Report on the 2013 GOA Herbicide Resistance Survey

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Keywords

Herbicide, resistance, survey, testing, Central West NSW, GOA

GRDC code

GOA00001

Background

Herbicide resistance could possibly be one of the greatest threats to the sustainability of our current grain production systems. The dominant minimum or zero tillage systems have evolved to place a heavy reliance on herbicides for weed control and, in many cases no other alternate methods of control are being employed. This reliance on herbicides has already led to the development of resistance in many cropping regions of the world with Australia being no exception.

So it lends to reason that the Central West of NSW is not immune from the development of resistance either but the extent of which resistance exists in the region has not yet been quantified. Prior to this survey the only formal confirmation of resistance was through ad-hoc resistance testing by advisors or growers mainly in situations where herbicides had previously failed. Or more informally, resistance was only diagnosed by "gut feel" or educated guess by advisors.

This survey is the first attempt to quantify both the extent and types of resistance presently in the Central West of NSW.

In contrast, other cropping regions in Australia have undertaken a number of surveys and these have started to form sound evidence of the level and types of herbicide resistance present. In other regions this evidence would be crucial in convincing growers for the need to address the issue of resistance and provide the insight into the characterisations of resistance to make informed decisions for its management.

Local advisors within the Central West region have suggested there is an inherent lack of recognition and acceptance by many growers of the presence of herbicide resistance in the region and the threat and severity it poses. Partly because of this many growers are not often prepared to act to prevent, slow or combat resistance. GOA's engagement with local stakeholder groups expressed a clear need and desire to assess levels of resistance within the region and the products that were affected. In doing so it would help raise awareness of the issue through hard evidence, the presence of herbicide resistance.

Aims

The aims of this survey were to establish what the current level and types of herbicide resistance are in the Central West of NSW. In doing so, this would raise awareness of the issue to foster a change in attitude and following on from this, a greater willingness to address the issue. The information provided from this survey would aid growers and agronomists to make more informed decisions around herbicide choices or in fact even management strategies beyond simple herbicide rotations

Methodology

In November of 2013, all growers and agronomists on the GOA contacts list were offered an opportunity to submit weed seeds of any of four common species for testing for resistance to a range of common herbicides. The four weed species were:

- Annual Ryegrass (ARG)
- Wild or Black Oats (BO)
- Wild radish (WR)
- Sow or Milk thistle (ST)

These species were chosen as they were either already noted as key weeds in the region that were either commonly demonstrating herbicide resistance such as ARG and BO, or those that are thought likely to develop herbicide resistance such as WR and ST.

Samples were limited to two per grower in an effort to survey a wider cross section of the GOA region. Weed seeds were to be sampled from cropping paddocks in the local region with no stipulation as to their suspected resistance status. That is they could be taken from paddocks regardless of whether they were suspected resistant or not.

Samples were to be collected in accordance with commercially accepted sampling instructions provided by Plant Science Consulting[1], the commercial testing service that undertook the herbicide testing on the seed samples provided. The herbicide testing was carried out to industry accepted standards in calibrated spray cabinets with control populations introduced to ensure confidence in testing procedures.

A range of herbicides specific to the weed species were applied and these are listed in tables 1 and 2 below. The herbicide types and rates used were developed with input from a number of sources with an aim to characterise the resistance status of each population to commonly used products and if for some products at multiple rates to examine any rate responsiveness. The options tested are not completely exhaustive as cost would have been prohibitive but the results do serve to give a significant characterisation of the resistance status of the populations tested.

Details regarding the herbicide history from the paddocks were also collected when samples were submitted for testing. Information such as the suspected resistance status, length of farming history and details regarding the number of herbicide applications of each mode of action that had been applied in the last 10 years.

A total of 123 weed seed samples were collected, 79 ARG, 41 BO and 3 WR. No ST samples were received. The WR samples have been combined into Dr Christopher Prestons project monitoring WR for resistance and the results have not been included in this report.

GOA returned the individual results growers or agronomists who submitted the samples so as to allow them to make informed decisions regarding the management of those weeds. This report however concentrates on the combined results and the broader overall impact of the survey.

[1] www.plantscienceconsulting.com/seedtest

Table 1. Herbicides and rates tested on annual ryegrass samples

Herbicide tested	Common trade names	Rate applied L/ha or g/ha
Haloxyfop	Verdict®	0.1L
Clethodim	Select®	0.35 L
Clethodim	Select®	0.5 L
Butoxydim	Factor®	180 g
Pinoxaden	Axial®	0.3 L
Triasulfuron	Logran®	35 g
Iodoulfuron-methyl sodium	Hussar OD®	0.1 L
Imazamox & Imazapyr	Intervix®	0.6 L
Atrazine	Gesaprim®	2000 g
Trifluralin	Triflur X®, Treflan®	2 L
Glyphosate 540 g/L	Round Up Powermax®	1 L
Glyphosate 540 g/L	Round Up Powermax®	1.5 L
Glyphosate 540 g/L	Round Up Powermax®	2 L

Table 2. Herbicides and rates tested on wild/ black oat samples

Herbicide tested	Common trade names	Rate applied L/ha or g/ha
Clodinofop	Topik®	0.1 L
Haloxyfop	Verdict®	0.1L
Clethodim	Select 500®	0.35 L
Clethodim	Select 500®	0.5 L
Pinoxaden	Axial®	0.2 L
Mesosulfuron-methyl	Atlantis OD®	0.33 L
Flamprop-M-methyl	Mataven 90®	1.8 L

The herbicide resistance testing reported upon two measurements:

1. The percentage of the treated population that survived the herbicide quoted as % survival
2. A rating of the herbicide effect on the surviving plants as follows
    - "R" indicates surviving plants were significantly stunted and only recovered with a few tillers
    - "RR" indicates plants suffered stunting in the order of 40-70% relative to untreated plants
    - "RRR" rating which indicates survivors showed virtually no herbicide effect.

This report concentrates mainly on the percentage survival to the individual herbicides as this gives a better indication of the potential frequency of resistance in the region. The second measurement aims to offer some insight into the relative "strength" of the resistance in the surviving plants.

The industry accepted terminology for herbicide resistance is that:

1. "Developing Resistance"-Survival of up to 19% but no more of the treated population
2. "Resistant" -Survival of 20% or more of the treated population

However, to simplify the reporting of the findings of this survey, any populations with survival greater than 10% is termed resistant. This approach has been taken for the following reasons-

- To report on the levels of both "developing resistance" and "resistant" would serve only to complicate the message, lengthen the report unduly and potentially confuse the reader.
- We cannot be 100% certain that very low levels of survival are not just an anomaly in testing
- Differences in resistance between 10 to 20% survival would be indistinguishable in paddock situations and therefore irrelevant to managing such populations
- Growers could expect a higher % survival to herbicides applied outside the "ideal" conditions under which testing so even these lower level of survival would be considered commercially unacceptable.

Results

Ryegrass

79 samples of annual ryegrass were submitted to the survey. Of the samples submitted, one seed lot was unviable (poor germination) so 78 samples underwent testing. 91% of samples were collected and logged by advisors and only seven samples were collected and submitted by growers.

The distribution of the sample locations is detailed in Figure 1 below and provides a reasonable cross section of the GOA region, with the notable exceptions of the western area beyond Trangie and Warren and the north eastern areas around Coolah where no samples were submitted.

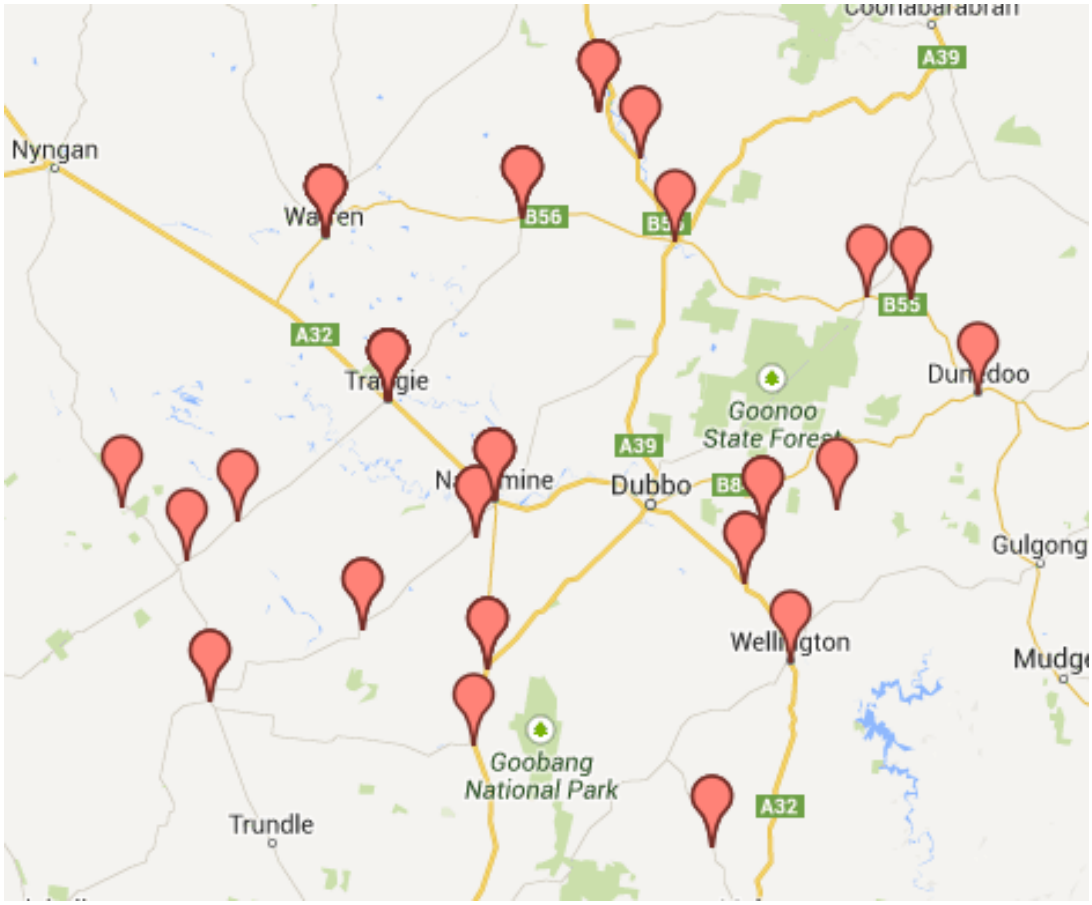

Figure 1: Locations from where ryegrass samples were taken from.

NB Multiple samples may have been taken from the general locality marked by only a single pin marker

There appeared to be little clear correlation between the patterns of past herbicides usage or the length of cropping history indicated in the survey questions to the demonstrated resistance to those herbicides. However a number of populations demonstrated resistance to a particular herbicide where there has been no or very little previous use indicated. For example,

- 15 populations demonstrated resistance to Axial® where it was indicated it had not been used in the past
- Two of the four populations demonstrating resistance to Trifluralin where it was not reported to have been used in the past.
- Two populations had been reported to have not used a Group B applied however demonstrated 100% resistance to Logran®
- 22 samples reported having less than two Group B applications yet all demonstrated resistance, 12 of which demonstrated 100% resistance to Logran®.

Of all the samples submitted, 30% of samples were predicted to be “possibly resistant”, 70% of samples were predicted to be “resistant” but no samples submitted were predicted to be “susceptible” in the submission questionnaires.

There were no samples submitted that were susceptible to all the herbicides tested. In other words there was not one sample that was not resistant to at least one of the herbicides tested. Table 3 below details the demonstrated resistance of the submitted ARG samples to individual herbicides tested.

Table 3: Number of AGR samples demonstrating resistance to the various herbicides and rates tested

Herbicide and rate tested	No of samples >10% survival	% of samples with > 10% survival
Verdict 0.1L/ha	59	75%
Select 0.35L/ha	17	22%
Select 0.5L/ha	6	8%
Factor 180g/ha	1	1%
Axial 0.3L/ha	47	60%
Logran 40g/ha	77	99%
Hussar 200 g/ha	64	82%
Intervix 0.6L/ha	44	56%
Atrazine 900WG 2000g/ha	7	9%
Triflur X 2L/ha	4	5%
Glyphosate 540 1L/ha	5	6%
Glyphosate 540 1.5L/ha	4	5%
Glyphosate 540 2L/ha	3	4%

Only ten samples, or 13%, demonstrated resistance to only one of the herbicides tested. **Multiple herbicide resistance**, where a sample demonstrates a resistance to more than one herbicide types, was found in 86% of the samples tested as detailed in Table 4 below. 54% of samples demonstrated resistances to four or more herbicide groups or sub-groups and the largest single group, constituting 37% of the samples demonstrated resistance to only four groups or subgroups.

Note that for the purpose of this table Fops, Dims and Dens are considered as subgroups of Group A because it has been previously shown that differential levels of control can be expected when using these herbicides. Similarly for the Group B's they are in two subgroups being the sulfonylureas (SU's) and the imidazolinones.

Table 4: Number of ARG sample populations demonstrating resistance to multiple herbicide groups and sub groups.

No. of herbicide groups or sub groups with demonstrated resistance#	No of samples	% of samples submitted
1	10	13%
2	15	19%
3	10	13%
4	29	37%
5	12	15%
6	1	1%
7	1	1%

Herbicides groups and subgroups considered- Fop, Dim (Select® only), Den, SU, Imi, Triazines and Glycines.

Further analysis of the levels of cross resistance due to the significant number of resistance combinations over such a number of herbicides is quite difficult and potentially unsound. However it is interesting to note some of the variations within a particular herbicide group and/or over a number of rates.

Group A's - Fops, Dims & Dens

- 59 (75%) samples were resistant to Verdict but only 17 of those were resistant to Select® @ 350ml/ha and only six to the higher rate of 500ml/ha.
- Of the samples resistant to Select®, all were also resistant to Verdict.
- Only one sample demonstrated low level resistance (10% survival) to Factor and it was already resistant to both Select® and Verdict®.
- 47 (60%) samples demonstrated resistance to Axial®, of these 100% demonstrated resistance to Verdict but only 17 populations were also resistant to Select® (350ml/Ha)

Group B's - SU's and imidazolinones

- Logran® was all but ineffective with 77 of the 78 (99%) samples demonstrating resistance
- 64 (82%) of the samples demonstrated to be also resistant to both Hussar OD® and Logran®
- 43 samples were resistant to Intervix® and both Logran® and Hussar OD®.

Group M

- Only five samples demonstrated resistance to Glyphosate 540
- Three of these demonstrated significant levels of cross resistance to more than two other herbicide groups or sub groups

This is only a small sample of resistant populations so any characterisation of a glyphosate resistant population based on this would be not well founded. However it should be noted that for most populations tested glyphosate is still effective but for some of the more resistant populations tested (multiple resistances) found it was one of the last few effective options left.

Wild Oats

41 samples of wild or black oats were submitted to the survey. All samples were collected and submitted by advisors.

The distribution of the sample locations is detailed in Figure 2 below where a reasonable cross section of the GOA region has been taken in by this survey but noticeably the area around and east and north east of Dubbo had no samples submitted.

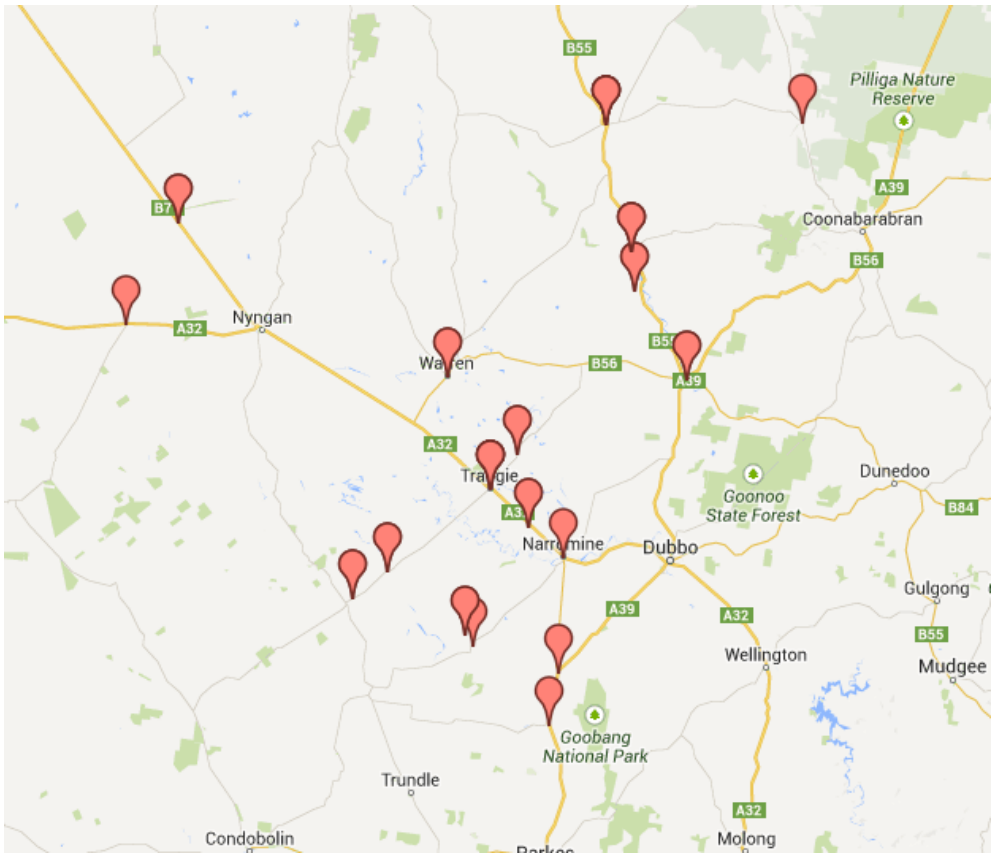

Figure 2: Location of wild oats sampling locations.

NB Multiple samples may have been taken from the general locality marked by only the one pin marker

There appears to be little clear correlation between the patterns of past herbicides usage or the length of cropping history indicated in the survey questions to the demonstrated resistance to those herbicides. But similarly to the ARG samples, a number of herbicides demonstrated resistance where it has been indicated that no applications of that herbicide had been applied. For example,

- 16 samples demonstrated resistance to Mataven® where no Mataven® had been applied.
- Four samples demonstrated resistance to Axial® where no Axial® had been applied

Of all the samples submitted, 56% of samples were predicted to be “resistant”, 41% were predicted to be “possibly resistant” and none were predicted to be “susceptible”, and two samples did not indicate their suspected resistance status on the submission questionnaires.

Despite the strong predictions for the samples to be resistant, 29% of the samples demonstrated no resistance to any of the herbicides tested with five of these predicted to be “resistant” and the balance only “possibly” resistant.

73% of the samples submitted demonstrated some level of resistance to the herbicides tested as detailed in table 5 below. The most common resistance in these samples has been demonstrated to be Topik® (56%) but closely followed by Mataven® 90 (51%). Resistance to Axial® has been demonstrated to be 37%, nearly three times greater than that for Verdict® at 12%. Interestingly

there was no demonstrated resistance to Select® in the samples tested and only two samples resistant to Atlantis OD®.

Table 5: Number of samples demonstrating resistance to the various herbicides and rates tested.

Herbicide and rate tested	No of samples >10% survival	% of samples with > 10% survival
Topik 0.1L	23	56%
Verdict 520 0.1L	5	12%
Select 0.5L	0	0%
Axial 0.2L	15	37%
Atlantis 0.33L	2	5%
Mataven 90 1.8L	21	51%

Table 6 below details the level of multiple resistances in the samples submitted. 22% of samples demonstrated to be resistant to two herbicide groups or sub groups and a similar number (22%) were three herbicides groups or sub groups. A much smaller number were resistant to four, at 7% of the samples submitted.

Table 6: Number of sample populations of black oats demonstrating resistance to multiple herbicide groups and sub groups.

No. of herbicide sub groups with demonstrated resistance#	No of samples	% of samples submitted
0	11	27%
1	9	22%
2	9	22%
3	9	22%
4	3	7%

Herbicides groups and subgroups tested- Fop, Dim, Den, SU, Grp Z.

Of the six herbicides tested on these samples they all belong to only three different herbicide groups, Group A, B's and Z.

- Of the 23 samples that demonstrated resistance to Topik® only five were resistant to Verdict® which is also a Fop herbicide, but 15 demonstrated to be resistant to Axial®, a Den herbicide.
- All samples resistant to Axial® were also resistant to Topik®
- In those cross resistant samples, the level of resistance demonstrated to Verdict® and Axial® was much lower than that was shown to Topik® in the majority of cases - but not all
- As shown above there was no demonstrated resistance to Select®.
- There were only two samples with Atlantis OD® resistance at a low level - for one sample it was the only resistance demonstrated, the other sample was also resistant to Topik®, Axial® and Mataven® as well but susceptible to Verdict®.
- 15 of the 21 samples resistant to Mataven® were also resistant to Topik®, 11 were resistant to both Topik® and Axial®.
- Six samples were resistant to Mataven® but demonstrated no cross resistance to the other herbicides tested.

Discussion

Acknowledgement should be first given to the collection method and the potential bias that may have given to the samples submitted to this survey. This was not a random survey of the region and most likely growers and advisors would most likely sampled weed seeds from more suspicious populations than not. This is highlighted in the sample submission questionnaire where all samples were either suspected to be “resistant” or “possibly” resistant. Therefore it should be considered the outcomes and information presented from this survey maybe a “worst case scenario”.

In defence of this approach, testing is carried out through seed testing and that the target populations for this survey are from cropping paddocks. Therefore it is most likely the only seed available to sample would be those from plants at maturity that have survived in crop herbicide applications. This scenario would by definition support a theory they are possibly resistant to at least some herbicides. Another way to put it is that susceptible plants would have been controlled by herbicide applications and simply not available to sample- suggesting seed tests will always bias towards a greater level of resistance whether the collection method is random or directed.

A “Quick Test” which samples live seedling plants from populations not already “selected” for a resistance by a herbicide application and as such may not have as stronger bias for resistance applied.

Given this bias towards sampling populations suspected of resistance the finding of some levels of resistance is no surprise. That said though for both ARG and BO, the samples that were submitted have overwhelmingly demonstrated a high level of resistance to many of our common herbicides. Nearly all (99%) the samples of ARG and 73% of BO samples submitted were resistant to at least one of the herbicides tested.

The higher level of susceptibility in the BO samples is interesting given that 27% of the samples submitted showed complete susceptibility to the range of herbicides tested yet sufficient survivors were available to sample from (assuming the paddock was treated for BO) which does fly in the face of the above comments. There are a number of potential explanations however no clear evidence in the data set is available to confirm as to which one. BO is well noted for seed dormancy and possibly the plants sampled germinated after the paddock was treated with herbicides. Alternatively does the presence of susceptible plants at maturity suggest reduced effectiveness of the herbicides through poor application or the impact of plant stresses? This could suggest that more attention and care should be paid to application of BO herbicides to ensure the highest level of control.

Multiple resistances

As detailed above in tables 4 & 6, multiple resistances are common with only 13% of ARG samples and 22% of BO samples demonstrating resistance to just a single herbicide group or subgroup. The clear majority of the samples submitted from both species demonstrated multiple resistances. Therefore within this set of samples, multiple resistances are significantly more common than no resistance or just single product resistance. Put another way, samples with no resistance or single product resistance is a rarity.

It was found that the levels and types of cross resistances, as discussed below, for each population tended to be unique. Hence the reader should exercise caution as what is demonstrated in this data will not necessarily be applicable to all populations. Further to this the complexity of relationships between the large numbers of herbicides tested can be overwhelming and the results potentially completely unrelated.

Source of resistance

As detailed in the results above there are a number of samples that have demonstrated resistances to a number of products where it has been indicated that the particular herbicide group has not previously been used.

For example there were-

- 15 populations of ARG that demonstrated resistance to Axial® where use of Axial® was not indicated,
- Two of the four populations of ARG demonstrating resistance to Trifluralin had not reported its application,
- Two populations of ARG had not reported the use of a Group B herbicide yet demonstrated 100% resistance to Logran®
- Four BO samples demonstrated resistance to Axial® where its use was not reported
- 16 BO samples demonstrated resistance to Mataven® where its use was not reported.

This phenomenon is not uncommon and potentially could occur through a number of mechanisms.

- The population is naturally resistant to the herbicide, explained by simple genetic variability within species
- Some resistances can simply be selected for indirectly. That is selection for resistance by use of one particular herbicide may at the same time select for a resistance for another unrelated herbicide.
- Resistant plants or seeds can be introduced into your system, carried in on machinery and stock or in seed, wind or flood waters.

There is no way to confidently identify which mechanism has resulted in the outcomes in the examples listed above but their occurrences do highlight challenges for management of herbicide resistance. There are unfortunately no defence growers and advisors can employ to manage the natural presence of resistance in a population. For the second mechanism there is also little that can be done to avoid this other than sound and informed chemical rotations. However for the third mechanism sound farm hygiene for machinery, seed and stock can slow the spread or incursions of resistant weeds.

Knowing your resistance status

For the set of samples provided to this survey we have detailed the frequencies of individual resistances as well as a number of generalisations about levels of cross resistances between some herbicides. However it should be again noted these apply to this set of samples only and may not apply beyond these in the wider farming systems or other regions. There is no unique and distinct characterisation of a “resistant” ARG or BO plant- each one can be different.

As growers and advisors this survey showed our predictive skill for identifying resistance without testing may be variable. For ARG, all samples were predicted to either possibly or definitely resistant and that was shown to be true. For BO however 29% of the samples that were predicted to be possibly or definitely resistant were not resistant to any of the products tested.

So given our questionable skill at predicting resistance and that it can occur somewhat independent of past herbicide resistance testing is a must. The results from this survey have demonstrated populations where some products have retained effectiveness when it could easily be assumed that they would be ineffective and visa-versa. To highlight this point;

- 56% of BO samples demonstrated resistance to Topik®, but only 12% demonstrated resistance to Verdict® with both herbicides in the same herbicide group
- 99% of ARG samples demonstrated resistance to Logran®, only 82% demonstrated resistance to Hussar OD® and 56% to Intervix®- all Group B herbicides
- 51% of samples demonstrated resistance to Mataven® but only one quarter of those had Mataven® applied previously.

This highlights the value in herbicide resistance testing not so much to confirm that a particular product has not worked due to resistance, but to identify which products may still work in the future.

Worthy of a mention

As discussed above, the identification of resistance to a number of key products such as the Fop herbicides and Group B herbicides such as Logran® have come as no surprise. Alarmingly though the testing has highlighted some cases of resistance to a few "less used" products thought to be safe and still effective and which many growers would be potentially relying on for herbicide control in the future.

Atrazine and trifluralin have had much less reliance or use in the region compared to many other products for the control of these weeds. Experience from other regions also indicated that resistance can take much longer to develop and are much less common. However six populations (10%) of ARG samples demonstrated resistance to atrazine with another six populations demonstrating 5% survival in testing which could indicate very early stages of developing resistance. There were also four populations of ARG that demonstrated resistance to trifluralin.

Similarly Intervix®, as a product mainly utilised in Clearfield canola and hence only a small proportion of our annual cropping area, demonstrated resistance in 56% of the ARG samples.

Identification of resistance to Select® in the region is not a total surprise but for many populations of ARG it was the only reliable in crop selective herbicide available and is therefore a key in the management of ARG. But nearly a quarter of the samples submitted (22%) of ARG samples demonstrated resistance at the lower application rate of 350 ml/ha. All of these populations were already resistant to Verdict®, Axial®, Logran®, and Hussar OD®. Increasing the application rate to 500ml/ha did decrease the survival to 8% of the populations but this for many populations is the last lever to pull for ryegrass control.

Mataven® is often talked of as an alternate herbicide product for the control of BO and in recent times had very little use with only six samples indicated to have received any applications of Mataven® in the past 10 years. However 51% of the BO populations demonstrated resistance to

Mataven®. As an alternate product to control Topik® resistant BO only 36% of the Topik® resistant populations would be susceptible. There are currently questions over the continued manufacture and supply of Mataven® but its usefulness on these populations would be limited.

Finally, herbicides play a pivotal role in our current minimum-till or zero till farming systems. Possibly the most important product in the northern farming region is glyphosate. This herbicide is invaluable in the control of weeds in our fallow systems which are essential to conserve out of season rainfall to achieve profitable crop yields. It is also important for managing pre-planting flushes of weeds, potentially the largest germination of winter weeds. Loss of the effectiveness of this herbicide will seriously challenge the sustainability of this otherwise profitable system.

Testing of the ARG samples submitted to this survey has revealed 6% of them demonstrated resistance to Glyphosate 540 @ 1lt/ha. Higher application rates improved control but only marginally. Although this is only a small proportion of the samples tested the importance of the product and lack of comparable alternatives is reason enough to be very alarmed by these results.

Conclusion

As intended, the survey included weed seed samples from a healthy cross section of the GOA region of the Central West of NSW. Both AGR and BO were submitted to the survey with ARG samples attributing approximately two thirds of the total samples and BO one third.

Testing revealed that herbicide resistance was common place and that in the vast majority of the samples submitted multiple resistances were most dominant. In a number of cases the broadness of multiple resistances was such that there are only a few potentially effective herbicide options left that might control those weeds.

This survey has proven herbicide resistance is not an issue confined to Western Australia or other regions only. This survey has identified many populations in the Central West of NSW that rival and possibly surpass the severity of Western Australian herbicide resistance. It should no longer be considered “someone else’s issue” and that “it’s not that bad”.

The survey was also invaluable in identifying what some of the most challenged herbicide groups are in terms of effectiveness. But the survey also served a warning to growers particularly for what many have thought to be “safe” and “effective” herbicide options such as Select®, Atrazine and Trifluralin and even glyphosate with clear signs of resistance developing.

The results of this survey are damning and its strength may be the force that has been needed to change attitudes, to acknowledge the issue of herbicide resistance which of course is the first step to better manage the issue of herbicide resistance into the future.

Acknowledgements

This survey would not have been possible without the support and funding of GRDC. GOA would like to acknowledge this as well as the efforts of growers and advisors who participated in this survey.

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