Lessons on Managing Glyphosate Resistance from Australia:
When on a Good Thing, Don't Stick With It

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Flexibility and diversity in crops and weed management strategies are needed to slow or deter the evolution of herbicide resistant weeds. As described in this paper, avoiding use of glyphosate at least one year every few years may be crucial to maintaining its effectiveness, especially for horseweed (*Conyza*) and ryegrass.

The first cases of resistance in weeds to glyphosate documented anywhere in the world were in ryegrass from under orchards in Australia in 1996 (Powles, et al. 1998, Lorraine-Colwill, et al. 1999). Resistance has since been found in goosegrass (*Eleusine indica*) in oil palm plantations in Malaysia (1997), ryegrass in California in 1998 with Brazil and Chile following in 2001-03, horseweed (*Conyza canadensis*) in connection with Roundup Ready soy production in 2000, and other *Conyza* species in Spain and South Africa in 2003-04. Buckhorn plantain (*Plantago lanceolata*) was also documented as showing resistance in South Africa in 2003 (www.weedscience.com). Resistance cases encountered thus far in several of these species lower glyphosate's effectiveness in the cropping, and it is an herbicide that is not easily replaced. Glyphosate is a key herbicide with an excellent profile for human health and the environment. In California, glyphosate can be especially important for the adoption of reduced tillage, thereby helping to reduce dust in our air, which is a looming regulatory issue.

Resistance to glyphosate has attracted considerable attention outside agriculture because of its use in transgenic Roundup Ready crops, especially soybeans, corn, and cotton. There is a great deal of concern that such crops will further intensify the use of glyphosate and result in more resistance in weeds to glyphosate. Resistance in horseweed, for example, has been associated with Roundup Ready soybeans in eastern states, but appeared so quickly that much of the selection had probably occurred earlier due to previous pre-season applications of glyphosate. Further, many other cases of resistance to glyphosate have not involved the use of transgenic crops, which serves to emphasize that conventional use of glyphosate products is also sufficient to select for resistance. Resistance management for glyphosate is thus needed independent of GM crops. However, glyphosate resistant corn and cotton are already grown commercially in California, with alfalfa soon to follow, and Roundup Ready rice under investigation at least elsewhere in the US, so we should be careful in these systems. Given the history of resistance to glyphosate in horseweed and ryegrass in California and elsewhere, these are key weeds for which we should adopt strategies to avoid glyphosate resistance, including avoiding glyphosate as their sole means of control.

Given the importance of glyphosate (the active ingredient of Roundup) for weed management systems around the world and especially with the detection of resistance to glyphosate in California (as described in the previous presentations by Shrestha, Lanini, and Jasieniuk), it is important to consider what lessons can be learned from Australia and applied elsewhere to slow resistance to this important and popular herbicide. Most of what I can tell you about resistance to glyphosate in Australia is due to the work of my colleague Dr. Chris Preston at the University of Adelaide in South Australia, who also visited Davis in late 2005.

There are now more than 44 populations documented with resistance to glyphosate in Australia, scattered across four states in the southern part of the continent. There has been a rapid increase in the number of populations found since 2000, almost all of them from reports by growers that glyphosate seemed to be working more poorly than in the past. First were two from orchards receiving quarterly applications of glyphosate, but there were ten populations by 2001 and 34 by 2002. The good news is that resistance to glyphosate is still confined to intensive uses. Of the 34 confirmed populations, the majority are in fallows or horticulture (Table 1).

Table 1. Confirmed glyphosate-resistant annual ryegrass populations in Australia.

Situation	Number of resistant populations	States
Orchard, vineyard	9	NSW,SA, WA
No-till grain cropping	6	NSW,Vic, SA, WA
Chemical fallow	18	NSW
Fencelines, firebreak, irrigation ditches, etc.	11	NSW, SA, WA

Where the herbicide history is known, these populations are characterised by persistent use of glyphosate for many years, the use of few or no other effective herbicides, and little or no tillage. Although it is tempting to assume that tillage delays resistance by killing any weeds that survive the glyphosate application, modelling results suggest that the absence of tillage also accelerates resistance by not turning susceptible weeds seeds into the soil as part of the "seed bank" that can sprout in later years and breed with resistant plants (especially obligate outcrossers like ryegrass), thereby slowing resistance.

Glyphosate resistance genetics and resistance management

Research by Chris Preston and colleagues shows that resistance to glyphosate in Australian annual ryegrass is probably due mostly to one gene and is relatively low, about 3 fold in the heterozygotes (weeds carrying one copy of the resistance gene) and 7 fold in the resistant homozygotes (carriers of 2 copies of the resistance allele) (Lorraine-Colwill et al. 2001). This resistance seems to have something to do with the translocation of glyphosate (Lorraine-Colwill et al. 2003). By "relatively low", I mean by comparison to resistance for most other kinds of herbicides, where resistance is generally on the order of

10-40 fold. Preston has also found a few cases of resistance by an altered (mutated) target enzyme EPSPS (discussed by Jasieniuk), which can "stack" by crossing with the other gene to provide resistance more like 15 fold, which would become much more serious.

From research at Monsanto, the genetics of resistance to glyphosate in *Conyza* associated with Roundup Ready soybeans seem similar to those in ryegrass. Even before the commercial introduction of glyphosate, *Conyza* was naturally somewhat tolerant to it, so even a relatively low resistance of 3-7 fold resistance can make a difference, in contrast to weed species that are much more sensitive. Resistance evolved very soon after the introduction of Roundup Ready soybeans, starting in about 4 years. As in the several other cases of resistance that have nothing to do with GM crops, resistance to glyphosate in *Conyza* was probably was building to conventional use of glyphosate over many years prior to the commercial release of GM soy.

Given that resistance to other herbicides has evolved resistance in as little as 4 years in Australia, it seemed odd that resistance had taken so long to evolve to glyphosate, even after adjusting for the relatively low advantage of resistance to glyphosate sprays. Simulation models that Chris Preston and I developed, which matched resistance evolution quite well for other herbicides, indicated that resistance should be evolving much faster to glyphosate.

This led Chris and I to suspect that there are strong fitness costs to glyphosate resistance, that is, in the absence of glyphosate treatment, resistant weeds are poorly fit (e.g., could have lower seed set, poorer pollen production, etc.) compared to susceptible plants in the same field. This fitness penalty will slow the evolution of herbicide resistance and also decrease the number of resistant individuals in the population when the herbicide is not used. Preston's subsequent research showed that the fitness disadvantages depend on circumstances and are enhanced in competition with other plants, but the frequency of resistance in a population declines quite rapidly, from 45% to about 11% in three years The fitness penalty is at least 40%. It has also been noted that glyphosate resistance does not seem to spread as rapidly in the field as do other resistances in Australia, which also supports the idea of a fitness cost.

It should be possible to exploit the fitness penalty associated with glyphosate resistance. This is likely to be achieved best by employing a rotation that does not require glyphosate to be applied to a field in every year, perhaps skipping at least one year out of every three. In the years when glyphosate is not used, the frequency of resistant individuals will decrease due to selection against them. This will delay the onset of resistance, especially if undertaken before resistance becomes common, and will be especially important to avoid the accumulation and spread of both resistance genes. This conclusion is consistent with the field observation in Australia that resistance has not evolved in populations where glyphosate has not been persistently used, but where there has instead been more variety in weed management practices.

It's probably also important to avoid spraying large weeds, but rather to spray them when they are small. Spraying weeds when they are smaller may be less likely to select for weeds that have the small resistance advantage conferred by a glyphosate resistance allele. If weeds are sufficiently large and tolerant that a few will survive even when susceptible, plants with a 3 fold advantage in resistance are more likely to survive and increase the frequency of resistance. On the other hand, more general work in resistance management suggests that altering the rates or concentrations of herbicides is unlikely to make much difference to the selection for resistance (Preston and Roush. 1999).

In California, we need to avoid excessive use of glyphosate. especially for Roundup Ready alfalfa. Alfalfa is grown on a large acreage in California, so selection pressures could be extensive. Alfalfa is also a perennial crop that won't be rotated with another crop (and other weed management practices) for 3-4 years. It will be tempting to use glyphosate often, and indeed labels will allow glyphosate apoplications 3-4 times a year in Roundup Ready alfalfa. I want to emphasize that it was similar circumstances, 3-4 uses per year under orchards, which generated the very first cases of glyphosate resistance anywhere in the world. We need to be especially careful in California with management practices for *Conyza* and ryegrass, which have already shown a facility for evolving resistance to this important herbicide. Monsanto has accepted the need for resistance management and offers very useful information on a new website, **http://www.weedresistancemanagement.com/.**

In Australia, playing off a popular commercial advertisement for fly spray, advice given for resistance management is "When on a Good Thing, Don't Stick With It". Glyphosate is a good thing, but we have to be careful not to use it too much. Resistance can be managed, but we need to vary herbicides and other weed control practices to keep weeds "off balance", and thereby make it harder for weeds to adapt to any particular control tactics. With care and restricted use, resistance to glyphosate can be delayed, perhaps indefinitely.

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