

The link between biomass and speed of fluorescence effects by some herbicides on *Amaranthus retroflexus* L. weeds

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Abstract: Background: Using the biomass endpoint as a reference, the rates of change in the fluorescence parameters demonstrated how rapidly the photosystem II-inhibitor (PSII-inhibitor) bentazon; a mixture of PSII-inhibitors—phenmedipham, desmedipham, and ethofumesate (a very-long-chain fatty acid {VLCFA} inhibitor); and the acetolactate synthase inhibitor (ALS-inhibitor) nicosulfuron affected the plants.

Objective: The objective was to investigate how fluorescence parameters (F_v/F_m , Area above the Kautsky curve, and Performance Index (PI)) responded prior to biomass harvest. **Methods:** Two greenhouse experiments were conducted with *Amaranthus retroflexus* plants as models. ED_{20} , ED_{50} , and ED_{80} parameters derived from log-logistic dose-response curves were estimated using meta-analysis to investigate inherent variability. **Results:** For PSII-inhibitors, either applied alone or

in combination with a VLCFA-inhibitor, F_v/F_m exhibited a negative linear slope with herbicide dose. The intercepts were close to the theoretical 0.8, one to two days after spraying (DAS). At three to four DAS, the response to PSII, and PSII combined with a VLCFA-inhibitor could be described by a log-logistic dose-response curve. For nicosulfuron, the F_v/F_m parameter showed a slope of zero and an intercept close to 0.8 across all DAS. Area and PI were imprecise in assessing the ED_x values. Meta-analyses indicated that, in some cases, the variability of ED_x was too high for these parameters to be considered reliable endpoints. **Conclusions:** The variability of all fluorescence parameters, besides F_v/F_m , was too high compared to the biomass endpoint. The study emphasized that combining independent dose-response curve experiments and analyzing ED_x -derived parameters is essential to capture the extent of dose-response variability.

Keywords: Bentazon; Phenmedipham; Desmedipham; Ethofumesate; Nicosulfuron; F_v/F_m , Area, PI

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1. Introduction

Amaranthus retroflexus L. (redroot pigweed) is a widespread annual and competitive C_4 weed that can cause drastic crop yield losses (Cao et al., 2022). *Amaranthus retroflexus* exerts negative effects on various crops, including sunflower (*Helianthus annuus*) (Soltani, 1994), maize (*Zea mays* L.), soybean (*Glycine max* L.) (Horak, Loughin, 2000), and sugar beet (*Beta vulgaris* L.) (Brimhall et al., 1965).

The herbicides bentazon and nicosulfuron, along with Betanal Progress®—a commercial mixture of ethofumesate, phenmedipham, and desmedipham—are commonly used in Iran for the control of *A. retroflexus*. (Hesami, 2019).

The commercially available mixture consists of post-emergent herbicides that target a wide range of annual broadleaf weeds and inhibit the growth of certain monocot species (Hesami, 2019). It has been tested and approved specifically for controlling broadleaf weeds in sugar beet. Nicosulfuron, a key herbicide in corn production (Zand et al., 2010), which is extensively applied to control broadleaf and some monocot weed species in various crops (Shahbazi et al., 2015).

Bentazon, a benzothiadiazole, is a PSII inhibitor. It is a post-emergence herbicide used to control dicot weeds in several crops, including maize, beans, and soybeans. Bentazon is frequently applied in soybean for managing *A. retroflexus* and other broadleaf weeds (Aghajani et al., 2001). It typically induces changes in the chloroplasts, pigment ratios, and levels of chlorophyll-protein complexes within the photosynthetic apparatus (Cobb, Read, 2010). However, it is more readily translocated within the plant compared to desmedipham and phenmedipham. Desmedipham and phenmedipham are phenyl carbamates and PSII inhibitors. They rapidly penetrate the leaves but have restricted translocation via the xylem. As with most PSII inhibitors, weed mortality ultimately results from light-induced reactive oxygen species (ROS) damage (Fufezan et al., 2002; Rutherford, Krieger-Liszkay, 2001).

Ethofumesate, a benzofuran, inhibits the biosynthesis of very long-chain fatty acids (VLCFA) (Székács, 2021). It interferes with the development of meristems, slowing cell division and restricting cuticle formation (Kohler, Branham, 2002). Therefore, ethofumesate may alter the absorption characteristics of other herbicides when used in mixtures.

Nicosulfuron is a sulfonylurea herbicide widely used to control both perennial and annual weeds. It inhibits acetolactate synthase (ALS), thereby suppressing the formation of branched-chain amino acids such as valine, leucine, and isoleucine (Cobb, Read, 2010; Hennigh et al., 2010; Lum et al., 2005). The inhibition of branched-chain amino acid synthesis also negatively impacts the photosynthetic apparatus over time (Cobb, Read, 2010; Lum et al., 2005; Yuan et al., 2014). According to Wang et al. (2022) nicosulfuron reduced plant growth and development, disrupted antioxidant systems, and ultimately decreased chlorophyll content, thereby impairing photosynthetic productivity.

Evaluation of changes in the chlorophyll fluorescence Kautsky curve and its derived parameters has been used as an early indicator of herbicide effects on plants, before visible symptoms of herbicide damage appear. For instance, PSI and PSII inhibitors, and herbicides with other modes of action that generate ROS, can be studied using chlorophyll fluorescence (Christensen et al., 2003). This technique is non-destructive, highly sensitive to changes, fast, and easy to evaluate, providing valuable insights into the functioning of photosynthetic apparatus (Frankart et al., 2003).

The objective of this study was to investigate how rapidly herbicides with different modes of action affected Kautsky curve parameters prior to the harvest of weed biomass. More specifically, we aimed to determine which fluorescence parameters were influenced by the herbicides tested, and how rapidly and precisely the herbicides impacted these parameters. Finally, we summarized the independent experiments to investigate the presence of a variation in the differences within ED_x parameters (ED_{20} , ED_{50} , and ED_{80}).

2. Material and Methods

Completely randomized experiments, with four replicates for each herbicide or herbicide-mixture were conducted in a greenhouse at the Faculty of Agriculture, Gonbad Kavous University, Iran in 2022. For each herbicide, two independent experiments were performed to assess the variability of the effective dose endpoints (ED_{50} , ED_{50} , and ED_{80}).

2.1 Plant material

Amaranthus retroflexus seeds were collected from Gorgan, Golestan province, Iran. Seeds were placed in trays containing a peat and moss mixture in a 1:1 ratio. After emergence, three seedlings were transplanted into 15-cm diameter pots filled with a mixture of soil, sand, and peat (1:1:1 v/v). The pots were sub-irrigated daily. The average temperature was 30 °C (min: 28 °C and max: 32 °C) with a relative humidity of 75% within the greenhouse complex.

2.2 Herbicide application

The herbicides used in this study, along with their formulations, active ingredient concentrations, and application doses, are summarized in Table 1.

Amaranthus retroflexus was sprayed at the recommended 3-4 leaf stage, with a 200 L ha⁻¹ spray solution at a pressure of 300 kPa using an 8002 flat fan nozzle.

Amaranthus retroflexus plants were harvested 14 days after spraying (DAS). Dry matter biomass was measured after oven drying at 75 °C for 48 hours.

2.3 Chlorophyll fluorescence parameters and biomass measurements

Chlorophyll fluorescence was measured at one, two, three, four and five DAS using a portable chlorophyll fluorometer (Handy PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). Leaves were dark-adapted for 30 minutes using leaf clips, and measurements were taken with a 650 nm light pulse at an intensity of 3,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 10 seconds (Maxwell, Johnson, 2000). Fluorescence parameters including F_v/F_m , Area and performance index (PI) were recorded to monitor the effects of various herbicide treatments.

F_v/F_m represents the maximum quantum efficiency of PSII photochemistry and indicates the level of photoinhibition or stress, with a theoretical value of approximately 0.8 in non-stressed plants (Maxwell, Johnson, 2000). F_0 denotes initial fluorescence, F_m represents maximum capacity, and F_v (calculated as $F_m - F_0$) reflects variable fluorescence, corresponding to the potential for photochemical quenching.

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

PI is one of the comprehensive parameters in chlorophyll fluorescence analysis, especially used for assessing the health and efficiency of PSII (Strasser et al., 2000). This parameter provides more specified information compared to the F_v/F_m ratio (Strasser et al., 2000). The PI index reflects the general ability of PSII to absorb, transfer, and

Table 1 - Applied doses of bentazon (Basagran®), nicosulfuron, and Betanal Progress® used on *Amaranthus retroflexus*

	(Basagran®) Bentazone	Nicosulfuron	Betanal Progress®
Ingredient (g ai /L)	480 soluble liquid (SL)	4% oil dispersion (OD)	274 emulsifiable concentrate (EC): contained ethofumesate 112, phenmedipham 91, desmedipham 71
Dose (g ai /ha)	1,440, 1,080, 720, 360	120, 80, 60, 40, 20	822, 616.5, 411, 205.5

Dose-response curves included untreated controls.

utilize light energy and measures the total performance of the electron transport through PSII (Ceusters et al., 2019).

$$PI_{ABS} = \frac{RC}{ABC} \cdot \frac{\phi PO}{1 - \phi_{PO}} \cdot \frac{\Psi_0}{1 - \Psi_0}$$

RC/ABS represent the density of active reaction centers per absorbed light energy, ϕ_{PO} equal as F_v/F_m define maximum quantum yield of PSII, Ψ_0 shows electron transport efficiency (Strasser et al., 2000).

Area refers to the geometric area between the Kautsky curve and the maximum fluorescence level. This parameter indicates the total number of electrons that are transferred from QA⁻ to the next electron transport chain. In other words, Area reflects the overall capacity of the electron transport chain on the acceptor side of PSII (Strasser et al., 2000).

2.4 Statistical analysis

The changes in Kautsky curve parameters in response to different herbicides were initially modeled using linear regression of the parameter values against herbicide doses over time. When the dose-response relationship appeared to follow a sigmoidal pattern, a three-parameter log-logistic model was fitted to the data (Eq. 1) (Ritz et al., 2015; Streibig et al., 1993).

$$U = C + \frac{D - C}{1 + \exp \{b[\log(z) - \log(ED_{50})]\}} \quad \text{Eq. 1}$$

In the model, U represents the response endpoint at a dose z . D is the upper asymptote corresponding to the response in the absence of herbicide (untreated control). C denotes the lower asymptote at infinitely high herbicide doses. In this study, the lower limit C was assumed to be zero. Thus, the ED_{50} is defined as the dose resulting in a 50% reduction of the maximum response ($D/2$), and b represents the slope around the ED_{50} value. At certain sampling times, the log-logistic dose-response model

(Eq. 1) was appropriate for describing the variation of Kautsky curve parameter endpoints over time.

Dose-response analyses were performed using R software (Version 4.4.0). For mixed linear regression models applied at early DAS, the *lme4* package (Version 1.1-37) combined data from the two independent experiments. For the log-logistic regression model, the *drc* package (Version 3.0-1) was employed (Ritz et al., 2015). Additionally, the *metafor* package (Version 4.6-0) was used to conduct meta-analyses and summarize ED_x (ED_{20} , ED_{50} , and ED_{80}) values across the two experiments (Ritz et al., 2019).

Biomass and log-logistic dose-response Kautsky curve parameters were based on ED_{20} , ED_{50} , and ED_{80} . From the first to the fifth DAS, some Kautsky curve parameters moved from linear to sigmoid dose-response relationships, allowing the derivation of ED_x . Log-logistic regression was applied to Kautsky curve parameters at five DAS, nine days before the final biomass harvest.

The linear or log-logistic nonlinear regressions were assessed through graphical analysis of residuals. A two-step approach was used to combine the two independent experimental runs with the same herbicide (Ritz et al., 2019). The first step involved separately fitting the individual dose-response curves fitting the individual dose-response curves separately to obtain the relevant regression parameters (ED_{20} , ED_{50} , and ED_{80}) and their associated standard errors. In the second step, meta-analysis was applied using the *metafor* R package to combine estimates from individual experimental runs into a pooled estimate (Ritz et al., 2019).

3. Results and Discussion

In addition to the correlation analysis of the three Kautsky curve parameters (Figure 1), the results enable a comparison between the fluorescence-derived endpoints and the biomass ED_x values measured at 14 DAS. Biomass served as the reference endpoint against which the other Kautsky curve-derived parameters were evaluated.

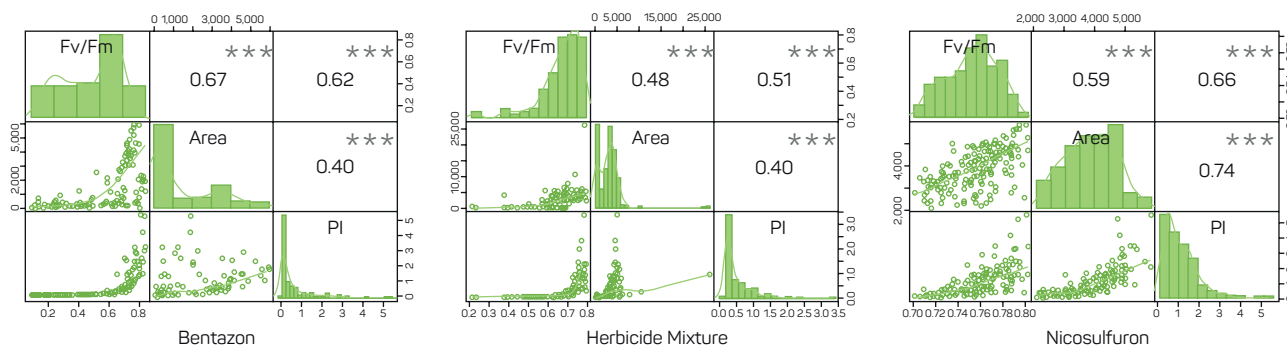


Figure 1 - Correlations among Kautsky curve parameters in *A. retroflexus* treated with bentazon, a mixture of ethofumesate, phenmedipham, and desmedipham, and nicosulfuron. The histograms along the diagonal display the distribution of each respective Kautsky curve parameter. Correlation coefficients for herbicides are presented in the upper right graphs. Three stars for all correlation coefficients indicate a highly significant correlation ($p < 0.001$). The sizes of the correlation coefficient font also indicate the magnitude. However, the correlations between F_v/F_m , Area, and PI were non-linear for Bentazon and the Mixture

3.1 Correlation analyses

The Kautsky curve offers a wide range of derived parameters, many of which are highly correlated. From the numerous parameters suggested based on the shape and characteristics of the Kautsky curve (Ritz, Streibig, 2009), the present study focused on F_v/F_m , Area, and PI, measured during the first five DAS (Figure 1). These parameters were selected for their ability to reveal herbicidal effects shortly after spraying (Christensen et al., 2003).

The Kautsky parameters exhibited strong inter-correlations (Figure 1). However, for bentazon (a PSII inhibitor) and the mixture (combining PSII and VLCFA inhibition), the distribution of the measurements was non-linear; therefore, the correlation coefficients should not be considered. In contrast, the correlations observed for nicosulfuron were all nearly linear, thus satisfying the assumptions required for reliable linear correlation analysis.

Nicosulfuron, is an ALS inhibitor that disrupts the biosynthesis of branched-chain amino acids. Therefore, its primary mode of action is not directly related to photosynthesis processes (Hennigh et al., 2010).

3.2 Bentazon

3.2.1 F_v/F_m and Biomass

The F_v/F_m endpoint exhibited a rapid response at the first and second DAS. For both DAS the intercepts were close to the theoretical value of 0.8. The slope showed a significantly negative trend ($p < 0.05$) in the first experiment at one DAS

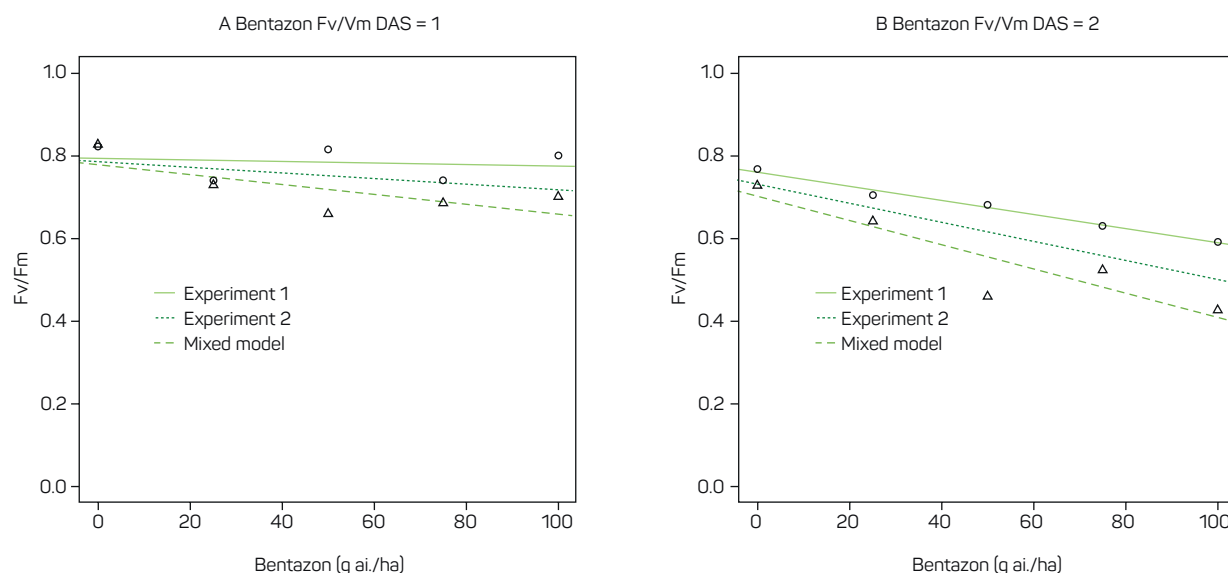
(Figure 2A), and a highly significant negative slope ($p < 0.001$) in the second experiment at two DAS (Figure 2B).

For the first experimental run, F_v/F_m at three DAS could be described by Eq 1, and the same applies for the second experiment at four DAS (data not shown).

The y and x axes in Figure 3 were scaled to allow direct comparison of the shapes and positions of the graphs between endpoints. The biomass dose-response curves 14 DAS were similar for both experiments (Figure 3A and B). The untreated controls for F_v/F_m were close to the theoretical value of 0.8 (Figure 3C and D). Although the response curves for both experiments were similar, the responses' distributions were not as clear as those observed for biomass.

The ED_{20} , ED_{50} , and ED_{80} values were derived from the fittings of Eq 1, and the pooled mean was calculated using a meta-analysis (Ritz et al., 2019) (Figure 4). F_v/F_m appeared to be more sensitive than biomass, exhibiting lower ED values (compare Figure 4A and B). It is common for different endpoints to exhibit varying sensitivities. For both biomass and F_v/F_m (Figure 4A and B), the differences between the different ED_x within the endpoints were all highly significant ($p < 0.001$). The question arises as to why this is so rarely addressed in literature.

F_v/F_m was measured on the fifth DAS. The clear separation among the ED_{20} , ED_{50} , and ED_{80} values in Figure 4 indicates low variability in the relative slopes among the dose-response curves. For both biomass and F_v/F_m endpoints, the differences among ED_x values were highly significant ($p < 0.001$), suggesting a consistent pattern in herbicidal responses across dose levels.



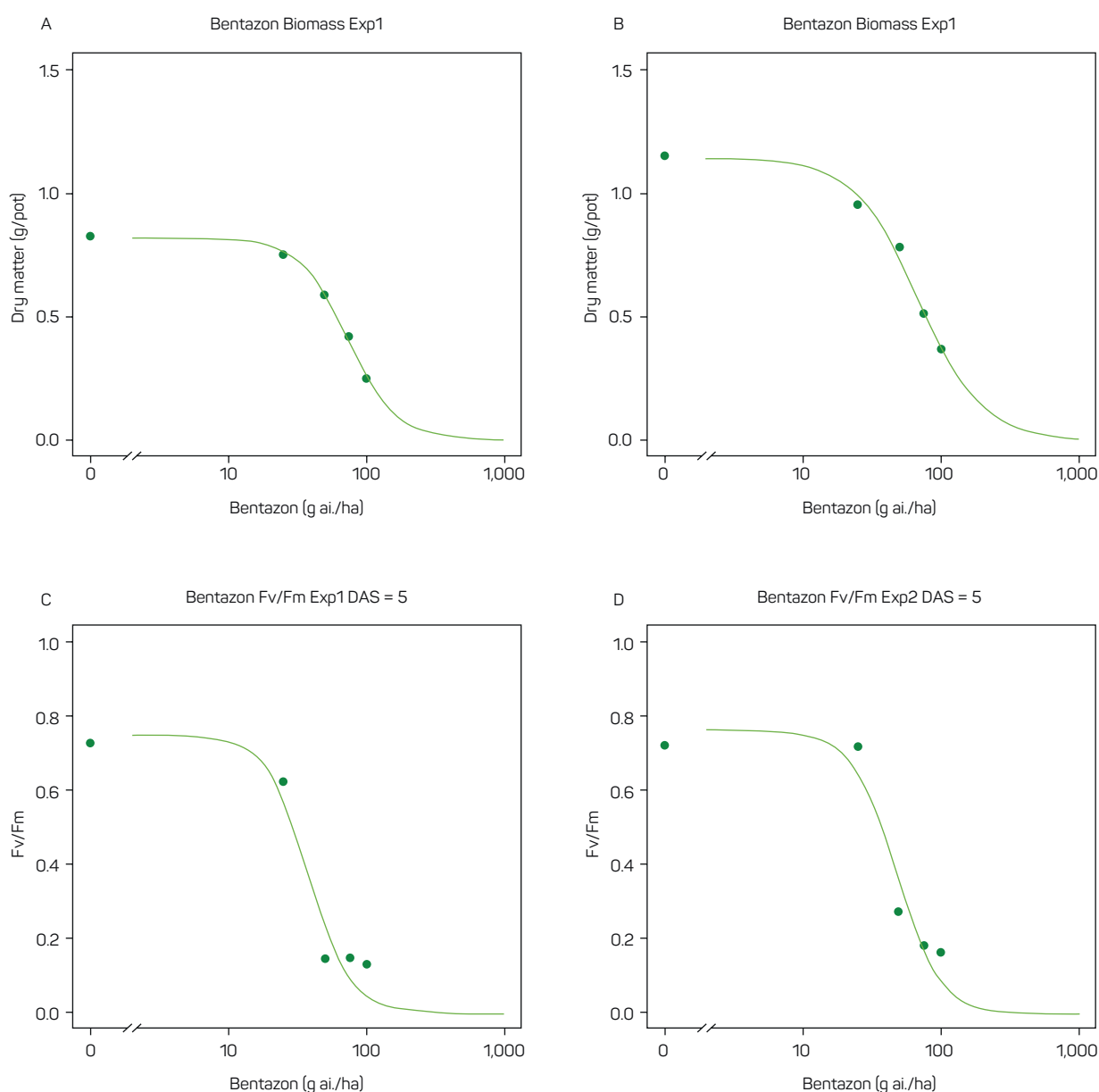
DAS: Days After Spraying

Figure 2 - Mixed linear regression of F_v/F_m values in response to bentazon doses. At 1 DAS (A), the intercept was close to the theoretical value of 0.8 for unstressed plants, and the mixed model showed a significant negative slope ($p < 0.05$). At 2 DAS (B), the negative slope was more pronounced ($p < 0.001$), and the intercept deviated slightly more from the theoretical 0.8 value

Generally, ED_{50} is the most precise estimate among the effective dose levels. However, from an agronomic perspective, it merely reflects general toxicity and may not represent a practically useful level of weed control. For managing *A. retroflexus*, end-users might be more interested in higher effective doses such as ED_{90} or ED_{95} , which correspond to agriculturally relevant control levels. Estimating such high-end ED_x values, however, requires sufficient data to establish robust lower asymptotes. Without those data, the uncertainties associated with ED_{90} or ED_{95} can be extremely large and, in many cases, not significantly different from zero (as illustrated for nicosulfuron in Figure 9).

3.2.2 Area and PI

Unlike F_v/F_m , which has a theoretical reference value of approximately 0.8 in the untreated control, the Area and PI parameters lacked consistent reference values between the two independent experiments (Figure 5). Linear regressions of Area versus dose revealed substantial differences in intercepts as early as one DAS (data not shown). From three DAS onward, the dose-response relationships could be adequately described using Eq. 1, although the models exhibited considerable standard errors for the upper limit parameter. Moreover, a significant ED_{50} was observed in the first experiment, while the second did not yield a significant ED_{50} estimate (data not shown).

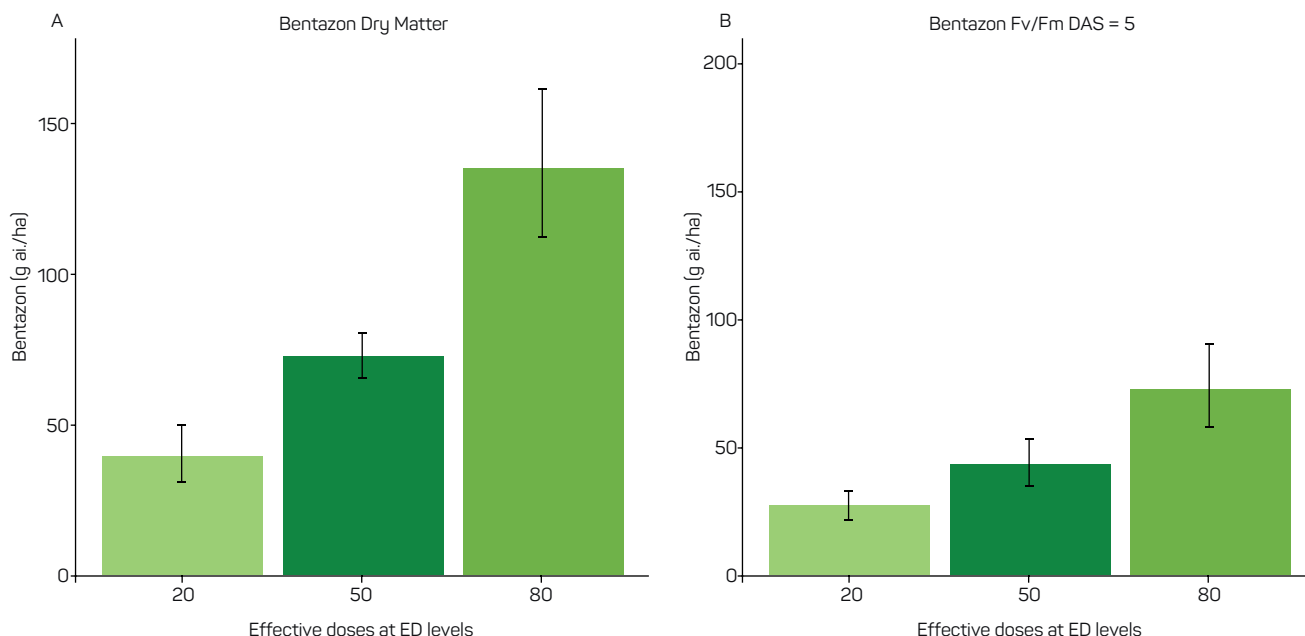


DAS: Days After Spraying

Figure 3 - Graphs A and C display biomass and F_v/F_m data from Experiment 1, while graphs B and D show the corresponding data from Experiment 2. To enable comparison of the curve positions along the dose axis, the y-axes for biomass and F_v/F_m are standardized, respectively. The graphs present the mean values of the dose-response observations

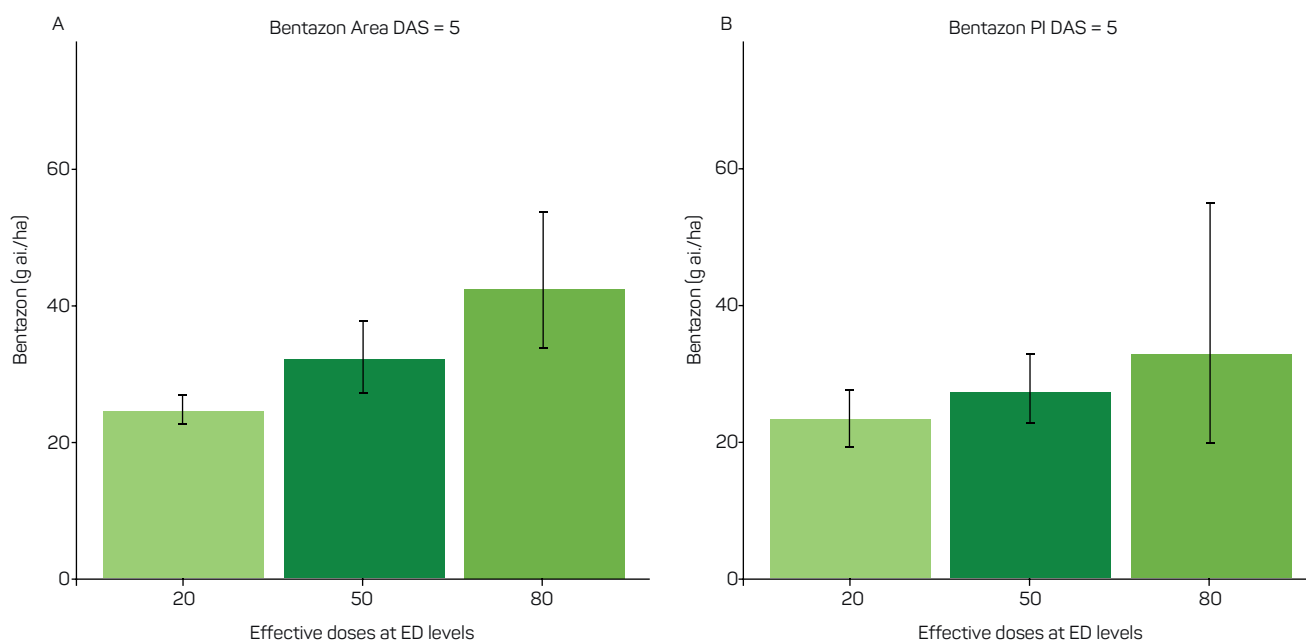
For Area, ED_{20} was different from ED_{50} and ED_{80} ($p < 0.001$), whereas no significant difference was found between ED_{50} and ED_{80} . For PI, no significant

differences were observed among ED_{20} , ED_{50} , and ED_{80} due to the large 95% confidence intervals (Figure 5B).



DAS: Days after spraying

Figure 4 - Meta-analysis of ED_x values for dry matter revealed highly significant differences ($p < 0.001$) (Figure A). Similarly, the ED_x values for F_v/F_m were all significantly different from each other ($p < 0.01$) (Figure B). The 95% confidence intervals were asymmetrical around the ED_x estimates due to the back-transformation from logarithmic values



DAS: Days after spraying

Figure 5 - For the Area parameter (Figure A), ED_{20} differed significantly from both ED_{50} and ED_{80} ($p < 0.001$). In contrast, no significant differences were observed among ED_{20} , ED_{50} , and ED_{80} for the Performance Index (PI), as indicated by the large confidence intervals (Figure B). The 95% confidence interval bars were asymmetrical around the ED_x estimates due to the back-transformation from logarithmic values

3.3 The Mixture (Ethofumesate, desmedipham, and phenmedipham)

The mixture comprises two contact herbicides—desmedipham and phenmedipham, both acting as PSII inhibitors—and ethofumesate, which exhibits limited movement within the plant and inhibits VLCFA synthesis (Székács, 2021).

3.3.1 Kautsky Parameters and Biomass

Similar to bentazon, the F_v/F_m intercepts for the mixture were close to the theoretical maximum of 0.8. The linear regressions showed significantly negative slopes ($p < 0.001$) (Figure 6A and B), indicating that the herbicide doses began affecting the plants as early as one and two DAS.

The meta-analysis of biomass harvested at 14 DAS showed that ED_{20} was significantly different from ED_{50} and ED_{80} ($p < 0.01$) (Figure 7A). However, no significant differences were observed between ED_{20} and ED_{50} , or between ED_{50} and ED_{80} . (Figure 7A). This lack of significance was likely due to insufficient data points on the declining portion of the dose-response curve (data not shown).

The three Kautsky curve parameters in Figure 7B–D showed no significant differences among ED_{20} , ED_{50} , and ED_{80} within each endpoint (F_v/F_m , Area, and PI), as the combined confidence intervals were too wide. Nevertheless, the two-step meta-analysis for the ED_x estimates revealed a common trend: higher ED_x values were generally associated with wider confidence intervals, except for Area (Figure 7C). This pattern likely resulted from the

concentration of data points in the upper portion of the dose-response curves (data not shown).

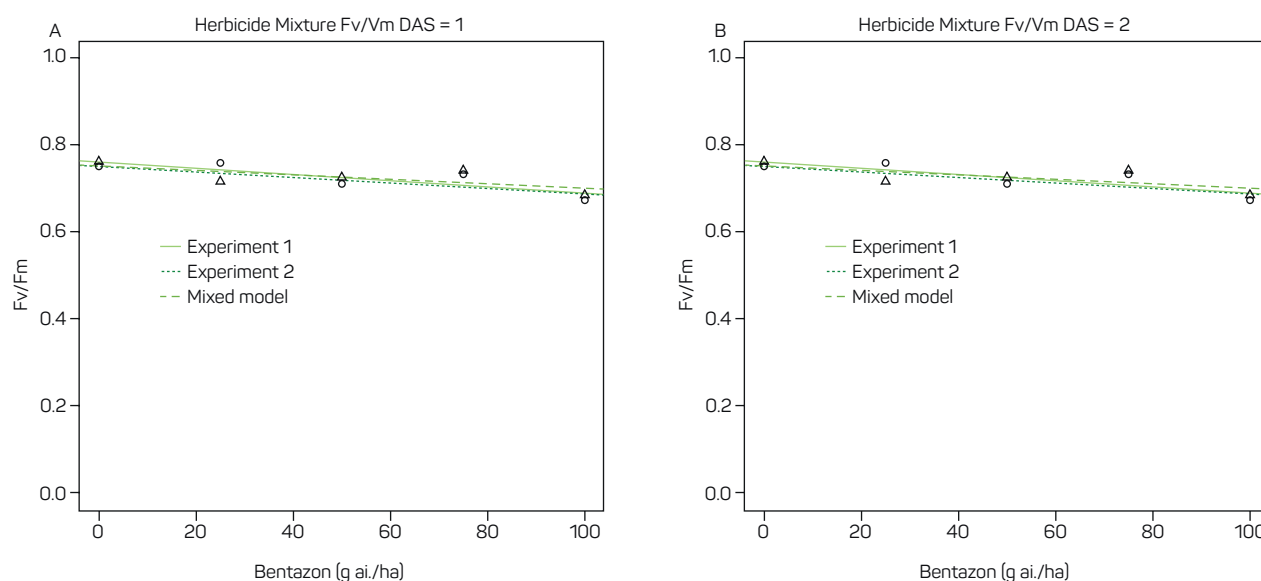
3.4 Nicosulfuron

Nicosulfuron is readily absorbed by the aerial parts of plants, and it is systemically translocated via the phloem. As an ALS inhibitor, it does not produce rapid control of plants, as it requires time to deplete the concentration of branched-chain amino acids, affecting the entire plant (Cobb, Read, 2010). Consequently, the rapid effects on certain Kautsky parameters, observed with bentazon and the mixture, which contains PSII inhibiting herbicides, were not immediately evident following the application of Nicosulfuron.

Nicosulfuron causes chlorosis in the meristematic growth points, which worsens as plants become deficient in essential branched-chain amino acids (Cobb, Read, 2010). Full control usually takes three to four weeks, ultimately leading to the plant's demise.

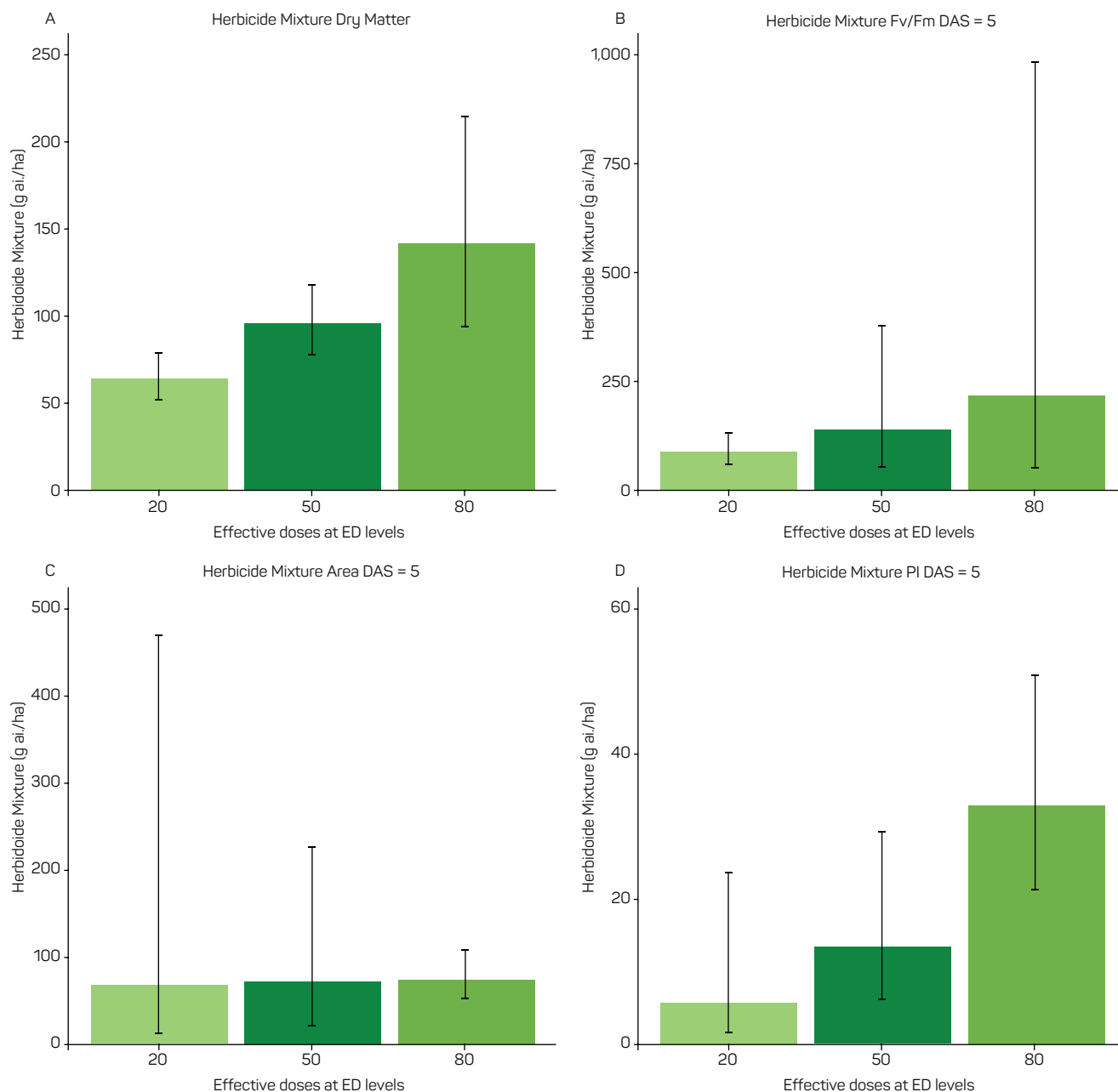
Figures 8A and B demonstrated that nicosulfuron did not significantly alter the linear dose-response regressions. The F_v/F_m remained virtually unaffected across all five DAS, with the slopes not differing from zero. Similarly, Kargar et al. (2019) reported that the F_v/F_m parameter was not influenced by mesosulfuron-methyl + iodosulfuron in wild oat.

Figures 9A and B illustrated that biomass harvested at 14 DAS only represented the upper part of the dose-response curve, and the confidence intervals of the regression lines were rather wide. As a result, the EDs for biomass were not significantly different from each other (Figure 9C). This was



DAS: Days after spraying

Figure 6 - The mixed linear regressions of F_v/F_m on the dose of the herbicide mixture (ethofumesate, phenmedipham, and desmedipham) at one DAS (Figure 6A) and two DAS (Figure 6B) showed highly significant negative slopes ($p < 0.001$). In both cases, the intercepts were close to the theoretical value of 0.8 for unstressed plants



DAS: Days after spraying

Figure 7 - The meta-analysis of biomass harvested at 14 DAS showed that ED_{20} differed from ED_{50} and ED_{80} ($p < 0.01$) (Figure 7A), although no significant differences were found between ED_{20} and ED_{50} or between ED_{50} and ED_{80} . The confidence intervals for F_v/F_m (Figure 7B), Area (Figure 7C), and PI (Figure 7D) at five DAS were too wide to detect differences among the EDs, due to high variability between the independent experiments. The 95% confidence interval bars were asymmetrical around the ED_x estimates due to the back-transformation from logarithmic values

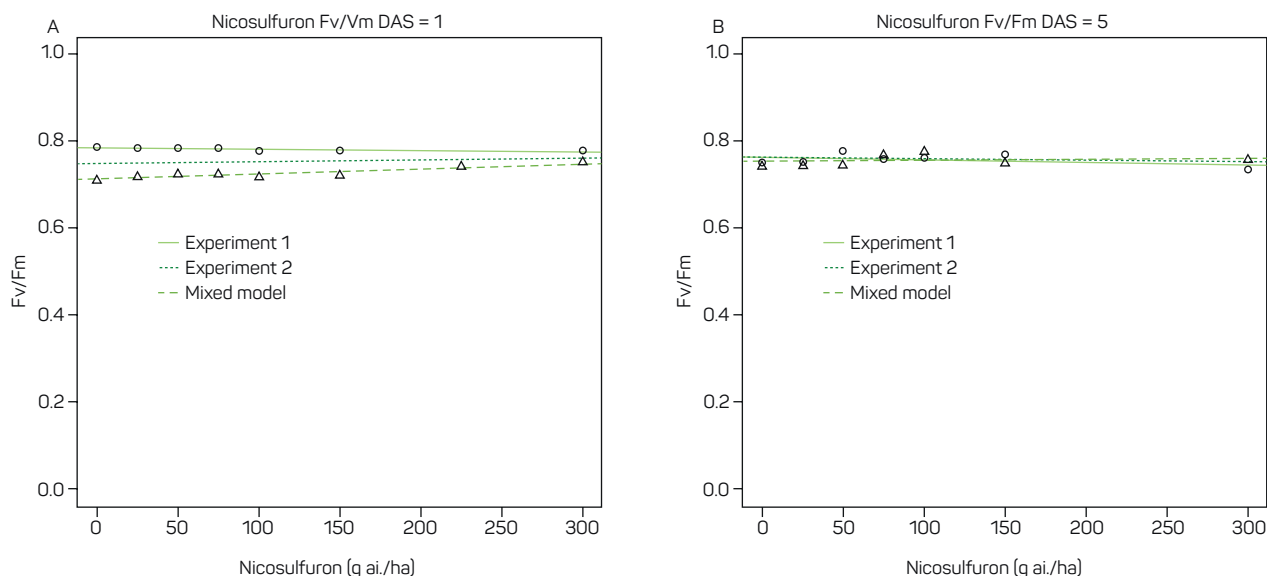
likely due to the pronounced difference in the relative slopes between the two experiments (Figures 9A and B), which caused wide confidence intervals of the biomass dose-response curves. However, the difference between ED_{20} and ED_{80} was significantly different ($p < 0.01$) as shown in Figure 9C.

The Kautsky parameters, Area and PI, were unstable between the two experiments (Data not shown). However, PI could be fitted with a log-logistic model, though the

distribution of the endpoints and the resulting regression parameters were not convincing enough to establish a stable upper limit.

4. Conclusions

The Kautsky curve parameters were highly correlated; however, the correlations of PSII inhibitor parameters were



DAS: Days after spraying

Figure 8 - The mixed linear regressions of F_v/F_m on nicosulfuron dose at one DAS (Figure 8A) and five DAS (Figure 8B) showed no significant slopes; F_v/F_m remained virtually unaffected. The intercepts were close to the theoretical value of 0.8, indicating no visible stress response in the early days after application

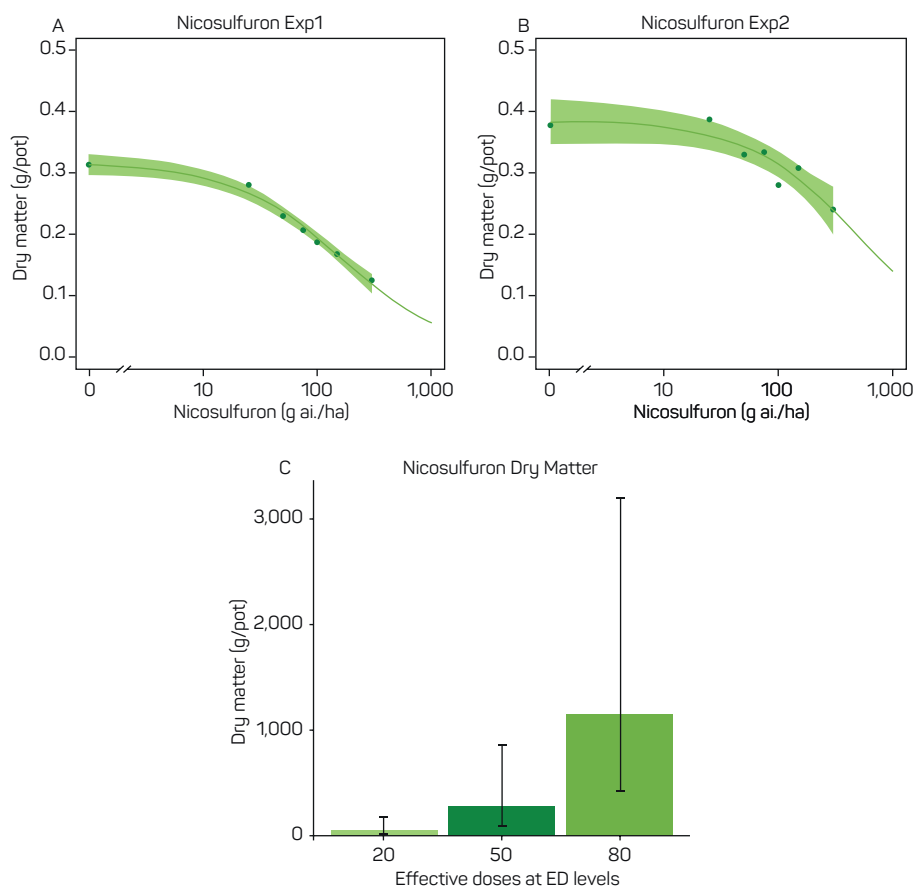


Figure 9 - The regression confidence interval from biomass experiment 1 (Figure 9A) was considerably narrower than that from biomass experiment 2 (Figure 9B). Although both plots share similar y- and x-axis scales, the relative slopes differ. This variation is reflected in meta-analysis (Figure 9C), where the confidence intervals are relatively wide. Nonetheless, the differences between ED_{20} and ED_{80} were statistically significant ($p < 0.01$). The 95% confidence interval bars in Figure 9C were asymmetrical around the ED_x estimates due to the back-transformation from logarithmic values.

nonlinear within the first five DAS. Linear correlations were found for the non-ROS-generating nicosulfuron. It can be tentatively concluded that herbicides with direct or indirect effects on plants' ROS generation can be detected with F_v/F_m within the first five DAS. Furthermore, F_v/F_m has a well-known theoretical upper value of around 0.8 in untreated controls, provided the plants are not stressed.

The other Kautsky curve-derived parameters, including Area above the Kautsky curve and the Performing Index (PI), were variable and poor indicators of an early effect. Numerous combinations of Kautsky-derived parameters are mentioned in the literature, but a literature search by the authors on correlations among Kautsky curve-derived parameters, such as in Figure 1, was not found.

This paper demonstrates that the quality of the dose-response endpoint distribution is crucial for understanding and comparing variations within and between experiments. In experiments for the research, development, and registration of new pesticides, end users are primarily concerned with the extremes: the sensitivity of a crop (ED_{20}) and the control of weeds (ED_{80}). Importantly, we show these should be based on the variation of results derived from combining multiple independent experiments.

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Author's contributions

All authors read and agreed to the published version of the manuscript. ZA, and JS: conceptualization of the manuscript and development of the methodology. ZA, ARK, and ANM: data collection and arrangement. JS: data analysis. ZA, and JS: data interpretation. ZA, JS, ARK, and ANM: writing the original draft of the manuscript. JS, and ZA: writing, reviewing and editing.

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