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Response of *Eleusine indica* and *Paspalum distichum* to glyphosate following repeated use in citrus groves

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ABSTRACT

Eleusine indica L. Gaertn. and *Paspalum distichum* L. are annual and perennial grasses, respectively that are widely distributed in turf and perennial cropping systems throughout Spain. Often, glyphosate is used between rows of perennial crops for control of these grasses, but variable responses have been observed. Sensitivity to glyphosate in each species was examined under greenhouse, laboratory and field conditions. *In vitro* tests on whole plants of both *P. distichum* and *E. indica* revealed no differences in sensitivity to glyphosate for areas with long-term use (treated; T) and no history of use (not treated; NT). The NT population of *P. distichum* (ED_{50} 73.1 g ae ha^{-1}) was 11.6% more sensitive to glyphosate than NT *E. indica* (ED_{50} 81.6 g ae ha^{-1}). No differences between T and NT populations of both species were observed for foliar retention of glyphosate as well as accumulation of shikimate. However, glyphosate retention and shikimate accumulation were up to 64 and 24.4% greater, respectively in *P. distichum* compared to *E. indica*. Within 96 h after treatment (HAT), foliar absorption of ^{14}C-glyphosate was similar among T and NT populations, but 8.8% higher for *P. distichum* compared to *E. indica*. Retention of ^{14}C-glyphosate in treated leaves of *P. distichum* was approximately 55% lower compared to *E. indica*. Translocation from the treated leaf into other shoot tissue (2.8-fold) and roots (8.5-fold) was higher for *P. distichum* versus *E. indica*. This would suggest that differences in *E. indica* versus *P. distichum* response to glyphosate are based upon differential retention in treated leaves and reduced movement out of treated tissue in other shoot and root tissue. In separate field experiments in citrus orchards, glyphosate and other herbicides were applied to assess control of *E. indica* and *P. distichum* over two years. Flazasulfuron and cycloxidim resulted in 90% or greater control of both species by 60 days after treatment (DAT). Only glufosinate, oxyfluorfen, paraquat and iodosulfuron resulted in >85% control of *E. indica*. These corresponding treatments ranged in effectiveness from 73 to 92% on *P. distichum*. Integration of effective herbicides with modes of action different than glyphosate should be used for management of *E. indica* and *P. distichum* and may delay the selection for resistance to glyphosate.

1. Introduction

In the Mediterranean region, no-till practices are adopted commonly to conserve soil resources in perennial cropping systems such as olive (*Olea europaea* L.) groves, *Citrus* spp. orchards and grape (*Vitis vinifera* L.) vineyards (Cerda et al., 2015). In the absence of tillage, living cover crops consisting of barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and legumes such as vetch (*Vicia* spp.) and lupins (*Lupinus* spp.) are established to deter weed establishment, build soil organic matter, and reduce soil erosion (Gomez et al., 2011; Hartwig and Ammon, 2002). In some cases, grass weeds are allowed to develop in open canopy areas to conserve soil. Growth of cover crops or naturally established weeds is controlled by mowing, non-selective herbicides or animal grazing.

The herbicide glyphosate is frequently applied beneath perennial crops in Spain to manage cover crops or other vegetation (Costa, 1997). Lacking residual activity, glyphosate is non-selective and controls a broad-spectrum of annual and perennial plant

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species (Baylis, 2000). According to Duke and Powles (2008), glyphosate is the most widely sold herbicide in the world.

Glyphosate inhibits the chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19), which catalyzes the conversion of shikimate-3-phosphate and phosphoenolpyruvate to EPSP and inorganic phosphate via the shikimic acid pathway (Geiger and Fuchs, 2002; Reddy et al., 2008). Inhibition of this enzyme prevents biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan as well as other important secondary compounds including auxins and allelochemicals (Harring et al., 1998; Schönbrunn et al., 2001).

Despite the effectiveness of glyphosate, repeated applications within and over numerous years as well as over large areas has resulted in selection of numerous glyphosate-resistant (GR) biotypes (Owen, 2001; Thill and Lemerle, 2001). To date, there are 32 GR biotypes worldwide; five (*Conyza bonariensis* L. Cronq., *Conyza canadensis* L. Cronq., *Conyza sumatrensis* (Retz) E.H. Walker, *Lolium multiflorum* Lam., and *Lolium rigidum* Gaudin) of which are found in Spain (Heap, 2015). In addition, there are other weed species which are difficult to control with glyphosate (Cruz-Hipólito et al., 2009). Two of these species include *Paspalum distichum* L. and *Eleusine indica* L. Gaertn.

P. distichum is a perennial grass introduced from tropical regions of the Americas. *P. distichum* is spread both by seed and rhizomes (Manuel and Mercado, 1977). Aguiar et al. (2005) reported that *P. distichum* exhibits invasive characteristics where water is available such as ditch banks, riparian areas and irrigated crops in the Mediterranean basin. Infestations are commonly reported in perennial crops throughout Spain (Costa, 1997).

Left uncontrolled, established stands of *P. distichum* form monocultures (Guillerm et al., 1990). Mechanical cultivation is effective on seedlings prior to formation of rhizomes, but cultivation spreads perenniating plants by cutting rhizomes into smaller propagules (Huang et al., 1987; Manuel and Mercado, 1977). Moist soil conditions at the time of cultivation renders mechanical control ineffective. Control with glyphosate is challenging; Okuma and Chikura (1985) recommended rates up to 4.9 kg ha^{-1}. Alternative herbicides are necessary to reduce the selection pressure resulting from repeated applications of glyphosate.

E. indica is a summer annual species in the *Poaceae* family. Plants thrive in sub-tropical areas at approximately 50° latitude. Exhibiting a C4 process for photosynthesis, plants are also considered troublesome in temperate areas with hot summers. In climates lacking a killing frost, some *E. indica* plants can survive longer than 1 year. It is an important weed of cultivated crops (*Zea mays* L., upland *Oryza sativa* L., *Saccharum officinarum* L. and many fruit and vegetable orchards), lawns, and golf courses (Holm et al., 1977; Lourens et al., 1989). Eke and Okereke (1990) found 10–16 *E. indica* seedlings competing with a *Z. mays* plant reduced plant biomass approximately 52% compared to *Z. mays* lacking competition.

Once established, goosegrass plants tiller extensively and adapt to frequent mowing. Timely mechanical tillage and herbicide application can be effective for control. However, one consequence in utilizing herbicides is the propensity of some populations to evolve resistance (Vidal et al., 2006; Jalaludin et al., 2010). Recently, glyphosate resistance based upon a Pro-106 point mutation in EPSPS has been identified in a population from Malaysia, with resistant biotypes surviving rates up to 5-fold higher than sensitive populations (Ng et al., 2003; Heap, 2015).

Exclusive, long-term use of glyphosate in irrigated citrus crops for control of *P. distichum* and *E. indica* has led to concerns for selection of resistant biotypes. The specific objectives of this research were: (a) to assess whole plant sensitivity of *E. indica*, and *P. distichum* species to glyphosate based upon comparing T and NT populations; (b) to identify if physical (leaf retention) or physiological (shikimic acid accumulation, ^{14}C-glyphosate absorption and translocation) characteristics may explain differential responses between species; and (c) to determine if herbicide alternatives to glyphosate result in effective control of one or both species.

2. Materials and methods

2.1. Plant production

All seeds from both the treated (T) and non-treated (NT) populations of *E. indica* and *P. distichum* species were collected from mature plants in summer 2009. Plants were considered T if they originated from fields where glyphosate had been applied annually for at least five years. *E. indica* was collected from a field containing citrus crops in the Huelva province (Southern Spain); *P. distichum* was collected from a citrus field located in Castellón province (Eastern Spain). The NT populations of *E. indica* and *P. distichum* were obtained from fields in close proximity to the corresponding T populations, where no documented use of glyphosate was found.

All seeds were germinated in 663 cm^2 trays containing peat saturated at field capacity, then placed in growth chambers. Growing conditions included air temperatures of 28/18 °C (day/night) with a photoperiod of 16 h, 850 μmol m^{-2} s^{-1} photosynthetic photon flux density, and 80% relative humidity. Both T and NT seedlings from each species were transplanted into pots (3 plants per pot) containing a 1:2 (v/v) ratio of sand:peat and placed in a growth chamber under the conditions described above.

2.2. Dose–response assays

Glyphosate applications were made at the BBCH 13–14 stage (3–4 leaves) (Hess et al., 1997). A laboratory spray chamber (DeVries Manufacturing, Hollandale, MN) equipped with TeeJet 8002 flat fan nozzle (Spraying Systems Co., Wheaton, IL) tips delivered 200 L ha^{-1} at 200 kPa at a height of 50 cm. Glyphosate (Roundup Energy SL, Monsanto, Spain) rates included: 0, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 g ae ha^{-1}. A typical field use rate of glyphosate (1X dose) is 360 g ha^{-1}. Experimental design was completely randomized with four replications of each treatment, where each replicate utilized three plants. Plants were harvested 21 days after treatment (DAT), and immediately weighed to determine fresh weight. Data were expressed as ED_{50} (effective dose to reduce plant fresh weight by 50%) and compared to untreated plants. Assays were conducted twice and results combined.

2.3. Spray retention assays

At the BBCH 13–14 stage, *P. distichum* and *E. indica* were sprayed with 300 g ae ha^{-1} of glyphosate and 100 mg L^{-1} Na-fluorescein using conditions as described above. Once the foliage had dried (20–25 min), shoot tissue was cut at ground level. The tissue was immersed in 50 mL of 5 mM NaOH for 30 s to remove spray solution. Fluorescein absorbance was determined using a spectrofluorometer (Hitachi F-2500, Tokyo, Japan) with excitation wavelength of 490 nm and absorbance at 510 nm. Dry biomass of plant tissue was recorded following exposure to 60 °C for 48 h. The experimental design was completely randomized with four replications, where one replicate included three plants of each population and species. Assays were conducted twice and results combined. Spray retention was expressed as mL spraying solution per gram dry matter (González-Torralva et al., 2010).