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Effectiveness of alternative herbicides on three *Conyza* species from Europe with and without glyphosate resistance

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ABSTRACT

Glyphosate has been applied in European countries for over a decade between rows in olive groves and grape vineyards to control *Conyza* species [hairy fleabane (*C. bonariensis*), horseweed (*C. canadensis*) and Sumatran fleabane (*C. sumatrensis*)], however poor control has been observed in recent years. Glyphosate susceptible (GS) or resistant (GR) populations were assayed in each species. In addition, *Conyza* spp. control with alternative herbicides (alone or in mixture with glyphosate) over two years was also assessed. The GS populations of the three species were controlled with glyphosate field doses (1080 g ae ha^{-1}). The GR hairy fleabane, horseweed and Sumatran fleabane populations were 15.0, 15.7 and 19.8 times more resistant, respectively, than their respective GS population. The shikimic accumulation of GS populations was 4–6 times higher compared with the GR *Conyza* populations, confirming the glyphosate resistance of the latter ones. The increase in the glyphosate dose did not control the GR *Conyza* populations, despite providing a higher dry growth reduction. Glufosinate and flazasulfuron, alone or mixed with glyphosate, were the effective options to control GR and GS populations of hairy fleabane and Sumatran fleabane. However, the GR horseweed population might have evolved multiple resistance to glyphosate and flazasulfuron in Hungary. The other herbicides (PSI, auxinic and PPO) showed an additive effect together with the control provided by glyphosate in the GS and GR populations; however generally, these herbicides could be applied alone at the rosette stage. Effective herbicides with modes of action different from glyphosate, except flazasulfuron for controlling horseweed, should be used to delay the selection of herbicide resistance in perennial crops in Europe.

1. Introduction

Conyza species [hairy fleabane (*C. bonariensis*), horseweed (*C. canadensis*) and Sumatran fleabane (*C. sumatrensis*)] are common weeds invading a great variety of agronomic crop systems worldwide (Travlos and Chachalis, 2010, 2013), due to their rapid adaptation to undisturbed (non-tillage) and plant-free soils (Brown and Whitwell, 1988). *Conyza* species have evolved resistance to herbicides in many countries (100 cases), and are listed among the most problematic weeds (Matzrafi et al., 2015; Heap, 2018). Glyphosate is responsible for 13, 42 and 8 cases of resistance in hairy fleabane, horseweed and Sumatran fleabane, respectively, reported in annual and perennial crops, orchards, forests, pastures, roadsides, railways, industrial sites and nurseries around the world (Heap, 2018).

Glyphosate [*N*-(phosphonomethyl)-glycine] is the world's most successful post-emergence and non-selective herbicide (Duke, 2018). In Spain it has been widely used to control weeds in citrus orchards, olive groves, grape vineyards, and others perennial and annual crops (González-Torralva et al., 2010, 2014), as well as in path borders, railway lines, recreation areas and derelict sites (Urbano et al., 2007). This herbicide is absorbed through leaves and other young-green tissues, and translocated via phloem into meristematic tissues (Preston and Wakelin, 2008). Glyphosate is a potent inhibitor of 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) by blocking the biosynthesis of phenylalanine, tryptophan, tyrosine and others aromatic compounds in susceptible plants (Maeda and Dudareva, 2012).

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Glyphosate-resistant weeds are able to survive glyphosate exposure due to target-site (mutations in the gene encoding the EPSPS or gene duplication) (Heap and Duke, 2018) or non-target-site (degradation to non-toxic compound, impaired translocation, poor absorption and/or vacuolar compartmentation) (Ghanizadeh and Harrington, 2017) resistance mechanisms, alone or in association (Sammons and Gaines, 2014). The resistant weeds will not be killed by using glyphosate alone.

The herbicide combinations with different modes of action, either in sequential application or tank-mix, can contribute to improve the control of resistant weeds (Tornisielo et al., 2013), and additionally, may also avoid or delay evolution of resistance (Ganie and Jhala, 2017). Improvements in weed control have been observed in glyphosate-resistant species by applying glyphosate tank-mix combinations (Riley and Bradley, 2014), in some cases, the control was improved by 25–30% compared to glyphosate alone (Eubank et al., 2008).

The objectives were to characterize glyphosate resistant *Conyza* species, collected in perennial crop systems from different European countries, and to propose alternative chemical strategies for their management.

2. Material and methods

2.1. Plant material and experimental conditions

Seeds of *Conyza* species (hairy fleabane, horseweed and Sumatran fleabane) resistant (GR) and susceptible (GS) to glyphosate (Table 1), were planted in plastic containers (10 × 10 cm x 6.3 cm) filled with peat moistened and covered with parafilm. Plastic containers were kept in a controlled condition room [28/18 °C (day/night), 16 h photoperiod, 850 mmol $m^{-2} s^{-1}$ light density and 60% relative humidity] until germination. Seedlings were transplanted into 250 cm^3 pots (1 plant pot^{-1}) filled with sand/peat (1:1 v/v). Pots were placed in the controlled condition room again and watered daily until the herbicide treatments.

Herbicides were applied in a treatment chamber (Devries Manufacturing, Hollandale, Minnesota) equipped with a TeeJet 8002EVS flat fan nozzle calibrated to deliver 200 L ha^{-1} at 200 kPa at a height of 50 cm. Plants with six-eight true leaves (rosette stage or BBCH16-18 stage) were used for the different herbicide treatments.

2.2. Whole-plant glyphosate dose-response

The glyphosate (Roundup Energy 45% w/v, Monsanto, Spain) doses applied on GR and GS *Conyza* plants were the following: 0, 31.25, 62.5, 125, 250, 500, 1000 and 2000 g ae ha^{-1}. Once treated, the plants were taken to a greenhouse at a temperature regime of 26/18 °C day/night, and watered as necessary. Mortality was recorded 3 weeks after application (WAA), and above-ground biomass was harvested and dried individually in an oven (JP Selecta S.A., Barcelona, Spain) at 75 °C for 72 h. The experiments had a completely random design using 8 plants per dose and were repeated twice. Plant mortality (PM = number of dead plants after treatment/total treated plants * 100) and dry weight reduction (DWR) data were expressed in percentage relative to the non-treated control, and submitted to non-linear regression analysis using the following log-logistic equation: $Y = d/1 + (x/g)^b c$ (Ritz et al., 2015).

2.3. Accumulation of shikimate

Leaf discs (4 mm in diameter) were taken from young leaves of both GR and GS *Conyza* plants at the rosette stage to give a total sample of 50 mg. Shikimic acid accumulation was determined according to Shaner et al. (2005) using the following glyphosate concentrations: 0, 10, 50, 100, 500 and 1000 μM. Absorbance of samples was measured in a Beckman DU-640 spectrophotometer at 382 nm. The test was performed in triplicate per glyphosate concentration in a completely random design. The shikimic accumulation was determined by constructing a standard curve with known shikimate concentrations. Results were expressed in mg of shikimic acid μg^{-1} fresh tissue.

2.4. Alternative chemical control

Alternative chemical control experiments were carried out in a greenhouse during two cropping seasons (2014–2015 and 2015–2016) in the spring. Ten herbicides were applied alone or in mixture with glyphosate at a single dose (recommended field dose) (Table 2). *Conyza* seedlings were transplanted in 3-L pots (five plants pot^{-1}) filled with sand/peat (1:1 v/v), kept in the controlled condition room and watered daily until the herbicide treatments. Once treated, pots were taken to the greenhouse and watered as necessary. The experiment had a random block design with 6 repetitions plus a set of control plants for each *Conyza* species. Both the PM and DWR were assessed as in the dose-response assays 4 WAA, i.e., data were expressed as percentage of untreated controls. The variance stability tests of control rate data showed no difference for both cropping seasons, and data were pooled and subjected to analysis of variance. Differences with $p < 0.05$ were considered significant and Tukey's test was conducted for means comparison. Statistical analysis was conducted the Statistix 9.0 software (Analytical Software, Tallahassee).

3. Results and discussion

3.1. Confirmation of glyphosate-resistant populations in Conyza spp.

Dose-response assays revealed variable glyphosate susceptibility between GR and GS *Conyza* populations, both in terms of PM and DWR. All GS populations died at the dose recommend to the farmers in European countries in olive groves and vineyards (1080 g ha^{-1}). On the other hand, the GR populations were not injured at this dose. The resistance indexes (RI = R/S) based on the LD_{50} (15.2, 12.6 and 8.1) and DWR_{50} (15.0, 15.7 and 19.8) values were higher in the GR than the GS populations of hairy fleabane, horseweed and Sumatran fleabane, respectively (Table 3).

Additionally, differential levels of shikimate accumulation were

Table 1
Features of *Conyza* populations used in this research.

Species	Status	Country/Location	Crops	Herbicide application	$Dose_{(years)}$	Coordinates
C. bonariensis	Resistant	Spain/Antequera	Orchard	Glyphosate + Auxinic synthetic	[a]$1080_{(10)}$ + [b]$400_{(5)}$	37.057224, −4.515618
	Susceptible	Spain/Antequera	Railway	Mechanical control	NH	37.058480, −4.504785
C. canadensis	Resistant	Hungary/Badacsony	Vineyard	Glyphosate + ALS inhibitor	[a]$1800_{(20)}$ + [c]$750_{(7)}$	46.790435, −17.490739
	Susceptible	Hungary/Balaton	No crop	Mechanical control	NH	48.085674, −20.303097
C. sumatrensis	Resistant	France/Mazan	Vineyard	Glyphosate	[a]$1080_{(15)}$	44.047650, −5.129497
	Susceptible	France/Mazan	Vineyard	Organic crop	NH	44.069471, −5.153672

[a] Glyphosate g ae ha^{-1}.
[b] 2,4-D ae ha^{-1}.
[c] Flazasulfuron g ai ha^{-1}; subscript of years = application history of the respective herbicide at the indicated dose, NH = no history of herbicide application.