Diflufenican resistance in wild radish (*Raphanus raphanistrum* L.): its discovery and consequences for the lupin industry

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**Summary** We reported the first population of wild radish resistant to diflufenican, a Group F herbicide which targets the enzyme phytoene desaturase, in 1999. This was the first documented case of diflufenican resistance in any plant in the world. Since then, sixteen Western Australian wild radish populations have been confirmed resistant to diflufenican. It is highly likely that there are more wild radish populations, already resistant or developing resistance to diflufenican, that have not been detected.

To date, diflufenican is the herbicide of choice for wild radish control in lupins and farmers whose paddocks are infested with diflufenican-resistant wild radish are already finding it difficult to manage the resistant population. This is further exacerbated in cases where multiple resistance to both diflufenican and triazines occurred. If this multiple resistance becomes widespread, it will have serious consequences for the lupin industry. This paper reports the discovery of diflufenican resistance in Western Australia and discusses why diflufenican resistance is a threat to the lupin industry followed by suggestions on how growers can confront this threat.

**Keywords** Diflufenican resistance, wild radish, lupin.

**INTRODUCTION**

A significant challenge to the success of growing lupins is the control of broadleaf weeds, especially wild radish (*Raphanus raphanistrum* L.) which is an extremely well-adapted weed of Australian agriculture. In Western Australia (WA), lupins have been grown for many years in rotation with wheat in the northern agricultural region. In some farms this lupin-wheat rotation has been going on for over 20 years. The lupin-wheat rotation however, builds up wild radish numbers very quickly.

Wild radish is the most troublesome weed in lupins. Both dormancy and prolonged viability of its seed are responsible for the persistent seed banks of wild radish and contribute to the annual recurrence of this weed. Its prolific seed production, apart from recharging the seed bank, is also a concern because contamination of the harvested lupin grain can result in dockages being applied by marketing agencies. More recently however, it is its increasing levels of resistance to herbicides commonly used for wild radish control that is posing a real challenge to its management into the future.

This paper looks at the discovery of diflufenican resistance in wild radish, its cross resistance to picolinafen and the presence of populations with multiple resistance across several modes of action. The paper also discusses why diflufenican resistance is a threat to the lupin industry and how lupin growers can confront this threat.

**THE HERBICIDE DIFLUFENICAN**

Today, a total of at least fifteen diflufenican products are available across Australia. This recent increase in diflufenican products is the result of expiry of the diflufenican patent in November 2001. Diflufenican being a Group F herbicide, is a carotenoid biosynthesis inhibitor that was first marketed as Brodal® in 1987 mainly for wild radish control in lupins. Prior to the introduction of Brodal, the use of triazines, especially simazine, was the only chemical option for controlling wild radish in lupins. However, simazine on its own has never given satisfactory control of wild radish in most years because its activity is too dependent on soil moisture. It was the introduction of Brodal as a post-emergence spray for the control of wild radish in lupins that has become a great success story. Farmers however, are aware that the activity of diflufenican very much depends on the dosage, size of the weed at the time of spraying and whether the wild radish has been unduly moisture stressed. Despite this, its use has been extended to include mixtures containing diflufenican and another herbicide of a different mode of action, to broaden its use in cereals and pastures. Tigrex® (diflufenican + MCPA) and Jaguar® (diflufenican + bromoxynil) were the only mixtures available before the expiry of the diflufenican patent. Today, ten of the diflufenican products are straight diflufenican and five are mixtures containing diflufenican and another herbicide, such as MCPA (Group I) or bromoxynil (Group C).
HISTORY AND CONFIRMATION OF DIFLUFENICAN RESISTANCE

In 1999, the first population of wild radish resistant to diflufenican was confirmed (Cheam et al. 2000a, Cheam et al. 2000b). This was the first reported case of diflufenican resistance in any plant in the world. The plants that survived showed herbicide symptoms and their growth was retarded, the severity of the retardation increased at higher rates. At the highest recommended rate of Brodal (500 g a.i. L⁻¹) at 200 mL ha⁻¹, the percentage of plants that survived ranged from 70 to 80% at four weeks after spraying. The survivors flowered and set seed successfully in the field.

This first resistant population was collected from a farm in the northern agricultural region of WA. An examination of the history of herbicide use on the farm revealed that the wild radish population had been exposed to only four applications of diflufenican. Prior to this confirmation, there had been missed opportunities to test for resistance in suspected populations because poor weather conditions had been too frequently blamed for the poor herbicide activity.

In studies involving diflufenican and triazines, triazine compounds have been shown to widen the window of application of diflufenican and increase the level of efficacy (Sermon 1998, Burgess 1999). Because of this, it may be difficult to detect the survival of wild radish plants within a population that have already evolved resistance to diflufenican in a wet year due to the simazine activity (Cheam et al. 2000a).

To date, out of the fifty or so samples of wild radish collected from various parts of WA, sixteen populations have been confirmed to be resistant to diflufenican (Cheam 2003). Most of them came from the northern agricultural region and only four applications of diflufenican were needed for resistance to develop in some populations. Apart from these confirmed cases, it is likely that there are populations out there that are developing resistance to this herbicide. This resistance problem is likely to be exacerbated by the recent spate of diflufenican products available on the market following the expiry of the diflufenican patent.

CROSS RESISTANCE TO OTHER GROUP F HERBICIDES

Two other Group F herbicides currently recommended for wild radish control in lupins and chickpea are picolinafen (Sniper®) and isoxaflutole (Balance®), respectively. Although both herbicides have been placed under the general grouping F under the Australian herbicide classification system, the international Herbicide Resistance Action Committee (HRAC) classification system, places both diflufenican and picolinafen under Group F1 and isoxaflutole under Group F2 (Schmidt 2002). Unlike the Australian system, the HRAC system classifies herbicides not only according to their modes of action and similarity of symptoms or chemical classes, but also according to their site(s) of action. Picolinafen and diflufenican target the same enzyme phytoene desaturase (PDS) whereas isoxaflutole inhibits the enzyme 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD).

Based on the more elaborate HRAC classification system, cross resistance of the diflufenican resistant wild radish to picolinafen was therefore suspected. Experiments confirmed there was cross resistance to picolinafen (Cheam and Lee 2002a, Cheam and Lee 2002b).

Picolinafen at 37.5 g a.i ha⁻¹, the highest recommended label rate, readily killed the susceptible population with few survivors. No survivors were recorded at 112.5 g a.i. ha⁻¹, which is three times the highest recommended rate. In contrast, at 37.5 g a.i. ha⁻¹, 82% of the resistant population survived and at 112.5 g a.i. ha⁻¹, there was still 27% survival in the resistant population. Based on the LD50 ratio, the resistant population was 3.4-fold more resistant to picolinafen than the susceptible population (Table 1).

This is the first documented case of cross resistance of diflufenican resistant wild radish to picolinafen. This confirmation sent out an important message that it is essential to test for cross resistance to picolinafen in all cases of diflufenican resistant wild radish so as to avoid unnecessary control failures.

As expected, there was no cross resistance to isoxaflutole.

MULTIPLE RESISTANCE ACROSS OTHER HERBICIDE GROUPS

The challenge ahead for the control of wild radish lies in our ability to control populations that have developed multiple-herbicide resistance across several modes of action. The first documented wild radish population resistant to diflufenican was also resistant to the Group B herbicides, the acetolactate synthase-inhibiting herbicides (Cheam et al. 2000a). This particular population had been exposed to only five applications of Group B herbicides. Determination of the resistance status of the remaining fifteen diflufenican resistant populations in our collection revealed that the

<table>
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<th>Table 1.</th>
<th>The concentration of picolinafen required to kill 50% (LD50) of the resistant and susceptible populations of wild radish.</th>
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<tr>
<td></td>
<td>LD50 (g a.i. ha⁻¹)</td>
</tr>
<tr>
<td>Resistant (R)</td>
<td>114.3</td>
</tr>
<tr>
<td>Susceptible (S)</td>
<td>33.7</td>
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[Fourteenth Australian Weeds Conference]
most common combination involved Groups F and B. Another population collected by Walsh et al. (2004), besides being resistant to Groups F and B, was also resistant to 2,4-D amine (Group I). Of the different combinations, the one that poses the greatest threat to lupin growers is the multiple resistance to Groups F and C. One population in our collection had multiple resistance to diflufenican and triazines. Walsh et al. (2004) also collected one population resistant to diflufenican, metribuzin and 2,4-D. With multiple resistance to diflufenican and triazines, growing lupins or TT-canola using current varieties may not be possible.

MULTIPLE RESISTANCE TO GROUPS F AND C: CONSEQUENCES AND SOLUTIONS

Given the importance of diflufenican and triazines in Australian agriculture, especially for controlling wild radish in lupins, the evolution of resistance to both herbicide groups is considered a significant development that should be closely watched. In this regard, short and long-term studies were undertaken on one of these confirmed populations in our collection (Cheam et al. 2003).

The short-term approach involved the screening of herbicides to determine which are still effective on the population and which are not. The long-term approach involved field trials to run down the wild radish seed bank as quickly as possible.

The herbicide screening work reinforced the concerns that once wild radish has evolved multiple resistance to diflufenican and triazines, there is a lack of alternative herbicides for its control in the lupin phase of a cropping rotation. All three herbicides, diflufenican, picolinafen and simazine (on their own and in mixtures) currently recommended for wild radish control in lupins, were found to be no longer effective on the resistant population. The common mixture, diflufenican + metribuzin, also failed to control the resistant population R compared with the susceptible S (Table 2).

In the wheat cropping phase however, the population can still be controlled with a good range of herbicides, one of which is the product Jaguar (diflufenican + bromoxynil). As diflufenican and bromoxynil are classified under Group F and C, respectively, based on the present Australian herbicide classification system, the interpretation of the guidelines would suggest that the mixture is unlikely to be effective. This is because the population is already resistant to both F and C groups of herbicides. However, under the HRAC classification system, which takes into consideration the site of herbicide action, bromoxynil is placed under Group C3 and the triazines and metribuzin under C1 (Schmidt 2002). Unlike the triazines, bromoxynil has a different binding behaviour at the binding protein D1 in photosystem II. This protein is also called the herbicide or Qb binding protein (Pfister et al. 1981, Kyle 1985). Trebst (1987) grouped herbicides that bind in the Qb niche into two families based on their interaction with amino acids at this site: the triazine/urea family which shows a strong interaction with Ser 264 and the phenol family that interacts strongly with His 215. Bromoxynil, being a nitrile, belongs to the phenol family. According to Trebst (1991), mutations in triazine resistance lead to an increased sensitivity to phenol-type herbicides. This, together with the fact that bromoxynil has an additional mode of action involving membrane disruption (Schmidt 2002) probably accounts for the effectiveness of the bromoxynil + diflufenican mixture.

Another significant result was the effectiveness of the mixtures diflufenican + MCPA and picolinafen + MCPA, despite the resistance of the population to one of the components in the mixture. This was clearly evident in the suppression of the wild radish biomass rather than plant mortality. Therefore, once a herbicide is no longer effective on a population due to resistance, mixing it with the appropriate herbicide having a different mode of action may result in control of the resistant population, as shown in this study. This approach should be taken seriously, since it allows the continuous use of the herbicide to which the population has evolved resistance.

The long-term experiment highlighted the difficulty in achieving an acceptable level of wild radish seed bank control within a short period of time. The ability to control the population at the outset is crucial. A single break year of very little seed set was found to have a dramatic impact on reducing the wild radish seed bank. Any seed set management tactic which prevents fresh seed input would be beneficial because of the slow exhaustion of the wild radish seed bank due to its long-term seed dormancy and longevity. Despite three years of intensive

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<th>Herbicide and rate (g a.i. ha⁻¹)</th>
<th>% Survival</th>
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<tr>
<td>Diflufenican (100)</td>
<td>55.1</td>
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<tr>
<td>Diflufenican + simazine (50 + 500)</td>
<td>47.9</td>
</tr>
<tr>
<td>Diflufenican + metribuzin (50 + 75)</td>
<td>22.0</td>
</tr>
<tr>
<td>Picolinafen (37.5)</td>
<td>60.2</td>
</tr>
<tr>
<td>Picolinafen + simazine (18.7 + 500)</td>
<td>52.3</td>
</tr>
<tr>
<td>Picolinafen + metribuzin (18.7 + 75)</td>
<td>44.0</td>
</tr>
<tr>
<td>Atrazine (1000) + 1% Ulvapron</td>
<td>100</td>
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</tbody>
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Table 2. Response of susceptible S and multiple resistant R (diflufenican and triazines) populations of wild radish to herbicides commonly used in the lupin phase.
control, involving selective herbicides in the first year, and non-selectives in conjunction with cultivation and mowing and green and brown manuring in the second and third year in a pasture situation, the level of seed bank depletion is still inadequate. Monitoring of this long-term experiment is still in progress, following the implementation of more control options.

CONCLUSIONS

It is certain that the development of herbicide resistance in wild radish will continue as long as herbicides remain the tool of choice for weed control. However, this resistance can be slowed down if herbicides are used judiciously and in combination with other weed control practices. The limited choice of herbicides for wild radish control in lupins will undoubtedly increase the selection for resistance to the two key herbicides, diflufenican and triazines, currently in use. As reported here, the consequences of this multiple resistance to the lupin industry will be disastrous.

With the expiry of the diflufenican patent in 2001 combined with the prolonged use of triazines for the control of wild radish in lupins and TT-canola, there can be no doubt that more populations of wild radish evolving multiple resistance to diflufenican and triazines will continue to increase. In areas where wild radish is still susceptible to both herbicides, preservation of their effectiveness should be given high priority. In this regard, it will be wise to avoid using products containing diflufenican in the wheat phase of the lupin-wheat rotation because of our current dependence of this herbicide in the lupin phase. Likewise, because of simazine use in the lupin phase, the lupin/TT-canola rotation should be avoided to prevent the use of triazines in successive years. Therefore, using herbicides sustainably is a real challenge and all precautions must be taken to preserve the efficacy of the various herbicides still available for the control of wild radish.

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REFERENCES