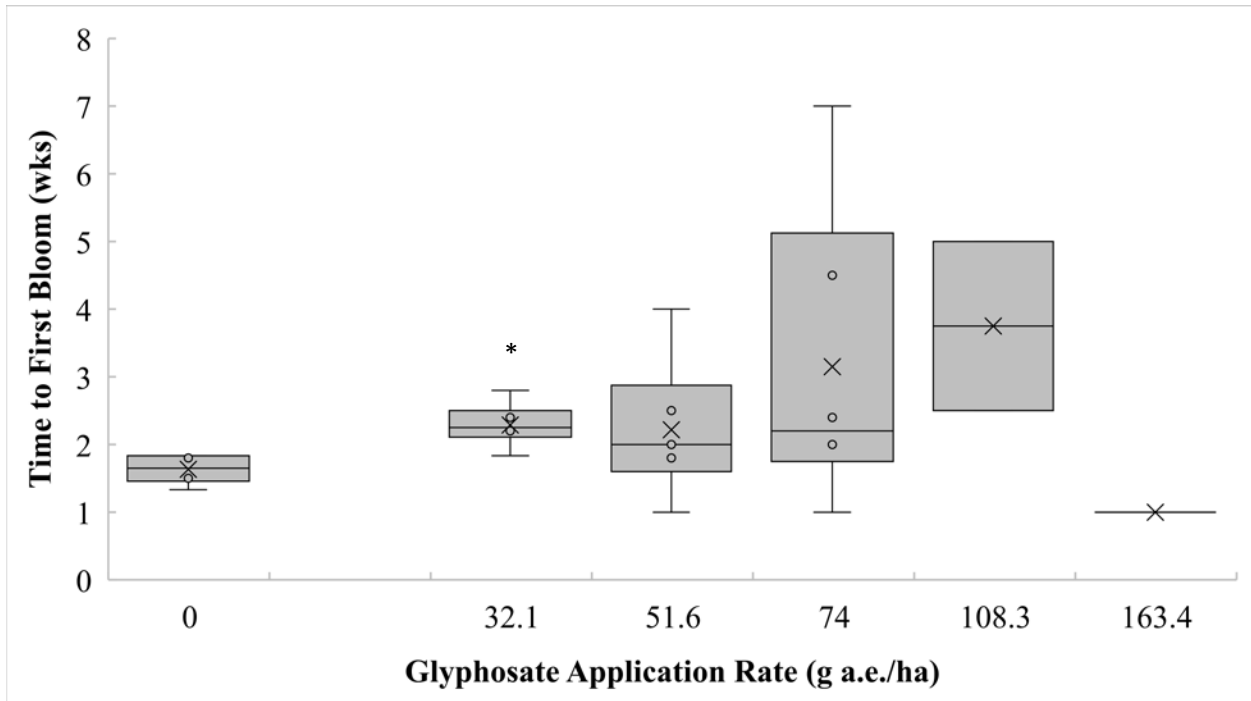


Figure 35. *T. pratense* average time to first bloom in PHEN experiment. No significant differences based on Wilcoxon exact comparisons to control at family wise error rate of 0.05, except at the first treatment level ($p=0.0043$). X indicates means, box enclose 25th to 75th percentiles, and whiskers (- - - - |) extend to furthest data points from the median falling within 1.5 times the interquartile range.

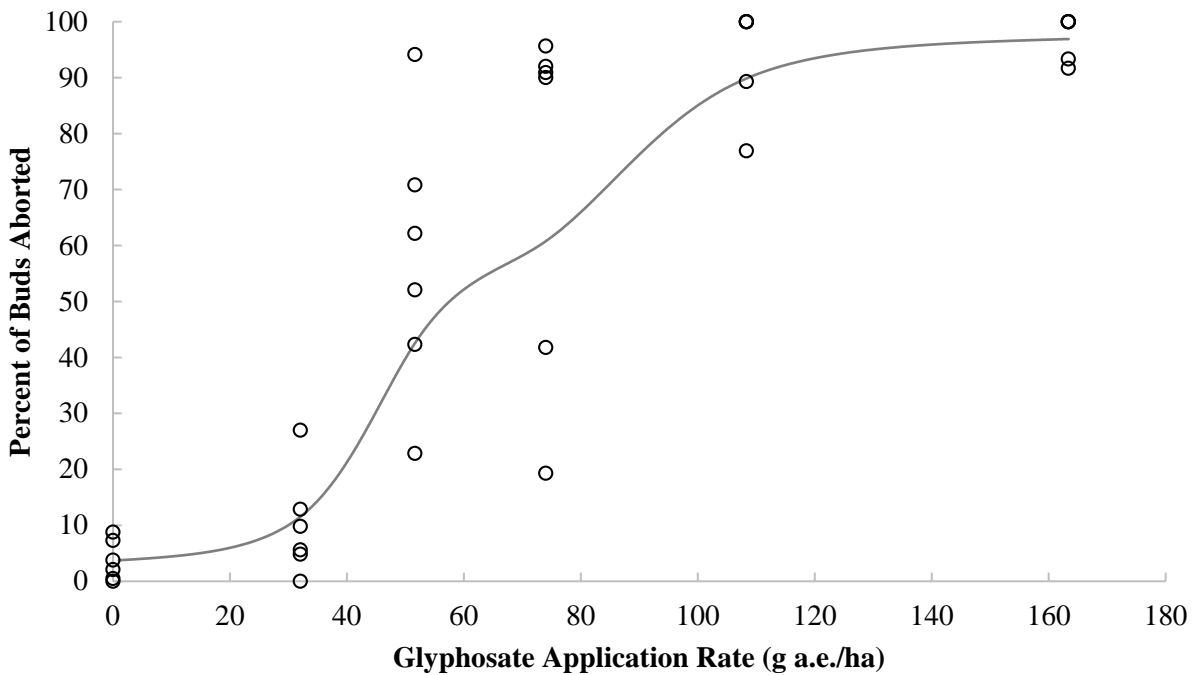


Figure 36. Percent of *T. pratense* buds aborted in the PHEN experiment with nonparametric regression model generated by spline interpolation over rate.

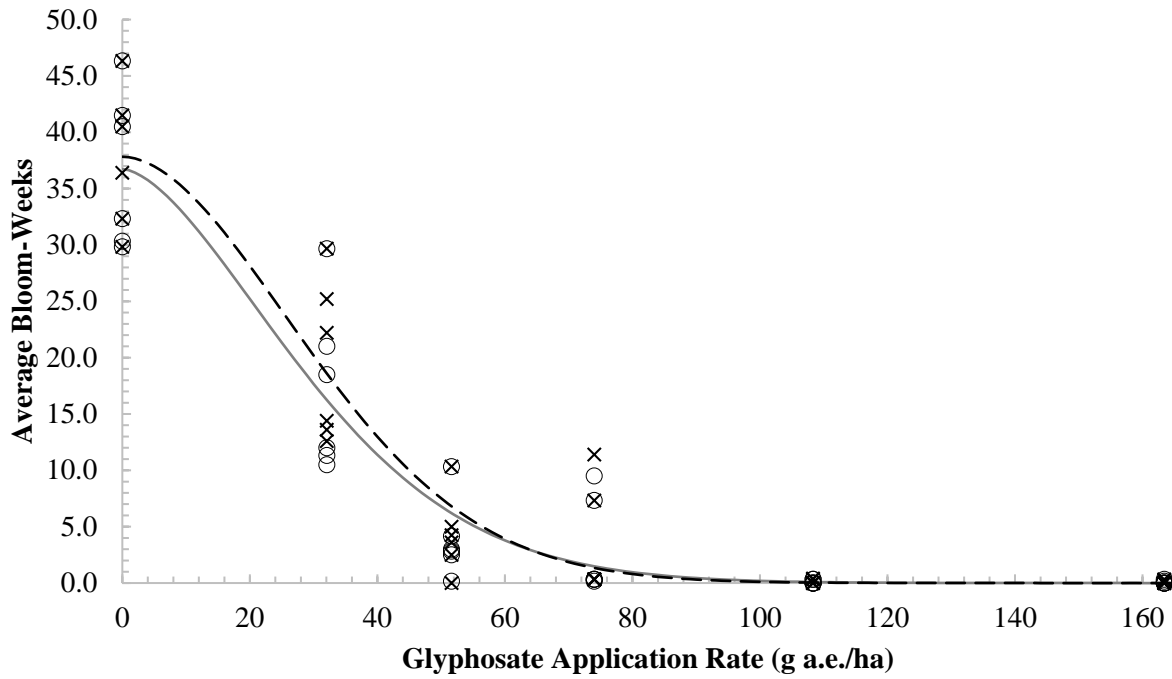


Figure 37. Average bloom-weeks per *T. pratense* PHEN experimental plant (o) and surviving and reproductive plant (x) with best-fitting log Gompertz models, solid and dashed lines, respectively.

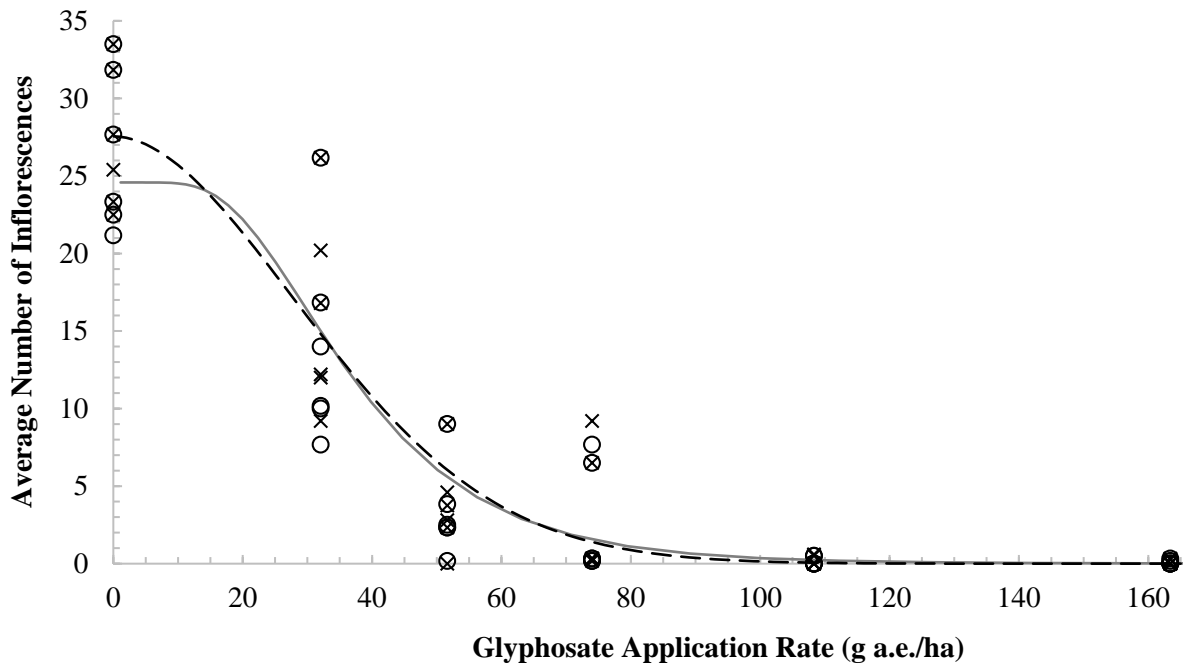


Figure 38. Average number of bloomed inflorescences per *T. pratense* PHEN experimental plant (o) and surviving and reproductive plant (x) at 8 weeks post-spray with best-fitting models, lognormal (solid line) and log Gompertz (dashed line), respectively.

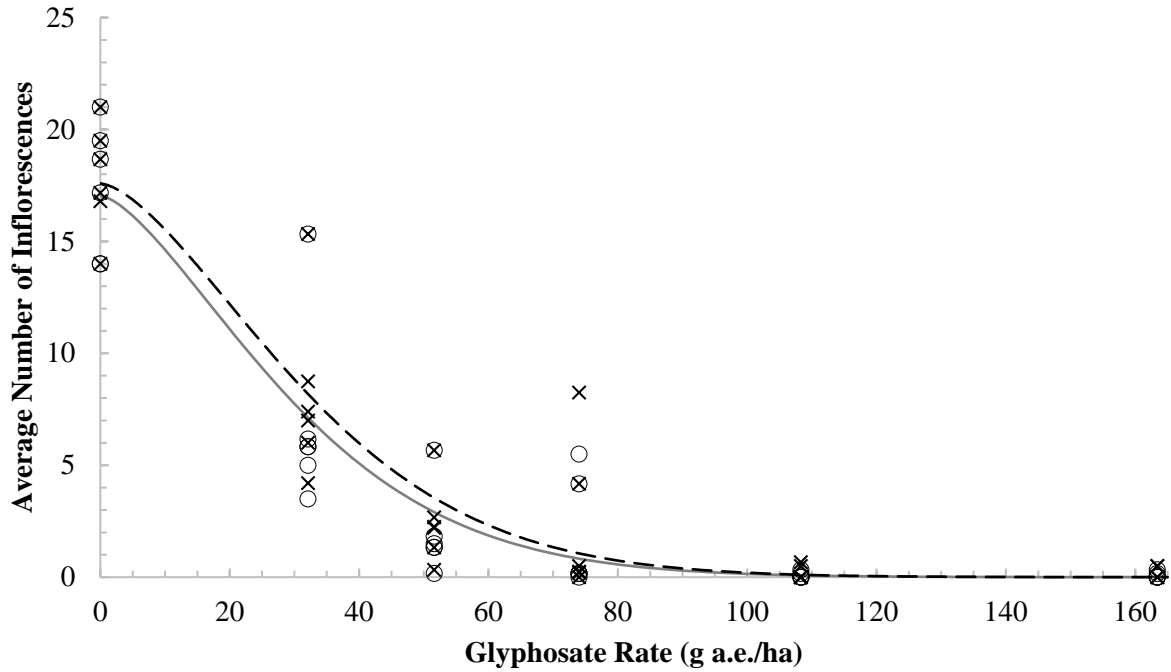


Figure 39. Average number of bloomed inflorescences per *T. pratense* PHEN experimental plant (o) and surviving and reproductive plant (x) at 5 weeks post-spray in PHEN experiment with best-fitting models, lognormal (solid line) and log Gompertz (dashed line), respectively.

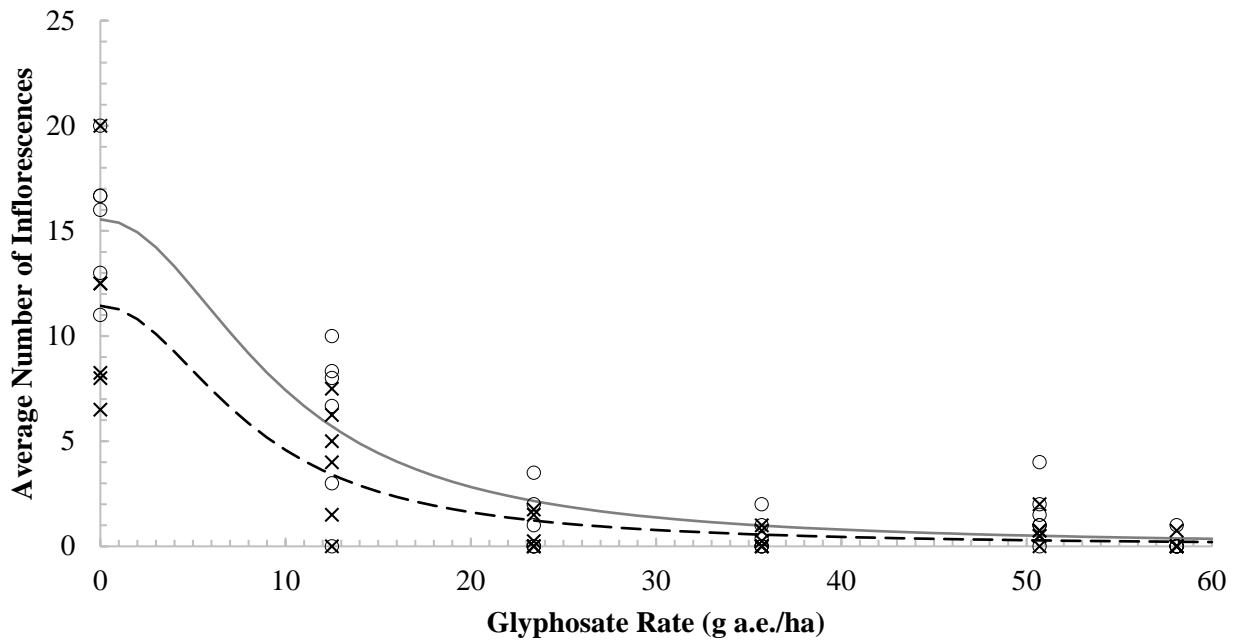


Figure 40. Average number of bloomed inflorescences per *T. pratense* PHEN experimental plant (o) and surviving and reproductive plant (x) at 5 weeks post-spray in PHEN experiment with best-fitting logistic models, experimental plants (solid line) and surviving and reproductive plants (dashed line).

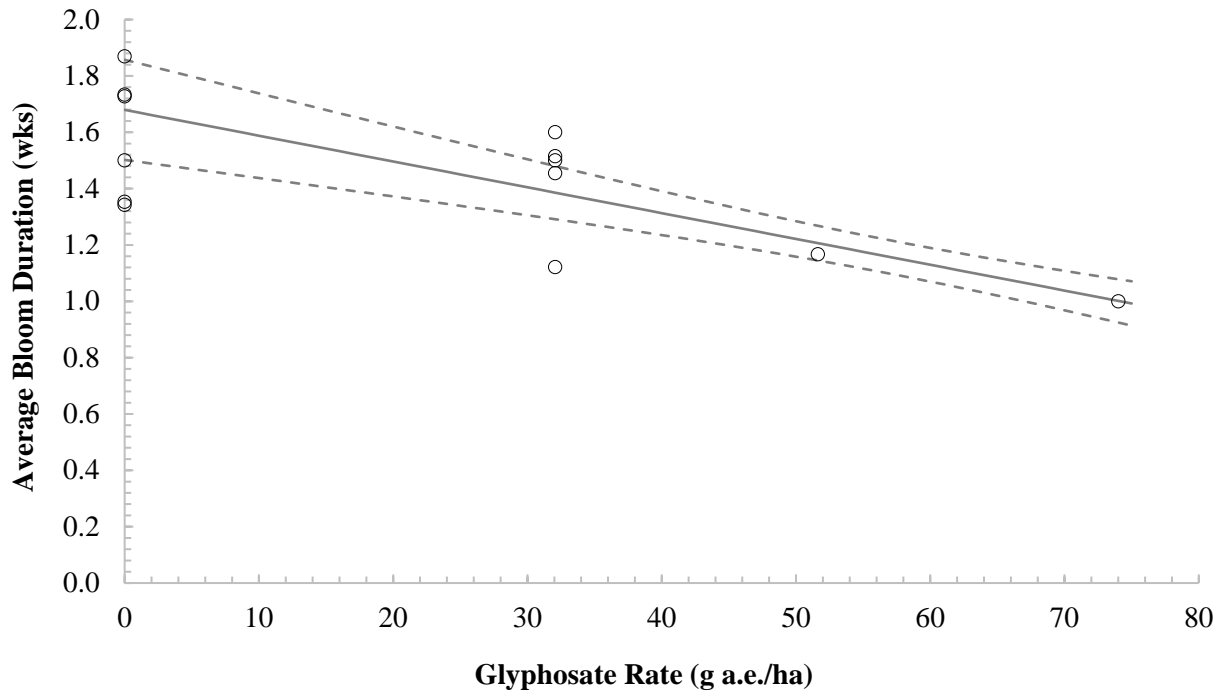


Figure 41. Average *T. pratense* bloom duration in the PHEN experiment with linear model and 95% confidence limits.



Figure 42. Malformations observed in *T. pratense* inflorescences.

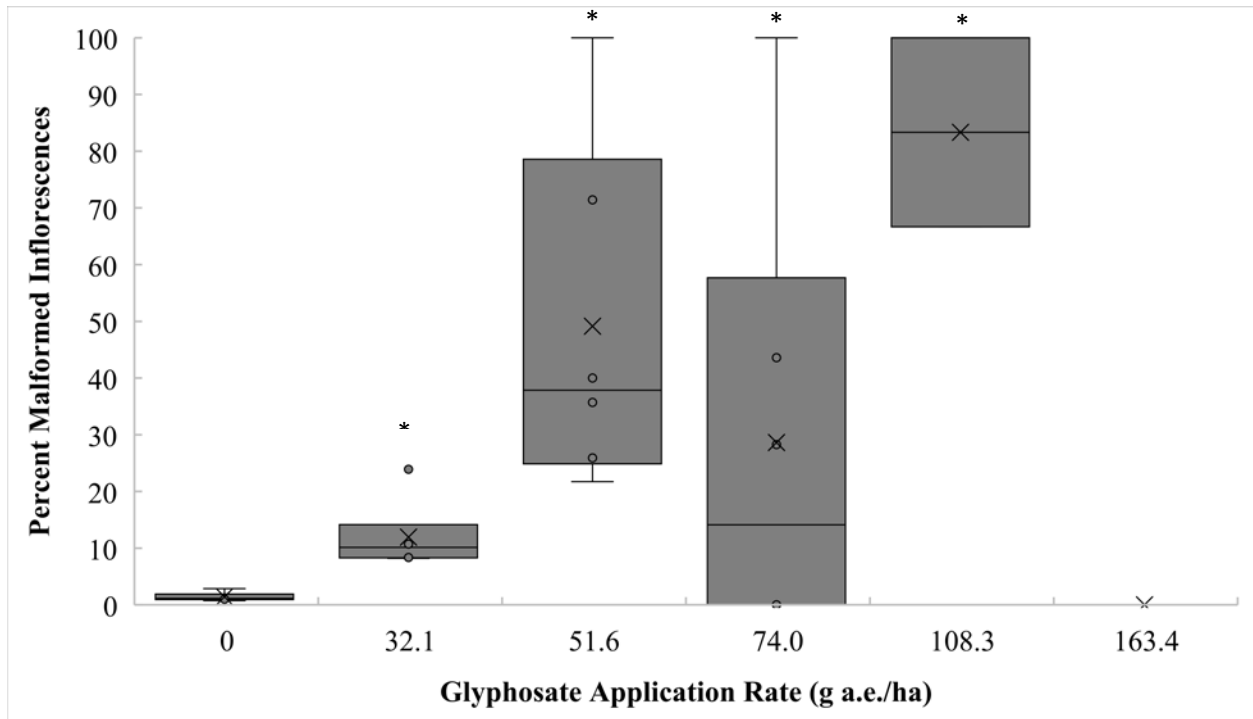


Figure 43. Percent malformed *T. pratense* inflorescences in the PHEN experiment. Asterisks indicate significant difference from control ($p < 0.01$) based on Fisher exact test. X indicates means, box enclose 25th to 75th percentiles, and whiskers (----|) extend to furthest data points from the median falling within 1.5 times the interquartile range.

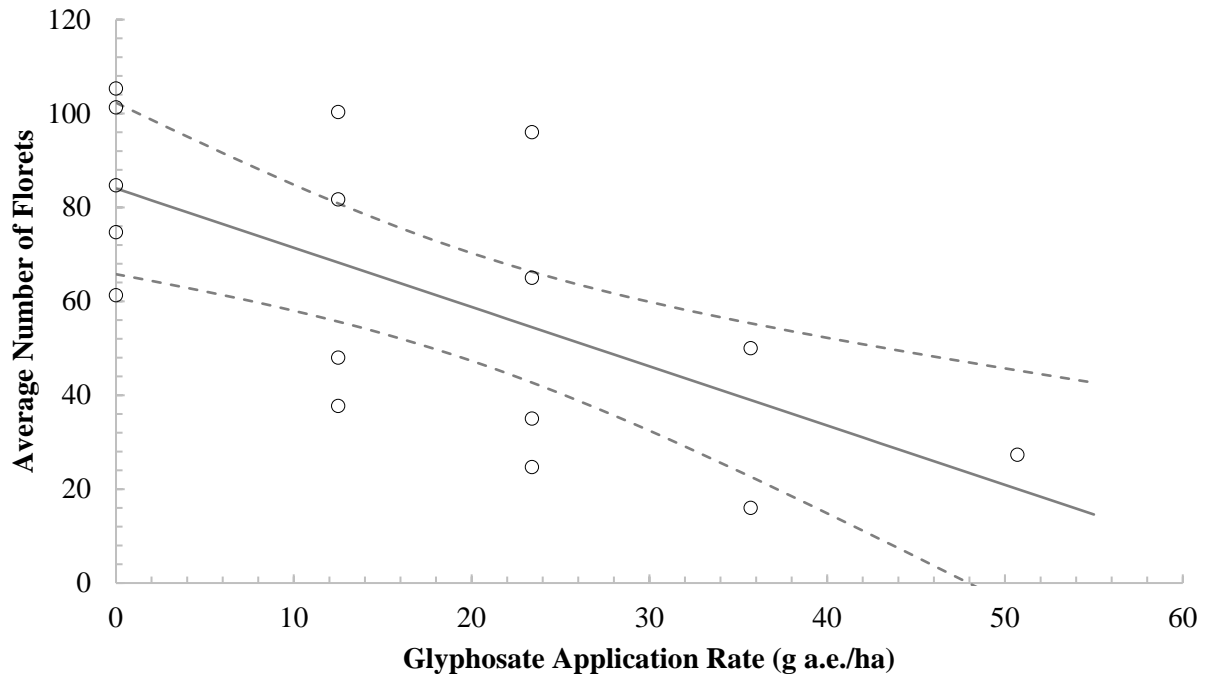


Figure 44. Average number of *T. pratense* florets per inflorescence in INFLO experiment with linear model and 95% confidence limits.

Appendix A – Preliminary Species, Growth Media and Methods Assessments

For the experiments, a short list of local wildflower candidate species were selected (*Centaurea cyanus*, *Chamerion angustifolium*, *Impatiens capensis*, *Monarda punctata*, *Prunella vulgaris*, *Rudbeckia hirta*, *Solidago canadensis* and *Trifolium pratense*). These species are entomophilous and also have been documented to provide pollen and nectar for bees (Table A-1). Due to time limitations in the greenhouse, candidate species are either annuals or perennials that are known to flower in their first year.

Two crop species were also selected for comparison with local wild species (*Cucumis sativus* and *Brassica napus*). Cucumber (*C. sativus*) because it produced the lowest endpoints in the Tier II vegetative vigour study used by the U.S. EPA in their most recent assessment of glyphosate (U.S. EPA 2008). The 4-week cucumber growth ER25 was 82.9 g a.i./ha, and the corresponding NOEL was 54.9 g a.i./ha. It will be interesting to see if reproductive effects due to glyphosate exposure are found in this species. Oilseed rape (*Brassica napus*) was selected because it is known to be of nutritional value to bees (Diekötter et al. 2010) and is also expected to be somewhat sensitive to glyphosate, based on low growth endpoints for a related *Brassica sp.* (U.S. EPA 2008).

Initial experimentation investigated the feasibility of using these wild and crops species in the definitive experiments, by growing these species in the greenhouse to determine: time to flower, number of flowers produced, amount of pollen produced, duration of blooming, and other factors that might affect their overall suitability to the definitive experiments.

Scientific Name (Family)	Common Name	Southern Ontario Coefficient of Conservatism^a	Southern Ontario Wetness Index^a	Southern Ontario Weediness Index^a	Native status, Lifespan and Southern Ontario Physiognomy^b	Habitat	Mutualism with Pollinators	Comment
<i>Centaurea cyanus</i> (Asteraceae)	Cornflower	Not assessed, but declining in some of its global range (Penet et al. 2012)	Obligate upland	-1 (Little or no impact on natural areas)	Non-native annual forb	Fields, roadsides, open habitat (Gleason and Cronquist 1991 cited in U.S. EPA 2012a)	Entomophilous, mostly pollinated by bees (Carreck and Williams 2002). <i>Centaurea sp.</i> pollen found in honeybee pollen loads (Dimou and Thrasyvoulou 2007). Nectar is consumed by bees (Hintermeier 2011)	U.S. EPA recommended non-crop test species (U.S. EPA, 2012a) Also on OECD list of potential non-crop test species (OECD 2006a). Seed source: Richters.
<i>Chamerion angustifolium</i> (Onagraceae)	Fireweed	3 (Found in a wide variety of plant communities, including disturbed sites)	Facultative (equally likely to occur in wetlands or non-wetlands)	Not assessed	Native annual/perennial forb	Successional plant communities, streamsides, upland habitat, logged or burned areas, disturbed sites	Entomophilous, visited by many bee species (Benham 1969 cited in Myerscough 1980). Pollen found in honeybee pollen loads (McLellan 1976; Girard et al. 2012). Nectar is consumed by bees and other arthropoda, as reviewed by Myerscough (1980).	Seed source: Prairie Moon Nursery.
<i>Impatiens capensis</i> (Balsaminaceae)	Jewelweed, spotted touch-me-not	4 (typically associated with a specific plant community, but tolerant to moderate disturbance)	Facultative wetland (Usually occurs in wetlands, but occasionally found in non-wetlands)	Not assessed	Native annual forb	Moist habitats, along water ways, successfully colonizes a range of habitats (Waller 1979)	Zoophilous, particularly reliant on bees and humming birds, as reviewed by Waller (1979) and Walters and Stiles (1996). Pollen and nectar collected by bees (Wilson and Thomson 1991).	Seed source: Yuko's Open-Pollinated Seeds.
<i>Monarda punctata</i> (Lamiaceae)	Spotted beebalm, spotted horsemint, horsemint	9 (high degree of fidelity to a narrow range of synecological parameters)	Obligate upland	Not assessed	Native annual/perennial forb	Prairies, plains, meadows, pastures, savannahs	Entomophilous (Baskett et al 2011). Nectar and pollen collected by bees (Hilty, 2014).	. Seed source: Prairie Moon Nursery.

Scientific Name (Family)	Common Name	Southern Ontario Coefficient of Conservatism^a	Southern Ontario Wetness Index^a	Southern Ontario Weediness Index^a	Native status, Lifespan and Southern Ontario Physiognomy^b	Habitat	Mutualism with Pollinators	Comment
<i>Prunella vulgaris ssp. vulgaris</i> (Lamiaceae)	Common selfheal, lawn prunella	Not assessed	Facultative (equally likely to occur in wetlands or non-wetlands)	-1 (Little or no impact on natural areas)	Native annual forb	Disturbed habitats, reported for the sp. (Boutin et al. 2004), lawns (NPIN 2014)	Entomophilous (Qu and Widrlechner 2011). Pollen collected by bees (Girard et al. 2012). It is also a nectar source.	U.S. EPA recommended non-crop test species, germination may be inhibited by darkness, larger seeds more likely to germinate (U.S. EPA, 2012a) Also on OECD list of potential non-crop test species (OECD 2006a). Seed Source: Richters.

Scientific Name (Family)	Common Name	Southern Ontario Coefficient of Conservatism^a	Southern Ontario Wetness Index^a	Southern Ontario Weediness Index^a	Native status, Lifespan and Southern Ontario Physiognomy^b	Habitat	Mutualism with Pollinators	Comment
<i>Rudbeckia hirta</i> (Asteraceae)	Black-eyed susan, prairie sun, brown-eyed Susan	0 (found in a wide variety of plant communities, including disturbed sites)	Facultative upland (occasionally occurs in wetlands, but usually occurs in non-wetlands)	Not assessed	Native biennial/perennial forb	Disturbed habitat (Boutin et al. 2004)	Insects feed on nectar and pollen, particularly bees, flies and butterflies (Nuffer 2007; Girard et al. 2012).	U.S. EPA recommended non-crop test species (U.S. EPA, 2012a). May bloom longer with some afternoon shade No special treatments required (U.S. EPA 2012). Stratification may be required. Also, on OECD list of potential non-crop test species (OECD 2006a). Seed Source: Richters.

Scientific Name (Family)	Common Name	Southern Ontario Coefficient of Conservatism^a	Southern Ontario Wetness Index^a	Southern Ontario Weediness Index^a	Native status, Lifespan and Southern Ontario Physiognomy^b	Habitat	Mutualism with Pollinators	Comment
<i>Solidago canadensis</i> (Asteraceae)	Canada goldenrod	1 (found in a wide variety of plant communities, including disturbed sites)	Facultative upland (occasionally occurs in wetlands, but usually occurs in non-wetlands)	Not assessed	Native perennial forb	Pasture, secondary succession (Boutin et al. 2004)	Entomophilous (Coladonato 1993). Important source of nectar for honeybees (Amtmann 2010). Solidago species are an important source of pollen and nectar for bees and other insects in late summer and early fall (Mader et al. 2011)	U.S. EPA recommended non-crop test species (U.S. EPA, 2012a). Also on OECD list of potential non-crop test species (OECD 2006a). Stratification of two months recommended by White et al. (2008). Seed Source: Wild About Flowers.
<i>Trifolium pratense</i> (Fabaceae)	Wild red clover	Not assessed	Facultative upland (occasionally occurs in wetlands, but usually occurs in non-wetlands)	-2 (sometimes causes problems, but infrequently or in localized areas)	Non-native annual/biennial/perennial forb	Fields, roadsides, arable land (U.S. EPA 2012a)	Entomophilous, with pollen and nectar collected by bees (Palmer-Jones et al. 1966).	U.S. EPA recommended non-crop test species (U.S. EPA 2012a). Scarification possibly required. Also on OECD list of potential non-crop test species (OECD 2006a). Seed Source: Richters.

Scientific Name (Family)	Common Name	Southern Ontario Coefficient of Conservatism^a	Southern Ontario Wetness Index^a	Southern Ontario Weediness Index^a	Native status, Lifespan and Southern Ontario Physiognomy^b	Habitat	Mutualism with Pollinators	Comment
<i>Cucumis sativus</i> (Cucurbitaceae)	Cucumber	NA	NA	NA	NA	Field crop, garden vegetable	Entomophilous (Mussen and Thorp 1997, dos Santos et al. 2008). Pollen and nectar consumed by bees (Mussen and Thorp 1997).	U.S. EPA recommended crop test species (U.S. EPA 2012a,b). Historically used in toxicity testing (U.S. EPA 2012a, OECD 2006a). Seed Source: Greta's Organic Gardens – Spacemaster variety.
<i>Brassica napus</i> (Brassicaceae)	Oilseed rape, turnip, rutabaga	Not assessed	Obligate upland	-1 (Little or no impact on natural areas)	Annual forb	Field crop, garden vegetable	Anemophilous and entomophilous (Kevan and Eisikowitch 1990). Pollen and nectar consumed by bees (Cresswell 1999, Cook et al. 2003).	U.S. EPA recommended crop test species (U.S. EPA 2012a,b). Historically used in toxicity testing (U.S. EPA 2012a, OECD 2006a). Seed Source: Johnny's Selected Seeds – Dwarf variety.

^a Source: Oldham et al. (1995)

^b Sources: USDA Plant Database (2014) and Oldham et al. (1995)

The soil medium initially used had a base of 30% clay and 70% sand. Organic matter was added to achieve approximately 3% by mass (in accordance with standardized tests) in the form of manure compost, and a peat and shrimp compost blend. A slow release fertilizer for annual flowering species was added following label recommended application rates.

It was clear early on in these growth trials (summer of 2014) that the soil medium was not ideal for seed germination. In particular, *Monarda punctata* (bee balm), *Prunella vulgaris* (self-heal) and *Rudbeckia hirta* (black-eyed Susan) had prohibitively low germination in this soil. This was primarily attributed to the high clay content (30%), and so subsequent trials were conducted with 20% clay and 80% sand. This ratio seemed to improve germination (qualitative observation) in subsequent growth trials. However, despite improved germination, the following candidate species were dropped from the project because either they did not flower, or the time to flower was excessive:

1. *Solidago canadensis* (Asteraceae), Canada goldenrod – too long to flower
2. *Prunella vulgaris* (Lamiaceae), self-heal – never flowered

Although growth of *Cucumis sativus*, cucumber, was successful. It was decided to drop the comparison of wild and crop species for the project, due to an insufficient number of species for such analysis overall.

The data from my growth trials are summarized in Table A-2. Notably, we cannot directly compare results across media because there were also differences in growing conditions related to timing.

In early November of 2014 four species in three different growth media were grow. The species were *Centaurea cyanus* (cornflower), *Monarda punctata* (bee balm), *Rudbeckia hirta* (black-eyed Susan), *Trifolium pratense* (red clover). The purpose of these trials was two-fold:

1. To test the difference in inflorescence production among three soil types: low organic matter (3%, from compost) with slow release fertilizer, low organic matter (3%, from peat) with slow release fertilizer, and

high organic matter (8%, from compost) with no slow release fertilizer.

2. To test if clipping inflorescences (to collect pollen) would have a significant impact on overall flower production.

The latter was of interest, because I was looking to maximize the amount of pollen collected. If I could clip inflorescences without having an impact on overall flower production, it would stand to reason that I could take this approach in the toxicity experiments.

The *Monarda punctata* experiment was aborted, because the species took too long to flower, and when it did flower, only a couple out of dozens of plants flowered.

For the other three species, the data are summarized in Tables A-2 through A-4. I used the PROC GENMOD procedure in SAS Software® (SAS Version 9.3) to test for significant differences among the groups in terms of the total number of bloomed inflorescences produced by termination (20 weeks from planting). “Clipping” or “not clipping”, and the three different media were treated as class variables, and the count response variable was assumed to be Poisson (with expected over-dispersion for species that can produce many flowers). The results of the analysis are summarized in Tables 4-6 through 4-8 for the three species.

For *Centaurea cyanus*, the statistical analysis suggests that both the medium and whether the inflorescences are clipped are significant factors in predicting the number of inflorescences that bloomed. Based on the significant model parameters presented in Table A-7, we can infer that clipping increased the number of blooming inflorescences. We can also infer that fertilizer increased the number of bloomed inflorescences, and that compost rather than peat produced more inflorescences in the low OM media.

For *Rudbeckia hirta*, the statistical analysis suggests that only medium was a significant factor in predicting the number of inflorescences that bloomed. Based on the significant model parameters

presented in Table A-8 we can infer that like *Centaurea cyanus*, *Rudbeckia hirta* produced the most bloomed inflorescences in the compost-containing low OM soil with slow release fertilizer. The medium producing the lowest number of bloomed inflorescences, which was significant in the model, was the high OM soil with no fertilizer.

For *Trifolium pratense*, the statistical analysis was more complex. An interaction effect was identified, whereby the combination of low OM (compost) and clipping resulted in a significant increase in bloomed inflorescence, while for the other two media, clipping had no significant effect on the number of bloomed inflorescences, based on the significance of the interaction parameters in the model. In contrast to the results for the other two species tested, the medium producing the lowest number of bloomed inflorescences, which was significant in the model was the low OM soil with peat and slow release fertilizer.

On the basis of these results, I decided that I would not clip inflorescences over time from the same plant, to avoid the confounding factor of augmenting inflorescence production, as illustrated with the *Centaurea cyanus*. Instead, as suggested by my lab mate, Simon Grafe (M.Sc.), I opted to sacrifice a subset of each replicate through the desired portion of the bloom period.

Also, on the basis of these results, I decided that I could use a low organic matter soil (as specified by standard terrestrial plant toxicity tests), but that I would need slow release fertilizer (which is allowed in standard terrestrial plant toxicity tests). Because I had differing results with peat and compost as the organic matter medium, I opted to use a mix of the two. Qualitatively, better germination was observed in the soils containing peat as the organic matter source.

Plant date	Total No. of Plants	Soil	Time to Flower (weeks) ^a	Peak Bloom (weeks) ^b	Last Week of Observation (weeks)	Average No. Bloomed Inflorescences Produced at termination per Plant ^c
June 13, 2014	18	30% clay, 70% sand, 3% OM + slow release fertilizer	6	6-10	10	13 (3.2)
June 20, 2014	20	20% clay, 80% sand, 8% OM (no fertilizer)	7	7-9	9	9 (4.0)
November 5, 2014	35	20% clay, 80% sand, 3% OM (compost), slow release annual flower fertilizer	9	13-16	17	Clipped: 35 (11) Not clipped: 30 (9.2)
November 5, 2014	35	20% clay, 80% sand, 3% OM (peat), slow release annual flower fertilizer	9	13-16	17	Clipped: 27 (5.8) Not clipped: 20 (5.8)
November 5, 2014	35	20% clay, 80% sand, 8% OM (compost)	9	13-16	17	Clipped: 8.4 (2.7) Not clipped: 9.4 (3.4)

^a Weeks from planting when first blooming inflorescences were recorded.

^b Period during which the highest number of full bloom inflorescences/plant occurred.

^c Bracketed value is the standard deviation

Plant date	Total No. of Plants	Soil	Time to Flower (weeks) ^a	Peak Bloom (weeks) ^b	Last Week of Observation (weeks)	Average No. Bloomed Inflorescences Produced at termination per Plant ^c
June 13, 2014	18 pots	30% clay, 70% sand, 3% OM + slow release fertilizer	Very poor germination; experiment aborted.			
June 20, 2014	20	20% clay, 80% sand, 8% OM (no fertilizer)	9	Not determined	11	1.1 (1.0)
November 5, 2014	35	20% clay, 80% sand, 3% OM (compost), slow release annual flower fertilizer	14	>19	20	Clipped: 3.4 (1.5) Not clipped: 3.1 (0.9)
November 5, 2014	35	20% clay, 80% sand, 3% OM (peat), slow release annual flower fertilizer	14	>19	20	Clipped: 2.5 (1.0) Not clipped: 3.3 (1.3)
November 5, 2014	35	20% clay, 80% sand, 8% OM (compost)	14	>19	20	Clipped: 1.0 (0) Not clipped: 1.1 (0.3)

^a Weeks from planting when first full bloom inflorescences were recorded.

^b Period during which the highest number of full bloom inflorescences/plant occurred.

^c Bracketed value is the standard deviation

Plant date	Total No. of Plants	Soil	Time to Flower (weeks)^a	Peak Bloom (weeks)^b	Last Week of Observation (weeks)	Average No. Bloomed Inflorescences Produced at termination per Plant^c
May 23, 2014	21	30% clay, 70% sand, 3% OM + slow release fertilizer	7	≥8	10	3.2 (3.0)
June 20, 2014	19	20% clay, 80% sand, 8% OM (no fertilizer)	9	9-17	17	4.1 (3.0)
November 5, 2014	35	20% clay, 80% sand, 3% OM (compost), slow release annual flower fertilizer	14	14-20	20	Clipped: 12 (12) Not clipped: 5.6 (6.4)
November 5, 2014	35	20% clay, 80% sand, 3% OM (peat), slow release annual flower fertilizer	17	17-20	20	Clipped: 4.8 (6.5) Not clipped: 5.1 (7.2)
November 5, 2014	35	20% clay, 80% sand, 8% OM (compost)	14	14-20	20	Clipped: 13 (10) Not clipped: 11 (11)

^a Weeks from planting when first full bloom inflorescences were recorded.

^b Period during which the highest number of full bloom inflorescences/plant occurred.

^c Bracketed value is the standard deviation

Parameter	Class	Degrees of Freedom	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	p-value	F-value	p-value	Chi-Square	p-value
Intercept		1	3.2316	0.0573	3.1193	3.344	3176.9	<.0001				
clipped	n	1	-0.1772	0.06	-0.2949	-0.0596	8.73	0.0031	8.75	0.0039	8.75	0.0031
clipped	y	0	0	0	0	0	.	.				
medium	highcomp	1	-0.9536	0.0946	-1.139	-0.7681	101.51	<.0001	125.86	<.0001	251.72	<.0001
medium	lowcomp	1	0.3381	0.0656	0.2096	0.4667	26.57	<.0001				
medium	lowpeat	0	0	0	0	0	.	.				
Scale		0	1.4173	0	1.4173	1.4173						

***Rudbeckia hirta* bloomed inflorescence production as a function of medium and clipping treatment: analysis of maximum likelihood parameter estimates and likelihood ratio statistics for Type 3 analysis**

Parameter	Class	Degrees of Freedom	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	p-value	F-value	p-value	Chi-Square	p-value
Intercept		1	1.0178	0.0661	0.8882	1.1473	237.09	<.0001				
clipped	n	1	0.0733	0.0723	-0.0684	0.2149	1.03	0.3108	1.03	0.3134	1.03	0.311
clipped	y	0	0	0	0	0	.	.				
medium	highcomp	1	-1.0221	0.1172	-1.2519	-0.7924	76.04	<.0001	66.34	<.0001	132.68	<.0001
medium	lowcomp	1	0.1276	0.0777	-0.0247	0.2799	2.7	0.1005				
medium	lowpeat	0	0	0	0	0	.	.				
Scale		0	0.5768	0	0.5768	0.5768						

***Trifolium pratense* bloomed inflorescence production as a function of medium and clipping treatment: analysis of maximum likelihood parameter estimates and likelihood ratio statistics for Type 3 analysis**

Parameter	Medium Class	Clipping Class	Degrees of Freedom	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	p-value	F-value	p-value	Chi-Square	p-value
Intercept			1	2.068	0.2429	1.592	2.5441	72.49	<.0001				
medium	highcomp		1	0.6664	0.285	0.1078	1.2249	5.47	0.0194	7.88	0.0009	15.76	0.0004
medium	lowcomp		1	1.0321	0.2866	0.4704	1.5937	12.97	0.0003				
medium	lowpeat		0	0	0	0	0	.	.				
medium*clipped	highcomp	n	1	0.0662	0.2249	-0.3745	0.507	0.09	0.7684	3.39	0.0233	10.17	0.0172
medium*clipped	highcomp	y	0	0	0	0	0	.	.				
medium*clipped	lowcomp	n	1	-0.8383	0.2767	-1.3807	-0.2959	9.18	0.0025				
medium*clipped	lowcomp	y	0	0	0	0	0	.	.				
medium*clipped	lowpeat	n	1	-0.0986	0.3445	-0.7738	0.5766	0.08	0.7748				
medium*clipped	lowpeat	y	0	0	0	0	0	.	.				
Scale			0	2.2655	0	2.2655	2.2655						

Of those species that were grown to flowering in the summer of 2014 (*Centaurea cyanus*, *Trifolium pratense*, *Rudbeckia hirta*, *Cucumis sativus*), only pollen harvested from *Trifolium pratense* (red clover) was sufficient for amino acid analysis (≥ 5 mg dw). However, in these early trials I only collected inflorescences at one time point, the last week of observation (Table A-3), and I was attempting pollen collection for the first time.

At the end of 2014, we received some glyphosate tolerant canola seed (Monsanto product). Earlier this year I began growing these seeds to determine growth characteristics and pollen production, as I had done for the other candidate species.

The amount of pollen that I harvested from growth trials is summarized in Table A-10 below. For amino acid analysis (which is being carried out at UC Davis), a minimum of 5 mg dw pollen is recommended. I was able to get sufficient pollen from all species, except *Rudbeckia hirta* for two growth mediums (Table A-10). However, this is likely attributed to my collection technique. The *Rudbeckia* pollen is quite sticky, and unbeknownst to me was sticking to the bottom of my sieve. I did not realize this until I was washing the sieve after processing the second sample. Qualitatively I would say that almost half of the pollen had remained stuck to the sieve. Sample size going forward was based on this estimate.

Table A-8 Amounts of pollen collected from test species				
Species	Soil Type	No. plants	Total No. Inflorescences	Dry Mass of Pollen (mg)
<i>Brassica sp.</i> (GM canola)	Low OM (peat + compost) with slow release fertilizer	39	NR	73.2
<i>Centaurea cyanus</i>	Low OM (compost) with slow release fertilizer	17	600	45.2
	Low OM (peat) with slow release fertilizer	17	457	36.7
	High OM (compost) no fertilizer	17	143	14.9
<i>Rudbeckia hirta</i>	Low OM (compost) with slow release fertilizer	19	65	6.2
	Low OM (peat) with slow release fertilizer ^a	20	50	1.8
	High OM (compost) no fertilizer ^a	20	16	0.9
<i>Trifolium pratense</i>	Low OM (compost) with slow release fertilizer	18	222	108.6
	Low OM (peat) with slow release fertilizer	19	87	20.3
	High OM (compost) no fertilizer	18	231	65.9

^a The pollen of this species sticks to the sieve, and I missed a good quantity of it on the underside for these two samples.

All samples described in Table 4-9 except for the canola sample were sent to UC Davis (Molecular Structure Facility) for standard total amino acid analysis (ion-exchange chromatography with a Hitachi amino acid analyzer). The results for amino acids essential to honey bees (and humans) are summarized in Table 4-10 below. Phenylalanine will be the amino acid of greatest interest, because it is known to be essential to honey bees, and presumably other palynivorous species, and because it is one of the three amino acids inhibited by glyphosate (tyrosine is not essential, and methods for extracting tryptophan from pollen are dubious). Results in Table A-10 suggest that variability between soil types is relatively low, and that phenylalanine and other essential amino acids are highest in *Trifolium pratense* pollen, followed by *Centaurea cyanus*, and lastly, *Rudbeckia hirta*.

Rudbeckia hirta seems to have lower essential amino acid content than the other two species.

While *Trifolium pratense* generally appears to have the highest essential amino acid content of the three species (Table A-10).

Based on the results presented in this section I ran a couple of preliminary toxicity experiments with *Rudbeckia hirta* and *Trifolium pratense* at 0, 10 and 45 g acid equivalent/ha of glyphosate (using the end-use product RoundUp® WeatherMax). Results were presented at SETAC North America's 36th Annual Meeting (Rodney et al. 2015).

Species	Growth Medium	Threonine (mg/g)	Valine (mg/g)	Methionine (mg/g)	Isoleucine (mg/g)	Leucine (mg/g)	Phenylalanine (mg/g)	Histidine (mg/g)	Lysine (mg/g)	Arginine (mg/g)
<i>Centaurea cyanus</i>	low OM, compost	12.1	13.3	2.1	11.2	18.8	11.5	10.7	21.0	11.8
	low OM, peat	12.2	13.7	2.3	16.5	19.4	11.8	10.9	21.9	11.8
	high OM, compost	10.7	11.9	2.0	10.1	16.8	10.2	9.2	18.6	10.4
<i>Trifolium pratense</i>	low OM, compost	15.9	18.4	3.5	15.7	27.1	16.2	8.2	25.8	15.8
	low OM, peat	16.7	19.2	3.0	16.5	28.1	17.0	8.6	26.3	17.0
	high OM, compost	15.7	18.2	3.2	15.6	26.6	16.1	8.1	25.0	16.1
<i>Rudbeckia hirta</i>	pooled	9.1	9.0	1.7	8.5	16.2	9.2	7.8	15.8	7.9

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Appendix B – Pollen Amino Acid Results from Preliminary Testing with *R. hirta* and *T. pratense*

<i>T. pratense</i> pollen tyrosine (tyr) and phenlalanine (phen) concentrations from plants treated with glyphosate^a			
Replicate	Nominal treatment level (g a.i./ha)	Tyr (mg/g)	Phe (mg/g)
2.0	0.0	10.5	14.2
5.0	0.0	9.8	15.5
6.0	0.0	12.0	13.8
3.0	10.0	8.9	12.2
8.0	10.0	10.9	14.7
9.0	10.0	11.2	15.2
1.0	45.0	10.7	14.3
4.0	45.0	11.1	15.3
7.0	45.0	11.6	15.5

^a Pollen was collected weekly, dried (35°C), pooled and sieved, and frozen prior to analyses, which were conducted at UC Davis by ion-exchange chromatography with a Hitachi amino acid analyzer.

SAS output presented below shows no significant effects of treatment on tyrosine or phenylalanine concentrations in sieved *T. pratense* pollen.

'Dataset 43 tyr'

The GLM Procedure

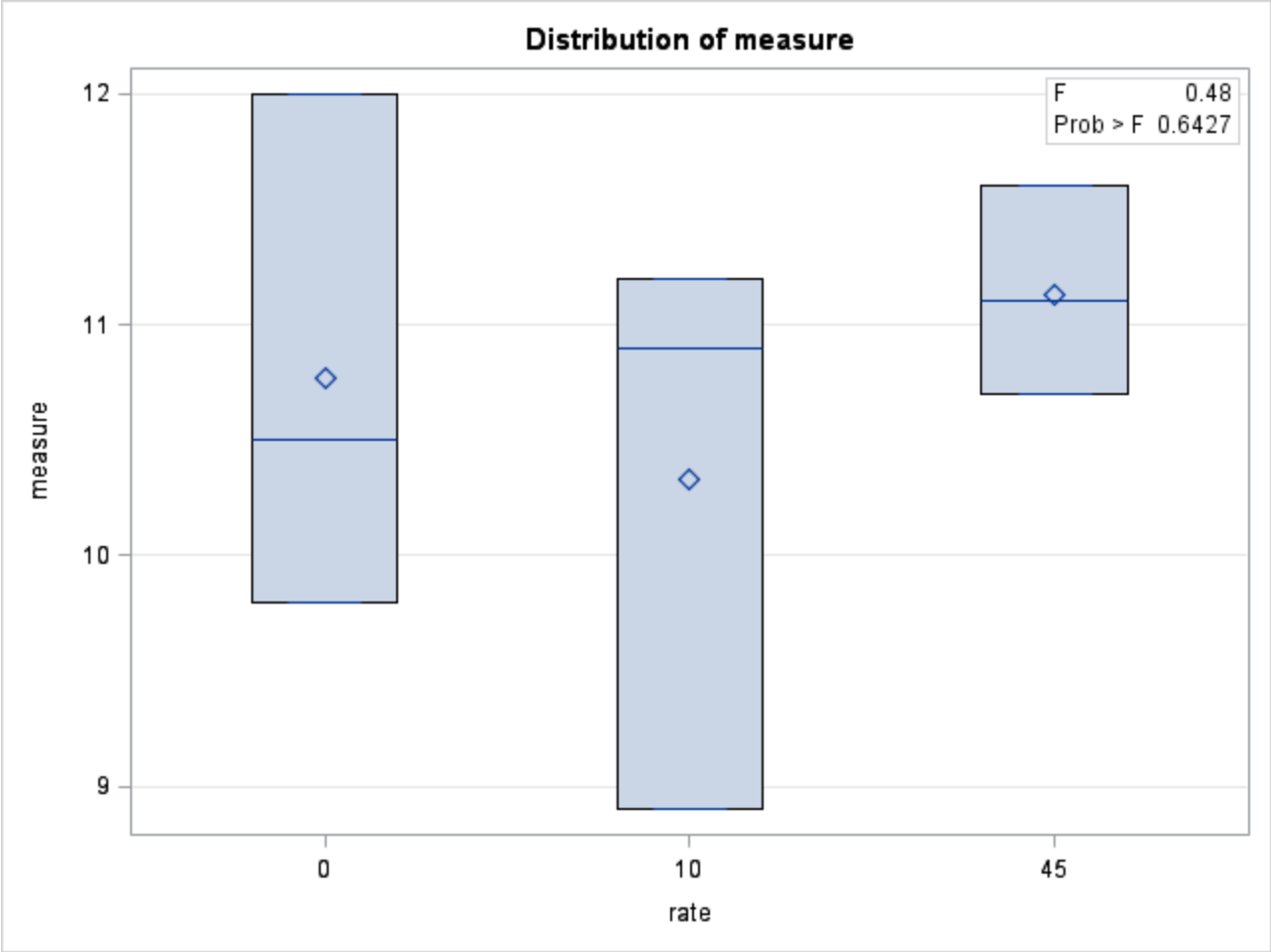
Dependent Variable: measure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.96222222	0.48111111	0.48	0.6427
Error	6	6.06000000	1.01000000		
Corrected Total	8	7.02222222			

R-Square	Coeff Var	Root MSE	measure Mean
0.137025	9.353555	1.004988	10.74444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rate	2	0.96222222	0.48111111	0.48	0.6427

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rate	2	0.96222222	0.48111111	0.48	0.6427



'Dataset 43 tyr'

The GLM Procedure

Levene's Test for Homogeneity of measure Variance ANOVA of Squared Deviations from Group Means					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	1.3614	0.6807	1.50	0.2961
Error	6	2.7209	0.4535		

The GLM Procedure

Brown and Forsythe's Test for Homogeneity of measure Variance
ANOVA of Absolute Deviations from Group Medians

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.4067	0.2033	0.34	0.7250
Error	6	3.5933	0.5989		

Welch's ANOVA for measure

Source	DF	F Value	Pr > F
rate	2.0000	0.52	0.6381
Error	3.2807		

'Dataset 43 tyr'

The GLM Procedure

Dunnett's t Tests for measure

Note: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	1.01
Critical Value of Dunnett's t	2.86275
Minimum Significant Difference	2.3491

Comparisons significant at the 0.05 level
are indicated by ***.

rate Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
45 - 0	0.3667	-1.9824	2.7158
10 - 0	-0.4333	-2.7824	1.9158

'Dataset 43 tyr'

**The UNIVARIATE Procedure
Variable: RESID**

Moments			
N	9	Sum Weights	9
Mean	0	Sum Observations	0
Std Deviation	0.87034476	Variance	0.7575
Skewness	-0.2773563	Kurtosis	-0.7610023
Uncorrected SS	6.06	Corrected SS	6.06
Coeff Variation	.	Std Error Mean	0.29011492

Basic Statistical Measures			
Location		Variability	
Mean	0.00000	Std Deviation	0.87034
Median	-0.03333	Variance	0.75750
Mode	.	Range	2.66667
		Interquartile Range	1.00000

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	0	Pr > t 	1.0000
Sign	M	-0.5	Pr >= M 	1.0000
Signed Rank	S	0.5	Pr >= S 	1.0000

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.976174	Pr < W	0.9418
Kolmogorov-Smirnov	D	0.148529	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.021332	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.148615	Pr > A-Sq	>0.2500

Quantiles (Definition 5)	
Quantile	Estimate
100% Max	1.2333333

'Dataset 43 phe'

The GLM Procedure

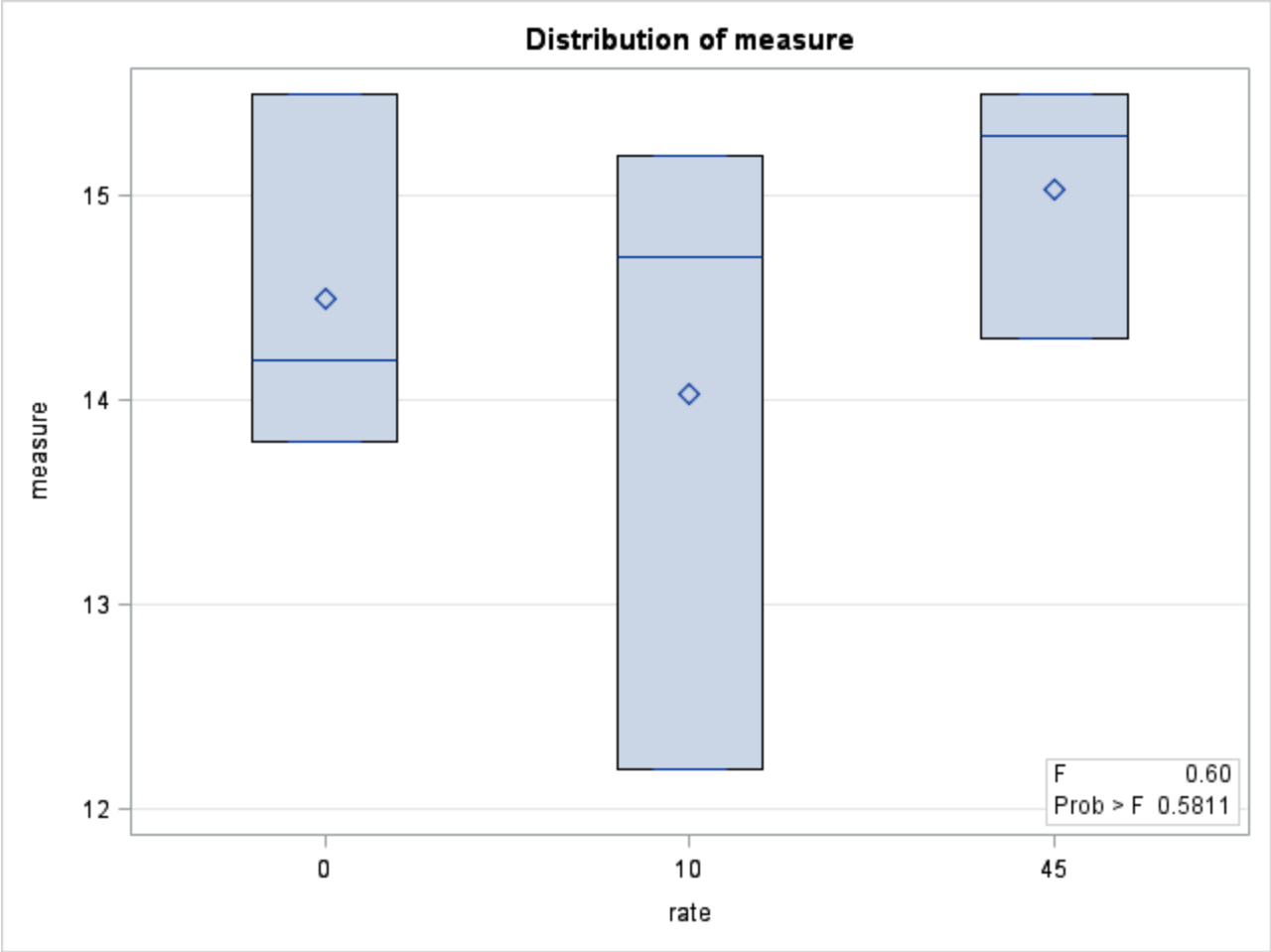
Dependent Variable: measure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.50222222	0.75111111	0.60	0.5811
Error	6	7.57333333	1.26222222		
Corrected Total	8	9.07555556			

R-Square	Coeff Var	Root MSE	measure Mean
0.165524	7.736327	1.123487	14.52222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rate	2	1.50222222	0.75111111	0.60	0.5811

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rate	2	1.50222222	0.75111111	0.60	0.5811



'Dataset 43 phe'

The GLM Procedure

Levene's Test for Homogeneity of measure Variance ANOVA of Squared Deviations from Group Means					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	3.5853	1.7926	2.16	0.1965
Error	6	4.9790	0.8298		

Brown and Forsythe's Test for Homogeneity of measure Variance ANOVA of Absolute Deviations from Group Medians					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.5756	0.2878	0.35	0.7188
Error	6	4.9467	0.8244		

The GLM Procedure

Dunnett's t Tests for measure

Note: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	1.262222
Critical Value of Dunnett's t	2.86275
Minimum Significant Difference	2.6261

Comparisons significant at the 0.05 level are indicated by ***.

rate Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
45 - 0	0.5333	-2.0927	3.1594
10 - 0	-0.4667	-3.0927	2.1594

'Dataset 43 phe'

The UNIVARIATE Procedure Variable: RESID

Moments			
N	9	Sum Weights	9
Mean	0	Sum Observations	0
Std Deviation	0.97296797	Variance	0.94666667
Skewness	-0.6842538	Kurtosis	-0.1068993
Uncorrected SS	7.57333333	Corrected SS	7.57333333
Coeff Variation	.	Std Error Mean	0.32432266

Basic Statistical Measures

Location		Variability	
Mean	0.000000	Std Deviation	0.97297
Median	0.266667	Variance	0.94667
Mode	.	Range	3.00000

Basic Statistical Measures			
Location		Variability	
		Interquartile Range	1.36667

Tests for Location: $\mu_0=0$				
Test	Statistic		p Value	
Student's t	t	0	Pr > t	1.0000
Sign	M	0.5	Pr >= M	1.0000
Signed Rank	S	0.5	Pr >= S	1.0000

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.942602	Pr < W	0.6095
Kolmogorov-Smirnov	D	0.163542	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.036998	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.253232	Pr > A-Sq	>0.2500

<i>R. hirta</i> pollen tyrosine (tyr) and phenlalanine (phen) concentrations from plants treated with glyphosate^a			
Replicate	Nominal treatment level (g a.i./ha)	Tyr (mg/g)	Phe (mg/g)
12	0	6.116614	9.337547
13	0	5.336137	8.116211
17	0	5.924339	9.351071
10	10	5.399648	8.588517
14	10	5.270607	8.19447
16	10	6.277418	9.555599
11	45	5.58544	8.820609
15	45	5.87101	9.288314
18	45	5.995548	9.442458

^a Pollen was collected weekly, dried (35°C), pooled and sieved, and frozen prior to analyses, which were conducted at UC Davis by ion-exchange chromatography with a Hitachi amino acid analyzer.

SAS output presented below shows no significant effects of treatment on tyrosine or phenylalanine concentrations in sieved *R. hirta* pollen.

'Dataset 71 tyr'	
------------------	--

The GLM Procedure

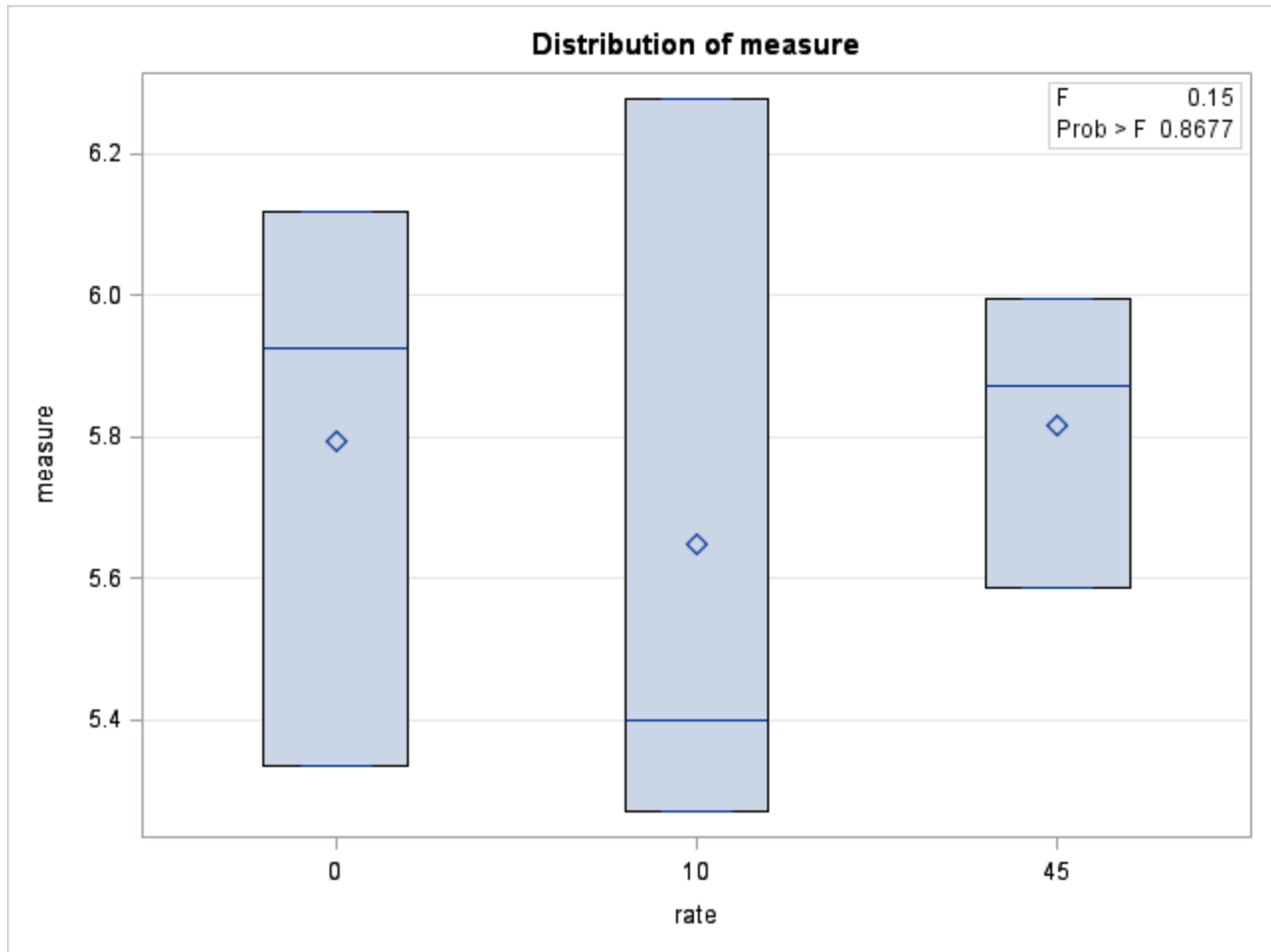
Dependent Variable: measure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.04937270	0.02468635	0.15	0.8677
Error	6	1.01938141	0.16989690		
Corrected Total	8	1.06875411			

R-Square	Coeff Var	Root MSE	measure Mean
0.046197	7.164739	0.412186	5.752973

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rate	2	0.04937270	0.02468635	0.15	0.8677

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rate	2	0.04937270	0.02468635	0.15	0.8677



'Dataset 71 tyr'

The GLM Procedure

Levene's Test for Homogeneity of measure Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.0437	0.0219	1.65	0.2690
Error	6	0.0796	0.0133		

Brown and Forsythe's Test for Homogeneity of measure Variance
ANOVA of Absolute Deviations from Group Medians

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.0605	0.0302	0.27	0.7716
Error	6	0.6701	0.1117		

Dunnett's t Tests for measure

Note: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.169897
Critical Value of Dunnett's t	2.86275
Minimum Significant Difference	0.9635

Comparisons significant at the 0.05 level are indicated by *.**

rate Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
45 - 0	0.0250	-0.9385	0.9884
10 - 0	-0.1431	-1.1066	0.8203

'Dataset 71 tyr'

The UNIVARIATE Procedure
Variable: RESID

Moments

N	9	Sum Weights	9
Mean	0	Sum Observations	0
Std Deviation	0.35696313	Variance	0.12742268
Skewness	0.39875976	Kurtosis	-0.6327308
Uncorrected SS	1.01938141	Corrected SS	1.01938141
Coeff Variation	.	Std Error Mean	0.11898771

Basic Statistical Measures

Location		Variability	
Mean	0.000000	Std Deviation	0.35696
Median	0.053678	Variance	0.12742
Mode	.	Range	1.08442
		Interquartile Range	0.42779

Tests for Location: Mu0=0

Test	Statistic	p Value
Student's t	t 0	Pr > t 1.0000
Sign	M 0.5	Pr >= M 1.0000
Signed Rank	S -1.5	Pr >= S 0.9102

Tests for Normality

Test	Statistic	p Value
Shapiro-Wilk	W 0.953071	Pr < W 0.7238
Kolmogorov-Smirnov	D 0.186477	Pr > D >0.1500
Cramer-von Mises	W-Sq 0.036315	Pr > W-Sq >0.2500
Anderson-Darling	A-Sq 0.233589	Pr > A-Sq >0.2500

'Dataset 71 phe'

The GLM Procedure

Dependent Variable: measure

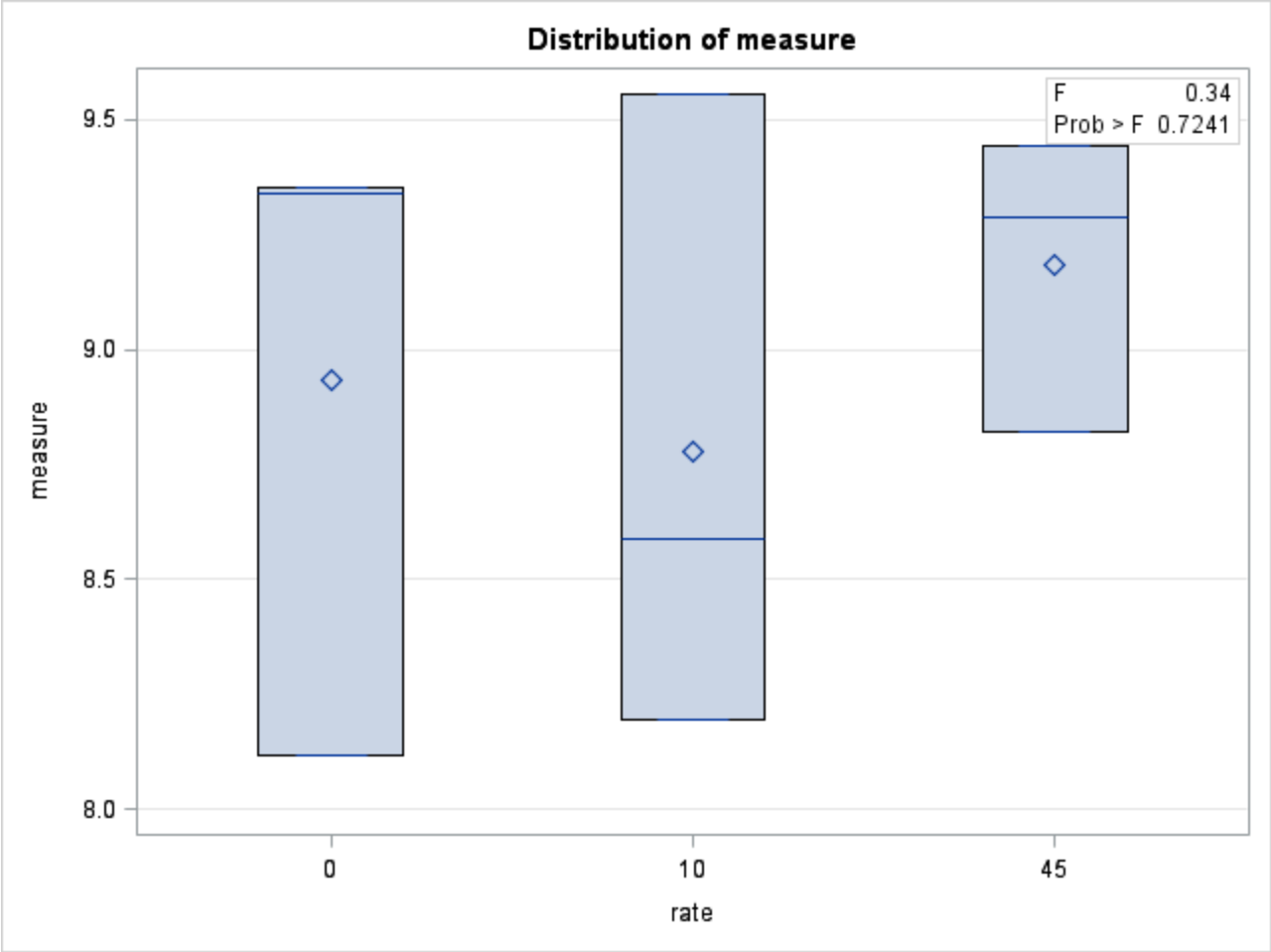
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.24951081	0.12475540	0.34	0.7241
Error	6	2.19637330	0.36606222		
Corrected Total	8	2.44588411			

R-Square **Coeff Var** **Root MSE** **measure Mean**

0.102013 6.747990 0.605031 8.966088

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rate	2	0.24951081	0.12475540	0.34	0.7241

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rate	2	0.24951081	0.12475540	0.34	0.7241



'Dataset 71 phe'

The GLM Procedure

**Levene's Test for Homogeneity of measure Variance
ANOVA of Squared Deviations from Group Means**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.1365	0.0683	1.22	0.3597
Error	6	0.3363	0.0560		

**Brown and Forsythe's Test for Homogeneity of measure Variance
ANOVA of Absolute Deviations from Group Medians**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
rate	2	0.1043	0.0521	0.20	0.8246
Error	6	1.5701	0.2617		

Dunnett's t Tests for measure

Note: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.366062
Critical Value of Dunnett's t	2.86275
Minimum Significant Difference	1.4142

**Comparisons significant at the 0.05 level
are indicated by ***.**

rate Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
45 - 0	0.2489	-1.1654	1.6631
10 - 0	-0.1554	-1.5696	1.2588

'Dataset 71 phe'

The UNIVARIATE Procedure
Variable: RESID

Moments

N	9	Sum Weights	9
Mean	0	Sum Observations	0
Std Deviation	0.52397201	Variance	0.27454666
Skewness	-0.2019556	Kurtosis	-0.9864388
Uncorrected SS	2.1963733	Corrected SS	2.1963733
Coeff Variation	.	Std Error Mean	0.17465734

Basic Statistical Measures

Location		Variability	
Mean	0.000000	Std Deviation	0.52397
Median	0.104520	Variance	0.27455
Mode	.	Range	1.59480
		Interquartile Range	0.76579

Tests for Location: Mu0=0

Test	Statistic	p Value
Student's t	t 0	Pr > t 1.0000
Sign	M 0.5	Pr >= M 1.0000
Signed Rank	S 0.5	Pr >= S 1.0000

Tests for Normality

Test	Statistic	p Value
Shapiro-Wilk	W 0.967839	Pr < W 0.8756
Kolmogorov-Smirnov	D 0.134611	Pr > D >0.1500
Cramer-von Mises	W-Sq 0.030519	Pr > W-Sq >0.2500
Anderson-Darling	A-Sq 0.190587	Pr > A-Sq >0.2500

Appendix C – *In vitro* Pollen Germination Medium Preparations

Directions followed:

- (1) Dissolve sucrose and polyethylene glycol using a stir bar with half the allocated water in a beaker.
- (2) Measure and added remaining ingredients into beaker. Water should be pipetted from the pre-measured water to wash ingredients into the flask.
- (3) Balance pH to 6.0 to 6.25 for *C. cyanus* or 5.8-6.0 for *T. pratense* using KOH, adding 5 μ L at a time, or HCl, adding 1 μ L at a time.

Recipe for <i>Centaurea cyanus</i> pollen germination medium (Developed by Krista Neilly)	
Ingredient	Amount in 150 mL of Water (mg)
Sucrose	7500
Boric acid	15
Potassium nitrate	20
Magnesium sulphate	15.5
Iron sulphate	10
Polyethylene glycol	30000
Calcium nitrate	40
Yeast extract	10

Recipe for <i>Trifolium pratense</i> pollen germination medium	
Ingredient	Amount in 100 mL of Water (mg)
Sucrose	20000
Boric acid	5
Polyethylene glycol	15
Calcium nitrate	200
Yeast extract	20

C. cyanus medium: Started at pH 5.28, added 11 μ L of KOH, final pH was 6.19.

T. pratense medium: Started at pH 5.66, added 133 μ L of KOH, and 2 μ L of HCl, final pH was 5.83.

Appendix D – Example SAS Code

Example Code for Binary Data

```
/*Dataset 93*/
data dataset93ca;
input group rate affected $ count;
cards;
1      0      no      48
1      0      yes     0
2      37     no      48
2      37     yes     0
3      52.8   no      47
3      52.8   yes     1
4      74.2   no      41
4      74.2   yes     2
5      113.8      no      38
5      113.8      yes     5
6      162.1      no      30
6      162.1      yes     10
;

ods graphics on;
PROC FREQ data=dataset93ca;
Title 'Dataset 93';
Title 'Cochran-Armitage Test for Trend';
tables affected*group/trend measures cl plots=mosaicplot;
exact trend/maxtime=60;
weight count/zeros;
run;
ods graphics off;
quit;

/*Dataset 93*/
Data dataset93probit;
input rep rate n affected;
cards;
130  0      12      0
134  0      12      0
145  0      12      0
151  0      12      0
135  37     12      0
136  37     12      0
143  37     12      0
144  37     12      0
```

```

133  52.8 12  0
137  52.8 12  0
142  52.8 12  1
148  52.8 12  0
131  74.2 12  1
132  74.2 12  0
140  74.2 12  0
150  74.2 7   1
139  113.8      11
141  113.8      12
149  113.8      11
153  113.8      9
138  162.1     12
146  162.1     12
147  162.1      6
152  162.1     10
;

```

```

PROC PROBIT log10 optc data=dataset93probit;
Title 'Dataset 93';
title 'NORMAL/PROBIT';
MODEL affected/n=rate / d=normal scale=pearson INVERSECL
ALPHA=0.05 LACKFIT;
run;

```

```

PROC PROBIT log10 optc data=dataset93probit;
Title 'Dataset 93';
title 'LOGISTIC';
MODEL affected/n=rate / d=logistic scale=pearson INVERSECL
ALPHA=0.05 LACKFIT;
run;

```

```

PROC PROBIT log10 optc data=dataset93probit;
Title 'Dataset 93';
title 'GOMPERTZ';
MODEL affected/n=rate / d=gompertz scale=pearson INVERSECL
ALPHA=0.05 LACKFIT;
run;
quit;

```

Example Code for Continuous Data

```
/*Dataset #52 surviving plants*/

Data dataset52surv;
INPUT RATE Y1 Y2 Y3 Y4;
IF RATE=0 THEN LOG_RATE=.;
ELSE LOG_RATE=LOG10(RATE);
CARDS;
0      3.888      4.994      5.308      4.434
37     4.090      4.893      4.331      4.501
52.8   3.385      3.670      3.154      4.203
74.2   3.429      2.988      2.824      2.270
113.8  2.037      2.935      1.447      3.924
162.1  2.662      2.138      1.422      2.190
;

Title 'Dataset #52 surviving plants';
PROC PRINT DATA= dataset52surv;
VAR RATE LOG_RATE Y1-Y4;
DATA dataset;
SET dataset52surv END=LAST;
ARRAY Y[4] Y1-Y4;
DO I=1 TO 4;
MEASURE=Y[I];
OUTPUT;
KEEP RATE LOG_RATE MEASURE;
END;

IF LAST THEN DO;
MEASURE=.; LOG_RATE=.;
DO RATE=0 TO 165 BY 1;
OUTPUT;END;
END;

DATA logdataset;
SET dataset52surv END=LAST;
ARRAY Y[4] Y1-Y4;
DO I=1 TO 4;
MEASURE=Y[I];
OUTPUT;
KEEP RATE LOG_RATE MEASURE;
END;
```

```

IF LAST THEN DO;
MEASURE=.; RATE=.;
DO LOG_RATE=0.05 TO 2.5 BY 0.05;
OUTPUT;END;
END;

/*NLMIXED LINEAR*/

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LINEAR Y=(((-C0*0.1)/EC10)*RATE)+C0';
PARMS EC10=30 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC10>0, THETA>=0, VAR>0;
Y=(((-C0*0.1)/ EC10)* RATE)+C0;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
RUN;
QUIT;

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LINEAR Y=(((-C0*0.25)/EC25)*RATE)+C0';
PARMS EC25=50 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC25>0, THETA>=0, VAR>0;
Y=(((-C0*0.25)/ EC25)* RATE)+C0;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
RUN;
QUIT;

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LINEAR Y=(((-C0*0.5)/EC50)*RATE)+C0';
PARMS EC50=100 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC50>0, THETA>=0, VAR>0;
Y=(((-C0*0.5)/EC50)* RATE)+C0;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
PREDICT Y OUT=LINEAR;
RUN;
QUIT;

PROC PRINT DATA=LINEAR;
RUN;
QUIT;

DATA LINEARRESID;
SET LINEAR;
R=MEASURE-Pred;
IF MEASURE>=0;
run;

```

```

PROC UNIVARIATE NORMAL DATA=LINEARRESID;
VAR R;
CLASS RATE;
BY RATE;
RUN;

```

```

PROC UNIVARIATE NORMAL DATA=LINEARRESID;
VAR R;
HISTOGRAM/NORMAL;
RUN;

```

```

PROC PLOT DATA=LINEARRESID;
PLOT R*RATE='*';
RUN;
QUIT;

```

```

PROC GLM DATA=LINEARRESID;
CLASS RATE;
MODEL R=RATE;
MEANS RATE/HOVTEST=BF;
MEANS RATE/HOVTEST=LEVENE;
RUN;
QUIT;

```

```

/*NLMIXED EXPONENTIAL*/

```

```

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: EXPONENTIAL Y=C0*exp(LOG(0.9)*(RATE/EC10))';
PARMS EC10=30 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS EC10>0, C0>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0*exp(LOG(0.9)*(RATE/EC10));
end;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
RUN;
QUIT;

```

```

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: EXPONENTIAL Y=C0*exp(LOG(0.75)*(RATE/EC25))';
PARMS EC25=50 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS EC25>0, C0>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;

```

```

else do;
Y=C0*exp(LOG(0.75)*(RATE/EC25));
end;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
Run;
Quit;

PROC NL MIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: EXPONENTIAL Y=C0*exp(LOG(0.5)*(RATE/EC50))';
PARMS EC50=100 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS EC50>0, C0>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0*exp(LOG(0.5)*(RATE/EC50));
END;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
PREDICT Y OUT=EXPONENTIAL;
Run;
Quit;

PROC PRINT DATA=EXPONENTIAL;
RUN;
QUIT;

DATA EXPONENTIALRESID;
SET EXPONENTIAL;
R=MEASURE-Pred;
IF MEASURE>=0;
run;

PROC UNIVARIATE NORMAL DATA=EXPONENTIALRESID;
VAR R;
CLASS RATE;
BY RATE;
RUN;

PROC UNIVARIATE NORMAL DATA=EXPONENTIALRESID;
VAR R;
HISTOGRAM/NORMAL;
RUN;

PROC PLOT DATA=EXPONENTIALRESID;
PLOT R*RATE='*';
RUN;
QUIT;

```

```

PROC GLM DATA=EXPONENTIALRESID;
CLASS RATE;
MODEL R=RATE;
MEANS RATE/HOVTEST=BF;
MEANS RATE/HOVTEST=LEVENE;
RUN;
QUIT;

```

```

/*NLMIXED LOG LOGISTIC*/

```

```

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LOGISTIC  $Y=C0/(1+(0.1/0.9)*(RATE/EC10)**SIGMA)$ ';
PARMS EC10=30 C0=4.5 SIGMA=2 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC10>0, SIGMA>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0/(1+(0.1/0.9)*(RATE/EC10)**SIGMA);
end;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);

RUN;
QUIT;

```

```

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LOGISTIC  $Y=C0/(1+(0.25/0.75)*(RATE/EC25)**SIGMA)$ ';
PARMS EC25=50 C0=4.5 SIGMA=2 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC25>0, SIGMA>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0/(1+(0.25/0.75)*(RATE/EC25)**SIGMA);
end;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);

RUN;
QUIT;

```

```

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: LOGISTIC  $Y=C0/(1+(0.5/0.5)*(RATE/EC50)**SIGMA)$ ';
PARMS EC50=100 C0=4.5 SIGMA=2 THETA=0.5 VAR=1.5;
BOUNDS C0>0, EC50>0, SIGMA>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;

```

```

else do;
Y=C0/(1+(RATE/EC50)**SIGMA);
END;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
PREDICT Y OUT=LOGISTIC;
RUN;
QUIT;

PROC PRINT DATA=LOGISTIC;
RUN;
QUIT;

DATA LOGISTICRESID;
SET LOGISTIC;
R=MEASURE-Pred;
IF MEASURE>=0;
run;

PROC UNIVARIATE NORMAL DATA=LOGISTICRESID;
VAR R;
CLASS RATE;
BY RATE;
RUN;

PROC UNIVARIATE NORMAL DATA=LOGISTICRESID;
VAR R;
HISTOGRAM/NORMAL;
RUN;

PROC PLOT DATA=LOGISTICRESID;
PLOT R*RATE='*';
RUN;
QUIT;

PROC GLM DATA=LOGISTICRESID;
CLASS RATE;
MODEL R=RATE;
MEANS RATE/HOVTEST=BF;
MEANS RATE/HOVTEST=LEVENE;
RUN;
QUIT;

/*NLMIXED LOG GOMPERTZ*/

PROC NLMIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: GOMPERTZ Y=C0*EXP(LOG(0.9)*(RATE/EC10)**SIGMA)';

```

```

PARMS EC10=30 SIGMA=2 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, EC10>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0*EXP(LOG(0.9)*(RATE/EC10)**SIGMA);
END;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
Run;
Quit;

PROC NL MIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: GOMPERTZ Y=C0*EXP(LOG(0.75)*(RATE/EC25)**SIGMA)';
PARMS EC25=50 SIGMA=2 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, EC25>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0*EXP(LOG(0.75)*(RATE/EC25)**SIGMA);
END;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
Run;
Quit;

PROC NL MIXED DATA=dataset MAXITER=500;
TITLE 'MODEL: GOMPERTZ Y=C0*EXP(LOG(0.50)*(RATE/EC50)**SIGMA)';
PARMS EC50=100 SIGMA=2 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, EC50>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
end;
else do;
Y=C0*EXP(LOG(0.50)*(RATE/EC50)**SIGMA);
END;
MODEL MEASURE~NORMAL(Y, (Y**THETA)*VAR);
PREDICT Y OUT=GOMPERTZ;
RUN;
QUIT;

PROC PRINT DATA=GOMPERTZ;
RUN;
QUIT;

DATA GOMPERTZRESID;
SET GOMPERTZ;

```

```

R=MEASURE-Pred;
IF MEASURE>=0;
run;

PROC UNIVARIATE NORMAL DATA=GOMPertzRESID;
VAR R;
CLASS RATE;
BY RATE;
RUN;

PROC UNIVARIATE NORMAL DATA=GOMPertzRESID;
VAR R;
HISTOGRAM/NORMAL;
RUN;

PROC PLOT DATA=GOMPertzRESID;
PLOT R*RATE='*';
RUN;
QUIT;

PROC GLM DATA=GOMPertzRESID;
CLASS RATE;
MODEL R=RATE;
MEANS RATE/HOVTEST=BF;
MEANS RATE/HOVTEST=LEVENE;
RUN;
QUIT;

/*NLMIXED LOGNORMAL*/

PROC NLMIXED DATA=logdataset MAXITER=500;
TITLE 'MODEL: LOGNORMAL Y=C0*PROBNORM((LOG_EC10 -
LOG_RATE)/sigma + 1.2816)';
PARMS LOG_EC10=0.3 SIGMA=0.5 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, LOG_EC10>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
DER.LOG_EC10=0; DER.SIGMA=0;
DER.C0=1;
END;
IF RATE NE 0 THEN DO;
Z=(LOG_EC10-LOG_RATE)/SIGMA;
EX=Z+1.2816;
PX=PROBNORM(EX);
Y=C0*PX;
EEX=C0*0.398942/SIGMA*EXP(-EX*EX/2);
DER.LOG_EC10=EEX;

```

```

DER.SIGMA=-Z*EEX;
DER.C0=PX;
END;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
RUN;
QUIT;

PROC NL MIXED DATA=logdataset MAXITER=500;
TITLE 'MODEL: LOGNORMAL Y=C0*PROBNORM((LOG_EC25 -
LOG_RATE)/sigma + 0.6745)';
PARMS LOG_EC25=0.6 SIGMA=0.5 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, LOG_EC25>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
DER.LOG_EC25=0; DER.SIGMA=0;
DER.C0=1;
END;
IF RATE NE 0 THEN DO;
Z=(LOG_EC25-LOG_RATE)/SIGMA;
EX=Z+0.6745;
PX=PROBNORM(EX);
Y=C0*PX;
EEX=C0*0.398942/SIGMA*EXP(-EX*EX/2);
DER.LOG_EC25=EEX;
DER.SIGMA=-Z*EEX;
DER.C0=PX;
END;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
RUN;
QUIT;

PROC NL MIXED DATA=logdataset MAXITER=500;
TITLE 'MODEL: LOGNORMAL Y=C0*PROBNORM((LOG_EC50 -
LOG_RATE)/sigma + 0.0)';
PARMS LOG_EC50=0.9 SIGMA=0.5 C0=4.5 THETA=0.5 VAR=1.5;
BOUNDS SIGMA>0, C0>0, LOG_EC50>0, THETA>=0, VAR>0;
IF RATE=0 THEN DO;
Y=C0;
DER.LOG_EC50=0; DER.SIGMA=0;
DER.C0=1;
END;
IF RATE NE 0 THEN DO;
Z=(LOG_EC50-LOG_RATE)/SIGMA;
EX=Z+0.0;
PX=PROBNORM(EX);
Y=C0*PX;
EEX=C0*0.398942/SIGMA*EXP(-EX*EX/2);

```

```

DER.LOG_EC50=EEX;
DER.SIGMA=-Z*EEX;
DER.C0=PX;
END;
MODEL MEASURE~NORMAL(Y,(Y**THETA)*VAR);
PREDICT Y OUT=LOGNORMAL;
Run;
Quit;

PROC PRINT DATA=LOGNORMAL;
RUN;
QUIT;

DATA LOGNORMALRESID;
SET LOGNORMAL;
R=MEASURE-Pred;
IF MEASURE>=0;
run;

PROC UNIVARIATE NORMAL DATA=LOGNORMALRESID;
VAR R;
CLASS RATE;
BY RATE;
RUN;

PROC UNIVARIATE NORMAL DATA=LOGNORMALRESID;
VAR R;
HISTOGRAM/NORMAL;
RUN;

PROC PLOT DATA=LOGNORMALRESID;
PLOT R*RATE='*';
RUN;
QUIT;

PROC GLM DATA=LOGNORMALRESID;
CLASS RATE;
MODEL R=RATE;
MEANS RATE/HOVTEST=BF;
MEANS RATE/HOVTEST=LEVENE;
RUN;
QUIT;

```

Appendix E – Analytical Data

Sample ID	Client ID	Batch	Analyzed Date	Glyphosate (mg/Filter)	Measured (g /ha)	Nominal (g a.e./ha)	Percent of Nominal
W171376-01-A	CC 1A	BC00439	2017-09-07	0.037	38.7	40	96.8
W171377-01-A	CC 1B	BC00439	2017-09-07	0.037	39.1	40	97.9
W171378-01-A	CC 1C	BC00439	2017-09-07	0.038	40.2	40	100.5
W171379-01-A	CC 2A	BC00439	2017-09-07	0.046	48.6	47.5	102.3
W171380-01-A	CC 2B	BC00439	2017-09-07	0.043	45.0	47.5	94.8
W171381-01-A	CC 2C	BC00439	2017-09-07	0.045	47.6	47.5	100.1
W171382-01-A	CC 3A	BC00439	2017-09-07	0.052	54.3	55	98.7
W171383-01-A	CC 3B	BC00439	2017-09-07	0.053	55.4	55	100.6
W171384-01-A	CC 3C	BC00439	2017-09-07	0.050	52.8	55	96.0
W171385-01-A	CC 4A	BC00439	2017-09-07	0.063	66.1	62.5	105.7
W171386-01-A	CC 4B	BC00440	2017-09-08	0.064	66.9	62.5	107.1
W171387-01-A	CC 4C	BC00440	2017-09-08	0.061	63.8	62.5	102.0
W171388-01-A	CC 5A	BC00440	2017-09-08	0.077	80.8	70	115.4
W171389-01-A	CC 5B	BC00440	2017-09-08	0.075	78.9	70	112.7
W171390-01-A	CC 5C	BC00440	2017-09-08	0.061	64.4	70	92.0
W171391-01-A	TP1 A	BC00440	2017-09-08	0.012	12.8	10	128.4
W171392-01-A	TP1 B	BC00440	2017-09-08	0.011	11.6	10	116.0
W171393-01-A	TP1 C	BC00440	2017-09-08	0.012	13.1	10	130.9
W171394-01-A	TP2 A	BC00440	2017-09-08	0.022	22.7	20	113.6
W171395-01-A	TP2 B	BC00440	2017-09-08	0.019	20.4	20	102.2
W171396-01-A	TP2 C	BC00441	2017-09-11	0.026	26.9	20	134.7
W171397-01-A	TP3 A	BC00441	2017-09-11	0.033	35.1	30	117.2
W171398-01-A	TP3 B	BC00441	2017-09-11	0.037	39.4	30	131.2
W171399-01-A	TP3 C	BC00441	2017-09-11	0.031	32.6	30	108.7
W171400-01-A	TP4 A	BC00441	2017-09-11	0.051	53.9	40	134.7
W171401-01-A	TP4 B	BC00441	2017-09-11	0.047	49.5	40	123.6
W171402-01-A	TP4 C	BC00441	2017-09-11	0.046	48.8	40	122.1
W171403-01-A	TP5 A	BC00441	2017-09-11	0.055	58.1	50	116.2
W171404-01-A	TP5 B	BC00441	2017-09-11	0.056	59.3	50	118.7
W171405-01-A	TP5 C	BC00441	2017-09-11	0.054	56.8	50	113.6
W171406-01-A	GLYD1-1	BC00442	2017-09-12	0.031	32.2	30	107.3
W171407-01-A	GLYD1-2	BC00442	2017-09-12	0.029	30.5	30	101.7
W171408-01-A	GLYD1-3	BC00442	2017-09-12	0.030	31.4	30	104.5
W171409-01-A	GLYD2-1	BC00442	2017-09-12	0.045	47.8	45	106.2
W171410-01-A	GLYD2-2	BC00442	2017-09-12	0.043	45.2	45	100.5
W171411-01-A	GLYD2-3	BC00442	2017-09-12	0.047	49.2	45	109.4
W171412-01-A	GLYD3-1	BC00442	2017-09-12	0.075	78.9	67.5	116.9
W171413-01-A	GLYD3-2	BC00442	2017-09-12	0.073	77.2	67.5	114.4
W171414-01-A	GLYD3-3	BC00442	2017-09-12	0.074	77.7	67.5	115.0
W171415-01-A	GLYD4-1	BC00442	2017-09-12	0.117	122.9	101	121.7
W171416-01-A	GLYD4-2	BC00444	2017-09-13	0.105	110.7	101	109.6
W171417-01-A	GLYD4-3	BC00444	2017-09-13	0.112	118.1	101	116.9
W171418-01-A	GLYD5-1	BC00444	2017-09-13	0.172	180.6	152	118.8
W171419-01-A	GLYD5-2	BC00444	2017-09-13	0.151	158.9	152	104.5
W171420-01-A	GLYD5-3	BC00444	2017-09-13	0.155	163.3	152	107.4
W171421-01-A	GLY-TP1-1	BC00444	2017-09-13	0.032	33.5	30	111.5
W171422-01-A	GLY-TP1-2	BC00444	2017-09-13	0.032	33.5	30	111.5
W171423-01-A	GLY-TP1-3	BC00444	2017-09-13	0.028	29.3	30	97.5

Sample ID	Client ID	Batch	Analyzed Date	Glyphosate (mg/Filter)	Measured (g /ha)	Nominal (g a.e./ha)	Percent of Nominal
W171424-01-A	GLY-TP2-1	BC00444	2017-09-13	0.048	50.5	45	112.2
W171425-01-A	GLY-TP2-2	BC00444	2017-09-13	0.052	54.9	45	122.1
W171426-01-A	GLY-TP2-3	BC00447	2017-09-14	0.047	49.5	45	109.9
W171427-01-A	GLY-TP3-1	BC00447	2017-09-14	0.073	77.0	67.5	114.1
W171428-01-A	GLY-TP3-2	BC00447	2017-09-14	0.070	73.2	67.5	108.5
W171429-01-A	GLY-TP3-3	BC00447	2017-09-14	0.068	71.8	67.5	106.3
W171430-01-A	GLY-TP4-1	BC00447	2017-09-14	0.108	113.4	101	112.3
W171431-01-A	GLY-TP4-2	BC00447	2017-09-14	0.098	103.3	101	102.3
W171432-01-A	GLY-TP4-3	BC00447	2017-09-14	0.103	108.2	101	107.1
W171433-01-A	GLY-TP5-1	BC00447	2017-09-14	0.148	155.3	152	102.2
W171434-01-A	GLY-TP5-2	BC00447	2017-09-14	0.156	164.2	152	108.0
W171435-01-A	GLY-TP5-3	BC00447	2017-09-14	0.162	170.7	152	112.3
W171436-01-A	GLY-RH1-1	BC00448	2017-09-14	0.040	41.7	30	138.9
W171437-01-A	GLY-RH1-2	BC00448	2017-09-14	0.033	34.3	30	114.3
W171438-01-A	GLY-RH1-3	BC00448	2017-09-15	0.033	35.1	30	117.2
W171439-01-A	GLY-RH2-1	BC00448	2017-09-15	0.050	52.2	45	116.0
W171440-01-A	GLY-RH2-2	BC00448	2017-09-14	0.051	53.2	45	118.3
W171441-01-A	GLY-RH2-3	BC00448	2017-09-14	0.050	53.0	45	117.9
W171442-01-A	GLY-RH3-1	BC00448	2017-09-14	0.065	68.2	67.5	101.0
W171443-01-A	GLY-RH3-2	BC00448	2017-09-14	0.071	75.1	67.5	111.3
W171444-01-A	GLY-RH3-3	BC00448	2017-09-14	0.075	79.1	67.5	117.2
W171445-01-A	GLY-RH4-1	BC00448	2017-09-14	0.110	116.0	101	114.8
W171446-01-A	GLY-RH4-2	BC00449	2017-09-15	0.100	105.7	101	104.6
W171447-01-A	GLY-RH4-3	BC00449	2017-09-15	0.114	119.8	101	118.6
W171448-01-A	GLY-RH5-1	BC00449	2017-09-15	0.160	167.9	152	110.5
W171449-01-A	GLY-RH5-2	BC00449	2017-09-15	0.155	163.5	152	107.6
W171450-01-A	GLY-RH5-3	BC00449	2017-09-15	0.147	154.7	152	101.8

Appendix F– Environmental Data

Table F-1. Environmental Data from experiments investigating phenology (PHEN) and inflorescence characteristics (INFLO), means with standard deviations in parantheses						
Species	Experiment	Minimum Temperature (°C)	Maximum Temperature (°C)	Minimum Relative Humidity (%)	Maximum Relative Humidity (%)	PAR (μmol photons m⁻²s⁻¹)
<i>Rudbeckia hirta</i>	PHEN	20.8 (2.5)	31.1 (6.2)	25.5 (8.1)	59.1 (14.9)	272 (137)
<i>Centaurea cyanus</i>	PHEN	20.7 (2.5)	30.7 (5.5)	25.5 (7.6)	60.4 (13.8)	293 (187)
<i>Trifolium pratense</i>	PHEN	15.7 (2.1)	29.4 (6.8)	27.5 (8.2)	57.7 (9.0)	451 (331)
<i>Centaurea cyanus</i>	INFLO	18.2 (3.3)	40.0 (6.3)	37.0 (14.7)	81.6 (6.5)	475 (289)
<i>Trifolium pratense</i>	INFLO	16.9 (3.0)	41.8 (6.4)	28.5 (12.1)	81.8 (10.3)	538 (393)

Appendix G – Select Statistical Output

Table G-1. Statistical analyses performed on plant toxicity datasets

Species	Experiment	Measure of Effect	Dataset #	Experimental Units	Hypothesis Test	Rate-response Model
<i>Rudbeckia hirta</i>	PHEN	Mortality	50	24	Cochran-Armitage	No model selected.
<i>Rudbeckia hirta</i>	PHEN	Average above ground biomass per experimental plant	52	24	Not tested, distinct rate-response	Nonlinear (NLMIXED), Exponential
<i>Rudbeckia hirta</i>	PHEN	Average above ground biomass per surviving plant	52	24	Not tested, distinct rate-response	Nonlinear (NLMIXED), Exponential
<i>Rudbeckia hirta</i>	PHEN	Aborted buds/total buds	60	24	Cochran-Armitage	Non-parametric fit (GAM), spline interpolation
<i>Rudbeckia hirta</i>	PHEN	Average bloom-weeks per experimental plant	66	24	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Rudbeckia hirta</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	66	24	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Rudbeckia hirta</i>	PHEN	Average bloom duration	63	21	Not tested, distinct rate-response	Nonlinear (NLMIXED), Linear
<i>Rudbeckia hirta</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant	58	24	ANOVA F-test	Not modeled.
<i>Rudbeckia hirta</i>	PHEN	Average number of bloomed inflorescences per experimental plant	58	24	Not tested, distinct rate-response	Nonlinear (NLMIXED), Linear
<i>Rudbeckia hirta</i>	PHEN	Percent of surviving plants producing inflorescences	93	24	Cochran-Armitage	Binomial (PROBIT), Normal/probit
<i>Rudbeckia hirta</i>	PHEN	Average time to bloom of blooming plants	55	24	Not tested, distinct rate-response	Generalized linear (GENMOD), Polynomial
<i>Rudbeckia hirta</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	69	24	Cochran-Armitage	Non-parametric fit (GAM), spline interpolation

Species	Experiment	Measure of Effect	Dataset #	Experimental Units	Hypothesis Test	Rate-response Model
<i>Centaurea cyanus</i>	PHEN	Mortality	74	36	Not tested, distinct rate-response	Binomial (PROBIT), Log Gompertz
<i>Centaurea cyanus</i>	PHEN	Average above ground biomass per experimental plant	76	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Centaurea cyanus</i>	PHEN	Average above ground biomass per surviving plant	76	32	Not tested, distinct rate-response	Nonlinear (NLMIXED), Linear
<i>Centaurea cyanus</i>	PHEN	Percent of surviving plants producing inflorescences	94	32	Fisher exact	Not modeled.
<i>Centaurea cyanus</i>	PHEN	Aborted buds/total buds	82	36	Not tested, distinct rate-response	Non-parametric fit (GAM), spline interpolation
<i>Centaurea cyanus</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	85	32	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Centaurea cyanus</i>	PHEN	Average bloom-weeks per experimental plants	85	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plants at 5 weeks	80	30	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plants at 8 weeks	80	32	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per experimental plant at 5 weeks	80	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Logistic
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per experimental plant at 8 weeks	80	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Centaurea cyanus</i>	PHEN	Average Time to Bloom of Blooming plants	78	31	Not tested, distinct rate-response	Generalized linear (GENMOD), polynomial

Species	Experiment	Measure of Effect	Dataset #	Experimental Units	Hypothesis Test	Rate-response Model
<i>Centaurea cyanus</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	87	31	Cochran-Armitage	Non-parametric fit (GAM), spline interpolation
<i>Centaurea cyanus</i>	INFLO	Mortality	75	54	Not tested, distinct rate-response	Binomial (PROBIT), Log Gompertz
<i>Centaurea cyanus</i>	INFLO	Average above ground biomass per experimental plant	77	54	Not tested, distinct rate-response	Nonlinear (NLMIXED), Lognormal
<i>Centaurea cyanus</i>	INFLO	Average above ground biomass per surviving plant	77	54	Not tested, distinct rate-response	Non-parametric fit (GAM), spline interpolation
<i>Centaurea cyanus</i>	INFLO	Average number of bloomed inflorescences per experimental plant	81	54	Not tested, distinct rate-response	Nonlinear (NLMIXED), Exponential
<i>Centaurea cyanus</i>	INFLO	Average number of bloomed inflorescences per surviving and reproductive plant	81	53	Not tested, distinct rate-response	Nonlinear (NLMIXED), Exponential
<i>Centaurea cyanus</i>	INFLO	Average inflorescence dry weight	89	44	Williams	Non-parametric fit (GAM), spline interpolation
<i>Centaurea cyanus</i>	INFLO	Average number of reproductive florets per inflorescence	90	46	Jonckheere-Terpestra	Non-parametric fit (GAM), spline interpolation
<i>Centaurea cyanus</i>	INFLO	Pollen count	91	38	Jonckheere-Terpestra	Not modeled.
<i>Centaurea cyanus</i>	INFLO	Pollen germination	92	18	Cochran-Armitage	Non-parametric fit (GAM), spline interpolation
<i>Trifolium pratense</i>	PHEN	Mortality	13	36	Cochran-Armitage	Not modeled.
<i>Trifolium pratense</i>	PHEN	Average above ground biomass per experimental plant	15	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Logistic
<i>Trifolium pratense</i>	PHEN	Average above ground biomass per surviving plant	15	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Logistic
<i>Trifolium pratense</i>	PHEN	Average bloom-weeks per experimental plants	33	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz

Species	Experiment	Measure of Effect	Dataset #	Experimental Units	Hypothesis Test	Rate-response Model
<i>Trifolium pratense</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	33	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per experimental plant 5 weeks	23	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per experimental plant 8 weeks	23	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), lognormal
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant 5 weeks	23	34	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant 8 weeks	23	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Gompertz
<i>Trifolium pratense</i>	PHEN	Aborted buds/total buds	26	36	Not tested, distinct rate-response	Non-parametric fit (GAM), spline interpolation
<i>Trifolium pratense</i>	PHEN	Average bloom duration	30	13	Not tested, distinct rate-response	Nonlinear (NLMIXED), Linear
<i>Trifolium pratense</i>	PHEN	Average Time to Bloom of Blooming plants	19	28	Wilcoxon exact	Not modeled. No distinct trend.
<i>Trifolium pratense</i>	PHEN	Percent of surviving plants producing inflorescences	95	36	Cochran-Armitage	Binomial (PROBIT), Log logistic
<i>Trifolium pratense</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	37	28	Fisher exact	Not modeled. No distinct trend.
<i>Trifolium pratense</i>	INFLO	Mortality	100	36	No mortality	
<i>Trifolium pratense</i>	INFLO	Average above ground biomass per experimental plant	16	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Exponential
<i>Trifolium pratense</i>	INFLO	Average number of bloomed inflorescences per experimental plant	24	36	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Logistic

Species	Experiment	Measure of Effect	Dataset #	Experimental Units	Hypothesis Test	Rate-response Model
<i>Trifolium pratense</i>	INFLO	Average number of bloomed inflorescences per surviving and reproductive plant	24	33	Not tested, distinct rate-response	Nonlinear (NLMIXED), Log Logistic
<i>Trifolium pratense</i>	INFLO	Average inflorescence dry weight	40	14	ANOVA F-Test	Not modeled.
<i>Trifolium pratense</i>	INFLO	Average number of reproductive florets per inflorescence	42	16	Not tested, distinct rate-response	Nonlinear (NLMIXED), Linear
<i>Trifolium pratense</i>	INFLO	Pollen count	44	24	Jonckheere-Terpstra	Not modeled.
<i>Trifolium pratense</i>	INFLO	Pollen germination	45	24	Cochran-Armitage	Not modeled. No significant trend.

Table G-2. Cochran-Armitage step-down trend test results for binary datasets

Species	Experiment	Binary Measure of Effects	Dataset #	One-sided p-value ^a					Comment
				Initial	Step 1	Step 2	Step 3	Step 4	
<i>Rudbeckia hirta</i>	PHEN	Mortality	50	<.0001	0.0005	0.0254	No mortality in remaining treatment groups		Input data corrected for design effect based on Tarone's test
<i>Rudbeckia hirta</i>	PHEN	Aborted buds/total buds	60	<.0001	<.0001	0.0681	0.2828	NA	Input data corrected for design effect based on Tarone's test
<i>Rudbeckia hirta</i>	PHEN	Percent of surviving plants producing inflorescences	93	<.0001	0.0007	0.0283	0.1095	NA	No significant design effects.
<i>Rudbeckia hirta</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	69	<.0001	<.0001	<.0001	<.0001	0.0043	No significant design effects.
<i>Centaurea cyanus</i>	PHEN	Mortality	74	Not tested, distinct rate-response: See rate-response model					
<i>Centaurea cyanus</i>	PHEN	Aborted buds/total buds	82	Not tested, distinct rate-response: See rate-response model					
<i>Centaurea cyanus</i>	PHEN	Percent of surviving plants producing inflorescences	94	Due to non-monotone response: By Fisher exact test, with Bonferroni correction for family-wise Type I error rate of 0.05. Single test alpha = 0.01. All tests p>0.01, except 117.2 g a.e./ha (p = 0.0034)					
<i>Centaurea cyanus</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	87	<.0001	<.0001	<.0001	<.0001	0.0063	Input data corrected for design effect based on Tarone's test
<i>Centaurea cyanus</i>	INFLO	Mortality	75	Not tested, distinct rate-response: See rate-response model					
<i>Centaurea cyanus</i>	INFLO	Pollen germination	92	0.0425	0.0371	0.0333	0.2751	NA	Input data corrected for design effect based on Tarone's test

Species	Experiment	Binary Measure of Effects	Dataset #	One-sided p-value ^a					Comment
				Initial	Step 1	Step 2	Step 3	Step 4	
<i>Trifolium pratense</i>	PHEN	Mortality	13	0.3679	NA	NA	NA	NA	No significant design effects.
<i>Trifolium pratense</i>	PHEN	Aborted buds/total buds	26	Not tested, distinct rate-response: See rate-response model					
<i>Trifolium pratense</i>	PHEN	Percent of surviving plants producing inflorescences	95	<.0001	<.0001	<.0001	<.0001	0.0047	No significant design effects.
<i>Trifolium pratense</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	37	Due to non-monotone response: By Fisher exact test, with Bonferroni correction for family-wise Type I error rate of 0.05. Single test alpha = 0.01. All tests p<0.01, except 163.4 g a.e./ha (p = 1)					
<i>Trifolium pratense</i>	INFLO	Mortality	100	No mortality					
<i>Trifolium pratense</i>	INFLO	Pollen germination	45	0.1669	NA	NA	NA	NA	NA

^a last p-value presented if >0.05 represents step at which no significant difference was obtained by Fisher's exact comparison of highest group considered and control (or Cochran-Armitage if adjusted values used such that Fisher exact test could not be applied), unless otherwise noted.

Table G-3. PROC PROBIT lack fit test results and selected models for binary dataset

Species	Experiment	Binary Measure of Effects	Dataset #	Pearson Chi-square p-value			Selected Model	Model Parameters	
				Normal/probit	Log Logistic	Log Gompertz		μ	σ
<i>Rudbeckia hirta</i>	PHEN	Mortality	50	<.0001	<.0001	<.0001	None	NA	NA
<i>Rudbeckia hirta</i>	PHEN	Aborted buds/total buds	60	<.0001	<.0001	<.0001	None	NA	NA
<i>Rudbeckia hirta</i>	PHEN	Percent of surviving plants producing inflorescences	93	0.5952	0.5832	0.5791	Normal/probit	2.43	0.327
<i>Rudbeckia hirta</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	69	0.0042	0.0021	0.0007	None	NA	NA
<i>Centaurea cyanus</i>	PHEN	Mortality	74	0.0177	0.2159	0.4804	Log Gompertz	2.05	0.152
<i>Centaurea cyanus</i>	PHEN	Aborted buds/total buds	82	<.0001	<.0001	<.0001	None	NA	NA
<i>Centaurea cyanus</i>	PHEN	Percent of surviving plants producing inflorescences	94	Not modeled.					
<i>Centaurea cyanus</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	87	<.0001	<.0001	<.0001	None	NA	NA
<i>Centaurea cyanus</i>	INFLO	Mortality	75	0.1825	0.1837	0.1868	Log Gompertz	2.05	0.337
<i>Centaurea cyanus</i>	INFLO	Pollen germination	92	<.0001	<.0001	<.0001	None	NA	NA
<i>Trifolium pratense</i>	PHEN	Mortality	13	Not modeled.					
<i>Trifolium pratense</i>	PHEN	Aborted buds/total buds	26	<.0001	<.0001	<.0001	None	NA	NA
<i>Trifolium pratense</i>	PHEN	Percent of surviving plants producing inflorescences	95	0.1845	0.2097	0.092	Log Logistic	1.74	0.150

Species	Experiment	Binary Measure of Effects	Dataset #	Pearson Chi-square p-value			Selected Model	Model Parameters	
				Normal/probit	Log Logistic	Log Gompertz		μ	σ
<i>Trifolium pratense</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	37	Not modeled.					
<i>Trifolium pratense</i>	INFLO	Mortality	100	Not modeled. No mortality.					
<i>Trifolium pratense</i>	INFLO	Pollen germination	45	Not modeled.					

^a last p-value presented if >0.05 represents step at which no significant difference was obtained by Fisher's exact comparison of highest group considered and control (or Cochran-Armitage if adjusted values used such that Fisher exact test could not be applied), unless otherwise noted.

Table G-4. PROC NL MIXED goodness-of-fit and hypothesis test results for continuous data

Species	Exp.	Measure of Effect	Dataset #	Akaike information criterion (AIC)					Selected Model	Selected Model Parameters			Hypothesis Test
				Linear	Exponential	Log Logistic	Log Gompertz	Lognormal		Co	ER25	σ	
<i>Rudbeckia hirta</i>	PHEN	Average above ground biomass per experimental plant	52	58.6	57.4	57.8	58.9	57.4	Exponential	4.90	45.3	NA	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average above ground biomass per surviving plant	52	53.2	52	53	53.8	52.7	Exponential	4.82	53.5	NA	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average bloom-weeks per experimental plant	66	121.4	123.1	121.2	121.9	120.7	Lognormal	12.5	71.7	0.319	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	66	116.1	117.2	116.2	116.7	115.7	Lognormal	12.5	82.7	0.353	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average bloom duration	63	50	51.9	50.5	50	50.9	Linear	3.93	61.2	50.9	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average number of bloomed inflorescences per experimental plant	58	74.9	75.4	77.7	75.4	75.1	Linear	3.98	105	NA	Not tested.
<i>Rudbeckia hirta</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant	58	Not modeled.									ANOVA F-test, p>0.05
<i>Centaurea cyanus</i>	PHEN	Average above ground biomass per experimental plant	76	126.4	139.8	120.2	121.5	120	Lognormal	7.18	57.7	0.191	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average above ground biomass per surviving plant	76	124.3	123.8	125.3	125.8	125.1	Linear	7.32	86.4	NA	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	85	Did not converge	210.1	194.7	195.1	194.5	Lognormal	25.3	65.3	0.183	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average bloom-weeks per experimental plants	85	195.9	222	179.8	172.1	175.6	Log Gompertz	24.8	63.3	6.09	Not tested.

Species	Exp.	Measure of Effect	Dataset #	Akaike information criterion (AIC)					Selected Model	Selected Model Parameters			Hypothesis Test
				Linear	Exponential	Log Logistic	Log Gompertz	Lognormal		Co	ER25	σ	
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plants at 8 weeks	80	246.8	257.7	239	239.3	238.7	Lognormal	47.1	75.1	0.154	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per experimental plant at 5 weeks	80	Did not converge.	197.7	147.1	Did not converge.	Did not converge.	Log Logistic	20.5	60.0	6.60	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per experimental plant at 8 weeks	80	243.3	273.1	228.5	221.6	223.6	Log Gompertz	47.9	59.3	3.31	Not tested.
<i>Centaurea cyanus</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plants at 5 weeks	80	188	193.3	184.5	185.2	184.4	Lognormal	21.3	59.9	0.195	Not tested.
<i>Centaurea cyanus</i>	INFLO	Average above ground biomass per experimental plant	77	151.7	138.4	138.8	138.7	138.7	Lognormal	6.19	10.8	0.594	Not tested.
<i>Centaurea cyanus</i>	INFLO	Average above ground biomass per surviving plant	77	155	146.5	138	138	138	None	NA	NA	NA	Step-down Williams, $p < 0.05$ for all treatment levels
<i>Centaurea cyanus</i>	INFLO	Average number of bloomed inflorescences per experimental plant	81	Did not converge.	246.8	248.6	277.3	248.5	Exponential	21.0	7.12	NA	Not tested.
<i>Centaurea cyanus</i>	INFLO	Average number of bloomed inflorescences per surviving and reproductive plant	81	Did not converge.	268.7	268.9	308.3	268.9	Exponential	21.6	9.19	NA	Not tested.
<i>Centaurea cyanus</i>	INFLO	Average inflorescence dry weight	89	328.5	334.7	369.7	318.1	318.1	None	NA	NA	NA	Step-down Williams, $p < 0.05$ for all treatment levels
<i>Centaurea cyanus</i>	INFLO	Average number of reproductive florets per inflorescence	90	205.5	205	204.8	205	204.8	None	NA	NA	NA	Jonckheere-Terpstra step-down test, $p < 0.05$ for all treatment levels

Species	Exp.	Measure of Effect	Dataset #	Akaike information criterion (AIC)					Selected Model	Selected Model Parameters			Hypothesis Test
				Linear	Exponential	Log Logistic	Log Gompertz	Lognormal		Co	ER25	σ	
<i>Centaurea cyanus</i>	INFLO	Pollen count	91	Not modeled.									Jonckheere-Terpstra, $p>0.05$
<i>Trifolium pratense</i>	PHEN	Average above ground biomass per experimental plant	15	159.9	137.8	136.4	139.4	136.9	Log Logistic	13.8	24.0	1.67	Not tested.
<i>Trifolium pratense</i>	PHEN	Average above ground biomass per surviving plant	15	156.7	132.1	130	133.9	130.7	Log Logistic	13.9	25.4	1.71	Not tested.
<i>Trifolium pratense</i>	PHEN	Average bloom duration	30	-2.7	-1	-4.4	-4	-8.3	Linear	1.68	45.8	NA	Not tested.
<i>Trifolium pratense</i>	PHEN	Average bloom-weeks per experimental plants	33	Did not converge	174.6	178.3	166.9	170.3	Log Gompertz	36.7	17.1	1.65	Not tested.
<i>Trifolium pratense</i>	PHEN	Average bloom-weeks per surviving and reproductive plant	33	Did not converge	182	183.6	171.1	175.4	Log Gompertz	37.8	19.7	1.85	Not tested.
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per experimental plant 5 weeks	23	Did not converge	135.1	141.1	132.3	135.4	Log Gompertz	17.1	15.2	1.49	Not tested.
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per experimental plant 8 weeks	23	Did not converge	164.3	164.4	157.3	158.6	Lognormal	24.6	26.7	0.201	Not tested.
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant 5 weeks	23	Did not converge	151.4	155.1	149.3	152.1	Log Gompertz	17.6	17.1	1.56	Not tested.
<i>Trifolium pratense</i>	PHEN	Average number of bloomed inflorescences per surviving and reproductive plant 8 weeks	23	Did not converge	170.8	169	160.8	162.9	Log Gompertz	27.6	21.3	1.88	Not tested.
<i>Trifolium pratense</i>	INFLO	Average above ground biomass per experimental plant	16	144	143.1	200.9	143.1	143.1	Exponential	10.9	35.1	NA	Not tested.

Species	Exp.	Measure of Effect	Dataset #	Akaike information criterion (AIC)					Selected Model	Selected Model Parameters			Hypothesis Test
				Linear	Exponential	Log Logistic	Log Gompertz	Lognormal		Co	ER25	σ	
<i>Trifolium pratense</i>	INFLO	Average number of bloomed inflorescences per experimental plant	24	Did not converge	125.7	122.1	125.0	123.5	Log Logistic	11.4	4.73	2.01	Not tested.
<i>Trifolium pratense</i>	INFLO	Average number of bloomed inflorescences per surviving and reproductive plant	24	Did not converge	144.4	143.4	145.9	144.5	Log Logistic	15.5	5.59	2.04	Not tested.
<i>Trifolium pratense</i>	INFLO	Average inflorescence dry weight	40	Not modeled.									ANOVA F-test, $p>0.05$
<i>Trifolium pratense</i>	INFLO	Average number of reproductive florets per inflorescence	42	152.1	152.2	162.1	154.1	154.2	Linear	84.0	16.6	NA	Not tested.
<i>Trifolium pratense</i>	INFLO	Pollen count	44	Not modeled.									Jonckheere-Terpstra step-down test, $p>0.05$

Table G-5. PROC GENMOD modeling results for average time to first bloom of blooming plants (normal error assumption, identity link)

Species	Exp.	Dataset #	Akaike information criterion (Type III p-values for parameters)			Selected Model	Selected Model Parameters		
			Rate	Rate, Rate ²	Rate ²		intercept	B1	B2
<i>Rudbeckia hirta</i>	PHEN	55	33.5594 (<.0001)	30.1314 (0.4435, 0.0198)	28.7186 (<.0001)	Rate ²	3.02	0.0001	NA
<i>Centaurea cyanus</i>	PHEN	78	53.3973 (<.0001)	37.7697 (0.1685, <.0001)	37.6661 (<.0001)	Rate ²	2.54	0.0002	NA
<i>Trifolium pratense</i>	PHEN	19	Not modeled. No significant differences based on Wilcoxon exact comparisons to control at family wise error rate of 0.05, except at the first treatment level (p=0.0043).						

Table G-6. PROC GAM modeling results for nonparametric regressions

Species	Experiment	Measure of Effect	Dataset #	p-value for rate parameter	Intercept parameter	Linear(rate) parameter
<i>Rudbeckia hirta</i>	PHEN	Mortality	50	0.332	Not retained.	
<i>Rudbeckia hirta</i>	PHEN	Aborted buds/total buds	60	<.0001	-4.12673	0.01701
<i>Rudbeckia hirta</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	69	<.0001	-2.41274	0.0167
<i>Centaurea cyanus</i>	PHEN	Aborted buds/total buds	82	<.0001	-3.9397	0.02845
<i>Centaurea cyanus</i>	PHEN	Number of malformed inflorescences per total bloomed inflorescences	87	<.0001	-4.05616	0.02099
<i>Centaurea cyanus</i>	INFLO	Average above ground biomass per surviving plant	77	<.0001	-0.16823	-0.00330
<i>Centaurea cyanus</i>	INFLO	Average inflorescence dry weight	89	<.0001	74.62224	-0.47371
<i>Centaurea cyanus</i>	INFLO	Average number of reproductive florets per inflorescence	90	0.0005	-0.08344	-0.0004196
<i>Trifolium pratense</i>	PHEN	Aborted buds/total buds	26	<.0001	-3.27935	0.0502