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Sumatran Fleabane (*Conyza sumatrensis*) Resistance to Glyphosate in Peach Orchards in Turkey

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***Abstract*. Glyphosate has been widely used to control annual, perennial, and biennial weeds including *Conyza* species. *Conyza sumatrensis* (Sumatran fleabane) is considered a highly invasive and troublesome weed worldwide, including in European and Mediterranean regions. In Turkey, the use of glyphosate in orchards has recently increased; however, extensive use of glyphosate has resulted in poor control of *C. sumatrensis* in several peach orchards. The objectives of this research were to determine if *C. sumatrensis* is resistant to glyphosate and identify alternative herbicides with different modes of action that can be used instead of glyphosate. Two dose response studies were conducted in the greenhouse to evaluate the response of four *C. sumatrensis* populations to glyphosate, chlorsulfuron, and metribuzin. Glyphosate isopropyl amine and glyphosate potassium was applied at 0, 0.25, 0.5, 1, 2, 4, and 8 times the use rate of 1080 g a.e./ha (a.e. indicates acid equivalent) when the plants were at rosette (5–6 true leaves) and vegetative (20–22 cm tall) stages. Effects of both glyphosate formulations were combined. The resistant populations showed higher resistance 3.8 to 6.6 and 5.3 to 7.8 times at rosette stage and vegetative stage, respectively, compared with the susceptible population. Furthermore, glyphosate-resistant populations were treated with chlorsulfuron and metribuzin at 0, 0.25, 0.5, 1, 2, 4, and 8 times use rate of 7.5 and 350 g a.i./ha, respectively at the rosette stage. The glyphosate-resistant populations exhibited 2.4 to 3.8 times more resistance to chlorsulfuron, but were adequately controlled with metribuzin.**

Glyphosate [N-(phosphonomethyl)-glycine] is a systemic, nonselective, postemergence herbicide that controls more weed species than any other herbicide (Duke, 2018; Heap and Duke, 2018). It has been used to control annual, perennial, and biennial species of grasses, sedges, and broadleaf weeds (Dinelli et al., 2006). Glyphosate inhibits the enzyme 5-enolpyruvlshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of shikimate-3-phosphate and phosphoenolpyruvate to form 5-enolpyruvil-shikimate-3-phosphate (Fernandez et al., 2015; González-Torralva et al., 2012). Inhibition of EPSPS prevents the biosynthesis of phenylalanine, tryptophan, tyrosine, and other aromatic compounds in sensitive plants (Amaro-Blanco et al., 2018; Tahmasebi et al., 2018). In Turkey, glyphosate is the most widely used herbicide and is registered on more than 70 crops, including peach (Torun, 2017). In the past 5 years, the total amount of glyphosate sold in Turkey was ≈1.1 million kg of acid equivalent (Ministry of Agriculture and Forestry, 2018).

The application of glyphosate in crop and noncrop areas has resulted in decreased efficacy on several populations of three widespread species of the genus *Conyza* (Amaro-Blanco et al., 2018). These species include *C. bonariensis* (hairy fleabane), *C. canadensis* (horseweed), and *C. sumatrensis* [Sumatran fleabane (Syn. *C. albida*)]; there are at least 13 hairy fleabane, 42 horseweed, and 8 Sumatran fleabane cases of resistance reported in field crops, orchards, forests, pastures, urban areas, and nurseries around the world (Heap, 2018; Mylonas et al., 2014). Several glyphosate-resistant *Conyza* species have been reported in European and Mediterranean countries including France (Fernandez et al., 2015), Spain (Amaro-Blanco et al., 2018), Greece (Margaritopoulou et al., 2018), and Israel (Matzrafi et al., 2015). These species are native to the Americas (Amaro-Blanco et al., 2018) and considered as invasive and troublesome species in many parts of the world (Matzrafi et al., 2015). They are common weeds in orchards, row crops, roadsides, abandoned fields, and wasteland (Amaro-Blanco et al., 2018; Sansom et al., 2013) and occur in more than 70 countries (Holm et al., 1997). Currently, these *Conyza* species have become established in new territories including the Mediterranean basin (Amaro-Blanco et al., 2018) and are invading a variety of cropping systems (Tahmasebi et al., 2018).

In 2015, peach growers in Çanakkale Province of Turkey complained about a lack of glyphosate control of *Conyza* species. To date the only report of poor *Conyza* species control with glyphosate in Turkey was reported in citrus orchards in Adana, Mersin, and Hatay of Mediterranean region (Dogan et al., 2016). No research has been conducted to confirm and determine the level of resistance in these populations.

There are ≈56,000 ha of cherry, apple, pear, peach, and nectarine orchards in the Çanakkale Province in northwestern Turkey, which is considered one of the most important fruit and vegetable production areas in Turkey (TUIK, 2018). Currently, *C. sumatrensis* is considered as the most common troublesome weed in these orchards. Because of the poor control of *C. sumatrensis* with glyphosate, the objectives of this study were to confirm and identify the level of glyphosate resistance in *C. sumatrensis* and to determine the effect of chlorsulfuron (an acetolactate inhibitor) and metribuzin (a photosynthetic inhibitor) on glyphosate-resistant populations, which some farmers use to solve the problem despite their not being registered for use in orchards.

Materials and Methods

Plant material. Conyza seeds were collected from peach orchards where farmers reported a lack of control with glyphosate and from noncrop areas in the Çanakkale Province in northwestern Turkey. Herbicide application records were obtained from farmers (Table 1). Seeds were collected from three peach orchards that had been established for at least 10 years and from noncrop areas where glyphosate was not used at Çanakkale. Before the experiments commenced, plant species were identified by the Düzce University Herbarium (Table 1). The populations EYSAL-1, EYSAL-2, and EYYAP-3 were selected for this study because they were under the highest glyphosate selection pressure according to growers' records and herbicide use history. The susceptible population, KEPKO-1 was taken from a noncrop area with no recorded glyphosate use.

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Styrofoam seedling trays of 228 individual cells were filled with a sterilized mixture of 1:1:2 parts by volume of white sod peat, black peat, and white peat, then covered with a layer of vermiculite to preserve soil moisture. Each cell was seeded with ≈100 *C. sumatrensis* seeds, and trays were placed in a germination chamber under conditions of 25 ± 1 °C and 90 ± 3% relative humidity for 72 h. Three days after planting, the trays were transferred to the greenhouse under the following conditions: temperature 35/30 ± 3 °C day/night with 16/8-hour day/night periods; relative humidity was 65 ± 5% during the day and 70 ± 3% during the night. Seedling emergence was ≈35% to 45% for all populations; once the cotyledons reached 1 cm in height, plants were thinned to one plant per cell and uniform plants were selected for the dose response study. Plants were irrigated daily to maintain adequate soil moisture and fertilized weekly 0.8 L/m^2 with a solution containing 0.40 $mg \cdot L^{-1}$ nitrogen, 0.20 $mg \cdot L^{-1}$ phosphorus, and 0.40 $mg \cdot L^{-1}$ potassium.

Glyphosate dose–response study. EYSAL-1, EYSAL-2, EYYAP-3, and KEPKO-1 populations were treated with glyphosate at the rosette stage when plants had five or six true leaves and at the vegetative stage when plants were 20 to 22 cm in height. Glyphosate rates were 0, 0.25, 0.5, 1, 2, 4, and 8 times a typical use rate of 1,080 g a.e./ha (Table 2). Each population was separately treated with two formulations of glyphosate either isopropyl amine salt or potassium salt. Treatments were applied with a motorized backpack sprayer (SP126; Oleo-Mac Inc., Piano, Italy), calibrated to deliver 250 $L \cdot ha^{-1}$ at 166 kPa pressure using a Lechler ST-110-02 standard flat spray nozzle (Lechler Inc., Charles, IL). Injury ratings were recorded at 7, 14, and 21 d after treatment (DAT) based on a scale of 0 = no injury and 100 = mortality. Aboveground biomass were harvested at 21 DAT and dried at 65 °C for 72 h and weighed.

Chlorsulfuron and metribuzin dose–response study. EYSAL-1, EYSAL-2, EYYAP-3, and KEPKO-1 populations were treated with chlorsulfuron and metribuzin to determine whether these herbicides could be used to control glyphosate-resistant *C. sumatrensis* populations. Chlorsulfuron inhibits acetolactate synthase (ALS), and metribuzin inhibits photosynthesis at site A of Photosystem II (PSII). Plants were treated at the rosette stage with 0, 0.25, 0.5, 1, 2, 4, and 8 times a typical use rate of chlorsulfuron and metribuzin, 7.5 and 350 g a.i./ha, respectively. Experiments were conducted and data collected as described in the previous study.

Experimental Design and Data Analysis. All dose–response experiments were replicated 10 times, each experimental unit had 10 individuals, and studies were conducted twice. Data from glyphosate isopropyl amine and glyphosate potassium were combined because of their insignificant difference in variance analysis, but each application time regarding glyphosate growing level was analyzed separately. Data were analyzed using analysis of variance and nonlinear regression analysis to determine the herbicide rate required to cause 50% visible injury (GR_{50}) and 50% dry weight reduction (GD_{50}) as described by Seefeldt et al. (1995). Dose–response curves of the visible injury and dry weight for different populations were plotted as a percentage of the untreated control. GR_{50} and GD_{50} values were calculated, using the following [sigmoidal logistic, three parameters; SigmaPlot (ver. 11.0) software (Systat Software Inc., San Jose, CA] equation:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}$$

In the model, if $b > 0$, then a describes the upper limit of y. $X_0 = GR_{50}$ or GD_{50} (depending on visible injury or dry weight) and b describes the slope of the curve in GR_{50}, and GD_{50} (Seefeldt et al., 1995). Resistance index (RI) levels of glyphosate-resistance of all resistant *C. sumatrensis* populations were calculated by dividing of the GR_{50} and GD_{50} of the resistant populations by GR_{50} and GD_{50} of the susceptible control (Matzrafi et al., 2015; Mylonas et al., 2014). The results were considered as low ($2 \leq RI < 4$), medium ($4 \leq RI < 10$), and high ($10 \leq RI$) resistance levels to glyphosate (Mei et al., 2018).

Results and Discussion

Glyphosate dose–response study. Glyphosate injury symptoms were apparent on all *C. sumatrensis* populations, and visible injury increased as glyphosate rates increased across all treatments; however, the severity of symptoms was more visible in the KEPKO-1 population. Additionally, the duration to develop symptoms was shorter with KEPKO-1 population. Initial glyphosate injury symptoms were chlorosis and leaf curling followed by necrosis and stunting, but injured plants showed some recovery with slow growth within 14 DAT. The recovery was more apparent in EYYAP-3, EYSAL-1, and EYSAL-2 populations, respectively, with no observed recovery in the KEPKO-1 population. Symptoms were more severe when plants were treated at the rosette stage compared with the vegetative stage. *C. sumatrensis* visual injury and dry weight data were similar for isopropyl amine salt or potassium salt formulations of glyphosate; therefore, the data were combined (Figs. 1 and 2). When EYSAL-1, EYSAL-2, and EYYAP-3 populations were treated with glyphosate at rosette stage (smaller plants), GR_{50} rates based on visual injury symptoms were 4401, 3046, and 5346 g a.e./ha, respectively, whereas KEPKO-1 was 800 g a.e./ha. When EYSAL-1, EYSAL-2, and EYYAP-3 populations were treated with glyphosate at the vegetative stage (larger plants), GR_{50} rates were 6277, 5060, and 7460 g a.e./ha, respectively, whereas KEPKO-1 was 950 g a.e./ha (Fig. 1). The GR_{50} values for EYSAL-1, EYSAL-2, and EYYAP-3 clearly showed that these populations are more resistant to glyphosate compared with KEPKO-1. The range of glyphosate RI for EYSAL-1, EYSAL-2, and EYYAP-3 populations was 3.8 to 6.6 (low to medium) for rosette stage plants and 5.3 to 7.8 (medium) for vegetative stage plants (Table 3).

Table 1. Collection dates, geographical coordinates, location details, and herbicide use history for the four populations of *Conyza sumatrensis* used in this study.

Population	Collection date	Coordinate	Habitat	Herbicide use[z]
EYSAL-1	23 Aug. 2016	40°12′02.7″N; 26°32′49.2″E	Peach orchard	Glyphosate >5 years
EYSAL-2	25 Aug. 2016	40°11′59.5″N; 26°32′47.4″E	Peach orchard	Glyphosate >5 years
EYYAP-3	5 Sept. 2016	40°11′59.3″N; 26°32′34.1″E	Peach orchard	Glyphosate >8 years
KEPKO-1	25 Aug. 2016	40°06′45.5″N; 26°24′14.2″E	Empty area	None

[z]Glyphosate has been used in the orchard sites multiple times per year at over recommended doses.

Table 2. Glyphosate, chlorsulfuron, and metribuzin: main characteristics, use rate, and application period.

Herbicide	Group[z]	MOA[y]	Rate[x]	Application period
Glyphosate potassium salt	9/G	EPSPS	1,323 g a.i./ha = 1,080 g a.e./ha	Postemergence
Glyphosate isopropyl amin salt	9/G	EPSPS	1,440 g a.i./ha = 1,080 g a.e./ha	Postemergence
Chlorsulfuron	2/B	ALS	7.5 g a.i./ha	Postemergence
Metribuzin	5/C	PSII	350 g a.i./ha	Postemergence

[z]Herbicide group according to Weed Science Society of America and the Herbicide Resistance Action Committee.
[y]MOA: inhibitors of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), acetolactate synthase (ALS), and inhibitors of photosynthesis at photosystem II site A (PSII).
[x]Recommended herbicide use rate in Turkey, a.i. = active ingredient, a.e. = acid equivalent.
MOA = mode of action.

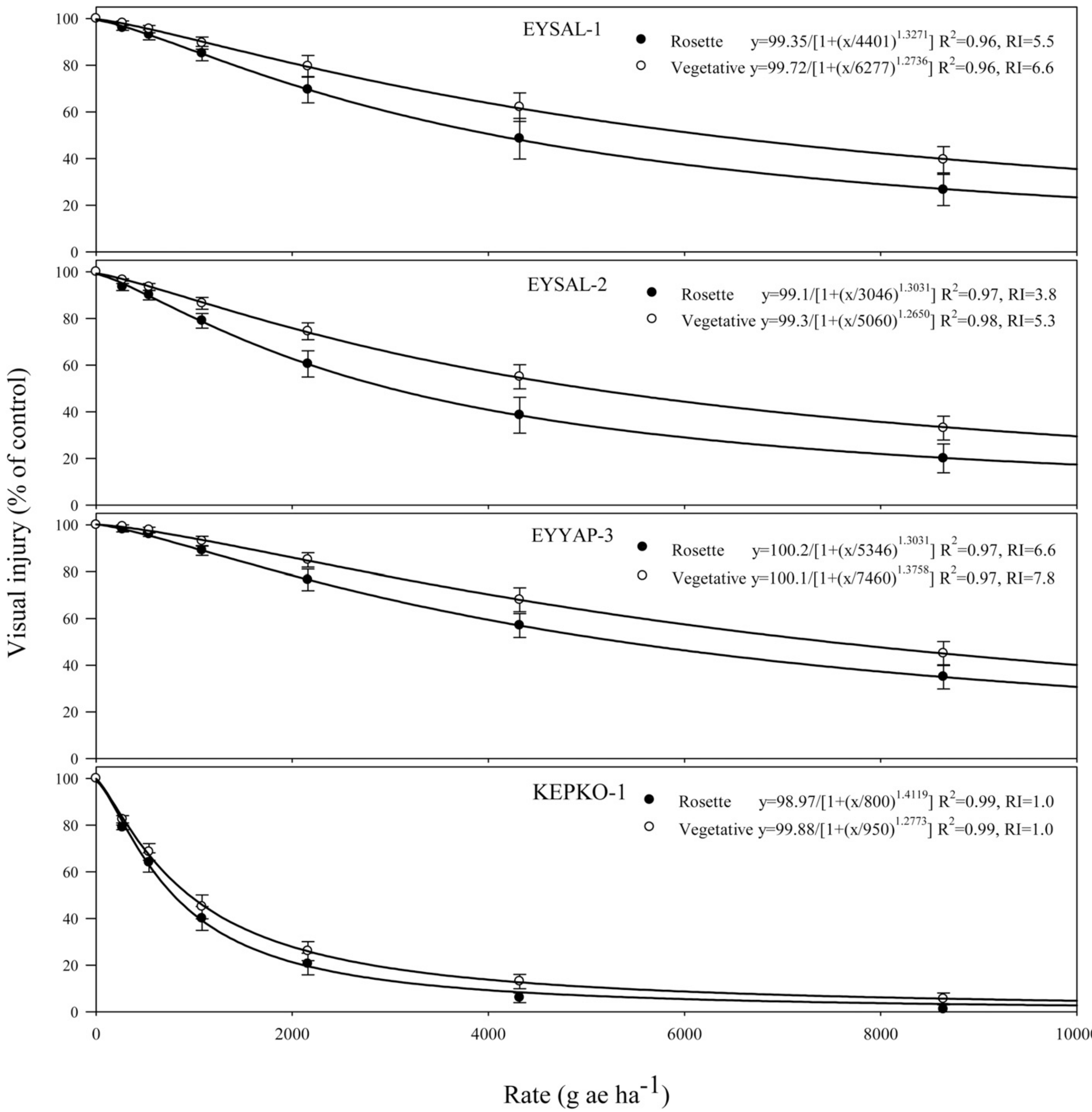

Fig. 1. Percent of control by visual injury at 21 d after treatment with glyphosate applied to four *Conyza sumatrensis* populations at rosette and vegetative stages. Resistance index (RI) was calculated by the ratio of the GR_{50} value of the resistant population (EYSAL-1, EYSAL-2, and EYYAP-3) to the GR_{50} of the susceptible population (KEPKO-1).

The reduction in *C. sumatrensis* dry weight in all populations after treatment with glyphosate showed similar patterns to visual injury ratings. When EYSAL-1, EYSAL-2, and EYYAP-3 were treated with glyphosate at the rosette stage, GD_{50} rates were 1502, 1821, and 1570 g a.e./ha, respectively and 1099 g a.e./ha in the KEPKO-1. When EYSAL-1, EYSAL-2, and EYYAP-3 were treated with glyphosate at the vegetative stage, GD_{50} rates were 4923, 5519, and 7925 g a.e./ha, respectively, whereas KEPKO-1 was 1,660 g a.e./ha (Fig. 2). The glyphosate resistance index range was 1.36 to 1.65 (low) for the rosette stage plants and 2.96 to 4.77 (low to medium) for the vegetative stage plants (Table 3).

Dose–response studies confirmed resistance of *C. sumatrensis* to glyphosate, and observed RI was similar to some earlier literature that reported the RI ranging between 6.1, and 8.38 (González-Torralva et al., 2012, 2014; Mei et al., 2018), but was less than some other earlier findings that the RI ranging 19.8 and 37.3 (Mylonas et al., 2014; Tahmasebi et al., 2018).

Regardless of formulation and rates, glyphosate injury symptoms were more severe in smaller (i.e., younger plants; rosette stage) than in larger (i.e., older plants; vegetative stage). The increase in glyphosate injury for younger plants was not surprising and similar to other previous reports (Hennigh et al., 2005; Schuster et al., 2007; Waite et al., 2013) because younger plants are metabolically more active, making them generally more susceptible to glyphosate (Waite et al., 2013). Similarly, the decrease in glyphosate injury at larger size may be due to the morphological and anatomical properties, such as a thicker cuticle, characterizing more mature plants (Waite et al., 2013; Wanamarta

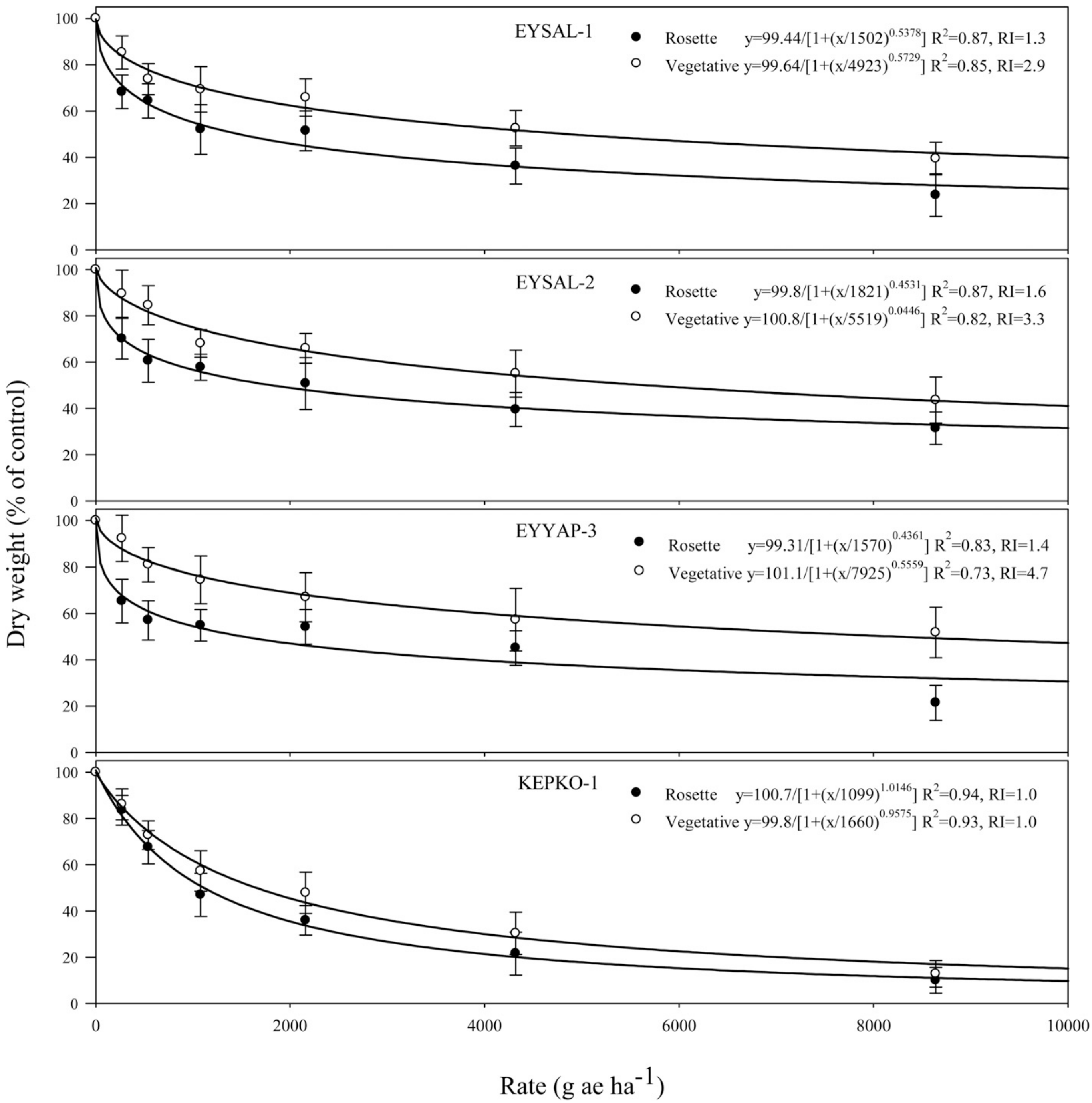

Fig. 2. Percent of control by dry weight at 21 d after treatment with glyphosate applied to four *Conyza sumatrensis* populations at rosette and vegetative stages. Resistance index (RI) was calculated by the ratio of the GD_{50} value of the resistant population (EYSAL-1, EYSAL-2, EYYAP-3) to the GD_{50} of the susceptible population (KEPKO-1).

Table 3. Glyphosate required to cause 50% visual injury (GR_{50}) and 50% dry weight reduction (GD_{50}) for the four *Conyza sumatrensis* populations at 21 d after treatment at two growth stages.

	Rosette stage				Vegetative stage			
			Resistance index[y]				Resistance index	
Population	GR_{50}[z]	GD_{50}[z]	GR50	GD50	GR_{50}[z]	GD_{50}[z]	GR50	GD50
EYSAL-1	4,401 (g a.e./ha)	1,502	5.50	1.36	6,277 (g a.e./ha)	4,923	6.60	2.96
EYSAL-2	3,046 (g a.e./ha)	1,821	3.80	1.65	5,060 (g a.e./ha)	5,519	5.32	3.32
EYYAP-3	5,346 (g a.e./ha)	1,570	6.68	1.42	7,460 (g a.e./ha)	7,925	7.85	4.77
KEPKO-1	800.0 (g a.e./ha)	1,099	1.00	1.00	950.0 (g a.e./ha)	1,660	1.00	1.00

[z]GR_{50} (glyphosate rate required to cause 50% visual injury) and GD_{50} (glyphosate rate required to cause 50% dry weight reduction) values were calculated from dose–response study. Glyphosate was applied at 0, 270, 540, 1080, 2160, 4320, and 8640 g a.e./ha (a.e. = acid equivalent).

[y]Resistance index was calculated as the ratio of the GR_{50} or GD_{50} value of the resistant population to the GR_{50} or GD_{50} of the susceptible population (KEPKO-1).

and Penner, 1989). This phenomenon has been confirmed in *Conyza bonariensis* and *C. canadensis*, which was most likely achieved via sequestration of the herbicide molecule (Shaner et al., 2012). The mechanisms of glyphosate resistance include reduced uptake and/or translocation, enhanced detoxification of the glyphosate molecule, expression of an insensitive form of EPSPS, amplification of the EPSPS gene, or two codon changes in EPSPS (Dill, 2005; Sammons et al., 2018; Shaner, 2014).

Chlorsulfuron and metribuzin dose–response study. Overall, chlorsulfuron visual injury increased as rates increased; injury symptoms were apparent on all populations, but the severity of symptoms was greater on KEPKO-1, the susceptible population. Chlorsulfuron symptoms were chlorosis and leaf malformation followed by necrosis. When EYSAL-1, EYSAL-2, and EYYAP-3 populations were treated with chlorsulfuron at the rosette stage, GR_{50} rates were 11.9, 9.1, and 14.1 g a.i./ha, respectively, whereas KEPKO-1 was 3.7 g a.i./ha (Fig. 3). The GR_{50} values for EYSAL-1, EYSAL-2, and EYYAP-3 illustrated that these populations are more resistant to chlorsulfuron compared with the susceptible KEPKO-1 population. The range of chlorsulfuron resistance index (RI) for EYSAL-1, EYSAL-2, and EYYAP-3 populations was 2.4 to 3.8 for rosette stage plants (Table 4).

The reduction in *C. sumatrensis* dry weight for all populations after treatment with chlorsulfuron showed response patterns similar to visual injury ratings. When EYSAL-1, EYSAL-2, and EYYAP-3 at the rosette stage were treated with chlorsulfuron, GD_{50} rates were 112.5, 93.3, and 23.8 g a.i./ha, respectively, and 8.8 g a.i./ha in the KEPKO-1 (Fig. 3). The chlorsulfuron resistance index range was 2.6 to 12.7 for rosette stage plants (Table 4). RI, based on dry weight reduction (GD_{50}), clearly showed that the glyphosate-resistant populations were not effectively controlled by chlorsulfuron at tested rates; however, visual symptoms by chlorsulfuron application were more severe than glyphosate symptoms. Injured plants did not show same recovery as glyphosate-treated plants did. In similar studies conducted with

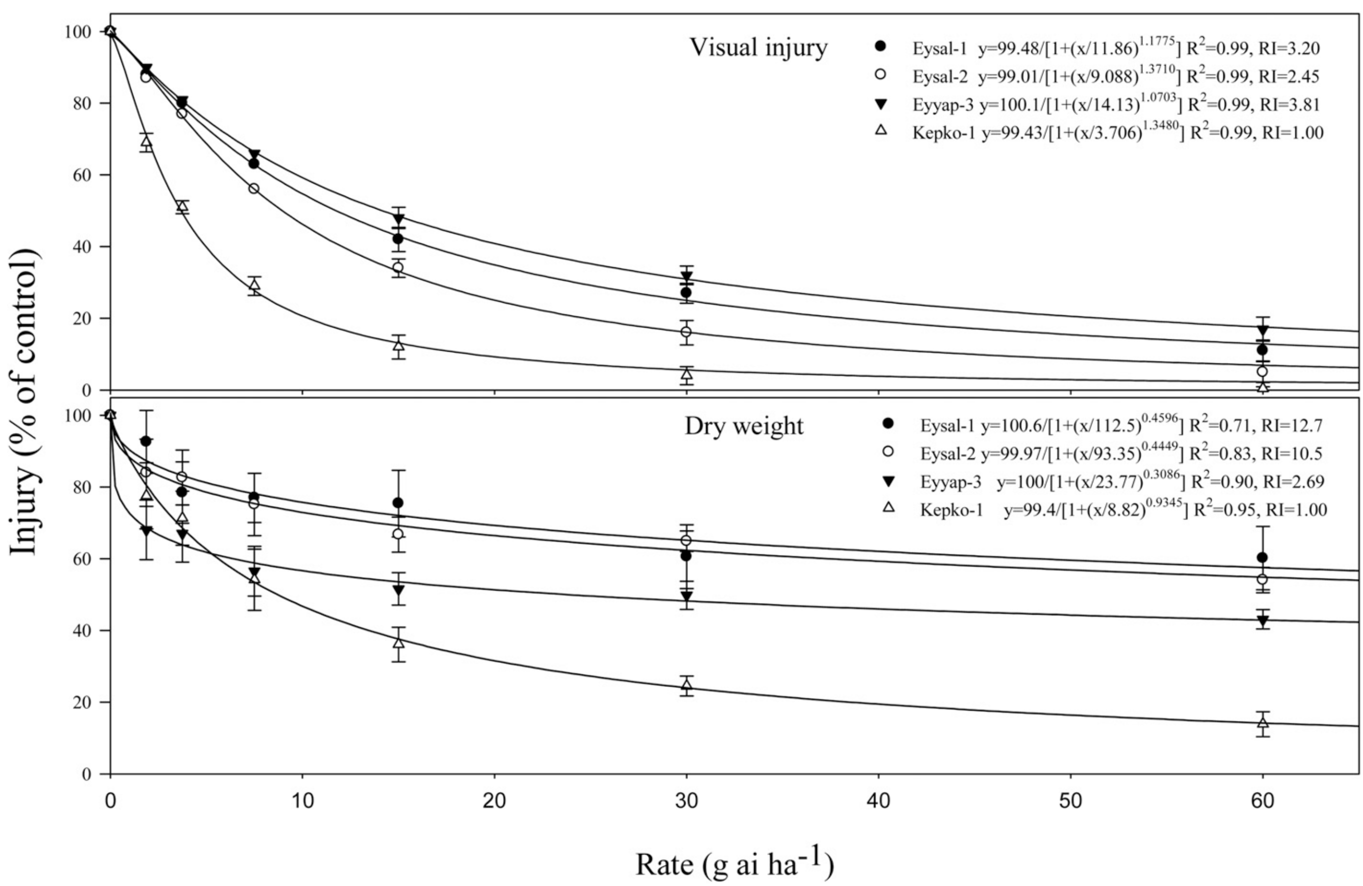

Fig. 3. Percent of control by injury at 21 d after treatment with chlorsulfuron applied to four *Conyza sumatrensis* populations at rosette stage. Resistance index (RI) was calculated by the ratio of the GR_{50} or GD_{50} value of the resistant population (EYSAL-1, EYSAL-2, EYYAP-3) to the GR_{50} or GD_{50} of the susceptible population (KEPKO-1).

Table 4. Chlorsulfuron required to cause 50% visual injury (GR_{50}) and 50% dry weight reduction (GD_{50}) for the four *Conyza sumatrensis* populations at 21 DAT at rosette stage.

	Chlorsulfuron				
				Resistance index[y]	
Population	GR_{50}[z]		GD_{50}[z]	GR_{50}	GD_{50}
EYSAL-1	11.86	(g a.i./ha)	112.5	3.20	12.7
EYSAL-2	9.088	(g a.i./ha)	93.35	2.45	10.5
EYYAP-3	14.13	(g a.i./ha)	23.77	3.81	2.69
KEPKO-1	3.706	(g a.i./ha)	8.820	1.00	1.00

[z]GR_{50} (herbicide rate required to cause 50% visual injury) and GD_{50} (herbicide rate required to cause 50% dry weight reduction) values were calculated from dose–response study. Chlorsulfuron was applied at 0, 1.875, 3.75, 7.5, 15, 30, and 60 g a.i./ha. KEPKO-1 was the most susceptible population in the dose–response studies.

[y]Resistance index was calculated as the ratio of the GR_{50} or GD_{50} value of the resistant population to the GR_{50} or GD_{50} of the susceptible population.

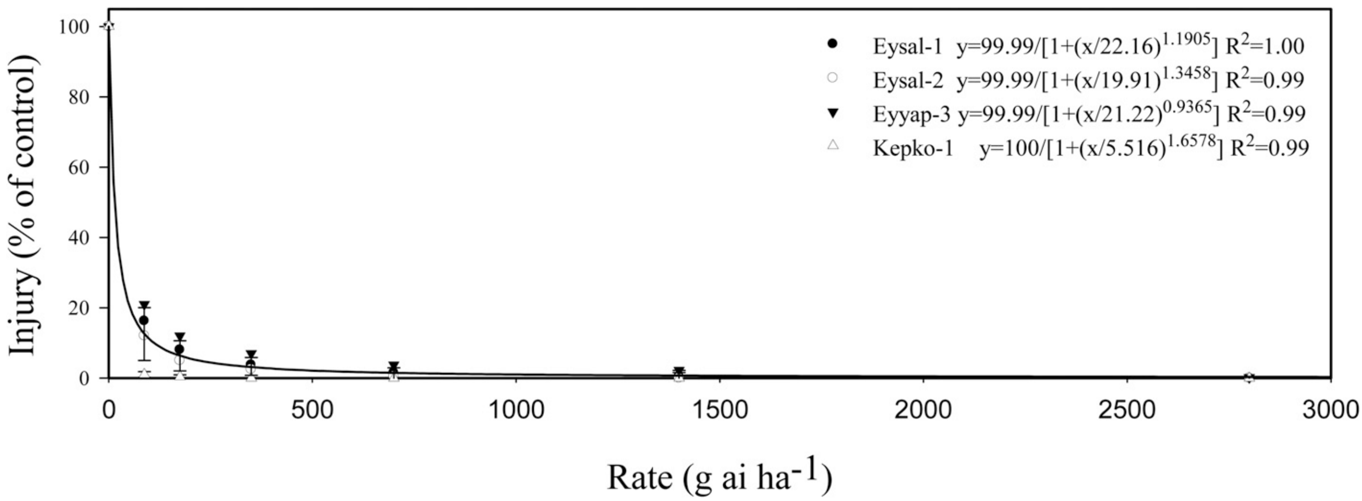

Fig. 4. Percent of control by injury at 21 d after treatment with metribuzin applied to four *Conyza sumatrensis* populations at rosette stage. Plants showed no resistance, thus no RI was calculated for metribuzin.

ALS inhibitors, *C. canadensis* populations were resistant to cloransulam, chlorimuron, imazethapyr, and bispyribac with the ranging 70, 40, 9.1, and 580, respectively (Zheng et al., 2011). In addition, *C. sumatrensis* populations are resistant to imazapyr, imazethapyr, and amidosulfuron with the ranging 4, 3.7, and 2, respectively, but not to chlorsulfuron (RI = 1.2) (Osuna and Prado, 2003).

Metribuzin visual injury increased as rates increased, and injury symptoms were apparent in all populations. Symptoms were chlorosis and leaf distortion followed by necrosis and full plant desiccation; visual injury from metribuzin developed rapidly within 1 to 7 DAT depending on the rate. Plants showed no recovery at observations. When EYSAL-1, EYSAL-2, and EYYAP-3 populations were treated with metribuzin at rosette stage, GR_{50} rates were 9.5, 12.3, and 10.3 g a.i./ha, respectively, whereas KEPKO-1 was 5.5 g a.i./ha (Fig. 4). These rates represent 2.7%, 3.5%, 2.9%, and 1.5% of the use rate; the GR_{50} values for all populations showed that these populations are not resistant to metribuzin.

The reduction in *C. sumatrensis* dry weight in all populations after treatment with metribuzin was similar to visible injury ratings. When EYSAL-1, EYSAL-2, EYYAP-3, and KEPKO-1 at the rosette stage were treated with metribuzin, GD_{50} rates were 0.042, 0.011, 0.161, and 0.033 g a.i./ha, respectively (Fig. 4). Regardless of the population or application rate, metribuzin provided > 99% control on rosette stage *C. sumatrensis* plants.

Conclusions

Our findings demonstrate that *C. sumatrensis* populations collected from several peach orchards from the Çanakkale province of Turkey are resistant to glyphosate. The study also shows that younger plants were more sensitive to glyphosate than older plants. With this in mind, chemical management practices should focus on early stages of *C. sumatrensis* when glyphosate is the only option. Results illustrate that *C. sumatrensis* populations in northwestern Turkey have resistance to glyphosate and chlorsulfuron but are still susceptible to metribuzin. Consequently, metribuzin, which has a photosynthetic inhibitor mode of action, can be effectively used as an alternative herbicide to control glyphosate-resistant *Conyza sumatrensis* in peach orchards but needs to be registered and tested for fruit quantity and quality.

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