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Mini-review

Resistance to glyphosate from altered herbicide translocation patterns

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Abstract: Glyphosate-resistant weeds have evolved as a result of the intensive use of glyphosate for weed control. An alteration in the way glyphosate is translocated within the plant has been identified as a mechanism of glyphosate resistance in populations of *Lolium rigidum* Gaud., *L. multiflorum* Lam. and *Conyza canadensis* (L.) Cronq. In these resistant plants, glyphosate becomes concentrated in the leaves rather than being translocating throughout the plant. This type of resistance is inherited as a single dominant or semi-dominant allele. Resistance due to reduced translocation appears to be a common mechanism of resistance in *L. rigidum* and *C. canadensis*, probably because it provides a greater level of resistance than other mechanisms. This type of glyphosate resistance also appears to reduce the fitness of plants that carry it. This may influence how glyphosate resistance can be managed.
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1 INTRODUCTION

Glyphosate is the world's most widely used herbicide. Much of its popularity comes from the unique properties of the herbicide.[1] Glyphosate is a broad-spectrum herbicide able to control a wide range of perennial as well as annual plant species. The ability of glyphosate to control perennial plants is a result of the extensive translocation of glyphosate from its site of application, usually the leaves, to all parts of the plant including the roots.[2] Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, a key enzyme in the shikimate pathway in plants.[3] This inhibition leads to a reduction in products of the pathway and a build-up of shikimate, although it is not clear which of these effects might be responsible for toxicity.

Since its introduction in 1974, glyphosate has been widely used for the control of weeds prior to crop seeding, for inter-row spraying and in orchards, and for weed control in non-cropped areas.[1] Glyphosate-resistant crops containing a resistant EPSP synthase, or a resistant EPSP synthase and a glyphosate oxidase, have been grown for over a decade now in several countries.[4] In 2006, about 80 million ha of crops around the world were planted to glyphosate-resistant crops.[5] However, the success of glyphosate-resistant crops has greatly increased the selection pressure for the evolution of glyphosate-resistant weeds.[6,7]

The widespread and intensive use of glyphosate for weed control has resulted in the selection of herbicide-resistant weeds in a number of countries. To date, there are glyphosate-resistant populations present in 12 different weed species (Heap I, http://www.weedscience.org/in.asp, accessed 9 March 2007). Several mechanisms endowing resistance to glyphosate have been documented in glyphosate-resistant weeds. These include target-site mutations in *Eleusine indica* (L) Gaertn.[8] and *Lolium rigidum* Gaud.,[9] and non-target-site resistance mechanisms in *L. rigidum*,[10] *Conyza canadensis* (L.) Cronq.[11] and *L. multiflorum* Lam.[12] This paper will focus on the present understanding of non-target-site resistance to glyphosate, particularly that caused by reduced translocation of the herbicide.

2 GLYPHOSATE RESISTANCE DUE TO REDUCED HERBICIDE TRANSLOCATION

Glyphosate is relatively poorly absorbed through leaves, but herbicide that is absorbed is rapidly and extensively translocated out of the leaf.[2] Glyphosate translocation follows source-to-sink patterns similar to photosynthate, suggesting that phloem translocation plays a major role. Glyphosate translocates in the xylem as well as in the phloem, although the rapid reloading of glyphosate back into the phloem means it tends to accumulate in sinks rather than at leaf tips.[2] The extensive phloem translocation of glyphosate means that the herbicide must be taken into cells and kept there against a concentration gradient. The transporter responsible for glyphosate uptake has not been determined, but may be a phosphate transporter.[13–15] In addition to rapid absorption into cells, there also needs to be a means of glyphosate

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unloading from the phloem. This latter process has been poorly studied.

Early studies with one population of *L. rigidum* from Australia determined that resistance to glyphosate was not due to a less sensitive EPSP synthase, reduced absorption of glyphosate or to more rapid detoxification of glyphosate inside the plant. Instead there was a significant difference in the pattern of glyphosate translocation around the plant.[10] This difference in translocation resulted in the accumulation of large amounts of radiolabelled glyphosate in the tips of the treated leaves of resistant plants, whereas glyphosate was readily translocated out of the treated leaf in susceptible plants. Less radiolabelled glyphosate was found in the untreated leaves or in the roots in the resistant plant.[10]

Further studies with other glyphosate-resistant populations from Australia[16] showed the same patterns of glyphosate translocation in most, but not all, glyphosate-resistant populations (Table 1). In these experiments, plants were sprayed with unlabelled glyphosate, and radiolabelled glyphosate was then applied to a single leaf. Most resistant plants accumulated more radiolabelled glyphosate in the treated leaf and less in the stem than did susceptible plants. There were no consistent differences in the amount of glyphosate accumulated in roots between the resistant and susceptible populations. Of the 11 glyphosate-resistant *L. rigidum* populations examined, two populations showed a glyphosate translocation pattern that is characteristic of susceptible plants. One of these populations, SLR 77, is known to have a target-site mutation at Pro 106 within EPSP synthase that probably provides resistance.[9] The second population, NLR 75, does not have this mutation and must either have a mutation elsewhere within EPSP synthase or another mechanism of resistance. Reduced glyphosate translocation has also been observed in a glyphosate-resistant *L. rigidum* population from South Africa. Additionally, this population has a target-site mutation.[17]

Glyphosate translocation has been examined in two populations of *L. multiflorum* from Chile. In one population, no significant difference was observed in glyphosate translocation patterns between resistant and susceptible plants.[18] In the other population, greater retention of glyphosate in the treated leaf tip of resistant plants was observed.[12] The latter population also had reduced glyphosate absorption through the abaxial leaf surface, suggesting that two mechanisms of resistance may occur in this population.

Glyphosate resistance in *C. canadensis* first appeared in Delaware in 2000 and has subsequently occurred on a large number of fields across much of the soybean and cotton growing areas of the USA.[19] Feng *et al*.[11] examined translocation of glyphosate in eight susceptible and three resistant populations of *C. canadensis*. There were two resistant populations from Delaware and one from Tennessee in this study. Plants were sprayed with radiolabelled glyphosate, and the amount translocated from the shoots to roots was measured. Much less glyphosate translocated from the leaves to the roots in all the resistant populations compared with the susceptible populations. Autoradiography confirmed reduced translocation of glyphosate in leaves of resistant plants. Koger and Reddy[20] examined four resistant and four susceptible populations of *C. canadensis* from four different states, Delaware, Mississippi, Tennessee and Arkansas. Radiolabelled glyphosate was spotted on to individual leaves and the amount that translocated to other parts of the plant was measured. In each pair, more glyphosate was measured in the roots and crown of glyphosate-susceptible plants compared with glyphosate-resistant plants.

Table 1. Distribution of ^{14}[C]glyphosate in plant parts of susceptible (S) and resistant (R) populations of *Lolium rigidum* 48 h after application to a single leaf[a]

	^{14}[C]Glyphosate present 48 h after application (% of absorbed) (± SE)				
Population	Treated leaf	Untreated leaves	Stem	Roots	n[b]
VLR 1 (S)	15 (±2)	12 (±1)	29 (±1)	44 (±2)	22
SLR 4 (S)	14 (±2)	12 (±1)	27 (±2)	48 (±2)	20
NLR 70 (R)	45 (±4)	10 (±2)	16 (±1)	27 (±2)	22
NLR 71 (R)	27 (±4)	20 (±5)	15 (±1)	38 (±3)	8
NLR 72 (R)	25 (±6)	11 (±5)	19 (±1)	45 (±6)	4
NLR 75 (R)	16 (±4)	6 (±1)	31 (±6)	37 (±1)	5
NLR 76 (R)	48 (±16)	9 (±3)	10 (±4)	13 (±3)	3
NLR 79 (R)	39 (±12)	5 (±1)	20 (±6)	24 (±5)	5
NLR 84 (R)	38 (±11)	5 (±1)	21 (±6)	25 (±5)	5
SLR 76 (R)	27 (±7)	19 (±5)	15 (±1)	39 (±5)	5
SLR 77 (R)	19 (±3)	9 (±2)	22 (±1)	50 (±2)	10
SLR 78 (R)	40 (±4)	9 (±2)	23 (±3)	28 (±3)	5
WLR 50 (R)	34 (±4)	8 (±2)	19 (±3)	39 (±3)	8

[a] Data collated from reference 16 and Wakelin and Preston unpublished data.
[b] Number of individuals. Each run included standard R and S populations.

In both *L. rigidum* and *C. canadensis*, a reduction in glyphosate translocation appears to be a common mechanism of resistance. However, it is not the only mechanism of glyphosate resistance, as mutations within EPSP synthase also occur.[8,9,17] Reduced translocation of herbicides has been a rarely reported mechanism of resistance to other herbicides in weeds, only being well documented so far with paraquat resistance.[21–23] As glyphosate is such a widely translocated herbicide in plants, a reduction in translocation to sensitive areas, such as the shoot meristem, could have a large effect on the mortality of plants. This may explain why reduced translocation is such an effective mechanism of resistance to glyphosate, but not to other herbicides. In addition, glyphosate-resistant plants with reduced translocation are more resistant to glyphosate than are those with a mutation at Proline 106 of the EPSP synthase.[9] Therefore, these plants are more likely to be selected for resistance by use of the herbicide.

3 INHERITANCE OF REDUCED-TRANSLOCATION-BASED GLYPHOSATE RESISTANCE

The inheritance of glyphosate resistance has been studied in several populations using crosses and back-crosses to the susceptible population.[24,25] In *L. rigidum*, resistance mostly appears to be single gene and encoded on a nuclear chromosome (Table 2). In one population with weaker glyphosate resistance, the number of genes contributing to resistance is less clear. What is perhaps surprising is the observed variation in the level of dominance between populations, in spite of all populations having the same resistance mechanism. Resistance was either dominant or partially dominant across the herbicide concentrations used. It may be that there are different alleles or different genes conferring the same type of resistance. Comparing the dose–response curves shows that the heterozygote has high levels of survival at herbicide rates used in the field,[25] ensuring selection for resistance will readily occur.

The inheritance of resistance has only been determined in a single population of glyphosate-resistant *C. canadensis* from Delaware.[26] In this population, glyphosate resistance was shown to be inherited as a single gene, nuclear encoded and partially dominant. Resistance that is single gene and largely dominant will be easily selected in the field. Therefore, we should expect to see this type of resistance appear in other weed species as well.

4 POTENTIAL CONSEQUENCES OF REDUCED TRANSLOCATION OF GLYPHOSATE

While reducing the translocation of glyphosate within the plant allows for survival of the plant when treated with the herbicide, such a mechanism may lead to negative consequences for resistant plants in the absence of the herbicide. As yet, the exact mechanism whereby resistant plants reduce translocation has not been identified in any of the species discussed. Lorraine-Colwill *et al*.[27] speculated that a change in a chloroplast transporter may be involved. Later, speculation concerned the possible involvement of a plasma membrane transporter.[10] Similarly, Feng *et al*.[11] suggested that changes in the cellular distribution of glyphosate in glyphosate-resistant *C. canadensis* was important, also pointing to a change in activity or specificity of a transporter. As discussed earlier, it is believed that glyphosate may be moved into cells on a phosphate pump.[13–15] If glyphosate resistance is caused by a change in activity or specificity of a cell transporter, such as a phosphate pump, this is likely to affect the normal activity of that transporter. For example, the distribution of phosphates within cells or around the plant is likely to be different in resistant plants. Any alteration to the activity of such a pump could have a profound negative impact on growth and development of the plant.

The fitness of glyphosate-resistant populations has been investigated only in *L. rigidum*. A study with one glyphosate-resistant population indicated the resistant population produced less, but larger, seed than the susceptible population.[28] Such small differences in life history traits may be important under field conditions.

Another series of experiments planted a segregating population of *L. rigidum* created from a cross between a glyphosate-resistant and a glyphosate-susceptible population. Seedlings from a segregating F_2 population were planted into wheat crops at three sites, and no glyphosate was applied. At the end of the season, seed was collected from plants surviving in the field. A proportion of this seed was planted into crops in subsequent years, and the remainder kept. At the end of 3 years, the frequency of resistant individuals from each year at each site was determined by treatment with 450 g ha^{-1} glyphosate and compared with the original F_2 population (Table 3). The frequency of glyphosate-resistant individuals declined in the population with time at all sites, although environment had an effect on the rate of decline. Further work with four different populations of *L. rigidum* using the same type of approach also showed a decline in resistance frequency with time.[29]

Table 2. Summary of inheritance of glyphosate resistance in ten glyphosate resistant *Lolium rigidum* populations.[a]

Resistant population	Level of dominance	Genome	No of genes[b]
NLR 70	Semi	Nuclear	1
NLR 71	Full	Nuclear	1
NLR 72	Semi	Nuclear	1
NLR 75	Full	Nuclear	1
NLR 76	Full	Nuclear	nd
NLR 79	Full	Nuclear	nd
NLR 84	Semi	Nuclear	nd
SLR 76	Full	Nuclear	1
SLR 78	Full	Nuclear	1
WALR 50	Full	Nuclear	Unclear

[a] Collated from references 24 and 25 and Wakelin and Preston unpublished data.
[b] nd = not determined.

Table 3. Change in frequency of glyphosate resistance as measured by survival (±SE, $n = 3$) of a segregating F_2 population sown into a crop and not treated with glyphosate[a]

	Survival of populations after treatment with 450 g ha^{-1} glyphosate (%) Site		
Population	SA	NSW	WA
Original F_2	45 (±2)	45 (±2)	45 (±2)
After year 1	11 (±2)	18 (±2)	31 (±11)
After year 2	5 (±2)	10 (±1)	19 (±2)
After year 3	2 (±0)	4 (±0)	11 (±1)

[a] From Preston, Dellow, Matthews, Neve and Powles unpublished data.

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All of these experiments were conducted on populations of *L. rigidum* with the translocation mechanism of resistance. This limited body of evidence suggests that this mechanism of resistance carries a significant fitness penalty in *L. rigidum*. This has both academic and practical interest. A significant fitness penalty for glyphosate resistance may help explain why glyphosate resistance proved so difficult to select and why resistance is occurring much more rapidly with the advent of glyphosate-resistant crops. A large fitness penalty will tend to keep resistance alleles at low frequencies in populations in the absence of selection by the herbicide.[30] It will also mean that susceptible escapes, owing to timing or placement of herbicide application, may dilute resistance more effectively, requiring more intensive selection for resistance to evolve. Lastly, a large fitness penalty will mean that the frequency of resistance alleles will decline if the herbicide is not used over one or more seasons.

A large fitness penalty may also be exploited in the management of glyphosate-resistant weeds. Such a penalty means that management strategies, such as rotation of herbicides, crop competition and seed set control of escapes, will have more impact on reducing the selection for resistance. Therefore, more integrated management approaches to weed management, rather than relying on glyphosate alone, may significantly delay the onset of resistance. In this context it is significant that glyphosate resistance has yet to appear in situations where glyphosate is not relied upon alone for weed management.[7] However, other mechanisms of glyphosate resistance are known, and the relative fitness of these has not been explored.

5 CONCLUSION

To date, glyphosate resistance has evolved in populations of 12 species in various parts of the world. Glyphosate resistance has occurred in systems where glyphosate was intensively used and was often the dominant or only method of weed control. These sites include orchards, vineyards, non-cropped areas and glyphosate-resistant crops.[7] So far, both target-site and non-target-site resistance mechanisms have been identified, but, at least in *L. rigidum* and *C. canadensis*, non-target-site resistance is more common. In spite of the apparent fitness penalty carried by this type of resistance, the greater level of resistance in the field compared with target-site resistance means it will be favoured under intense glyphosate selection. Glyphosate resistance also appears to be inherited as a single, dominant to partially dominant allele, again favouring selection in the field.

Non-target-site glyphosate resistance appears to carry a significant fitness penalty as measured in the field. This reduces the ability to select glyphosate resistance, but also may be exploited to delay glyphosate resistance. Integrated systems that do not rely solely, or mainly, on glyphosate for weed control seem to offer the best management option. As there is more than one glyphosate resistance mechanism known, it should come as no surprise that populations with multiple mechanisms of glyphosate resistance will evolve.[17] These multiple-resistant populations may turn out to be more difficult to manage.

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