Research Article

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Tolerance of two *Bifora radians* Bieb populations to ALS inhibitors in winter wheat

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Abstract

BACKGROUND: *Bifora radians*, an annual weed in winter wheat, is distributed mainly in the Mediterranean area, Asia Minor and the Caucasus. It infests winter-sown crops of the Central Anatolia and Middle Black Sea regions of Turkey. Field experiments in heavily *B. radians*-infested fields were conducted over 3 years in Samsun, Turkey, to determine the response of *B. radians* to ALS-inhibiting herbicides, because growers had complained of a decrease in herbicide effect.

RESULTS: The efficacy of ALS inhibitors on a putatively tolerant population sprayed annually with ALS inhibitors and an adjacent allegedly sensitive population was estimated at the ED_{50} and ED_{90} response levels. The recommended rates of herbicides controlled 90% of the weed (ED_{90}) in the sensitive population at the early stage of *B. radians* development, but not in the tolerant population. The relative potencies ($ED_{x(tolerant)}/ED_{x(sensitive)}$) of herbicides on the two populations were estimated by assuming years as being random effects. The relative potency was on average about 1.7, irrespective of the ED_x levels.

CONCLUSION: Although the relative potencies were not large, they were large enough to be noted by growers. In field experiments it would be important to establish tools demonstrating when farmers recognise loss of herbicide efficacy. There has been no indication of evolution of resistant biotypes so far, but continuous spraying favours biotypes with increased levels of tolerance.

Keywords: dose response; efficacy; herbicide-tolerant population

1 INTRODUCTION

Bifora radians Bieb, in the *Apiaceae*, is a competitive annual dicot weed of winter-sown crops in the Central Anatolia and Middle Black Sea regions of Turkey.[1,2] It is reported to be a problem weed in Ukraine, the Caucasus, the Crimea, Greece and Italy.[3] *B. radians* is erect, 30–50 cm tall, branched and thermophilic and prefers nitrogen-rich alkaline soils.

Turkey is one of the top ten producers of wheat in the world and produces most of the wheat in the central regions of the country.[4] Wheat fits well in rotation with maize (*Zea mays* L.), sugar beet (*Beta vulgaris* L.) and sunflower (*Helianthus annuus* L.). Weed surveys, conducted in wheat fields of Central Anatolia and Middle Black Sea regions during 1985 to 1998, found more than 70% wheat fields infested with *B. radians*.[5] *B. radians* reduces wheat yield by 13–39%, depending on infestations, wheat cultivars and seeding rates.[6] Its aggressive growth habit and allegedly allelopathic effects allow the species to compete strongly with wheat and other crops.

Herbicides with 2,4-D and MCPA have been used against dicot weeds, but have little or no effect on *B. radians*, with the consequence that this weed species has gained importance and is predominating in wheat fields.[6] Acetolactate synthase (ALS)-inhibiting herbicides and mixtures are registered in wheat to control *B. radians* (Table 1). As a result of intensive use of these herbicides, the first case of resistance to chlorsulfuron was reported in *Lactuca serriola* L. in 1987, just 5 years after its introduction into the market.[7] More than 107 species have now evolved resistant biotypes to the ALS inhibitors,[8] and multiple resistances have also been reported.[9]

Growers in the wheat belt of the Black Sea region noticed that ALS inhibitors were gradually losing their potency on *B. radians*. There is limited information on *B. radians* as a weed and its response to ALS inhibitors, let alone its putative tolerance development. Wheat crops of the region are part of the crop rotation in the area, and it is likely that intensive use of ALS-inhibiting herbicides causes flora shift in favour of less sensitive *B. radians* biotypes. Two adjacent fields with heavy infestation of *B. radians* were used: one had often been sprayed with ALS herbicides, and the other had received only half the number of ALS herbicide sprays over the years. The two objectives of this study were to compare the efficacy of the herbicides at different effect levels (ED_{50} and ED_{90}) between the two localities and then to estimate the relative potency between locations and test whether it differed from 1.00.

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Table 1. Herbicides and recommended field rates

Herbicide	Formulated product	Field rate of active ingredients (g ha^{-1})
Chlorsulfuron	Glean 75 DF	7.5
Thifensulfuron-methyl + tribenuron-methyl	Harmony extra	18.75 (12.5 + 6.25)
Triasulfuron + dicamba	Lintur 70 WG	87.62 (5.12 + 82.5)
Mesosulfuron-methyl + iodosulfuron-methyl-sodium	Atlantis WG	8 (6.67 + 1.33)
Tribenuron-methyl	Granstar WG	7.5

2 MATERIALS AND METHODS

2.1 Experimental site

Field experiments were conducted from 2005 to 2008 in the Havza (40° 51′ N, 35° 40′ E) district of Samsun Province in Turkey. The soil consisted of 14% sand, 45% silt, 41% clay and 1.9% organic matter, with a pH of 7.9. It has a severe continental climate, with cold winters and warm summers, and with an annual precipitation of 400 mm.

A field with high infestation of *B. radians* (20–40 plants m^{-2}) had received nine applications of sulfonylurea herbicides between 1990 and 2004, of which chlorsulfuron was the first one used in 1990 to control this species. Wheat had been grown in this field in rotation with maize (*Zea mays* L.), sugar beet (*Beta vulgaris* L.) and sunflower (*Helianthus annuus* L.) since 1990, and thus had been intensively sprayed with ALS inhibitors and other herbicides. The population of *B. radians* was denoted as tolerant.

A control experiment was conducted in a nearby field, also heavily infested with *B. radians*, 500 m from the field described above. This field had received only four applications of sulfonylurea herbicides, as opposed to the nine applications in the other field, and its population of *B. radians* was denoted as sensitive. The application time and other management practices were similar for the two experiments.

2.2 Field experiments

At the beginning of the experiment in 2005, the previous crop had been sugar beet and then wheat for the next 3 years. The fields were ploughed to a depth of 35 cm with a mouldboard plough, followed by two passes of a rototiller in the autumn. Wheat cultivar 'Bezostaja' was seeded with a double-disc drill on 5 November 2005, 10 November 2006, and 27 October 2007. The seed rate was 200 kg ha^{-1} to obtain the desired density of 250 plants m^{-2}. The experimental sites were fertilised according to soil test recommendations prior to and after wheat seeding. The field experiments were randomised complete blocks with four replicates, and an experimental unit was 2.1 by 8 m.

Treatments consisted of five herbicides, which are listed in Table 1 together with their recommended rates. The mesosulfuron-methyl + iodosulfuron-methyl-sodium mixture was applied with the safener mefenpyr-diethyl. The herbicides were applied with a compressed CO_2 backpack sprayer with 8004 flat-fan nozzles at 3 bar and calibrated to deliver a carrier volume of 200 L ha^{-1}. The herbicides were applied at the 2–4-, 4–6- and 6–8-true-leaf stages of *B. radians*, hereafter referred to as the two-, four- and six-true-leaf stages (Table 2). The date of spraying and the developmental stages of the crop among years are shown in Table 2. In order to cover the response range, the herbicide rates were untreated control and 0.5, 1.0, 1.5, 2.0, 3.0 and 6.0 times the recommended field rate of the products in Turkey (Table 1). The control experiment 500 m away was cultivated and seeded as in the experiment above, and the herbicide rates were untreated control and 0.5, 1.0, 1.5 and 2.0 times the recommended field rate of the products.

The above-ground biomass of *B. radians* was sampled from plots by using four 1 m^{-2} permanent quadrats 10 days after treatment (DAT). The samples were dried at 60 °C for 72 h. Visual assessment of percentage *B. radians* control was assessed at 14, 28 and 56 DAT, based on a scale of 0–100%, where 0% represents no control and 100% is complete plant kill.

2.3 Data analyses

Preliminary analyses of dose–response curves within years and stages of *B. radians* development showed that the responses were best described with a three-parameter asymmetric sigmoid Weibull curve, which has a slowly descending slope at small rates and a rapidly decreasing slope close to the lower limit of zero:[10]

$$y = D \cdot \exp\{-\exp[b \cdot \log(x) - \log(e)]\} \quad (1)$$

where y is the biomass (g m^{-2}), D is the upper limit (g m^{-2}) and b is the slope of the regression line around the inflection point, denoted by e. For visual measurement, the upper limit D was fixed at 100 in model 1. As the commonly used ED_{50} (effective dose required to cause 50% effect) is not a 'natural' parameter of the Weibull model, the ED_{50} and ED_{90} effect levels were derived using the delta method.[11] In order to stabilise the variance, a transform-both-sides technique was used.[12] The analysis of dose–response curves was conducted with the open-source program **R**, using the add-on package *drc,* which is described elsewhere.[11]

The simultaneous fitting of the 45 dose–response curves within each field yielded a total of 90 parameters. The fits were checked by residual plots.[12] Because of the large number of dose–response

Table 2. Herbicide application timing, dates of application and growth stage of weed and wheat at spraying

Number of true leaves of *B. radians*	2005–2006		2006–2007		2007–2008	
	Date of Spraying	Wheatgrowth stage[a]	Date of spraying	Wheat growth stage	Date of spraying	Wheat growth stage
2–4	17 February	16–17	21 February	17–18	22 February	17–18
4–6	3 March	24–25	8 March	25–26	16 March	24–25
6–8	18 March	29–30	22 March	30–31	21 March	28–29

[a] Zadok's two-digit code system for growth stages in wheat.

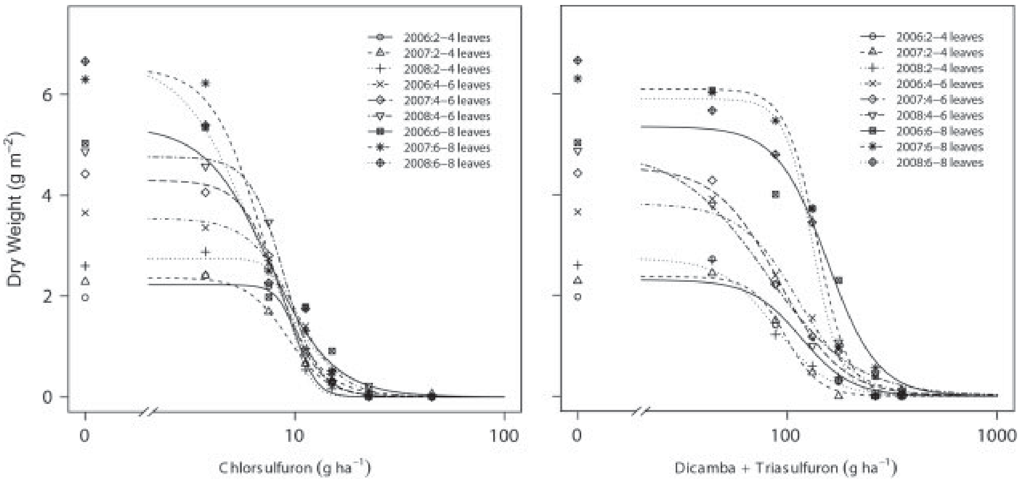

Figure 1. Three-parameter Weibull dose–response curves of the biomass of an alleged tolerant population of *B. radians*. Note that the untreated controls are the same for both graphs.

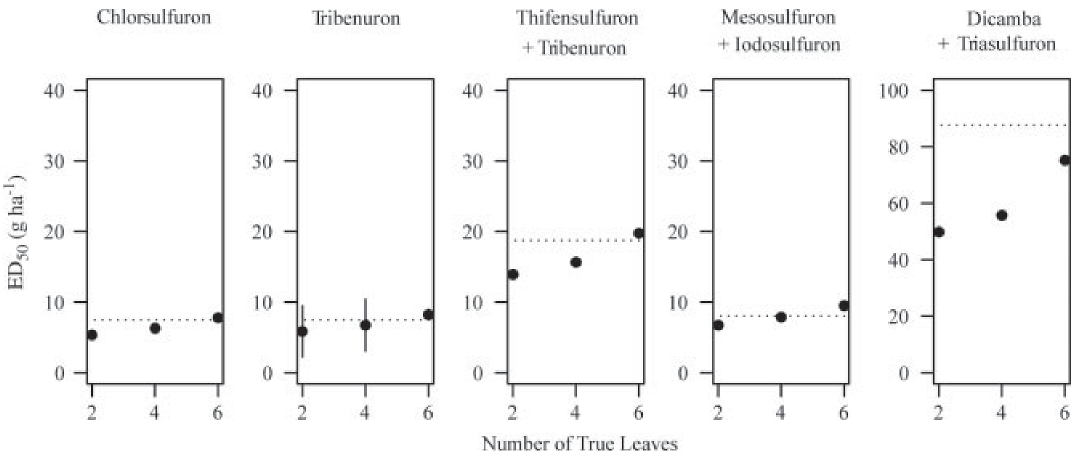

Figure 2. ED$_{50}$ in response to herbicides applied at various developmental stages of a *B. radians* tolerant population at 28 DAT, based on visual assessment. The 95% confidence intervals are shown. The horizontal dotted line represents the recommended field rate.

curves to be fitted simultaneously, it was not possible to apply a non-linear mixed model with years as random effects. The number of parameters was so large that convergence of the regression fit would either fail or not be satisfactory. Consequently, dose–response curves were fitted without any random effects, and subsequently the derived regression parameters of ED$_{50}$ and ED$_{90}$ were used for further analysis in the mixed model below:

$$\mathrm{ED}_x = A_i + B_j + C_k + \varepsilon \tag{2}$$

where the population A_i ($i = 1, 2$) and the herbicide ED$_x$ B_j ($j = 1, 2, 3, 4, 5$) are fixed effects, C_k is the random effect of year ($k = 1, 2, 3$) and ε is the error term. The standard errors of the ED$_{50}$ and ED$_{90}$ parameters were used as weights in model (2).

3 RESULTS AND DISCUSSION

The dose–response curves of the tolerant population on chlorsulfuron and the triasulfuron + dicamba mixture in Fig. 1 show that the choice of equation (1) with a rapid decrease close to the lower limit of zero was able in most cases to fit to the biomass at high herbicide rates.

Development of efficacy was evaluated at the ED$_{50}$ and ED$_{90}$ levels by visual assessment at 14, 28 and 56 DAT (Figs 2, 3 and 4). By comparing the herbicides in Fig. 2, the pattern of efficacy was the same for all herbicides. The number of leaves of the weeds increased ED$_{50}$, depending upon the herbicides. The ED$_{50}$ for the two herbicides chlorsulfuron and tribenuron on their own and for the mesosulfuron + iodosulfuron mixture did not change much. For the thifensulfuron + tribenuron and dicamba + triasulfuron mixtures there was a marked increase in ED$_{50}$ as a function of the number of true leaves. These two herbicide mixtures had ED$_{50}$ values mostly below the recommended rates given in Table 1. Also, it is noted that the 95% confidence intervals for tribenuron were large compared with the others. The pattern of ED$_{50}$ was the same for the assessment 14 and 56 DAT (data not shown).

The agronomically interesting ED$_{90}$ levels in Fig. 3 were all above the recommended rates, and, the larger the weeds, the higher the rates. The most pronounced difference was for the three mixture products. Consequently, the species could not be effectively controlled at the recommended herbicide rates. A comparison of the ED$_{50}$ and ED$_{90}$ control levels in Figs 2 and 3 clearly shows that the confidence intervals in Fig. 3 are far bigger than those in Fig. 2. This indicates that the ED$_{50}$ levels were more precisely estimated than the ED$_{90}$ levels, a well-known phenomenon in

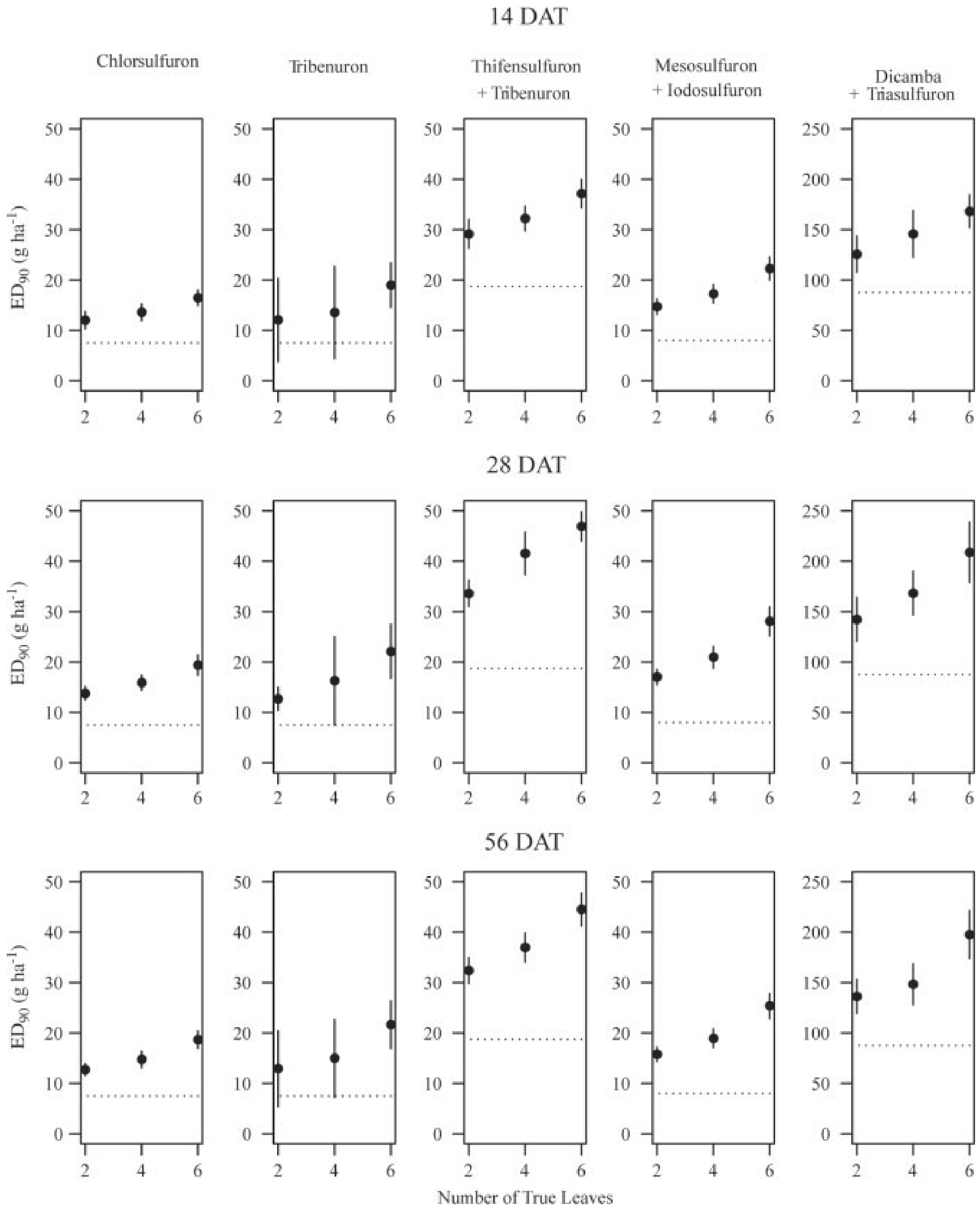

Figure 3. ED_{90} in response to herbicides applied at various growth stages of a *B. radians* tolerant population at 14, 28 and 56 DAT, based on visual assessment. Symbols as in Fig. 2.

regression; the further away from the mid-section, the higher is the variability of predicted responses. A comparison of the ED_{90} for chlorsulfuron and the triasulfuron + dicamba mixture in Fig. 3 with the biomass in Fig. 1 illustrates that the results are almost identical; up to 15–20 g ha^{-1} of chlorsulfuron were required for effective control of *B. radians*. This is more than twice the recommended rate of 7.5 g ha^{-1}. The same applies to the triasulfuron + dicamba mixture: 150–200 g ha^{-1} were required, which were almost double the recommended rate of 88 g ha^{-1}.

For the population that had not been continuously sprayed with ALS inhibitors (Fig. 4), the ED_{90} values were very close to the recommended rate, particularly at the two-true-leaf stage. As was the case for the tolerant population, the assessment 14 DAT was rather similar to the later assessments at 28 and 56 DAT. Notably, the change in ED_{90} values in responses to the developmental stage of the weed was less pronounced compared with those in Fig. 3. The relationship between the ED_{50} (data not shown) and the ED_{90} (Fig. 4) was the same as for the tolerant population.

In order to compare the differences between the populations, the relative potency between the herbicides was calculated, with the allegedly sensitive populations as reference (Table 3). The relative potency at both efficacy levels was in all cases larger than 1.00, indicating that there was a difference in the sensitivity between the two populations. At the ED_{50} levels, the relative

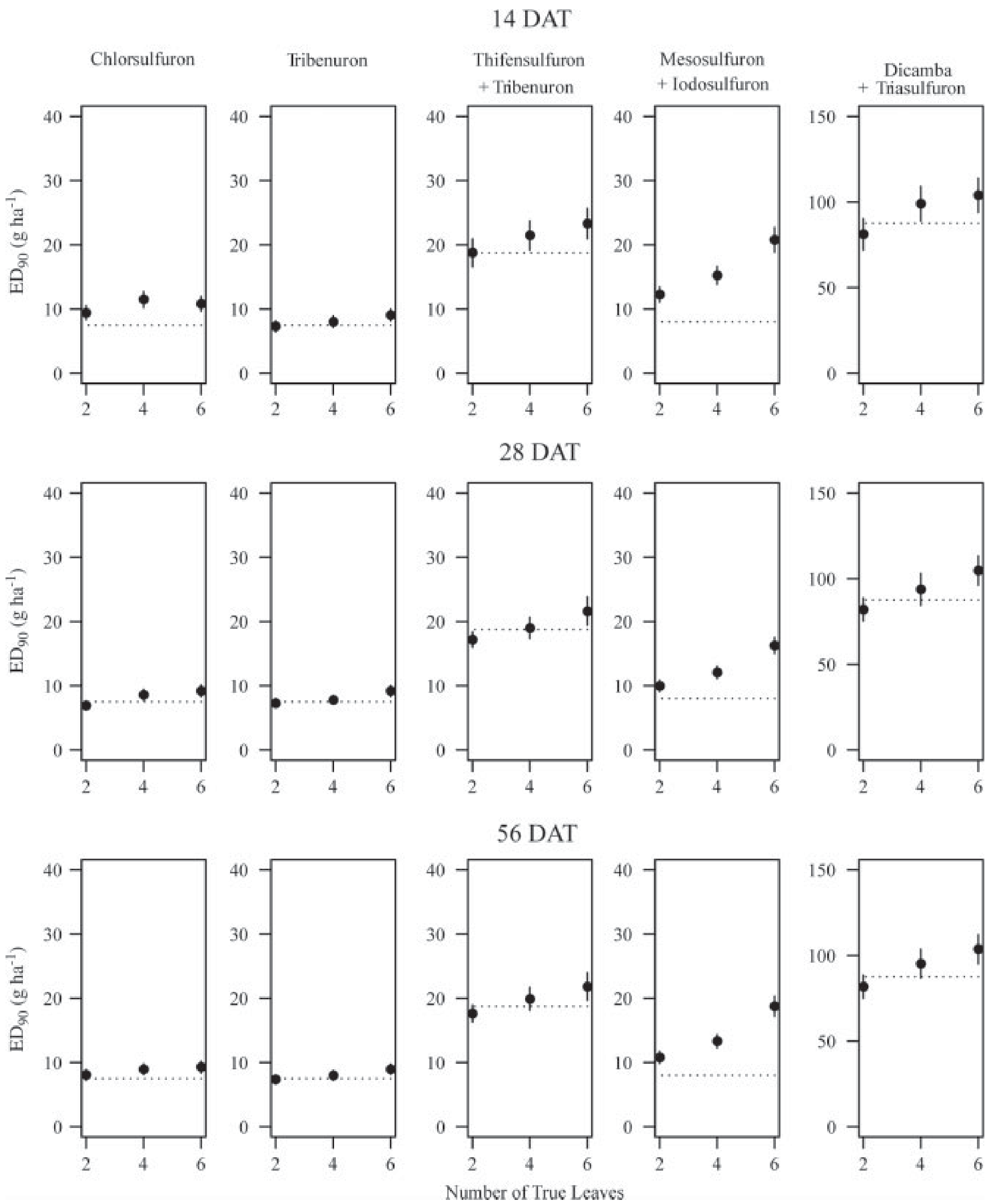

Figure 4. ED_{90} in response to herbicides applied at various stages of a *B. radians* sensitive population at 14, 28 and 56 DAT, based on visual assessment. Symbols as in Fig. 2.

potencies for more than half the comparisons were significantly different from 1.00. For the relative potencies at the ED_{90} level, the size was in all instances greater than 1.00 also, but not significantly different from 1.00. This corresponded to the variability in Figs 3 and 4 compared with Fig. 2. At ED_{90} levels, the variability was higher than at ED_{50} At the ED_{90}, the relative potencies were 1.4–2.4 (Table 3), not a dramatic change, but apparently enough to alert growers of the region because of poor control of *B. radians* at recommended rates. In some cases the relative potency increased marginally when spraying was done later on older weeds (Table 3), but in no instance was this trend significantly different. With these rather small relative potencies, a genuine ALS resistance could be ruled out. If rates are to be increased further in the coming years because of poor performance, the tolerance may turn into genuine evolved resistance.

In spite of the widespread use of the ALS herbicides for almost three decades, emergent herbicide tolerance in *B. radians* indicated a shift in general sensitivity owing to selection for natural tolerance. The evolution of genuine resistance associated with strong selection pressure on *B. radians* is not yet a well-researched area, perhaps because this species has not been reported to be a problem weed in temperate regions, where the number of weed scientists is high. Consequently, the findings cannot be compared with other experiments, but it is possible to compare them with publications dealing with relative herbicide potency in other weed species. Tolerance to auxin herbicides fluroxypyr and

Table 3. Relative potency ($Weed_{tolerant}/Weed_{sensitive}$) at ED_{50} and ED_{90}. If the relative potency is greater than 1.00, tolerant weeds require higher rates than sensitive weeds. Note that the relative potency at ED_{90} is not different from 1.00 in any case. NS = not significantly different from 1.00

Herbicide	Development stage	Relative potency at ED_{50}	95% Confidence interval	Relative potency at ED_{90}	95% Confidence interval
Chlorsulfuron	2–4	1.9	1.5–2.3	1.6	NS
Chlorsulfuron	4–6	1.8	1.5–2.1	1.6	NS
Chlorsulfuron	6–8	2.3	1.8–2.7	2.0	NS
Dicamba + triasulfuron	2–4	1.4	NS	1.7	NS
Dicamba + triasulfuron	4–6	1.2	NS	1.6	NS
Dicamba + triasulfuron	6–8	1.5	NS	1.9	NS
Mesosulfuron-methyl + iodosulfuron-methyl-sodium	2–4	1.5	1.3–1.7	1.5	NS
Mesosulfuron-methyl + iodosulfuron-methyl-sodium	4–6	1.5	1.2–1.7	1.4	NS
Mesosulfuron-methyl + iodosulfuron-methyl-sodium	6–8	1.5	1.1–1.8	1.4	NS
Thifensulfuron-methyl + tribenuron-methyl	2–4	2.0	1.3–2.6	1.8	NS
Thifensulfuron-methyl + tribenuron-methyl	4–6	1.7	NS	1.9	NS
Thifensulfuron-methyl + tribenuron-methyl	6–8	2.0	NS	2.0	NS
Tribenuron-methyl	2–4	1.7	NS	1.8	NS
Tribenuron-methyl	4–6	1.8	NS	1.9	NS
Tribenuron-methyl	6–8	2.0	1.8–2.2	2.4	NS

mecoprop has been reported in *Galium* spp.[13,14] Froud-Williams and Ferris-Kaan[15] reported the ED_{50} of mecoprop on selected *G. aparine* populations from four localities in the United Kingdom. Using the most sensitive population as reference, the range of relative potencies was between 1.0 and 4.7; however, there were no descriptions of how they arrived at the ED_{50} and no estimates of standard errors. Hill and Courtney[16] also looked at populations of *G. aparine* from five European countries. On the basis of fluroxypyr ED_{50}, the relative potencies, using the most sensitive population as reference, ranged from 1.0 to 1.8, but again with no report on their associated standard errors. Hubner *et al.*[17] compared the sensitivity in *G. aparine* to mecoprop in Norwegian localities and two accessions from Belgium and Germany. On the basis of the most sensitive accession, the relative potencies ranged from 1.00 to 1.98, but again with no associated standard error.

Although the present comparison was among different herbicides and two populations of *B. radians* with different spray and crop histories, the range of relative potencies in *G. aparine* corresponded to that shown in Table 3. Taking the average relative potencies at ED_{50} and ED_{90} in Table 3, the authors arrived at the very same relative potencies, 1.72 and 1.77 respectively. This indicated that, even though the relative potency at the ED_{90} level was not significantly different from 1.00, the overall relative potencies at the ED_{50} and the ED_{90} levels did not change much and thus were not different from each other. Consequently, the changes between the tolerant and sensitive *B. radians* populations were of the same order of magnitude as in the *G. aparine* papers.

B. radians has a diverse genetic background that gives it the ability to adapt to different environments with high reproductive capacity, rapid seed bank turnover and genetic and phenotypic plasticity.[18] Therefore, intelligent use of crop rotation, growing wheat cultivar with high competitive ability and timely control with herbicides of different modes of action are important to avoid undue selection of tolerant biotypes. The challenge ahead is to establish how much difference in relative potency is necessary to alert growers to reduced effects on problem weeds. In the greenhouse, precise measurements are possible, but in the field, with yearly variation, imprecise estimates are usually obtained. One avenue to take is to use inexpensive designs with logarithmic sprayers to obtain the response curves and subsequently calculate the potencies of different weed populations suspected to have changed their sensitivity to the herbicides.[19]

In summary, visual assessment of herbicide efficacy at 14 DAT gave the same results as assessment at 28 or 56 DAT. The relationship between ED_{50} and ED_{90} was the same for the sensitive and the tolerant populations. Obviously, the changes in efficacy of the mixture with dicamba were more sensitive to the developmental stage of the weed than in the case of the other herbicides. The relative potencies showed that the allegedly tolerant populations required up to twice the field rate in order to get the same efficacy. These relative potencies seem small, but in the field it is enough to make growers aware of the changes in potency when biotypes of *B. radians* slowly become more tolerant to the registered herbicides. The challenge ahead is to establish how much difference in relative potency is necessary in the field to alert growers to reduced effects. The principles in this paper of using the relative potency with associated standard errors could by used for other problem weeds and finally provide evidence of which size of relative potencies would indicate emerging evolution of resistant weed biotypes.

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