

RESPONSE OF IMIDAZOLINONE-RESISTANT SUNFLOWER TO VARIOUS DRIFT RATES OF GLYPHOSATE, GLUFOSINATE AND INDAZIFLAM

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ABSTRACT

Imidazolinone (IMI) herbicides are used in sunflower due to the need for sunflower broomrape control, and IMI - resistant sunflower has become common in Turkey. Cereal fields and orchards are often in close vicinity to sunflower fields, and herbicide drift from these areas can adversely affect sunflower. Fields experiments were conducted at Edirne and Ankara, Turkey, in 2018 and 2019 to quantify the sunflower (IMI - resistant) response to various simulated drift rates of glyphosate, glufosinate, and indaziflam.

Herbicides were applied to sunflower at 12.5, 6.25, 3.125, and 1% of recommended rates. Crop injury was visually evaluated at 7, 14, and 28 d after treatment (DAT), and plant responses to herbicides were assessed at harvest. Sunflower was injured by all rates of glyphosate applied, with 15 to 100% (in Edirne) and 9 to 84% (Ankara) injury at 28 DAT. Glufosinate - related injury was 5 to 58% in Edirne and 7 to 43% in Ankara at 7 DAT, and decreased with time. In contrast, indaziflam caused no significant crop injury or yield losses. The recommended rates of 6.25% and 12.5% of glyphosate killed all sunflower plants in 2019, while in 2018 the yield loss was 100% only at recommended rate of 12.5% glyphosate. Lower rates of glyphosate reduced yield by 2 to 87% in 2018 and 18 to 62% in 2019. On the other hand, the two highest rates of glufosinate resulted in a yield reduction of 9 and 6% in 2018, respectively, but not in 2019. Injury at early stages after exposure is a good indicator of the impact of glyphosate drift on sunflower yield.

Keywords: sunflower yield; off-target movement; phytotoxicity, total herbicide.

INTRODUCTION

Sunflower is favoured in most of the agricultural areas of Turkey because the climate and soil provide suitable growth conditions. The production area of most oilseed crops in Turkey has declined in the past 5 years, while the area allocated to cotton and sunflower production has increased by 11 and 30%, respectively (TUIK, 2020). Although sunflower seed yield increased in Turkey during this period, only about 65% of domestic demand was supplied by domestic production (TUIK, 2020), and Turkey is one of the biggest sunflower seed importers globally (Konyalı, 2017). Furthermore, sunflower importation is expected to continue in the next decades and is estimated to reach US\$2.5 billion in 2030-2031 (Güleş et al., 2016). To reduce the gap in supply, efforts have been made to

improve agronomic practices and use new agrichemicals and new sunflower varieties have been introduced. Imidazolinone (IMI) - resistant sunflower varieties have been commercially available in Turkey for nearly two decades, and the seed technology enables the use of IMI to control weeds, especially broomrape, without crop damage (Demirci and Kaya, 2009). Expansion of IMI - resistant sunflower fields has occurred in areas dominated by wheat production and by orchards and vineyards, which has led to a new and unexpected problem for sunflower crops: herbicide drift. Orchards and vineyards are among the fields where total herbicides, including glyphosate, glufosinate, and indaziflam, are extensively used.

Glyphosate and glufosinate have also long been used to control weeds along roads and railways that are near or adjacent to IMI - resistant sunflower fields (PPPD, 2020).

Glyphosate is a prominent herbicide for the control of annual and perennial weed species, as well as brushes, in orchards, vineyards, and non-croplands in Turkey. Indaziflam, which is a relatively new herbicide, was registered to control annual narrow-leaf and broadleaf weeds in orchard areas (PPPD, 2020).

Drift is a phenomenon that frequently occurs during or soon after application of a pesticide via movement of droplets or vapour from the area of application to unintended targets (Felsot et al., 2011). The severity of drift varies depending on the environmental conditions, including relative humidity, temperature, wind direction, wind velocity, air stability; herbicide properties, such as volatility; and application procedures, including boom height, spray pressure, particle size, nozzle type, and spray angle (Cederlund, 2017). Herbicide drift not only results in yield reduction of sensitive crops, but it also negatively affects biodiversity, the aquatic environment, human and livestock health (Qi et al., 2020).

The impacts of herbicide drift on sunflower have been known for a long time, but the incidence has become higher in recent years with increased herbicide use. Studies on drift have raised awareness about the problem and its impacts on plant physiology. For example, when sunflower seedlings were exposed to 6% glyphosate, the translocation of Fe and Mn from root to shoot was almost inhibited and the seedlings later showed severe Fe and Mn deficiency symptoms (Eker et al., 2006). In another study, Lanini and Carrithers (1998) found that sunflower yield loss was 32 and 11% when glyphosate was applied at 112.09 g ha⁻¹ active ingredient (ai) at the two-leaf stage and the flowering stage, respectively. In comparison with glyphosate, glufosinate ammonium resulted in more apparent injury symptoms on sensitive plants, and drift reduced biomass accumulation in plants or delayed the maturity of the crop following herbicide application (Davis et al., 2011). Moreover, glufosinate significantly reduced chlorophyll content 3 days after treatment (DAT), but the plants recovered from the adverse impacts of the herbicide at

14 DAT (Reddy et al., 2011). Carpenter and Boutin (2010) tested sub-lethal doses of glufosinate ammonium on crops and wild species, including sunflower, and found sunflower had higher tolerance of glufosinate than some of the other crops tested, including broccoli, cucumber, buckwheat, and garden lettuce. Additionally, their results showed that some of dicot weed species, such as *Capsella bursa-pastoris* (L.) Medik., *Melilotus officinalis* (L.) Lam., *Phytolacca americana* L., and *Solanum dulcamara* L., were more sensitive than sunflower to glufosinate. Little research has been published on the response of crops to indaziflam. Among the existing studies, Guerra et al. (2014) reported that indaziflam was highly phytotoxic to crops including millet, cucumber, beet, soybean, sorghum, maize, and cotton, but it had a very limited detrimental effect on sunflower. Even at the highest application rate (100 g ha⁻¹ ai), indaziflam did not cause substantial injury to the sunflower.

Although there are a few studies to determine the effect of these herbicides on sunflower, they are narrow in scope and comprehensive research on this topic is lacking. The aim of this study was to determine the response of IMI - resistant sunflower varieties to glyphosate isopropylamine salt, glufosinate ammonium, and indaziflam at various drift rates.

MATERIAL AND METHODS

Field studies were conducted in 2018-2019 at the Experiment Station of Trakya Agricultural Research Institute (TARI) in Edirne, Turkey (41°38'54.7"N, 26°36'02.4"E) and İkizce Research and Experiment Station (İRES) of Field Crops Central Research Institute in Gölbaşı, Ankara, Turkey (39°36'42.7"N, 32°40'30.6"E). The soil in experimental fields was silty loam with 0.9% organic matter and pH of 5.9 at TARI and clay loam with 0.7% organic matter and pH of 7.77 at İRES.

IMI - resistant sunflower varieties were sown using a pneumatic seed drill on March 27, 2018, in Edirne (cv. 64 LC 108) and May 3,

2019, in Ankara (cv. Colombi). Pendimethalin was applied pre-emergence at 1.35 kg ha⁻¹ ai on March 29, 2018, followed by imazamox post-emergence at 50 g ha⁻¹ ai in Edirne, whereas benfluralin was applied pre-emergence

at 375 g ha⁻¹ ai on May 3, 2019, in Ankara according to the recommendations of local agronomists to control weeds. The climatic conditions of the experimental fields are presented in Figure 1.

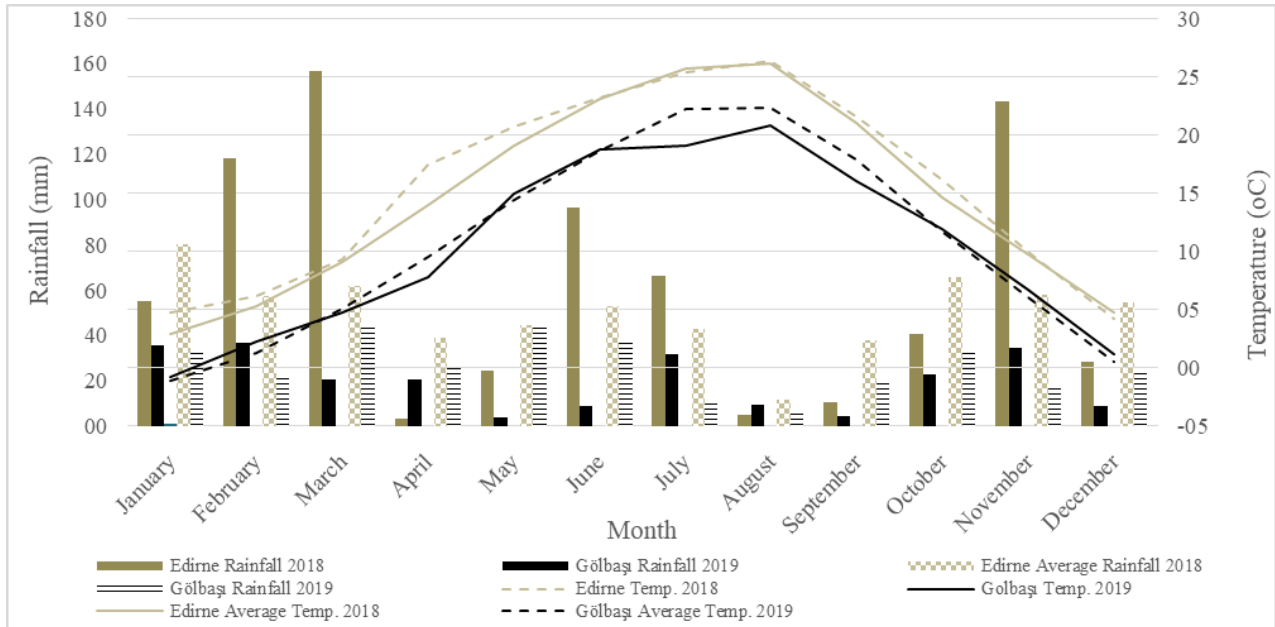


Figure 1. Average monthly precipitation and temperatures in the experimental fields (MGM, 2020)

The experiments were conducted for each of the three herbicides as randomized complete block designs with four replications. Plots were 2.1 m wide and 10 m long, and consisted of four rows of sunflower plants with 0.7 m spacing. Two crop rows between plots were left as an alley to avoid cross-contamination during herbicide applications. Herbicide rates were 1, 3.125, 6.25, and 12.5% of the recommended use rates of 1440, 600, and 50 g ha⁻¹ ai for glyphosate isopropylamine salt, glufosinate ammonium, and indaziflam, respectively (PPPD, 2020). Herbicides were applied using a motorized backpack sprayer in Edirne on June 11, 2018, when sunflower plants had seven to eight true leaves (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie: BBCH 17-18), and using a backpack compressed-CO₂ sprayer in Ankara on May 23, 2019, when plants had six to seven true leaves (BBCH 16-17). Both types of sprayer had a portable boom mounted with five flat-fan nozzles (Teejet XR11002) and calibrated to deliver 195 L ha⁻¹ for all

experiments. Herbicide treatments were applied at wind speeds of less than 3.7 and 4.6 km/h in 2018 and 2019, respectively. Air temperature and relative humidity during applications in Edirne and Ankara were respectively 26°C and 57% and 17°C and 44%.

Crop injury was visually rated in terms of stunting, necrosis, chlorosis, and bleaching on a scale of 0 to 100%, where 0 indicates no injury and 100 is complete plant death, at 7, 14, and 28 DAT and at harvest. At physiological maturity, 10 plants from the middle rows of each plot were randomly selected for assessment and then harvested by hand on September 5, 2018, and August 27, 2019. The heights of the selected plants were measured from the soil surface to the tip of the plant, and the sunflower head diameter (SHD) was measured from one edge of the sunflower head to the other. Mature seeds were manually separated from immature seeds, and sunflower seed yields were adjusted to 10% moisture. The 1000-seed weight (TSW) was then calculated.

All data were calculated as percentages based on the non-treated control, and ANOVA was conducted for each herbicide and location/year. Means separation was done with the use of Fisher's Protected LSD test at the 5% level of probability using SPSS (SPSS, 2004). Prior to analyses, visual crop injury data were transformed using arcsine of the square root to normalize the variances within treatments. Data obtained from the untreated controls were not included in the ANOVA. In the ANOVA model, the drift rate was considered a fixed factor, and site-year was considered a random factor. There was a year-by-glyphosate/glufosinate rate interaction for sunflower injury at 7, 14, and 28 DAT; therefore, data from each year were analysed separately. The year-by-indaziflam rate interaction was not significant for yield components and yield; therefore, the main effects are presented and discussed.

RESULTS AND DISCUSSION

Effects of glyphosate

Glyphosate-related injury on sunflower included chlorosis and necrosis, especially

affecting the new leaves, followed by stunting. Sunflower injury increased with increased glyphosate rates at 7, 14, and 28 DAT (Figure 2). The injury increased in parallel with plant growth during the growing season and resulted in the death of the sunflowers. At 7 DAT, glyphosate at the 14.4 g ha⁻¹ ai rate resulted in 4 and 6% injury, whereas 180 g ha⁻¹ ai resulted in 41 and 64% injury in Edirne and Ankara, respectively. Similarly, injury at 14 DAT from glyphosate exposure at rates of 14.4-180 g ha⁻¹ ai ranged from 7 to 54% in Edirne and from 9% to 77% in Ankara. Sunflower injury caused by glyphosate drift rates increased at 28 DAT and reached 84 and 100% at 180 g ha⁻¹ ai in Edirne and Ankara, respectively. That drift rate prevented surviving plants from forming heads. The response of sunflowers to glyphosate rates in 2018 and 2019 varied by climatic conditions and location (Figure 1). Previous research indicated that glyphosate had higher herbicidal activity at a moderate temperatures, as in Ankara, compared with warmer or cooler temperatures as in Edirne (Tanpipat et al., 1997).

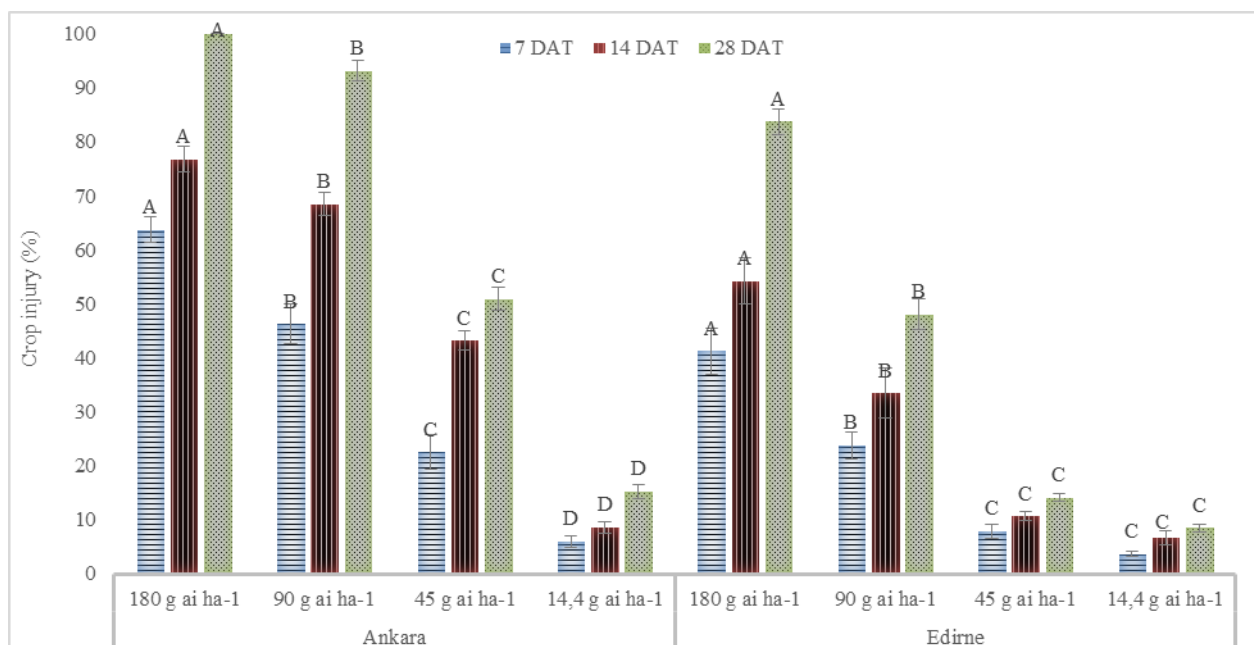


Figure 2. Sunflower injury caused by glyphosate drift rates 7, 14, and 28 DAT; means followed by the same letters on bars are not different ($p > 0.05$); vertical bars indicate \pm standard error of the mean values

A year-by-treatment interaction was found for sunflower yield and yield component; therefore, data from each year were analysed

separately, except for TSW. Depending on the application rates, the severity of glyphosate injury varied with regard to the

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yield and yield components, SHD, plant height (PH), and TSW. The yield and all yield components were significantly reduced by at all glyphosate rates. SHD was the yield component that was the most sensitive to glyphosate, while TSW was the least sensitive. SHD decreased by 1, 29, and 67% at 14.4, 45, and 90 g ha⁻¹ ai in Edirne and by 39 and 50% at 14.4 and 45 g ha⁻¹ ai in Ankara, respectively (Table 1). PH was the other important yield parameter affected by glyphosate application. Glyphosate at 14.4, 45, and 90 g ha⁻¹ ai resulted in height being reduced by 1, 3, and 33% in Edirne,

respectively; however, the reductions caused by 14.4 and 45 g ha⁻¹ ai were 29 and 48%, respectively, in Ankara. Changes in TSW were associated with glyphosate rate, but not by year. Therefore, the data were pooled and evaluated. Glyphosate at 14.4 g ha⁻¹ ai reduced sunflower yield 18% in Ankara and 2% in Edirne. The 45 g ha⁻¹ ai glyphosate decreased sunflower yield with 56% in Ankara and 46% in Edirne. Glyphosate at 90 and 180 g ha⁻¹ ai killed all the sunflower plants in Ankara, whereas only the highest glyphosate rate killed the plants in Edirne.

Table 1. Effect of glyphosate drift rates on height of plants, yield components, sunflower yield (% of nontreated), and growth stage (BBCH) during 2018 and 2019

Rate g ha ⁻¹ ai	PH		TSW ^a	SHD		Yield ^b		BBCH ^c	
	2018	2019	2018-2019	2018	2019	2018	2019	2018	2019
14.4	99 ^d a	71 a	99 a	99 a	61 a	98 a	82 a	92	92
45	97 a	52 b	84 b	71 b	50 b	54 b	44 b	87	85
90	67 b	-	78 b	33 c	-	12 c	-	83	-
180	-	-	-	-	-	-	-	-	-

^a One-thousand seed weight (g) data from 2108 and 2019 were pooled;

^b Average yield of non-treated plots were 2172.90 kg ha⁻¹ in Edirne and 1729.1 kg ha⁻¹ in Ankara;

^c Average BBCH stage of non-treated plots was 97 in Edirne and Ankara;

^d Means followed by the same letter are not different (P>0.05);

- means that all sunflower died.

The negative impact of glyphosate on PH was not only reported in sunflower (Vital et al., 2017) but also in other crops such as wheat (Davis et al., 2013; Roider et al., 2007), rice (Davis et al., 2011), soybean (Ellis and Griffin, 2002), cotton (Ellis and Griffin, 2002), safflower (Asav, 2022), and grain sorghum (Hale et al., 2019). Moreover, similar to our findings, the suppressive effect of glyphosate was previously reported to be higher for PH than for seed weight (Davis et al., 2011; Davis et al., 2013; Roider et al., 2007). To our knowledge, the available literature contains no studies on the impact of glyphosate on sunflower head size. However, our results showed that the response of IMI-resistant sunflower to glyphosate rates was greater in terms of SHD than PH and TSW. These reductions were attributed to plant physiology being impaired by glyphosate. Nutrient deficiency leading to dry matter

reduction and low chlorophyll content in sunflower was caused by glyphosate prevents metals such as Fe and Mn from being taken up by the plant Eker et al. (2006). Moreover, the changes in sunflower physiology due to glyphosate exposure may include a decrease in the number of leaves, smaller leaf area and stem diameter, and a reduced number of nodes (Vital et al., 2017).

As a function of the impact on yield components, sunflower yield was negatively affected by glyphosate to various degrees, depending on the rate and site. Yield loss in Ankara was higher than in Edirne due to environmental conditions. Similar results with different crop varieties were reported by Davis et al. (2013) and Felix et al. (2012). Also, Lanini and Carrithers (1998) reported that glyphosate at a rate of 112.09 g ha⁻¹ ai reduced sunflower yield 32 and 11% when plants were treated at the two-leaf and

flowering stages, respectively. The higher crop injury observed in our study compared with that of Lanini and Carrithers (1998) may be because of the environmental and climatic conditions from the experimental fields.

A strong positive correlation was found between SHD and yield in both sites (Figure 3), which was unsurprising because a wider sunflower head can have more seeds. The high negative correlation was observed

between visible injury at 28 DAT and sunflower yield, with coefficient of correlation values of -0.931 and -0.993 in Edirne and Ankara, respectively. Indeed, these correlations accorded with the real sunflower yield losses (Table 1). Visible injury may be considered a good indicator of the impact of glyphosate drift on sunflower yield, similar to the results of Lassiter et al. (2007) for peanut.

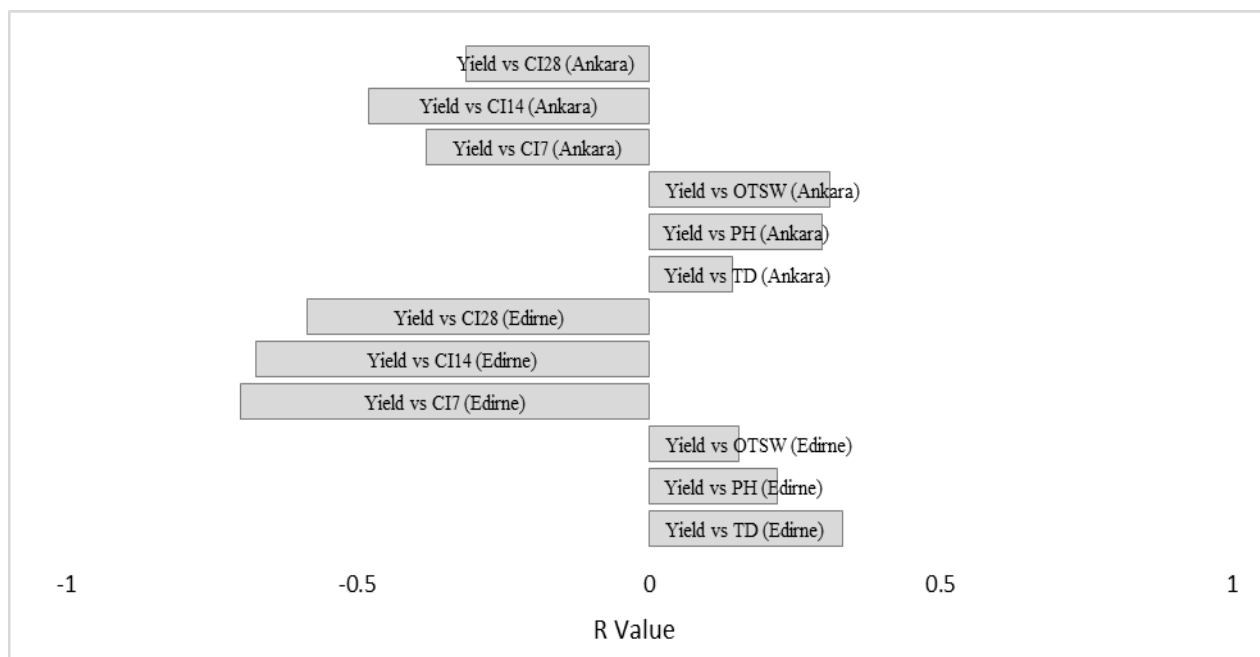


Figure 3. The Pearson correlation coefficient for yield components and yield in Ankara and Edirne for sunflower treated with 4 rates of glyphosate, CI7: Crop Injury 7 DAT, CI14: Crop Injury 14 DAT, CI28: Crop Injury 28 DAT

Effects of Glufosinate

Glufosinate injury to sunflower seedlings varied depending on the rates and sites, and declined as time went by (Figure 4). The symptoms of injury increased as glufosinate drift rates increased. Glufosinate created injury symptoms such as chlorosis, necrosis, and dry and brittle leaves soon after herbicide application, and the intensity of injury increased up to 7 DAT. In contrast to the glyphosate effects, injury symptoms diminished over time and no visible injury symptoms were seen on sunflower plants at harvest. The two highest rates of glufosinate resulted in severe injury to sunflower seedlings in both experimental fields. The injury rates caused by glufosinate applied at 75 g ha⁻¹ ai was 43, 21, and 3% in Ankara

and 58, 35, and 10% in Edirne at 7, 14 DAT, and 28 DAT, respectively (Table 2). Glufosinate caused a transient sunflower injury when it was applied at 18.75 g ha⁻¹ ai in Ankara and Edirne, while the lowest rate of glufosinate had no visually apparent adverse effects on the sunflower seedlings. Glufosinate was observed to be more effective in Edirne than in Ankara, similar to the study of Coetzer et al. (2001), who indicated that glufosinate was more active under conditions of higher temperature and relative humidity. Similar to our results in Ankara, Hale et al. (2019) reported that glufosinate resulted in severe injury at 14 or 28 DAT in grain sorghum when it was applied at 60 g ha⁻¹ ai, but the plants recovered up to the harvest.

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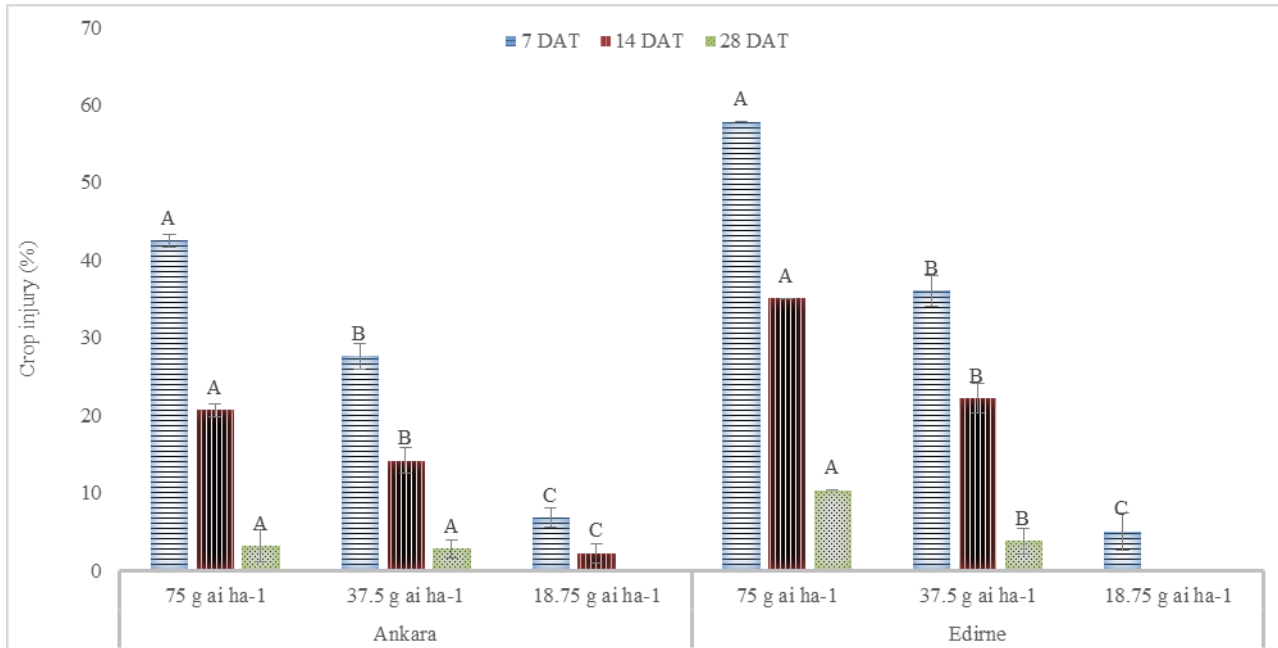


Figure 4. Sunflower injury caused by glufosinate drift rates 7, 14, and 28 DAT; means followed by the same letters on bars are not different ($P > 0.05$); vertical bars indicate \pm standard error of the mean values

Table 2. Effect of glufosinate drift rates on height of plants, yield components and yield during 2018 and 2019 (% of nontreated)

Rate g ha ⁻¹ ai	PH ^a	TSW ^a	SHD ^a	Yield ^b	
	2018-2019	2018-2019	2018-2019	2018	2019
6	101 ^c a	102 a	100 a	101 a	99 a
18.75	100 a	100 a	98 a	99 a	101 a
37.5	98 a	99 a	98 ab	94 b	98 a
75	93 b	99 a	94 b	91 b	97 a

^a PH, TSW, and SHD data from 2108 and 2019 were pooled because of insignificant year-by-treatment interactions;

^b Average yield of non-treated plots were 2135.18 kg ha⁻¹ in Edirne and 1684.47 kg ha⁻¹ in Ankara;

^c Means followed by the same letter are not different ($P > 0.05$).

The year-by-rate interaction was not significant for glufosinate in terms of yield components, SHD, PH, and TSW; therefore, these data were combined over the years. In Edirne, glufosinate slightly reduced the height of plants and the head diameter compared with the non-treated control (Table 2). The SHD and PH were significantly reduced as the glufosinate rate increased, and this difference could be due to growth reduction as reported in the previous research (Davis et al., 2011; Hale et al., 2019). No meaningful change in TSW was seen based on the glufosinate rate. Davis et al. (2013) found that glufosinate at 31 and 62 g ha⁻¹ ai reduced wheat seed weight by 4-8% depending on the application time; however Hensley (2009)

reported that the herbicide had no impact on rice seed weight, similar to our results.

A year-by-treatment interaction was found for sunflower yield; therefore, sunflower yield data from each year were analysed separately. A significant reduction in sunflower yield was observed at the 37.5 and 75 g ha⁻¹ ai rates of glufosinate, with 6 and 9% in 2018, respectively; however, severe yield reduction was not associated with glufosinate rates in 2019 (Table 2). Similar results were found by Davis et al. (2013) and Johnson et al. (2012).

The correlations between sunflower yield components and sunflower yield in Edirne were quite low in statistical analysis, but the negative correlations between crop injury at

7, 14, and 28 DAT and sunflower yield were strong, especially in Edirne (Figure 5). However, in Ankara, no significant high correlations were observed between sunflower yield components and sunflower yield (Table 2). Johnson et al. (2012) found a moderate negative correlation between visible injury and peanut or soybean yield at

7 or 14 DAT, similar to our results in Edirne. According to the R values, the correlations between yield components and yield were weaker than the correlation between yield and visible injuries. In contrast to the glyphosate, crop injury may be a poor predictor for estimate the sunflower yield loss caused by glufosinate drift.

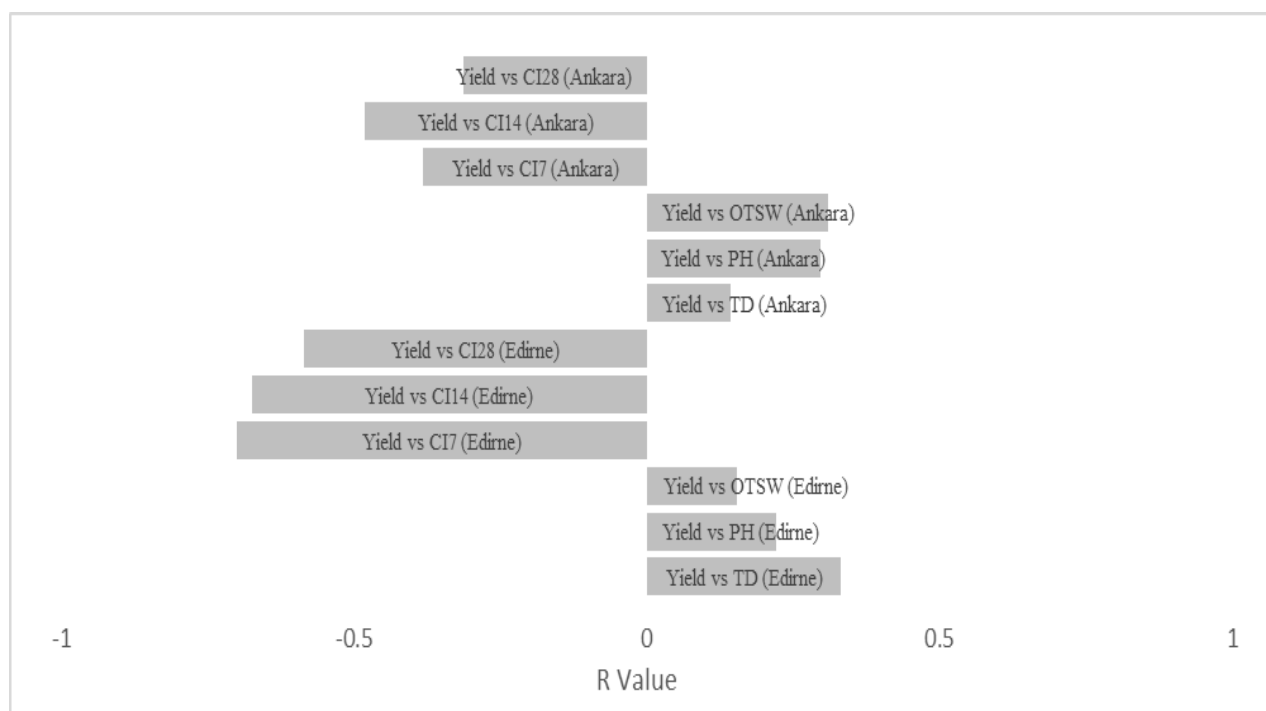


Figure 5. The Pearson correlation coefficient for yield components and yield in Ankara and Edirne, sunflower treated with 4 rates glufosinate

Indaziflam results

Indaziflam was a non-destructive herbicide for sunflower because the indaziflam-treated seedlings were at least as healthy as the plants in the control plots (Table 3). In contrast to the other two herbicides studied in the experiments, indaziflam did not cause injury to sunflower seedlings at 7, 14, and 28 DAT. These findings are expected given the mode of action of the herbicide. Indaziflam prevents the biosynthesis of cellulose, the main component of cell walls (Brabham et al., 2014). The efficacy of indaziflam on plant tissue is very limited or completely

absent when it is applied to the surface of a developed leaf because cellulose synthesis has already occurred. However, some plants, such as pepper, soybean, squash, and tomato, are sensitive to indaziflam even if it is applied post-emergence (Jeffries et al., 2014). Our findings accord with those of Guerra et al. (2014), who indicated that sunflower had high tolerance to indaziflam residue in the soil, and the I_{50} value was $>100 \text{ g ha}^{-1} \text{ ai}$ in soil. In addition, Torres et al. (2018) reported that indaziflam applied pre-emergence at $6 \text{ g ha}^{-1} \text{ ai}$ had limited impact on sunflower dry biomass, leaf area, and chlorophyll content.

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*Table 3. Effect of indaziflam drift rates on height of plant, yield and yield components during 2018 and 2019
(% of nontreated)*

Rate g ha ⁻¹ ai	PH ^a	OTSW ^a	SHD ^a	Yield ^{ab}
0.5	99.87	99.75	100.55	100.98
1.5625	99.00	98.81	98.78	100.92
3.125	98.62	97.65	98.65	97.66
6.25	96.75	99.80	98.19	97.60

^a PH, TSW, SHD, and yield data from 2108 and 2019 were pooled because of insignificant year-by-treatment interactions;

^b Average yield of non-treated plots were 2098.15 kg ha⁻¹ in Edirne and 1746.72 kg ha⁻¹ in Ankara.

CONCLUSIONS

The severity of herbicide drift depended on the specific herbicide and its application rate, as well as the region owing to environmental conditions varying between them. Sunflower growth was reduced by increasing drift rates of glyphosate regardless of the year or rate, and severe crop injury and yield losses were observed in both areas.

Glufosinate drift rates resulted in slight yield losses in sunflower even if injury symptoms were seen after herbicide application at a high rate. Sunflower plants recovered almost completely from the harmful impacts of glufosinate at 28 DAT, and an adverse effect was only apparent with regard to sunflower yield in Edirne.

Compared with glyphosate and glufosinate, indaziflam is a reliable herbicide for controlling weeds in field nearby or adjacent to sunflower fields.

Glyphosate and glufosinate should be applied under suitable environmental conditions, and anti-drift nozzles should be used or shield units should be mounted on the boom if these herbicides are applied to control weeds in orchards and vineyards. In this study, the rates of glyphosate and glufosinate were adjusted based on the annual weeds; however, they may commonly be used at higher rates to control perennial weeds and be applied multiple times. Therefore, glyphosate and glufosinate require special attention during application when drift conditions have been observed.

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