High Frequency of Chlorsulfuron-Resistant Wild Radish (Raphanus raphanistrum) Populations across the Western Australian Wheatbelt

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Research

High Frequency of Chlorsulfuron-Resistant Wild Radish (*Raphanus raphanistrum*) Populations across the Western Australian Wheatbelt[1]

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Abstract: In 1998, field populations of wild radish suspected of being resistant to chlorsulfuron were collected and screened for resistance to this herbicide using the Quick-Test technique. This test successfully identified chlorsulfuron-resistant populations of wild radish. Detailed dose–response experiments with the progeny of these populations confirmed their resistance and validated the use of the Quick-Test for a dicot species. Subsequently in 1999, a random survey was conducted employing this test to establish the current extent of chlorsulfuron resistance in wild radish populations. The survey covered more than 200 fields in the northern, central, and eastern wheatbelt regions of Western Australia. Wild radish plants were collected from wheat crops in 133 of these fields. The Quick-Test method was used to screen these plants with the acetolactate synthase (ALS)-inhibiting herbicide chlorsulfuron. Overall, 21% of randomly collected wild radish populations were found to be resistant to chlorsulfuron.

Nomenclature: Chlorsulfuron; wheat, *Triticum aestivum* L.; wild radish, *Raphanus raphanistrum* L.
Additional index words: Herbicide resistance, Quick-Test, resistance survey.
Abbreviations: ALS, acetolactate synthase; WA, Western Australia.

INTRODUCTION

In Western Australia (WA), growers rate wild radish (*Raphanus raphanistrum*) second only to annual ryegrass (*Lolium rigidum* Gaud.) as the most problematic weed of crop production (Alemseged et al. 2000). It is widely dispersed across the WA wheatbelt, where it competes vigorously with crops for water, light, and nutrients. Code and Reeves (1981) found that in northeast Victoria, 10 and 200 wild radish plants/m^2 reduced wheat (*Triticum aestivum*) yields by 20 and 50%, respectively. Moore (1979) reported similar yield reductions due to wild radish competition in WA. Furthermore, wild radish infestations hinder harvest because their fibrous stems block machinery, and seed pods contaminate the harvested grain, incurring dockage when the grain is sold. Wild radish also can act as an alternative host to a number of important plant pests and diseases of both crops and pastures (Cheam and Code 1995).

Chlorsulfuron, the first acetolactate synthase (ALS)-inhibiting herbicide introduced in 1982, is toxic to wild radish and other important crop weeds. As a result, chlorsulfuron and the closely related triasulfuron have been used extensively for wild radish control. Resistance to chlorsulfuron was first reported in 1987 in a population of prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al. 1990). Since then there has been a steady increase in the number of weed biotypes that have developed ALS herbicide resistance (reviewed by Saari et al. 1994). By 1999, ALS herbicide resistance had developed in 83 weed species worldwide (Heap 2000), including 14 in Australia (Adkins et al. 1997; Boutsalis and Powles 1995). Resistance develops particularly rapidly to ALS herbicides and in some instances has developed with as few as four applications (Gill 1995). The extensive use of ALS herbicides throughout the WA wheatbelt has led to widespread resistance. Since the first report of ALS resistance in annual ryegrass (Christopher et al. 1992), many thousands of ryegrass populations now exhibit resistance to ALS-inhibiting herbicides and other herbicide groups (Gill 1995). A survey conducted across the WA wheatbelt in 1999 by Llewellyn and Powles (unpublished data), determined that 38% of randomly selected annual ryegrass populations were resistant to chlorsulfuron. Similarly, at least 100 wild radish populations from WA farms exhibited resistance to chlorsulfuron and other ALS-inhibiting herbicides (Hashem

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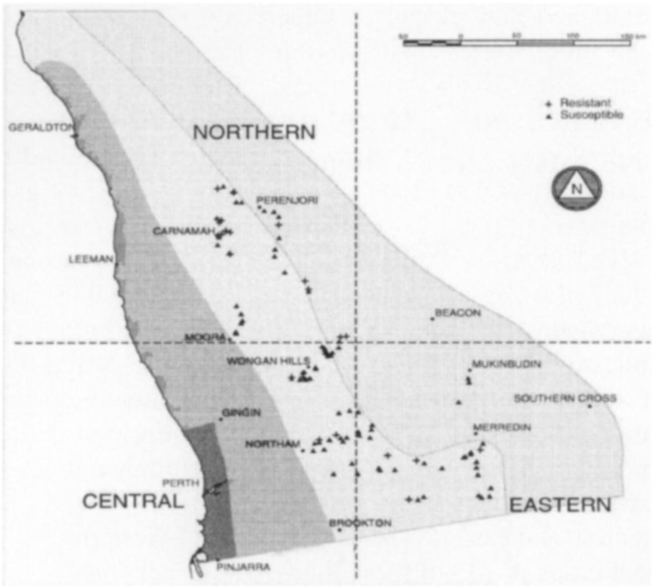

Figure 1. Survey region and collection sites for wild radish populations treated with chlorsulfuron. The survey region embraced some 8,000 km² of the WA wheatbelt covering latitudes 29°00′S to 32 °00′S and longitudes 115°00′E to 118°00′E.

2001). However, the current frequency of resistant wild radish populations throughout the WA wheatbelt is unknown.

The development of the Quick-Test procedure[3] for herbicide resistance allows for a more rapid screening of weed populations than the conventional seed-based techniques (P. Boutsalis, personal communication). Although this test was developed for monocot weed species, the method also may be applicable to dicot weed species. However, the reliability of screening populations of dicot weed species for resistance using this method is still unknown. The objectives of this research were to establish the effectiveness of the Quick-Test in screening for herbicide resistance in a dicot weed species and to determine, with the aid of this method, the current frequency of herbicide resistance in randomly sampled wild radish populations from the WA wheatbelt.

MATERIALS AND METHODS

Validation of the Quick-Test. In 1998, three suspected resistant populations of wild radish were screened for resistance to chlorsulfuron using the Quick-Test procedure (Boutsalis 2001). Wild radish plants (ranging in growth stages from 1.3 to 2.2) (Madafiglio et al. 1999) were dug up from crop fields, shaken to remove excess soil, placed in plastic bags, and posted to our laboratory by overnight express. On receipt, root material was trimmed and the foliage was pruned leaving only the two youngest leaves. The seedlings were transplanted (four plants/pot) into 15-cm-diam plastic pots containing potting mix (50% composted pine bark, 25% peat, and 25% river sand). Seedlings were watered, fertilized, and maintained in a greenhouse (25/18 C) at the University of Western Australia's Nedlands campus. Once the transplants had re-established and were growing vigorously (7 to 10 d after transplanting) they were treated with 15 g/ha chlorsulfuron plus 0.2% (v/v) of a nonionic surfactant.[4] Chlorsulfuron was applied in 220 L/ha water, delivered in two passes at 200 kPa using a cabinet sprayer equipped with two flat fan nozzles.[5] Plants were returned to the greenhouse immediately after the treatment. Ten days later, the survivors were trimmed to the two youngest leaves and allowed to regrow for 7 to 10 d, after which they were treated with chlorsulfuron at 15 g/ha.

Plants that survived both herbicide treatments were classified tentatively as herbicide resistant. To fully establish and quantify resistance, the surviving plants were grown to maturity in the greenhouse in enclosures that prevented cross-pollination. Hand pollination between plants within each population was carried out at intervals throughout the flowering period, and seeds were collected from the plants at maturity. In the following growing season (1999), these seeds were germinated and seedlings were established in the same greenhouse in 15-cm-diam plastic pots containing potting mix (12 seeds/pot at 1 cm deep). The two suspected resistant wild radish populations (designated RR2 and RR3) and a known susceptible population (RR1) were treated at growth stage 1.2 to 1.3 (Madafiglio et al. 1999) with chlorsulfuron at rates of 0, 7.5,

Table 1. Fields surveyed, wild radish populations collected, and number and proportion of resistant wild radish populations across the Western Australian wheatbelt.

Region	Fields surveyed	Wild radish fields: Infested	Wild radish fields: Chlorsulfuron resistant
	no.		
Northern	64	50	19 (38%)
Central	74	51	5 (10%)
Eastern	67	34	5 (15%)
Total	205	135	29 (21%)

[3] Quick-Test procedure developed and patented by Novartis Crop Protection, WST-149, Stein 432, Switzerland.

[4] Wetter TX (1,040 g/L octyl phenol ethoxylate). Monsanto Co., 800 N Lindbergh Boulevard, St. Louis, MO 63167.

[5] Hardi 4110-10 flat fan nozzles, Hardi International, 1500 West 76th Street, Davenport, IA 52806.

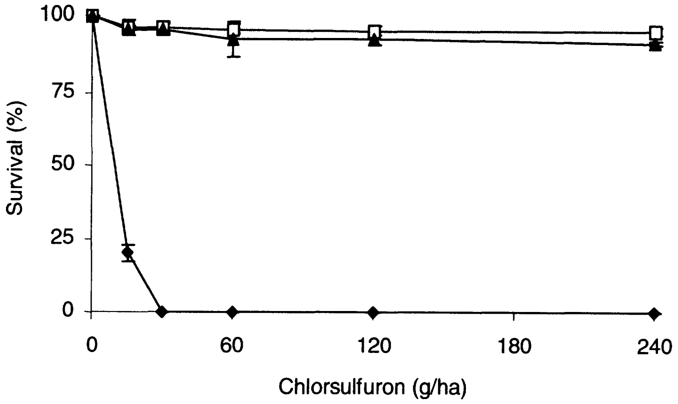

Figure 2. Percent survival of two resistant (RR2 □, RR3 ▲) and a susceptible (RR1 ♦) biotype of wild radish in response to the application of chlorsulfuron. Standard errors were calculated for comparison of differences in mortality rates between populations at each application rate.

15, 30, 60, and 120 g/ha. When this experiment was repeated 2 wk later, rates were altered slightly to 0, 15, 30, 60, 120, and 240 g/ha. Herbicide treatments were applied as described above. Mortality was scored 35 d after treatment and was expressed as a percentage of the number of established plants in each pot prior to treatment. The experiments were established as a randomized design with four replicates, where each pot constituted a single treatment replicate. Average mortality values were calculated from the mean responses from the four replicates and were plotted as a function of herbicide rate. Standard errors were calculated for comparison of differences in mortality between populations at each application rate.

Chlorsulfuron Resistance Survey. Random surveying of wild radish populations for herbicide resistance was conducted across the northern, central, and eastern regions of the WA wheatbelt (Figure 1). The survey was conducted during the growing season months of June and July 1999, shortly after application of postemergence herbicide treatments. Wild radish plants were collected by randomly selecting wheat fields and then searching an area of approximately 500 m^2 in each field. Within these areas, wild radish seedlings were exhumed and placed in paper bags for transplanting the following day. At the time of collection, wild radish plant growth stages ranged from 1.1 to 2.2 (Madafiglio et al. 1999). At each survey site, a hand-held global positioning system (GPS) unit[6] determined the latitude and longitude coordinates. Visual ratings of wild radish plant densities were recorded for each surveyed field; densities were determined to be in one of the following five categories: None (no visible plants in survey area), very low (< 1 plant/m^2), low (1 to 5 plants/m^2), medium (6 to 10 plants/m^2), and high (> 10 plants/m^2). Collected wild radish seedlings were transported to our laboratory via overnight express post where they were processed and screened for resistance as described above.

RESULTS AND DISCUSSION

Validation of the Quick-Test. Two suspected chlorsulfuron-resistant populations (RR2 and RR3) collected from wheat fields in 1998 (Mullewa region of WA) were identified using the Quick-Test as being resistant. Detailed testing in 1999 established these populations as highly resistant (Figure 2) with over 90% of plants surviving 16 times the recommended rate of chlorsulfuron. As expected, plants of the known susceptible population were killed at the recommended rate (15 g/ha) of chlorsulfuron. The dose–response experiments verified the effectiveness of the Quick-Test in identifying chlorsulfuron-resistant populations of wild radish.

The effectiveness and the flexibility of the Quick-Test (Boutsalis 2001) for rapidly identifying herbicide resistance in dicot weed species will provide major benefits in the diagnosis of herbicide resistance. This test, when compared with conventional seed testing procedures (Moss 1995), provides a substantially faster, yet similarly accurate, method of determining the

[6] Lowrance Globalnav 212 Handheld GPS unit. Lowrance Electronics Inc. P.O. Box 129, Catoosa, OK 74015-0129.

Table 2. Proportions of the total wheat fields surveyed and categorized into four wild radish density classes. Numbers in parentheses indicate number of resistant populations.

Region	Proportion wild radish in density class					
	None	Very low (< 1 plant/m^2)	Low (1–5 plants/m^2)	Medium (6–10 plants/m^2)	High (> 10 plants/m^2)	Average
	%					
Northern	14	15 (2)	23 (12)	10 (2)	2 (2)	Low
Central	33	12 (3)	17 (1)	4 (1)	1	Very low
Eastern	24	32 (2)	11 (3)	6	1	Very low
Total	71	59	51	20	4	

resistance status of a weed population. The Quick-Test as used in this research identified two chlorsulfuron-resistant weed populations in less than 1 mo after resistance was first suspected in the field. This potentially enables herbicide resistance management decisions to be made during the growing season in which the problem is first identified. Clearly, this is not possible using testing procedures that rely on the collection of mature seed at the end of the growing season.

Potential exists to use the Quick-Test to screen a comprehensive variety of dicot weed species with a range of herbicides. In 1999, in addition to wild radish, we used this test to identify chlorsulfuron-resistant populations of other weed species, including Indian hedge mustard (*Sisymbrium orientale* Torn), capeweed (*Arctotheca calendula* L.) and fumitory (*Fumaria densiflora* DC.) (data not presented). Other dicot weeds that have been successfully screened for herbicide resistance using this technique include common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), and hairy beggarticks (*Bidens pilosa* L.) (Boutsalis 2001). Additionally, the Quick-Test was used in 1999 to identify several randomly selected populations of wild radish as resistant to atrazine (data not presented). Therefore, it is likely that this test is suitable for use in a variety of combinations of dicot weed species and herbicides.

Chlorsulfuron Resistance Survey. *Extent of infestations.* Wild radish plants were collected from 133 of 206 (65%) fields surveyed across the wheatbelt regions of WA (Figure 1; Table 1). The vast majority (83%) of these wild radish infestations occurred at low to very low plant densities, generally less than 5 plants/m^2. Although wild radish plant densities were low throughout the survey area, slightly higher populations were observed in wheat crops in the northern region. The average plant density in wheat fields in the central and eastern regions was less than 1 plant/m^2, whereas the average density in the northern region was 1 to 5 plants/m^2 (Table 2). More populations were in the higher density categories for the northern region than for the other two regions.

Herbicide resistance in randomly collected wild radish populations. Twenty-one percent of the 133 randomly collected wild radish populations were resistant. In the northern survey region, 38% of populations were resistant to chlorsulfuron (Table 1). This was more than double the frequency of resistant populations in the central region and almost four times the frequency of populations in the eastern region. Recently, similarly high proportions of around 50% of annual ryegrass populations in this region also were identified as chlorsulfuron resistant (Llewellyn and Powles, unpublished data), suggesting that growers in this area have imposed a high selection pressure for chlorsulfuron resistance. A similar survey conducted in North America identified resistance to chlorsulfuron in 70% of randomly collected Russian thistle (*Salsola iberica*) populations from Washington State (Stallings et al. 1994). Although a very high frequency of resistance was identified in this survey, given results from previous studies it is likely that this frequency will increase further with continued reliance on ALS-inhibiting herbicides.

The Quick-Test is clearly an effective tool for determining the resistance status in dicot weed species. The use of this test will undoubtedly improve the efficiency of diagnosing and, therefore, of managing suspected resistant populations. The Quick-Test may become an invaluable tool for preventing the already extensive levels of chlorsulfuron resistance from increasing. The early detection of isolated patches of resistant weeds in a field using this test procedure will allow procedures to be employed that prevent the spread of resistant individuals. Results from this survey indicate that the extent of chlorsulfuron resistance in randomly selected wild radish populations from the WA wheatbelt is a major concern. Therefore, there is an urgent need to alter management practices in order to prolong the use of this and related ALS-inhibiting herbicides.

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