

The effects of reduced doses and application timing of metribuzin on redroot pigweed (*Amaranthus retroflexus* L.) and wild mustard (*Sinapis arvensis* L.)

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Received: 13.05.2009

Abstract: Minimum doses of metribuzin that provide satisfactory control (>90%) of redroot pigweed (*Amaranthus retroflexus* L.) and wild mustard (*Sinapis arvensis* L.), 2 troublesome weeds of potato crops planted in spring and autumn, respectively, were assessed. Greenhouse experiments were conducted using 100%, 80%, 60%, 40%, 20%, and 0% of the registered dose (525 g a.i. ha⁻¹) of metribuzin applied to the weeds grown in plastic pots. Dry weight data from the experiments were subjected to nonlinear regression analysis and the effective doses of metribuzin that caused a 90% reduction in dry weight (ED₉₀) were estimated. Metribuzin at doses of 240.8 and 293.2 g a.i. ha⁻¹ resulted in a 90% reduction in the dry weight of *A. retroflexus* when applied at the BBCH 12-15 and BBCH 16-19 growth stages, respectively. Metribuzin at doses of 183.7 and 256.2 g a.i. ha⁻¹ provided 90% control of *S. arvensis* at the BBCH 12-15 and BBCH 16-19 growth stages.

Key words: Dose-response curve, metribuzin, minimum dose, satisfactory control efficiency, weed development stage

Metribuzinin azaltılmış doz ve uygulama zamanlarının, kırmızı köklü horozibiği (*Amaranthus retroflexus* L.) ve yabancı hardal (*Sinapis arvensis* L.)'a etkisi

Özet: Bu çalışma ile, ilkbahar ve sonbahar patates dikim alanlarında sorun olan kırmızı köklü horozibiği (*Amaranthus retroflexus* L.) ve yabancı hardal (*Sinapis arvensis* L.)'in mücadelesinde tatmin edici kontrol (% > 90) sağlayan, minimum metribuzin dozları belirlenmiştir. Denemeler kontrollü sera koşullarında, plastik saksılar içerisinde yetiştirilen yabancı otlara metribuzinin önerilen dozunun (525 g a.i. ha⁻¹) % 100, % 80, % 60, % 40, % 20 ve % 0 oranındaki dozlarının uygulanması ile gerçekleştirilmiştir. Çalışmalar sonucunda elde edilen bitki kuru ağırlıkları kullanılarak doza-tepki eğrileri çizilmiş ve bu eğriler yardımı ile yabancı otların mücadelesinde % 90 (ED₉₀) oranında başarı sağlayan herbisit dozları tahmin edilmiştir. Yapılan çalışmalar sonucunda, *A. retroflexus*'ün BBCH 12-15 gelişme dönemindeki mücadelesinde 240.8 g e.m. ha⁻¹, BBCH 16-19 gelişme döneminde ise 293.2 g e.m. ha⁻¹ metribuzinin % 90 kontrol sağlayacağı belirlenmiştir. Diğer yabancı ot türü *S. arvensis*'de ise, BBCH 12-15 gelişme döneminde 183.7 g e.m. ha⁻¹, BBCH 16-19 gelişme döneminde ise 256.2 g e.m. ha⁻¹ metribuzinin % 90 oranında kontrol sağlayacağı belirlenmiştir.

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Introduction

Most of the benefits of pesticides are based only on direct crop returns; however, excessive and continuous use of agrochemicals negatively affects agricultural production, reduces agricultural sustainability, damages the environment, and is harmful to human health. To facilitate the development and implementation of an effective pesticide use policy, these costs must be examined (Pimentel et al. 1992). Herbicides significantly assist the maintenance and security of crop yields, and are therefore compulsory in modern arable farming. However, there is an urgent need to optimize herbicide use in order to minimize the possible adverse effects on the environment, and to ensure that herbicides remain an effective and valuable tool to farmers in the future (Kudsk and Streibig 2003). On the other hand, low commodity prices, crop damage, the problem of herbicide-resistant weeds, and rising concern about the effects of herbicides on the environment and human health are forcing growers to reconsider how best to manage weeds (Blackshaw et al. 2006).

Reducing the label rates of herbicides is becoming an important tool. Registered doses of herbicides are set to ensure adequate weed control of a wide spectrum of weed species, weed densities, growth stages, and environmental conditions (Zhang et al. 2000). This shows that herbicide rates can vary and can be reduced according to the weed spectrum, density, growth stages, and environmental conditions. Not surprisingly, numerous studies report that satisfactory weed control can often be obtained when herbicides are used at doses below label recommendations (Steckel et al. 1990; Doğan and Hurlle 1997; Vitta et al. 2000; Walker et al. 2002; Cheema et al. 2003; Auskalnis and Kadzys 2006; Barros et al. 2007). Previous studies indicate that many weeds, such as *Polygonum convolvulus* L., *Fallopia convolvulus* (L.) Á.Löve, *Galium spurium* L., *Thlaspi arvense* L., *Chenopodium album* L., *Avena sativa* L., and *Brassica napus* L., can be controlled with reduced doses of herbicides (Kjær 1994; Andersson 1995; Ketel et al. 1996; Brain et al. 1999). Crop competitiveness is also important in weed management; potato (*Solanum tuberosum* L.) grows rapidly and suppresses weeds that emerge 4 weeks

after planting (Thakral et al. 1989). This is why pre-planting and early post-emergence herbicides constitute an important part of weed management in potato production areas.

Potato, with 19,327,261 ha of production in 2007, is the fourth largest fresh crop in the world, following wheat, rice, and corn (FAO 2008). In addition to its large production area, potato has a wide spectrum of usage areas, an important issue in terms of reducing the use of herbicides. Metribuzin is one of the most commonly used herbicides in Turkey and the Turkish Republic of Northern Cyprus for controlling weeds in potato, and currently farmers do not consider using minimum doses.

It is crucial that growers are informed about the adverse effects of herbicides, and the importance and practicability of using reduced doses. This is also important for ensuring that herbicides remain an effective tool for farmers in the future. Therefore, the present study aimed to evaluate the use of post-emergence metribuzin at reduced doses to control redroot pigweed (*Amaranthus retroflexus* L.) and wild mustard (*Sinapis arvensis* L.), 2 troublesome weeds of potato crops planted in spring and autumn that cause substantial yield losses.

Materials and methods

Materials

Metribuzin was tested in this study, the most common herbicide for potato fields in Turkey and the Turkish Republic of Northern Cyprus. Metribuzin (Sencor WP 70, 700 g a.i. kg⁻¹, WP, BAYER) is a pre-emergence and post-emergence herbicide for potato, and 525 g a.i. ha⁻¹ is the recommended dose. It belongs to the triazinones group, which affects photosystem II site absorption by roots and shoots, but is transported only in the xylem (Monaco et al. 2002). Symptoms of herbicide injury appear quickly and susceptible plants die due to a build-up of highly reactive molecules that destroy cell membranes (Peterson et al. 2001). The test weeds, redroot pigweed (*Amaranthus retroflexus* L.) and wild mustard (*Sinapis arvensis* L.), are 2 troublesome weeds of potato planted in spring and autumn, respectively, and cause substantial yield losses (Üstüner and Günçan 2002; Danijela and Zoran 2004). Clay-loam soil, with moderate organic matter content

(2.3%), moderate lime content (12.2%), and a pH of 7.8, was collected from untreated potato fields for use in pot trials.

Experimental design, treatments, and measurements

Minimum doses of metribuzin were tested on *A. retroflexus* and *S. arvensis* at the BBCH 12-15 and BBCH 16-19 growth stages. The experiments, for both weeds and leaf stages, were conducted separately and repeated 2 times. The experiments were carried out in 700-mL plastic pots with 6 replicates, each containing ~800 g of soil. The pots were arranged in a completely randomized block design in controlled greenhouses.

Soil was sifted with a 2-mm sieve and air-dried for 72 h (Rainbolt et al. 2001). For each rate, 10 seeds were planted in each pot; when the weeds reached the mentioned growth stages, 2 healthy and uniform weeds were left. Pots taken out from the greenhouses and 6 replicates of each treatment were arranged in a 5-m² area. The herbicide was applied at 100%, 80%, 60%, 40%, 20%, and 0% (control) of the recommended dose. The herbicide was delivered at a spray volume of 300 L ha⁻¹ using 304-kPa spray pressure. Pots were transferred into greenhouses after the herbicide was applied, regularly checked, and irrigated to maintain growth.

Plant height, herbicide symptoms, and the number of leaves were determined on post-application days 1, 3, 5, 7, 14, 21, and 28 (Uygur 1991). On day 28 the plants were cut at the soil surface and their fresh weight was determined. In order to determine dry weight, samples were dried at 105 °C for 24 h (Anderson 1930).

Statistical Analysis

Biomass data from the 2 experiments (individually and combined) were subjected to nonlinear regression analysis in order to describe the relationship between the weeds and herbicide doses. The log-logistic model was used to plot curves using the following equation that relates response *Y* (dry weight) to the herbicide rate (*x*) (Streibig et al. 1993):

$$Y = C + \frac{D - C}{1 + \exp\{b^*[\log(x) - \log(ED_{50})]\}}$$

where *C* is the lower limit, *D* is the upper limit, *b* is the slope, and *ED*₅₀ is the dose causing a 50% response. Thus, the effective dose of herbicide that caused a 90% reduction in the biomass (*ED*₉₀) was estimated from the dose-response curve (*ED*₉₀ = *ED*₅₀ × 9^{1/*b*}). ANOVA was used to obtain a lack-of-fit test because of replicates, comparing the 4-parameter logistic model to the more general one-way ANOVA model. *P* values greater than 0.05 indicate non-significant differences between the model and ANOVA, showing that the regression model is correct. R software was used for dose-response analysis (Ritz and Streibig 2007). To determine the effect of metribuzin on the true leaf number, the data obtained from the 2 experiments were combined and then analyzed. The true leaf number of the weeds was subjected to ANOVA and the means were separated using Duncan's multiple range test at *P* < 0.05.

Results

Effect on the true leaf number

When metribuzin was applied at reduced doses to *A. retroflexus* at the BBCH 12-15 growth stage, the mean true leaf number was 3.93. On post-application day 3 there were no significant differences between the metribuzin doses (Table 1), and the true leaf number of the plants exposed to the 3 highest doses (525, 420, and 315 g a.i. ha⁻¹) reached the maximum, and then remained constant, whereas there was a tendency for a decrease in weed control efficacy at the 2 lowest doses (210 and 105 g a.i. ha⁻¹), as compared to the control. On post-application day 28 the highest true leaf number was obtained from the control plants, which was nearly 2-fold that of the plants exposed to the lowest metribuzin dose (105 g a.i. ha⁻¹). These results show that *A. retroflexus* was very susceptible to metribuzin at the BBCH 12-15 growth stage and that 60% of the registered dose of metribuzin (315 g a.i. ha⁻¹) had a similar effect on *A. retroflexus* as did the registered dose. Additionally, 210 g a.i. ha⁻¹ of metribuzin produced a significant effect on *A. retroflexus*, and the true leaf number was 2.35-fold less than that observed with the control. Therefore, 210 g a.i. ha⁻¹ of metribuzin might be sufficient for controlling *A. retroflexus* at the BBCH 12-15 growth stage.

Table 1. Changes in the true leaf numbers of the *Amaranthus retroflexus* L. and *Sinapis arvensis* L. exposed to the reduced doses of metribuzin.

Metribuzin g a.i. ha ⁻¹	Average leaf numbers of the 2 experiments						
	day 1	day 3	day 5	day 7	day 14	day 21	day 28
<i>A. retroflexus</i> / BBCH 12-15 growth stages							
525	4.0 NS	4.1 NS	4.1 (b)	4.1 (cd)	4.1 (cd)	4.1 (c)	4.1 (d)
420	3.8 NS	4.0 NS	4.0 (b)	4.0 (d)	4.0 (d)	4.0 (c)	4.0 (d)
315	3.9 NS	4.0 NS	4.0 (b)	4.0 (d)	4.0 (d)	4.0 (c)	4.0 (d)
210	4.0 NS	4.2 NS	4.2 (ab)	4.4 (bc)	4.4 (c)	4.4 (c)	4.9 (c)
105	3.9 NS	4.1 NS	4.3 (ab)	4.8 (b)	5.4 (b)	5.6 (b)	6.5 (b)
Control	4.0 NS	4.2 NS	4.5 (a)	5.2 (a)	6.0 (a)	6.6 (a)	11.5 (a)
<i>A. retroflexus</i> / BBCH 16-19 growth stages							
525	8.1 NS	8.6 (b)	8.9 (c)	8.9 (c)	8.9 (d)	8.9 (d)	8.9 (d)
420	8.2 NS	9.1 (b)	9.1 (c)	9.1 (c)	9.1 (d)	9.1 (d)	9.1 (d)
315	8.3 NS	8.9 (ab)	8.9 (c)	8.9 (c)	8.9 (d)	8.9 (d)	8.9 (d)
210	8.2 NS	9.1 (ab)	9.1 (c)	9.6 (c)	12.1 (c)	13.9 (c)	15.1 (c)
105	8.1 NS	10.8 (ab)	10.8 (b)	12.9 (b)	15.1 (b)	19.4 (b)	25.2 (b)
Control	8.2 NS	12.5 (a)	12.5 (a)	16.6 (a)	22.6 (a)	32.0 (a)	39.3 (a)
<i>S. arvensis</i> / BBCH 12-15 growth stages							
525	3.8 NS	3.8 (b)	4.2 (b)	4.2 (b)	4.2 (c)	4.2 (bc)	4.2 (bc)
420	3.7 NS	3.7 (b)	4.1 (b)	4.1 (b)	4.1 (c)	4.1 (bc)	4.1 (bc)
315	3.7 NS	3.7 (b)	4.2 (b)	4.2 (b)	4.2 (c)	4.2 (bc)	4.2 (bc)
210	3.8 NS	3.8 (b)	4.2 (b)	4.2 (b)	4.2 (c)	4.2 (bc)	4.2 (bc)
105	3.7 NS	3.7 (b)	4.5 (a)	4.5 (a)	4.6 (b)	4.6 (b)	4.7 (b)
Control	3.8 NS	4.1 (a)	4.6 (a)	4.6 (a)	5.1 (a)	6.6 (a)	7.2 (a)
<i>S. arvensis</i> / BBCH 16-19 growth stages							
525	7.3 NS	7.7 NS	7.8 (b)	7.8 (b)	7.8 (b)	7.8 (c)	7.8 (c)
420	7.7 NS	7.8 NS	7.8 (b)	7.8 (b)	7.9 (b)	7.9 (c)	7.9 (c)
315	7.6 NS	7.6 NS	7.7 (b)	7.8 (b)	7.8 (b)	7.8 (c)	7.8 (c)
210	7.5 NS	7.6 NS	7.7 (b)	7.8 (b)	7.8 (b)	7.9 (c)	8.0 (c)
105	7.5 NS	7.6 NS	7.8 (b)	8.0 (ab)	8.2 (b)	8.6 (b)	9.2 (b)
Control	7.5 NS	7.7 NS	8.3 (a)	8.5 (a)	10.3 (a)	11.1 (a)	11.8 (a)

Values followed by the same letter or letters are not significantly different at a 5% level (Duncan multiple range test)

The mean true leaf number of *A. retroflexus* (exposed to metribuzin at the BBCH 16-19 growth stage) was 8.09 on the day of herbicide application. The true leaf number of the plants exposed to different doses began to differ from each other on day 3. The true leaf number of the plants exposed to the 3 highest doses (525, 420, 315 g a.i. ha⁻¹) reached the maximum on post-application day 3, and then remained constant. On post-application day 5 there were no significant differences between the 3 highest doses. The true leaf number of the plants exposed to 210 g a.i. ha⁻¹ of metribuzin was 1.70-

fold greater than that of the plants exposed to 315 g a.i. ha⁻¹ of metribuzin. Nevertheless, 7 days after herbicide application there were no significant differences between 210 g a.i. ha⁻¹ of metribuzin and the 3 highest doses. This means that 210 g a.i. ha⁻¹ of metribuzin negatively affected *A. retroflexus*, but could not stop its growth at the BBCH 16-19 growth stage; therefore, at the BBCH 16-19 growth stage it seems necessary to increase the metribuzin dose (compared to the BBCH 12-15 growth stage) to achieve greater efficacy, i.e. from 210 to 325 g a.i. ha⁻¹.

Metribuzin was applied to *S. arvensis* at the BBCH 12-15 growth stage. During the first 3 days following application of the herbicide only the control plants' true leaf number significantly increased at all metribuzin doses. From day 3 to 5 the true leaf number of the weeds continued to increase, and then remained constant at the 4 highest doses (525, 420, 315, and 210 g a.i. ha⁻¹). The increase observed during the initial post-application days may have been due to the growth habit of *S. arvensis* and timing of herbicide application. Roughly, on the day of herbicide application the weeds were just before the growth stage; therefore, the plants produced new true leaves and grew until metribuzin showed its effect. In addition to the 4 highest doses, the lowest dose (105 g a.i. ha⁻¹) of metribuzin also had an effect similar to that of the other higher doses. This means that *S. arvensis* is very susceptible to metribuzin at the BBCH 12-15 growth stage. The mean true leaf number of the weeds exposed to the lowest metribuzin dose was nearly 35% less than that in the control plants. This is also an important parameter that highlights the sensitivity of *S. arvensis* to metribuzin at the BBCH 12-15 growth stage.

S. arvensis (BBCH 16-19 growth stage) plants had a mean 7.45 true leaves on the day metribuzin was applied. During the first 3 days post-application, no significant differences were observed between the treatments. On post-application day 5 the true leaf number of the control plants was significantly higher than that of the plants exposed to metribuzin. This implies that the effect of metribuzin on *S. arvensis* began on post-application day 5. There were no significant differences between the leaf number obtained with the 4 highest doses (525, 420, 315, and 210 g a.i. ha⁻¹) and, not surprisingly, the control plants had the highest number of leaves.

Dose-response models and minimum doses

The parameters (Table 2) of the dose-response curves (Figure 1) fitted for metribuzin show that both *A. retroflexus* and *S. arvensis* had higher slopes (*b*) at the BBCH 12-15 growth stage than at the BBCH 16-19 growth stage. Figure 1 shows mean dry weight measurements for the 2 experiments and the fitted curve. At earlier leaf stages in *A. retroflexus* and *S. arvensis* lower herbicide doses were sufficient to achieve acceptable control (90% reduction in the dry weight [ED₉₀]).

Table 2. Parameter estimates of non-linear regression analysis of metribuzin, effective doses causing 90% reduction in the dry weight and P-values for the comparison of model with ANOVA.

Weed species / leaf stage	Experiment	Parameter estimates				ED ₉₀	P-value
		C	D	<i>b</i>	ED ₅₀		
<i>A. retroflexus</i> / BBCH 12-15 growth stages	1	27.9 ± 5.8	233 ± 8.3	3.00 ± 0.7	113.6 ± 7.4	236.2 ± 27.1	0.948 NS
	2	30.7 ± 7.7	203 ± 10.5	3.07 ± 0.9	120.3 ± 12.0	245.8 ± 37.8	0.956 NS
	Combined	29.3 ± 4.3	218 ± 6.8	3.03 ± 0.6	116.6 ± 6.8	240.8 ± 20.9	0.909 NS
<i>A. retroflexus</i> / BBCH 16-19 growth stages	1	119.5 ± 7.3	2474 ± 99.5	2.01 ± 0.6	98.7 ± 10.2	294.3 ± 25.3	0.992 NS
	2	135.7 ± 12.3	2133 ± 91.4	2.27 ± 0.7	111.6 ± 11.0	292.7 ± 24.7	0.999 NS
	Combined	127.4 ± 10.1	2303 ± 69.0	2.13 ± 0.5	104.8 ± 7.6	293.2 ± 15.8	0.996 NS
<i>S. arvensis</i> / BBCH 12-15 growth stages	1	14.7 ± 0.9	135 ± 1.0	2.84 ± 0.3	85.4 ± 1.8	185.4 ± 10.6	0.618 NS
	2	14.7 ± 2.8	152 ± 2.8	2.63 ± 0.7	78.9 ± 5.8	182.0 ± 18.8	0.842 NS
	Combined	14.8 ± 1.7	144 ± 2.7	2.73 ± 0.4	82.1 ± 3.5	183.7 ± 18.4	0.624 NS
<i>S. arvensis</i> / BBCH 16-19 growth stages	1	94.3 ± 4.6	410 ± 6.5	1.68 ± 0.5	67.6 ± 6.1	250.0 ± 29.1	0.568 NS
	2	105.8 ± 7.3	446 ± 4.6	1.96 ± 0.3	84.7 ± 3.3	260.3 ± 27.3	0.997 NS
	Combined	100.2 ± 7.4	426 ± 5.6	1.83 ± 0.4	77.2 ± 5.1	256.2 ± 18.4	0.827 NS

C: the lower limit, D: the upper limit and *b*: the slope

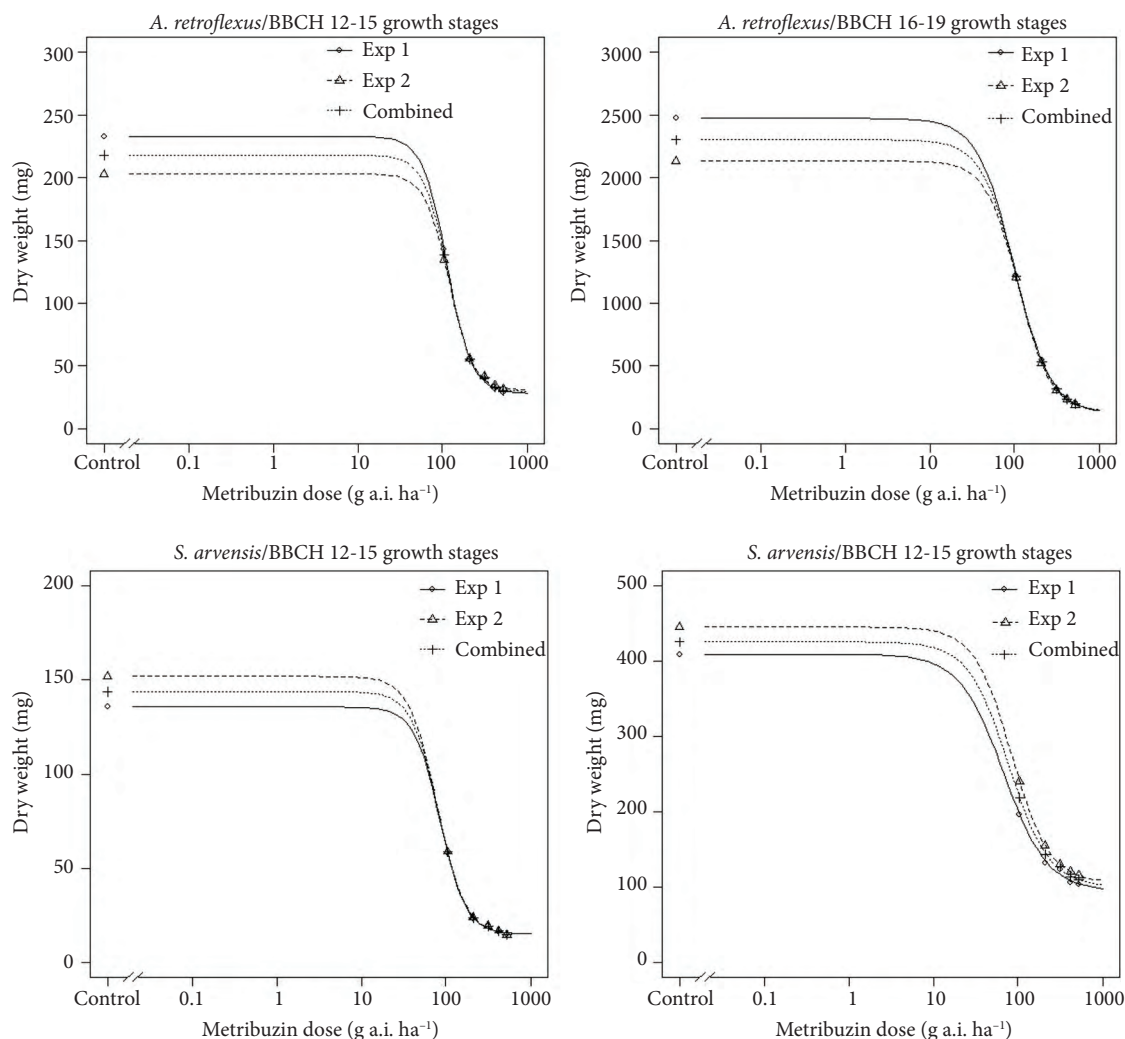


Figure 1. Relationships between metribuzin doses and dry weight of *A. retroflexus* and *S. arvensis* at BBCH 12-15 and BBCH 16-19 growth stages separately.

At the BBCH 16-19 growth stage the weeds were more tolerant to metribuzin, indicating its ineffectiveness at low doses; therefore, the slope was steeper at the BBCH 16-19 growth stage for both weeds. For *A. retroflexus* satisfactory control efficacy (>90%) was observed with 240.8 g a.i. ha⁻¹ at the BBCH 12-15 growth stage and with 293.2 g a.i. ha⁻¹ at the BBCH 16-19 growth stage. Metribuzin doses of 183.7 and 256.2 g a.i. ha⁻¹ resulted in satisfactorily control of *S. arvensis* at the BBCH 12-15 and BBCH 16-19 growth stages, respectively.

Discussion

Redroot pigweed (*Amaranthus retroflexus* L.) and wild mustard (*Sinapis arvensis* L.), which are major problem-causing weeds in potato crops planted in spring and autumn respectively, were successfully controlled by early post-emergence metribuzin application under Mediterranean conditions. The results show that doses of metribuzin lower than those recommended by the manufacturer (525 g a.i. ha⁻¹) could be used in potato cultivation when *A. retroflexus* and/or *S. arvensis* are predominant. The results also show that metribuzin affected the weeds within 3-5 days of application and stopped weed

growth. *S. arvensis*, with a lower ED₅₀ and slope (*b*), was more sensitive to metribuzin than *A. retroflexus*. The present findings support the results of Medd et al. (2001), who reported that present weed species affect herbicide efficiency. To reduce the metribuzin dose, early application timing (BBCH 12-15 growth stage), when weeds are more sensitive, is necessary. This result is in accordance with the findings of Ketel and Lotz (1997) and Riethmuller-Haage et al. (2007), who reported that the metribuzin dose required for satisfactory control changes according to leaf area and the number of leaves. As plants grow, competitive capacity increases, decreasing the effects of herbicides (Dogan and Hurlle 1997; Auskalnis and Kadzys 2006; Reithmuller-Haage et al. 2007).

The present results also support the previous finding (Steckel et al. 1990; Dogan and Hurlle 1997; Vitta et al. 2000; Walker et al. 2002; Cheema et al.

2003; Auskalnis and Kadzys 2006; Barros et al. 2007) that satisfactory weed control can often be obtained when herbicides are used at doses below label recommendations. According to the results of the present study, it is clear that metribuzin doses can be reduced when *A. retroflexus* and/or *S. arvensis* are the predominant weeds in potato cultivation, reducing production costs. Additional research should be conducted on the effects of reduced metribuzin doses on different weed species.

Acknowledgements

The authors thank their colleagues at the Çukurova University Weed Science Laboratory for their valuable assistance during the study and the Research Fund of Çukurova University (Scientific Research Projects Unit) for its financial support.

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