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A risk calculator for glyphosate resistance in *Lolium rigidum* (Gaud).

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Abstract
BACKGROUND – Glyphosate resistance has been confirmed in 58 populations of *Lolium rigidum*, a major weed of crops in southern Australia. Extensive use of glyphosate in conjunction with minimal soil disturbance has been identified as high risk for resistance to that herbicide. Land managers need a simple method to rapidly assess the risk of resistance occurring as a result of past and proposed future management practices. Modelled on risk assessment nomographs, a simple calculator for indicating the risk of evolved glyphosate resistance in *L. rigidum* is described.
RESULTS: The calculator uses the generations since first use and the frequency of use of glyphosate in combination with historical cultivation levels as critical factors for determining the risk of glyphosate resistance evolution. Based on the management history of a field, a land manager can graphically determine a glyphosate resistance risk for that field.
CONCLUSION: The calculator enables the farmer or the advisor to assess the risk of weeds population becoming resistant and modify practices accordingly to manage for sustainable glyphosate use. The risk calculator could be modified for other herbicides and different weed species.
Keywords: herbicide resistance, annual ryegrass, risk management

1. INTRODUCTION

Glyphosate has herbicidal action against nearly all of the 78 worst weeds worldwide[1] and is the most widely used herbicide in Australia.[2] Initially, cost restricted glyphosate use to mainly horticultural and industrial situations. However, a reduction in this cost has enabled glyphosate to become widely used as a knockdown, ie preplant broadspectrum, herbicide in Australian agriculture.[3] Glyphosate provides an effective means of controlling a broad range of weeds and has allowed the widespread adoption of minimum tillage farming practices.[4]

Lolium rigidum Gaud. is a common grass weed across southern Australia within broadacre crop production, horticulture and other disturbed environments. Populations resistant to one or more herbicide modes of action have been identified.[5,6,7]

Glyphosate resistance was first reported in *L. rigidum* in northern Victoria, Australia in an intensive cropping regime with a history of reduced cultivation.[8,9] Powles *et al.*[10] reported the second instance of glyphosate resistant *L. rigidum* in an orchard in central New South Wales, Australia. There are currently 58 confirmed glyphosate resistant *L. rigidum* populations in Australia,[11] with nearly half of these occurring under broadacre crop production.

A combination of three key factors drives the evolution of glyphosate resistance in Australian *L. rigidum* populations:[12] a prolonged history of glyphosate use; the frequent use of glyphosate; and little or no cultivation. Both initial glyphosate resistant populations had lengthy histories of glyphosate use,[8,10] while repeated use of glyphosate for chemical fallows has been identified as the main cause of resistance in broadacre farming on the Liverpool Plains, New South Wales.[13]

Resistance to glyphosate places greater dependence on alternative herbicides or other weed management strategies and has implications for the long-term viability of minimum tillage farming systems in Australia. Managing resistance through reactive control strategies can be expensive, time-consuming and option-limiting.

Proactive strategies, such as the use of alternative herbicides, can incur similar costs to reactive strategies in the year imposed. However, proactive strategies can be interspersed throughout the rotation, whereas reactive management strategies require replacement of glyphosate for several years. The decision to implement a proactive strategy would be improved if based on a decision tool that allows the operator to rate the level of resistance risk, and to weigh this risk against the cost of the alternative tactical options. One potential, simple to use tool is a nomograph.

2. METHODOLOGY

Nomographs were first developed by d'Ocagne in 1899 and have been used in early twentieth century engineering, aeronautical and military applications.[14] The mathematically derived structure of the completed nomograph is generally simple in appearance and easy to use. It allows a user to obtain an answer rapidly by connecting points between axes, instead of needing to complete mathematical calculations.[15]

Fine[16] first proposed a mathematical model for occupational health and safety risk assessment nomographs. This model has since been expanded.[17,18] Qualitative categories for probabilities, exposure and consequences were assigned numerical scores and the nomograph constructed by solving mathematical equations. A risk assessment score can be easily determined from the nomograph.. The resulting risk of occurrence of glyphosate resistance may be determined using a similar method.

The three key factors considered to have the greatest influence on the development of glyphosate resistance were used as the basis for developing the resistance nomograph. These are the history of glyphosate use, the frequency of use of glyphosate and the use of cultivation. Other factors such as weed density, size of area to be treated and the resistance gene frequency, though important, were considered not to exert the same level of influence and were not used in the development of the risk calculator.

10.1.1. 2.1 Number of generations since first glyphosate use

The histories of the glyphosate resistant biotypes examined in this exercise suggest that resistance is first noted after many years of glyphosate use. Detection of herbicide resistance in the field tends not to occur until 10-30% of the weed population is resistant. This may be several years after resistance first evolves.

Time since first use and frequency of use have been treated separately to account for potential fitness penalties. It has been reported[19,20] that glyphosate resistance within a population of *L. rigidum* can decline in the absence of selection pressure. It follows that one or more years without glyphosate use may reduce the risk of resistance developing.

10.1.2. 2.2. Frequency of use

The frequency of use needs to be considered in terms of the life cycle of the weed of concern. For *L. rigidum*, which has an annual life cycle, the same generation of that species is treated regardless of the number of herbicide applications during the season. Multiple applications only serve to increase the percentage of the population that is treated by picking up later germinators, or to increase the selection pressure upon individuals that receive several spray applications during the one season. The rate of development of resistance in *L. rigidum* shows a pattern of resistance evolution after about 15 years, or 15 generations, of continuous glyphosate selection pressure.[21]

10.1.3. 2.3 Cultivation

The trend towards less soil disturbance at sowing (e.g. conservation tillage, minimum tillage, no-till farming) has reduced, or removed, the potential for cultural control of weeds at sowing. Reduced cultivation places greater pressure on glyphosate, as individuals that have survived a pre-season glyphosate application may escape the physical disturbance or be too large and advanced for effective control by selective herbicides later in the season. Without adequate soil disturbance for weed management, survivors of pre-season herbicides are therefore more likely to reach maturity and set seed.

10.1.4. 2.4 Assigning scores for risk calculations

In developing the calculator model, it has been assumed that a logarithmic relationship exists between the risk of glyphosate resistance occurring and both the frequency of use and the number of *L. rigidum* generations since first treatment with glyphosate. In developing a risk calculator for occupational health and safety, Fine[16] employed logarithmic scales that differed only slightly for several factors. The scale used for the probability factor for glyphosate resistance risk was arbitrarily chosen for assigning ratings to the number of generations since first treatment with glyphosate (Table1). It has been reported that a 40% and 20% decrease in frequency of resistant *L. rigidum* plants in four resistant populations occurred after one and two years absence of glyphosate selection pressure respectively.[20] For this risk calculator, it has been assumed that the level of resistance would not return immediately to the initial level after the next use of glyphosate and that increasingly intermittent glyphosate use decreases the risk of glyphosate resistance occurring. The factors used for frequency of glyphosate use were chosen to reflect the expected long term nett effect of different frequencies of use. A linear relationship was assumed between the percentage soil disturbance and the level of *L. rigidum* control achieved from a single cultivation and so a linear score was assigned to this factor.

A detailed explanation of the mathematical derivations of nomographs is given by Allcock.[23] The relevant determinants used in the construction of this risk calculator are explained below. While the order in which the factors are treated during the construction of the nomograph may alter the scale on each vertical axis and the spacing between the axes, it does not alter the final risk score.

The *L. rigidum* resistance risk calculator was constructed by employing two nomographs consecutively to allow all three factors to be taken into account. Frequency of use and generations since the first use of glyphosate were used to derive a combined score in the first nomograph. This was then used, together with the level of soil disturbance, in the second nomograph to provide an overall risk score. The risk score (R) for a particular situation can be calculated by multiplying scores for the frequency of use (F) by the number of generations since the first use of glyphosate (L) and by the level of cultivation (C).

$$F \ x \ L \ x \ C = R$$

This equation can then be solved in two stages, each involving three variables, by forming two equations

$$F \ x \ L = T \quad \text{and} \quad T \ x \ C = R$$

where T represents the product of the first two factors as displayed on a tie-line (T). Logarithmic transformation allows these equations to be converted to a form appropriate for use in a nomograph

$$\log(F) + \log(L) - \log(T) = 0$$
$$\log(T) + \log(C) - \log(R) = 0$$

A construction matrix[23] can be formed for each nomograph in the standard form

$$\begin{bmatrix} -\delta_1 & \mu_1 \cdot f_1(a) & 1 \\ 0 & \dfrac{-\mu_1 \cdot \mu_3 \cdot f_2(b)}{\mu_1 + \mu_3} & 1 \\ \delta_3 & \mu_3 \cdot f_3(c) & 1 \end{bmatrix} = 0$$

where δ_1 is the distance between the first two axes, δ_3 is the distance between the central and right hand axes, μ is the scale factor used on each axis and f_1 (a), f_2 (b) and f_3 (c) are the functions for each of the factors. The distances between axes and the scale in each axis are related according to

$$\delta_1 \cdot \mu_3 = \delta_3 \cdot \mu_1$$

The finished size of each of the nomographs was arbitrarily chosen to be 10x10cm to produce a finished risk calculator to fit on a single page. The axes spacing and scale factors were determined to provide construction matrices for both nomographs and the outside axes were then drawn parallel to the central axis according to the derived values of δ_1 and δ_3.

Graduations were marked along the outside axes according to the derived scale factors μ_1 and μ_3. A diagonal line was then drawn from the lowermost value on one outside axis to the uppermost value on the other outside axis to determine the position of the corresponding value on the central axis, which can be derived numerically by solving the original equation. This axis was then graduated according to the value derived in the construction matrix.

The risk calculator was then constructed based on the combined nomographs to allow rapid estimation of the risk of resistance occurring (Figure 1). The most extreme situations extend outside the range of the tie-line and the risk score axis of the completed risk calculator due to practical limitations.

In the case of the combination of high risk practices, it is likely that resistance has already occurred. Combinations of factors may result in a value in excess of the maximum or minimum on the tie-line or risk score. In these cases, the maximum or minimum can be assumed to reflect the status of the population. Calculations were completed in order to provide a functional scale on the resistance risk axis for factor combinations of most interest.

2.5 Extension of the risk calculator

An extension of the risk calculator can be to refine glyphosate usage, estimate the risk of resistance occurring and determine which weed management options provide an acceptable risk, particularly in regard to the sustainability of glyphosate. Where the extended herbicide resistance calculator is employed to assess the sustainable use of glyphosate, consideration needs to be given to the resistance levels present in the weed population to alternative herbicides. If survivors of a glyphosate application can not be controlled by alternative herbicides due to the presence of multiple herbicide resistance, there is an increased risk of the glyphosate resistant plants reaching maturity and contributing seed to the seedbank. There will be many cases where seed samples from that particular field have never been tested for resistance, so a best guess is needed based on observed levels of control. If the total weed population is low, it can be argued that all herbicide groups are still working effectively. For *L. rigidum*, however, results from a previous study[24] and from a commercial seed testing service[5] suggest that a majority of *L. rigidum* populations are resistant to at least one herbicide group. Therefore, if the resistance status of the *L. rigidum* population is not known, it should be assumed that there is resistance to one herbicide group. From results obtained over more than 15 years by a commercial herbicide testing service,[5] there appears to be an inverse relationship between total number of herbicide mode of action groups and percentage of samples with resistance. Based on these observations, risk scores for this model were assigned for the number of herbicide groups to which the *L. rigidum* is known to be resistant (Table 2).

Having defined chemical options for weed control, consideration then needs to be given to alternative integrated weed management (IWM) options. These include grazing, fodder conservation, green manure crops, chaff carts to collect weed seed at harvest, spraytopping where appropriate, and crop residues burning to reduce the amount of weed seed entering the seed bank. For this model it has been assumed that each of these options potentially provide similar levels of weed control. Therefore, when multiple options are used, there should be a logarithmic increase in the cumulative level of weed control obtained. Risk scores similar to those used by Fine[16] have been assigned.

The length and structure of the field rotations used will impact upon alternative weed control options. Mixed farming enterprises have options to include grazing (both continuous and strategic stocking) in the field rotation, whereas animal based IWM strategies are less available in continuous cropping situations.

With these variables defined, it is then possible to estimate the sustainability of glyphosate under the current and proposed management practices. The final line in the calculator gives an indicator value for the sustainability of glyphosate use (Figure 2).

2.6 Example use of the calculator

To demonstrate the calculator, it is assumed that glyphosate has been used many times in the last 10 years on a moderate *L. rigidum* population and that there has been full soil disturbance at sowing. A line is drawn through the first two points and continued to the tie line, as illustrated in Figure 3. A straight line is then drawn from this point to the third point to determine resistance risk score.

A high risk value indicates that there is risk of glyphosate resistance occurring unless sufficient IWM options are available to minimize seed set in the current season and manage the weed population in the coming season. To determine if sufficient IWM techniques can be used to maintain the sustainable use of glyphosate, the risk score value is transferred to the second calculator to indicate that the intended farm practices on that field are sustainable.

It is also assumed that the *L. rigidum* in this field has been tested for resistance and that no resistance has been detected. It is assumed also that the farmer has in place a long rotation that includes several alternative methods of weed control. A line can be drawn connecting the points on the first two lines, and is then extended to meet the tie line (Figure 4). A second line can then be drawn from here through the third point to the last axis to provide a sustainability rating for the field.

3. DISCUSSION

Glyphosate resistance has evolved over similar time periods in horticulture and broadacre crops. Weed control in horticultural and amenity situations is generally undertaken with non-selective herbicides and can occur throughout the season, whereas in broadacre production, glyphosate use is restricted to pre-season application, in fallows and via shielded sprayers in crops such as lupins.[25]

Modelling research[26,27] suggests that proactive management of resistance is a better strategy to adopt if resistance is expected to occur before a critical break-even point. The risk calculator is not only a tool for assessing the risk of glyphosate resistance occurrence, but also as a method for visualising the value of proactively using IWM practices within a resistance management plan framework. Increasing the number of IWM techniques employed for weed control can provide for greater sustainability of glyphosate use.

The calculator is not designed to provide a definitive value for resistance, but rather an indicative rating to assist land managers in selecting suitable weed management strategies to minimise glyphosate resistance. Extreme situations, such as annual use of glyphosate for lengthy periods and with minimal soil disturbance, are difficult to assess accurately using this calculator but should be ranked as high risk.

There are limitations in the accuracy of this risk calculator. The lack of adequate field history details over an extended period, for example, increases the degree of subjectivity in the determination of each of the factor levels used in calculating the risk score but the methodology is still useful. The use of the risk score determined using this calculator is also subject to interpretation by the land manager. An acceptable level of risk for one person may not be acceptable for another person and therefore the responses are likely to vary between land managers.

The proposed calculator is a tool that can be easily used by advisors and land managers to assess the risk of glyphosate resistance evolving and to assess the sustainability of management practices to minimise this risk. The calculator can be reproduced on a small card that can be conveniently carried in a pocket or vehicle. Although the focus of this study has been on managing glyphosate resistance in *L. rigidum*, similar risk calculators could be developed for resistance to other herbicide modes of action or for other weed species. The assumptions would need to be re-evaluated based the characteristics of the alternative herbicide and the biology and ecology of the alternative weed. In all cases the calculator can be refined as knowledge improves.

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Table 1. Scores assigned for calculation of resistance risk. The factor scales were based on probability factors for generations of use and frequency, and a linear scale assigned for level of soil disturbance.

Factor	Classification	Score
Generations since first use of glyphosate	1 generation	1
	5 generations	3
	10 generations	5
	15 generations	7
	20 generations	9
	25 generations	10
Frequency of use	Every generation	10
	1 in 2 generations	5
	1 in 3 generations	3
	1 in 5 generations	1
	1 in 10 generations	0.5
Cultivation	Full soil disturbance	1
	¾ soil disturbance	2
	½ soil disturbance	3
	¼ soil disturbance	4
	Minimum soil disturbance	5

Table 2. Scores assigned, based on herbicide groups with resistance and IWM methods used, for calculation of sustainability of glyphosate under current and proposed management practices

Factor	Classification	Score
Herbicide groups with resistance	0	0.5
	1	2
	2	6
	3	9
	4	10
Additional IWM methods used	1	10
	2	7
	3	4
	4	2
	5	1