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Restricted Herbicide Translocation Was Found in Two Glyphosate-resistant Italian Ryegrass (*Lolium multiflorum* Lam.) Populations from New Zealand

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ABSTRACT

Glyphosate resistance has been found in two populations of Italian ryegrass (*Lolium multiflorum*) following many years of glyphosate application in New Zealand vineyards. Dose-response experiments showed that both glyphosate-resistant Italian ryegrass populations had 10-fold levels of resistance to glyphosate compared to a susceptible population. Possible mechanisms of glyphosate resistance target site mutation at position Pro-106 of *5-enolpyruvyl-shikimate-3-phosphate synthase* gene and alterations in glyphosate absorption/translocation in these populations were investigated. Genotyping assays demonstrated that there was no point mutation at Codon 106 of the *5-enolpyruvyl-shikimate-3-phosphate synthase* gene in either of the resistant populations. Glyphosate-resistant and susceptible populations did not differ in ^{14}C-glyphosate absorption. However, in both resistant populations, much more of the absorbed ^{14}C-glyphosate was retained in the treated leaf than occurred in the susceptible population. Significantly more ^{14}C-glyphosate was found in the pseudostem region of susceptible plants than resistant plants. Based on these results, it was suggested that alterations in glyphosate translocation patterns plays a major role in glyphosate resistance for Italian ryegrass populations from these New Zealand vineyards.

Keywords: EPSP synthase mutation, Glyphosate, Herbicide resistance, Mechanism of resistance, Weeds.

INTRODUCTION

Glyphosate is an important agrochemical because it controls a wide range of annual and perennial weeds, becomes inactive on contact with soil and has minimal environmental impact (Powles and Yu, 2010). Continuous applications of glyphosate over the last three decades have led to the evolution of glyphosate resistance in a number of different weed species in various parts of the world (Sammons and Gaines, 2014). Glyphosate inhibits the enzyme 5-EnolPyruvyl-Shikimate-3-Phosphate Synthase (EPSPS) which is responsible for the synthesis of three aromatic amino acids and plant metabolites (Powles and Preston, 2006). Both target site and non-target site mechanisms of resistance have been found to confer glyphosate resistance to weed species. The target site mechanism of resistance could be due to a mutation at position Pro-106 in *EPSPS* gene (modified target site mechanism of resistance) (Baerson *et al*., 2002) or *EPSPS* gene over-production (EPSPS gene amplification) (Gaines *et al*., 2010). The modified target site mechanism of resistance has been reported for *Lolium rigidum* (Wakelin and Preston, 2006), *Lolium*

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multiflorum (Perez-Jones *et al*., 2007) and *Eleusine indica* (Ng *et al*., 2003). The *EPSPS* gene amplification has been reported for several weed species including *Lolium multiflorum* (Sammons and Gaines, 2014)

The non-target site mechanism of resistance involves alterations in glyphosate translocation, restricting glyphosate movement to its target site (restricted herbicide translocation) (Preston *et al*., 2009). Restricted herbicide translocation has been reported in glyphosate-resistant populations of *Lolium rigidum* (Wakelin *et al*., 2004), *Conyza canadensis* (Koger and Reddy, 2005), and *Lolium multiflorum* (Perez-Jones *et al*., 2007). Previous studies have suggested that both target site and non-target site mechanisms of resistance to glyphosate can occur simultaneously in a weed species (Ghanizadeh *et al*., 2015b; Bostamam *et al*., 2012; Yu *et al*., 2007).

Recently, glyphosate-resistant Italian ryegrass has been reported from two different vineyards in New Zealand (Ghanizadeh *et al*., 2013). The herbicide exposure records showed that glyphosate had been applied 3-4 times annually for the previous 10 years in both vineyards. As the modified target site mechanism of resistance and restricted herbicide translocation have been the two most common mechanisms of resistance to glyphosate (Sammons and Gaines, 2014), the objective of this study was to determine if the two glyphosate-resistant populations of Italian ryegrass found in New Zealand had evolved either the target site (mutation at Codon 106) or non-target site (alterations in glyphosate absorption/translocation) mechanisms.

MATERIALS AND METHODS

Plant Material

Live plants of two suspected glyphosate-resistant Italian ryegrass populations (populations A and P) were obtained from separate vineyards (10 km apart) in Marlborough, New Zealand, and transplanted into pots. Plants of Italian ryegrass (population SI) were obtained from grazed pastures at Massey University in Manawatu for comparison as they were unlikely to have been sprayed with glyphosate.

The clones of plants that had been proven to be resistant (populations A and P) and Susceptible (population SI) to glyphosate in dose-response experiments were used for the *EPSPS* genotyping study. To obtain seeds from the glyphosate-resistant populations for physiological studies (restricted herbicide translocation studies), plantlets from the same glyphosate-resistant populations were grown together in a glasshouse within pollen-proof cloth in order to prevent contamination by pollen from other populations. Seeds of Italian ryegrass (population SI), which were known from previous studies to be susceptible to glyphosate (Ghanizadeh *et al*., 2015a), were also obtained from a commercial source.

Glyphosate Dose-Response

A conventional dose-response study was conducted comparing glyphosate-resistant populations of Italian ryegrass (populations A and P) with a susceptible population (population SI) to determine the magnitude of resistance. All plants were split into plantlets consisting of two to three tillers and each was established in separate polythene planter bags (700 mL) filled with potting mix (50% bark, 30% fibre, 20% Pacific Pumice (7mm) and slow-release fertilizer (Woodace, Lebanon, PA)). The plants were left to develop in an unheated glasshouse at Massey University with automated overhead irrigation. When plants had an average of five tillers, they were sprayed with different rates of glyphosate (Roundup 360 Pro, an isopropylamine salt) on 16 December, 2012, using a laboratory track sprayer calibrated to deliver 230 L ha^{-1} of spray solution. Population SI received glyphosate rates of 0, 22.5, 45, 90, 180 and 360 g ae (acid equivalent) ha^{-1}, while populations A and P

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received 0, 360, 720, 1,440 and 2,880 g ae ha^{-1}. All herbicide treatments contained 0.1% organosilicone surfactant (Pulse Penetrant (Nufarm, Auckland, New Zealand)). The daily maximum and minimum temperatures in the 2 weeks following application averaged 26.8 and 15.6°C, respectively. The foliage of all plants was removed from pots at ground level 5 weeks after treatment and fresh weight of this plant material was determined. The effect of each herbicide treatment was calculated as a percentage of the fresh weight of untreated plants for that population.

The glyphosate dose-response experiment was conducted in a randomized complete block design with 10 replicates of each rate. This experiment was then repeated, and the daily maximum and minimum temperatures of the second experiment in the 2 weeks following glyphosate application averaged 26.1 and 12.2°C, respectively. As similar results were obtained from both dose-response experiments, only results from one experiment are presented below.

EPSPS Genotyping

The clones of plants that had been proven to be glyphosate-resistant (populations A and P) and susceptible (population SI) to glyphosate were used for this study. For DNA extraction, the young expanding leaf of five tillers from each population was harvested. Segments of 10 mm were cut and put into separate 1.5 mL Eppendorf tubes. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc.), following the method described by the manufacturer of the kits. PCR amplification and sequencing of conserved region of EPSPS (~ 350 bp fragments) was carried out using the method described by Ghanizadeh *et al*. (2015b). Briefly, for PCR amplification, 2 µL (30 ng) of template genomic DNA was mixed with a PCR reaction mixture containing forward primer (5'-CAAAAAGAGCTGTAGTCGT-3') and reverse primer (5'-CAAGGAACTCAAGTATTGGC-3') and amplification was carried out with one cycle of 30 seconds at 98°C for denaturation, followed by 35 cycles of 10 seconds at 98°C for denaturation, 15 seconds at 55°C for annealing and 1 minute at 68°C for elongation, followed by a final cycle of 7 minutes at 68°C. PCR products were then visualized on ethidium bromide (1 µg mL^{-1}) stained 1% agarose gels. For sequencing, 5 µL of each PCR product was mixed with a sequencing reaction mixture containing forward (5'-CAAAAAGAGCTGTAGTCGT-3') or reverse primer (5'-ACATTCGCACCTAGTTGTTT-3'). Sequencing was performed using an AB3130xl automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequence data were assembled compared and analyzed using DNA Baser Sequence Assembler software (Version 3.51).

Absorption and Translocation

The seeds of each population were germinated using the method described by Ghanizadeh *et al*. (2015b) in a controlled environment cabinet (20/30°C, 16 hours dark/8 hours light at 40 µmol m^{-2} s^{-1}). The seedlings were each positioned through an 8 mm hole in the lid of a 230 mL plastic container and grown hydroponically in 130 mL nutrient solution (Hoagland and Arnon, 1938). Each container had four seedlings, and these were placed in a growth cabinet at an average temperature of 21.4°C with a 12 hour photoperiod (100 µmol m^{-2} s^{-1}), provided by four 40-W white fluorescent tubes, at a relative humidity of 65-70%. Additional nutrition solution was added as needed to account for evaporation losses.

Absorption and translocation studies conducted on these seedlings used ^{14}C-glyphosate labelled at the phosphonomethylene site with a specific activity of 2.041 GBq $mmol^{-1}$ (obtained from PerkinElmer, 940 Winter St, Waltham

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Massachusetts, 02451, USA). When plants were at the three-leaf stage, they were thinned to one plant per container and sprayed with 180 g ae ha^{-1} of glyphosate (Roundup 360 Pro) and 0.1% organosilicone surfactant (Pulse Penetrant) using a laboratory track sprayer at 250 L ha^{-1} of spray solution at 200 kPa. Immediately after treatment, 1 µL of ^{14}C-glyphosate solution (containing 0.7 kBq of radioactivity and 0.004 µmol of glyphosate) was applied to the midpoint of the second leaf using a micropipette. The ^{14}C-glyphosate was smeared on the adaxial side of the treated leaf. Plants were then returned to the growth cabinet.

Plants were harvested 48 and 72 Hours After Treatment (HAT) because significant differences in glyphosate translocation patterns were recorded in previous studies at 48 and 72 HAT between glyphosate-resistant and susceptible populations of *Lolium rigidum* (Wakelin *et al*., 2004; Yu *et al*., 2007) and *Lolium multiflorum* (Nandula *et al*., 2008). The plants were divided into four sections: the treated leaf, the root, the pseudostem region (defined as the region of leaf sheaths in a vegetative grass (Beecher *et al*., 2015)) and untreated leaves. The treated leaves were washed in 10 mL of 0.1% Triton X-100 solution (Sigma-Aldrich) in order to remove any non-absorbed radioactivity from the leaf surface. Radioactivity in the 10-mL leaf wash solution was quantified by mixing 1 mL of the solution with 10 mL of Ultima Gold scintillation cocktail (PerkinElmer) in 20 mL scintillation vials.

The ^{14}C-glyphosate in each divided plant section was extracted using the method of Ghanizadeh *et al*. (2015b). Briefly, each section was digested in 0.5 mL of sodium hypochlorite solution (100 g L^{-1}). Then, the supernatant in each tube was diluted by a factor of 20 times with distilled water. One mL aliquot from each tube was mixed with 5 mL of liquid scintillation cocktail (Optiphase HiSafe 3, Wallac, Finland) in 20 mL scintillation vials. The radioactivity of each sample (leaf wash and plant sections) was quantified using a liquid scintillation counter (Tri-Carb 2900TR, PerkinElmer).

The percentage of glyphosate in each plant section was calculated proportional to the sum of radioactivity in all plant sections (treated leaves, root, the pseudostem region and untreated leaves), using the following equation:

$$\text{Glyphosate (\%)} = [\frac{X}{X+\text{leaf wash}}] \times 100$$

Where, X is the sum of radioactivity in all plant sections.

The absorption and translocation experiments were conducted in a completely randomized design with five replicates and were repeated for each population.

Data analysis

A three-parameter logistic model was fitted to data of the sprayed potted plant study:

$$Y = [d/1+\exp(b(\log(x)-\log(GR50)))]$$

Where, *Y* is plant fresh weight as a percentage of the untreated control, *d* is the upper limit, *x* is herbicide rate, GR_{50} is the rate of herbicide corresponding to 50% reduction in fresh weight, and *b* is the slope around GR_{50}. Biomass data were fitted to this model using the statistical software R (Version 2.15.2) with its dose-response curve (drc) package (Knezevic *et al*., 2007).

For the absorption and translocation experiments, a two-way ANOVA was conducted and the results showed that the data from the two runs of the experiment were not significantly different ($P > 0.05$) and the data were, therefore, pooled. A normality test was conducted in order to investigate the distribution of the data. The data for leaf absorption and radioactivity levels within the pseudostem region and untreated leaves were normally distributed ($P > 0.05$), whereas the data from the treated leaf and root tissue were arcsine square root transformed prior to analysis. Differences between the three populations in absorption of the ^{14}C-glyphosate and also the percentage of radioactivity found within each of the four tissue types were examined

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using a one-way ANOVA. Means were separated using Fisher's protected tests at the 5% level of probability.

RESULTS

Dose-Response Experiments

Dose-response experiments were conducted to conform and evaluate the magnitude of glyphosate resistance in two populations of Italian ryegrass (populations A and P) (Figure 1). A consistent response was recorded for plants of each population within each replicate suggesting that plant materials provided from each vineyard were homogenous in glyphosate resistance. The plants of the susceptible population (SI) were all controlled at the glyphosate rate of 720 g ae ha^{-1}; whereas, for resistant populations (A and P), plants were completely controlled only once at glyphosate rate of 2,880 g ae ha^{-1}.

The glyphosate-resistant populations (A and P) had higher GR_{50} values than the susceptible population (SI, Table 1). The GR_{50} for the susceptible population (SI) was calculated as 155 g ae ha^{-1} while the GR_{50} for the resistant populations (A and P) were 1603 and 1528 g ae ha^{-1}, respectively. By comparing the GR_{50} values of each Marlborough population against the GR_{50} value of the Manawatu population, populations A and P appeared to be 10.3 and 9.9 times more resistant to glyphosate than population SI, respectively (Table 1).

Sequencing of the Conserved Region of the *EPSPS* Gene

The *EPSPS* gene of the glyphosate-resistant and susceptible populations was partially sequenced in order to investigate any changes in the *EPSPS* nucleotide sequence (Table 2). The predicted amino acid sequence of the susceptible population of Italian ryegrass (SI) and one of the glyphosate-resistant populations (population P) was similar to the consensus sequence. However, substitutions of GGC to GGG at Position 98 and GCT to GCA at Position 100 were recorded for population A (glyphosate-resistant). These nucleotide

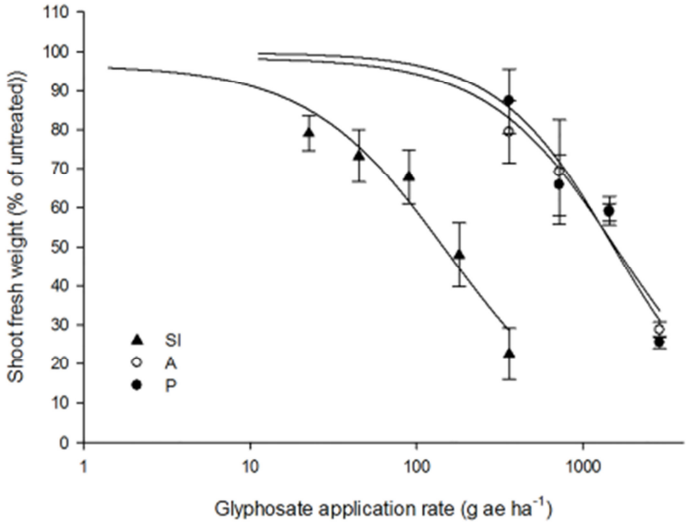

Figure 1. Fitted dose response curves (on logarithmic dose scale) of fresh weight measured 5 weeks after treatment in the sprayed pot experiment for three populations: SI (Glyphosate-susceptible): From Manawatu pasture; A and P (Glyphosate-resistant): From Marlborough vineyards) of Italian ryegrass, treated with glyphosate-resistant as potted plants. Vertical bars represent ±standard error of the mean.

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Table 1. Parameters (see footnote) estimated from the nonlinear regression analysis of a glyphosate dose–response experiment of three Italian ryegrass populations 5 weeks after application of glyphosate.[a]

Population	*d*±*SE*	*b*±*SE*	GR_{50}±*SE*	R/S GR_{50} ratio	R^2
Manawatu SI	96.6 ± 6.5	1.0 ± 0.2	155 ± 134	-	0.96
Marlborough A	98.5 ± 6.5	1.1 ± 0.3	1603 ± 196	10.3	0.97
Marlborough P	99.7 ± 6.8	1.2 ± 0.3	1528 ± 299	9.9	0.97

[a] *d*= The upper limit; *b*= The slope around the GR_{50}; *SE*= Standard Error; GR_{50}= The rate of herbicide (g ae ha^{-1}) required to reduce shoot fresh weight by 50%; R/S GR_{50}= Resistant/Susceptible GR_{50} ratio, R^2= Coefficient of determination.

Table 2. Nucleotide sequence in 5-enolpyruvylshikimate-3-phosphate synthase DNA isolated from glyphosate susceptible and resistant population of Italian and perennial ryegrass. Changes to codons from the consensus sequence are highlighted.

Amino acid number	97	98	99	100	101	102	103	104	105	106	107	108
Amino acid	Leu	Gly	Asn	Ala	Gly	Thr	Ala	Met	Arg	Pro	Leu	Thr
Consensus sequence	TTG	GGC	AAC	GCT	GGA	ACT	GCG	ATG	CGG	CCA	TTG	ACG
SI (S)	TTG	GGC	AAC	GCT	GGA	ACT	GCG	ATG	CGG	CCA	TTG	ACG
A (R)	TTG	**GGG**[a]	AAC	**GCA**[a]	GGA	ACT	GCG	ATG	CGG	CCA	TTG	ACG
P (R)	TTG	GGC	AAC	GCT	GGA	ACT	GCG	ATG	CGG	CCA	TTG	ACG

[a] Silent mutations.

changes are silent changes though and do not result in amino acid substitutions at these codons (Bostamam *et al*., 2012).

Pattern of Glyphosate Absorption and Translocation

The average quantity of applied ^{14}C-glyphosate recovered from each Italian ryegrass population was over 85%. The leaf absorption of ^{14}C-glyphosate between the resistant populations (populations A and P) and the susceptible population (SI) was not significantly different at 48 and 72 HAT. The percentage of ^{14}C-glyphosate absorption for populations SI, A, and P was 27, 28, and 33%, respectively, at 48 HAT (Table 3). At 72 HAT, the amount of glyphosate absorption for populations SI, A, and P was 31, 33, and 36% of applied ^{14}C-glyphosate, respectively (Table 3).

Although glyphosate absorption was similar in all three Italian ryegrass populations, different patterns of the proportion of the ^{14}C-glyphosate translocation were observed between the glyphosate-resistant and susceptible populations. Significantly less of the ^{14}C-glyphosate was translocated out of the treated leaf blade section to the rest of the plant in the glyphosate-resistant populations (populations A and P) compared to the susceptible population (SI).

At 48 HAT, 59 and 56% of the ^{14}C-glyphosate remained in the treated leaf blade of populations A and P, respectively, in contrast to 31% for population SI (Table 4). A greater percentage of the ^{14}C-glyphosate moved to the pseudostem region for population SI (39%) compared to populations A and P in which only 15% and 18% of the ^{14}C-glyphosate, respectively, was translocated to the pseudostem region. No significant differences were recorded for all three populations of Italian ryegrass in the amount of the ^{14}C-glyphosate movement into the untreated leaves and roots at 48 HAT (Table 4). A similar pattern was observed at 72 HAT and a greater amount of

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Table 3. Absorption of ^{14}C-glyphosate in three Italian ryegrass populations 48 and 72 Hours After Treatment (HAT).

Population	^{14}C-glyphosate absorption (% of applied)	
	48 HAT	72 HAT
SI (Susceptible)	27.4	30.7
A (Resistant)	28.1	32.6
P (Resistant)	32.8	36.2
P values	0.56	0.23

Table 4. Distribution of the ^{14}C-glyphosate in three Italian ryegrass populations 48 and 72 Hours After Treatment (HAT).[a]

Population	^{14}C distribution (% of absorbed radiolabelled glyphosate)							
	Treated leaf		Untreated leaves		Pseudostem		Root	
	48 HAT	72 HAT	48 HAT	72 HAT	48 HAT	72 HAT	48 HAT	72 HAT
SI (Susceptible)	31.3b	27.4b	15.6	11.3	38.9a	47.8a	14.2	13.5
A (Resistant)	59.1a	50.9a	13.1	15.2	15.1b	23.1b	12.7	10.8
P (Resistant)	56.1a	51.6a	13.4	11.2	18.4b	28.4b	12.1	8.8
P values	<0.01	<0.01	0.285	0.36	<0.01	<0.01	0.77	0.59

[a] Mean values within each column followed by the same letters are not different at 5% probability according to Fisher's protected tests.

the ^{14}C-glyphosate was moved out of the treated leaf blade in the susceptible population (SI) compared to the resistant ones (A and P).

DISCUSSION

The results of this study confirmed that the two Italian ryegrass populations collected from Marlborough vineyards (A and P) were resistant to glyphosate. Glyphosate resistance can be conferred by a target site modification at Pro106 position on the EPSPS enzyme in weed species (Sammons and Gaines, 2014). Previous studies showed that a change in proline at Position 106 to serine, threonine, alanine, or leucine conferred resistance to glyphosate in *Lolium rigidum* (Bostamam *et al*., 2012; Wakelin and Preston, 2006) and *Eleusine indica* (Ng *et al.,* 2003). However, the result of our study showed that glyphosate resistance in populations A and P was not conferred by this target site modification mechanism of resistance, as no nucleotide substitution at Codon 106 was observed for both glyphosate-resistant populations of Italian ryegrass.

Reduced glyphosate absorption has been reported in some glyphosate-resistant biotypes of *Lolium multiflorum* (Nandula *et al*., 2008) and *Sorghum halepense* (Vila-Aiub *et al*., 2012). However, in both cases, reduced glyphosate absorption was not the main mechanism of glyphosate resistance. In the present study, no differences were found between glyphosate-resistant and susceptible Italian ryegrass in glyphosate absorption at both studied time courses. The amount of glyphosate absorption in our study was slightly less than levels reported for *Lolium rigidum* (Wakelin *et al*., 2004), however, nearly identical amounts of glyphosate absorption have been recorded for *Lolium multiflorum* (Perez-Jones *et al*., 2007) and *Digitaria insularis* (Carvalho *et al*., 2012).

Results from previous studies have shown that glyphosate metabolism was not the mechanism of glyphosate resistance in *Lolium multiflorum* (González-Torralva *et al*., 2012). Likewise, glyphosate was not

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metabolised to any significant extent in either resistant or susceptible plants of *Lolium rigidum* investigated by Lorraine-Colwill *et al*. (2002). As enhanced glyphosate metabolism has not yet been reported as a mechanism for resistance in *Lolium spp*., it was assumed that the radiolabelled materials extracted from different parts of the plants in this study were still the intact ^{14}C-glyphosate molecules.

Prevention of absorbed herbicide within the plant from reaching the target site (restricted herbicide translocation) is another possible mechanism of resistance which confers glyphosate resistance to weed species (Koger and Reddy, 2005; Lorraine-Colwill *et al*., 2002; Wakelin *et al*., 2004; Yu *et al*., 2007). In the present study, different patterns of the ^{14}C-glyphosate translocation were observed between the glyphosate-resistant and glyphosate susceptible populations of Italian ryegrass. Almost half of the ^{14}C-glyphosate was retained in the treated leaf of populations A and P, whereas in the population SI, over 70% of the ^{14}C-glyphosate was translocated out of the treated leaf at the studied time courses. Similar results have been reported in glyphosate-resistant Italian ryegrass from Brazil and USA (Perez-Jones *et al*., 2007; Ge *et al*., 2012). The movement of the ^{14}C-glyphosate to roots and untreated leaves among the three Italian ryegrass populations was not significantly different, while a greater percentage of the ^{14}C-glyphosate was accumulated in pseudostem of the population SI compared to the populations A and P.

The pseudostem region of the treated grasses consisted not only of the meristematic tissues responsible for leaf production and elongation, but also the basal portion of the treated leaf and other leaves. Greater movement of glyphosate to active growth points (meristematic tissues) is crucial for glyphosate efficacy and accumulation of glyphosate in this region disrupts meristematic activity, which leads to growth cessation and plant death (Powles and Yu, 2010).

In our study, in glyphosate-resistant populations (A and P) more ^{14}C-glyphosate was retained in the treated leaf while less ^{14}C-glyphosate was translocated to the pseudostem region at 48 and 72 HAT. In contrast, in the glyphosate susceptible population (SI), a greater percentage of the ^{14}C-glyphosate was translocated to the pseudostem region at 48 and 72 HAT. Thus, having more glyphosate accumulating in the meristematic region in the susceptible population compared with resistant plants is a possible explanation of how differences in resistance were occurring. Greater accumulations of glyphosate mostly within the pseudostem region were also reported for susceptible *Lolium rigidum* plants, whereas for resistant populations, glyphosate was mainly retained in the treated leaf (Adu-Yeboah *et al*., 2014; Bostamam *et al*., 2012).

Restricted glyphosate movement in resistant plants was postulated to be due to the existence of a cellular pump which actively pumped glyphosate to apoplastic spaces in resistant plants (Lorraine-Colwill *et al*., 2002). However, in a study on a glyphosate-resistant *Conyza canadensis* (Canadian fleabane) population, it was noted that the glyphosate-resistant plants were able to rapidly shift a large amount of absorbed glyphosate from the cytoplasm to vacuoles, while in susceptible plants rapid transportation into vacuoles was not recorded (Ge *et al*., 2010). Also, a positive correlation between the levels of resistance to glyphosate and extent of vacuole sequestration of glyphosate in glyphosate-resistant *Lolium* spp. from different countries has been documented (Ge *et al*., 2012). Yuan *et al*. (2007) suggested that transporters associated with vacuolar membranes such as ATP-Binding Cassette (ABC) transporters might have roles in the vacuolar sequestration of glyphosate. Ge *et al*. (2014) have since provided evidence for the existence of a tonoplast transporter which actively shifts glyphosate into vacuole in Canadian fleabane.

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The restricted herbicide translocation usually confers a moderate level of glyphosate resistance (Preston *et al*., 2009) and this is in agreement with the moderate glyphosate resistance levels found in several weed species with restricted herbicide translocation mechanism of glyphosate resistance (reviewed by Sammons and Gaines, 2014). In the present study, for both glyphosate-resistant populations of Italian ryegrass (*Lolium multiflorum*) a moderate level of resistance to glyphosate was recorded which was similar to the level of glyphosate resistance in *Lolium spp.* with the restricted herbicide translocation reported by other authors (Adu-Yeboah *et al*., 2014; Bostamam *et al*., 2012; Perez-Jones *et al*., 2007; Ge *et al*., 2012; Nandula *et al*., 2008; Wakelin *et al*., 2004; Yu *et al*., 2007).

In conclusion, in the present study, the possible presence of the target site mechanism of glyphosate resistance at Codon 106 of the *EPSPS* gene was investigated and no nucleotide substitution at this codon was observed for both glyphosate-resistant populations of Italian ryegrass (populations A and P). The non-target site mechanism of glyphosate resistance (alterations in glyphosate absorption/translocation) was also investigated for Italian ryegrass and only an alteration in glyphosate translocation (restricted herbicide translocation) was observed for all studied glyphosate-resistant populations (A and P). The restricted herbicide translocation could explain the level of glyphosate resistance recorded for both studied glyphosate-resistant Italian ryegrass populations.

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محدودیت انتقال علف کش در دو جمعیت علف هرز چچم یکساله (*Lolium multiflorum*) مقاوم به علف کش گلایفوزیت از کشور نیوزیلند مشاهده گردید

ح. غنی زاده، ک. س. هارینگتون، ت. ک. جمز، د. ج. وولی، و ن. و. الیسون

چکیده

مقاومت به علف کش گلایفوزیت در دو جمعیت علف هرز چچم یکساله از کشور نیوزیلند اخیرا گزارش شده. نتایج بدست آمده از آزمایشات محلول پاشی با دوزهای مختلف علف کش گلایفوزیت نشان داد که هر دو جمعیت یافت شده در مقایسه با یک جمعیت حساس به گلایفوزیت، ده مرتبه نسبت به علف کش گلایفوزیت مقاوم تر هستند. در این تحقیق دو مکانیسم تغییر در اسید آمینه پرولین ۱۰۶ در آنزیم مورد هدف گلایفوزیت و محدودیت انتقال علف کش با استفاده از علف کش نشاندار شده مورد بررسی قرار گرفت. در بررسی انجام شده برروی آنزیم-5 انول پیرویل شیکیمات- ۳ فسفات سنتاز(EPSPS)، هیچ گونه جهش در اسید آمینه پرولین ۱۰۶ یافت نگردید. همچنین، هر سه جمعیت، مقادیر مشابهی از علف کش نشاندار شده را جذب کردند. بررسی الگوی انتقال علف کش در هر سه جمعیت نشان داد که علف کش گلایفوزیت به مقدار معنی داری در محل برگ گیاهان مقاوم به گلایفوزیت تجمع می یابد، در صورتی که این میزان تجمع در برگ گیاهان حساس بسیار کمتر می باشد. در گیاهان حساس به علف کش گلایفوزیت مقادیر بسیار زیادی از علف کش نشاندار شده در محل ساقه یافت گردید، در صورتی که میزان علف کش نشاندار شده تجمع یافته در ساقه گیاهان مقاوم به گلایفوزیت، به میزان معنی داری کمتر از گیاهان حساس بود. بنابراین نتایج این تحقیق نشان داد که مکانیسم محدودیت انتقال علف کش نقش مهمی در ایجاد مقاومت به گلایفوزیت در دو جمعیت مقاوم ایفا می کند.

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