Plant Physiology and Biochemistry 98 (2016) 72–80

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Physiological, morphological and biochemical studies of glyphosate tolerance in Mexican Cologania (*Cologania broussonetii* (Balb.) DC.)

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ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form
29 October 2015
Accepted 16 November 2015
Available online 30 November 2015

Keywords:
Absorption/translocation
Cover crop
Glyphosate tolerance
Glyphosate metabolism
Morphological characteristics
Shikimic acid

ABSTRACT

In recent years, glyphosate-tolerant legumes have been used as cover crops for weed management in tropical areas of Mexico. Mexican cologania (*Cologania broussonetii* (Balb.) DC.) is an innate glyphosate-tolerant legume with a potential as a cover crop in temperate areas of the country. In this work, glyphosate tolerance was characterized in two Mexican cologania (a treated (T) and an untreated (UT)) populations as being representatives of the species, compared in turn to a glyphosate-susceptible hairy fleabane (S) (*Conyza bonariensis* (L.) Cronq.) population. Experiments revealed that T and UT Mexican cologania populations had a higher tolerance index (TI), and a lower shikimic acid accumulation and foliar retention than the hairy fleabane S population. Absorption and translocation, leaf morphology and metabolism studies were only carried out in the Mexican cologania T population and the hairy fleabane S population. The latter absorbed 37% more ^{14}C-glyphosate compared to the Mexican cologania T at 96 h after treatment (HAT). Mexican cologania T translocated less herbicide from the treated leaf to the remainder of the plant than hairy fleabane S. The Mexican cologania T presented a greater epicuticular wax coverage percentage than the hairy fleabane S. This morphological characteristic contributed to the low glyphosate absorption observed in the Mexican cologania. In addition, the Mexican cologania T metabolized glyphosate mainly into AMPA, formaldehyde and sarcosine. These results indicate that the high glyphosate tolerance observed in Mexican cologania is mainly due to the poor penetration and translocation of glyphosate into the active site, and the high glyphosate degradation into non-toxic substances.

1. Introduction

Weeds reduce crop productivity, displace native species, contribute to land degradation and increase production costs. A cover crop is constituted by a living vegetal that keeps the soil covered, protects it from erosion, and prevents nutrient loss (Hartwig and Ammon, 2002). Cover crops are also involved in the elimination of weeds and they reduce the spread of numerous pathogens (Renard and Franqueville, 1991). Cover crops allow for the selective use of herbicides within integrated weed management programs in some agricultural systems, drastically reducing germination, emergence and/or growth of weed populations (Lal et al., 1991; Buhler et al., 2001). The use of cover crops for weed displacement and soil protection against erosion presents some limitations, such as the interference of weeds at the early stages of cover crop growth (Cameron et al., 1991). At that point, the use of herbicides with good toxicological and environmental profiles, such as glyphosate, could facilitate crop establishment (Domínguez-Valenzuela, personal observation).

Herbicide tolerance is a natural, hereditary mechanism that allows a plant species to survive and reproduce after herbicide application; this survival is due to inherent morphological and/or physiological characteristics. Therefore, herbicide-tolerant populations have never been susceptible (Cruz-Hipolito et al., 2009). Resistant populations of weed species are naturally present and occur at low densities. Herbicide resistance happens when resistant weeds are able to survive and complete their life cycles when the herbicide is applied at recommended field doses. Repeated

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http://dx.doi.org/10.1016/j.plaphy.2015.11.009
0981-9428/

selection pressure through herbicide applications selects and promotes the expansion of the resistant population (Beckie, 2006). Herbicide tolerance and resistance are phenomena of interest although weed resistance to herbicides is a serious problem due to the loss of active ingredients. Therefore, herbicide tolerance in a cover crop would be desirable (Cameron et al., 1991).

Glyphosate (N-phosphonomethyl glycine) is currently the most important herbicide (Duke and Powles, 2008), and it is used for broad-spectrum weed control in glyphosate-resistant crop. Glyphosate is systemic, non-residual, non-selective, and also has an acceptable toxicity profile. It is easily transported from the leaves to meristematic tissues, rapidly reducing photosynthesis and increasing shikimate levels (Duke et al., 2003). Glyphosate acts by inhibiting 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19), responsible for the biosynthesis of chorismate, an intermediate in the shikimic acid pathway that leads to the synthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan) (Amrhein et al., 1980; Siehl, 1997; Shaner et al., 2005; Wiersema et al., 2013).

Like many herbicides, the continuous use of glyphosate has led to the evolution of resistant weed biotypes. However, many other species have some natural tolerance degree, which could be related to the same resistance mechanisms found in resistant weed biotypes (Duke, 2011). New weed management technologies are a priority in sustainable agriculture (Hartwig and Ammon, 2002). In Mexico, some suspected glyphosate-tolerant species could be being used as cover crops. Nevertheless, this tolerance has still not been confirmed (Dominguez-Valenzuela, personal observation).

Mexican cologania (*Cologania broussonetii* (Balb.) DC.), is a perennial legume from America, distributed in Central and South America. It grows in temperate regions of Mexico (Vibrans, 2009a), and has morphological and physiological characteristics that could be useful in weed management. In previous unpublished studies by this research group, some level of tolerance to glyphosate has been observed in Mexican cologania. Beneficial morphological and physiological characteristics and glyphosate tolerance permit its use as a cover crop to control weeds such as hairy fleabane in fruit orchards with temperate climates. Glyphosate applications may favor the establishment of Mexican cologania and the selective suppression of weeds.

Hairy fleabane (*Conyza bonariensis* (L.) Cronq.) is an annual weed that originated in South America, which is commonly found along roadsides, pastures and abandoned farm fields (Vibrans, 2009b). This weed has a prolific seed production, producing more than 50,000 seeds per plant (Holm et al., 1997). Seed dormancy up to 3 years has been documented in the soil of some crops (Wu et al., 2007). In addition, hairy fleabane is a very common weed species hard to control in perennial crops of Mexican temperate regions with high population densities.

Glyphosate tolerance/resistance in triggered through two major mechanisms, Non-Target Site Resistance (NTSR) and Target Site Resistance (TSR). The NTSR mechanisms include the reduced absorption and/or translocation of the herbicide, vacuolar sequestration, glyphosate metabolism and others (Cruz-Hipolito et al., 2009; Sammons and Gaines, 2014), so that less herbicide reaches the action site. Enhanced metabolism can also cause tolerance or resistance to herbicides, but whether this happens with glyphosate is unclear (Duke, 2011). Glyphosate is enzymatically metabolized to other non-toxic compounds such as glyoxylate, sarcosine, formaldehyde and aminomethyl phophonate (AMPA) (Sammons and Gaines, 2014). Glyphosate degradation to non-toxic compounds has been documented in several cases (Cruz-Hipolito et al., 2011; Alarcón-Reverte et al., 2013; Sammons and Gaines, 2014). The TSR mechanisms are due to a loss of affinity between the binding protein (EPSPS) and the herbicide, overexpression of this protein and spontaneous mutations that occur randomly, mainly in the Pro106 amino acid position (González-Torralva et al., 2014; Sammons and Gaines, 2014), and recently the Thr102 position was described (Yu et al., 2015).

Glyphosate tolerance can involve inherent morphological and histological characteristics. The main barrier to herbicide absorption is the cuticle (Heredia-Guerrero et al., 2014). The main entry points for herbicides are the guard cells of the stomata, trichomes and leaf veins in broadleaf species. A thicker leaf cuticle, some trichomes and/or stomata limit herbicide absorption in an aqueous solution such as glyphosate (Devine et al., 1992; Heredia-Guerrero et al., 2014).

Mexican cologania is used as a cover crop in Mexican temperate areas to suppress weeds. During cover crop establishment, weed control is needed and this can be implemented by applying a certain dose of glyphosate that is toxic to weeds like hairy fleabane and others species, but not to Mexican cologania in fruit orchards with temperate climates. Therefore, we to understand why this differential glyphosate sensitivity occurs among these species.

The purpose of this study was to determine the efficacy of glyphosate in two Mexican cologania populations and a hairy fleabane susceptible population by dose–response assays under growth chamber conditions. Physical (foliar retention), physiological (accumulation of shikimic acid, absorption and translocation of ^{14}C-glyphosate), morphological (leaf morphology) and biochemical (metabolism) characteristics of glyphosate tolerance were examined in Mexican cologania and hairy fleabane plants.

2. Material and methods

2.1. Plant material and experimental conditions

Mexican cologania T population seeds were collected in the plots of experimental fields from University of Chapingo, Mexico (19°29′43″N, 98°52′44″W), that had survived sporadic applications of glyphosate at the recommended field dose, 720 g acid equivalent (ae) ha^{-1}. Mexican cologania UT population seeds were also collected from glyphosate untreated experimental fields. Mexican cologania was considered as being a representative of the entire species. The objective of comparing the UT population with respect to T population was to identify possible differences in the innate glyphosate tolerance of this species. Hairy fleabane S population seeds never treated with herbicide were collected in a public lands from Tequila, Mexico (20°53′03″N, 103°48′18″W). The seeds of both species were collected in 2009 and 2010, respectively.

Mexican cologania T and UT populations seeds were germinated in 15 cm Petri dishes containing two layers of filter paper moistened with distilled water and sealed with parafilm. The hairy fleabane S population was planted in 663 cm^3 trays with peat saturated at field capacity and covered with a plastic layer until emergence. Both species were placed in a growth chamber at 28/18 °C (day/night) with a photoperiod of 16 h, a light density of 850 mmol m^{-2} s^{-1}, and 60% relative humidity.

Once germinated, the seedlings of both species were transplanted into 250 mL pots containing (one plant per pot) a mixture of peat/sand (1:1 v/v). Pots were watered to saturation and placed in a growth chamber under the conditions described above. Irrigation was suspended for one day after transplanting to stimulate root growth. Subsequently, pots were watered daily.

All glyphosate (Roundup® Energy 45% w/v, Monsanto, Spain) applications, except for absorption and translocation, were performed in a spray chamber (Devries Manufacturing, Hollandale, Minnesota) equipped with a Tee Jet 8002EVS flat fan nozzle with a pressure of 200 kPa, a height of 50 cm and an application volume of 200 L ha^{-1}.