

RESEARCH ARTICLE

Conyza Species in Citrus Orchards and Vineyards of Turkey and their Management with Glyphosate

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ABSTRACT

Conyza species are considered one of the most aggressive weeds in the Aegean and Mediterranean regions of Turkey in terms of their invasive character and their ability to spread to agricultural and nonagricultural areas, including citrus orchards and vineyards. This study was conducted to determine the distribution of *Conyza* species in citrus orchards and vineyards in these regions and the efficacy of glyphosate when used at different growth stages. The majority of citrus fields and nearly half of the vineyard fields of Turkey were visited in 2015. Due to their similar appearance, the identification of *Conyza* species at the early stages of growth cannot straightforward; therefore, the species *C. canadensis*, *C. bonariensis*, and *C. sumatrensis* were identified by using PCR and DNA sequence analysis. A total of 252 citrus orchards (131) and vineyards (121) were included in the field survey conducted in the Aegean and Mediterranean regions, and 203 *Conyza* species were collected. The molecular data revealed that *C. bonariensis* was the most common *Conyza* species in nearly half of the citrus orchards, followed by *C. canadensis* at 33%. *C. canadensis* was the most dominant *Conyza* species in more than half of the vineyards, while *C. bonariensis* and *C. sumatrensis* were found in 36 and 13 vineyards, respectively. Sequence information of *Conyza* species was submitted to NCBI with GenBank accession numbers (*Conyza* (*Erigeron*) *canadensis* accession number: OM302550.1, *C. bonariensis* accession number: OM302551.1, *C. sumatrensis* accession number: OM302548.1). Glyphosate was more effective when it was applied at the earlier rosette stages for both susceptible and resistant biotypes. Therefore, late treatments could be the main reason for glyphosate having low efficacy against non-resistant populations of *Conyza* spp.

Keywords: Citrus orchard; DNA sequence analysis; Glyphosate; Horseweed; Vineyard

INTRODUCTION

Conyza spp. are among the annual weeds native to the American continent and are frequently found in some annual crops, including sunflower, soybean, corn, wheat and cotton, perennial plants, including vineyards and orchards, other agricultural fields and uncultivated areas (ISC 2022). The weed is commonly localized to field margins and escapes from herbicide treatments. As a typical member of Asteraceae, it may produce a high number of seeds containing white bristly pappus to help with wind dispersal (Shrestha et al. 2010a). The plant may create a rich soil seed bank, and its seeds may germinate when they reach sufficient amounts of water and temperature even if their seeds have a short life in the soil and no dormancy (Zinzolker et al. 1985; Wu et al. 2007). Additionally, horseweed has allelopathic chemicals that inhibit the seed germination and shoot growth

of other weeds without auto toxicity (Hu and Zhang 2013). With the help of these factors, *Conyza* spp. may replace other native weed species and become the most dominant weed in a new or disturbed environment such as post-fire forest and orchard. Actually, *Conyza* species have commonly been found in post-fire plantations (Moser and Wohlgemuth 2006; Pino et al. 2013; Kuenzi et al. 2008; Viedma et al. 2017) because horseweed seeds disperse over a wide range of fields depending on the prevailing wind direction and speed (Dauer et al. 2006), and fire can stimulate germination of horseweed (Aran et al. 2013). Moreover, dried weeds may increase the risk of broadcast burning and fire.

Similar to other annual and perennial crops, citrus plants are also sensitive to weed competition. On the other hand, there are a few perennial crops as old as the vine, but they are sensitive to weed competition. The competition was more destructive at the early stage of perennial crops

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such as vineyards and citrus orchards (Olmstead et al. 2012; Singh and Sharma 2008). One of the horseweed plants resulted in 20 and 30% declines in the shoot weight and stem length of the 12-month grapevine sapling, respectively (Alcorta 2009). This rivalry is a consequence of the struggle for survival because weed species have to be more competent than crop plants to reach water, nutrients, space and light; therefore, they have been endowed with many mechanisms like releasing allelopathic chemicals (Radosevich et al. 1997). Although citrus growers frequently apply well-known weed control treatments, such as hoeing, flaming, mulching, and chemical control, some treatments are superior to others like herbicide use, especially under drought conditions (Soares et al. 2021). Drought time in the growing seasons of citrus trees is a period that is the most sensitive to water deficits because of weed competition and is considered the critical period of weed interference (Goncalves et al. 2018). The sensitivity of vineyard plants to weeds, especially horseweed, may decrease over time, even when the weed density is 18 horse-weed per square meter, resulting in no grape yield loss (Shrestha et al. 2010b). Horseweed occurrence in grape fields resulted in fewer grape leaves and less dry matter accumulation (Alcorta et al. 2011). Additionally, mature weeds may create trouble during harvest and decline the crop quality of grapes if their seeds are included in the bunch of grapes. Moreover, weeds may harbour some pests and diseases, as well as rodents (Elmore 1996).

Conyza species are among the most abundant and common weeds in the grape fields and citrus orchards of Turkey (Kaçan and Boz 2015; Arıkan et al. 2015). Several weed control treatments may be employed to reduce their adverse impacts on vineyards and citrus orchards, but glyphosate and its mixtures are generally preferred by growers due to their apparent impacts on weeds (Doğan et al. 2022). Therefore, the use of glyphosate has been increasing year by year for the reason mentioned above and in harmony with the increasing number of herbicide-tolerant fields (Benbrook, 2016). However, herbicides, including glyphosate, may result in some problems, including runoff, drift, residue, and resistance (Asav and Serim 2018; Serim and Maden 2014; Serim 2022; Doğan et al. 2022). Consequence of this trend, GR accessions have been found in fields in which glyphosate is incessantly used in orchards and vineyards (Doğan et al. 2022).

Most orchard growers misidentified the *Conyza* species at the early stages and described all of them as one species. However, this attitude of the growers was not acceptable in terms of sustainable herbicide use practices because the response of these species to total herbicides varied even if they belonged to the same genus. Hairy fleabane absorbed 29-51% more glyphosate than horseweed, and

the translocation rate of glyphosate in hairy fleabane was twofold that in horseweed (Moretti and Hanson 2017). Interestingly, they were very similar to each other in terms of leaf morphology, but they were not similar. Therefore, glyphosate retention on leaves of *Conyza* species varied; for instance, retention on tall fleabane was highest, while hairy fleabane was higher than horseweed (Gonzalez-Torralva et al. 2010). This structural difference significantly changed their response to glyphosate. Identification of *Conyza* species may help orchard growers control them with herbicides. The producer of these crops has often complained about the insufficiency of glyphosate to control horseweeds even though they consecutively used glyphosate at higher rates.

Few studies have been conducted to determine the weed flora of vineyards and citrus orchards of Turkey using the Flora of Turkey; however, no research has attempted to identify *Conyza* species in these fields using molecular techniques and their management with glyphosate. The aims of the study were (1) to identify *Conyza* species collected from citrus orchards and vineyards in Turkey using PCR-based molecular markers and using the Flora of Turkey, (2) to determine the distribution of *Conyza* species in these fields, and (3) to evaluate the efficacy of glyphosate on GR and GS *Conyza* species when applied at various *Conyza* growth stages.

MATERIALS AND METHODS

Survey field and collection

A total of 252 agricultural areas belonging to 131 citrus orchards and 121 vineyards in the Mediterranean and Aegean regions were included in this survey program, covering 97% of Turkey's citrus orchards and 48% of its vineyards in 2015. The coordinates of the sampling points from which the seed samples of *Conyza* species were collected and some important agronomic treatments were also noted. Seed collection points were selected based on the presence of *Conyza* species and without any data on glyphosate use. The locations of the sampling points were at least 3-4 km apart. *Conyza* seed heads were cut using secateurs, and the heads were placed in paper bags. The seeds in each location were taken from one plant, but the seeds in other plants with morphological variations were also collected. *Conyza* seeds collected from the fields were placed on laboratory benches to dry for 1–2 weeks and then cleaned manually. They were stored in a cooler until use in molecular analysis and bioassays.

Identification of the species

The *Conyza* species were identified by using the Flora of Turkey and the East Aegean Islands (Davis 1965-1985;

Davis et al. 1988) (Figs. 1-2), and their location data were recorded.

Molecular analysis

Seeds of *Conyza* spp. were germinated on sterile filter paper placed in petri dishes, then they were transferred to pots filled with sterilized soil, and the seedlings were grown until 2-4 true leaf stages in the greenhouse. DNA extraction was performed by using young leaves of one plant (100 mg) according to the manufacturer's protocols (Qiagen GmbH, Hilden, Germany). The PCR amplification, cycling programs, and the process related to PCR products such as separation and staining were performed following the method described by Doğan et

al. (2022), with small modifications, using specific primers (Table 1). Amplification was implemented in a thermal cycler (Qiagen 5R). Agarose gel was visualized under UV light (Vilber Lourmat Quantum S5).

Sequence analysis

Fragments obtained from PCR assays were bidirectionally sequenced by Genoks Company (Ankara, Turkey). The original forward and reverse sequences were assembled using CodonCode Aligner 3.0 and BioEdit software to estimate the quality of the generated sequence traces. The neighbor-joining (NJ) method, a phylogenetic method involving tree generation from a distance matrix, was implemented by using the BLAST program at NCBI (National Center for Biotechnology Information) based on a maximum sequence difference of 0.05.

Growth stage studies

The sensitivity of different biotypes to glyphosate based on plant growth stage was determined in the screen house at Ege University Agricultural Faculty and greenhouse at Adnan Menderes University Agricultural Faculty during May–October 2017. AT8 (hairy fleabane) and AT42 (horseweed) from citrus orchards in Adana; HT10 (tall fleabane), HT13 (horseweed), and HT16 (hairy fleabane) from citrus orchards in Hatay; MT17 (hairy fleabane) and MT23 (horseweed) from citrus orchards in Mersin; MB5



Fig 1. *Conyza* species in vineyards.



Fig 2. *Conyza* species in orchard fields.

(hairy fleabane) and MB12 (horseweed) from vineyards in Mersin represented resistant populations; and AT33 (hairy fleabane) and MT4 (horseweed) represented sensitive populations in these studies. The GS seeds of tall fleabane was not used in this study because they had lost their germination ability. The seeds were planted in soil-filled pots as for the screening tests and grown to 2- to 4-leaf, 5- to 7-leaf, 10- to 12-leaf, and late rosette stages. Herbicide was applied to the GR and GS biotypes at 1,323 and 2,646 g a.i. ha⁻¹ rates. The pots were regularly watered with tap water as needed. For each accession, a non-treated control was also added to the experiments. The seedlings were clipped at ground level at 21 DAT and put in an oven at 65°C for 3 days. The biomass data were expressed as percentages of untreated controls for each population by growth stage combination.

The experiment was designed in a randomized complete block design with five replications for both studies. All relative dry biomass data obtained from the experiment were analysed by ANOVA using a 2 × 11 × 5 (two herbicide rates by 9 GR + 2 GS accessions by five replicates) factorial design. The differences between the means of treatments were compared using Duncan's multiple comparison test (P<0.05).

RESULTS AND DISCUSSION

Identify of the *Conyza* species

In the molecular identification of *Conyza* species, the chloroplast gene regions (rbcL, matK, and trnL-trnF) provided weaker identification than the nuclear ITS region, which was superior in all cases. The ITS region was able to positively identify 100% of the sequences queried against preexisting specimen accessions. We detected three interspecific length polymorphisms among the three studied species; *C. canadensis* has a 626 bp length, *C. bonariensis* has a 570 bp length and *C. sumatrensis* has an identical 649 bp length. Comparative nucleic acid sequences of the studied species are shown in Fig. 3.

According to NCBI BLAST data, while it showed 94.28%-100% similarity with a total of 99 sequences from 52 different species covering 550 bp of the 626 bp length of *C. canadensis*, the similarity rate with 16 *C. canadensis* sequences was 99.52%-100%. For *C. bonariensis*, it showed 96.14%-100% similarity with a total of 96 sequences from 52 different species covering 551 bp of the 570 bp length. Finally, we determined an identity at the rate of 93.84-100% per 99 sequences from 52 different species covering 548 bp of the 649 bp long *C. sumatrensis*, while the similarity rate with 16 *C. sumatrensis* sequences of the studied species was 98.26-100%. Phylogenetic trees of the species are given in Figs. 4-6.

The ITS region can be safely recommended for rapidly identifying the studied species belonging to the genus *Conyza*. The whole ITS region was annotated into the parts ITS1, 5.8S and ITS2 (*Conyza* (*Erigeron*) *canadensis* accession number: OM302550.1, *C. bonariensis* accession number: OM302551.1, *C. sumatrensis* accession number: OM302548.1).

Distribution of horseweed species

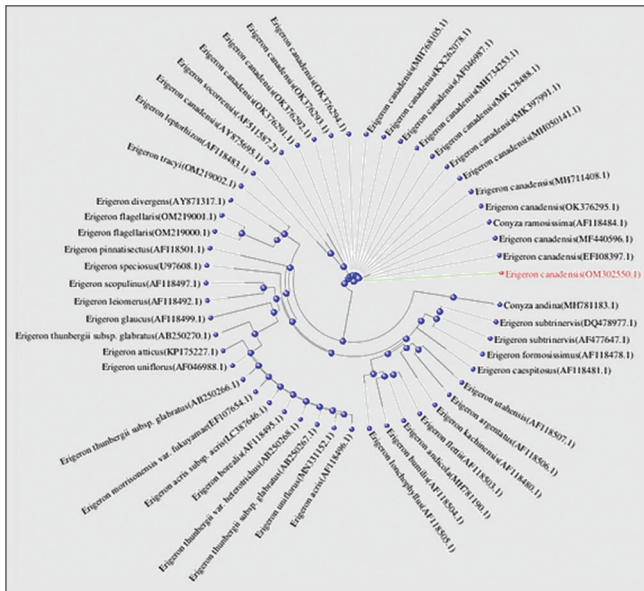
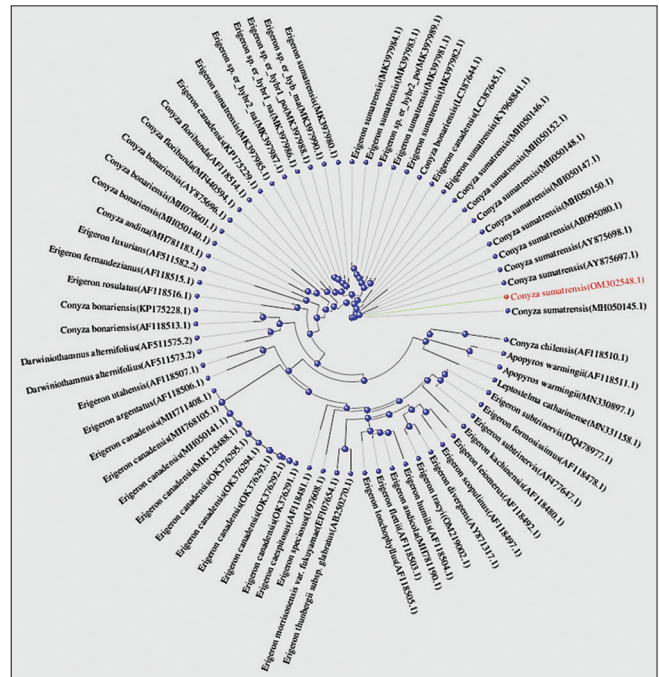
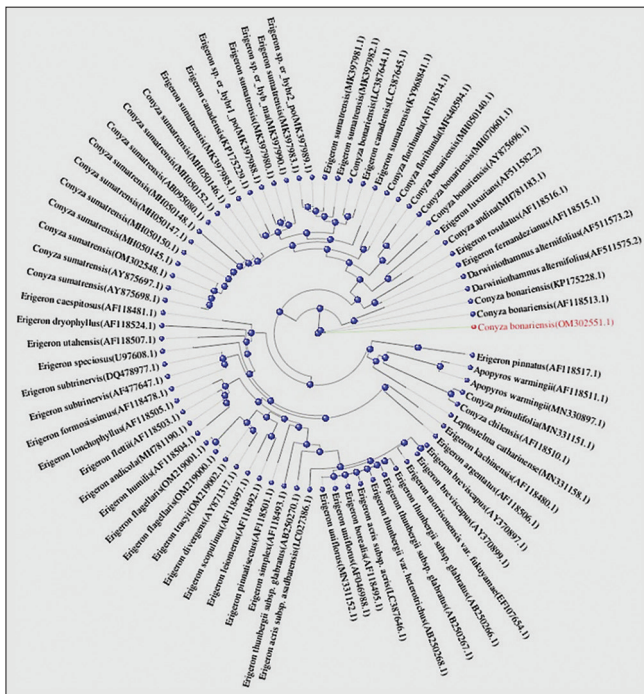
A total of 252 accessions collected from two regions of Turkey were tested to determine their taxonomic classes (Fig. 7; Tables 2 and 3). In orchards, hairy fleabane was the most common *Conyza* spp. followed by horseweed and tall fleabane, while horseweed was the most prominent in vineyard fields. Adana province had the most orchard fields with *Conyza* spp., while İzmir province had the fewest. For vineyard fields, Manisa province had the highest number of accessions (44), followed by Denizli province and Mersin province, which had 17 populations each. The orchard fields harboured a larger number of *Conyza* spp. compared with vineyard fields because most orchard fields are located in the Mediterranean region, where plants produce more than one generation per season owing to climatic conditions. Tall fleabane was found only in the vineyard fields of the Aegean region but not in the Mediterranean region.

ERIBO	1	TCGAAGCTGCAAGCTGAACGACCCGCGAAGCATGTTAAACAACCATGCCAGGATGCGTCGAGATCAGTTTCATCGCTCTGGCACACCGTTGATGTCCTGCTAGTTGGCCCTACG	120
ERIFL	31	A.....T.....G.....T.....	150
ERICA	1	A.....C...TG.....T.....A.....	118
ERIBO	121	GTTATCTTGTGGTCGATTGACGTAAACAAACCCAGGACGATGTCGAAGAACTTTAAATTGAAGATTGCCCTCTATGAAGTCCCGTTCGCGTGTGCTCATGGGGTGTGGC	240
ERIFL	151	T.....A.....T.....C.....C.....	270
ERICA	119	.CC.....T.....A.....C.....C.....C.....	238
ERIBO	241	ATCTTTGTAATCACAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGACTTGGTGTGAATTGCAGAAATCCCGTGAACCATCGAGTTTGTG	360
ERIFL	271		390
ERICA	239		358
ERIBO	361	AACGCAAGTTGCGCCGAAGCCATTTCGGCTGAGGGCAGCTGCTGCCGCGGTACGCGTCCGCCCAACATTCTTTGGGATGCTT-GGCTGGGAGCGGATATTGGCCCTCC	479
ERIFL	391	C.....	509
ERICA	359	A.....T..AC.....	478
ERIBO	480	GTITTAACCGAGCGGTTGGCCAAATAAAGCACCTCTTGATGGCGCAAGACTATTGGTGACACAACCATGATATTGTTGAGTGCTCG	570
ERIFL	510	T.....G..G.....C.....T.....	600
ERICA	479	T.....G.....A.....C.....A.....C.....C.....	568

Fig 3. Nucleic acid sequences of *Conyza* spp. (*ERIBO: *Conyza bonariensis*'s Bayer and EPPO Code, ERIFL: *Conyza sumatrensis*'s Bayer and EPPO Code, ERICA: *Conyza canadensis*'s Bayer and EPPO Code).

Table 1: Primers used in the molecular identification of *Conyza* spp

Region	Primer	References
trnL-F	5'-CGAAATCGGTAGACGCTACG-3'	Taberlet et al. 2007
trnL-R	5'-GGGGATAGAGGGGACTTGAAC-3'	Taberlet et al. 2007
rbcl-F	5'-ATGTCACCACAAACAGAGACTAAAGC -3'	Hollingsworth et al. 2009
rbcl-R	5'-GTAAATCAAGTCCACCCRCG -3'	Hollingsworth et al. 2009
ITS2-F	5'-GCTGCGTTCTTCATCGATGC-3'	White et al. 1990
ITS3-R	5'-GCATCGATGAAGAACGCAGC-3'	White et al. 1990
ITS1-F	5'-TCCGTAGGTGAACCTGCGG-3'	White et al. 1990
ITS4-R	5'-TCCTCCGCTTATTGATATGC-3'	White et al. 1990

Fig 4. Phylogenetic trees of *Erigeron (Conyza) canadensis*.Fig 6. Phylogenetic trees of *Conyza sumatrensis*.Fig 5. Phylogenetic trees of *Conyza bonariensis*.

Horseweed was commonly found in Adana, Mersin, Hatay, Antalya, and Denizli, and its abundance was higher in vineyard fields than in citrus fields. Hairy fleabane, another common *Conyza* species, was found in Adana, Mersin, Hatay, and Antalya, and it occurred more frequently in orchard fields than in vineyard fields. The less common *Conyza* species, tall fleabane, was found in Antalya, Muğla, Manisa, and Denizli. Horseweed and hairy fleabane biotypes were more common in the southern regions of Turkey, whereas tall fleabane biotypes were more common in the northern regions of Turkey. Some of the *Conyza* accessions collected from Mersin, Adana and Hatay were resistant to glyphosate applied.

Conyza species were more widespread in agricultural fields that were left fallow or were no-tillage for a long time and in non-agricultural fields in Turkey. Although these species are generally abundant in citrus orchards and vineyard fields, the data about their distribution are limited or lacking

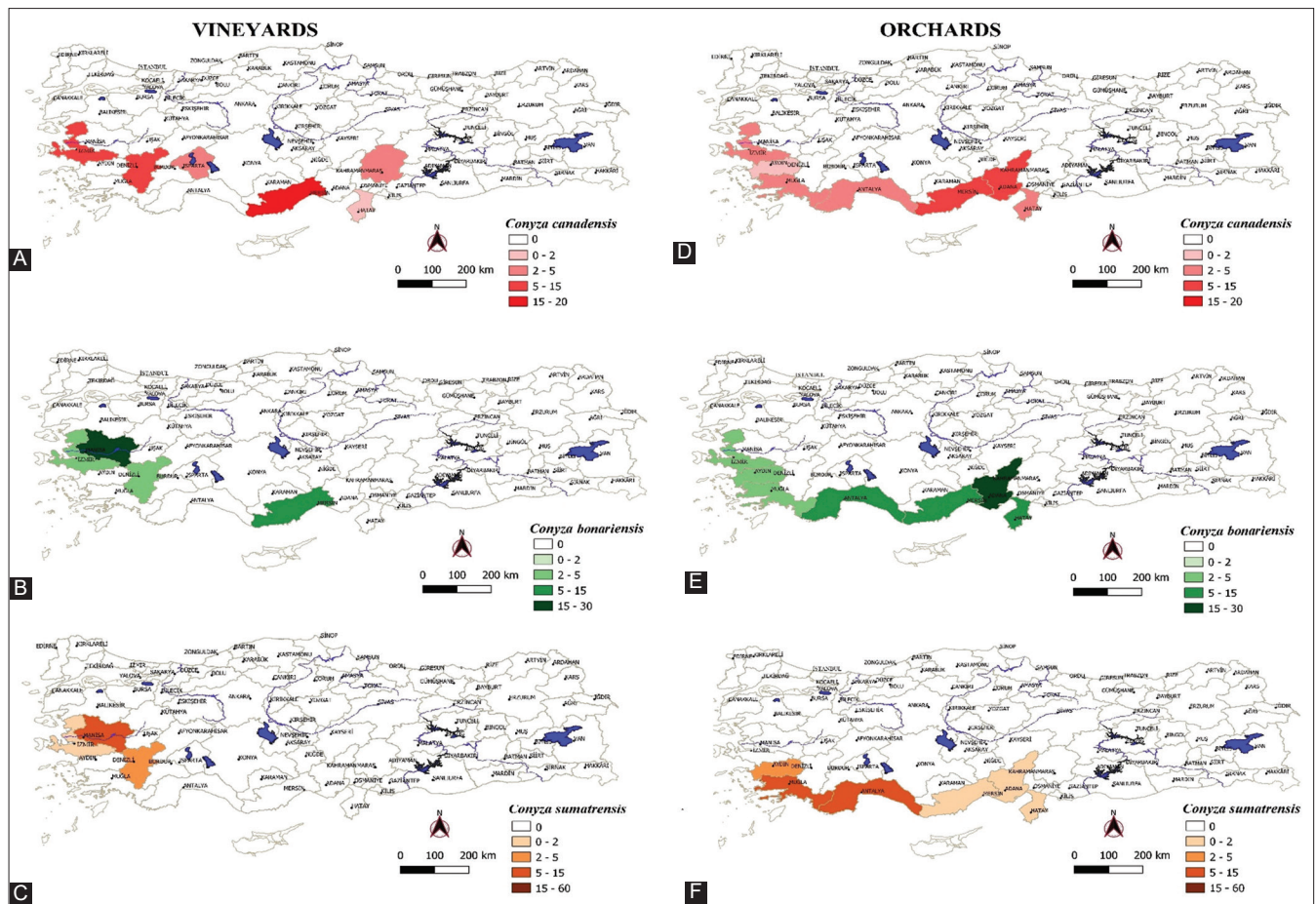


Fig 7. Distribution of *Conyza* species in vineyards and orchard fields of Turkey (A: *C. canadensis* in vineyards; A: *C. bonariensis* in vineyards; A: *C. sumatrensis* in vineyards; D: *C. canadensis* in orchards; E: *C. bonariensis* in orchards; F: *C. sumatrensis* in orchards).

Table 2: Distribution of the *Conyza* accessions in orchard fields of Turkey

Region	Province	<i>C. canadensis</i>	<i>C. bonariensis</i>	<i>C. sumatrensis</i>	Population Number
Mediterranean	Adana	13 (30%)	28 (67%)	1 (3%)	42
	Mersin	14 (52%)	11 (44%)	1 (4%)	26
	Hatay	5 (26%)	13 (68%)	1 (6%)	19
	Antalya	4 (21%)	9 (47%)	6 (32%)	19
	Total	36 (34%)	61 (58%)	9 (8%)	106
Aegean	Muğla	3 (25%)	3 (25%)	6 (50%)	12
	Aydın	1 (20%)	3 (25%)	3 (40%)	7
	İzmir	3 (50%)	3 (50%)	-	6
	Total	7 (%)	9 (%)	9 (%)	25
Total		43 (33%)	70 (53%)	18 (14%)	131

Table 3: Distribution of the *Conyza* accessions in vineyard fields of Turkey

Region	Province	<i>C. canadensis</i>	<i>C. bonariensis</i>	<i>C. sumatrensis</i>	Population Number
Mediterranean	Mersin	18 (71%)	7 (29%)	-	25
	K.Maraş	4 (67%)	2 (33%)	-	6
	Hatay	1 (33%)	2 (67%)	-	3
	Isparta	5 (100%)	-	-	5
	Total	28 (72%)	11 (29%)	-	39
Aegean	Manisa	24 (48%)	18 (34%)	9 (18%)	51
	Denizli	14 (67%)	4 (20%)	3 (13%)	21
	İzmir	6 (60%)	3 (30%)	1 (10%)	10
	Total	44 (54%)	25 (30%)	13 (16%)	82
Total		72 (59%)	36 (30%)	13 (11%)	121

because most of the weed survey have focused on all the weeds in these fields not a specific genus in Turkey. In some survey studies conducted in vineyard and orchard fields, *Conyza* spp., especially horseweed, were found to be some of the most common weed species (Kaçan and Boz 2015). Mengüç (2019) also reported that horseweed was the most abundant *Conyza* species, followed by hairy fleabane and tall fleabane, in a survey conducted in the southern Marmara region.

The closely related species belonging to the same genus like *Conyza* have been in similar habitats; however, their geographical distributions might be significantly different. This geographical difference between the expansion fields of horseweed and hairy fleabane might be seen on the plant maps (TUBIVES 2022; IPA 2022). Milovic (2004) compared the *Conyza* species in Croatia according to herbarium data and the literature and indicated that horseweed commonly spread to the coastal and continental fields of Croatia, while others were only distributed throughout the coastal regions of Croatia, similar to our results.

The climatic conditions have changed the spread patterns of the plant taxon. Hairy fleabane and Sumatran fleabane populations have commonly been found in coastal regions and found at a narrow range in the terrestrial regions of Australia, but horseweed populations have been found in the coastal fields contrary to Turkey and Croatia (ALA 2022). The thing limits their expanding fields is mainly weather conditions, especially the warm climate caused by global warming.

Changes in the climate of plant growth may closely affect weeds and their spread in agricultural and non-agricultural fields. Another side effect of global warming is increasing forest fire (Calda et al. 2020), and this impact has been obviously shown in the Mediterranean forests for the last twenty years (Avcı and Korkmaz 2021; Sarris et al. 2014; Cardil et al. 2015). Actually, the forest trees create a darker shade environment for the weeds grown under them compared to bare soil. As a result of the shade,

a decrease in the temperature that provides unsuitable growing conditions for *Conyza* spp. was observed under the forest trees. Forest trees are vulnerable to wild fire caused by sudden occurrence many times; therefore, after a wildfire, all the field was covered by ashes and lost their shade. These areas are generally sensitive to the infestation of *Conyza* spp. Following the infestation process, the field has completely been covered by the members of this genus.

Growth stage studies

The efficacy of glyphosate, similar to other herbicides, is related to choosing application parameters, including application technique, the timing of treatment and climatic factors. Moreover, using glyphosate for a long period could also result in the selection of resistant biotypes (Travlos and Chachalis 2010). ANOVA results showed that the interactions of experimental sites and accessions with glyphosate efficacy were not significant; therefore, all data were pooled (Table 4). There were no significant differences between the efficacy of glyphosate applied locations, Aydın and İzmir, so the results were pooled, but the main impacts are also presented in Table 4. The efficacy of glyphosate at 1,323 and 2,646 g ai ha⁻¹ on GR and GS *Conyza* accessions at four growth stages varied depending on growth stages and application rates (Fig 8). The seedlings at the earliest stage, 2-4 true leaves, were the most sensitive to glyphosate at 1.323 g ai ha⁻¹ with 29-37% relative biomass and at 2.646 g ai ha⁻¹ with 12-24% relative biomass, while the latest growth stage was the least sensitive to the herbicide with 70-78% and 56-68% relative biomass at the same rates, respectively. Significant differences between the growth stages on glyphosate efficacy were observed between the 7-8 leaf stage and the 10-12 leaf stages in both experiments. Parallel to the GR accessions, the impact of glyphosate on GS *Conyza* accessions changed from 99 to 72%, depending on the growth stage and application rates. The early stages of the accessions were more sensitive to herbicide, and the high rate of glyphosate resulted in less dry matter accumulation.

The responses of GS and GR hairy fleabane and horseweed to glyphosate rates were investigated by researchers based

Table 4: Relative biomass of GR and GS hairy fleabane and horseweed accessions in various growth stages as affected by glyphosate treatments at 1,323 and 2,646 g ai ha⁻¹ (percentage of untreated control)

Accession	Growth stage	1,323 g ai ha ⁻¹			2,646 g ai ha ⁻¹		
		Aydın	İzmir	Average	Aydın	İzmir	Average
GR	2-4 true leaves	37	29	33 ^{c*}	24	12	18 ^b
	7-8 true leaves	43	35	39 ^c	24	18	21 ^b
	10-12 true leaves	59	60	60 ^b	61	58	60 ^a
	Before bolting	78	70	74 ^a	68	56	65 ^a
GS	2-4 true leaves	5	3	4.0 ^b	4	1	2.5 ^b
	7-8 true leaves	4	6	5.0 ^b	3	6	4.5 ^b
	10-12 true leaves	14	24	19.0 ^a	13	17	15.0 ^a
	Before bolting	18	28	23.0 ^a	14	14	14.0 ^a

*Means followed by different letters show significant differences ($p \leq 0.05$)



Fig 8. Response of GR and GS hairy fleabane accessions to glyphosate applied at 1,323 (Glyphosate-1) and 2,646 (Glyphosate-2) g ai ha⁻¹ in various growth stages (S: glyphosate sensitive, R: glyphosate resistant, 1: 2-4 true leaves, 2: 7-8 true leaves, 3: 10-12 true leaves, 4: before bolting).

on growth reduction or chlorophyll content (Travlos and Chachalis, 2010; Urbano et al. 2007; Mylanos et al. 2019; VanGessel et al. 2009; Koger et al. 2004). They indicated that the early stages of hairy fleabane and horseweed were critical to control these weeds using glyphosate, similar to our results.

CONCLUSION

The identification ability of the chloroplast gene regions in terms of molecular classification of *Conyza* species was more incapable than that of nuclear ITS regions. ITS region may define all the sequences as favourable compared to the recorded sequence data. Additionally, some significant changes on the base resulted in easy discrimination of the species. The data indicated that ITS regions (ITS1, 5.8S, and ITS2) may be used to rapidly identify the members of the *Conyza* genus. Discriminating weed species at early stages of their growth using verified genetic sequences is valuable for creating convenient management practices. These data will also be used in phylogenetic studies, especially barcoding studies based on the ITS region.

Turkey is placed in a location with a high degree of biodiversity, not only in natural fields but also in agricultural fields with high endemism rates. Citrus orchards and vineyards are among the most weed-abundant fields in terms of plant species. Therefore, weeds are commonly controlled by repeatedly using total herbicides, especially glyphosate, without considering biodiversity in these fields.

Using glyphosate repeatedly in the same fields resulted in an increase in the frequency of the HR accessions and a decrease in biodiversity. Additionally, the distribution of *Conyza* members in these fields may change over time because their response to glyphosate changes is not the same. Another significant result of this treatment is changing weed flora and replacing herbicide-sensitive weeds with herbicide-resistant weeds. This alteration may result in a vicious cycle in which the more herbicide used to control herbicide-resistant weeds, the less biodiversity is observed. Correct identification of *Conyza* species in these fields is important before applying glyphosate, especially when weeds are at the beginning of their growth.

Weed control scores were higher when applied at earlier rosette stages at both sensitive and resistant biotypes. For this reason, it can be concluded that late treatments could be the main reason for the inefficiencies of glyphosate against *Conyza* species with non-resistant populations. However, there is also a lack of other components of good agricultural practices, such as equipment, water volume, and water quality can lead to low effectiveness. Based on the results of the studies presented here, management strategies such as mechanical control, soil tillage, and alternative herbicides should be applied to the sites where resistance is confirmed but also not yet confirmed areas. If glyphosate use is a necessity, especially for other weeds, treatments should be performed at earlier growth stages of *Conyza* species by following good agricultural practices. The locations near the glyphosate-resistant *Conyza* accessions

found should be considered potential infestation areas. These fields are generally vulnerable to weed infestations, such as *Conyza* species when they are empty caused by wildfire. The wildfire that occurred in the Mediterranean region in 2021 may provide an opportunity for members of this genus to invade. The climatic conditions of the region also accelerate the invasion of *Conyza* species compared to other regions of Turkey. Therefore, these fields should be generally surveyed regularly by weed scientists, and integrated weed management should be implemented to manage these invasive weed species in these areas.

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Authors' contributions

Mehmet Nedim Doğan created project idea, managed project, provided plant material, interpreted the data, drafted and wrote-reviewed & edited the article; Emine Kaya-Altop carried out the laboratory experiment, performed data analysis, created the Figures and Tables, and interpreted the data; Süleyman Gürdal Türkseven performed the laboratory experiments, and provided plant material; Ahmet Tansel Serim provided plant material, drafted and wrote-reviewed & edited the article.

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